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Local adaptation of *Pinus leiophylla* under climate and land use change models in the Avocado Belt of Michoacán

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Abstract

Climate change and land use change are two main drivers of global biodiversity decline, decreasing the genetic diversity that populations harbour and altering patterns of local adaptation. Landscape genomics allows measuring the effect of these anthropogenic disturbances on the adaptation of populations. However, both factors have rarely been considered simultaneously. Based on a set of 3660 SNPs from which 130 were identified as outliers by a genome–environment association analysis (LFMM), we modelled the spatial turnover of allele frequencies in 19 localities of *Pinus leiophylla* across the Avocado Belt in Michoacán state, Mexico. Then, we evaluated the effect of climate change and land use change scenarios, in addition to evaluating assisted gene flow strategies and connectivity metrics across the landscape to identify priority conservation areas for the species. We found that localities in the centre-east of the Avocado Belt would be more vulnerable to climate change, while localities in the western area are more threatened by land conversion to avocado orchards. Assisted gene flow actions could aid in mitigating both threats. Connectivity patterns among forest patches will also be modified by future habitat loss, with central and eastern parts of the Avocado Belt maintaining the highest connectivity. These results suggest that areas with the highest priority for conservation are in the eastern part of the Avocado Belt, including the Monarch Butterfly Biosphere Reserve. This work is useful as a framework that incorporates distinct layers of information to provide a more robust representation of the response of tree populations to anthropogenic disturbances.

KEYWORDS
climate change, conservation genomics, genomic offset, habitat loss, land use change, local adaptation

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1 | INTRODUCTION

Climate change and habitat loss due to land use change are two of the main drivers of global biodiversity decline (Hansen et al., 2001; Oliver & Morecroft, 2014), causing reductions in the distribution range of species and decreasing the amount of genetic diversity they harbour. A recent study estimated that on average, 10%–16% of global genetic diversity has been lost due to decreasing habitat (Exposito-Alonso et al., 2022). Moreover, projections for the year 2070 estimate that between 6.2% and 10.7% of habitat will be lost per decade for amphibians, mammals, and bird species causing the upcoming loss of 2%–5% of genetic diversity in these groups (Exposito-Alonso et al., 2022; Powers & Jetz, 2019). The decrease of intraspecific variation may have considerable effects on ecological communities and ecosystem processes (Des Roches et al., 2018) as it represents a significant threat to species persistence, since standing genetic diversity could fuel the adaptation to future environmental change (Barrett & Schluter, 2008; Frankham et al., 1999; Laikre et al., 2020).

Rapid climate change is altering the patterns of local adaptation by creating novel climates and disrupting genotype–environment associations that populations have developed throughout their evolutionary history (Gougherty et al., 2021). In consequence, populations may respond to these disturbances either by adjusting to the new conditions via phenotypic plasticity or through adaptive evolution. There is increasing evidence that local adaptation to variation in environmental conditions across species' distribution ranges is common (Gugger et al., 2021; Hoffmann & Sgró, 2011), suggesting that studying the evolutionary potential at the population level could allow understanding of how species respond to changing environments. Nevertheless, species with long generation times may not have enough time to adapt to rapidly changing environmental conditions, causing an *adaptation lag*, and future maladaptation (Browne et al., 2019; Kremer et al., 2012). Alternatively, populations could migrate to sites where they could become locally adapted; or they could receive, either naturally or mediated by humans, adaptative genetic variation through gene flow, creating novel genotype–environment relationships (Aitken & Whitlock, 2013; Rhoné et al., 2020).

The recent development of next-generation sequencing and the advances in landscape genomics have increased our ability to measure the effect of the pressures on adaptative processes caused by anthropogenic activities and climate change. Methods such as gradient forest (Ellis et al., 2012) have been recently used to estimate the genomic offset of populations to climate change (Aguirre-Liguori et al., 2021; Capblancq et al., 2020; Fitzpatrick & Keller, 2015; Martins et al., 2018). The genomic offset is obtained in three steps. First, a model that describes the association between the genetic composition of populations and their current climatic conditions is constructed. Second, this model is used to infer the expected genetic composition of the populations projected in future conditions so that populations can stay locally adapted. Finally, the genetic offset is measured as the Euclidian distance between the present and future genetic compositions and quantifies the amount of genetic change that a population would need to maintain the existing gene–environment relationships (Fitzpatrick & Keller, 2015). Higher values of genetic offset indicate that a population would need to respond more to the selection pressure generated by climate change and, therefore, that the population would be more vulnerable. By estimating the genetic offset of populations, it is possible to identify areas that should be prioritized for conservation and design robust management plans (Yu et al., 2022).

Genetic offsets are powerful tools that could indicate how populations will respond to climate change. However, studies that have analysed genetic offset usually do not consider other processes that could also affect or limit the response of populations by increasing the loss of genetic diversity, such as changes in land use and fragmentation of populations (Selwood et al., 2015). Therefore, it is important to estimate the joint impact of anthropogenic disturbances on the vulnerability of the populations to better predict how these will respond in the future. Yet, it is still not easy to predict how land use and habitat fragmentation will occur in the future, since this requires accurate models of different scenarios of habitat change, considering both environmental and socioeconomic variables that could determine the speed and magnitude of these land transitions.

Mexico is considered a centre of diversification for the *Pinus* and *Quercus* genera, harbouring around 47 of 121 pine species, and 161 of 450 oak species (38% and 35% of the worldwide total species, respectively; Perry et al., 1998; Valencia-Avalos, 2004). In the state of Michoacán, pine-oak forests potentially covered approximately 1,623,100 ha or 28% of the state's total area (Denvir et al., 2022), hosting a great diversity of flora and fauna and approximately 16 pine and 34 oak species (Leal-Nares & Villaseñor-Gómez, 2016). These forests provide fundamental ecosystem services, such as carbon sequestration and storage, water and air purification, regulation of the hydrological cycle, and a large number of cultural and aesthetic values (Denvir, 2023; Gómez-Tagle Chavez et al., 2019; Leal-Nares & Villaseñor-Gómez, 2016). Nevertheless, despite their high biological and social importance, in the last decades Michoacán's pine-oak forests have experienced a dramatic transformation to other land uses, most notably avocado cultivation. Michoacán is the principal producer of avocado in Mexico, with 244,705 ha of planted orchards by the year 2019 and 73% of the total production in the country, followed in importance by the contiguous state of Jalisco (Khan et al., 2021; Latorre-Cárdenas et al., 2023). In Michoacán, the rapid expansion of the areas dedicated to avocado cultivation has led to the loss of up to 8000 ha of temperate forest per year (Mas et al., 2017).

Moreover, it is projected that cultivation of avocado will continue to increase in the future by approximately 178,534 additional ha by the year 2050, making the pine-oak forest the most vulnerable ecosystem in the region due to the combination of climate change and avocado expansion (Denvir, 2023). Therefore, prioritizing the conservation of pine-oak forests is of vital importance not only for their invaluable biological worth but also because they could maintain the ecosystem services in the region.

Pinus leiophylla (Pinaceae) is a representative species of Michoacán's pine-oak forests with high ecological and economic value since it is an important source of wood and resin, and likewise is frequently used in restoration projects due to its ability to tolerate stress conditions, as water scarcity and frost (Martínez-Trinidad et al., 2002). In this work, we selected *P. leiophylla* as a study system to simultaneously consider land use and climate change in genomic offset scenarios and adaptative management proposals for this species.

The specific aims of this work were (1) to assess current genotype–environment associations in *P. leiophylla* populations in Michoacán, (2) to evaluate the vulnerability of populations simultaneously considering genomic offset on the face of climate change scenarios and the growing expansion of avocado crops, and (3) to lay out assisted gene flow strategies for the species' management.

2 | MATERIALS AND METHODS

2.1 | Study system and sample collection

Pinus leiophylla is present across highly heterogeneous environments from the Southwest of the United States through the Sierra Madre Occidental, the Trans-Mexican Volcanic Belt, and the Sierra Madre del Sur in Mexico (Perry, 1991). As all pines, *P. leiophylla* is wind pollinated, a trait that is often associated with high genetic variation, high effective population sizes, and low genetic differentiation between populations (Rodríguez-Banderas et al., 2009; Sork et al., 2013). In turn, these characteristics result in a high adaptative capacity and clear patterns of local adaptation of populations (González-Martínez et al., 2006).

Our sampling was conducted in the "Avocado Belt", which is a region of avocado production covering 62 municipalities in the state of Michoacán, with 244,705 ha of avocado crops (Latorre-Cárdenas et al., 2023). The area is located within the Trans-Mexicana Volcanic Belt and is topographically complex with mountains, plateaus, valleys, and hills, which result in a high climatic heterogeneity and a variety of soils including luvisols, acrisols, and andosols. Elevation ranges from 1300 to 3600 m, and the mean temperature and annual precipitation range from 10 to 24°C and from 800 to 1600 mm, respectively (Ramírez-Mejía et al., 2022).

We sampled 77 individuals from 19 localities encompassing the temperature and precipitation gradients (Figure 1, Table S1). At each site, we randomly chose 2–5 adult trees with a diameter at breast height (DBH) of at least 20 cm and separated by a minimum distance of 30 m from each other to avoid sampling related individuals. Sampling design was aimed at maximizing the number of localities along the environmental gradients of the region. Several studies have shown that as the number of sampled sites increases, fewer individuals per site are needed to obtain accurate estimates of genetic diversity (Aguirre-Liguori et al., 2020; Nazareno et al., 2017; Sork et al., 2013). Fresh leaf tissue was stored at −80°C until DNA extraction.

2.2 | DNA extraction, library construction, sequencing, and variant detection

Genomic DNA was extracted from approximately 30 mg of frozen tissue using the cetyltrimethylammonium bromide (CTAB) protocol described by Doyle and Doyle (1987). We assessed the purity and concentration of the extracted DNA using absorbance ratios at 260/280 and 260/230 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific) and fluorometric quantification (Qubit 2.0 BR assay; Invitrogen). For each individual that had an adequate quality and concentration of DNA, we collected >1 μg DNA that was sent to Floragenex (www.floragenex.com) for Rad tag sequencing.

Genomic libraries were created using the ddRAD-Seq protocol described by Truong et al. (2012). Briefly, genomic DNA of each sample was digested using the restriction enzymes *PstI* and *MseI*, and an adapter was ligated to the DNA fragments. The adapter-ligated fragments were sequenced using Illumina NovaSeq 6000. The sequenced samples were demultiplexed and trimmed for further analysis.

A de novo RAD reference genome was constructed using the individual that had the highest number of unique RAD sequences. Next, we used custom scripts to cluster identical sequences. The assembly for the reference individual was realigned against itself using BWA (Li & Durbin, 2009). BOWTIE (Langmead et al., 2009) was then used to align the reads of each individual to the RAD reference genome. SAMTOOLS (Li et al., 2009) and custom scripts were used to detect and filter SNPs that had a minimum sequencing depth per sample of 15×, individual per locus genotype quality scores of at least 20 and a minimum of 10x sequence coverage (Izaguirre-Toriz et al., 2024; ID 1076599—BioProject—NCBI).

Subsequently, we used PLINK v1.9 (Purcell et al., 2007) and vcftools v0.1.16 (Danecek et al., 2011) to retain diallelic SNPs that had minor allele frequencies (MAF) ≥ 0.025 and had less than 20% of missing data. We also removed SNPs that were in linkage disequilibrium (LD < 0.5) within a window size of 50 bp and a window shift of 5 (Purcell et al., 2007). We used HDplot (McKinney et al., 2017) to remove possible paralogs. We removed all regions that had observed heterozygosities (H_O) greater than 0.5 and a *D* value outside the range of −15 and 15, which could indicate potentially duplicate loci due to deviation of allelic ratios expectation. After these filtering procedures, we obtained 3660 SNPs.

2.3 | Spatial modelling and environmental variables

We obtained from Latorre-Cárdenas et al. (2023) the land use and land cover classification (LULC) map of the Avocado Belt for 2019 with a resolution of 30 m, consisting of six classes: (1) temperate forest, (2) avocado orchards, (3) water bodies, (4) agriculture and pasture areas (which included all types of croplands except the avocado orchards), (5) settlements, and (6) dry forest and shrublands of low and medium deciduous forests (Figure 1). In total, our study area included 786,812 ha of temperate forest. Based on the LULC layer

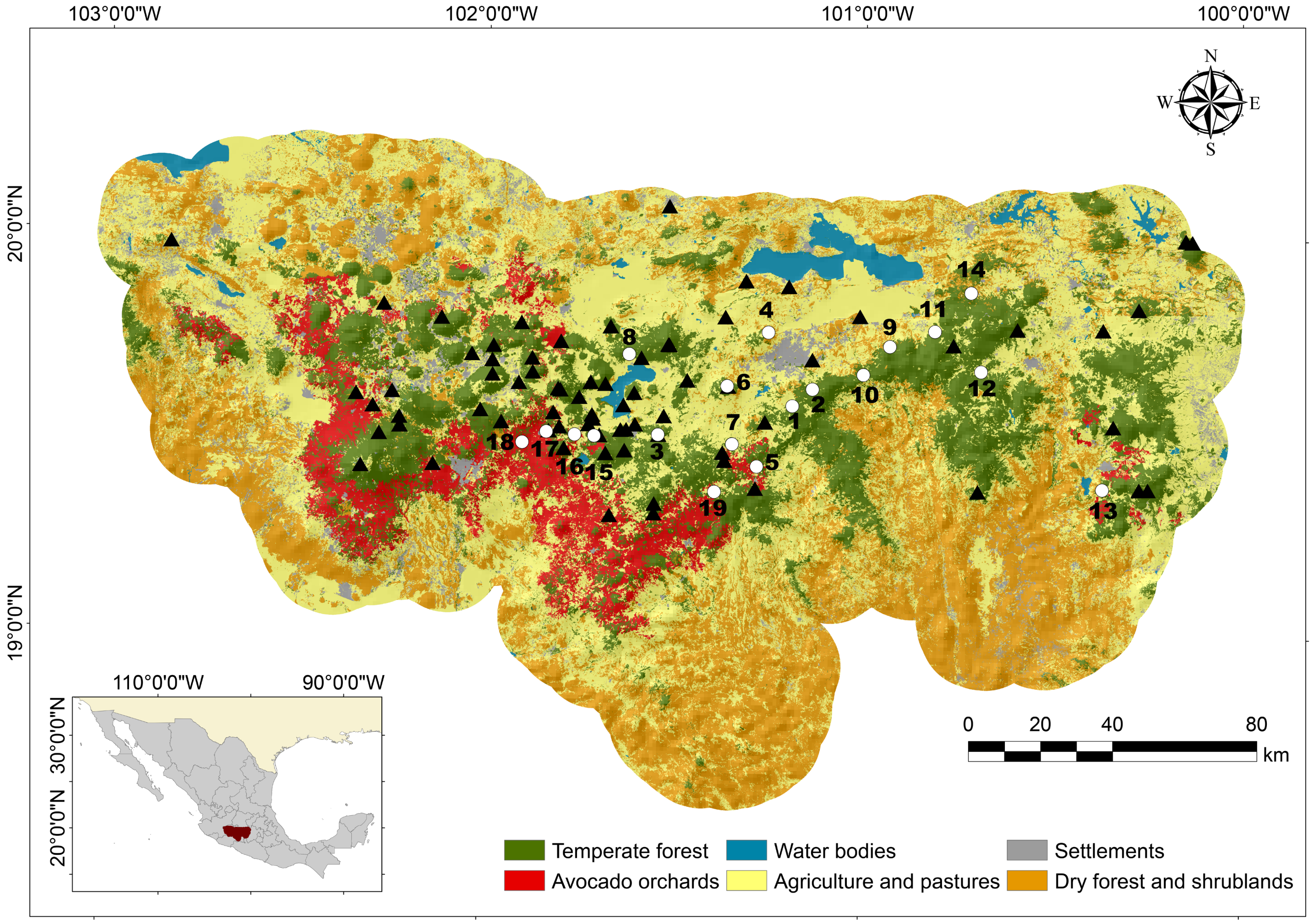

FIGURE 1 Land-use and land-cover map based on 2019 satellite images of the Avocado Belt located in the state of Michoacán, Mexico, showing six land-use classes. Also see Latorre-Cárdenas et al. (2023) for more details. White dots represent the 19 collected locations of *Pinus leiophylla* and black triangles show presence records of the species from the National Herbarium of Mexico (MEXU) database.

for 2019, we followed the procedure of Arima et al. (2022) to project the expansion of new avocado orchards by the year 2050 and created a temperate forest layer for 2050 subtracting from the 2019 forest layer the areas where avocado is projected to be established by 2050. For downstream analysis, we only considered the temperate forest patches that had an area equal to or greater than 10 ha to avoid including patches that are too small for prioritizing conservation areas and reduce the number of patches for further analysis (initially $n = 23{,}225$). These maps allowed us to evaluate the effects of fragmentation, habitat loss, and the avocado expansion on the patterns of genetic diversity distribution and connectivity of *P. leiophylla* in temperate forest patches.

We downloaded 19 bioclimatic variables from WorldClim (http://www.worldclim.com/version2) representing the mean observations between 1970 and 2000 with a resolution of 30 arc/s (Fick & Hijmans, 2017). For the future climate, we downloaded the 2041–2060 layers using the Miroc6 model under the 370 shared socioeconomic pathway (SSP) scenario, which is in an intermediate range of global climate projections, with a resolution of 30 arc/s (http://worldclim.com/version2). To remove the correlation among the climatic variables, we kept those that had Pearson correlation coefficients <0.8, isothermality (BIO3), minimum temperature of coldest month (BIO6), temperature annual range (BIO7), mean temperature of driest quarter (BIO9), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18), and precipitation of coldest quarter (BIO19).

2.4 | Genetic diversity and structure

We used the *basic.stats* function in the *hierfstat* package (Goudet, 2005) in R 3.4.1 (R Core Team, 2021) to obtain the per SNP site and mean observed heterozygosity (H_O), gene diversity (H_S), and the inbreeding coefficient (F_{IS}) per sampling locality. The number of private alleles was estimated with the package *poppr* version 2.9.3 (Kamvar et al., 2014). We also obtained the genetic differentiation among localities using the Weir and Cockerham F_{ST} (Weir & Cockerham, 1984). Finally, we evaluated if there is a correlation between the connectivity values of the forest patches (for seed and pollen dispersal scenarios, see below) and the values of gene diversity (H_S).

To determine the genetic structure, we first used the software Admixture v1.3 (Alexander et al., 2009) with K values ranging from 1 to 19. From this analysis, we selected the K value with the lowest cross-validation error. We also analysed genetic variation within and among localities with a nonparametric analysis of molecular variance (AMOVA) (Stewart & Excoffier, 1996) using Arlequin software v3.0 (Excoffier et al., 2005).

2.5 | Identification of outlier SNPs

We used a genome–environment association (GEA) test to identify potentially non-neutral SNPs, employing latent factors mixed models (LFMM; Frichot et al., 2013) implemented in the package *LEA* in R (Frichot & François, 2015). LFMM tests for correlations between allelic frequencies and environmental variables after controlling for genetic structure using latent factors. To select the optimal number of latent factors, we used $K=1$ as the number of inferred clusters (K) based on the admixture analysis (see Results). We ran LFMM analysis using the allelic frequencies of all SNPs and the nine bioclimatic variables described above. Five independent runs were made using 10,000 iterations with a burn-in of 5000. Adjusted p-values (q) were calculated using the genomic inflation factor (λ) procedure (Devlin & Roeder, 1999). For each run, we analysed the histograms of p-values to ensure that the confounding effect of population structure was controlled. Finally, to correct for multiple testing, the resulting p-values were adjusted using the false-discovery rate method with a significance threshold of $Q<0.01$. The outlier SNPs detected by LFMM were defined as the outlier set.

2.6 | Annotation and gene ontology

We used the Blast2Go v6.0.3 program to identify the putative gene functions and Gene Ontology (GO) terms using the flanking sequences of each SNP of the outlier set. We first performed a homology search using BLASTN and BLASTX on the NCBI non-redundant (nr) public database with an e-value threshold set to 1×10^{-5}. Second, the putative function of the sequences was assigned according to the highest BLAST hits and GO terms associated with the BLAST hits.

2.7 | Gradient forest

To model the current patterns of genetic variation of *P. leiophylla* through the Avocado Belt, we used Gradient Forest (GF), a machine-learning approach that models the turnover in genetic composition across the landscape (Fitzpatrick & Keller, 2015). GF identifies nonlinear associations between allele frequencies and environmental variables and determines how allele frequencies change along the gradient. We used the *gradient forest* package in R (Ellis et al., 2012) using the allele frequencies of each locality for the full set and for the outlier set of SNPs. We decided to incorporate both sets to seek for differences in genomic turnover patterns across the landscape between putatively adaptative SNPs and reference SNPs, as has been done in previous studies (Martins et al., 2018; Nielsen et al., 2021). To obtain the GF models, we used the same nine bioclimatic variables for the current climate (1970–2000). We ran gradient forest with 1000 regression trees per SNP and a variable correlation threshold of 0.5 (Fitzpatrick & Keller, 2015).

Once we had the GF models, we performed a principal components analysis (PCA) on the predictions and used the first three axes to obtain a red-green-blue colour palette. The colour scale was used to map the turnover in genotype–environment associations across the landscape, where similar colours indicate a similarity in the expected patterns of genetic composition (Fitzpatrick & Keller, 2015). We mapped the resulting prediction only considering actual forest fragments according to our LULC map. To estimate the difference in genetic composition patterns between the models obtained with the two datasets (full and outlier), we performed a Procrustes analysis with the *vegan* package in R (Oksansen et al., 2022).

2.8 | Local, forward, and reverse offsets

To assess the genomic offset of *P. leiophylla* localities under future climate change, we used the same nine bioclimatic variables for the years 2041–2060. First, we used the *predict* function in R to infer the expected genetic composition across the present and future landscapes. Next, we estimated the genomic offsets as the Euclidean distance between the genomic composition under the present and future scenarios (local offset). The genetic offset across the landscape was mapped to identify the regions of temperate forests that are expected to become vulnerable because of climate change. Finally, we used ArcMap program v10.1 to subtract the projected areas of expansion of avocado crops by 2050 to evaluate the effects occasioned by both, climate change and habitat loss.

Genomic offset predicts the risk of maladaptation in situ (local offset). However, organisms can migrate to more suitable areas within their current range for their survival (forward offset), or in turn, receive genotypes that are more preadapted to future climatic conditions from other localities (reverse offset). To provide a more realistic approach of the vulnerability of *P. leiophylla* localities to climate change, we quantified the forward and reverse offsets following the procedure of Gougherty et al. (2021). Briefly, to obtain the forward offset, we estimated the genetic offset of each grid cell within the current forest layer under current climatic conditions in relation to all other grid cells within the temperate forest layer for 2050 in future climate, and we determined the minimum offset value for each focal grid cell as the forward offset. In other words, forward offset values allow identifying areas where individuals could migrate so that their genomic vulnerability would be minimal under future conditions. In turn, for the reverse offset we contrasted each focal grid cell within the current forest layer under future climatic conditions, and all other grid cells within the temperate forest layer for 2050 in current climate conditions and identified the minimum value as the reverse offset. Therefore, this allows identifying all possible source areas from which individuals could migrate to a focal grid cell so that their genomic vulnerability would be minimal. Finally, we mapped the local, forward, and reverse offsets using a red-green-blue image with a histogram equalization performed on each band to ensure the visualization of the full range of colours.

2.9 | Isolation by distance and environment

To estimate the contribution of geographic distance and environmental variation on the patterns of genetic differentiation, we performed redundancy analyses (RDA) and partial redundancy analyses (pRDA) using a variance partitioning approach implemented in the *varpart* and *rda* functions of the package *vegan* in R (Oksansen et al., 2022). These analyses were performed using as dependent matrix the allelic frequencies of each locality, with the corresponding climate and geographic variables as explanatory matrices. To obtain the matrix of climatic variables, we performed a PCA with the same nine bioclimatic variables to avoid the redundancy between variables and used the first three axes, which explained 72% of the accumulated variation. The geographic matrix consisted of the geographic coordinates of the localities (latitude and longitude). We performed this analysis with (1) the full set of SNPs; (2) the outlier set; and (3) the SNPs with a positive R^2 obtained from the gradient forest analyses (see below). Significance was tested by performing 999 permutations.

2.10 | Connectivity and future land use change

Land use change associated with avocado crops has caused considerable habitat reduction of pine-oak forests through the Avocado Belt in recent years (Mas et al., 2017) altering the patterns of connectivity between natural populations restricted to forest patches. The degree of connectivity is given by attributes of the landscape (structural connectivity) and the organism (functional connectivity). High connectivity values mean that the organisms could move easily between the patches, thereby maintaining genetic diversity, avoiding inbreeding, and contributing to the recolonization process. To estimate how the expansion of avocado crops has affected the connectivity patterns between the patches of temperate forest within the Avocado Belt, we obtained the Composite Connectivity Index (CCI) values from Latorre-Cárdenas et al. (2023) using two different scenarios based on the seed and pollen dispersal capacity of pine and oak species. CCI considers the contribution of every focal patch (*f*) to the connectivity in adjacent patches and their contribution to connectivity in the total landscape (Latorre-Cárdenas et al., 2023). To forecast how connectivity patterns will be modified in future scenarios of habitat loss, we estimated CCI values on the projected remnants patches of forest for the year 2050 according to our model of avocado expansion following the same procedure described in Latorre-Cárdenas et al. (2023).

Next, we performed a weighted overlay technique in ArcMap program v10.1 to identify the priority areas for conservation that probably will be less affected by avocado expansion and that will have lower values of local offset and high connectivity. This technique uses overlays of different raster layers by giving a weight to each layer according to its importance. We reclassified each raster on a scale of four categories with the Jenks natural breaks classification method (Jenks, 1963), assigning the value of 1 to the areas with lowest local genomic offset and 4 to areas with higher values. For the connectivity layers, 1 corresponded to areas with higher connectivity and 4 to areas with the lowest connectivity. The mean overall weights were set to 50% for the values of local offset and 25% for each of the two dispersal (seed and pollen) scenarios. Next, we weighted the overlay of the three layers by summing the product of each category by its weight. Finally, the values obtained were converted into a map with four priority classes: 1 = high priority, 2 = intermediate priority, 3 = low priority, and 4 = not priority.

3 | RESULTS

3.1 | Genetic diversity and structure

Across localities the mean gene diversity was $H_S = 0.153$, ranging from 0.142 to 0.163, the mean observed heterozygosity was $H_o = 0.144$, ranging from 0.127 to 0.157 and the mean inbreeding coefficient was $F_{IS} = 0.06$, ranging from −0.08 to 0.065 (Table S2). The number of private alleles per locality ranged from zero to six. We did not detect a significant correlation between current connectivity indexes of the forest patches and the values of gene diversity (H_S) for either the seed or pollen dispersal scenarios (Figure S1), suggesting that the current generation of adult *P. leiophylla* trees does not show an effect of fragmentation and habitat loss on genetic diversity.

Genetic differentiation among localities was low, with a global F_{ST} value of 0.026. Accordingly, the AMOVA showed that most of the variation was found within localities (97.05%) and only a small but significant proportion of variation was found among them (2.95%, $p < .0001$) (Table S3). In turn, ADMIXTURE showed $K = 1$ as the optimal value for K. Together, these results supported the presence of a single genetic group in the area.

3.2 | Identification of outlier SNPs, annotation, and gene ontology

In total, we identified 130 SNPs (3.5% of the total) that were significantly associated with climate variables. The majority of these SNPs were associated with the mean temperature of driest quarter (BIO9), followed by the minimum temperature of coldest month (BIO6) and the precipitation of warmest quarter (BIO18) (Table S4).

Ten of the 130 outlier SNPs had a match in the BLAST search. The RAD_kmer_0003444 was associated with six climatic variables and was found to be homologous to a protein-coding sequence previously identified in other pines as *P. taeda*, *P. sylvestris*, and *P. pinaster*, and shown to be involved in the response to water-deficit stress (protein LP3-2) (Eveno et al., 2008; Padmanabhan et al., 1997). The other nine SNPs fell within proteins of unknown function. In addition, we obtained 19 GO terms associated with a broad range of biological processes such as biosynthesis, transport, metabolic processes, and localization.

3.3 | Gradient forest

According to the GF models, the full dataset explained a slightly higher proportion of variation (mean R^2 = 11.71%) than the outlier set (mean R^2 = 10.94%) (Table 1). The minimum temperature of coldest month (BIO6) and the precipitation of wettest quarter (BIO16) were consistently within the three most important environmental predictors in the two GF models, with similar R^2-weighted importance for both datasets (Figure S2).

The allele turnover showed different genotype–environment associations across the landscape for the two models. The turnover was stronger for the model constructed with the outlier set than for the full set. However, the Procrustes analysis revealed that the difference in predicted patterns of allele distribution was restricted to some patches in the east and west areas, showing a deviation between the full and potentially non-neutral genetic composition (Figure 2).

3.4 | Local, forward, and reverse offsets

First, we estimated the local genetic offset of localities, using the full and outlier datasets. We found that the estimates of local genomic offset for both datasets revealed a similar pattern between models, with greater values found in the outlier set model. However, we tested the correlation between the offsets estimated by both datasets and found that they were significantly correlated (R^2 = .79, $p < .05$, Figure S3). Therefore, henceforth we will focus only on the outlier set model.

Local genomic offset predictions presented the highest values in the centre-east regions, indicating that localities in these areas would be at higher risk due to future disruption of present genotype–environment associations. In contrast, we found that areas in the easternmost and some patches at the west and centre of the study area had lower local genomic offsets (Figure 3, Figure S4).

TABLE 1 Information from models based on the full and outlier SNP datasets used to perform the gradient forest analysis.

SNP sets	Number of SNPs	SNPs with $R^2 > 0$	Mean % R^2 (range)
Full set	3660	392	11.71 (0.016–48.76)
Outlier	130	15	10.94 (0.86–30.62)

Forward genetic offsets showed higher values at the eastern and northern parts of the area, indicating that these localities would be maladapted to all future climates within the future temperate forest range and that the impacts of climate change cannot be mitigated by moving to more suitable parts of the study area. In contrast, for the most part of the distribution, we found low values of reverse genetic offset, with moderate to higher values in some small patches at the centre-east and west. High values of reverse offset would imply that no locality in the current distribution range will be preadapted to future climates of these latter localities.

3.5 | Isolation by distance and environment

The RDA analysis revealed that geography did not explain any percentage of the genetic variation for the full set, or the outlier set, while for the set of SNPs with positive R^2 values in the gradient forest analysis, geography explained 2.9% of the variation. However, none of these components were significant ($p > .05$; Table 2). Environmental variation neither explained a large portion of the genetic variation for the full (1.4%) and the outlier SNP sets (3.5%) with none of these being significant. In contrast, for the SNPs with positive R^2, environmental variables explained 18.2% of the genetic variation ($p < .01$; Table 2). Geography alone, when controlling for environmental variation, explained a small proportion of the genetic variation for the full (1.4%), outlier (0.9%), and positive R^2 sets (5.3%), but none of these were significant ($p > .05$; Table 2). Environmental variation alone, when controlling for geography, accounted for a nonsignificant 3.4% of the variation for the full set ($p > .05$), while for the outlier and positive R^2 sets it explained 4.8% and 20.7%, respectively ($p < .05$; Table 2). This means that the pure effects of climate are greater on shaping the spatial pattern of genetic variation in outlier sets, while geography is negligible.

3.6 | Connectivity and future land use change

Using our model of avocado expansion by the year 2050, we estimated an expected distribution extent of temperate forest of 674,906 ha, with a loss of approximately 58,589 additional ha, compared to the present. These transformations will affect mainly the southwest area, where some of the *P. leiophylla* localities will be potentially less affected by climate change (lower values of local offset; Figure 3) and where Zurumucapio is located, the locality with the higher number of private alleles (Table S2). According to these results, one locality will be completely lost, while three other localities are in forest patches that will be reduced to less than 60% of their current area. The rest of the localities are in forest patches that will probably conserve over 90% of their current extension (Table S5), assuming avocado expansion as the only driver of deforestation.

Next, we analysed how the future expansion of avocado crops could impact the connectivity of the localities. We found that the connectivity patterns for the seed and pollen dispersal scenarios will be modified by future habitat loss (see Latorre-Cárdenas

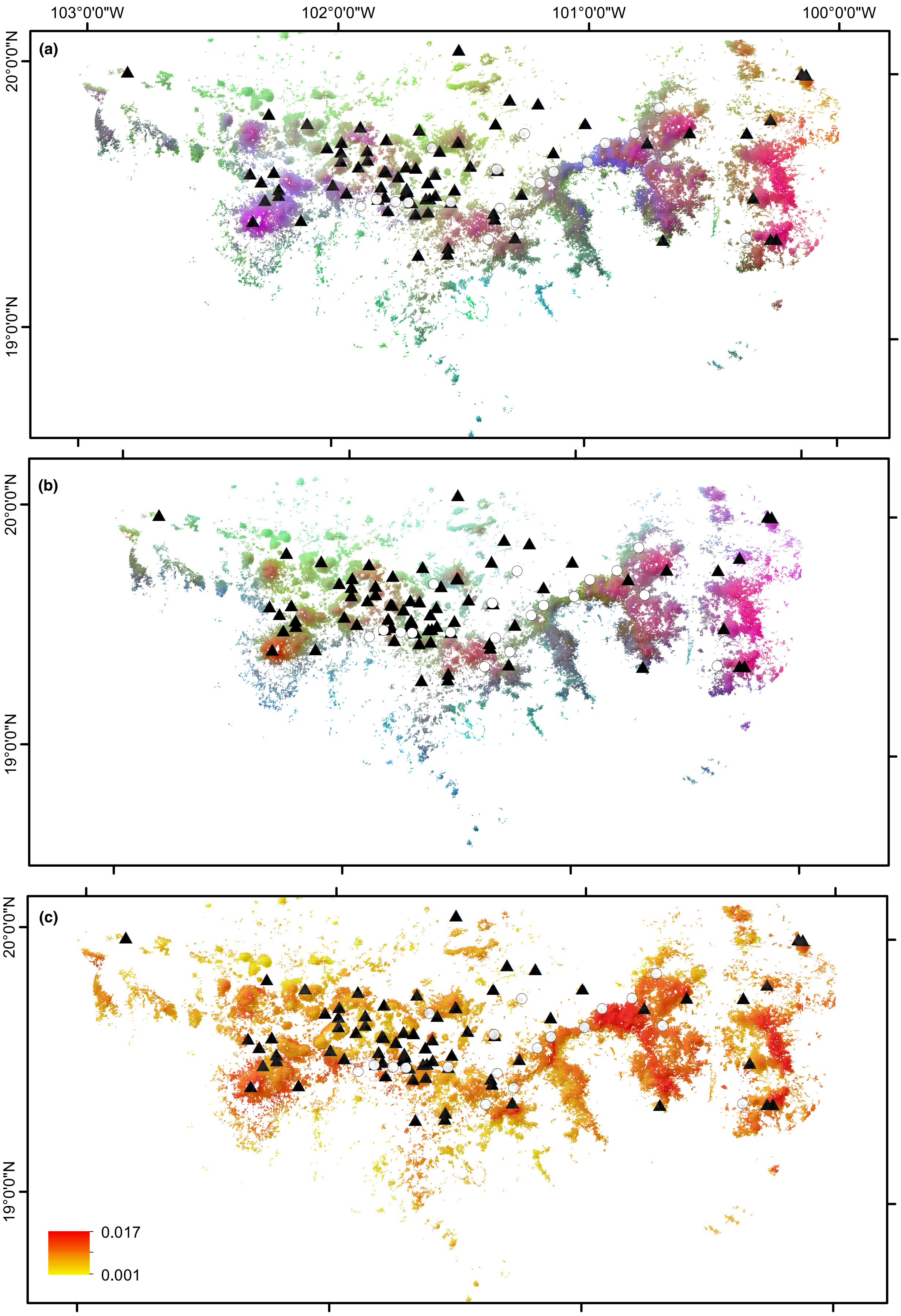
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FIGURE 2 Predicted spatial allele turnover of *Pinus leiophylla* from Gradient Forest analysis for (a) full and (b) outlier set of SNPs within temperate forest patches. Colours in (a) and (b) represent the genetic turnover based on the modelled relationships of allele frequencies with environmental and spatial variables. Similar colours represent localities with similar expected genetic composition (colours are not comparable between panels). (c) Procrustes residuals from comparison between the models from the outlier set and the full set of SNPs. Bright red indicates larger differences. White dots represent the 19 collected locations of *P. leiophylla* and black triangles show presence records of the species from the National Herbarium of Mexico (MEXU) database.

et al., 2023). According to our models, the central and eastern patches will present higher CCI values for both dispersal scenarios (Figure 4). Contrary to the present, the patches in the west area where the avocado expansion is projected to be more intense will show the lowest CCI values, meaning that they will be less connected and will have the lowest contribution to the total connectivity across the area.

Finally, we identified that the areas with the highest priority for conservation considering the future avocado extension, low local offset, and high connectivity are those located in the eastern patches, which encompass the Monarch Butterfly Biosphere Reserve, while the localities with the lower priorities are those located at the centre-west patches (Figure 5).

4 | DISCUSSION

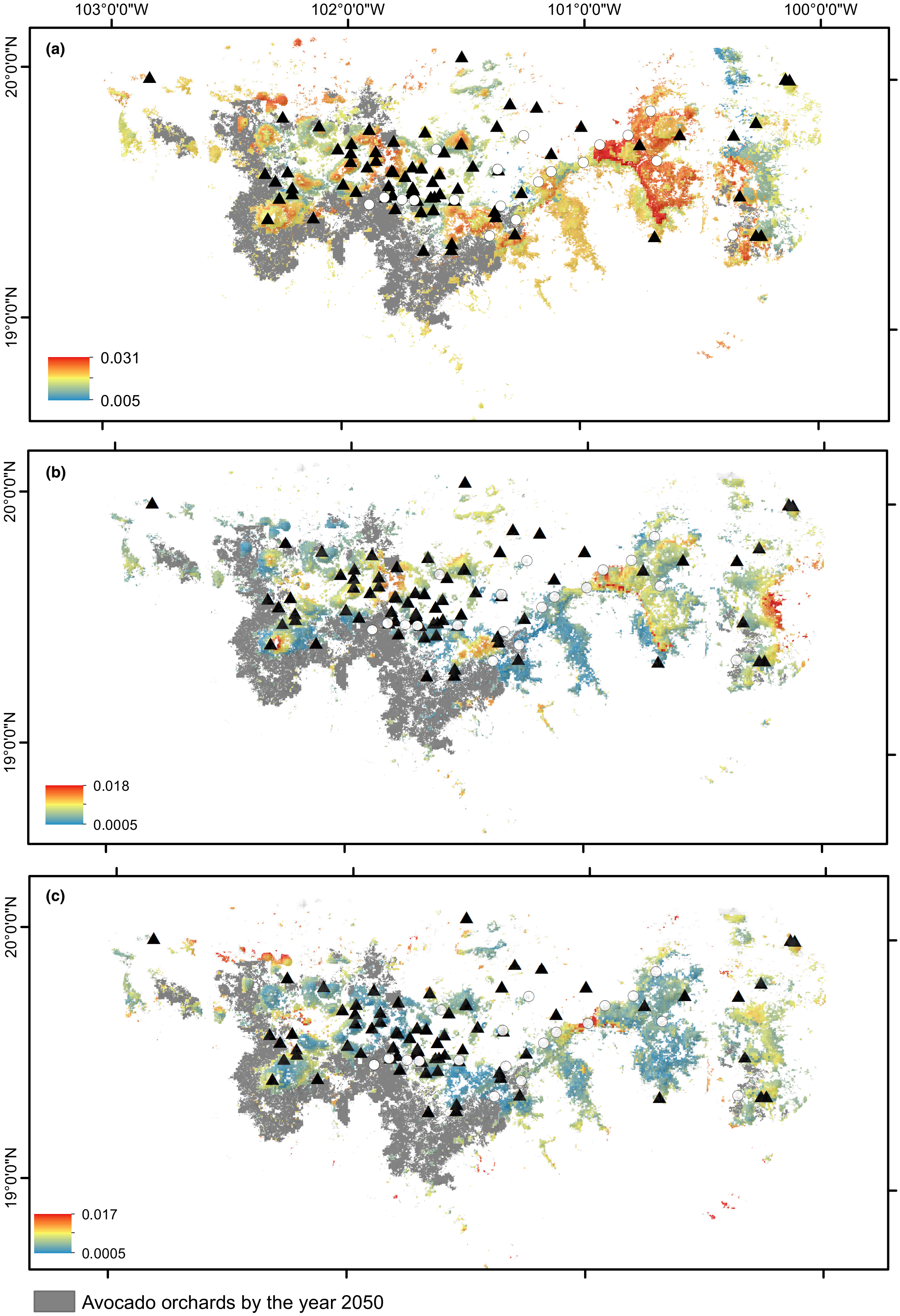
Climate change and land use change are two of the main drivers of local extinction, contributing to the ongoing loss of global biodiversity. Genomic data have improved our understanding of how organisms respond to such disturbances, but most studies do not consider both factors simultaneously. In this study, we analysed how climate change and land use change associated to the expansion of avocado cultivation will impact localities of *P. leiophylla* in the Avocado Belt in Michoacán. To our knowledge, this is the first time that the effects of climate change and land use change have been modelled together, providing more realistic information to evaluate the vulnerability of the localities and prioritizing conservation of those that will be less affected by 2050.

Our population genomics analysis of *P. leiophylla* revealed a very low level of differentiation ($F_{ST}=0.026$) with similar or higher gene diversity values ($H_S=0.153$) than other pines (Chhatre et al., 2013; Dauphin et al., 2021; Figueroa-Corona et al., 2022), indicating high rates of historical gene flow, as is common in wind-pollinated, outcrossing tree species (González-Martínez et al., 2006; Sork, 2018). This lack of structure reduces a confounding factor in our analysis to detect signals of local adaptation (Dalongeville et al., 2018). Moreover, previous studies have demonstrated that pines, despite having weak genetic differentiation, usually display strong patterns of local adaptation (Eckert et al., 2015; Lind et al., 2017). In this work, we performed a genotype–environment association analysis, which detected 130 putatively adaptative SNPs, whose genetic variance was mainly explained by the environment as opposed to the full set of SNPs (Table 2). The genomic context of these candidates revealed one protein related to water-deficit stress (protein LP3-2), which has been suggested to be involved in adaptative mechanisms in *P. albicaulis*, *P. pinaster*, and *P. halepensis* (Grivet et al., 2011; Lind et al., 2017).

The map resulting from the gradient forest analysis revealed patterns of genotype–environment associations across the distribution range of *P. leiophylla* within the Avocado Belt which followed an east–west direction, with similar trends for the full SNP set and the putatively adaptative set (Figure 2). However, differences between both sets are evident in some forest patches in the eastern and western parts. The discrepancies between the putatively adaptative and full models could indicate areas where local adaptation might be occurring. Although these areas do not present extreme climatic values compared to the rest of the distribution range of *P. leiophylla* in the Avocado Belt, the differences observed by comparing both models could indicate that localities might be adapting to intermediate climatic values that could be exerting a selection pressure. It is also possible that there could be local adaptation to other factors correlated with the variables we measured, such as soil type or biotic interactions that would be shaping the patterns of allele distribution seen in this study. For both of our GF models, the minimum temperature of the coldest month was the most important variable predicting the turnover of allele distribution. This variable is also a major determinant in the distribution of other conifer species (Akyol & Örücü, 2019; Yun et al., 2018), indicating that cold temperatures could play a role as a major factor in shaping allele distribution patterns, with some localities possibly being more resistant to colder temperatures. These results are comparable to those of Martins et al. (2018) who found patterns of spatial turnover that followed an east–west direction in an oak species along the Trans-Mexican Volcanic Belt and were similar between gradient forest models based on a full set of SNPs and only on SNPs associated to precipitation. However, for the oak species, precipitation seasonality was the main environmental variable explaining genomic variation.

Our projections of genomic offsets for the year 2050 were also highly correlated between the full set and the adaptative set as has been observed in studies with *Pseudotsuga menziesii* var. *menziesii*, *P. menziesii* var. *glauca*, *Pinus banksiana*, and *Picea rubens* (Lachmuth et al., 2023; Lind et al., 2023) as well as in animals (*Cyclograpsus punctatus*, *Parechinus angulosus*, *Scutellastragranularis*; Nielsen et al., 2021). One explanation for this correlation could be the fact that a vast number of adaptations are due to polygenic effects rather than to single loci (Fagny & Austerlitz, 2021; Savolainen et al., 2013). This could explain why the neutral and non-neutral data provide a similar pattern, but more studies are necessary to test this.

The genomic offset models show that localities of *P. leiophylla* located in the centre-east of the Avocado Belt are at greater risk of maladaptation because of climate change (Figure 3). However,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Avocado orchards by the year 2050

FIGURE 3 Local (a), forward (b), and reverse offset (c) of *Pinus leiophylla* estimated from the set of outlier SNPs within the predicted future temperate forest patches. The offset values were calculated for 2050 under the Miroc6_ssp370 climate scenario. Grey areas represent the projected extent of avocado orchards for the year 2050 according to our land use model. White dots represent the 19 collected locations of *P. leiophylla* and black triangles show the location of presence records of the species from the National Herbarium of Mexico (MEXU) database.

TABLE 2 Partitioning of genetic variation into environmental (env), geographic (geo), and their shared variance based on redundancy analysis (RDA).

	Full set	Outlier set	$R^2 > 0$ set
Combined fractions			
F ~ geo	−0.005 ns	−0.003 ns	.029 ns
F ~ env	0.014 ns	0.035 ns	.182**
Individual fractions			
F ~ geo.|env	0.014 ns	0.009 ns	.053 ns
F ~ env|geo	0.034 ns	0.048*	.207**
Shared	−0.019	−0.013	−.024
Residual	0.971	0.955	.763

Note: Adjusted R^2 are reported. Asterisks indicate statistical significance (*$p < .05$, **$p < .01$, ***$p < .001$, ns nonsignificant). F = dependent matrix of localities allele frequencies. RDA tests are of the form: F ~ dependent matrices|covariate matrices. Env = environment (PCs 1–3), geo = geography (x, y).

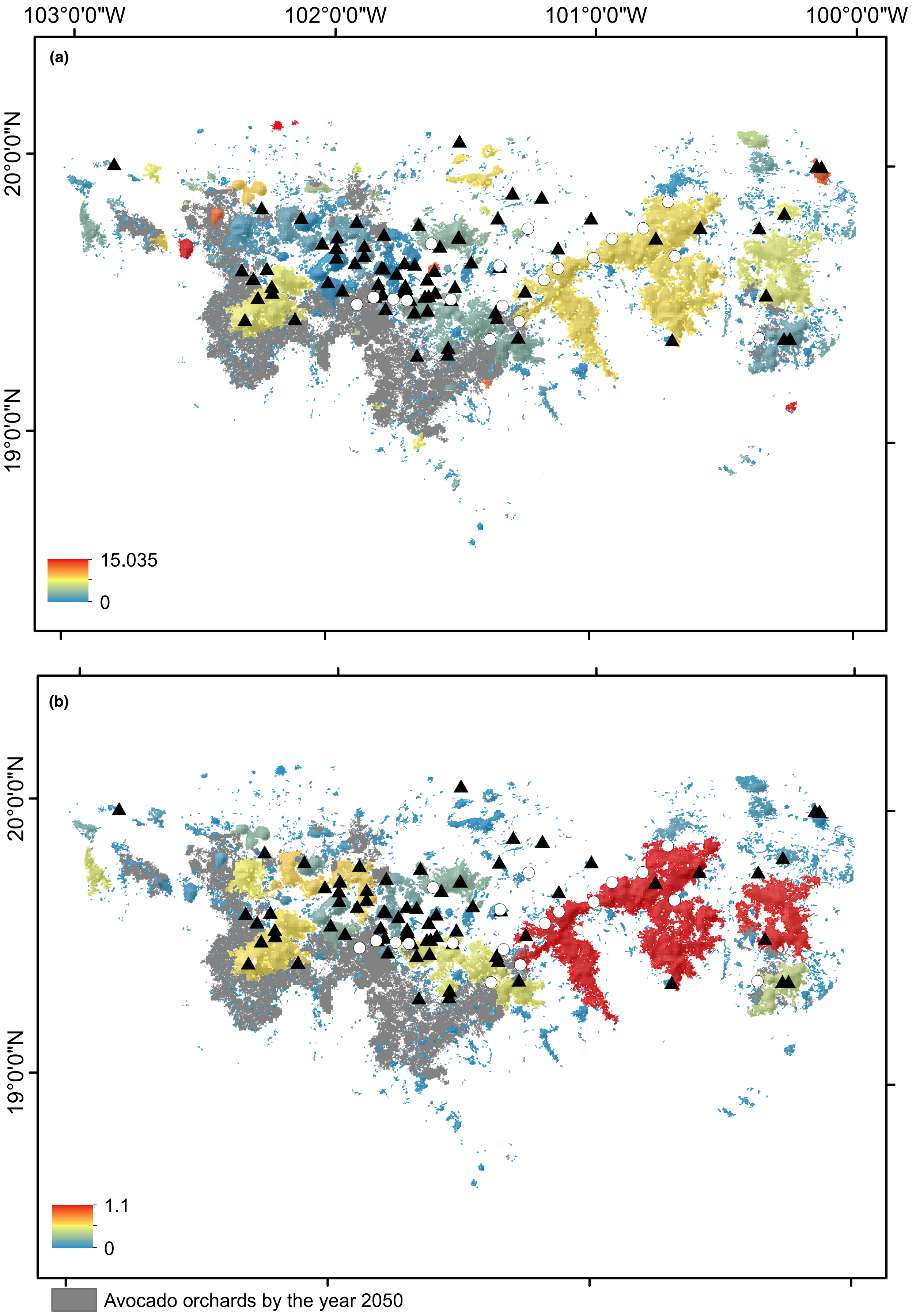
the reverse offset analyses suggest that the local offset of most localities could be mitigated by actions of assisted gene flow. Notwithstanding, these measures are context-specific and should be analysed with caution depending on each locality. For example, some localities with low local offset might not need assisted gene flow actions to persist, while for others it will be necessary to carry out strategies of assisted gene flow to avoid the risk of extinction. Additionally, *P. leiophylla* populations outside the study area could serve as sources of genetic diversity, diminishing the local offset for those localities that are predicted to be maladapted to future climate. Therefore, a complementary range-wide characterization of genomic variation in *P. leiophylla* would be advisable.

Land use change is another pressure expected to greatly affect forest tree species within the Avocado Belt (Arima et al., 2022; Charre-Medellín et al., 2021; Denvir, 2023). The projection of avocado cultivation expansion by the year 2050 indicates a more intensive transformation at the west and centre of the study region, probably causing the total loss of the forest patch where the Zurumucapio sample (Id. 18, Table S1) was obtained and reducing the original patch extension to less than 60% for three other localities. To safeguard the genetic diversity of these localities, it will be required to carry out strategies of assisted migration to new locations where they would have a low forward genetic offset. Another conservation strategy could be to promote among avocado producers the maintenance of forest strips that include individuals of *P. leiophylla* from the localities that are more threatened by the expansion of avocado orchards. In fact, the Zurumucapio locality is interesting because it grows at the upper limit of variables related to temperature and at the lowest limit of variables related to precipitation (Table S1). It is likely that these individuals are locally adapted to hotter and drier climates and might be interesting donors of adaptative genetic diversity. Also, at this locality the highest number of private alleles was found and could contain significant genetic diversity that could serve as a fuel to future adaptation to climate change.

Avocado expansion will also modify the current patterns of connectivity on the remaining patches. For both, the seed and pollen dispersal scenarios, we found that the central and western localities will be the least connected, as a result of the projected establishment of avocado crops. These alterations will leave those areas as isolated patches causing risk of inbreeding and experimenting with the negative effect of genetic drift; also, these patches cannot contribute to the regeneration process of the temperate forest, suggesting that conservation actions through human intervention might be necessary. However, we caution that our study excludes forest patches less than 10 ha and that even isolated trees could represent potential bridges for gene flow, maintaining connectivity between localities (e.g. Oyama et al., 2017).

Finally, our results indicate that the localities in the eastern part of the Avocado Belt will have low local genomic offset, a probable low reduction of their original patch extension and will maintain higher values of connectivity (Figure 4). This area is particularly relevant because it includes localities that could be well adapted to projected future environmental changes and will be less susceptible to loss by land use change, maintaining the connectivity between patches. Also, this area encompasses the Monarch Butterfly Biosphere Reserve (MBBR), an area that provides important ecosystem services (Galicia & Zarco-Arista, 2014) so it must be a conservation target to preserve viable localities of *P. leiophylla* that contribute to the persistence of temperate forests in the region and their ecological interactions. However, although it is a protected zone, illegal deforestation for timber sources and land use change mainly for avocado plantations remain a problem within the MBRR. By the year 2021, avocado crops occupied almost 1000 ha in the buffer zone of the reserve, representing a risk for its integrity (Sáenz-Ceja & Pérez-Salicrup, 2021), so more conservation policies must be implemented to ensure the future persistence of this area and prevent the continued expansion of avocado crops.

Although genomic offset has proven to be a powerful tool to incorporate genomic information to understand how species will respond on the face of climate change, this approach must be validated to estimate how related is the genomic offset with the real performance of the populations (Rellstab et al., 2021) taking into account their demographic history and genomic architecture (Láruson et al., 2022). In our study, we caution that we did not sample all the genomic diversity through the entire distribution range of *P. leiophylla*,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Avocado orchards by the year 2050

FIGURE 4 Composite Connectivity Index (CCI) values by the year 2050 for (a) seed dispersal scenario and (b) pollen dispersal scenario within the predicted temperate forest areas. Grey areas represent the projected extent of avocado orchards for the year 2050 according to our land use model. White dots represent the 19 collected locations of *Pinus leiophylla*, and black triangles show the location of presence records of the species from the National Herbarium of Mexico (MEXU) database.

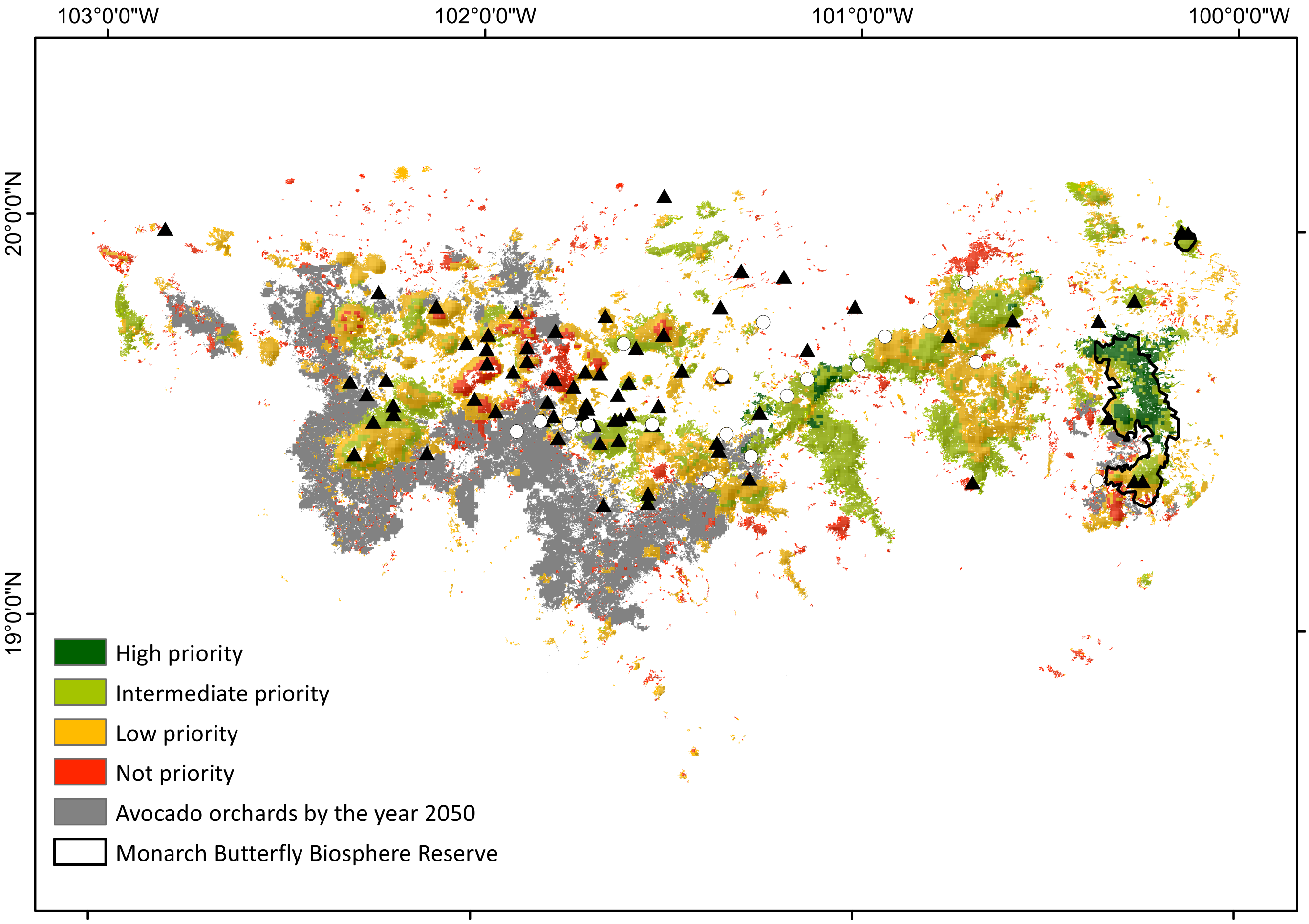

FIGURE 5 Prioritization of areas for conservation considering local offset, connectivity from seed and pollen dispersal and avocado orchards extent by 2050. Grey areas represent the projected extent of avocado orchards for the year 2050 according to our land use model. White dots represent the 19 collected locations of *Pinus leiophylla*, and black triangles show the location of presence records of the species from the National Herbarium of Mexico (MEXU) database.

limiting our ability to identify genetic variants that could contribute to climate change adaptation. Furthermore, it is advisable to perform common garden experiments with *P. leiophylla* to evaluate whether higher values of genomic offset in a locality indeed correlate with lowered performance under some environmental conditions, an aspect that remains a limitation of landscape genomics methods.

However, recent studies with other species have used such common garden experiments to test if genetic offsets are adequate to measure reductions in fitness. The results have shown that genomic offsets are better predictors of future maladaptation than climatic or geographic differences alone, showing a correlation between higher genomic offset values with lower values of population performance (Capblancq & Forester, 2021; Fitzpatrick et al., 2021; Lachmuth et al., 2023; Lind et al., 2023). Also, it is important to note that genetic offset only models the quantity of genomic change necessary to survive given the actual genotype–environment association that populations present. However, other factors could also be playing an important role in the fate of the populations, such as genetic drift or biotic interactions and should be considered in future studies (Aguirre-Liguori et al., 2021).

Nevertheless, since trees are long-lived species, genetic offsets offer an opportunity to estimate the effects of climate change (which is already causing a damage in global biodiversity) in taxa for which phenotypic information or common garden experiments are lacking and whose attainment will cost a lot of time and resources. We believe that this work is useful as a framework to incorporate distinct layers of information to provide a more robust representation of the response of the populations to future anthropogenic disturbances. Our results show that the genetic diversity of *P. leiophylla* within the Avocado Belt will be threatened by both climate change and land use change.

While some localities could be more affected by the effects of climate change, others could be more vulnerable to land use change. Overall, this would reduce the evolutionary potential of the species by erasing genetic diversity that could serve as a source of variability allowing adaption to a changing environment. Our results could serve as a basis to design conservation strategies and restoration projects for future forest management enhancing the persistence of this tree species and the temperate forest ecosystem in the region.

AUTHOR CONTRIBUTIONS

VI-T, JAA-L, and AG-R designed the study; VI-T and AG-R collected samples from natural populations; VI-T conducted genotyping and data analyses; MCL-C performed connectivity analysis; MCL-C and EYA conducted analysis of avocado expansion; VI-T, JAA-L, and AG-R wrote the manuscript draft. All authors completed and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Individual fastq data files are available at the SRA repository of NCBI under BioProject number PRJNA1076599. VCF and code are archived at Dryad: doi: 10.5061/dryad.gxd2547t6.

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SUPPORTING INFORMATION

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