Integrating genetics and suitability modelling to bolster climate change adaptation planning in Patagonian *Nothofagus* forests

Marchelli P[1,2]*, Thomas E[3]*, Azpilicueta, M M[1], van Zonneveld M[4], and Gallo L[1]

[1] Genética Ecológica y Mejoramiento Forestal, INTA EEA Bariloche, Modesta Victoria 4450, 8400 San Carlos de Bariloche, Argentina

[2] CONICET Argentina

[3] Bioversity International, Regional Office for the Americas, Cali, Colombia

[4] Bioversity International, Costa Rica Office.

* Both authors contributed equally to this work.

Corresponding author

Paula Marchelli

INTA EEA Bariloche Modesta Victoria 4450 (8400) Bariloche, Río Negro, Argentina

marchelli.paula@inta.gob.ar

pmarchelli@gmail.com

Tel: +54 294 4422731

ORCID 0000-0002-6949-0656

Abstract

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33 We investigated the impact of changes in past and future habitat suitability on the current

34 distribution and threats to the genetic diversity of two southern beeches (*Nothofagus*

35 *nervosa* and *N. obliqua*) in their eastern fragmented range in Patagonian Argentina. Our

36 goal was to develop a spatially-explicit strategy for guiding conservation and management

37 interventions in light of climate change. We combined suitability modelling under current,

38 past (Last Glacial Maximum ~21,000BP), and future (2050s) climatic conditions with

39 genetic characterization data based on chloroplast DNA, isozymes and microsatellites. We

40 show the complementary usefulness of the distribution of chloroplast haplotypes and

41 locally common allele richness calculated from microsatellite data for identifying the

42 locations of putative glacial refugia. Our findings suggest that contemporary hotspots of

43 genetic diversity correspond to convergence zones of different expansion routes, most

44 likely as a consequence of admixture processes. Future suitability predictions suggest that

45 climate change might differentially affect both species. All genetically most diverse

46 populations of *N. nervosa* and several of *N. obliqua* are located in areas that may be most

47 severely impacted by climate change, calling for forward-looking conservation

48 interventions. We propose a practical spatially-explicit strategy to target conservation

49 interventions distinguishing priority populations for (1) *in situ* conservation (hotspots of

50 genetic diversity likely to remain suitable under climate change); (2) *ex situ* conservation in

51 areas where high genetic diversity overlaps with high likelihood of drastic climate change;

52 (3) vulnerable populations (areas expected to be negatively affected by climate change);

53 and (4) potential expansion areas under climate change.

54

Introduction

Changes in climate are predicted to substantially alter the geographic distribution of plant species (Jump et al. 2009) with most envisaged movements towards higher elevations and latitudes following their ecological optima (Parmesan 2006). Distributional shifts in tree species populations are already taking place (Fisichelli et al. 2013; Lloyd et al. 2011), but uncertainty remains about their ability to effectively colonize new habitats in pace with a rapidly changing climate, particularly in light of widespread habitat fragmentation (Alfaro et al. 2014; Hampe et al. 2013).

Alternatively to migration, tree populations are generally able to endure a certain degree of change in habitat conditions (Hamrick 2004). They may persist either if climatic changes remain within the range of their phenotypic plasticity or if they succeed in modifying their local phenotypic optimum through genetic adaptation (Aitken et al. 2008; Alfaro et al. 2014). A pre-requisite for maintaining evolutionary potential is the availability of a high number of genetic variants within populations to allow natural selection to progressively filter out the best-adapted genotypes (Jump et al. 2008; Reed and Frankham 2003). Loss of genetic diversity, for example through bottlenecks induced by fragmentation or overexploitation, increases the probability of a decrease in the adaptive potential of populations, and may in some cases even lead to extirpation (Jump and Peñuelas 2005).

4

78   Responses of forests to past climate change can provide useful insights into plausible

79   scenarios of their responses to future change (Petit et al. 2008). Paleo-studies have

80   evidenced vegetation shifts associated with past climatic changes in different areas around

81   the globe, including the southern Patagonian Andes, the focus of the present study (e.g.

82   (Compagnucci 2011; Folguera et al. 2011). However, the scarceness and discontinuity of

83   paleoecological records in Patagonia (Iglesias et al. 2014; Quattrocchio et al. 2011), and the

84   inability to distinguish between tree species with similar pollen morphology (Heusser

85   1984), complicate detailed reconstructions of past species distributions. With the advent of

86   intraspecific molecular phylogeographies new powerful methods became available to make

87   inferences about the location of putative refugia (Petit et al. 1997), with several studies

88   conducted in southern beeches (*Nothofagus* spp.) supporting persistence in multiple refugia

89   (e.g. (Acosta and Premoli 2010; Azpilicueta et al. 2009; Marchelli et al. 1998; Mathiasen

90   and Premoli 2010; Soliani et al. 2012). While the combination of palaeobotanical and

91   genetic data is the most appropriate approach for reconstructing past habitat suitability

92   (Magri et al. 2006), the use of ecological niche models (ENM) in conjunction with genetic

93   characterization data are a good alternative to unravel past range dynamics of species when

94   paleoecological records are scarce (e.g. Thomas et al. 2012, 2015; Waltari et al. 2007).

95

96   Tree species in the *Nothofagus* genus are characteristic elements of southern South

97   American temperate forests. *Nothofagus nervosa* (Phil.) Dim. et Mil. (= *N. alpina*) and *N.*

98   *obliqua* (Mirb.) Oerst. are native to the Valdivian rain forest which is most extensively

99   distributed west of the Andes, in Chile. In Argentina these forests are constrained to small

100   and fragmented patches located along the foothills of the Andes following a steep rain

101   shadow. To the east there is a transition to steppe vegetation. Particularly the transitional

102 zone between Patagonian forest and steppe is expected to undergo rapid and pronounced

103 shifts in distribution and species composition as a consequence of projected climate change

104 (Allen and Breshears 1998; Suarez and Kitzberger 2010). Notably the precipitation gradient

105 is predicted to become sharper due to expected drops in rainfall (Castañeda and González

106 2008) and increases in extreme climatic events, like severe droughts (Rusticucci and

107 Barrucand 2004). Ongoing climate change in Patagonia has already resulted in significant

108 warming, decreasing precipitation, and a general glacier recession over the past century

109 (Masiokas et al. 2008).

110

111 Owing to their superb wood quality *N. nervosa* and *N. obliqua* have been extensively

112 logged, mainly during the first half of the 20th century. The first protective measures were

113 initiated with the creation of National Parks in 1937, and the contemporary distribution

114 ranges of both species now largely coincide with either National Parks or Provincial

115 Reserves (Sabatier et al. 2011). Genetic studies have previously been used to inform the

116 development of protection measures (Gallo et al. 2009) and management practices

117 (Chauchard et al. 2012), as well as to identify genetic zones for seed sourcing (Azpilicueta

118 et al. 2013). However, assessments of the potential impact of climate change on current

119 distributions and potential responses of these species are still missing.

120

121 Here we reconstruct past (Last Glacial Maximum (LGM) ~21,000BP) and future (2050s)

122 habitat suitability scenarios of *N. nervosa* and *N. obliqua* to investigate the possible impacts

123 of suitability shifts on their genetic diversity. As both species have sympatric distributions,

124 but occupy differential niches along the precipitation gradient, owing to their inherent

125 temperature and hydric tolerances, we believe they can serve as a model for gauging the

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126   expected impact of climate change on the eastern Patagonian forests. Combining ENM with

127   ecogeographical analysis and genetic characterization data obtained from three marker

128   types (isozymes, nuclear microsatellites and chloroplast DNA) we (i) validate past

129   distributions and range dynamics; (ii) identify contemporary hotspots of genetic diversity

130   (i.e. areas of high genetic diversity) and (iii) assess the potential impact of expected climate

131   change on the suitability distributions of both species. Our final objective is to integrate the

132   obtained results in a spatially-explicit strategy to (i) enhance the adaptive capacity of

133   vulnerable populations in light of climate change; (ii) ensure the effective conservation of

134   the species' genetic resources, through a combination of translocations and *in situ*/*ex situ*

135   conservation activities; and (iii) promote their adequate use in tree planting projects,

136   including restoration.

137

138   **Methods**

139   *Studied species*

140   *Nothofagus nervosa* and *N. obliqua* are wind pollinated, outcrossing, anemochorous and

141   deciduous forest trees. *Nothofagus obliqua* typically grows between 650 and 800 m a.s.l. and

142   *N. nervosa* between 800 and 1000 m a.s.l. Both species hybridize naturally (Gallo et al. 1997),

143   especially within the narrow altitudinal fringe where they coexist. A recent taxonomic

144   revision (Heenan and Smissen 2013) renamed both species to *Lophozonia nervosa* (=*L.*

145   *alpina*) *and L. obliqua.* However, as this change is still controversial (Hill et al. 2015), here

146   we will use the 'old' names, in line with others (e.g. Worth et al. 2014).

147

148   *Sampled populations and genetic analyses*

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149   To obtain a comprehensive representation of the extant genetic diversity of both species, we

150   combined previously published data based on three different genetic markers: (i) maternally

151   inherited chloroplast DNA, and biparentally inherited (ii) isozymes and (iii) microsatellites.

152   The low mutation rate of the chloroplast genome and its clonal inheritance permit its use in

153   past range dynamics studies (Petit et al. 1997). Here we use existing chloroplast DNA

154   marker data (Azpilicueta et al. 2009; Marchelli and Gallo 2006) to validate habitat

155   suitability scenarios during the LGM. Sampled populations covered the overall

156   distributions of both species in Chile and Argentina (57 locations; between two and 20

157   individuals per population, Table S1, Online resource).

158

159   Neutral nuclear markers like microsatellites can provide complementary indications about

160   past and current range dynamics (Thomas et al. 2012, 2015; Galluzzi et al. 2015). In

161   particular, they can be useful to identify populations that bear the marks of prolonged

162   genetic isolation. Nuclear SSR characterization data were obtained through genotyping at 7

163   loci, 321 and 307 adult trees of *N. nervosa* and *N. obliqua,* respectively (Azpilicueta et al.

164   2013) (Table S1). Based on this dataset we calculated and mapped richness of locally

165   common alleles (LCA) according to the circular neighborhood methodology implemented

166   by (Thomas et al. 2012; van Zonneveld et al. 2012). LCA are alleles that occur only in a

167   limited area of a species' distribution (here <10% of the sampled populations) but reach

168   high frequencies (here >5%) in those areas. High LCA richness can be indicative for the

169   level of genetic isolation of populations (Frankel et al. 1995) and hence be a useful tool for

170   identifying the location of putative species-specific refugia that may have formed under

171   past climate conditions (Galluzzi et al. 2015; Thomas et al. 2012).

172

173   For the identification of areas of high genetic diversity (hotspots) east of the Andes, in

174   addition to the chloroplast and SSR data mentioned above, we used isozyme

175   characterization data from a total of eighteen populations (nine of each species, Table S1,

176   Online resource), representing the species' distribution along the eastern ecotonal range. A

177   total of 1,164 seeds of *N. nervosa* (115-166 seeds per population) and 1,151 of *N. obliqua*

178   (47-232), collected as a bulk from 40-50 trees per population were genotyped for 8 and 7

179   isozyme loci respectively (Azpilicueta and Gallo 2009; Marchelli and Gallo 2004).

180

181   Priorization of conservation activities should ideally be guided by measures of allelic

182   richness (Petit et al. 1998) which are highly sensitive to past demographic changes (Leberg

183   1992). Rarefaction provides unbiased estimates of allelic richness with great precision and

184   statistical power (Leberg 2002). Accordingly, we estimated the allelic richness (R_g) in each

185   population after rarefaction to the lowest sample size (g) for all three datasets (cpDNA,

186   isozymes and nuclear SSRs) (Pons and Petit 1995). For priorization and identification of

187   the most diverse populations (hotspots of genetic diversity) we calculated a standardized

188   allelic richness (R_{gst}) as shown in equation (1). For population *j*, R_{gst} was obtained dividing

189   *Rg* by the average *Rg* of the species for each marker and then summing up across markers.

190   Since chloroplast DNA has a lower mutation rate, but polymorphism revealed ancient

191   lineages, we doubled its contribution to the overall richness score. Hence R_{gst} for population

192   *j* was estimated as:

193    $$Rgst_j = \left(\frac{Rgiso_j}{\overline{Rgiso}}\right) + \left(\frac{Rgssr_j}{\overline{Rgssr}}\right) + 2 * \left(\frac{Rgcp_j}{\overline{Rgcp}}\right) \quad (1)$$

194

195 where $Rgiso_j$ is the allelic richness for isozymes, $Rgssr_j$ is the allelic richness for

196 microsatellites and $Rgcp_j$ is the haplotype richness for chloroplast DNA for population *j*.

197

Current distribution and ecogeographic analyses

199 We revised, for each species, those populations that currently occur under extreme

200 temperature or rainfall conditions. To do this, we identified outlier populations with annual

201 mean temperature or annual precipitation values above the third quartile + 1.5 *

202 interquartile range (IQR) and below the first quartile – 1.5 IQR for these variables. Climate

203 data was derived from 30 seconds resolutions climate layers from Worldclim

204 (www.worldclim.org).

205

Ecological Niche Modelling

207 We characterized the spatial distribution of favorable habitat conditions of both species under

208 current, past, and future climatic conditions by means of suitability mapping based on

209 ensembles of modelling algorithms, implemented in R package BiodiversityR (Kindt and

210 Coe 2005). Modelling algorithms considered were maximum entropy (MAXENT), boosted

211 regression trees (BRT, including a stepwise implementation), random forests (RF),

212 generalized linear models (GLM; including stepwise selection of explanatory variables),

213 generalized additive models (GAM; including stepwise selection of explanatory variables),

214 multivariate adaptive regression splines (MARS), regression trees (RT), artificial neural

215 networks (ANN), flexible discriminant analysis (FDA), support vector machines (SVM), and

216 the BIOCLIM algorithm.

217

218   We applied two different strategies for past and future suitability modelling, in line with our

219   interest in identifying potential LGM refugia in both Chile and Argentina, and assessing the

220   impact of anthropogenic climate change in Argentina only. Acknowledging that the

221   remaining *Nothofagus* trees and forests have been influenced by past anthropogenic

222   disturbances (logging, clearing forest for agricultural land etc), model calibrations for

223   projections to LGM climate conditions made use of presence points from both Chile and

224   Argentina which included records from the remaining continuous *Nothofagus* forests, as well

225   as most isolated trees and populations. Presence points consisted of 432 and 407 unique

226   presence sites for *N. nervosa* and *N. obliqua*, respectively (our own observations,

227   complemented with data obtained from www.gbif.org). Background points were randomly

228   selected from the area enclosed by a convex hull polygon constructed around all presence

229   points and extended with a buffer corresponding to 10% of the polygon's largest axis. Model

230   calibrations for projections to LGM climate conditions were carried out at 2.5 arc minute

231   resolution using only climate layers obtained from the Worldclim database (i.e. averages

232   from 1960–1990) as explanatory variables. This modelling approach does not assume that

233   sites were the species are currently absent, or of which no observation data was included in

234   our dataset, means that the site is therefore unsuitable for the species. If such sites have

235   climate conditions falling within the fundamental niche constructed based on the climate

236   conditions observed at sampling sites in our dataset, they will be recognized as suitable.

237

238   By contrast, model calibrations intended for projections to future climate scenarios (2050s),

239   were carried out at 0.125arc minute resolution (~250mx250m at the equator) using aside from

240   climate layers also altitude, slope, aspect, terrain roughness, direction of water flow, and soil

241   type (FAO et al. 2012). As accurate maps of the current Argentinean distribution of both

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242   species exist (Sabatier et al. 2011), our objective was to find the ensemble model solution

243   that performed best at predicting existing distribution areas, as this model is also likely to

244   provide more reliable indications of how remaining forests might respond to future climates

245   (and hence impact the in situ conservation of genetic diversity). Regional-based modelling

246   has been found to yield more reliable results compared to whole range modelling in a

247   southern beech from Australia (Worth et al. 2014). There is growing evidence that more

248   accurate potential distributions are generally obtained based on higher rather than on lower

249   resolution layers, particularly when making projections to future climate scenarios

250   (Gillingham et al. 2012; Seo et al. 2009). The relevance of performing modelling at high

251   spatial resolution is furthermore justified by the mountain habitat with steep climatic

252   gradients of our target species, where changes in climate are manifested at relatively small

253   scale (Dobrowski, 2011). Accordingly, we constructed a dataset of presence points in

254   Argentina based on the centroids of all 0.125 arc minute grid cells contained in (at least 60%

255   spatial overlap) existing distribution maps, and complemented this with the datasets used for

256   LGM model calibration to also include isolated trees. A set of absence points was obtained

257   from all cells located outside of current distribution maps and contained in the area delimited

258   by the latitudinal stretch between 35.85 and 44.4 decimal degrees south, and the longitudinal

259   stretch between 70.66 decimal degrees west and the border between Argentina and Chile.

260

261   For both modelling approaches, collinear explanatory variables were removed based on

262   iterative calculations of variance inflation factors (VIF), retaining only variables with VIFs

263   smaller than 5. The resulting sets of explanatory variables, as well as presence, background

264   and absence points used for model calibrations are given in Table S1. As spatial

265   autocorrelation among species presence points is known to bias model evaluations based on

12

266 cross-validation, we evaluated the ability of all individual modelling algorithms to cope with

267 spatial autocorrelation by calculating calibrated Area Under Curve (cAUC) values and

268 comparing these with a geographical null model (see Hijmans 2012). We compared the

269 cAUCs of each of the distribution models with the cAUCs of the geographical null model

270 resulting from ten iterations, by means of Mann-Whitney tests. Only models that gave cAUC

271 values that were significantly higher than the null model were retained for constructing

272 ensemble model combinations. In a next step, we calculated calibrated and non-calibrated

273 AUC values for all possible ensemble combinations of the retained models. Each ensemble

274 combination was constructed as the weighted average of its individual composing models,

275 using the cAUC values as weights. The ensemble that yielded the highest sum of calibrated

276 and non-calibrated AUC values was considered to be the most appropriate scenario for

277 projecting to past and future climate conditions, respectively

278

279 Suitability scores of individual projections were restricted to the maximum training

280 sensitivity plus specificity threshold obtained from model calibration under current climate

281 conditions. For model projections to LGM climate conditions we calculated average habitat

282 suitability values from two models (MIROC and CCSM; Braconnot et al. 2007). For

283 characterizing future climate conditions, we used 19 and 31 downscaled climate models for

284 the period 2040-2069 based on the SRES-A2 scenario of greenhouse gas emissions, obtained

285 from CMIP3 and the representative concentration pathway 4.5 obtained from CMIP5,

286 respectively (Ramirez-Villegas and Jarvis 2010).

287

288 *Zoning of populations to steer management interventions*

289   We used the following spatially-explicit classification to identify needs and opportunities

290   for the conservation and use of *N. nervosa* and *N. obliqua* genetic resources in Argentina in

291   light of expected climate change. The different zones are not mutually exclusive, but

292   intended to facilitate identification of the dominant conservation and management needs in

293   each area. The percentage thresholds do not have a specific biological meaning, but were

294   chosen from a practical perspective.

295   1. **Priority populations for *in situ* conservation:** populations with high genetic

296   diversity (hotspots) in areas that are likely to remain suitable under climate change

297   (according to at least 75% of future climate models)

298   2. **Priority populations for *ex situ* conservation and translocation**: Populations with

299   high genetic diversity in areas with high likelihood of drastic climate change

300   (expected to become unsuitable according to at least 75% of future climate models),

301   putting at risk their mid to long-term persistence. We also included populations

302   currently growing under extreme climate conditions, but located in areas expected

303   to become unsuitable in the future in this category. In the absence of scientific

304   evidence about whether or not these populations hold unique adaptive traits

305   underlying their local persistence, following the precautionary principle, we believe

306   their genetic resources should be safeguarded until further knowledge comes

307   available.

308   3. **Vulnerable populations**: Populations located in areas expected to be negatively

309   impacted by climate change (areas that are expected to become unsuitable according

310   to more than 50% of future climate models)

311   4. **New populations**: Natural migration, translocation, restoration or tree planting

312   opportunities in areas identified to become suitable under future climate (areas that

14

313   are expected to become suitable according to more than 50% of future climate

314   models).

315

316 **Results**

317 *LGM distribution*

318 Modelling results suggest that the distribution of suitable habitat of both species during the

319 LGM may have been much more extensive than at present (Fig 1). In general, LGM

320 modelling (Online Resource, Table S2) suggests a shift towards northern latitudes for the

321 eastern forests, possibly related with the extent of the ice-cap (Fig 1). Suitable areas of both

322 species during the LGM may have shown considerable overlap, most notably along the

323 Coastal Mountains and Central Valley in Chile, and to lesser extent in areas currently

324 occupied by steppe-like ecosystems in Argentina. The models suggest that suitable habitat

325 of *N. obliqua* may have been more restricted and fragmented than of *N. nervosa*, and that

326 vegetation fragments may have extended further north on the coastal side of the Andes. The

327 fragmented nature of suitable habitat conditions west of the Andes for *N. obliqua* is

328 mirrored by an abundance of exclusive haplotypes, much more so than for *N. nervosa*. A

329 more limited number of haplotypes are found east of the Andes, but for each of the species

330 some haplotypes are more abundant there than on the western side (Fig 1a and b). In

331 general, the distributions of haplotypes suggests that range expansions have taken place

332 across the Andes for both species.

333

334 The distribution of LCAs shows divergent patterns for both species (Fig 2). However, while

335 most of the current distribution ranges of both species in Argentina appear to have been

336 unsuitable during the LGM there seems to be a tendency for high LCA scores to be

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337 associated with different LGM suitable habitat fragments. The highest LCA scores were

338 consistently observed in the vicinities of suitable habitat (on one or the other side of the

339 Andes) with decreasing values at higher distances, which could be indicative of the

340 directions of range expansions during the warming period of the Holocene.

341

342 *Current distribution and ecogeographic analyses*

343 The annual mean temperature range for the studied *N. nervosa* and *N. obliqua* populations

344 was 6.9-8.6 ℃ 8.3–9.9 ℃, respectively (Table 1). Annual rainfall ranged between 936-

345 1461mm and 600-1474 mm for *N. nervosa* and N. *obliqua*, respectively. We found two

346 outlier populations under current climate conditions. The *N. nervosa* population of

347 Boquete, west of Lolog Lake occurs under much colder conditions than all other

348 populations, whereas the *N. obliqua* population of Pilolil persists under conditions below

349 the rainfall ranges (Table 1).

350

351 *Future distribution and genetic diversity*

352 Projections to future climatic scenarios developed for the third and fifth phase of the

353 Coupled Model Intercomparison Project (CMIP3 and CMIP5) of the World Climate

354 Research Programme were consistent for both species (Fig 3, Online Resource, Fig S1).

355 Therefore, in the following we focus on the projections obtained for CMIP5. The results

356 suggest that *N. nervosa* might be impacted more severely by climate change than *N.*

357 *obliqua*. No single area in Argentina exists where all future climate model projections

358 unanimously predict habitat suitability by 2050s for *N. nervosa.* In addition, in some areas

359 along the eastern distribution range, no single future climate model supports the potential

360 persistence of the species (Fig. 3a). Only a very tiny fraction of its current distribution is

16

361 expected to remain suitable according to at least half of all future climate model projections

362 (Fig. 3b).

363

364 Hotspots of genetic diversity of *N. nervosa* (Table 1) are located in areas that run the risk of

365 becoming unsuitable in the future (Fig 3a and 3b). The few areas that may remain suitable

366 tend to be located along the western edge of the current distribution, which is probably

367 because of the higher elevation of these areas and the fact that they are expected to receive

368 the largest amounts of precipitation.

369

370 For *N. obliqua* the predicted impact is less dramatic with potential suitability losses for the

371 northern (Epulauquen), eastern (Pilolil) and southern-most populations (Quila Quina) (Fig.

372 3c and 3d). In spite of the reduced surface areas occupied by these three populations, they

373 are highly relevant from a conservation point of view: Epulauquen and Quila Quina due to

374 their high genetic diversity and exclusive haplotype variants and Pilolil for being located at

375 the lower end of the species´ precipitation range in Argentina (Table 1). More

376 optimistically, a general increase in suitable areas is expected for this species, both at lower

377 and higher elevations, and in most of the areas that may become unsuitable for *N. nervosa*.

378 Interestingly, new potential areas are identified for *N. obliqua* towards southern latitudes

379 (around 42° S) where the species does not exist presently (Fig 3c and 3d).

380

381 Figure 4 shows the spatial representation of our zoning approach that combines the results

382 of the suitability modelling, the ecogeographical analysis and genetic characterization data.

383

384 **Discussion**

17

385 *Historical range dynamics and the distribution of genetic diversity*

386 Modeled distributions, both past and future, are in agreement with known species

387 preferences and physiological tolerances, as well as with the colder and wetter climate

388 predicted by fossil pollen (Moreno et al. 1999). At the LGM, *N. nervosa* is likely to have

389 been distributed more broadly than *N. obliqua*, in accordance with its higher tolerance to

390 low temperatures. On the other hand, a warmer and dryer climate would favor *N. obliqua,*

391 which is less vulnerable to cavitation than *N. nervosa* (Varela et al. 2010) and tolerates

392 warmer temperatures.

393

394 Numerous genetic studies have postulated the existence of multiple glacial refugia for

395 different Patagonian species (e.g. Arana et al. 2010; Azpilicueta et al. 2009; Marchelli et al.

396 2010; Marchelli and Gallo 2006; Mathiasen and Premoli 2010; Sérsic et al. 2011; Soliani et

397 al. 2015). Our finding of relatively large suitable areas during the LGM for both species

398 along the coastal mountain ranges and central valley in Chile, is in agreement with the

399 higher genetic diversity and multiple paleorecords found in this region (Heusser et al. 1996;

400 Moreno 1997). The more fragmented nature of suitable LGM habitat of *N. obliqua* than of

401 *N. nervosa* on the coastal side of the Andes is clearly matched by an overabundance of

402 exclusive haplotypes which tend to accumulate towards the northern end of the former

403 species´ distribution range. The restricted distributions of coastal haplotypes in *N. obliqua*,

404 and some in *N. nervosa* may hint at prolonged isolation of different populations during the

405 LGM. Differential expansion dynamics during the re-colonization from different refugia

406 (Magri et al. 2006), as a result of geographical barriers and differential competitive

407 advantages, may have contributed to further shaping the current distribution of haplotypes.

408 Some haplotypes are situated in areas that were unsuitable during the LGM and hence may

409 be the result of more recent forest expansion from nearby refugia.

410

411 While the scarceness of pollen records has until present constrained the confirmation of

412 cryptic refugia east of the Andes (Iglesias et al. 2014), the results of our suitability models

413 give support to the possible existence of eastern refugia. The overabundance of some

414 (exclusive) haplotypes in both species particularly towards the southeastern end, suggests

415 that a prolonged isolation of populations may also have occurred there. Combined

416 interpretation of the distribution of LCAs and haplotypes provides useful insights about the

417 locations of potential refugia and the direction of post-glacial expansion. Most of the

418 current distribution of both species is located in areas that were covered by ice during the

419 last glaciation and hence appear to be the result of post-glacial range expansion. Opposing

420 migration routes can be suggested in *N. nervosa* with haplotype I originating east of the

421 Andes and haplotype II in the west. Patterns of decreasing LCA richness could imply that

422 haplotype I is the result of genetic isolation of one or more populations in a southern

423 refugium followed by range expansion. Interestingly, the areas where the different putative

424 expansion routes meet are contemporary hotspots of genetic diversity (Fig 3a), probably as

425 a consequence of admixture of divergent linages (Petit et al. 2003).

426

427 In *N. obliqua* a possible westward expansion of haplotype I from a southeastern refugium is

428 supported by a decreasing trend in LCA from east to west, and by two exclusive

429 haplotypes. Haplotype II is found on both sides of the Andes, and probably had a wide

430 distribution prior to the LGM. The high genetic diversity scores found at the northernmost

431 population might be the consequence of admixture processes with western range

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432 expansions. No other convergence zones for *N. obliqua* were found east of the Andes.

433 Therefore, secondary hotspots of genetic diversity (indicated by high values of allelic

434 richness, Fig 3c) might be explained by the fact that both are located close to the putative

435 refugia where the genetic diversity of their source populations was concentrated during the

436 glaciation, as suggested by high LCA richness in both cases.

437

438 Climate relicts are populations that persist in isolated enclaves of suitable climate

439 surrounded by areas with adverse conditions (Hampe and Jump 2011). The long-lasting

440 persistence of populations in such enclaves may have been accompanied by strong climate-

441 driven selection pressures (Jansson and Dynesius 2002). For *N. obliqua*, notably the

442 northern (Epulauquen) and eastern (Pilolil) edge populations might correspond to these

443 criteria. They occur at the extremes of the species´ current climatic niche with Pilolil

444 receiving the lowest level of annual precipitation. Therefore these populations may be of

445 relevance for climate change adaptation strategies of *N. obliqua*. However, experimental

446 field trials such as provenance or progeny trials are necessary to confirm or refute this

447 hypothesis.

448

449 *Ensuring conservation and adaptation in light of climate change*

450 Based on the criteria of our spatially-explicit zoning approach, priority populations for *in*

451 *situ* conservation were only identified for *N. obliqua* (Fig 4b). However, for both species

452 future climate projections identified suitable habitat at higher altitudes west of some of the

453 diversity hotspots, implying that any *in situ* conservation strategy should promote

454 ecological connectivity to expedite natural migration of high diversity populations to these

455 suitable areas. This is particularly significant for *N. nervosa* whose populations seem to be

456 threatened most by climate change.

457

458 We identified numerous priority populations of both species holding high genetic diversity

459 and/or growing in extreme climates that are located in areas with elevated risks of drastic

460 climate change. Many of the targeted populations were classified as priority sites for

461 conservation attending to the high species richness, regional and micro-endemism and

462 presence of threatened species (populations indicated in Table 1; Rusch et al. 2008). Also a

463 hotspot of genetic diversity for other tree species was detected at latitude 39° S, coinciding

464 with some of our populations (Souto et al. 2015). The genetic resources of these

465 populations should be safeguard, either as *ex situ* collections in seed banks, or as live

466 collections through translocation to areas that will remain suitable in the future. Aside from

467 the priority regions indicated in Fig 4, also populations with high LCA richness (Fig 2) and

468 geographically located closest to putative LGM refugia should be targeted for germplasm

469 collection. The genetic uniqueness of such populations through prolonged isolation and

470 differentiation in LGM refugia should largely outweigh their low diversity for long-term

471 conservation purposes (Petit et al. 2003).

472

473 We characterized *vulnerable populations* as those occurring in areas that are likely to

474 become unsuitable according to at least half of future climate projections. We chose this

475 threshold from a pragmatic perspective, but acknowledge that this is a simplistic approach

476 as it does not consider all factors determining the persistence of populations. Vulnerability

477 of a species to climate change is a function of intrinsic and extrinsic factors combining

478 sensitivity, adaptability and exposure (Pacifici et al. 2015; Williams et al. 2008) and will

21

479 ultimately depend on life history traits such as high reproductive rates, short life span and

480 dispersal capacity. In the case of *Nothofagus*, adapting to a changing climate is conditioned

481 by the trade-off between having a long life span and high reproductive rate on the one hand

482 and low dispersal capacity on the other (Donoso 1993; Marchelli et al. 2012). A rapid

483 adaptation to climate change can be favored by the wide range of germination temperatures

484 observed in *Nothofagus* seeds during dormancy alleviation (Arana et al. 2016).

485

486 If the trends indicated by future suitability models are correct, it could imply that the

487 current mixed forests may gradually be replaced by pure *N. obliqua* stands. This is

488 supported by the fact that recruitment at some localities is already now largely dominated

489 by saplings of *N. obliqua* (Sola et al. 2015; Sola et al. 2016). However, this might also be

490 the legacy of past logging which was more intense towards *N. nervosa* and might have

491 promoted this differential recruitment. In addition, natural hybridization is highly frequent

492 at these localities. Since hybrids have a prolonged growing season and higher susceptibility

493 to early frosts (Crego 1999), they might be favored by a warming climate. A successional

494 change towards a hybrid zone might already be occurring in the mixed forest and could as

495 such increase the adaptive potential of these populations (Becker et al. 2013; Lexer et al.

496 2004; Rieseberg et al. 2003).

497

498 Translated to management practices, favouring the adaptive capacity and migratory

499 potential of populations characterised as vulnerable to climate change would imply (i)

500 broadening the genetic bases of populations (e.g. through introduction of new genes as

501 seeds from (multiple) areas); (ii) eliminating or reducing other threats and pressures they

502 are currently exposed to (e.g. presence of livestock); and (iii) enhancing ecological

22

503   connectivity between fragmented patches and with areas that will become suitable in the

504   future. Prior to initiation of movement of germplasm in natural forests, the fitness of

505   introduced germplasm and hybrids produced by intra and interspecific crosses should

506   ideally be tested under experimental conditions, so as to avoid possible deleterious effects

507   of maladaptation and genetic contamination.

508

509   Large areas of suitable habitat are predicted to appear in the future for both species, but

510   most significantly *N. obliqua*, outside of the species' current distribution ranges (Fig 3). In

511   the area around 42° S, provenance trials for both species have demonstrated good survival

512   and growth (Gallo et al. 2000), a basic measure of adaptation (Thomas et al. 2014).

513   Therefore, these areas might be considered for translocation from northern areas that

514   presently hold similar climatic conditions than those predicted for the future in the south. In

515   the case of *N. nervosa*, germplasm from the population of Boquete, west of Lolog lake

516   which occurs under much colder conditions than all other populations may be useful for

517   planting endeavours at these southern latitudes, as it may be more resistant against colder

518   temperatures that are more likely to occur there, at least in short term. The fact that figure

519   3b does not identify habitat suitability for *N. nervosa* in the area around 42° S suggests that

520   the threshold of at least half of future climate model projections predicting habitat

521   suitability may be too high.

522

523   Although the usefulness of species distribution modelling for guiding conservation and

524   management decision is increasingly being recognized (Van Zonneveld et al. 2014), some

525   caution is indeed important when using it to guide decision making. These models have

526   been designed for predicting habitat suitability only and it is much less clear how suitability

23

527 scores relate to population stability and persistence under rapid environmental change

528 (Guisan et al. 2013; Oliver et al. 2012). Suitability modelling may overestimate climate

529 change impacts on tree species distributions because they do not necessarily fully capture a

530 species´ adaptive capacity, which can be deducted from provenance trials established

531 outside the current species niches (Van Zonneveld et al. 2009). In a next phase suitability

532 model outcomes should therefore be complemented with information from existing and

533 possibly new provenance trials for matching the most appropriate reproductive material to a

534 given planting site (Thomas et al. 2014). However, assisted colonization can have

535 unintended and unpredictable consequences (Fady et al. 2016; Ricciardi and Simberloff

536 2009) and caution is in place before initiating a massive introduction of these two species to

537 areas where they could be considered as exotics.

538

539 To end with a positive note, concrete conservation policies have already allowed the

540 protection of two of the most diverse populations of *N. nervosa* (Hua Hum and Boquete) both

541 of them located in vulnerable areas (Gallo et al. 2009). Similar efforts are being pursued for

542 the *N. obliqua* population Lagunas of Epulauquen (Azpilicueta et al. 2014). In accordance

543 with the protection of both species within National Parks and Provincial Reserves, and the

544 new legislation in Argentina that promotes their use in restoration and afforestation activities,

545 a domestication program was initiated some years ago.

546

547 **Data Archiving Statement**

548 Genotypic data for the three genetic markers are provided as supplementary material (Table

549 S3).

550

24

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1: Sampled populations

Table S2 Suitability modelling metrics

Figure S1 Future (2050s) habitat suitability of *Nothofagus nervosa* and *Nothofagus obliqua*

with CMIP3.

Table S3 : Genotypic data for the three markers

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806 Figure Legends

807 Fig 1: LGM suitability distribution of *Nothofagus nervosa* (a) and *Nothofagus obliqua*

808 combined with chloroplast DNA haplotypes (b)

809

810 Fig 2: Distribution of locally common alleles at microsatellite markers for *Nothofagus*

811 *nervosa* (a) *and Nothofagus obliqua* (b).

812

813 Fig 3: Future (2050s) habitat suitability of *Nothofagus nervosa* and *Nothofagus obliqua* (a

814 and c) showing all areas identified as suitable by at least one of 31 future CMIP5 climate

815 model projections in combination with standardized allelic richness; (b and d) showing

816 only areas identified as suitable by at least 15 of 31 future climate model projections.
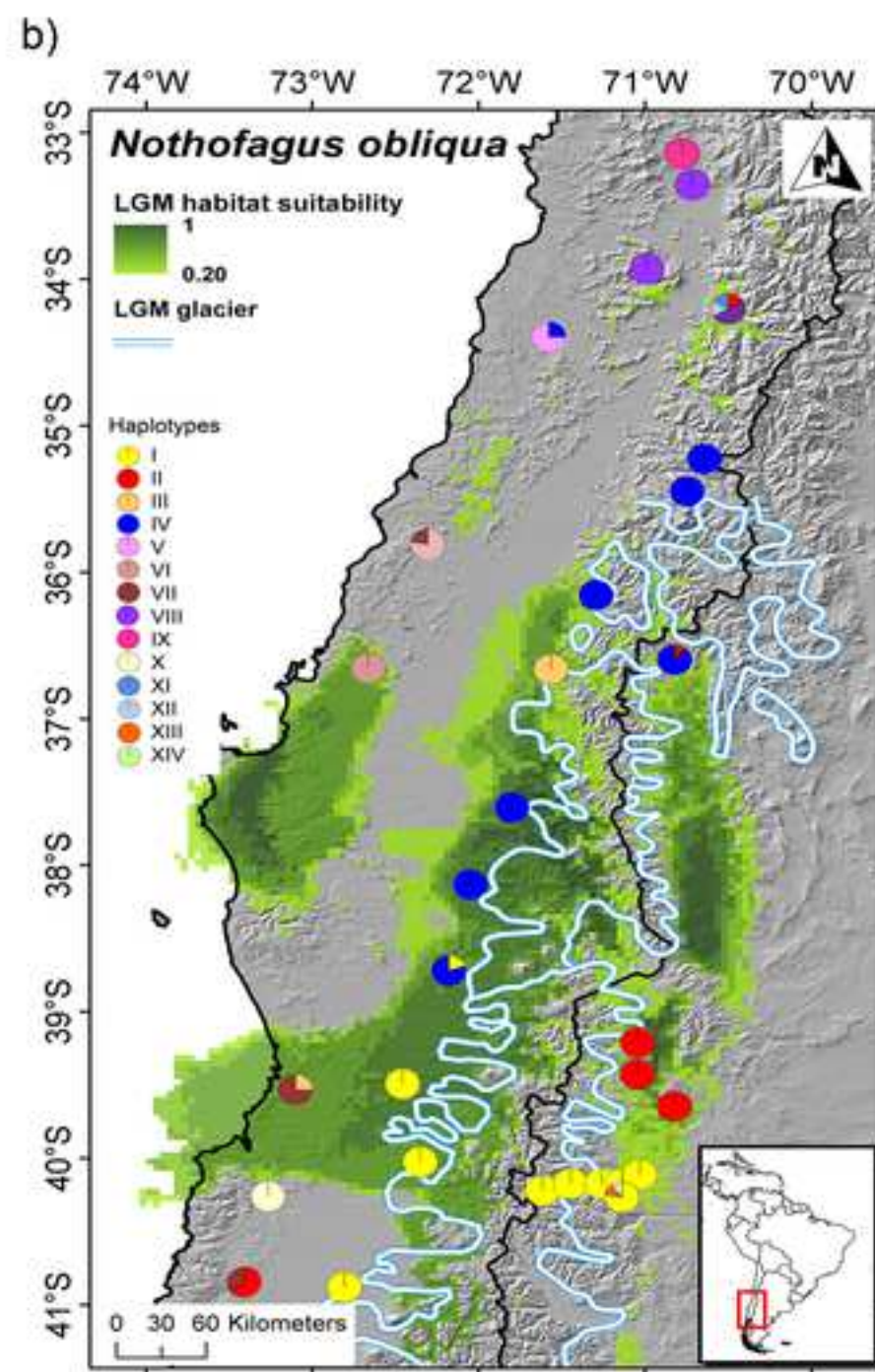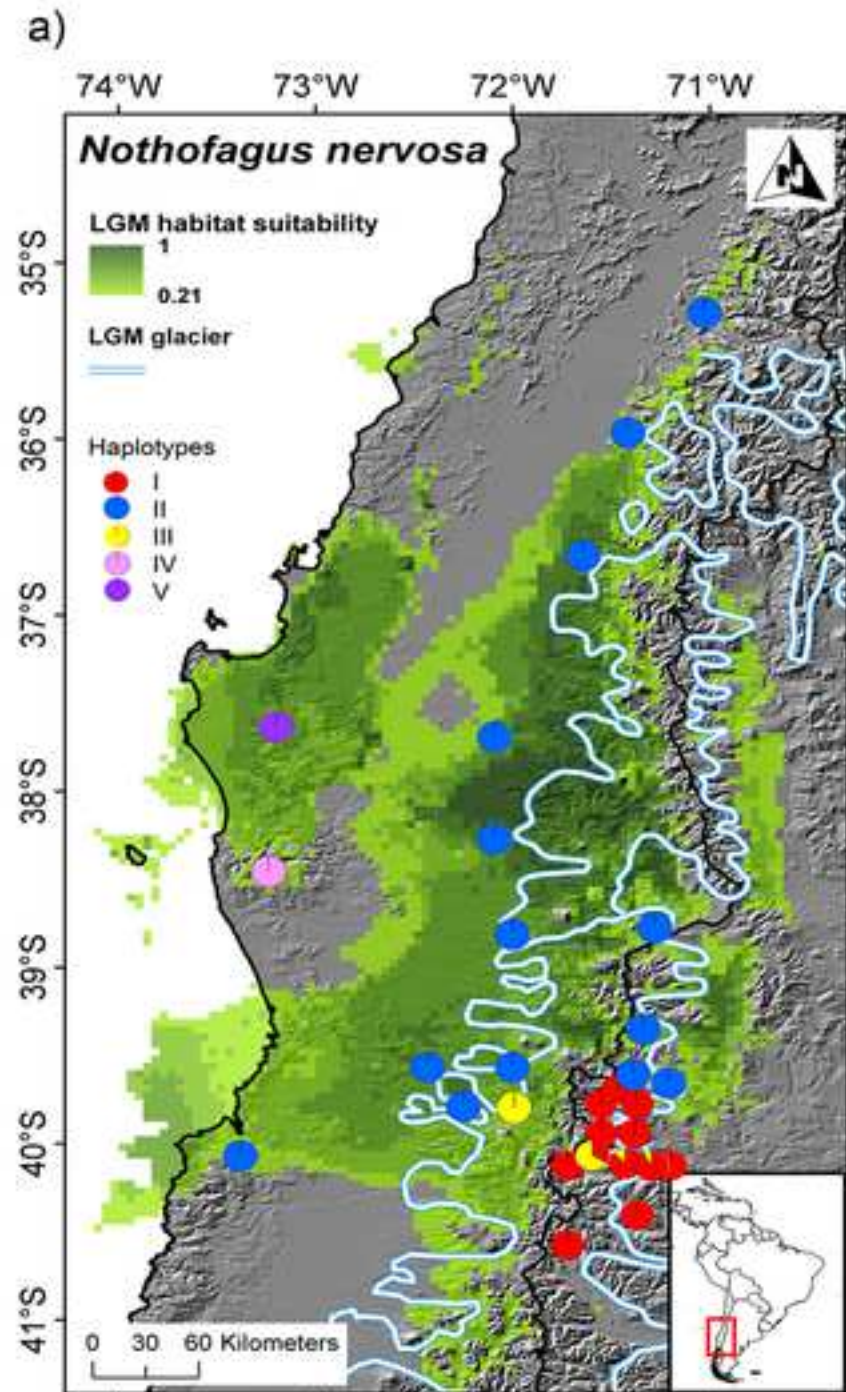
817 Fig 4 Zoning of the current distribution of *Nothofagus nervosa* and *Nothofagus obliqua* in

818 Argentina based on the expected impacts of climate change and the location of hotspots of

819 genetic diversity and populations growing under extreme climate conditions, to guide

820 management interventions. Hatched areas marked in red and purple are priority populations

821 for the collection of germplasm (for conservation in *ex situ* collections or translocation) and

822 *in situ* conservation, respectively. Unhatched populations marked in red, followed by those

823 marked in yellow are particularly vulnerable to climate change and require interventions to

824 enhance their adaptive and migratory capacity. Areas with potential for natural migration,

825 translocation, restoration or plantation are highlighted in different tones of green.

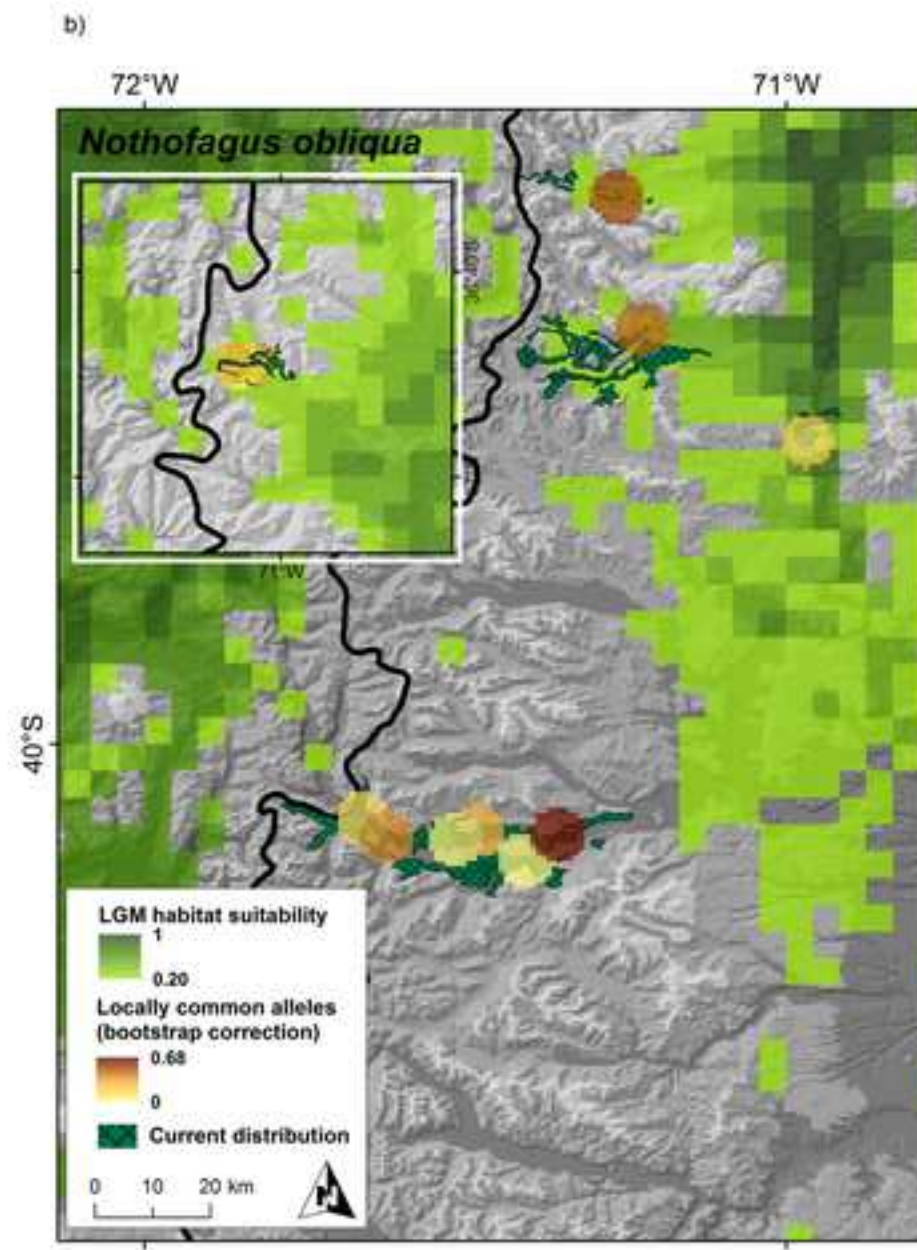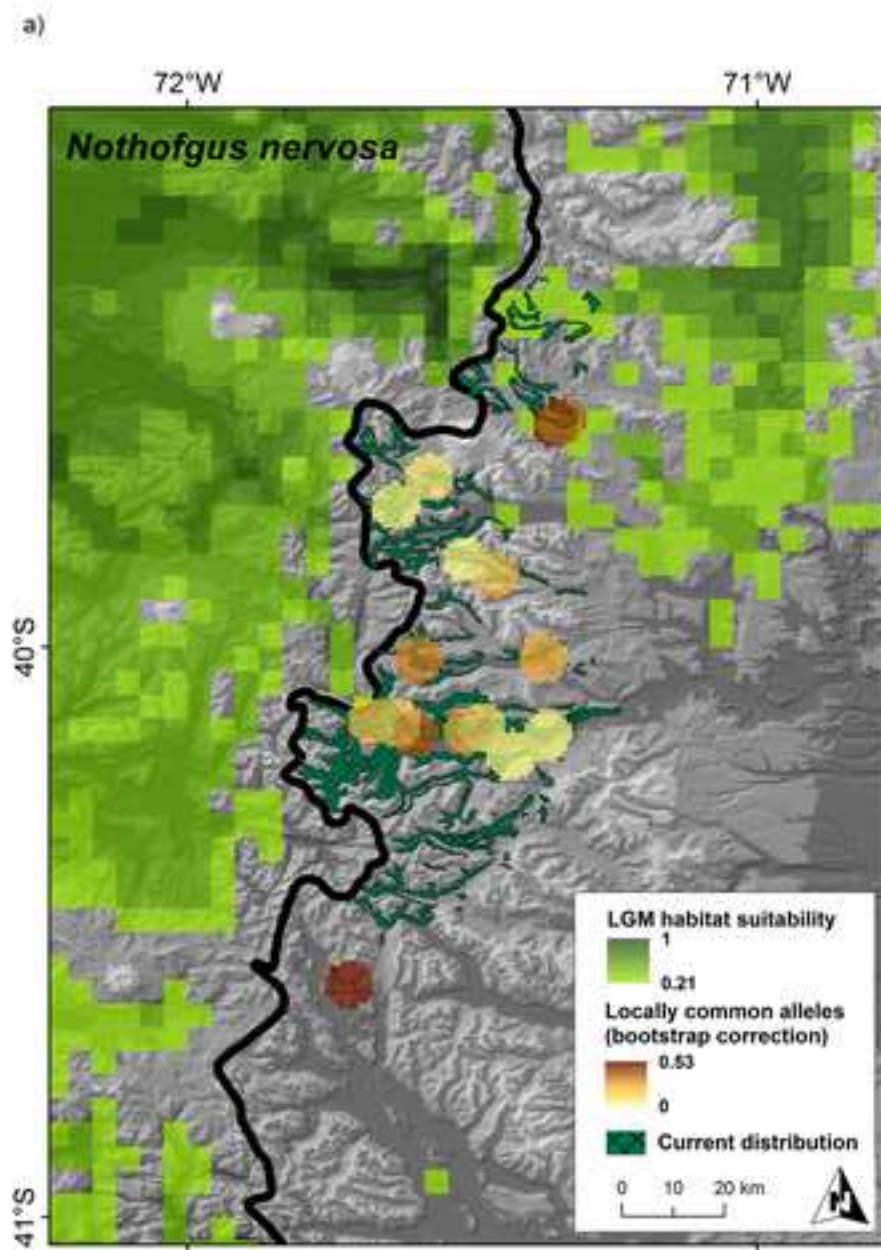826 Percentages indicate the proportion of models predicting suitability.

Table 1: Current climate conditions in each population of *Nothofagus nervosa* and *Nothofagus obliqua,* allelic richness (R_g) for the three markers after rarefaction to the lowest sample size and standardized allelic richness (R_{gst}).
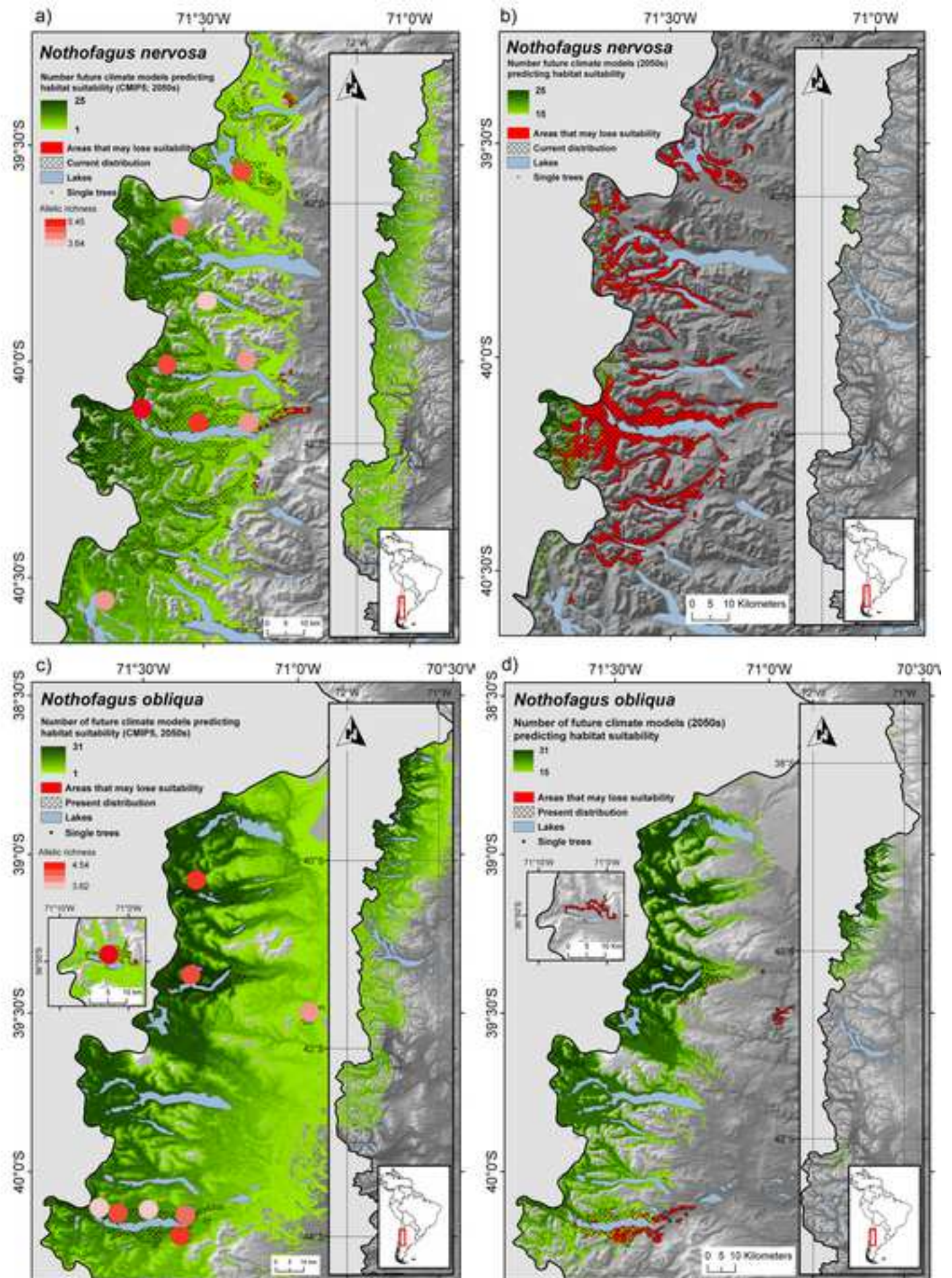
Population	Annual Mean temperature	Annual precipitation (mm)[a]	$R_{g\ cp}$	$R_{g\ iso}$	$R_{g\ SSR}$	R_{gst}
N. nervosa						
(1) Espejo[c]	8.1	1461	1	14.73	27.86	3.74
(2) Bandurrias	8.2	945	1	18.82	20.79	3.72
(3) Quilanlahue	8.3	1093	1.25	14.00	27.13	4.12
(4) Hua hum[c]	8.6	1395	1.727	20.97	28.53	5.45
(5) Pto Arturo	8.1	936	1	15.00	26.93	3.72
(6) Boquete[c]	**6.9[b]**	1141	1	18.51	27.24	3.94
(7) Currhue[c]	8	1090	1	14.00	26.56	3.64
(8) Lanin[c]	8.6	1285	1	17.60	25.44	3.82
(9) Tromen[c]	7.7	942	1	16.34	28.84	3.87
N. obliqua						
(10) Bandurrias	9.4	1009	1	11.58	29.33	3.86
(11) Yuco	8.7	1179	1	12.02	21.7	3.62
(12) Nonthue	9.5	1413	1	12.73	28.32	3.91
(13) Hua Hum[c]	9.6	1474	1	12.14	25.86	3.78
(14) Quila Quina	9.2	1067	1.191	14.91	24.06	4.29
(15) Quillen	8.6	863	1	12.80	29.61	3.96
(16) Ñorquinco	8.9	1025	1	15.75	29.7	4.19
(17) Pilolil[c]	9.9	**600[b]**	1	12.52	27.04	3.85
(18) Epulauquen[c]	8.3	960	1.166	14.88	32.33	4.54

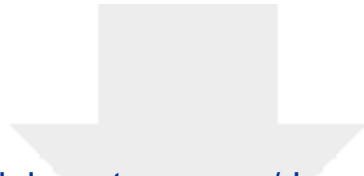[a] Climate data is derived from the downscaled 2.5 minutes resolution Worlclim dataset (http://www.worldclim.org). [b] These are outlier values according to the 1.5 *x* interquartile range. [c] Populations identified as priority sites for conservation (Rusch et al., 2008). R_{gcp}: chloroplast DNA. R_{giso}: isozymes and R_{gSSR}: microsatellites.

a) *Nothofgus nervosa*

b) *Nothofagus obliqua*

LGM habitat suitability
1
0.21
Locally common alleles
(bootstrap correction)
0.53
0
Current distribution
0 10 20 km

LGM habitat suitability
1
0.20
Locally common alleles
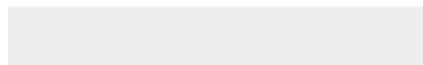(bootstrap correction)
0.68
0
Current distribution
0 10 20 km
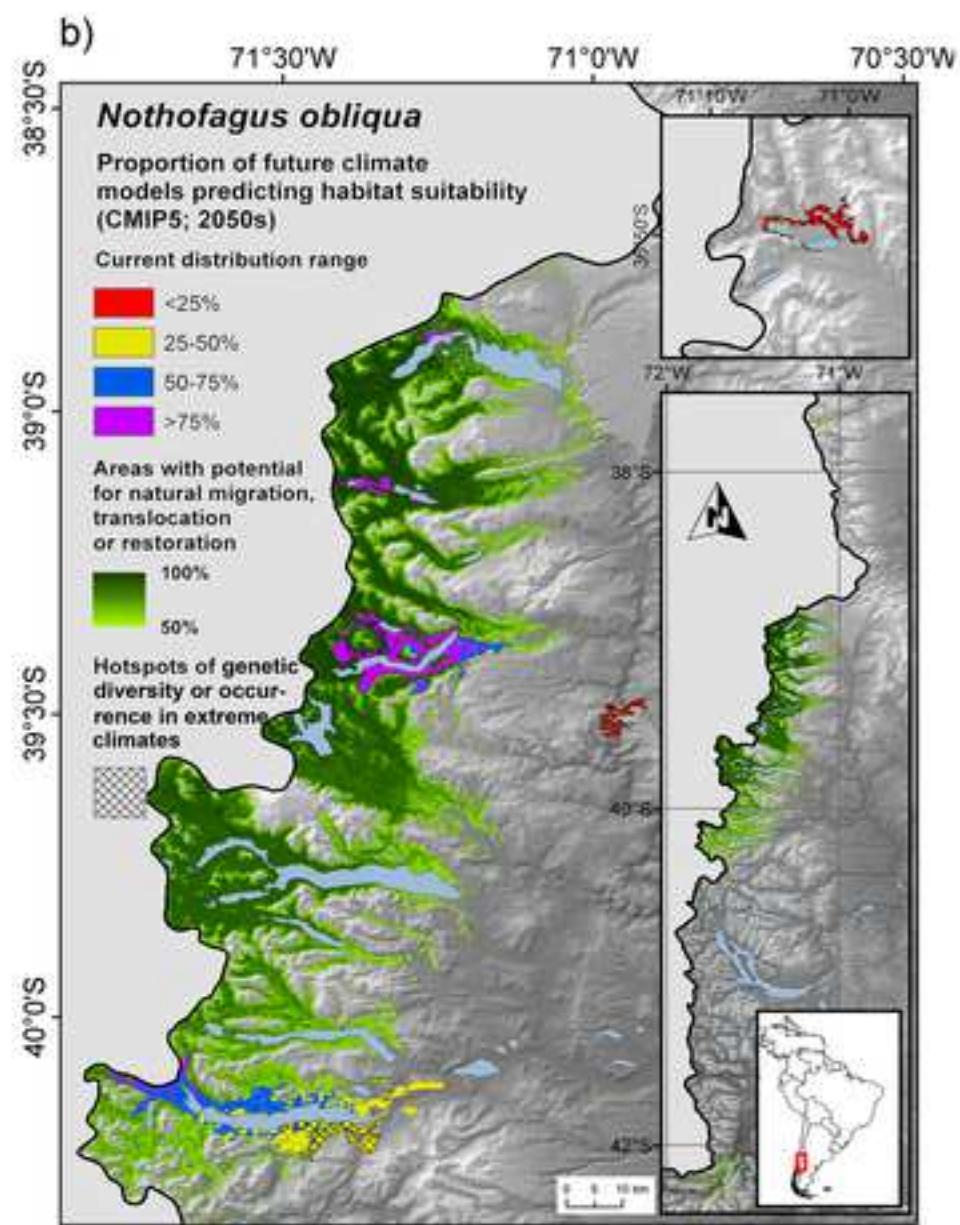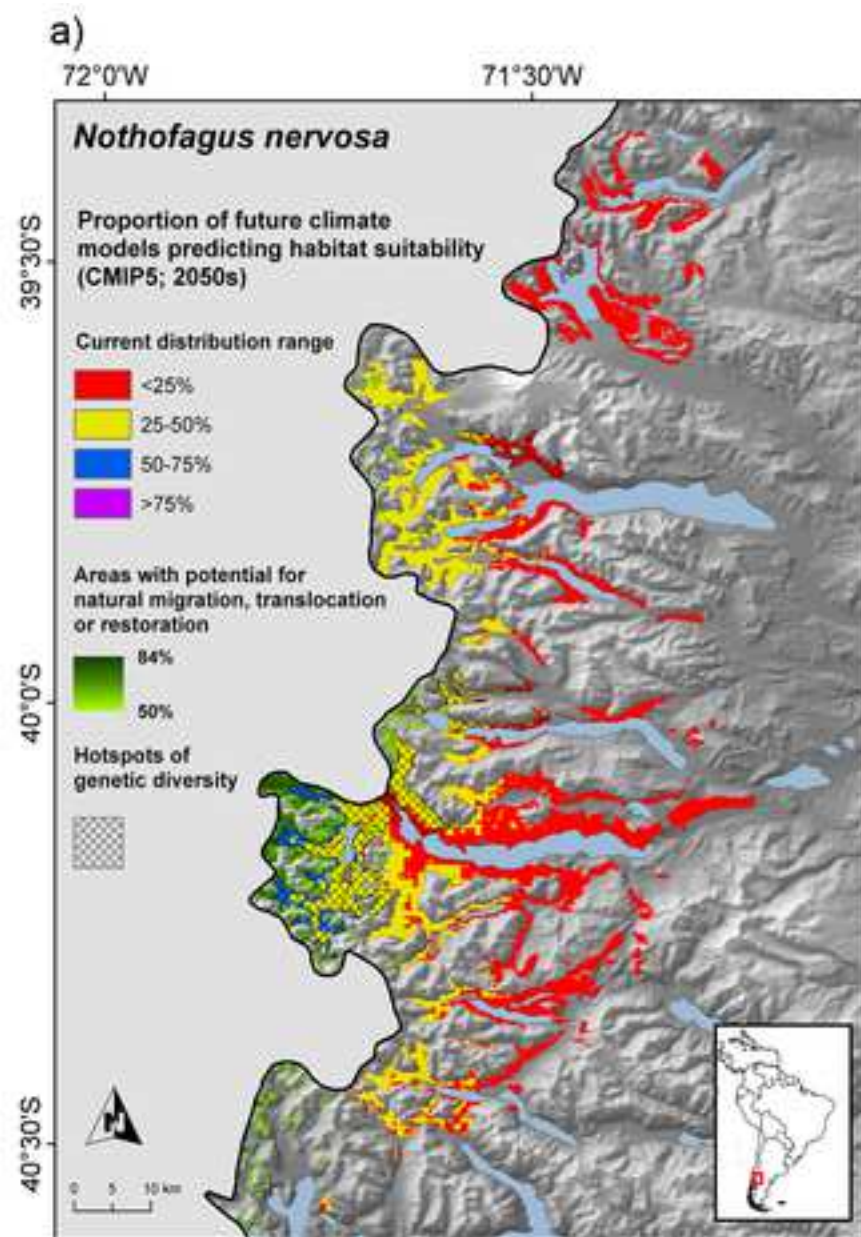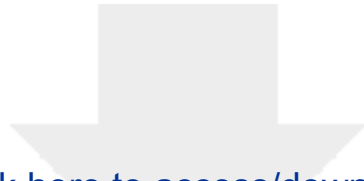
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Supplementary Material
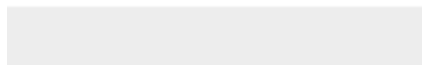Supplementary Material Marchelli-Thomas.docx

Click here to access/download
Supplementary Material
Suplementary Material Marchelli-Data markers.xlsx

Dear Dr David Neale

Editor -in-chief of Tree Genetics and Genomes

Herewith we submit the revised version of our manuscript "Integrating genetics and suitability modelling to bolster climate change adaptation planning in Patagonian *Nothofagus* forests" (Marchelli P, Thomas E, Azpilicueta MM, van Zonneveld M and Gallo LA) after the Minor revision requested.

Please find below (in red) the answers to the points addressed by the reviewer

Looking forward to a final decision

Sincerely

Paula Marchelli

Dear Authors,
first of all I would like to apologise for the delay in sending an answer about the suitability of this paper to be published in TGG. It was incredibly difficult to get the reports from the invited reviewers. In fact I got the comments by one reviewer only who did a good job providing some comments and suggestions useful to improve the paper. I think that you can easily deal with the comments so I ask for minor revision.
best regards
GG Vendramin

Reviewer #1: The manuscript entitled "Integrating genetics and suitability modelling to bolster climate change adaptation planning in Patagonian Nothofoagus forests" by Marchelli et al. integrates previously published genetic information (derived from plastidial DNA, nuclear microsatellites, and isozymes), and spatially explicit distribution model (Ecological Niche Modelling - ENM-) analyses to identify contemporary hotspots of intraspecific genetic diversity and to assess the potential impact of expected climate change on the suitability distributions of Nothofagus nervosa and Nothofagus obliqua in Argentina. Ultimately, the aim of the authors is to propose a conservation strategy for the species including in situ, ex situ and re-forestation initiatives in light of the obtained results.
The combination of molecular data and ENM became popular a decade ago, and there are many papers published for both American and European species (see for instance Premoli et al. 2010, Palaeogeography Palaeoclimatology Palaeoecology 298(3-4):247-256; Magri et al. 2007, Molecular Ecology, 5259-5266; etc.). In my opinion, these methodologies are still valid, and, if correctly applied, are may aid in solid strategy definition for the conservation of forest species (although they shouldn't be the only criteria contemplated in conservation planning).
Overall, I consider the manuscript suitable for publication in TGG. The manuscript is clear and concise: the Introduction describes the state of the art, the Material and Methods are correctly addressed, the Results are clearly presented (Tables and Figures are relevant), and the Discussion is well structured.
Molecular information was previously published, but I like the way it was incorporated into the

present paper, by computing relevant diversity indexes to infer conservation priorities. Particularly, I liked the way the ENM analysis was explained in material and methods. I appreciate the effort made by the authors to establish a priorization of conservation activities based on genetic results previously published (and re´-analyzed and re-interpreted here), and on the ENM results that are new to this paper. The four categories used here to define conservation strategies - (1) priority populations for in situ conservation; (2) priority populations for ex situ conservation and translocation; (3) vulnerable populations, and (4) New populations - are outstanding. This is an example of basic research with an applied component, as is essential to support the conservation activities defined by forest managers and regulators, and I, therefore, recommend its publication in Tree
Genetics and Genomes.

We are grateful for the positive and encouraging comments of the reviewer.

However, I have some "very" minor points the authors should consider before publication to improve the manuscript:
1. I suggest to remove or relocate the paragraph in page 10, lines 198-203. Maybe I missed something, but it makes no sense in the context, after the explanation of the standardized allelic richness parameter. The same for the paragraph in page 16, lines 341-348 (and associated Table 1). If this information is relevant to the paper, the authors should organize it better, maybe including a separate section in Material and Methods, with a sentence explaining why this is important.

We add a title to this section (Current distribution and ecogeographical analyses) in Material and Methods as well as in the Results section. We consider this information relevant to account for the current conditions under which the populations are distributing.

2. I am not a native English speaker, however, I found some minor typographic errors, and/or sentences that are vague throughout the text. A an example (not being exhaustive):
- Page 3, lines 33-34: "We investigated the impact of changes in past and future habitat suitability on the current distribution and threats to the genetic diversity of two southern beeches".
- "Modelling" is used in the title, while "modeling" is mostly used throughout the text (not always).
- I'd used the term "priorization" instead of "prioritization".
- Page 7, lines 140-141: "Nothofagus nervosa and N. obliqua". After a full stop, however, use the full genus name.
- Page 12, line 246: "beech" instead of "beach"
- Page 19, lines 410-412. This sentence is awkward. Which is the hypothesis the authors refer to?
- The way bibliography is referred in the text should be revised to comply to the instructions of the journal (some references have "," after et al., some have et al. in italics, etc.).
- Page 31, lines 808-809: "LGM suitability distribution of Nothofagus nervosa(a) and N.obliqua combined with chloroplast DNA haplotypes (b)".

We corrected all these typos.

3) Page 20, lines 443-445: This sentence is too vague. Probably the genes contained in those population are the same than in others. I think I understand what the authors mean, but this type of sentences should be avoided (see also, for instance, page 22, lines 500-502).

We deleted the vague sentences.

4) Page 22, lines 491-493: In the same line of my previous comments, what does it mean that natural hybridization is "elevated"? The higher fitness found for hybrids in warmer climates should be supported by a reference. To my knowledge, this sentence is rather speculative.

We changed "elevated" by "highly frequent" and we relativized our speculation for a higher fitness of hybrids in warmer climates.

5) Figure 4: What do the percentages in the legend refer to? This should be clarified in the figure caption.

We add a comment on the caption to address this information.