

Conservation macrogenetics reveals the potential hidden consequences of the 2019–2020 Black Summer fires on Australian biodiversity

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Abstract

The use of genetic analyses has become ubiquitous in conservation planning and management. Typically, such analyses are employed at the species-level, though as genetic data accrue, it is now possible to consider the genetic composition of multiple species across landscapes. Such macrogenetic perspectives can reveal the potential genetic ramifications of extreme disturbance events, such as the catastrophic Australian ‘Black Summer’ wildfires of 2019–2020. This event severely impacted forested habitats and fauna across much of eastern Australia – but whether there were differential impacts upon genetically distinct populations, or a significant erosion of high diversity populations across species, was not known. Here, we present a conservation macrogenetics framework to examine the potential genetic impacts of this large-scale disturbance. Using hundreds of samples spanning dozens of frog, mammal, and reptile species, we first demonstrate how reduced-representation sequencing can be aggregated across species to describe the distribution of genetic diversity across a landscape. We then show that, whilst variable across the study area, these unprecedented fires generally burned areas where genetic diversity of sampled taxa was higher than for areas remaining unburned. Additionally, areas with high concentrations of evolutionarily distinct and short-range species were disproportionately represented in burned regions, and potential cross-taxonomic adverse effects were greatest in Australia’s southeast and central eastern seaboard regions. More broadly, our work demonstrates how the conservation genetics principles applied at a species level can be expanded to landscapes, whilst accounting for the challenges that arise when aggregating across taxonomic groups, thus improving our understanding of the overall impacts of large-scale disturbance events upon genetic diversity.

Article impact statement

SNPs can be reliably used in macrogenetic contexts to infer the potential genetic impacts of disturbance events across landscapes.

1. Introduction

Biodiversity across the globe is in peril. Species from all realms of life are declining, and as many as half of known species potentially face imminent extinction (Finn et al., 2023). The primary drivers of this biodiversity crisis are anthropogenic in nature, and key threats are ongoing (Ceballos et al., 2015, 2017). In particular, the multifaceted threat of climate change, including the associated increasing probability and severity of extreme disturbance events, is an especially important pressure upon ecological communities worldwide (Urban, 2015; Kelly et al., 2020; Dirzo et al., 2022; Habibullah et al., 2022). To understand and address these threats, various methods across the domains of conservation biology are used to limit erosion of biodiversity. Among these is population genetics, which is becoming ubiquitous in conservation settings (Hoban et al., 2022), reflecting the premise that the maintenance of genetic diversity within species is critical to their short- and long-term persistence (DeWoody et al., 2021). As genetic data amass, the opportunity to describe broad, landscape-scale patterns of genetic diversity across multiple species has emerged. This aggregative use of data, or ‘conservation macrogenetics’, has the potential to enhance global efforts to stem biodiversity loss by identifying regions of high value that can be prioritised for protection and management (Leigh et al., 2021; Schmidt, Hoban, & Jetz, 2023).

At the species level, an understanding of genetic diversity may confer improved conservation outcomes for several reasons. Generally, higher genetic diversity in individuals and populations is a hallmark of greater evolutionary adaptive potential to environmental change (Fernandez-Fournier et al., 2021; Kardos et al., 2021; Forester et al., 2022) and elevated fitness and persistence in the face of threatening processes (Reed & Frankham, 2003; Willoughby et al., 2017; Doyle et al., 2019; Scott et al., 2020). Consequently, the impacts of reduced genetic diversity can be substantial. Studies have found that often, across a variety of taxa, threatened species tend to have lower genetic diversity than those that are non-threatened (Willoughby et al., 2015; Li et al., 2016; Canteri et al., 2021). Exceptions to this general pattern exist: low genetic diversity does not in all cases necessitate elevated threat status (Schmidt, Hoban, Hunter, et al., 2023), and high genetic diversity is not in itself an infallible protection against population declines (Roycroft et al., 2021). However, retaining existing genetic diversity within wild populations to aid their conservation over the longer term is a precautionary approach of great value (Frankham, 2005, 2015; Kardos et al., 2021), and it is essential that it be prioritised given the global loss of diversity that has been observed over recent decades (Shaw et al., 2025). Indeed, for this reason, international policy targets intended to halt the further loss of biodiversity specify the retention of genetic diversity as a key aim (e.g., Target 4 of the UN Convention on Biological Diversity Kunming-Montreal Global Biodiversity Framework; (CBD, 2022)).

Partly in response to such targets, alongside advances in genetic sequencing and analysis methodologies, a wealth of high-quality genetic data have been generated for thousands of species (Leigh et al., 2021; Hoban et al., 2022). This provides an opportunity for comparative macrogenetics to guide the retention of biodiversity across broad spatial scales. An understanding of the distribution of genetic diversity in fine detail across landscapes provides a means to direct conservation action to areas or regions where the cross-taxonomic benefits are likely to be greatest (Paz-Vinas et al., 2015; Leigh et al., 2021; Nielsen et al., 2023; Schmidt, Hoban, & Jetz, 2023). Several studies have already demonstrated the potential of conservation macrogenetics by describing macrogenetic trends relevant to landscape-scale conservation efforts. For example, Almeida-Rocha et al. (2020) showed that species inhabiting anthropogenically disturbed landscapes had lower genetic diversity, while Schmidt et al. (2024) revealed that the global protected area network does not adequately protect genetically diverse populations. However, most macrogenetic studies undertaken thus far, including those mentioned above, do not utilise more contemporary and data-rich genomic markers such as single nucleotide polymorphisms (SNPs), instead opting for historically more available markers such as microsatellites. Whilst microsatellite data are useful, these markers may not be appropriate to answer all macrogenetic questions (Paz-Vinas et al., 2021). As the quantity of SNP data increases (Holderegger et al., 2019), more comprehensive assessments of genetic diversity are possible (Schmidt et al., 2024). Thus far, the estimation of genetic diversity from SNP data has been limited by an inherent lack of comparability of estimates across – and even within – species (Schmidt et al., 2021; Sopniewski & Catullo, 2024). However, recent developments that improve both the comparability and reliability of genetic diversity estimates from SNP data (Schmidt et al., 2021; Sopniewski & Catullo, 2024) have made their use in macrogenetics feasible.

To demonstrate how SNP data can be used in a macrogenetic context, we investigate the potential impacts of an extreme, large-scale, stochastic disturbance event on the genetic diversity of co-occurring species across an impacted landscape. Specifically, we explore how the Australian ‘Black Summer’ wildfires of 2019 and 2020, which burned ~97,000 km² across southern and eastern Australia (Ward et al., 2020), may have affected the genetic diversity of more than 30 vertebrate species. This climate change-fuelled event (Abram et al., 2021) was historically unprecedented (Boer et al., 2020; Collins et al., 2021), and accordingly, the landscape-scale implications for biodiversity have been explored from multiple angles (Driscoll et al., 2024). Studies have included the estimation of the degree of species’ distributional overlap with fire (Ward et al., 2020), mortality estimates (Legge et al., 2022), and potential ecological niche impacts (Sopniewski et al., 2024). However, the repercussions of these fires on landscape genetic diversity across taxonomic groups is unknown. If regions with high or unique genetic diversity were impacted, the ramifications of these fires could be more severe than initially thought, potentially affecting recovery of remnant populations and reducing their ability to withstand ongoing threatening processes (Willoughby et al., 2015). Hence, a

136 macrogenetic analysis will provide a more comprehensive understanding of the genetic consequences
137 of this disturbance across the fire-affected landscape.

2. Materials and Methods

2.1. Genomic data background

In response to the Australian 2019–2020 Black Summer fires, a project was initiated in 2020 to determine the genetic composition of potentially affected species. Coordinated by the Centre for Biodiversity Analysis at the Australian National University, with support from the National Environmental Science Program (NESP) Threatened Species Hub, BioPlatforms Australia, and tissue collections held by field ecologists and Australian museums, scientists who specialise in a suite of vertebrate species led genetic assessments for likely-impacted species. Vertebrate species that had known or suspected strong population substructure or were likely a complex of undescribed taxa, and/or had sufficient tissue samples available for broadscale genetic analysis, were selected (Catullo & Moritz, 2021; Catullo et al., 2021). Sufficient genetic samples were available (see below) for 20 frog, six mammal, and seven reptile species. These species were distributed across the study area encompassing the eastern part of South Australia and Karta Pintingga (Kangaroo Island), much of Victoria and eastern New South Wales, and the south-eastern region of Queensland (Figure SM1). Sequencing was performed in a similar manner for each species, and sample data were uploaded to BioPlatforms Australia (BPA). Briefly, DNA extractions were performed either using published methods, commercially available kits, or commercially by Diversity Arrays Technology Pty Ltd. (Canberra) (DArT). Extracted samples were submitted to DArT for reduced representation genomic library preparation using proprietary techniques (Kilian et al., 2012). Libraries were then sequenced at the Biomolecular Resource Facility at the Australian National University. All raw data are publicly available via the BPA, and have also been uploaded to the Sequence Read Archive (BioProject PRJNA1345431).

Following sequencing, population genetic analyses were performed for each species following a standardized pipeline (Catullo et al., 2021). This included admixture analysis, principal components analysis, and heterozygosity calculation, with all computations completed using the ‘dartR’ package (Gruber et al., 2018) in R (R Core Team, 2024). A genetic summary for each species was used by the relevant specialists (coauthors, as listed in Table SM1) to prepare assessments. An example of the population genetic analyses conducted is provided in Supplementary Material (pg. 54). Each assessment also described populations with significant genetic divergence that should be considered conservation units (CUs) within each species (Palsbøll et al., 2007). These CUs, which for clarity we simply refer to as ‘populations’, formed the basis of all following analyses. Each of these species’ assessments were compiled into a report for government agencies to guide conservation management, although due to the sensitivity of some of these data, the report is not publicly available. However, to date these data have contributed to published population genetics work for a subset of species (Heard et al., 2023; Parkin et al., 2024; Lin et al., 2025; Schembri et al., 2025).

2.2. Spatial projections of conservation units

We created a spatial projection for the distribution of each population using species distribution modelling (SDM). For each species, we downloaded occurrence records from the Atlas of Living Australia (ALA, 2024), and for frogs we added additional records from FrogID (Rowley & Callaghan, 2020). For species that had recently undergone taxonomic revision, we used the most recent species name but ensured that we checked former species names for likely records (for example, we re-assigned *Limnodynastes dumerilii* records that were likely to be the more recently resurrected *L. superciliaris* based on their location). We cleaned each species' dataset using broad guidelines for data filtering (Gueta & Carmel, 2016). Specifically, using R, we first removed records without location data. We then cropped any records from outside of a species' known distribution (buffered by one degree), using distributions from the Australian Frog Atlas (Cutajar et al., 2022) for frogs and IUCN distribution polygons (IUCN, 2024) for mammals and reptiles. We also removed records not recorded to at least two decimal places, and thinned records such that only one record was present in any 30-arcsecond grid cell (the resolution of environmental data used for subsequent modelling). All spatial data preparation was conducted using the 'terra' package in R (Hijmans et al., 2022).

We then created an SDM for each species. Generally, SDMs are most accurate when variables are chosen with reference to the biology of the species in question (Guisan et al., 2013), but in instances where multiple SDMs for many species are being constructed (such as here) this is often not feasible. Hence, for each species, we initially used a large suite of ecological predictors that included elevation, each of the 19 bioclimatic variables from the WorldClim 2.0 dataset (Fick & Hijmans, 2017), topographic wetness index (Gallant & Austin, 2012), mean vegetation height (Ticehurst et al., 2023), and gross primary productivity (Li & Xiao, 2019), following Sopniewski et al. (2024). Climatic layers from WorldClim were downloaded at 30-arcsecond resolution, and other layers were reprojected to match this resolution. For each species, we then reduced this set of ecological predictors to the largest uncorrelated set for that species as follows. First, using the 'terra' package, all variables were cropped to that species' distribution (using the species distribution polygon described above). Second, the variance inflation factor (VIF) for each cropped variable was calculated to determine the degree of collinearity amongst predictors present, and the variable with the highest VIF score was removed. This was repeated until only a set of variables with a VIF score < 10 remained. Third, we used Maxent (Phillips et al., 2017), implemented via the 'dismo' package (Hijmans et al., 2017) to construct an SDM. For each species, we selected 10% of the available background cells, within that species' distribution, as pseudo-absences. As modelling can be affected by preferential sampling bias, we created a bias layer for each taxonomic class (frogs, mammals, and reptiles), replicating methods used by Sopniewski et al. (2022). For each species, this bias layer was used to weight the selection of pseudo-absences accounting for the sampling density of that species' class. Finally, for each species,

we randomly selected 20% of occurrence records to withhold for model evaluation and ran a Maxent model with the remaining 80%. Using the withheld occurrence records, we then evaluated each species' model using the *evaluate* function from the 'dismo' package. From this result, we were able to derive model performance statistics and environmental suitability thresholds to make binary suitable/unsuitable predictions.

Based on the SDM constructed for each species, we predicted the distribution of each individual *population* within the bounds of its entire *species*' distribution, using the lineage range estimation method described by Rosauer et al. (2015). We first loaded all locations for each genotyped sample for that species, as well as the associated SDM prediction, into R. We then generated a cost-distance layer from the sequenced locations for each population, with cost defined as the suitability value (as determined from the SDM) subtracted from 1, such that cells with a low suitability had a higher cost. For each population, a weight layer was then generated according to the inverse cube of this cost, which was then multiplied by the model likelihood such that each cell within the SDM was assigned to a particular population. We then created binary presence/absence raster layers for each population, where all cells with a suitability value higher than the maximum sum of sensitivity and specificity (maxSSS) values derived from the species' SDM were included in the predicted distribution of that population. Each of these population predictions was then assessed by species specialists (Table SM1), and any erroneous predictions were amended. Figures representing the final predicted distributions of each population are presented in the Supplementary Material (pgs. 20-53).

2.3. Genetic analysis pipeline

Using conservation macrogenetic principles, our goal was to describe the distribution of genetic diversity across the study area by amalgamating genetic data across multiple species. We described this composition in two ways. First, we calculated genetic diversity, which we measured using expected heterozygosity (H_E). Second, we developed a novel metric comparable to phylogenetic endemism - a measure of range-restricted genetic distinctiveness (Rosauer et al., 2009) - which we term 'weighted distinctiveness' (WD, described in Section 2.3.2 below).

2.3.1. Population genetic statistics

We obtained the raw short read sequences for each sample described above (Section 2.1) in FASTQ format and followed Sopniewski & Catullo (2024) to prepare our data for analysis, primarily using 'Stacks' v2.0 (Catchen et al., 2013; Rochette et al., 2019). First, we trimmed barcodes, removed uncalled bases, and discarded low quality reads using *process_radtags*. Following this, we used 'Trimmomatic' v0.39 (Bolger et al., 2014) to filter adapter sequences with the parameters "ILLUMINACLIP:TruSeq3-SE:2:20:10; LEADING:5; SLIDINGWINDOW:4:5; MINLEN:68". Loci were then constructed *de novo* using *ustacks*, setting "M" to 4 and using default parameters for "m"

(3) and “N” ($M + 2$) (Schmidt et al., 2021; Sopniewski & Catullo, 2024). As recommended by Sopniewski & Catullo (2024), we treated each population independently for all subsequent analyses. Continuing with the ‘Stacks’ pipeline, we constructed a catalogue of loci using *cstacks*, setting “n” to 4. For catalogue construction, we used either every unique sample or 20 randomly selected unique samples (whichever was smaller). All samples then had their loci matched to the catalogue using *sstacks* before being transposed, using *tsv2bam*, for population genetics statistics calculations.

For heterozygosity statistics calculated using ‘Stacks’ to be comparable, both fixed and variable sites should be used, sample sizes should be kept both low and equal, and missing data should be omitted (Schmidt et al., 2021; Sopniewski & Catullo, 2024). Accordingly, we developed a pipeline for the calculation of population genetic statistics that accounted for these requirements. For each population, we created ‘population maps’ (lists of individual samples used to calculate population genetic statistics in the ‘Stacks’ pipeline) for groups of exactly five unique samples – a threshold chosen to minimise the effects of sample size upon diversity estimates whilst adequately representing population-wide genetic diversity (Schmidt et al., 2021; Sopniewski & Catullo, 2024). To capture any geographic variation of heterozygosity within each population (i.e., any differential diversity resulting from isolation by distance), we spatially biased these population maps such that one population map for each individual sample was created, each time selecting the four unique samples that represented its closest geographic neighbours. We then called SNPs from the catalogue using *gstacks*, before calculating population genetic statistics using *populations*, with the call rate filter set to 1 (no missing data allowed) (Schmidt et al., 2021; Sopniewski & Catullo, 2024). We excluded any populations with fewer than five unique individuals from further analyses.

2.3.2. Weighted distinctiveness calculation

To represent range-restricted evolutionary uniqueness of populations we developed a metric termed ‘weighted distinctiveness’ (WD). This metric considered the evolutionary ‘distinctiveness’ of a population relative to its broader species (as measured by the unique length of its phylogenetic branch) and weighted that distinctiveness by the population’s range size. Accordingly, a population that has been historically isolated from other populations and exists in only a small area would be allocated a high value. Although similar to phylogenetic endemism (Rosauer et al., 2009), this measure differs in that it considers the evolutionary distinctiveness of a population only in the context of its species, rather than the position of that species in a broader phylogenetic tree. We deemed this finer-scale approach to be more appropriate for our aims, which were to describe the distribution of genetic distinctiveness at a population-scale across a landscape, which would be confounded by the inclusion of long ancestral branches in an all-taxon phylogeny, to which our subset of taxonomically varied species was not suited.

First, for each species with more than one population (Table SM1), we constructed a maximum likelihood phylogenetic tree in ‘IQTree’ (Minh et al., 2020) using the output from ‘Stacks’ (Supplementary Methods S1). For species, we included each population for which data existed in the phylogenetic tree (even if the number of available samples was insufficient for heterozygosity to be computed), to ensure the appropriate calculation of evolutionary distinctiveness (Table SM1). Next, we converted each species’ phylogeny into an ultrametric tree using the R package ‘ape’ (Paradis & Schliep, 2019). For each population, we randomly selected one individual to retain and dropped all other tips, then rescaled the tree such that their branches summed to 100. This ensured that each species’ phylogeny had a maximum possible evolutionary distinctiveness (ED) value of 100, and therefore each species contributed an equal total ED to landscape-scale analyses. We then calculated the ED value for each population (the proportion of the species’ entire evolutionary history that was held within that population) using the ‘picante’ package (Kembel et al., 2010). Species for which only one population existed were assigned an ED value of 100. Weighted distinctiveness (WD) values were then calculated for each population by dividing its ED by the size (extent of occurrence; EOO) of its modelled distribution (measured in square kilometres) using the ‘terra’ package in R.

2.4. ‘Stacking’ of genetic composition metrics

Once we had calculated comparable genetic diversity and WD values for each population, we ‘stacked’ these together to describe variable cross-taxonomic genetic patterns across the landscape. First, we defined genetic diversity (H_E) for each population. We represented this in two ways: (1) a mean value for the entire population, calculated as the mean result of each population map repetition for that population, and (2) a spatial projection of genetic diversity. To create the spatial projection, we first added the value of each population map result to the geographic location of the ‘focal sample’ upon the previously modelled population distribution raster layer. We then interpolated these values across the population layer using the *interpIDW* function of the ‘terra’ package. This produced a raster layer expressing the ‘raw’ value of H_E for each population.

Though these values were comparable across species, in the sense that they were not ‘biased’ estimates of heterozygosity as described by Schmidt et al. (2021) and Sopniewski & Catullo (2024), this was not the case ecologically (i.e., the meaning of an absolute value of heterozygosity is contextual). Heterozygosity is partly determined by life history, ecological strategy, and relative population size, thus the broad, systemic differences we observed between taxa are to be expected (Romiguier et al., 2014; Ellegren & Galtier, 2016). That is, it is not logical to expect a similar value of H_E in a frog and a mammal, given the substantial differences in their ecological traits. Accordingly, we rescaled each raster layer to express ‘relative’ H_E , rather than raw H_E . Specifically, for each population, we divided each raster layer by the maximum H_E value observed for its species. This meant that for each species, the population with the highest H_E had a value of 1, with all other

populations having a relative value <1 , rendering these layers comparable. Using the ‘terra’ package, we overlaid all species’ rasters to obtain the mean *relative* H_E value. One potential concern with this approach was that undue importance may be given to ‘single population species’ (short-range endemic species, or species for which we only had sufficient data for one population), because these by default have a uniform value of 1. Such species are indeed of high conservation value by virtue of having only one population, however their inclusion could obscure true, cross-taxonomic, landscape-scale trends in genetic diversity. Accordingly, we created an additional ‘stacked H_E ’ layer, omitting any species represented by only a single population. We also created a ‘stacked WD’ layer for each population using their calculated WD values. As such, we produced three descriptive raster layers: two showing where, across species, populations with higher genetic diversity tended to be concentrated (including and excluding species represented by a single population), and another showing areas of congregation for populations with smaller ranges and unique evolutionary history.

2.5. Assessing the macrogenetic impacts of the Black Summer fires

We obtained a raster layer of areas burned by the Black Summer fires from the AUS GEEBAM Fire Severity Dataset (2019–2020) (DAWE, 2020), which details fire extent and severity over the period between 1 July 2019 and 13 February 2020. Cells within the study area were classified as either unburned, mildly burned (GEEBAM value 3) or severely burned (GEEBAM values 4 and 5) (DAWE, 2020). Our analyses considered regions ‘burned’ (mild and severe fire), as well as ‘severely burned’ (only severe fire). To explore differences between burned and unburned regions of the landscape, we generally refer to the median and range of values of areas (mean values with confidence intervals were not appropriate, given the uneven skew and non-normal distribution of our data). These were always calculated by cell and weighted by the area of each cell. In the main text, we generally define the study area as cells where at least three study species were present (given we were interested in exploring cross-taxonomic patterns) (Figure SM2), though we present results for other species-richness thresholds in the Supplementary Material. Aside from comparing the genetic characteristics of burned and unburned regions, we also determined where in the study area the potential ramifications of the fires may have been most acute. To do so, we categorised each burned cell according to its 10% quantile for both H_E and WD separately, then combined these two values for each cell. This allowed us to determine which cells in the burned landscape were most important in terms of genetic diversity and WD.

3. Results

3.1. Genetic composition of the landscape

Our final dataset comprised 33 vertebrate species represented by 63 independent populations (Table SM1). Twenty of these species were frogs (12 of which were listed as threatened on the IUCN Red List as of 2020), six were mammals (three threatened), and seven were reptiles (none threatened). Of the 33 species, 13 were represented by only a single population. Species richness per grid cell within the study area ranged from a single species to 12 (Figure SM1). Our broad study area (i.e., the area in which we predicted the occupancy of at least one species in our dataset) encompassed 1,055,257km², with 375,747km² of this area holding at least three species. Hereafter, we refer to the latter as the ‘restricted study area’ and primarily refer to this region as we aimed to describe trends in diversity and potential impacts across multiple species. Additionally, in some instances we discuss the ‘multi-population-species restricted study’ area when species represented by only a single population are omitted, and this subset covered an area of 293,863km².

Within the restricted study area, we found genetic diversity (heterozygosity) to generally follow both a longitudinal and latitudinal gradient, being lowest in the north and inland regions of the study area, and highest in the south and coastal areas (Figure 1). This pattern was evident both when species with a single population were included and excluded, though it was especially prominent in the latter analysis (Figure 1b), as many of the single-population species we considered were present in the northern region of the study area (Figure SM3). Trends in genetic diversity across the landscape were broadly similar for other species richness thresholds (rather than those used to define the restricted study area) (Figure SM4).

We found that landscape patterns of weighted distinctiveness (WD) differed from those we observed for genetic diversity (Figure 2). Though the south-east of the region was also identified as an important region for WD, we also found Karta Pintingga (Kangaroo Island) to the southwestern periphery of the study region and northeastern NSW/southeastern Qld to harbour high mean values of WD (Figure 2b). The mountaintops of the northeastern region of the study area held by far the greatest aggregate levels of WD (Figure 2), driven by the many short-range endemic species present there, such as mountain frogs of the genus *Philoria* (Figure 2), the Wollumbin Pouched Frog (*Assa wollumbin*), and range-restricted populations of the pouched frog (*Assa darlingtoni*). The lowest values for WD were observed in the inland regions west of the Great Dividing Range, where several widespread species reside. Intermediate WD values (those inside the bounds of the 10% and 90% deciles) were far lower than the values in the northeastern region, and landscape patterns only became evident when these values were log-transformed. This revealed a subtle pattern of decreasing WD with latitude and distance from the coast (Figure SM5).

3.2. Potential impacts of the Black Summer fires

Approximately 16.4%, or 61,441km², of our restricted study area was burned during the Black Summer fires (15.9% or 46,585km², excluding single-population species). Of the burned area, 44% (27,168km²) was burned at high severity (Figure 3), with a similar proportion (44% or 20,457km²) severely burned when single-population species were excluded.

We generally found the Black Summer fires disproportionately burned areas with higher genetic diversity and weighted distinctiveness (Figure 4). For genetic diversity (excluding single-population species), relative H_E had an area-weighted mean value of 0.825 (SE 0.0001) and area-weighted median value of 0.807 (Figure 4a,b). Both values were higher amongst all burned regions (average 0.886, SE 0.0003 and median 0.903) and higher again for only severely burned regions (average 0.900, SE 0.0003 and median 0.917). In fact, the median relative H_E values for both all burned and severely burned areas were higher than the upper quartile for the region, meaning that more than half of burned areas were among the most genetically diverse regions in the landscape. Importantly, this pattern was not affected by our species richness threshold choice for inclusion in the restricted study area; relative H_E values were consistently higher in burned regions (and higher again in severely burned regions) for species richness thresholds between one and five (Table SM2). Although more varied throughout the distribution of values, we observed a similar pattern for log-transformed weighted distinctiveness (Figure 4c,d). The mean (-6.87 [SE 0.004]) and median (-7.18) log-transformed WD of burned regions was higher than the restricted study area as a whole (-7.53 [SE 0.002] and -7.62, respectively). In contrast to genetic diversity, we did not find WD to be higher in severely burned areas (relative to all burned areas), observing a mean value of -6.83 (SE 0.005) and median of -7.14.

3.3. Assessment of variable potential genetic impacts across the landscape

In addition to finding that across the study area the Black Summer fires burned areas harbouring high genetic diversity and weighted distinctiveness, our landscape ‘stacking’ approach also revealed specific regions where the impacts of the fires may have been most pronounced. The southeast of the study area had the highest relative H_E across taxa (species with multiple populations only), whereas the fires that burned in the southwest and northwest of the study area held lower genetic diversity (Figure 5a). For WD, areas with higher values (i.e., evolutionarily unique and range-restricted populations) were also burned in the southeast (Figure 5b), in addition to large parts of the New England Tablelands and eastern escarpment in the north of the study area. When both measures (genetic diversity and weighted distinctiveness) were considered, it was clear that the fires had the greatest potential impacts in broad regions (Figure 5c) which hosted a high proportion of severe fires (Figure 3). Though these results are centred around the multi-population-species restricted study area,

425 we observed congruent results with species richness thresholds ranging from one to five (Figures
426 SM6-9), complementing our proposition that the potential effects were greatest in these regions.

4. Discussion

4.1. The genomic impact of Australia's Black Summer fires

Australia's Black Summer fires impacted hundreds of native species. Whilst species responses to these fires were variable (e.g., Legge et al. 2022; Ensbey et al. 2023; Driscoll et al., 2024), several studies have demonstrated adverse effects for taxa, including frogs (Beranek et al., 2023; Heard et al., 2023; Lin et al., 2025), mammals (Woinarski et al., 2023), and reptiles (Letnic et al., 2023), as well as across entire landscapes (Ward et al., 2020; Sopniewski et al., 2024). Our results extend this body of work. We show that Australia's Black Summer fires generally burned in areas characterised by high genetic diversity and concentrations of evolutionarily distinctiveness. We also demonstrated that the potential consequences of these fires were uneven across the fire footprint, as genetic diversity was highest in the southeast, while weighted distinctiveness was highest in the northeast. Collectively, these results enhance our understanding of the potential impacts to biodiversity from this globally significant disturbance event.

Our finding that the fires generally burned in regions with higher genetic diversity (Figure 4) is concerning, given that these areas are key to the recovery and persistence of affected species. Relative to others with lower genetic diversity, the local populations that contribute to the higher aggregate diversity we observed within burned areas are likely to have greater fitness and viability (DeWoody et al., 2021; Kardos et al., 2021), and be subject to lower levels of inbreeding, thus reducing the cumulative load of fixed deleterious alleles within their populations (Keller & Waller, 2002; Robinson et al., 2019). Regardless of any threatening processes, it is these high-diversity populations that are the most valuable for ensuring the long-term viability of the species, both *in situ* (DeWoody et al., 2021; Fernandez-Fournier et al., 2021; Harrisson et al., 2014) or by acting as a source of genetic diversity for conservation translocations or captive breeding (Whiteley et al., 2015; Ralls et al., 2020). Further, we found that genetic diversity was slightly higher in severely burned regions than those only mildly burned. For species negatively impacted by the fires, other studies have also shown or suggested that consequences were often more pronounced in severely burned areas (Legge et al., 2022; Letnic et al., 2023; Driscoll et al., 2024;), meaning the long-term ramifications of these fires could be particularly bleak.

It should also be considered that where fires burned in more genetically diverse areas of the landscape, the populations most exposed to this disturbance were perhaps those best placed to face it. As mentioned previously, these populations are the most likely to have higher abundance, effective population size, metapopulation connectivity, fitness, and general capacity to rebound following a demographic reduction such as that caused by a major disturbance (Reed & Frankham, 2003; Willoughby et al., 2017; Doyle et al., 2019; Scott et al., 2020). Accordingly, in the instances where

fires did burn in more diverse areas, less genetically diverse populations that may have a lower capacity to rebound following the disturbance were less impacted. Though this perspective should be considered, the consequences for a species collectively would likely be greater if mortality were high enough in more genetically diverse populations as to cause cumulative loss of diversity through a genetic bottleneck (Banks et al., 2013; Kardos et al., 2021). As a result, the total breadth of genetic diversity present within a species, and its capacity to persist through subsequent threatening events, could be reduced.

We also found weighted distinctiveness to be higher in burned than unburned regions (Figure 4), but the consequences are less nuanced than for genetic diversity. Our WD metric measures the distinct evolutionary history of a population relative to its species, thus capturing adaptations and genetic variability unique to that population. For these reasons – as well as the fact that once lost, they cannot be recovered – evolutionarily distinct populations are important to conserve (Moritz, 1994, 2002; Cadotte & Davies, 2010; Dufresnes et al., 2023). Further, WD prioritises populations with small, restricted distributions, that is, populations that already have a higher susceptibility to local habitat or environmental changes and disturbance events (Manes et al., 2021), amplifying any potential consequences resulting from these fires. Burned areas with high WD, such as those in the north of the study area, are therefore likely some of the most adversely affected. Notably, however, several populations with particularly high WD had relatively low overlap with fire (Table SM1), highlighting that by nature of having smaller ranges, areas of high impact related to WD will be localised.

4.2. The opportunities and challenges of a macrogenetic approach to population genetics

Here, we have shown how the aggregation of population genetic statistics across landscapes can elucidate macrogenetic spatial patterns. In the context of the Black Summer fires, the relationships between fire and the genetic composition metrics we considered were unsurprising given that these fires burned predominately in native forests (Davey & Sarre, 2020), and natural environments generally harbour greater genetic diversity than disturbed or modified areas (Miraldo et al., 2016; Schmidt et al., 2020). Further, the study area included extensive areas of Australia's Gondwanan rainforests – a region noted for its high concentrations of short-range endemic populations and species with distinct evolutionary histories (Rosauer et al. 2015; Kooyman et al., 2020) – some of which were substantially burned. Hence, our findings that the fires tended to burn in areas of the landscape with higher genetic diversity and WD for this selection of frog, mammal, and reptile species are perhaps to be expected. However, this does not diminish the importance of this result. Rather, it highlights these areas as important candidates for focused conservation action following these fires. This could involve broad, landscape-scale interventions, such as the targeting of post-fire weed management and feral animal control, to reduce other threatening processes whilst ecosystems recover.

500 Additionally, and perhaps more importantly, our results identify where limited resources could be
501 directed for the active conservation of species not represented in our dataset but potentially affected,
502 such as targeted research or monitoring. We observed non-random patterns of diversity and
503 uniqueness across the landscape, and it is likely these patterns exist more broadly. The drivers of intra-
504 and interspecific differences in genetic diversity – both natural (Figuerola-Ferrando et al., 2023;
505 Paz-Vinas et al., 2015) and anthropogenic (Almeida-Rocha et al., 2020; Schmidt et al., 2020; De Kort
506 et al., 2021) – can act similarly upon sympatric species across landscapes, resulting in the non-random
507 spatial patterns of cross-taxonomic diversity we observed. These results could be used to infer
508 potential impacts for the many sympatric species that we were unable to include in our study, and to
509 initiate management actions post fire. For instance, where species have a large distribution,
510 monitoring that targets areas where genetic diversity is likely to be highest could be used as a proxy
511 for monitoring the genetic state of the species as a whole.

512
513 Though our results have great utility, the use of macrogenetics in isolation is likely insufficient to
514 guide the broadscale identification of priority areas for conservation management. Indeed, a frequent
515 criticism of such methods is a potential disconnect between aggregate landscape patterns and the *in*
516 *situ* realities for single species (Santini et al., 2021). An example of this disparity is apparent in our
517 own results. The pouched frog, *Assa darlingtoni*, had an estimated 22% of its distribution (Ward et al.,
518 2020) and 36% of its environmental niche (Sopniewski et al., 2024) burned in the 2019–2020 fires.
519 However, the high-value, genetically diverse populations of *A. darlingtoni* we identified experienced
520 fire to a far lesser degree, with the greatest fire overlap occurring in the least diverse population (Table
521 SM1, Supplementary species Figures). Whilst not a positive outcome for this low-diversity
522 population, it is likely that a greater proportion of the species' total genetic diversity has been spared
523 from the impacts of this fire event. This differs to our landscape-scale findings, which imply that more
524 genetically diverse areas were burned more often, and is a fortunate result, given that Beranek et al.
525 (2023) found *A. darlingtoni* occupancy to be adversely affected in areas burned during the Black
526 Summer fires. The benefits of macrogenetic approaches to informing conservation actions are thus
527 best used in conjunction with on-ground, species-specific actions (Zizka et al., 2021).

528
529 While our study demonstrates how common population genetic data can be used to uncover
530 macrogenetic patterns across a landscape, it is emblematic of perhaps the largest obstacle to
531 conservation macrogenetic studies: the lack of discoverable and reusable genomic data (Hoban et al.,
532 2022). Despite a rapid increase in the accessibility of genetic data for conservation research
533 (Hohenlohe et al., 2021; Hoban et al., 2022), it remains challenging to amass data that are both
534 sufficiently representative of the genomic diversity of species present in any given landscape and
535 available in comparable forms. Our study is large in the context of conservation genomics,
536 particularly considering the use of SNP data: our final dataset included samples from 1,205 unique

individuals, representing over 30 vertebrate species. However, this pales in comparison to the true diversity of the study area, where hundreds of species from the three vertebrate Classes we examined had distributions within the fire footprint (Ward et al., 2020), as did thousands of bird, freshwater fish, invertebrate, and plant species (Driscoll et al., 2024). Given that genetic analyses in conservation assessments are increasingly utilised (Willi et al., 2022), and that it is commonplace to upload genetic sequences to the public domain accompanying publication (Crandall et al., 2023), more extensive analyses and studies such as ours should become more feasible. To make such studies a reality, the ongoing collection and deposition of genetic samples in public museums remains key and should be a priority amongst conservation practitioners and researchers (Catullo et al., 2021; Forbes et al., 2025), and it is critical that museums and curators are adequately funded to support curation of samples and associated metadata, which, concerningly, is not generally the case in Australia (Shaw et al., 2024).

Though Australia's Black Summer fires were historically unprecedented, similar events have since occurred elsewhere in the world and are predicted to increase in frequency (Kelly et al., 2020; Cunningham et al., 2024). To direct conservation actions to where they will be most effective, novel tools are needed to understand biodiversity at scale, both in response to, and in preparation for, sudden disturbance events and other processes that can trigger population declines and extinctions. Conservation macrogenetics is a promising avenue to achieve this (Leigh et al., 2021; Schmidt, Hoban, & Jetz, 2023), and here we have demonstrated how it can be comprehensively deployed in response to a major disturbance event. If it can be ensured that genetic information continues to accumulate, studies such as our own can be deployed in numerous contexts to support multilateral efforts to halt the global reduction of biodiversity.

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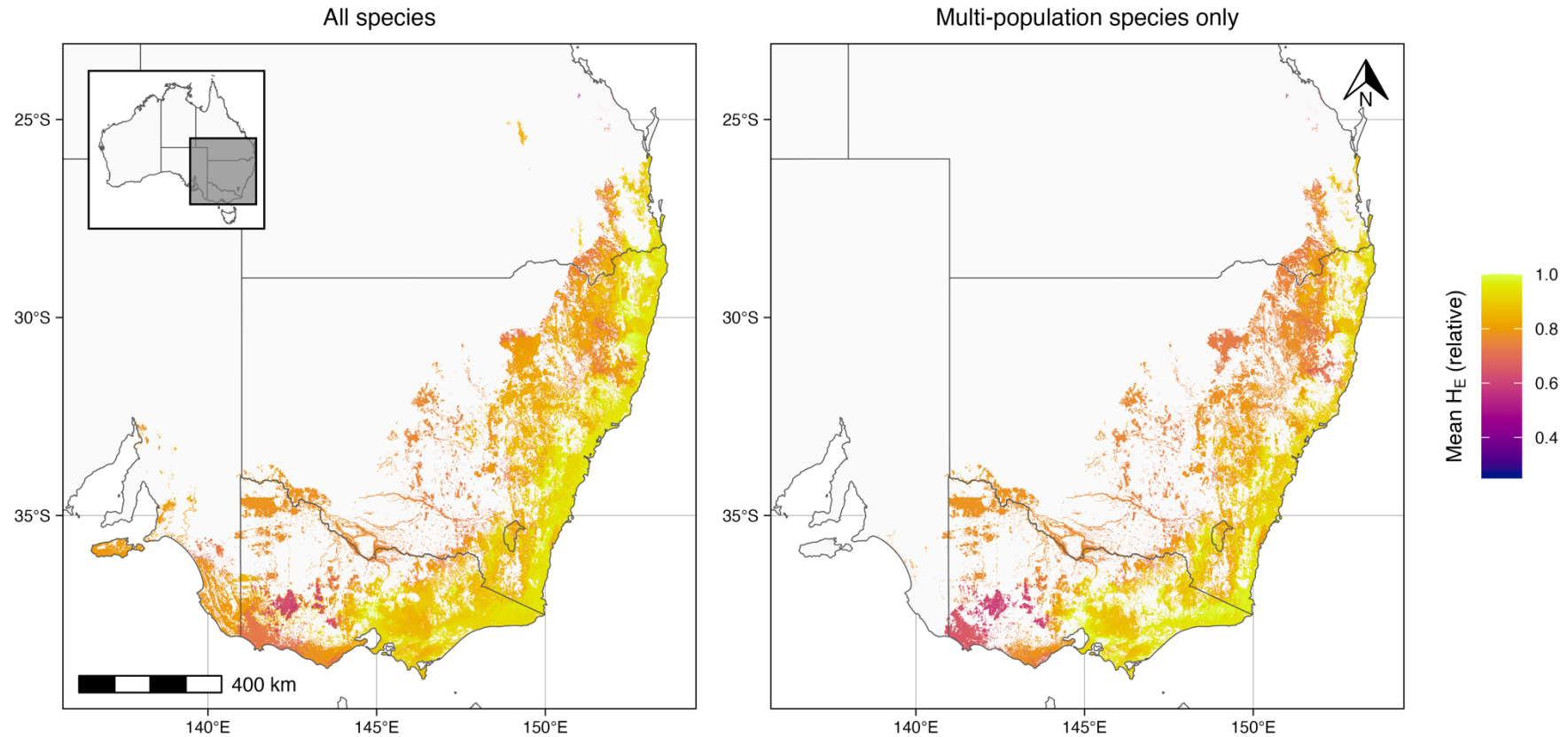
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1012

1013 **Figure 1.** Mean relative expected heterozygosity (H_E) across the restricted study area when all species are considered (a), and when only species with more than one
 1014 population included in the study are present (b). Only cells with an occupancy of three or more species are shown. Lighter, yellow cells are indicative of higher
 1015 relative H_E . A general gradient whereby H_E increases from both north to south and west to east can be observed, particularly in (b) when single-population species are
 1016 excluded, indicating a concentration of high relative genetic diversity towards the south-east of the study area.

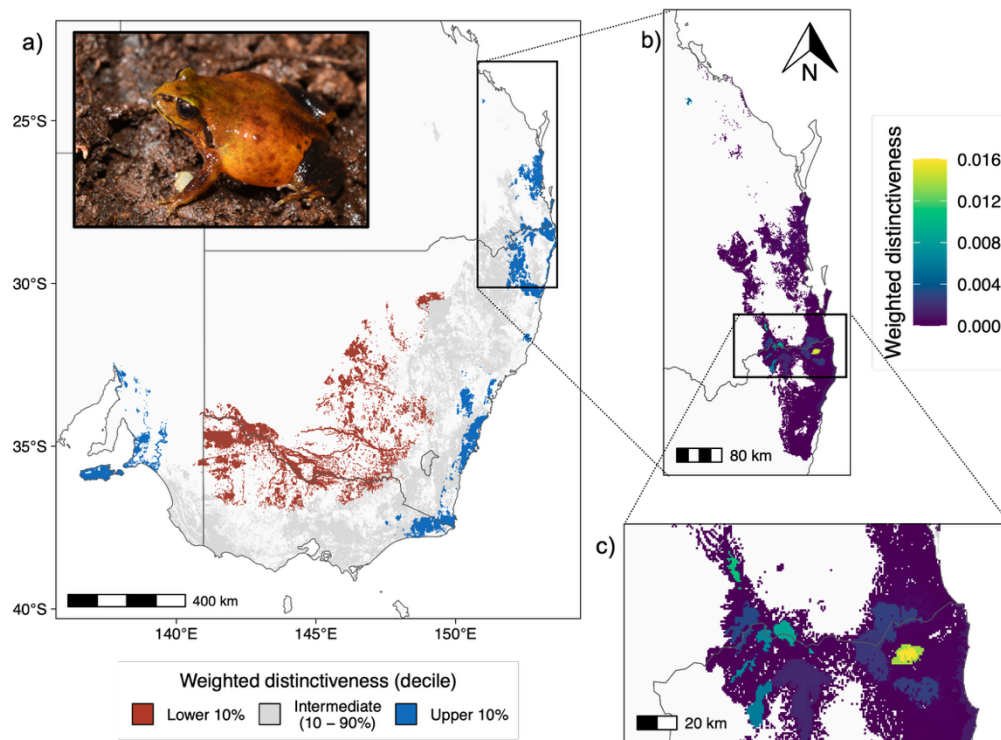
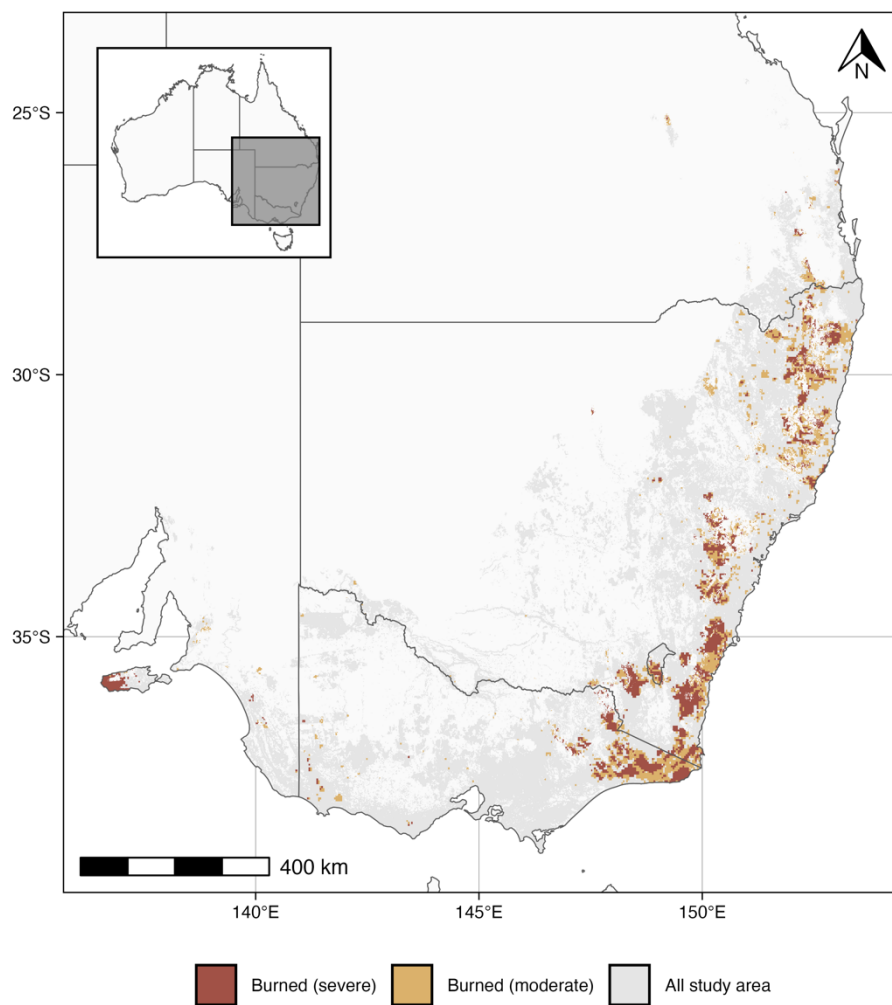
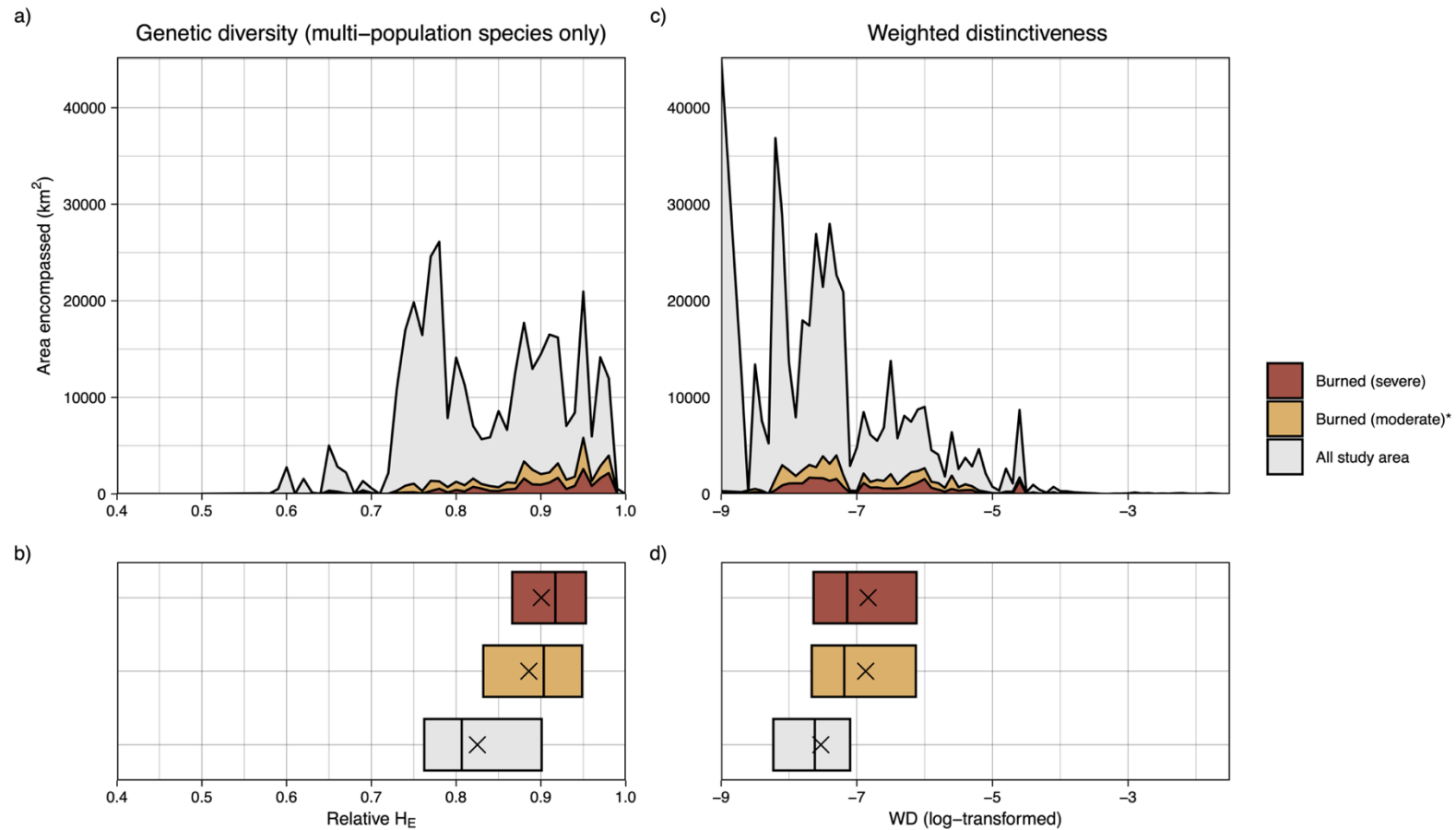


Figure 2. Mean weighted distinctiveness across the study area (restricted to cells with a species richness of at least 3). For clarity, only the upper (blue) and lower (red) deciles are shown in (a), with grey indicating cells with a value between these bounds. High values indicate that, in general, the populations present in that area are more evolutionarily distinct (compared to other populations of their species in the dataset) and have small distributions; low values denote the opposite. The highest values are found along the coastal fringes of the study area, though are especially high to the northeast of the study area (b). It is here the highest WD values are observed, with some cells (generally in elevated areas, such as Wollumbin) supporting very high, outlying values (c). This pattern is primarily driven by the presence of many short-range, mountain-dwelling species within this region, including the mountain frogs of the genus, *Philoria* (*P. kundagungan* pictured. Photo credit: Jodi Rowley).



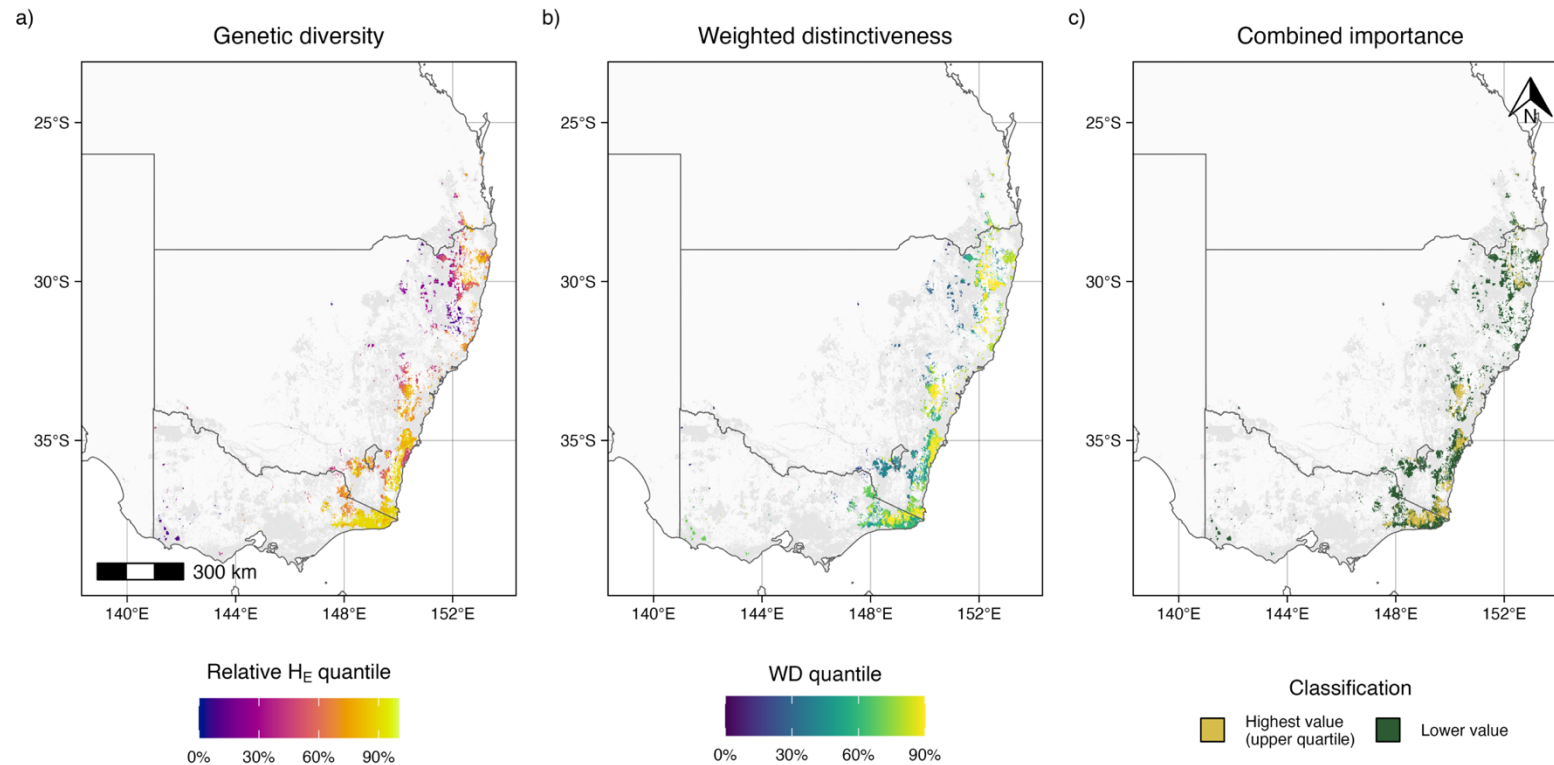
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1030 **Figure 3.** Regions burned during the Black Summer fires by severe (red) and moderate (yellow) fires
 1031 within the restricted study area (species richness of at least 3).



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1033 **Figure 4.** Representation of genetic diversity (a,b) and log-transformed weighted distinctiveness (c,d) across the landscape where species richness is ≥ 3 (multi-
 1034 population species only for genetic diversity) and in burned regions. Panels a) and c) show the area in the entire study area (grey), the moderately burned area
 1035 (yellow) and the severely burned area (red), with each metric rounded to 2 significant figures to aid visualisation. Panels b) and d) show the area-weighted
 1036 interquartile range (boxes), median (line), and mean (crosses) for each landscape classification. Both H_E and WD are higher in burned regions compared to the study
 1037 area as a whole, and highest in severely burned areas.



1038

1039 **Figure 5.** Relative importance of areas burned within the multi-population-species restricted study area during the Black Summer fires for a) genetic diversity, b)
 1040 weighted distinctiveness, and c) a combination of these two measures, calculated for the study area restricted to cells where species richness is at least 3 amongst
 1041 species with multiple populations. In a) and b), cells are coloured according to the 10% quantile they fall within, with more yellow cells (in both cases) indicating
 1042 higher relative H_E and WD, respectively. These quantiles were calculated amongst only burned cells restricted to the displayed study area and corrected for cell size.
 1043 In c), these raw measures were first normalised and averaged before an area-corrected 75th percentile was calculated. Burned cells within the upper quartile of
 1044 importance when both genetic diversity and weighted distinctiveness are calculated are shown in yellow—this shows the southeast of the study area and patches of
 1045 the tablelands to the north had the most acute potential impacts of the fires.