

# From genes to ecosystems: Analyzing *Ormosia microphylla* endangerment driven by multiple dimensions

**Xiao-ming Tian**

Hunan Botanical Garden

**Bin Yuan**

Zhejiang University

**Cun Mou**

Hunan Botanical Garden

**Guang-feng Xiang**

Hunan Botanical Garden

**Lu Zhu**

Hunan Botanical Garden

**Gao-fei Li**

Hunan Botanical Garden

**Chao Liu**

Hunan Botanical Garden

**Xiang-peng Li**

Hunan Botanical Garden

**Fu-liang Hu**

Zhejiang University

**Hao Lv**

**lvhao0203@163.com**

Hunan Botanical Garden

---

## Research Article

**Keywords:** anthropogenic logging, *Ormosia microphylla*, plant conservation, population genetics, seed spreader

**Posted Date:** September 2nd, 2025

**DOI:** <https://doi.org/10.21203/rs.3.rs-7231308/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.  
[Read Full License](#)

**Additional Declarations:** No competing interests reported.

---

**Version of Record:** A version of this preprint was published at BMC Plant Biology on June 30th, 2026.  
See the published version at <https://doi.org/10.1186/s12870-026-09412-x>.

# Abstract

## Background

The ecologically and economically important species *Ormosia microphylla* faces a high extinction risk. However, systematic studies on the multiple threats faced by these populations and mechanisms underlying the interactions among threats remain limited. To formulate effective conservation strategies, we conducted population surveys across seven natural populations within its main distribution range.

## Results

Overall, 71% of the studied populations exhibited unstable age structures. Population genomic analyses classified the populations into three genetic clusters that were all characterized by low genetic diversity. The slowest decline rate was predicted for the HN (individuals from Tongdao and Cengbu; lacking young individuals) cluster than for the GX (individuals from Nandan) and HG (individuals from Jianhe, Liping, Jingzhou, and Tangbaocun; lacking mature trees or dominated by senescent individuals) clusters. However, the GX cluster possessed a more stable age structure. Selective sweep analysis further revealed enhanced fatty acid biosynthesis and metabolism pathways in the GX cluster. Further, saplings predominated within the HG cluster. Although all populations produced viable seeds, seed production declined annually and germination was restricted by the seed coat. Illegal logging (tree stumps) evidence was observed in all populations, with populations exhibiting more stumps having fewer mature adult trees. Finally, soil nutrients were not significantly correlation with seedling number, whereas bird diversity was positively correlated with seedling numbers.

## Conclusions

These results help reconcile the apparent contradictions between field observations and genetic predictions and highlight the critical importance of curbing illegal logging and monitoring bird diversity for the recovery and persistence of *O. microphylla* populations.

## Background

Biodiversity is the cornerstone of Earth's life-support systems and establishes ecosystem functions through mechanisms such as functional complementarity and resilience stabilization [1]. Driven by habitat loss, overexploitation, and climate change, the escalating extinction crisis threatens this foundational framework [2]. According to the International Union for Conservation of Nature (IUCN) Red List [3], 43% of plant taxa face the risk of extinction. This trend is particularly prominent in tropical and subtropical regions. The decrease in plant diversity disrupts carbon sequestration and nutrient cycling [4] and destabilizes mutualistic networks, such as plant–pollinator interactions. These changes in turn trigger cascading impacts on ecosystem services that are critical to human well-being, including food

security and climate regulation [5]. Therefore, protecting plant diversity is important for global ecological protection and sustainable development.

*Ormosia* plants have attracted considerable attention as an important group within Fabaceae because of their beautiful red seeds and high-quality wood [6]. This genus is widely distributed in tropical and subtropical regions and plays an important role in forest ecosystems [7]. Its seeds are often spread by birds and form a complex interaction network with various animals and plants, which is important for maintaining biodiversity [8]. However, factors such as habitat loss and climate change are increasingly impacting the habitats of *Ormosia* [9, 10]. For example, *Ormosia microphylla*, a representative *Ormosia* taxon, has shown a sharp population decrease and reduced distribution range [11]; thus, it has been listed as an Endangered species by the IUCN. The Endangered status of *O. microphylla* reflects the conservation challenges affecting *Ormosia* and highlights the urgency of protecting plant diversity.

Current research on the decline of *O. microphylla* populations has focused on analyzing single factors. Meanwhile, systematic studies on the multiple threats faced by these populations and mechanisms underlying the interactions among threats are still limited [12]. This hinders the design of holistic conservation strategies that align with the 2030 targets of the Global Biodiversity Framework [13, 14].

Therefore, we aimed to explore protection strategies for *O. microphylla*, improve the conservation status of this Endangered species, and provide a scientific reference for protecting plant diversity and sustainable ecosystem development [15].

## Results

### Population surveys of *O. microphylla*

We conducted population surveys of *O. microphylla* at four sites in Hunan, two sites in Guizhou, and one site in Guangxi; we found a total of 1,329 individuals of *O. microphylla* (Fig. 1A). Among them, Jianhe (JH) in Guizhou had the largest number of individuals, with 1,020 individuals found. Liping (LP) in Guizhou had the smallest number of individuals, with only one individual at a height of 25 m and diameter at breast height of 155 cm (Fig. 1B, Table S1). In the other populations, the number of *O. microphylla* individuals did not exceed 100. By combining the cluster analysis and survey results, the seven *O. microphylla* populations were classified into four categories (Figs. 1B and S1): Category 1 only included the populations from Cengbu (CB) and LP, wherein the individuals had heights greater than 5 m; Category 2 included the population from Tongdao (TD), wherein more than 60% of the individuals were young trees with heights ranging from 1.3 to 2.5 m; Category 3 included the populations from Jingzhou (JZ) and Tangbaocun (TBC), wherein more than 60% of the saplings had heights ranging from 0 to 1.3 m; and Category 4 included populations from Nandan (ND) and JH, wherein the age structure of the trees was suitable for sustainable development.

### Population structure analyses

To clarify the genetic relationships among the 7 populations, 62 individual leaves were collected for genome sequencing (Fig. 1C). A principal component analysis (PCA) ellipse was drawn based on the sequencing results, and the 62 individuals were categorized into 3 genetic clusters (Fig. 1C). All individuals from ND were individually defined as the GX genetic cluster, which separated from the other two groups along the PC1 axis. Individuals from JH, LP, JZ, and TBC were defined as the historical analysis of the HG genetic cluster. Individuals from TD and CB were defined as the HN genetic cluster (Fig. 1C and D). Each population occupied a different geographic distribution area. These sequencing results may support the potential for past genetic exchange between populations within each genetic cluster. However, genetic exchange was not detected between each genetic cluster (Fig. S2). A comparison of the nucleotide diversity of the three genetic clusters showed that all three had a nucleotide diversity below 0.005, with the HN genetic cluster showing the highest diversity, followed by the HG genetic cluster (Fig. 1E). This was also illustrated by the linkage disequilibrium results within the populations (Fig. 1F). Analysis of the population history showed a persistent decline in the population number of *O. microphylla* over the last 10 years. The predicted population of the GX genetic cluster had fewer individuals than the HG and HN populations (Fig. 1G). However, the number of individuals in the GX genetic cluster of *O. microphylla* was higher than that in the HN genetic cluster.

## Selective sweep analysis

Integrated analysis of the population age structure and genomic population structure revealed that the GX population exhibited the most stable age structure compared to the HN (lacking young individuals) and HG (lacking mature trees or dominated by senescent individuals) populations. To investigate the contribution of genetic adaptation to these age structure differences, we performed selective sweep analysis using GX as the reference population against HG and HN. Employing a combined threshold approach (top 5% of  $\pi$ ,  $F_{ST}$ , and XPCLR values), we identified 282 genes under selection (Fig. 2A). Compared to those in GX, selected genes in HG exhibited  $F_{ST} > 0.19$ ,  $0.29 < \theta_{\pi} < 1.49$ , and  $XPCLR \geq 6.71$ , while selected genes in HN showed  $F_{ST} > 0.21$ ,  $0.43 < \theta_{\pi} < 1.40$ , and  $XPCLR \geq 8.03$  (Fig. S3). Among the 165 selected genes in HG, significant enrichment was observed in the cutin, suberine, and wax biosynthesis (ko00073) pathways ( $p\text{-adjust} < 0.05$ ) relative to those in GX. Additional pathways, including RNA polymerase (ko03020) and Phenylpropanoid biosynthesis (ko00940), were also enriched (Fig. 2B a). For the 117 selected genes identified in HN, KEGG annotation revealed enrichment in pathways such as other glycan degradation (ko00511), phenylpropanoid biosynthesis (ko00940), and cutin, suberine, and wax biosynthesis (ko00073, Fig. 2B b) relative to those in GX.

Similarly, to elucidate the mechanisms underlying the stable age structure of GX, we used HG and HN as reference populations and identified 176 and 150 selected genes in GX, respectively (Fig. 2C). Genes selected in GX were predominantly enriched in linoleic acid metabolism (ko00591), fatty acid biosynthesis (ko00061), and fatty acid metabolism (ko01212) pathways relative to those in HG. In contrast, genes selected in GX showed primary enrichment in galactose metabolism (ko00052), other

types of O-glycan biosynthesis (ko00514), and protein export (ko03060) pathways (Fig. 2D) relative to those in HN.

## Seed number and seed vigor of representative populations

Based on the above findings, we subsequently focused on the CB, TBC, and TD populations because they belonged to different age groups. The TD and CB populations belonged to the HN genetic cluster, and the TBC population belonged to the HG genetic cluster.

In this study, we determined the plant seed number and seed vigor in three representative populations—TD, TBC, and CB. The results showed that populations of different genetic clusters could naturally produce fruit. However, the lack of adult trees within the populations or the decline in the reproductive capacity of old trees could cause a reduction in seed numbers. Seeds were collected from all three populations selected in this study. However, the number of seeds collected varied, and the total seed number declined annually (Fig. 3). The TBC population had the highest seed number each year from 2019 to 2024. The TD population had a significantly higher seed number than the CB population from 2019 to 2023, and the seed number in 2024 was not significantly different from that of the CB population. Meanwhile, the CB population annually produced only a small number of seeds ( $p < 0.05$ , Fig. 3A). The 2023 count showed higher numbers of adult and old trees in TD (9) and CB (10) than in TBC (5). The TD and CB populations had significantly lower individual fruiting rates in 2024 than did the TBC population ( $p < 0.05$ , Fig. S4).

Seed vigor was similar in different populations, although the seed numbers of the different populations varied. The sowing of seeds obtained from the population showed that seed viability in each region ranged from 20–37% and was not significantly different from each other ( $p > 0.05$ , Fig. 3B). Breaking the seed coat (T2) promoted the germination of *O. microphylla* seeds. Three treatments were used to treat the *O. microphylla* seeds. All three treatments significantly increased the germination rate of *O. microphylla* seeds ( $p < 0.05$ ; Fig. 3C). Among them, the 45 °C water immersion treatment (T1) and concentrated sulfuric acid immersion treatment (T2) were the most effective and increased seed germination by 40%. This result also highlights the importance of the soil environment and seed dispersal in the germination of *O. microphylla* seeds.

## External risks affecting *O. microphylla* populations

### Illegal logging

All three representative populations selected were subjected to varying degrees of logging, with the TD population being the most severely logged. Stumps of *O. microphylla* were observed within all three populations examined. The number of stumps in the TD population was significantly higher than that of the TBC and CB populations ( $p < 0.05$ , Fig. 4A). This also implies that the TD population may have

experienced the greatest degree of anthropogenic disturbance among the populations studied. This result also indicated that all three groups had larger population sizes in the past.

## Soil conditions

Twelve soil variables were measured, including pH. PCA showed that the soil conditions of the TD and TBC populations were similar and significantly different from those of the CB population (Fig. 4B). The ANOVA significance analysis of the 12 indicators did not reveal significant differences in pH and total phosphorus among the three regions ( $p > 0.05$ , Fig. S5). Except for pH,  $\text{NO}_3\text{-N}$ , and total phosphorus, the soil composition of CB was significantly higher than that of the TBC and TD populations ( $p < 0.05$ ). This implies that the soils in the CB area were of higher quality than those in the other two sites. Meanwhile, the soil conditions in the TBC area were more suitable than those in the TD area because the contents of seven components, including nitrate nitrogen, humus, fulvic acid, and humin, were significantly higher in the TBC soil than in the TD soil ( $p < 0.05$ ).

## Bird diversity

In the bird observation experiment, 56 bird species were identified (Table S2). The total number of birds did not significantly differ among the three sites ( $p > 0.05$ , Fig. S6). PCA was performed to analyze the species composition of birds at the three sites (Fig. 4C, a). The PC1 loading was 50.11%, and the PC2 loading was 26.49%. Ellipse plotting with PC1 and PC2 as the axes showed that the bird species compositions of the TD and TBC areas were similar, although both differed significantly from that of the CB area. Further analysis of bird diversity at the three sites showed that the Shannon and Simpson indices of birds in the CB and TBC were significantly higher than those in the TD ( $p < 0.05$ , Fig. 4C b, c). In contrast, the Shannon and Simpson indices did not significantly differ for the birds in the CB and TBC. The bird diversity in the TD group was substantially lower than that in the CB and TBC groups.

## Discussion

*O. microphylla* currently faces a high extinction risk due to synergistic anthropogenic and genetic threats. Given its ecological significance in biodiversity maintenance and economic significance as timber, we conducted comprehensive population surveys across seven naturally occurring populations in three Chinese provinces where *O. microphylla* is almost exclusively distributed. All populations exhibited critical vulnerability, necessitating urgent identification of extinction drivers to formulate science-based conservation strategies.

## Unstable age structure in most populations

Usually, plant populations in the field present a certain capacity for natural regeneration and a suitable age structure [16, 17]. However, the field survey results showed that the age structure of *O. microphylla* at

more than 70% of the surveyed sites was not conducive to population expansion and stable reproduction. These populations were characterized by an over-representation (> 60%) of young trees and saplings with a tree height between 0 and 2.5 m or old trees with a tree height > 5 m. Given the overabundance of young trees and saplings within the population, *Ormosia* trees may be unable to obtain sufficient nutrients for interspecies competition. *Ormosia* spp. are sun-loving plants, and their slow growth relative to other companion species may prevent them from obtaining sufficient sunlight to develop into large trees [18]. Moreover, when old trees are overrepresented in the population, plant productivity is degraded and the plant cannot produce a sufficient number of seeds to sustain the population [19, 20]. The results of the 6 consecutive years of seed number counts supported this hypothesis and suggested that sustained population decline is almost unavoidable. The results of many surveys of threatened plants show that populations of these plants usually have a poor age structure and are usually characterized by an overabundance of old trees and a low number of young trees [21, 22]. This differs from the dynamics of rare *Ormosia* plants and thus implies that *O. microphylla* populations with a sufficient number of young trees were still naturally regenerating at some point in the past [23].

## Inconsistencies between the population genetic analyses and field survey data

Population genetics techniques have been widely employed to assess genetic risks in endangered plants, including genetic diversity and adaptive potential. Our analyses revealed uniformly low genetic diversity across all *O. microphylla* populations, which represented a significant threat to species persistence [24]. Despite the well-established age structure and natural regeneration of the ND (belonging to the GX cluster) and JH (belonging to the HG cluster) *O. microphylla* populations, their low nucleotide diversity raises concerns regarding their long-term survival [25]. The results of the population genetics analyses also suggest that populations from different genetic clusters will all experience sustained population decline in the future. Population history analysis suggests that this decline occurred 10 years ago. However, the population decline of many threatened plants occurred 1000 years ago, suggesting that *O. microphylla* populations have been strongly disturbed in recent years [26, 27]. In addition, gene flow is lacking between the different genetic clusters, which is not conducive to the genetic diversification of populations [28]. Habitat fragmentation also reduces the gene flow in populations within genetic clusters [29]. This low nucleotide diversity and limited gene flow within a population may cause the accumulation of deleterious alleles and reduce its adaptability to environmental stress [30].

Selective sweep analyses indicated positive selection in the GX cluster for genes enriched in linoleic acid metabolism, fatty acid biosynthesis and metabolism, and other types of O-glycan biosynthesis pathways, potentially reflecting local adaptation [31, 32]. Enhanced fatty acid and linoleic acid metabolism may contribute to the membrane structure, energy storage, and signaling processes, while linoleic acid may be involved in membrane stabilization and support stress tolerance under challenging conditions [33]. Elevated seed lipid biosynthesis may also enhance seed disperser attractiveness [34]. However, both the

HN and HG clusters were enriched in the cutin, suberin, and wax biosynthesis pathways, suggesting enhanced seed coat constraints relative to the GX cluster [35] despite the contribution of these pathways to seed protection. The HG cluster exhibited more significant enrichment ( $p\text{-adjust} < 0.05$ ) than HN ( $p\text{-adjust} = 0.46$ ), which was supported by the seed germination experiments; however, the differences were not significant. Dormancy imposed by hard seed coats in *Ormosia* typically requires scarification or animal digestion for germination, which was experimentally validated [36]. Thus, dormancy is recognized as a reproductive barrier in endangered plants, and intensified wax biosynthesis in HN and HG population may exacerbate physical dormancy, potentially limiting sapling recruitment.

Although population genetic insights partially explain the population declines and unstable age structures, discrepancies with empirical field data emerged. (1) Endangered plants usually undergo a process of population decline [37, 38]. The population history analysis showed that the number of individuals in the HN genetic cluster was greater than that in the GX genetic cluster. This result contradicts the field survey data. (2) The selected genes of the HG population were significantly enriched in the cutin, suberine, and wax biosynthesis pathways, suggesting that the number of saplings may be too low. In reality, the HG population was dominated by saplings at three out of four sites. These findings imply that external factors may be significantly accelerating the decline in the *O. microphylla* population.

## External risks may explain the contradiction result

External risks, including human logging and declining bird diversity, may directly explain these contradictions [39]. Given the economic value of *O. microphylla*, overharvesting may also be a key factor contributing to its population decline [40, 41]. Traces of logging were found in all three populations (TBC, TD, and CB), and individuals with tree heights of 2.5–5.0 m were lacking in all three populations. The TD area with the highest number of stumps only had one adult tree. The number of stumps may not accurately reflect the extent of logging because stumps may erode over time due to rain, microorganisms, and wind [42, 43]. However, the available results are sufficient to demonstrate deforestation of *O. microphylla*, and they explain the presence of a certain number of young trees but a lack of adult trees in some populations. These substantial economic gains are driving illegal harvesting and logging and thus pose a serious threat to global biodiversity. Therefore, a sound regulatory system for the efficient management and conservation of biodiversity is urgently required [44]. Such systems will benefit not only *O. microphylla* but also fish, mammals, tropical timber species, and other taxa.

Additionally, in the TD population, although a certain number of seeds was observed annually, the number of saplings was extremely limited. Seed germination rates were similar in different populations of *O. microphylla*, and germination rates were significantly higher after treatment of the seed coat with acid treatment or hot water soaking. This is similar to the findings in other *Ormosia* plants such as *O. microphylla*, which exhibits seed coat limitation [45]. Additionally, this implies that the TD population lacks suitable conditions for the germination of *O. microphylla* seeds. However, the soil conditions in TD were similar to those in TBC, suggesting that soil was not responsible for the lack of saplings. Considering the bird-dependent seed dispersal of *O. microphylla*, we suggest that the significantly lower bird diversity in TD than in the other areas may be a key factor limiting population development, although

the population can normally produce seeds [46, 47]. Similarly, the higher bird diversity in the TBC area (HG population) may explain why the strong seed coat restrictions did not result in a lack of saplings. These results support the hypothesis that bird diversity may influence the sustainability of the *O. microphylla* populations by influencing the number of saplings. The decline in the number of *O. microphylla* individuals was accompanied by a decline in seed production, implying that birds feeding on *O. microphylla* seeds have difficulty obtaining sufficient food, which further exacerbates the decline in bird diversity. These results emphasize the importance of biological interactions in biological conservation, and the maintenance of these interactions may help achieve better biological conservation benefits.

## Future measures to protect *O. microphylla*

*O. microphylla* faces serious internal and external threats, and the interaction between these two risks exacerbates the decline in population size. However, regardless of the reasons that led to the initial population decline, we can conduct conservation studies on the existing populations of *O. microphylla*. Based on our findings, we propose the following conservation recommendations: (1) human intervention, such as transplanting *O. microphylla* or felling companion trees, should be considered in the future to help increase its population number [48]; (2) artificial pollination should be performed to improve genetic diversity; (3) regulations on illegal logging activities should be strengthened [49, 50]; and (4) seed dispersers should be protected to indirectly promote population recovery [51–53]. Implementing these measures can help to alleviate the survival pressures faced by *O. microphylla* and guarantee the long-term survival of its population. Many threatened taxa that are as economically valuable as *O. microphylla*, including plant and animal taxa, may present similar population declines. Therefore, to determine plant threat status and develop effective conservation programs with predictable benefits, we call for a more detailed assessment of future risk factors that includes more varied parameters than just the impacts on the plants themselves.

## Materials and methods

### Study area overview

The study area is located in south-central and southwestern China (107°14'E–110°19'E, 25°7'N–26°28'N), including four sites in Hunan Province, that is, Chengbu (CB, 110°19'E, 26°22'N), Tongdao (TD, 109°56'E, 26°10'N), Jingzhou (JZ, 109°42'E, 26°28'N), and Tangbaocun (TBC, 110°02'E, 25°49'N); two sites in Guizhou Province: Liping (LP, 109°08'E, 26°12'N), Jianhe (JH, 108°36'E, 26°38'N); and one site in Guangxi: Nandan (ND, 107°14'E, 25°07'N). The altitudes of the study sites vary from 564 m to 970 m. The vegetation type is subtropical evergreen broad-leaved forest. The region has a typical warm and humid subtropical monsoon climate with abundant light. The average annual temperature is 16–22 °C, the average temperature of the warmest month (July) is 25–30 °C, and the average temperature of the coldest month (January) is 5–12 °C. The average annual precipitation is 1,200–1,800 mm, with

approximately 85% concentrated between April and October. The average annual relative humidity is above 75%.

In this study, a field survey was conducted from April to July each year from 2019 to 2024. For each sample site, we randomly selected 62 robustly growing mother trees of *O. microphylla* from each sample site. Plant materials were collected from natural populations in the above regions. The species was formally identified by Dr. Lihong Yan (anlh0424@163.com), and voucher specimens (voucher No. OMi-2024-001 to OMi-2024-062) have been deposited at Hunan Botanical Garden (Hunan Province, China). Permission for field sampling was obtained from the local forestry authorities. All collections and experimental research on plants complied with institutional, national, and international guidelines, as well as local legislation on biodiversity conservation.

## Quadrat and population surveys

Field surveys were conducted from April to July (2019–2024) to investigate the population structure of seven natural populations of *O. microphylla* in ND, JH, LP, TD, JZ, TBC, and CB. The distribution ranges of the seven natural populations were determined through field surveys combined with records from local foresters. To cover all *O. microphylla* individuals at the distribution sites, we referred to the method of Ohsawa (1984)M Ohsawa [54] for the population structure survey and made some adjustments in light of the actual situation. We set 20 × 20 m to 30 × 30 m sample plots based on the distribution range of the natural population, and 13 quadrats were established. The number and height of *O. microphylla* in each quadrat were recorded. Individuals were classified into four height categories: (1) 0 m < tree height ≤ 1.3 m; (2) 1.3 m < tree height ≤ 2.5 m; (3) 2.5 m < tree height ≤ 5 m; and (4) 5 m < tree height. These height categories were grouped into four age classes: sapling (tree height ≤ 1.3 m), young tree (1.3 m < tree height ≤ 2.5 m), adult tree (2.5 m < tree height ≤ 5 m), and old tree (tree height > 5 m).

## Population genomic analyses

At the seven sampling sites, we randomly selected healthy growing trees of *O. microphylla* to collect leaves for genome sequencing. A total of 62 trees were sampled. An Illumina Bipartite Library was constructed for each sample. Based on these data, we performed variation, population structure, chain imbalance, gene flow, and population history analyses.

The reference genome (*Ormosia henryi*) was 2.64 G. The comparison rates of the 62 samples ranged from 98.53–98.98%, the reference genome coverage ranged from 42.75–47.34%, and the average sequencing depth ranged from 6.316× to 13.56×. Based on the comparison results, the samples were tested to detect mutations using GATK (Version: 4.6.0.0; Parameters: HaplotypeCaller, CombineGVCFs, and GenotypeGVCFs, <https://github.com/broadinstitute/gatk>) to detect variants in the samples. The identified single nucleotide polymorphisms (SNPs) and indels were filtered separately using GATK (version 4.6.0.0; parameters: VariantFiltration) according to the hard-filtering criteria officially recommended by GATK. To measure the specific variations in each sample, the filtered results were assessed for variations. Information on base transitions, inversions, inversion transition ratios, pure genotypes, heterozygous genotypes, and heterozygous ratios was obtained for each sample.

Population genetic structure was determined using ADMIXTURE (version: 1.3.0; parameter: -cv inputFile K, <https://dalexander.github.io/admixture/index.html>), with K values ranging from two to nine.

Genome-wide linkage disequilibrium (LD) analysis was performed using PopLDdecay (version: v3.41; parameters: default parameters, <https://github.com/BGI-shenzhen/PopLDdecay>).

TreeMix (<https://bitbucket.org/nygcresearch/treemix/wiki/Home>) was used to infer population differentiation and gene flow from a set of population histories. Moreover, TreeMix 1.3.1 was used to return a maximum likelihood (ML) tree for a population by obtaining allele frequencies from multiple populations and inferring possible hybridization events.

In this study, we used smc++ (version: latest; base mutation rate: 4e-9; years per generation: 50) to infer the effective population size of the population to which the individual belonged at various times using individual weight sequencing data.

## Selective sweep analyses

To identify key genomic regions subject to selection, this study compared three populations divided according to sequencing results and combined three indices ( $\pi$ -ratio,  $F_{st}$ , and XPCLR) for selective sweep analysis. The genomes were divided into equal-sized intervals by sliding windows. VCFtools (version: 0.1.16; parameters: -weir-fst-pop, -window-pi, [https://vcftools.sourceforge.net/man\\_latest.html](https://vcftools.sourceforge.net/man_latest.html)) was used to calculate the  $F_{st}$  values and  $\pi$ -ratio between each window of the two compared populations, and the Python version of XPCLR (version: 3.42, <https://github.com/hardingnj/xpclr>) was used to calculate the XPCLR values of the two compared populations between each window on each chromosome. Then, the intersection region of the three indices (all top 5%) was identified. Subsequently, for the selected genomic regions, KEGG enrichment analysis was performed to identify sets of functional genes and metabolic regulatory pathways with associations.

## Seed number determination

We collected the fruits of adult and old *O. microphylla* trees from October to November between 2019 and 2024 to determine the seed number and viability of different *O. microphylla* populations. The plants for fruit collection were selected as described in Section 2.2. Five branches were randomly selected from each individual, and the fruit from each branch was collected. After fruit picking, the seeds were stripped in the laboratory and the total number of seeds was recorded. The average seed number was expressed as the average number of seeds per adult and old *O. microphylla* tree.

## Seed vitality

We conducted a sowing test from November 2019 to February 2021 in the Conservation Nursery of Hunan Provincial Botanical Garden to determine the viability of *O. microphylla* seeds in terms of germination rate. Owing to the limited number of seeds collected each year, a sowing trial was conducted over two years.

The collected seeds were sown immediately in November 2019, and 30 *O. microphylla* seeds from TBC, TD, and CB were randomly selected. The selected seeds were treated for 3 days by soaking in cold water soaking. Then, the seeds were evenly sown on the planting bed, covered with 1 cm of soil, and then covered with film to maintain warmth. During the experiment, seeds were watered with tap water [55]. Seed germination was measured in April 2020.

To compare the germination of *O. microphylla* seeds under different pretreatment methods, we conducted a sowing test in February 2021. The following four methods were applied to pretreat the seeds before sowing: (1) no soaking (CK); (2) soaking in hot water at 45 °C for 6 h (T1); (3) scratching the seed coat (T3); and (4) soaking in concentrated sulfuric acid for 3 h (T4). The collected seeds of *O. microphylla* were mixed well. For pretreatment, randomly selected seeds were evenly sown on the planting bed, covered with 1 cm of soil, and then covered with a film to maintain warmth. Thirty seeds were sown for each treatment. Seed germination was determined in April 2021.

Germination rate (%) = (number of normal germinated seeds/total number of treated seeds) × 100

## Detection of traces of human logging

We recorded the number of *O. microphylla* logged within each quadrat as an indicator of the degree of anthropogenic disturbance to the *O. microphylla* population. As Chinese government regulations do not allow logging in natural forests, the discovery of these stumps also indicates that illegal logging is taking place.

## Soil composition testing

We conducted field sampling in June 2024 at TBC, TD, and CB using the standardized protocol for soil sampling. Three standardized sample plots measuring 20 × 20 m were randomly deployed at the three sample sites, and nine sample plots were established. Within each quadrat, we used the five-point mixing method to collect samples, randomly selected five soil cores (diameter 5 cm, soil depth 30–60 cm), and mixed the soil samples from the three quadrats to form a composite sample. Nine soil samples were collected (three sampling sites × three replicates). The soil samples were placed in self-sealing bags and refrigerated for immediate transportation to the laboratory after being sieved through a 2-mm sieve to remove stone particles and plant roots.

The soil pH, dry matter, organic matter, NO<sub>3</sub>-N, NH<sub>4</sub>-N, available phosphorous, total nitrogen, total carbon, total phosphorus, and humus, including humic acid, fulvic acid, and humin were measured to assess the soil conditions.

Soil pH was determined using the methods of Hu et al. (2021a) [56] with some adjustments. Briefly, soil sample solutions (1:2.5, ratio of soil sample to deionized water) were assessed using a potentiometric method with an acidimeter (Sartorius PB-10). The dry matter content of the soil was determined based on soil dried to a constant weight using the drying and weighing method with an electric blast-drying oven (Boante BAT240-LGF).

The organic matter content in the soil was determined by the potassium dichromate oxidation-external heating method (the ratio of the soil sample to the solution was 1:40), which was heated by an oil bath at 170–180 °C, cooled, washed with water, and titrated with ferrous sulfate solution [57].

The NO<sub>3</sub>-N and NH<sub>4</sub>-N contents of the soil samples were determined using an ultraviolet spectrophotometer (Shanghai Mepuda UV-1800PC). Fresh soil samples were extracted with a 1 mol/L potassium chloride solution for NO<sub>3</sub>-N and 2 mol/L potassium chloride solution for NH<sub>4</sub>-N [58]. Soil samples were air-dried and sieved to extract the available phosphorus content using ammonium fluoride–hydrochloric acid leachate (1:10 soil sample-to-leachate ratio). After shaking, centrifugation, and filtration, the available phosphorous content was determined using the molybdenum antimony colorimetric method [59].

The total carbon and nitrogen contents of the soil were determined by dry combustion using an elemental analyzer (Thermo Fisher Scientific) [60].

The soil total phosphorus content was determined according to the methods of Pan et al. (2023) [61] with some adjustments. The soil samples were decomposed using sodium hydroxide fusion (1:8 ratio of soil sample to sodium hydroxide solids). Molybdenum antimony anticolorant was added under acidic conditions. The total phosphorus content of the soil samples was determined using an ultraviolet spectrophotometer (Shanghai Mepuda UV-1800PC).

The soil humus content, including humic acid, fulvic acid, and humin, was determined as described by Li et al. (2018) [62] with some adjustments according to the actual situation [63]. Humic acid and fulvic acid in the soil samples were extracted using a 0.1 mol/L sodium pyrophosphate–sodium hydroxide mixture and the ratio of soil sample to mixture was 1:20. The humic and fulvic acid contents were determined by oxidation, precipitation, and filtration with potassium dichromate, and the humin content was determined using difference calculations.

## Bird diversity statistics

Between April 2022 and May 2023, a bird diversity survey was conducted at three sites: TD, TBC, and CB. The sample line method was used for bird observations, with 3–5 km long sample lines set up at each site, totaling 312 sample lines. Observations were performed under clear and windless weather at a walking speed of 1–2 km/h along the set sample lines, and each observation lasted from 07:00 to 09:00 or from 16:00 to 18:00. The names and numbers of birds that appeared and called within 30 m of the left and right sides of the sample line were recorded. The Shannon–Wiener (H) and Simpson diversity indices (D) were calculated for each of the three sites [64].

$$H = - \sum_{i=1}^S (P_i \ln P_i)$$

$$D = 1 - \sum_{i=1}^S P_i^2$$

where  $S$  is the species number and  $P_i$  is the relative abundance of species  $i$ .

## Data analysis

The seven sample plots were clustered according to the community structure of *O. microphylla*. Tukey's test was used to test the significance of the variability in *O. microphylla* fruiting and seed vitality among the different treatment groups, external risk variables, and bird diversity. A  $p$  value of 0.05 was selected as the threshold for significant differences. PCA was performed to assess the population genetics of *O. microphylla* within the seven sample plots and the bird species composition in the three representative sample plots. All data processing and plotting were performed using SPSS version 26 (SPSS Inc., Chicago, IL, USA) and Origin (Origin Pro 2025, OriginLab, Northampton, Massachusetts, USA).

## Declarations

### ***Ethics approval and Consent to participate***

This study did not require ethical approval and did not involve human research.

### ***Consent to Publish declaration***

Not applicable.

### ***Competing Interest***

The authors declare no competing interest.

### ***Funding***

This study was funded by the 2023 Central Finance Second Batch of Subsidized Projects for Conservation of National Key Wildlife and Animals.

### ***Author Contributions***

X.M.T., B.Y., F.L.H., and H.L. designed the research; C.M., G.F.X., L.Z., and G.F.L. collected the sample; C.L. and X.P.L. performed the research; X.M.T. and B.Y. analyzed the data; and X.M.T., B.Y., F.L.H. and H.L. wrote the paper. All authors read and approved its content.

### ***Availability of data and material***

The whole-genome sequencing data for *O. henryi* (No. GWHFICR000000000.1) and DNA sequencing data for the *O. microphylla* population (No. PRJCA037388) reported in this paper have been deposited in the Genome Warehouse at the National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation. The accession number is publicly accessible at <https://ngdc.cncb.ac.cn/gwh>.

## **Clinical Trail Number**

Not applicable.

## **Acknowledgements**

Not applicable.

## **References**

1. Hamilton J, Humphries MM, Bennett EM: A mixed method evaluation of ecosystem services and services-to-ecosystems illuminates culturally important trees in a settled landscape. *Ecosystems and People* 2025, 21(1).
2. Šmejkal M, Kalous L, Auwerx J, Gorule PA, Jarić I, Dočkal O, Fedorčák J, Muška M, Thomas K, Takács P et al: Wetland fish in peril: A synergy between habitat loss and biological invasions drives the extinction of neglected native fauna. *Biological Conservation* 2025, 302.
3. IUCN: Table 1a: Number of species evaluated in relation to the overall number of described species, and number of threatened species by major groups of organisms. *IUCN Red List Summary Statistics* 2024, 1.
4. Li Q, Chen Y, Xu L, Cui X, Xu H, Wang L, You C, Tian X, He X, Liu Y: Loss of plant functional group mediates microbial community assembly in litter decomposition of alpine fir forest. *Global Ecology and Conservation* 2025, 58.
5. Larigauderie A, Prieur-Richard A-H, Mace GM, Lonsdale M, Mooney HA, Brussaard L, Cooper D, Cramer W, Daszak P, Díaz S et al: Biodiversity and ecosystem services science for a sustainable planet: the DIVERSITAS vision for 2012–20. *Current Opinion in Environmental Sustainability* 2012, 4(1):101-105.
6. Liu P-P, Yu E-P, Tan Z-J, Sun H-M, Zhu W-G, Wang Z-F, Cao H-L: Genome Assemblies of Two *Ormosia* Species: Gene Duplication Related to Their Evolutionary Adaptation. *Agronomy* 2023, 13(7).
7. Liu B, Weng H, Ye X, Zhao Z, Zhan C, Ahmad S, Xu Q, Ding H, Xiao Z, Zhang G, Chen S: Simulation of Potential Geographical Distribution and Migration Pattern with Climate Change of *Ormosia microphylla* Merr. & H. Y. Chen. *Forests* 2024, 15(7).
8. Pizo MA, Fontanella ABA, Canassa G, Espindola WD, Gussoni COA, de C. Guaraldo A, Carlo TA: Decoding Darwin's puzzle: avian dispersal of mimetic seeds. *Ecology* 2020, 101(6).
9. Galetti M: Seed dispersal of mimetic fruits: parasitism, mutualism, aposematism or exaptation? *CABI* 2002: 177–191.
10. Nereu M, Silva JS, Timóteo S: The disruption of birds' double mutualistic interactions in novel ecosystems. *Proceedings of the Royal Society B: Biological Sciences* 2024, 291(2033).
11. Wei L, Wang G, Xie C, Gao Z, Huang Q, Jim CY: Predicting suitable habitat for the endangered tree *Ormosia microphylla* in China. *Scientific Reports* 2024, 14(1).

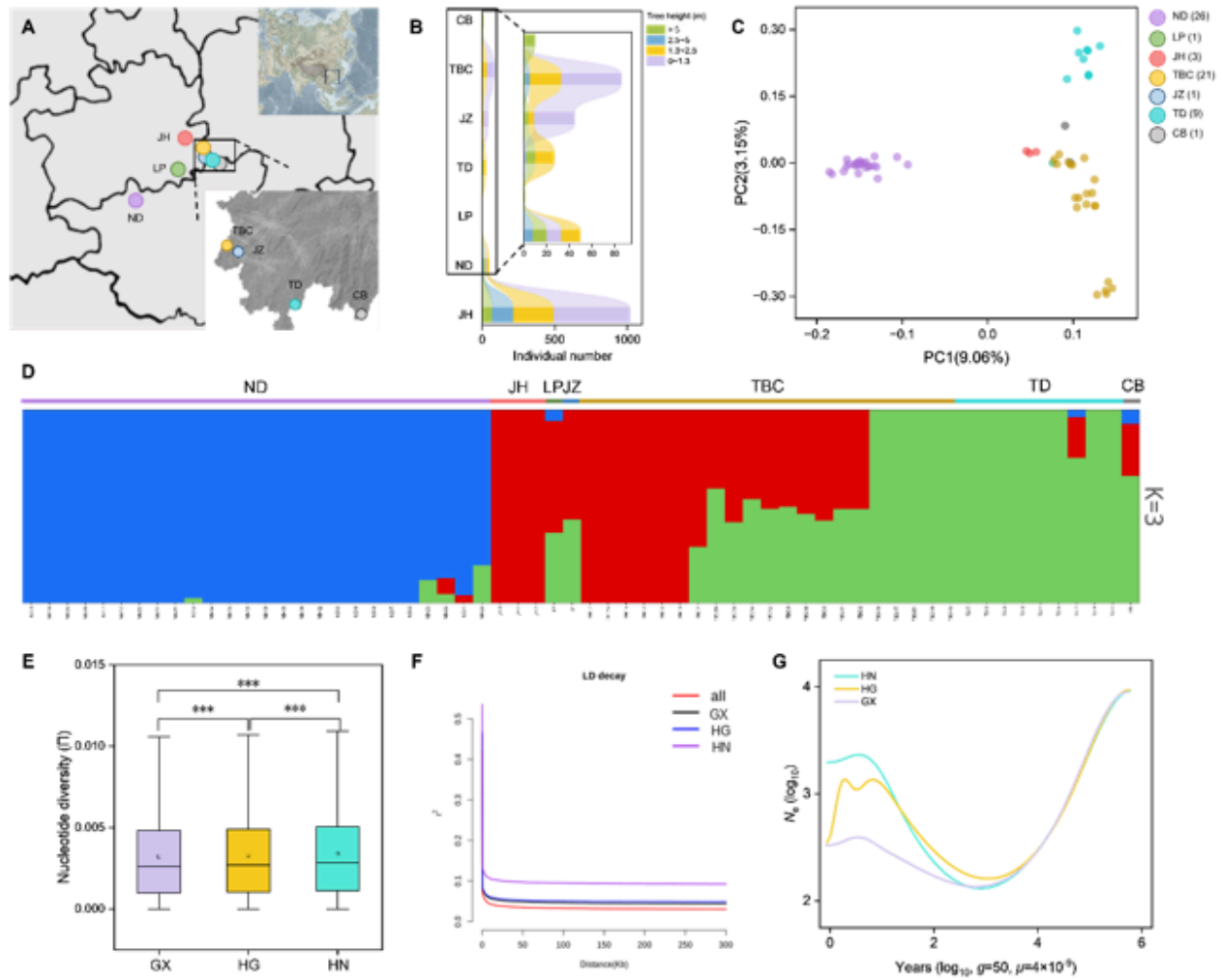
12. Huang P, Xiao Y, Sun Y, Huang H, Gong Z, Zhu Y: Distribution changes of *Ormosia microphylla* under different climatic scenarios. *Scientific Reports* 2025, 15(1).
13. CBD: 15/5. Monitoring framework for the Kunming-Montreal Global Biodiversity Framework. *Conference of the Parties to the Convention on Biological Diversity Montreal, Canada* 2022.
14. Hébert K, Jousse M, Serrano J, Karger DN, Blanchet FG, Pollock LJ: Five recommendations to fill the blank space in indicators at local and short-term scales. *Biological Conservation* 2025, 302.
15. Lebel Vine M, Walczak M, Lebel Vine G, Fragman-Sapir O, Leschner H, Ur Y, Ron M, Ben-Natan D, Shemesh B, Singer A, Sapir Y: Are local species prioritization lists sufficient for protecting endangered plants? Israeli red list as a test case. *Conservation Science and Practice* 2024, 6(12).
16. Fricker JM, Chen HYH, Wang JR: Stand age structural dynamics of North American boreal forests and implications for forest management. *International Forestry Review* 2006, 8(4):395-405.
17. Zuo X, Xu K, Yu W, Zhao P, Liu H, Jiang H, Ding A, Li Y: Estimation of Forest Phenology's Relationship with Age-Class Structure in Northeast China's Temperate Deciduous Forests. *Forests* 2024, 15(12).
18. Zhang D, Yuan W, Chen C, Zhu J, jiang B: Preliminary Study on Growth Regularity of Man-made *Ormosia henryi* Forest. *Journal of Zhejiang Forestry Science and Technology* 2003, 23(3):9-11,27.
19. Luo W, Xu H, Li Y-d, Luo T-s, Lin M-x, Chen D-x: The Population Structure and Distribution Pattern of *Cinnamomum rigidissimum* in Jianfengling, Hainan Island. *Forest Research* 2010, 23(5):787-790.
20. Liu P, Zhang J: Structure and Dynamics of *Populus Euphratica* Population in Guazhou Oasis, Northwest China. *Journal of Desert Research* 2012, 32(2):407-412.
21. Wang Y, Wu P, Wang R, Ma X, Zhou X: Community characteristics of *Cyclobalanopsis chungii* forest in Mingqing nature reserve. *Journal of Fujian Agriculture and Forestry University Natural Science Edition* 2011, 40(1):37-42.
22. Guo M, Yang N, Liu H, Tang S, Fan Z, Zou T: Spatial distribution pattern and quantitative dynamics of the endemic plant *Camellia rubituberculata* in Guizhou Province. *Guihaia* 2019, 39(10):1359-1369.
23. Yang K: Ecological Characteristics of the Populations of the Precious Tree Species *Ormosia henryi* in Shaowu Jiangshi Provincial Nature Reserve. *Chinese Wild Plant Resources* 2023, 42(12):65-69.
24. Kahilainen A, Puurtinen M, Kotiaho JS: Conservation implications of species–genetic diversity correlations. *Global Ecology and Conservation* 2014, 2:315-323.
25. Lanfear R, Kokko H, Eyre-Walker A: Population size and the rate of evolution. *Trends in Ecology & Evolution* 2014, 29(1):33-41.
26. Wang R, Liu C-N, Segar ST, Jiang Y-T, Zhang K-J, Jiang K, Wang G, Cai J, Chen L-F, Chen S et al: Dipterocarpaceae genomics reveal their demography and adaptations to Asian rainforests. *Nature Communications* 2024, 15(1).
27. Yang Y-X, Wang M, Wu X-Y, Zhou Y-N, Qiu J, Cai X, Li Z-H: The chromosome-level genome assembly of an endangered herb *Bergenia scopulosa* provides insights into local adaptation and genomic vulnerability under climate change. *GigaScience* 2024, 13.

28. Bohm S, Kelly N, Postuma M, Wagemaker NCAM, ter Haar S, Scheper J, Vergeer P: Small populations, big challenges: Genetic, demographic, and landscape context collectively shape population performance of a perennial herb. *Biological Conservation* 2025, 305.
29. Stevens K, Harrisson KA, Hogan FE, Cooke R, Clarke RH: Reduced gene flow in a vulnerable species reflects two centuries of habitat loss and fragmentation. *Ecosphere* 2018, 9(2).
30. O'Grady JJ, Brook BW, Reed DH, Ballou JD, Tonkyn DW, Frankham R: Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation* 2006, 133(1):42-51.
31. Zhao H, Yang A, Kong L, Xie F, Wang H, Ao X: Proteome characterization of two contrasting soybean genotypes in response to different phosphorus treatments. *AoB PLANTS* 2021, 13(3).
32. Liu B, Wang X, Li K, Cai Z: Spatially Resolved Metabolomics and Lipidomics Reveal Salinity and Drought-Tolerant Mechanisms of Cottonseeds. *Journal of Agricultural and Food Chemistry* 2021, 69(28):8028-8037.
33. Abdelmoiz EF, Karim R, Farid R, Fatima ezzahra A, Abderrahim A, Abdelkarim F-M, Bouchra B: Unveiling regional and altitudinal lipidomic analyte signatures of the argan tree (*Argania spinosa* L.) for environmental adaptation. *Journal of Plant Physiology* 2025, 311:154523.
34. Xie P, Shi J, Tang S, Chen C, Khan A, Zhang F, Xiong Y, Li C, He W, Wang G et al: Control of Bird Feeding Behavior by Tannin1 through Modulating the Biosynthesis of Polyphenols and Fatty Acid-Derived Volatiles in Sorghum. *Molecular Plant* 2019, 12(10):1315-1324.
35. Gou M, Hou G, Yang H, Zhang X, Cai Y, Kai G, Liu C-J: The MYB107 Transcription Factor Positively Regulates Suberin Biosynthesis. *Plant Physiology* 2016, 173(2):1045-1058.
36. Dai L, Chen Y, Wei X: Hard Seed Characteristics and Seed Vigor of *Ormosia hosiei*. *Agriculture* 2023, 13(5):1077.
37. Zhu S, Wei X-F, Lu Y-X, Zhang D-W, Wang Z-F, Ge J, Li S-L, Song Y-F, Yang Y, Yi X-G et al: The jacktree genome and population genomics provides insights for the mechanisms of the germination obstacle and the conservation of endangered ornamental plants. *Horticulture Research* 2024, 11(8).
38. Wang Y, Yang Y, Han Z, Li J, Luo J, Yang H, Kuang J, Wu D, Wang S, Tso S et al: Efficient purging of deleterious mutations contributes to the survival of a rare conifer. *Horticulture Research* 2024, 11(6).
39. Arnold I, Marchand G, Hayoz-Andrey A, Serres-Hänni A, Arlettaz R, Humbert J-Y: Relaxation of management intensity promotes butterfly communities in mountain grasslands. *Biological Conservation* 2025, 304.
40. Lapola DM, Pinho P, Barlow J, Aragão LEOC, Berenguer E, Carmenta R, Liddy HM, Seixas H, Silva CVJ, Silva-Junior CHL et al: The drivers and impacts of Amazon forest degradation. *Science* 2023, 379(6630):eabp8622.
41. Yuan J, Wang G, Zhao L, Kitchener AC, Sun T, Chen W, Huang C, Wang C, Xu X, Wang J et al: How genomic insights into the evolutionary history of clouded leopards inform their conservation. *Science Advances* 2023, 9(40):eadh9143.

42. Oliva J, Romeralo C, Stenlid J: Accuracy of the Rotfinder instrument in detecting decay on Norway spruce (*Picea abies*) trees. *Forest Ecology and Management* 2011, 262(8):1378-1386.
43. Vergara PM, Carreño-Chovan C, Quiroz M, Alaniz AJ, Fierro A, Saavedra M, Hidalgo-Corrotea CM, Zúñiga AH, Carvajal MA, Borquez C, Moreira-Arce D: The internal decay of wood is driven by the interplay between foraging Magellanic woodpeckers and environmental conditions. *Science of The Total Environment* 2024, 955.
44. Albert JS, Carnaval AC, Flantua SGA, Lohmann LG, Ribas CC, Riff D, Carrillo JD, Fan Y, Figueiredo JJP, Guayasamin JM *et al*: Human impacts outpace natural processes in the Amazon. *Science* 2023, 379(6630):eabo5003.
45. Ge M, Wei X: Spermosphere bacterial community at different germination stages of *Ormosia henryi* and its relationship with seed germination. *Scientia Horticulturae* 2024, 324.
46. Peres CA, vanRoosmalen MGM: Avian dispersal of "mimetic seeds" of *Ormosia lignivalvis* by terrestrial granivores: Deception or mutualism? *Oikos* 1996, 75(2):249-258.
47. Foster MS, Delay LS: Dispersal of mimetic seeds of three species of *Ormosia* (Leguminosae). *Journal of Tropical Ecology* 1998, 14:389-411.
48. Zhu Z, Chelli S, Tsakalos JL, Bricca A, Canullo R, Cervellini M, Pennesi R, De Benedictis LLM, Cesaroni V, Bottacci A, Campetella G: How effective are different protection strategies in promoting the plant diversity of temperate forests in national parks? *Forest Ecology and Management* 2025, 584.
49. Belhabib D, Le Billon P: Fish crimes in the global oceans. *Science Advances* 2022, 8(12):eabj1927.
50. zu Ermgassen EKHJ, Bastos Lima MG, Bellfield H, Dontenville A, Gardner T, Godar J, Heilmayr R, Indenbaum R, dos Reis TNP, Ribeiro V *et al*: Addressing indirect sourcing in zero deforestation commodity supply chains. *Science Advances* 2022, 8(17):eabn3132.
51. Corral A, Valerio LM, Cheung KC, dos Santos Ferreira BH, Guerra A, Szabo JK, Reis LK: Plant-bird mutualistic interactions can contribute to the regeneration of forest and non-forest urban patches in the Brazilian Cerrado. *Urban Ecosystems* 2021, 24(1):205-213.
52. Speziale KL, Lambertucci SA, Gleiser G, Tella JL, Hiraldo F, Aizen MA: An overlooked plant-parakeet mutualism counteracts human overharvesting on an endangered tree. *Royal Society Open Science* 2018, 5(1).
53. Camargo PHSA, Carlo TA, Brancalion PHS, Pizo MA: Frugivore diversity increases evenness in the seed rain on deforested tropical landscapes. *Oikos* 2022, 2022(2).
54. Ohsawa M: Differentiation of Vegetation Zones and Species Strategies in the Subalpine Region of Mt. Fuji. *Vegetatio* 1984, 57(1):15-52.
55. Li C, Ding J, Huang W, Tian B, Siemann E, Zhang J: Differences in seed properties and germination between native and introduced populations of *Triadica sebifera*. *Journal of Plant Ecology* 2020, 13(1):70-77.
56. Hu H, Umbreen S, Zhang Y, Bao M, Huang C, Zhou C: Significant association between soil dissolved organic matter and soil microbial communities following vegetation restoration in the Loess

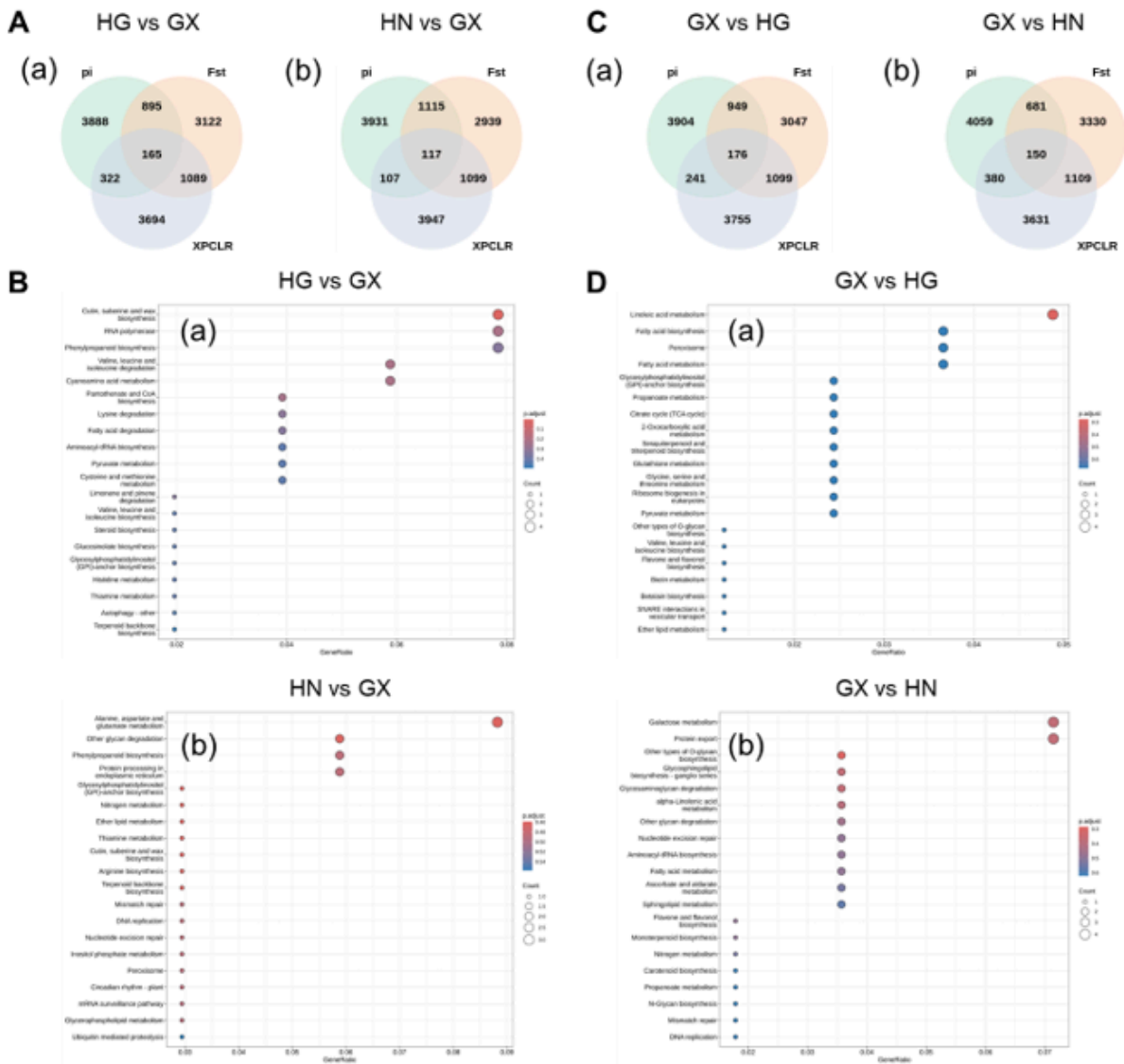
- Plateau. *Ecological Engineering* 2021, 169.
57. Nelson DW, Sommers LE: Total Carbon, Organic Carbon, and Organic Matter. In: *Methods of Soil Analysis*. 1996: 961-1010.
58. Hu W, Ran J, Dong L, Du Q, Ji M, Yao S, Sun Y, Gong C, Hou Q, Gong H et al: Aridity-driven shift in biodiversity–soil multifunctionality relationships. *Nature Communications* 2021, 12(1):5350.
59. Hu Z, Delgado-Baquerizo M, Fanin N, Chen X, Zhou Y, Du G, Hu F, Jiang L, Hu S, Liu M: Nutrient-induced acidification modulates soil biodiversity-function relationships. *Nature Communications* 2024, 15(1):2858.
60. Querejeta JI, Ren W, Prieto I: Vertical decoupling of soil nutrients and water under climate warming reduces plant cumulative nutrient uptake, water-use efficiency and productivity. *New Phytologist* 2021, 230(4):1378-1393.
61. Pan C, Sun C, Yu W, Guo J, Yu Y, Li X: Mixed planting enhances soil multi-nutrient cycling by homogenizing microbial communities across soil vertical scale. *Land Degradation & Development* 2023, 34(5):1477-1490.
62. Li HY, Zhou XG, Wu FZ: Effects of root exudates from potato onion on *Verticillium dahliae*. *Allelopathy Journal* 2018, 43:217-222.
63. Zhang Y, Wang C, Gao Y, Zhao L, Xi B, Tan W: Structure and composition of rhizosphere-soil humic acid and fulvic acid as affected by the land-use change from paddy to upland fields. *Sustainable Horizons* 2024, 10.
64. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara B, Simpson G, Solymos P, Stevens H, Wagner H: Vegan: Community Ecology Package. R Package Version 22-1 2015, 2:1-2.

## Figures



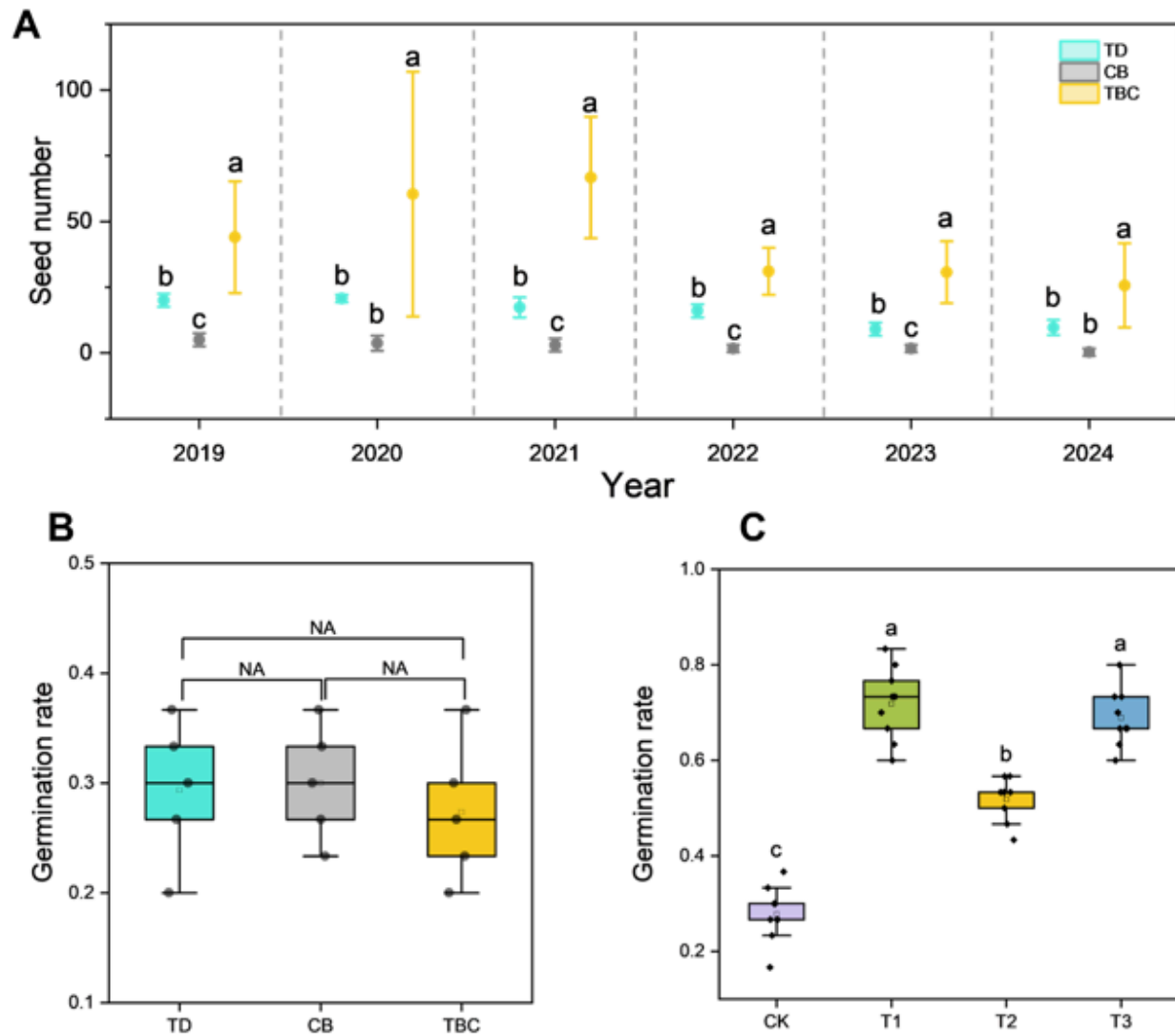
**Figure 1**

Population of *O. microphylla*. **(A)** Distribution of seven populations of *O. microphylla*. **(B)** Age structure of seven populations of *O. microphylla*. **(C)** PCA of the sequencing results of *O. microphylla*. **(D)** Population genetic structure analysis of *O. microphylla* after individual weight sequencing (K = 3). **(E)** Nucleotide diversity in GX, HG, and HN genetic clusters. **(F)** Linkage disequilibrium analysis of GX, HG, and HN genetic clusters. T-test for all variables, \* \* \* $p < 0.001$ . **(G)** Population history analysis of GX, HG, and HN genetic clusters. JH, Jianhe population; LP, Liping population; ND, Nandan population; TBC, Tangbaocun population; JZ, Jingzhou population; TD, Tongdao population; and CB, Chengbu population.



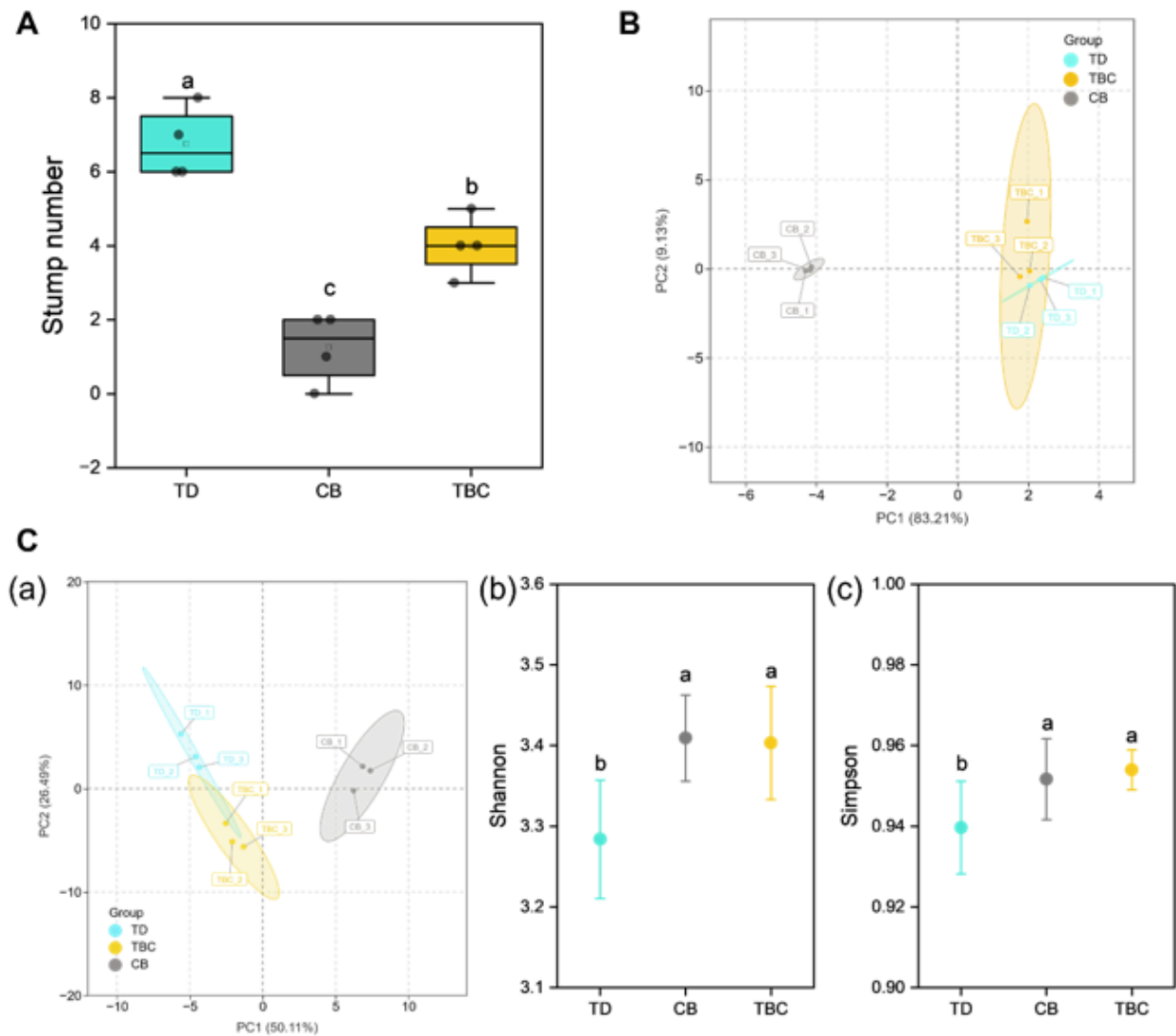
**Figure 2**

Analysis of gene selective swapping. (A) Screening of selected genes using HG (a) and HN (b) populations, respectively, with the GX population as the background population; (B) KEGG annotations of selected genes from the HG (a) and HN (b) populations screened with the GX population as the background population; (C) Screening of selected genes using the GX population, respectively, with HG (a) and HN (b) populations; (D) Screening of selected genes with the HG (a) and HN (b) populations, respectively, with the KEGG annotations of selected genes from the GX population screened with the HG (a) and HN (b) populations as background population.



**Figure 3**

Firmness and seed vitality of *O. microphylla*. **(A)** Statistics on the number of *O. microphylla* seeds for six consecutive years. TD, Tongdao population; CB, Chengbu population; TBC, Tangbaocun population. Different letters represent significant differences at  $p < 0.05$ . **(B)** Germination rate of *O. microphylla* seeds. TD, Tongdao population; CB, Chengbu population; TBC, Tangbaocun population. T-test for all variables. **(C)** Germination rate of *O. microphylla* seeds under different treatments. CK, natural treatment group; T1, water immersion treatment at 45 °C; T2, skin coat laceration treatment; T3, concentrated sulfuric acid immersion treatment. Different letters represent significant differences at  $p < 0.05$ .



**Figure 4**

External risks of *O. microphylla*. (A) Number of stumps within the population; (B) PCA of soil conditions; (C) bird species diversity. TD, Tongdao population, CB, Chengbu population, and TBC, Tangbaocun population. Different letters represent significant differences at  $p < 0.05$ .

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SOmicrophylla20250712.docx](#)