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Abstract: Extensive knowledge of the ecological and genetic consequences of implementing management practices (i.e. logging) in natural ecosystems is of fundamental importance to conservation action. Accordingly, characterization of forest genetic resources in managed vs non-managed stands may inform management decisions to ensure the long-term persistence of genetic diversity. The main objective of this study was to evaluate the impact of management practices on the genetic diversity and spatial genetic structure of contrasting forests, through an age-class sampling design and the use of microsatellite markers. We evaluated the impact of logging in three populations of Nothofagus pumilio, a dominant tree species in Patagonian temperate forests, by comparing managed and non-managed stands in each population. Selective extraction of best-featured individuals, i.e. those with forestry aptitude, such as higher trees with straight trunks and good sanitary conditions, was performed between 1990 and 2004. One of the studied sites was located in a state-protected area while the others were on private land affected by grazing. At each managed stand over-mature trees (MF-O), adult remnant trees (MF-A) and seedlings representing forest regeneration (MF-R) after silvicultural management were sampled. In non-managed stands age classes were restricted to adults (CF-A) and seedlings (CF-R). A minimum of 30 individuals per age class were collected, totalling 454 samples which were genotyped at six microsatellite loci.
Non-significant differences in genetic diversity were found between managed and natural woods in all populations. A trend towards decreasing frequencies or even allele loss among remnant adults of logged stands can however be interpreted as a sign of impact, probably a consequence of genetic drift. Each site showed particular, different outcomes with respect to genetic structure. While in Pop 1 (Huemules, 42°S) significant genetic differentiation was found between management treatments, admixture of genetic clusters (Bayesian clustering and DAPC analysis) occurred in Pop 2 (Guacho Lake, 43°S) and no genetic structure was found in Pop 3 (Engaño Lake, 43°S). Post-harvest genetic contact between contrasting stands is likely. A Landscape Interpolation Analysis showed clusters of individuals (shared genotypes) spatially restricted for

managed stands (significant in Pop 3), whereas a random spatial distribution characterized control forests. Therefore, it is possible that management affected and disrupted the genetic structure.
The different genetic patterns revealed for each population call for site-by-site interpretation. Differential intensity and frequency of management practices, presence/absence of livestock in the forest, and evolutionary history may all have had combined effect on current genetic diversity.

Harri Mäkinen, PhD

Editor-in-Chief

Forest Ecology and Management

Ref: Minor Revision of the Manuscript: Logging by selective extraction of best trees:

does it change patterns of genetic diversity? The case of Nothofagus pumilio

By: Carolina Soliani , Giovanni G. Vendramin, Leonardo Gallo and Paula Marchelli.

Dear Sir,

We greatly appreciate the comments made by the reviewers and the possibility to resubmit this revised version of our manuscript in order to get it published. The suggestions continue to improve our manuscript.

We followed all the recommendations and made corrections in the text as suggested, as we specify in a separate file named Reply to Reviewers.

Looking forward to your answer.

Yours sincerely,

Dr. Carolina Soliani

*Reply to reviewers

Harri Mäkinen, PhD

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By: Carolina Soliani , Giovanni G. Vendramin, Leonardo Gallo and Paula Marchelli.

We greatly appreciate reviewers' positive comments, which continue to improve our manuscript. All the minor comments handwritten by the reviewer in the file were incorporated in this revised version. Concerning the brief comments state by Reviewer 1 in the response letter we answer beneath each of them.

Reviewer #1: The revised version of the paper addresses my major comments. I think there are only a few clarifications needed. As stated in my first review the low effect of management on genetic diversity could be related either to relatively recent management activities in the "management plots" or due to a recent discontinuation of management activities in the control plots.

We do agree with the fact that management is recent and we want to clarify that control plots, to the best of our knowledge, were never managed. We included this information in the text.

More information is now provided in the Material and Methods section. However, it is not entirely clear for how long management has been discontinued. Actually, the information is provided in the discussion (Line 429), but should also be mentioned in Material and methods. Also, control plots were subject to indirect human impact (grazing).

Already in our previous version, we provided detailed information about years of management in the Material and Method section, as show below:

"A recent forest inventory of Chubut province compiled information on the areas with potential aptitude for wood extraction, based on an analysis of satellite images and field surveys (Bava *et al.*, 2006). This document also included a summary of the logging activities carried out in *N. pumilio* forests during the past decades, describing the intervened forests at a regional level. Logging has been registered since 1971, with peaks of most intensive activity from 1980 to 1985 (Bava *et al.*, 2006). The last date registered for forest management was in the 1990s for populations 1 (Futaleufú region) and 3 (Río Pico region), and in 2004 for population 2 (Río Pico region)".

As mention in the paragraph above the last date registered for forest management was in the 1990s and 2004 for each region, and no activity was performed since then.

While a lot of the discussion focusses on the differences between management and control plots, the relatively small effect of management should be explained and discussed in a bit more detail. The relatively recent start of the interventions seems to be the main reason.

We discussed a bit more about this issue by stating that impact could become more evident in future generations due to alterations in the mating system. Anyway, we consider that throughout the manuscript we already emphasize that the impact of logging on genetic diversity was low.

Even though the effect in the adult tree regeneration is small, the mating pattern could be affected resulting in a decrease of genetic variation in future tree generations. Future studies could analyze the genetic variation in the seedling generation and gene flow patterns. I think a short outlook section stating the potential future impacts on genetic variation and potential analyses could be provided.

In the Final Remarks section, we included this consideration made by the reviewer.

Abstract
Lines 39 -41: results on a Landscape Interpolation Analysis are summarized. However, significance was only detected in one plot. I think this sentence should be slightly rephrased. Done

Considering the handwritten edits there are only three that we didn't agree:

1-

after shelterwood exploitation, and hypothesized that seed or pollen dispersal from surrounding areas contributed to maintaining genetic diversity. The effective pollen dispersal distance was recently estimated as being below 50 m in *Nothofagus alpina* (=*nervosa*) (Marchelli *et al.*, 2012), a related wind-pollinated species from the genus. However, less frequent long-distance seed dispersal ? I think SGS is mainly to restricted seed dispersal. events should not be disregarded. Each year, from September to December, pollen release determines maximal pollen concentration in the air across middle latitudes in Patagonia (Bianchi & Olabuenaga, 2006). Simultaneously, west winds (i.e. westerlies) reach their maximum speed and are capable of carrying this pollen in low concentrations to sites as far away as the Atlantic coast (1,100 km from the nearest pollen release source) (Gassmann & Pérez, 2006). Therefore, in the evaluated plots, both short- and long-distance pollination events can contribute to maintaining or renewing the genetic composition of nearby populations.

Describe that this is mainly the result of restricted seed dispersal.

Pollen dispersal is expected to be over much longer distances.

In this part of the discussion, we were explaining that the observed cluster admixture could be the result of gene flow, mainly through pollen, between control and managed plots in one population. The reviewer refers to SGS and seed dispersal, which is not under discussion on this section, and therefore we do not follow his intention with this comment.

2-

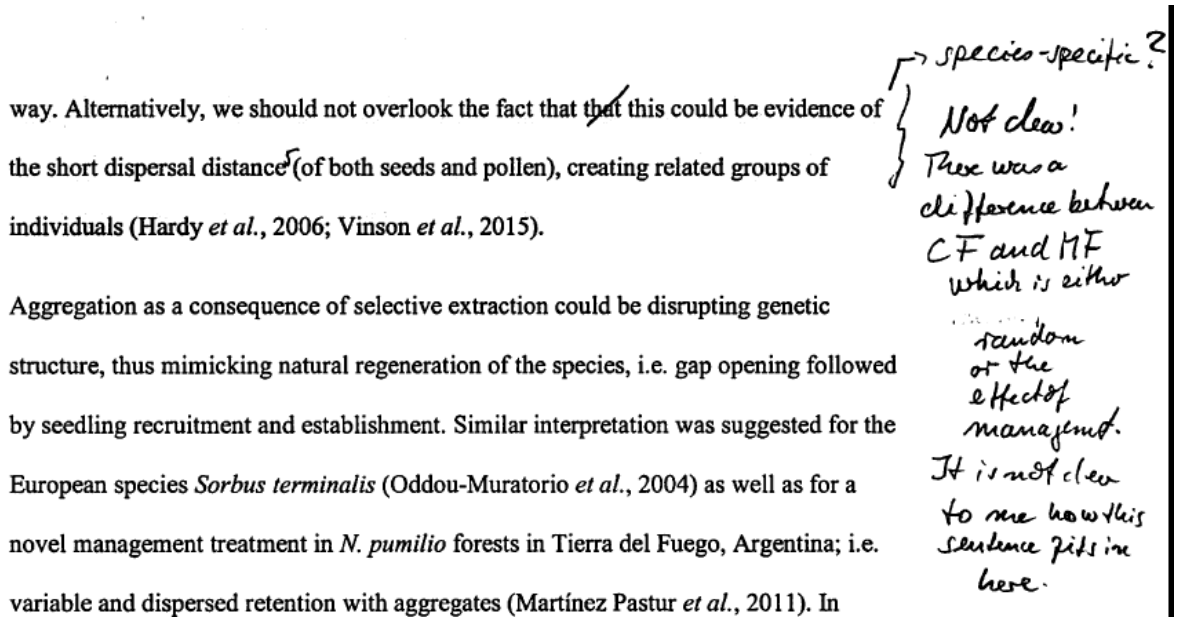
distribution obtained from 10,000 permutations of individual locations. To represent SGS patterns graphically, kinship coefficients were averaged in distance classes established *a priori* (50, 80, 110, 140, 170, 200, 230, 260, 290, 320 and >320 m) and plotted against distance.

The strength of the spatial pattern was evaluated by the *Sp* statistic that represents the rate of decrease in pairwise kinship coefficients between individuals with the logarithm

A minimum of pairwise comparisons and variable distance classes could have been defined.

Referring to this second comment, we added a statement about the minimum number of pairwise comparisons. If the reviewer refers to the analysis of variable distance classes with equal number of pairwise comparison in each class, this analysis is already included in the supplementary material.

3-


way. Alternatively, we should not overlook the fact that ~~that~~ this could be evidence of the short dispersal distance (of both seeds and pollen), creating related groups of individuals (Hardy *et al.*, 2006; Vinson *et al.*, 2015).

Aggregation as a consequence of selective extraction could be disrupting genetic structure, thus mimicking natural regeneration of the species, i.e. gap opening followed by seedling recruitment and establishment. Similar interpretation was suggested for the European species *Sorbus terminalis* (Oddou-Muratorio *et al.*, 2004) as well as for a novel management treatment in *N. pumilio* forests in Tierra del Fuego, Argentina; i.e. variable and dispersed retention with aggregates (Martínez Pastur *et al.*, 2011). In

Since the effect of management is not strong enough to distinguish from the species familiar structure due to restricted gene flow, we considered appropriate to discuss all the alternatives. Therefore, we didn't erase the last sentence which is the one that the reviewer doesn't like: "Alternatively, we should not overlook the fact that this could be evidence of the short dispersal distances (of both seeds and pollen), creating related groups of individuals (Hardy *et al.*, 2006; Vinson *et al.*, 2015)".

Finally, we want to stress that all minor corrections received were considered and we hope that this new version reach the standards for publication in Forest Ecology and Mangement.

Looking forward to your answer,

Yours sincerely,

Dr. Carolina Soliani

Logging by selective extraction of best trees: does it change patterns of genetic diversity? The case of *Nothofagus pumilio*

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Abstract

Extensive knowledge of the ecological and genetic consequences of implementing management practices (i.e. logging) in natural ecosystems is of fundamental importance to conservation action. Accordingly, characterization of forest genetic resources in managed *vs* non-managed stands may inform management decisions to ensure the long-term persistence of genetic diversity. The main objective of this study was to evaluate the impact of management practices on the genetic diversity and spatial genetic structure of contrasting forests, through an age-class sampling design and the use of microsatellite markers. We evaluated the impact of logging in three populations of *Nothofagus pumilio*, a dominant tree species in Patagonian temperate forests, by comparing managed and non-managed stands in each population. Selective extraction of best-featured individuals, i.e. those with forestry aptitude, such as higher trees with straight trunks and good sanitary conditions, was performed between 1990 and 2004. One of the studied sites was located in a state-protected area while the others were on private land affected by grazing. At each managed stand over-mature trees (MF-O), adult remnant trees (MF-A) and seedlings representing forest regeneration (MF-R) after silvicultural management were sampled. In non-managed stands age classes were restricted to adults (CF-A) and seedlings (CF-R). A minimum of 30 individuals per age class were collected, totalling 454 samples which were genotyped at six microsatellite loci.

Non-significant differences in genetic diversity were found between managed and natural woods in all populations. A trend towards decreasing frequencies or even allele loss among remnant adults of logged stands can however be interpreted as a sign of impact, probably a consequence of genetic drift. Each site showed particular, different

outcomes with respect to genetic structure. While in Pop 1 (Huemules, 42°S) significant genetic differentiation was found between management treatments, admixture of genetic clusters (Bayesian clustering and DAPC analysis) occurred in Pop 2 (Guacho Lake, 43°S) and no genetic structure was found in Pop 3 (Engaño Lake, 43°S). Post-harvest genetic contact between contrasting stands is likely. A Landscape Interpolation Analysis showed clusters of individuals (shared genotypes) spatially restricted for managed stands (significant in Pop 3), whereas a random spatial distribution characterized control forests. Therefore, it is possible that management affected and disrupted the genetic structure.

The different genetic patterns revealed for each population call for site-by-site interpretation. Differential intensity and frequency of management practices, presence/absence of livestock in the forest, and evolutionary history may all have had combined effect on current genetic diversity.

Keywords: *Nothofagus pumilio*, logging, age classes, microsatellites, genetic diversity, spatial genetic structure.

1. Introduction

Forest ecosystems have been increasingly influenced by human activities which alter the natural evolution of populations by impacting their genetic diversity and structure (Rajendra *et al.*, 2014). Logging, in particular, might result in impoverishment of forest stands, altering within-population genetic variation, the key to adaptation (Finkeldey & Ziehe, 2004). The analysis of genetic diversity trends could reveal signs of impact when comparing pre- and post-intervention forest stands (e.g. El-Kassaby *et al.*, 2003), and is crucial to the understanding of population evolution in space and time (Jump *et al.*, 2012). One sign of logging could be a decrease in allelic richness or modifications in heterozygote proportions, which are expected results in a remnant population due to the effects of genetic drift in small or reduced populations (Cornuet & Luikart, 1996). In addition, the effects of logging can be seen as a reduction in allele frequencies or loss of variants between the adult cohort and its regeneration (Rajora *et al.*, 2000). The genetic consequences of logging should also be evaluated in relation to other factors like the type of management practice, its frequency and duration, and post-management activities. Intensive forest management practices, e.g. clear-cut, would more directly affect the next generation, leading to a fragmented forest represented by fewer genetic variants, while selective extraction of best-featured individuals would probably decrease stand performance. Subsequent generations would probably not be capable of mitigating the effect of logging if they inherited a depauperate gene pool from their parents.

Impact on the spatial structure, i.e. the amount and distribution of genetic variation between and within local populations and individuals of a species, might be conditioned by both genetic and demographic processes (Jump *et al.*, 2012). Spatial structure is highly dependent on the mating system, but is also modeled by evolutionary forces such

as gene flow and genetic drift (Templeton, 2006). A disrupted spatial structure could benefit from gene flow from neighboring sites, through the contribution of new or lost genetic variants. Moreover, limited dispersion and density-dependent mortality events could also impact the genetic structure by affecting population demography, generating unbalanced proportions of genotypes (e.g. Hampe *et al.*, 2010). However, different outcomes are expected depending on the time that has elapsed since the last intervention. Due to long generation times, late reproductive maturity, high outcrossing rates and a very long life span (Petit & Hampe, 2006), the genetic impact could be overlooked in a tree population when we analyze current genetic variation. In addition, post-logging activities might also impose different pressures if conceived under a non-sustainable management scheme. The presence of livestock within the forests constitutes additional selective pressures for the seedlings emerging each year. Uncontrolled grazing could lead to damage in plant tissues and increased mortality. Moreover, the lack of post-clearing management in these ecosystems would not favor forest recovery if grazing pressure overcame the capacity of remnant trees to regenerate.

In Patagonia, Argentina, selective extraction was implemented over many decades (Bava *et al.*, 2006; Bava & Rechene, 2004; González *et al.*, 2006). The removal of best-featured individual trees (stem straightness and best sanitary conditions) may be expected to result in changes in allelic richness or modifications in the spatial distribution of alleles. The influence of logging on genetic structure and the factors affecting recruitment of natural regeneration is not yet well understood in temperate Nothofagus forests.

The main objective of this study is to evaluate the impact of management practices on the genetic diversity of a dominant forest tree. *Nothofagus pumilio* (Poepp. & Endl.)

Krasser reaches the upper altitudinal limit of the forests (treeline), but also inhabits other extreme areas like the boundary between forest and steppe in Patagonia (Donoso Zeger, 2006). Its ecologic characteristics, such as inhabiting different climatic conditions in terms of temperature and water availability (i.e. gradients within its natural distribution), may reflect an important adaptive potential, which could be relevant in the current context of climate change. *Nothofagus pumilio* has historically been one of the most exploited native species in Patagonia. In this study, we selected managed and non-managed stands of *Nothofagus pumilio* and estimated genetic diversity within stands and genetic differentiation between stands. We also evaluated spatial genetic patterns among individuals both within and among stands. We aim to answer the following questions: i) Would extraction of the best individual trees have an impact on the genetic diversity of the adult cohort? ii) Do levels of genetic diversity in over-mature and remnant adult trees reflect the extraction of best-featured individuals? iii) Would the variability of regeneration in managed stands mirror that of the remnant adult cohort? iv) Is spatial structure affected by individual extraction in managed stands? Comparisons of managed versus non-managed stands at the within-population level and between cohorts at within-stand level (adults vs. regeneration) were made by applying bi-parentally inherited molecular markers (nuclear microsatellites, nSSRs). This research seeks to contribute to our understanding of the possible impact of forest management on genetic diversity, with the expectation that this knowledge can help delineate future forestry actions.

2. Material and methods

2.1. *Ecological features of the species and sampled locations*

Nothofagus pumilio (Poepp. & Endl.) Krasser is a native, cold-tolerant species of temperate Andean forests, growing at altitudes higher than 1000 m a.s.l. and up to the treeline (reaching 2000m a.s.l. in some places). It is a strictly outcrossing species, is wind-pollinated and has limited seed dispersal, although long distance events can also occur (Gassmann & Pérez, 2006). Seedling recruitment close to mother trees characterizes *N. pumilio* (Rusch, 1993). Mature *N. pumilio* forests are characterized by a phase of understory regeneration when the oldest trees fall, and the gaps created allow for the settlement of an enormous number of seedlings which come mainly from seeds produced by nearby trees (Heinemann *et al.*, 2000).

Due to its excellent properties, *N. pumilio* wood is recognized as one of the best in terms of quality, as it is able to inhibit the action of pathogens for a long time (González *et al.*, 2006). Exploitation of natural stands has occurred since the early 20th century (Martínez Pastur *et al.*, 2010), with the extraction of large volumes of wood from primary forests. This exploitation mainly consisted of the uncontrolled extraction of the best trees: those with very straight stems, right-angled branches and good height and sanitary conditions. Selection was based on these much-appreciated forestry features.

Sampling sites are located in one of the most-exploited regions in Argentina (Chubut 42º - 44ºS), where selective extraction (locally known as "floreo") has been the most common forestry practice over past decades. *Nothofagus pumilio* forests dominate Andean ecosystems in the Chubut region. The climate in this area is characterized by hard winters with mean temperatures between -5°C and +5°C, and dry summers with mean temperatures of 10°C to 15°C. The precipitation regime is Mediterranean, winter being the rainy season (in the form of rain, and snow on the highest mountains; Bianchi & Cravero, 2010).

148 Plant material was collected in three paired stands representing managed (MF) and non-
149 managed control forests (CF) (the latter resembling the natural forest condition),
150 totaling six sampling locations (Figure 1). Sample sites were named as Pop 1-Huemules
151 (Rivadavia Mountain Range), Pop 2- Lago Guacho and Pop 3- Lago Engaño (Table 1).
152 Therefore, in each population a managed (MF) and a non-managed (CF) stand was
153 sampled.

154 A recent forest inventory of Chubut province compiled information on the areas with
155 potential aptitude for wood extraction, based on an analysis of satellite images and field
156 surveys (Bava *et al.*, 2006). This document also included a summary of the logging
157 activities carried out in *N. pumilio* forests during the past decades, describing the
158 intervened forests at a regional level. Logging has been registered since 1971, with
159 peaks of most intensive activity from 1980 to 1985 (Bava *et al.*, 2006). The last date
160 registered for forest management was in the 1990s for populations 1 (Futaleufú region)
161 and 3 (Río Pico region), and in 2004 for population 2 (Río Pico region). The only
162 population currently closed to livestock is population 2. In terms of severity (repeated
163 interventions in the same patches, ecosystem degradation) and frequency of exploitation
164 events, Pop1 (Huemules) is the most affected population, with a total volume of
165 extracted wood for the entire region of 284,451 m^3 (91% of which corresponds to *N.*
166 *pumilio*) (Bava *et al.*, 2006).

167 In order to select control forests we used the following criteria: closeness to managed
168 stands, absence of signs of tree extraction (e.g. presence of stumps, forestry paths) and a
169 similar forest structure to managed stands (i.e. multi-aged *N. pumilio* forests with
170 natural pulses of regeneration). Therefore, the control forests were never managed or
171 exploited.

172 Figure 1

173 In order to understand in depth the patterns of variation within and among stands, an
174 explicitly defined age-structured sampling was applied in each population. Plant
175 material from three groups representing different age classes was collected in managed
176 forests: over-mature trees (MF-O), adult remnant trees not selected for extraction (MF-
177 A) mainly because of sanitary problems or low forestry aptitude, and seedlings
178 representing forest regeneration (MF-R) following silvicultural management (Table 1).
179 Reports from the same geographic region indicate that seedlings of *N. pumilio* grow
180 approximately 30 cm (overall height) in the first 10 years of life (growth rate is 3 cm/yr;
181 Loguercio, 1995). Therefore, to ensure that sampled regeneration was the progeny of
182 the remaining trees, the total height of collected seedlings did not exceed 30 cm.

183 In the non-managed neighboring forest (control) adults (CF-A) and seedlings (CF-R)
184 representing natural regeneration were sampled. A minimum of 30 individuals in each
185 age class, approximately 30-50 m apart, were sampled from each site, totalling 454
186 individuals (Table 1). We did not follow a particular scheme in the sampling, trees were
187 rather chosen at random.

188 2.2. *DNA protocols*

189 Total DNA was extracted from dormant buds following Dumolin *et al.* (1995) using an
190 extraction buffer based on ATMAB (2% ATMAB = Alkyltrimethylammonium
191 bromide, EDTA 0.5 M pH= 8, Tris/HCL 1M ph= 8, NaCl 5M, 1% DTT, 2% PVP
192 40,000). SSR amplification conditions and PCR thermal profiles are described in
193 (Soliani *et al.*, 2010). The M13 protocol (Schuelke, 2000) was applied and the SSR
194 fragments were visualised on a MEGABACE 1000 (GE Healthcare) automatic

sequencer. The six polymorphic loci (*Npum*3, *Npum*9, *Npum*10, *Npum*13, *Npum*17a, *Npum*18) (Soliani *et al.*, 2010) were amplified in all the individuals from six stands (two stands per population, one corresponding to logging intervention and the second to the control). After excluding the samples with missing data at more than three loci, 432 individuals were analysed in total.

In order to assign fragments to bins we first explored our peak panel for each marker and then, aided by the "Autobinning" function on MEGABACE Fragment Profiler v2.2 (GE Healthcare), we labelled alleles based on our microsatellite motifs to create bin sets.

2.3. *Data Analysis*

2.3.1. *Genetic diversity and differentiation in each population*

Genetic diversity levels at both within-stand (age structured sampling design) and between management treatments (managed *vs*. non-managed) were estimated by calculating allelic richness (A_R) after rarefaction to a common sample size (El Mousadik & Petit, 1996) using FSTAT (Goudet, 2001). Allele frequencies and effective number of alleles (N_e), observed (H_O) and expected (H_E) heterozygosity were also calculated. Deviations from Hardy-Weinberg proportions in each population, locus by locus, was evaluated with GenAlEx 6.5 (Peakall & Smouse, 2006) and its significance calculated with a Chi-Squared test.

To detect populations which have experienced a relatively recent reduction in effective population size, BOTTLENECK (Cornuet & Luikart, 1996) with the Two-Phased Mutation Model (TPM) and Stepwise Mutation Model (SMM) was used. Microsatellite loci do not evolve at the same rate: 3- to 5-bp repeats are thought to evolve mainly

under the single-step model (SMM) while those with shorter repeats (2-bp) are supposed to mainly evolve according to a multi-step mutation model (TPM) (Di Rienzo *et al.*, 1994). As we screened di- and tri-nucleotide repeat microsatellites, we tested both models. Departures from the mutation-drift equilibrium were tested using the Wilcoxon signed rank test.

A hierarchical analysis of molecular variance (AMOVA) was performed to evaluate the proportion of genetic variation explained by a) age class-structured stands, and b) management treatments within populations (GenAlEx 6.5; Peakall & Smouse, 2006). Statistical significance was obtained based on 1000 permutations. Differentiation coefficients were reported as standardized values calculated via AMOVA, following the method implemented by Meirmans (2006).

The frequency of null alleles was estimated using FreeNA (Chapuis & Estoup, 2007). To evaluate the possible bias introduced by the presence of null alleles in the differentiation coefficients, F_{ST} was recalculated by implementing the "exclusion null alleles" (ENA) method (FreeNA; Chapuis & Estoup, 2007). Confidence intervals (95% level) were obtained through a bootstrap re-sampling procedure. In addition, presence of null alleles was taken into account in the estimation of inbreeding coefficients (F_{IS}) using INEST 2.0 (Chybicki 2014). Inbreeding was evaluated across age classes and populations considering management treatments separately, implementing an Individual Inbreeding Model (IIM) through a Bayesian approach. By using a Gibbs sampler, IIM estimates a Deviance Information Criterion (DIC) for each tested model. The model with the lowest DIC best fits the data. The software compares null alleles (*n*), inbreeding (*f*) and genotyping error (*b*) models in the data. Since we want to ascertain whether inbreeding was a significant component of the full model (*nfb*) in our

populations, we made comparisons with the *nb* model. Support is given to an inbreeding effect when the lowest DIC is found in the *nfb* model. Once the model is identified, the mean inbreeding coefficient and its 95% confidence intervals (after post-proccesing *.hyp file) are obtained.

2.3.2. *Bayesian clustering and multivariate analysis*

The individual-based genetic structure and admixture patterns were evaluated by implementing a Bayesian cluster analysis using STRUCTURE (Pritchard *et al.*, 2000) on the LOCPRIOR model (Hubisz et al., 2009) with admixture and correlated allele frequencies as described by Falush *et al.* (2003).

Six independent runs for each K (from 1 to 10) were performed with a 10,000 burn-in period and 100,000 repetitions, and the optimal number of clusters was evaluated based on the rate of change in the log probability of data between successive K values (ΔK) (Evanno *et al.*, 2005). Membership coefficients to each inferred cluster were post-processed using CLUMPP (Jakobsson & Rosenberg, 2007) and edited with DISTRUCT (Rosenberg, 2004).

Even if successful detection of the optimum number of genetic clusters is needed in a population genetic study, it could also be of great importance to get a real representation of relatedness between clusters (Jombart *et al.*, 2010). Discriminant Analysis of Principal Components (DAPC) can help to obtain the best discrimination of individuals into pre-defined groups. DAPC finds principal components which best fit the two conditions around cluster relationships: to summarize and detect differences between clusters and to minimize differences within clusters (Jombart *et al.*, 2010). We

performed DAPC with the Adegenet package in R software (R Development Core Team, 2011) considering age classes and management condition as predefined groups.

2.3.3. *Spatial genetic structure and its heterogeneity between management treatments*

These analyses were carried out in *N. pumilio* Pop 1 and Pop 3, where individual geographic coordinates were available.

Spatial Genetic Structure (SGS) was assessed in each treatment and population by obtaining kinship coefficients *F* (Loiselle *et al.*, 1995) with Spagedi 1.4 (Hardy & Vekemans, 2002). *F* coefficients for all pairs of individuals were regressed on the logarithm of spatial distance. SGS was tested by comparing the regression slope *b* to its distribution obtained from 10,000 permutations of individual locations. To represent SGS patterns graphically, kinship coefficients were averaged in distance classes established *a priori* (50, 80, 110, 140, 170, 200, 230, 260, 290, 320 and >320 m) and plotted against distance. The recommended minimum number of pairwise comparisons (30) was not reached in the first distance class of some plots.

The strength of the spatial pattern was evaluated by the *Sp* statistic that represents the rate of decrease in pairwise kinship coefficients between individuals with the logarithm of distance (Vekemans & Hardy, 2004). *Sp* has the desirable characteristic of being comparable between stands in a single study (i.e. silvicultural management *vs*. control) and between studies. *Sp* is calculated as $Sp=b_F/(F_1-1)$, where b_F is the regression slope of the kinship estimator F_{ij} computed across all pairs of individuals against their geographical distances, and F_1 is the average kinship coefficient between individuals of the first distance class (0–50 m). Given our sampling design, in which we selected trees 30 to 50m apart to avoid half- and full-sibs, *Sp* was obtained considering the mean F_1 value in the first distance class (0-50m), which is about the effective dispersal distance

288 reported for related Nothofagus species (Marchelli *et al.*, 2012; Veblen *et al.*, 1996).
289 The statistical significance of F_1 and b_F was tested based on 1000 permutations of
290 individual locations with SPAGeDi. In order to test whether management treatments
291 within populations had significantly different effects on SGS, mean values of *b* and
292 95% jackknife confidence intervals over loci were obtained and plotted.

293 In addition, to evaluate random *vs* structured spatial distribution of multi-locus
294 genotypes, autocorrelation analysis (Smouse & Peakall, 1999) and heterogeneity tests
295 (Smouse *et al.*, 2008) were performed. Distance classes of 50m and also the even size
296 class option in GenAlEx 6.5 (Peakall & Smouse, 2006) were used. Both methods are
297 described in detail in the supplementary material.

298 Finally, to estimate the Allelic Aggregation Index (AAI) the program Alleles in Space
299 (Miller, 2005) was run. AAI can be considered a measure of stand structure by
300 describing the presence of random, clumped, or uniform spatial distribution of
301 individuals, under the null hypothesis that each genotype (codominant data) is
302 distributed at random across the landscape (no aggregation). The index is expressed by
303 the R_j value, such that an $Rj=1$ is random, $Rj<1$ is a clumped or aggregated spatial
304 distribution and $Rj>1$ represents a spatially uniform distribution. As a global test
305 statistic for the entire dataset, R_j^{AVE} was calculated over all alleles and loci. The
306 significance of each test was evaluated through a randomization procedure where
307 individuals and genotypes are randomly redistributed among individual sampling
308 locations (Miller, 2005). A graphical representation of landscape distribution of
309 genotypes was performed by implementing Landscape Shape Interpolation and
310 Monmonier's algorithm in AIS. The first routine is a 3-d graphical representation of
311 patterns of diversity across the sampled landscape, which contains peaks in areas where

312 there are large genetic distances, and allows qualitative characterization of all areas of a
313 sampled site. The Monmonier algorithm identifies genetic barriers, interpreted spatially
314 as the point where genetic distances are maximal.

315

316 **3. RESULTS**

317 3.1. *Genetic diversity and differentiation coefficients*

318 Different trends in genetic variation were evident although no significant differences
319 were found between managed (MF) and control (CF) populations. A slight increase in
320 allelic richness (A_R) and a higher number of rare alleles (frequencies ≤5%) were
321 observed in CF-A of population 2 (Lago Guacho) with respect to MF-O and MF-A
322 (Table 1). An opposite trend shows private alleles (frequency ≥5%), with more alleles in
323 MF than in CF, in Pop 1 (Huemules) and Pop 3 (Lago Engaño). Accordingly,
324 regeneration from the MF had greater allelic richness than the CF. We also observed a
325 tendency towards loss of alleles or a decrease in frequency (more evident in rare alleles,
326 <10%) in old growth (MF-O) and remnant adults (MF-A) of managed forests in all
327 populations. These results could be a sign of the impact of logging on the adult cohorts.

328 Table 1

329 Even though a genetically impoverished population is expected after logging, no signs
330 of recent bottlenecks were observed (no significant Wilcoxon sign rank test in any age
331 class throughout the populations). Deviations from expected Hardy-Weinberg
332 proportions were detected at locus *Npum*10 in all populations and age classes, and is
333 probably related to the existence of null alleles. The most affected age class showing
334 departure from equilibrium was MF-A Pop 2 (4 loci out of 6), and all age classes from

CF in Pop 1 (Table S1). In contrast, Pop 3 showed expected HW proportions at almost all loci for both treatments and all age classes.

The differentiation coefficient (F_{ST}) corrected for null alleles was similar to the uncorrected value after implementing ENA correction (Table S2). As no impact of null alleles was observed, the original genotype data were used for further analyses. F_{ST} between MF and CF in Pop 2 was significantly different from zero after 1000 bootstrap re-sampling over loci (CI 95%). When analyzing the effects of null alleles on the inbreeding coefficients, we found that the *nfb* model best explained the data (inbred population) in CF of Pop 3 (F=0.078 [0.016-0.168]) as well as in MF of Pop 1 (F=0.077 [0.017-0.179]). After a detailed analysis of age classes, we found that MF-O (F=0.156 [0.02-0.276]) in Pop 1 and CF-A (F=0.140 [0.0009-0.2684]) in Pop 3 were the groups affected most by inbreeding. In all other cases (i.e. age classes), inbreeding coefficients were not significantly different from zero.

The proportion of genetic variance partitioned between the two treatments (i.e. managed *vs*. control) was low but significant in Pop 1 (5%, F_{RT}=0.048 p=0.001) (Table 2), and the standardized genetic differentiation was even larger (F'_{RT}=0.141). In the remaining populations, management treatments did not explain a significant proportion of the total variance. Notwithstanding, the variation explained by age classes was moderate and significant in Pop 2 (F'_{SR}=0.065 p=0.001), whereas it was low but still significant in Pop 1 (F'_{SR}=0.019 p=0.001).

Table 2

3.2. *Population structure through Bayesian clustering and multivariate analysis*

357 Optimum clustering with STRUCTURE was found at K=2 in all populations after
358 considering the rate of change in the log probability of data between successive *K*
359 values (*ΔK)* and the mean value of the log-likelihoods of 10 runs at each K. Inferred
360 clusters clearly reflect contrasting management in Pop 1 (Figure 2). However, moderate
361 levels of gene flow between stands are evident as greater levels of admixture were
362 observed when cluster partitioning was increased (for example at *K*=3, see figure S1). A
363 surprising result in Pop 2 is that regeneration from the control forest seems to represent
364 variation in the logged forest more closely, and vice versa (Figure 2). The latter could
365 be the result of gene flow between managed and control stands in this population.
366 Finally, no pattern of genetic structure was found in Pop 3. Discriminant Analysis of
367 Principal Components (DAPC) supports these results. The highest proportion of
368 variation (PC1) mirrors forest logging in Pop 1, in agreement with the K=2 of
369 STRUCTURE. A similar genetic composition of old-growth and remnant adults is evident
370 in this population, as well as the occurrence of genetic exchange between adults of CF
371 and regeneration of MF. Meanwhile, PC2 discriminates individuals by age since adults
372 and regeneration are separated in both MF and CF treatments. In Pop 2 it seems that
373 PC1 explains logging (at least partially), but it also reflects gene flow between stands
374 since regeneration is admixed. PC2 partially discriminates age classes in Pop 2. Finally,
375 in Pop 3 eigenvalues for both PC1 and PC2 are similar in magnitude, making it difficult
376 to associate the genetic disposition of variants with a single causal factor (Figure 2).

377 Figure 2

378 3.3. *Spatial patterns*

379 3.3.1. *Fine-scale genetic structure associated with management treatments*

380 The mean number of pairwise comparisons per distance class was 66 for Pop 1-MF, 19
381 for Pop 1-CF, 106 for Pop 3-MF and 34 for Pop 3-CF. Non-significant spatial genetic
382 patterns were detected in all treatments and populations evaluated. Therefore, simple
383 linear correlations between pairwise kinship coefficients (F_{ij}) and geographic distances
384 were plotted (Figure 3A). On the other hand, the mean jackknife regression slopes (*b*-
385 slope) and their 95% confidence intervals within stands illustrated that *N. pumilio* Pop 3
386 CF had a stronger and significantly different SGS pattern (more negative *b)* than the
387 other stands evaluated (Pop 1-MF, Pop 1-CF, Pop 3-MF) (Figure 3B). The *Sp* statistic
388 ranged from 0.0009 in MF-Pop 1 and 0.0016 in MF-Pop 3 to 0.0120 in CF-Pop 3, the
389 latter being the highest value. In CF of Pop 1, *Sp* was interpreted as zero since the *b*-
390 slope of the regression analysis was positive after jackknife procedure; consequently,
391 the calculated value of the statistic was negative.

392 Spatial autocorrelation and heterogeneity tests were non significant (Supplementary
393 material, Appendix 1, Table S1, Fig S2).

394 Figure 3

395 Different patterns of allele (genotypes in the case of co-dominant markers) distribution
396 emerged as a representation of contrasting management treatments from the allelic
397 aggregation index analysis (AAIA) and the Landscape Shape Interpolation (LSI). We
398 observed aggregation of genotypes in MF stands (*Rj* much lower than CF), while the
399 unmanaged forests have a more random distribution (higher *Rj* values) (Figure 4). The
400 global test carried out by calculating Rj^{ave} over alleles and loci was significant in CF-
401 Pop 3 Rj^{ave}=1.1, $p<0.01$, a value that could indicate uniform distribution of genotypes
402 throughout the landscape (no aggregation).

403 Figure 4

4. Discussion

4.1. *Variation patterns under different management treatments*

We assessed genetic diversity and genetic structure of contrasting *N. pumilio* stands in one of the most important forestry regions in Argentina (42-44°S). In each population we found a different picture when comparing selectively logged with control forest patches. As reviewed in several publications, the effects of management on natural forests varied widely according to the type of treatment, having multiple effects (positive, negative or neutral) on genetic diversity and the mating system (Rajendra *et al.*, 2014). The lack of a unique outcome in the three forests analysed is probably a consequence of local conditions (intensity and duration of management, time elapsed since the last intervention, additional practices, presence of livestock, etc), but could also be related to differences in the evolution of its gene pool throughout history (e.g. Soliani *et al.* 2015).

Forest management has been very intense for the last 50 years in the Chubut region, with a peak in the extraction of wood from 1981 to 1985, and a decrease towards 2006. About 60% of the harvested trees corresponded to *Nothofagus pumilio*, and the greatest number of registered logging events were recorded in the Futaleufú Norte region (where Pop 1, known as Huemules, is located) (Bava *et al.*, 2006). Furthermore, large areas of forests degraded due to fire or overgrazing further aggravate ecosystem conditions after logging, particularly in the Futaleufú Norte region (Bava *et al.*, 2006). On the other hand, the recent creation of a state-protected area with livestock restrictions (Reserva

426 Forestal Lago Guacho) where Pop 2 is located (Bava et al., 2006) favours successful
427 recruitment.

428 Considering the repeated cycles of intervention in the management history of the region,
429 we predicted a negative impact with decreasing genetic diversity from unmanaged to
430 managed stands. Even though it is not possible to disregard changes in genetic
431 parameters due to a single intervention event, the effect may become more evident in
432 the long term (see Vinson *et al.* 2015 and references therein). For example, an impact on
433 the mating system can only be observable in future generations. In this sense, the time
434 elapsed since the start of interventions (around 1971) in the region fails to encompass
435 two generations of *Nothofagus pumilio*, estimated to be in the range of 50 to 70 years
436 (Veblen *et al.*, 1996). Moreover, due to multiple factors probably affecting the current
437 genetic diversity of each population we should interpret our results with caution.

438 A tendency towards allele/genotype loss and lower genetic diversity has been described
439 in situations where human intervention has altered the complexity and biodiversity of
440 the system after wood extraction, especially in tropical ecosystems (Pautasso 2009 and
441 references therein). However, until now, different and even opposite trends in genetic
442 variation were described for temperate forests when comparing the type and strength of
443 management practices. For instance, beech forests (*Fagus sylvatica*) in central Europe
444 subjected to dissimilar logging intensity showed no significant differences in genetic
445 diversity (Buiteveld *et al.*, 2007). On the contrary, the loss of rare alleles and lower
446 allelic richness were observed in forest fragments from an ancient intervention (s. XV)
447 with respect to continuous *F. sylvatica* forests in Spain (Jump & Peñuelas, 2006). In our
448 case, population 2 (Guacho Lake) resembles the latter pattern of variation, since adults
449 from the CF had higher allelic richness and a greater number of rare alleles compared to

MF. Besides, both Bayesian (STRUCTURE) and discriminant analysis (DAPC) clearly allowed discernment of adult age classes from MF and CF. Although together these results support a possible negative impact of logging in this location, the amount of allelic richness (A_R) was not significantly different between managed and unmanaged adult classes. On the other hand, regeneration cohorts seem not to follow the trend, showing admixture of the same genetic clusters in both MF and CF. We presumed this could be due to genetic exchange between the plots through gene flow. Recently, Sola *et al.* (2016) working on a South American mixed *Nothofagus* forest, reported a lack of impact at species level after shelterwood exploitation, and hypothesized that seed or pollen dispersal from surrounding areas contributed to maintaining genetic diversity. The effective pollen dispersal distance was recently estimated as being below 50 m in *Nothofagus alpina* (=*nervosa*) (Marchelli *et al.*, 2012), a related wind-pollinated species from the genus. However, less frequent long-distance events should not be disregarded. Each year, from September to December, pollen release determines maximal pollen concentration in the air across middle latitudes in Patagonia (Bianchi & Olabuenaga, 2006). Simultaneously, west winds (i.e. westerlies) reach their maximum speed and are capable of carrying this pollen in low concentrations to sites as far away as the Atlantic coast (1,100 km from the nearest pollen release source) (Gassmann & Pérez, 2006). Therefore, in the evaluated plots, both short- and long-distance pollination events can contribute to maintaining or renewing the genetic composition of nearby populations.

Levels of genetic diversity were slightly higher in MF than CF in Pop 1 and Pop 3, not only in the adult cohort but also when comparing regeneration in both treatments. Of particular importance in a temperate forest species is the fact that logging remnants may still belong to large populations that can be self-maintained and whose genetic

474 composition is not substantially affected by the action of erosive evolutionary forces
475 (e.g. genetic drift; Dubreuil *et al.*, 2010). Alternatively, a diverse gene pool could also
476 reflect the historical imprint, such as the admixture of postglacial colonization routes
477 (Soliani *et al.*, 2015). In long-lived species the short time lapse since forestry
478 management began may not have erased past genetic patterns. Even so, we should
479 interpret the results with caution because of the relatively low number of individuals
480 assessed in each age cohort and/or the low number of markers employed (e.g.
481 Westergren *et al.*, 2015). However, logging in Pop 1 was clearly detected by the PC1 in
482 the discriminant analysis of principal components (Fig.2), interpreted as distinctive
483 characteristics between MF and CF forest patches.

484 An associated problem with the selective extraction of adult-fertile individuals is the
485 decrease in pollen donors and seed producers, increasing mating events between
486 relatives (Dubreuil *et al.*, 2010; Jump&Peñuelas, 2006). In the most intensively logged
487 site, Pop 1-MF (Bava *et al.*, 2006), we detected an inbreeding coefficient significantly
488 different from zero, suggesting that the lower density favors non-random mating (3 out
489 of 6 markers showed high values of F_{IS}). The significance of the F_{IS} coefficient was
490 mainly explained by the genetic variation found in the over-mature age class individuals
491 (higher F_{IS} value), which are probably major contributors to the pollination and seed
492 production of the population. Conversely, a significant inbreeding coefficient in CF-A
493 Pop 3 could indicate the occurrence of a higher level of inbreeding, depicting a
494 particular familial structure at that location (4 out of 6 markers showed high F_{IS} values;
495 see next section). The long-term presence of livestock has been a common feature in
496 both Pop 1 and Pop 3, a fact that could impede forest regeneration more directly. Over-
497 grazing, particularly affecting young seedlings could confound our interpretations, thus

provoking a bias in estimations of inbreeding. Furthermore, it would be worth considering that the analysed stands were exploited relatively recently, and as we have already discussed, not enough time has elapsed to complete two generations in this tree species (Rajendra *et al.*, 2014). The consequences of management, if any, in the genetic structure will be more clearly seen in the next and subsequent generations.

4.2. Spatial genetic structure and the effects of logging

We detected, as a general outcome, an absence of spatial genetic structure (SGS), with few exceptions. The lack of a significant relationship between genetic and geographic distances was observed both in managed and control forests and autocorrelation analysis did not detect a clear pattern in the spatial disposition of genetic variation.

Our sampling encompassed wider areas of forest (>2 ha) although we did not perform a complete census of the patches. To look for possible impacts on the forest structure we checked available records of forest density from the surveys of the corresponding state institution (Dirección General de Bosques y Parques, Chubut Government). We noticed that the current number of trees per hectare in the MFs did not substantially differ from those of CF sites. Notwithstanding, the lack of historical records precluded the comparison of density after selective logging. If logging was mainly focused on the best individual phenotypes, then a notable decrease in tree density should not be expected because the extraction did not generate large treeless areas in the forest.

Accordingly, heterogeneity tests for contrasting management treatments (MF vs CF) did not reveal significant differences in genetic variation in relation to space (Supplementary material Appendix 1, Table S3, Fig S2). Even so, the absence of

significant differences in this analysis does not completely clarify whether there was a real impact as a result of management.

However, interesting results emerged on performing a Landscape Interpolation in AIS (see 3-D plots and Monmonier's algorithm). We found a high, significant Rj^{ave} in CF-Pop 3 (Rj^{ave}=1.1 $p<0.01$), although Rj^{ave} values were not significant in either MF or CF of Pop 1 and MF of Pop 3. However, based on the calculated values there seems to be a random spatial distribution of genotypes in CF (higher *Rj* values) compared to MF (lower *Rj* values). Our results suggest that in logged *N. pumilio* stands there are some spatially restricted clusters of individuals (shared genotypes). As a sign of the impact of management, genetic structure has probably been affected and/or disrupted in some way. Alternatively, we should not overlook the fact that this could be evidence of the short dispersal distances (of both seeds and pollen), creating related groups of individuals (Hardy *et al.*, 2006; Vinson *et al.*, 2015).

Aggregation as a consequence of selective extraction could be disrupting genetic structure, thus mimicking natural regeneration of the species, i.e. gap opening followed by seedling recruitment and establishment. Similar interpretation was suggested for the European species *Sorbus terminalis* (Oddou-Muratorio *et al.*, 2004) as well as for a novel management treatment in *N. pumilio* forests in Tierra del Fuego, Argentina; i.e. variable and dispersed retention with aggregates (Martínez Pastur *et al.*, 2011). In addition, this could be one reason why we did not find significant differences in genetic diversity between MF and CF, or even higher diversity in MF, since incoming dispersal vectors (pollen and/or seeds) could be ensuring the reproduction and renewal of managed plots. The positive genetic correlation in the 400 m distance class found in one of the analysed sites (Huemules, Fig S2) would also support this hypothesis, since

canopy opening could have favoured the arrival of pollen even from great distances. Genetic variability could be preserved thanks to the connection with neighboring populations (Hamrick *et al.*, 1992). Nevertheless, if the population is ancient and has a diverse gene pool, probably a relic from the glaciations or a product of admixture of different lineages (Soliani *et al*, 2015), it might not be severely affected by the impact of limited logging.

We also explored fine-scale (within stand) spatial genetic structure in both MF and CF by obtaining the *Sp* statistic, whose values are comparable with those obtained previously in the same species (Mathiasen & Premoli, 2013) and in beeches from Europe (e.g. Piotti *et al.*, 2013; Sjölund & Jump, 2015). The *Sp* values in *N. pumilio* fit with the expected values for a wind pollinated species (Vekemans & Hardy, 2004). As proposed for fine-scale patterns of variation, the statistic should mirror the ecological features and demographic history of the species (Jolivet *et al.*, 2011; Valbuena-Carabaña *et al.*, 2007). Of particular relevance in our study is the higher and significantly different (from the other plots) *Sp* value detected in CF-Pop 3 (Lago del Engaño). However, we could not confirm the higher strength of SGS since within stand *Sp* values were not significant. This unmanaged plot was the most severely affected by overgrazing. Consequently, it probably suffers more severe restriction to dispersal, or its spatial genetic structure is determined by the crossing of few reproductive trees, conforming families (also explained by a significant inbreeding coefficient).

We cannot be heedless of the possibility that the relatively low number of pairwise comparisons biases these results. Better assessment of SGS can be obtained by increasing the sample size and/or scoring more markers (Cavers *et al.*, 2005; Hardy *et al.*, 2006; Vekemans & Hardy, 2004).

568 *4.3. Final remarks*

569 Trends in genetic diversity among individuals from selective logged forest patches and
570 control woods allowed evaluation of the possible impact of management. To our
571 knowledge, this is the first time that several populations of the species *Nothofagus*
572 *pumilio* have been evaluated together with site location features and inferences on
573 population dynamics, resulting in a comprehensive analysis of the problem. Although a
574 preliminary work performed by Godoy & Gallo (2004) reported a significant difference
575 in the distribution of one Mdh-b allele in a single plot (control vs. managed) in Tierra
576 del Fuego, Argentina (55°S), this is the first attempt using age-class sampling and a
577 multi-locus approach. Here we report the absence of significant modifications in the
578 patterns of genetic diversity at neutral makers or genetic structure of the forests. Tree
579 populations are known to have high genetic diversity, which, together with the short
580 time lapse since the management practices, could temper the real impact of logging in
581 these forests. Nevertheless, the mating system could had been affected resulting in a
582 decrease of genetic variation in future tree generations. Future studies should focus on
583 the genetic variation in the seedling generation and gene flow patterns.

584 The possibility of adaptation of *N. pumilio* after natural or anthropogenic disturbances
585 will ultimately depend on its adaptive genetic variation. The species can face adverse
586 conditions using a combination of genetic based and plastic responses (Premoli, 2003;
587 Premoli & Brewer, 2007). In the current scenario of climate warming, which is already
588 affecting Patagonian forests (e.g. Suárez & Kitzberger, 2010), it is necessary to have
589 thorough knowledge and carry out research as to the consequences (ecological and
590 genetic) of implementing management practices. In southern Patagonia the combination
591 of a long-term warming trend and a significant decrease in precipitation (Castañeda &

592 González, 2008) has affected growth in *N. pumilio*, evidenced by differential inter-
593 annual ring-width (Masiokas & Villalba, 2004). A decreasing performance of tree
594 growth could affect individual fitness, which will ultimately influence the genetic
595 structure of natural populations. As crucial structural components of forests, tree
596 population survival may be key to ensuring the conservation of natural ecosystems and
597 their biodiversity, with the aim of promoting their sustainability (Sjölund & Jump,
598 2013).

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606 **Table 1**. Sampling sites of *Nothofagus pumilio* representing stands with selective extraction of individuals (MF) and natural forest (CF).

607 Geographic coordinates of each stand are indicated by Latitude (Lat.) and Longitude (Long.).

Pop.	**Treatment**[1]	**Lat. (S)**	**Long. (W)**	**Age class**	**N**	A_R	$Na_{<5\%}$	H_O	H_E	F_{IS}[IC 95%]
Huemules (Hm)	Hm_MF ˆ	42°49'44"	71°27'41"	O	30	42	14	0.530	0.677	0.0769* [0.0171-0.1791]
				A	30	40	14	0.465	0.645	
				R	39	34	13	0.454	0.607	
	Hm_CF	42°50'14"	71°28'46"	A	35	34	13	0.565	0.666	0
				R	35	31	14	0.458	0.593	
L. Guacho (G)	G_MF†	43°49'35"	71°27'41"	O	30	29	14	0.437	0.536	0
				A	30	31	7	0.494	0.582	
				R	30	37	12	0.578	0.648	
	G_CF	43°48'53"	71°29'41"	A	30	32	19	0.463	0.592	0
				R	40	35	7	0.473	0.602	
L. Engaño (Eg)	Eg_MF ˆ	43° 51' 24,81"	71° 32' 36,16"	O	30	24	9	0.439	0.547	0
				A	30	21	5	0.428	0.506	
				R	30	32	11	0.483	0.518	
	Eg_CF	43° 49' 52,19"	71° 35' 3,93"	A	30	22	8	0.360	0.546	0.0779* [0.0158-0.1680]

				R	30	30	9	0.458	0.513	

[1]Last date of registered management extraction in 1990s ˆ and 2004†

N: number of sampled individuals; A_R: allelic richness with rarefaction number based on a common sample size for each population; $Na_{<5\%}$: number of alleles with frequencies under 5% (considered as rare alleles); H_O observed and H_E expected heterozygosis; F_{IS}: inbreeding coefficient estimated in each management treatment and population using INEst; values significantly different from zero are indicated (*). O: over-mature; A: remnant adult; R: regeneration (MF); A: adult; R: regeneration (CF).

613 **Table 2.** Analysis of molecular variance (AMOVA) between management treatments in

614 each population and among age classes within populations.

Source	*df*	SS	Est. Var.	Percent	Stat	Sign.	F'_{RT}
Pop 1-Hm							
Between treatments	1	18.53	0.100	5%	**F_{RT}=0.048**	p=0.001	0.141
Among age class/treat.	3	12.75	0.039	2%	**F_{SR}=0.019**	p=0.001	
Within age class	293	571.4	1.950	93%	**F_{ST}=0.066**	p=0.001	
Pop 2-G							
Between treatments	1	5.607	0.000	0%	**F_{RT}=-0.013**	***ns***	0.0
Among age class /treat.	3	26.78	0.126	6%	**F_{SR}=0.065**	p=0.001	
Within age class	279	507.3	1.818	94%	**F_{ST}=0.053**	p=0.001	
Pop 3-Eg							
Between treatments	1	2.997	0.009	1%	**F_{RT}=0.006**	***ns***	0.012
Among age class /treat.	3	5.305	0.003	0%	**F_{SR}=0.002**	***ns***	
Within age class	277	449.0	1.621	99%	**F_{ST}=0.007**	***ns***	

615 *df:* degrees of freedom; SS: sum of squares; Est. Var.: estimated variance; Stat: statistic

616 value; sign: significance after 1,000 permutations; F'_{RT}: standardized differentiation

617 coefficient; treat.: treatment.

FIGURE LEGENDS

Figure 1. Sampling locations in Chubut forestry region (42-44°S). Maps of individual-sampled trees are shown for Pop 1 and Pop 3. The explicitly defined aged-structured sampling design is represented with different symbols in each population. Since managed and non-managed stands in Pop 3 are more distant, two separate maps are presented for better visualization**.**

Figure 2. Genetic structure in the evaluated populations represented as A) Individual membership coefficients for genetic demes with Bayesian clustering (STRUCTURE) and B) Discriminant Analysis of Principal Components (DAPC). MF-O: over-mature trees, MF-A: adult remnant trees not selected for extraction, and MF-R: regeneration of managed forest; CF-A: adults and CF-R: seedlings of non-managed forest.

Figure 3. Spatial correlations using pairwise kinship coefficient (F_{ij}) (Loiselle *et al.*, 1995) and distance classes. A) Linear regression of pairwise kinship coefficients against geographic distances (m), 95% confidence intervals are indicated with dashed lines. B) Mean jackknife regression slopes (*b*-slope) and their 95% confidence intervals within stands and treatments.

Figure 4. Graphical representation of genotype distribution with Landscape Interpolation Analysis. Allelic Aggregation Index (AAI) R_j and genetic barriers obtained with Monmonier's algorithm (top right corner of each graph) are shown. a) Pop 1- MF; b) Pop 1-CF; c) Pop 3-MF; d) Pop 3-CF. X-axis corresponds to latitude coordinates (South) and Y-axis corresponds to longitude coordinates (West).

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Highlights

- Impact of selective extraction of best trees in natural *Nothofagus pumilio* forests.
- A trend to loss of alleles in remnant adults of logged stands could be a signal of the impact.
- Post-harvest genetic contact through gene flow is suspected between contrasting stands.
- Clusters of trees spatially restricted (agglomeration) was detected in managed stands.
- Time elapsed since the last intervention could be not enough to measure the real impact.

Figure 1

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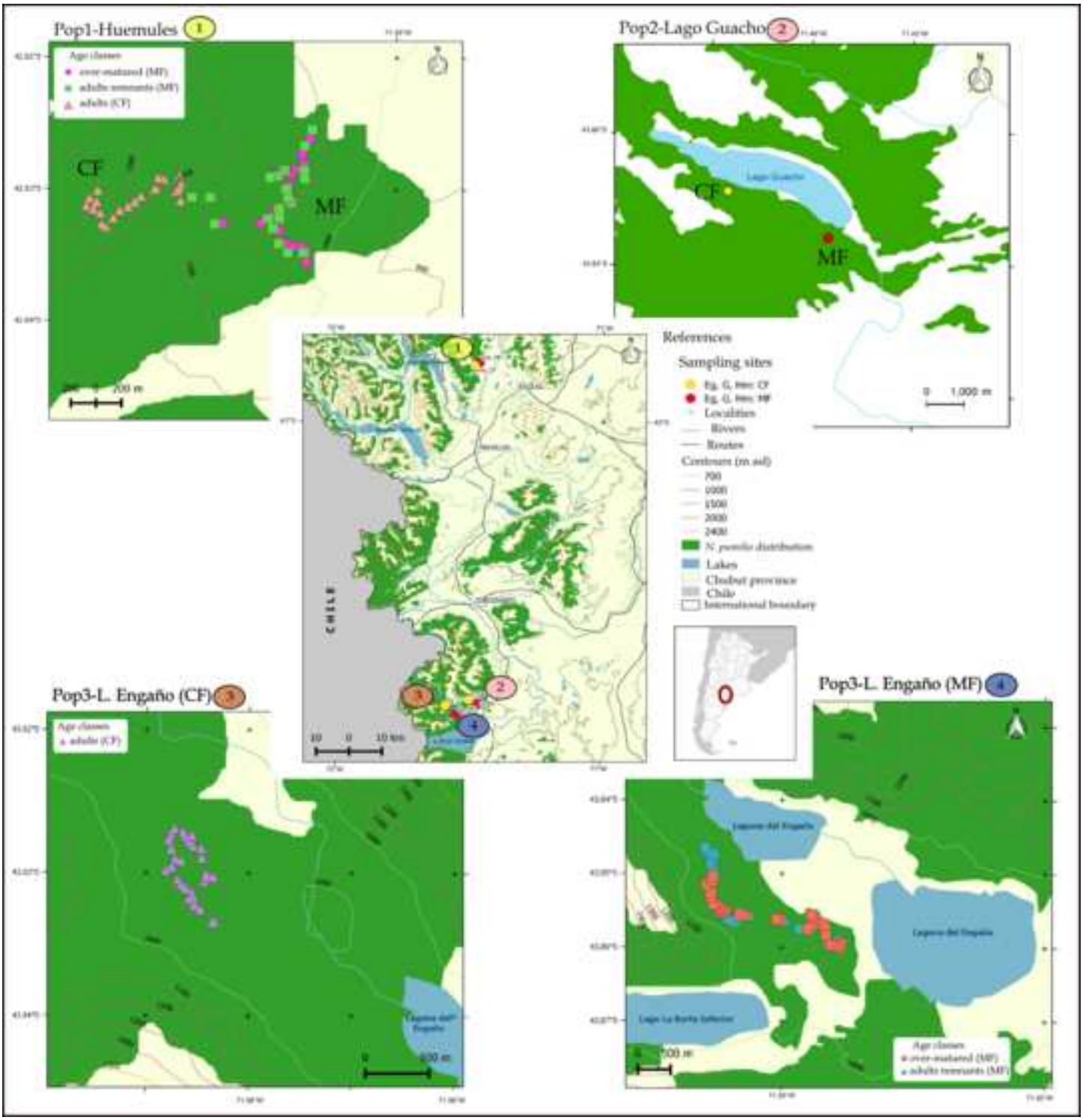

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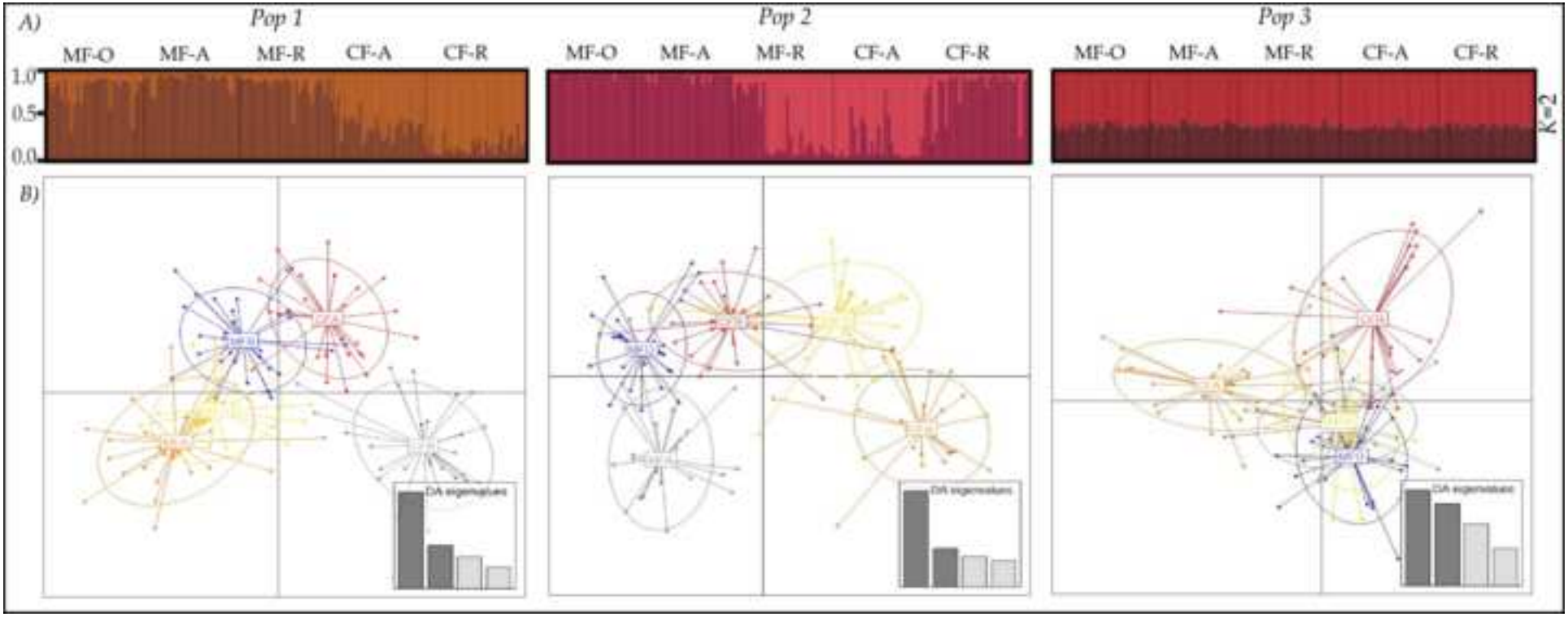

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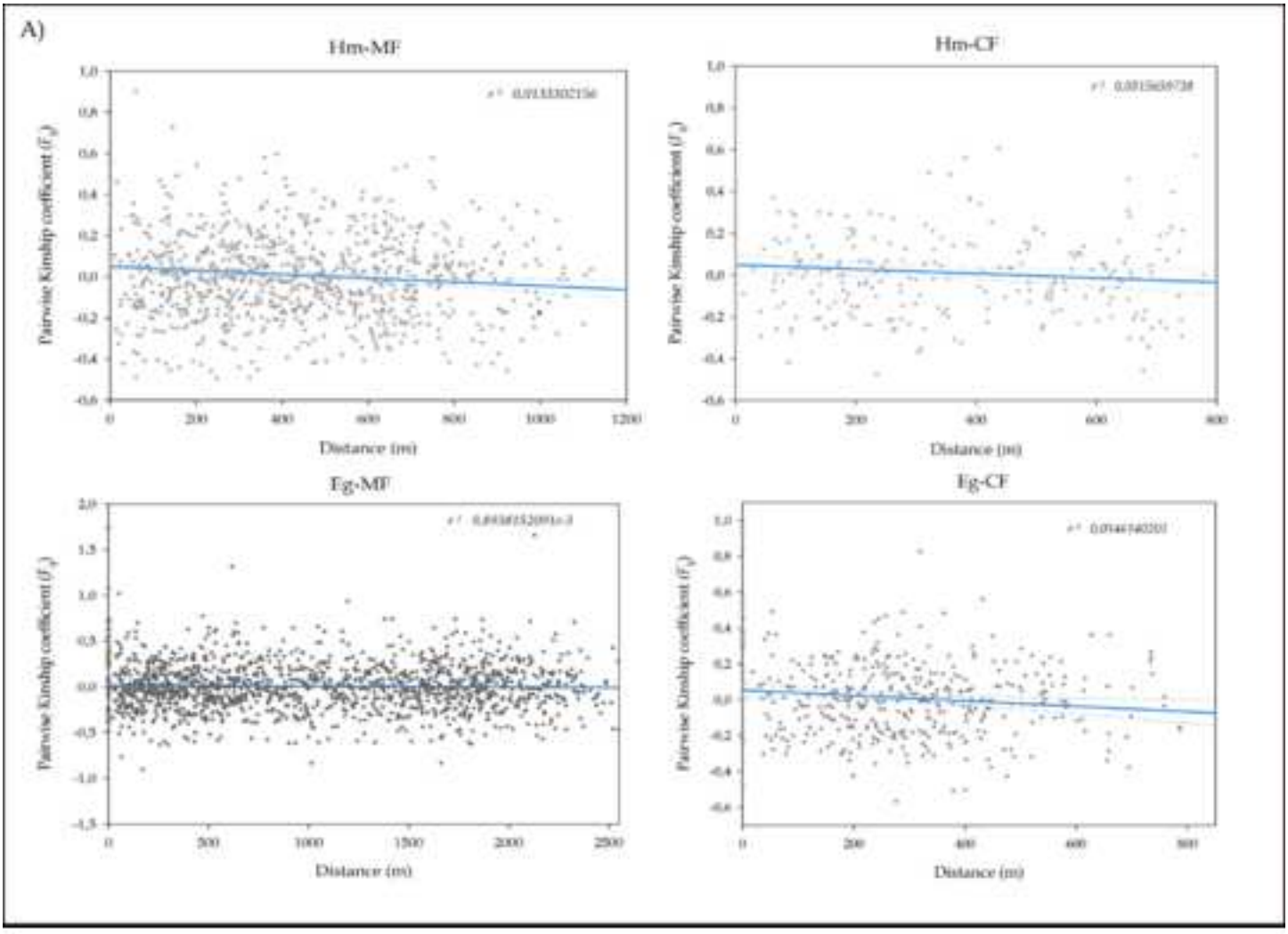

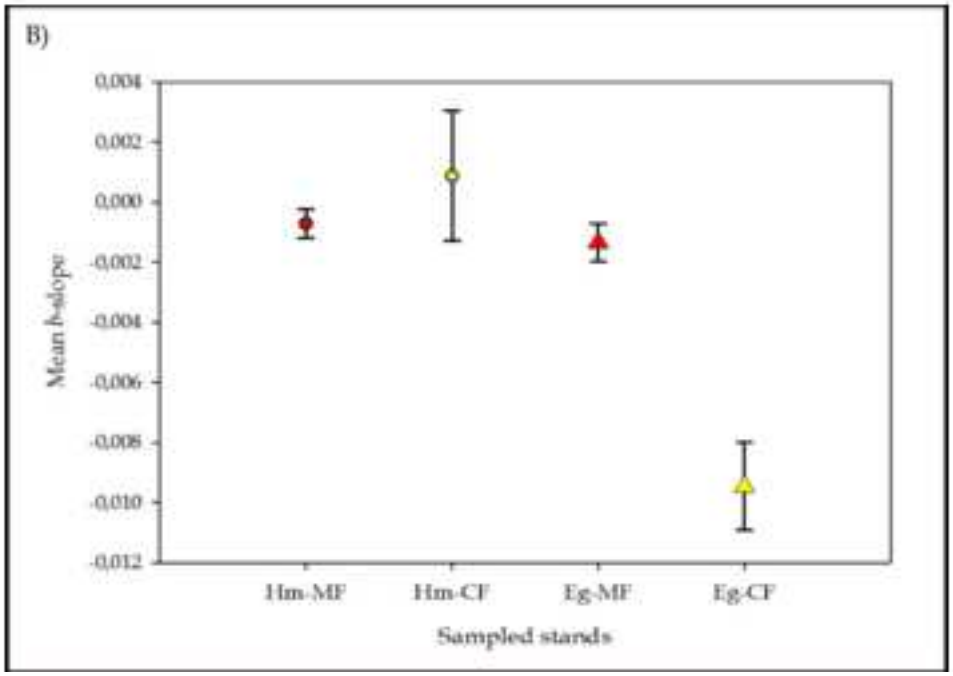

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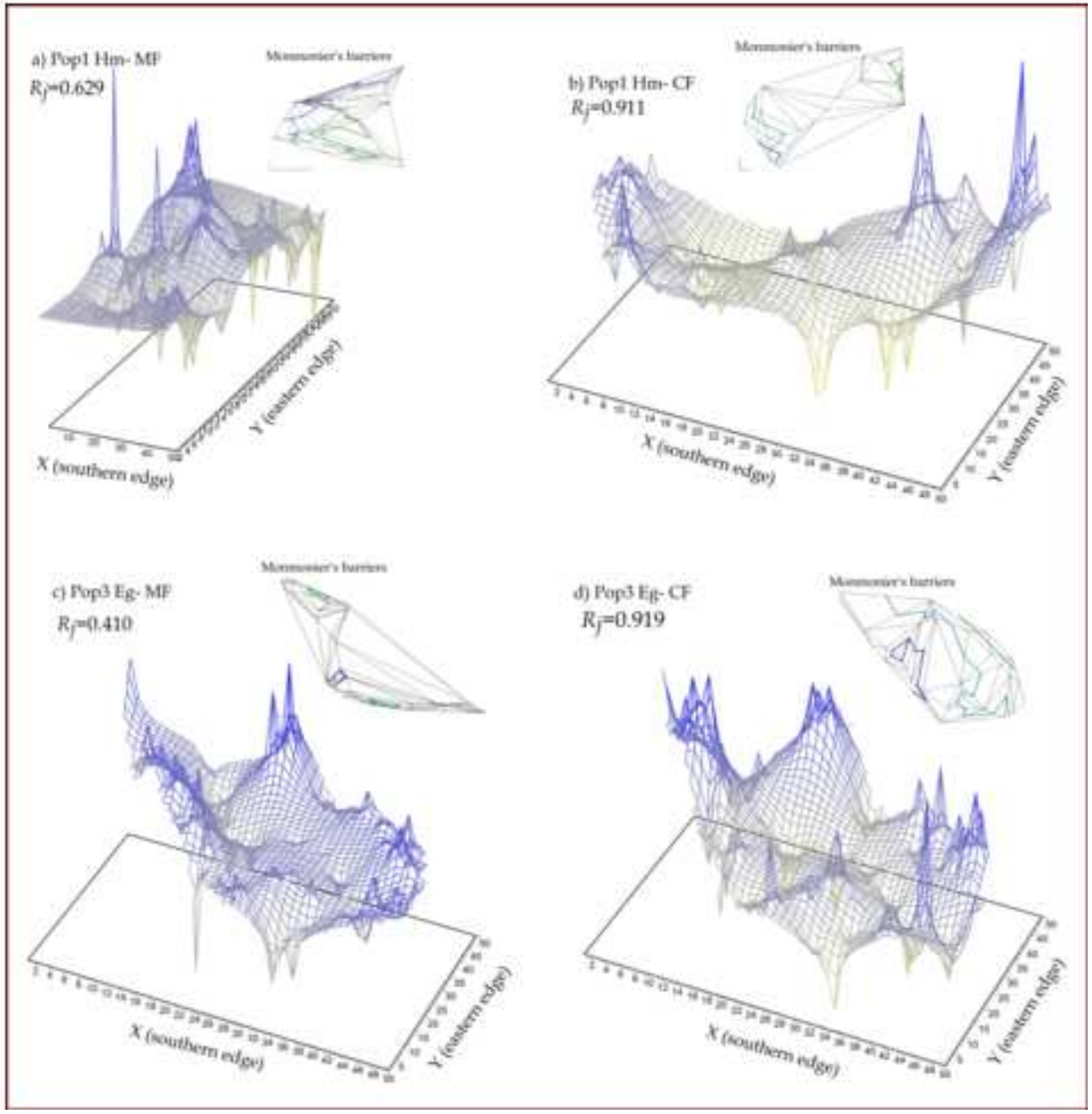

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