



## Cumberland Plain Woodland Knowledge Infrastructure

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### Executive summary

Ecological restoration is an essential tool in ensuring the persistence of native vegetation on the Cumberland Plain Woodland, especially in the face of a rapidly changing climate. Genetic information can help ensure material used in restoration is of appropriate provenance and sufficiently genetically diverse. Failure to adequately consider genetic diversity and we may end up with populations that exhibit low or no recruitment and are unable to adapt to change.

These guidelines provide advice on how to source genetically diverse climate-ready seed for woodland species of the Cumberland Plain. While initially planned as a targeted study on the Cumberland Plain Woodland threatened ecological community, as data came to light, the scope of the project expanded to be applicable to all woodland communities of the Cumberland plain.

Fifteen common woodland species, suitable for restoration of the Cumberland Plain, were sampled from across their distribution in New South Wales and population genetic analyses undertaken. Detailed guidelines for optimised seed collections and establishment of seed production areas are provided for eleven of these species, with the four remaining species proving unsuitable for such guidelines based upon the data gathered for this report. Eight species for which we present specific guidelines here have been added to the publicly available Restore and Renew webtool, allowing restoration practitioners to identify appropriate seed sourcing regions for specific restoration projects not only within the Cumberland Plain, but across NSW. However, the genetic patterns observed for the other three species violated the assumptions of the model employed by the webtool and so they could not be deployed there at this time (but will be in an upgrade of the webtool that is currently being developed).

To show the practicalities of implementing the guidelines, a case study establishing a seed production area is presented.

Key findings of the restoration genetic study include showing that for all species investigated, gene flow is continuous between plant community types on the Cumberland Plain and that the 'local' genetic neighbourhood that includes the Cumberland Plain is geographically much larger than the Cumberland Plain. Therefore, targeted mixing of seed from differing communities from both within and outside the Cumberland Plain will have no negative consequences and increase the accessibility and genetic diversity of seed. Furthermore, we observed no evidence of a relationship between patch size and genetic diversity, suggesting that even small patches of remnant vegetation on the Cumberland Plain may hold important diversity and should not be dismissed because of their small size when collecting seed.

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The objective of this pilot project was to demonstrate the utility of Restore and Renew style investigations and resulting knowledge infrastructure for guiding evolutionarily informed restoration for 10 keystone Cumberland Plain Woodland species. Restoration genomics has stepped into a new phase whereby we can now gather relevant genomic information quickly and cost-effectively, making it feasible for us to go beyond the initial scope of the project (namely to include 10 new CPW species into the Restore and Renew webtool) but to also generate additional species-specific information on restoration strategies and ex-situ collection developments.

Similar analysis could be undertaken for most species commonly used in restoration across the Cumberland Plain. Furthermore, the outcomes of this project have application far beyond the Cumberland Plain. The data and guidelines generated for at least eight species will be made freely accessible on the Restore & Renew webtool, thus providing valuable information that can guide genetically informed restoration of these species across NSW.

## Acknowledgements

We acknowledge the Traditional Custodians of the land on which the plant species in this study are found on and pay respects to Elders past and present, including the traditional custodians of the Cumberland Plain, the Darug, Dharawal and Gundungurra peoples. We acknowledge the many collectors who facilitated the sampling of plant material for this project, in particular Daniel Clarke (Arcane Botanica Pty Ltd), Joel Cohen (BGoS), and Richard Dimon (BGoS). We acknowledge those who have provided technical assistance in adding the species targeted in this study to the Restore and Renew Webtool, Dr Jason Bragg (BGoS), Karina Guo (BGoS) and Miranda Jordan (BGoS). We acknowledge Dr Mitchell Obrien for development of scripts used for genetic analysis within this study.

## Key terms

**Adaptability/Adaptive potential:** The ability of a population to survive and reproduce under changing environmental conditions.

**Allele:** Alternate versions of DNA sequence at a given location within the genome.

**Allelic diversity:** The number of alleles present within a population of a species. One measure of genetic diversity (see below).

**Climate-ready:** Provenances that come from areas where current climatic conditions match those predicted to occur in a target area at a future point under a particular model of climatic change.

**Ecological restoration:** Assisting ecosystems that have been disturbed or cleared to restore ecological function.

**Gene flow:** The movement of genetic material such as differing alleles (see above) between individuals or a group of individuals by processes such as dispersal of seed and pollen.

**Genetic bottleneck:** A sharp reduction in genetic diversity caused by a reduction in population size or by sampling of seed from too few individuals.

**Genetic distinctiveness:** How different the genetic diversity of a population is compared to the genetic diversity in other populations of the same species.

**Genetic diversity:** The totality of genetic variation present in a population and a determinant of adaptability and adaptive potential.

**Genetic neighbourhood:** An area within the distribution of a species where there are no restrictions to gene flow other than the distance between individuals, such that alleles can be shared between all individuals within the area given sufficient time.

**Genetic structure:** The amount and distribution of genetic variation within and between populations across the landscape.

**Genetic swamping:** Loss of rare alleles as a result of repeated crossing with individuals from a different genetic neighbourhood of the same species or a different species via hybridisation.

**Heterozygosity:** The average proportion of DNA sites in the genome where the two copies (or alleles) are different across a sample of individuals. Heterozygous refers to having two different copies of DNA at a particular location within the genome, as opposed to homozygous (see below) where the two copies at a location are the same.

**Homozygous:** Having two identical copies of DNA at a particular location within the genome, as opposed to heterozygous where the two copies at a location are different. See above.

**Inbreeding:** The mating of individuals that are genetically closely related within a population leading to the production of progeny.

**Inbreeding depression:** a decrease in fitness of progeny from closely related parents due to an increase in homozygosity with the genome.

**Isolation by distance:** The term used to describe the change of shared genetic material across geographic space due to dispersal ability limiting the mating between individuals. Importantly this often does not lead to discrete population structure and local adaptation if populations are continuous across the landscape.

**Maternal line:** All offspring from the same mother plant.

**Maintenance of maternal lines:** The practice of keeping seed or other material collected from a particular mother plant identifiable and separate from that of other mother plants through the process of collection through to propagation and planting. Upon planting, maternal lines should be traceable to individuals, but should be interplanted to maximise interbreeding and mixing of genetic diversity in progeny.

**Mother plant:** An individual plant from which seed has been collected.

**Outbreeding:** The mating of individuals that are not genetically closely related leading to the production of progeny.

**Outbreeding depression:** A reduction of fitness of offspring resulting from the interbreeding of parents that are highly genetically distinct.

**Population:** A group of individual plants growing in the same place at the same time and interbreeding freely.



**Private alleles:** Alleles observed only in a single population, thus being private to that population.

**Progeny:** Offspring of a plant, typically seed.

**Provenance:** A source area for propagules (seed, cuttings etc.) used in restoration activities.

**Seed Production Area:** Areas where native plants of known provenance are planted to harvest seed for restoration or other purposes.

**Site:** a specific locality where genetic samples or seeds have been collected.

**Sustainability/Self-sustaining:** The ability of a planting to survive and reproduce in the long-term with minimal further active investment.

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# 1. Introduction

## 1.1 Context

The native vegetation of Cumberland Plain, a subregion of the Sydney Basin Bioregion located in Western Sydney, has been heavily cleared since European settlement, with less than 13% of the pre-1750 extent remaining (Tozer, 2003). What remains is highly fragmented, with compromised connectivity among remnants (NSW Department of Planning and Environment, 2022).

Given the extent of loss and degradation of native vegetation across the Cumberland Plain, much of the remaining vegetation is listed as threatened, with the area supporting twenty-four threatened ecological communities, including five that are largely restricted to the Cumberland Plain (Cumberland Plain Woodland, Shale Sandstone Transition Forest, Shale Gravel Transition Forest, Cooks River Castlereagh Ironbark Forest and Moist Shale Woodland). Like many other threatened ecological communities occurring in heavily cleared landscapes, ecological restoration is an essential tool in ensuring the persistence of these communities, especially in the face of a rapidly changing climate.

The Cumberland Plain Conservation Plan (CPCP) (NSW Department of Planning and Environment, 2022), a strategic conservation plan that aims to ‘support Western Sydney’s biodiversity and growth’, commits to undertaking ecological restoration in priority parts of the landscape to improve and enhance connectivity across the Cumberland Plain.

The CPCP recognises the importance of genetic information in ensuring material used in ecological restoration is of appropriate provenance and sufficiently genetically diverse to be resilient in the long term. The plan identifies the need for a restoration implementation strategy that will establish best practice principles and methodologies to, among other things, ‘ensure the long-term sustainability of restoration considers genetic diversity’. A priority conservation action in the first five years of the CPCP is to ‘partner with the Royal Botanic Gardens & Domain Trust to deliver seed sourcing and seed banking guidance, to inform the CPCP restoration program’.

This report delivers guidelines for optimised seed collections and establishment of seed production areas for 11 keystone woodland species suitable for restoration of the Cumberland Plain Woodland identified through consultation with restoration practitioners and expert botanists. We also describe general principles for ensuring ecological restoration on the Cumberland plain is genetically diverse and able to respond to future environmental change.

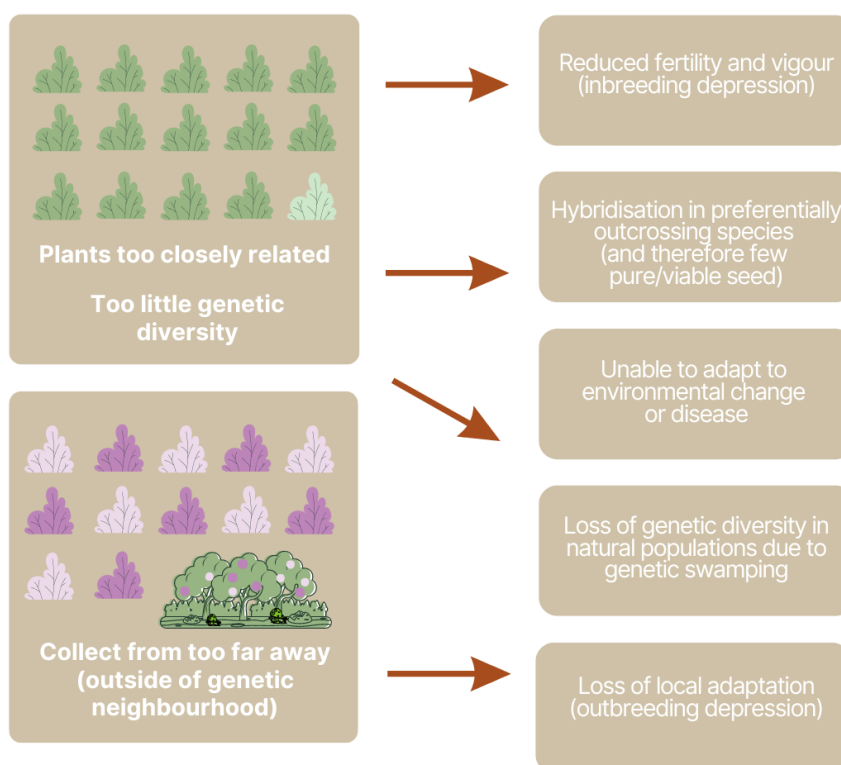
The project was funded independently from the CPCP research strategy and was aimed as a pilot for the rapid development of genetic guidelines for the critically endangered Cumberland Plain Woodland in support of restoration activities. However, the species-specific guidance provided within this plan and recommendations for future directions can be applied across all threatened ecological communities of the Cumberland Plain. The species studied occur across multiple communities within the Cumberland Plain, with none restricted to Cumberland Plain Woodland, and the results from the genetic analyses found no differentiation across the different ecological communities.

## 1.2 Relevance of genetic information to ecological restoration

Sourcing the correct material is amongst the most important steps for ecological restoration projects (Harrison et al. 2021), particularly when it comes to restoring threatened ecological communities in heavily cleared and highly fragmented landscapes such as the Cumberland Plain. Genetic information can guide seed sourcing strategies to help ensure material used in restoration are of appropriate provenance and sufficiently genetically diverse to be self-sustaining. Fail to adequately consider genetic diversity and we may end up with populations that exhibit low or no recruitment and are unable to adapt to change (Figure 1). Striving to restore self-sustaining populations minimises the need for ongoing human intervention and thus the expenditure of time and money (Suding et al., 2015). There are many influences on the sustainability of restored populations, but key concerns are overall fertility and the presence of sufficient adaptive potential for the restored population to survive through environmental change.

Mating among close relatives in populations with insufficient genetic diversity, termed inbreeding, can cause a further decline in genetic diversity due to the loss of rare alleles, and thus adaptive potential. Additionally, inbreeding can result in inbreeding depression, a reduction in overall population fitness due to increased genome-wide homozygosity (and hence increased impact of deleterious recessive alleles). Inbreeding depression can in turn result in reduced

reproductive success (e.g., reduced seed production) and loss of vigour in offspring (e.g., reduced seed/seedling viability).



**Figure 1:** A simplified overview of the key genetic risks for restored populations and nearby remnant native vegetation.

The negative impacts of a low number of sufficiently distinct individuals may not always be obvious in restored populations in the short term and may be caused by collecting seed from too few populations, too few maternal plants, or from populations that are highly selfing or have lost genetic diversity due to loss and fragmentation of habitat. For example, a site may be restored with thousands of trees of a particular species which have good survival and growth rates, but if they were all propagated from seed sourced from a single maternal plant (a common situation in eucalypts) then all individuals may be too closely related to produce viable progeny.

Outbreeding depressions, where the fitness of individuals that arise from the crossing of genetically distinct parents is reduced has long been a concern when mixing material from a wide set of source populations. However, due to a lack of real-world examples in common and widely distributed species, sentiment in the field of restoration genetics has been moving away from this concern (Hancock et al., 2023). In this report we address this concern using genetic neighbourhoods, defined areas where there is no evidence for interruptions to geneflow across the landscape. Per the decision tree of Frankham et al., (2011) all the species investigated in this report have low probability of showing outbreeding depressions, especially if seed is only sourced from within a single genetic neighbourhood, therefore we have minimal concern about this possible issue.

Hybridisation, especially in preferentially outcrossing species, is another potential consequence of a restored population having too few genetically distinct individuals. Unintentional hybridisation is particularly relevant for restoration of eucalypts, as natural eucalypt populations are generally highly outbred, with high levels of heterozygosity and genetic diversity. This is thought to be due to a significant decrease in fitness of individuals that are the result of inbreeding (Nickolas et al., 2019). A restored population of eucalypts that does not contain sufficiently distantly related individuals, even if there are thousands of plants as described above, is likely to show decreased progeny fitness. As this decrease in fitness in the progeny of inbreeding parents is so sharp, the representation of hybrids in a population may increase where inter-compatible species co-occur as these hybrids may show a smaller



decrease in fitness than non-hybrid inbred progeny. So not only are the pure recruits from the two narrowly sourced species going to be less fit, but there will start to get an increase in hybrids as well.

While not the only component of adaptability, genetic diversity is one of the most important mechanisms for a population to respond to future environmental change or challenges. Climate change is a serious and increasing threat to native species and ecosystems in Western Sydney and is expected to be an ongoing challenge to the effectiveness of ecological restoration of the Cumberland Plain (NSW Department of Planning and Environment, 2022). Under climate change, local conditions are likely to shift and a species that was collected from multiple populations from across a large geographic range is more likely to possess greater genetic diversity and include individuals that were already experiencing the new conditions a site may be shifting to. Whereas the probability of individuals collected from only a single location being pre-adapted is much lower. Similarly, considering disease (e.g., myrtle rust), a genetically diverse restored population is more likely to contain some individuals with a genetic makeup that helps them to withstand the disease than a genetically depauperate population.

An additional strategy, beyond maximising genetic diversity, to enhance the likelihood of a restored population being able to adapt to a changing climate is to incorporate, when possible, 'climate-ready' material sourced from areas currently experiencing conditions that match those predicted to occur at the restoration site in the future. The occurrence of climate-ready populations is going to be most likely for widespread species, whereas for species with narrow ranges and/or populations near the extremes of a species climatic niche, there may not be any populations that are climate-ready from which to source propagules. Adaptation of restored populations of these species will be dependent upon capturing sufficient genetic diversity.

While sampling from a broad geographic range is important for maximising genetic diversity, and for incorporating climate-ready material, it is important to not collect too broadly to avoid unintended negative consequences of highly distinct genetic groupings being brought together such as an outbreeding depression. For example, the introduction of plants from a different genetic neighbourhood can cause a loss of genetic diversity in natural populations of the same species due to genetic swamping, particularly when the number of planted individuals is considerably greater than the remaining natural individuals.

An additional area where genetic information is especially relevant to ecological restoration is in increasing the availability of genetically diverse seed. Genetic information can help overcome limits to seed availability by (i) guiding the design of genetically diverse and representative seed production areas, and (ii) increasing the geographic range of seed collection, thereby increasing the number of remnants seed may be collected from. Defining genetic neighbourhood boundaries in the absence of genetic information is challenging and research has shown that generalisations have their limitations (Rossetto et al., 2020). Underestimating the size of the genetic neighbourhood may unnecessarily reduce options for seed sourcing. For example, previous guidelines for restoration of the Cumberland Plain (Department of Environment and Conservation (NSW), 2005), made in the absence of genetic information, recommended all species (other than those with fleshy fruits) be collected either from the same remnant being restored or adjacent, or at the very most from 'nearby remnants that were formerly contiguous.' Such collections unnecessarily reduce options for increasing genetic diversity and may suffer the negative consequences summarised in Figure 1. This study has revealed genetic neighbourhoods that are considerably larger and broadened the geographic range seed can safely be collected from, addressing one of the key issues identified in The Australian Native Seed Survey Report published in 2020; that the supply of seed from 'local' provenances is limited and thus a cause of concern for restoration practitioners (Hancock et al., 2020). We show that for most of the study species, gene flow naturally occurs continuously across hundreds of kilometres, and therefore it is highly unlikely for mixing of seed sources to induce outbreeding depression. Rather, increased genetic diversity in restored plantings will increase reproductive success, fitness of progeny and adaptability.

### 1.3 We need genetic data rather than generalisations

To summarise the key genetic considerations when striving to restore self-sustaining populations (Figure 2), we need to:

- minimise the occurrence of inbreeding and hybridisation by maximising the number and distinctiveness of unique individuals.

- maximise genetic diversity by sourcing seed from multiple individuals from multiple populations across a broad geographic range.
- facilitate adaptation to a changing climate by supplementing, when possible, with climate-ready material.
- avoid unintended negative consequences by sourcing seed primarily from within the same genetic neighbourhood as the site being restored.

To achieve these objectives, we need species specific genetic data rather than generalisations, especially when it comes to restoring threatened ecological communities, because the consequences of using inappropriate material can be dire for both restored vegetation and remnant native vegetation. However, restoration genomics has stepped into a new phase. Until 10–15 years ago, genetic considerations were typically inferred or generalised and obtaining genetic data was considered too complex, expensive, difficult to manage, and hard to interpret. However, technology has changed such that we can now gather relevant genomic information quickly and cost-effectively, making it feasible for restoration practitioners to access the information they need to design effective ecological restoration projects.



**Figure 2:** A simplified overview of the key genetic considerations when striving to restore self-sustaining populations.

## 1.4 Restore and Renew: making genomic and climatic information freely available to restoration practitioners

The Restore and Renew project responds to the need for ecological restoration practitioners to incorporate the latest science into their toolkit, helping them to restore diverse, resilient, and adaptable ecosystems. Resilient ecosystems need to contain populations of species that are not only adapted to the local geology, climate and soil, but to future conditions as well. Restore and Renew acquires empirical knowledge on genetic diversity, climate suitability (current and future) and distributional patterns across multiple species to deliver restoration guidance to practitioners in easy to use publicly available web tools.

Results for the eight species studied in this project that proved suitable have been made publicly available via the open access Restore and Renew webtool and will be able to guide optimised seed collections and establishment of seed production areas for these taxa, within the Cumberland Plain, and across their distribution in NSW.

## 1.5 How to use these guidelines

Below we provide a brief description of each of the guideline's sections to help you understand their use and application.

In the **introduction** we set the context of the current study and outline the relevance of genetic information to ecological restoration.

The **methods** section introduces the target species, provides a brief outline of the methods used, and refers to research publications for more detailed information.

In the **findings and implications section** we share general landscape patterns of diversity, identify regions that can provide climate-ready seed for use on the Cumberland Plain, and outline important considerations when undertaking genetically informed restoration on the Cumberland Plain. We then go on to present **species-specific patterns of diversity**, including definition of genetic neighbourhoods, a biologically meaningful measure of provenances that can be safely mixed in a restoration planting. We provide **guidelines for optimised seed collection** for 11 species that ensure restored plant populations are sufficiently genetically diverse to be self-sustaining in the long term, while also being sufficiently adapted to both current and future climate conditions. A summary of results is provided for a further four species with genetic results too complex to facilitate generation of guidelines.

A **case study** demonstrates the application of these results to the design of a genetically diverse climate-ready seed production area for 11 species.

And finally, a **future directions** section describes how the methodology used and knowledge infrastructure established can be applied to additional species to further improve ecological restoration across the Cumberland Plain.

## 2. Summary of methodology

### 2.1 Selecting target species

When selecting target species for this study we focussed on Cumberland Plain Woodland (CPW), the most widespread (Tozer, 2003) of the five threatened ecological communities constrained to the Cumberland Plain. The community primarily occurs on soils derived from the Wianamatta Shale with smaller areas on well drained alluvium both of which receive lower annual rainfall than much of the rest of the Sydney Basin (<950 mm) due to a rain shadow that overlies large parts of the Cumberland Plain (Tozer, 2010). Previous work has treated CPW as two very similar, but distinct, floristic classes: Shale Hills Woodland and Shale Plains Woodland (Tozer, 2003), although they are not regarded separately in the legislation.

As part of the Coastal Valley Grassy Woodlands of eastern NSW and south-east Queensland, CPW is an open woodland community with minimal shrub layer and abundant grasses (Tozer, 2010). The two eucalypt species that dominate the vegetation type, *Eucalyptus moluccana* and *Eucalyptus tereticornis*, along with the many of the other characteristic trees, shrubs, and grasses, are widespread in eastern Australia, with few rare or endemic plant species

known from the community (Tozer, 2003). This is despite the CPW being isolated from other areas of Coastal Valley Grassy Woodlands by the elevated sandstones of the Woronora Plateau and Illawarra Escarpment to the south and Wollemi and Yengo wilderness to the north, which support contrasting plant communities to the Cumberland Plain.

Protected areas including areas of largely intact CPW include Scheyville National Park, Wianamatta Regional Park, Western Sydney Parklands, Mulgoa Nature Reserve, Prospect Nature Reserve and the Australian Botanic Gardens, Mount Annan, with smaller areas present in many other reserves and green spaces across western Sydney. However, the ecological community is highly fragmented as shown in Figure 3, with many patches consisting of little more than a stand of remnant trees. Thus, undertaking restoration activities with the aim of increasing connectivity between patches is important to ensure long term sustainability of this community and the species which live within it.

Twelve species commonly found within or associated with CPW and identified as candidates for use in ecological restoration were chosen (Table 1). We targeted relatively common species that are easy to sample across NSW. These species included canopy trees, understorey shrubs and grasses, three growth forms often targeted in restoration activities, but that come with different life histories (life span, pollination syndrome, etc.) that may lead to differing best practices for genetically informed restoration. There is also a large variance in the species native distributions, ranging from transcontinental for *Oplismenus hirtellus* and *Themeda triandra*, to localised around the Sydney Basin for *Acacia decurrens* and *Acacia parramattensis* (Figure 4).

As is the nature of landscape genetic studies, issues of sampling and taxonomy arose for four species which prevented development of optimised seed sourcing guidelines and addition to the Restore and Renew Webtool (Table 1). Therefore, three additional species (*Breynia oblongifolia*, *Dodonaea viscosa* and *Eucalyptus baueriana*), for which sampling could be completed promptly were chosen to supplement the original twelve (Table 1). Of the total 15 species, eight proved suitable for the model applied on the Restore and Renew Webtool and will be made available as indicated in Table 1. Three further species (*Acacia falcata*, *Bursaria spinosa* and *Dodonaea viscosa*) for which we provide specific seed sourcing guidelines did not model well in the current implementation of the webtool but may be added in the next upgrade to functionality.

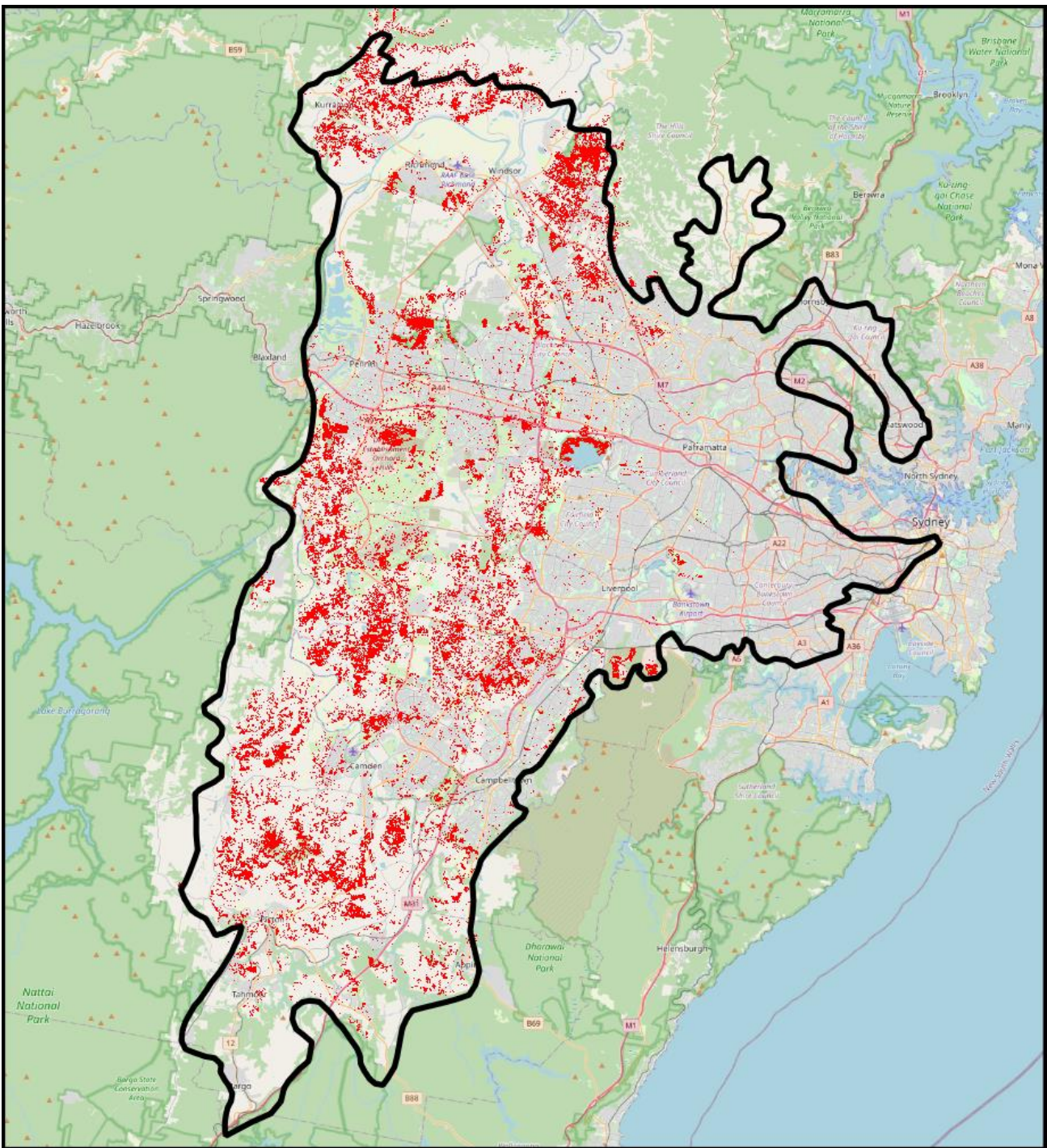
## 2.2 Landscape patterns of genetic diversity

Geneflow between the CPW and other communities in the wider Sydney Basin is quantified to investigate whether sourcing propagules from communities other than CPW for restoration of the community is appropriate. As the detail of this work is outside the scope of this report, work is currently underway to publish it as a scientific research article (Fahey et al., in prep), however the findings are relevant here and are highlighted in the results of this report.

## 2.3 Climate matching and identification of climate-ready provenances

To inform sourcing of climate-ready material, we investigated both moderate and severe climate projections for the year 2070 as implemented on the Restore and Renew climate matching webtool (Rossetto et. al. 2019). This climate matching tool highlights regions where the current climate matches the predicted future climate of a location based upon a set of climatic factors, including annual rainfall and annual temperature. However, as we were not targeting a specific site, but rather the entire Cumberland Plain, we used an alternate site matching technique to what is currently implemented on the Restore and Renew webtool, identifying areas that experience average annual rainfall and temperatures that are within the range of what is predicted to be experienced across the Cumberland Plain in the year 2070 due to climate change. Work is underway to implement this methodology on the Restore and Renew climate matching tool which will provide access to the maps provided in this report in an interactive format.



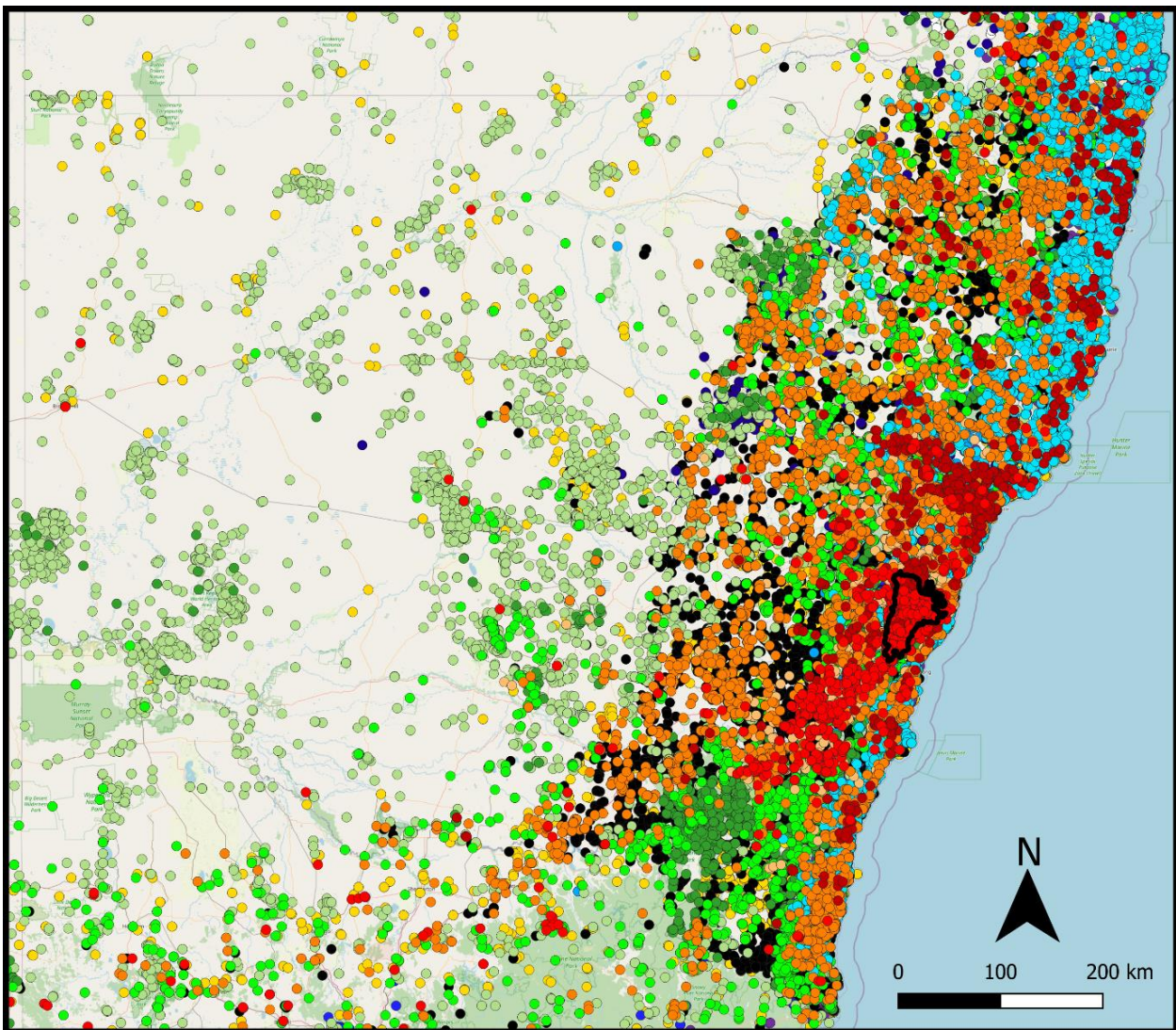


**Figure 3:** The current extent of Cumberland Plain Woodland (red) within the Cumberland Plain IBRA subregion (black outline), showing the highly fragmented nature of the remaining areas of this vegetation type.



**Table 1:** Fifteen target species sampled and used in landscape genetic studies that inform this report. Species for which specific restoration guidelines were developed and those added to the Restore and Renew Webtool are indicated.

| Scientific name               | Common names              | Species specific guidelines developed          | Species added to Restore and Renew Webtool |
|-------------------------------|---------------------------|------------------------------------------------|--------------------------------------------|
| <i>Acacia decurrens</i>       | Sydney Green Wattle       | Yes                                            | Yes                                        |
| <i>Acacia falcata</i>         | Sickle Wattle             | Yes                                            | No                                         |
| <i>Acacia implexa</i>         | Hickory Wattle, Lightwood | Yes                                            | Yes                                        |
| <i>Acacia parramattensis</i>  | Parramatta Wattle         | Yes                                            | Yes                                        |
| <i>Breynia oblongifolia</i>   | Coffee Bush               | Yes                                            | Yes                                        |
| <i>Bursaria spinosa</i>       | Boxthorn, Sweet Bursaria  | Yes                                            | No                                         |
| <i>Daviesia ulicifolia</i>    | Gorse Bitter Pea          | No – Taxonomic issues                          | No                                         |
| <i>Dodonaea viscosa</i>       | Sticky Hop-bush           | Yes                                            | No                                         |
| <i>Eucalyptus baueriana</i>   | Blue Box                  | Yes                                            | Yes                                        |
| <i>Eucalyptus eugenioides</i> | Thin-leaved Stringybark   | No – Sampling issues                           | No                                         |
| <i>Eucalyptus fibrosa</i>     | Red Ironbark              | Yes                                            | Yes                                        |
| <i>Melaleuca decora</i>       | White Feather Honeymyrtle | Yes                                            | Yes                                        |
| <i>Microlaena stipoides</i>   | Weeping Grass             | No – Natural genetic patterns have broken down | No                                         |
| <i>Oplismenus hirtellus</i>   | Australian Basket-Grass   | No – Taxonomic issues                          | No                                         |
| <i>Themeda triandra</i>       | Kangaroo Grass            | Yes                                            | Yes                                        |



**Figure 4:** Map of New South Wales showing the location of the Cumberland Plain in black outline overlying the records of the 15 species targeted in this study. Each species corresponds to a different coloured dot, making it clear that the Cumberland Plain Woodland cannot be considered in isolation from the wider distribution of the species which form the community.

## 2.4 Population genetic analyses and guidelines

Sample collection and population genetic analyses were undertaken using the Restore and Renew workflow developed by the Research Centre for Ecosystem Resilience (Botanic Gardens of Sydney) (Rossetto et al., 2019), with relevant species-specific information added to the publicly available Restore and Renew Webtool ([restore-and-renew.org.au](https://restore-and-renew.org.au)). This webtool uses species-specific genetic data to calculate the genetic neighbourhood for a selected site and species. Practitioners select the site that they want to restore, and the tool uses the genetic data to calculate the area recommended for sourcing material. The tool also provides information on areas that currently experience the predicted future climate of the selected site, helping practitioners investigate where genetically diverse seed can be sourced for restoration of a particular location and where climatically adapted populations may be found. As part of future implementations of the webtool, recommended source site and maternal line numbers will also be provided.

We provide our findings on the reproductive biology; patterns of genetic diversity and population structuring for each species and use that to highlight key considerations for seed sourcing for that species. To investigate what level of seed collecting effort is required to represent a minimum of 90% of genetic diversity within an identified source region,

a United Nations' Convention on Biological Diversity target (United Nations Convention on Biological Diversity, 2021), four seed sourcing scenarios were investigated for each species as follows:

*Cumberland Plain provenances only:* Whereby seed is collected only from within the Cumberland Plain, irrespective of whether a species genetic neighbourhood extends beyond the Cumberland Plain. This approach is insufficient to capture high levels of genetic diversity to provide good adaptive capacity.

*Genetic neighbourhood:* Whereby seed is collected from areas that are within the same genetic neighbourhood as the site being restored. This scenario, with sampling within and outside the Cumberland Plain, is recommended over the *Cumberland Plains provenances* scenario as it will capture greater genetic diversity.

*Moderate climate ready:* Whereby 50% of seed is collected from areas that are currently experiencing climatic conditions that match those that are predicted to occur in the Cumberland Plain in the year 2070 under a moderate (rcp 4.5) climate change scenario.

*Severe climate ready:* Whereby 50% of seed is collected from areas that are currently experiencing climatic conditions that match those that are predicted to occur in the Cumberland Plain in the year 2070 under a severe (rcp 8.5) climate change scenario.

Required seed sourcing effort for each scenario was determined using a random sampling technique of genotyped samples over 100 iterations for each combination of site and sample number, with the choice of site numbers being the lowest that always resulted in the capture of >90% of all alleles present in the subset of the species distribution being targeted for seed sourcing, thus allowing for flexibility in the site choice optimisation (Dimon et al., in prep). This approach is easily replicable for different regions and suites of taxa where genetic data is available or can be generated, with the possibility of being automated and used to optimise specific restoration or seed collection projects.

Optimised sourcing schemes are provided for two seed collecting protocols: no maintenance of maternal lines at a site i.e. mixing seed from all mothers as a single collection, and when five maternal lines per site are maintained. The collection of a single maternal line at a site is treated as not meaningfully different to mixing seed from multiple mothers in a single collection as there is no practical way to control what proportion of seed from each mother is used in any given planting with mixed collections, an undesirable outcome when the goal of reaching a target threshold of genetic diversity represented in a restoration site or seed production area. The testing of two collecting strategies showcases the benefits of maintaining maternal lines (van der Merwe et al., 2023), in that for all species and scenarios, by maintaining five maternal lines per source site, the number of sites needed to capture 90% of diversity was significantly reduced, thus decreasing the required seed collecting effort and cost. While the number of maternal lines could be adjusted to allow for the need of greater or fewer sites, as we are undertaking empirical optimisation here, we are only able to test a number of maternal lines equal to the number of genotyped individuals we have per site.

### 3. Findings and implications

#### 3.1 Landscape patterns of diversity

##### 3.1.1 No restricted geneflow between Cumberland Plain Woodland and other ecological communities

We found no evidence for restricted geneflow between CPW and other vegetation types on the Cumberland Plain or surrounding areas (see Figure 5) (Fahey et al., in prep). This suggests that at the scale of NSW Plant Community Types, local 'ecotypes' better adapted for each vegetation type do not exist, and therefore there is no need to restrict seed sourcing for the restoration of CPW to only other remnant patches of CPW. This broadens the pool of locations for seed sourcing, allowing for greater genetic diversity within restored populations, and thus greater population adaptability and sustainability through climatic and environmental changes.

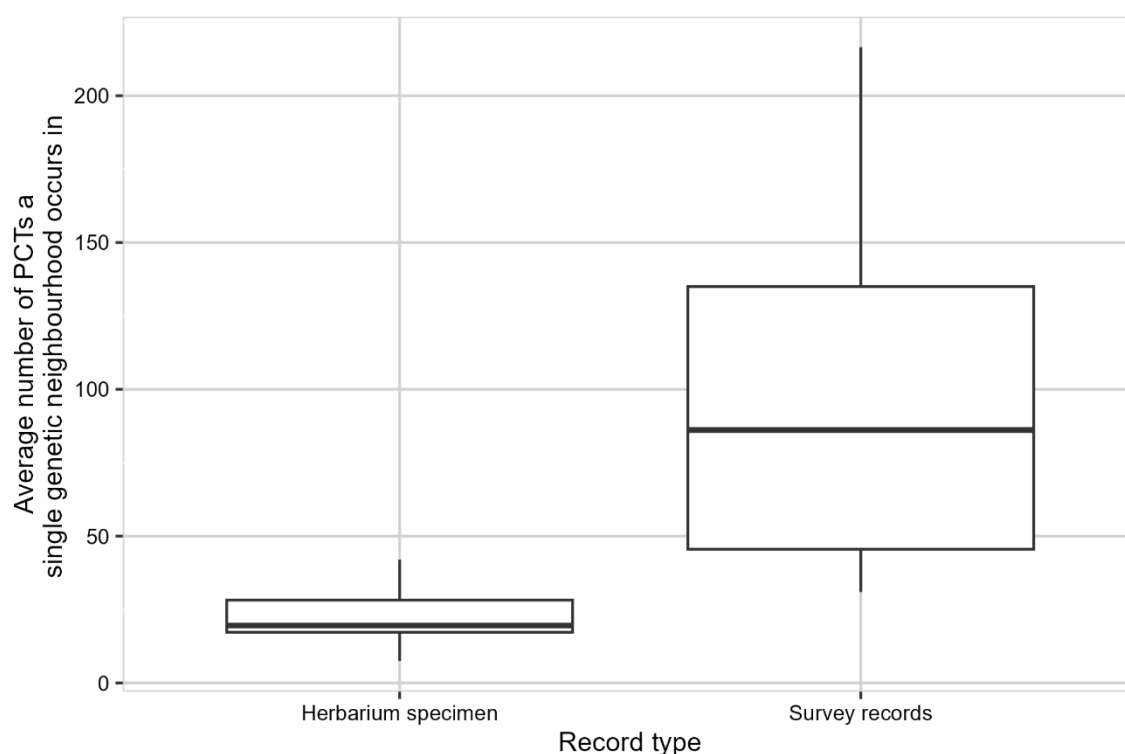
Overall, we see that genetic neighbourhoods for common and widespread CPW plant species are generally large, stretching hundreds to thousands of kilometres across latitudes, but often being more compressed in the longitudinal direction, due in part to the distribution of the species being limited to the higher rainfall areas near the coast, but also

the nature of the Great Dividing Range as a barrier to gene flow. Other important barriers that segregated genetic neighbourhoods for multiple species were the Illawarra Escarpment and Woronora Plateau to the south of the Cumberland Plain the Wollemi and Yengo wilderness to the north of the Cumberland Plain and the Hastings-Macleay Rivers on the Mid-North Coast (Figure 6), although no species showed a border between genetic neighbourhoods at all these barriers.

For most target species, the Cumberland Plain represented a smaller subsection of a more widely distributed genetic neighbourhood, with isolation by distance (IBD), where the geographic distance between populations is a major correlate of genetic divergence, being the most common pattern inferred. Thus, it can be surmised that populations of the target species in the CPW, and the Cumberland Plain more broadly, are not isolated from gene flow with populations in surrounding regions, in particular the Central Coast, Hunter Valley, Southern Highlands, and Illawarra regions, where very similar plant communities to CPW are common. This suggests that there is limited chance of outbreeding depression in the target species resulting from introducing seed from these regions to the Cumberland Plain in restoration plantings, a positive finding if climate-ready provenances are to be sought.

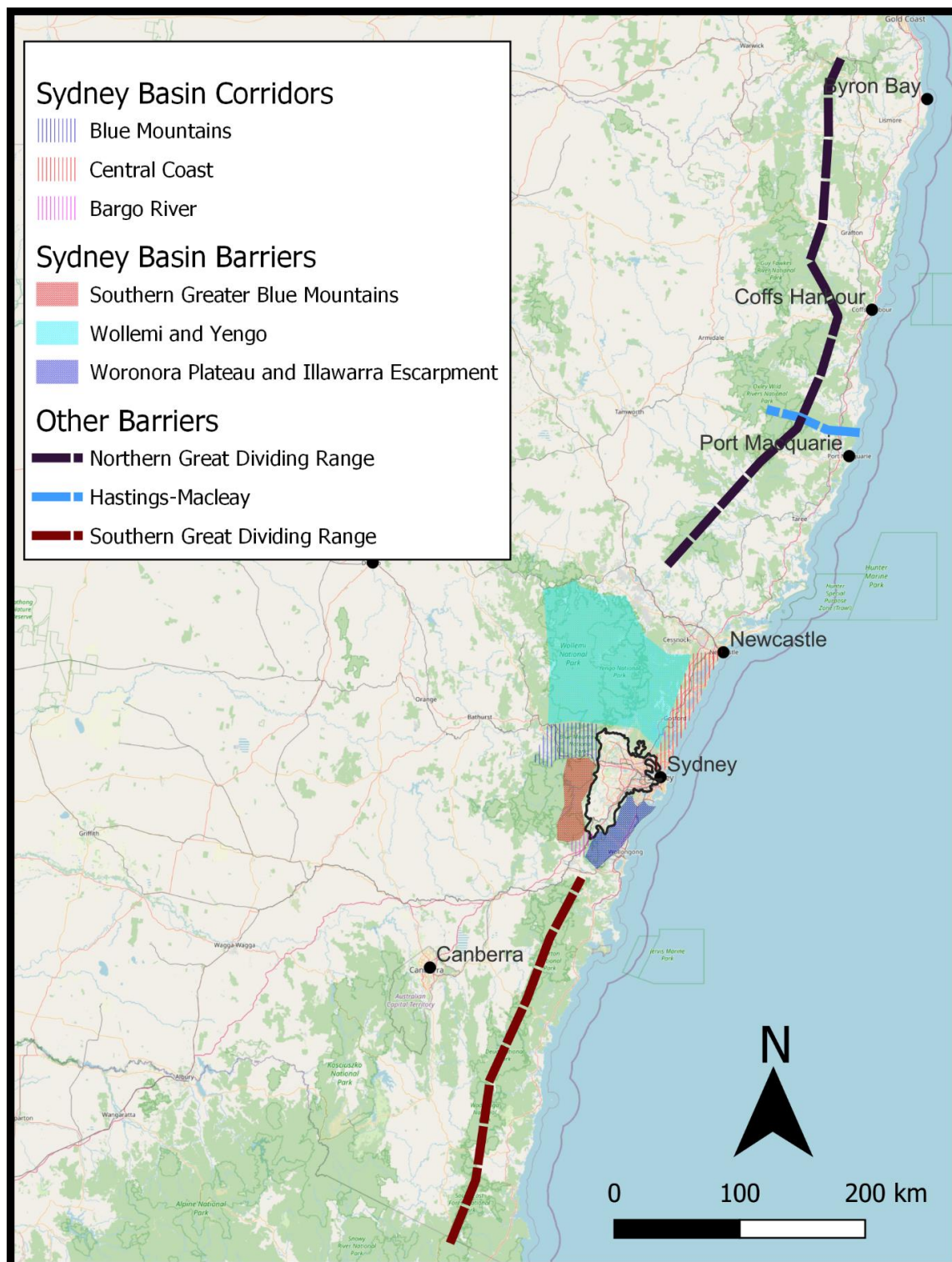
### 3.1.2 Genetically distinct sites typically/may hold less genetic diversity

When individual sites on the Cumberland Plain were examined for their relative genetic diversity and distinctness across species, we saw a trade-off between these two factors, with genetically distinct sites mostly not being highly genetically diverse. This suggests that when we collect from genetically distinct sites, we may capture rare and unique alleles, but potentially at the cost of common alleles that make up a large proportion of overall genetic diversity, implying that a balance of genetically distinct and genetically diverse sites is needed.



**Figure 5:** Boxplot of the average number of Plant Community types a genetic neighbourhood occurs in across the 11 CPW species for which genetically informed restoration guidelines have been developed in this report. The occurrence of a species is divided between herbarium specimen records and survey records, as the former have more accurate identifications, but are much less numerous than the latter. Note that for no species does a genetic neighbourhood only occur in a single PCT, therefore seed sourcing should not be constrained to a particular PCT when undertaking restoration activities.





**Figure 6:** Map of eastern New South Wales showing the location of major biographic barriers that correspond to breaks between genetic neighbourhoods in the Cumberland Plain species investigated in the report. Note that the majority correspond to upland areas that support contrasting vegetation to the lower elevation Cumberland Plain. Also shown are the three corridors where gene flow between populations on the Cumberland Plain and areas to the north (Hunter Valley via the Central Coast), south (Southern Highlands via the Bargo River corridor) and west (Central West via the Blue Mountains) is largely unrestricted.

### 3.1.3 No observed relationship between patch size and genetic diversity

We observed no evidence of a relationship between patch size and genetic diversity for the sites where multiple species were sampled on the Cumberland Plain (Figure 7), suggesting either that how we have measured fragmentation (patch size) is not appropriate, or that fragmentation was recent enough that its effects on patterns of genetic diversity have not yet fully resolved. This finding suggests that even small patches of remnant vegetation on the Cumberland Plain may hold important diversity and should not be dismissed because of their small size when collecting seed.

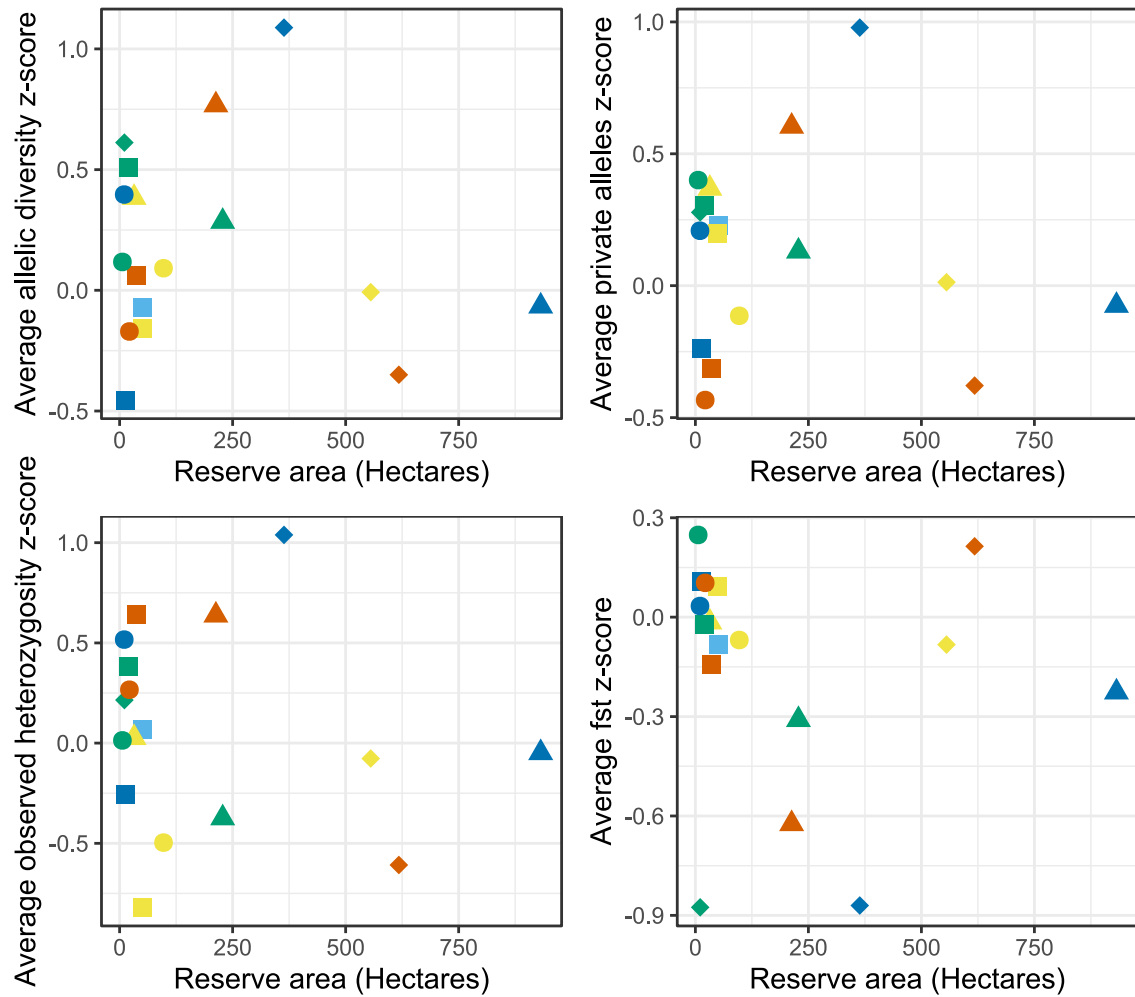
### 3.1.4 Location of climate-ready provenances for restoration of the Cumberland Plain

As shown in Figure 8, under a moderate climate model (rcp4.5 as implemented on the Restore and Renew webtool (Rossetto et al., 2019)), due to the strong climate gradient on the Cumberland Plain, portions of the northern Cumberland Plain can provide climate-ready seed for the Cumberland Plain. Additional future climate matched regions under this scenario are present in the Lower Hunter Valley, southern Mid-North Coast and Northern Rivers regions. This means for many species, sampling from within the genetic neighbourhood to which the Cumberland Plain belongs can include a large proportion of climate-ready collection sites, in which case the difference between the moderate climate-ready seed sourcing scenario and the genetic neighbourhood scenario is intentionally locating 50% of seed source sites within the climate-ready areas.

Under severe climate change projections (rcp8.5 as implemented on the Restore and Renew webtool (Rossetto et al., 2019)) there was substantially less suitable area for sourcing climate-ready provenances in NSW (Figure 8), and we include specific guidelines for seed sourcing when this scenario is implemented. These are based upon an assumption of targeted a 50/50 mix of provenances from the local genetic neighbourhood and climate-ready areas.

**Figure 7:** (next page): Graphs showing the relationships between three measures of genetic diversity (allelic diversity, number of private alleles, and observed heterozygosity) and one measure of genetic distinctiveness (average  $F_{st}$ ), and patch size for seventeen reserves and parklands from which we collected three or more target species. As genetic measures cannot be directly compared between species, for each measure for each species a z-score index was calculated for each site compared to all sampled sites on the Cumberland Plain, ranking how above or below average for that measure the site was for that species. The higher the index, the more diverse or distinct the site was. This index was then averaged across each species collected at a site to find the values shown in the graphs.

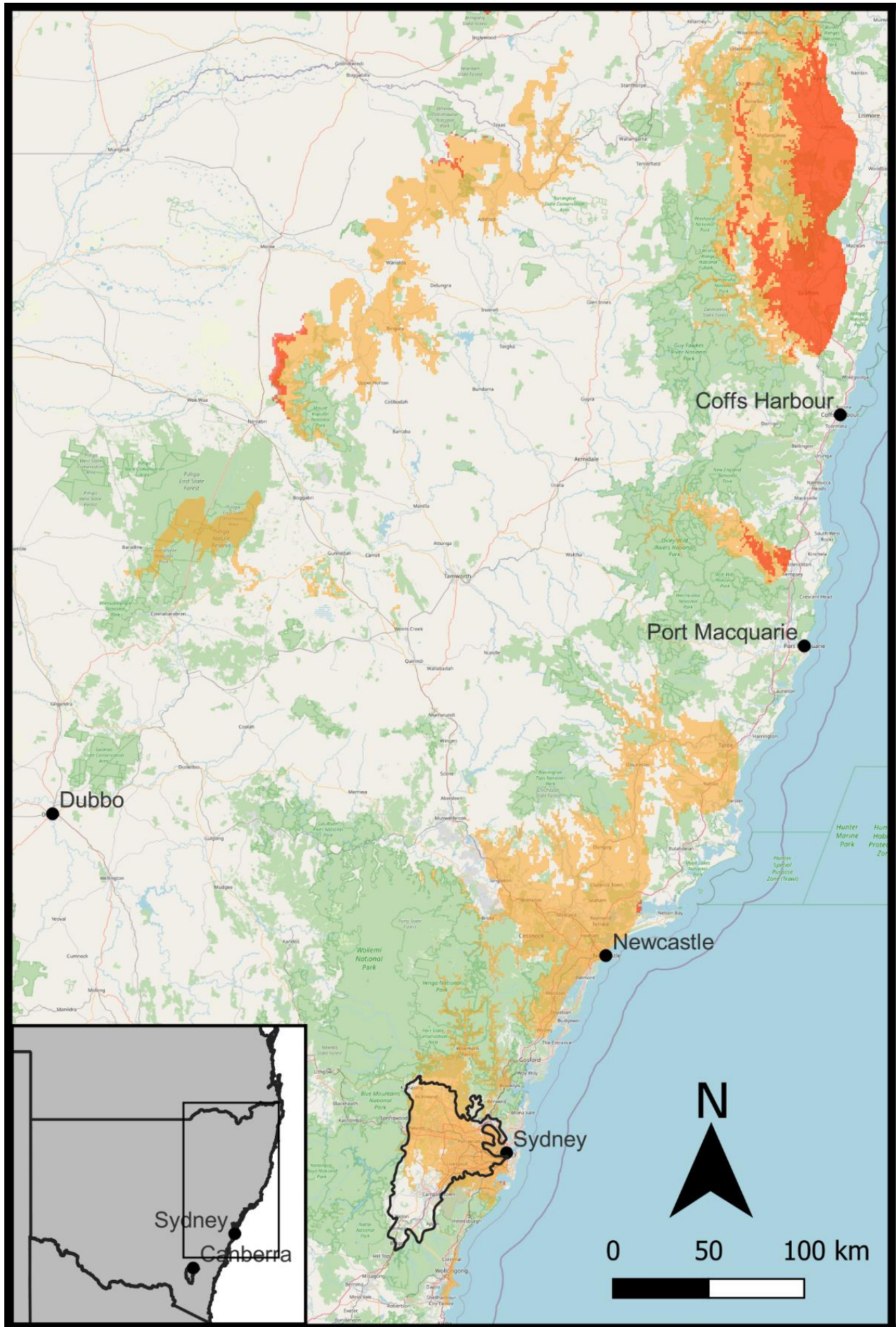
**Figure 8:** (page after next): Map showing areas where current climate conditions match modelled climate conditions on the Cumberland Plain in the year 2070 under moderate climate change (orange) and severe climate change (red) projections. Under moderate climate change, there is significant areas from where climate-ready provenances can be sought for ecological restoration on the Cumberland Plain, including large proportions of the northern Cumberland Plain itself. Under severe climate change, the area from where climate-ready provenances can be sought for ecological restoration on the Cumberland Plain, is largely limited to the Northern Rivers region of in NSW.



#### Site

- Bill Anderson Reserve, Kemps Creek
- Bushland along Redbank Creek, North Richmond
- Deepwater Park, Milperra
- Dr Charles Mckay Reserve, Mount Druitt
- Gordon Lewis Oval Reserve, Appin
- Hannaford Oval Bushland, Wilton
- Karne Street Reserve, Riverwood
- Lansdowne Nature Reserve, Lansdowne
- Lin Gordon Reserve, Thirlmere
- ▲ Mulgoa Nature Reserve, Mulgoa
- ▲ Noorumba Reserve and St Helens Park, Rosemeadow
- ▲ Remanent Bushland, Australian Botanic Gardens, Mount Annan
- ▲ Scheyville National Park
- ◆ Shanes Park, Shanes Park
- ◆ Timbertop Reserve, Prospect
- ◆ Western Sydney Regional Park
- ◆ Windsor Downs Nature Reserve, Windsor Downs







## 3.2 Important considerations when undertaking genetically informed restoration

### 3.2.1 Distribution of seed source sites and mother plant location within sites

Sites from which seed is sourced should be spread as evenly across the target genetic neighbourhood as possible. Based upon the species covered in this report and other investigated in the Restore and Renew program, we recommend 20 km as a minimum distance between sites for any species, however there may be circumstances where this is not possible, for example when sourcing climate-ready seed from a limited climate ready area. In such cases, sites should be located as far apart as possible within the target area to maximise the genetic distinctiveness of each site.

The within site location of mother plants is also an important consideration, as individuals in close proximity are likely to be related, especially for species with limited dispersal capacity such as eucalypts. Additionally for species that spread vegetatively such as *Acacia implexa* and *Acacia parramattensis*, separate stems that appear to be different individuals could be genetically identical clones, thus defeating the purpose of sampling from multiple mother plants. Based upon our findings for the target species in this report, we recommend choosing mother plants that are at least 50 m apart at a site if possible, to avoid these issues.

### 3.2.2 Avoid planted, naturalised and hybrid populations

During this study, some sites had to be excluded from analyses for three main reasons: they had been intentionally planted in the past and mistaken for natural stands, they represented naturalised populations that had recently escaped cultivation, or they contained many hybrid individuals.

Planted and naturalised sites should be avoided for seed sourcing as they often contain lower genetic diversity than natural, wild sites and may have originated from a different genetic neighbourhood to that targeted.

Eucalypts pose the classic example of hybrids being common, and due to the frequent reduced fitness of hybrid individuals, the proportion of hybrids in seed collections will be higher than in the mature individuals at a site. Hybrids should be avoided in restoration plantings due to this common reduction of fitness as this may reduce the success and sustainability of restored populations. Collection of hybrid seed can be minimised by avoiding sourcing seed from sites where species known to frequently hybridise, such as *Eucalyptus fibrosa* and *Eucalyptus siderophloia*, co-occur, and screening seedlings based upon either morphology or genotyping to exclude hybrid individuals.

### 3.2.3 Tracking of maternal lines

It is strongly recommended that to maximise genetic diversity in restoration sites or seed production areas (SPAs), all maternal lines are tracked and kept separate throughout the entire processes of seed collection to restoration planting. Mixing seed from multiple mothers in a single collection removes the ability to control the proportion of seed from each mother and consequently removes any ability to intentionally maximise genetic diversity or to track unintentional loss of genetic diversity. Maintaining separate maternal lines also considerably reduces the number of collection sites needed to capture adequate genetic diversity, thus decreasing the required seed collecting effort and cost (van der Merwe et al. 2023).

In restoration plantings and SPA's, maternal lines should be interplanted to allow for maximum interbreeding of maternal lines, but the identity of each planted individual should still be recorded. This recommendation applies to both single maternal line per site and multiple maternal lines per site strategies. In the latter case, both the source site and mother plant should be tracked. For species that are not planted individually such as grasses, maternal lines should be kept separate as seed collections and tested individually for viability but mixed in equal proportion before sowing.

### 3.2.4 Monitoring for success of restoration planting

An important aspect of genetically informed restoration is the monitoring of ongoing growth, survival, and reproductive success of plantings. The ability to achieve this is greatly increased when maternal lines are tracked and the identity of planted individuals is recorded, as this allows the success of maternal lines and source sites to be determined from simple surveys of survival and growth of individuals within plantings. We recommend that such surveys are

undertaken at intervals dependant on the life history of the species, as fast-growing, short-lived species such as *Themeda triandra* and *Acacia falcata* will grow and reach reproductive capacity over shorter timelines, and therefore correctional activities will need to be implemented sooner, than for long-lived tree species such as eucalypts and melaleucas.

Additionally, the recruitment of new individuals into the restored area should be surveyed once the planting reaches reproductive age to measure the trajectory of the population overtime. Genetic survey of seed or recruited individuals from restored plantings can be used when practical to confirm that the initial genetically informed seed sourcing strategy has resulted in a population that is producing sufficiently genetically diverse offspring to ensure its long-term survival, growth and adaptation to local conditions. If all seed and offspring are being produced by maternal lines that are particularly vigorous and fecund or different maternal lines are not interbreeding, problems such as a loss of genetic diversity, inbreeding depression or poor adaptation to changing environmental conditions may result. Such processes may not be readily apparent based upon the success of planted individuals or the number of newly recruited individuals alone, however, genetic surveys are a tool that allows such issues to be identified early and corrective actions undertaken to allow for a self-sustaining and healthy restored population to be achieved.

### 3.2.5 Monitoring of seed production areas

Within a SPA, individuals belonging to different maternal lines should be interplanted to facilitate outcrossing, either randomly or in an optimised pattern, as a goal of a genetically informed SPA is to produce seed that contains a high level of genetic diversity and unique genetic individuals. Survival and growth of individuals should be monitored to identify any maternal lines that are not performing well that should be replaced. An additional level of monitoring that can be implemented when maternal lines are tracked is genotyping of seed coming out of an SPA to maximise the diversity of seed used in downstream restoration projects. This provides a method to track whether maternal lines are mixing as expected within the SPA, whether genetic swamping by select genotypes is occurring and to measure what, if any, geneflow is occurring with populations outside the SPA such as nearby remnant patches of vegetation. In taxa that are prone to hybridisation, it also allows for the detection of hybrid seed being produced in the SPA that can then be removed prior to use in restoration plantings.

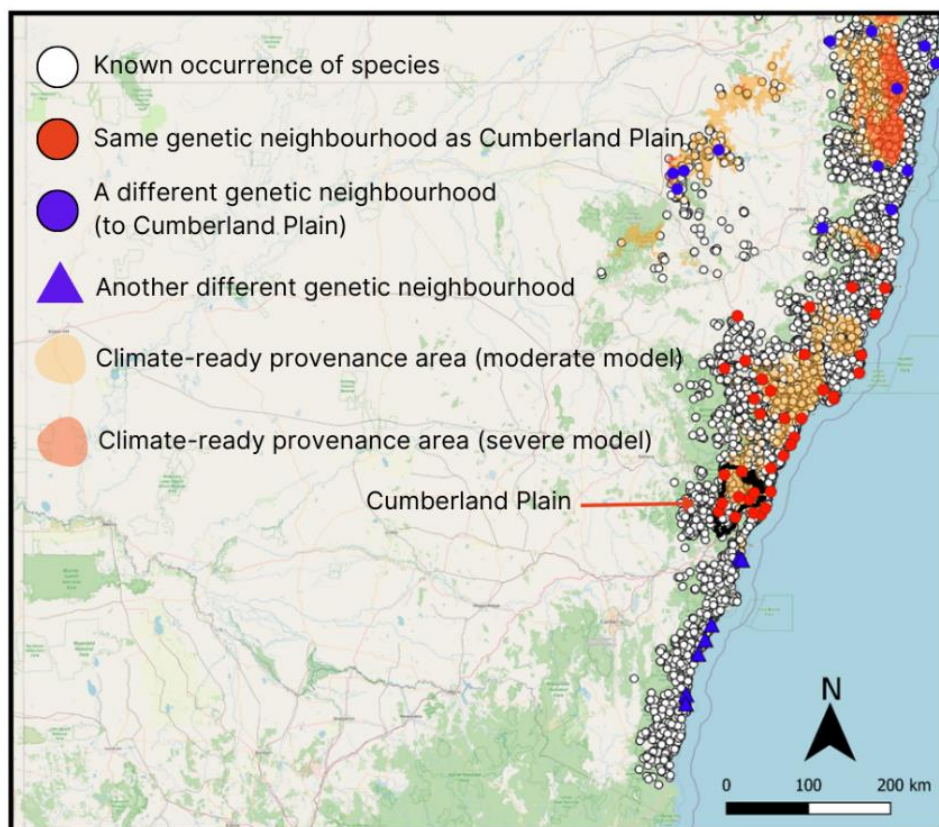
## 4. Species specific patterns of diversity and guidelines for use in restoration

### 4.1 How to interpret species-specific figures and tables

In this section we describe species specific patterns of diversity and provide seed collection guidelines for 11 species. Figure and table captions are self-explanatory; however we provide a brief introduction below.

#### 4.1.1 Genetic neighbourhood maps

Genetic neighbourhoods are mapped across NSW for each species, with the Cumberland Plain area identified. The known occurrence of each species (herbarium and survey records) is identified by white circles. The location of sites sampled for genetic analysis are represented by coloured shapes; solid red circles are sites found to be within the same genetic neighbourhood as the Cumberland Plain and other colours and shapes are as per the figure caption. Each map also depicts the areas where current climate conditions match modelled climate conditions on the Cumberland Plain in the year 2070 under moderate climate change (orange) and severe climate change (red) projections (as per Figure 8, Page 17).



### 4.1.3 Sampling effort tables

For each species we provide a table of the minimum sampling effort required to ensure capture of at least 90% of observed genetic diversity under different seed sourcing scenarios, including when seed is sourced only from within the Cumberland Plain, or from across the genetic neighbourhood. The table also depicts sampling effort required to source climate ready seed under modelled moderate and severe climate change scenarios. We favour the option of maintaining five maternal lines per source site (see discussions regarding the value of maintaining maternal lines on page 18), but also present the option of having a single maternal line from each site to allow for situations where maternal lines have not been tracked or seed collections have been pooled.

The number and location of sites you would need to collect seed from to capture genetically diverse seed if collecting seed from only 1 individual per site or if not maintaining separate collections for each mother plant (i.e. pooling seed for sites)

The number and location of sites you would need to collect seed from to capture genetically diverse seed when collecting from five mother plants and maintaining separate collections for each mother plant.

Seed sourced only from within Cumberland Plain

Seed sourced from within same genetic neighbourhood as the Cumberland Plain

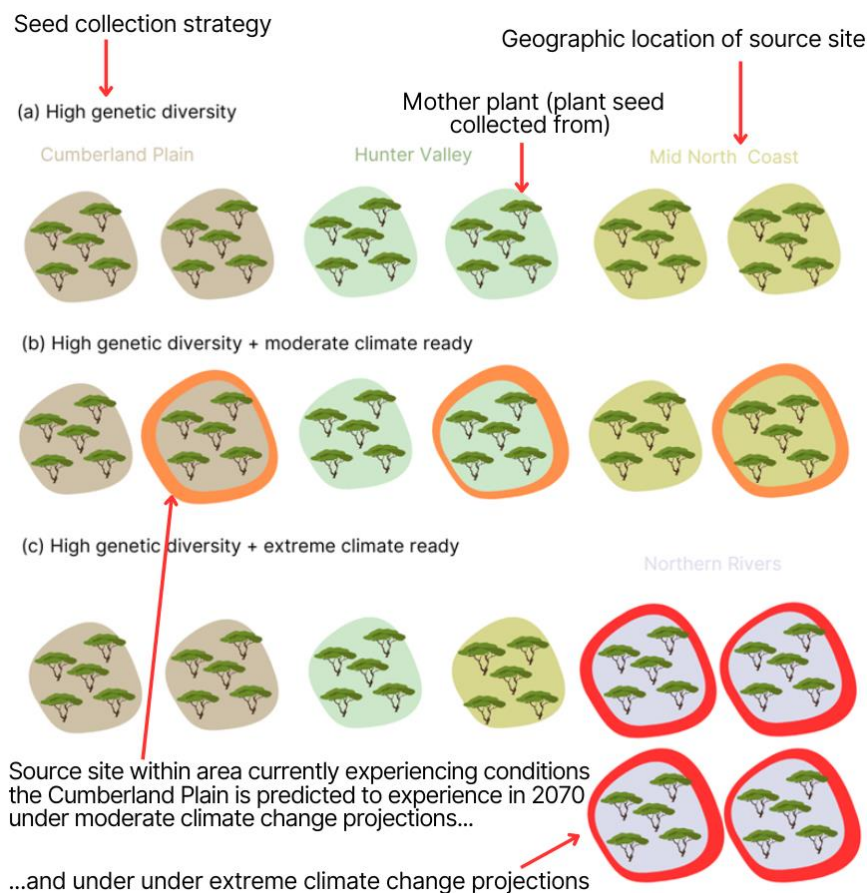
| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                                             | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site?                                                                                                                                    |
|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >13 sites x 1 mother trees per site = >13 maternal lines.                                                                                                                                                      | 4 sites x 5 mother trees per site = 20 maternal lines total                                                                                                                                                                                         |
| Genetic neighbourhood  | 16 sites x 1 mother trees per site = 16 maternal lines.                                                                                                                                                        | 5 sites x 5 mother trees per site = 25 maternal lines total (Depicted schematically in Figure 9a.)                                                                                                                                                  |
| Moderate climate ready | 16 sites – at least 8 located within climate matched areas (as shown in orange in Figure 8), 8 otherwise spread across northern genetic neighbourhood. 16 sites x 1 mother trees per site = 16 maternal lines. | 5 sites – at least 3 located within climate matched areas (as shown in orange in Figure 8), 2 otherwise spread across northern genetic neighbourhood. 5 sites x 5 mother trees per site = 25 maternal lines. (Depicted schematically in Figure 9b.) |
| Severe climate ready   | NA – no sites suitable                                                                                                                                                                                         | NA – no sites suitable                                                                                                                                                                                                                              |

Sourced from sites currently experiencing climate conditions matching those modelled to occur on the Cumberland Plain in the year 2070 under moderate and severe climate change projections.



#### 4.1.4 Schematic representation of optimised seed source strategy

A schematic representation of an ideal seed collection strategy, where maternal lines are maintained, is depicted for each species. The minimum collection effort required to source genetically diverse seed suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area is depicted in the first row (a). Each shape represents a seed collection site, whereby colour is representative of geographic location. Seed collection sites within the Cumberland Plain are brown, within the Hunter Valley in green, and so on as described in the figure. Each tree represents a maternal plant. In all cases seed is sourced from at least five maternal plants within each site. When possible, collection strategies that incorporate 50% from the Cumberland Plain specific genetic neighbourhood material and 50% moderate (b) and extreme (c) climate ready material are depicted. Source sites surrounded by an orange or red shape are to be located within areas identified as currently experiencing conditions the Cumberland Plain will experience in 2070 under moderate climate change (orange) and severe climate change (red) projections.



## 4.2 *Acacia decurrens*



*Acacia decurrens* is a tall shrub to small tree that grows chiefly south from the Hunter Valley to the A.C.T. and is common in the Sydney region. It has one of the smallest natural distributions of the species investigated in this report (Figure 9), although it is widely planted as a windbreak and for timber and has naturalised outside of its natural distribution. Our research has provided insight into the natural distribution of this species. This species has been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

### 4.2.1 Genetic structuring and neighbourhoods

- Two geographically discrete genetic neighbourhoods were observed for *Acacia decurrens* (Figure 9), with the break between them aligned with a small occurrence gap between populations on the Cumberland Plain and north thereof, and those in the Southern Highlands from Moss Vale south and west.
- Genetic evidence suggests this species has seen a substantial range expansion post European arrival after being brought into cultivation and naturalising.
- Based upon our findings, we suggest the western boundary of the species natural range is between Goulburn and Collector, north of Lake George, with populations west of that boundary likely to result from recent human activity.
- Populations from the Yass Valley, Lithgow and Cessnock districts (see yellow points in Figure 9) were genetically near identical to populations on the Cumberland Plain and therefore were identified as having recent origins on the Cumberland Plain, having been dispersed by human activity post European arrival.
- Despite forming part of the same genetic neighbourhood as populations on the Cumberland Plain, sampling sites west of Belford in the Hunter Valley and south of Ilford in the Mid-West were sufficiently genetically distinct and diverse that they could not be ruled out as representing natural populations falling within the northern genetic neighbourhood in these regions.

### 4.2.2 Reproductive biology

- There was no evidence for any clonality in our samples of *Acacia decurrens*.
- There was no notable signal of inbreeding observed in this species.
- Overall, this suggests a primarily outcrossing reproductive system and seed dominated dispersal.

### 4.2.3 Genetic diversity

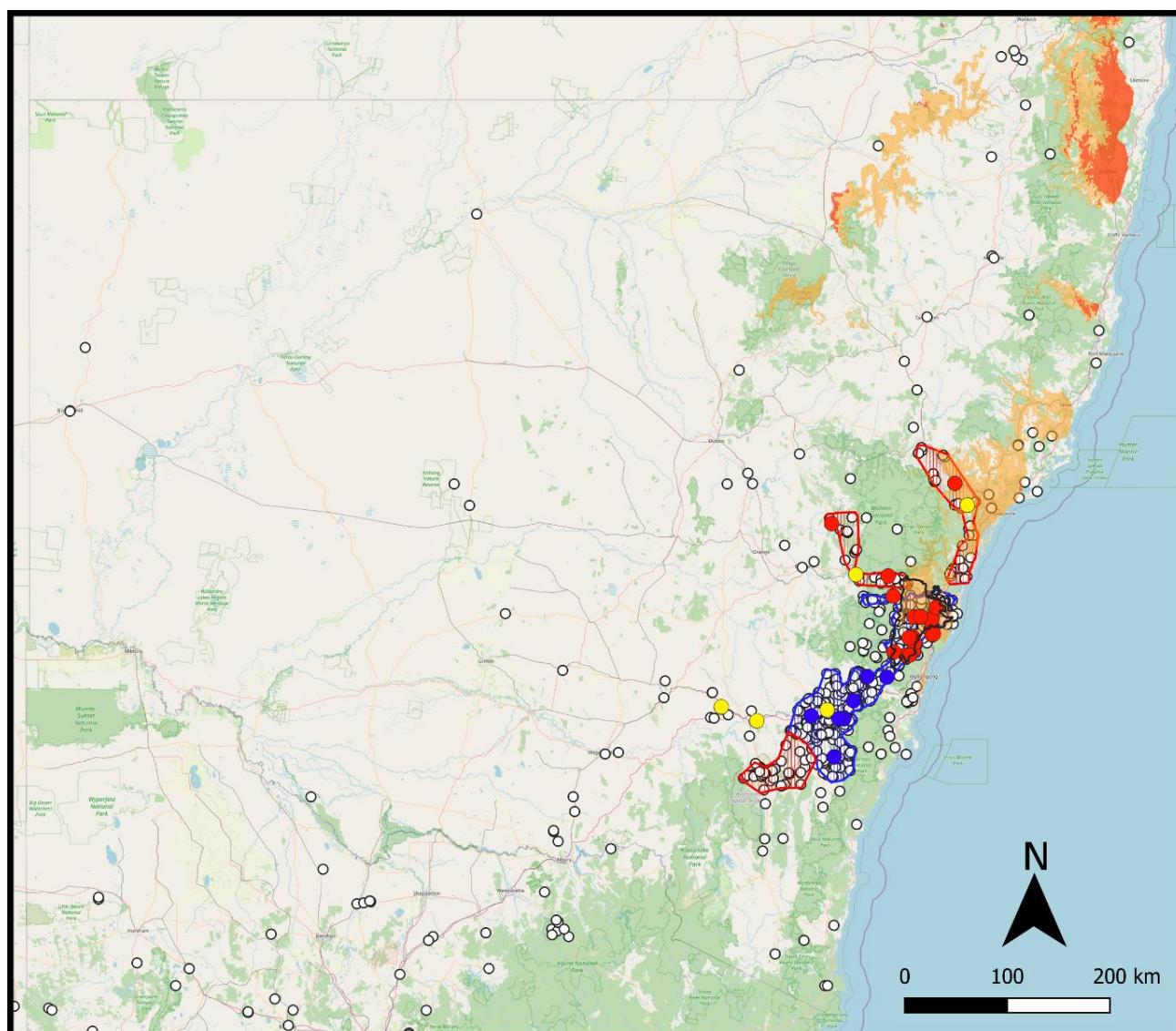
- Allelic diversity and heterozygosity were high at all sampled sites for *Acacia decurrens*.
- Across the species distribution, genetic diversity was greatest in Cumberland Plain populations.

#### 4.2.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 2 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios.

- Apart from maximising genetic diversity captured, this species presents very few issues for seed sourcing if seed is sourced from within the pre-European distribution of the species (see Figure 9). Given the uncertain provenance of material outside of the species natural range, it is important to avoid collecting seed from planted or naturalised populations.
- To maximise genetic diversity, we recommend sourcing seed from five sites within the Cumberland Plain genetic neighbourhood (which includes the Cumberland Plain, Blue Mountains and Hunter Valley geographic locations), maintaining five maternal lines per site (25 maternal lines in total) (Figure 10).
- To make plantings climate ready under a moderate climate change scenario, three sites can be sourced from within the climate matched areas (orange shading in Figure 9) of the northern Cumberland Plain and the lower Hunter Valley (Figure 10).
- It is not possible to use climate ready provenances under a severe climate model for this species as there are no populations in climate matched areas (red shading in Figure 9). This increases the importance of achieving high genetic diversity in restoration plantings as this will maximise adaptive potential and the chance of population persistence under severe climate change.





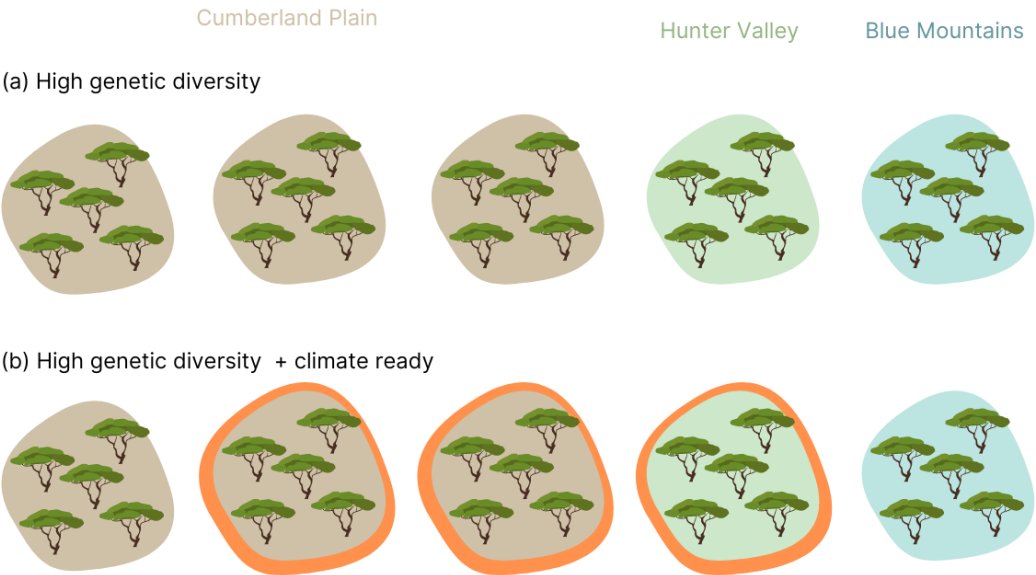
**Figure 9:** Sites sampled for genetic analysis (coloured circles) of *Acacia decurrens* indicating genetic neighbourhoods, with red circles being those belonging to the Cumberland Plain genetic neighbourhood, blue circles representing sites belonging to southern genetic neighbourhood and yellow circles representing sites that were genetically related to populations on the Cumberland Plain and therefore considered to represent recent range expansion due to human activity. The confident native range of the species is indicated by the blue striped region, with the red striped regions being plausibly natural areas of occurrence. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models. Note that this species has been brought into cultivation and escaped in regions well outside its native range as indicated in the figure.



**Table 2:** Sampling effort required to ensure capture of at least 90% of observed genetic diversity under different seed sourcing scenarios for *Acacia decurrens*. Note there are no populations in climate ready conditions for this species under severe climate change models. Additionally, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                                              | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site?                                                                                                                                      |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >13 sites x 1 mother plants per site = >13 maternal lines.                                                                                                                                                      | 4 sites x 5 mother plants per site = 20 maternal lines total                                                                                                                                                                                          |
| Genetic neighbourhood  | 16 sites x 1 mother plants per site = 16 maternal lines.                                                                                                                                                        | 5 sites x 5 mother plants per site = 25 maternal lines total (Depicted schematically in Figure 10a.)                                                                                                                                                  |
| Moderate climate ready | 16 sites – at least 8 located within climate matched areas (as shown in orange in Figure 9), 8 otherwise spread across northern genetic neighbourhood. 16 sites x 1 mother plants per site = 16 maternal lines. | 5 sites – at least 3 located within climate matched areas (as shown in orange in Figure 9), 2 otherwise spread across northern genetic neighbourhood. 5 sites x 5 mother plants per site = 25 maternal lines. (Depicted schematically in Figure 10b.) |
| Severe climate ready   | NA – no sites suitable                                                                                                                                                                                          | NA – no sites suitable                                                                                                                                                                                                                                |

**Seed source strategy for *Acacia decurrens***



**Figure 10:** Schematic representation of seed collection effort required for *Acacia decurrens* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, and (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 9). Severe climate ready provenances are not available for *A. decurrens*. Each circle represents a collection site and each tree represents a maternal line.

### 4.3 *Acacia falcata*



*Acacia falcata* is a widespread and easily collected small, upright shrub with a preference for disturbed habitat. It occurs north from Bermagui into Queensland and west to Denman and Murrurundi (Figure 11). All sampled populations are highly inbred, with minimal gene flow between populations. Several planted populations, identified by their close relationship to sites in very disparate regions and location beside highways, were unintentionally sampled and excluded from analyses, however this suggests there is already some human impacts on the patterns of genetic diversity for this species. Due to the high levels of inbreeding observed, which contribute to a lack of genetic structuring, this species could not be modelled for addition to the Restore and Renew Webtool.

#### 4.3.1 Genetic structuring and neighbourhoods

- Due to the lack of outcrossing and gene flow across the landscape, no genetic neighbourhoods could be established across the NSW portion of *Acacia falcata*'s distribution. Thus, due to the extremely high level of genetic structuring with each site being a distinct lineage, this species is treated differently to others in this report.
- While a simplification of reality, as all collected sites were highly genetically distinct from one another with no geographic pattern to relative distinctness, to determine sampling effort required to meet diversity capture targets we look at the species entire NSW distribution.
- Four populations collected from along highways appeared to be planted, with a source population on the south coast, and were excluded from further analyses.

#### 4.3.2 Reproductive biology

- *Acacia falcata* showed a strong signal for both inbreeding and clonal reproduction at many sites sampled, implying there is little mixing of genetic diversity between populations.
- The species has a single stemmed growth form and there are no recorded observations of resprouting or vegetative reproduction, implying the observed clonality and inbreeding is due to selfing and/or apomixis (the production of clonal seed).
- As a short-lived coloniser of disturbed sites (Benson & McDougall, 1996), it is likely that movement of genetic material across the landscape is related to rare seed dispersal events with limited interbreeding of populations.

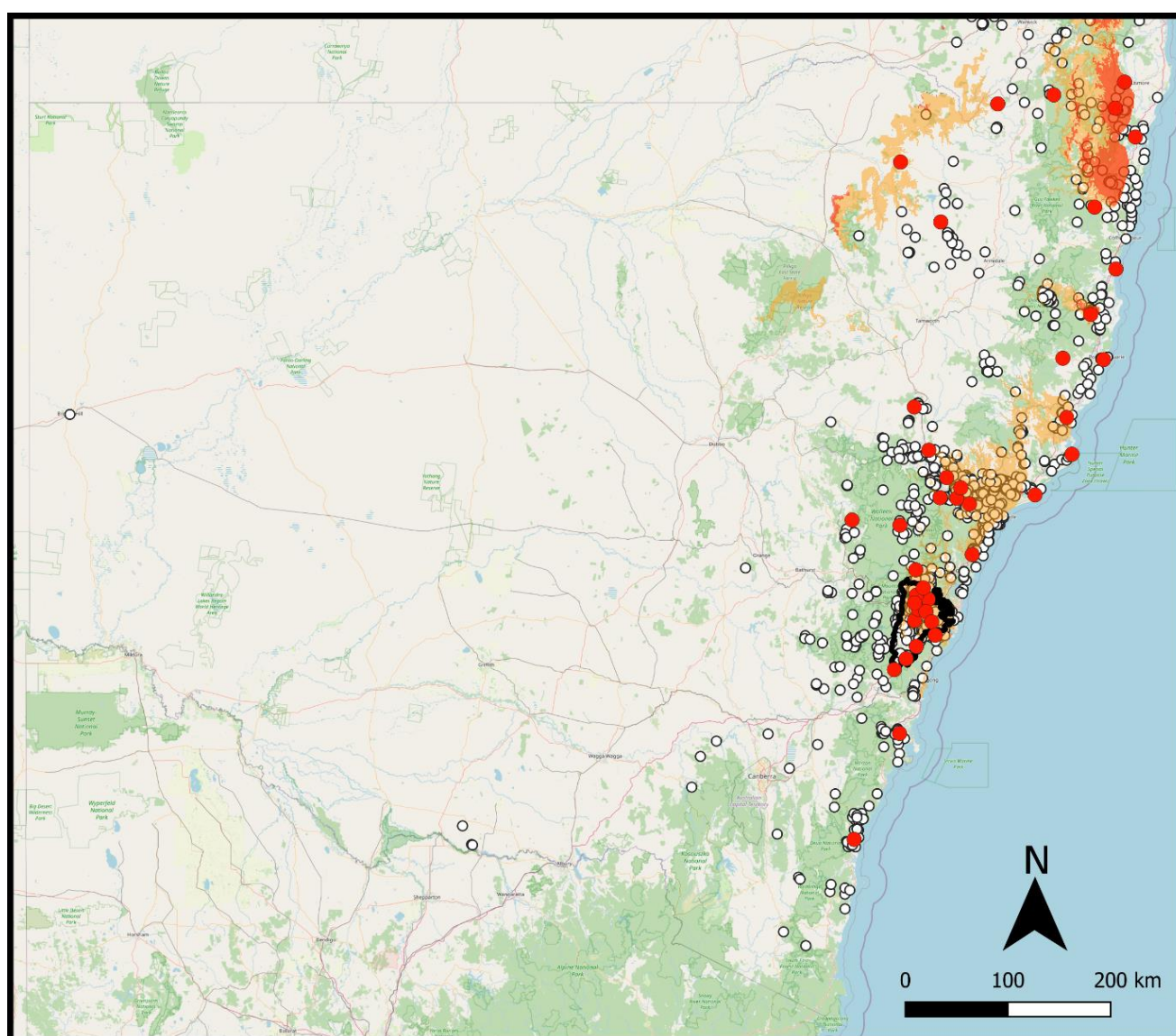
#### 4.3.3 Genetic diversity

- Due to the high level of inbreeding observed for this species, genetic diversity at all sites was extremely low.
- Populations with higher diversity were observed at Yarravel Nature Reserve on the mid-north coast and several sites on the Cumberland Plain.

#### 4.3.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 3 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios.

- As seed may be clonal (genetically identical to the parent and produced via apomixis) and/or highly inbred, the benefit of sampling from an increased number of sites vs more maternal lines per site is greater for this species than others in this report, as that is the only way to confidently increase the captured genetic diversity.
- Overall, for *Acacia falcata*, we recommend a lower investment of effort into optimal sourcing of seed for use in restoration plantings and seed production area establishment, as the improvements to restoration success and sustainability are anticipated to be low per unit of investment.
- Under a moderate climate change model, sampling from the northern Cumberland Plain and lower Hunter Valley provides climate ready provenances (orange shading in Figure 11).
- Under a severe climate change model, climate ready samples can be sourced from populations in the northern river's region of NSW (red shading in Figure 11).



**Figure 11:** Sites sampled for genetic analysis of *Acacia falcata* are shown with redpoints, with no genetic neighbourhoods established for this species. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models.



**Table 3:** Sampling effort required to ensure capture of at least 90% of observed genetic diversity under different seed sourcing scenarios for *Acacia falcata*. Note that there was no relative genetic structuring observed for this species due to the highly inbred sites leading to high genetic distinctness of all sites, and therefore its entire NSW distribution is treated as a single entity. Additionally, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                               | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                      |
|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >11 sites x 1 mother plants per site = >11 maternal lines.                                                                                                                                       | 8 sites x 5 mother plants per site = 40 maternal lines total                                                                                                                                         |
| NSW wide distribution  | 29 sites x 1 mother plants per site = 29 maternal lines.                                                                                                                                         | 19 sites x 5 mother plants per site = 95 maternal lines total                                                                                                                                        |
| Moderate climate ready | 29 sites – at least 15 located within climate matched areas (as shown in orange in Figure 11), 14 otherwise spread across distribution. 29 sites x 1 mother plants per site = 29 maternal lines. | 19 sites – at least 10 located within climate matched areas (as shown in orange in Figure 11), 9 otherwise spread across distribution. 19 sites x 5 mother plants per site = 95 maternal lines total |
| Severe climate ready   | 29 sites – at least 15 located within climate matched areas (as shown in red in Figure 11), 14 otherwise spread across distribution. 29 sites x 1 mother plants per site = 29 maternal lines.    | 19 sites – at least 10 located within climate matched areas (as shown in red in Figure 11), 9 otherwise spread across distribution. 19 sites x 5 mother plants per site = 95 maternal lines total    |



## 4.4 *Acacia implexa*



*Acacia implexa* is a tall shrub to tree that is known to vigorously sucker from its root system, which lead to clonal individuals being collected at several sites during this study. The species is widespread in NSW, on the inland slopes of the Great Dividing Range (GDR ) from the Victorian border to the Queensland border, and on the South Coast, Sydney Basin and Hunter Valley. Despite many records of the species from the coastal side of the GDR north of the Hunter Valley, we found most to be the morphologically similar *Acacia floribunda* or *Acacia melanoxydon*. Thus, we believe the extent of *A. implexa* populations in northern coastal parts of NSW is significantly over estimated due to incorrect species identifications in vegetation surveys. This species has been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

### 4.4.1 Genetic structuring and neighbourhoods

- Two poorly differentiated genetic neighbourhoods were identified for *Acacia implexa* (Figure 12), one from inland of the Great Dividing Range and the other from the coastal side of the GDR in the southern half of the state. Due to our lack of samples from the northern coastal regions of NSW, we cannot say what genetic neighbourhood these regions belong to, should populations be located.
- Several populations in the Upper Hunter and at Colo on the northern extent of the Cumberland Plain are intermediate between the two broad genetic neighbourhoods, suggesting an intergradation in this broad region.

### 4.4.2 Reproductive biology

- *Acacia implexa* is known to sucker extensively, and therefore it is no surprise that we found clonal individuals at many of the sites sampled for genetic analysis.
- There was no signal of inbreeding at any of the sampled sites.
- Overall, this suggests a primarily outcrossing reproductive system and dispersal via both vegetative suckering and seed.

### 4.4.3 Genetic diversity

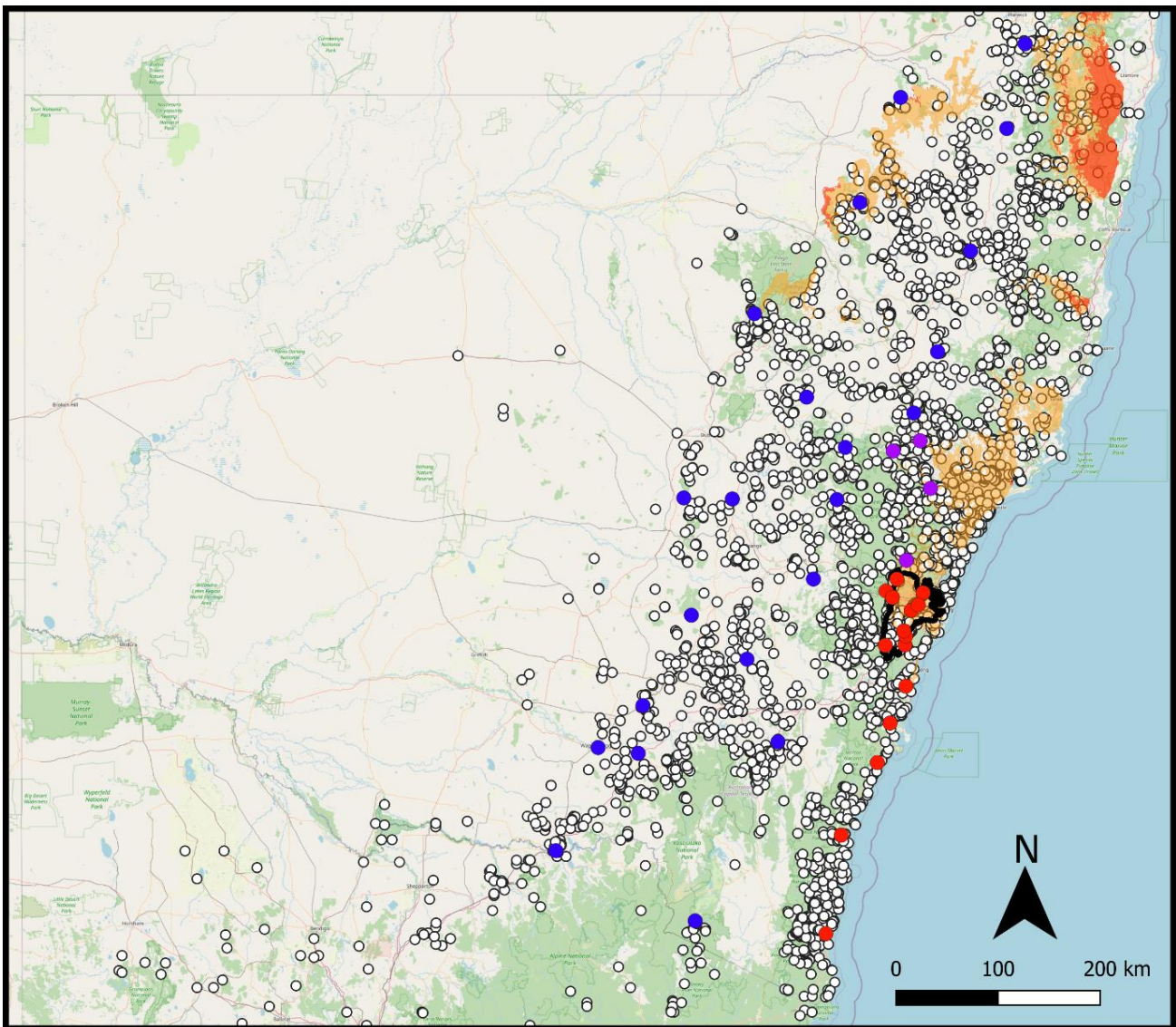
- Within site heterozygosity was high for all sites for *Acacia implexa*.
- Genetic diversity was spread evenly across the landscape, with no identifiable regions that supported notably high or low diversity.

### 4.4.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 4 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios.

- Special care will need to be taken to ensure seed is coming from genetically distinct mothers at each site, given the extensive vegetative reproduction the species undertakes. This can be achieved by only collecting from a single stem at each patch of the species and collecting from patches with at least 50m of separation.
- Our findings suggest that seed is most likely outcrossed as clonality appears primarily due to vegetative spread, and thus the risk of inbred seed being collected is of low concern for this species. This could be confirmed with genotyping of seed/seedlings of known genotyped mothers.
- While identification of this species has proved problematic, the seeds and associated funicle, which is white and folded below the seed, are the most diagnostic trait for the species, clearly distinguishing it from the otherwise similar *A. melanoxyton* (orange funicle wrapped around the seed). Therefore, misidentification of seed collections can be effectively managed by identification based upon the seed itself.
- For *Acacia implexa*, we advocate sourcing seed from eight sites across the South Coast, Cumberland Plain and Hunter Valley, maintaining five maternal lines per site (40 maternal lines in total) (Figure 13)
- To make plantings climate ready under a moderate climate change scenario, four sites can be located within the orange climate matched area in Figure 12.
- Under a severe climate change model, climate ready seed is more difficult to source, with climate matching showing provenances in the Northern Rivers region being suitable. However, this is the region where we were unable to locate populations of the species, and so the relationship of any populations that may be located and used for seed sourcing in this area to the populations on the Cumberland Plain is unclear.





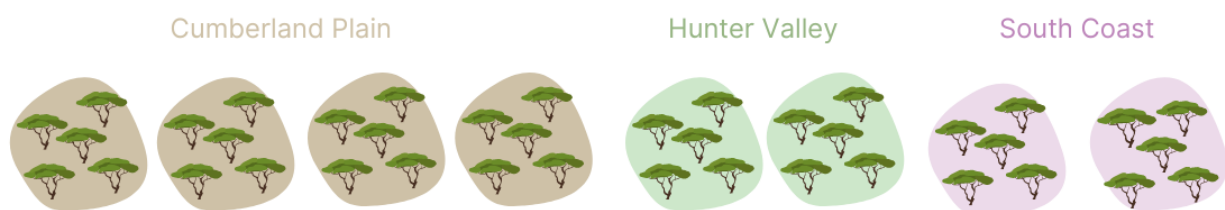
**Figure 12:** Sites sampled for genetic analysis of *Acacia implexa* indicating genetic neighbourhoods, with red circles being those belonging to the neighbourhood present on the Cumberland Plain, blue shapes representing sites belonging to other genetic neighbourhoods, and purple sites being mixed populations which partially belong to the neighbourhood present on the Cumberland Plain. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models. Note the lack of sampling sites on the north coast of NSW where we failed to find the species at many sites where records exist.

**Table 4:** Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Acacia implexa*. Note that there were no populations that occurred in climate ready conditions for this species under serve climate change models. Additionally, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

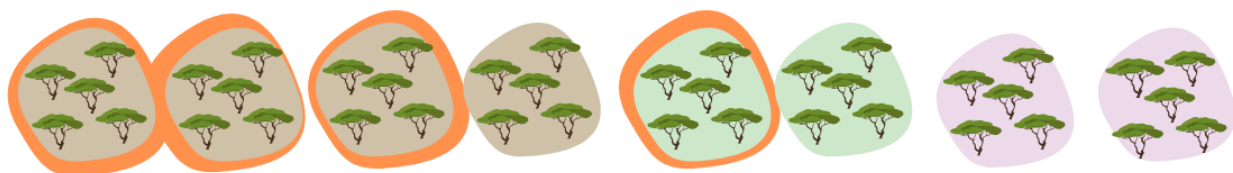
| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                                      | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                                                                     |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >9 sites x 1 mother plants per site = >9 maternal lines.                                                                                                                                                | 5 sites x 5 mother plants per site = 25 maternal lines total                                                                                                                                                                                        |
| Genetic neighbourhood  | 14 sites x 1 mother plants per site = 14 maternal lines.                                                                                                                                                | 8 sites x 5 mother plants per site = 40 maternal lines total (Depicted schematically in Figure 14a.)                                                                                                                                                |
| Moderate climate ready | 14 sites – at least 7 located within climate matched areas (as shown in orange in Figure 12), 7 otherwise spread across coastal neighbourhood. 14 sites x 1 mother plants per site = 14 maternal lines. | 8 sites – at least 4 located within climate matched areas (as shown in orange in Figure 12), 4 otherwise spread across coastal neighbourhood. 8 sites x 5 mother plants per site = 40 maternal lines total. (Depicted schematically in Figure 13b.) |
| Severe climate ready   | NA – insufficient sampling to make recommendations                                                                                                                                                      | NA – insufficient sampling to make recommendations                                                                                                                                                                                                  |

### Optimised Seed source strategy for *Acacia implexa*

#### (a) High genetic diversity



#### (b) High genetic diversity + climate ready



**Figure 13:** Schematic representation of seed collection effort required for *Acacia implexa* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, and (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 11). Each circle represents a collection site and each tree represents a maternal line.



## 4.5 *Acacia parramattensis*



*Acacia parramattensis* is an erect shrub to small tree that appears to spread vegetatively via root suckering. It has a limited natural distribution, occurring through the Sydney Basin and Southern Highlands, as far south-west as the ACT. We also located outlying populations west of Cooma near Coornartha Nature Reserve and ~3 km east of Wallendbeen on Burley Griffin Way. While we hypothesise these populations likely result from human mediated dispersal, the genetic data was not conclusive either way for these populations being natural vs recently dispersed by humans. Therefore, we include them in our analyses and guidelines for the species. This species has been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

### 4.5.1 Genetic structuring and neighbourhoods

- Sampled sites from across the entire distribution of *Acacia parramattensis* did not form multiple genetic clusters, and evidence for isolation by distance was weak, leading to the conclusion there is only the one genetic neighbourhood which encompasses the entire distribution of the species (Figure 14).
- This included populations identified and sampled from west of Cooma and east of Wallendbeen that are well outside the established distribution of the species, which otherwise extends only as far south and west as Canberra. We hypothesise these populations may be due to human mediated dispersal of the species, but due to the species lack of genetic structuring, this hypothesis could not be tested with our genetic data.

### 4.5.2 Reproductive biology

- Clones were identified at several sites, likely the result of root-suckering (Benson & McDougall, 1996).
- There was no evidence for widespread inbreeding for this species.
- Overall, this suggests a primarily outcrossing reproductive system and dispersal via both vegetative suckering and seed.

### 4.5.3 Genetic diversity

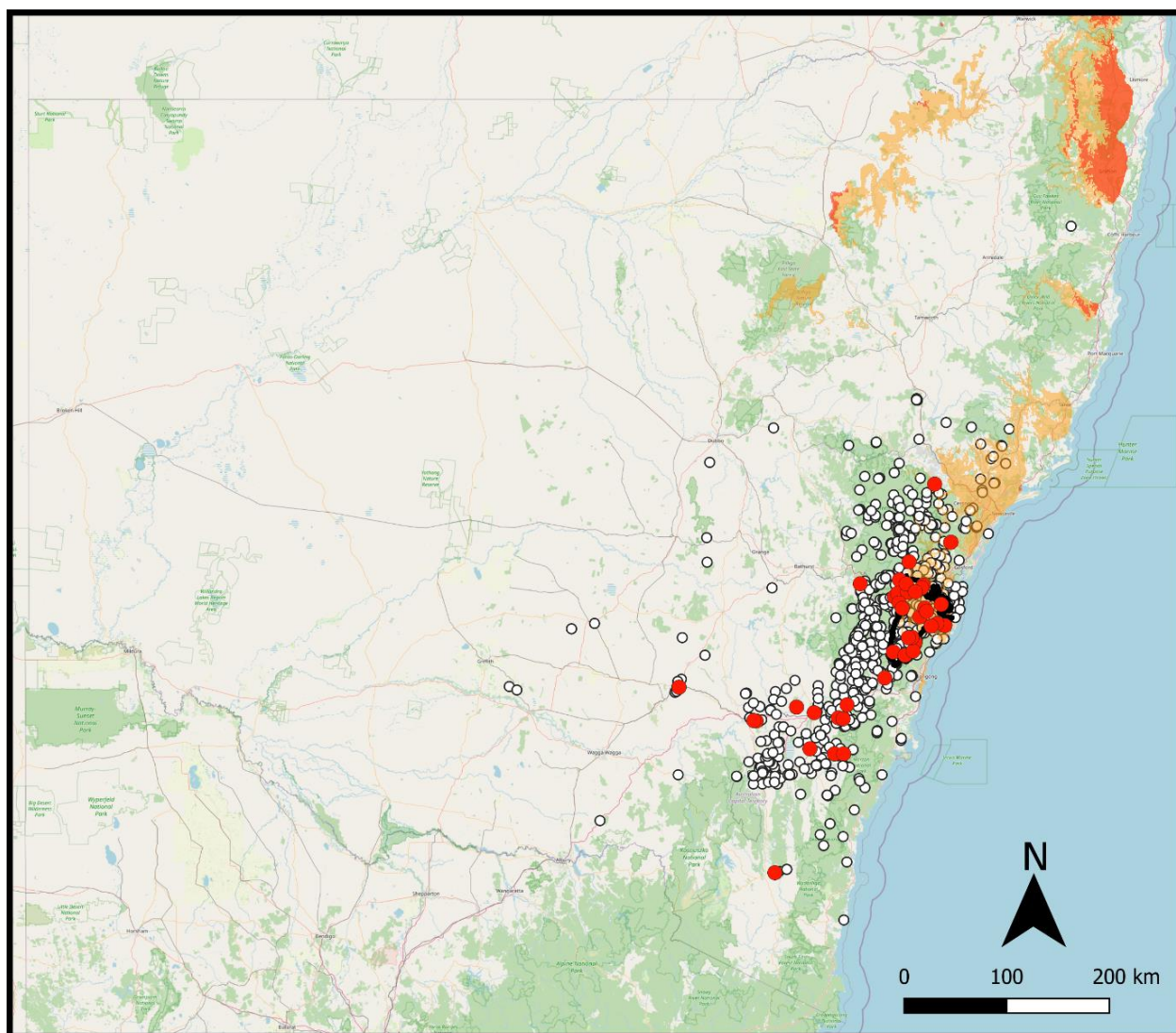
- Within site allelic richness and heterozygosity was moderate for *Acacia parramattensis*.
- This diversity was evenly spread across the landscape with no regions supporting notably high or low genetic diversity identified.

### 4.5.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 5 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios.

- As some clonal individuals were recorded, care should be taken when sourcing seed that mother plants that are adequately separated (>50m) are chosen.

- Otherwise, this species presents no other major concerns when sourcing seed apart from maximising the amount of genetic diversity captured.
- For *Acacia parramattensis*, we advocate sourcing seed from eight sites across the species entire distribution, maintaining five maternal lines per site (40 maternal lines in total) (Figure 15).
- To make plantings climate ready under a moderate climate change scenario, four sites can be sourced from within the climate matched areas (orange shading in Figure 14) of the northern Cumberland Plain and the lower Hunter Valley.
- It is not possible to use climate ready provenances under a severe climate model for this species as there are no populations in climate matched areas (red shading in Figure 14). This increases the importance of achieving high genetic diversity in restoration plantings as this will maximise adaptive potential and the chance of population success under severe climate change.

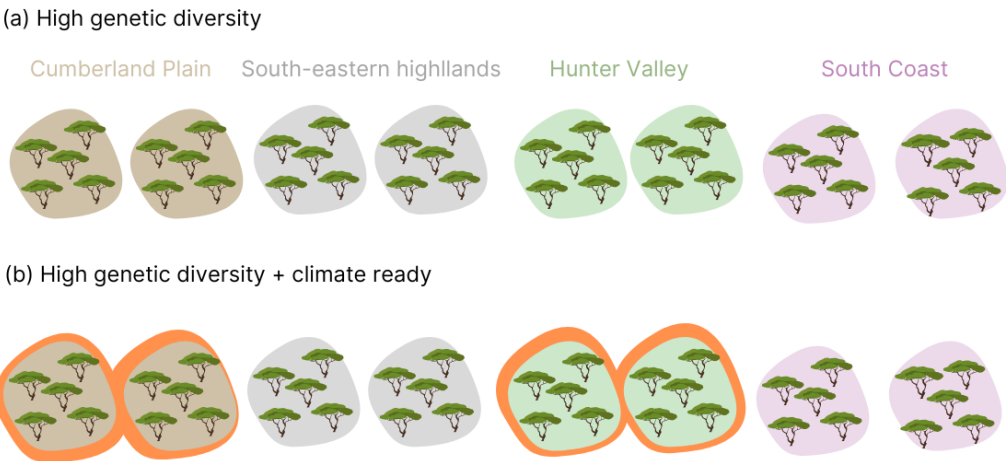


**Figure 14:** Sites sampled for genetic analysis of *Acacia parramattensis* indicating genetic neighbourhoods, with red circles being those belonging to the only neighbourhood observed across the species distribution. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models.

**Table 5:** Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Acacia parramattensis*. Note that there was only one genetic neighbourhood for this species and no populations that occurred in climate ready conditions for this species under serve climate change models. Additionally, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                               | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                                                           |
|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >17 sites x 1 mother plants per site = >17 maternal lines.                                                                                                                                       | 7 sites x 5 mother plants per site = 35 maternal lines total                                                                                                                                                                              |
| Genetic neighbourhood  | 22 sites x 1 mother plants per site = 22 maternal lines.                                                                                                                                         | 8 sites x 5 mother plants per site = 40 maternal lines total (Depicted schematically in Figure 15a)                                                                                                                                       |
| Moderate climate ready | 22 sites – at least 11 located within climate matched areas (as shown in orange in Figure 14), 11 otherwise spread across distribution. 22 sites x 1 mother plants per site = 22 maternal lines. | 8 sites – at least 4 located within climate matched areas (as shown in orange in Figure 14), 4 otherwise spread across distribution. 8 sites x 5 mother plants per site = 40 maternal lines total (Depicted schematically in Figure 15b.) |
| Severe climate ready   | NA – no sites suitable                                                                                                                                                                           | NA – no sites suitable                                                                                                                                                                                                                    |

**An optimised seed source strategy for *Acacia parramattensis***



**Figure 15:** Schematic representation of seed collection effort required for *Acacia parramattensis* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, and (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 14). Each circle represents a collection site and each tree represents a maternal line.



## 4.6 *Breynia oblongifolia*



*Breynia oblongifolia* is a common and widespread understorey shrub species of eastern Australia and occurs across a range of habitat types ranging from wet sclerophyll forests to woodlands. The species has an obligate pollination mutualism with *Epicephala* moth species (Finch et al., 2018). This species has been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

### 4.6.1 Genetic structuring and neighbourhoods

- Three genetic neighbourhoods were identified within NSW for *Breynia oblongifolia*, however they were all part of a larger genetic latitudinal cline along the east coast which showed an isolation by distance pattern (Figure 16).
- The breaks between the three neighbourhoods aligned with biogeographic features, namely the Illawarra Escarpment and the Hastings and Macleay rivers. Thus, the Cumberland Plain populations of *Breynia oblongifolia* formed the southern extent of the central NSW genetic neighbourhood.

### 4.6.2 Reproductive biology

- All sampled sites were predominately outcrossing and showed no evidence for inbreeding.
- Clonal samples were found a small number of sites, but all were collected near one another (within 5m) and therefore likely to be the result of suckering, a behaviour widely noted for the species when in cultivation, rather than clonal reproduction.
- Overall, this suggests a primarily outcrossing reproductive system and seed dominated dispersal.

### 4.6.3 Genetic diversity

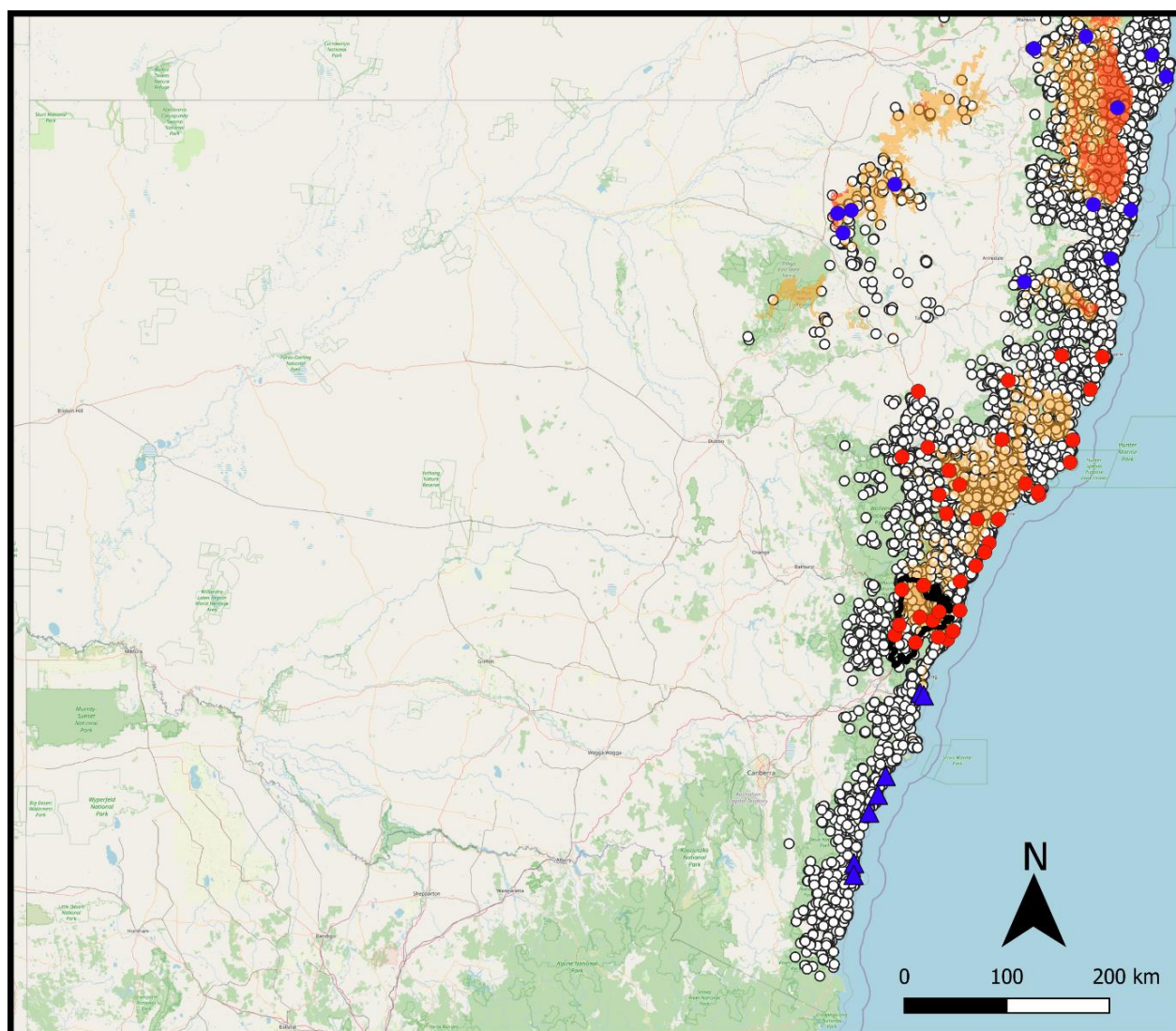
- Allelic diversity and heterozygosity within sites were moderate to high for *Breynia oblongifolia*.
- The observed genetic diversity was uneven across the landscape, being highest in the south coast of NSW and lower to the north.



#### 4.6.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 6 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios.

- Overall, apart from maximising genetic diversity captured, we identify minimal special considerations when using *Breynia oblongifolia* in restoration activities on the Cumberland Plain.
- For *Breynia oblongifolia*, we advocate sourcing seed from six sites across the area between the Illawarra escarpment and Hastings River, maintaining five maternal lines per site (30 maternal lines in total) (Figure 17).
- To make plantings climate ready under a moderate climate change scenario, three sites can be sourced from within the climate matched areas (orange shading in Figure 16) of the northern Cumberland Plain, the lower Hunter Valley and Mid-north Coast.
- To make plantings climate ready under a severe climate change scenario, we recommend increasing the number of sites to eight, as the climate ready sites will be located within the northern genetic neighbourhood and not local to the Cumberland Plain. Four sites should be sourced from within the climate matched areas (red shading in Figure 16) of the Northern Rivers region and four from across the central NSW genetic neighbourhood between the Cumberland Plain and Hastings River.



**Figure 16:** Sites sampled for genetic analysis of *Breynia oblongifolia* indicating genetic neighbourhoods, with red circles being those belonging to the neighbourhood present on the Cumberland Plain and blue shapes representing sites belonging to other genetic neighbourhoods. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models.

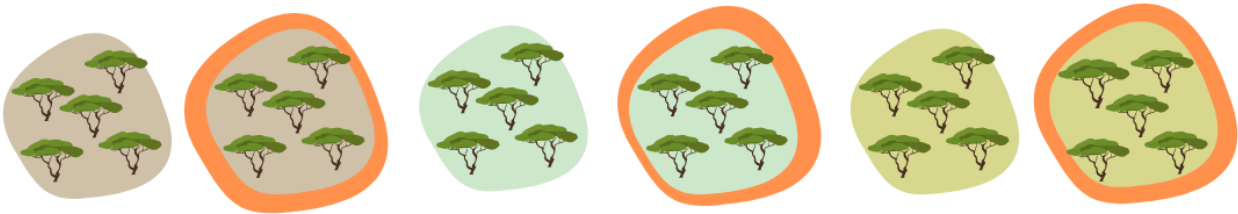
**Table 6:** Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Breynia oblongifolia*. Note, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is no maintenance of maternal lines                                                                                                                   | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                                                                            |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >8 sites x 1 mother plants per site = >8 maternal lines.                                                                                                                                                               | 4 sites x 5 mother plants per site = 20 maternal lines total                                                                                                                                                                                               |
| Genetic neighbourhood  | 21 sites x 1 mother plants per site = 21 maternal lines.                                                                                                                                                               | 6 sites x 5 mother plants per site = 30 maternal lines total (Depicted schematically in Figure 17a.)                                                                                                                                                       |
| Moderate climate ready | 21 sites – at least 11 located within climate matched areas (as shown in orange in Figure 16), 10 otherwise spread across central genetic neighbourhood. 21 sites x 5 mother plants per site = 21 maternal lines total | 6 sites – at least 3 located within climate matched areas (as shown in orange in Figure 16), 3 otherwise spread across central genetic neighbourhood. 3 sites x 5 mother plants per site = 30 maternal lines total (Depicted schematically in Figure 17b.) |
| Severe climate ready   | 24 sites – at least 12 located within climate matched areas (as shown in red in Figure 16), 12 otherwise spread across central genetic neighbourhood. 24 sites x 5 mother plants per site = 24 maternal lines total    | 8 sites – at least 4 located within climate matched areas (as shown in red in Figure 16), 4 otherwise spread across central genetic neighbourhood. 8 sites x 5 mother plants per site = 40 maternal lines total (Depicted schematically in Figure 17c.)    |

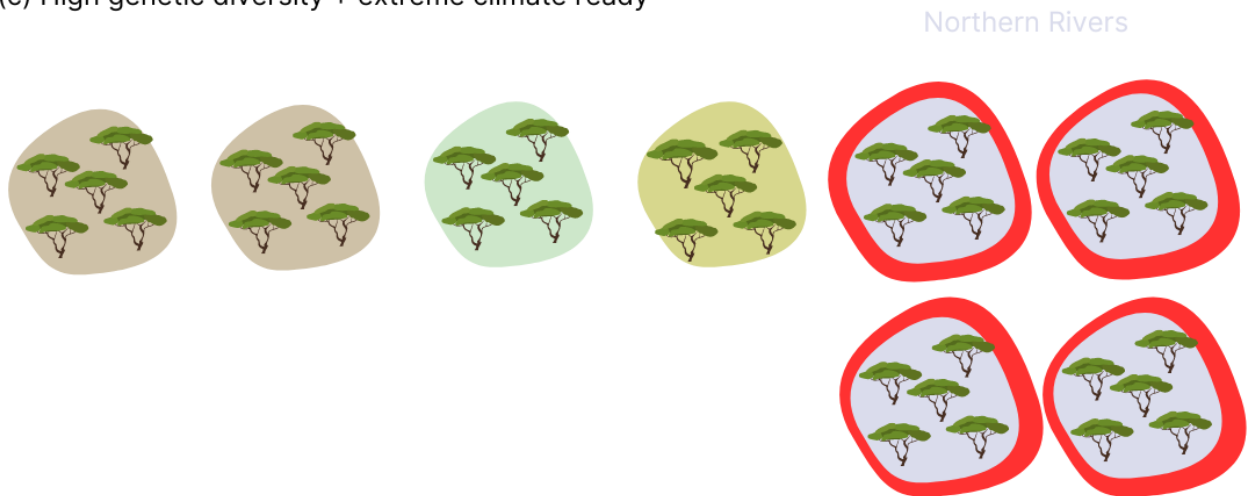
## (a) High genetic diversity



## (b) High genetic diversity + moderate climate ready



## (c) High genetic diversity + extreme climate ready



**Figure 17:** Schematic representation of seed collection effort required for *Breynia oblongifolia* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 16), and (c) incorporating severe climate readiness into the above scenario by sampling from severe climate matched sites (red shading in Figure 16). Each circle represents a collection site and each tree represents a maternal line.



## 4.7 *Bursaria spinosa*



*Bursaria spinosa* is a spinescent shrub to small tree that can often dominate the understorey of eucalypt woodlands in the absence of fire. It occurs across the mesic parts of south-eastern and eastern Australia. There are two subspecies of *Bursaria spinosa* identified primarily based upon leaf shape, size and colour and petal size, both of which occur in NSW and were sampled for this study. *Bursaria spinosa* subspecies *spinosa* is the more common of the two, occurring across the distribution of the species apart from the highland areas of south-eastern NSW and Victoria where *Bursaria spinosa* subspecies *lasiophylla* occurs instead. These subspecies have been treated by some as distinct species, but they are noted to intergrade with each other, and our genetic data do not support them as being reproductively isolated. Due to the existence of these divergent lineages that are not discretely distributed geographically, this species could not be modelled on the Restore and Renew Webtool.

### 4.7.1 Genetic structuring and neighbourhoods

- Two levels of genetic structuring were observed for *Bursaria spinosa*.
- The broadest level of genetic structure was between two lineages somewhat congruent with the two subspecies, however, coastal populations from the Sydney Basin north, which are currently considered to represent the subspecies *spinosa* were found to be part of the same lineage as subspecies *lasiophylla* populations from the southern highlands and south coast of NSW (Green triangles in Figure 18).
- All populations inland of the Great Divide along with those on the Cumberland Plain and some north coast populations formed the second major lineage believed to correspond to the typical subspecies *spinosa*.
- Within these two broad lineages two and four genetic neighbourhoods were identified respectively.
- The neighbourhood that included all Cumberland Plain sites also encompassed all Sydney Basin and central west sites, as well as some from the southern Hunter Valley.

### 4.7.2 Reproductive biology

- There was no evidence for any clonality in our samples of *Bursaria spinosa*.
- There was no notable signal of inbreeding observed in this species.
- Overall, this suggests a primarily outcrossing reproductive system and dispersal via seed.

### 4.7.3 Genetic diversity

- Allelic richness and heterozygosity were generally moderate to high within sites for *Bursaria spinosa*.
- However, sites belonging to the north coast neighbourhood (green triangles in Figure 18) showed notable lower genetic diversity than sites belonging to all other neighbourhoods.

#### 4.7.4 Seed sourcing for ecological restoration within the Cumberland Plain

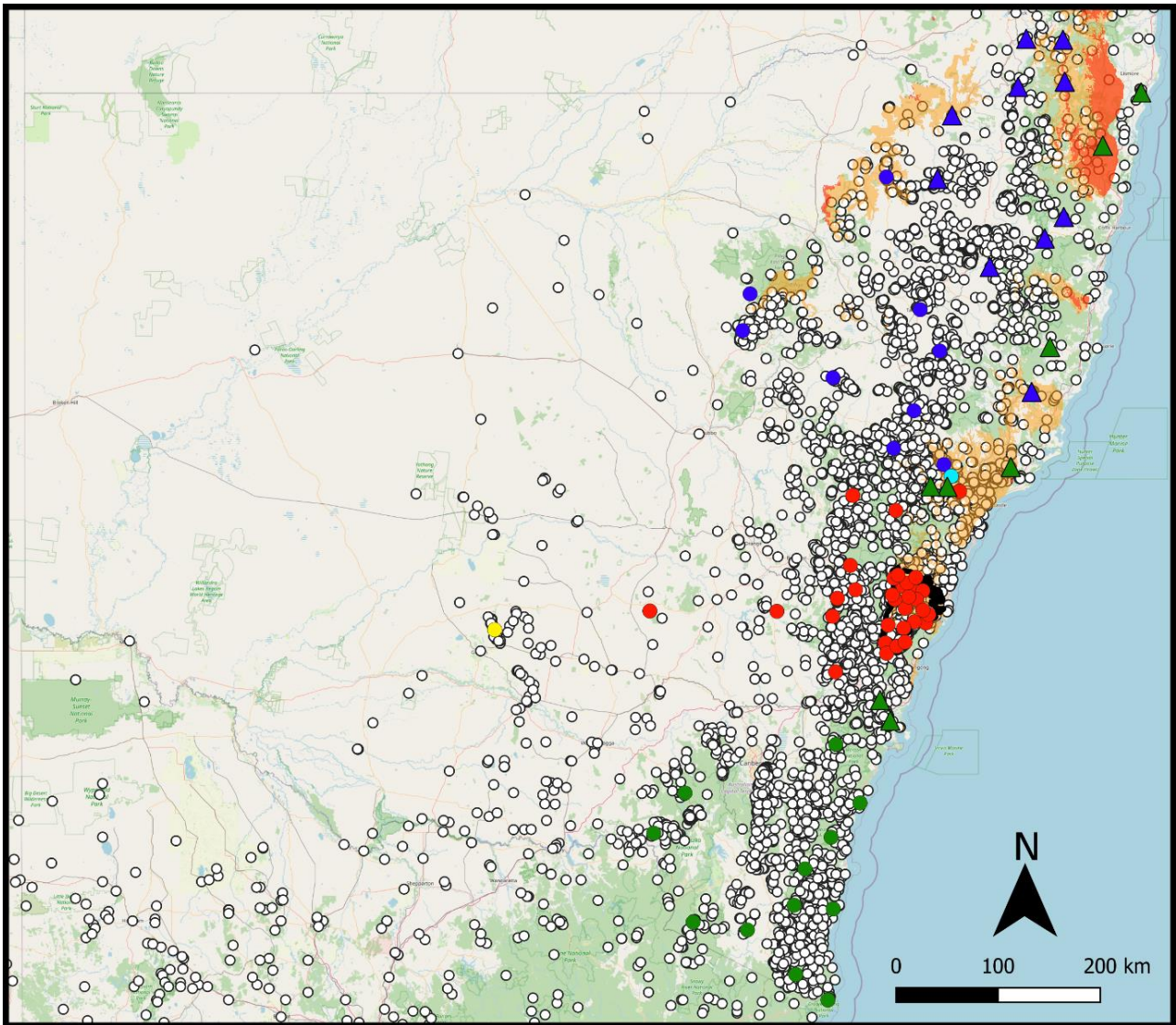
Table 7 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios.

- Care is needed if seed is sourced from the Hunter Valley as representatives of both major lineages as well as multiple neighbourhoods within the typical subspecies lineage were identified in this region (see Figure 18). These lineages cannot be identified with confidence by morphology alone.
- Outside this region, *Bursaria spinosa* presents no other major concerns when sourcing seed apart from maximising the amount of genetic diversity captured.
- For *Bursaria spinosa*, we advocate sourcing seed from eight sites across the Cumberland Plain, Blue Mountains and central west district, maintaining five maternal lines per site (40 maternal lines in total) (Figure 19).
- To make plantings climate ready under a moderate climate change scenario, four sites can be sourced from within the climate matched areas (orange shading in Figure 18) of the northern Cumberland Plain while avoiding the lower Hunter Valley where multiple genetic lineages occur. However, we do not advocate for this approach as it is not possible to adequately space the four required sites (i.e. 20 km apart) in the climate ready area of the Cumberland Plain.
- Given that populations in the severe climate ready area are more closely related to subspecies *lasiophylla* than the subspecies *spinosa* populations on the Cumberland Plain, we recommend against using the severe climate ready seed sourcing approach for *Bursaria spinosa*.

**Table 7:** Table 7: Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Bursaria spinosa*. Note that genetic neighbourhoods are not geographically distinct and do not match subspecies, so genotyping is the only method to identify which neighbourhood maternal lines belong to on the north coast of NSW. For this reason, we do not provide climate ready seed sourcing solutions for this species. Additionally, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                                | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                   |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >19 sites x 1 mother plants per site = >19 maternal lines.                                                                                                                                        | 5 sites x 5 mother plants per site = 25 maternal lines total                                                                                                                                      |
| Genetic neighbourhood  | 22 sites x 1 mother plants per site = 22 maternal lines.                                                                                                                                          | 8 sites x 5 mother plants per site = 40 maternal lines total (Depicted schematically in Figure 19a.)                                                                                              |
| Moderate climate ready | NA – due to complex genetic patterns north of the Cumberland Plain and the potential for co-occurring subspecies, we refrain from providing climate ready provenancing solutions for this species | NA – due to complex genetic patterns north of the Cumberland Plain and the potential for co-occurring subspecies, we refrain from providing climate ready provenancing solutions for this species |
| Severe climate ready   | NA – due to complex genetic patterns north of the Cumberland Plain and the potential for co-occurring subspecies, we refrain from providing climate ready provenancing solutions for this species | NA – due to complex genetic patterns north of the Cumberland Plain and the potential for co-occurring subspecies, we refrain from providing climate ready provenancing solutions for this species |

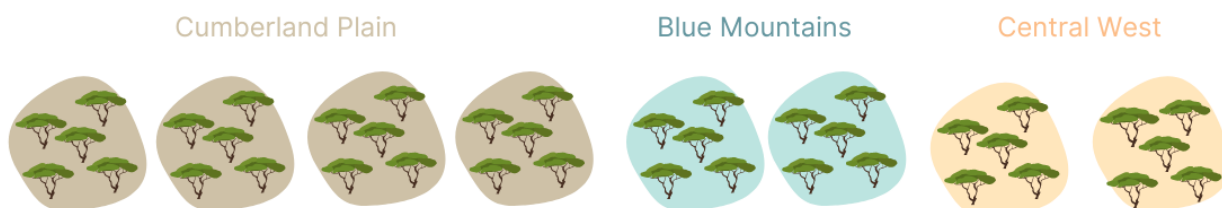




**Figure 18:** Sites sampled for genetic analysis of *Bursaria spinosa* indicating genetic neighbourhoods, with red circles being those belonging to the neighbourhood present on the Cumberland Plain, blue shapes representing sites belonging to other genetic neighbourhoods which were most closely related to the neighbourhood present on the Cumberland Plain, and other coloured points representing more distinct genetic neighbourhoods. Note that there is overlap in the distributions of the major lineages for this species, which partially correspond to subspecies. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models.

## An optimised seed source strategy for *Bursaria spinosa*

(a) High genetic diversity



**Figure 19:** Schematic representation of seed collection effort required for *Bursaria spinosa* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area. Each circle represents a collection site and each tree represents a maternal line.



## 4.8 *Dodonaea viscosa*



*Dodonaea viscosa* is a very widespread species (Figure 20), occurring across much of Australia, and subtropical and tropical areas of Asia, Africa, and the Pacific. It grows as a shrub to small tree and has highly variable leaf morphology, although it can be recognised by the sticky surface of its leaves. There are seven subspecies of *Dodonaea viscosa* identified primarily based upon leaf shape and size, six of which occur in NSW and were sampled for this study. *Dodonaea viscosa* subspecies *viscosa* does not occur in NSW and was not sampled for this study. Due to the existence of these divergent lineages that are not discretely distributed geographically, this species could not be modelled on the Restore and Renew Webtool.

### 4.8.1 Genetic structuring and neighbourhoods

- We find seven highly distinct genetic lineages across NSW, however there was only a partial correlation with subspecies.
- Two subspecies with distinct ecological ranges were genetically distinct from the other subspecies, subspecies *angustissima* (which itself was split into two discrete genetic lineages) from the arid zone subspecies *burmanniana* from the north-east corner of the state.
- Subspecies' *angustifolia*, *cuneata*, *mucronata* and *spathulata* were intermixed between the four remaining lineages.
- All populations from the Cumberland Plain were part of the most widespread and commonly observed lineage which included samples of subspecies' *angustifolia*, *cuneata* and *spathulata*.

### 4.8.2 Reproductive biology

- There was no evidence for any clonality in our samples of *Dodonaea viscosa*.
- There was no notable signal of inbreeding observed in this species.
- Overall, this suggests a primarily outcrossing reproductive system and dispersal via seed.

#### 4.8.3 Genetic diversity

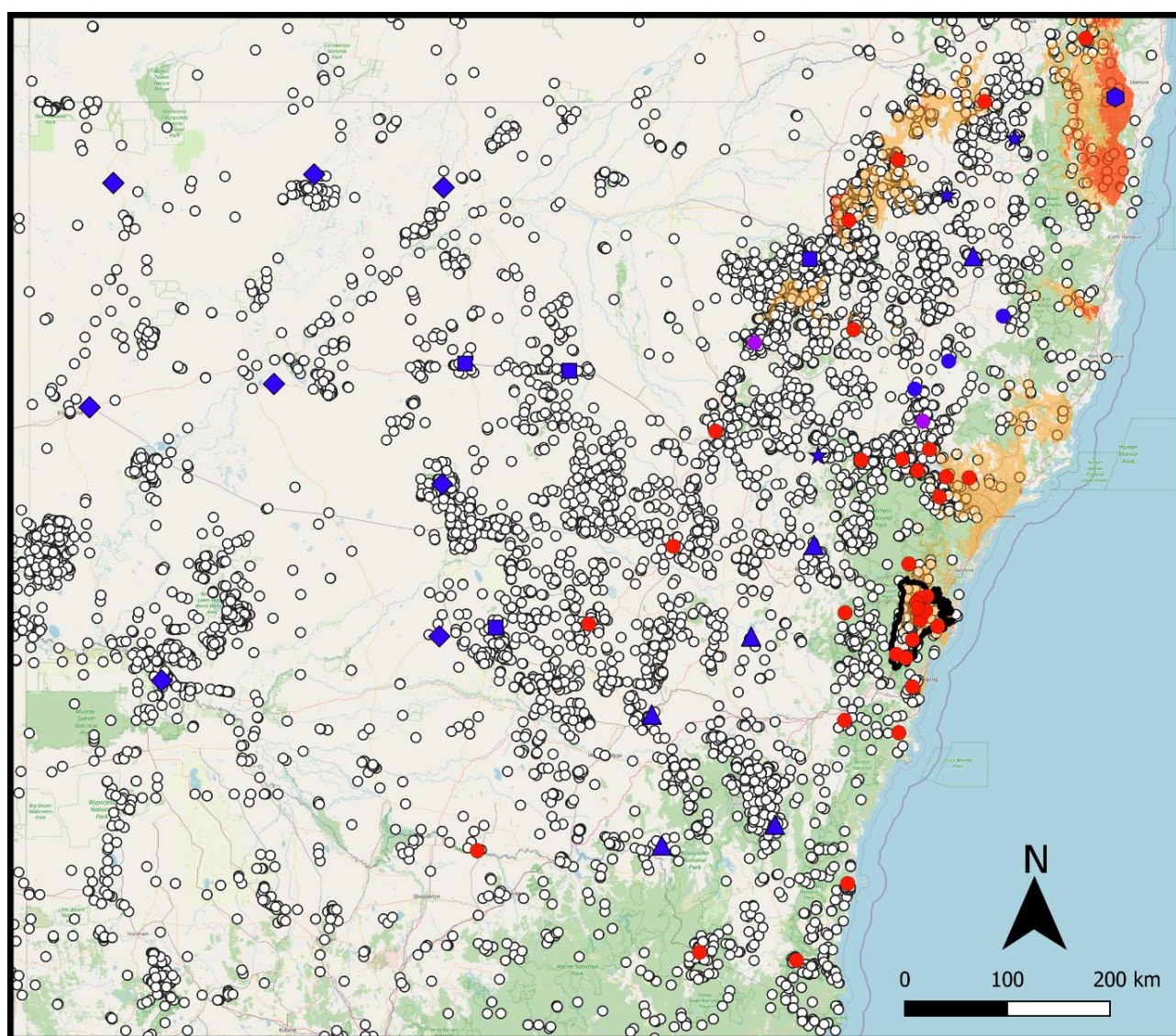
- Allelic diversity and heterozygosity varied greatly between sampling sites for *Dodonaea viscosa*.
- There were several genetically discrete but geographically overlapping lineages observed for this species, and there was a strong association between which lineage/s were present at a sampling site and the genetic diversity of that site.
- The most common and widespread lineage showed lower genetic diversity on average than several of the rarer lineages, potentially reflecting the processes that gave rise to these lineages such as polyploidisation.

#### 4.8.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 8 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios.

- As there are several highly discrete genetic lineages within *Dodonaea viscosa* that do not align with subspecies classification and have overlapping distributions, identification of genetic neighbourhoods and thus local provenances is not viable for this species without genetic data. Thus, we make general observations on how to capture representative genetic diversity, but the results of this approach will be less reliable for this species than others covered in this report.
- For restoration on the Cumberland Plain, only subspecies *angustifolia* and *cuneata* should be used, as they are native to the Cumberland Plain and are likely better adapted to the environmental conditions in the region. However, the use of only these two subspecies does not guarantee that seed belongs to the same genetic lineage as natural populations on the Cumberland Plain.
- Limiting seed sourcing to the lower Hunter Valley, Cumberland Plain and south coast appears to be the most reliable approach to limit the chance that seed is not part of the genetic lineage local to the Cumberland Plain. Within these regions, we advocate sourcing seed from six sites, maintaining five maternal lines per site (30 maternal lines in total) (Figure 21).
- To make plantings climate ready under a moderate climate change scenario, three sites can be sourced from within the climate matched areas (orange shading in Figure 20) of the northern Cumberland Plain and the lower Hunter Valley.
- Under a severe climate change model, it becomes more complex to source climate ready seed, as most populations in the suitable source region (Figure 20) of the Northern Rivers are subspecies *burmanniana*. This subspecies is genetically distinct from all other sample subspecies and inhabits different habitats (wet sclerophyll forests) than subspecies *angustifolia* and *cuneata* that occur on the Cumberland Plain (dry sclerophyll forests and woodlands). However, a previous study showed that subspecies *burmanniana* may be widely interbreeding with other subspecies (Christmas et al., 2018), therefore potentially reducing the risk of using it for restoration on the Cumberland Plain. We do not provide sample and site number for this scenario as we have only a single site sampled within the climate ready region which is also our only samples of subspecies *burmanniana*, meaning any guidelines would be based on insufficient data.





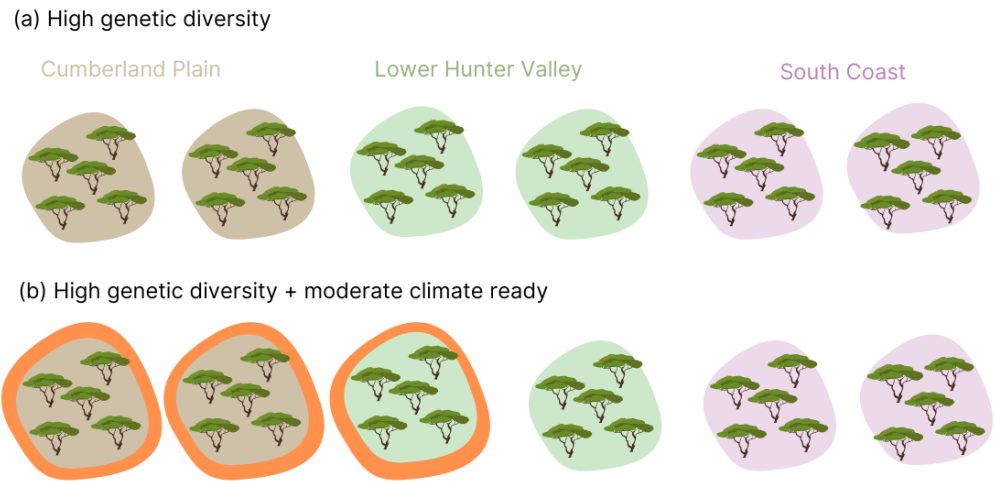
**Figure 20:** Sites sampled for genetic analysis of *Dodonaea viscosa* indicating genetic neighbourhoods, with red circles being those belonging to the neighbourhood present on the Cumberland Plain, blue shapes representing sites belonging to other genetic neighbourhoods, and purple sites being mixed populations which partially belong to the neighbourhood present on the Cumberland Plain. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models. Major neighbourhoods are poorly related to morphological subspecies, and many have overlapping distributions.



**Table 8:** Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Dodonaea viscosa*. Note that genetic neighbourhoods are not geographically distinct and do not match subspecies, so genotyping is the only method to identify which neighbourhood maternal lines belong to. For this reason, along with the presence of subspecies *burmanniana*, which has a different environmental niche than other subspecies, in the climate ready regions, we do not provide climate ready seed sourcing solutions for this species under a severe climate change scenario. Additionally, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                                                                                                            | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                                                                                                                                       |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >10 sites x 1 mother plants per site = >10 maternal lines.                                                                                                                                                                                                                    | 6 sites x 5 mother plants per site = 30 maternal lines total                                                                                                                                                                                                                                                          |
| Genetic neighbourhood  | 18 sites x 1 mother plants per site = 18 maternal lines.                                                                                                                                                                                                                      | 6 sites x 5 mother plants per site = 30 maternal lines total (Depicted schematically in Figure 21a.)                                                                                                                                                                                                                  |
| Moderate climate ready | 18 sites – at least 9 located within climate matched areas of the northern Cumberland Plain and lower Hunter Valley (as shown in orange in Figure 20), 9 otherwise spread across the Cumberland Plain and South Coast. 9 sites x 1 mother plant per site = 18 maternal lines. | 6 sites – at least 3 located within climate matched areas of the northern Cumberland Plain and lower Hunter Valley (as shown in orange in Figure 20), 3 otherwise spread across the Cumberland Plain and South Coast. 6 sites x 5 mother plants per site = 30 maternal lines. (Depicted schematically in Figure 21b.) |
| Severe climate ready   | NA – due to complex genetic patterns north of the Cumberland Plain and the potential for co-occurring subspecies, we refrain from providing climate ready provenancing solutions for this species                                                                             | NA – due to complex genetic patterns north of the Cumberland Plain and the potential for co-occurring subspecies, we refrain from providing climate ready provenancing solutions for this species                                                                                                                     |

**An optimised seed source strategy for *Dodonaea viscosa***



**Figure 21:** Schematic representation of seed collection effort required for *Dodonaea viscosa* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, and (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 20). Each circle represents a collection site and each tree represents a maternal line.

## 4.9 *Eucalyptus baueriana*



A tree of riparian habitats in eucalypt woodlands, *Eucalyptus baueriana* occurs in several discrete areas of south-eastern Australia, including parts of the South Coast and Cumberland Plain of NSW. Three subspecies of *Eucalyptus baueriana* are known from Victoria, however only *Eucalyptus baueriana* subspecies *baueriana* is known from NSW. We were able to sample the Victorian distribution of this species to cover its entire known distribution. This species is a riparian specialist, growing in gallery forest along waterways in woodland communities. This species has been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

### 4.9.1 Genetic structuring and neighbourhoods

- Within NSW, there was only a single genetic neighbourhood observed for *Eucalyptus baueriana*, despite the patchy nature of its distribution as a riparian species (Figure 22).
- A second genetic neighbourhood was confined to Victoria and therefore not of relevance to this report.
- Samples were collected from three sites on the Cumberland Plain where the species had been planted, and of these, one site belonged to the genetic neighbourhood that only occurred within Victoria, suggesting the seed had been sourced interstate, while the other two sets of samples belonged to the NSW neighbourhood, although one of these sets of samples appeared to originate from populations on the south coast. The final of these three populations was not closely related to any sampled wild site.

### 4.9.2 Reproductive biology

- Clones were identified at a single site for *Eucalyptus baueriana*, on the Deddick River in Victoria, where only 14 individuals are known to exist (Fahey et al., 2022). Given this population is highly restricted and in habitat not typical for the species, the finding of these clones does not imply clonality is common nor widespread for this species.
- There was no notable signal of inbreeding observed in this species.
- Overall, this suggests a primarily outcrossing reproductive system and dispersal via seed.
- As is common in eucalypts, hybrids were identified in our dataset and removed before calculations of genetic diversity.

### 4.9.3 Genetic diversity

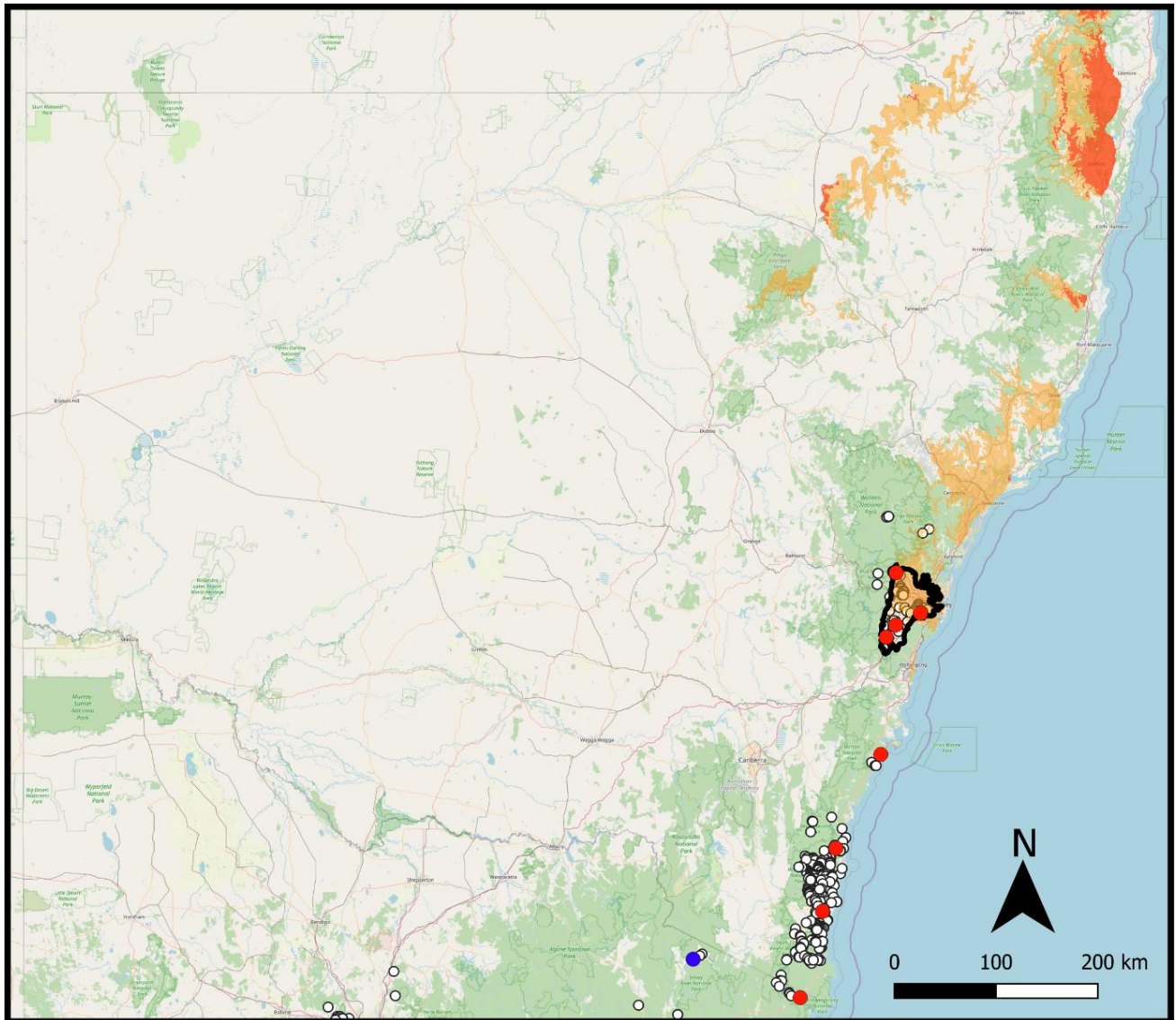
- Within site allelic diversity was high for *Eucalyptus baueriana*, however heterozygosity was lower than what has been observed for other *Eucalyptus* species which generally have high heterozygosity.
- Populations on the Cumberland Plain exhibited lower levels of genetic diversity than south coast populations of this species.

#### 4.9.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 9 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios.

- Care will need to be paid to avoiding sampling from hybrid mothers, but also all seedlings should be screened based on morphology and/or genetics to identify and remove hybrid individuals as these will be more common in the seed collections than the mature individuals due to selective pressures as the plants age.
- Otherwise, we find minimal consideration for seed sourcing for *Eucalyptus baueriana* if genetic diversity is considered.
- For *Eucalyptus baueriana*, we advocate sourcing seed from five sites across the Cumberland Plain and South Coast, maintaining five maternal lines per site (25 maternal lines in total) (Figure 23).
- To make plantings climate ready under a moderate climate change scenario, three sites can be sourced from within the climate matched areas (orange shading in Figure 22) of the northern Cumberland Plain. These should be located one each at the three suitable populations of the species:
  - The Georges River between Liverpool and East Hills
  - Ropes Creek in Ropes Crossing and North St Marys
  - Redbank Creek, North Richmond
- It is not possible to use climate ready provenances under a severe climate model for this species as there are no populations in climate matched areas (red shading in Figure 22). This increases the importance of achieving high genetic diversity in restoration plantings as this will maximise adaptive potential and the chance of population success under severe climate change.



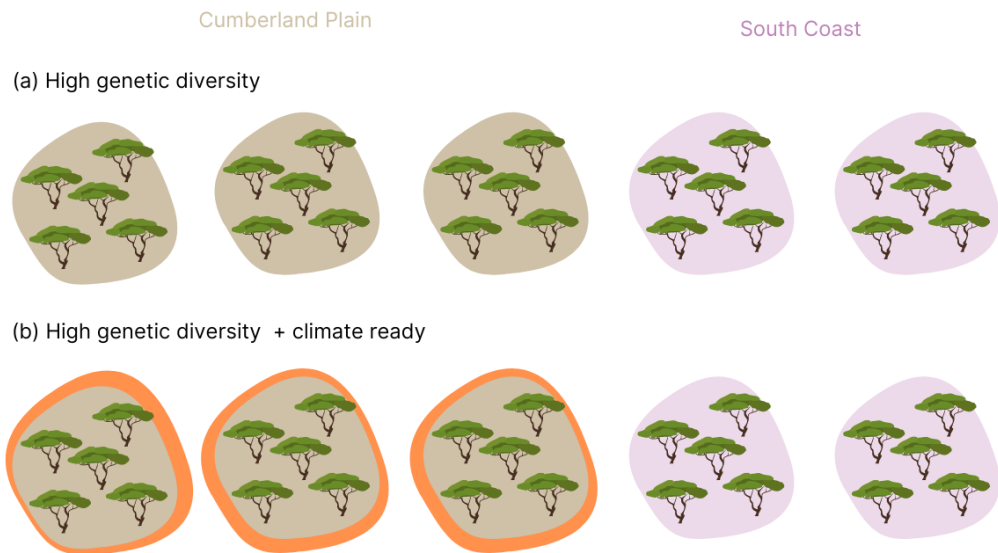


**Figure 22:** Sites sampled for genetic analysis of *Eucalyptus baueriana* indicating genetic neighbourhoods, with red circles being those belonging to the neighbourhood present on the Cumberland Plain and blue points (only present in Victoria) representing sites belonging to other genetic neighbourhoods. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models. Note several collections from the Cumberland Plain are excluded as they were realised to be cultivated post-collecting and therefore not relevant to establishing natural patterns of genetic variation across the species distribution.

**Table 9:** Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Eucalyptus baueriana*. Note that as the Cumberland Plain represents the northern limit of the species distribution, we do not provide climate ready seed sourcing solutions for the severe climate change model for this species. Additionally, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in both the Cumberland Plain provenance only and genetic neighbourhood scenarios and therefore we cannot provide a specific target number of sites for these scenarios.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                                     | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                                       |
|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >5 sites x 1 mother plants per site = >5 maternal lines.                                                                                                                                               | 4 sites x 5 mother plants per site = 20 maternal lines total                                                                                                                                                          |
| Genetic neighbourhood  | >7 sites x 1 mother plants per site = >7 maternal lines.                                                                                                                                               | 5 sites x 5 mother plants per site = 25 maternal lines total (Figure 23a.)                                                                                                                                            |
| Moderate climate ready | 8 sites – at least 4 located within climate matched areas (as shown in orange in Figure 22), 4 otherwise spread across distribution within NSW. 8 sites x 1 mother plants per site = 8 maternal lines. | 5 sites – at least 3 located within climate matched areas (as shown in orange in Figure 22), 2 otherwise spread across distribution within NSW. 5 sites x 5 mother plants per site = 25 maternal lines. (Figure 23b.) |
| Severe climate ready   | NA – no sites suitable                                                                                                                                                                                 | NA – no sites suitable                                                                                                                                                                                                |

### Seed source strategy for *Eucalyptus baueriana*



**Figure 23:** Schematic representation of seed collection effort required for *Eucalyptus baueriana* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, and (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 22). Each circle represents a collection site and each tree represents a maternal line.



## 4.10 *Eucalyptus fibrosa*



*Eucalyptus fibrosa* is an ironbarked eucalypt species with a patchy distribution in woodland and dry forest habitats, occurring in areas of the South Coast, Sydney Basin, Hunter Valley, Central West, Northern Inland Slopes and North West Plains regions within NSW. In our investigation we included populations variously regarded as *Eucalyptus fibrosa* subspecies *nubilis* or *Eucalyptus nubilis* under a broad definition of the species. The distribution of this entity varies between authorities, but we find support for *Eucalyptus fibrosa* subspecies *fibrosa* being limited to coastal areas including the Cumberland Plain and Hunter Valley in NSW, with *Eucalyptus fibrosa* subspecies *nubilis* being genetically identifiable and including all populations from Goulburn River National Park west. No samples were included from the Capertee Valley area and so the assignment of this population to a subspecies is unclear. This species has been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

### 4.10.1 Genetic structuring and neighbourhoods

- Two well differentiated genetic neighbourhoods were observed for *Eucalyptus fibrosa*, corresponding to the two subspecies of *Eucalyptus fibrosa*, subspecies *fibrosa* on the coast and subspecies *nubilis* inland (Figure 24).
- One neighbourhood consisted of all populations of subspecies *fibrosa* from the South Coast, Sydney Basin, Hunter Valley and North Coast regions.
- Despite the large gap in the distribution of *Eucalyptus fibrosa* subspecies *fibrosa* between the Hunter Valley and northern NSW, there was no evidence for a large genetic divergence between these areas.
- The second included all inland populations of subspecies *nubilis* from Goulburn River National Park west.

### 4.10.2 Reproductive biology

- There was no evidence for any clonality in our samples of *Eucalyptus fibrosa*.
- There was no notable signal of inbreeding observed in this species.
- Overall, this suggests a primarily outcrossing reproductive system and dispersal via seed.
- As is common in eucalypts, hybrids, most commonly with *Eucalyptus siderophloia*, were identified in our dataset and removed before calculations of genetic diversity. These hybrids were most prevalent in the North Coast region where *Eucalyptus fibrosa*, *Eucalyptus siderophloia* and *Eucalyptus tetrapleura* co-occur, as has been noted in a previous study (Rutherford et al., 2019).

### 4.10.3 Genetic diversity

- Within site allelic diversity and heterozygosity was high for *Eucalyptus fibrosa*.
- A signal for subspecies *nubilis* being less genetically diverse than subspecies *fibrosa* was observed, but the degree of this difference was only minor.



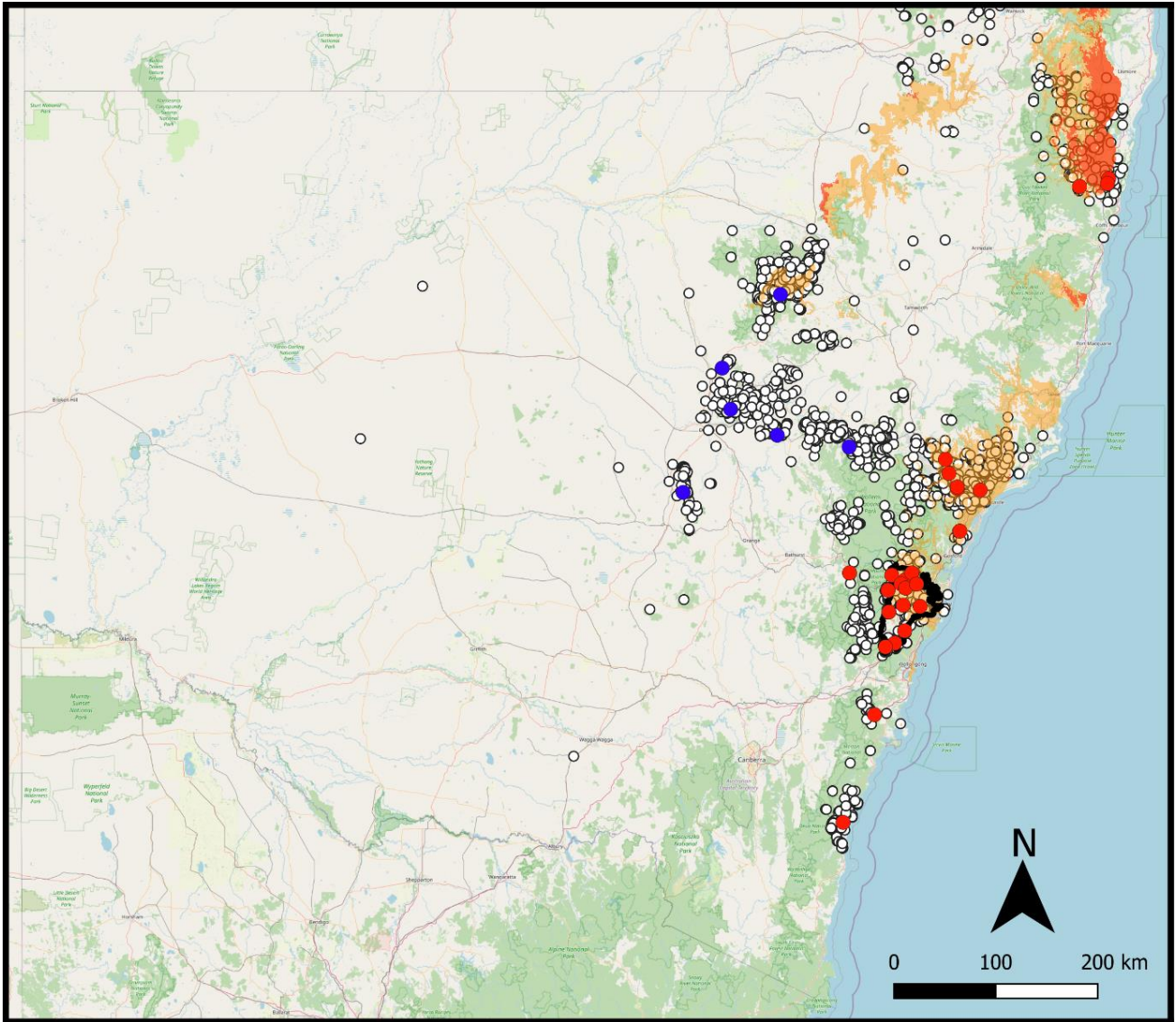
#### 4.10.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 10 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios

- Care will need to be paid to avoiding sampling from hybrid mothers, but also all seedlings should be screened based on morphology and/or genetics to identify and remove hybrid individuals.
- Given their high propensity for hybridising, *Eucalyptus fibrosa* seed from sites where *Eucalyptus siderophloia* also occurs should be avoided to limit the use of hybrid seed.
- Otherwise, we find minimal consideration for seed sourcing for *Eucalyptus fibrosa* if genetic diversity is considered.
- For *Eucalyptus fibrosa*, we advocate sourcing seed from five sites across coastal regions of NSW, maintaining five maternal lines per site (25 maternal lines in total) (Figure 25).
- To make plantings climate ready under a moderate climate change scenario, three sites can be sourced from within the climate matched areas (orange shading in Figure 24) of the northern Cumberland Plain, and the lower Hunter Valley.
- To make plantings climate ready under a severe climate change scenario, three sites can be sourced from within the climate matched areas in the Northern Rivers region (red shading in Figure 24).

**Table 10:** Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Eucalyptus fibrosa*. Note, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                                              | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                                            |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >14 sites x 1 mother plants per site = >14 maternal lines.                                                                                                                                                      | 4 sites x 5 mother plants per site = 20 maternal lines total                                                                                                                                                               |
| Genetic neighbourhood  | 18 sites x 1 mother plants per site = 18 maternal lines.                                                                                                                                                        | 5 sites x 5 mother plants per site = 25 maternal lines total (Figure 25a).                                                                                                                                                 |
| Moderate climate ready | 18 sites – at least 9 located within climate matched areas (as shown in orange in Figure 24), 9 otherwise spread across coastal genetic neighbourhood. 18 sites x 1 mother plants per site = 18 maternal lines. | 5 sites – at least 3 located within climate matched areas (as shown in orange in Figure 24), 2 otherwise spread across coastal genetic neighbourhood. 5 sites x 5 mother plants per site = 25 maternal lines (Figure 25b). |
| Severe climate ready   | 18 sites – at least 9 located within climate matched areas (as shown in red in Figure 24), 9 otherwise spread across coastal genetic neighbourhood. 18 sites x 1 mother plants per site = 18 maternal lines.    | 5 sites – at least 3 located within climate matched areas (as shown in red in Figure 24), 2 otherwise spread across coastal genetic neighbourhood. 5 sites x 5 mother plants per site = 25 maternal lines (Figure 25c).    |

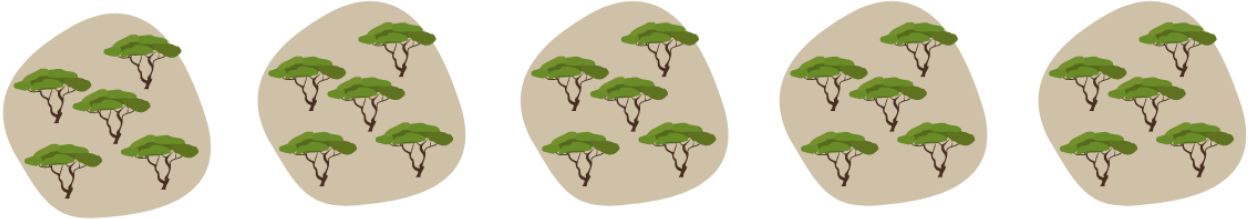


**Figure 24:** Sites sampled for genetic analysis of *Eucalyptus fibrosa* indicating genetic neighbourhoods, with red circles being those belonging to the neighbourhood present on the Cumberland Plain and blue circles representing sites belonging to other genetic neighbourhoods. Smaller white points represent records of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models. Note the large disjunction in the species distribution from the Hunter Valley to the north of Coffs Harbour which does not align with a break between genetic neighbourhoods.

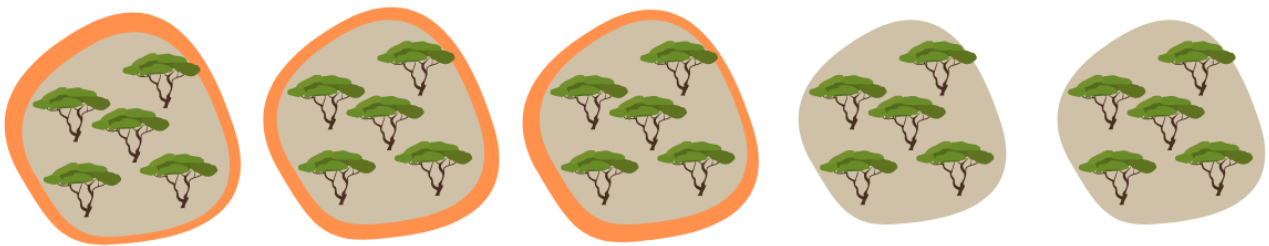
## Seed source strategy for *Eucalyptus fibrosa*

Coastal NSW

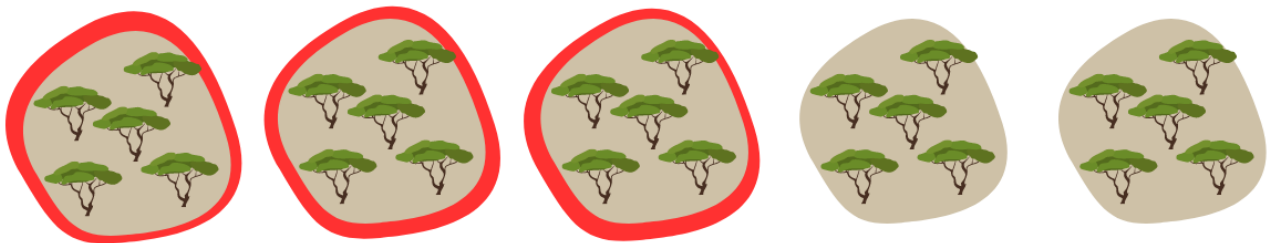
(a) High genetic diversity



(b) High genetic diversity + moderately climate ready



(c) High genetic diversity + severe climate ready



**Figure 25:** Schematic representation of seed collection effort required for *Eucalyptus fibrosa* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 24), and (c) incorporating severe climate readiness into the above scenario by sampling from severe climate matched sites (red shading in Figure 24). Each circle represents a collection site and each tree represents a maternal line.



#### 4.11 *Melaleuca decora*



A small tree with papery bark, *Melaleuca decora* occurs in woodlands on heavy soils, often in swampy areas. It occurs from the Shoalhaven district north to the Hunter Valley. While there are scattered records of this species in northern NSW (Figure 26), we were unable to sample from this region as the species occurs in swampy areas which were rendered inaccessible by high rainfall in 2022 and thus, we limit our comments to the populations south of the large disjunction in the species distribution between northern NSW and the Hunter Valley. This species has been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

##### 4.11.1 Genetic structuring and neighbourhoods

- *Melaleuca decora* was nearly panmictic where sampled, however the most westerly sampled site in the Hunter Valley at Hollydeen was highly distinct from other populations.
- Therefore, we identify two genetic neighbourhoods, one including all populations other than Hollydeen and the other only including the Hollydeen population.

##### 4.11.2 Reproductive biology

- There was no evidence for any clonality in our samples of *Melaleuca decora*.
- There was no notable signal of inbreeding observed in this species.
- Overall, this suggests a primarily outcrossing reproductive system and dispersal via seed.

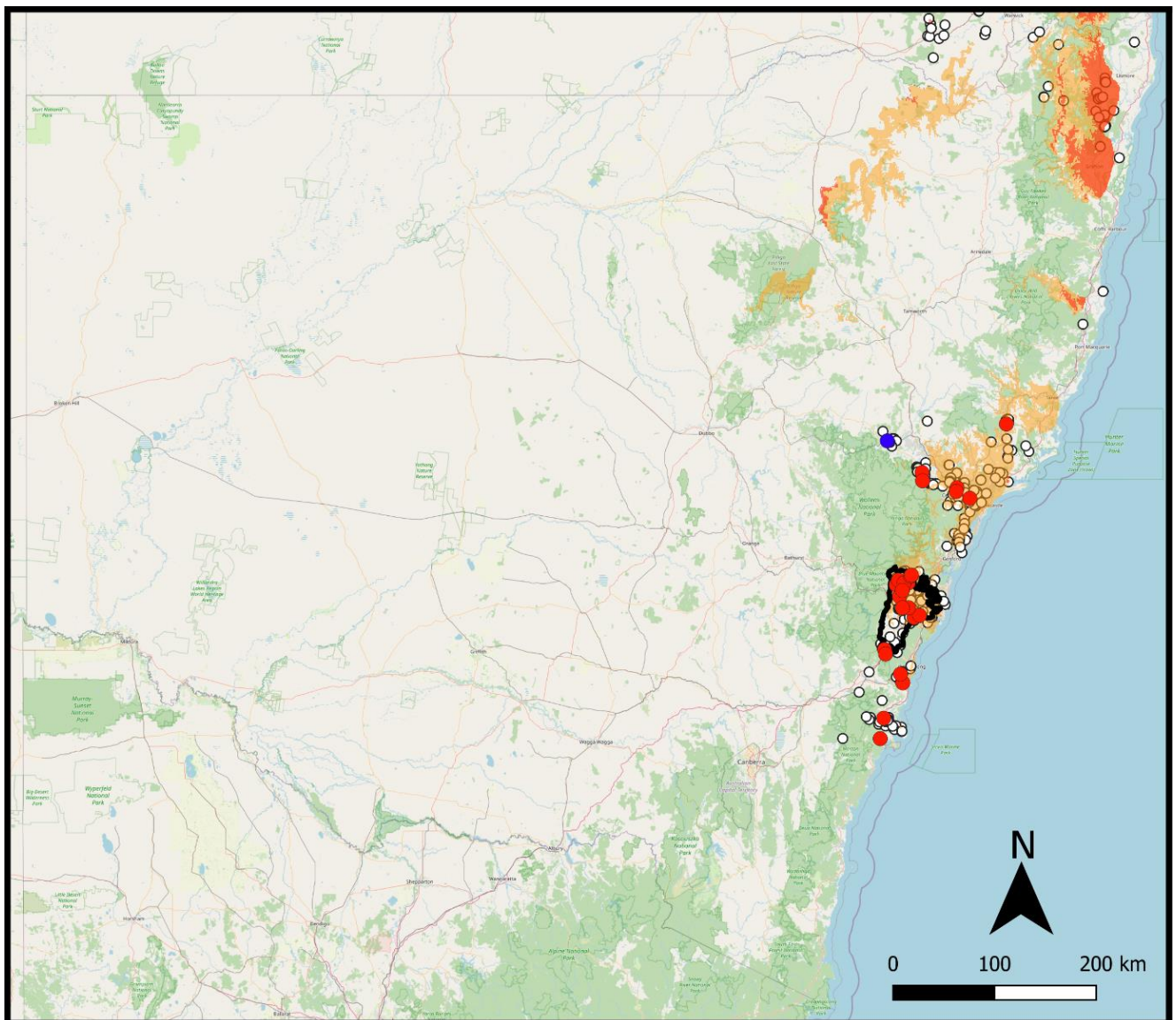
##### 4.11.3 Genetic diversity

- Allelic diversity and heterozygosity were high at all sampled sites for *Melaleuca decora*.
- One site from Hollydeen in the Hunter Valley showed significantly lower genetic diversity than the remaining sites and was the most genetically divergent. As this is the western most population and somewhat isolated from the other Hunter Valley populations which are 40 km east near Bulga, it may be that the low diversity of this site is due to a genetic bottleneck caused by limited gene flow with other populations and a small population in the area rather than adaptation to the local conditions of the site.

#### 4.11.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 11 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios

- We find minimal consideration for seed sourcing for *Melaleuca decora* if genetic diversity is considered.
- For *Melaleuca decora*, we advocate sourcing seed from six sites across the Cumberland Plain, Illawarra, South Coast and lower Hunter Valley, maintaining five maternal lines per site (30 maternal lines in total) (Figure 27).
- To make plantings climate ready under a moderate climate change scenario, three sites can be sourced from within the climate matched areas (orange shading in Figure 26) of the northern Cumberland Plain and the lower Hunter Valley.
- Under a severe climate change model, seed from the northern NSW populations could provide climate ready provenances, however our lack of genotyped samples means we are not able to make recommendations on the best sampling scheme to achieve this.



**Figure 26:** Sites sampled for genetic analysis of *Melaleuca decora* indicating genetic neighbourhoods, with red circles being those belonging to the neighbourhood present on the Cumberland Plain, and blue circles representing sites belonging to other genetic neighbourhoods. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models. Note the lack of sampling sites on the north coast of NSW where access to sites the species has been recorded was limited by flooding during the field season when collecting took place.



**Table 11:** Table 11: Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Melaleuca decora*. Note, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                             | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                                |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >12 sites x 1 mother plants per site = >12 maternal lines.                                                                                                                                     | 4 sites x 5 mother plants per site = 20 maternal lines total                                                                                                                                                   |
| Genetic neighbourhood  | 18 sites x 1 mother plants per site = 18 maternal lines.                                                                                                                                       | 6 sites x 5 mother plants per site = 30 maternal lines total (Figure 27a)                                                                                                                                      |
| Moderate climate ready | 18 sites – at least 9 located within climate matched areas (as shown in orange in Figure 26), 9 otherwise spread across distribution. 18 sites x 1 mother plants per site = 18 maternal lines. | 6 sites – at least 3 located within climate matched areas (as shown in orange in Figure 26), 3 otherwise spread across distribution. 6 sites x 5 mother plants per site = 30 maternal lines total (Figure 26b) |
| Severe climate ready   | NA – insufficient sampling to make recommendations                                                                                                                                             | NA – insufficient sampling to make recommendations                                                                                                                                                             |

**An optimised seed source strategy for *Melaleuca decora***

(a) High genetic diversity



(b) High genetic diversity + moderate climate ready



**Figure 27:** Figure 27: Schematic representation of seed collection effort required for *Melaleuca decora* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, and (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 26). Each circle represents a collection site, and each tree represents a maternal line.



## 4.12 *Themeda triandra*



*Themeda triandra* is a clumping grass species that was once extremely common in grassy habitats of NSW, but that does not cope well with grazing, which has reduced its abundance in many regions. It has the largest distribution of species sampled in this study, occurring not only in every state and territory in Australia, but also through much of Africa, tropical Asia, and the Pacific. Only populations in eastern NSW were targeted in this study. Ploidy levels are known to vary amongst populations of *Themeda triandra* (Ahrens et al., 2020), which impacts the findings we make here. This species has been added to the Restore and Renew webtool, allowing for appropriate areas for seed sourcing for site specific restoration projects to be explored.

### 4.12.1 Genetic structuring and neighbourhoods

- Two strongly differentiated genetic neighbourhoods were identified for *Themeda triandra*, although dozens of intergrades between them were observed in a broad buffer region between the two, including on the southern half of the Cumberland Plain (Figure 28).
- One neighbourhood occurred to the north-east of a line between Batemans Bay and the Cowra district, the second occurred south-west of a line between the southern Cumberland Plain and the Pilliga as well as the western side of the Great Dividing Range in northern NSW.

- The Cumberland Plain populations belonged to the north-eastern neighbourhood but included small numbers of individuals that were crossed between the two neighbourhoods.
- This creates a broad buffer zone between Batemans Bay and the southern Cumberland Plain, north-west to Cowra and the Pilliga, where allotetraploid individuals arising from interbreeding of the two neighbourhoods were collected.

#### 4.12.2 Reproductive biology

- Clones were observed at many sites for *Themeda triandra*, almost certainly due to vegetative spread.
- There was no evidence for widespread inbreeding for this species.
- Overall, this suggests a primarily outcrossing reproductive system and dispersal via seed, with localised vegetative spread.

#### 4.12.3 Genetic diversity

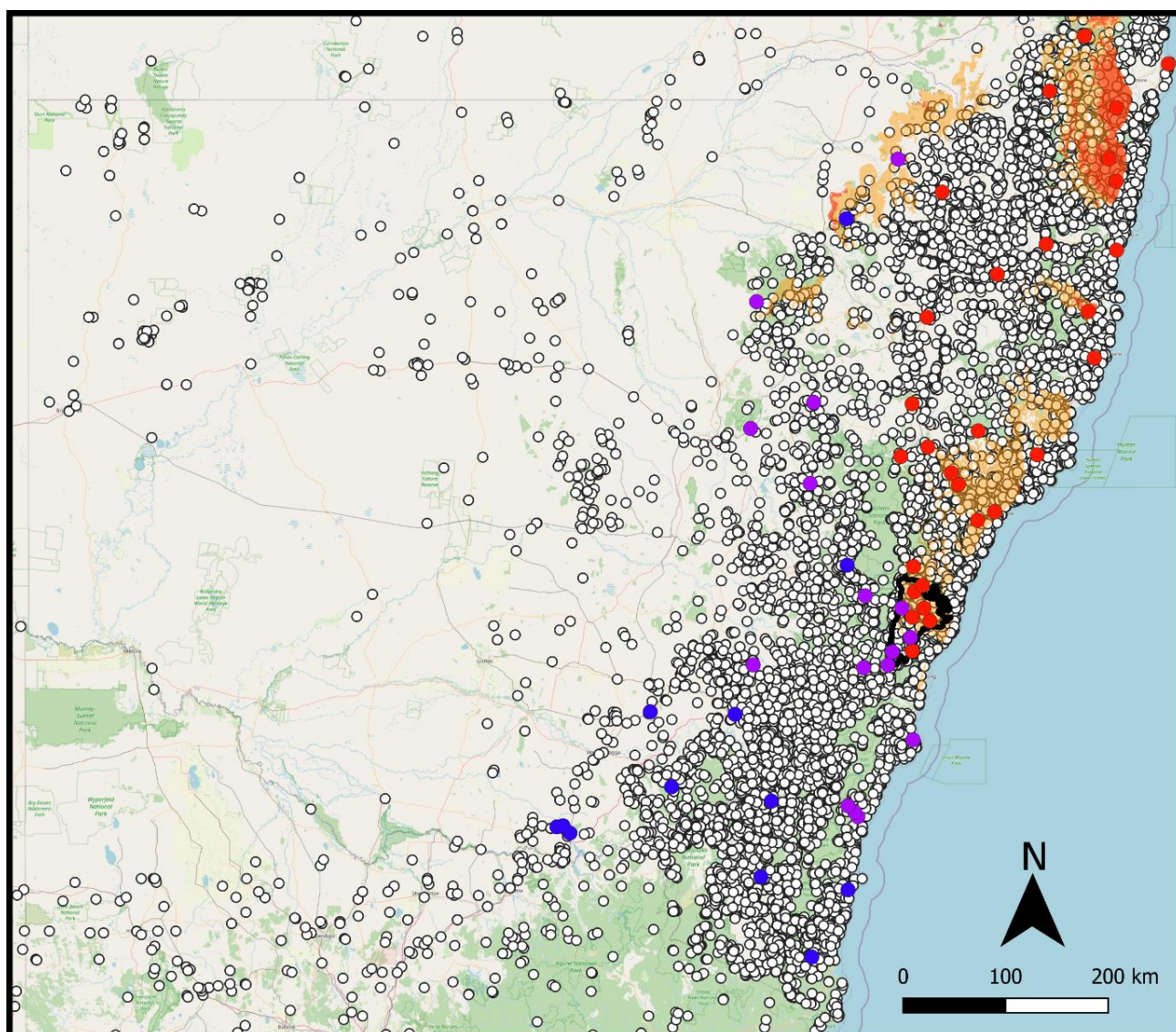
- Within site allelic richness and heterozygosity varied greatly between sites for *Themeda triandra* due to ploidy variation.
- Diversity was higher in populations of the south-western neighbourhood identified below, with lower diversity in the north-eastern neighbourhood.

#### 4.12.4 Seed sourcing for ecological restoration within the Cumberland Plain

Table 12 describes the sampling effort required to capture at least 90% of observed genetic diversity under different seed sourcing scenarios

- As some vegetatively spread clonal individuals were recorded, care should be taken when sourcing seed that maternal lines within a site are adequately separated (>50m).
- As we observed intergrades between the two neighbourhoods on the southern Cumberland Plain, we recommend sourcing seed only from the northern Cumberland Plain and north thereof for restoration on the Cumberland Plain under all scenarios (Figure 29).
- For *Themeda triandra*, we advocate sourcing seed from six sites from the northern Cumberland Plain, Hunter Valley and North Coast of NSW, maintaining five maternal lines per site (30 maternal lines in total) (Figure 29).
- To make plantings climate ready under a moderate climate change scenario, three sites can be sourced from within the climate matched areas (orange shading in Figure 28) of the northern Cumberland Plain, the lower Hunter Valley and Mid-north Coast.
- To make plantings climate ready under a severe climate change scenario, three sites can be sourced from within the climate matched areas in the Northern Rivers region (red shading in Figure 28).





**Figure 28:** Sites sampled for genetic analysis of *Themeda triandra* indicating genetic neighbourhoods, with red circles being those belonging to the neighbourhood present on the Cumberland Plain, blue circles representing sites belonging to other genetic neighbourhood and purple circles representing mixed populations where some or all individuals are allotetraploids arising from interbreeding of the two neighbourhoods. Smaller white points represent records of the species to indicate the known distribution of the species and are overlaid with areas that meet the criteria for sourcing climate ready provenances for the Cumberland Plain under moderate (orange) and severe (red) climate change models.



**Table 12:** Table 12: Sampling effort required to ensure capture of at least 90% of observed genetic diversity under four different seed sourcing scenarios for *Themeda triandra*. Note, more sites than we sampled are needed to capture 90% of observed genetic diversity using one mother plant per site in the Cumberland Plain provenance only scenario and therefore we cannot provide a specific target number of sites for this scenario.

| Seed source scenario   | Collection effort to capture 90% of genetic diversity when there is maintenance of a single maternal line per site                                                                                               | Collection effort to capture 90% of genetic diversity when there is maintenance of five maternal lines per site                                                                                                |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cumberland Plain only  | >6 sites x 1 mother plants per site = >6 maternal lines.                                                                                                                                                         | 4 sites x 5 mother plants per site = 20 maternal lines total                                                                                                                                                   |
| Genetic neighbourhood  | 18 sites x 1 mother plants per site = 18 maternal lines.                                                                                                                                                         | 6 sites x 5 mother plants per site = 30 maternal lines total (Figure 29a)                                                                                                                                      |
| Moderate climate ready | 18 sites – at least 9 located within climate matched areas (as shown in orange in Figure 28), 9 otherwise spread across northern genetic neighbourhood. 18 sites x 1 mother plants per site = 18 maternal lines. | 6 sites – at least 3 located within climate matched areas (as shown in orange in Figure 28), 3 otherwise spread across distribution. 6 sites x 5 mother plants per site = 30 maternal lines total (Figure 29b) |
| Severe climate ready   | 18 sites – at least 9 located within climate matched areas (as shown in red in Figure 28), 9 otherwise spread across northern genetic neighbourhood. 18 sites x 1 mother plants per site = 18 maternal lines.    | 6 sites – at least 3 located within climate matched areas (as shown in red in Figure 28), 3 otherwise spread across distribution. 6 sites x 5 mother plants per site = 30 maternal lines total (Figure 29c)    |

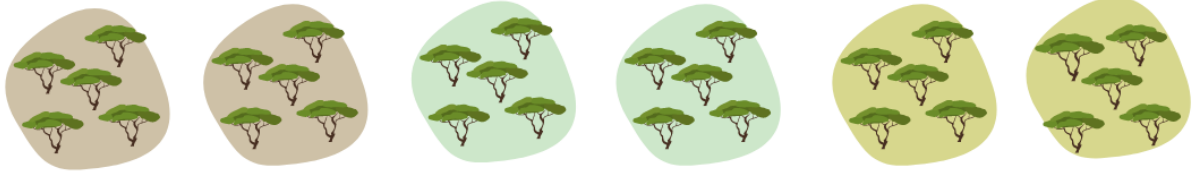
## An optimised seed source strategy for *Themeda triandra*

(a) High genetic diversity

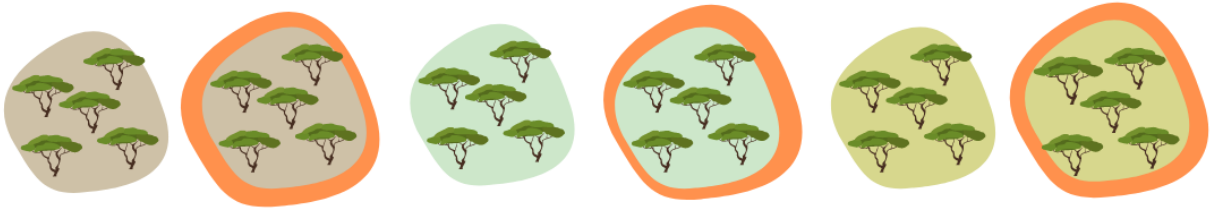
Northern Cumberland Plain

Hunter Valley

North Coast

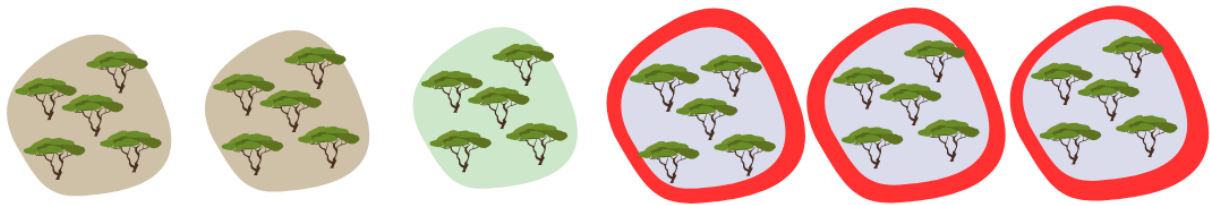


(b) High genetic diversity + moderate climate ready



(c) High genetic diversity + extreme climate ready

Northern Rivers



**Figure 29:** Schematic representation of seed collection effort required for *Themeda triandra* to source (a) genetically diverse seed (90% of total genetic diversity) suitable for use in restoration within the Cumberland Plain genetic neighbourhood or in the establishment of a Cumberland Plain seed production area, (b) incorporating moderate climate readiness into the above scenario by sampling from moderate climate matched sites (orange shading in Figure 28), and (c) incorporating severe climate readiness into the above scenario by sampling from severe climate matched sites (red shading in Figure 28). Each circle represents a collection site and each tree represents a maternal line.

### 4.13 Complex species

Several of the initial target species proved unsuitable for the webtool and use in the detailed optimisation analyses. The most common issue encountered was taxonomic inconsistencies with the genetic data. For both *Daviesia ulicifolia* and *Opilsenus hirtellus*, we found the genetic data did not support the current taxonomy of the species and therefore additional targeted sampling will be needed for use in the development of accurate guidelines. Limited sampling, caused by taxonomic identification complexities, also restricted the use of the available data for *Eucalyptus eugenoides*. In the future, further targeted sampling would allow for all three species to be added to the Restore and Renew webtool and inclusion in guidelines for restoration on the Cumberland Plain.

In contrast, for *Microlaena stipoides*, the final species for which analyses could not be completed or guidelines developed there is limited scope for developing specific restoration guidelines as our data provide evidence that patterns of genetic diversity in sampled populations already reflect extensive human movement of material rather than natural evolutionary patterns.

#### 4.13.1 *Daviesia ulicifolia*



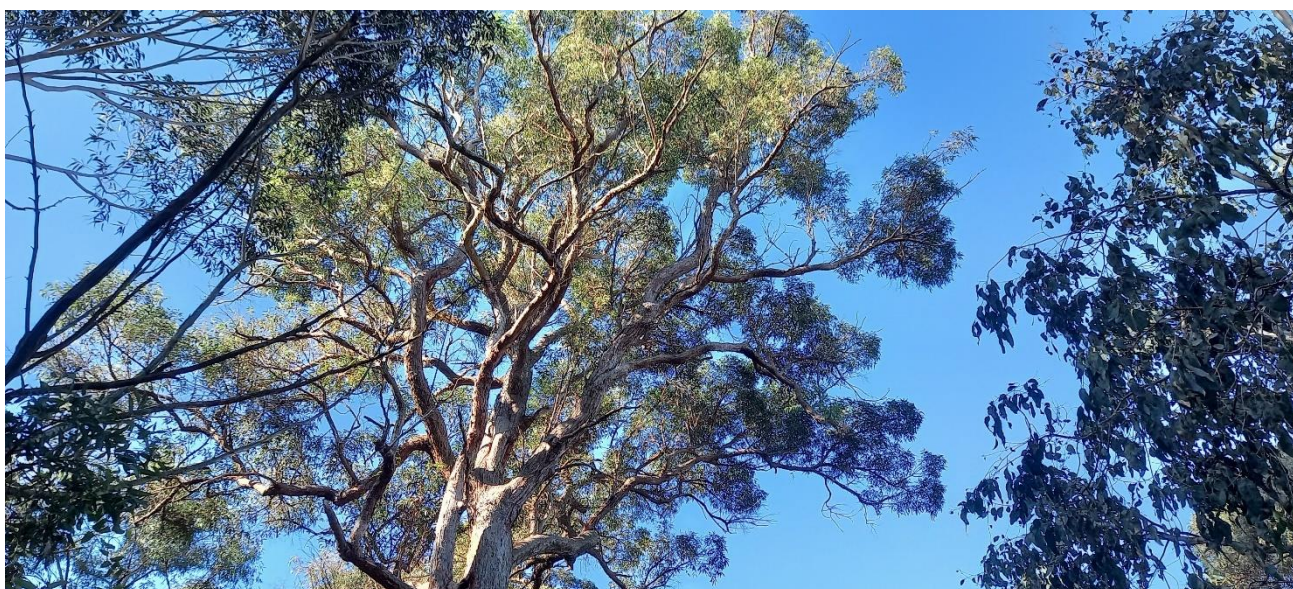
*Daviesia ulicifolia* subspecies *ulicifolia*

*Daviesia ulicifolia* subspecies *stenophylla*

There are five recognised subspecies of *Daviesia ulicifolia* in NSW, and while we only sampled four, lacking the capacity to sample subspecies *aridicola* from the far south-west of the state, the genetic data conclusively showed that there was no gene flow between subspecies despite co-occurrence at several of our sampled sites, including between the two subspecies that occur on the Cumberland Plain, subspecies *ulicifolia* and subspecies *stenophylla* (both pictured above). The genetic data provide strong evidence that all subspecies should be elevated to species, an issue that has been raised with taxonomists at the National Herbarium of NSW, Botanic Gardens of Sydney. While it would be possible to develop restoration guidelines for individual subspecies without this taxonomic change, as we were treating the species as a single entity when sampling, we lack sampling depth for individual subspecies to develop such guidelines. Thus, *Daviesia ulicifolia* was not further analysed nor included in any generalised recommendations and findings, and outcome that could be rectified with further sampling and sequencing.



#### 4.13.2 *Eucalyptus eugenioides*



Identification of stringybarked eucalypts is challenging and after inspection of the genetic data we concluded that many specimens were identified incorrectly and likely belong to a species other than *Eucalyptus eugenioides*. However, this meant that for this species we did not have enough samples for a thorough analysis. The stringybark eucalypts remain a challenging group of species from a taxonomic and identification perspective and further work is needed to understand the diversity of the group. However, if further sampling to establish a stable taxonomy and distribution for the species can be undertaken, it will be possible to complete a landscape genetic study of this species, develop guidelines and add it to the Restore and Renew webtool.



#### 4.13.3 *Microlaena stipoides*

Initial investigations of patterns of genetic diversity and divergence of populations of this species showed high levels of recent geneflow over large distances. While we cannot be certain the nature of this geneflow, we hypothesise it may be due to human mediated dispersal of the species. *Microlaena stipoides* is used both horticulturally and in remediation in circumstances such as road construction, which may have led to natural genetic patterns already having broken down. Given the ubiquity of *Microlaena* in the landscape, there seems to be limited negative

consequences for this loss of natural genetic patterns for this species. Thus, we did not pursue further study of this species and have excluded it from all analyses.

#### 4.13.4 *Oplismenus hirtellus*



A similar taxonomic issue to *Daviesia ulicifolia* was observed for *Oplismenus hirtellus*. Previously what is now considered one species was regarded as two distinct species with overlapping distributions in NSW, *O. aemulus* and *O. imbecilis*, which could be differentiated based upon morphology. While this taxonomy is no longer current nationally, our genetic data support the distinction of these two entities as species which do not experience gene flow and so it would be inappropriate to give restoration guidelines for *Oplismenus hirtellus* as a whole. As we have insufficient samples of each entity to build restoration guidelines, we do not provide these and we exclude these species from our cross-species analyses, although further sampling and sequencing could correct this.



## 5. Case study: Design of a genetically diverse climate-ready Seed Production Area

To illustrate how the recommendations provided in this report can be put into practice, we provide a fully designed project to establish a seed production area at the Australian Botanic Garden Mount Annan, Botanic Gardens of Sydney. We propose to include all 11 target species from this report that we could develop specific guidelines for in our hypothetical Seed Production Area (SPA) (Table 13). We implement these species-specific guidelines as presented in this report for each species to identify seed sourcing site and maternal line numbers, and appropriate geographic area in which to collect seed. For species where climate ready material under a moderate climate change model can be sourced, we include 50% climate ready maternal lines in our SPA.

For ten of the eleven target species we propose planting two individuals per maternal line within each plot, with a minimum of two plots per species, giving a range of plantings per species of 100 to 160 individuals (5-8 source sites \* 5 maternal lines per site \* 4 plants per maternal line) (see Table 13). These individuals would be germinated in the nursery and planted out as tubestock in a randomised planting scheme in two plots, each containing two representatives of every maternal line, as demonstrated in Figure 30: a graphical workflow of the process of SPA establishment for *Acacia decurrens*.

For the final species, *Themeda triandra*, we instead propose to plant small areas of single maternal lines to test the viability and vigour of each line, and then plant a larger area with seed from each maternal line mixed in even proportions pre-sowing.

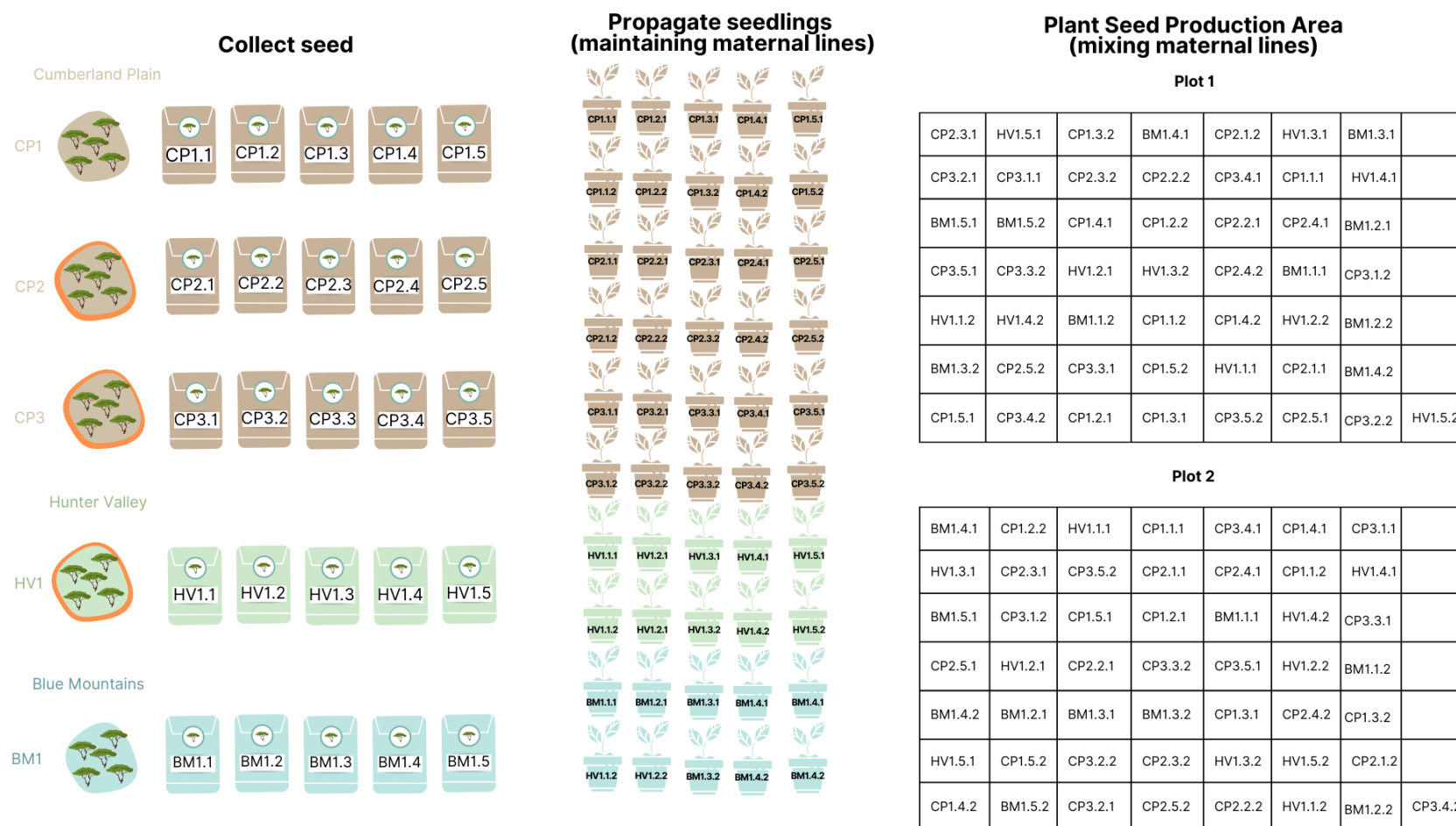


**Table 13:** Seed sourcing and planting strategies for eleven CPW species to be included in hypothetical SPA located at ABG Mount Annan.

| Species                                                                        | Area to source seed from                                                               | Number of source sites and maternal lines                     | Climate ready source sites included                          |
|--------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------|--------------------------------------------------------------|
| <i>Acacia decurrens</i>                                                        | Cumberland Plain and Hunter Valley                                                     | 5 sites * 5 maternal lines per site = 25 maternal lines total | Yes, 3 sites to be located within the climate matched area.  |
| <i>Acacia falcata</i>                                                          | Entire distribution of species within NSW                                              | 29 sites * 1 maternal line per site = 29 maternal lines.      | Yes, 15 sites to be located within the climate matched area. |
| <i>Acacia implexa</i>                                                          | South Coast, Cumberland Plain and Hunter Valley                                        | 8 sites * 5 maternal lines per site = 40 maternal lines total | Yes, 4 sites to be located within the climate matched area.  |
| <i>Acacia parramattensis</i>                                                   | Entire distribution of species                                                         | 8 sites * 5 maternal lines per site = 40 maternal lines total | Yes, 4 sites to be located within the climate matched area.  |
| <i>Breynia oblongifolia</i>                                                    | Area between the Illawarra Escarpment and Queensland Border                            | 8 sites * 5 maternal lines per site = 40 maternal lines total | Yes, 4 sites to be located within the climate matched area.  |
| <i>Bursaria spinosa</i>                                                        | Cumberland Plain, Hunter Valley and Central West                                       | 8 sites * 5 maternal lines per site = 40 maternal lines total | Yes, 4 sites to be located within the climate matched area.  |
| <i>Dodonaea viscosa</i>                                                        | Eastern NSW apart from Northern Rivers area where subspecies <i>Burmanniana</i> occurs | 6 sites * 5 maternal lines per site = 30 maternal lines total | Yes, 3 sites to be located within the climate matched area.  |
| <i>Eucalyptus baueriana</i>                                                    | Cumberland Plain and South Coast                                                       | 5 sites * 5 maternal lines per site = 25 maternal lines total | Yes, 3 sites to be located within the climate matched area.  |
| <i>Eucalyptus fibrosa</i>                                                      | Cumberland Plain, Hunter Valley, Mid-North Coast and Northern Rivers                   | 5 sites * 5 maternal lines per site = 25 maternal lines total | Yes, 3 sites to be located within the climate matched area   |
| <i>Melaleuca decora</i>                                                        | Cumberland Plain and Hunter Valley                                                     | 6 sites * 5 maternal lines per site = 30 maternal lines total | Yes, 3 sites to be located within the climate matched area   |
| <i>Themeda triandra</i> - Direct seed sowing with equally mixed maternal lines | Coastal and inland slope areas from the Cumberland Plain north                         | 6 sites * 5 maternal lines per site = 30 maternal lines total | Yes, 3 sites to be located within the climate matched area   |

## Seed Production Area design for *Acacia decurrens*

High genetic diversity + moderately climate ready



**Figure 30:** Figure 30: Schematic representation of the establishment process for a genetically diverse, moderately climate ready, seed production area using as an example *Acacia decurrens*.

## 6. Conclusions and future directions

### 6.1 Demonstration of the value of Restore and Renew and associated knowledge infrastructure

This project demonstrated the utility of the Restore and Renew approach and associated knowledge infrastructure for guiding genetically informed restoration of 11 Cumberland Plain woodland species, informing restoration practices and seed sourcing in the following ways:

- **Identifying genetic neighbourhoods:** We identified genetic neighbourhoods, thus providing a biologically meaningful measure of local provenances for restoration of the Cumberland Plain.
- **Providing guidelines on minimum seed sourcing effort:** Our large genetic datasets have identified the minimum seed sourcing effort required to meet genetic diversity capture targets. While these targets are high, we show that by careful choice of source sites within a genetic neighbourhood or region, along with the maintenance and even use of multiple maternal lines per source location, we can minimise the number of sites where seed needs to be collected and thus the effort required to collect.
- **Increasing suitable seed supply and diversity:** Identification of genetic neighbourhoods increases seed availability for restoration of the Cumberland Plain as genetic neighbourhoods were far larger than previous distance-based, generalised guidelines for sourcing local provenance material estimated. This increase in suitable area for seed sourcing has the flow-on effect of increasing the genetic diversity and hence adaptability of seed that can be used in restoration on the Cumberland Plain.
- **Climate proofing restorations:** Using regional climate matching and multiple future climate projections, we have identified regions where climate-ready seed may be collected for use on the Cumberland Plain.

### 6.2 Future restoration genomic research for the Cumberland Plain

The methodology used and knowledge infrastructure established can be applied to additional species to further improve ecological restoration across the Cumberland Plain. Our research has shown that species specific genetic data rather than generalisations are needed, especially when it comes to restoring threatened ecological communities, because the consequences of using genetically depauperate or inappropriate material can be dire for both restored vegetation and remnant native vegetation.

Restoration genomics has stepped into a new phase whereby we can now gather relevant genomic information quickly and cost-effectively, making it feasible for us to generate genetic guidelines similar to the ones provided in this report for many more species. It is not inconceivable that similar analysis could be undertaken for most, if not all, species commonly used in restoration across the Cumberland Plain.

We intend to continue to expand the Restore and Renew program both in terms of the number of species represented from the Cumberland Plain and beyond, and the ecological communities and regions from where the species come. We also hope to be able to undertake additional targeted sampling for the various *Daviesia ulicifolia* subspecies, the two *Opilismenus* species and *Eucalyptus eugenioides*, so that species-specific guidelines can be developed, and all three taxa can be added to the Restore and Renew webtool.

### 6.3 Benefits beyond the Cumberland Plain

The genetic data and guidelines generated by this project have application far beyond the Cumberland Plain. The data and guidelines generated for at least eight species will be made freely accessible on the Restore and Renew webtool, thus providing valuable information that can guide genetically informed restoration of these species across NSW.

The methodology used and knowledge infrastructure demonstrated in this project can be applied in many different contexts, including any regional or ecosystem context and to most species used in ecological restoration, with the ability to expand datasets as new species, samples and analyses are collected, genotyped, and developed. The tools and guidelines generated are mostly not location or project specific, allowing the costs and workloads of generating these to be shared across different regional projects.



## 7. References

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