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1 **Title**

2 Ecology and management history drive spatial genetic structure in Scots pine

3

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18

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20 *Pinus sylvestris*, spatial genetic structure, genetic diversity, forest management, life stages.

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29 **Abstract**

30 Forest management practices that remove trees from stands can promote substantial changes in  
31 the distribution of genetic diversity within and among populations at multiple spatial scales. In  
32 small and isolated populations, elevated inbreeding levels might reduce fitness of subsequent  
33 generations and threaten forest resilience in the long term. Comparing fine-scale spatial genetic  
34 structure (SGS) between life stages (e.g. adult and juvenile cohorts) can identify when populations  
35 have undergone disturbance, even in species with long generation times. Here, we studied the  
36 effects of historical and contemporary forest management, characterized by intense felling and  
37 natural regeneration respectively, on genetic diversity and fine-scale SGS in adult and juvenile  
38 cohorts. We examined fragmented Scots pine (*Pinus sylvestris* L.) stands in the Scottish  
39 Highlands, and compared them with a remote, unmanaged stand. A total of 777 trees were  
40 genotyped using 12 nuclear microsatellite markers. No difference was identified in allelic richness  
41 or gene diversity among stands or life stages, suggesting that historical and contemporary  
42 management have not impacted levels of genetic variation. However, management appears to  
43 have changed the spatial distribution of genetic variation. Adult genotypes from managed stands  
44 were more spatially structured than in the unmanaged stand, a difference mediated by contrasts  
45 in tree density, degree of fragmentation of stands at the time of establishment and rate of gap  
46 creation. Surprisingly, juveniles were less spatially structured than adults in the managed stands,  
47 suggesting a historical erosion of the structure of the adult cohort but contemporary recovery to  
48 natural dynamics, and indicating a high capacity of the species to recover after disturbance. Here  
49 we showed how using the spatial component of genetic diversity can help to detect both historical  
50 and contemporary effects of disturbance in tree populations. Evaluation of successional change is  
51 important to adequately detect early responses of tree populations to forest management practices.  
52 Overall, our study suggests that combining sustainable management with forest conservation  
53 practices that ensure larger effective population sizes is key to successfully maintaining genetic  
54 diversity in Scots pine.

55

56 **1. Introduction**

57 A prolonged history of forest exploitation based on the harvesting of trees has resulted in  
58 widespread modification of Europe's forests, impacting genetic diversity within and among  
59 populations (FAO, 2014). Currently, 15% of European forest is under management (Forest  
60 Europe, 2015) but, despite a substantial shift toward sustainable practices over the past 25 years  
61 (FAO, 2015), the consequences of historical management practices such as extensive felling on  
62 the distribution of genetic diversity in tree species remain largely uncertain. Genetic diversity  
63 plays an essential role in underpinning forest resilience by facilitating evolutionary processes, and  
64 it is key in forest responses to disturbances, such as habitat loss, fragmentation or pathogen attack  
65 (Schaberg et al., 2008; Cavers and Cottrell, 2014). Consequently, understanding how historical  
66 and contemporary forest management have shaped patterns of genetic diversity allows evaluation  
67 of the potential resilience of European forests and informs the development of adaptive  
68 management plans.

69

70 The impact that tree removal can have on population genetics has been addressed through  
71 exploration of levels of neutral genetic variation, revealing changes in gene frequencies (Schaberg  
72 et al., 2008) and loss of alleles (Adams et al., 1998; Rajora et al., 2000; Kettle et al., 2007; Ortego  
73 et al., 2010), yet many studies have failed to detect significant effects (Bradshaw, 2004; García-  
74 Gil et al., 2015; Rajora and Pluhar, 2003; Schaberg et al., 2008; Young et al., 1996). Some authors  
75 attribute the lack of effect to the long generation time in trees, because changes in genetic diversity  
76 after disturbance may take many generations (Lowe et al., 2005). However, changes in tree  
77 distribution and age structures can alter the spatial organisation of genetic variation, even when  
78 overall levels of variation are maintained, allowing us to explore the genetic legacy of forest  
79 management (Piotti et al., 2013; Sjölund and Jump, 2015).

80

81 In naturally regenerated tree populations, genotypes are not distributed randomly. Typically,  
82 individuals become less genetically similar as the distance between them increases (Jump and  
83 Peñuelas, 2007; Paffetti et al., 2012; Vekemans and Hardy, 2004), causing a phenomenon known

84 as spatial genetic structure (SGS). Restricted dispersal results in offspring being more likely to  
85 establish close to the mother tree (Jump et al., 2012; Pandey et al., 2012). Consequently, the  
86 dispersal strategy of pollen and seed will strongly influence the extent and magnitude of SGS  
87 within a species. For example, plants with animal dispersed pollen usually show greater SGS than  
88 those with wind dispersed pollen (Vekemans and Hardy 2004). Furthermore, individual density  
89 is usually inversely correlated with SGS. For example, the extent of SGS in low density  
90 populations of *Acer pseudoplatanus* is nine times greater than in high density populations  
91 (Vekemans and Hardy 2004).

92

93 The ecological determinants of SGS (such as recruitment frequency, seed and pollen dispersal  
94 distance, and individual density) are commonly modified by forest management practices that  
95 remove trees. Consequent changes in SGS may alter local mating patterns and the distribution of  
96 genetic diversity in subsequent generations (Smouse and Peakall, 1999). Furthermore, different  
97 forest management practices, such as felling, coppicing or thinning, will differentially impact  
98 selection of individuals and seedling establishment potentially leading to a broad range of genetic  
99 impacts (Cottrell et al., 2003; Paffetti et al., 2012; Piotti et al., 2013; Sjölund and Jump, 2015).  
100 Distinguishing the effects of forest management on SGS is, therefore, a challenging task.

101

102 SGS of plant populations is dynamic and can change across life stages. In individuals that  
103 reproduce sexually, seedlings might be affected by compensatory mortality and competitive  
104 thinning, post dispersal, thereby altering spatial distribution patterns with age (Ng et al., 2004).  
105 Most studies found greater SGS in early regeneration stages than in mature individuals (González-  
106 Martínez et al., 2002; Hardesty et al., 2005; Ng et al., 2004; Soto et al., 2007; Troupin et al.,  
107 2006). The successional component of SGS (e.g. comparing SGS between adult and juvenile  
108 cohorts) has mainly been studied in order to understand the natural development of SGS (Berens  
109 et al., 2014; González-Martínez et al., 2002; Jones and Hubbell, 2006). Such changes in SGS have  
110 rarely been used to assess the influence of forest management practices (but see Jones et al., 2006;  
111 Leclerc et al., 2015; Troupin et al., 2006).

112

113 This study focuses on the remaining fragmented Scots pine (*Pinus sylvestris* L.) forests of the  
114 Scottish Highlands (known as Caledonian pine forests), which are believed to be the only native  
115 pine forests in the UK. These fragmented remnants represent a valuable system in which to study  
116 the impacts of historical forest management practices because numerous records of management  
117 history exist. To understand the effects of historical and contemporary forest management  
118 practices, we investigated genetic diversity and fine-scale SGS in adult and juvenile cohorts in  
119 two native managed pine forests and compared these with a remote, unmanaged stand. We  
120 selected two life stages that were established in distinct periods with contrasting forest  
121 management systems: (1) adult trees that established during 19<sup>th</sup> Century, characterised by high  
122 browsing pressure by deer and after a period of intense felling (hereafter historical management);  
123 and (2) juveniles that established during the last two decades, characterised by conservation  
124 policies promoting natural regeneration (hereafter contemporary management). Specifically we  
125 sought to determine: 1) did historical management practice impact genetic diversity and SGS –  
126 comparing mature managed and unmanaged stands?, and 2) how has contemporary management  
127 practice affected diversity and SGS – comparing adults and juveniles from managed stands?. We  
128 hypothesised that in the absence of effects of historical management, mature managed stands  
129 would display similar values of genetic diversity and SGS as those in an unmanaged stand, while  
130 in the absence of effects of contemporary management, stronger SGS would be found in the  
131 juvenile stages, and similar values of genetic diversity will be evident in both juvenile and adult  
132 cohorts.

133

## 134 **2. Material and methods**

### 135 2.1. Study species

136 Scots pine is a wind-pollinated outcrossing conifer and is the most widely distributed pine species  
137 in the world, with a range that crosses Eurasia, going from the Arctic circle in Norway in the north  
138 to the south of Spain and south of Turkey and from the west coast of Scotland to the far east of  
139 Russia (Carlisle and Brown, 1968). Populations from southern Europe, Scotland and Asia Minor

140 generally represent isolated occurrences. In Scotland this species occurs at the western limit of its  
141 global distribution and constitutes the iconic species of the Caledonian pine forest. Scots pine is  
142 typically a pioneer species (together with birch and aspen) that readily regenerates after natural or  
143 human disturbances, if competition and grazing pressure are low (Mátyás et al., 2004). It grows  
144 well on most soils, nevertheless, due to shade and competition intolerance, it is often restricted to  
145 poor soils (Steven and Carlisle, 1959). It is a monoecious species, and female flowering can start  
146 at the age of 15 to 30 years, in open to closed stands respectively (Mátyás et al., 2004). Pollen  
147 movement is predominantly over tens of metres within a stand (Robledo-Arnuncio et al., 2004b),  
148 but it may reach 100 km (Robledo-Arnuncio, 2011). Seeds are primarily wind and gravity  
149 dispersed, and typically travel up to 100 metres (Mcvean, 1963).

150

## 151 2.2. Study sites and history of forest management

152 From a peak distribution around 6,000 years ago, Scots pine in Scotland has been in decline for  
153 millennia, with a major retreat 4,000 years ago, initially attributed to a climate shift to wetter  
154 conditions (Bennett, 1984), although human and grazing pressures may have also played a  
155 significant role (Tipping et al., 2008). The exploitation and reduction in Scots pine extent has been  
156 particularly intense from the 18<sup>th</sup> Century onwards (Hobbs, 2009), mainly characterized by felling  
157 and selective logging to provide construction timber (Smout, 2003). The general decrease in forest  
158 extent, together with poor natural regeneration in the Caledonian pine forest (due to extensive  
159 browsing pressure by deer and sheep), kept this forest at low tree density for many years (Mcvean,  
160 1963) and strongly suppressed regeneration during the last 200 years (Steven and Carlisle, 1959).  
161 During the last few decades, however, forest management has moved to protect and expand the  
162 remaining Caledonian pine forest (Forestry Commission, 2016).

163

164 We selected two study sites in Scotland, Abernethy (57°20'N, 3°61'W) and Glen Affric  
165 (57°15'N, 5°00'W). Nowadays, these sites constitute some of the largest ancient pine forest in  
166 Scotland covering areas of 2452 ha and 1532 ha, respectively (Mason et al., 2004). In each site,  
167 an old open native stand was selected, where trees are expected to have been established through

168 natural regeneration of native provenance. Hereafter these stands will be referred to as managed  
169 stands. The fire regime in the UK is largely human driven (Davies et al., 2008), but tree mortality  
170 through large fires is uncommon in Scotland. In addition, wind-blow and snow can cause some  
171 casualties through the year, and fungi and insects will be minor effects. However, intense forest  
172 disturbance in recent centuries can be attributed mainly to forest management practices.

173

174 The study site in Abernethy is located at 370 m a.s.l., with south westerly prevailing winds and  
175 densities of 160 stems ha<sup>-1</sup>. Stand composition is formed by Scots pine, with presence of *Juniperus*  
176 *communis*. The understory is predominantly *Calluna vulgaris*, *Vaccinium myrtillus* and small  
177 patches of *V. vitis-idaea*. Historical exploitation at Abernethy has taken place over millennia and  
178 high felling and browsing pressure during the 18<sup>th</sup> Century are reflected in the progressive  
179 contraction of the extent of Abernethy forest in historical maps from 1750 until 1830 (Smout et  
180 al., 2005, Summers et al. 2008). By 1858, the forest is represented by only scattered trees in the  
181 study site and followed by enclosure of the forest as deer forest occurred in 1869 (O'Sullivan,  
182 1973). In the 1980s the area was designated a National Natural Reserve. Seasonal grazing by  
183 sheep was stopped in 1990 and deer fences were removed (Beaumont et al., 1995). Since then,  
184 culling of deer has kept the population stable and compatible with forest regeneration. By 1992  
185 the percentage of seedlings with evidence of browsing had fallen from 72% to 43% with an  
186 increase of 20% in the number of established seedlings and saplings (Beaumont et al., 1995).

187

188 The study site in Glen Affric is located at 260 m a.s.l., on the south west of Loch Affric, where  
189 the prevailing winds are south westerly, and stand density is 103 stems ha<sup>-1</sup>. Stand composition is  
190 Scots pine and the vegetation layer is predominantly *C. vulgaris* with small patches of *V. vitis-*  
191 *idaea* and *V. myrtillus*. Evidence from pollen records from West Glen Affric, where our stand is  
192 located, show a sustained low tree cover around these sites for several thousand years as a result  
193 of prolonged human impact, with the recent expansion of the forest when the present tree cohort  
194 developed around 1880 (Shaw, 2006). Historical documents report felling of trees during the 18<sup>th</sup>  
195 and 19<sup>th</sup> Centuries (Smout et al., 2005) with the decline evident in pollen records. Following a



196 period of intensive sheep and deer grazing in the late 20<sup>th</sup> Century a major effort was made to  
197 protect and restore the remaining native pine forest (Bain, 2013). Glen Affric was initially  
198 declared as a Caledonian Forest Reserve in 1961 by the Forestry Commission (Bain 2013) and  
199 later, in 1984, a National Natural Reserve.

200

201 To compare our heavily managed stands with an unmanaged case, and since unmanaged stands  
202 do not exist in Scotland, pre-existing samples from a boreal site in Western Siberia were used  
203 (60°54'N, 68°42'E). These samples were taken from within a continuous population with  
204 extensive areas of natural forest, with a stand density of 470 stems ha<sup>-1</sup>. These forests have never  
205 been altered by humans, but are subject to regular natural disturbance by fire on roughly 50 year  
206 timescales. In these boreal forests, competition forces Scots pine to forest edges and onto poor  
207 quality sites such as sandy soils or bogs, and it is outcompeted on better soils by *Pinus sibirica*,  
208 *Larix sibirica* and *Populus tremula*. As a result even mature individuals may be small. Hereafter  
209 this stand will be referred to as the unmanaged stand.

210

211 In Scots pine, genetic variation is partitioned predominantly within rather than among  
212 populations, and levels of within-population genetic diversity across the range of Scots pine are  
213 similarly high (Wachowiak et al., 2014, 2011). Previous studies of diversity across the range of  
214 this species have shown that genetic differentiation among even distant populations of Scots pine  
215 is low (Naydenov et al., 2007; Provan et al., 1998; Prus-Glowacki and Stephan, 1994; Wang et  
216 al., 1991) but see (Forrest, 1982; Prus-Glowacki et al., 2012). Some authors attribute this  
217 homogeneity to common ancestry, as well as extensive gene flow (Chybicki et al., 2008) and lack  
218 of major physical barriers (Naydenov et al., 2007). As absolute genetic diversity levels in the  
219 managed and unmanaged stands are of similar magnitude, and the physical capacity for gene  
220 movement should be similar in each, we can assume that the primary driver of genetic structure  
221 will have been the presence or absence of significant human intervention. Therefore, this  
222 comparison can meaningfully inform on the processes that are likely responsible for the observed  
223 spatial pattern of genetic diversity at fine scales.

224

### 225 2.3. Sample collection, life stages and stand structure

226 We selected stands of 200 m × 200 m in Abernethy and Glen Affric, respectively. Sampling  
227 strategy was designed to account for short distance classes in order to detect fine-scale SGS,  
228 choosing individuals randomly and assuring sufficient numbers of pairwise comparisons in each  
229 distance class, as recommended by Cavers et al (2005). We sampled needles from two life stages,  
230 juveniles and adults. Sample size per stand in each life stage varied from 131 to 181 (Table 1). All  
231 individuals were mapped using a GARMIN 62s handheld GPS and diameter was measured at  
232 breast height (d.b.h.). The d.b.h. was used as a proxy of age, defining juveniles as individuals with  
233 d.b.h. below 10 cm. Existing data from trunk cores from nearby adult pines in Abernethy  
234 (Summers et al., 2008) were used to calibrate the relationship between d.b.h. and age.

235

236 The unmanaged study site was sampled in three sub-stands of 50 x 50 m along a linear transect of  
237 300 m, which were combined to give a single stand sample for subsequent analysis. All sampled  
238 individuals were mapped, measured at d.b.h. and tree height classified as short (<2m) or tall (>2m).  
239 Juveniles were defined as short individuals with d.b.h. below 10 cm. Sample size in each life stage  
240 varied from 57 to 73 (Table 1). Thirty random trunk sections from adult pines were taken from  
241 the unmanaged site to calibrate the d.b.h.-age relationship. We evaluated the relationship between  
242 d.b.h. and tree age, and whether this relationship varied among sites using a linear model in R  
243 3.0.1 (R Core Team 2013). We chose d.b.h. as the response variable and tree age and site  
244 (Abernethy and unmanaged) were the predictor variables. The interaction between the predictor  
245 variables was tested and compared with a model without interactions by using the Akaike  
246 Information Criterion.

247

### 248 2.4. Microsatellite analyses

249 Total genomic DNA was extracted from 50 mg silica gel dried needles using QIAGEN DNeasy  
250 96 Plant Kit (QIAGEN Ltd. Crawley, UK) following the manufacturer's protocol. All individuals  
251 were genotyped at twelve nuclear microsatellite markers (SSR): psy12, psy116, psy117, psy136,

252 psy142, psy144, psy157 (Sebastiani et al., 2011), SPAC7.14, SPAC12.5 (Soranzo et al., 1998),  
253 PtTX4001, PtTX4011 (Aukland et al., 2002) and SsrPt\_ctg4698 (Chagné et al., 2004), combined  
254 in two multiplexes of six loci each. Multiplex 1 consisted of primers psy12, psy117, psy142,  
255 psy144, PtTX4001 and PtTX4011 at concentrations of 3  $\mu$ l, 2  $\mu$ l, 2  $\mu$ l, 2  $\mu$ l, 3  $\mu$ l and 2  $\mu$ l  
256 respectively. Multiplex 2 consisted of primers psy116, psy136, psy157, SPAC7.14, SPAC12.5  
257 and SsrPt\_ctg4698 at concentrations of 2  $\mu$ l each. Reactions were carried out in a final volume of  
258 10  $\mu$ l with 1X of QIAGEN Type-it Multiplex PCR Master Mix, 1  $\mu$ M of each multiplex and 25  
259 ng of template DNA. Annealing temperature for both multiplexes was 56°C. Polymerase chain  
260 reactions (PCR) were performed in Veriti™ Thermal cycler (Applied Biosystems, Bleiswijk,  
261 Netherlands), with the following programme: 1 cycle at 95°C for 4 min followed by 35 cycles at  
262 95°C for 45 s, 56°C for 45 s, 72°C for 45 s, and a final step at 72°C for 5 min. PCR products were  
263 analysed by DNA Sequencing and Services, Dundee, UK, using an Applied Biosystems 3730  
264 DNA Sequencer with reference to a LIZ 500 size standard. Fragment analysis results were scored  
265 using GENEMARKER V.2.6.0. (SoftGenetics, State College, PA, USA). FLEXIBIN (Amos et  
266 al., 2007) was used to check discrete classes of raw allele sizes and MICRO-CHECKER (Van  
267 Oosterhout et al., 2004) to check genotyping errors and null allele frequencies. Several markers  
268 showed evidence of null alleles at very low frequencies (maximum frequency of 0.04, data not  
269 shown), which is far below to the threshold at which null alleles can result in a significant  
270 underestimate of expected heterozygosity, estimated as 0.2 (Belletti et al., 2012; Chapuis and  
271 Estoup, 2007). Therefore, all markers were kept for further analysis.

272

## 273 2.5. Genetic diversity and spatial genetic structure analysis

274 Genetic diversity estimators within stands and life stages were estimated using FSTAT 2.9.3.2  
275 (Goudet, 1995): mean number of alleles per locus ( $A$ ), rarefied allelic richness ( $A_R$ ) (rarefied to 57  
276 individuals for each stand and life stage), expected heterozygosity ( $H_E$ ) and inbreeding coefficient  
277 ( $F_{IS}$ ). We conducted ANOVAs to test for differences in  $A$ ,  $A_R$ , and  $H_E$  between stands and life  
278 stages in R 3.0.1 (R Core Team 2013). We calculated  $F_{ST}$  among stands and life stages in  
279 ARLEQUIN v3.5 (Excoffier and Lischer, 2010), and the differentiation index  $D$  (Jost, 2008)

280 implemented in the R package DEMETics (Gerlach et al., 2010). In both cases, significance values  
281 were determined for a 5% nominal level after Bonferonni correction.  $F_{ST}$  estimates differences in  
282 allele frequencies among stands, whereas differentiation index  $D$  measures the fraction of allelic  
283 variation among them.

284

285 We implemented fine scale SGS analyses in SPAGeDi 1.4b (Hardy and Vekemans, 2002). In order  
286 to test for significance in genetic relatedness, the Kinship coefficient of Loiselle et al., 1995 ( $F_{ij}$ )  
287 was estimated as  $F_{ij}=(Q_{ij}-Q_m)/(1-Q_m)$ , where  $Q_{ij}$  is the probability of identity in state for random  
288 gene copies from two individuals  $i$  and  $j$ , and  $Q_m$  is the average probability of identity by state for  
289 gene copies coming from random individuals from the sample. A regression between the Kinship  
290 coefficient  $F_{ij}$  and the logarithm of pairwise geographic distances of individuals was computed.  
291 Standard errors of the regression slope were computed using a jackknife procedure over loci. The  
292 significance of the slope of the regression was tested using 10,000 permutations of locations  
293 among individuals. To visualize the SGS, we plotted average pairwise estimates of relatedness as  
294 a function of distance to generate spatial genetic autocorrelograms. Analyses were conducted for  
295 each stand and life stage separately across the full distance range.  $SGS_{MAX}$  was also calculated for  
296 each stand and life stage, which is the greatest distance at which the Kinship coefficient of a given  
297 distance class  $F(d)$  is significant at  $p<0.05$  (Jump et al., 2012). We also calculated the  $Sp$  statistic,  
298 as suggested by Vekemans and Hardy (2004), to allow comparability among stands and life stages  
299 with other studies. The  $Sp$  statistic was determined as  $-b_F/(1 - F_l)$ , where  $b_F$  is the regression slope  
300 of kinship coefficient estimate ( $F$ ) on distance classes and  $F_l$  is the kinship coefficient for adjacent  
301 individuals in the first distance interval.

302

303 Because the number of pairs within each distance class should ideally exceed 50 pairs of  
304 individuals, we set the distance intervals of at least 10 metres (Cavers et al., 2005; Jump and  
305 Peñuelas, 2007). Overall, we established 10 distance classes for the managed stands (0-10, 10-20,  
306 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100), and 8 distances classes in the  
307 unmanaged stand (0-10, 10-20, 20-30, 30-60, 60-70, 70-80, 80-90, 90-100). Distance classes

308 between 30 and 60 metres were combined in the unmanaged stand to ensure sufficient numbers of  
309 pairs in the class. We also tested other distance class options and longer final distances up to 200  
310 metres, and found they revealed similar and no more informative results. In addition, in the  
311 unmanaged stand, analysis of each sub-stand was also conducted separately, and the same results  
312 were obtained.

313

### 314 **3. Results**

#### 315 3.1. Stand structure

316 Tree diameter distribution for managed stands was bimodal, with the highest frequencies for  
317 juvenile individuals at diameters between 0 and 10 cm (Fig. 1). A gap of adult individuals with  
318 diameter classes between 10 to 30 cm and 10 to 25 cm occurred in Abernethy and Glen Affric,  
319 respectively (Fig. 1). Contrastingly, tree diameter distribution in unmanaged stand was more  
320 skewed towards smaller diameters. There was no gap in the distribution in this case, instead there  
321 was a gradual decrease in the numbers of individuals with increasing diameter class (Fig. 1).

322

323 We found that d.b.h. was dependent on age and site ( $F=29.85$ ,  $R^2=0.31$ ), showing strong  
324 differences among age ( $t=3.81$ ,  $p<0.001$ ), and among sites ( $t=-6.03$ ,  $p<0.001$ ). However, we did  
325 not find significant interactions between age and study site (Fig. 2). The relationship between  
326 d.b.h. and age suggested that differences in age profiles in the two sites were smaller than  
327 differences in tree size (e.g. trees with different d.b.h. could have a similar age).

328

#### 329 3.2. Genetic diversity

330 Genetic diversity parameters did not significantly differ between managed and unmanaged stands  
331 (Table 1). Among the twelve nuclear loci analysed, the number of alleles ( $A$ ) in the managed stands  
332 ranged from 3 to 31 and 4 to 29 per locus for Abernethy and Glen Affric respectively for both life  
333 stages combined (multilocus average of 9.92 for each site).  $A$  ranged from 3 to 31 in the  
334 unmanaged stand, with a multilocus average of 9.83 again for both life stages combined. For  
335 rarefied allele richness ( $A_R$ ) in the managed stands, multilocus estimates obtained mean values of

336 8.99 and 8.83 for Abernethy and Glen Affric respectively and 8.95 for the unmanaged stand both  
337 life stages combined, based on a minimum number of 126 individuals. Expected heterozygosity  
338 levels ( $H_E$ ) showed multilocus estimates of 0.58 in Abernethy and 0.56 in Glen Affric, and similar  
339 values of 0.58 for the unmanaged stand for both life stages combined (See Table 1 for genetic  
340 diversity estimators on each site and life stage & Appendix, Table A1, for detailed information of  
341 each microsatellite). Neither  $A$ ,  $A_R$  or  $H_E$  significantly differed between managed vs. unmanaged  
342 stands (all  $p$ -values  $> 0.05$ ). However, some differences appeared in the inbreeding coefficient  
343 ( $F_{IS}$ ) which was significant and higher for both managed stands, indicating significant departure  
344 from Hardy–Weinberg equilibrium, whereas it was not significant for the unmanaged stand (Table  
345 1).  $F_{ST}$  values indicated low but significant differences among the two managed stands ( $F_{ST}=0.004$ ,  
346  $p<0.001$ ), and higher differences when comparing them with the unmanaged stand ( $F_{ST}=0.03$  and  
347  $F_{ST}=0.04$ ,  $p<0.001$ , for Abernethy vs. unmanaged and Glen Affric vs. unmanaged respectively),  
348 indicating that despite remarkably similar overall levels of genetic diversity, their genetic  
349 composition differs to some extent.

350

351 When comparing life stages within stands, neither  $A$ ,  $A_R$  or  $H_E$  significantly differed (all  $p$ -values  
352  $> 0.05$ ).  $F_{ST}$  values indicated no significant differences among life stages in Abernethy and the  
353 unmanaged stand, however low but significant  $F_{ST}$  occurred among life stages in Glen Affric. In  
354 agreement, differentiation index  $D$  showed the same pattern, although values were consistently  
355 larger (See Appendix, Table A2).

356

### 357 3.3. Spatial genetic structure

358 We found significant SGS in all managed stands for both life stages which extended up to 40  
359 metres further than the unmanaged stand (Table 1 and Fig. 3). The kinship coefficient for the first  
360 distance class  $F_{(1)}$  and the  $S_p$  statistic also reflected the relationship between the extent of SGS and  
361 historical management, which was larger for managed than for unmanaged stands (Table 1).

362

363 When comparing SGS among life stages within stands, both  $SGS_{MAX}$  and  $F_{(I)}$  were larger for adult  
364 than for juvenile stages in the managed stands (e.g.  $SGS_{MAX}$  extended up to 20 metres further in  
365 adults than juveniles) (Table 1 and Fig. 3). In contrast, SGS was larger for juveniles than for adults  
366 for the unmanaged stand, with significant SGS only at distances of less than 10 metres in the  
367 juvenile stage (Table 1 and Fig. 3). In the managed site of Glen Affric, we found that at 50-80 m  
368 trees were less genetically similar than expected compared with a random distribution of  
369 genotypes (see significant negative values of Kinship coefficient at distances between 50 and 80  
370 metres in Glen Affric in Fig. 2). The minimum number of pairwise comparisons per distance class  
371 in the managed stands for each life stage was 106 individuals, whereas it was 63 individuals in the  
372 unmanaged stand. The  $Sp$  values did not reflect the same relationship between the extent of SGS  
373 with contemporary management as  $SGS_{MAX}$  and  $F_{(I)}$  did. Thus, in the managed stand,  $Sp$  value was  
374 not significantly different between adults and juveniles in Abernethy, whereas it increased from  
375 adults to juveniles in Glen Affric (Table 1).

376

#### 377 **4. Discussion**

378 We found two main results: 1) although overall levels of genetic diversity were strikingly similar,  
379 more extensive spatial structuring of genetic diversity was found in the mature managed stands  
380 when compared with the unmanaged one; 2) in contrast to expectations, a reduced extent of spatial  
381 genetic structure was found in the early stages of regeneration of the managed stands compared  
382 with the adult cohorts, again despite no difference in overall levels of genetic diversity between  
383 life stages. These patterns suggest that both historical and contemporary management can  
384 significantly alter spatial genetic structure of Scots pine. Here, we combine ecological information  
385 with historical data on the stands to better understand the mechanisms that are likely responsible  
386 for these differences in spatial genetic structure.

387

##### 388 4.1. Impact of historical forest management practices

389 Notable differences in size profiles appeared between managed and unmanaged stands, (e.g. mean  
390 d.b.h. generally bigger in managed stands (Fig. 1)). However, the d.b.h.-age relationship was

391 different among managed and unmanaged stands (Fig. 2), linked to the growth-retarding effect of  
392 the bog habitat of the latter. Hence, the contrast in age profiles –a more important comparison for  
393 SGS analysis– was much smaller than for size profiles (e.g. small trees from the unmanaged stand  
394 often had similar ages to much larger trees from the managed one). The age profile of the stands  
395 was strongly reflective of their distinct histories, with large, old trees present in the managed sites  
396 plus a pulse of recent regeneration, whilst a much wider range of ages was present in the  
397 unmanaged one, with fewer very old trees. The structure in the unmanaged site is likely to reflect  
398 the natural fire disturbance dynamics to which it is exposed. These dynamics are likely in turn to  
399 affect genetic structure, with a higher turnover in the unmanaged stand –shown by the diverse, but  
400 generally young age profile– indicating a higher potential for gene dispersal and therefore erosion  
401 of spatial structure.

402

403 Genetic diversity of both mature managed sites, as indicated by allelic richness and expected  
404 heterozygosity, did not differ significantly from the unmanaged stand but instead was remarkably  
405 similar (e.g.  $H_E$ : 0.57-0.59 vs.  $H_E$ : 0.58, respectively). Although average diversity levels were  
406 lower than those reported in mainland European populations of Scots pine using nuclear SSR ( $H_E$ :  
407 0.62-0.85) (Scalfi et al. 2009; Naydenov et al. 2011; Nowakowska et al. 2014; García-Gil et al.  
408 2015) differences might be explained by the number of markers used and their specific levels of  
409 polymorphism. Thus, for example, selecting two of the three markers used by Scalfi et al. 2009,  
410 SPAC 7.41 and SPAC 12.5, the mean values of genetic diversity in our study would increase to  
411 even higher values of 0.90. Also, the markers with the lowest values of diversity in our study,  
412 psy144 and psy12, had very similar low values in mainland European populations (Sebastiani et  
413 al., 2011) (see Appendix Table A1). Previous studies in Scottish populations of Scots pine have  
414 also reported relatively high levels of genetic variation using other molecular markers (Forrest,  
415 1982, 1980; Kinloch et al., 1986; Provan et al., 1998; Sinclair et al., 1998; Wachowiak et al., 2013,  
416 2011).

417



418 High levels of genetic variation at the population level suggests that effective population size has  
419 been sufficiently high to restrict effects of genetic drift despite intensive management and  
420 geographical isolation of populations. Scots pine is a wind-pollinated tree with wind-dispersed  
421 seed, and achieves high levels of gene flow by: (1) long seed wings, up to four times as long as  
422 the seed (Steven and Carlisle, 1959), (2) low seed mass (Castro, 1999) (here 2.9 to 12.64 mg), on  
423 average smaller than other pine species (9.1 to 233 mg) (Wall and Vander, 2003), and (3) extensive  
424 pollen flow, from 17-22 m (Robledo-Arnuncio et al., 2004b) and up to 100 km in small fragments  
425 (Robledo-Arnuncio, 2011) (similar to other wind-pollinated tree species). Therefore, it appears  
426 that gene flow has been sufficient to prevent erosion of genetic diversity.  $F_{IS}$  values, an indirect  
427 measure of inbreeding, were not high in the managed sites although they were significantly higher  
428 than in the unmanaged site (0.05-0.06 vs. 0.01 respectively), suggesting that although gene flow  
429 has prevented loss of genetic diversity at the population level, fine scale alterations to gene flow  
430 might have taken place. Drastic reduction of population sizes can induce higher rates of selfing  
431 and mating between relatives (Robledo-Arnuncio et al., 2004a). The small size of the population  
432 at the time of establishment of the current adult cohorts, as indicated by historical data (Shaw,  
433 2006; Summers et al., 2008), might explain this pattern.

434

435 Consistent differences in SGS were found in the mature managed stands which showed greater  
436 extent and magnitude of structure, as indicated by  $SGS_{MAX}$  up to 40 metres and higher  $F_{(I)}$ ,  
437 compared with the unmanaged one. The extent of SGS of the mature managed stands was also  
438 larger than the values reported for Scots pine (Chybicki et al., 2008) and to other *Pinus* species,  
439 which had typically values below 15 metres (De-Lucas et al., 2009; González-Martínez et al.,  
440 2002; Jones et al., 2006; Marquardt and Epperson, 2004; Parker et al., 2001; Troupin et al., 2006;  
441 Williams et al., 2007). It should be noted, however, that SGS estimates in Parker et al. 2001 and  
442 Jones et al. 2006 were based on allozyme markers, and the need for caution when comparing SGS  
443 extent with different molecular markers has been previously highlighted (Jump and Peñuelas,  
444 2007).

445

446 Values of SGS extent more comparable to those in our managed stands were observed in  
447 fragmented populations of *Pinus pinaster* (~ 20 metres) (De-Lucas et al., 2009). The high values  
448 of  $SGS_{MAX}$  in the managed stands are likely a consequence of the drastic reductions in the number  
449 of seed and pollen donors, which are two of the main drivers of SGS (e.g. due to felling practices).  
450 The larger extent of SGS observed in Glen Affric may arise from local differences in historical  
451 management, with a prolonged limited tree cover due to human activities (Shaw, 2006), which is  
452 also reflected in the lower density of the site. The very short spatial scale of genetic structure in  
453 the mature unmanaged stand was remarkably similar to that in undisturbed continuous populations  
454 of *Pinus pinaster* which displayed either weak or no relatedness, with maximum values of  $SGS_{MAX}$   
455 of 10 metres (De-Lucas et al. 2009). As these populations have contrasting local contexts, the  
456 studied unmanaged stand being part of the extensive core Eurasian population whereas the  
457 undisturbed population of *P. pinaster* being a distributional edge population, the similarity in SGS  
458 values observed seems likely to be due to their common unmanaged state. Therefore, it seems  
459 clear that tree density, degree of fragmentation of stands at the time of establishment and rate of  
460 gap creation play a major role in the formation of SGS in populations.  $S_p$  values for the mature  
461 managed stands (0.0045 and 0.0098) were remarkably higher than for the non-managed stand (-  
462 0.0006). Similarly, De-Lucas et al., (2009) found differences in the  $S_p$  values between fragmented  
463 and continuous populations of *P. pinaster*, although they were generally higher than the values  
464 reported in this study.

465

#### 466 4.2. Impact of contemporary forest management practices

467 In the managed stands, there were no differences among life stages in the levels of allelic richness  
468 or gene diversity, suggesting contemporary management has not impacted genetic variation.  
469 However, we found higher relatedness – as higher SGS intensity and extent – in adults than in  
470 juveniles, with a greater discrepancy in the Glen Affric site. In contrast, the unmanaged site had  
471 stronger relatedness in the juvenile stage than in adults, as is usually found in natural tree  
472 populations. Natural populations often show greater SGS in the early stages of regeneration, due  
473 to the higher spatial aggregation of trees (Rozas et al., 2009; Szwagrzyk and Czerwczak, 1993).

474 This pattern has been reported in other species of *Pinus* (González-Martínez et al., 2002), in  
475 *Quercus* (Hampe et al., 2010), tropical trees (Hardesty et al., 2005; Ng et al., 2004) and other plant  
476 species (Yamagishi et al., 2007). Nevertheless, a few studies have found opposite and greater SGS  
477 in adult life stages, such as in *Jacaranda copaia* (Jones and Hubbell, 2006), where it was attributed  
478 to very low recruitment and high mortality rates, or in the tropical tree *Dicorynia guianensis*,  
479 linked to overlapping of generations in the adult cohort (Latouche-Hallé et al. 2003). A subsequent  
480 study of the latter species found stronger SGS in saplings (Leclerc et al., 2015), suggesting that  
481 earlier observations were probably specific to the particular study cohort. Stronger SGS in adults  
482 than in late juveniles was also found for *Prunus africana* and it was attributed to a reduction in  
483 gene flow due to past logging (Berens et al., 2014). In our study, the most probable explanation  
484 seems to be the influence of changes in contemporary management. In the managed populations  
485 of Scots pine investigated here, high felling pressure at the time of establishment of the adult  
486 cohort, together with the high browsing pressure has suppressed regeneration for decades, which  
487 is also reflected in the absence of individuals estimated between 25 and 100 years old (Fig. 2). In  
488 the last 25 years, there has been a deliberate policy to encourage regeneration in the pine forest  
489 (Mason et al., 2004), with a consequent increase in forest densification. This appears to have  
490 maintained diversity levels, increased gene flow and produced a more randomized distribution of  
491 genotypes in the new generation.

492

493 The observed reduction in juvenile *SGS* shows an erosion of the structure present in the adult  
494 cohort and contemporary recovery to natural dynamics, reflecting the high capacity of the species  
495 to recover after disturbance. Overall, *Sp* was higher in Glen Affric than in Abernethy, as for *SGS*.  
496 Although the spatial extent of *SGS* was higher in adults at Glen Affric, *Sp* was slightly higher in  
497 the juvenile stage. This means more distant pairs of juveniles were less related than they would be  
498 by chance (juveniles showed a lack of relatedness among individuals at 50-80 m separation). The  
499 biological cause of this trend is not clear but, together with  $F_{ST}$  values that showed a small but  
500 significant difference among juveniles and adults, it may indicate introgression from populations  
501 not present in our sample.

502

### 503 4.3. Conclusions

504 In this study we investigated how historical and contemporary forest management have shaped  
505 patterns of genetic diversity and spatial distribution of genotypes of Scots pine. We provide  
506 evidence to show that although overall levels of genetic diversity in historically managed  
507 populations can be similar to unmanaged populations and as high as continental populations,  
508 spatial genetic structure can be considerably altered. Our results suggest that intense management  
509 practices that remove trees from the stand, such as felling, could alter fine-scale patterns of gene  
510 flow and increase genetic relatedness of individuals at fine scales with implications for inbreeding  
511 levels and, potentially, long-term adaptability. As a consequence, the extent of family clusters can  
512 be modified, as for instance in our study which increased up to 40 metres in managed sites. From  
513 a practical point of view, to ensure a broad sample of genetic variability, guidelines for seed  
514 collection should aim for minimum sampling distances between mother trees of at least 40m.

515

516 The reduction of SGS observed in juveniles following contemporary management to promote  
517 regeneration, indicates a high capacity of the species to recover after intense forest management.  
518 Here, we suggest that combining sustainable management with forest conservation practices that  
519 ensure larger effective population sizes it is key to successfully maintain genetic diversity in Scots  
520 pine. This capacity of the early stages of regeneration to capture gene flow might have  
521 implications for forest resilience and will be particularly important in the context of climate  
522 change (Alfaro et al., 2014; Fady et al., 2015; Hoffmann and Sgrò, 2011; Millar et al., 2007) under  
523 which selection pressures are expected to change.

524

525 Here we showed how investigating the spatial component of genetic diversity alongside tree  
526 demographic structure can help to detect both historical and contemporary effects of disturbances  
527 in tree populations. The effects of forest management were not reflected in overall levels of  
528 genetic diversity, but they were manifested in SGS, as has been seen in previous studies (Paffetti  
529 et al. 2012; Leclerc et al. 2015; Sjölund and Jump 2015). Therefore, incorporating a spatial

530 component when evaluating the effects of forest management practices is highly recommended.  
531 The evaluation of successional change is also essential to properly assess genetic dynamics within  
532 populations and to adequately detect early responses to forest management practices.

533

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541

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## 798 **Tables**

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800 **Table 1:** Summary of multilocus genetic diversity and SGS estimators of Scots pine for each  
801 study site and life stage.

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## 803 **Figures**

804

805 **Fig. 1:** Tree diameter (d.b.h.) distribution of Scots pine in the three study sites: Abernethy (ABE),  
806 Glen Affric (GLA) and the unmanaged stand (UNM). Juvenile stem diameter was measured at 10  
807 cm height. Data are presented in intervals of 5 cm.

808 **Fig. 2:** Relationship between d.b.h. and age of Scots pine for the managed site of Abernethy  
809 (ABE) and the unmanaged site (UNM). Lines of best fit are represented by solid lines and 95%  
810 CI by dashed lines. Dots represent observed values.

811 **Fig. 3:** Genetic spatial autocorrelograms of Scots pine derived from 12 microsatellite loci,  
812 represented for each study site: Abernethy (ABE), Glen Affric (GLA) and the unmanaged stand  
813 (UNM); and life stage (adult and juvenile) using the kinship coefficient  $F_{ij}$  and consecutive 10 m  
814 distance classes (note that for the unmanaged stand distance classes were combined between 30  
815 to 60 metres). Shaded areas represent 95% confident intervals obtained from 10,000 permutations  
816 of genotypes among locations. Black bars around mean  $F_{ij}$  values represent standard errors  
817 derived through jackknifing over loci.

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## 820 **Appendix**

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822 **Table A1:** Genetic diversity estimators of Scots pine for each locus, study site and life stage.

823 **Table A2:** Pairwise  $F_{ST}$  values (below diagonal) and differentiation index  $D$  (Jost, 2008) (above  
824 diagonal) of Scots pine among study sites and life stages.

825 **Table 1:** Summary of multilocus genetic diversity and SGS estimators of Scots pine for each study site and life stage.

Population	Life stage	<i>N</i>	Genetic diversity estimators				Spatial genetic structure estimators			
			<i>A</i>	<i>A<sub>R</sub></i>	<i>H<sub>E</sub></i>	<i>F<sub>IS</sub></i>	<i>F<sub>(l)</sub></i>	<i>SGS<sub>MAX</sub></i> (m)	<i>b<sub>F</sub> ± SE</i>	<i>Sp ± SE</i>
Abernethy	Adult	181	9.50	7.11	0.587	0.052**	0.0291***	20	-0.0044 ± 0.0006***	0.0045 ± 0.0028
	Juvenile	170	9.25	6.72	0.583	0.080**	0.0183***	18	-0.0028 ± 0.0009**	0.0029 ± 0.0023
Glen Affric	Adult	165	8.92	6.79	0.568	0.063**	0.0298***	40	-0.0097 ± 0.0023***	0.0098 ± 0.0010
	Juvenile	131	9.25	6.74	0.561	0.049**	0.0156***	20	-0.0118 ± 0.0027***	0.0119 ± 0.0006
Unmanaged	Adult	57	7.58	6.51	0.576	0.012	-0.0033	0	0.0006 ± 0.0005	-0.0006 ± 0.0005
	Juvenile	73	8.17	6.94	0.582	0.021	0.0067	5	-0.0017 ± 0.0010*	0.0018 ± 0.0011

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827 *N*, sample size; *A*, mean number of alleles per locus; *A<sub>R</sub>*, rarefied allelic richness; *H<sub>E</sub>*, expected heterozygosity; *F<sub>IS</sub>*, inbreeding coefficient. *F<sub>(l)</sub>*, Kinship coefficient for first  
828 distance class (0-10m); *SGS<sub>MAX</sub>*, greatest distance at which the Kinship coefficient of a given distance class *F(d)* is significant at *p*<0.05; *b<sub>F</sub> ± SE*, regression slope of the Kinship  
829 coefficient *F<sub>ij</sub>* computed among all individuals against geographical distances ± standard error; *Sp ± SE*, *Sp* statistic ± standard error. Significant *P*-values are indicated as \**P*  
830 < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001. *P*-values for *F<sub>IS</sub>* are obtained after 10,000 permutations of gene copies within individuals of each stand.

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834 **Table A1:** Genetic diversity estimators of Scots pine for each locus, study site and life stage.

Locus	Life stage	Abernethy					Glen Affric					Unmanaged				
		<i>N</i>	<i>A</i>	<i>A<sub>R</sub></i>	<i>H<sub>E</sub></i>	<i>F<sub>IS</sub></i>	<i>N</i>	<i>A</i>	<i>A<sub>R</sub></i>	<i>H<sub>E</sub></i>	<i>F<sub>IS</sub></i>	<i>N</i>	<i>A</i>	<i>A<sub>R</sub></i>	<i>H<sub>E</sub></i>	<i>F<sub>IS</sub></i>
<b>PtTX4001</b>	Adult	181	11	9.28	0.8306	-0.028	165	9	7.59	0.7783	0.03	57	7	6.22	0.5951	-0.002
	Juvenile	170	12	9.32	0.8430	-0.06	131	11	8.79	0.8074	0.054	73	9	6.22	0.5073	0.028
<b>PtTX4011</b>	Adult	181	7	4.61	0.5920	0.099*	165	7	5.12	0.5423	0.213***	57	6	5.66	0.6717	0.204*
	Juvenile	170	6	4.73	0.6144	0.22***	131	6	5.05	0.6094	0.097	73	5	4.96	0.6922	0.3*
<b>psy144</b>	Adult	181	5	3.08	0.1166	-0.042	165	5	3.12	0.1380	-0.054	57	2	1.88	0.0517	-0.018
	Juvenile	170	5	2.88	0.0804	-0.024	131	5	3.2	0.1581	-0.067	73	3	2.39	0.1293	-0.06
<b>psy117</b>	Adult	181	8	6.32	0.7820	0.054	165	10	6.97	0.7907	-0.004	57	8	7.03	0.8224	-0.065
	Juvenile	170	8	5.98	0.7600	0.133**	131	8	6.56	0.7580	0.016	73	7	6.8	0.8247	-0.025
<b>psy142</b>	Adult	181	5	4.15	0.6466	0	165	6	5.22	0.6669	0.019	57	4	3.51	0.6479	-0.084
	Juvenile	170	6	4.34	0.6632	0.104*	131	6	5.07	0.6551	0.01	73	5	4.32	0.6411	-0.155*
<b>psy12</b>	Adult	181	3	2.17	0.3193	0.163*	165	3	2.18	0.2727	-0.096	57	2	2	0.3354	0.059
	Juvenile	170	3	2.17	0.3539	0.087	131	3	2.23	0.2386	0.393***	73	2	2	0.2314	-0.017
<b>psy116</b>	Adult	181	7	5.95	0.7862	-0.03	165	6	5.5	0.7736	0.011	57	6	5.5	0.7399	-0.092
	Juvenile	170	8	5.95	0.7720	0.063	131	7	5.42	0.7512	-0.024	73	6	5.87	0.7598	-0.01
<b>psy157</b>	Adult	181	5	4.23	0.3652	0.002	165	6	4.52	0.3483	-0.009	57	4	3.99	0.3892	-0.128
	Juvenile	170	5	4.19	0.3517	0.064	131	5	4.05	0.2984	-0.024	73	5	4.39	0.5168	-0.087
<b>CTG4698</b>	Adult	181	8	6.24	0.6044	0.019	165	8	5.17	0.5635	-0.043	57	5	5	0.6500	0.049
	Juvenile	170	6	5.34	0.6124	-0.034	131	6	5.27	0.5721	-0.068	73	5	4.64	0.6065	-0.016
<b>SPAC7.14</b>	Adult	181	29	19.08	0.9174	0.194***	165	26	18.6	0.9150	0.236***	57	22	17.95	0.9023	0.09*
	Juvenile	170	28	17.13	0.9093	0.179***	131	28	17.83	0.9072	0.21***	73	28	22.47	0.9513	0.097**
<b>SPAC12.5</b>	Adult	181	21	16.15	0.8989	-0.007	165	17	14.62	0.9058	0.098***	57	22	16.58	0.8475	0.048
	Juvenile	170	19	15.33	0.8956	0.054*	131	22	14.58	0.8814	0.005	73	19	15.85	0.8629	0.032
<b>psy136</b>	Adult	181	5	4.06	0.1877	0.438***	165	4	2.82	0.1166	0.216***	57	3	2.76	0.2607	-0.01
	Juvenile	170	5	3.23	0.1451	0.108	131	4	2.82	0.0897	-0.029	73	4	3.35	0.2578	-0.01

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836  $N$ , sample size;  $A$ , mean number of alleles per locus;  $A_R$ , rarefied allelic richness;  $H_E$ , expected heterozygosity;  $F_{IS}$ , inbreeding coefficient. Significant  $P$ -values are indicated as

837  $*P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ .  $P$ -values for  $F_{IS}$  are obtained after 10,000 permutations of gene copies within individuals of each stand.

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848 **Table A2:** Pairwise  $F_{ST}$  values (below diagonal) and differentiation index  $D$  (Jost, 2008) (above diagonal) of Scots pine among study sites and life stages.

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	ABE Adults	ABE Juveniles	GLA Adults	GLA Juveniles	UNM Adults	UNM Juveniles
ABE Adults	-	-0.00134	0.01367***	0.01694***	0.09089***	0.08407***
ABE Juveniles	-0.00085	-	0.01925***	0.01836***	0.09777***	0.09615***
GLA Adults	0.00531***	0.00504***	-	0.01223**	0.08486***	0.08469***
GLA Juveniles	0.00794***	0.00712***	0.00514***	-	0.09852***	0.09642***
UNM Adults	0.04973***	0.05174***	0.04434***	0.05228***	-	0.00843
UNM Juveniles	0.04923***	0.05132***	0.04586***	0.05382***	-0.00254	-

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851 ABE refers to Abernethy, GLA refers to Glen Affric, UNM refers to the unmanaged stand. Significant  $P$ -values are indicated as \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

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Fig. 1

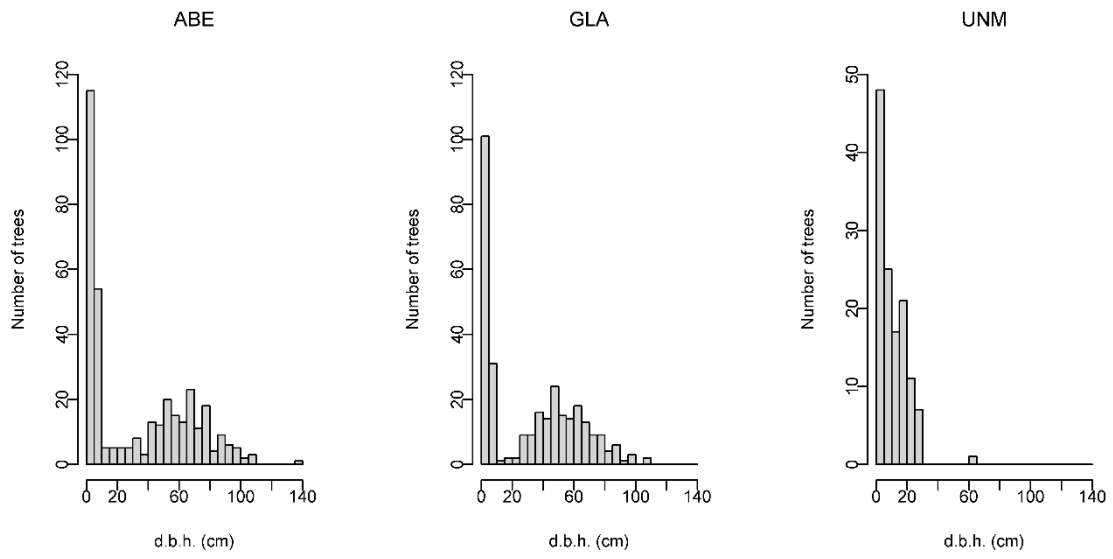


Fig. 2

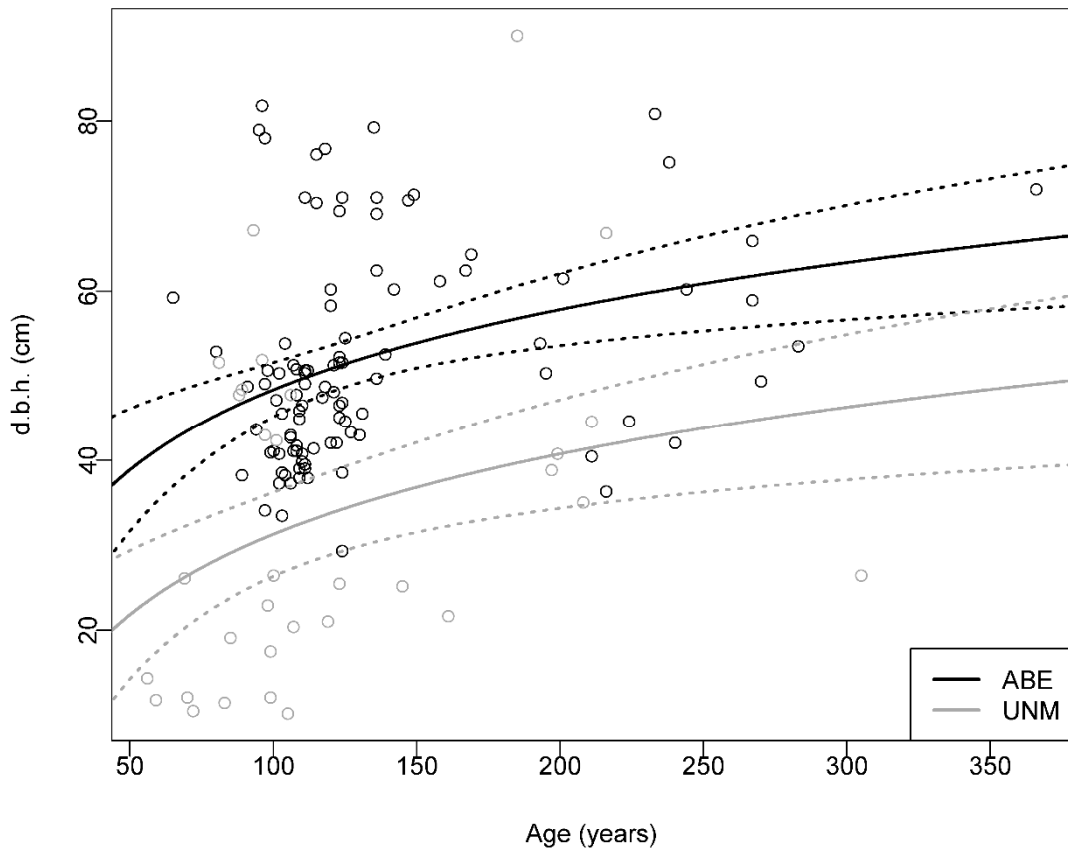


Fig. 3

