DEVELOPMENT OF CROP WILD RELATIVE CONSERVATION STRATEGIES FOR NORWAY

by

Jade Phillips

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Abstract

Climate change and anthropogenic activities threaten our global food security. One area of research that may help combat a future food crisis is the utilization of the genetic diversity available in wild plants. Crop wild relatives (CWR) are one such resource. They are the wild taxa most closely related to crops and from which diverse traits could be transferred to the crop. This project uses Norway as an example, to contribute towards methodologies to identify those CWR populations that are most important for conservation and use. This involves the creation of a priority list of CWR for Norway, *in situ* and *ex situ* diversity analysis of CWR populations, gap analysis and ecogeographic land characterization methodologies, predictive climate change analysis for CWR distributions and genetic diversity studies of taxa using Amplified Fragment Length Polymorphisms (AFLPs). Comprehensive *in situ* and *ex situ* national recommendations for the conservation of CWR in Norway are detailed. These include the incorporation of management plans for CWR populations within the Færder national park in Norway, the first instance of such conservation activities in Scandinavia. The scientific methods used and developed will help Norway meet its international obligations for conservation and use of genetic diversity of CWR and will contribute to the regional and global efforts to systematically conserve and utilize the diversity found in CWR.

For Grandad,

enjoy your long walk amongst the flowers.

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List of abbreviations

AFLP Amplified fragment length polymorphism

AMOVA Analysis of molecular variance

CBD Convention on Biological Diversity

CCAFS Climate Change, Agriculture and Food Security

CCVA Climate Change Vulnerability Analysis

CWR Crop wild relative

ELC Ecogeographic Land Characterisation

ESPC European Strategy for Plant Conservation

EURISCO European Search Catalogue for Plant Genetic Resources

GBIF Global Biodiversity Information Facility

GCM Global Circulation Model

GIS Geographical Information System

GP Gene Pool

GRS Geographical representativeness score

GSPC Global Strategy for Plant Conservation

IBERS Institute of Biological, Environmental and Rural Sciences

ICARDA International Center for Agricultural Research in the Dry Areas

INDC Intended Nationally Determined Contribution

IPCC Intergovernmental Panel on Climate Change

IPGRI International Plant Genetic Resources Institute

ITPGRFA International Treaty on Plant Genetic Resources for Food and Agriculture

IUCN International Union for the Conservation of Nature

LVO Landskapsvernområde

NGS Next-generation sequencing

NP National Park

PA/s Protected area/s

PCA Principal component analysis

PCoA Principal coordinate analysis

PCR Polymerase chain reaction

PGR Plant genetic resource

PGRFA Plant genetic resources for food and agriculture

RAPD Random amplification of polymorphic DNA

RCP Relative Concentration Pathway

RFLP Restriction fragment length polymorphism

SDM Species distribution model

SNP Single nucleotide polymorphism

TG Taxon Group

TVA Trait-based variability analysis

UK United Kingdom of Great Britain and Northern Ireland

VIF Variance inflation factor

Declaration

The work in chapter 3 and chapter 4 have been published. The work in chapter 5 has been submitted for publication. Co-author, Dr Nigel Maxted, has agreed for me to include the published work within this thesis by signing the declaration below. The wording of the chapters is largely identical to the manuscripts prepared for publication, however that text was written by me and all chapters presented here were written in their entirety by me. Details of co-author contributions can be found at the beginning of chapter 3, 4 and 5.

Chapter 3:

Phillips, J., Asdal, Å., Magos Brehm, J., Rasmussen, M. & Maxted, N. (2016) *In situ* and ex *situ* diversity analysis of priority crop wild relatives in Norway. *Diversity and Distributions,* 22, 1112-1126.

Author contributions: Conceived and designed the study: J.P., A.A., N.M; Data collation and preparation: J.P., A.A; Performed analysis: J.P; Interpreted results: J.P., A.A., J.M.B; Wrote the paper: J.P; Critically reviewed the paper: J.P., A.A., J.M.B., M.R., N.M.

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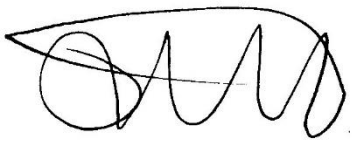
Signed:

Dr Nigel Maxted  Date: 02/05/17

Jade Phillips  Date: 5.5.2017

CHAPTER 1.

Introduction

Every species is worth conserving, every species has a value and place in the global ecosystem, however, the conservation of a resource only becomes important if the resource has or acquires recognized value (Hoisington *et al*., 1999). Biodiversity is a key component of our planet and vital for a range of ecosystem services that are essential to the human population. Throughout the globe there are 25 biodiversity hotspots containing high concentrations of endemic species, including 44% of vascular plants yet covering only 1.4% of land surface area (Myers *et al*., 2000). The agricultural landscape (including arable land, permanent crops and permanent pastures) covers over 37% of global land area (FAO, 2016). Undoubtedly, our food is one of the resources of highest value to us as a species. We depend on fewer than a dozen of the approximately 300,000 species of flowering plants for 80% of our calorific intake (McCouch *et al*., 2013). Just four crops, rice, wheat, maize and potato, provide more than 60% of our food. In addition, more and more wheat and maize are being grown as feed for our animals (Keyzer *et al*., 2005; Shiferaw *et al*., 2011) further increasing the pressures on our agricultural system. With a population that is expected to increase by 34% to 9.1 billion by 2050 (FAO, 2009a) we will need to increase food production by up to 70–100% to meet these demands (Royal Society of London, 2009; World Bank, 2008).

Resources are already limited, therefore conserving and utilising the materials we have available to help ensure our food security is becoming more urgent. Agrobiodiversity is an important concept in terms of bridging the research and implementation gaps between food security and conservation. It refers to species and varieties of crops and livestock as well as their wild relatives that contribute to agriculture (Qualset *et al*., 1995). Understanding,

conserving and harnessing this diversity is essential if we are to sustainably continue producing food and feeding the population.

The loss of flora and fauna is well documented but much less is widely known about the loss of genetic diversity (Nabham, 2009). We capitalize on only a fraction of the genetic diversity that resides within each of the species used to feed us (McCouch *et al*., 2013). Yet food production and food security depend on the wise use and conservation of agricultural biodiversity and genetic resources (Esquinas-Alcazar, 2005). Unexploited genetic material from landraces, rare breeds and wild relatives will be [and have been] important in allowing plant pre-breeders and breeders to respond to new challenges (Godfray *et al*., 2010). For example, 90% of wheat varieties worldwide are susceptible to the Ug 99 race of fungus, however transfer of resistance genes from wheat relatives into cultivars is already underway and proving successful (Singh *et al*., 2011). This use of wild genetic diversity can not only help to combat diseases and pests but can also improve productivity and adapt crops to climatic changes.

Genetic diversity is needed as global food supplies have become more similar in composition meaning there is a heightened interdependence between countries upon food and plant genetic resources (PGR; Khoury *et al*., 2014). This also implies that attention needs to be focused on the stable and long-term production of these staple crops to match the foreseeable increase in demand (Khoury *et al*., 2014). This global demand has created a situation where high production, which is based on uniform crops, has been given priority over more reliable, diversified methods (Esquinas-Alcazar, 2005). Industrialised large-scale farming meets the immediate hunger needs of the population but risks long-term security due to low adaptability of crops to environmental fluctuations. The dangers of having monocultures are highlighted most notably by the Irish potato famine in 1845 where the crops were attacked by the spores of *Phytophthora infestans*, leading to large scale famine. Other crops such as banana are also at

risk as they are genetically restricted leading to only a few cultivated varieties which are particularly susceptible to diseases, pests and ecological changes (Perrier *et al*., 2011).

Increasing the genetic diversity of our crops will also provide wider ecosystem service benefits both in the present and the future (Jackson *et al*., 2007). It is the underlying genetic diversity of these wild species, and others that is vital for playing a role in enhancing the provision of many services concurrently in multifunctional and sustainable agriculture (Hajjar and Hodgkin, 2007). For example, drought tolerant varieties will increase productivity of crops but also prevent soil erosion and increase soil organic matter (Millennium Ecosystem Assessment 2005). Furthermore, a study by Isbell *et al.* (2011) has shown that many species will be needed to maintain ecosystem multifunctionality at large spatial-temporal scales in a changing world. Ecosystem-based approaches to adaptation also harness the capacity of nature to buffer human communities against the adverse impacts of climate change (Jones *et al*., 2012) as well as being shown to be the most cost-effective defence against a varying climate (Martin and Watson, 2016).

Making use of and maintaining agricultural biodiversity can help improve food security (Millennium Ecosystem Assessment, 2005) for which PGR are essential as they contain useful traits for adapting our crops to future challenges. To help do this we should be moving from species conservation to conservation of genetic diversity.

1.1 What is a crop wild relative?

Crop wild relatives (CWR) are a PGR that have an indirect use derived from their relatively close genetic relationship to a crop. This is a broad definition of a CWR and as such includes a broad number of species. For example, using this definition Maxted and Kell (2009) estimate there are around 50 to 60,000 CWR globally, with Kell *et al*. (2008) finding that 80% of the

European and Mediterranean Flora contains CWR. In such a case, a more specific definition can be used:

A crop wild relative is a wild plant taxon that has an indirect use derived from its relatively close genetic relationship to a crop; this relationship is defined in terms of the CWR belonging to Gene Pools 1 or 2, or Taxon Groups 1 to 4 of the crop (Maxted *et al*., 2006).

This takes account of the main reason to conserve CWR, i.e. for their use as plant breeding material. The Harlan and de Wet (1971) Gene Pool (GP) concept referred to in the above definition, is used to determine a wild species' relatedness to a crop. This is done by establishing the relative crossing ability between the crop itself and the wild relative within the primary, secondary or tertiary GP (see Figure 1.1). CWR in the primary GP (GP1b) can be easily transferred to the crop, which belongs to GP1a. This primary genepool is often composed of landrace material. CWR in the secondary GP (GP2) can be crossed with some limited success and those in the tertiary GP (GP3) require biotechnological approaches to facilitate gene transfer (Harlan and de Wet, 1971). However, it is only possible to define CWR by the GP concept when extensive information is available on patterns of genetic diversity and relative crossing ability (Maxted *et al*., 2006), which is only known for the well-studied crops (Heywood, 2008). For CWR taxa in which we have little or no genetic diversity data, the Taxon Group (TG) concept can be used to assist in setting conservation priorities (Maxted *et al*., 2006). This follows traditional taxonomic relationships between taxa, with TG1a representing the crop, TG1b including the CWR belonging to the same species as the crop, TG2 includes CWR in the same section as the crop, TG3 contains CWR in the same subgenus and TG4 represents those CWR in the same genus as the crop. These methods of defining CWR are now commonplace and have been used to prioritize CWR for individual species, as well as CWR for conservation at national and global levels (Vincent *et al*., 2013).

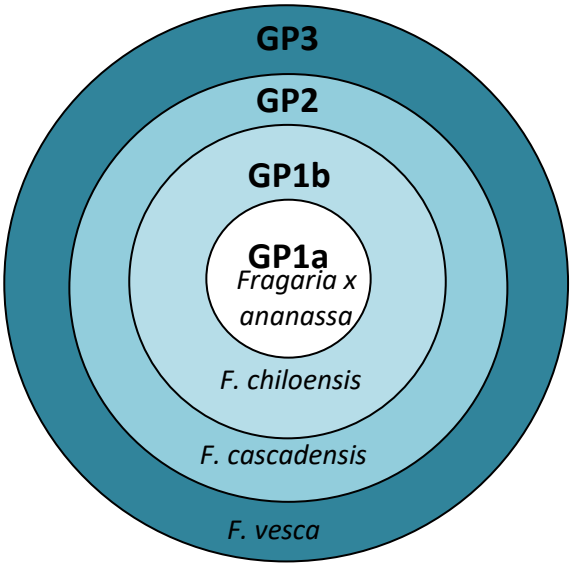


Figure 1.1: An example of the Harlan and de Wet Genepool concept (1971). GP1: *Fragaria x ananassa* Duchesne ex Rozier (crop), GP1B: *F. chiloensis* (L.) Mill, GP2: *F. cascadensis* K. E. Hummer, GP3 *F. vesca* L. (Vincent *et al.* 2013).

CWRs are found throughout the globe, from the Arctic Circle to the southern tip of South America. Key areas of CWR richness are found in those regions where cultivation of wild species is said to have originated. For example, cultivation of wheat, barley and oats first took place in the near east, which is one of the most species rich locations for these and other important grains (Vavilov, 1949; Vincent *et al*., 2013). Nikolai Vavilov is widely acknowledged as the person to first make this link and identify the centres of origin of cultivated plants (Vavilov, 1949). He also made the link between high levels of species diversity and the potentially high levels of genetic diversity within the wild species. In present day St Petersburg, Russia, the Vavilov Institute of Plant Genetic Resources conserves and utilises the seeds that he helped to collect. Vavilov began utilising wild species related to wheat (*Aegilops* L., *Secale* L., *Agropyron* Gaertn.) to improve the cultivated crop in Russia. Norman Borlaug continued this work and is said to have initiated the Green Revolution by

incorporating genes from wild wheat relatives for dwarfing and pest and disease resistance into cultivated wheat in the 1940s and 1950s (World Food Prize, 2016). Building on the work of Vavilov, a more recent study looking at 173 crop complexes has shown that West Asia has the most CWR present (Vincent *et al*., 2013). However, when country size and number of CWR are considered, the highest concentration of CWR are in Lebanon, Israel, Greece and the Mediterranean islands which are also likely to contain high numbers of endemic CWR compared to the mainland countries (Vincent *et al*., 2013).

From these early examples, the use of CWR in breeding has continued to rise, with the number of publications detailing use of CWR in breeding increasing from 2% in 1970 to 38% in 1999 (Maxted and Kell, 2009). CWR have had extensive periods of interaction between their environment (IPGRI, 1994) and because of this these species have outstanding characteristics in climatic and edaphic adaptation (Harlan, 1975). Plant breeders acknowledge that there is a wide range of useful genetic diversity available in CWR (Feuillet *et al*., 2008) with the most widespread use of CWR for pest and disease resistance (Maxted and Kell, 2009). For example, oat wild relatives have been used for rust resistance (Prescott-Allen and Prescott Allen, 1986) and a gene from the potato wild relative *Solanum venturii* Hawkes & Hjert. was introduced to a cultivated variety conferring blight-resistance (Jones *et al*., 2014). Other traits, including improvement of drought tolerance in cultivated barley from wild barley (*Hordeum vulgare* L. subsp. *spontaneum* (K. Koch) Thell.) (Lakew *et al*., 2011), size and shape of fruit in tomato (Tanksley and McCouch, 1997) and yield improvement in sorghum (Hajjar and Hodgkin, 2007) have also been harnessed from CWR. There is also widespread use of wild forage species in improvement of pasture lands (Pecetti *et al*., 2008; Abberton, 2011; Helgadóttir *et al*., 2016). Hajjar and Hodgkin (2007) and Maxted and Kell (2009) have compiled an extensive list of other such traits being used from CWR for crop improvement. Furthermore,

technological advances are meaning that CWR are becoming more available for use (Meilleur and Hodgkin, 2004; Jones *et al*., 2014) which will be essential to open up the wild genepool to plant pre-breeders and breeders helping to feed our growing population in the future.

The value of CWR in helping to increase crop yields has been estimated at $115 billion per year globally (Pimentel *et al*., 1997) and we should assume that this has increased since. A more recent estimate values the wild genepools of 29 priority crops, identified by the Millennium Seed Bank, Kew, to be potentially worth $120 billion, with a current value of $42 billion (PwC, 2013). Furthermore, the cost of pest and diseases to crops is huge, with annual worldwide potato losses due to blight being conservatively estimated at $6.7 billion (Fry, 2008). With the recent transfer of blight resistance from a potato wild relative to the cultivar (Jones *et al*., 2014) the cost of this loss to farmers will be reduced. There are also the indirect values associated with CWR including their role as ecosystem services (De Groot *et al*., 2002; Ford-Lloyd *et al*., 2011; Jaradat, 2015), as mentioned above, therefore we can only assume that these global valuations are under estimates of the true value of such resources.

1.2 Aim of *in situ* and *ex situ* CWR conservation

Conservation of CWR can be undertaken in two complementary processes as defined by the Convention on Biological Diversity (UN, 1992): *in situ*, 'the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings where they have developed their distinctive properties' and *ex situ* conservation, 'the protection of components of biological diversity outside their natural habitats.' Complete and effective conservation of PGR cannot be successful without utilising both methods.

In situ conservation tends to be targeted within current protected areas (PAs), such as nature reserves or national parks (NP). However, often CWR tend to be conserved only passively within these areas (Iriondo *et al*., 2008; Maxted *et al*., 1997a) i.e. they are protected only because of incidentally being present within the reserve. The CWR populations are unlikely to be actively managed unless they are threatened species, or they are the reason why the reserve was set up in the first place. For *in situ* conservation to be effective, active conservation of CWR populations needs to take place. This would include the management and monitoring of CWR within the PA to ensure the populations are maintaining appropriate sizes and meet the guidelines set out by Iriondo *et al*. (2012). These guidelines allow the definition of a genetic reserve, in which active long-term conservation involving the management and monitoring of genetic diversity takes place (Maxted *et al*., 1997a).

However, there are problems with targeting current PAs for *in situ* conservation of CWR. Firstly, many PAs were designated on areas of land that contain climax vegetation or have important landscape value, not CWR value. Furthermore, the management of PAs does not address the conservation of genetic variation in individual species (Hunter *et al*., 2012), the main aim of CWR conservation. Jain (1975) stressed that none of the progenitors of major food crops occur as climax vegetation, thus increasing the need to identify conservation solutions for disturbed ecosystems. Allem (1997) and Jarvis (2015) also identified the importance of disturbed areas and road verges as major suppliers of biological diversity and PGR to society. Conservation of agrobiodiversity needs to be taken up by local landowners and communities, with agreements and management plans drawn up to maintain the current populations (Maxted *et al*., 1997a). Most traditional farmers prefer to maintain varieties within their fields so they can evolve within their environment and along with changing management practises (Nabham, 2009). Furthermore, under climate change a static approach of establishing

isolated reserves surrounded by highly unnatural landscape is not effective (Ramirez-Villegas *et al*., 2014). Hence, identifying areas and/or populations outside of PAs for conservation action will also be a major part of *in situ* conservation of CWR.

The designation of the most appropriate crop wild relative populations (MAWPs; Maxted *et al*., 2015) could be an alternative (or indeed work within the traditional genetic reserve designation) to conserve populations. These MAWPs are the most valuable in terms of containing distinct or complementary genetic diversity or specific traits of interest and therefore allow conservation efforts to be directly targeted upon specific populations (Maxted *et al*., 2015). This level of conservation will require more detailed information upon populations of interest and therefore may be more appropriately applied once PAs have been identified as containing important populations.

In situ conservation not only benefits the target species but also the ecosystem as it allows the populations to continue to thrive in their natural habitats and adapt to changing environmental conditions, which *ex situ* conservation alone cannot achieve (Maxted *et al*., 2015). It also allows the generation of new variation which may be important with the future effects of a changing climate. There are only a few examples of active *in situ* conservation of CWR (see Maxted *et al*., 2016 for more examples) for example: Teosinte (*Zea diploperennis* Iltis, Doebley & R. Guzm) in the MAB Sierra de Manatlan Biosphere Reserve in Mexico (Sánchez-Velásquez, 1991), wild *Solanum* species in the Laguna de los Pozuelos Natural Monument and Los Cardones NP in Argentina (Marfil *et al*., 2015) and *Beta patula* Aiton species in Maderia (Pinheiro de Carvalho *et al*., 2012). However there is the scientific background laid for the establishment of further *in situ* genetic reserve networks in Cyprus (Phillips *et al*., 2014), England (Fielder *et al*., 2015), Finland (Fitzgerald, 2013), Jordan (Magos Brehm *et al*., 2016), Norway (Phillips *et al*., 2016), Oman (Al Lawati *et al*., in press), Portugal (Magos Brehm,

2009), Spain (Rubio Teso *et al*., 2013) among other countries (see Iriondo *et al*., 2016). There is also work on regional (Maxted *et al*., 2015; Weibull *et al*., 2016; Kell *et al*., in prep) and global networks (Maxted and Kell, 2009) of *in situ* genetic reserves for PGR.

There are still fundamental questions around *in situ* conservation, such as how many reserves or populations would be optimal to protect the entire genetic diversity of the taxon in question. This may vary depending upon the life history of the species or the potential diversity that may be present within the total area of study. For example, Schoen and Brown (1993) noted that one out-breeding population contained over 80% of species genetic variation whilst Neel and Cummings (2003) found that 67-85% of alleles were conserved in five populations if they were selected randomly without knowledge of genetic diversity patterns. Dulloo *et al*and collaborators (2008) recommended a minimum of five *in situ* populations conserved whilst Fielder *et al*. (2015) expanded on this and recommended five populations conserved that represent the full ecogeographic range. We suggested (Phillips *et al*. 2016) that a minimum of five populations should be protected within a PA complementary network, with Heywood (2008) stating that once taxa are found in five actively managed reserves, population genetic theory suggests there would be little need of further duplication. Both Fielder *et al*. (2015) and Phillips *et al*. (2016) agree that the guidelines set out by Iriondo *et al*. (2012) should be followed for effective *in situ* conservation which includes ensuring that minimum standard population sizes are large enough to sustain long-term population viability. In addition, Iriondo *et al*. (2012) stresses that reserves must capture as much genetic diversity of each target taxon as possible, conserving at least the alleles that are common, widespread and localized *sensu* Marshal and Brown (1975), but they stop short of suggesting a number of populations or reserves required.

Ex situ conservation offers long-term, secure conservation and allows plant pre-breeders and breeders full control and access to the PGR resources they require. However, this form of conservation involves removal of the species from its natural habitat and therefore halts the evolution of the taxa which will limit the future beneficial impact of the species upon the ecosystem as a whole (Ford-Lloyd *et al*., 2013). *Ex situ* conservation most notably takes the form of seed storage under cool dry environments but it can also involve in vitro conservation and botanical garden conservation and depends upon the nature of the seed being conserved (i.e. orthodox seeds or recalcitrant seeds).

The Svalbard Global Seed Vault is one of the largest seed banks with its main aim being to: "provide insurance against both incremental and catastrophic loss of crop diversity held in traditional seed banks around the world" (The Crop Trust, 2016). The seeds within this vault are duplicates from collections in other seed banks worldwide and the vault acts as a store or back-up of seeds in-case of complete loss of the collections elsewhere. A recent example of the importance of such a seed bank is when the International Center for Agricultural Research in the Dry Areas (ICARDA) requested that it remove some of its seed deposits from the vault due to loss of collections at its seed bank in Aleppo, Syria. They hope to replace the seeds in Svalbard once they have been duplicated in the field (Norwegian Ministry of Agriculture and Food, 2015).

One global project which is helping to conserve CWR *ex situ* is the 'Adapting Agriculture to Climate Change project' (Dempewolf *et al*., 2014) which includes the creation of the Harlan and de Wet inventory (Vincent *et al*., 2013). This is focused on the conservation and use of the wild relatives of 29 crops of major importance to food security which are included in Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA; FAO, 2001). The project will identify CWR of these priority crops that are missing from *ex situ*

collections, collect them from the wild, evaluate this material for use in crop improvement programmes and make the products and results available to users (Dempewolf *et al*., 2014). The project will improve the levels of CWR represented within seed banks which currently makes up only up 10% of accessions within Europe as recorded in EURISCO (Dias *et al*., 2011). More recent analysis of collections suggests that of 1076 globally important CWR, 71% are high priority for collecting and 29% of those taxa assessed have no germplasm accessions at all (Castañeda-Álvarez *et al*., 2016). Furthermore over 95% are insufficiently represented in gene banks in regard to their full range of geographic and ecological variation (Castañeda-Álvarez *et al*., 2016).

The number of populations to conserve *ex situ* to capture the full range of genetic diversity is still undecided and may vary species by species. Marshall and Brown (1995) propose a minimum of 50 sites are sampled to adequately conserve the genetic diversity of a taxon *ex situ*, however this is rarely achieved as shown by Vincent *et al*. (2013). In this study over 74% (1247) of 1667 taxa had 50 or less *ex situ* accessions and of these over 75% (939) had less than ten *ex situ* accessions. Parra-Quijano *et al*. (2011) have suggested another method that involves ensuring *ex situ* collections are representative of the full ecogeographic range of a taxon which can be used a proxy for genetic diversity. This is picked up by Phillips *et al*. (2016) who propose for each taxon the conservation of populations from five different ecogeographic zones as a minimum (see Parra-Quijano *et al*. 2012b) for more information on ecogeographic zones). By ensuring that the full range of ecogeographic diversity is conserved *ex situ* we should confident that any important adaptive traits that may be potentially useful are under long term protection.

The Second FAO report on the State of Food and Agriculture (FAO, 2016) highlights the use of *ex situ* methods to act as a backup of material that can be harnessed in the face of climate

change, whilst *in situ* conservation is important for allowing 'evolution to keep step with environmental changes'. The Millennium Ecosystem Assessment (2005) highlights that there are benefits gained through the better integration of both *ex situ* and *in situ* conservation methods. The positive and negatives of both methods are complementary to each other and therefore conservation of PGR will not be successful without the integration of both into national, regional and global conservation strategies. Clearly *ex situ* conservation has been more widely implemented than *in situ* conservation activities for PGR, as illustrated by the wide network of agricultural centres and seed banks worldwide, including the CGIAR network (www.cgiar.org) and the Svalbard Global Seed Vault. This is surprising considering the high cost associated with continued duplication and germination testing and the resources required to conserve these seeds outside their natural environment (van Hintum, 2002). Yet the benefits of being able to easily access the resources to utilise them in breeding projects outweighs these negatives and is the main purpose of conservation of PGR. *In situ* conservation is less developedthan *ex situ* conservation (Maxted *et al*., 2016) but by establishing an *in situ* network of genetic reserves, they could act in the same way as the gene bank network and be managed by those gene banks that have a vested interest in the resources present within the reserves. In this way, *in situ* resources will be just as easy to access as those in *ex situ* storage.

1.3 International legislation for PGR conservation

The conservation of PGR is widely recognized at the international level and numerous initiatives have been set out to try and protect these resources for future food security. The Convention on Biological Diversity (CBD; UN, 1992) is the most important international political instrument dealing with biodiversity loss and although genetic diversity is clearly included in the convention, practical implementation has failed to recognize this sufficiently (Laikre *et al*., 2010). The CBD Strategic Plan (2010 Strategic Plan for Biodiversity 2011-

2020) states that "*By 2020, the loss of genetic diversity of cultivated plants and domestic farm animals in agricultural ecosystems and of wild relatives is halted and strategies have been developed and implemented for safeguarding the genetic diversity of other priority socio-economically valuable species as well as selected wild species of plants and animals*". The convention has been ratified by 196 parties who must now prepare national reports on their progress towards meeting the CBD strategic plan. Furthermore, the Nagoya Protocol (https://www.cbd.int/abs/about/default.shtml/), an additional agreement to the CBD, ensures the fair and equitable sharing of benefits arising from the utilization of genetic resources and came into force in 2014.

The Global Strategy for Plant Conservation (GSPC; UN, 2012) is also part of the CBD and specifically mentions CWR conservation within Target 9: "*70 percent of the genetic diversity of crops including their wild relatives and other socio-economically valuable plant species conserved*." This target is aimed to be completed by signatories by 2020. Furthermore, as part of the ITPGRFA (FAO, 2001) a proposed list of crops of global significance under Annex 1, will require collection and protection to guarantee food security. Although some major crops are missing from this list, such as Soybean (*Glycine max* (L.) Merr) it is a major step forward in conserving those globally important priority PGR.

PGR are not only important to conserve as part of the specific targets above but they can also help to contribute to other global goals. The Sustainable Development goals (2015-2030) (UN, 2015b) build on the Millennium Development goals (2005-2015; UN, 2005) and highlight the need to eradicate extreme poverty and hunger and to protect and enhance food security, for which PGR play major roles. Food security is an international commitment with the legislation above aiming to ensure that it remains a priority on the international agenda. It is not only the

work of one country that will help to secure our food but the collaboration and cooperation of all nations which will determine the future of our food security.

At a regional level within Europe the Biodiversity Strategy (EU, 2011) aims to stop global biodiversity loss by 2020, with Action 9 and Action 10 encouraging the protection of genetic resources by farmers and foresters and the development of the conservation of genetic resources.

1.4 Threats to CWR

The implications ofnot conserving PGR are perhaps much larger than not conserving other wild species. General threats to species are caused by the conflict between supply and demand for natural resources (Stuart and Adams, 1990). This includes, but is not limited to industrialization, urbanization, deforestation, pollution, intensive agriculture, invasive alien species, over exploitation, habitat destruction, changes in agricultural practices and climate change (Bilz *et al*., 2011; Kell *et al*., 2011). In a recent analysis by Maxwell *et al*. (2016) it was found that overexploitation and intensive agriculture were the biggest threats to biodiversity with over 70% of threatened or near-threatened study species at risk. Change in agricultural practises is also one of the most cited threats to CWR (Kell *et al*., 2011) with intensification and unsustainable farming of both livestock and arable land the greatest threat to CWR in Europe (Bilz *et al*., 2011). Furthermore, due to human development and population growth, overexploitation and agricultural expansion will also increase (Maxwell *et al*., 2016). In general, 44.9% of 1826 vascular plant species are assessed as threatened at the European level (Bilz *et al*., 2011). For CWR, 11.5% of 572 species are threatened at the European level (Kell *et al*., 2011). Furthermore, CWR tend to be found in disturbed, pre-climax communities, habitats not traditionally conserved and yet habitats that are the most affected by increasing

levels of anthropogenic change (Jain, 1975; Jarvis *et al*., 2015). CWR are therefore likely to be disproportionately impacted by threats to biodiversity (Maxted and Kell, 2009).

These threats contribute to the main problem facing CWR which is genetic erosion (Maxted *et al*., 2002). Along with the loss of species there will be a loss of genetic diversity which will be faster than the loss of the former as there will be genetic erosion from the extant species (Maxted *et al*., 1997b). As well as other contributing factors, fragmented populations may result from this loss of species resulting in negative effects such as inbreeding, genetic drift and limited geneflow contributing to genetic erosion (Bijlsma *et al*., 2000; Bijlsma and Loeschcke, 2012). The fewer populations lost today the more resilient they will be in the future (Sætersdal *et al*., 1998). The Millennium Ecosystem Assessment (2005) already recognises that genetic diversity has declined globally; particularly among domesticated species. This makes domesticated species more vulnerable to stochastic changes in their surrounding environment, thus threatening food productivity. Furthermore, the narrowing of the food commodities that are being consumed globally (FAO, 2009a; Khoury *et al*., 2014) means that threats to one crop could exasperate the global food crisis.

Climate change is likely to be the greatest threat in many, if not most regions (Thomas *et al*., 2004) and predicting such effects upon terrestrial plant communities is crucial because of the ecosystem services vegetation provides (Franklin *et al*., 2016). Projections show that even under the most optimistic emission scenarios, climate change impacts on biodiversity will be increasingly severe over the next century and beyond (IPCC, 2014). Surface temperature and precipitation are expected to increase at northern latitudes (Solomon, 2007) and an already poleward range expansion across many species is being seen (Thomas *et al*., 2012). The IPCC predicts that in the short-term (2016-2035) the global mean surface temperature change is expected to be between 0.3-0.7˚C with the highest prediction set at 4.8˚C for the year 2100

(Prather *et al*., 2013). These climatic changes will influence agricultural production as well as leading to fragmentation of populations which may cause further genetic problems as mentioned above.

Many regions throughout the world are projected to experience climate change-induced reductions in crop yields and additional challenges are mounting (for example, pests, water supply and soil degradation) (Müller & Robertson, 2014; Rosenzweig *et al*., 2014). In a recent global study from 1980-2008, maize and wheat exhibited negative impacts due to climate change for several major producers and a global net loss of 3.8% and 5.5% respectively (Lobell *et al*., 2011). Furthermore, climate change may be contributing to ~10% stagnation in wheat and barley production since the 1990s (Moore & Lobell, 2014) and yield gains from technological advances are likely to have been offset by warming from 1981–2002 (Lobell & Field, 2007). In a specific study on the CWR of peanut (*Arachis* L.), potato (*Solanum*) and cowpea (*Vigna* L.), it was found that 16–22% of species are predicted to go extinct by 2055 with most species losing over 50% of their range sizes (Jarvis *et al*., 2008). However, climate change may not have negative impacts on agriculture everywhere. Burke *et al*. (2015b) showed that agricultural activity peaked at an average annual temperature of 13˚C, therefore Europe could benefit as warming tends to harm productivity in countries which already have high average temperatures. Extreme weather events, such as the El Niño Oscillation (Rosenzweig *et al*., 2001) and storm damage may result in the destruction of crops and will have implications for food production. We should be looking to adapt to a more uncertain world where, in particular regions the risk of crop failure on a year-to-year basis is likely to increase (Parry *et al*., 2005; Ford-Lloyd *et al*., 2013).

CWR represent one of the most critical assets to address climate change, because they hold so much promise for crop improvement now and in the future (Ford-Lloyd *et al*., 2011). Genetic

diversity improves resilience in species (FAO, 2016) but must be properly conserved and utilised. The effects of climate change will have direct consequences for how *in situ* and *ex situ* management of CWR is undertaken and will require a more dynamic approach. Dispersal is likely to be the most important factor for plants needing to migrate under climate change (Mokany *et al*., 2013) therefore to accommodate these changing plant distributions *in situ* conservation will need to adopt new strategies. This may include upgrading the current, highly static PA system to satisfy targets for both current and future projected occurrences (Midgley *et al*., 2003). Ramirez *et al*. (2014) propose a shift from a static PA approach to a landscape development strategy with improved connectivity between reserves across the Andean countries. Corridors between reserves could be created which follow temperature gradients ensuring that species always have a suitable climatic habitat to move through (Nuñez *et al*., 2013) as well as increasing the heterogeneity in vegetation structure which favours species richness at local and landscape levels (Zapata & Robledano, 2014). *In situ* conservation sites should be established in areas where CWR habitats are likely to remain suitable under climate change (Magos Brehm *et al*., 2016). Maintenance of large populations should also remain a key conservation priority as they support higher levels of genetic diversity and genetic variation (Christmas *et al*., 2016). For *ex situ* conservation of seeds, the effect of climate change may mean targeting collection of populations at the trailing edge of species distributions to try and capture the genetic diversity that is under threat. These populations could be prioritised following the methods described by Magos Brehm *et al*. (2016) which incorporate both the conservation of ecogeographic diversity and climatically vulnerable populations. Further *ex situ* measures such as relocation may also be an option but the problems associated with this such as identifying suitable habitat, pollinator and edaphic conditions may be an issue (Barber *et al*., 2016). Targeted *ex situ* conservation will also have

to focus upon the needs of plant pre-breeders and breeders and the material and traits that they will require to adapt our crops to climate change.

At the Paris climate change talks in 2015 an agreement was made between 195 countries to curb global warming to below 2.0˚C above pre-industrial levels with a specific aim to limit the temperature increase to 1.5˚C (UNFCCC, 2015). Countries have submitted Intended National Determined Contributions (INDCs) that set out measures to help meet this temperature target. Some of the measures proposed include the development of new crop varieties that will allow for a decrease in the use of pesticides and varieties that can withstand water stress (UNFCCC, 2015). Such developments in crop improvement will no doubt benefit from increased conservation and use of PGR. The actions proposed by these plans are not yet enough to meet the 2.0˚C limit (UNFCCC, 2015) but do pave the way to achieving this target.

Although the precise effect of climate change upon biodiversity is unknown, we should be looking to adapt to a more uncertain world where in particular regions the risk of crop failure on a year-to-year basis is likely to increase (Parry *et al.*, 2005). The above threats will be acting simultaneously upon biodiversity therefore by attempting to maintain diversity within the ecosystem we can also help ensure we have a robust and sustainable food system.

1.5 Floristic background to Norway

The population of Norway is just over five million people with the mainland of Norway stretching from 58˚ north to 71˚ north and covering an area of 304, 148 km^2 (Norway Statistics, 2016). Climatic and solar conditions as well as day length vary significantly from the south to the north which tends to favour thermophylious species having their northern distribution limit and cold-loving species having their southern limits within Norway. The country has substantial north-south and east-west climate gradients, with the inland areas having a typical

continental climate and the coastline characterised by a maritime climate (Norwegian Ministry for Agriculture and Food, 2008). The country has been ice-free for less than 10,000 years therefore there tends to be few endemics present (Kålås *et al*., 2006), however this may also mean that species tend to have a restricted occurrence and therefore may harbour important and unique genetic adaptations or traits due to *in situ* glacial refugia (Eidesen *et al*., 2013). The topography of Norway is dominated by mountains and glaciers with a coastline that is defined by islands and fjords (Norwegian Environment Agency, 2015). Although Norway is not a traditional centre of diversity for crop species its location on the north western periphery of Europe may mean that the species present there harbour unique genetic diversity and adaptive traits.

Floristically, Norway has 3148 recorded wild plant species and subspecies of which 1463 (46.5%) are native with the rest regarded as introduced and 43.5% of those introduced taxa being persistent i.e. they have reproducing populations (Kålås *et al*., 2006). The number of introduced species is estimated to be 1719 species (Gederaas *et al*., 2012) of which 135 are listed on the Norwegian black list of species. Norway has an online species observation system (www.artsdatabanken.org) that allows people to register sightings of species throughout the country. This is helping to document biodiversity within Norway with currently over 11.5 million taxon records (Valland, 2014). Furthermore, in Norway the Nature Types index attempts to classify the ecological variation found throughout the country which has helped to create a red list for the ecosystem and habitats (Lindgaard & Henriksen, 2011). Many of the most urban areas are the richest in botanical terms, with the highest concentrations of rare and vulnerable species found around Oslofeltet and south of Østfold in Kristiansand and Stavanger, due to the calcareous nature of the soils (Kålås *et al*., 2006). The soil and topography also impacts agriculture with the main regions for field crops such as cereals, potatoes and

vegetables, found around Oslofjord, in the south east and far south western regions. Forage production however, can be found in all parts of the country where soil conditions are favourable to growing grass (Norwegian Ministry for Agriculture and Food, 2008) and in northern Norway grassland occupies more than 90% of cultivated land (Volden *et al*., 2002). Agricultural activities take up 3.4% of land area, with mountain and mountain plateaus making up 45.2% and forests covering 37.4% of the Norwegian mainland (Norway Statistics, 2016).

1.5.1 Threats to CWR in Norway

CWR in Norway are subject to much the same threats as mentioned previously. In a study by the Norwegian Directorate for Nature Management as part of compiling the Nature Index (Nybø *et al*., 2011) to measure the level of biodiversity within Norway, it was noted that of all the major ecosystems, open lowland and forest are, overall, in a poor state (NI =0.43-0.44). These two habitats are the location of priority CWRs including *Carum carvi* L. and *Rubus chamaemorus* L., amongst others. The red list for ecosystems and habitat types (Lindgaard & Henriksen, 2011) in Norway shows that changes in management practices i.e. disappearance of grazing and therefore allowing the land to become overgrown, plus the use of more fertilizer and new cultivation methods, is one of the most serious threats to semi-natural grassland (classified as vulnerable) and hay meadow habitats (endangered). For hay meadow habitats there is now a specific action plan for their management (Svalheim & Asdal, 2011) which will go some way to improve the condition of this ecosystem. This is important for conservation of CWR in Norway as many tend to be found in lowland areas associated with agricultural practises (Kålås *et al*., 2006). Furthermore, these lowland agricultural systems also contain 35% of the threatened red list assessed species (Kålås *et al*., 2006) with the largest numbers of red listed plant species found in dry grasslands and long-established pastures (Nybø *et al*., 2011; see Table 1.1 for list of CWR assessed by red list). The majority of threatened or near-

threatened species are found in south eastern Norway, mainly Oslo, Vestfold, Telemark, Ostfold and Buskerud. This may be because more thermophilous species are found in south eastern Norway, which is also where the greatest diversity of habitat types that are quite rare in Norway is found. This is also the most populous region, however findings show that this has little influence on the main pattern of wild species distribution (Kålås *et al*., 2006) in Norway.

Invasive species are also a problem for the Norwegian flora and have shown an increase of 54% over the last ten years (Nordic Gene Bank, 2006). The predicted change in climate within Norway to increased precipitation, a longer growing season as well as shorter and milder winters is expected to benefit alien species as the majority come from warmer climates (Gederaas *et al*., 2012). Northern, alpine and continental native species will be at a disadvantage (Gederaas *et al*., 2012). Furthermore, mountainous species will also be negatively affected as they occupy small niches and are adapted to extreme climatic conditions which may make them especially vulnerable if they are exposed to additional impacts (Nybø *et al*., 2011). However, the effects of climate change on agriculture in Northern areas is expected to be positive (Olesen and Bindi, 2002). The effect of temperature rise will likely be a longer growing season with higher mean temperatures allowing farmers to increase harvest and yields (Uleberg *et al*., 2014). This in turn could lead to an estimated 250% increase in GDP per capita by 2100 for Norway as the annual average temperature increases due to climate change (Burke *et al*., 2015b). However, farmers will need to adapt to this change by ensuring their crops are robust enough to withstand climatic changes which will require the harnessing of all available genetic variation, for which the gene pool of wild populations and landraces is important (Uleberg *et al*., 2014). Although the overall effect for agriculture in Norway may be positive there will still be challenges in the short term such as unstable winters, increased precipitation and more weeds and diseases (Uleberg *et al*., 2014).

Table 1.1: National Red List status of priority CWR within Norway. 13% (27) of the priority list CWR were assessed, with 7% (14) assessed as threatened. Within the checklist 11% (274) were assessed and 6% (154) were threatened (Kålås *et al*., 2006).

Taxa	National Red List status
Allium fistulosum L.	EN B2ab(i,ii,iii,iv,v)
Allium scorodopraum L.	NT
Allium senescens montanum (F.W.Schmidt) Holub	EN B1ab(i, ii, iii, iv, v)+2ab(i,ii,iii,iv,v)
Alopecurus pratensis alpestris (Wahlenb.) Selander	NT
Arnica montana L.	VU A2bc+3c;B2ab(ii,iii,iv,v)
Artemisia maritima L.	NT
Elymus fibrosus (Schrenk) Tzvelev	EN B1ab(ii,iii,iv,v)+2ab(ii,iii,iv,v)
Lactuca sibirica (L.) Benth. Ex Maxim	NT
Lathyrus palustris L.	VU A4bc
Phleum phleoides L.	EN B1ab(I,ii,iii,iv,v)+2ab(I,ii,iii,iv,v)
Poa arctica caespitans	NT
Poa bulbosa L.	EN B2ab(iii)
Poa lindebergii Tzvelev	VU A2abc+3c
Poa x jemtlandica (Almq) K Richt	NT
Rorippa islandica (Oeder ex Murray) Borbas	EN B1b(iii)c(v)+2b(iii)c(v)
Rosa pimpinellifolia L.	EN B1ab(ii,iii,iv)+2ab(ii,iii,iv)
Rosa pseudoscabriuscula (R.Keller) Henker & G. Schulz	DD
Rosa rubiginosa L.	NT
Rubus caesius L.	NT
Rubus hallandicus	NT
Rubus septrentrionalis W.C.R.Watson	NT
Trifolium campestre Schreb	NT
Trifolium fragiferum L.	EN B2ab(ii,iii,iv,v)+2ab(ii,iii,iv,v)C1
Trifolium montanum L.	VU D2
Vicia lathyroides L.	EN B2ab(i,ii,iii,iv,v)
Vicia orobus DC.	NT
Vicia pisiformis L.	EN B1ab(ii,iii,iv,v)+2ab(ii,iii,iv,v)

1.5.2 Current in situ and ex situ conservation actions in Norway

As Norway is a signatory to the previously mentioned legislative initiatives it has a responsibility to undertake appropriate actions to meet such targets. These actions will take place at the national level and have been incorporated into the Nature Diversity Act (2001-12-

21 nr 1525) which is the most important instrument for expanding protection of the natural environment. Under this Act it states:

*'Management objectives for species are to maintain their genetic diversity for the long term…the genetic diversity of domesticated species shall be managed in such a way that it helps to secure the future resources base.' (*Chapter 2, Section 5)

'*Ex situ conservation measures shall be implemented if this will promote the species' survival in the natural environment.'* (Chapter 3, Section 27)

'That protected areas shall promote the conservation of... (b) species and genetic diversity...(f) natural environments that reflect human use through the ages (cultural landscapes) and facilitation of forms of use that help to maintain biological, geological and landscape diversity.' (Chapter 5, Section 33)

These explicit goals can be achieved through the identification of gaps in current *in situ* and *ex situ* CWR conservation combined with improved recognition of CWR in national and regional policy, ultimately leading to systematic CWR diversity conservation (Maxted *et al*., 2015).

The legislation also designates different types of protected areas which encompass about 17% of mainland Norway (Norwegian Environment Agency, 2016) including NPs, nature reserves, natural monuments and landscape protected areas as well internationally defined areas such as Ramsar sites (www.ramsar.org). The landscape protected areas are particularly interesting in the CWR context as these sites tend to cover areas of traditionally managed agricultural land associated with anthropogenic influences, a habitat which has been shown to favour CWR (Maxted & Kell, 2009, Jarvis *et al*., 2015). Although Norway has no formal *in situ* CWR conservation these taxa are conserved passively in the above mentioned protected areas, but

this ‘is not enough to guarantee the continuation of these populations’ (Maxted *et al*., 1997a, Hunter *et al*., 2012) hence the proposal for the establishment of a network genetic reserves with active management guidelines for CWR. Furthermore, according to the Norwegian Environment Agency (2016) about 30% of the total number of PAs are under threat, hence establishment of genetic reserves and the surveying associated with these may help to improve this situation.

Ex situ conservation is done between the Nordic Genetic Resources Centre (NordGen) and the National Programme for Plant Genetic Resources managed by the Norwegian Genetic Resource Centre. NordGen is responsible for the conservation of seed propagated crops and the potato collection, whilst vegetatively propagated crops are the responsibility of the Norwegian Genetic Resource Centre which coordinates activities regarding the conservation and utilisation of national genetic resources. Seed storage is within freezers at -18ºC and is situated in Alnarp, Sweden and duplicated in Arslev, Denmark and in the Svalbard Global Seed Vault. Information about the conserved germplasm is managed through the SESTO database (www.nordgen.org/SESTO). There are 2291 accessions stored that originate from Norway with 1512 which are CWR (Table S1.1). Of the priority CWR there are 1457 accessions stored in NordGen (Table S1.1). Use of *ex situ* material is coordinated by Graminor AS, a company jointly owned by private and governmental institutions. Current breeding programmes include cereals, forage grasses and clovers, potato,fruit and berries (Norwegian Ministry for Agriculture and Food, 2008). The Norwegian government also owns and manages the Svalbard Global Seed Vault with the Crop Trust providing the funding. The vault is a back-up for seed collections from around the world which can be deposited free-of-charge in the vault.

The CWR taxa present within Norway are nationally and globally important due to numerous reasons including: the climatic gradients found across the country which vary from north to

south and east to west (Norwegian Ministry for Agriculture and Food, 2008); the location of the country on the north west of Europe meaning many species may find themselves at the limits of their distributional ranges; the relatively recent glacial periods within Norway which may lead to important populations found within glacial refugia (Eidesen *et al*., 2013) and the distribution and presence of important temperate forage species such as *Trifolium* L., *Festuca* L. and *Lolium* L. across the country. Furthermore, Norway is obligated under the international treaties mentioned above to increase protection and use of their genetic resources which can only begin with national level strategies.

1.6 Background to methods

The preparation of a national strategy for PGR conservation is one tool to help ensure future food security by meeting the targets set out in the above legislation. A strategy will always need to involve local stakeholders who will ensure its appropriateness and effectiveness (Magos Brehm *et al*., 2016). Furthermore, the results from any strategy must be dynamic and should be updated as and when new data become available.

1.6.1 Checklist and inventory

Checklists and inventories are the foundation for the formulation of conservation strategies (Maxted *et al*., 2013) and provide the data for monitoring and allowing us to determine what exists, where and how to conserve it. Countries will always need to establish their own CWR lists of taxa that are most relevant to their own crops, floras, national capacities and priorities (Meilleur & Hodgkin, 2004). A checklist is a list of CWR taxa present within a country whilst the inventory is created after prioritization and provides extra information on these taxa such as nomenclature and ecogeography (Maxted *et al*., 2013).

Due to limited resources for conservation of PGR, prioritization is a key component to help in the focusing of resources to where they are most needed, therefore it is usually necessary to reduce the list of taxa within the checklist to a more manageable number. Maxted *et al*. (1997b) propose numerous measures that can be applied to aid prioritization with Kell *et al*. (2015) and Vincent *et al*. (2013) highlighting the three most commonly used criteria: relative socio-economic importance of the related crop, potential use for crop improvement and threatened status. Other criteria may include whether the area of interest is a center of diversity for specific CWR (Phillips *et al*., 2014), recent change in population range (Fielder *et al*., 2015) native status of the crop (Magos Brehm *et al*. 2008; Taylor *et al*., 2013; Fitzgerald, 2013; Landucci *et al*., 2014) and more. Iriondo *et al*. (2016) reviews the criteria used for prioritization in numerous national strategies and concludes that even commonly used criteria can be different in the way in which they are conceived and implemented, highlighting that there is no right or wrong way to creating an inventory of CWR.

1.6.2 Ecogeographic study

Ecogeographic studies are recognized as the basic planning tools for *in situ* and *ex situ* CWR conservation (Hodgkin & Guarino, 1997; Maxted *et al*., 1997b). An ecogeographic study is the process of gathering and synthesizing taxonomic, geographic and ecological data, the results of which are predictive and can be used to assist in the formulation of collecting and conservation priorities (Maxted *et al*., 1995). This may involve, amongst others, the identification of the target taxa, delimitation of the study area and the identification of data sources. The ecogeographic study can then utilize Geographic Information Systems (GIS) to formulate *in situ* and *ex situ* conservation needs.

One key step within the ecogeographic study is the gathering of occurrence data for the priority taxa. This requires the collecting of location information from herbaria, gene banks, online

resources and others to capture as fully as possible the distribution of the taxa. Increasingly, online resources such as the Global Biodiversity Information Facility (GBIF; www.gbif.org) are providing easier access to a range of location data sources. This data can then be utilized for spatial analysis which can contribute significantly to improved understanding and monitoring of biodiversity (Scheldeman & van Zonneveld, 2010). Species distribution models (SDM) can be created to identify areas that may be suitable for populations to persist in and prioritise areas for conservation. This analysis can be conducted using GIS tools (Guarino *et al*., 2002) which use the conditions at points where the species has been found in order to construct a statistical model of its adaptation ranges, based on a set of user-defined environmental variables (Guisan & Zimmermann, 2000). The models can help identify the bioclimatic space where species could persist (Pacifici *et al*., 2015) both currently and in the future.

The accuracy and predictive ability of the spatial analyses can be dependent upon the reliability of location data that is available. There is no minimum defined number of presence points needed to create species distribution models, but it is generally accepted that the more the better the predictions will be. For example van Zonneveld *et al*. (2009) used a minimum of 50 presences for widely distributed species whereas Scheldeman *et al*. (2007) used 10 presences for rare species and Phillips *et al*. (2014) used a minimum of 10 presences for CWR taxa within Cyprus. Specifically, for studies using MaxEnt a minimum of 30 records should be used for a stable performance but MaxEnt can still be used for exploratory modelling when sample size varies between 10 and 30 occurrences (Wisz *et al*., 2008). Hernandez *et al*. (2006) found that model accuracy does increase with larger sample sizes but useful models could still be produced with as few as 5-10 observations, therefore the appropriate number of presences to use will depend upon the amount of data available and the needs of the project in question.

Preliminary spatial analyses include determining the spread of taxa richness (i.e. the number of different taxa) within the area of interest. This can help give an overview of where the diversity is located. However, this depends upon the number and spread of location points used, therefore it is also necessary to create a sampling bias map to determine how this may be affecting the results. Further analyses include a complementarity analysis to create an effective reserve network for *in situ* conservation. This will not only optimize conservation at levels from landscape to individual species, but also is cost effective and practical to implement (Bolliger *et al*., 2011). This can be partly achieved through the use of an iterative approach to select reserves (Rebelo, 1994). The cell with the highest taxon number is selected first, then these taxa are excluded from the analysis as it is repeated until all taxa have been selected (Rebelo, 1994). Hence both species richness and difference in species composition at each site is considered and therefore locations are identified that conserve a high number of different species between sites (Scheldeman & van Zonneveld, 2010). A consideration to be made here concerns the size of the potential reserves in the complementary network. Phillips *et al*. (2014) use a 5 km^2 area for reserves in Cyprus whereas Maxted *et al*. (2008a) use 100 km^2 grids for analysis at the regional level in Africa. The size of reserves will also depend upon who is planning to implement the network and their available resources. The grid cell approach to complementarity analysis allows the determination of a complementary network that can include all taxa regardless of their location within or outside of a PA, whereas the PA approach restricts the analysis to within PAs. Establishing complementary *in situ* conservation within the current PA network is more cost effective and may only require minimal adjustments to existing management plans (Maxted & Kell, 2009). It is possible to do these analyses using tools in the CAPFITOGEN package (Parra-Quijano *et al*., 2016) and see section 1.6.5).

1.6.3 Predicted distribution

The predictive capacity of SDM can be harnessed to undertake a gap analysis study (Margules, 1989). This may be used to identify *in situ* locations where species are predicted to occur but are not yet actively conserved, and populations/areas that are underrepresented in *ex situ* collections. The potential distribution maps are created from ecogeographic variables such as climatic, edaphic and geophysical factors which are used to predict where a species may be located. Such data are feely available from sources such as WorldClim (Hijmans *et al*., 2005) and CCAFS (http://www.ccafs-climate.org/data/ -see 'Baseline data') with national experts often able to provide more local and detailed data.

MaxEnt (maximum entropy) software is one approach used to create a predictive distribution map for the species under current and future climatic conditions by calculating the species realized niche and probability of occurrence using an algorithm for maximum entropy (Phillips *et al*., 2004; *et al*2006). MaxEnt has fared well in evaluations in comparison to other programmes (Anderson *et al*., 2006; Elith *et al*., 2011), has been widely applied in predicting species distributions (Elith *et al*., 2011; Costa *et al*., 2010) and for use with wild relatives from single crop studies such as sweet potato (Khoury *et al*., 2015) and bean (Ramirez-Villegas *et al*., 2010) to global analysis of multiple CWR (Jarvis *et al*., 2008; Conolly *et al*., 2012; Castañeda-Álvarez *et al*., 2016). However, it can be sensitive to the number of environmental predictors used (Phillips & Dudík, 2008). Therefore prior to analysis it is necessary to carefully select the environmental variables. This can be done using bibliographic searches, expert opinion or through purely statistical methods (although the latter is not recommended without expert involvement; Parra-Quijano *et al*., 2016). Other modelling algorithms may also be used including, but not limited to: Domain (Carpenter *et al*., 1993), Bioclim (Booth *et al*., 2014), Generalized Linear Models (GLM) and Generalized Additive Model (GAM) (Guisan *et al*.,

2002) and Random Forest (Bradter *et al*., 2013). Once predictions are made it is desirable to ground-truth the areas of interest to confirm the presence of the taxa.

1.6.4 Climate change analysis

Potential distribution maps can also be created for future climatic scenarios which is particularly important for the long-term conservation of CWR. There is an established strong correlation between climate and the distribution of both flora and fauna from the equator to the poles (von Humboldt & Bonpland, 1807; Woodward & Williams, 1987). As climate changes the distribution of species will change, thus knowing how and where species may move to is essential if we are to create a robust conservation strategy. The most recent IPCC report (IPCC, 2014) provides detailed future climatic data that can be accessed through CCAFS and WorldClim and can be used in climate modelling. General Circulation Models (GCM) form the basis of these future predictions with multiple models and scenarios to select from. The Representative Concentration Pathway (RCP) scenarios are from the most recent IPCC report and represent emission, concentration and land-use trajectories to the year 2100 (Van Vuuren *et al*., 2011). RCP 2.6 is the scenario which relates most closely to the agreed maximum temperature rise set out by the Paris Agreement (UNFCCC, 2016) of 1.5°C. Whilst RCP 6.0 represents a temperature rise of 2.5-3.5°C which is predicted to be reached if the INDC proposals are followed (UNFCCC, 2015). Climatic models are specific statistical predictions regarding the details of how the climate may change and are also used along with the RCP scenarios (Flato *et al*., 2013). Models can be used independently (although this is not recommended if you do not account for uncertainties within the model) or combined in an ensemble approach (Meehl *et al*., 2007) which generates more uncertainty in predictions but without which could greatly under estimate the severity of worst-case scenarios (Burke *et al*.,

2015a). Consultation with experts is recommended when choosing the appropriate model/s to use.

When modelling the potential species distribution we can use a correlative model which assumes that species distributions are in equilibrium with their climate and which have worked well in predicting taxon range shifts (Guisan & Thuiller, 2005). However, this method ignores the roles of inter-specific interactions, habitat, geomorphology and human interactions (Guisan & Thuiller, 2005). Other methods that can be used include Trait-based Vulnerability Assessments (TVAs) which combine sensitivity, adaptive capacity and exposure assessments for taxa and Mechanistic models which require detailed information upon physiological, demographic and distribution data of taxa (Foden & Young, 2016).

When determining how taxa will be affected by climate change the IUCN recommends that a Climate Change Vulnerability analysis (CCVA) is carried out (Foden *et al*., 2013; Foden and Young, 2016). This involves measuring the sensitivity, exposure and low adaptive capacity of a species (Foden *et al*., 2013) to determine which species are most vulnerable to extinction. This method requires detailed information upon a species' ecology, biology and physiological traits (Foden *et al*., 2013) which can often be hard to gather, especially when needing to assess a wide range of different species. Population changes for species can be inferred from projected changes in suitable habitat (Nenzén & Araújo, 2011) which in the absence of more specific information is deemed an allowable assumption when assessing a species' vulnerability in the face of climate change (Foden & Young, 2016). An essential step is to consider whether habitat patches are large enough to support viable subpopulations and whether patches are likely to be colonised by individuals in the future with such considerations being species specific (Foden & Young, 2016).

1.6.5 CAPFITOGEN tools

The CAPFITOGEN tools were developed to help strengthen the capabilities of national PGR programs (Parra-Quijano *et al*., 2016). The tools bring together a range of statistical analyses and GIS functions aimed at enabling the conservation and efficient use of PGR (Parra-Quijano *et al*., 2016). The CAPFITOGEN tools utilise the "R" software (R CoreTeam, 2012) which is freely available and open-source allowing continued development of the tools. The tools can be used to help formulate both *in situ* and *ex situ* conservation targets. This includes, but is not limited to, the identification of complementary networks of *in situ* reserves both selecting grid cell areas and specific PAs (using the Complementa tool), the use of the Representa tool for the establishment of gaps within *ex situ* collections and the creation of ecogeographic maps to study the ecogeographic diversity within the area of interest (Parra-Quijano *et al*., 2016).

Ecogeographic Land Characterization (ELC) maps (Parra-Quijano *et al*., 2012b) can be used to study potential plant adaptation ranges, allowing the identification of distinct populations for conservation. Ecogeographic diversity can be used as a proxy for genetic diversity (Greene & Hart, 1999) with the assumption being that the conservation of maximum ecogeographic diversity will result in the conservation of maximum genetic diversity (Maxted *et al*., 2013) both *in situ* and *ex situ*. ELC maps also have the advantage of being low cost, easy-to-use and appropriate for large numbers of species and populations (Parra-Quijano *et al*., 2012b). If populations are conserved in each of the different ELC zones it is possible to assume that a wide range of genetic diversity is also conserved (Parra-Quijano *et al*., 2012c). ELC maps can be created as generalist maps for a range of different species with one study showing that ELC maps for some species i.e. leguminous species, perform better than other species i.e. grasses (Parra-Quijano *et al*., 2012b). Parra-Quijano *et al*. (2012b) concluded that the maps provided a satisfactory rendition of adaptive scenarios, but they suggest specific maps should be created

for individual species as efficiency at detecting favourable and marginal environments varies per plant species. This is more important if the map is not validated by experts and ecogeographic variables are not properly selected (Parra-Quijano *et al*., 2016). An ELC map has also been used for assigning conservation priorities within Jordan (Magos Brehm *et al*., 2016) in which 20 species were studied and *in situ* and *ex situ* conservation priorities for populations were determined. The ELC map can be used within the Complementa and Representa tools. In the latter, the tool can help identify gaps relating to the ecogeographic diversity within germplasm collections, thus allowing collectors to target those ecogeographic and hence genetic diversity gaps within their collections. Within the Complementa tool the complementary network can be selected to target both species and ecogeographic diversity for conservation within the proposed network.

1.6.6 Genetic diversity analyses

Although ELC maps are thought to act as a proxy for genetic diversity (Greene & Hart, 1999) it is still useful to undertake detailed genetic studies of populations. These can help determine a genetic baseline against which change can be measured and enable the establishment of the number and location of populations in which to conserve and identify traits of importance for crop improvement (Maxted *et al*., 2013). Genetic diversity studies are also a requirement for the establishment of a genetic reserve network (Iriondo *et al*., 2012).

The selection of populations for genetic diversity studies will depend upon the aims of the conservation strategy but should utilise all previous diversity analyses and expert knowledge to assist. The number of populations to study can vary dependent upon needs but the number of individuals per population will need to be a balance between gathering enough reliable information and the resources available. In a study by Khanlou *et al*. (2011) they used 75 individuals per sample of white clover, but the authors acknowledge that this is more than the

norm in genetic diversity studies of white clover. In another white clover study, Hargreaves *et al*. (2010) used between 12-32 individuals per population in order to determine diversity between island and mainland populations. Other genetic diversity studies tend to use 20 individuals per population as the norm (Guthridge *et al*., 2001; Fjellheim & Rognli, 2005; Johnson *et al*., 2011; Magos Brehm *et al*., 2012; Fielder, 2015).

For studying the genetic diversity within and between populations a range of molecular markers can be used depending upon the needs of the study and resources available. AFLPs (Amplified Fragment Length Polymorphisms) are frequently used in conservation planning (Maxted *et al*., 2006; Watson-Jones *et al*., 2006; Collins *et al*., 2012; Hargreaves *et al*., 2010; Magos Brehm *et al*., 2012). They are neutral dominant markers that can amplify many loci at a time and they tend to be more reliable than other markers such as RAPDs (Random Amplified Polymorphic DNA) and SSRs (Simple Sequence Repeat). They are cheap, relatively easy to use, and can be used on a range of species without prior knowledge of the genome. However, they only allow the study of neutral genetic diversity which is not affected by natural selection and does not provide any evolutionary ability to the individual. Furthermore, as AFLPs include both coding and non-coding regions a higher level of genetic diversity may be recorded (Dagher-Kharrat *et al*., 2007). Microsatellites (or SSRs) are highly polymorphic and co-dominant meaning a high level of allelic diversity can be identified. However, these markers have not been identified in the majority of CWR (Schlötterer, 2004) and therefore may not be appropriate for multi-species analyses. Single nucleotide polymorphisms (SNPs) allow us to focus upon adaptive genetic diversity within populations as they may be found within coding and non-coding regions of genes. However, the best approach for large scale SNP identification requires a fully-sequenced genome which is not available for the majority of crop plants (Ganal *et al*., 2009) l*et al*one their wild relatives.

Although markers that detect neutral genetic diversity (AFLPs, SSRs) are used it is important to determine how this diversity may or may not relate to adaptive traits within the individual. Schoen and Brown (1993) tentatively suggest that some variation which is potentially useful may be identified. Studies by Johnson *et al*. (2011) on *Poa* L. species and Richardson *et al*. (2009) on *Pinus* L. species found that there is a potential correlation between AFLP diversity and adaptive traits that may be linked to climate. Reed and Frankham (2003) go further and show there is a significant correlation between heterozygosity and fitness through its link with population size but they also suggest that this correlation may not be strong due to the neutral nature of markers. Furthermore, in an earlier study Reed and Frankham (2001) found there was no correlation between heritability and molecular heterozygosity, therefore interpretations of genetic diversity and adaptive ability need to be made cautiously. Neutral markers can still provide information on the level of genetic diversity between populations, which may allow us to determine which populations contain the highest levels of diversity. If those populations are targeted for conservation, then we should be confident that the majority of genetic diversity is protected. With a lack of information on the adaptive traits conferred from genetic diversity it will be better to conserve more genetic diversity as it will allow populations a larger capacity to adapt (Reed & Frankham, 2003).

1.7 Aim and objectives

The project will develop the scientific background and the methodologies needed for conservation of PGRs at the national level, including GIS techniques and genetic diversity studies, to produce a clear and focused strategy for the conservation of CWR in Norway. The strategy is a set of suggestions that will require the support and involvement of local stakeholders to implement. The methods used here can be applied to other countries and at the

regional and global levels. This is the first such national strategy and is a starting block in the future of sustainable and effective, *in situ* and *ex situ* CWR conservation in Norway.

The following objectives will help achieve this:

- A detailed diversity analysis of both *in situ* and *ex situ* conservation priorities for CWR to assist in the creation of a conservation strategy for CWR in Norway.
- A climate change analysis for priority CWR which will contribute to a long-term conservation strategy for Norway.
- Genetic diversity studies of a subset of the priority CWR species to help inform conservation priorities for Norway.

1.8 Overview of thesis

Chapter 2: Methodology

The methodology used in chapters 3, 4 and 5 is described in detail here. The criteria and processes used to prioritise the CWR in Norway is detailed first. Following this the use of GIS to create species distribution models both in the present and future predicted climate is described. The assessment process of how populations may shift and decline or increase in distribution is described. Along with this the IUCN Red List categories are applied to help prioritise those taxa that may require urgent conservation. Next a description of the field work that was conducted to collect leaf samples from CWR populations around Norway for use in genetic diversity studies is described. The laboratory methods used for the AFLP procedure and the statistical methods used to determine the levels of genetic diversity are detailed.

Chapter 3: *In situ* and *ex situ* diversity analysis of priority crop wild relatives in Norway (Phillips *et al*., 2016).

Norway has both national and international commitments to the systematic, long-term conservation of CWR. This can be achieved by ensuring both *in situ* and *ex situ* protection and utilisation of the broad range of genetic diversity of CWR present within the country. The creation of a CWR checklist and subsequently a priority list of CWR within Norway is detailed. The diversity analysis procedure is then described and involved predictive species distribution modelling in MaxEnt, the use of CAPFITOGEN software to undertake *in situ* complementarity analyses and identify *ex situ* collecting priorities, as well as the creation of ELC maps. This resulted in a priority list of 204 CWR within Norway. Recommendations for both *in situ* and *ex situ* conservation are described based upon the results of the diversity analyses. The priority taxa are important at both national and global levels for food security and the methodology used can be applied at national, regional and global levels for similar *in situ* and *ex situ* diversity analyses. The recommendations put forward will help Norway to meets its international obligations for conservation and use of the genetic diversity of CWR.

Chapter 4: Climate change and national crop wild relative conservation planning (Phillips *et al*., 2017).

Climate change is likely to be one of the key factors affecting our future food security. To mitigate any potential negative impacts, we will require our crops to more genetically diverse. Such diversity is available in CWR, the wild taxa relatively closely related to crops and from which diverse traits can be transferred to the crop. Conservation of such genetic diversity resides within the nation where they are found therefore, national level conservation recommendations are fundamental to global food security. In this chapter, the potential impact

of climate change on CWR richness in Norway is studied. A predicted 1.5°C and 3.0˚C temperature rise was modelled for the years 2030, 2050, 2070 and 2080. The application of the IUCN red list criterion A3(c) to priority CWR diversity in Norway was used to indicate the potential threat level of taxa. Based on these climate change predictions recommendations for conservation and management of *in situ* and *ex situ* priority CWR are suggested. The methods developed here can be applied within other countries and at global levels to improve the effectiveness of long-term conservation actions and help ensure global food security in changing environments.

Chapter 5: Genetic diversity studies of priority crop wild relatives in Norway using AFLPs: Implications for conservation (Phillips *et al*., submitted)

CWR are an essential source of genetic diversity due to their close relationship to cultivated crops and the relative ease of trait transfer. Understanding the genetic diversity of CWR will allow us to better manage and sustain our natural resources. Genetic diversity studies were carried out upon three CWR taxa *C. carvi*, *T. repens* and *T. pratense* using Amplified Fragment Length Polymorphisms (AFLPs). Descriptive statistics were calculated to help determine the patterns of genetic diversity within and amongst populations. Management plans for the *in situ* and *ex situ* conservation of the broadest range and most important genetic diversity were suggested. The creation of management plans for priority CWR in Færder NP and its potential for being designated the first genetic reserve in Norway are discussed.

Chapter 6: Discussion

Collation of the results and recommendations from the previous chapters are discussed. *In situ* and *ex situ* priorities for CWR conservation in Norway are highlighted. Achievements of the project including the progress made in Færder NP and the initiation of the Nordic initiative for

conservation of CWR are detailed. Limitations of the project are discussed and proposals for solving these and improving upon the work are explored. Further areas of research are suggested that will help to improve conservation of CWR in Norway. Conclusions are drawn with a focus upon the essential need for stakeholder engagement throughout the project and the broader impact of the national strategy for Norway on CWR conservation and use.

CHAPTER 2.

Methodology

To help meet the needs and aims for the conservation of CWR both *in situ* and *ex situ* within Norway a targeted national strategy was developed. The *Resource book for the preparation of national plans for conservation of CWR* (Maxted *et al*., 2013) contains a detailed description of the process and methodology required for the CWR conservation planning. The format suggested in the resource book helped inform the creation of the national strategy for Norway. Other resources that have been used here include the Training Manual on Spatial Analysis of Plant Diversity and Distribution (Scheldeman & van Zonneveld, 2010) and the CAPFITOGEN manual (Parra-Quijano *et al*., 2016). It was important to use both objective (statistical analysis) and subjective (expert opinion) methods for the creation and validation of the Norwegian national strategy. Local stakeholders were involved at each stage of the process which added value and improved the likelihood of the strategy being implemented, as suggested by Magos Brehm *et al*. (2016) and Phillips *et al*. (2014). The methodology used is a scientific baseline for further work and as such the results are dynamic and can be updated as and when new data become available. Furthermore, the national strategy and methodology used can and should be incorporated into both regional and global objectives for conservation of CWR.

2.1 National CWR checklist

A complete CWR checklist [with taxa names and authorities] was created for Norway (Table S2.1). This was initially derived from the Crop Wild Relative Catalogue for Europe and the Mediterranean (Kell *et al*., 2008). The checklist was updated and harmonized with the Flora of Norway (Lid & Lid, 2005) and cross checked with national experts to ascertain the commonly

used taxonomy for the Norwegian flora. The CWR checklist is dynamic and as taxonomy and species distributions' change the list should be updated.

2.2 Prioritization and Inventory

Within Norway the CWR checklist was prioritized using the following criteria (Phillips *et al*., 2016 and chapter 3):

- Those CWR within the same genera as crops of high economic value were ranked (1 to 24, highest monetary value to lowest), as given by gross production value (current million US$) for global production value (2013), European value (2013) and Norwegian value (2013) (FAO, 2013);
- CWR present in Annex 1 of ITPGRFA and not previously included;
- CWR highlighted as being of specific importance to Norwegian research (e.g. *Phleum* species), culture (e.g. *C. carvi*) and environment as ascertained from local experts and literature and which were not previously included;
- Taxa within the Harlan and de Wet inventory (Vincent *et al*., 2013) which contains 29 priority crops of global importance and which were not previously included on the above criteria.

Only indigenous taxa according to the Flora of Norway (Lid & Lid, 2005) and/or those populations of introduced taxa that have stable populations (present for at least 10 years, following the same criteria as the Norwegian Red list (Kålås *et al*., 2006)) were kept in the prioritized list. Crops were ranked (1 to 24, highest monetary value to lowest) and the associated wild taxa were matched to these crops. The other criteria were then applied. The prioritized list was validated by Norwegian experts at the Natural History Museum Oslo and the Forest and Landscape Institute via discussions and email contact where taxa were removed

or added if deemed appropriate and now forms the basis of the Norwegian Inventory of CWR (Table S2.2).

2.3 Ecogeographic study

The flora of Norway (Lid & Lid, 2005) was used as the taxonomic classification for the national strategy. Some common synonyms are included in the inventory to help with identification and ease of use. Microsoft Excel and Microsoft Access were used to create the basic database for all accession information gathered from the ecogeographic study. All passport information was gathered from GBIF (GBIF, 2013), which includes herbaria, observational and gene bank data. Data for *ex situ* accessions were initially gathered from the SESTO database (August 2015; www.nordgen.org/sesto/; see Table S1.1). This data was cross-checked with the GBIF database to ensure that all *ex situ* data was being utilised and was then used to produce an *ex situ* database of the priority CWR (Table S1.1). Only geo-referenced passport data was collected which included the longitude and latitude locations using the decimal degrees (D.D) WGS 1984 coordinate system. Over 96% of occurrence records were accurate to three or more decimal places with the remaining 4% of data that had two or less decimal places (Phillips *et al*. 2016). This was deemed appropriate to use in distribution modelling for this study as removal of such data would have resulted in the loss of large amounts of information, including the removal of some taxa from the study altogether (Phillips *et al*., 2016).

Only unique records were kept and those presence points which were the same species and the same coordinates were deleted. In the case of duplicate data collected at different times, the most recent observations were kept. Erroneous data such as incomplete entries and ambiguous species names, for each taxon were removed before proceeding with the analysis. DIVA-GIS

(Hijmans *et al*., 2004) software was used to check for outlier occurrence data by locating those presence points outside the country boundaries of Norway which were then consequently removed from further analysis.

2.4 Diversity analyses

Figure 2.1 is a representation of the main processes that were conducted in the creation of the species distribution models (SDM) and resulting conservation strategy for Norway. Species distribution modelling required the use of specific software including the commonly used, freely available, DIVA-GIS, CAPFITOGEN and MaxEnt. Arcmap 10.2 (ESRI, 2011) was also used but is not freely available. All maps created were in the geographic projection WGS 1984 World Mercator. This geographic projection was used due to being widely recognised as the standard system in navigation and is the reference coordinate system for the Global Positioning System. The distances measured by this projection are true along the equator but area measurements become distorted at the poles, however angles and shapes are essentially true to their measures when studying at small scales. Due to the small size of areas which are used in this study (4 x 8 km^2) this coordinate system is still valuable and with agreement from Norwegian stakeholders was deemed appropriate to use here.

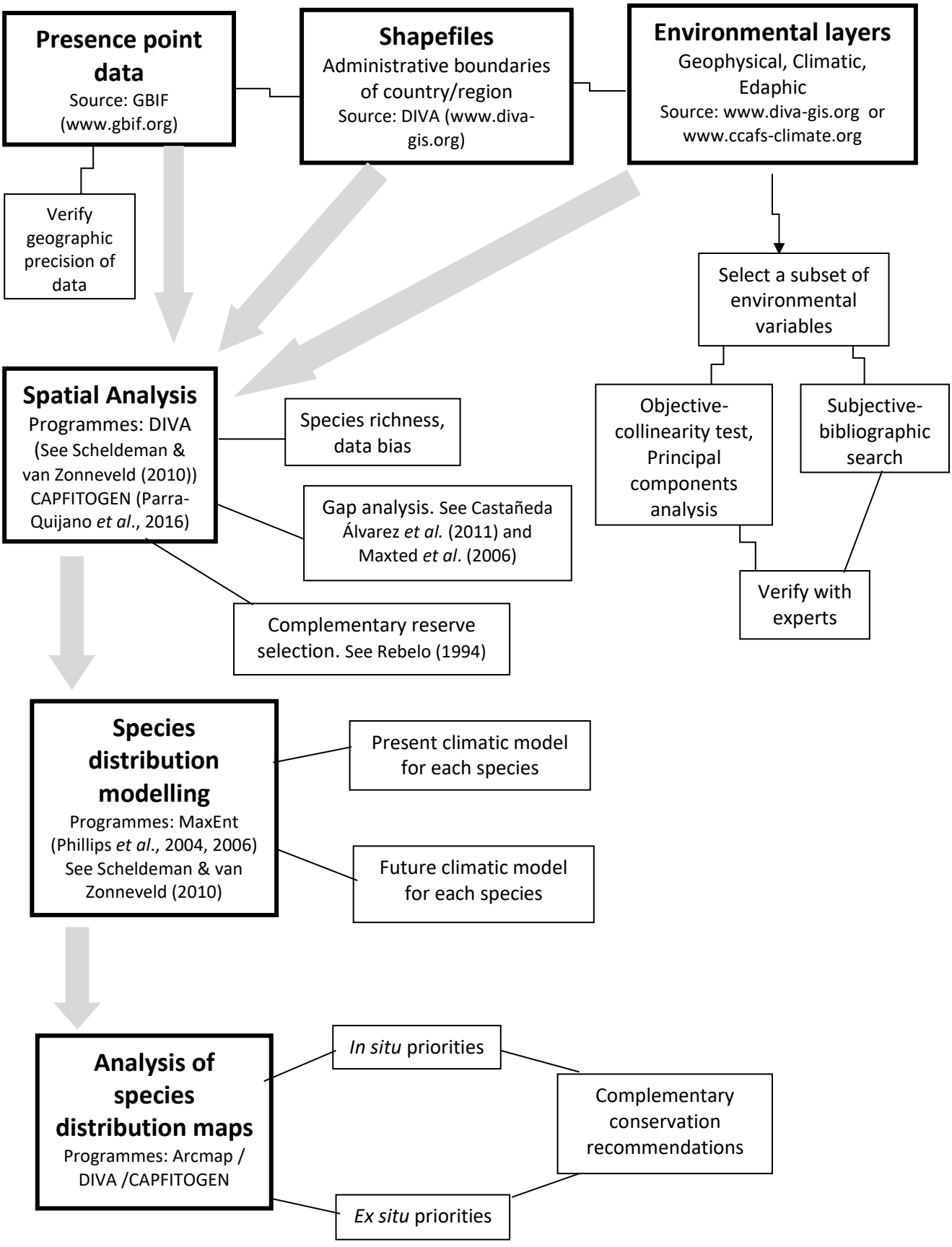


Figure 2.1: Schematic of the main procedures used in creating SDM and predictive climatic models in this study.

2.4.1. Ecogeographic Land Characterization maps

The ELC mapas tool of the CAPFITOGEN package (Parra-Quijano *et al*., 2016) was utilised to create a generalist ELC map for the priority CWR. This generalist map can be applied to several plant species occurring in a territory based on a single, initial effort i.e. generation of the map (Parra-Quijano *et al*., 2012b). Experts on plant distribution within Norway were consulted on which variables most contributed to plant adaptation within the landscape (specifically the priority CWR), therefore validating the use of such a generalist map.

To create the ELC map, first a subset of variables from bioclimatic, geophysical and edaphic components was selected from an initial 103 variables (Table S2.3). These variables represented the main factors related to abiotic adaptation according to Lobo *et al*. (2001). This was done by using a combination of objective and subjective methods to decide upon the variables to use (Cowling *et al*., 2003). The environmental data was combined with the presence point data for the priority taxa in Norway and the environmental values at each point were extracted in R using packages *rgdal* (Bivand *et al*., 2014) and *raster* (Hijmans & van Etten, 2014). The bioclimatic variables and geophysical variables were analyzed separately, standardized in R and a test for collinearity (Dormann *et al*., 2013) between the variables was done (Box S2.1). This was necessary to remove redundant variables because multi-collinearity may violate statistical assumptions and may alter model predictions (Dormann *et al*., 2013). The collinearity test removed variables with high (>5) variance inflation factors (VIF) and resulted in a subset of bioclimatic variables (Table S2.3) and all the geophysical variables. Edaphic variables were not run through collinearity due to large amounts of missing data as not all edaphic variables selected had measurements made across the whole of Norway (i.e. some coastal areas and islands were missing data). The resulting bioclimatic and geophysical variables and all the edaphic variables were run separately through a principal components

analysis (PCA) in SPSS 22 (IBM Corp, 2013) where variables were tentatively selected based on the highest loadings (>0.3) (Tabachnick & Fidell, 2001). Experts in Norway from the Forest and Landscape Institute in Ås and Natural History Museum in Oslo, were consulted via email contact on which of the resulting variables were thought to most likely affect adaptation of plant species in Norway. Eight variables were used to create the ELC map (Table S2.3) along with the following parameters in the ELC mapas tool: 8 clusters as a maximum number of clusters allowed by component (bioclimatic, edaphic and geophysical); elbow method as it can process large amounts of data and is recommended for large countries; latitude, and a resolution of 2.5 arc-minutes (equivalent to approximately 4 x 8 km^2).

The resulting ELC map was used to inform both *in situ* and *ex situ* conservation priorities. The total number of ELC categories that each taxon was found in and predicted to be found within was calculated. ELC categories within PAs were determined for *in situ* conservation and the CAPFITOGEN tool Representa was used for identifying *ex situ* conservation needs. See below for further details.

2.4.2 Species richness and occurrence data bias

A species richness map was created for the priority CWR in Norway. The map was created in DIVA-GIS following the procedure set out by Scheldeman and von Zonneveld (2010). Each grid cell was equivalent to approximately 4 x 8 km^2 at the equator. The richness for those presence points inside PAs was also studied and followed the same procedure. Sampling bias was mapped following Scheldeman and von Zonneveld (2010) with each grid cell equivalent to 4 x 8 km^2 at the equator.

2.4.3 Predicted distribution and gap analysis

The predicted distribution maps for the priority CWR within Norway used GIS layers composed of climatic, edaphic and geophysical variables that were obtained from freely available data sources (Table S2.3). The data and methods used to select the variables were the same as those used in the creation of the ELC map. However, when experts were consulted on the appropriateness of the resulting variables they were asked to validate the variables on which they thought were the most important for predicting plant distribution. This therefore resulted in a different selection of final variables to the ELC map (Table S2.3).

The presence point data in '.csv' format for the priority taxa and the environmental layers selected by the above method in 'ASCII' format were run in MaxEnt. All environmental layers had the same extent and grid size (2.5 arc-map minutes). In MaxEnt the following settings were used: Jack-knife was selected to measure variable importance; random test percentage was set at 30, meaning 30% of records were used to 'test' the model and 70% were used to 'train' the model; Equal training sensitivity and specificity was used as the threshold rule meaning the proportion of presences incorrectly predicted was the same as the proportion of absences incorrectly predicted as it is an accurate threshold for predictive accuracy (Liu *et al*., 2005); all other settings remained as default.

The models were evaluated by the area under the receiver operating curve (ROC), commonly known as AUC. This measures the ability of a model to discriminate between sites where a species is present *versus* those where it is absent (Hanley & McNeil, 1982). Models with an AUC >0.7 are considered acceptable and such predictions based on presence-only data can be sufficiently accurate to be used in conservation planning (Pearce & Ferrier, 2000). AUC <0.5 indicates performance is worse than random (Anderson *et al*., 2006). For taxa with AUC <0.7 their SDM should be treated with extra caution.

2.4.3.1 In situ gap analysis

A gap analysis was undertaken that used the results from the observed species richness and the predicted distribution. In DIVA-GIS the predicted distribution of species richness was subtracted from the observed species richness to produce a new grid file showing those areas where there are gaps in the current observation data. For *in situ* conservation the predicted distribution map was compared with the current PA system (UNEP, WCMA and IUCN, 2015) to determine which taxa were predicted to be passively conserved. Passive conservation refers to those taxa that coincidentally occur within an established PA and are therefore protected but not necessarily managed. To determine how well the ecogeographic diversity of the priority CWR was passively conserved *in situ*, the ELC categories within PAs were determined. The values of the points that corresponded to the ELC categories were extracted from the PA layer to create a table with corresponding PAs and ELC category points. A visual inspection was carried out on the map as points were located in the centre of the grid cells, therefore if some PAs contained only a section of a grid cell this may have been excluded from the analysis. For each taxon the percentage of ELC diversity conserved within the entire PA network was also calculated by comparing the total number of ELC categories the taxon was present in to the number of ELC categories in PAs that the taxon was found in.

2.4.3.2 Ex situ gap analysis

A priority CWR richness map and a predicted distribution map was created for those taxa with *ex situ* data. A gap analysis map was created for those taxa with *ex situ* accessions, following the methodology of Maxted *et al*. (2008a) and Parra-Quijano *et al*. (2011). A separate CWR richness map and predicted distribution map was created for those taxa without *ex situ* accessions. For each taxon the number of ELC zones the taxon was observed within, predicted to be within and collected from was studied. The percentage of ecogeographic diversity

conserved per taxon with *ex situ* accessions was calculated. The ELC zones where the species was predicted to be found but had not yet been collected from were determined. Further gap analysis for those taxa with *ex situ* accessions was then undertaken using the CAPFITOGEN tool Representa (Box S2.2). A comparison was made between the *ex situ* accession data only and all other data collected from GBIF, including herbarium and observational data which indicated the presences of populations that had not been collected. The frequency of both these sources of data within the ELC zones in Norway was also compared. ELC zones for which there were no germplasm collections but for which the herbarium and observational data suggested species presences, were gaps in the *ex situ* collections and should be targeted immediately for collection. ELC zones were then classified according to this and given a priority level for collection, where class 1 is the highest priority and class 13 is the lowest (see Parra-Quijano *et al*., 2016). The ELC zones with the highest number of species portioned by class were identified to ascertain which ELC zones to target for collecting a high number of underrepresented taxa. The ELC zones that contained the highest number of taxa with no *ex situ* accessions were also determined.

2.4.4 Complementarity analysis

The CAPFITOGEN tool Complementa (Box S2.3) was used to perform complementarity analysis to create a network of sites for potential genetic reserves which followed the Rebelo (1994) approach. This was done for all taxa presence points across mainland Norway creating a grid cell complementary network, which used an area of 4 x 8 km^2 per grid cell (i.e. reserve). A separate analysis was carried out for only those presence points located within the current PA network in Norway and created a PA complementary network.

A further analysis was performed to determine how many populations of each taxon were conserved within the grid cell complementary network and the PA complementary network.

The point information within each of the aforementioned areas was extracted to determine which taxa were located there and thus how many populations could potentially be actively conserved. The ELC zones covered by the PA complementarity analysis were also extracted to determine if the complementary network can conserve both species richness and ecogeographic diversity.

2.4.5 Climate change assessment

For the climate change studies the same methods used in section 2.4.3 to create species distribution models were followed, including the same environmental variables and the same settings in MaxEnt. We consulted with experts within Norway from the Natural History Museum, Oslo and the Forest and Landscape Institute, to determine the environmental variables which would commonly affect all the priority CWR and thus create multispecies' climate change maps. Future climatic layers were used instead of present climatic variables, and were obtained from CCAFS (http://www.ccafs-climate.org/data/) where the data is based upon the most recent IPCC report (IPCC, 2014) up to the year 2080. Geophysical and edaphic variables are not expected to change under future climate therefore no data was available for them; hence only bioclimatic variables were used. These included: Isothermality, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation and precipitation seasonality (Table S2.3).

The Delta Method IPCC AR5 empirical statistical downscaling was used for the most recent Relative Concentration Pathway (RCP) climate scenarios under the years 2030, 2050, 2070, 2080 so that both short and long-term effects of climate change could be studied. The Norwegian Earth System Model, NorESM1-M (Bentsen *et al*., 2013) which was based upon the Community Climate System Model version 4 (CCSM4; Gent *et al*., 2011) was used due to its specificity to climatic processes that are particularly important at northern latitudes (Bentsen

et al., 2013). Data was used for two RCP scenarios, RCP 2.6 and RCP 6.0, which represent the agreed maximum temperature rise set out by the Paris agreement (1.5˚C) and the more likely temperature rise from the INDC proposals of 2.5-3.5˚C (UNFCCC, 2015), respectively. Once data for each of the four study years was obtained, models were created separately for each taxon in MaxEnt following a similar procedure to that in section 2.4.3. All layers had the same extent and resolution of 2.5 arc-map minutes.

Evaluation of the model accuracy was done using the average Area under the ROC (Receiver Operating Characteristic) Curve of the test data (AUC_{Test}) and standard deviation of the AUC_{Test} data (STAUC; Ramirez-Villegas *et al*., 2010) for each taxon. Models with an AUC_{Test} > 0.7 and STAUC < 0.15 are considered accurate and stable (Ramirez-Villegas *et al*., 2010).

2.4.5.1 Climate change diversity analyses

Taxa richness maps were created for taxa under both unlimited migration, where taxa can move unrestricted to where the climate is suitable and no migration scenarios, where taxa are unable to expand from their current distribution. This was done for both RCP scenarios and the four study years. Analyses were undertaken in ArcMap 10.2 using Python scripting to automate and streamline the process. Change in taxa richness was studied by comparing present predicted distribution and future predicted distribution to create a new map showing where taxa distribution has changed.

Taxon loss and gain was calculated for each grid cell where the number of taxa found per grid cell was compared to the current taxa richness per grid cell for both migration scenarios. The turnover rate for each climate scenario and each year was calculated following the method set out by Thuiller *et al*. (2005) and Thomas *et al*. (2004) where:

$$\text{Taxa turnover} = 100 \times \frac{L + G}{}$$

SR + G

SR is the current taxa richness, L is loss of taxa per grid cell and G is gain of taxa per grid cell. This was calculated for both RCP scenarios per study year and the average was calculated for each year.

To determine the threat level of the taxa we compared the number of grid cells where a taxon was present under both no migration and unlimited migration and present and future climatic scenarios. The level of threat for the priority taxon was then assessed using the IUCN red list criterion A3(c) (IUCN, 2001; Thuiller *et al*., 2005). This used the projected geographic range loss of a taxon as a proxy for population reduction to assign a threat category by the following parameters: Extinct (EX) is a taxon with a projected range loss of 100 %, Critically Endangered (CR) has a projected range loss of >80%, Endangered (EN) has a range loss of >50% and Vulnerable (V) had a range loss of >30% (IUCN, 2001). The remaining taxa were considered Least Concern (LC) in terms of climate change impacts.

2.5 Genetic diversity studies

Genetic diversity studies were conducted to assess the extent of genetic diversity of ten priority CWR in Norway. The taxa selected were: *Allium ursinum* L., *Brassica rapa* subsp. *campestris* L., *C. carvi, Festuca pratensis* (Huds.) P. Beauv., *Phleum pratense* L., *Ribes uva-crispa* L., *R. chamaemorus, Rubus idaeus* L., *Trifolium pratense* L., *Trifolium repens* L. Taxa were chosen based upon consultation with Norwegian stakeholders at the Forest and Landscape Institute and the Natural History Museum Oslo who selected a broad range of taxa that were of interest to their work. For example, *P. pratense* is one of the most widely used forage species in Norway and Scandinavia and *R. chamaemorus* is a traditionally used wild species important in Norwegian food. The species chosen are also widely distributed across the length of Norway

so the difference in genetic diversity between wide ranging populations could be studied. *A. ursinum* and *B. rapa* were not distributed across the length of Norway but they are important species in global agriculture and have had their genetic diversity studied in other similar studies (for example Fielder, 2015).

From June to August 2014 and in May and July 2015, sampling was carried out for the above ten CWR. Twenty-six sites were visited which ranged from islands in the south of Norway to the Arctic in the north (Figure 2.2 and Table S2.4). Not all ten CWR taxa were collected from each site. These sites were chosen firstly based upon the results of the grid cell complementarity analysis (see chapter 3) as this analysis was based upon known occurrences and meant it was likely that the species would be found at these locations. Once these locations were known we then targeted collecting within PAs near these grid cell sites (at this point in the project the CAPFITOGEN Complementa tool had not been adequately developed to use the PA complementary network to identify our collection sites within PAs). We chose sites that were spread across the length and breadth of Norway and were deemed suitable by local experts. We also had to consider the accessibility of sites, which was particularly important in the north of Norway. Here we tried to focus collecting upon areas within different ELC zones, not necessarily within PAs as these were often difficult and impractical to access.

The sites covered a wide range of geographical variation in longitude, latitude and altitude (Table S2.4). From each site one leaf sample was collected (approximately 100 mg fresh weight minimum) from 20 different individuals within a population for each species (if that species was present at the site). As the sites varied in size individuals were collected from different populations within that site to try and get a representation of the range of genetic diversity within the area. The different sites were deemed far enough away from each other that geneflow would be limited between them. Leaf samples were immediately stored in

airtight plastic bags containing drying indicator silica gel (Chase & Hills, 1991). A total of 2289 individual samples were collected from the wild.

The genetic diversity between wild and cultivated/semi-wild populations of *T. pratense* was also analysed, to determine if there was a difference in the levels of genetic diversity between cultivated and wild populations. The cultivated populations were grown out in glasshouses at Bioforsk, Grimstad, Norway. Twenty cultivars were grown and leaf samples from twenty individuals per cultivar were sampled, resulting in 400 samples (Table S2.5). These leaf samples were stored and handled in the same way as the wild samples were above. Ten percent of all samples, wild and cultivated were replicated following the guidelines from Bonin *et al*. (2004). The total number of samples for genetic diversity analysis was 3034.

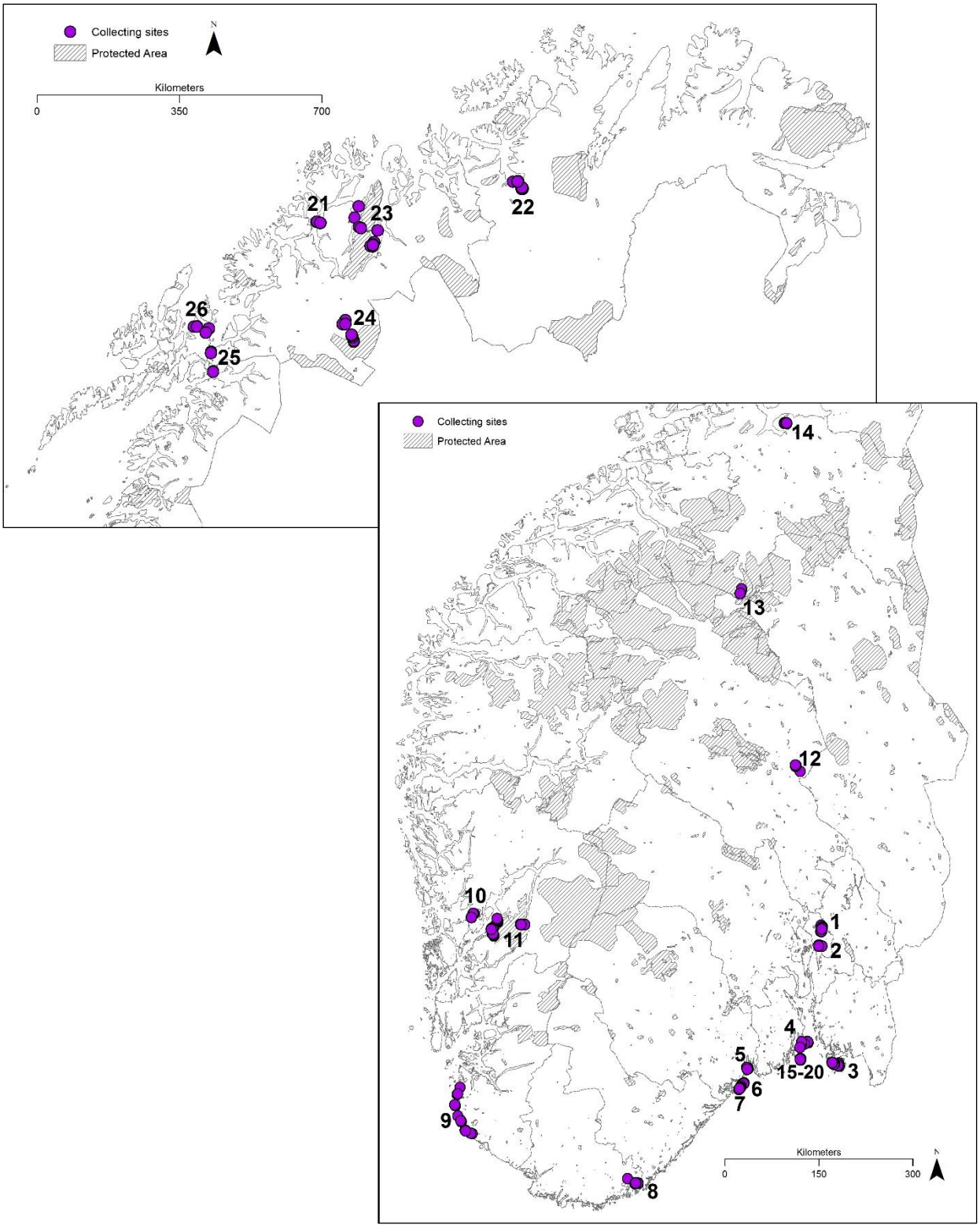


Figure 2.2: Location of the 26 collecting sites (see Table S2.4 for more details) for the ten CWR in Norway. The PA network is shown. Multiple points at each site represent the breadth of the locations visited within one site to collect the ten species.

2.5.1 Molecular marker genotyping

Molecular work was undertaken at IBERS, Aberystwyth University. Genomic DNA was extracted from the dried leaves using the DNeasy 96 Plant Kit (Qiagen, 2012). The AFLP method was completed according to IBERS standard protocol (Skøt *et al*., 2005), based on Vos *et al*. (1995). AFLP reactions were run on the ABI 3730X1 capillary sequencer. Pre-amplification was done to identify appropriate primers that produced markers distributed throughout the genome of each individual species.

Three primer combinations were initially tested on samples from each species that were collected during 2014, the primers used were: ACG-CTG, AGC-CGC and ATC-CAC. The results from these samples were 'good' except ACG-CGC did not work with *R. chamaemorus* (K. Skøt, 2016, personal communication). After this initial testing the extracted DNA from these samples was stored in the freezer until the following year when all samples had been collected. Following extraction of the DNA from the collections made in summer 2015, further primer testing was carried out, see Table 2.1 for results.

Table 2.1: The primer combinations tested on all species after extraction of DNA from all samples collected over both the years 2014 and 2015. *represents primers used in final AFLP analysis.

Primer	Result
ACG-CTG*	GOOD
AGC-CGC	POOR
ATC-CAC	GOOD
AAC-CAC	POOR
ACA-CTA*	GOOD
AGA-CGA	POOR
ATG-CAG	AVERAGE
ATG-CAT	POOR
ACG-CAT	GOOD
AAG-CTC	POOR
AGT-CAC	POOR
ATC-CTA	AVERAGE
ACA-CAT	POOR

ACT-CTA	AVERAGE

Initial analysis of the AFLP electropherograms and the calculation of the dataset error rates (see section 2.5.2 for details on how this was done) showed that the AFLP procedure had not been successful. Dataset error rates were >50% for all species samples. Due to budget limitations, in autumn 2016 three species samples were reanalysed, these included *T. pratense, T. repens* and *C. carvi*. For *T. pratense* new pre-amplifications were used. For *T. repens* and *C. carvi* the original pre-amplifications were used. For all three species and two primer combinations, 3µl of pre-amplification product was used for the selective amplifications and the same PCR protocol above was used with 25 cycles. 1µl of selective amplification product was mixed with 10µl formamide/size standard and run on the ABI3730.

2.5.2 Analysis of the AFLP electropherograms

AFLP electropherograms were visualised for the three species in the freely available software Peak Scanner (Applied Biosystems, 2006). The size and fluorescence of AFLP peaks was detected using an 'Analysis Method' with the following settings as described by Arrigo *et al*. (2009): light smoothing of electropherograms was selected; default parameters for the 'sliding window' analysis were selected; peaks were filtered using 50 relative fluorescent units (rfu) as a minimum; and the size standard used was GS500(-250)LIZ. The resulting Peak Scanner table was imported into RawGeno (Arrigo *et al*., 2009), where peaks were automatically scored as present (1) or absent (0). In RawGeno the scoring of peaks was limited to blue dye only and samples were analysed separately for each species and each primer. The binning parameters were optimized prior to analysis with samples analysed using a maximum bin width of both 2.0 and 1.5. Using a larger bin width increased the likelihood of technical homoplasy (Arrigo *et al*., 2009), therefore a maximum bin width of 1.5 and a minimum bin width of 1.0 were used.

The scoring range for the peaks was from 50 to 500 base pairs with low fluorescence bins set at 100 rfu. After peak scoring, replicates were separated from the samples and the dataset and loci error rates were calculated.

Approximately 10% of samples for each species were manually checked for quality of binning to ensure peaks were being correctly scored. These were then compared to the RawGeno output. RawGeno detected a higher number of peaks and a lower dataset error rate. The automated scoring process using RawGeno has been shown to provide results that are as accurate as those scored manually or within commercial software such as Genemapper (Arrigo *et al*., 2009; Herrmann *et al*., 2010). Furthermore, the automated procedure can increase reproducibility of the dataset and limit genotyping errors to technical factors (Arrigo *et al*., 2009)

For each taxon 10% of the collected samples were replicated randomly. Error rates were calculated separately for each taxon based upon the replicates resulting in a locus and dataset error rate (Bonin *et al*., 2004; Pompanon *et al*., 2005). This procedure followed that of Bonin *et al*. (2004, 2007) where the loci error rates were calculated based upon the ratio of the total number of mismatches between samples and replicates to the number of replicated individuals and for the dataset by the number of mismatches between sample and replicate divided by the number of peaks per sample (shown as a percentage).

2.5.3 Statistical analysis

Genetic diversity and population structure metrics were calculated at species level using AFLP-SURV (Vekemans *et al*., 2002) and GenAlEx software (Peakall & Smouse, 2006; Peakall & Smouse, 2012). Allele frequencies were calculated using AFLP-SURV's Bayesian method with non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999) due to the

dominant nature of AFLP markers. The following descriptive statistics were calculated in AFLP-SURV: proportion of polymorphic loci at the five percent confidence level expressed as a percentage (PLP), expected heterozygosity (H_E), mean expected heterozygosity within populations (H_W) and Wright's fixation index (F_{ST}) (Lynch and Milligan, 1994). Principal coordinate analysis (PCoA) was performed in GenAlEx 6.5 to determine patterns of variation between individuals based on pairwise genetic data and between populations based upon Nei's genetic distance. An Analysis of Molecular Variance (AMOVA) using 999 permutations was also undertaken in GenAlEx to determine the distribution of genetic variation within and among populations. A Mantel test was carried out in GenAlEx to assess if genetic diversity was correlated with geographic distance. Pairwise F_{ST} values (transformed to $F_{ST}/(1-F_{ST})$) were compared to log-transformed geographic distances with 999 permutations. The geographic distance was calculated in GenAlEx using latitude and longitude decimal degree coordinates and utilising a modification of the Haversine formula developed by R. W. Sinnott (1984). This closely approximates the output of Garmin GPS software and the distances calculated are returned in km's (see Peakall and Smounse 2012). SPSS 24 was used to perform correlation analyses between the environmental variables in the ELC zones (Table S2.3) and the level of heterozygosity for each population per species. Stepwise Regression analysis was also undertaken in SPSS for each species to determine if the environmental variables had any significant predictive relationship with the level of heterozygosity within populations. Finally, the number of private (unique) alleles within each population was calculated in SPSS, to help determine how distinct populations were from each other.

CHAPTER 3.

In situ and *ex situ* diversity analysis of priority crop wild relatives in Norway

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Jade Phillips, Åsmund Asdal, Joana Magos Brehm, Morten Rasmussen, Nigel Maxted

Author contributions:

Conceived and designed the study: J.P., A.A., N.M.

Data collation and preparation: J.P., A.A.

Performed analysis: J.P.

Interpreted results: J.P., A.A., J.M.B.

Wrote the paper: J.P.

Critically reviewed the paper: J.P., A.A., J.M.B., M.R., N.M.

3.1 Abstract

This study aims to contribute directly to Norway's national and international commitments to systematic, long term conservation of CWR by ensuring both the *in situ* and *ex situ* protection and availability of a broad range of CWR genetic diversity within the country. We created a priority list of CWR within Norway based upon four main criteria including economic value from national to global level of associated crops and inclusion in Annex 1 of the ITPGRFA. Species presence data was gathered from GBIF and used for predictive species distribution modelling in MaxEnt. CAPFITOGEN software was utilised to create an ELC map and to identify complementary *in situ* genetic reserves and *ex situ* collecting priorities which target the full range of ecogeographic diversity of taxa. An inventory of 204 priority CWR within Norway was compiled. A grid cell complementary network of 19 *in situ* areas (4 x 8 km^2) conserved 201 priority CWR and a separate analysis identified a PA complementary network of 23 reserves that conserved 181 priority taxa. For *ex situ* conservation, 177 taxa did not have *ex situ* accessions and of the 24 with accessions, 15 had the minimum of five populations conserved throughout their ecogeographic range. We present the first comprehensive national recommendations for *in situ* and *ex situ* conservation of 204 priority CWR in Norway. Proposals target the conservation of the ecogeographic diversity of the priority CWR and hence their genetic diversity. Both the priority taxa and the methodology used are applicable at regional and global scales with the recommendations not only helping Norway to meet its international obligations for conservation of genetic diversity of CWR but also ensuring this genetic diversity is available for use in tackling global food security.

3.2 Introduction

The global population is expected to reach 9.3 billion by 2050 (UN, 2015b). This will intensify the strain on existing agricultural systems and the environment through a need for higher food productivity which the FAO (2009b) states will need to increase by 70% globally before 2050. In combination with an increase in population there is evidence of increased homogeneity of the world's food supplies (Flores-Palacios, 1998) and of a decrease in genetic diversity (Hoisington *et al*., 1999; Esquinas-Alcazar, 2005; Gepts, 2006; van de Wouw *et al*., 2009) this will be a threat to food security at the national, regional and global levels due to mal-adaptation of crops to the changing environment. CWR are a PGR pre-breeders and breeders are looking towards to help tackle this food security challenge.

CWR are wild plant taxa that have an indirect use derived from their relatively close genetic relationship to a crop. These wild taxa are located throughout the world, with particularly species rich areas found near the Vavilov centres of diversity (Vincent *et al*., 2013). However, those populations in more peripheral locations within less favourable habitats are also important as they will have developed unique adaptive genetic diversity. This broad range of genetic diversity has been utilised in modern plant breeding with the most common use of wild relatives as a source of pest and disease resistance along with other characteristics such as drought and salt tolerance, also being harnessed (Prescott-Allen & Prescott Allen, 1986; Hajjar & Hodgkin, 2007; Maxted & Kell, 2009).

Pimentel *et al*. (1997) estimates that 30% of the increase in crop yields since 1945 has been made through crossing with wild relatives, representing a worldwide value of US$115 billion per year and we can only assume that this has increased by today's standards. The future value of benefits from CWR has also been estimated at potentially $196 billion for the wild

genepools of 32 major crops (PwC, 2013). Furthermore, the direct and indirect uses of CWR through the ecosystem services they provide will increase their value for the economy. Despite the high value attributed to CWR and our dependence on fewer than a dozen of the approximately 300,000 species of flowering plants for crops (McCouch *et al*., 2013) they still lack concerted conservation efforts. Policy initiatives are being put in place with the CBD (UN, 1992), the ITPGRFA (FAO, 2001; www.planttreaty.org) and the GSPC (www.biodiv.org/programmes/cross-cutting/plant/) stressing the need for effective conservation of PGR. Although these goals have been set out at an international level, 'food security is a national responsibility' (FAO, 2009b) and therefore they must be implemented at the national level if conservation actions are to be effective. National CWR priorities have been developed for Cyprus (Phillips *et al*., 2014), England (Fielder *et al*., 2015), Finland (Fitzgerald, 2013), Italy (Panella *et al*., 2014), Jordan (Magos Brehm *et al*., 2016), Portugal (Magos Brehm *et al*., 2008), Spain (Rubio Teso *et al*., 2013), the USA (Khoury *et al*., 2013), among other countries.

Norway is also seeking to act upon such international legislation to develop national CWR conservation priorities. The CWR taxa present within their borders are nationally and globally important due to the position of the country on the north western periphery of Europe. The country has a substantial north-south and east-west climate gradient (Norwegian Ministry for Agriculture and Food, 2008) and has been ice-free for less than 10,000 years (Kålås *et al*., 2006). This means that species tend to have a restricted occurrence which may lead to unique genetic adaptations or traits due to *in situ* glacial refugia (Eidesen *et al*., 2013).

Norway has 3,148 recorded plant species of which 2,250 are described as native (Kålås *et al*., 2006). The most botanically rich areas are found in the calcareous areas around Oslofeltet and south east Norway. The country is composed of 37% mountains whereas the global average is

27% (Nybø *et al*., 2011). This is important in the context of climate change, as it is likely to be the greatest threat in many, if not most regions (Thomas *et al*., 2004) with mountainous species likely to have higher rates of species loss (Thuiller *et al*., 2005). In Norway the annual mean temperature is expected to rise 2.3-4.6 ˚C by 2100 and the growing season is expected to become 1-2 months longer in lowland and 2-4 months longer in high mountain areas (www.environment.no). These changes may increase crop yields at high latitudes (Olesen and Bindi, 2002) and species diversity from the south but could negatively impact populations at their northern limit which may become restricted in their distribution.

In Norway, national conservation legislation is implemented through the Nature Diversity Act (2009) and is the most important instrument for expanding protection of the natural environment. Under this Act it designates different types of PAs which encompass about 17% of mainland Norway (www.environment.no) including NPs, nature reserves and landscape protected areas. The latter is particularly interesting in the CWR context as these sites include areas of agricultural land which tends to be associated with CWRs (Maxted & Kell, 2009; Jarvis *et al*., 2015). Although Norway has no formally recognised *in situ* CWR conservation these taxa are conserved passively in the above mentioned PAs, but this 'is not enough to guarantee the continuation of these populations' (Maxted *et al*., 1997a; Hunter *et al*., 2012) hence the need to assess the suitability of a system of complementary *in situ* genetic reserves with active management guidelines and *ex situ* collecting needs.

The aim of this research is to contribute directly to Norway's national and international commitments to systematic, long term conservation of CWR by ensuring both the *in situ* and *ex situ* protection and availability of a broad range of CWR diversity within the country. The specific objectives include the recommendation for a network of *in situ* genetic reserves to conserve taxa richness and ecogeographic diversity, as well as identification of areas outside

formal PAs for *in situ* conservation actions. Proposals are made for where and what *ex situ* material should be collected to ensure the full range of ecogeographic diversity is conserved and available for use. The methodology is based on freely available and open source software and can be applied to CWR conservation efforts in other nations as well as at regional and global scales.

3.3 Methods

3.3.1 National CWR checklist and inventory

CWR checklists and inventories are the foundation for the formulation of conservation strategies (Maxted *et al*., 2015). A CWR checklist was created for Norway (Table S2.1) that was initially derived from the Crop Wild Relative Catalogue for Europe and the Mediterranean (Kell *et al*., 2008), updated and harmonized with the Flora of Norway (Lid & Lid, 2005) and subsequently prioritized using the following criteria:

- Those CWR within the same genera as crops of high economic value were ranked (1 to 24, highest monetary value to lowest), as given by gross production value (current million US$) (from FAO stat, http://faostat3.fao.org/home/E) for global production value (2013), within Europe (2013) and within Norway (2013);
- CWR present in Annex 1 of ITPGRFA and not previously included;
- CWR highlighted as being of specific importance to Norwegian research (e.g. *Phleum* species), culture (e.g. *C. carvi*) and environment as ascertained from local experts and literature and which were not previously included;Taxa within the Harlan and de Wet inventory (Vincent *et al*., 2013) which contains 29 priority crops of global importance and which were not previously included on the above criteria.

Only indigenous taxa according to the Flora of Norway (Lid & Lid, 2005) and/or those populations of introduced taxa that have stable populations (present for at least 10 years, following the same criteria as the Norwegian Red list (Kålås *et al*., 2006)) were kept in the prioritized list. Crops were ranked (1 to 24, highest monetary value to lowest) and the associated wild taxa were matched to these crops. The other criteria were then applied. The prioritized list was validated by Norwegian experts via discussions and email contact where taxa were removed or added if deemed appropriate and now forms the basis of the Norwegian Inventory of CWR (Table S2.2).

3.3.2 In situ diversity analyses

For all the taxa in the CWR inventory geo-referenced occurrence records were gathered from GBIF (GBIF, 2013). Spatial duplicates, in other words, records from the same species with the same coordinates, were deleted (most recent records were kept when these were collected at different times). Erroneous data such as incomplete entries and ambiguous species names were removed before proceeding with the analysis. Over 96% of occurrence records were accurate to three or more decimal places. DIVA GIS (Hijmans *et al*., 2004) software was used to check for outlier observations, those locations outside the mainland boundaries of Norway, and such points were removed from further analysis.

Taxon richness and sampling bias maps were created for the priority CWR in Norway, for the entire distributional range of taxa and for the PA network using DIVA-GIS. For *in situ* conservation the CAPFITOGEN tool (version 2.0; Parra-Quijano *et al*., 2016) Complementa was used to perform a grid cell complementarity analysis to create a network of sites (at 4 x 8 km^2 each) for potential genetic reserves for active *in situ* conservation using the Rebelo (1994) approach (see Box S2.3 for settings used in Complementa). The 4 x 8 km^2 size of sites was deemed effective for this study by Norwegian stakeholders due to the heterogeneity of the

landscape restricting use of a larger size and the limitations on resources preventing smaller scale reserves. Both larger (Maxted *et al*., 2008a) and smaller (Phillips *et al*., 2014) sizes of reserves have been used in other studies. The PA network was analysed separately (Box S2.3) and identified a PA complementarity network for *in situ* conservation. Further analysis explored the number of populations that would be conserved within the complementary reserve networks, with five populations being the minimum threshold set out by Brown and Briggs (1991) and Dulloo *et al*. (2008) to minimise the loss of population fitness.

3.3.3 Potential distribution modelling

The potential distribution of a species can be used to infer the full geographical range of a species' natural occurrence, which is often important due to lack of presence point data which may not cover the entire distributional range of that taxon (Scheldeman & van Zonneveld, 2010). The potential distribution map was modeled on GIS layers covering climatic, edaphic and geophysical variables. These initial 105 variables were obtained from freely available sources (Table S2.3) and were reduced in number to remove redundant and correlated variables using the following procedure. The environmental data values at each taxon presence point were extracted in R using packages *rgdal* (Bivand *et al*., 2014) and *raster* (Hijmans and van Etten, 2014). The bioclimatic and geophysical variables were standardized in R and a test for collinearity (Dormann *et al*., 2013) among the variables was done (Box S2.1). This was necessary to remove redundant variables because multi-collinearity may violate statistical assumptions and may alter model predictions (Heikkinen *et al*., 2006). The collinearity test removed variables with high (>5) variance inflation factors (VIF; Table S2.3). The edaphic variables were not run through collinearity due to a large amount of missing data. Principal components analysis (PCA) was performed in SPSS (IBM Corp, 2013) on the resulting bioclimatic and geophysical variables and all the edaphic variables, to determine relationships

among variables and define the final selection. The resulting uncorrelated variables were used in consultation with experts in Norway to validate the final selection of variables they felt were most important for predicting plant distribution. Consequently, thirteen environmental variables were used in creating the predicted distribution map (Table S2.3).

The maximum entropy (MaxEnt) algorithm (Phillips *et al*., 2004;*et al* 2006) was used to create the potential distributions for each priority taxon with the observational data (see chapter 2, section 2.4.3 for MaxEnt settings). Models were evaluated by the area under the receiver operating curve (ROC), known as AUC. Models with an AUC >0.7 are considered acceptable and such predictions based on presence-only data can be accurate to be used in conservation planning (Pearce & Ferrier, 2000). A value of < 0.5 indicates a model performance worse than random (Anderson *et al*., 2006). The potential distribution map was used with the observed distribution map for individual taxa, to undertake a gap analysis that determined how many taxa were predicted to be conserved *in situ* within the current PA network in Norway.

3.3.4 Ecogeographic Land Characterization maps

An ELC map can be used to identify useful ecogeographic zones which represent adaptive scenarios for plants (Parra-Quijano *et al*., 2012b). By helping to ensure conservation of the full range of ecogeographic diversity it is assumed that the full extent of genetic diversity will also be captured (Maxted *et al*., 1995, Thomson *et al*., 2001). The ELC mapas tool of CAPFITOGEN (Parra-Quijano *et al*., 2016) was used to create the ecogeographic map. Eight variables were selected (Table S2.3) following the methodology used in the above potential distribution modelling, with the following parameters selected in the ELC mapas tool: 8 clusters as a maximum number of clusters allowed by component (bioclimatic, edaphic and geophysical); elbow method, as it can process large amounts of data and is recommended for large countries; latitude; and a resolution of 2.5 arc-minutes (equivalent to 4 x 8 km^2). To

determine how well the ecogeographic diversity was passively conserved *in situ* and the likelihood of active conservation measures being taken, the ELC zones within PA were determined. The ELC zones covered by both the complementarity analyses were also extracted to determine if the complementary network can conserve both species richness and ecogeographic diversity.

3.3.5 Ex situ diversity analyses

Georeferenced accession data was obtained from the SESTO database at NordGen (August 2015; www.nordgen.org/sesto/; Table S1.1) and cross-checked with the GBIF data. This confirmed that all the *ex situ* data was recorded within the GBIF database which then allowed the identification of taxa with and without *ex situ* accessions. A priority CWR richness map was created for those taxa without *ex situ* collections to determine where collections should take place to target a high number of taxa in minimal visits. A gap analysis map was created following the methodology of Maxted *et al*. (2008a) and Parra-Quijano *et al*. (2012c) for those taxa with *ex situ* accessions. This highlighted the areas where the species was likely to be found but had not yet been collected from.

The CAPFITOGEN tool Representa (Parra-Quijano *et al*. (2016) and Box S2.2 for parameters selected) was used to undertake a gap analysis of *ex situ* collections. A comparison was made between the *ex situ* accession data only and all other data indicating presences of populations. The frequency of both these sources of data within the ELC zones was also compared. For each taxon with *ex situ* accessions the ELC zones to collect from were prioritised into classes, following the methods described by Parra-Quijano *et al*. (2016). The ELC zones with the highest number of species portioned by class were identified to ascertain which ELC zones to target to collect the highest number of underrepresented taxa. This was also done for taxa without *ex situ* accessions.

3.4 Results

3.4.1 CWR checklist, inventory and ecogeographic study

A complete CWR checklist containing 2538 CWR was created for Norway (Table S2.1). This list is dynamic and as taxonomies and species distribution changes the list should be updated. The priority list was composed of 204 CWR taxa (Table S2.2) of which 44% were forage and 43% were food. The 'other' category contained 13% of taxa that were related to medicinal taxa, ornamentals, forestry taxa, materials etc. Some taxa were classed in more than one category of CWR, for example some *Brassica* L. and *Fabaceae* L. species.

For the 204 priority CWR taxa 382,605 presence points were collected from GBIF (Table S2.2). After removal of erroneous data and duplicates 304,461 unique presence points were used. These were for 201 of the priority CWR as the following three taxa did not have any presence points in mainland Norway: *Rosa inodora* Fries, *Poa arctica* L. subsp. *microglumis* Nannf, *Festuca rubra* L. subsp. *megastachys* Gaudin. These taxa were recorded within the Flora of Norway as found on the mainland and were therefore prioritized for immediate recording and conservation actions. The majority of CWR have not been assessed within the Norwegian Red List (Kålås *et al*., 2006), but of those assessed within the checklist at least 55% are threatened (Table 3.1).

Table 3.1: Percentage and number of CWR within the checklist and priority list that have been red listed assessed and those that have been assessed as threatened in the Norwegian Red List (Kålås *et al.,* 2006). Threatened categories include critically endangered (CR), endangered (EN) and vulnerable (VU).

Total number of taxa	Norwegian Red List	
	% assessed (number)	% threatened as CR, EN, VU (number)
Checklist (2538)	11% (274)	6% (154)
Priority list (204)	10% (27)	7% (14)

3.4.2 In situ diversity analysis

The areas with the highest taxa richness were found around Oslo and the south east coast of Norway, with up to 131 different taxa present (Figure 3.1). The areas with the lowest taxa richness included the coast around Alesund and the Nordland and Finnmark regions where only one priority taxon was found. The most taxa rich PAs were those in the Oslo and Østfold region, Kristiansand and the islands in Vestfold. The PAs that showed the lowest taxa richness tended to be in Finnmark and Troms (Figure S3.1). The 10 km^2 area in the city of Oslo had the highest number of observations with 1881 (Figure 3.2). Much of mainland Norway had low numbers of observations, notably in Nordland and Finnmark. The pattern of observations matched with the pattern of species richness, which was not unsurprising but may have implications for interpretation of the diversity analysis results.

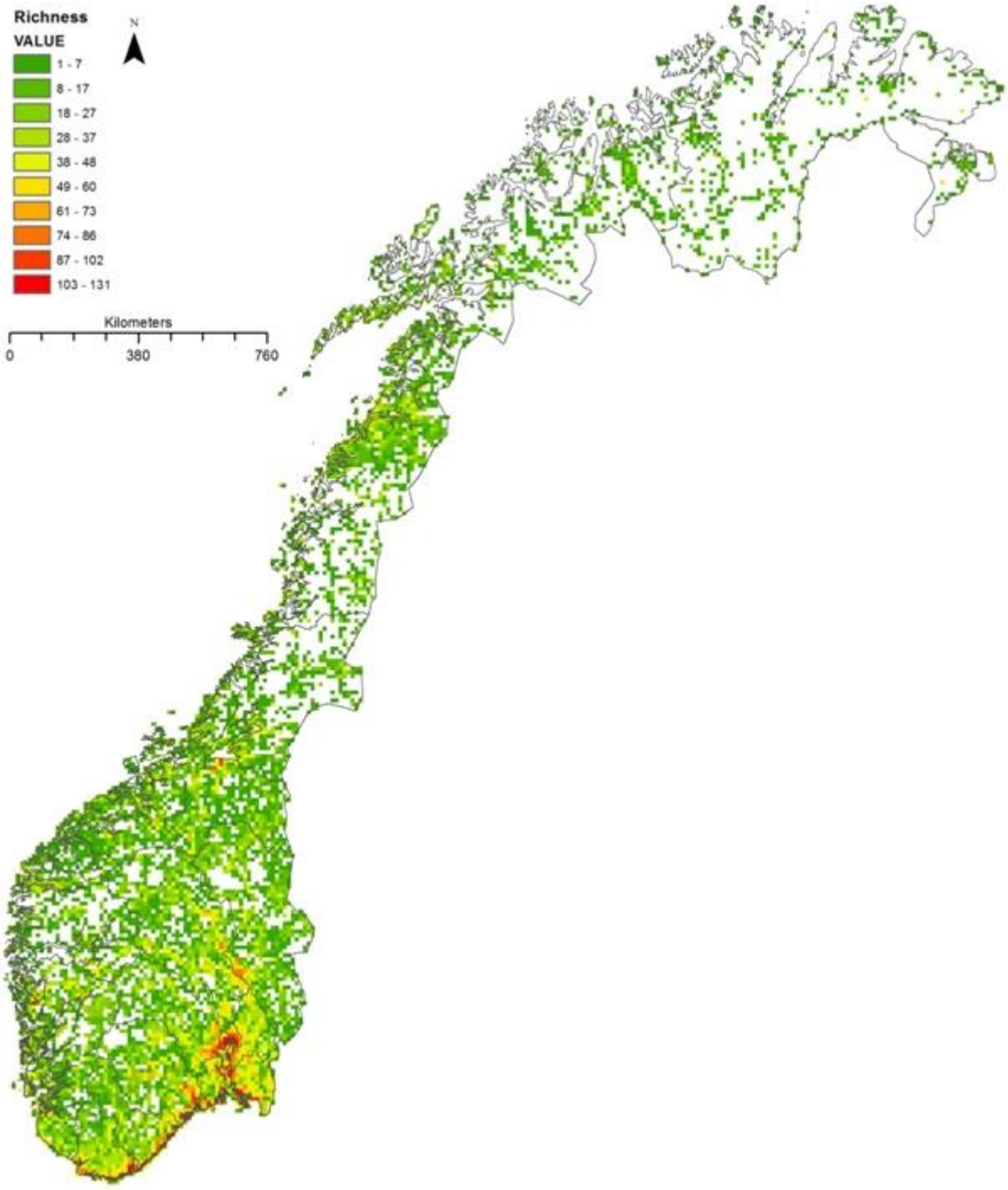

Figure 3.1: Taxa richness of 201 priority CWR in Norway. All grid cells are equivalent to 10 km^2 at the equator. Map drawn to geographic coordinate system WGS 1984.

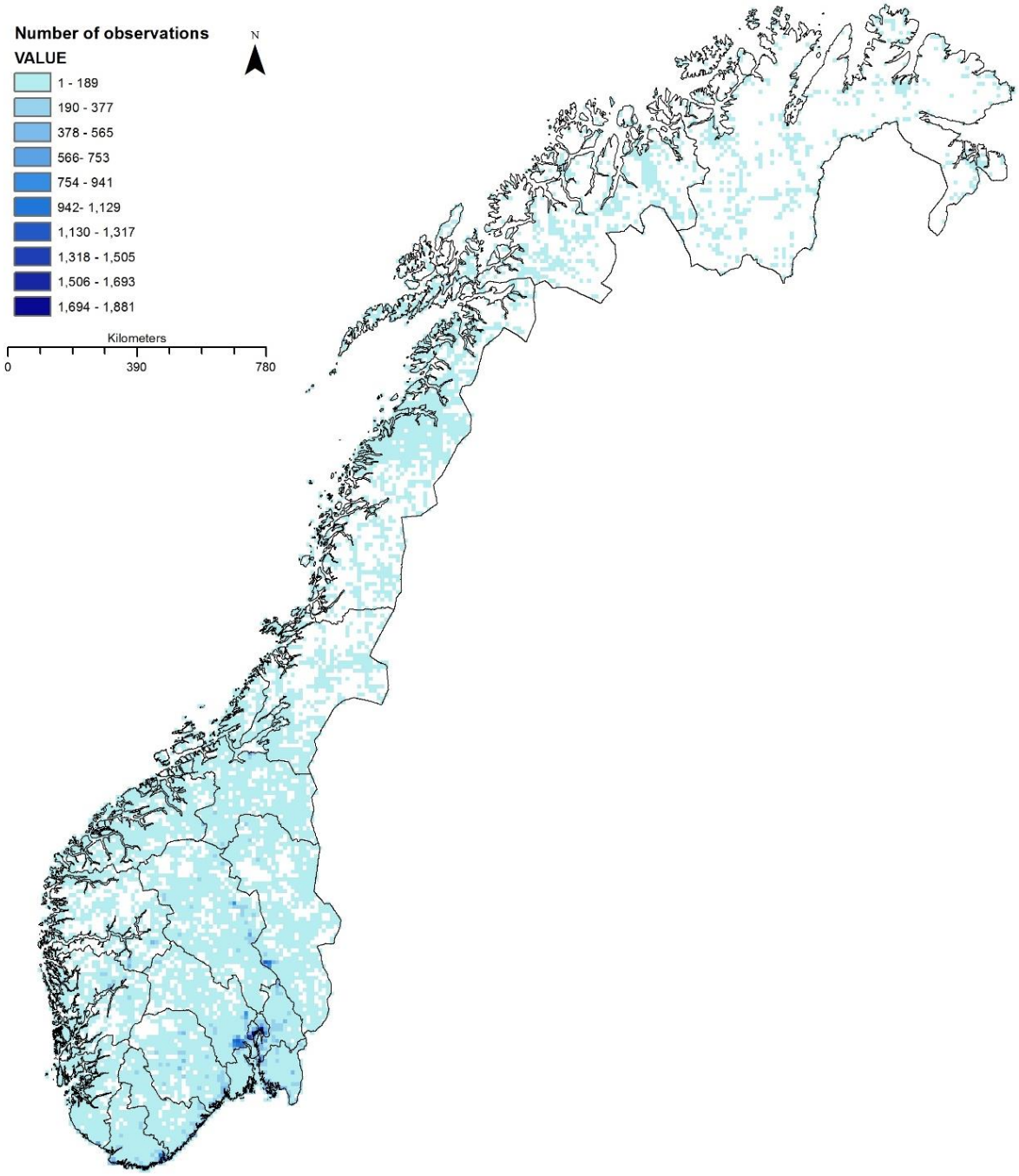

Figure 3.2: Sampling bias of observation data obtained from GBIF. All grid cells are equivalent to 10 km^2 at the equator. Map drawn to geographic coordinate system WGS 1984.

Complementary reserve analysis using the Complementa tool in CAPFITOGEN produced a grid cell network of 19 areas (4 x 8 km^2) that conserved all 201 priority taxa (Figure 3.3). Of these 19 grid cells, 13 contained PAs (Table S3.1). Complementary cell one was located in the county of Oslo and contained 131 taxa. The second complementary cell was found in the Vest-Agder region and conserved 20 taxa that were different to those found in cell one, with 130 taxa conserved in total. Fifty-four percent (109 taxa) of the priority taxa had five or more populations within the complementary network (Table S3.2). Within the 19 complementary grid cells, 18 of 27 ELC zones were conserved (Table S3.1).

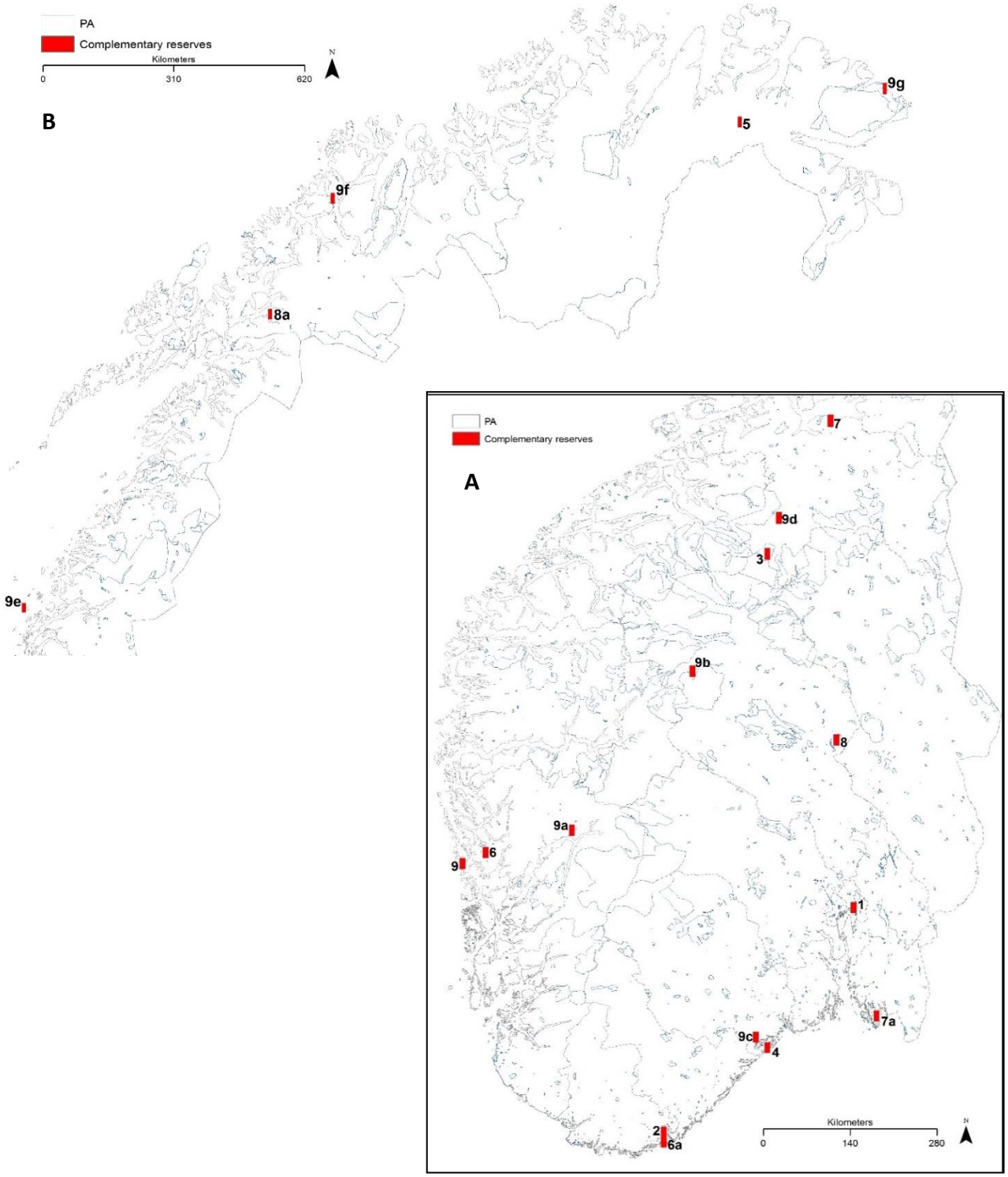

Figure 3.3: The grid cell complementary network of 19 areas (4 x 8 km^2) which conserve 201 priority CWR in Norway. A) Southern Norway, B) Northern Norway. Numbers refer to priority cell order (number one is higher priority than number two). Letters refer to reserves with the same number of additional taxa but different numbers of total taxa, with 'a' containing more taxa than 'b' etc. Created using the Complementa tool from CAPFITOGEN. Map drawn to geographic coordinate system WGS 1984.

The PA complementarity analysis (Figure 3.4) identified 23 PAs that conserved 181 taxa (the remaining 20 taxa are not found within a PA). The top priority PA that conserved 105 (58%) of the 181 taxa was Kristiansand kommuneskog in the Vest-Agder region (Table S3.3) and Hardangervidda NP was the second priority reserve, which conserved 21 taxa that were different to the first reserve, with 101 taxa conserved in total. Fifty percent (91 taxa) of the 181 taxa had more than five populations conserved within the PA complementary network (Table S3.2). The PA complementary network covered 17 of the 27 ELC zones (Table S3.3) and conserved an average of 23% of ecogeographic diversity per taxon (Table S3.5).

Figure 3.4: The PA complementarity network of 23 reserves (shaded) for 181 priority CWR taxa in Norway. A) Southern Norway, B) Northern Norway. Numbers refer to priority order (number one is the first reserve location with the highest number of taxa, number two has the highest number of additional taxa et.). Letters refer to reserves with the same number of additional taxa but different numbers of total taxa, with 'a' containing more than 'b' etc. Created using the Complementa tool in CAPFITOGEN. Map drawn to geographic coordinate system WGS 1984.

The predicted distribution of 187 priority taxa was mapped (Figure 3.5). During the modeling process 14 taxa (Table S3.6) were not analyzed due to lack of presence points and could not be included in further predictive studies. The predicted distribution map (Figure 3.5) showed an increase in taxa richness along the south east coast. A comparison between observed (Figure 3.1) and predicted (Figure S3.5) taxa richness showed that there were gaps in the current knowledge of priority CWR distribution (Figure S3.2). The Vestfold and Østfold regions were particularly high in potential taxa richness but lacked observational data or *ex situ* collections. For evaluation of the models, the AUC score was >0.9 for 63% of taxa; 32% had an AUC >0.7; and 5% had an AUC between 0.646 and 0.699 (Table S3.4).

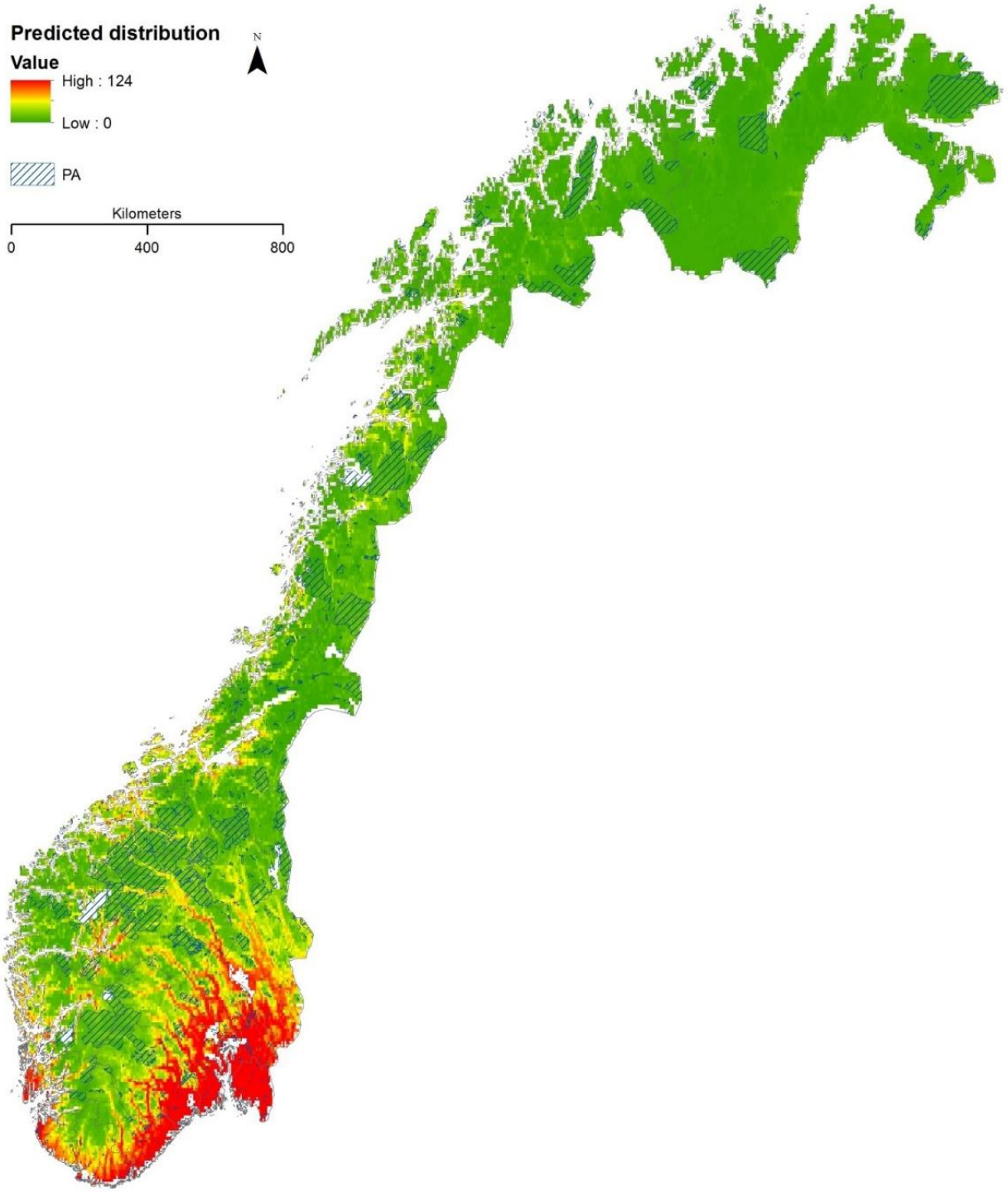

Figure 3.5: The predicted distribution of 187 priority CWR in Norway under the current climatic conditions. Red areas indicate taxon-rich areas with up to 124 taxa found there, and green areas indicate low taxon richness. All grid cells equal to 4 x 8 km^2.

There were 34 908 (11%) presence points inside an existing PA with 181 (90%) of the 201 priority CWR found within at least one of 898 different PA in Norway. The remaining 20 taxa are listed in Table 3.2 and were not located within any protected areas analyzed. From the predicted distribution maps for the missing taxa, there were nine that were predicted to be in a PA, although they have not been observed there (Table 3.2). For the remaining taxa there was not enough data available to produce a predicted distribution map.

Table 3.2: Priority taxa outside PAs according to occurrence data used in this study and those taxa predicted to be inside PAs.

Taxa outside PA	Number of presence points	Predicted to be inside a PA
Allium victorialis L.	12	Yes
Elymus fibrosus(Schrenk) Tzvelev	8	Yes
Festuca baffinensis Polunin	1	n/a
Festuca brachyphylla Schult	1	n/a
Festuca hyperborea Holmen ex Fred	1	n/a
Festuca rubra L. *commutata* Gaudin	30	yes
Peucedanum ostruthium(L.) W.D.J.Koch	32	Yes
Poa abbreviata R.Br.	1	n/a
Poa lindebergii Tzvelev	0	n/a
Poa arctica R. Br. *Tromsensis* Nannf	2	n/a
Poa bulbosa L.	9	n/a
Poa arctica R. Br. *caespitans* Simmons ex Nannf.	1	n/a
Rosa balsamica Besser	2	n/a
Rosa corymbifera Borkh.	6	Yes
Rubus dissimulans Lindeb	16	Yes
Rubus fissus Lindl	18	n/a
Rubus norvegicus A.Pedersen	28	n/a
Rubus scissus W.C.R.Watson	24	Yes
Rubus septentrionalis W.C.R.Watson	12	Yes
Vicia tenuifolia Roth	15	Yes

3.4.3 ELC map

The ELC map contained 27 zones based on the eight variables selected (Table S2.3). The average values within each ELC zone are found in Table S3.6. All ELC zones apart from zone four were found within the PA network. The average ecogeographic diversity conserved within the entire PA network per taxon was 48% and the PA complementary network conserved an average ecogeographic diversity per taxon of 23% (Table S3.5). Two taxa were not found within any ELC zones (*Festuca brachyphylla* Schult and *Rubus hallandicus* (Gabr. Ex Aresch.) Neuman) due to their area of distribution lacking ecogeographic information at the scale used here.

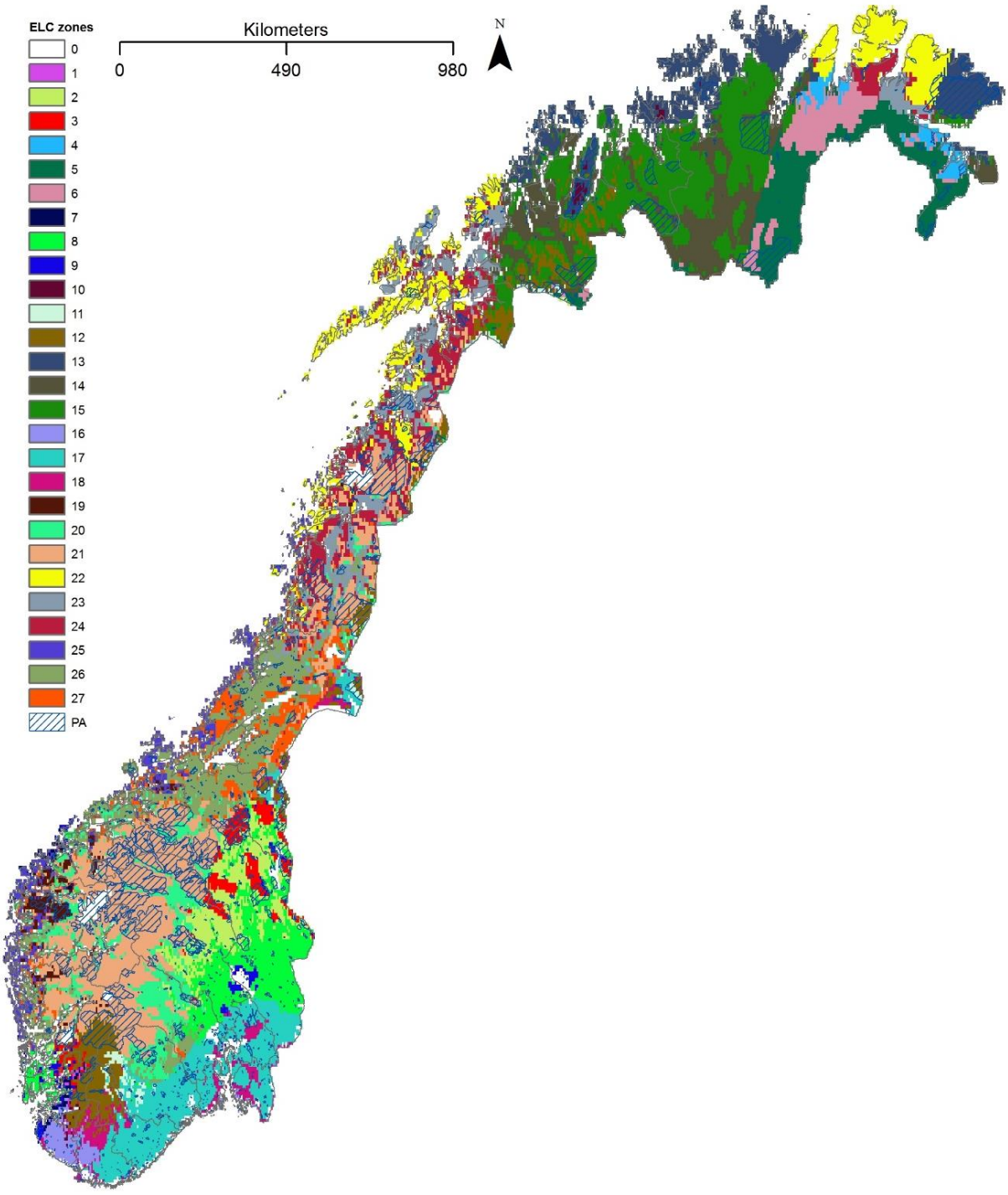

Figure 3.6: The ELC map for Norway composed of 27 ELC zones each representing a unique combination of environmental variables. See Table S3.6 for average values in each zone. Zone 0 refers to those areas where information for some of the components making up the map are missing. Created in CAPFITOGEN using the ELC mapas tool. Cell size is equivalent to 4 x 8 km^2 and is drawn to geographic coordinate system WGS 1984.

3.4.4 Ex situ diversity analyses

There were 24 (12%) priority taxa with *ex situ* accessions (Table 3.3) the remaining 177 (88%) taxa did not have any *ex situ* accessions according to the data used. Of these 24 taxa, two (*Allium fistulosum* L., *Allium scorodoprasum* L.) were assessed for the Norwegian Red List (Kålås *et al*., 2006) with *A. fistulosum* being the only taxon listed as threatened. Fifteen taxa (63%) with *ex situ* accessions had more than the suggested minimum number of five populations conserved (Table 3.3) (Brown and Briggs, 1991). For the 177 taxa without *ex situ* collections the areas of highest taxa richness were the Oslo region and the south of Norway but also on the west coast of the Nordland region (Figure S3.3).

Table 3.3: The 24 priority taxa with *ex situ* accessions. The number of accessions (i.e. populations) for each taxon, the number of ELC zones taxa have been collected from, which ELC zones taxa have been collected from and which ELC zones the taxa have been observed in but not collected from (excluding ELC zones -9999 and 0).

Taxa	Number of *ex situ* accessions	Number of ELC zones collected from	Which ELC zones collected from	Total number of ELC zones taxa is present within
Agrostis capillaris	86	16	2, 4, 5, 8, 9 11, 13, 14, 15, 16, 17, 18, 20, 21, 23, 26	27
Allium fistulosum	3	2	16, 17	7
Allium schoenoprasum	4	1	19	18
Allium scorodoprasum	1	n/a	n/a	9
Alopecurus pratensis	40	12	2, 4, 5, 8, 13, 14, 15, 17, 20, 21, 23, 24	24
Artemisia absinthium	1	n/a	n/a	12
Asparagus officinalis	3	n/a	n/a	5
Bromus inermis	17	6	2, 8, 17, 20, 21, 26	16
Carum carvi	53	12	5, 8, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27	26

Cichorium intybus	1	n/a	n/a	9
Dactylis glomerata	163	18	2, 7, 8, 9, 11, 12, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27	23
Daucus carota	2	1	17	10
Festuca ovina	9	4	2, 8, 9, 17	27
Festuca pratensis	95	17	2, 4, 5, 8, 9, 14, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27	24
Festuca rubra	115	17	2, 3, 5, 8, 9, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 26, 27	27
Lactuca serriola	1	1	18	6
Lolium perenne	5	3	8, 9, 16	15
Pastinaca sativa	1	1	9	8
Phalaris arundinacea	55	15	1, 2, 8, 9, 11, 12, 16, 17, 18, 19, 20, 21, 23, 25, 26	26
Phleum pratense	189	21	2, 4, 5, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27	27
Poa pratensis	123	18	2, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 17, 18, 20, 21, 22, 23, 26	27
Trifolium hybridum	13	5	8, 16, 17, 18, 20	24
Trifolium pratense	88	15	2, 8, 9, 11, 12, 14, 17, 18, 20, 21, 23, 24, 25, 26, 27	26
Trifolium repens	114	15	2, 8, 9, 11, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 26	27

As shown in Figure 3.7, ELC zones three and 19 could be targeted first for *ex situ* collections as they contained 14 taxa with the highest priority for collection (class 1). The ELC zone to target for collection of the highest number of taxa without *ex situ* accessions was category 17 (Figure S3.4) with 153 taxa present. ELC zones four and ten had the lowest number of taxa (33 and 30 respectively) but should still be targeted for collection to ensure the full ecogeographic range of populations is conserved.

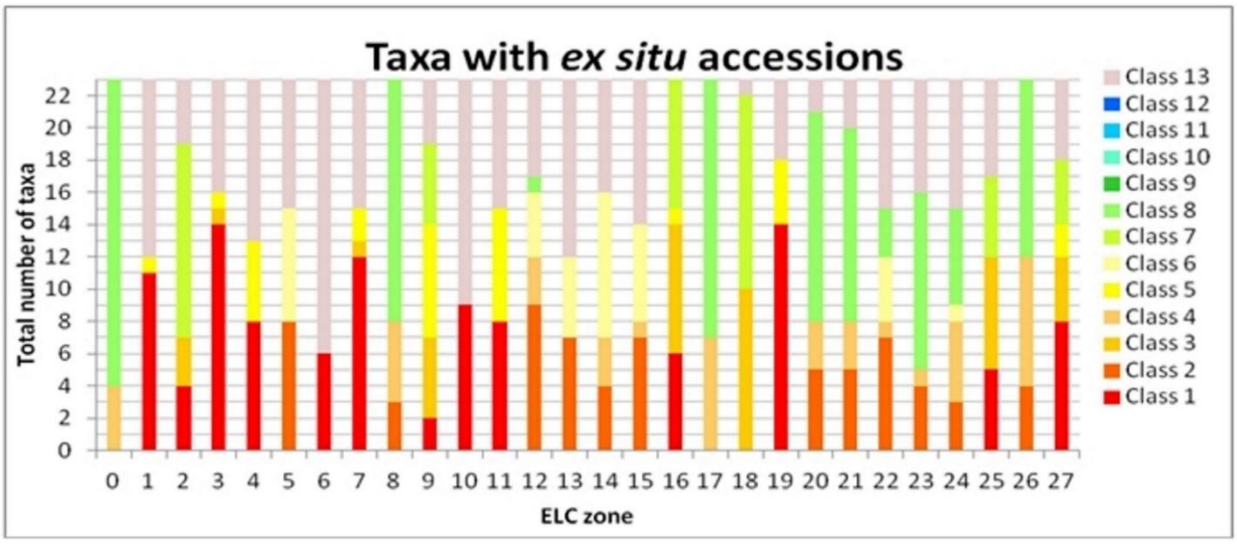

Figure 3.7: The number of taxa and priority level (class one is the highest priority level) for collecting within the ELC zones, for a total of 23 taxa that currently have *ex situ* accessions. (Asparagus only had three *ex situ* accessions which were located in areas of the ELC map which did not contain environmental data; therefore in this analysis it was assumed the taxa had no *ex situ* collections).

Of the 15 taxa with *ex situ* accessions and more than five populations conserved, 13 are conserved in five different ELC zones (Table S3.8). *Festuca ovina* L. and *Lolium perenne* L. only have populations conserved within four and three ELC zones respectively (i.e. 14.8% and 20% of ecogeographic diversity conserved within *ex situ* accessions).

3.5 Discussion

The interdependence among countries for food supplies and plant genetic resources has long been acknowledged and is now even more important due to increased homogeneity of staple human food crops across the world (Flores-Palacios, 1998; Khoury *et al*., 2014). Therefore, one of the primary criteria in creating the inventory of 204 CWR for Norway was to take a global as well as national approach to the selection of priority taxa. Some taxa on the list are particularly important to Norwegian stakeholders i.e. *P. pratense*, and Norwegian culture, such as *Arnica montana* L. There are also taxa important at regional levels (*R. chamaemorus*) and at

the global scale such as *Festuca* and *Brassica* L. species which are important forage, fodder and food crops.

The criteria used for prioritisation of Norwegian CWR are specific to Norway but include some commonly cited criteria such as: economic value, presence in Annex 1 of the ITFGRFA and importance to local and national stakeholders (Iriondo *et al*., 2016). These criteria are also common to other national strategies such as Cyprus' (Phillips *et al*., 2014) and Finland's (Fitzgerald, 2013). Finland's strategy also used threat status as a criterion for prioritizing CWR however as agreed with stakeholders this was not used here as threatened taxa are already conserved by other means in Norway. This highlights the differences that may occur between countries with respect to prioritising CWR for conservation and that there cannot be one method that suits all.

All taxa presence points were obtained from GBIF as national stakeholders agreed this was the most complete source of data for this project. Less than 4% of the occurrence data used was accurate to two or less decimal places. This data was deemed appropriate to use, along with the other 96% of data for these analyses, as removal of it would lead to a loss of information due to elimination of species from the analyses. Other sources of data may complement this and provide data for the three taxa that did not have any presence points on the mainland but are included in the Flora of Norway. For these taxa it is a priority to confirm their existence *in situ* as should be done for all other taxa to increase the coverage of observational data. The accuracy of the GIS methods used in this analysis is only as reliable as the input data which may not have been generated for the purpose of biogeographical studies (Chapman, 2005) and may be subject to a sampling bias (Araujo & Guisan, 2006; Loiselle *et al*., 2008). Furthermore, due to the common nature of CWR they may have been over looked in terms of botanical recording.

The use of MaxEnt in species distribution modelling is now commonplace (Costa *et al*., 2010; Ramirez-Villegas *et al*., 2010; Elith *et al*., 2011) and it has fared well in evaluations in comparison to other programmes (Anderson *et al*., 2006). Species distribution modelling may produce bias results if the data itself is biased, with MaxEnt being no exception to this (Merow *et al*., 2013; Fourcade *et al*., 2014). Furthermore, MaxEnt uses presence only data which may add to this bias, therefore any results in this study should be interpreted with this in mind. The CAPFITOGEN tools have been developed to help strengthen the capabilities of national plant genetic resource programmes and the use of these tools allowed multiple taxa to be assessed over a short time frame (Parra-Quijano *et al*., 2016).

3.5.1 In situ diversity analysis

The Oslo region and the south have a naturally high level of floristic diversity (Nordic Gene Bank, 2006) due to the limestone bedrock, unique environmental patterns and fertile soils (Moen, 1998). Therefore, it is not unexpected from our results that this region has a high level of CWR richness, as many of the priority CWR taxa are thermophilous species which tend to be concentrated in this region of Norway. However, it is concerning that areas close to the city contain important populations of CWR as it puts them under threats such as habitat degradation. It also poses a difficulty for the establishment of genetic reserves, especially considering climate change and the potential of species distributions to shift (Midgley *et al*., 2003; Kelly & Goulden, 2008). The low levels of taxa richness in Northern Norway is due to the high latitude and environmental conditions such as cooler temperatures and a shorter growing season which limits growth of all plant taxa, including CWR. The low numbers of people and difficult terrain may lead to a lack of surveying and hence a lack of knowledge on the species present which could lead to sampling bias.

The predicted taxa richness map (Figure 3.5) could not be created for all taxa due to lack of data, suggesting these taxa (Table S3.6) will need to be priorities for further surveying. The predicted distribution map does not account for factors such as land use or taxon dispersal ability therefore some areas of high or low species richness may be inaccurate. The Vestfold region is highlighted as an area of potentially high species richness, yet our presence point data does not show this. Consultation with national experts confirms this region is a hotspot of taxa richness and should be targeted for surveying and consequent conservation actions. The 10% of taxa not found within a PA should be targeted for further surveying and *ex situ* collection. Populations of all conserved taxa will need to be large enough to meet the levels expected for successful *in situ* conservation (see Iriondo *et al*. (2012)).

The importance of having a network of complementary reserves means that a fully representative reserve system with maximum efficiency in terms of land and number of different species can be achieved with minimal costs (Maxted *et al*., 2008b). Two different genetic reserve networks are proposed. In terms of the PA complementary network, large PA such as the Hardangervidda NP (number two priority in Figure 3.4) are highlighted as priorities. Conservation throughout the whole area is not practical due to its large size (6500 km^2), or necessary as taxa will not be distributed throughout the entire area due to inhospitable environments such as glaciers. The proposed PA complementary network may be more appropriate for *in situ* genetic reserve conservation than the proposed grid cell complementary network of 19 (4 x 8 km^2) areas which is not entirely focused upon current PAs. The former will also avoid the high start-up cost of acquiring land for new reserves and may only require minimal adjustments to existing management plans (Maxted and Kell, 2009).

From the two different complementary network approaches, there are six sites that overlap (Table 3.4). These locations may be promising areas to initially target for *in situ* conservation

actions within PAs as, according to our data it is more likely that priority taxa will be found there and these particular locations are spread across the whole of Norway. This suggests that a wide range of ecogeographic diversity (and hence genetic diversity) might be conserved (Table 3.4).

Table 3.4: List of PAs that are found within both complementary network analyses (Figure 3.3 and Figure 3.4) and the ELC zones found within both networks. Zone 0 refers to those areas where information for some of the components making up the map is missing.

Complementary network		Protected areas	ELC zones
Grid cell network	PA network		
1	4a	Hovedøya landscape PA	0
2	1	Kristiansand kommuneskog	16, 17, 18
3	4	Dovrefjell-Sunndalsfjella NP	20, 21
6a	8b	Oksy-Ryvingen Landscape PA	17
7a	3 and 7	(nearby) Sandysalta nature reserve and Ytre Hvaler NP	0, 16
9g	8k	Persfjorden-Syltefjorden landscape protected area	13

Conserving a high number of different taxa within the complementary network is necessary as well as protecting an appropriate number of populations to ensure the full range of genetic diversity found within and among populations is conserved (Brown & Briggs, 1991). For taxa that lack the minimum of five populations conserved (Table S3.2) they should be targeted for further surveying to determine the location of populations. If populations are not located within PA then *in situ* conservation outside of PA, specific taxon-wide legislative protection measures and collection of seed for *ex situ* conservation could take place.

The PA complementarity network analysis accounts for taxa richness and ecogeographic diversity that may be used as a proxy for genetic diversity, which is required in the designation of a CWR genetic reserve (Greene & Hart, 1999; Iriondo *et al*., 2012; Parra-Quijano *et al*., 2012a). For taxa with narrow distributions such as *Poa lindebergii* Tzvelev, 100% of ecogeographic diversity is conserved within the PA complementary network, whereas for a common species with a wide distribution such as *Deschampsia flexuosa* (L.) Trin, 59% of ecogeographic diversity is conserved. *Vicia pisiformis* L. has no ecogeographic diversity conserved within the PA complementary network yet 100% of its ecogeographic diversity is conserved within the entire PA network (Table S3.5). This means that for some taxa the complementary network is not appropriate for conserving the full range of ecogeographic diversity. There may be a need for *in situ* species specific conservation strategies within Norway. Furthermore, there will need to be genetic diversity studies upon priority taxa to determine the correlation with ecogeographic diversity and to inform specific management strategies for populations.

Although PAs offer formal protection of species they may not be appropriate for all taxa in all environments. CWR tend to be associated with pre-climax communities and areas experiencing anthropomorphic change, such as field margins and road verges (Maxted & Kell, 2009; Jarvis *et al*., 2015), habitats which tend to be more common outside of PA. In Norway, many of the PAs were set up to conserve unique environmental features such as glaciers or fjords. Along with CWR populations near cities, common habitats may need less formal conservation strategies that heavily involve local stakeholders and land owners. The need for conservation outside of PAs may become increasingly important due to the effects of climate change which is predicted to have a significant impact on the Arctic flora (McCarthy *et al*., 2001; Arctic Climate Impact Assessment, 2004). Protection of the most appropriate CWR

populations (MAWPs; Maxted *et al*., 2015) may be a complementary approach that should be taken for these taxa outside PAs. This will allow prioritization of the most valuable populations whether inside or outside a PA which could then, for example, be protected via separate legislation. The grid cell complementary network of 19 areas (Figure 3.3) could also be used to locate important populations outside of the reserve network which could complement those conserved within the proposed PA complementary network (Figure 3.4).

3.5.2 Ex situ diversity analysis

Ex situ accessions are considered a backup and complementary source of material which should be representative of populations *in situ* (Maxted & Kell, 2008). This material should be made available to plant pre-breeders and breeders for use in crop improvement which is becoming increasingly important due to climate change. The gaps in the *ex situ* accessions for the 201 priority CWR should be prioritized for immediate collection. The locations for collecting may be based upon the taxa richness map (Figure S3.3) so a large number of taxa can be collected in minimal visits. However, to collect the genetic diversity of taxa it would be more appropriate to collect taxa from different ELC zones to ensure the full range of genetic diversity is conserved (Figure S3.4 and Table S3.8). Priority ELC zones could initially be those with high numbers of taxa richness for the missing *ex situ* accessions, such as category 17 which is predominantly distributed throughout the south east of Norway (Figure 3.6). This collecting methodology was shown by Parra-Quijano *et al*. (2012c) to be an efficient method for improving the representativeness of *ex situ* collections. Furthermore, to expand on this, the use of the Representa tool in CAPFITOGEN enables the targeting of ELC zones with the highest number of high priority taxa (Figure 3.7; Parra-Quijano *et al*., 2016). By guaranteeing that a minimum number of five populations (Brown and Briggs, 1991) are conserved per

ecogeographic zone for each taxon, we ensure that the full range of naturally occurring genetic diversity is conserved both within and between populations in ELC zones.

The following are conservation recommendations from this scientific assessment for *in situ* and *ex situ* conservation of 204 priority CWR within Norway:

3.5.3 In situ conservation priorities:

- Target the three taxa without presence point records for immediate surveying efforts (*R. inodora, P. arctica* subsp *microglumis, F. rubra* subsp *megastachys).*

- Assess the suitability of the six locations (Table 3.4) that overlap between the complementarity analyses to become a CWR genetic reserve network. They should include active *in situ* management and monitoring with subsequent establishment of CWR genetic reserves according to Iriondo *et al*. (2012).

- Use the grid cell complementary network (Figure 3.3) to target locations for conservation of CWR populations outside of PA.

- For the taxa found outside of PA (Table 3.2) increase surveying efforts to determine if this is accurate. If so, conserve populations outside of PA, by creating a new PA if appropriate and collect for *ex situ* conservation.

- Target the 90 taxa with less than five populations conserved within the PA complementary network (Table S3.2), for further surveying.

- *In situ* conservation priorities should target areas that are gaps in taxa distribution data for immediate surveying, such as Vestfold and Østfold regions (Figure S3.2).

3.5.4 Ex situ conservation priorities:

- Collect the 177 taxa without *ex situ* accessions. Figure S3.3 can be used to target locations with high taxa richness for collecting and Figure S3.4 should be used to target those ELC zones with the highest number of taxa. Table S3.8 should be used to ensure the full range of ecogeographic diversity, with a minimum of five different ELC zones are collected (where possible) and/or a minimum of five populations targeted.

- Ensure those 24 taxa with *ex situ* accessions have collections made from the full range of ecogeographic diversity, with a minimum of five populations within each ELC zone conserved where possible.

- All *ex situ* material should be duplicated as appropriate in national and/or regional genebanks and a backup of material should be stored in the Svalbard Global Seed Vault. Accessions should be regenerated as appropriate to ensure germination rates are kept at correct levels. Accessions should be made available to plant pre-breeders and breeders in accordance with the ITPGRFA so that the genetic diversity can be harnessed for crop improvement.

Update and review the priority list of CWR and conservation recommendations every five years or as deemed appropriate.

3.6 Conclusion

This study provides recommendations for *in situ* and *ex situ* conservation of 204 priority CWR within Norway. The outcomes should be regarded as provisional and interim and therefore can and should be updated when additional data and knowledge becomes available. There is an intrinsic link between both *in situ* and *ex situ* conservation activities proposed and neither can be done without the other, this is even more important due to climate change and the potential

of populations to move or become extinct. Furthermore, cooperation with local stakeholders such as landowners, PA managers, plant breeders and policy makers is essential at all levels. Both the priority taxa and the methodology used are applicable at regional and global scales with the recommendations not only helping Norway to meet its international obligations for conservation of genetic diversity of CWR but also ensuring this genetic diversity is available for use in tackling global food security.

CHAPTER 4.

Climate change and national crop wild relative conservation planning

The work presented in this chapter has been published in Ambio.

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Jade Phillips, Joana Magos Brehm, Bob van Oort, Åsmund Asdal, Morten Rasmussen and Nigel Maxted

Author contributions:

Conceived and designed the study: J.P., J.M.B.

Data collation and preparation: J.P.

Performed analysis: J.P.

Interpreted results: J.P., J.M.B., B.O.

Wrote the paper: J.P.

Critically reviewed the paper: J.P., J.M.B., B.O., A.A., M.R., N.M.

4.1. Abstract

Climate change is likely to be one of the principal factors affecting our future food security. To mitigate negative impacts, we will require our crops to be more genetically diverse. Such diversity is available in CWR, the wild taxa relatively closely related to crops and from which diverse traits can be transferred to the crop. Conservation of such genetic resources resides within the nation where they are found; therefore national-level conservation recommendations are fundamental to global food security. We investigate the potential impact of climate change on CWR richness in Norway. The consequences of a 1.5°C and 3.0˚C temperature rise was studied for the years 2030, 2050, 2070, 2080 and then compared to the present climate. The results indicate a pattern of shifting CWR richness from the south to the north, with increases in taxa turnover and in the numbers of threatened taxa. Recommendations for *in situ* and *ex situ* conservation actions over the short and long term for the priority CWR in Norway are presented. The methods and recommendations developed here can be applied within other nations and at regional and global levels to improve the effectiveness of conservation actions and help ensure global food security.

4.2 Introduction

Many regions throughout the world are projected to experience climate change-induced reductions in crop yields and additional associated challenges are mounting (e.g. pests, water supply, and soil degradation) (Müller & Robertson, 2014; Rosenzweig *et al*., 2014). The IPCC (2014) predicts that in the short-term (2016 to 2035) the global mean surface temperature change is expected to be between 0.3-0.7˚C with the highest prediction set at 4.8˚C for the year 2100. At the Paris climate change conference (UNFCCC, 2016) a global action plan to limit warming to below 2.0˚C increase was agreed. To help achieve this target Intended Nationally

Determined Contributions (INDCs) were submitted by individual countries and if followed are predicted to limit global warming to approximately a 3.0˚C temperature increase (UNFCCC, 2015). While some locations may see crop yield increases (Olesen & Bindi, 2002; Uleberg *et al*., 2014), the global average negative effects of climate change on many aspects of food security (McCarthy *et al*., 2001) and the interdependence of most countries on imports and exports of food (FAO, 2009a) mean it is becoming increasingly important to make our crops more climate resilient.

CWR are a key resource in meeting this challenge as they are often found in a wide range of habitats, under variable environmental conditions. They are wild taxa closely related to our cultivated crops and, as such, tend to contain higher levels of genetic diversity (Tanksley & McCouch, 1997; Buckler *et al*., 2001; Maxted & Kell, 2009). The value of CWR in climate change adaptation is highlighted in a report by the FAO (2015) who recommend consolidating collections of wild species, including CWR, because of an increased adaptive capacity inherent in a greater genetic diversity, and the need to adapt agriculture to climate change. Some examples of the use of CWR in cultivar development include the transfer of cold tolerance from wild *Malus baccata* (L.) Borkh. to *M. domestica* Borkh. (Cummins & Aldwinckle, 1979) and the improvement of drought tolerance in cultivated barley from wild barley (*Hordeum vulgare* L. subsp. *spontaneum* (K. Koch) Thell. (Lakew *et al*., 2011; see Maxted & Kell (2009) for more examples). Furthermore, the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture (2011) stresses the importance of expanding the programme on *ex situ* conservation to ensure maintenance of diversity of species including those that are adapted to extreme conditions and from those areas expected to be highly affected by climate change. The report also places emphasis on *in situ* conservation of

genetically diverse populations to allow evolution and thus permit the continued generation of adaptive traits.

Specific studies on CWR have confirmed that they might be negatively affected by climatic change, with a high proportion of the species studied losing over 50% of their range size by 2055 (Jarvis *et al*., 2008). Broader-scale studies on terrestrial species have identified trends for distribution shifts to higher latitudes and elevations (Thomas *et al*., 2012) as well as increasing IUCN threat level due to climate change (Thuiller *et al*., 2008). However, few if any, studies have been done at a national level regarding the effects of climate change on the distribution of CWR. Norway is particularly interesting in respect of climate change effects upon species distribution as it is located on the periphery of Europe with the mainland stretching, from south to north, from 50° N to 71° N as well as having notable north-south and east-west climate gradients (Norwegian Ministry for Agriculture and Food, 2008). At northern latitudes surface temperature and precipitation are expected to increase (Solomon, 2007), with the arctic warming at a faster rate than the global average (Arctic Climate Impact Assessment, 2004). Initially, these changes may increase crop yields at high latitudes (Olesen & Bindi, 2002), due to an extension of the growing season, possibly up to 1-3 months (Hanssen-Bauer *et al*., 2009). These effects of climate change will have important consequences for northern flora with areas potentially experiencing an increase in species diversity (Sætersdal *et al*., 1998) but some species populations may become restricted at their northern limit. Furthermore, currently well-adapted northern species may be increasingly challenged by rising incidences of new pests and diseases.

Recommendations were recently identified for the *in situ* and *ex situ* conservation of CWR at the national level within Norway (Phillips *et al*., 2016). That study pinpointed 204 priority CWR of which 44% were forage taxa, 43% were food taxa and 13% were ‘other’ taxa such as

medicinal or forestry species (Phillips *et al*., 2016). The Norwegian priority taxa are important from the global to the national level, due to their economic value, presence within the ITPGRFA (FAO, 2001; www.planttreaty.org) importance to Norwegian research and/or within the Harlan and de Wet inventory of 173 globally important crops (Vincent *et al*., 2013). To determine actions required to mitigate the negative effects of climate change, species distribution models (SDM), which use environmental data to identify taxon-specific ecological niches (Phillips *et al*., 2004; Phillips *et al*., 2006) that may be suitable for populations to persist in, can be utilised. However, SDM assume that nature is static or on a linear path of change, often ignoring fecundity, dispersal, soil specificity and preference (Midgley *et al*., 2003) and it is therefore necessary to use the results with caution. Here we aim to contribute towards the knowledge base on climate-smart conservation planning for CWR conservation by developing methods to identify priority taxa and genetic diversity that may be under threat. Due to climate change it will be important to consider both incremental (adjustments made enabling the continuation of current practices) and transformational (fundamental changes in conservation practices) (Walker *et al*., 2004; Nelson *et al*., 2007; Stafford Smith *et al*., 2011) adaptation plans to ensure long term flexibility and effectiveness of conservation strategies. The current PA system throughout the globe (including NPs and nature reserves) is static (Peters & Myers, 1991), often severely restricted in the future potential for expansion and may not have been established with the future effects of climate change in mind. In addition it is highly likely that the rate of climate change will exceed the potential of populations to track climate by natural migration and adaptation (Midgley *et al*., 2003; Jump & Penuelas, 2005), therefore increasing the need for *ex situ* conservation. Both *in situ* and *ex situ* conservation recommendations are made to ensure that this diversity is available for use by plant pre-breeders to help develop

crops that are sufficiently robust enough to withstand predicted changes in climate not only within Norway but globally.

4.3 Methods

4.3.1 Species data sources

The priority CWR inventory of Norway containing 204 taxa was used as the basis for the climate change analysis (Table S2.2). Taxa occurrence records were gathered from GBIF (GBIF, 2013) and are the same as those used in chapter 3 (Phillips *et al*., 2016). During predictive distribution modelling the same 14 taxa as in chapter 3 were excluded from the final analysis due to lack of presence points (Table S3.6). Hence the following evaluation was done upon 187 of the priority CWR, with a total of 304 372 presence points utilised.

4.3.2 Species distribution modelling

The potential distribution of taxa under both the present and future climate scenarios was mapped. The present bioclimatic variables used were obtained from freely available sources and were the same as those used in chapter 3 (Phillips *et al*., 2016), which consisted of bioclimatic, geophysical and edaphic factors, each with the same extent and raster grid cell size (0.0416, approximately equal to 4 x 8 km^2; Table S2.3). Present day climate refers to an interpolation of observed data which was representative of the years 1960-1990 (Hijmans *et al*., 2005). Environmental variables in the model were reduced in number from 105 to 13 variables in order to help minimise redundant or correlated variables which may affect the validity of the SDM. This was done by testing for collinearity (Dormann *et al*., 2013) between variables and running a principal components analysis (PCA) in SPSS 22 (IBM Corp, 2013) to produce a list of uncorrelated variables. Experts within Norway were then consulted on which of these variables they considered most important for predicting plant distribution in Norway. The

maximum entropy (MaxEnt) algorithm (Phillips *et al*., 2004; *et al*2006) was used to model the potential distributions for each priority taxon individually under both present and future bioclimatic variables (see chapter 3, Phillips *et al*. (2016) for MaxEnt settings). MaxEnt has been used robustly for predictions of climate modelling (Ramirez-Villegas *et al*., 2010; Warren *et al*., 2013) and has performed well in comparison tests with similar programs (Anderson *et al*., 2006; Elith *et al*., 2011).

The future bioclimatic variable data was obtained for the climatic variables only, as edaphic and geophysical variables were unlikely to change with climate change. The climatic variables used were: isothermality, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation and precipitation seasonality (Table S2.3). These data were gathered from CCAFS (www.ccafs-climate.org/data/) where climate data was available from numerous models and scenarios based upon the most recent IPCC report (IPCC, 2014). The Norwegian Earth System Model, NorESM1-M (Bentsen *et al*., 2013), which was based upon the Community Climate System Model version 4 (CCSM4; Gent *et al*., 2011), was used due to its specificity to climatic processes that are particularly important at northern latitudes (Bentsen *et al*., 2013). These models were driven by the two relative concentration pathway scenarios (RCP 2.6, RCP 6.0), representative of the potential future variability and pathways of greenhouse gas emissions (Prather *et al*., 2013). RCP 2.6 was used in this study as it represents the agreed maximum temperature rise set out by the Paris agreement (1.5˚C) (Rogelj *et al*., 2012; UNFCCC, 2016). RCP 6.0 represents the more likely development from the implementation of global INDC proposals of a 2.5-3.5˚C temperature increase by 2100 (Rogelj *et al*., 2012; UNFCCC, 2015). Analysis was undertaken for the years 2030, 2050, 2070, 2080 and compared to the present to allow visualization of the long-term pattern of distribution change of CWR.

Evaluation of the models' accuracy was done using two validation metrics suggested by Ramirez-Villegas *et al*. (2010), the Area under the ROC Curve of the test data (AUC_{Test}) and standard deviation of the AUC_{Test} data (STAUC) for each taxon. Models with an $AUC_{Test} > 0.7$ and STAUC < 0.15 are considered accurate and stable (Ramirez-Villegas *et al*., 2010).

4.3.3 Species richness, turnover and threat level

To determine the potential impacts of climate change upon the priority taxa they were assessed under unlimited migration, with populations able to move to where the climate is suitable, and no-migration scenarios, where populations cannot move from their present distribution, with the reality being that species will likely fall between these extremes (Higgins *et al*., 2003). Outcomes were analysed in ArcMap 10.2 (ESRI, 2011) using Python scripting to automate and streamline the process.

Species richness was calculated under unlimited migration for the 187 taxa using DIVA-GIS (Hijmans *et al*., 2004) and Spatial Analyst tools in Arc Map 10.2, for present and future climate scenarios. The broad patterns of species richness throughout Norway were compared among the years studied. Change in taxon richness was studied under unlimited migration by comparing future with present potential taxon distributions, which allowed patterns in the direction of taxon distributional changes to be analysed.

Species loss and gain were assessed by the number of species found per grid cell and compared to the current species richness per grid cell for both unlimited and no-migration scenarios. The turnover rate (T) was then calculated for the unlimited-migration scenario following Thuiller *et al.* (2005):

$$T = 100 \times \frac{(L+G)}{(SR+G)}$$

where SR was the current species richness, L was loss of taxa per grid cell and G was gain of taxa per grid cell. Turnover rate was calculated for both RCP scenarios per study year. Turnover was determined for mainland Norway (32241 grid cells), which excluded Jan Mayen and Svalbard.

To determine the extent of taxon range we compared the number of grid cells where a taxon was present under both no-migration and unlimited-migration and present and future climatic scenarios. The level of threat for the priority taxa was then assessed using the IUCN Red List criterion A3(c) (IUCN, 2001). This used the projected geographic range loss of a taxon as a proxy for population reduction to assign a threat category by the following parameters: Extinct (EX) is a taxon with a projected range loss of 100 %, Critically Endangered (CR) with a projected range loss of >80%, Endangered (EN) with a range loss of >50% and Vulnerable (V) with a range loss of >30% (IUCN, 2001). The remaining taxa were considered Least Concern (LC) in terms of climate change impacts.

4.4 Results

The potential effects of climate change showed a change in distribution for 187 priority CWR in Norway. Under the unlimited-migration scenario, taxon-richness increased across Norway from a predicted richness of 124 taxa under the current climate (Figure 3.5) to a maximum of 150 taxa in the most taxon-rich areas for some of the scenarios (Figure 4.1, 4.2). Taxa tended to spread from the south east of Norway towards the west and the north with the mountainous regions preventing further westward dispersal. Taxon richness also increased from the west

coast and moved both eastwards and northwards, with the RCP 6.0 scenarios showing a larger area of Norway with increased taxon richness. This pattern of distribution change was reflected in Figure S4.1 and Figure S4.2, where gain in taxon richness tended to increase further northwards under both RCP scenarios, from the year 2030 to 2080. There was also a slight loss of taxon richness in the south east, which was more apparent in the RCP 6.0 scenarios, although this region was still the area with highest taxon richness overall.

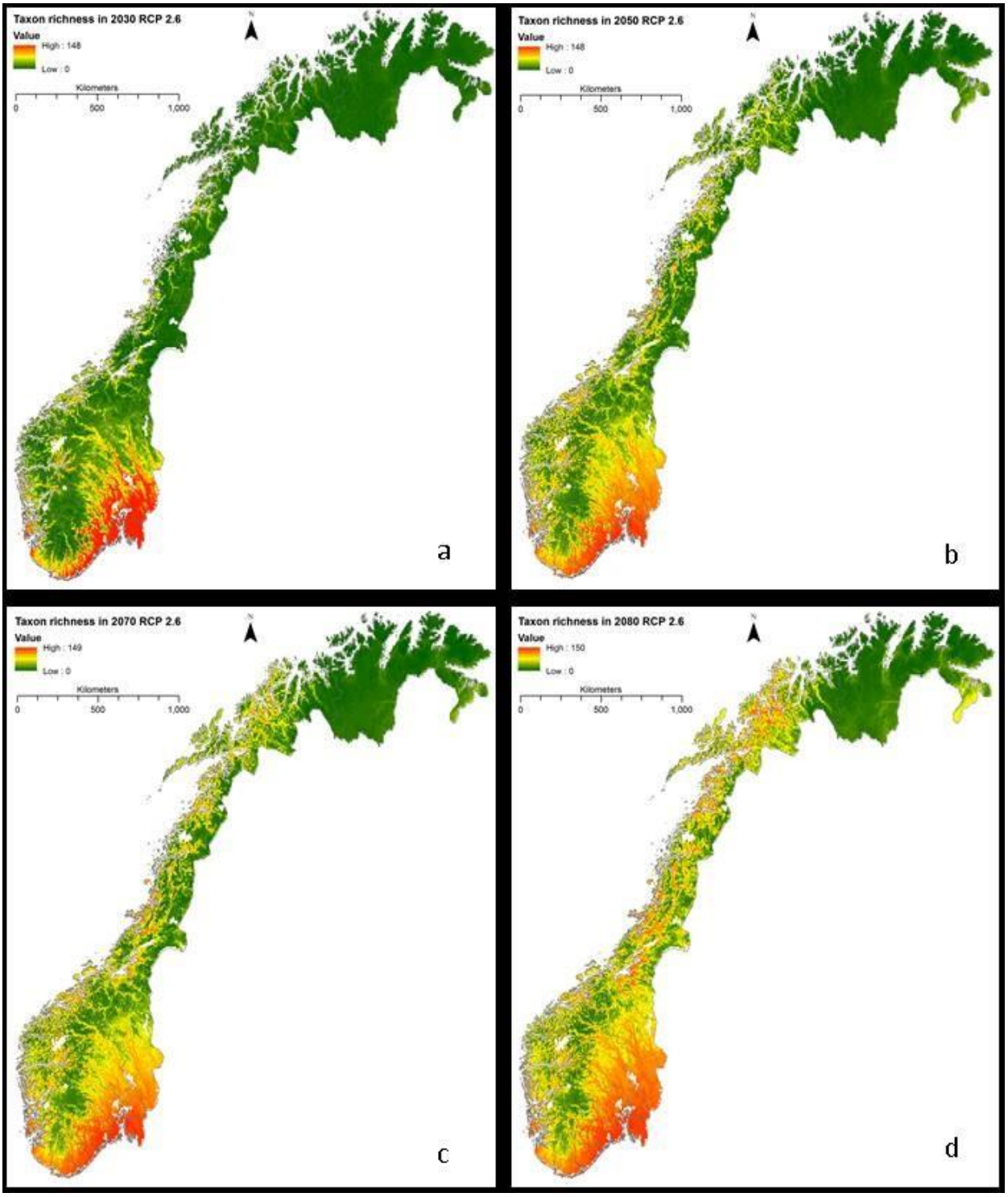


Figure 4.1: The average predicted taxon richness of 187 priority CWR in Norway under RCP 2.6 for the years a) 2030, b) 2050, c) 2070, d) 2080. Raster grid cell size 0.0416, approximately equal to 4 x 8 km^2.

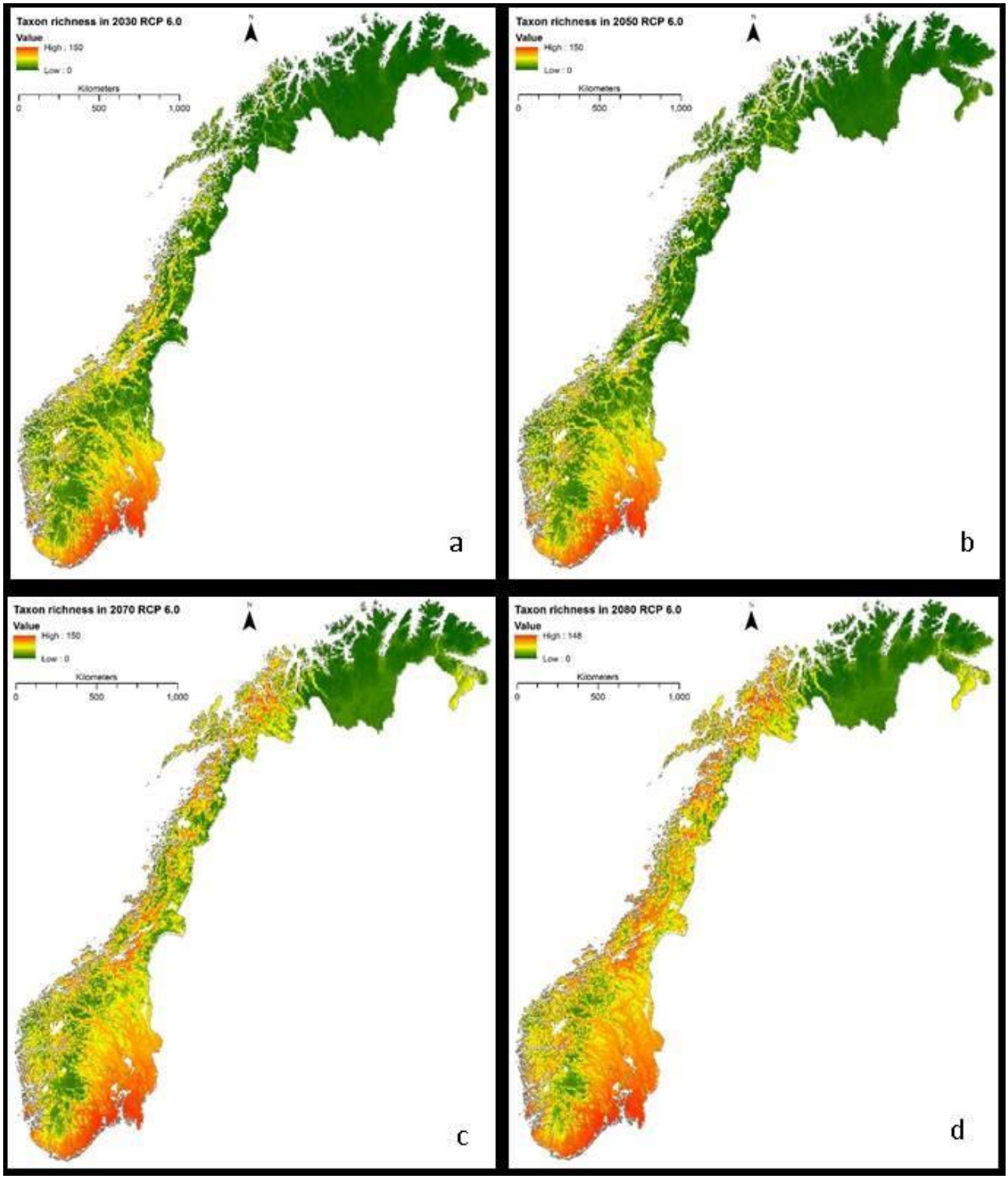


Figure 4.2: The average predicted taxon richness of 187 priority CWR in Norway under RCP 6.0 for the years a) 2030, b) 2050, c) 2070, d) 2080. Raster grid cell size 0.0416, approximately equal to 4 x 8 km^2.

The pattern of taxon turnover also reflected this distribution change (Figure 4.3, 4.4). From 2030 to 2080, under unlimited migration and RCP 2.6, the percentage turnover rate of taxa per pixel increased, from 29% to 68% (Table S4.1). Under RCP 6.0 turnover of taxa per pixel increased from 50% to 72% (Table S4.1). The area of Norway with a turnover of 100% tended to increase from 2030 to 2080, with the south east and southern coast maintaining a low turnover rate and a large proportion of the mainland showing an increased turnover rate (Figure 4.3, 4.4).

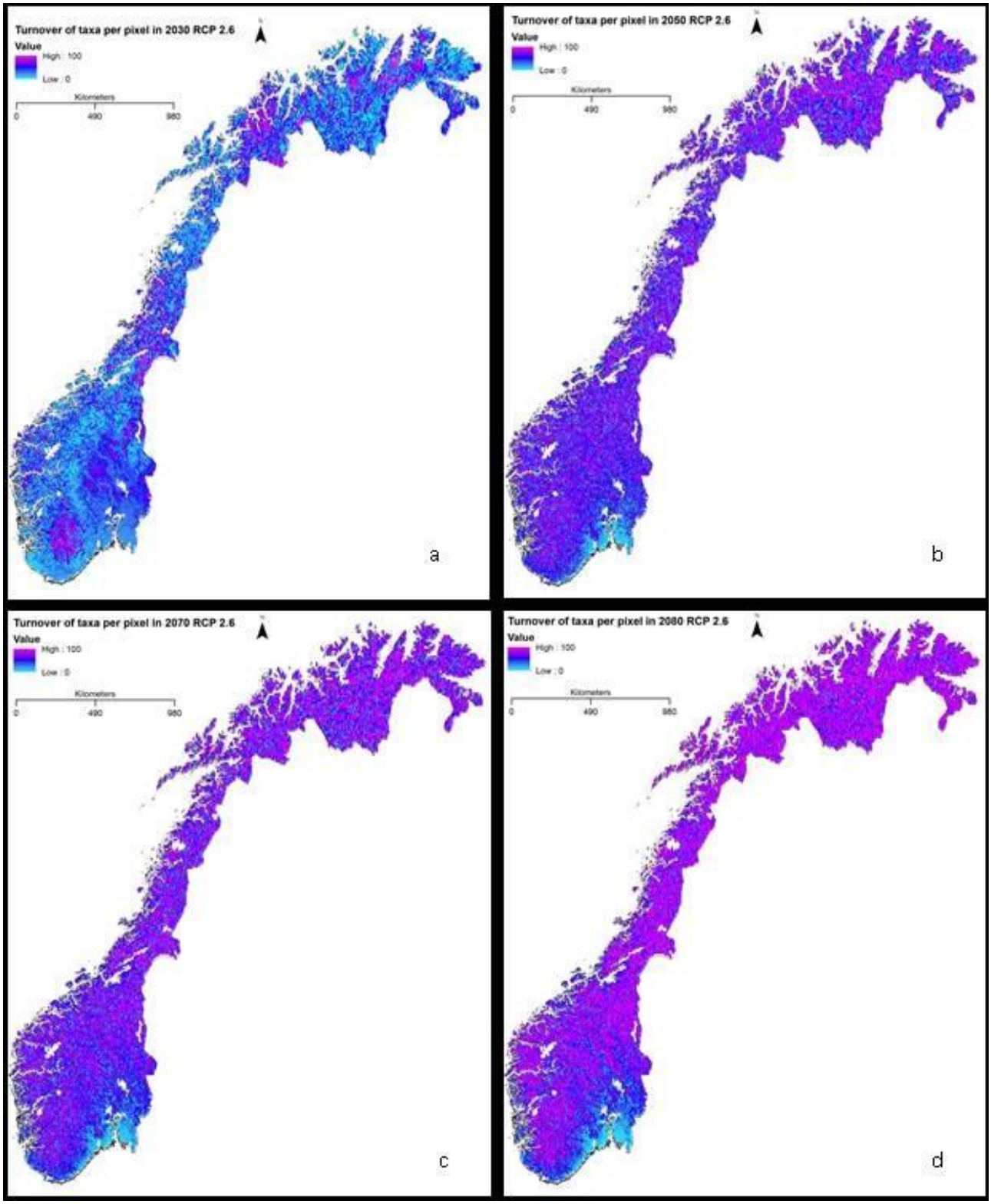


Figure 4.3: The average turnover of taxa per grid cell under RCP 2.6 for year a) 2030, b) 2050, c) 2070, d) 2080. Value is in percent, with 100 representing a complete turnover of all taxa within that cell. Zero means the taxa within the cell stay the same. Raster grid cell size 0.0416, approximately equal to 4 x 8 km^2.

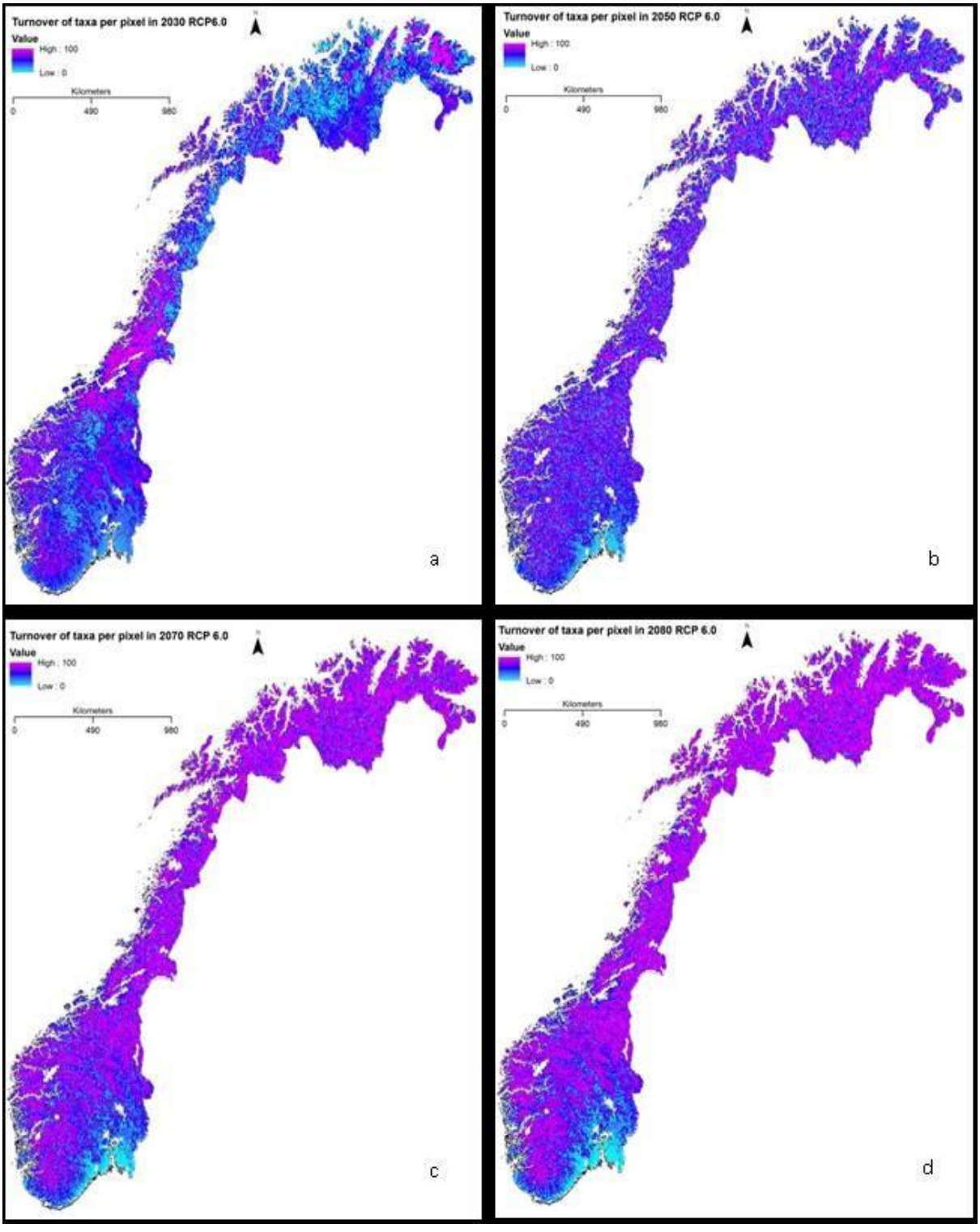


Figure 4.4: The average turnover of taxa per grid cell under RCP 6.0 for year a) 2030, b) 2050, c) 2070, d) 2080. Value is in percent with 100 representing a complete turnover of all taxa within that cell. Zero means the taxa within the cell stay the same. Raster grid cell size, approximately equal to 4 x 8 km^2.

Climate change affected individual taxa under both unlimited-migration and no-migration scenarios from the year 2030 to 2080. Under no migration, the number of taxa that lost area decreased under both RCP scenarios from the year 2030 to 2080 (Figure 4.5). For unlimited migration, the number of taxa that lost area under RCP 2.6 reduced from 25% in 2030 to 12% in 2080, whereas under RCP 6.0 the number of taxa that lost area increased from 11% to 13% (Figure 4.5). Under unlimited migration, there was an increase in the number of taxa gaining area under RCP 2.6 but a decrease in the number of taxa gaining area in RCP 6.0 from 2030 to 2080 (Figure 4.6). Under no migration, none of the taxa gained area as they cannot expand their distribution, only lose area or maintain their current distribution.

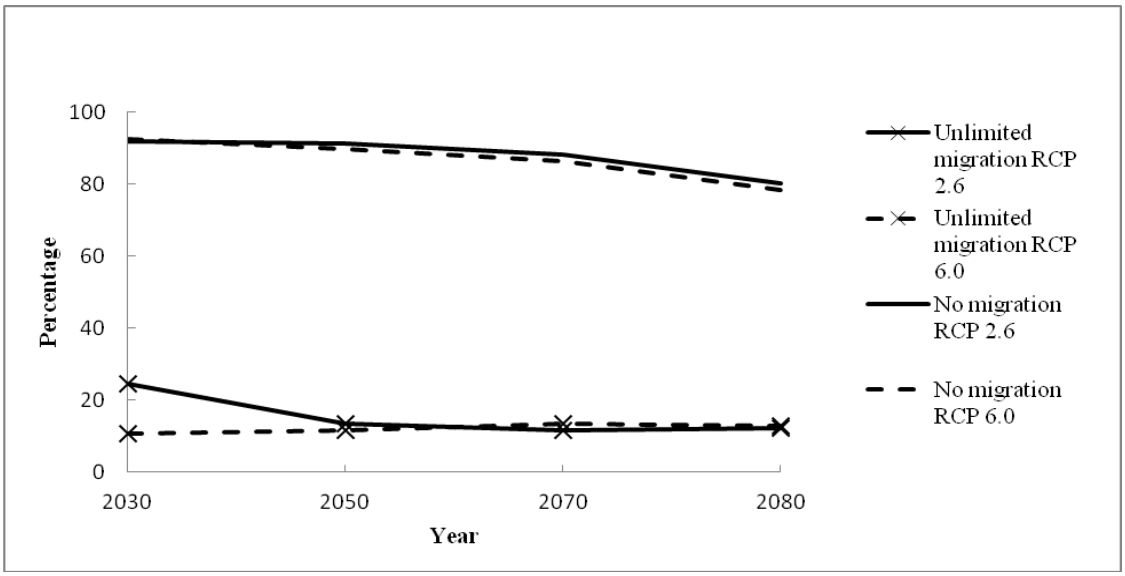


Figure 4.5: Percentage of taxa that lose area. Modelled under unlimited migration and no-migration scenarios and RCP 2.6 and RCP 6.0.

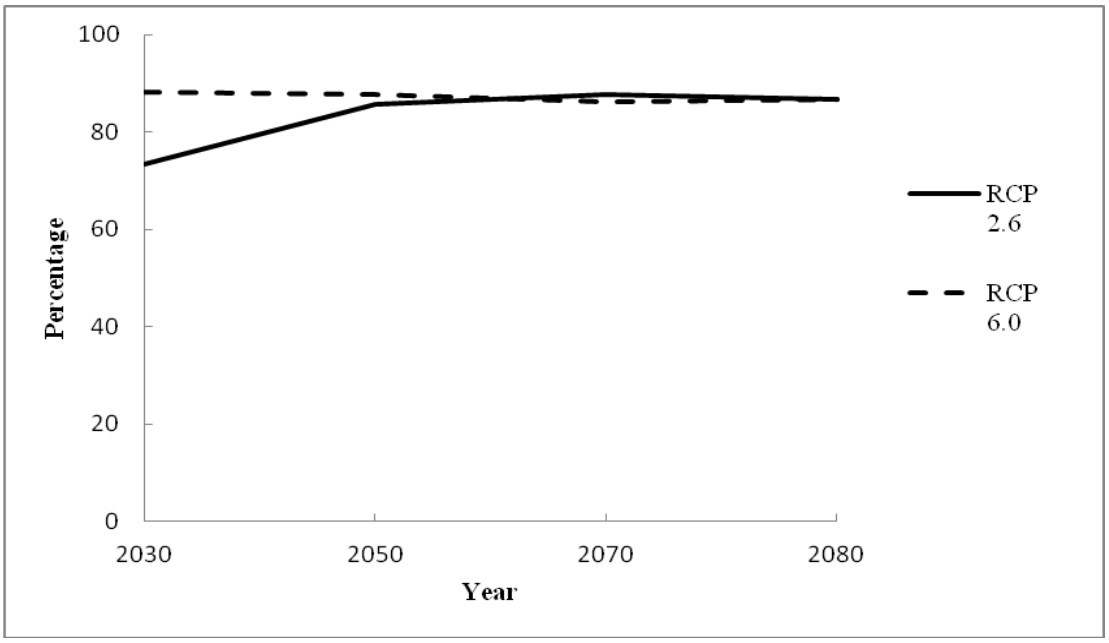


Figure 4.6: Percentage of taxa that gain area under unlimited migration and RCP 2.6 and RCP 6.0. Taxa under no-migration gain no area as they are unable to expand from their current distribution.

The decrease in geographic range size for taxa was related to the level of threat using the IUCN Red List category A3(c) (IUCN, 2001) (threat status of taxa under current climate is from Kålås *et al*. (2006)). As expected, under no migration there was a higher number of taxa assessed as threatened with the most being 12% of taxa under RCP 6.0 (Figure 4.7). For unlimited migration, the highest number of taxa threatened was 11% in the year 2080 for RCP 6.0 (Figure 4.7). There tended to be higher numbers of taxa threatened under RCP 6.0 than RCP 2.6.

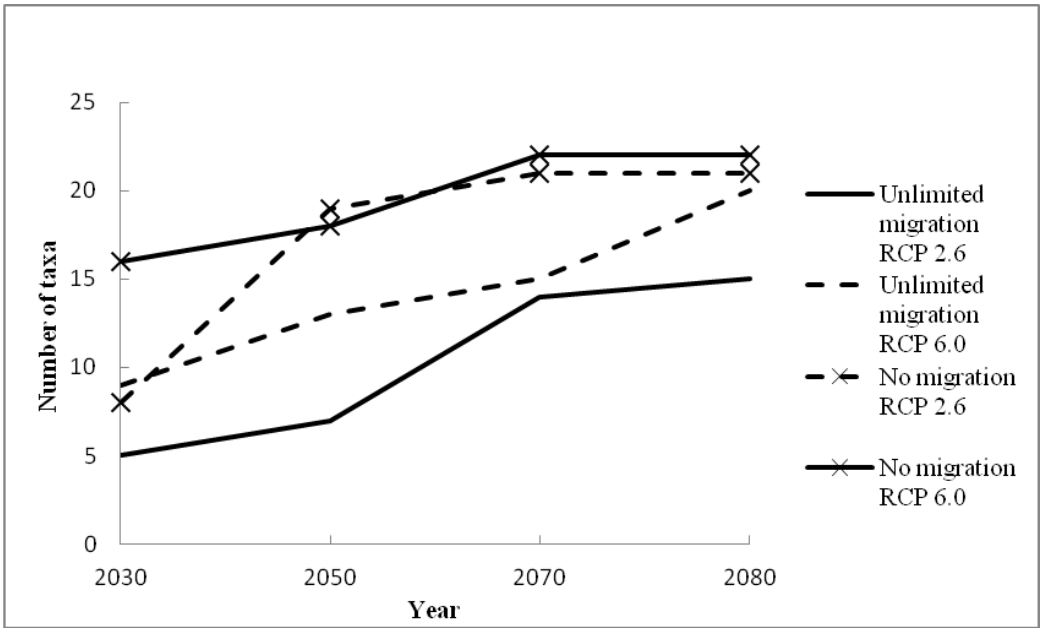


Figure 4.7: The predicted number of threatened taxa as determined by the IUCN category A3(c). For the full list of threatened taxa see Table S4.2.

Fourteen taxa have been assessed as threatened under current climatic conditions, with data for further predictive modelling lacking for four taxa and fifteen taxa assessed as not threatened in the future, according to this study (Table S4.2). Under both migration scenarios the severity of threat to the taxa tended to increase along with the number of threatened taxa. The number of Critically Endangered (CR) and Extinct (EX) taxa tended to increase (Figure 4.8) from 2030 to 2080 under both RCP scenarios. Furthermore, *Alopecurus pratensis* L. subsp *alpestris* (Wahlenb.) Selander is predicted to go extinct in 2070 if there is no migration and in 2080 if there is unlimited migration (Table S4.2). Thirty-one taxa were assessed as threatened under the predicted climate change scenarios (Table S4.2).

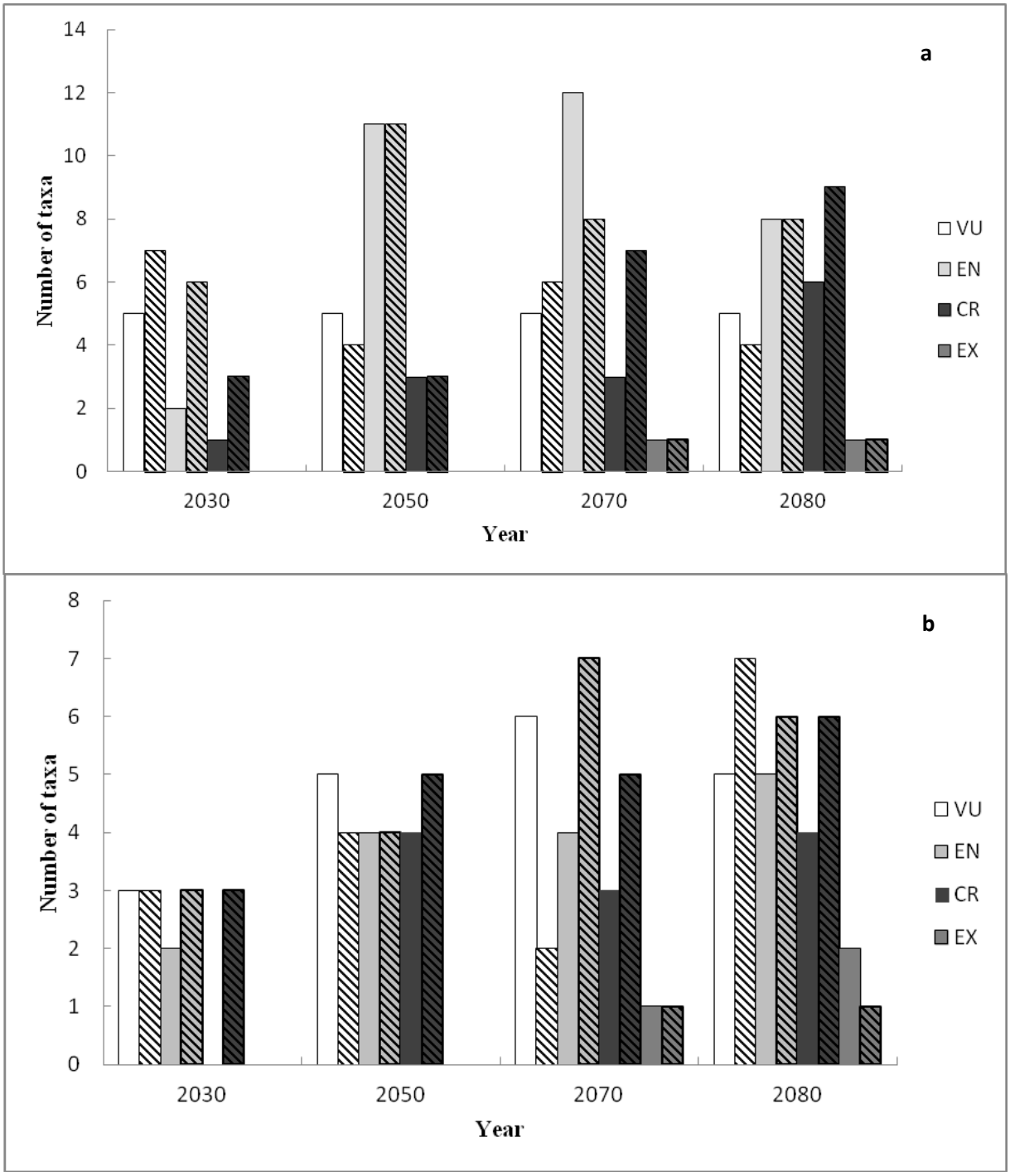


Figure 4.8: The number of taxa threatened per year under a) no-migration and b) unlimited migration for RCP 2.6 and RCP 6.0 (diagonal line bars), as determined by the area lost based upon IUCN criterion A3(c).

For evaluation of the models' performance, 93% of the SDM for each species had an AUC > 0.7 (average), 7% had an AUC > 0.5. All models had STAUC < 0.15 apart from one taxon which had STAUC 0.1506 (*Fragaria ananassa* Duchesne) (Table S3.4).

4.5 Discussion

The climate change analysis of the priority CWR for Norway shows a trend of increasing CWR richness under both RCP scenarios, from the present to the year 2080. Taxon richness appears to spread from present areas of richness in the south east, to the inland regions of Norway and northwards. This suggests that current limiting factors to plant growth in the north, such as all-year-round low temperatures (Olesen & Bindi, 2002) and others are expected to become less pronounced as the climate changes, leading to an increase in taxon richness. This is supported by Sætersdal *et al*. (1998) and Parmesan and Yohe (2003), who find that in the northern hemisphere temperate regions during cold periods, the geographic ranges of most species are restricted to one or a few refugia in the south and with subsequent warming each species expands its range with increasing species richness, mainly northward. This may also be associated with a gradual lengthening of the growing season in the north due to increasing temperatures, as well as increasing shrub abundance in the arctic (Tømmervik *et al*., 2004). This could be positive for farmers, who may be able to extend and increase the production of food and forage within Norway, thereby improving food security at national level. Furthermore, the CWR populations that successfully migrate or adapt with climate change may be key in aiding farmers to adapt their crops to future climatic changes. Leading-edge populations (in this case, those populations spreading northwards) are expected to be stable or increasing (Nekola, 1999) and show positive demographic rates (Foden *et al*., 2009). This is compared to trailing-edge populations, which often have reduced genetic variation (Lesica & Allendorf, 1995). Furthermore, the diversity found at the leading and trailing edges may be

unique (Hampe & Petit, 2005) and could help underpin efforts of plant breeders to develop varieties adapted to new conditions (Jarvis *et al*., 2008).

When this pattern of shifting distributions is compared with the turnover of taxa per grid cell, locations in the south tend to maintain low levels of taxon turnover. This suggests that species composition in the south may remain stable in comparison with much of the mainland, where the turnover rate increases. However, it is important to note the study does not model species that may move into Norway from the south and east, but it should be expected that such taxa will, provided they can reach the region (Henningsson & Alerstam, 2005; Huntley *et al*., 2008; Hof *et al*., 2012), and monitoring of such changes should be undertaken. A high turnover rate isseen in Thuiller *et al*. (2005), who show that the Boreal region (which covers southeast Norway) and southern Fennoscandia (which incorporates southern Norway) could in principal gain many species from further south. There appears to be a larger change in the turnover of taxa per grid cell under the lower temperature increase of RCP 2.6 (29% to 68%), than RCP 6.0 (50% to 72%), perhaps suggesting that the 1.5˚C rise in temperature is more favourable to the priority CWR taxa than a 3.0˚C increase.

This high turnover rate and change in taxon richness is important for how CWR populations may be conserved in Norway. With such a dynamic change in taxa distribution predicted and the expectation that the effects of climate change will be felt sooner (Stafford Smith *et al*., 2011) it will be necessary to create flexible conservation strategies for CWR. This will mean using both *in situ* and *ex situ* conservation actions for specific taxa as well as at larger multi-taxa conservation scales. In terms of *in situ* conservation, the current PA network in Norway encompasses large areas with unique landscape conservation value, such as mountain plateaus which are often dominated by climax communities that do not tend to support CWR diversity (Jarvis *et al*., 2015). These reserves may require incremental changes in management

strategies, such as increased levels of species monitoring to account for new CWR taxa that are predicted to spread into the reserves. However, multiple authors suggest that new reserves may be needed to purposely account for climate change impacts (Sætersdal *et al*., 1998,; Araújo *et al*., 2004). This would require transformative management actions such as clustering reserves in areas of temporal overlap (Araújo *et al*., 2004), creating reserves in hotspots of future diversity (Heller & Zavaleta, 2009), conserving the 'core' of populations (Araújo *et al*., 2004) and improving connectivity between reserves by creating corridors so species can migrate (Halpin, 1997). Corridors and areas outside of formal PAs are important for CWRs (Jarvis *et al*., 2015) as many are common taxa with wide distributions (for example *Trifolium* sp., *Phleum* sp., *Rubus* sp.). For widespread taxa with well-connected populations a reduction of genetic diversity within populations is likely to contribute to population extinctions but is less likely to threaten the existence of the species (Jump and Penuelas, 2005). Corridors could be considered a short-term, incremental change, as connecting reserves could be flexible in their design and management for the protection for CWR outside of formal PAs, an approach suggested as critical in the face of climate change (Franklin *et al*., 1992; Lovejoy, 2005; Thomas *et al*., 2012). Close cooperation with landowners and relevant stakeholders would be essential if these situations were to arise. All CWR populations will benefit from an *in situ* conservation approach as it allows continued evolution of traits that may be required in the future. For all priority taxa but particularly for those predicted to lose many populations, *ex situ* conservation will be a complementary tool to aid conservation actions.

Ex situ conservation will be a vital tool for the conservation of CWRs in Norway. If CWR populations are monitored and changes in genetic diversity and/or population size are identified (i.e. a reduction below 5000 individuals per population as the minimum number recommended by Iriondo *et al*. (2012)), then collecting of seeds from these populations could be undertaken.

Taxa could be prioritized for *ex situ* conservation based upon their predicted level of threat using the IUCN criteria. For example, *Alopecurus pratensis* subsp. *alpestris* (Table S4.2), which is predicted to become extinct, could benefit from immediate collecting of seeds with regeneration and translocation of populations also a possibility, meaning a more transformational management plan will be required. The taxa that are predicted to lose area but not become threatened will require monitoring and collecting of seeds incrementally, perhaps on a less frequent timescale, when negative impacts are identified. It will also be necessary to ensure that a range of genetic diversity is collected, perhaps targeting ecogeographically diverse populations by using an ELC map, as done by Phillips *et al*. (2016). As well as protecting threatened populations, *ex situ* conservation will ensure that the genetic material is available for use by plant pre-breeders to adapt our crops to climate change.

The IUCN methodology used in this study only takes into account one red list criterion (A3(c)), which assesses the population size reduction, for which we have used the percentage of area lost as a proxy, following Thuiller *et al*. (2012). The use of the change in distribution extent as a proxy for population changes is an allowable assumption according to the IUCN. However, considerations such as whether the size of habitat patches can support viable populations must also be taken into account (Foden & Young, 2016). An alternative to this method may be to use the framework suggested by Foden *et al*. (2013;*et al*2016) for assessing three different dimensions of climate change vulnerability of populations. This will aid in a more taxon-specific management strategy allowing effective conservation of threatened populations.

Adaptation of populations as well as migration will be crucial for survival of CWRs *in situ* in the long-term. There is little evidence that adaptation alone will be able to keep pace with climatic changes (Jump & Penuelas, 2005; Thuiller *et al*., 2008). Furthermore, climate change is also expected to exceed the potential of populations to track climate by migration (Midgley

et al., 2003). Uncertainty surrounding the capacity of populations to adapt or migrate in response to climate change strengthens the need to apply both *in situ* and *ex situ,* as well as incremental and transformational conservation strategies to CWR in Norway. As well as uncertainties in how the taxa will respond there are uncertainties associated with the methods used to create the predictions. The models used in this study do not account for the soil or other habitat conditions that may remain unfavourable for the CWR taxa. It is also likely that the presence points used do not reflect the entire geographic range of the CWRs and may be subject to sampling bias (Araujo & Guisan, 2006; Loiselle *et al*., 2008). Maldonado *et al*. (2015) found that often diversity patterns overestimate species richness when data from large public databases is utilised. We tried to limit these effects by basing future predictions on a predicted distribution, created in MaxEnt, of taxon richness under the current climate, not only on the observed distribution data. Furthermore, over 96% of the GBIF records used had coordinates accurate to three or more decimal places. However in further studies, the use of ignorance maps (Rocchini *et al*., 2011; Ruete, 2015) as well as the use of tools such as GeoQual in the CAFITOGEN package (Parra-Quijano *et al*., 2016) may allow the filtering out of unreliable occurrence data.

4.6 Conclusion

For management strategies to be effective for CWR within Norway the continued monitoring of populations will be required (Stafford Smith *et al*., 2011). A monitoring programme for CWR within Norway should include thresholds that if met will mean taking the next course of action. These thresholds could include monitoring of population sizes with a focus upon those trailing-edge populations, for example if they fall below 5000 individuals (Iriondo *et al*., 2012) then conservation action will be required; use of the IUCN categories as thresholds to prioritise the collecting of threatened taxa (i.e. those that are predicted to become extinct or critically

endangered would be the first priorities to collect) and identifying leading-edge populations to allow the planning of conservation areas that allow populations to migrate to the nearest suitable location or PA. The strength of PAs is that they are already working now to protect biodiversity and to passively conserve CWR that happen to occur within them. Therefore the use of PAs in terms of climate change equates to a 'no-regrets' conservation decision (Stafford Smith *et al*., 2011), especially if new PAs are created. Informal PAs for CWR such as those acting as corridors (increasing connectivity from the south east to north west in Norway) in the landscape should be flexible in their location, design and management to allow for the uncertainties associated with the climate change predictions and species responses. *Ex situ* conservation is likely to be essential as a back-up to *in situ* resources by making the genetic diversity available to plant pre-breeders. *Ex situ* conservation can be used to incrementally collect populations that are showing negative responses to climate change by meeting the thresholds (including population size and IUCN threat level) set out in the monitoring programme.

Pittock and Jones (2000) state that climate change will not be a new stable equilibrium but an ongoing transient process that requires an ongoing adaptation process. This is clear from the results and recommendations presented above, which can enhance the previous work on a national CWR conservation strategy for Norway (Phillips *et al*., 2016). Although this study was conducted at a national level the methodologies and recommendations have applicability at regional and global levels. Furthermore, it is at the national level that such recommendations will need to be implemented. Unless we increase our knowledge on the impact of climate change upon CWR we will not be able to effectively conserve and utilise taxa to improve food security in the face of climate change.

CHAPTER 5.

Genetic diversity studies of priority crop wild relatives in Norway using AFLPs: Implications for conservation

The work presented in this chapter has been submitted to Conservation Genetics (April 2017)

Jade Phillips, Lindsey Leech, Joana Magos Brehm, Åsmund Asdal, Morten Rasmussen, Nigel Maxted

Author contributions:

Conceived and designed the study: J.P., J.M.B., A.A., N.M.

Data collation and preparation: J.P., A.A.

Performed analysis: J.P.

Interpreted results: J.P., L.L., J.M.B.

Wrote the paper: J.P.

Critically reviewed the paper: J.P., L.L., J.M.B., A.A., M.R., N.M.

5.1 Abstract

Understanding the genetic diversity of species will allow us to better manage and sustain our natural resources. CWR are an essential source of genetic diversity due to their close relationship to cultivated crops and the relative ease of trait transfer. These wild species are vital for improving the resilience of our cultivated crops and mitigating the impact of future challenges. Few studies have used genetic diversity assessments to inform conservation of CWR. Here we conduct genetic diversity studies using AFLPs for three priority CWR in Norway. Leaf material for *C. carvi*, *T. repens* and *T. pratense* was sampled from populations across 26 sites throughout Norway. RawGeno software was used to score the presence and absence of peaks. Descriptive statistics were calculated in AFLP-SURV and GenAlEx to determine genetic diversity patterns. Genetic diversity is partitioned mostly within rather than amongst populations for all three taxa. PCoA showed no obvious geographic partitioning of genetic diversity for either the individuals or populations. Southern populations contained a higher number of private alleles and had higher levels of heterozygosity than northern populations. The level of gene diversity differed between all ecogeographic zones. Based on the results from the AFLP analysis, *in situ* and *ex situ* conservation of the three taxa should initially focus on populations in the south of Norway due to the higher diversity and more distinct populations found there. Management plans have already been put in place for populations in the Færder NP, making it a potential candidate for the first CWR genetic reserve in Norway.

5.2 Introduction

The loss of species is well documented but the loss of genetic diversity is much less understood (Nabham, 2009). Understanding genetic diversity, how it is distributed and how we can utilise

it, is essential if we are to sustain resilient and robust food- and eco-systems. Such systems are vital for the survival of the human population. Food security, for example, depends on the wise use and conservation of genetic resources (Esquinas-Alcazar, 2005), yet we are only capitalising on a fraction of the genetic diversity that is available within each species (McCouch *et al*., 2013). Just four crops, rice, wheat, maize and potato provide us with more than 60% of our food (FAO, 2015). With an increasing population and increasing political and environmental instability we will need to make our food systems more robust. Harnessing the full extent of genetic diversity available will help us to achieve this and ensure our future food security.

Simply counting the number of species in an ecosystem does not account for how variable species may be, therefore measurements of genetic diversity are more valuable (Millennium Ecosystem Assessment, 2005). The characterisation of genetic variation and its distribution within and amongst populations is important for plant breeding and conservation focused on the management of genetic resources (Marshall & Brown, 1995; Nybom & Bartish, 2000; Herrmann *et al*., 2005; Bonin *et al*., 2007). CWR are one such genetic resource. They are the wild progenitors of our cultivated crops and often contain higher levels of genetic diversity as they have not been through the bottleneck of domestication (Tanksley and McCouch, 1997; Maxted *et al*., 2006). To make our crops more robust to environmental and human pressures we will need to harness this genetic diversity.

Hajjar and Hodgkin (2007), Maxted and Kell (2009) and Dempewolf *et al*. (2017) give a broad picture of how genetic material has been utilised for improving crops in response to both past and future challenges such as biotic and abiotic resistance, and climatic changes. For example, disease resistant genes from wild wheat relatives are already being transferred into wheat cultivars, of which 90% are susceptible to the Ug 99 fungus (Singh *et al*., 2011). This genetic

diversity is valuable for crop improvement with one estimate placing a value of potentially $120 billion, on the wild genepools of 29 globally important crops (PwC, 2013).

There are a few studies that have utilised genetic diversity assessments to inform conservation planning for CWR. Watson-Jones *et al*. (2006) used AFLPs for genetic diversity studies of *Brassica* species to establish a baseline level of diversity which can be monitored in the future. Genetic diversity studies have also identified *in situ* conservation sites for *Dianthus* species in Portugal (Magos Brehm *et al*., 2012), wild *Brassica* in Italy (Branca *et al*., 2012), *Beta* species in Madeira (Pinheiro de Carvalho *et al*., 2012) and for establishing conservation measures for multiple CWR in the UK (Fielder, 2015). Furthermore, there are standards in place for the designation of genetic reserves which include regular genetic monitoring of species and the identification of reserves that capture as much genetic diversity of target species as possible (Iriondo *et al*., 2012).

The countries that are signatories to international agreements on protection of diversity, such as the CBD (UN, 1992), the ITPGRFA (FAO, 2001; www.planttreaty.org) and the GSPC (www.biodiv.org/programmes/cross-cutting/plant/), also have a responsibility to conserve genetic diversity. Conservation of these resources *in situ* must begin at the national level to be effective, because each population is located in a particular country and is subject to national sovereignty. Norway has already begun to meet these targets with the creation of a national strategy for the *in situ* and *ex situ* conservation of priority CWR (Phillips *et al*., 2016). This strategy utilised ELC maps (Parra-Quijano *et al*., 2012b) as a proxy for identifying genetic diversity within Norway. It is assumed that, unless there is evidence to the contrary, the conservation of populations from the maximum diversity of locations will result in the most genetically diverse sample, as genetic diversity is partitioned in relation to ecogeographic diversity (Heywood, 2008). However, genetic diversity studies have not yet been conducted

for priority CWR in Norway to determine if this is the most appropriate approach. Plant populations in Norway may have lower genetic diversity than other countries due to the long migration distances for plants, but they may also be more distinct due to heavy glaciation in the past which may have formed glacial refugia for certain arctic species (Eidesen *et al*., 2013).

Here we investigate the genetic diversity of three Norwegian priority CWR using AFLPs. We aim to identify if genetic diversity is different within ecogeographical environments and how this will affect conservation strategies. We study the levels of genetic diversity from populations throughout Norway to identify locations suitable for *in situ* conservation and populations that may require *ex situ* conservation. We aim to add to the previously published recommendations for *in situ* and *ex situ* conservation of CWR in Norway (Phillips *et al*., 2016) by establishing the baseline genetic diversity data required for an effective establishment of *in situ* genetic reserves and continued genetic monitoring.

5.3 Methods

5.3.1 Sample collection

Genetic diversity studies were carried out for three priority CWR in Norway (see Table S2.2 for the full list of priority CWR) *C. carvi* (caraway), *T. pratense* (red clover) and *T. repens* (white clover). The taxa were chosen based upon consultation with Norwegian stakeholders at the Norwegian Forest and Landscape Institute and the Natural History Museum, Oslo. *C. carvi* (2x=20) is a biennial, outcrossing species and is an important herb and medicinal plant. *T. pratense* (2n=2x=14) is a perennial species and an obligate out-crosser with self-incompatibility. *T. repens* (2n=4x=32) is an allotetraploid, perennial species which is outbreeding and self-incompatible but also clonally propagated. Both *Trifolium* taxa are important forage species in the temperate world.

From June to August 2014 and in May and July 2015, sampling was carried out throughout Norway for the three species. A total of twenty-six sites were visited ranging from the south of Norway to the Arctic north (Table 5.1, Figure 5.1, Table S2.4). These sites were initially chosen based upon the results of the grid cell complementarity analysis, based on known occurrences, used to identify sites for *in situ* conservation (Phillips *et al*., 2016, Figure 5.1). The nearest PAs to these grid cell locations were then identified and, where possible, collection of the leaf material took place within these. All individual sites were deemed appropriate for sampling by local experts. The PA complementary network developed by Phillips *et al*. (2016) was also considered for potential collecting locations (Figure 5.1). PAs were targeted for collection of material as management plans resulting from the genetic diversity studies would be easier to implement within rather than outside a PA in Norway. The ecogeographic characteristics of the locations were also identified from the ELC map for Norway (see Phillips *et al*., 2016). This was to ensure collection was from a range of different ecogeographical areas (Table 5.1).

Table 5.1: The location of sites visited and which species were collected at each site. The nearest PA complementary grid cell and ELC zone are also listed. See Figure 5.1 for a map of the collecting sites, protected areas and complementary grid cells. 'NA' = not applicable, no samples collected from these sites. LVO = landskapsvernområde (protected landscape area).

Site	Species			Nearest protected area name	Nearest town	ELC zone	Nearest complementary grid cell
1	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Maridalen	Oslo	17	1
2	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Lindøya, Hovedøya	Oslo	17	1
3	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Ytre Hvaler NP-Viker and Pikesten site	Fredrikstad	16	7a
4	NA	*Trifolium pratense*	*Trifolium repens*	Færder NP	Tønsberg	16	1, 7a
5	NA	*Trifolium pratense*	*Trifolium repens*	Rognsflauane Geo Park	Langesund	17	9c, 4
6	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Stråholmen	Valle on mainland	18	4

7	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Jomfruland	Kragerø on mainland	18	4
8	NA	*Trifolium pratense*	*Trifolium repens*	Nedre Timenes	Kristiansand	17	2, 6a
9	NA	*Trifolium pratense*	*Trifolium repens*	Jærstrendene-Rogaland	Ogna and Bryne	18	n/a
10	NA	*Trifolium pratense*	*Trifolium repens*	Yddal	Femanger	26	6
11	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Folgefonna-Buerdalen LVO, Ænes, Buer, Hatteberg and Myrdalsvatnet	Odda	21	6
12	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Lågendeltaet bird reserve	Lillehammer	8	8
13	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Kongsvoll LVO, Dovrefjell NP	Kongsvoll	21	3, 9d
14	NA	*Trifolium pratense*	*Trifolium repens*	Bymarka	Trondheim	26	7
15	*Carum carvi*	NA	NA	Færder NP-Island of Hvaløy	Tønsberg	16	1
16	*Carum carvi*	NA	NA	Færder NP-Østre Bolærne	Tønsberg	16	1
17	*Carum carvi*	NA	NA	Færder NP-Skjellerøy island	Tønsberg	16	1
18	*Carum carvi*	NA	NA	Færder NP-Kjøleholmen	Tønsberg	16	1
19	*Carum carvi*	NA	NA	Færder NP-Southern Ekornholmen	Tønsberg	16	1
20	*Carum carvi*	NA	NA	Færder NP-South Årøy	Tønsberg	16	1
21	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Prestvannet	Tromso	15	9f
22	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Romsdal	Alta	14	n/a
23	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Lyngenalps	Lyngen	15	9f
24	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Dividalen LVO	Dividalen	15	n/a
25	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Kjerkvatnet	Skar	24	8a
26	*Carum carvi*	*Trifolium pratense*	*Trifolium repens*	Trondenes natur- og kultursti	Harstad	23	8a

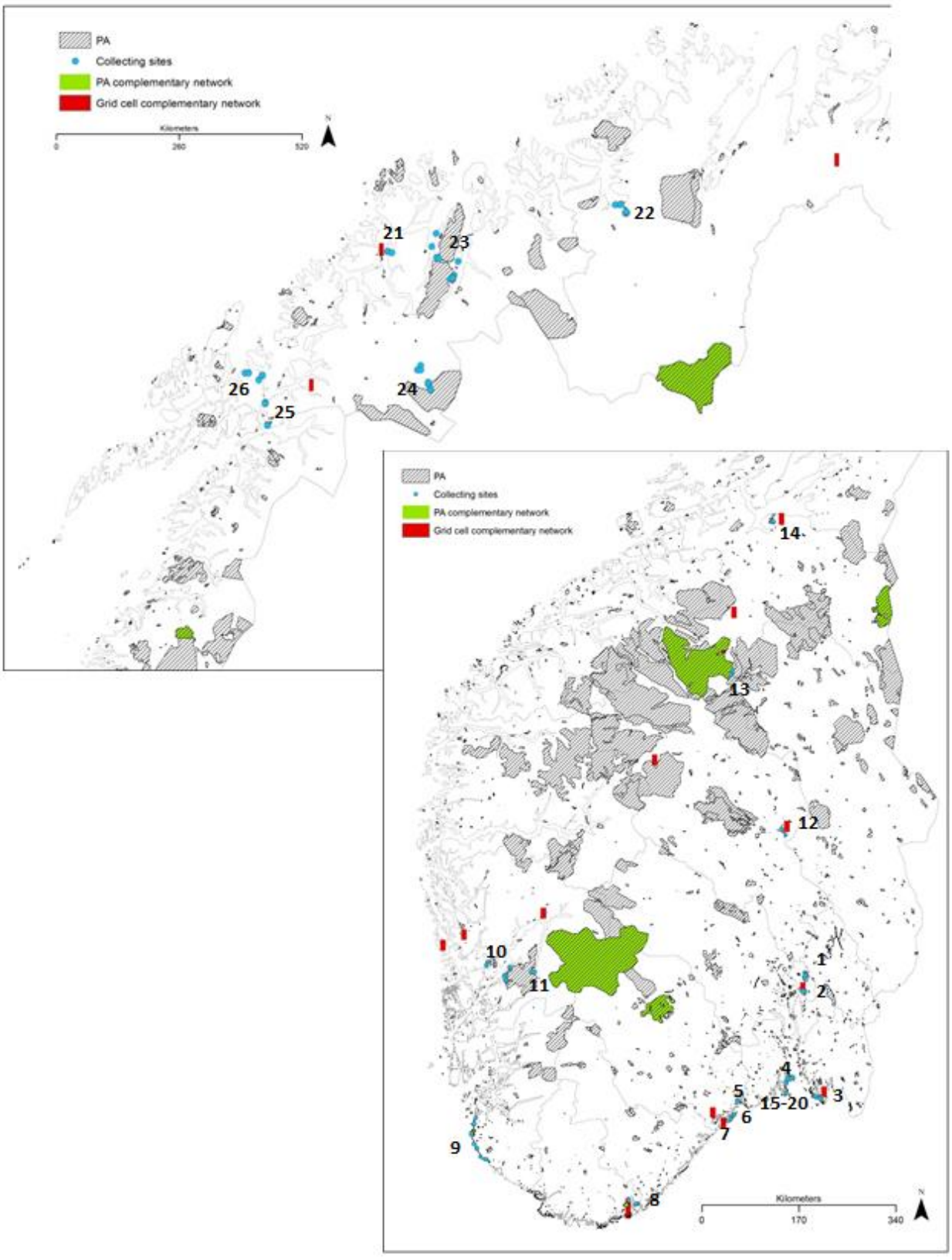


Figure 5.1: Location of the 26 collecting sites (see Table 5.1 for further site information) and the PA network in Norway. The green highlighted PAs are those in the PA complementary network and the red cells are those from the grid cell complementary network. See Phillips *et al*. (2016) for detailed information about the PA and grid cell complementary network.

From each site one leaf sample was collected (approximately 100 mg fresh weight minimum) from 20 different individuals for each species. As the sites varied in size, individuals were collected from different populations within that site to gather a representative range of genetic diversity within the area. The sites were deemed far enough away from each other that gene flow was expected to be very unlikely between them. Leaf samples from each individual were stored in separate airtight plastic bags containing drying indicator silica gel (Chase and Hills, 1991). A total of 1201 samples were used for molecular work (*C. carvi* = 389, *T. pratense* = 392, *T. repens* = 420).

5.3.2 Molecular marker genotyping

Genomic DNA was extracted from the dried leaves using the DNeasy 96 Plant Kit (Qiagen, 2012). The AFLP method was completed in accordance with IBERS standard protocol (Skøt *et al*., 2005) based on Vos *et al*. (1995) with the following adjustments: 3µl of pre-amplification product was used for the selective amplifications; the PCR protocol was used with 25 cycles and 1µl of selective amplification product was mixed with 10µl formamide/size standard. Pre-amplification identified appropriate primers to use that produced markers distributed throughout the genome of the individual species. Two primers were subsequently used in the AFLP analysis: ACG-CTG and ACA-CTA (Table 2.2). AFLP reactions were run on the ABI 3730 capillary sequencer.

AFLP electropherograms were visualised and peaks were scored using the freely available Peak Scanner (Applied Biosystems, 2006) software which detected the size and fluorescence of the AFLP peaks and with RawGeno software (Arrigo *et al*., 2009), which is implemented as an R CRAN package. In Peak Scanner the 'Analysis method' proposed by Arrigo *et al*. (2009) was used as it has provided robust results in the past (Holland *et al*., 2008; Herrmann *et al*., 2010). The Size Standard used was GS500(-250)LIZ. The resulting Peak Scanner table was

imported into RawGeno where samples were initially not filtered based on quality. Scoring of the peaks was based on the blue dye only and samples were analysed separately for each species and each primer. Prior to the final analysis samples were initially analysed using a maximum bin width of both 2.0 and 1.5 after which a maximum bin width of 1.5 and a minimum bin width of 1.0 was selected (using a larger bin width increased the likelihood of technical homoplasy (Arrigo *et al*., 2009)). The scoring range of the base pairs was between 50 to 500 with low fluorescence bins equal to 100 relative fluorescence units and low frequency bins set at 1.

Approximately 10% of samples per species were manually checked for quality of binning to ensure peaks were being correctly scored. The automated scoring process using RawGeno has been shown to provide results that are as accurate as those scored manually or within commercial software such as Genemapper (Arrigo *et al*., 2009). Furthermore, the automated procedure can increase reproducibility of the dataset and limit genotyping errors to technical factors (Arrigo *et al*., 2009).

5.3.3 Statistical analysis

The error rate was calculated based upon the methods of Bonin *et al*. (2004, 2007). For each species, a randomly selected 10% of the collected samples were subject to replicate AFLP analysis. Error rates were then calculated separately for each species based upon the replicates, yielding a per locus error rate and a dataset error rate (Bonin *et al*., 2004; Pompanon *et al*., 2005). Highly error prone loci (error rate > 0.14) were removed from the species datasets to reduce the dataset error rate.

Allele frequencies were calculated in AFLP-SURV 1.0 (Vekemans *et al*., 2002) using the Bayesian method with non-uniform prior distribution of allele frequencies (Zhivotovsky,

1999). Mating was assumed to be random due to the out-breeding nature of the three species and therefore not to deviate from Hardy-Weinberg equilibrium. In AFLP-SURV the following descriptive statistics were calculated: proportion of polymorphic loci at the 5% confidence level expressed as a percentage (PLP), expected heterozygosity (H_E), mean expected heterozygosity within populations (H_W) and Wright's fixation index (F_{ST}) (Lynch and Milligan, 1994). Principal coordinate analysis (PCoA) was performed in GenAlEx 6.5 (Peakall and Smouse, 2006, Peakall and Smouse, 2012) to determine patterns of variation between individuals based on pairwise genetic data and between populations based upon Nei's genetic distance. An AMOVA analysis using 999 permutations was also undertaken in GenAlEx to determine the distribution of genetic variation within and among populations. A Mantel test was carried out in GenAlEx to assess if genetic diversity was correlated with geographic distance. Pairwise F_{ST} values (transformed to $F_{ST}/(1-F_{ST})$) were compared to log-transformed geographic distances with 999 permutations. SPSS 24 (IBM Corp, 2013) was used to perform correlation analyses between the environmental variables in the ELC zones (Table S2.3) and the level of heterozygosity for each population per species. Stepwise Regression analysis was also undertaken in SPSS for each species to determine if the environmental variables had any significant predictive relationship with the level of heterozygosity within populations. Finally, the number of private (unique) alleles within each population was calculated in SPSS, to help determine how distinct populations were from each other.

5.4 Results

Table 5.2 summarises the results of the AFLP analysis. The dataset error rate was lowest for *C. carvi*, 14.7%, and highest for *T. pratense*, 29.9%, with *T. repens* having a dataset error rate of 21.0%. The number of loci analysed per species ranged from 285 for *C. carvi* to 385 for *T. repens*. *T. repens* has the highest level of within population heterozygosity (H_W = 0.06 ±

0.005) and *T. pratense* has the lowest level (H_W = 0.05 ± 0.005). Wright's F statistic (F_{ST}) shows that population differentiation for the three taxa is lowest for *T. pratense* (F_{ST} = 0.02 ± 0.117) and highest for *C. carvi* (F_{ST} = 0.10 ± 0.110). *C. carvi* and *T. repens* (F_{ST} = 0.06 ± 0.156) show moderate level of genetic differentiation among populations (Hartl and Clark, 1997). AMOVA supports this pattern of population differentiation with *T. pratense* containing the highest within population diversity (96%) and *C. carvi* showing the lowest level (83%). This high within population diversity is expected for outcrossing taxa such as the three studied here.

PCoA supports the above findings and shows that there is no obvious geographic portioning of genetic diversity for the three species (Figure 5.2). The Mantel tests show there is no significant isolation by distance (IBD) for *C. carvi* and *T. pratense* (Figure 5.3), though there is a significant level of IBD for *T. repens* (R^2 = 0.0498, P = 0.032).

Table 5.2: Summary of population genetic statistics from AFLP data. H_W ± standard error, F_{ST} ± standard error.

Species	Dataset error rate (%)	Highest loci error rate	Number of loci	Number of samples	Number of populations	PLP (%)	H_W	F_{ST}	Within population AMOVA (%)	Mantel test	
										R^2	*P*-value
C. carvi	14.7	0.086	285	372	20	16.8	0.05 ± 0.006	0.10 ± 0.110	83	0.003	0.317
T. pratense	29.9	0.105	383	372	20	15.1	0.05 ± 0.005	0.02 ± 0.117	96	0.020	0.066
T. repens	21.0	0.138	385	374	19	16.4	0.06 ± 0.005	0.06 ± 0.156	89	0.050	0.032

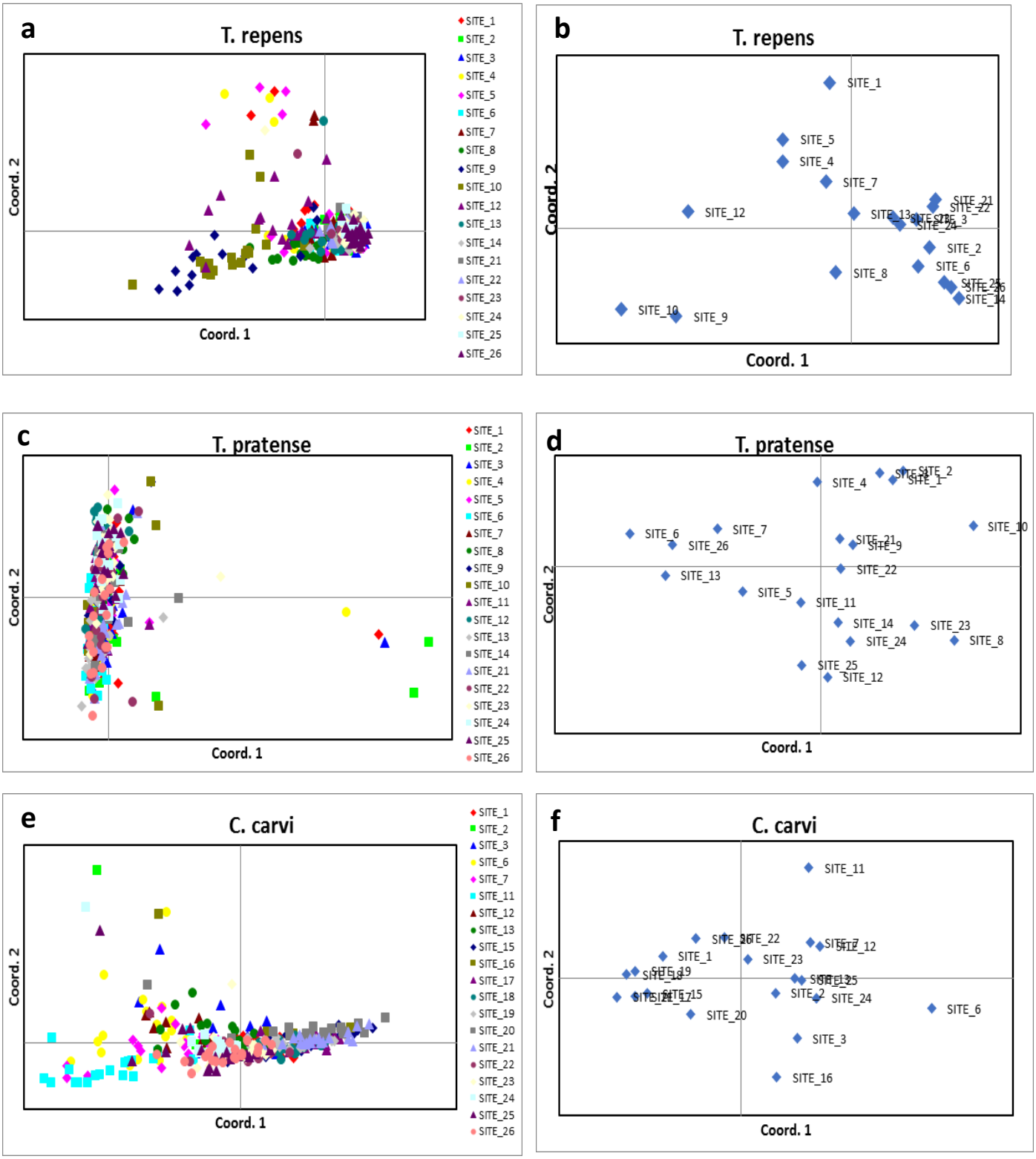
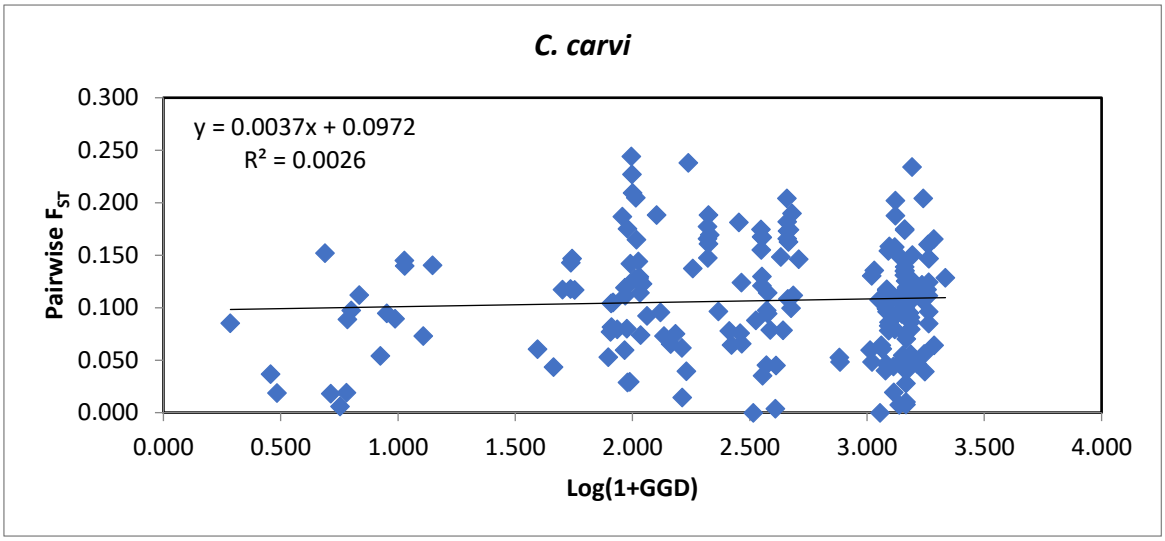
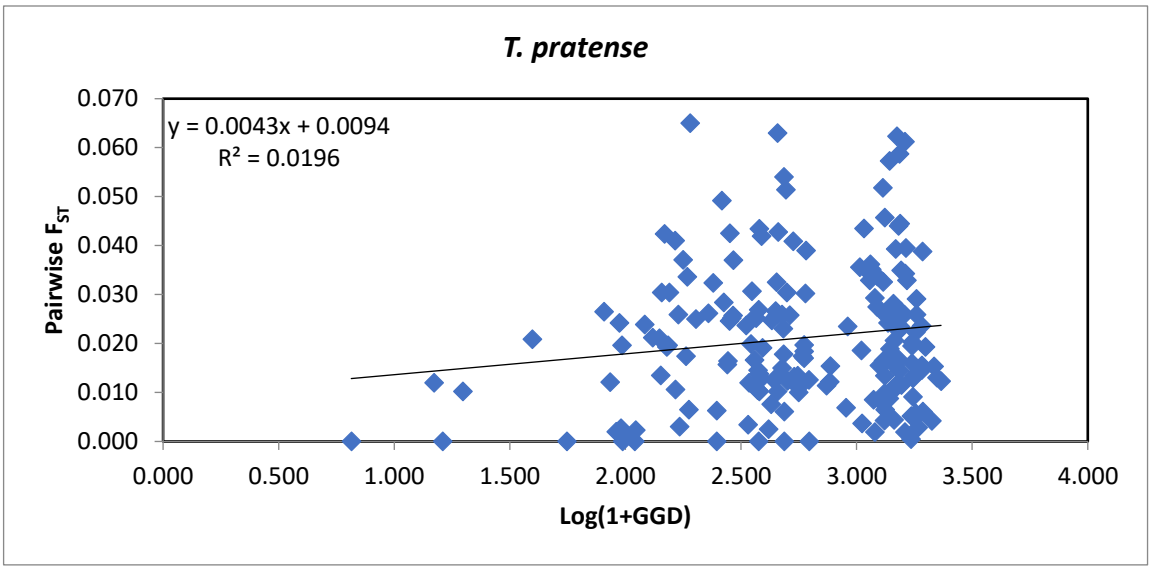


Figure 5.2: Principal coordinates analysis for *T. repens* (a and b), *T. pratense* (c and d) and *C. carvi* (e and f). a), c) and e) represent PCoA for individuals b), d) and f) represent PCoA for populations.





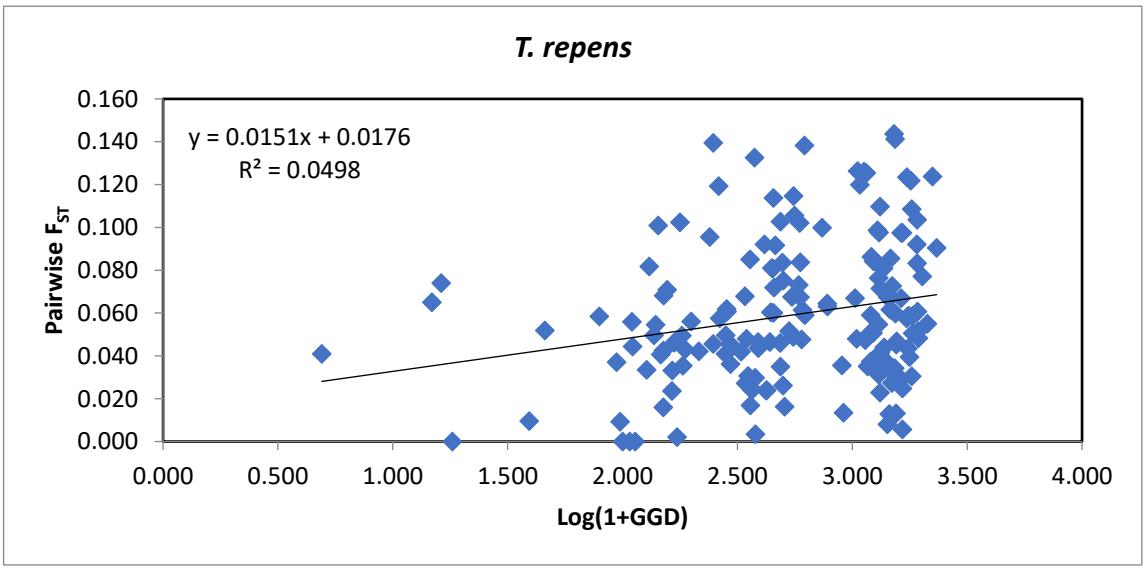


Figure 5.3: Mantel tests for the *C. carvi*, *T. pratense* and *T. repens* showing the relationship between genetic distance (F_{ST}) and geographic distance

5.4.1 Genetic distinctness among populations

A study of the number of private alleles (Table 5.3) within populations for the three species indicated southern populations (sites 1-20) were more distinct, with a higher number of private alleles found within these populations. The relationship between the number of private alleles and the level of heterozygosity is significant for all taxa (*C. carvi F* (1, 18) = 9.873, *P* <0.05; *T. repens F* (1, 18) = 11.332, *P* <0.005; *T. pratense F* (1, 18) = 23.897, *P* <0.005).

Table 5.3: The number of private alleles and the level of heterozygosity within each population at each site. 'NA' = not applicable, no data available. * = populations with the highest levels of diversity and private alleles and therefore recommended for further *in situ* and *ex situ* conservation.

	C. carvi		*T. pratense*		*T. repens*		ELC zone
	Number of private alleles	Level of heterozygosity	Number of private alleles	Level of heterozygosity	Number of private alleles	Level of heterozygosity	
SITE 1	1	0.02742	6*	0.07042*	15*	0.07926*	17
SITE 2	11*	0.06878*	14*	0.07733*	3	0.05048	17
SITE 3	7*	0.07627*	3	0.05583*	1	0.05804	16
SITE 4	NA	NA	3	0.04776	18*	0.07517*	16
SITE 5	NA	NA	7*	0.05028	14*	0.07136*	17
SITE 6	11*	0.08630*	1	0.03407	1	0.03766	18
SITE 7	10*	0.06791	1	0.03458	0	0.05170	18
SITE 8	NA	NA	3	0.04393	0	0.06537	17
SITE 9	NA	NA	0	0.04439	6*	0.10018*	18
SITE 10	NA	NA	2	0.05230*	3	0.08415*	26
SITE 11	5	0.07548*	0	0.04275	NA	NA	21
SITE 12	1	0.05065	1	0.03661	3	0.06988	8

SITE 13	3	0.04996	1	0.03530	2	0.05059	21
SITE 14	NA	NA	4*	0.05129	1	0.03213	26
SITE 15	0	0.04112	NA	NA	NA	NA	16
SITE 16	0	0.07402*	NA	NA	NA	NA	16
SITE 17	0	0.05019	NA	NA	NA	NA	16
SITE 18	0	0.03439	NA	NA	NA	NA	16
SITE 19	6*	0.04409	NA	NA	NA	NA	16
SITE 20	2	0.04752	NA	NA	NA	NA	16
SITE 21	0	0.04474	4*	0.05327*	2	0.04444	15
SITE 22	0	0.03699	0	0.03989	0	0.04464	14
SITE 23	0	0.05077	0	0.03845	0	0.03922	15
SITE 24	1	0.05369	3	0.03266	4*	0.04091	15
SITE 25	0	0.06207	0	0.03048	0	0.02853	24
SITE 26	2	0.04102	2	0.03193	1	0.03018	23
Total	60	-	55	-	74	-	-

5.4.2 Environmental diversity and genetic diversity

The level of gene diversity differs between and within all ELC zones for each species (Table 5.3). For *C. carvi* the ELC zone with highest gene diversity is ELC zone 18 and the lowest gene diversity is ELC zone 17. The highest gene diversity is found within ELC zone 17 for *T.*

pratense and ELC zone 18 for *T. repens*. The lowest gene diversity is found within ELC zone 24 for both *T. pratense* and *T. repens*. However, correlation analysis showed no significant linear relationships between the environmental variables used in the creation of the ELC zones and the level of gene diversity for each species (Table 5.4). Stepwise regression showed that latitude significantly contributed to the level of genetic diversity in *T. repens* populations (Table 5.5). For *C. carvi* and *T. pratense* using stepwise regression none of the variables had any significant effect upon genetic diversity, with a multiple regression analysis confirming this (Table 5.5).

Table 5.4: T-values based on Pearson correlation of environmental variables against level of gene diversity (H_w).

	T. pratense		*T. repens*		*C. carvi*	
	t value	significance	t value	significance	t value	significance
Bio 15	-1.059	0.309	0.420	0.682	-0.583	0.570
Bio 3	-0.878	0.396	-0.611	0.552	0.828	0.423
Average altitude	0.029	0.977	-0.796	0.441	-0.600	0.559
Average organic content	0.645	0.530	-0.167	0.870	-1.324	0.208
Average pH of topsoil	-0.350	0.732	-0.539	0.599	-0.733	0.476
Latitude	-1.792	0.096	-1.479	0.163	-1.457	0.169

Table 5.5: Stepwise regression analysis of six environmental variables against level of gene diversity (H_w) for *T. repens* and a multiple regression analysis for *C. carvi* and *T. pratense**= Predictor (constant) latitude.

Multiple regression						
Species	*R*	R^2	adjusted *R*	df (degrees of freedom)	*F*	Significance
C. carvi	0.500	0.250	-0.096	(6, 13)	0.723	0.639
T. pratense	0.581	0.337	0.031	(6, 13)	1.102	0.412
T. repens	0.586*	0.236	0.194	(1, 19)	5.575	0.030*

5.5 Discussion

Knowledge of genetic diversity within and among species is indispensable to optimally managing genetic resources for the improvement of cultivars as well as to maintain and restore biodiversity (Herrmann *et al*., 2005). Nevo (1998), Watson-Jones *et al*. (2006) , Hargreaves *et al*. (2010), Magos Brehm *et al*. (2012). and Fielder (2015) have shown how genetic diversity data can be used to establish baseline data for future monitoring of populations for conservation management. This investigation is the first genetic diversity study of three priority CWR within Norway. By undertaking this study the first baseline level of genetic diversity data for continued monitoring of these taxa has been established. This baseline data will be important for identifying *in situ* and *ex situ* conservation planning priorities. The consequent management of populations within potential genetic reserves as set out in the guidelines by Iriondo *et al*. (2012) and the targeted collection of material for *ex situ* conservation, will be more effective with knowledge on the genetic diversity of the populations to conserve.

The results of the AFLP analysis show that the genetic diversity is portioned mostly within rather than amongst populations for all three taxa. For *C. carvi* the results in this study are in contrast to Laribi *et al*. (2011) who showed that genetic diversity was lower within populations despite the species being outcrossing. The study by Laribi *et al*. (2011) used populations from three different countries and used RAPDs to analyse the genetic diversity, which may account for the differences between their results and the results shown here. The high within population diversity for *C. carvi* reflects the outbreeding life history of the species. The level of population differentiation (F_{ST}) for *C. carvi* suggests there are moderate levels of gene flow between populations and that the species can disperse widely. This knowledge of population differentiation can be used as a baseline for future studies and population monitoring. For example, Watson-Jones *et al*. (2006) suggests that if there is a higher F_{ST} than previously seen then populations are becoming more differentiated, meaning they are adapting to the specific environmental conditions around them and therefore the adaptive genetic diversity may be changing. Laribi *et al*. (2011) suggests that to conserve *C. carvi* we should conserve as many populations as possible as well as preserving the species' natural grassland habitat to prevent fragmentation and genetic drift. Due to most of the diversity being portioned within populations it may not be necessary to conserve a large number of populations but it will be important to conserve a large number of individuals within those populations due to the outbreeding nature of the species (Hamrick & Godt, 1990; Marshall & Brown, 1995; Hamrick & Godt, 1996; Hoban & Schlarbaum, 2014). Conserving, *C. carvi* populations across the species ecogeographic range in Norway will be important, along with habitat protection and the utilisation of PAs being one method to achieve this.

For the *Trifolium* species, studies on wild and cultivated populations have shown that the level of genetic diversity is mostly portioned within rather than among populations (Kölliker *et al*.,

2001; Gustine *et al*., 2002; Kölliker *et al*., 2003; Ulloa *et al*., 2003; Herrmann *et al*., 2005; Dias *et al*., 2008; Hargreaves *et al*., 2010; Ahsyee *et al*., 2014; Fielder, 2015) which is in concordance with the results obtained in this study. This within population diversity suggests that the conservation of large numbers of plants in a few populations would be most beneficial (Rao & Hodgkin, 2002; Christmas *et al*., 2016). The levels of population differentiation in the *Trifolium* species is lower in this study than that of others which also used AFLPs to study genetic diversity (e.g. Hargreaves *et al*., 2010; Fielder, 2015). This result may be because the populations found in Norway are far from their centres of diversity (in the Mediterranean) which tends to reduce levels of genetic diversity in species (Chowdhury & Slinkard, 2000; Grivet & Petit, 2003). There may also have been a bottleneck in the studied populations given their location within Norway, which is located on the north western periphery of Europe (Fjellheim & Rognli, 2005). In contrast, Williams (1987) describes both synthetic and natural white clover populations as being highly genetically heterogeneous, with Wedderburn *et al*. (2005) suggesting that this diversity may favour a genetic shift in response to environmental factors. With the base line genetic data gathered in this study, this potential for genetic shift can be monitored.

The homogenisation of populations is reflected in the PCoA which shows no obvious genetic clustering of populations or individuals for any of the three CWR species. This is a similar finding to Van Treuren *et al*. (2005) who found that there were low levels of population structuring within wild grassland populations, a habitat in which *C. carvi*, *T. repens* and *T. pratense* occur within. The results from the PCoA and Mantel tests suggest that site selection for conservation may be made independently of geographic distance for the three CWR species.

For the three species studied, results showed that the southern populations tended to be more distinct and have higher levels of heterozygosity than northern populations. This agrees with Beatty and Provan (2011) who found that the loss of southern populations may have deleterious consequences on genetic diversity since southern populations tend to be more genetically diverse. This pattern of genetic diversity may be due to the lack of physical barriers such as large areas of glaciation in the past, which may have allowed gene flow between populations, compared to those populations further north which experienced higher levels of glaciation (Eidesen *et al*., 2013). For conservation measures, it is important to protect the highest neutral genetic diversity as it may be the future target of natural selection (McKay & Latta, 2002). Focusing conservation efforts on the most genetically diverse populations will therefore be important. Tigerstedt (1994) found that the lowest levels of gene diversity were in wild white clover populations from Iceland that were under extreme conditions and hypothesizes that traits of high adaptive value may have been forced to uniformity by stabilizing selection. For conservation purposes, it may be important to targ*et al*leles that are common in specific populations because these may represent marginal populations harbouring alleles of adaptive significance (Marshall & Brown, 1975; Allard, 1992). The chances of finding new and unique alleles are the greatest in populations that are genetically distinct (Fjellheim & Rognli, 2005), with Nevo (1998) emphasising the *in situ* conservation of unique alleles and allele combinations. Furthermore, in a study by Gasi *et al*. (2016) it was found that a high percentage of rare alleles indicated presence of a considerable genetic diversity among apple accessions. In Norway, conservation of southern populations which contain higher genetic diversity and more unique alleles than northern populations should be favoured, at least for the three species studied.

It is generally assumed that genetic diversity is correlated with ecogeographic diversity (Maxted *et al*., 1995; Thomson *et al*., 2001), hence the conservation of populations within each ecogeographic zone and from all ecogeographic zones is required to ensure protection of the full range of genetic diversity. The results from this study show that gene diversity is different within each ELC zone, however there is no clear pattern of diversity within each zone for each species. The level of genetic diversity for the species studied does not show any correlation with specific environmental conditions, although for *T. repens* latitude had a significant effect upon genetic diversity and may be interesting to study further. This supports Greene *et al*. (2004) who found that RAPDs did not separate accessions into distinct classes that correspond to environmental conditions in *T. pratense*. Kölliker *et al*. (2001) also found no correlation between rainfall and altitude based on AFLP data for *T. repens*. Heaton *et al*. (1999) suggests this lack of correlation may be due to the homogenising influence of long distance gene flow, failure of primers to identify genetic differences due to limited sampling of the genome and sampling of neutral markers that are not associated with adaptation. However, other studies, including Nevo (1998), Hermann *et al*. (2005) and Watson-Jones *et al*. (2006) have found correlations between environmental factors and genetic diversity.

Although genetic diversity studies can be useful for determining which populations to conserve, we cannot use molecular marker data alone for identifying conservation units (Pearman, 2001). Whilst collecting samples in the field in Norway it was noted that the populations in the south and north had different phenotypic diversity, for example *T. pratense* populations in the north had larger flowers and leaflet sizes than southern populations. This has also been found when comparing wild and cultivated forms of clover from Iceland, Sweden and the UK (Collins *et al*., 2012). However, this phenotypic difference was not reflected in the populations genetic diversity (Collins *et al*., 2012), supporting the finding that plasticity can

allow genetically similar populations to occur in widely differing environments (Johns *et al*., 1997; McNeilly, 1997).

5.5.1 Issues with the use of AFLP data and the statistical analyses used

Neutral genetic diversity, as measured by AFLPs in this study, reflects evolutionary forces such as genetic drift, mutation and migration and is not under selection (Reed & Frankham, 2001; McKay & Latta, 2002). Adaptive genetic diversity reflects the fitness and adaptive potential of a species and is the process by which an organism will adapt to a new environment (Reed & Frankham, 2001; McKay & Latta, 2002; Reed & Frankham, 2003). AFLPs may not enable us to identify important traits for potential plant breeding but they allow us to establish a baseline level of genetic diversity. This is important for conservation management as we can compare it against future studies to determine if there are any changes or detrimental effects occurring within the populations. Furthermore, in long-term conservation plans it is preferable to assess neutral genetic diversity, as it is not possible to predict which adaptive genes will be required (Luikart *et al*., 2003). In future studies it may be beneficial to utilise next generation DNA sequencing (NGS) technologies, for example, to help understand crop genomes, identify useful genetic diversity for plant breeding and for genotyping of germplasm collections (Edwards *et al*., 2013). This is especially advantageous for crops and their wild relatives with complex and large genomes such as wheat and brassica, which are also some of the most widely utilised crops worldwide. This technology may also help with the identification and utilization of CWR material for targeted *ex situ* conservation.

The error rates for the AFLP datasets in this study ranged from 14.7% for *C. carvi* to 29.9% for *T. pratense*. Though procedures were put in place to minimize potential human and technical errors (including automated laboratory procedures and pre-selective amplification (Vos *et al*., 1995)), these error rates are higher than the level proposed by Bonin *et al*. (2004, 2007), Arrigo

et al. (2009) and Zhang and Hare (2012) of between 2% to 5% dataset error rates. Other similar studies using AFLPs including Pompanon *et al*. (2005) and Holland *et al*. (2008) have found high error rates of up to 15% and 18% respectively. Pompanon *et al*. (2005) suggests this could be due to poor quality of DNA and Holland *et al*. (2008) cites small sample sizes (<30 individuals) and the use of fully automated scoring being the cause of high error rates. However, when parameter settings are chosen carefully, automated scoring is still preferred as it removes subjectivity, is more time efficient, makes it easier to maintain consistency and eliminates human biases as well as being more repeatable (Holland *et al*., 2008). Our results showed that more alleles were scored and there was a lower dataset error rate using the automated software, RawGeno, than scoring manually. Furthermore, both Holland *et al*. (2008) and Zhang and Hare (2012) agree that it is not always best to choose the lowest error rates or to use error free data as it could lead to datasets with low information content. This may occur because to minimize the dataset error rate involves removing loci with high numbers of mismatches from the analysis, thus at some point there will be too few loci to detect population structure, which could also introduce a bias on *Fst* in either direction (Holland *et al*., 2008; Zhang & Hare, 2012). Most AFLP studies report average mismatch error rates of around 2%, so using error free data is not necessary but determining a threshold for data error is important (Zhang & Hare, 2012).

There are also controversies around the use of the Mantel test for analysing spatial patterns, including isolation by distance (IBD) (Legendre and Fortin, 2010; Diniz-Filho *et al*., 2013; Guillot and Rousset, 2013; Legendre *et al*., 2015). The Mantel test is less powerful than other statistical techniques at detecting spatial relationships (Legendre and Fortin, 2010). IBD predicts the presence of spatial autocorrelation in the response data and that prediction should be tested using other methods such as multivariate correlogram analysis, regression or

canonical redundancy analysis (Legendre and Fortin, 2010). The non-significant results from this study indicates that genetic variability is not structured, which deserves more research using methods such as Spatial Eigenfunction Analyses (SEA) or Redundency Analysis (RDA) (Legendre and Legendre, 2012), to see if a similar result as the Mantel test is produced (Diniz-Filho *et al*., 2013). Both Hargreaves *et al*. (2010) and Watson *et al*. (2006) have used the Mantel test to determine spatial patterns in CWR populations, therefore use of such a statistic to help formulate potential conservation strategies can be appropriate. However, it would be valuable for this study to develop this analysis following the previously mentioned methods. With the comparisons to other studies described above and knowledge of the life histories of the species we are content that with the data used, the conservation measures suggested are both valuable and practical to the management of CWR in Norway.

5.6 Conclusion

In situ conservation of at least 5000 individuals, as proposed by Iriondo *et al*. (2008), across a minimum of five populations (Brown & Briggs, 1991), selected from the most diverse populations (Table 5.3), is proposed as the most effective method for genetic conservation of *C. carvi*, *T. repens* and *T. pratense* in Norway. This is because the results show there is a larger proportion of diversity contained within rather than amongst populations. The results do not show a link between environmental conditions and genetic diversity. It may therefore be better to conserve a broader range of genetic diversity *in situ* rather than sampling a fraction of the population for *ex situ* conservation. As the southern populations for all three species show higher levels of genetic diversity and distinctness then it will be appropriate to begin initial conservation actions here. This is the case with the Færder NP, which covers an archipelago in the Oslo Fjord in the south of Norway and where the Norwegian priority CWR have been incorporated into management plans for that area (M. Rasmussen, 2017, pers. comms.). It will

still be necessary to conduct conservation on populations further north in Norway to cover the full range of phenotypic and ecogeographic diversity, although there were comparatively low levels of genetic diversity in northern populations. In all instances of *in situ* conservation the focus can be upon those populations already located within PAs that are easy to access and monitor.

Although *ex situ* conservation may not be the most effective conservation method for these populations, it will still be necessary to collect a subset of material for *ex situ* storage. This will enable easier access for plant breeders and will act as *ex situ* back-up which is a standard adjunct to *in situ* conservation. Collections from the most genetically diverse populations (see Table 5.3) in the south of Norway will ensure a broad range of diversity is captured in *ex situ* collections. The number of individuals required for *ex situ* conservation will ultimately depend upon the effective population size and resources available for conservation. Crossa *et al*. (2011) suggest collecting samples from 25 individuals from the largest possible number of sites, with Brown and Briggs (1991) suggesting a minimum of 10 individuals. However, due to the outbreeding nature of the species and the high within population diversity the sample should be as large as possible and should be adjusted and increased depending on the ease of sampling material, the total distribution of the species and the project needs (Brown & Briggs, 1991). *Ex situ* conservation for these three species may benefit from further genetic diversity studies targeting adaptive traits in species, or targeting those phenotypic traits that may be beneficial for plant pre-breeders. Conservation of CWR should be focused upon capturing the broadest range of genetic diversity possible using both *in situ* and *ex situ* approaches. The methods used in this study will help achieve this along with the effective monitoring and management of CWR in Norway which will be vital for future food security at national, regional and global levels.

CHAPTER 6.

Discussion

The work presented in the previous chapters contributes towards both food security and conservation needs in Norway, the wider Nordic region and globally. In the Adapting Agriculture to Climate Change project (Dempewolf *et al*., 2014) of the 1667 CWR taxa identified, Norway has 35 and the Nordic region has 46 CWR important for global food security (Vincent *et al*., 2013). Wild forages such as timothy, festuca and berry species, such as strawberry, are especially abundant in Norway and the Nordic region and are vital for global food security (Weibull *et al*., 2016). Although Norway is far from the centres of diversity for many staple crops its climatic and environmental variability may lead to important adaptations for CWR populations. Furthermore, the effects of climate change are being felt more rapidly in the Arctic region which has warmed at twice the rate of the global average over the last century (IPCC, 2014). This puts Norway and the other Arctic countries in a unique position at the forefront of climate change adaptation. This will involve not only developing crops to help continue feeding the human population but also the development of adaptive and robust conservation strategies to protect the diversity that underpins the food system.

6.1 The process of CWR diversity planning for Norway

As part of the diversity planning process for the conservation of CWR we need to gather information about the resources we have available to us. This began with the creation of a checklist of CWR within Norway which was completed with the close collaboration of local stakeholders and experts from the Natural History Museum Oslo and the Forest and Landscape

Institute. Due to the well documented flora of Norway (Lid & Lid, 2005) taxa were identified genus by genus to determine if they were classed as CWR, according to the definition used by Maxted *et al*. ((2006); see chapter 3 for more information).

It was not feasible to plan conservation actions for all taxa on the checklist due to limited resources and time, hence prioritisation of the species was necessary. Due to the dynamic nature of Norway's flora this process encountered some problems such as taxa that have populations present but not currently persistent (i.e. *Brassica rapa*). These issues were resolved with the help of local botanical experts who were able and willing to study the priority list and make appropriate comments on the taxa. The prioritisation process has been extensively covered by Maxted *et al*. (1997b) with Kell *et al*. (2015) identifying the three most important criteria to prioritize CWR. These three criteria include the socio-economic value of the associated crop, the potential for utilization of the CWR and the threat status of the CWR (Kell *et al*., 2015). Numerous national, regional (Weibull *et al*., 2016; Kell *et al*., in prep) and global (Dempewolf *et al*., 2014) studies have used a combination or subset of these criteria for prioritisation, illustrating there is no one right way to select priority CWR. Within Norway the prioritisation process is documented in chapter 3 and Phillips *et al*. (2016) and began by initially selecting crops and their associated wild relatives that were present within Norway which have the highest economic value in terms of global, European and Norwegian production value (current million US$). Threat level and potential for utilization were not considered in the prioritisation process as the number of CWR selected after application of the criteria in Phillips *et al*. (2016) was deemed appropriate by stakeholders at the time. However, future work could use the other criteria suggested by Vincent *et al*. (2013) and Kell *et al*. (2015) to prioritise the list further and create a sub-set of priority taxa for more detailed studies. Occurrence data was gathered from GBIF for the Norwegian CWR as this was deemed the

most complete source of presence information that was available for the taxa. Additional information about the use of the CWR, the relationship to the cultivated crop, red list status and more, was gathered to create the Norwegian Inventory of CWR (Table S2.2, chapter 3).

The conservation planning process for CWR can be separated out into *in situ* and *ex situ* priorities. In Norway, the focus was upon multi-species CWR conservation as decided by national stakeholders. One reason for this was that the CWR concept was still relatively little understood in the wider conservation and policy maker community. Therefore, there was a need to demonstrate a 'value-for-money' approach to conservation and to be more efficient with the resources available. Recent research has suggested that instead of focusing upon species level site selection for CWR, the focus should be upon the selection of the most appropriate CWR populations, called MAWPs (Maxted *et al*., 2012The premise behind MAWPs is that they are targeted for conservation regardless of their location inside or outside of a PA. As discussed below, analyses were conducted across both the whole of Norway and restricted to inside PAs only. The MAWPs were identified in the sense that these populations occurred in locations that contained high levels of CWR richness (i.e. high numbers of multiple taxa populations; Maxted *et al*. (2015)). As the focus was on multispecies conservation this meant they were the most appropriate populations to conserve for Norway.

6.1.1 In situ diversity analysis

The *in situ* diversity analysis process is documented in chapter 3 and Phillips *et al*. (2016). Perhaps the most valuable output for this project in terms of *in situ* analyses was the production of the *in situ* complementary networks for the priority CWR in Norway. Two methods were followed, a grid cell analysis (Figure 3.3) and a PA analysis (Figure 3.4). The grid cell analysis meant that the MAWPs were identified regardless of their presence inside a PA. Both methods were used so a comparison could be made between the complementary networks identified. In

this case six locations overlapped between both analyses (Table 3.4), suggesting that these locations contain particularly high taxa richness and could be prioritised over other locations for further *in situ* conservation actions. If resources and time are limited it may be appropriate to focus only upon the PA network, as conservation will be easier to implement and by the definition of a PA, conservation of the species is already taking place even if only passively (Maxted *et al*., 2008). Furthermore, for Norway the PA complementary network still conserves 90% of priority taxa. Within heavily populated countries such as the UK, focusing upon the PA network for conservation of CWR may initially be the only option to ensure conservation of these taxa (Fielder, 2015). However, by focusing upon PAs only this suggests MAWPs are not located outside of these areas. This will not be true, even in heavily populated areas and as such, a response to this would be to increase conservation of populations in areas such as road verges and field margins, habitats preferable for CWR (Jarvis *et al*., 2015). Within Norway, protected landscapes are an important category of PA. They often cover agricultural landscapes that are actively used and the maintenance of this is encouraged (Norwegian Environment Agency, 2017). These will be important areas to focus the *in situ* conservation of CWR upon, especially wild forage species which favour this habitat type in Norway. Conservation outside formal PAs and a more dynamic approach to conservation activities will become more important in the long term due to the effects of climate change and the increasing environmental and anthropogenic impacts upon wild populations.

The climate change diversity analysis is essential for long term conservation planning, with the results for Norway suggesting that CWR distribution may shift further northward in the future (see chapter 4 and Phillips *et al*., 2017). Although there are uncertainties associated with these predictions the information presented will be useful to inform management and monitoring plans for the priority CWR. The climate change analysis lends itself to being utilised most

effectively in the creation of robust and long-term management plans. The results can be used to create both incremental and transformative management plans for genetic reserves or for particular taxa and particular populations (see chapter 4 and Phillips *et al*., 2017). In this way, PA managers can be prepared for the future potential impacts of climate change without needing to expend large amounts of resources upon the creation of new PAs. This is an area of research that would benefit greatly from practical application and close collaboration between scientists and conservation managers as well as policy makers.

The genetic diversity work carried out in chapter 5 showed that the south of Norway may be more genetically diverse and contain more distinct genetic diversity than northern areas. This suggests that initially focusing *in situ* conservation in the southern regions may be a good area to target resources, which is also in accordance with the results of the complementarity analysis. Five of the six locations that overlap from the complementarity analyses are located in the south of Norway. It will still be necessary to conserve populations further north as they contain different genetic diversity to that found in the south, as supported by the genetic diversity results (Table 5.3), the ELC map (Figure 3.6) and complementarity analysis (Figure 3.3, 3.4) which identify several locations in the north for potential conservation actions. The genetic diversity studies increase the knowledge of the structure of *in situ* populations and will be valuable for PA managers who will need to create appropriate management plans for the taxa. Due to the high costs associated with genetic diversity studies it may be best to conduct this analysis once the populations and/or location of a genetic reserve have been established by the diversity analysis methods described in chapters 3 and 4. The use of the ELC map (Figure 3.6) to target populations for conservation is a more readily available tool that may be used as a proxy for genetic diversity when the latter information is not available (Parra-Quijano *et al*., 2012b). The work in chapter 5 revealed that the levels of genetic diversity for the three species

studied was different across ELC zones, again with those zones in the south showing higher levels of diversity than the north. Although this pattern of genetic diversity may not be reflected in different countries it does suggest that the use of an ELC map to decide which populations to conserve to capture the full range of diversity available is a valid assumption for the species studied. The genetic studies also show that most of the diversity is found within rather than between populations, with a similar pattern being found in meadow fescue populations across Norway (Fjellheim and Rognli, 2005). This suggests the conservation of a large number of individuals from a few populations would conserve the majority of genetic diversity, favouring the use of *in situ* conservation over *ex situ* conservation for the three species studied (*C. carvi*, *T. pratense* and *T. repens*). This may also be the case for other priority CWR in Norway, especially forage related species which are abundant in the Nordic countries. The most sustainable way of maintaining such diversity will be to promote their *in situ* conservation (Batello *et al*., 2008) as forage species tend to have large, widely distributed populations spread across a range of different environments.

6.1.2 Ex situ diversity analysis

For *ex situ* conservation, a gap analysis assessment is the priority as it gives an overview of the extent of *ex situ* conservation currently taking place. Within Norway, the gap analysis (see chapter 3 and Phillips *et al*., 2016) identified 88% of priority taxa without any *ex situ* accessions. An extension of this analysis utilising the ELC map and the Representa tool from CAPFITOGEN gave a more detailed assessment of the extent of diversity conserved *ex situ*. This showed that of the 24 taxa conserved *ex situ*, 15 had more than five populations conserved across their ecogeographic range. This shows that of the taxa conserved *ex situ* over half have an acceptable *ex situ* representation of their *in situ* diversity (i.e. minimum of five geographically distinct populations conserved according to Brown and Briggs (1991)). For the

taxa that did not have any *ex situ* accessions the most species rich areas to collect from are those regions in the south (Figure S3.3). This information can be used to inform future collecting missions to increase the representation of taxa conserved *ex situ*. Furthermore, the results from the Representa tool in CAPFITOGEN (Figure 3.7) can be used to inform focused collecting missions to increase the range of genetic diversity conserved *ex situ* by targeting collection of populations from under represented ELC zones (Parra-Quijano *et al*., 2012c). Predictive characterization methods can also use the environmental information from the ELC zones to identify potentially useful traits that individuals may contain (Thormann *et al*., 2014). This information can contribute towards enabling plant pre-breeders to select material for further breeding work.

For *ex situ* conservation priorities, climate change studies within Norway (chapter 4) identified taxa that may become threatened in the future. This threat level, based upon the IUCN category A3(c) (IUCN, 2001), can be used to focus *ex situ* collecting activities on those potentially threatened species. This can also be combined with the results from the gap analysis to further prioritise which taxa require immediate collection (Magos Brehm *et al*., 2016). The climate change results can allow the further prioritisation of *ex situ* collecting activities which is essential if resources and time are limited.

The genetic diversity work in chapter 5 was conducted on wild populations collected *in situ* and for which seeds were not collected for *ex situ* storage. AFLP studies were not conducted upon *ex situ* material, therefore the level of genetic diversity currently conserved *ex situ* (as measured by molecular markers) is unknown. However, the genetic results suggest that focusing *ex situ* collecting efforts on populations in the south of Norway may be the most efficient method of collecting the most genetically diverse and distinct populations. As demonstrated by the AFLP results the level of genetic diversity is different across the ELC

zones. The results from the genetic diversity studies also show most of the diversity is within populations rather than amongst. This favours the use of *in situ* conservation as large numbers of individuals will need to be conserved. It may not be efficient to conserve such a large number of individuals *ex situ* due to the high costs of traditional *ex situ* conservation methods (A. Palmé, 2016, pers comms.; although Li and Pritchard (2009) argue against this) and the need to regenerate material periodically which is time consuming and may have a negative impact on the genetic integrity of material (Hamilton, 1994; van Hintum *et al*., 2002). *Ex situ* conservation will require taking a sub sample of the *in situ* population to conserve, therefore it is not possible for the *ex situ* material to capture the full range of genetic diversity present within the population. If the recommendation by Brown and Briggs (1991) to conserve five different populations *ex situ* is followed, it will likely protect 90-95% of common alleles and represent the range of genetic diversity found *in situ*. However, Brown and Marshall (1975) recommend targeting alleles that are locally common, as common alleles will likely be represented within a collection. These locally common alleles will be found in populations adapted to specific environments and may be represented by the private alleles found within the populations (Slatkin & Takahata, 1985), strengthening the case for collection of accessions from southern populations in Norway. Crossa *et al*. (2011) recommend collecting seeds from at least 25 individuals of cross-fertilizing species (of which the three species studied are) but also that seeds should be collected from the largest possible number of sites. These collecting strategies will of course depend upon the resources available for conservation, which is therefore why the Brown and Briggs (1991) recommendation to conserve five populations is suggested as a minimum.

AFLPs were chosen for this study as they offered the most cost effective method of obtaining information upon the genetic diversity for the three CWR chosen. It may be more efficient to

utilise different molecular methods such as SNPs or whole genome sequencing (Bruford *et al*., 2017) that can identify adaptive genetic diversity within collections. SNPs were not available for the CWR studied and whole genome sequencing was not within the means of this project. These methods may help determine if there is a link between adaptive genetic diversity and the ELC zones, which may be more beneficial to plant pre-breeders for targeting future collecting missions.

6.1.3 What have we achieved in Norway?

For conservation priorities in Norway this study used a bottom-up approach (Maxted *et al*., 2013) whereby priorities were identified at the national level by relevant stakeholders. These national level priorities can now be incorporated into regional (Weibull *et al*., 2016), European (Maxted *et al*., 2015) and global (Maxted *et al*., 2012) conservation strategies for CWR.

Due to the engagement of stakeholders from the beginning and the opportunity to be involved with the creation of a new NP in Norway, the inclusion of the priority list of CWR into the protected area management plan for Færder NP was achieved (M. Rasmussen., 2017, pers. comm.). The process of collaboration began at the outset of this project (in 2013) before the detailed scientific background had been established or the required policy instruments to create a genetic reserve had been put in place. In conjunction with the creation of a Nordic CWR conservation programme (Weibull *et al*., 2016), Norwegian PA managers for Færder were involved in the first regional workshop held on Østre Bolærne in Færder NP (May 2015) for which both the scientific and conservation communities collaborated (Weibull *et al*., 2016). This example of stakeholder engagement from the outset meant conservation authorities could see the value that the priority CWR taxa had, not only at the national level but also at the regional level. Furthermore, PA managers were able to see that the level of work required to maintain these populations *in situ* would be minimal as, in this instance, populations had

already been documented and just required continued monitoring, which can follow the methods proposed by Iriondo *et al*. (2012).

Færder NP was selected as the first genetic reserve for CWR in Norway not directly based upon the diversity analyses (chapters 3, 4) but based upon the practicability of instigating conservation of such taxa. It happens that Færder NP shows up as a gap in the knowledge on the current distribution of CWR in Norway (Figure S3.2) due to the lack of occurrence information in GBIF at the time of data collection. Interestingly, local botanists have an in-depth knowledge of the flora on the archipelago, but due to the common nature of the priority CWR these taxa were often not included in survey notes. However, at workshops, local expert knowledge highlighted the area as already being rich in species due to the calcareous nature of the soils. Management plans include the reintroduction of grazing sheep on some of the islands to limit the natural succession into forest that is beginning to take place. This will encourage the priority CWR populations to increase as they tend to favour open grassland more than complex forest habitat (Jarvis *et al*., 2015). The application of the MAWP methodology (Maxted *et al*., 2012) can also be applied here. For a country as large and ecologically diverse as Norway, initially focusing on population conservation (i.e. MAWPs) instead of species conservation was not appropriate due to the large number of populations, but at the level of specific management plans and establishment of genetic reserves this concept can be more effectively applied. This population level management is emphasized by Iriondo *et al*. (2012), Maxted *et al*. (2012) and Fielder (2015).

Policy wise, the scientific work on the proposed genetic reserve began with the knowledge that issues regarding funding for the future monitoring of the taxa had not been addressed. Furthermore, as the conserved genetic resources are a resource this means having access to the material for use is a requirement. These issues have not yet been fully resolved in Norway

(although progress is being made; M. Rasmussen, 2017, pers. comms.) and until they are, official status as a genetic reserve may not be possible. These policy obstacles are not specific to Norway, with work on the UK CWR conservation strategy facing similar policy obstacles (Fielder, 2015). Moreover, these issues could not be resolved within the scope of this PhD thesis and will require collaboration between colleagues from the various ministries within Norway.

In general, CWR diversity planning for Norway has identified some essential needs for further work on the conservation of CWR. The increased surveying effort for species across Norway, within and outside of PAs will be essential to give a broader picture of the populations that may be important for future use. Climate change analysis supports the need for continued monitoring of taxa distributions, where and which taxa to focus monitoring upon. It is also important to note that selecting populations for conservation is always flexible and will change as food security needs change. It is likely that these findings are not only applicable within Norway but across other countries and regions. Simply increasing monitoring and surveying efforts of CWR across Norway may be a simple approach to a greater understanding of where and what CWR diversity is available. This knowledge can help to more fully identify populations that will be the most important to our future food security.

6.1.4 The Nordic Project

In 2015 work began on initiating the conservation of CWR at the Nordic level (Weibull *et al*., 2016). The project is focused on the *in situ* conservation of CWR as well as increasing knowledge of both *in situ* and *ex situ* conservation of CWR across the Nordic region. The project has identified 133 priority taxa based upon gross economic production values at the global and Nordic levels, crop relatedness expressed as genepool or taxon group levels and breeders estimates of wild forage value for use (Weibull *et al*., 2016).

The Nordic countries already have a regional collaboration since 1979 for the *ex situ* conservation of PGR in the form of NordGen (www.nordgen.no), of which Norway contributes material too. The new project aims to help integrate *in situ* conservation at the regional level into this collaboration and encourage the creation of national level strategies and policy framework to implement PGR conservation actions (Weibull *et al*., 2016). The Nordic project has already identified six recommendations that will be delivered to policy makers to further expand the conservation of CWR across the region (Nordgen, 2016). The Norwegian project and national stakeholders have contributed greatly towards to the Nordic project with policy actions one, three and four already being reached within Norway. This includes the creation of a national strategy for conservation of CWR, the use of *in situ* conservation for safeguarding CWR and the implementation of *in situ* conservation in at least one site (Færder NP). The remaining recommendations include the need to develop policy instruments at the national level for conservation and use of CWR and the establishment of complementary conservation across multiple sites. The final recommendation relates to the development of a common Nordic approach to CWR conservation and use.

This regional level project and the work being done at a national level in Norway are complementary to each other and will help strengthen the voice for conservation and use of CWR. These initiatives will also help towards meeting the CBD's strategic plan for 2011-2020 to halt the loss of genetic diversity, as well as the GSPC target 9 to conserve 70% of genetic diversity of crops including their wild relatives by 2020. Regarding the bigger picture, the regional and national projects for conservation and use of CWR will help towards meeting the Sustainable Development goals (UN, 2015a) to protect and enhance global food security.

6.2 Project limitations

There may never be a point at which we have enough data available to satisfy the needs of scientific analysis. Data availability and reliability is almost always something that can be improved upon. Within the Norwegian work, data was gathered from GBIF and filtered by number of decimal places to remove inaccurate data. The reliability of this data could be improved by utilising the CAPFITOGEN tool GEOQUAL (Parra-Quijano *et al*., 2016) which assesses the consistency of the data more completely. More occurrence data could also be gathered in the field to give a more complete view of the distribution of CWR across Norway. The under recording of common species such as many CWR means that potentially useful traits and genetic diversity within those populations are being missed by conservationists and plant breeders. These problems are not restricted to this project and are common when using species distribution modelling techniques (Anderson *et al*., 2006). The limits associated with the use of species distribution modelling software has been well discussed in other literature (Anderson *et al*., 2006; Araujo & Guisan, 2006).

In the climate change studies (chapter 4, and Phillips *et al*., 2017), there will be inherent uncertainty of predictions due to the nature of such work. For the Norwegian work, which used a single climatic model, the range of possible outcomes will be substantially underestimated (Burke *et al*., 2015a). It may be more beneficial to use multiple models to create predictions (see '6.3 Further work' section below), which may provide a wider range of climatic predictions but may be more credible to policy-makers (Burke *et al*., 2015b).

The genetic diversity studies proved to be the most challenging part of the project. Initially genetic studies were to be carried out upon ten priority CWR taxa and material was collected for all species. However, during the lab procedure and analysis of the AFLP outputs it was

apparent that the procedure had not been successful. This meant that we were only able to re-run the analysis upon a smaller (three) number of species, therefore severely limiting the breadth at which our analysis was done. The lack of reliable results (as illustrated by the high dataset error rates) was the biggest limitation to the interpretation of our results. The main source of error is as yet unidentified and could be isolated to the initial DNA quality being poor, problems in the lab procedure or the final analysis of the results and the choice of software. One solution to this could be to use larger samples sizes with Khanlou *et al*. (2011) suggesting a minimum of 30 individuals per population. Perhaps the best option would be to utilise different molecular techniques such as SNPs or by undertaking entire genome sequencing of the species (Bruford *et al*., 2017). The latter has already been done for crops such as *Brassica rapa* (Wang *et al*., 2011), barley (International Barley Genome Sequencing Consortium, 2012) and soybean (Cannon *et al*., 2009), amongst others. It would be beneficial in any future genetic diversity studies to focus research upon identifying adaptive diversity that both conservationists and plant pre-breeders can harness.

The work done for the conservation of CWR in Norway was never seen as just an academic exercise. This is illustrated by the successful incorporation of management plans for the taxa into Færder NP as well as the initiation of the Nordic CWR project. However, a major limitation for the continuation and further implementation of this work is the policy barriers that are in place. The lack of clarity on which government departments should be responsible for the conservation of CWR will prevent any further movement on this work. Within Norway there is also differences between each region as the PAs are managed by different authorities. Therefore, each region will have to decide if CWR conservation is a priority for them. With the establishment of Færder NP as a genetic reserve for CWR and impetus from the Nordic

project and global initiatives this will encourage regional authorities and the national government to act more decisively upon conservation of CWR.

6.3 Further work

- Species by species diversity analysis: specific *in situ* and *ex situ* conservation recommendations for individual species, including diversity analysis (chapter 3), climate change studies (chapter 4) and genetic diversity studies (chapter 5). Work on this has already begun with a project on *R. chamaemorus* across Norway and the Czech Republic (M. Rasmussen and V. Holubec, 2017, pers. comms.) which will identify the *in situ* and *ex situ* conservation needs for the species so future conservation missions can take place. The MAWPs can be identified for priority species and further climate change analysis could be developed which should include undertaking climate change vulnerability studies (CCVA; Foden *et al*., 2009). Species specific management plans can also be created that account for both incremental and transformational management strategies which will incorporate the potential effects of climate change. By creating species specific strategies, conservation can be more targeted towards the needs of plant pre-breeders who are looking for specific traits. This process could be done for other priority CWR in Norway as and when the funding and opportunities become available.

- Develop ELC maps for the future climatic conditions: these could be used along with the climate change analysis to help plant pre-breeders identify populations that they may require specific traits from in the future i.e. predictive characterization. The future ELC map could show how environmental conditions may change and whether this may influence plant population distribution. This could help determine if genetic diversity for

specific environmental adaptations may be lost which allows the targeting of those at-risk populations for conservation actions.

- Adaptive genetic diversity studies: although we have identified the levels of neutral genetic diversity within and between populations the usefulness of such genetic diversity remains unknown. We need to assay functional genes that matter for improving crops and will benefit farmers, not just conservationists as neutral genetic diversity does (Brown, 2008). As costs come down this could be done using SNPs or whole genome sequencing.

- Genetic diversity comparisons between CWR and cultivars: a comparison between cultivated and wild *T. pratense* was initially part of the original project, however due to the problems with the AFLP analysis this data was not appropriate to utilise. By determining how different wild and cultivated populations are it will help identify which populations we should be conserving and utilising, as we may only want to conserve those that are the most different from cultivars. This was the case with meadow fescue, in which western and southern populations were targeted for conservation as they were the most different to cultivars (Fjellheim and Rognli, 2005).

- Landscape protected areas: this type of PA could be very important for the establishment of CWR conservation as well as the engagement of local stakeholders within Norway. In landscape protected areas the maintenance of traditional agricultural activities are encouraged which may favour the establishment of CWR populations. It may be beneficial to carry out research on the occurrence of the priority CWR within these PAs.

A comparison between taxa richness as well as the levels of genetic diversity in such landscapes may help to determine if these areas should be the focus of *in situ* conservation actions in Norway.

- Policy changes: although outside the scope of this project, policy changes are an essential area of research that needs to be pursued if further active conservation and use of CWR is to take place. It would be beneficial to undertake studies on the economic value of CWR and how they contribute towards ecosystem services and add value to PAs. Some important aspects of policy research include: enabling the conservation and use of PGR outside of PAs, perhaps through agri-environment schemes; the designation of PAs as genetic reserves for PGR; the legislation required for the collecting of taxa from the wild to be used for breeding purposes.

- Education: it is impossible to care for something if you know nothing about it, therefore education will be an essential step in the continuation of PGR conservation and use. High profile programs such as the Adapting Agriculture to Climate Change project and the Svalbard Global Seed Vault are already engaging audiences about the value of PGR. At local and national levels actions such as including information boards in PAs that highlight the link between the wild 'weeds' and our food could be a simple but effective step to get the food security message across.

6.4 Conclusion

Our food systems underpin the workings of the planet but the lack of understanding of where our food comes from and how we should be effectively utilising the diversity available to us may lead to a food crisis in the future. In northern Europe climate change may present new opportunities for agriculture such as increased growing season length and crop productivity (Uleberg *et al*., 2014; Burke *et al*., 2015b). However, climate change will also have negative effects on ecosystem services and the distribution of wild species which will inevitably lead to the loss of genetic diversity from wild populations. As discussed throughout this study, genetic diversity underpins the resilience of our food system by allowing species to develop adaptations to changing conditions. The genetic diversity most valuable to us is harnessed by plant pre-breeders to enhance our food systems, however diversity is also lost through the domestication of species by the selection of a few traits that are beneficial to us now.

The work presented above contributes towards the Norwegian strategy for the *in situ* and *ex situ* conservation of CWR. The impending establishment of the first genetic reserve for CWR in Norway has shown that there is an appetite amongst conservationists, not just academics, for the protection of these resources. The link to plant pre-breeders will be established with projects such as the Adapting Agriculture to Climate Change work which is developing the pre-breeding of certain CWR. Once it is seen that harnessing the value of our natural genetic diversity is accessible and beneficial then the call for conservation of such resources will become that much stronger.

From the work above, by combining *in situ* and *ex situ* diversity analyses, climate change studies and genetic diversity work, an effective conservation strategy and the groundwork for further studies has been established. The project has helped increase the knowledge on

Norwegian CWR conservation and the potential availability of a rich source of breeding material for plant pre-breeders. The study has also helped to further develop methodologies needed to create national strategies in other countries and strategies at regional and global levels. Working from the local level and engaging with stakeholders directly has made it possible for this academic work to have a practical impact upon CWR conservation in Norway. Although we face great challenges from both politics and climate change this project has shown that there is an appetite for PGR conservation and use. This work is only the beginning of a more effective strategy to protect national and international food security for the future.

References

Abberton, M. (2011). New opportunities for forage species. Available online: http://www.dairyco.org.uk/resources-library/research-development/production-system/newopportunities-for-forage-species/#.VFtd3HnivIV [Accessed 1 April 2017]

Ahsyee, R. S., Ćalić, I., Zorić, M., Karagić, Đ. & Šurlan-Momirović, G. (2014). Enetic diversity in red clover (*Trifolium pratense* L.) using SSR markers. *Genetika,* 46**,** 949-961.

Al Lawati, A. H., Al Saady, N., Ghaloub, H. A., Patzelt, A., Phillips, J., Maxted, N., Al Balushi, A. H., Al-Maqbali, D., Al Naabi, A. S., Al Busaidi, K. A., Al Kharusi, M., Al Shukaili, M. S., Al Jabri, A. A. & Al Nabhani, H. M. (2017). Plant Agrobiodiversity Conservation Strategy for the Sultanate of Oman. Muscat, Sultanate of Oman: Oman Animal and Plant Genetic Resources Center, the Research council of the Sultanate of Oman. In press.

Allard, R. (1992). Predictive methods for germplasm identification. In: H. Stalker, and J. P. Murphy, eds., *Plant Breeding in the 1990s.* Wallingford, UK: CAB International.

Allem, A. C. (1997). Roadside habitats: a missing link in the conservation agenda. *Environmentalist,* 17**,** 7-10.

Alsos, I. G., Ehrich, D., Thuiller, W., Eidesen, P. B., Tribsch, A., Schonswetter, P., Lagaye, C., Taberlet, P. & Brochmann, C. (2012). Genetic consequences of climate change for northern plants. *Proceedings of the Royal Society B-Biological Sciences,* 279**,** 2042-2051.

Anderson, R. P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R. J., Huettmann, F., Leathwick, J. R., Lehmann, A., Li, J. & Lohmann, L. G. (2006). Novel methods improve prediction of species' distributions from occurrence data. *Ecography,* 29**,** 129-151.

Applied Biosystems. (2006). Peak Scanner. 1.0 ed. Foster City, USA: Applied Biosystems.

Araujo, M. B. & Guisan, A. (2006). Five (or so) challenges for species distribution modelling. *Journal of Biogeography,* 33**,** 1677-1688.

Araújo, M. B., Cabeza, M., Thuiller, W., Hannah, L. & Williams, P. H. (2004). Would climate change drive species out of reserves? An assessment of existing reserve-selection methods. *Global Change Biology,* 10**,** 1618-1626.

Arctic Climate Impact Assessment. (2004). *Impacts of a warming Arctic*. Cambridge, UK: Cambridge University Press. Available at: https://www.amap.no/documents/doc/arctic-arctic-climate-impact-assessment/796 [Accessed 6 April 2017]

Arrigo, N., Tuszynski, J. W., Ehrich, D., Gerdes, T. & Alvarez, N. (2009). Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. *Bmc Bioinformatics,* 10**,** 33.

Barber, Q. E., Nielsen, S. E. & Hamann, A. (2016). Assessing the vulnerability of rare plants using climate change velocity, habitat connectivity, and dispersal ability: a case study in Alberta, Canada. *Regional Environmental Change,* 16**,** 1433-1441.

Beatty, G. E. & Provan, J. (2011). Comparative phylogeography of two related plant species with overlapping ranges in Europe, and the potential effects of climate change on their intraspecific genetic diversity. *BMC Evolutionary Biology,* 11**,** 29.

Batello, C., Mannetje, L., Martinez, A., Suttie, J. (2008). *Plant Genetic Resources of Forage Crops, Pasture and Rangelands. Thematic Background Study.* Rome, Ital. FAO Report. Available at: http://www.fao.org/fileadmin/templates/agphome/documents/PGR/SoW2/thematicstudy_forage.pdf [Accessed 6 April 2017]

Bentsen, M., Bethke, I., Debernard, J. B., Iversen, T., Kirkevåg, A., Seland, Ø., Drange, H., Roelandt, C., Seierstad, I. A. and Hoose, C. (2013). The Norwegian earth system model, NorESM1-M—Part 1: description and basic evaluation of the physical climate. *Geosci.Model Dev,* 6**,** 687-720.

Bijlsma, R., Bundgaard, J. & Boerema, A. C. (2000). Does inbreeding affect the extinction risk of small populations?: predictions from Drosophila. *Journal of Evolutionary Biology,* 13**,** 502-514.

Bijlsma, R. & Loeschcke, V. (2012). Genetic erosion impedes adaptive responses to stressful environments. *Evolutionary Applications,* 5**,** 117-129.

Bilz, M., Kell, S. P., Maxted, N. and Lansdown, R. V. (2011). *European Red List of Vascular Plants.* Luxembourg, European Commission.

Bivand, R., Keitt, T. & Rowlingson, B. (2014). rgdal: Bindings for the geospatial data abstraction library. *R package version 0.8-16*.

Bolliger, J., Eggenberg, S., Ismail, S., Seidl, I. & Kienast, F. (2011). Balancing Forest-Regeneration Probabilities and Maintenance Costs in Dry Grasslands of High Conservation Priority. *Conservation Biology,* 25**,** 567-576.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C. & Taberlet, P. (2004). How to track and assess genotyping errors in population genetics studies. *Molecular ecology,* 13**,** 3261-3273.

Bonin, A., Ehrich, D. & Manel, S. (2007). Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular ecology,* 16**,** 3737-3758.

Booth, T. H., Nix, H. A., Busby, J. R. & Hutchinson, M. F. (2014). BIOCLIM: the first species distribution modelling package, its early applications and relevance to most current MAXENT studies. *Diversity and Distributions,* 20**,** 1-9.

Bradter, U., Kunin, W. E., Altringham, J. D., Thom, T. J. & Benton, T. G. (2013). Identifying appropriate spatial scales of predictors in species distribution models with the random forest algorithm. *Methods in Ecology and Evolution,* 4**,** 167-174.

Branca, F., Argento, S. & Tribulato, A. (2012). Assessing genetic reserves in Sicily (Italy): The Brassica wild relatives case study. In*:* N. Maxted., M. E. Dulloo., B. V. Ford-Lloyd., L. Frese., J. M. Iriondo & M. A. A. Pinheiro De Carvalho. eds., *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces.* Wallingford, UK: CAB International.

Brown, A. H. (2008). Indicators of genetic diversity, genetic erosion and genetic vulnerability for plant genetic resources for food and agriculture. *FAO, Rome*.

Brown, A. H. D. and Briggs, J. D. (1991). Sampling strategies for genetic variation in *ex situ* collections of endangered plant species. *In:* Falk, D. A. & Holsinger, K. E. (eds.) *Genetics and conservation of rare plants.* New York: Oxford University Press.

Bruford, M. W., Davies, N., Dulloo, M. E., Faith, D. P. & Walters, M. (2017). Monitoring Changes in Genetic Diversity. In: M. Walters. And R. J. Scholes. eds., *The GEO Handbook on Biodiversity Observation Networks.* Cham: Springer International Publishing.

Buckler, E. S., Thornsberry, J. M. & Kresovich, S. (2001). Molecular diversity, structure and domestication of grasses. *Genetics Research,* 3**,** 213-218.

Burke, M., Dykema, J., Lobell, D. B., Miguel, E. & Satyanath, S. (2015a). Incorporating climate uncertainty into estimates of climate change impacts. *Review of Economics and Statistics,* 97**,** 461-471.

Burke, M., Hsiang, S. M. & Miguel, E. (2015b). Global non-linear effect of temperature on economic production. *Nature*. 527, 235-239.

Cannon, S. B., May, G. D. & Jackson, S. A. (2009). Three sequenced legume genomes and many crop species: rich opportunities for translational genomics. *Plant Physiology,* 151**,** 970-977.

Carpenter, G., Gillison, A. N. & Winter, J. (1993). DOMAIN: a flexible modelling procedure for mapping potential distributions of plants and animals. *Biodiversity & Conservation,* 2**,** 667-680.

Castañeda-Álvarez, N. P., Khoury, C. K., Achicanoy, H. A., Bernau, V., Dempewolf, H., Eastwood, R. J., Guarino, L., Harker, R. H., Jarvis, A. & Maxted, N. (2016). Global conservation priorities for crop wild relatives. *Nature plants,* 2**,** 16022.

Chapman, A. D. (2005). Principles of Data Quality, version 1.0. Report for the Global Biodiversity Information Facility. Copenhagen.

Chase, M. W. & Hills, H. H. (1991). Silica Gel: An Ideal Material for Field Preservation of Leaf Samples for DNA Studies. *Taxon,* 40**,** 215-220.

Chowdhury, M. A. & Slinkard, A. E. (2000). Genetic diversity in grasspea (*Lathyrus sativus* L.). *Genetic Resources and Crop Evolution,* 47**,** 163-169.

Christmas, M. J., Breed, M. F. & Lowe, A. J. (2016). Constraints to and conservation implications for climate change adaptation in plants. *Conservation Genetics,* 17**,** 305-320.

Collins, R. P., Helgadottir, A., Frankow-Lindberg, B. E., Skøt, L., Jones, C. & Skøt, K. P. (2012). Temporal changes in population genetic diversity and structure in red and white clover grown in three contrasting environments in northern Europe. *Annals of Botany,* 110**,** 1341-1350.

Conolly, J., Manning, K., Colledge, S., Dobney, K. & Shennan, S. (2012). Species distribution modelling of ancient cattle from early Neolithic sites in SW Asia and Europe. *The Holocene,* 22**,** 997-1010.

Costa, G. C., Nogueira, C., Machado, R. B. & Colli, G. R. (2010). Sampling bias and the use of ecological niche modeling in conservation planning: a field evaluation in a biodiversity hotspot. *Biodiversity Conservation,* 19**,** 883-899.

Cowling, R. M., Pressey, R. L., Sims-Castley, R., Le Roux, A., Baard, E., Burgers, C. J. & Palmer, G. (2003). The expert or the algorithm?—comparison of priority conservation areas in the Cape Floristic Region identified by park managers and reserve selection software. *Biological Conservation,* 112**,** 147-167.

Crossa, J. & Vencovsky, R. (2011). *Basic sampling strategies: theory and practice. Collecting plant genetic diversity: Technical guidelines*.

Cummins, I. N. and Aldwinckle, H. S. (1979). Breeding tree crops. In: M. K. Harris. ed., *Biology and breeding for resistance to anthropods and pathogens in agricultural plants.* Texas Agricultural Experiment Station: Texas A and M University.

Dagher-Kharrat, M. B., Mariette, S., Lefevre, F., Fady, B., Grenier-De March, G., Plomion, C. & Savoure, A. (2007.) Geographical diversity and genetic relationships among Cedrus species estimated by AFLP. *Tree Genetics and Genomes,* 3**,** 275-285.

De Groot, R. S., Wilson, M. A. & Boumans, R. M. (2002). A typology for the classification, description and valuation of ecosystem functions, goods and services. *Ecological economics,* 41**,** 393-408.

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C. & Guarino, L. (2017). Past and Future Use of Wild Relatives in Crop Breeding. *Crop Science*.

Dempewolf, H., Eastwood, R. J., Guarino, L., Khoury, C. K., Mãlier, J. V. & Toll, J. (2014). Adapting Agriculture to Climate Change: A Global Initiative to Collect, Conserve, and Use Crop Wild Relatives. *Agroecology and Sustainable Food Systems,* 38**,** 369-377.

Dias, P. M. B., Julier, B., Sampoux, J.-P., Barre, P. & Dall'agnol, M. (2008). Genetic diversity in red clover (Trifolium pratense L.) revealed by morphological and microsatellite (SSR) markers. *Euphytica,* 160**,** 189-205.

Dias, S., Dulloo, M. E. & Arnaud, E. (2011). 33 The Role of EURISCO in Promoting Use of Agricultural Biodiversity. *Agrobiodiversity Conservation Securing the Diversity of Crop Wild Relatives and Landraces***,** 270.

Diniz-Filho, J. A. F., Soares, T. N., Lima, J. S., Dobrovolski, R., Landeiro, V. L., Pires de Campos Telles, M., Rangel, T. R., Bini, L. M. (2013) Mantel test in population genetics. *Genetic and Molecular Biology,* 36, 4, 475-485.

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B. & Leitão, P. J. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography,* 36**,** 27-46.

Dulloo, M. E., Labokas, J., Iriondo, J. M., Maxted, N., Lane, A., Laguna, E., Jarvis, A. & Kell, S. P. (2008). Genetic reserve location and design. In: J. M. Iriondo., N. Maxted, & M. E. Dulloo. eds., *Conserving plant genetic diversity in protected areas.* Wallingford: CABI Publishing.

Edwards, D., Batley, J. & Snowdon, R. J. (2013). Accessing complex crop genomes with next-generation sequencing. *Theoretical and Applied Genetics,* 126**,** 1-11.

Eidesen, P. B., Ehrich, D., Bakkestuen, V., Alsos, I. G., Gilg, O., Taberlet, P. & Brochmann, C. (2013). Genetic roadmap of the Arctic: plant dispersal highways, traffic barriers and capitals of diversity. *New Phytologist,* 200**,** 898-910.

Elith, J., Phillips, S. J., Hastie, T., Dudik, M., Chee, Y. E. & Yates, C. J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions,* 17**,** 42-57.

Esquinas-Alcazar, J. (2005). Protecting crop genetic diversity for food security: political, ethical and technical challenges. *Nature reviews. Genetics,* 6**,** 946-953.

ESRI, (2011). ArcGIS Desktop Release 10.2. Redlands California: Environmental Systems Research Institute.

EU, (2011). The EU Biodiversity Strategy to 2020. Belgium: European Union.

FAO, (2001). International Treaty on Plant Genetic Resources for Food and Agriculture. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO, (2009a). How to Feed the World in 2050. Rome: Food and Agriculture Organization.

FAO, (2009b). Declaration of the world summit on food security. Rome: Food and Agricultural Organization.

FAO, (2011). Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture. Rome: Food and Agriculture Organization of the United Nations.

FAO, (2015). Coping with climate change-the roles of genetic resources for food and agriculture. Rome.

FAO. (2016). *Agricultural Land* [Online]. The World Bank. Available: http://data.worldbank.org/indicator/AG.LND.AGRI.ZS [Accessed 29th March 2017].

FAO, (2016). The State of Food and Agriculture. Rome: Food and Agriculture Organization of the United Nations.

FAO Stat, (2013). *Food and Agriculture Organisation Statistics.*

Feuillet, C., Langridge, P. & Waugh, R. (2008). Cereal breeding takes a walk on the wild side. *Trends in genetics,* 24**,** 24-32.

Fielder, H. (2015). *Developing methodologies for the genetic conservation of UK crop wild relatives.* PhD, University of Birmingham.

Fielder, H., Brotherton, P., Hosking, J., Hopkins, J. J., Ford-Lloyd, B. & Maxted, N. (2015). Enhancing the conservation of crop wild relatives in England. *PloS one,* 10**,** e0130804.

Fitzgerald, H. (2013). The national crop wild relative strategy report for Finland. Jokioinen: MTT.

Fjellheim, S. & Rognli, O. A. (2005). Molecular diversity of local Norwegian meadow fescue (Festuca pratensis Huds.) populations and Nordic cultivars-consequences for management and utilisation. *Theoritically Applied Genetics***,** 640-650.

Flato, G., Marotzke, J., Abiodun, B., Braconnot, Chou, S. C., Collins, W., Cox, P., Driouech, F., Emori, E., Eyring, V., Forest, C., Gleckler, P., Guilyardi, E., Jakob, C., Kattsov, V., Reason, C. & Rummukainen, M. (2013). Evaluation of Climate Models. In: Stocker, T. F., D. Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P. M. eds., *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* United Kingdom and New York, USA: Cambridge University Press.

Flores-Palacios, X. (1998). Contribution to the Estimation of Countries' Interdependence in the Area of Plant Genetic Resources. Rome: Food and Agriculture Organization of the United Nations.

Foden, W. B., Butchart, S. H. M., Stuart, S. N., Vié, J.-C., Akçakaya, H. R., Angulo, A., Devantier, L. M., Gutsche, A., Turak, E. & Cao, L. (2013). Identifying the world's most

climate change vulnerable species: a systematic trait-based assessment of all birds, amphibians and corals. *PLoS One,* 8**,** e65427.

Foden, W. B., Mace, G. M., Vié, J.-C., Angulo, A., Butchart, S. H. M., Devantier, L., Dublin, H. T., Gutsche, A., Stuart, S. & Turak, E. (2009). Species susceptibility to climate change impacts. *Wildlife in a changing world–an analysis of the 2008 IUCN Red List of threatened species***,** 77.

Foden, W. B. & Young, B. E. (2016). *IUCN SSC Guidelines for Assessing Species' Vulnerability to Climate Change,* Cambridge, UK and Gland, Switzerland, IUCN Species Survival Comission.

Ford-Lloyd, B., Engels, J. M. M., Jackson, M. & Parry, M. (2013). Genetic resources and conservation challenges under the threat of climate change. *Plant Genetic Resources and Climate Change,* 4**,** 16.

Ford-Lloyd, B. V., Schmidt, M., Armstrong, S. J., Barazani, O., Engels, J., Hadas, R., Hammer, K., Kell, S. P., Kang, D. & Khoshbakht, K. (2011). Crop wild relatives—undervalued, underutilized and under threat? *Bioscience,* 61**,** 559-565.

Fourcade, Y., Engler, J. O., Rödder, D. & Secondi, J. (2014). Mapping species distributions with MAXENT using a geographically biased sample of presence data: a performance assessment of methods for correcting sampling bias. *PloS one,* 9**,** e97122.

Franklin, J., Serra-Diaz, J. M., Syphard, A. D. & Regan, H. M. (2016). Global change and terrestrial plant community dynamics. *Proceedings of the National Academy of Sciences of the United States of America,* 113**,** 3725-3734.

Franklin, J. F., Swanson, F. J., Harmon, M. E., Perry, D. A., Spies, T. A., Dale, V. H., Mckee, A., Ferrell, W. K., Means, J. E., Gregory, S. V., Lattin, J. D., Schowalter, T. D. & Larsen, D. (1992). Effects of global climatic change on forests in northwestern North America. *In:* Peters, R. L. & Lovejoy, T. E. (eds.) *Global Warming and Biodiversity.* New Haven: Yale University Press.

Fry, W. (2008). Phytophthora infestans: the plant (and R gene) destroyer. *Molecular plant pathology,* 9**,** 385-402.

Ganal, M. W., Altmann, T. & Röder, M. S. (2009). SNP identification in crop plants. *Current opinion in plant biology,* 12**,** 211-217.

Gasi, F., Kanlić, K., Stroil, B. K., Pojskić, N., Asdal, Å., Rasmussen, M., Kaiser, C. & Meland, M. (2016). Redundancies and Genetic Structure among *ex situ* Apple Collections in Norway Examined with Microsatellite Markers. *HortScience,* 51**,** 1458-1462.

GBIF. (2013). *Biodivesity occurrence data available through the GBIF Data Portal.*

Gederaas, L., Loennechen Moen, T., Skjelseth, S. & Larsen, L. K. (2012). Alien species in Norway-with the Norwegian Black List 2012. Norwegian Ministry of the Environment.

Gent, P. R., Danabasoglu, G., Donner, L. J., Holland, M. M., Hunke, E. C., Jayne, S. R., Lawrence, D. M., Neale, R. B., Rasch, P. J. & Vertenstein, M. (2011). The community climate system model version 4. *Journal of Climate,* 24**,** 4973-4991.

Gepts, P. (2006). Plant genetic resources conservation and utilization. *Crop Science,* 46**,** 2278-2292.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M. & Toulmin, C. (2010). Food Security: The Challenge of Feeding 9 Billion People. *Science,* 327**,** 812-818.

Greene, S., Gritsenko, M. & Vandemark, G. (2004). Relating Morphologic and RAPD marker varlation to collection site environment in wild populations of Red Clover (*Trifolium pratense* L.). *Genetic resources and crop evolution,* 51**,** 643-653.

Greene, S. L. & Hart, T. C. (1999). Implementing a geographic analysis in germplasm conservation. In: S. L. Greene & L. Guarino. eds., *Linking genetic resources and geography: emerging strategies for conserving crop biodiversity.* Madison, Wisconsin: American Society of Agronomy and Crop Science Society of America.

Grivet, D. & Petit, R. J. (2003). Chloroplast DNA phylogeography of the hornbeam in Europe: evidence for a bottleneck at the outset of postglacial colonization. *Conservation Genetics,* 4**,** 47-56.

Guarino, L., Jarvis, A., Hijmans, R. J. & Maxted, N. (2002). 36 Geographic Information Systems (GIS) and the Conservation and Use of Plant Genetic Resources. *Managing plant genetic diversity*.

Guisan, A., Edwards, T. C. & Hastie, T. (2002). Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling,* 157**,** 89-100.

Guisan, A. & Thuiller, W. 2005. Predicting species distribution: offering more than simple habitat models. *Ecology Letters,* 8**,** 993-1009.

Guisan, A. & Zimmermann, N. E. (2000). Predictive habitat distribution models in ecology. *Ecological Modelling,* 135**,** 147-186.

Guillot, G. & Rousset, F. (2012). Dismantling the Mantel tests. *Methods in Ecology and Evolution*, 4(4), 336-344.

Gustine, D. L., Voigt, P. W., Brummer, E. C. & Papadopoulos, Y. A. (2002). Genetic variation of RAPD markers for North American white clover collections and cultivars. *Crop science,* 42**,** 343-347.

Guthridge, K. M., Dupal, M. P., Kölliker, R., Jones, E. S., Smith, K. F. & Forster, J. W. (2001). AFLP analysis of genetic diversity within and between populations of perennial ryegrass (*Lolium perenne* L.). *Euphytica,* 122**,** 191-201.

Hajjar, R. & Hodgkin, T. (2007). The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica,* 156**,** 1-13.

Halpin, P. N. (1997). Global climate change and natural-area protection: management responses and research directions. *Ecological Applications,* 7**,** 828-843.

Hamilton, M. B. (1994). *Ex situ* conservation of wild plant species: time to reassess the genetic assumptions and implications of seed banks. *Conservation Biology,* 8**,** 39-49.

Hampe, A. & Petit, R. J. (2005). Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters,* 8**,** 461-467.

Hamrick, J. L. & Godt, M. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 351**,** 1291-1298.

Hamrick, J. L. & Godt, M. W. (1990). Allozyme diversity in plant species. *Plant population genetics, breeding, and genetic resources.***,** 43-63.

Hanley, J. A. & Mcneil, B. J. (1982). The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology,* 143**,** 29-36.

Hanssen-Bauer, I., Drange, H., Førland, E. J., Roald, L. A., Børsheim, K. Y., Hisdal, H., Lawrence, D., Nesje, A., Sandven, S., Sorteberg, A., Sundby, S., Vasskog, K. & Ådlandsvik, B. (2009). Klima i Norge 2100. Bakgrunnsmateriale til NOU Klimatilplassing. Oslo: Norsk klimasenter.

Hargreaves, S., Maxted, N., Hirano, R., Abberton, M., Skã¸T, L. & Ford-Lloyd, B. 2010. Islands as refugia of Trifolium repens genetic diversity. *Conservation Genetics,* 11**,** 1317-1326.

Harlan, J. R. (1975). Geographic patterns of variation in some cultivated plants. *Journal of Heredity,* 66**,** 182-191.

Harlan, J. R. & De Wet, J. M. (1971). Towards a rational classification of cultivated plants. *Taxon***,** 509-517.

Hartl, D. L. & Clark, A. G. (1997). *Principles of population genetics,* Sinauer associates Sunderland.

Heaton, H., Whitkus, R. & Gómez-Pompa, A. (1999). Extreme ecological and phenotypic differences in the tropical tree chicozapote (*Manilkara zapota* (L.) P. Royen) are not matched by genetic divergence: a random amplified polymorphic DNA (RAPD) analysis. *Molecular Ecology,* 8**,** 627-632.

Heikkinen, R. K., Luoto, M., Araújo, M. B., Virkkala, R., Thuiller, W. & Sykes, M. T. (2006). Methods and uncertainties in bioclimatic envelope modelling under climate change. *Progress in Physical Geography,* 30**,** 751-777.

Helgadóttir, Á., Østrem, L., Collins, R., Humphreys, M., Marshall, A., Julier, B., Gastal, F., Barre, P. & Louarn, G. (2016). Breeding Forages to Cope with Environmental Challenges in the Light of Climate Change and Resource Limitations. *Breeding in a World of Scarcity.* Springer.

Heller, N. E. & Zavaleta, E. S. (2009). Biodiversity management in the face of climate change: a review of 22 years of recommendations. *Biological Conservation,* 142**,** 14-32.

Henningsson, S. S. & Alerstam, T. (2005). Patterns and determinants of shorebird species richness in the circumpolar Arctic. *Journal of Biogeography,* 32**,** 383-396.

Hernandez, P. A., Graham, C. H., Master, L. L. & Albert, D. L. (2006). The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography,* 29**,** 773-785.

Herrmann, D., Boller, B., Widmer, F. & Kolliker, R. (2005). Optimization of bulked AFLP analysis and its application for exploring diversity of natural and cultivated populations of red clover. *Genome,* 48**,** 474-486.

Herrmann, D., Poncet, B. N., Manel, S., Rioux, D., Gielly, L., Taberlet, P. & Gugerli, F. (2010). Selection criteria for scoring amplified fragment length polymorphisms (AFLPs) positively affect the reliability of population genetic parameter estimates. *Genome,* 53**,** 302-310.

Heywood, V. H. (2008). Challenges of *in situ* conservation of crop wild relatives. *Turkish journal of Botany,* 32**,** 421-432.

Higgins, S. I., Lavorel, S. & Revilla, E. (2003). Estimating plant migration rates under habitat loss and fragmentation. *Oikos,* 101**,** 354-366.

Hijmans, R. J., Cameron, S., Parra, J., Jones, P. G. & Jarvis, A. (2005). WorldClim, version 1.3. Berkeley: University of California.

Hijmans, R. J., Guarino, L. & Mathur, P. (2004). *DIVA-GIS.Version 7.5.A geographic information system for the analysis of species distribution data*.

Hijmans, R. J. & Van Etten, J. (2014). raster: Geographic data analysis and modeling. *R package version,* 2**,** 15.

Hoban, S. & Schlarbaum, S. (2014). Optimal sampling of seeds from plant populations for ex-situ conservation of genetic biodiversity, considering realistic population structure. *Biological Conservation,* 177**,** 90-99.

Hodgkin, T. & Guarino, L. (1997). Ecogeographical surveys: a review. *Bocconea,* 7**,** 21-26.

Hof, A. R., Jansson, R. & Nilsson, C. (2012). Future climate change will favour non-specialist mammals in the (sub) arctic. *PloS one,* 7**,** e52574.

Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J. M., Skovmand, B., Taba, S. & Warburton, M. (1999). Plant genetic resources: what can they contribute toward increased crop productivity? *Proceedings of the National Academy of Sciences of the United States of America,* 96**,** 5937-5943.

Holland, B. R., Clarke, A. C. & Meudt, H. M. (2008). Optimizing automated AFLP scoring parameters to improve phylogenetic resolution. *Systematic Biology,* 57**,** 347-366.

Hunter, D., Maxted, N., Heywood, V., Kell, S. & Borelli, T. (2012). Protected Areas and the Challenge of Conserving Crop Wild Relatives. *PARKS. The International Journal of Protected Areas and Conservation,* 18.1**,** 87.

Huntley, B., Collingham, Y. C., Willis, S. G. & Green, R. E. (2008). Potential impacts of climatic change on European breeding birds. *PloS one,* 3**,** e1439.

IBM Corp, (2013). IBM SPSS statistics for windows. Armonk, NY: IBM Corp.

International Barley Genome Sequencing Consortium. (2012). A physical, genetic and functional sequence assembly of the barley genome. *Nature,* 491**,** 711-716.

IPCC, (2014). Climate Change 2014: Synthesis Report, Contribution of Working Groups l, ll and lll to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: R.K. Pachauri & L. A. Meyer. eds., Geneva, Switzerland.

IPGRI, (1994). ***In situ*** conservation of crop and agroforestry species. Prepared for the CGIAR Mid-Term Meeting, 23-26 May. New Dehli, India.

Iriondo, J. M., Fielder, H., Fitzgerald, H., Kell, S. P., Labokas, J., Magos Brehm, J., Negri, V., Phillips, J., Rubio Teso, M. L., Sensen, S., Taylor, N. & Maxted, N. (2016). National Strategies for the Conservation of Crop Wild Relatives. In: Maxted, N., Dulloo, M. E. & Ford-Loyd, B. V. eds., *Enhancing Crop Genepool Use: Capturing Wild Relative and Landrace Diversity for Crop Improvement.* Wallingford, UK: CAB International.

Iriondo, J. M., Maxted, N. & Dulloo, E. (2008). *Conserving Plant Genetic Diversity in Protcted Areas: Population Management of Crop Wild Relatives,* Wallingford, UK, CAB International.

Iriondo, J. M., Maxted, N., Kell, S. P., Ford-Lloyd, B. V., Lara-Romano, C., Labokas, J. & Magos Brehm, J. (2012). Quality standards for genetic reserve conservation of crop wild relatives. In: Maxted, N., Dulloo, M. E., Ford-Lloyd, B. V., Frese, L., Iriondo, J. M. & Pinheiro De Carvalho, M. A. A. eds., *Agrobiodiversity conservation: securing the diversity of crop wild relatives and landraces.* Wallingford: CABI Publishing.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., Scherer-Lorenzen, M., Schmid, B., Tilman, D. & Van Ruijven, J. (2011). High plant diversity is needed to maintain ecosystem services. *Nature,* 477**,** 199-202.

IUCN, (2001). IUCN Red List Categories and Criteria: Version 3.1. Gland, Switzerland and Cambridge, UK: IUCN Species Survival Commission. IUCN.

Jackson, L. E., Pascual, U. & Hodgkin, T. (2007). Utilizing and conserving agrobiodiversity in agricultural landscapes. *Agriculture, Ecosystems & Environment,* 121**,** 196-210.

Jain, S. K. (1975). Genetic reserves. *Crop genetic resources for today and tomorrow***,** 379-396.

Jaradat, A. A. (2015). Beyond Biodiversity: Ecosystem Services of Crop Wild Relatives. In: Redden, R., Yadav, S. S., Maxted, N., Dulloo, M. E., Guarino, L. & Smith, P. eds., *Crop Wild Relatives and Climate Change.* Hoboken, NJ, USA: John Wiley & Sons.

Jarvis, A., Lane, A. & Hijmans, R. J. (2008). The effect of climate change on crop wild relatives. *Agriculture Ecosystems & Environment,* 126**,** 13-23.

Jarvis, S., Fielder, H., Hopkins, J., Maxted, N. & Smart, S. (2015). Distribution of crop wild relatives of conservation priority in the UK landscape. *Biological Conservation,* 191**,** 444-451.

Johns, M. A., Skroch, P. W., Nienhuis, J., Hinrichsen, P., Bascur, G. & Munoz-Schick, C. (1997). Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. *Crop Science,* 37**,** 605-613.

Johnson, R. C., Kisha, T. J., Pecetti, L., Romani, M. & Richter, P. (2011). Characterization of Poa supina from the Italian Alps with AFLP Markers and Correlation with Climatic Variables. *Crop Science,* 51**,** 1627-1636.

Jones, H. P., Hole, D. G. & Zavaleta, E. S. (2012). Harnessing nature to help people adapt to climate change. *Nature Climate Change,* 2**,** 504-509.

Jones, J. D., Witek, K., Verweij, W., Jupe, F., Cooke, D., Dorling, S., Tomlinson, L., Smoker, M., Perkins, S. & Foster, S. (2014). Elevating crop disease resistance with cloned genes. *Philosophical transactions of the Royal Society of London.Series B, Biological sciences,* 369**,** 20130087.

Jump, A. S. & Penuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters,* 8**,** 1010-1020.

Kell, S., Qin, H., Chen, B., Ford-Lloyd, B., Wei, W., Kang, D. & Maxted, N. (2015). China's crop wild relatives: Diversity for agriculture and food security. *Agriculture, Ecosystems & Environment,* 209**,** 138-154.

Kell, S. P., Knüpffer, H., Jury, S. L., Ford-Lloyd, B. V. & Maxted, N. (2008). Crops and wild relatives of the Euro-Mediterranean region: making and using a conservation catalogue.

In: Maxted, N., Ford-Lloyd, B. V., Kell, S. P., Iriondo, J., Dulloo, E. & Turok, J. eds., *Crop Wild Relative Conservation and Use.* Wallingford, UK: CAB International.

Kell, S. P., Maxted, N. & Bilz, M. (2011). 28 European Crop Wild Relative Threat Assessment: Knowledge Gained and Lessons Learnt. *Agrobiodiversity Conservation Securing the Diversity of Crop Wild Relatives and Landraces***,** 218.

Kelly, A. E. & Goulden, M. L. (2008). Rapid shifts in plant distribution with recent climate change. *Proceedings of the National Academy of Sciences of the United States of America,* 105**,** 11823-11826.

Keyzer, M. A., Merbis, M. D., Pavel, I. & Van Wesenbeeck, C. F. A. (2005). Diet shifts towards meat and the effects on cereal use: can we feed the animals in 2030? *Ecological Economics,* 55**,** 187-202.

Khanlou, K. M., Vandepitte, K., Asl, L. K. & Bockstaele, E. V. (2011). Towards an optimal sampling strategy for assessing genetic variation within and among white clover (*Trifolium repens* L.) cultivars using AFLP. *Genetics and molecular biology,* 34**,** 252-258.

Khoury, C. K., Bjorkman, A. D., Dempewolf, H., Ramirez-Villegas, J., Guarino, L., Jarvis, A., Rieseberg, L. H. & Struik, P. C. (2014). Increasing homogeneity in global food supplies and the implications for food security. *Proceedings of the National Academy of Sciences,* 111**,** 4001-4006.

Khoury, C. K., Greene, S., Wiersema, J., Maxted, N., Jarvis, A. & Struik, P. C. (2013). An inventory of crop wild relatives of the United States. *Crop Science,* 53**,** 1496-1508.

Khoury, C. K., Heider, B., Castãeda-ÃLvarez, N. P., Achicanoy, H. A., Sosa, C. C., Miller, R. E., Scotland, R. W., Wood, J. R. I., Rossel, G., Eserman, L. A., Jarret, R. L., Yencho, G. C., Bernau, V., Juarez, H., Sotelo, S., De Haan, S. & Struik, P. C. (2015). Distributions, *ex situ* conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato Ipomoea batatas (L.) Lam., I. series Batatas]. *Frontiers in Plant Science,* 6**,** 251.

Kålås, J. A., Viken, A. & Bakken, T. (2006). *Norwegian Red List,* Norway, Artsdatabaken.

Kölliker, R., Herrmann, D., Boller, B. & Widmer, F. (2003). Swiss Mattenklee landraces, a distinct and diverse genetic resource of red clover (*Trifolium pratense* L.). *Theoretical and Applied Genetics,* 107**,** 306-315.

Kölliker, R., Jones, E., Jahufer, M. & Forster, J. (2001). Bulked AFLP analysis for the assessment of genetic diversity in white clover (*Trifolium repens* L.). *Euphytica,* 121**,** 305-315.

Laikre, L., Allendorf, F. W., Aroner, L. C., Baker, C. S., Gregovich, D. P., Hansen, M. M., Jackson, J. A., Kendall, K. C., Mckelvey, K., Neel, M. C., Olivieri, I., Ryman, N., Schwartz, M. K., Bull, R. S., Stetz, J. B., Tallmon, D. A., Taylor, B. L., Vojta, C. D.,

Waller, D. M. & Waples, R. S. (2010). Neglect of Genetic Diversity in Implementation of the Convention on Biological Diversity. *Conservation Biology,* 24**,** 86-88.

Lakew, B., Eglinton, J., Henry, R. J., Baum, M., Grando, S. & Ceccarelli, S. (2011). The potential contribution of wild barley (Hordeum vulgare ssp. spontaneum) germplasm to drought tolerance of cultivated barley (H. vulgare ssp. vulgare). *Field Crops Research,* 120**,** 161-168.

Landucci, F., Panella, L., Lucarini, D., Gigante, D., Donnini, D., Kell, S., Maxted, N., Venanzoni, R. & Negri, V. (2014). A prioritized inventory of crop wild relatives and wild harvested plants of Italy. *Crop Science,* 54**,** 1628-1644.

Laribi, B., Zoghlami, N., Lamine, M., Kouki, K., Ghorbel, A. & Mougou, A. (2011). RAPD based assessment of genetic diversity among annual caraway (Carum carvi) populations. *Eurasia J.Biosci,* 5**,** 37-47.

Legendre, P & Fortin, M.J. (2010) Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. *Molecular Ecology Research*, 10, 831-844.

Legendre, P. & Legendre, L. (2012) Numerical Ecology, 3rd edition. Elsevier, Amsterdam, pp990.

Legendre, P., Fortin, M. J. , Borcard, D. (2015) Should the Mantel test be used in spatial analysis? *Methods in Ecology and Evolution*, 6, 1239-1247.

Lesica, P. & Allendorf, F. W. (1995). When are peripheral populations valuable for conservation? *Conservation Biology,* 9**,** 753-760.

Li, D.-Z. & Pritchard, H. W. (2009). The science and economics of *ex situ* plant conservation. *Trends in plant science,* 14**,** 614-621.

Lid, J. & Lid, D. T. (2005). *Norsk flora,* Oslo, Det Norske Samlaget.

Lindgaard, A. & Henriksen, S. (2011). Norwegian red list for ecosystems and habitat types 2011. *Norwegian Biodiversity Information Centre, Trondheim*.

Liu, C., Berry, P. M., Dawson, T. P. & Pearson, R. G. (2005). Selecting thresholds of occurrence in the prediction of species distributions. *Ecography,* 28**,** 385-393.

Lobell, D. B. & Field, C. B. (2007). Global scale climate–crop yield relationships and the impacts of recent warming. *Environmental research letters,* 2**,** 014002.

Lobell, D. B., Schlenker, W. & Costa-Roberts, J. 2011. Climate trends and global crop production since 1980. *Science (New York, N.Y.),* 333**,** 616-620.

Lobo, J. M., Castro, I. & Moreno, J. C. (2001). Spatial and environmental determinants of vascular plant species richness distribution in the Iberian Peninsula and Balearic Islands. *Biological Journal of the Linnean Society,* 73**,** 233-253.

Loiselle, B. A., Jørgensen, P. M., Consiglio, T., Jiménez, I., Blake, J. G., Lohmann, L. G. & Montiel, O. M. (2008). Predicting species distributions from herbarium collections: does climate bias in collection sampling influence model outcomes? *Journal of Biogeography,* 35**,** 105-116.

Lovejoy, T. E. (2005). Conservation with a changing climate. *In:* T.E. Lovejoy and L. Hannah. eds., *Climate change and Biodiversity.* New Haven: Yale University Press.

Luikart, G., England, P. R., Tallmon, D., Jordan, S. & Taberlet, P. (2003). The power and promise of population genomics: from genotyping to genome typing. *Nature reviews genetics,* 4**,** 981-994.

Lynch, M. & Milligan, B. G. (1994). Analysis of population genetic structure with RAPD markers. *Molecular ecology,* 3**,** 91-99.

Magos Brehm, J. (2009). *Conservation of wild plant genetic resources in Portugal.* PhD, University of Birmingham.

Magos Brehm, J., Ford-Lloyd, B. V., Maxted, N. & Martins-Loução, M. A. (2012). Using neutral genetic diversity to prioritise crop wild relative populations: a Portuguese endemic case study for Dianthus cintranus Boiss. & Reut. subsp. barbatus R. Fern. & Franco. In: Maxted, N., Dulloo, M. E., Ford-Lloyd, B. V., Frese, L., Iriondo, J. M. & Pinheiro De Carvalho, M. A. A. eds., *Agrobiodiversity conservation: securing the diversity of crop wild relatives and landraces.* Wallingford, UK: CAB International.

Magos Brehm, J., Maxted, N., Ford-Lloyd, B. V. & Martins-Loução, M. A. (2008). National inventories of crop wild relatives and wild harvested plants: case-study for Portugal. *Genetic Resources and Crop Evolution,* 55**,** 779-796.

Magos Brehm, J., Saifan, S., Taifour, H., Abu Laila, K., Al-Assaf, A., Al-Oqlah, A., Al-Sheyab, F., Bani-Hani, R., Ghazanfar, S., Haddad, N., Shibli, R., Abu Taleb, T., Bint-Ali, B. & Maxted, N. (2016). Crop wild relatives, a priority in Jordan? Developing a national strategy for the conservation of plant diversity in Jordan using a participatory approach. In: N. Maxted., M.E. Dulloo., & B. V. Ford-Loyd. eds., *Enhancing Crop Genepool Use: Capturing Wild Relative and Landrace Diversity for Crop Improvement.* Wallingford, UK: CAB International.

Maldonado, C., Molina, C. I., Zizka, A., Persson, C., Taylor, C. M., Albán, J., Chilquillo, E., Rønsted, N. & Antonelli, A. (2015). Estimating species diversity and distribution in the era of Big Data: to what extent can we trust public databases? *Global Ecology and Biogeography,* 24**,** 973-984.

Marfil, C. F., Hidalgo, V. & Masuelli, R. W. (2015). *In situ* conservation of wild potato germplasm in Argentina: Example and possibilities. *Global Ecology and Conservation,* 3**,** 461-476.

Margules, C. R. 1(989). Introduction to some Australian developments in conservation evaluation. *Biological Conservation,* 50**,** 1-11.

Marshall, D. R. & Brown, A. H. D. (1975). Optimum sampling strategies in genetic conservation. In: O. Frankel, & J. Hawkes. eds., *Crop Genetic Resources for Today and Tomorrow*. Cambridge, UK.: Cambridge University Press.

Marshall, D. R. & Brown, A. H. D. (1995). A basic sampling strategy: theory and practice. *In:* Guarino, V. L., Rao, R. & Reid, R. (eds.) *Collecting Plant Diversity: Technical Guidelines.* Rome: CAB International.

Martin, T. G. & Watson, J. E. M. (2016). Intact ecosystems provide best defence against climate change. *Nature Climate Change,* 6**,** 122-124.

Maxted, N., Van Slageren, M. W. & Rihan, J. R. (1995). Ecogeographic surveys. *In:* Ramanatha Rao, V. & Reid, R. (eds.) *Collecting plant genetic diversity. Technical guidelines.* Wallingford, UK: CAB International.

Maxted, N., Ford-Loyd, B. V. & Hawkes, J. G. (1997a). *Plant Genetic Conservation. The in situ approach,* London, Chapman and Hall.

Maxted, N., Hawkes, J. G., Guarino, L. & Sawkins, M. (1997b). Towards the selection of taxa for plant genetic conservation. *Genetic Resources and Crop Evolution,* 44**,** 337-348.

Maxted, N., Guarino, L., Myer, L. & Chiwona, E. A. (2002). Towards a methodology for on-farm conservation of plant genetic resources. *Genetic Resources and Crop Evolution,* 49**,** 31-46.

Maxted, N., Ford-Lloyd, B. V., Jury, S. L., Kell, S. P. & Scholten, M. A. (2006). Towards a definition of a crop wild relative. *Biodiversity and Conservation,* 8**,** 2673-2685.

Maxted, N. & Kell, S. P. (2008). Linking *in situ* and *ex situ* conservation with use of crop wild relatives. *In:* Maxted, N., Ford-Lloyd, B. V., Kell, S. P., Iriondo, J. M., Dulloo, M. E. & Turok, J. (eds.) *Crop wild relative conservation and use.* Wallingford: CABI Publishing.

Maxted, N., Dulloo, E., Ford-Lloyd, B. V., Iriondo, J. M. & Jarvis, A. (2008a). Gap Analysis: A tool for complementary genetic conservation assessment. *Diversity and Distributions,* 14**,** 1018-1030.

Maxted, N., Iriondo, J. M., Dulloo, M. E. & Lane, A. (2008b). The integration of PGR conservation with protected area management. *In:* Iriondo, J. M., Maxted, N. & Dulloo, M. E. (eds.) *Conserving plant genetic diversity in protected areas.* Wallingford: CAB International.

Maxted, N. & Kell, S. P. (2009). Establishment of a Global Network for the *In Situ* Conservation of Crop Wild Relatives: Status and Needs. Rome, Italy: Food and Agriculture Organisation.

Maxted, N., Kell, S., Ford-Lloyd, B., Dulloo, E. & Toledo, A. (2012). Towards the Systematic Conservation of Global Crop Wild Relative Diversity. *Crop Science,* 52**,** 1-12.

Maxted, N., Magos Brehm, J. & Kell, S. (2013). *Resource Book for Preparation of National Plans of Crop Wild Relatives and Landraces,* Rome, Italy, Food and Agriculture Organization of the UN.

Maxted, N., Avagyan, A., Frese, L., Iriondo, J. M., Margos Brehm, J., Singer, A. & Kell, S. P. (2015). Concept for *in situ* conservation of crop wild relatives of crop wild relatives in Europe. Wild species conservation in genetic reserves working group, European Cooperative Programme for Plant Genetic Resources. Rome, Italy.

Maxted, N., Amri, A., Castañeda-Álvarez, N. P., Dias, S., Dulloo, M. E., Fielder, H., Ford-Lloyd, B. V., Iriondo, J. M., Brehm, J. M. & Nilsen, L. B. (2016). 10 Joining Up the Dots: A Systematic Perspective of Crop Wild Relative Conservation and Use. *Enhancing Crop Genepool Use: Capturing Wild Relative and Landrace Diversity for Crop Improvement***,** 87.

Maxwell, S. L., Fuller, R. A., Brooks, T. M. & Watson, J. E. (2016). Biodiversity: The ravages of guns, nets and bulldozers. *Nature,* 536**,** 143-145.

Mccarthy, J. J., Canziani, O. F., Leary, N. A., Dokken, D. J. & White, K. S. (2001). Climate Change 2001: Impacts, Adaptation, and Vulnerability, Contribution of Working Group II to the Intergovernmental Panel on Climate Change Third Assessment Report. Cambridge.

Mccouch, S., Baute, G. J., Bradeen, J., Bramel, P., Bretting, P. K., Buckler, E., Burke, J. M., Charest, D., Cloutier, S., Cole, G., Dempewolf, H., Dingkuhn, M., Feuillet, C., Gepts, P., Grattapaglia, D., Guarino, L., Jackson, S., Knapp, S., Langridge, P., Lawton-Rauh, A., Lijua, Q., Lusty, C., Michael, T., Myles, S., Naito, K., Nelson, R. L., Pontarollo, R., Richards, C. M., Rieseberg, L., Ross-Ibarra, J., Rounsley, S., Hamilton, R. S., Schurr, U., Stein, N., Tomooka, N., Van, D. K., Van Tassel, D., Toll, J., Valls, J., Varshney, R. K., Ward, J., Waugh, R., Wenzl, P. & Zamir, D. (2013). Agriculture: Feeding the future. *Nature,* 499**,** 23-24.

Mckay, J. K. & Latta, R. G. (2002). Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution,* 17**,** 285-291.

Mcneilly, T. (1997). Patterns of population differentiation. *Bocconea,* 7**,** 89-93.

Meehl, G. A., Covey, C., Taylor, K. E., Delworth, T., Stouffer, R. J., Latif, M., Mcavaney, B. & Mitchell, J. F. B. (2007). The WCRP CMIP3 multimodel dataset: A new era in

climate change research. *Bulletin of the American Meteorological Society,* 88**,** 1383-1394.

Meilleur, B. A. & Hodgkin, T. (2004). *In situ* conservation of crop wild relatives: status and trends. *Biodiversity & Conservation,* 13**,** 663-684.

Merow, C., Smith, M. J. & Silander, J. A. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography,* 36**,** 1058-1069.

Midgley, G. F., Hannah, L., Millar, D., Thuiller, W. & Booth, A. (2003). Developing regional and species-level assessments of climate change impacts on biodiversity in the Cape Floristic Region. *Biological Conservation,* 112**,** 87-97.

Millennium Ecosystem Assessment. (2005). Ecosystems and human well-being. *Washington, DC.*

Moen, A. (1998). *Nasjonalatlas for Norge: Vegetasjon,* Hønefoss, Statens Kartverk.

Mokany, K., Harwood, T. D. & Ferrier, S. (2013). Comparing habitat configuration strategies for retaining biodiversity under climate change. *Journal of Applied Ecology,* 50**,** 519-527.

Moore, F. & Lobell, D. B. (2014). The Fingerprint of Climate Trends on European Crop Yields. AGU Fall Meeting Abstracts, 0713.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature,* 403**,** 853-858.

Müller, C. & Robertson, R. D. (2014). Projecting future crop productivity for global economic modeling. *Agricultural Economics,* 45**,** 37-50.

Nabham, G. P. (2009). *Where our food comes from. Retracing Nikolay Vavulov's quest to end famine,* Washington DC, Island Press.

Neel, M. C. & Cummings, M. P. 2003. Effectiveness of conservation targets in capturing genetic diversity. *Conservation Biology,* 17**,** 219-229.

Nekola, J. C. 1999. Paleorefugia and neorefugia: the influence of colonization history on community pattern and process. *Ecology,* 80**,** 2459-2473.

Nelson, D. R., Adger, W. N. & Brown, K. 2007. Adaptation to environmental change: contributions of a resilience framework. *Annual review of Environment and Resources,* 32**,** 395.

Nenzén, H. K. & Araújo, M. B. 2011. Choice of threshold alters projections of species range shifts under climate change. *Ecological Modelling,* 222**,** 3346-3354.

Nevo, E. 1998. Genetic diversity in wild cereals: regional and local studies and their bearing on conservation *ex situ* and *in situ*. *Genetic Resources and Crop Evolution,* 45**,** 355-370.

NORDGEN, (2016). Crop wild relatives-actions needed to assure conservation of an important genetic resources.

Nordic Gene Bank, (2006). Spice and Medicinal Plants in the Nordic and Baltic Countries. Conservation of Genetic Resources. Alnarp.

Norwegian Environment Agency, (2015). *RE: Norway.* Available at http://www.miljodirektoratet.no/en/Areas-of-activity1/Species-and-ecosystems/ [Accessed 6 April 2016]

Norwegian Environment Agency. (2016). *Protected Areas*. Available at http://www.miljodirektoratet.no/en/Areas-of-activity1/Conservation-Areas/Management-of-protected-areas/ *[Accessed 6 April 2017]*

Norwegian Environment Agency. (2017). *Categories of protected areas* [Online]. Available: http://www.miljodirektoratet.no/en/Areas-of-activity1/Conservation-Areas/Categories-of-protected-areas/ [Accessed 1 April 2017].

Norwegian Ministry For Agriculture And Food. (2008). Country Report on the State of Plant Genetic Resources for Food and Agriculture in Norway. Rome, Italy: FAO.

Norwegian Ministry Of Agriculture And Food. (2015). *Svalbard Global Seed Vault returns seeds to Syrian Gene Bank.* Available at: https://www.regjeringen.no/en/aktuelt/svalbard-globale-frohvelv-returnerer-fro-til-genbank-i-syria/id2457931/ [Accessed 6 April 2017]

Nuñez, T. A., Lawler, J. J., Mcrae, B. H., Pierce, D., Krosby, M. B., Kavanagh, D. M., Singleton, P. H. & Tewksbury, J. J. (2013). Connectivity planning to address climate change. *Conservation Biology*, 27**,** 407-416.

Nybom, H. & Bartish, I. V. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in plant ecology, evolution and systematics,* 3**,** 93-114.

Nybø, S., Certain, G. & Skarpaas, O. (2011). The Norwegian Nature Index 2010. Oslo: Directorate of Nature.

Olesen, J. E. & Bindi, M. (2002). Consequences of climate change for European agricultural productivity, land use and policy. *European Journal of Agronomy,* 16**,** 239-262.

Pacifici, M., Foden, W. B., Visconti, P., Watson, J. E. M., Butchart, S. H. M., Kovacs, K. M., Scheffers, B. R., Hole, D. G., Martin, T. G. & Akçakaya, H. R. (2015). Assessing species vulnerability to climate change. *Nature Climate Change,* 5**,** 215-224.

Panella, L., Landucci, S., Torricelli, R., Gigante, D., Donnini, D., Venanzoni, R. & Negri, V. (2014). The national crop wild relative strategy for Italy: first steps to be taken. *PGR Secure*.

Parmesan, C. & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature,* 421**,** 37-42.

Parra-Quijano, M., Iriondo, J. M. & Torres, E. (2011). Chapter 6: Strategies for the collecting of wild species. In: L. Guarino, V., Rao, R. & Reid, R. eds., *Collecting Plant Genetic Diversity: Technical Guidlines-update.* Wallingford: CAB International.

Parra-Quijano, M., Iriondo, J. M., Frese, L. & Torres, E. (2012a). Spatial and Ecogeographic Approaches for Selecting Genetic Reserves in Europe. In: Maxted, N. & *et al*. eds., *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces.* CABI International.

Parra-Quijano, M., Iriondo, J. M. & Torres, E. (2012b). Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. *Genetic Resources and Crop Evolution,* 59**,** 205-217.

Parra-Quijano, M., Iriondo, J. M. & Torres, E. (2012c). Improving representativeness of genebank collections through species distribution models, gap analysis and ecogeographical maps. *Biodiversity and Conservation,* 21**,** 79-96.

Parra-Quijano, M., López, F., Iriondo, J. M., Torres, E. & Molina, A. (2016). CAPFITOGEN Tools User Manual Version 2.0. FAO, Rome: International Treaty on Plant Genetic Resources for Food and Agriculture.

Parry, M., Rosenzweig, C. & Livermore, M. (2005). Climate change, global food supply and risk of hunger. *Philosophical transactions of the Royal Society of London.Series B, Biological sciences,* 360**,** 2125-2138.

Peakall, R. & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular ecology notes,* 6**,** 288-295.

Peakall, R. & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics,* 28**,** 2537-2539.

Pearce, J. & Ferrier, S. (2000). Evaluating the predictive performance of habitat models developed Using Logistic Regression. *Ecological Modelling,* 133**,** 225-245.

Pearman, P. B. (2001). Conservation value of independently evolving units: Sacred cow or testable hypothesis? *Conservation Biology,* 15**,** 780-783.

Pecetti, L., Romani, M., De Rosa, L. & Piano, E. (2008). Selection of grazing-tolerant lucerne cultivars. *Grass and Forage Science,* 63**,** 360-368.

Perrier, X., De Langhe, E., Donohue, M., Lentfer, C., Vrydaghs, L., Bakry, F., Carreel, F., Hippolyte, I., Horry, J. P., Jenny, C., Lebot, V., Risterucci, A. M., Tomekpe, K., Doutrelepont, H., Ball, T., Manwaring, J., De Maret, P. & Denham, T. (2011). Multidisciplinary perspectives on banana (Musa spp.) domestication. *Proceedings of the National Academy of Sciences of the United States of America,* 108**,** 11311-11318.

Peters, R. L. & Myers, J. P. (1991). Preserving biodiversity in a changing climate. *Issues in Science and Technology;(United States),* 8.

Phillips, J., Kyratzis, A., Christoudoulou, C., Kell, S. & Maxted, N. (2014). Development of a national crop wild relative conservation strategy for Cyprus. *Genetic Resources and Crop Evolution,* 61**,** 817-827.

Phillips, J., Asdal, Å., Magos Brehm, J., Rasmussen, M. & Maxted, N. (2016). *In situ* and *ex situ* diversity analysis of priority crop wild relatives in Norway. *Diversity and Distributions,* 22**,** 1112-1126.

Phillips, J., Brehm, J. M., Van Oort, B., Asdal, Å., Rasmussen, M. & Maxted, N. (2017). Climate change and national crop wild relative conservation planning. *Ambio***,** 1-14.

Phillips, S. J., Dudík, M. & Schapire, R. E. (2004). A maximum entropy approach to species distribution modeling. Proceedings of the twenty-first international conference on Machine learning. ACM, 83.

Phillips, S. J., Anderson, R. P. & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling,* 190**,** 231-259.

Phillips, S. J. & Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography,* 31**,** 161-175.

Pimentel, D., Wilson, C., Mccullum, C., Huang, R., Dwen, P., Flack, J., Tran, Q., Saltman, T. & Cliff, D. (1997). Economic and environmental benefits of biodiversity. *Bioscience,* 47**,** 747-757.

Pinheiro De Carvalho, M. Â. A., Nóbrega, H., Freitas, G., Fontinha, S. & Frese, L. (2012). Towards the establishment of a genetic reserve for *Beta patula* Aiton. In: Maxted, N., Dulloo, M. E., Ford-Lloyd, B. V., Frese, L., Iriondo, J. M. & Pinheiro De Carvalho, M. A. A. eds., *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces.* Wallingford, UK: CAB International.

Pittock, A. B. & Jones, R. N. 2000. Adaptation to what and why? *Environmental monitoring and assessment,* 61**,** 9-35.

Pompanon, F., Bonin, A., Bellemain, E. & Taberlet, P. 2005. Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics,* 6**,** 847-846.

Prather, M., Flato, G., Friedlingstein, P., Jones, C., Lamarque, J. F., Liao, H. & Rasch, P. (2013). IPCC 2013: Annex II: Climate System Scenario Tables. *In:* Stocker, T. F., Quin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y.,

Bex, V. & Midgley, P. M. (eds.) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge, United Kingdom and New York, NY, USA.: Cambridge University Press.

Prescott-Allen, R. & Prescott Allen, C. (1986). *The first resource: Wild species in the North American economy,* New Haven, CT, Yale University.

PWC, (2013). Crop wild relatives: A valuable resource for crop development. Price Waterhouse Cooper.

QIAGEN, (2012). DNeasy Plant Handbook. US: Qiagen.

Qualset, C., Mcguire, P. & Warburton, M. (1995). In California:'Agrobiodiversity'key to agricultural productivity. *California agriculture,* 49**,** 45-49.

Ramirez-Villegas, J., Cuesta, F., Devenish, C., Peralvo, M., Jarvis, A. & Arnillas, C. A. (2014). Using species distributions models for designing conservation strategies of Tropical Andean biodiversity under climate change. *Journal for Nature Conservation,* 22**,** 391-404.

Ramirez-Villegas, J., Khoury, C., Jarvis, A., Debouck, D. G. & Guarino, L. (2010). A gap analysis methodology for collecting crop genepools: A case study with phaseolus beans. *PLoS ONE,* 5**,** e13497.

Rao, V. R. & Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. *Plant cell, tissue and organ culture,* 68**,** 1-19.

Rebelo, A. G. (1994). Iterative selection procedures: centres of endemism and optimal placement of reserves. In: B.J. Huntley., eds., *Botanical Diversity in Southern Africa.* Pretoria: National Botanic Institute.

Reed, D. H. & Frankham, R. (2001). How closely correlated are molecular and quantitative measures of genetic variation? a meta-analysis. *Evolution,* 55**,** 1095-1103.

Reed, D. H. & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation Biology,* 17**,** 230-237.

Richardson, B. A., Rehfeldt, G. E. & Kim, M. S. (2009). Congruent Climate-Related Genecological Responses from Molecular Markers and Quantitative Traits for Western White Pine (Pinus monticola). *International journal of plant sciences,* 170**,** 1120-1131.

Rocchini, D., Hortal, J., Lengyel, S., Lobo, J. M., Jimenez-Valverde, A., Ricotta, C., Bacaro, G. & Chiarucci, A. (2011). Accounting for uncertainty when mapping species distributions: the need for maps of ignorance. *Progress in Physical Geography,* 35**,** 211-226.

Rogelj, J., Meinshausen, M. & Knutti, R. (2012). Global warming under old and new scenarios using IPCC Climate Sensitivity Range Estimates. *Nature Climate Change,* 2**,** 248-253.

Rosenzweig, C., Iglesias, A., Yang, X. B., Epstein, P. R. & Chivian, E. (2001). Climate change and extreme weather events; implications for food production, plant diseases, and pests. *Global change and human health,* 2**,** 90-104.

Rosenzweig, C., Elliott, J., Deryng, D., Ruane, A. C., Muller, C., Arneth, A., Boote, K. J., Folberth, C., Glotter, M., Khabarov, N., Neumann, K., Piontek, F., Pugh, T. A., Schmid, E., Stehfest, E., Yang, H. & Jones, J. W. (2014). Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. *Proceedings of the National Academy of Sciences of the United States of America,* 111**,** 3268-3273.

Royal Society Of London, (2009). *Reaping the Benefits: Science and the Sustainable Intensification of Global Agriculture,* London, Royal Society.

Rubio Teso, M. L., Iriondo, J. M., Parra, M. & Torres, E. (2013). National strategy for the conservation of crop wild relatives of Spain. *PGR Secure.*

Ruete, A. (2015). Displaying bias in sampling effort of data accessed from biodiversity databases using ignorance maps. *Biodiversity Data Journal*.

Scheldeman, X. & Van Zonneveld, M. (2010). Training Manual on Spatial Analysis of Plant Diversity and Distribution. Rome, Italy: Bioversity International.

Scheldeman, X., Willemen, L., D'eeckenbrugge, G. C., Romeijn-Peeters, E., Restrepo, M. T., Motoche, J. R., Jiménez, D., Lobo, M., Medina, C. I. & Reyes, C. (2007). Distribution, diversity and environmental adaptation of highland papayas (*Vasconcellea* spp.) in tropical and subtropical America. *Biodiversity and Conservation,* 16**,** 1867-1884.

Schlötterer, C. (2004). The evolution of molecular markers—just a matter of fashion? *Nature Reviews Genetics,* 5**,** 63-69.

Schoen, D. J. & Brown, A. H. 1993. Conservation of allelic richness in wild crop relatives is aided by assessment of genetic markers. *Proceedings of the National Academy of Sciences of the United States of America,* 90**,** 10623-10627.

Shiferaw, B., Prasanna, B. M., Hellin, J. & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security,* 3**,** 307-327.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S., Singh, P. K., Singh, S. & Govindan, V. (2011). The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annual review of phytopathology,* 49**,** 465-481.

Skøt, L., Humphreys, M. O., Armstead, I., Heywood, S., Skøt, K. P., Sanderson, R., Thomas, I. D., Chorlton, K. H. & Hamilton, N. R. S. (2005). An association mapping approach to identify flowering time genes in natural populations of *Lolium perenne* (L.). *Molecular Breeding,* 15**,** 233-245.

Slatkin, M. & Takahata, N. (1985). The average frequency of private alleles in a partially isolated population. *Theoretical Population Biology,* 28**,** 314-331.

Solomon, S. (2007). *Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC,* Cambridge University Press.

Stafford Smith, M., Horrocks, L., Harvey, A. & Hamilton, C. (2011). Rethinking adaptation for a 4 degrees C world. *Philosophical transactions.Series A, Mathematical, physical, and engineering sciences,* 369**,** 196-216.

Norway Statistics. (2016). *Area of land and freshwater.* Available at: https://www.ssb.no/en/natur-og-miljo/statistikker/arealdekke/aar/2017-03-06 [Accessed 6 April 2017]

Stuart, S. N. & Adams, R. J. (1990). Biodiversity in SubSaharan Africa and its Islands: Conservation, Management and Sustainable Use. Gland, Switzerland: International Union for Conservation of Nature and Natural resources.

Svalheim, E. & Asdal, Å. (2011). Conservation of Biodiversity and Genetic Resources in Semi-Natural Grasslands in Norway. In: Maxted, N., Dulloo, E. M., Ford-Lloyd, B. V., Frese, L., Iriondo, J. & Pinheiro De Carvalho, M. A. A. eds., *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces.* Wallingford, UK: CAB International.

Sánchez-Velásquez, L. R. (1991). *Zea diploperennis*: mejoramiento genético del maíz, ecología y la conservación de recursos naturales. *Tiempos de Ciencia,* 24**,** 1-8.

Sætersdal, M., Birks, H. J. & Peglar, S. (1998). Predicting changes in Fennoscandian vascular-plant species richness as a result of future climatic change. *Journal of Biogeography,* 25**,** 111-112.

Tabachnick, B. G. & Fidell, L. S. (2001). *Using Multivariate Statistics,* Boston, Allyn and Bacon.

Tanksley, S. D. & McCouch, S. R. (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. *Science (New York, N.Y.),* 277**,** 1063-1066.

Taylor, N. G., Holubec, V., Chobot, K., Parra-Quijano, M., Maxted, N. & Kell, S. (2013). Systematic crop wild relative conservation planning for the Czech Republic. *Crop wild relative***,** 5.

R Core Team. (2012). r: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

The Crop Trust. (2016). FAQ About the Seed Vault. Available at: https://www.croptrust.org/our-work/svalbard-global-seed-vault/faq-about-the-vault/ [Accessed 6 April 2017]

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., De Siqueira, M. F., Grainger, A. & Hannah, L. (2004). Extinction risk from climate change. *Nature,* 427**,** 145-148.

Thomas, C. D., Gillingham, P. K., Bradbury, R. B., Roy, D. B., Anderson, B. J., Baxter, J. M., Bourn, N. A., Crick, H. Q., Findon, R. A., Fox, R., Hodgson, J. A., Holt, A. R., Morecroft, M. D., O'hanlon, N. J., Oliver, T. H., Pearce-Higgins, J. W., Procter, D. A., Thomas, J. A., Walker, K. J., Walmsley, C. A., Wilson, R. J. & Hill, J. K. (2012). Protected areas facilitate species' range expansions. *Proceedings of the National Academy of Sciences of the United States of America,* 109**,** 14063-14068.

Thomson, L., Graudal, L. & Kjaer, E. (2001). Selection and management of *in situ* gene conservation areas for target species. In: FAO, DFSC & IPGRI. eds., *Forest genetic resources conservation and management, in Managed natural forest and protected areas.* Rome, Italy: International Plant Genetic Resources Institute.

Thormann, I., Parra-Quijano, M., Endresen, D. T. F., Rubio-Teso, M. L., Iriondo, M. J. & Maxted, N. (2014). Predictive characterization of crop wild relatives and landraces. Rome, Italy: Bioversity International.

Thuiller, W., Lavorel, S., Araújo, M. B., Sykes, M. T. & Prentice, I. C. (2005). Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America,* 102**,** 8245-8250.

Thuiller, W., Alberta, C., Araujo, M. B., Berry, P. M., Cabez, M., Guisane, A., Hicklerf, T., Midgleyg, G. F., Paterson, J., Schurrh, F. M., Sykes, M. T. & Zimmerman, N. E. (2008). Predicting global change impacts on plant species' distributions: Future challenges. *Perspectives in Plant Ecology, Evolution and Systematics,* 9**,** 137-152.

Tigerstedt, P. (1994). Adaptation, variation and selection in marginal areas. *Breeding Fodder Crops for Marginal Conditions.* Springer.

Tømmervik, H., Johansen, B., Tombre, I., Thannheiser, D., Høgda, K. A., Gaare, E. & Wielgolaski, F. E. (2004). Vegetation changes in the Nordic mountain birch forest: the influence of grazing and climate change. *Arctic, Antarctic, and Alpine Research,* 36**,** 323-332.

Uleberg, E., Hanssen-Bauer, I., Van Oort, B. & Dalmannsdottir, S. (2014). Impact of climate change on agriculture in Northern Norway and potential strategies for adaptation. *Climatic Change,* 122**,** 27-39.

Ulloa, O., Ortega, F. & Campos, H. (2003). Analysis of genetic diversity in red clover (Trifolium pratense L.) breeding populations as revealed by RAPD genetic markers. *Genome,* 46**,** 529-535.

UN, (1992). The Convention on Biological Diversity. United Nations.

UN, (2005). Investing in Development: A Practical Plan to Achieve the Millennium Development Goals. New York: EarthScan.

UN, (2012). Global Strategy for Plant Conservation: The targets: 2011-2020. Montreal, Canada: Secretariat of the Convention on Biological Diversity.

UN, (2015a). Sustainable Development Knowledge Platform. Available at: https://sustainabledevelopment.un.org/ [Accessed 6 April 2017]

UN, (2015b). World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241.

UNEP-WCMC and IUCN (2015), Protected Planet: The World Databse on Protected Areas (WDPA) [On-line], Cambridge, UK: UNEP-WCMC and IUCN. Available at: www.protectedplanet.net

UNFCCC, (2015). Synthesis Report on the Aggregate Effect of the Intended Nationally Determined Contributions. Paris: United Nations.

UNFCCC, (2016). Report of the Conference of the Parties on its twenty-first session, held in Paris from 30 November to 13 December 2015. Paris: United Nations.

Valland, N. (2014). *RE: Ten million species observations in Norway.* Available at: http://artsdatabanken.no/Pages/166304 [Accessed 6 April 2017]

Van De Wouw, M., Kik, C., Van Hintum, T., Van Treuren, R. & Visser, B. (2009). Genetic erosion in crops: concept, research results and challenges. *Plant Genetic Resources: Characterization and Utilization,* 1**,** 1-15.

Van Hintum, T. J. L., Sackville Hamilton, N. R., Engels, J. M. M. & Van Treuren, R. (2002). Accession Management Strategies: Splitting and Lumping. In: Engels, J. M. M., Ramanatha Rao, V., Brown, A. H. D. & Jackson, M. T. eds., *Managing Plant Genetic Diversity.* IPGRI.

Van Treuren, R., Bas, N., Goossens, P., Jansen, J. & Van Soest, L. (2005). Genetic diversity in perennial ryegrass and white clover among old Dutch grasslands as compared to cultivars and nature reserves. *Molecular Ecology,* 14**,** 39-52.

Van Vuuren, D. P., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G. C., Kram, T., Krey, V. & Lamarque, J.F. (2011). The representative concentration pathways: an overview. *Climatic Change,* 109**,** 5-31.

Van Zonneveld, M., Koskela, J., Vinceti, B. & Jarvis, A. (2009). Impact of climate change on the distribution of tropical pines in Southeast Asia. *Unasylva 60***,** 24-28.

Vavilov, N. I. (1949). The origin, variation, immunity and breeding of cultivated plants. *Chronika Botanica,* 13**,** 1-54.

Vekemans, X., Beauwens, T., Lemaire, M. & Roldán-Ruiz, I. (2002). Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular ecology,* 11**,** 139-151.

Vincent, H., Wiersema, J., Dobbie, S., Kell, S. P., Fielder, H., Castañeda Alvarez, N. P., Guarino, L., Eastwood, R., León, B. & Maxted, N. (2013). A prioritised crop wild relative inventory as a first step to help underpin global food security. *Biological Conservation,* 167**,** 265-275.

Volden, B., Sveistrup, T. E., Jørgensen, M. & Haraldsen, T. K. 2002. Effects of traffic and fertilization levels on grass yields in northern Norway.

Von Humboldt, A. & Bonpland, A. (1807). Essai sur la geographie des plantes. Paris.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van De Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J. & Kuiper, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic acids research,* 23**,** 4407-4414.

Walker, B., Holling, C. S., Carpenter, S. R. & Kinzig, A. (2004). Resilience, adaptability and transformability in social--ecological systems. *Ecology and Society,* 9**,** 5.

Wang, X., Wang, H., Wang, J., Sun, R., Wu, J., Liu, S., Bai, Y., Mun, J.-H., Bancroft, I. & Cheng, F. (2011). The genome of the mesopolyploid crop species Brassica rapa. *Nature genetics,* 43**,** 1035-1039.

Warren, R., Vanderwal, J., Price, J., Welbergen, J. A., Atkinson, I., Ramirez-Villegas, J., Osborn, T. J., Jarvis, A., Shoo, L. P. & Williams, S. E. (2013). Quantifying the benefit of early climate change mitigation in avoiding biodiversity loss. *Nature Climate Change,* 3**,** 678-682.

Watson-Jones, S. J., Maxted, N. & Ford-Loyd, B. V. (2006). Population baseline data for monitoring genetic diversity loss for 2010: A case study for Brassica species in the UK. *Biological Conservation***,** 490-499.

Wedderburn, M., Barker, D. J., Chapman, D., Orr, S. & Dymock, N. (2005). Genetic differentiation in white clover (*Trifolium repens*) populations during 8 years of contrasting phosphorus supply in New Zealand hill country. *New Zealand Journal of Agricultural Research,* 48**,** 63-74.

Weibull, J., Fitzgerald, H., Lund, B., Palme, A. & Porbjornsson, H. (2016). Conservation and sustainable use of crop wild relatives: a Nordic initiative. *Sveriges Utsadesforenings Tidskrift***,** 53-58.

Williams, W. M. (1987). Genetics and breeding. In: Baker, M. J. & Williams, W. M. eds., *White Clover.* Wallingford, Oxon: CAB International.

Wisz, M. S., Hijmans, R., Li, J., Peterson, A. T., Graham, C. & Guisan, A. (2008). Effects of sample size on the performance of species distribution models. *Diversity and Distributions,* 14**,** 763-773.

Woodward, F. I. & Williams, B. G. (1987). Climate and plant distribution at global and local scales. *Vegetatio,* 69**,** 189-197.

World Bank. (2008). *World Development Report 2008: Agriculture for Development*. Washington, DC: World Bank. Available at: https://siteresources.worldbank.org/INTWDR2008/Resources/WDR_00_book.pdf [Accessed 6 April 2017]

World Food Prize. (2016). *About Norman Borlaug.* Available at: https://www.worldfoodprize.org/en/dr_norman_e_borlaug/dr_borlaug__the_world_food_prize/ [Accessed 6 April 2017]

Zapata, V. M. & Robledano, F. (2014). Assessing biodiversity and conservation value of forest patches secondarily fragmented by urbanisation in semiarid southeastern Spain. *Journal for Nature Conservation,* 22**,** 166-175.

Zhang, H. & Hare, M. P. (2012). Identifying and reducing AFLP genotyping error: an example of tradeoffs when comparing population structure in broadcast spawning versus brooding oysters. *Heredity,* 108**,** 616-625.

Zhivotovsky, L. A. (1999). Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular ecology,* 8**,** 907-913.