UNIVERSITÀ
DEGLI STUDI
FIRENZE

PhD COURSE IN
Sustainable Management of Agricultural and Forestry Resources

XXVII CYCLE

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Pinus nigra J.F. Arnold ssp. *nigra* var. *italica:* a study case of an isolated and altitudinally-marginal forest population in view of the *Global Change*

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Academic years 2012 / 2014

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Abstract

Marginal and Peripheral forest populations (MaPP), can be a very important resource to be investigated and preserved. Their genetic structure, their adaptability to particular local climate conditions and ecological dynamics could play a key role in developing mitigation strategies and actions for preserving species in view of the climate change effects on forest ecosystems. In addition, many of them are located in the Mediterranean region, an area well known as biodiversity hotspot.

The Black pine of Villetta Barrea (*Pinus nigra* ssp. *nigra* var *italica*) is a variety of the *nigra* subspecies and is naturally distributed only in Abruzzo Region near the village of Villetta Barrea. This MaPP is about 400 hectares and represents a geographically isolated population, due to its distance from the core of the natural distribution of Black pine in Europe. More in particular, it is split in two populations, an altitudinally marginal population growing in the Camosciara area and a lower-altitude stand near Villetta Barrea, nowadays registered as a seed stand. In addition, past studies on genetics and morphological traits of the population and comparison with Northern (*nigra* subspecies) and Southern (*laricio* subspecies) Italian populations, have pointed out the possibility that the Back pine of Villetta Barrea can be considered a real MaPP.

With the aim of studying the most probable interactions among MaPP, the Villetta Barrea population and the effects of Global change in the Mediterranean area, the whole population has been analysed as a study-case. Some of the widely used global climate data (WorldClim) were tested and compared with those derived from regional climate monitoring networks concerning accuracy and fitness to local studies. Dendrochronology was used to assess the growth trends and climatic tolerance of the species, whereas a study based on Species Distribution Modelling approach (SDM) was carried out in order to predict the spatial future development for the species under two different future climate

scenarios for the Mediterranean area. To detect the most important climate factors, general correlation function and moving correlation functions with a 30 years window were used. General correlation was used to consider the entire life of the trees whereas moving correlation functions were used to subdivide growth of the population in sub-periods and highlight particular periods. In both cases, averaged monthly temperatures and total monthly precipitations, starting from October of the year before the ring formation (t-1) and up to September of the reference year (t) were used as climate input data. After that a Species Distribution Model was built.

As a result, global climate data compared with local data demonstrated to be accurate but not enough to describe ecological niche of the MaPP. Errors were very high for annual precipitations with a mean value of +90% whereas mean annual temperature was overestimated of about +25%. The dendrochronological approach revealed different trends depending on the method and periods considered. Concerning the entire lifespan of the population, comparable to the available climate information (1901-2009), Black pine showed a positive and statistically significant correlation between ring-width and average temperatures of previous December, February and March (t-1) and negative correlation with temperatures of July, September and October of the current year (t). At the same time, the analysis with moving correlation functions suggested that, in the last decades, the population has reacted to very few climate factors and mainly to changes in temperatures, especially concerning September of the year of ring formation (both minimum and maximum temperature). The use of local data and dendrochronological information applied to SDM demonstrate that global warming could seriously affect the distribution of Black pine in Abruzzo. According to the first scenario where an increase of temperature between 1°C and 2°C and the reduction of -25% / -40% of total precipitations were simulated, models predicted a decrease of -72% of the suitable area for Black pine in Abruzzo. With a

second and heavier sceanario (+2°C / +4°C and -45% / - 60%) nearly the total loss (95%) was forecasted.

In conclusion, the dendrochronological study suggested that Villetta Barrea Black pine may be able to react to future climate change effects. Data demonstrated an ability to adapt to climate effects very quickly in the past. A quite low sensitivity to climate factors, especially concerning precipitations was detected whereas, in the last decades, very few correlations were found. In addition, SDM predicted the possibility for the species to migrate, highlighting three different zones which could be suitable in the heaviest scenario. In any case, changes in environmental conditions due to Global change effects are thought to be faster as ever before and, maybe, too fast to allow species to adapt to new environmental driving forces or to migrate. For this reason, in order to preserve the population, an *in situ* adaptive management protocol with the possibility to perform an assisted migration should be taken into account.

Key Words

European Black pine; Marginal and peripheral forest populations; Mediterranean area; Forest Genetic Resources management; dendrochronology; climate change; Species Distribution Models.

1. Introduction

1.1 Marginal and Peripheral forest populations

The spatial distribution and the genetic structure of forest populations is the result of decades of interaction between many factors, both biotic and abiotic (Jimenez et al., 1999; Guisan and Zimmermann, 2000; Guo 2014). Species adaptation to new environments, resilience to disturbances, genetic variability and stability, glacial refugia, competition abilities and, above all, human activities played a key role in that field and especially in Europe. However, in the last years, despite the fast arise of global information and knowledge, little attention has been paid to small forest populations located at the margin of the species ranges. Those populations, that could seriously be endangered by *Global Change* effects with possible loss of valuable genetic variability (Eckert et al. 2008) may play a key role in the future due to its important genetic structure and ecological dynamics (Hampe and Petit, 2005).

Even if marginality is a quite simple concept in geography, it can become more complex if linked to ecological localization or genetic aspects and to define forest populations as "marginal" many approaches are used and a lot of different aspects are connected. Marginal and Peripheral forest Populations (MaPP) can be defined as small groups of living trees located at the margins of the distribution including tree communities growing in particular environmental conditions or owning a particular genetic structure (Yeh and Layton, 1979). Anyway, in general, significant lower diversity (number of alleles and expected heterozygosity) is generally found in the most isolated and small size populations in contrast to central forests. In these cases the inter-population diversity can indicate both extensive gene flows or recent postglacial expansion which are both connected to MaPPs dynamics (Jimenez et al., 1999).

In recent years, many classifications and definitions were created to organize the knowledge about MaPPs and to highlight different genetic and ecological dynamics. More in general, four groups were identified:

- Geographical MaPPs;
- Ecological MaPPs;
- Altitudinal MaPPs;
- Artificial MaPPs.

However, despite definitions and classification rules, it is quite easy to find MaPPs sharing common traits. For example, a Geographical MaPP is very likely to be also ecologically marginal. At the same time, a population quite far away from the core of the distribution could be very similar for genetic and/or ecological qualities to populations which are located near the core of the distribution.

Geographically-marginal populations, also called *peripheral populations*, are the easiest to be detected and are located at the spatial edge of natural distributions. They may be genetically well distinct from other populations and geographical distance from the core of the natural distribution higher than a critic threshold is the main tool to detect them. Geographically marginal populations may be genetically divergent due to joint effects of genetic drift and natural selection. For that reason, they may be more likely to disappear in the future. In addition, geographically marginal populations may themselves be "strictly marginal" or "disjunct" (=isolated). The first ones are connected with the core of the distribution both spatially and genetically, while the latter are not. For strictly geographically-marginal populations, '*leading edge*' and '*rear edge*' populations can be identified (Figure 1), which are very important concepts to be considered particularly under changing environmental conditions. The first group represents populations which are expanding into new territories. They are generally younger groups than the others and the major driving forces are founder effect, long-distance dispersal, and adaptation to cold stresses. In general, in the Mediterranean area, leading edge populations are located at the northern side of the species range. Differently, rear-edge populations occupy the stable edges of the species range and are composed by groups persisting in a territory that is becoming more and more unsuitable for the species. They are generally older than the leading ones and the core of distribution and are characterized by a good genetic stability (Hampe and Petit, 2005). Main ecological issues of these populations are a warming climate, drought stresses and genetic drift and they may conserve important genetic diversity as a result of adaptation. During time, two scenarios can appear: i) range fragmentation (trailing edge) due to extinction of the species in such environment; ii) a small group of trees (and genotypes) which may persist in locally suitable environments (stable edges) as a result of adaptation to a new environment (Aparicio et al., 2012).

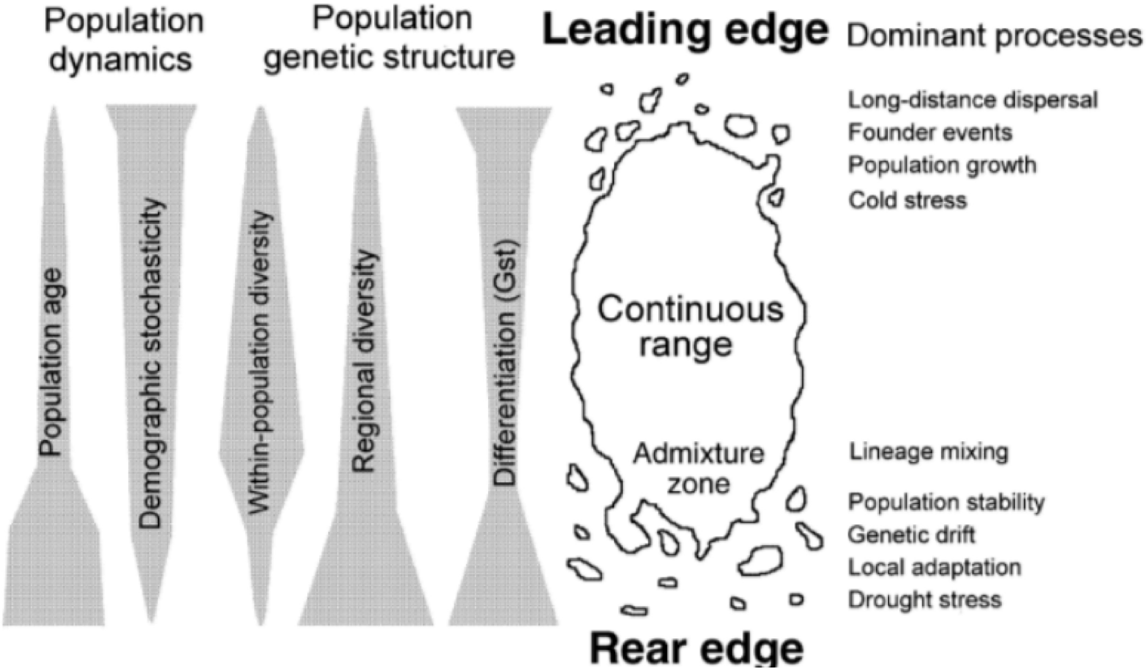


Figure 1: MaPP dynamics (form Hampe and Petit, 2005)

Ecologically-marginal populations belongs to the second group. Concerning geographical distribution, which is not the key issue which characterize them, they can be found almost anywhere in the range of a species, including the core. The discriminant characteristic is the species ecological niche coverage and the main qualities of these populations are ecological environment and inter-specific competition. Ecologically-marginal populations are adapted to grow in very particular environment with marginal climate condition with respect to the species *optimum.* Reduced precipitations during the growing season or the whole year, thin soils high inter- and intra-annual variability and extreme events (cold or hot temperatures) are the main ecological threats for such populations. Thus, exactly for their ability to adapt to particular environmental conditions, they can be a very important source of information to study the adaptation dynamics of species (Tigerstedt, 1994; Arana et al., 2009; Aparicio et al., 2012).

Altitudinally-marginal populations are distributed near the species altitudinal tolerance limit which may vary in different local climatic conditions and due to local conditions. They can be quite easily recognised on the ground due to the distribution and conformation of trees, usually small groups characterized by short and deformed trees living on rocks or inaccessible sites on top of mountains. Their biological trend is usually very slow due to cold stresses and, in global warming scenarios, they could become a new focal point for further species expansion.

Artificially marginal populations are the last group and are connected to human activities, with living trees constrained by human management, timber production and modification of the environment.

While MaPPs' classification and definition is quite easy, a deep analysis ad a good knowledge of the species charachters and the study area are compulsory to detect them correctly on the ground. MaPPs are also usually smaller in size and number of trees and range-wide patterns of populations and genetic diversity are usually shaped by past climate-driven dynamics (Hewitt 2000, 2004).Geography, ecology and genetic of populations, as well as the biotic and abiotic drivers, responsible for the direction and speed of range shifts, must be well understood. Generally, main approaches to identifying existing MaPPs include the use of geographic distances as thresholds when considering geographically marginal populations. Ecological distances such as the Mahalanobis Euclidean distance (Mahalanobis, 1936) may highlight ecologically-marginal populations. In addition, every case of MaPPs may be genetically distinct from the core population (Lawton 1993, Vucetich and Waite 2003) and the use of genetic tools play a key role and should be always performed to support geographical or ecological criteria. Isolation on one side and adaptation on the other are the main driving forces that can produce genetic diversity among populations. Another tool to detect MaPPs is the Principal Components Analysis (PCA) approach which is widely used to study environmental variability and to compress available information into few not-correlated variables which can be used for ecological modelling activities (Giannini et al., 2011; Marchi et al., 2013; Metzger et al., 2013; Cardenas et al., 2014).

The key role of marginal populations for biodiversity maintenance make them extremely important for the conservation of intra- and inter-specific biodiversity in the face of climate change (Soulé 1973, Hampe and Petit, 2005, Eckert et al. 2008). The vulnerability of MaPPs, the quality and ability to adapt and avoid extinction, the speed with which they can colonize new territories and their genetic importance relay on how such species are likely to change in response to future climate change.

1.2 Climate data and Global Change impacts in the Mediterranean area

Together with genetic information, climatic data have become the main tool to support management of forest genetic resources (FGR) in view of global change effects (Marris 2009; Borghetti et al., 2012). Climate change and species response are considered the driving factors that will modify species range in the future (Parmesan 1996; Martinez-Meyer 2005; Thuiller et al., 2005; Csaba et al., 2009; Schueler et al., 2014; Isaac-Renton et al., 2014). Relationships between climatic data and other research fields such as phenologycal studies (Aletà et al., 2009), dendrochronology (Amodei et al., 2012; Mazza et al., 2013), delineation of “Climatic Zones” (Tercek et al., 2012) and Species Distribution Modelling (Elith et al. 2009; Porfirio et al., 2014) were evaluated with the general aim to connect species' attitude and/or spatial distribution with climate environmental variables (Zaniewski et al., 2002; Pearson and Dawson 2003; Sinclair et al., 2010; Wang et al., 2012; Yu et al., 2014). Especially in the Mediterranean region, the climate change issue has quickly emerged in the last twenty years as the major environmental problem in agriculture and forestry fields. The effects may be evident especially in MaPPs, frequently smaller populations where climatic conditions can already extreme for the species (rear-edge MaPPs) and the effects of climate change are likely to be much faster and stronger than elsewhere.

The Mediterranean area is an important basin for biodiversity and many studies have focused on species' distribution, with the aim of (i) defining the present ecological niche of species at scales relevant for useful management plans (Guisan and Zimmermann 2000; Warren et al., 2008; Broennimann et al., 2012; McInerny and Etienne 2012) and (ii) predicting Global Change effects on organisms and populations (Willis et al., 2009; Sinclair et al., 2010; Attorre et al., 2011; Cheaib et al., 2012; Pellat et al., 2012; Forester et al., 2013). These studies require long-term climate baseline data, future predictions and, in some cases, information about past climate variability. In forestry, as in many others environmental disciplines, climatic data (temperatures and precipitations) are used as limiting factors for species distribution through GIS and statistical techniques. The most relevant problems are data access and spatial density/resolution on small areas for a comprehensive set of biologically-relevant climate variables.

To model future climate scenarios, source of interest are current and future levels of energy use from burning of fossil fuels and clearing of forests for cultivation which can have relevant effects on the global environment. The Intergovernmental Panel on Climate Change (ICPP), the main subject involved in Climate Change studies, was established in 1988 as a scientific intergovernmental body under the auspices of the United Nations. Every year, an annual report is published which describes the state of the art and modifications in climate conditions at the global scale. For future predictions, the Special Report on Emissions Scenarios (SRES) is the main reference where scenarios are developed, based on human activities and economy directions (Figure 2). Starting several years ago, with four storylines (A1, A2, B1, B2) different tendencies were explored: one set varying between strong economic values and strong environmental values A vs B), the other set between increasing globalization and increasing regionalization (1 vs 2). The storylines can be summarized as follows (Nakicenovic et al., 2000):

- A1: a future world of very rapid economic growth, global population that peaks in mid-century and declines thereafter, and rapid introduction of new and more efficient technologies.
- A2: a very heterogeneous world with continuously increasing global population and regionally oriented economic growth that is more fragmented and slower than in other storylines.
- B1: a convergent world with the same global population as in the A1 storyline but with rapid changes in economic structures toward a service and information

economy, with reductions in material intensity, and the introduction of clean and resource-efficient technologies.

- B2: a world in which the emphasis is on local solutions to economic, social, and environmental sustainability, with continuously increasing population (lower than A2) and intermediate economic development.

After determining the basic features of each of the four storylines, they were fully quantified using integrated assessment models, resulting in families of scenarios.

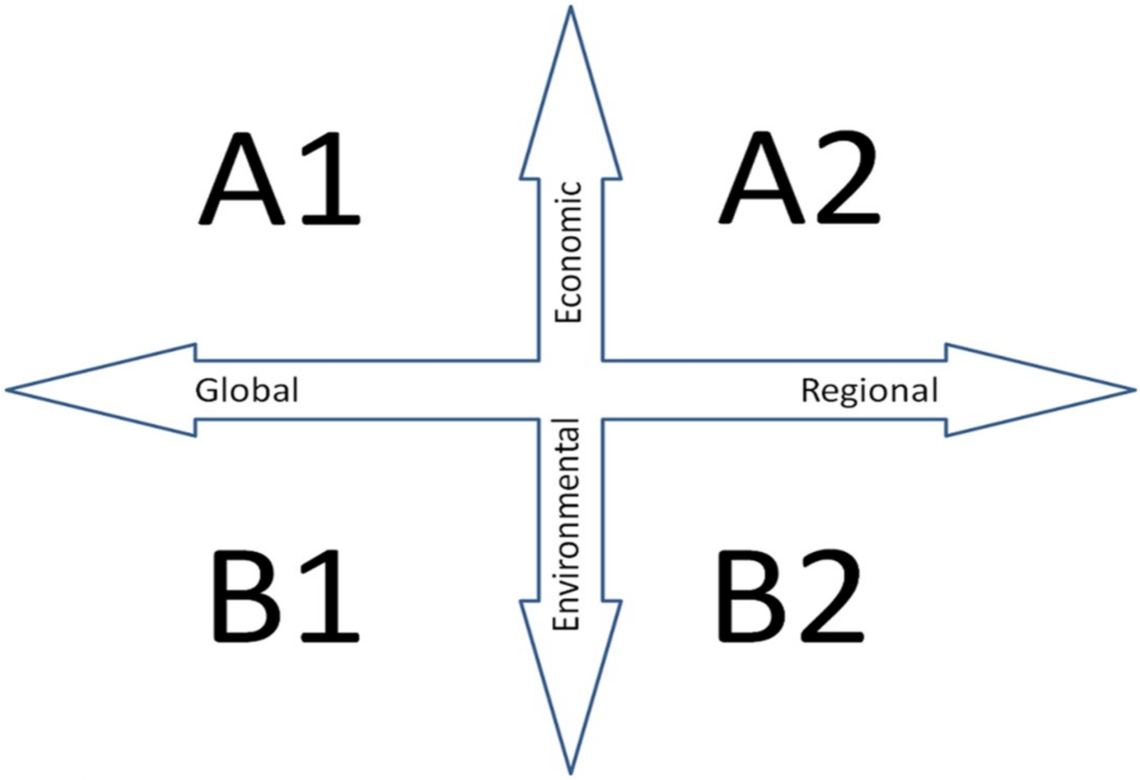


Figure 2: SRES scenarios

IICPP, in the last report (AR5), used a new classification for scenarios, following the Representative Concentrations Pathway (RCP) approach. Predictions are quite similar to the former ones but the increasing concentration of CO_2 in the atmosphere is expressed by the expected amount of solar radiation at soil level (W/m^2, Figure 3). In such new code, RCP2.6 corresponds to B1 scenario whereas RCP8.5 to A2. In any case, we must remember that CO_2 plays an important role in inhibiting the escape of the heat radiated by the earth. The sun beams short-wave radiation to the earth, which sends long-wave radiation back to space. Greenhouse gases in the earth's atmosphere (carbon dioxide, water vapour, methane, nitrous oxide, and the chlorofluorocarbons) absorb the outgoing radiation, thereby holding heat. It should be considered that this process occurs also naturally and without the natural greenhouse effect, our planet would be freezing. Instead, this process keeps the earth to its current mean temperature of about 15°C.

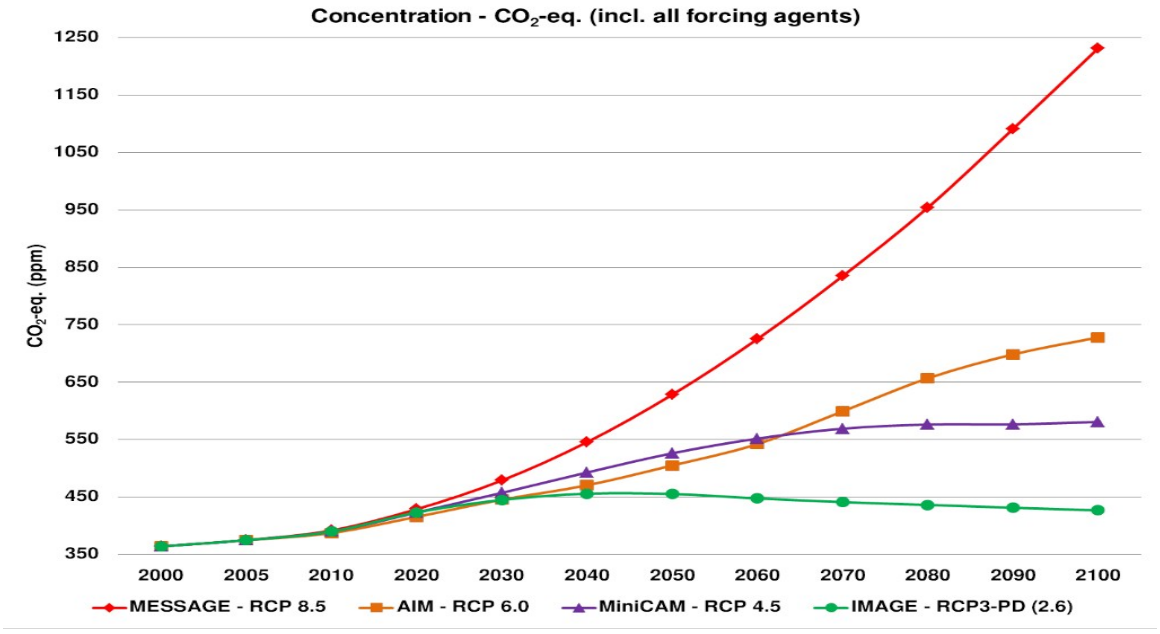


Figure 3: Representative Concentration Pathways

While ICPP provides background about climate change scenarios, other climatology groups developed many climatic models on predictions. As a result, several Global Circulation Models (GCMs) have been published and are often used to forecast the effects of greenhouse gas increases. GCMs are mathematical models used to predict climate change effects at global scale. The spatial resolution is generally quite low and about 5° x 5° of latitude and longitude (approximately 500 km at the equator) and is (obviously) too coarse to perform analysis on organisms, especially over complex and relatively small areas (such as the Mediterranean region). As a consequence, many downscaling techniques were developed to be applied to GCM results in order to get higher resolution results (Wilby and Wigley, 1997; Moriondo and Bindi, 2006; Ramirez-Villegas and Jarvis, 2010; Jones and Thornton, 2013). On the average, all model simulations for Europe agree that the range of temperature rise is expected to be higher in North Europe in comparison to the Mediterranean areas. Despite that prediction of temperature change varies widely, most of the models suggest that the winter temperature will increase mainly in northern Europe while in summer the major increase will affect southern Europe. All model simulations, however, have one common feature: temperature will increase considerably during the next decades. These results could be positively correlated with the observed general increase in North Europe in the 20th century. Concerning rainfall, most of the models agree on winter increase over North Europe and give some indications about increase in summer. On the contrary, all models suggest that the summer temperature in South Europe will decline whereas there are some indications about an increase in summer precipitations.

However, even if for continental Europe there is some agreement, it is very difficult to agree on future climate conditions in the Mediterranean basin (Giannakopoulosu et al., 2005; Giorgi and Lionello 2008; Giannakopoulosu et al., 2009). Detection of climate change on this scale is extremely difficult as the high variability in local environmental and local micro-climates tend to masks trends in the 'noise' of natural fluctuations. Moreover, the short period of observations makes the identification of clear trends difficult and creates uncertainty over the scale of natural variability. However, a lower temperature increase is expected over the sea and the coastal regions compared to the inland

Mediterranean areas. While global temperatures are expected to increase about 0.2°C/decade and climb by between 1.7 and 4°C by the year 2100, it is a widely shared opinion that temperatures will rise in the next 50-100 years especially in mountainous regions. Results from many simulations show a mean global warming in the range of 1.5 to 4.5°C by the end of the next century (Giannakopoulos et al., 2005, 2009). In addition, when the effects of other forces are included in the projections (e.g. sulphate aerosols), the best estimate for 2100 is a temperature increase in the range of 1.0 to 3.5°C. More in particular:

- the high latitudes are likely to have greater warming than the global mean and warming, especially in winter;
- the hydrological cycle is likely to intensify, bringing more floods and more droughts;

Concerning rainfalls, a common feature of many projections is that an increase of annual precipitation over much of the Mediterranean region north of 40 or 45° N is more likely, whereas to more southern latitudes will be lower. In addition, a very important role will be played by extreme events. Despite the uncertainties on how climate variability and extremes will change in the Mediterranean region, the overall picture suggests an increase in frequency of extreme events and, in particular, drought in the western Mediterranean. In general, warmer conditions in the Mediterranean region should lead to an increase in the occurrence of extremely high temperatures and a decrease in extremely low temperature events. In areas experiencing a general decrease in precipitations, drought is likely to become more frequent as the probability of dry days.

1.3 Management of FGR in view of Global Change

Forests are the main repository of terrestrial biological diversity. They provide a wide range of services for terrestrial species, are important carbon sink and have influence on mitigation of climate. Anyway, they are also one of the most threatened biological system by the climate change effects. Forests harbour most of planet's biodiversity and genetic diversity, which is a critical component of biodiversity. Even if genetic diversity is well known to be the power which allows the species to evolve and to survive during adverse situations, it is not properly considered due to its long-term relevance for human welfare. Resilience of forest ecosystems and reaction to disturbances are strictly connected to genetic variability and phenotypic plasticity of populations (Friedman, 1997).

Climate change may have different impacts on different forest ecosystems. Expected changes in tree growth will influence the competitive relationships between species, the potential species composition and the choice of species available (Lindner, 2000). Under climate change effects, it is likely that forest species will migrate or will have to adapt to new environmental conditions (Parmesan 1996, 2006). Anyway, even when species' adaptability is well know and proved, in the next decades, the main driving force will be speed in the changing environment that could bring populations to extinction. For example, while populations living under *optimum* climate conditions could “simply” reduce their growth trend, small and isolated populations with low gene flow and low genetic variability (genotypes) could disappear, with a huge loss of genetic variability (Hampe and Petit, 2005; Eckert et al. 2008). In such framework, to make a better use of existing potentials and to minimize negative impacts of climate change on forests, conservation strategies can be developed especially in southern Mediterranean area where a huge number “rear edge populations” is located as consequence of glacial refugia (Petit et al., 2003).

In general, the conservation of FGR can be divided into two main groups:

- group of the *ex-situ* conservation, to be divided in *static* (as seed storage and genotypes archive and collections) and dynamic (when genotypes are moved in a new environment but managed to adapt and react);
- group of the *in-situ* conservation.

The approach of the first group is generally followed when the aim is to move living genotypes to avoid interaction between species and environmental changes (for human activities or bio-ecological reasons) and to preserve them from local threats. The second is used when the aim is to support and enforce species adaptability (when available) in native zones. Among them, seed storage is the easiest method whereas genotypes archive and collections imply many efforts to select families and progeny. On the other side, *in-situ* conservation is followed when the population's size and genetic structure is thought to be able to adapt to new climate condition and/or to migrate to new surrounding territories (Palmberg-Lercheand and Hald, 2000). In such conditions, forest management is used to support population's natural dynamic to speed up selective processes and enforce colonization of new lands. An adaptive forest management (Stankey et al.,2005) or integral reserve in natural parks (e.g. Sasso Fratino or Camosciara area in Abruzzo) where no management is allowed, are two possible ways to be followed. Between those two extreme approaches there is the *ex-situ* dynamic conservation which is a way to move a representative sample or, as in case of the relict species *Abies nebrodensis* (Vendramin et al., 1997). In such case, 28 scions, one for each living genotype, were collected in the the original site “Vallone della Madonna” in Sicily and grafted on *Abies alba* rootstock in Tuscany where the species is actively managed to produce seeds to be planted (Ducci et al., 1999; Ducci, 2014). Unfortunately it is very expensive and many efforts are necessary to achieve the goal of adaptation. However, despite *in-situ* or *ex-situ* definitions, when an active action is made to allow species to move in new territories the general definition is *assisted migration*.

1.4 Aim of the study and thesis structure

In these background of MaPP dynamics, Climate change effects and forest management in view of *Global Change*, the Black pine of Villetta Barrea (*Pinus nigra* J.F.Arnold ssp. *nigra* var. *italica*) in its natural area (Abruzzo) has been studied. This population has been treated as a study case of an isolated MaPP due to geographical distance from core of the species' natural distribution across Europe. Literature reported also some adaptive and genetic traits in this population which aid to define it as a possible MaPP (Giacobbe, 1933, 1937; Gellini, 1968, Blasi et al., 2005; Bruschi et al., 2006). The possible effects of climate change will be forecasted and modelled with the aim of analysing the future development of the population. More in particular, different issues connected to MaPPs studies and management were considered:

1. How can a MaPP be classified and detected? And, if recognized, what kind of MaPP is the Black pine of Villetta Barrea?
2. Are global climate data reliable for local studies at higher spatial scale?
3. Does past forest management influence growth trends and population's response to climate factors?
4. Will the climate change effect influence the distribution of Black pine of Villetta Barrea in Abruzzo?

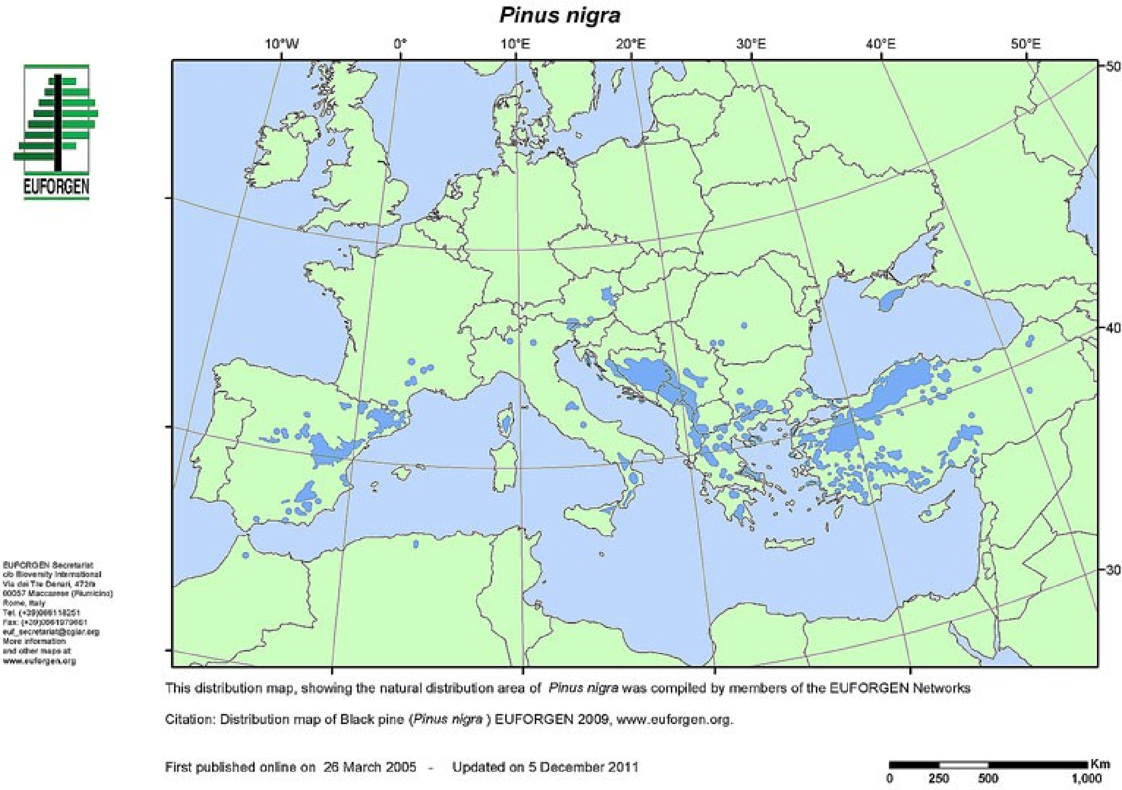
5. Will this MaPP need conservation strategies in the future? And could an adaptive management be useful to preserve this endemic population?

Black pine MaPP has been firstly analysed at European scale following the taxonomic division. Then Italian populations were described. Local climate data were interpolated to solve global data accuracy's problems and to compare different interpolation methods for local studies. Then such climate data were used to forecast future scenarios for Abruzzo region. Working on the field, due to already available data about some parts of the MaPP (Giacobbe, 1933; Biondi and Visani, 1993) more attention was paid to the description of the seed stand of Villetta Barrea (the unique for the subspecies in the whole Italy) following the management plan of the Villetta Barrea Municipality. Dendrochronology and ecological modelling approaches were used to consider adaptability of the species to climate change and to support forest adaptive management in view of the more likely future dynamics of the detected MaPP.

2. Target species and study area

2.1 The European Black pine in Europe and Italy

European Black pine (*Pinus nigra* J.F. Arnold 1785) can be considered a Tertiary relict species already growing in sites where it occurs at present since the Pliocene (Vidakovic, 1974). It continued its spreading during the Quaternary age (Gellini and Grossoni, 2003) and due to its wide and very discontinuous distribution, genetic and phenotypic variability is very high among populations. The species is actually distributed in the mountain regions of the Mediterranean basin, with a complex and fragmented range across all Europe (Figure 4). Taxonomy of the species follows the geographical distribution and the morphology of trees, even in there is no consensus between researchers (Arbez and Millier,1971). Provenances differs generally for needles length, total height of trees, colours and shape of stem and bark.



This distribution map, showing the natural distribution area of *Pinus nigra* was compiled by members of the EUFORGEN Networks

Citation: Distribution map of Black pine (*Pinus nigra*) EUFORGEN 2009, www.euforgen.org.

First published online on 26 March 2005 - Updated on 5 December 2011

Figure 4: Pinus nigra natural range

During 19th and early 20th century reforestation programmes Black pine spp. were intensively used in whole Europe because of its ability to grow well on open lands and in ecologically-demanding situations. The species was generally planted for soil protection and is quite common to find populations made with more than one single subspecies (Isajev et al., 2004). For that reasons, most of research activities on Black pine ssp. are aimed to identify autochthonous areas.

Even if at least fifteen subspecies were described by botanists in the time (Fenaroli and Gambi 1976) there is a general agreement on the division into six subspecies (Quézel and Médail, 2003), ranging from Spain to Turkey:

I. *Pinus nigra* ssp. *mauretanica,* located in northern part of Morocco and Algeria with a very small range;
II. *Pinus nigra* ssp. *salzmanni* which is distributed in Spain and South of France;
III. *Pinus nigra* ssp. *laricio is* in Corsica, Calabria and Sicily with a small and probably artificial stand of medieval age in Tuscany;
IV. *Pinus nigra* ssp. *nigra* occupies some areas of central and north-eastern side of Italy (Abruzzo and Friuli-Venezia-Giulia), Austria and Balkan's area up to Greece;
V. *Pinus nigra* ssp. *dalmatica* can be found on the Adriatic side of Croatia, and Serbia;
VI. *Pinus nigra* ssp. *pallasiana* is the last one, it growths in Turkey and Eastern Europe and covers the majority of the range.

As mentioned, concerning only the Italian peninsula, it is nowadays accepted that Black pine is present with the three mentioned subspecies. Both of them are very common across the Country as pure or mixed stand with other species as beech, oaks and chestnut. Anyway, it is very common to find single trees in open and abandoned lands due to its own auto-ecology and seed dispersal. Main accepted and discriminant differences between subspecies are leaves morphology and soil-growing types (Fukarek, 1958; Gellini, 1968; Arbez and Millier,1971; Debazac, 1971). Southern provenances (ssp. *laricio*) have longer and less rigid needles than northern and are use to grow on acids granitic or sandy soils. On the other side, spp. *nigra* can be found only on calcareous or dolomitic soils but is often smaller concerning diameters an total height (between 25-50 meters). Anyway, despite their ecological differences, both were generally used for the same goals, especially in overexploited, abandoned and cultivated lands and according to the last National Forest Inventory (INFC 2005; Gasparini and Tabacchi, 2011), Black pine ssp. in Italy cover 236,467 ha.

The genetic diversity of Black pine is strictly connected to morphological traits and have been often studied at European level aimed to detect differences among populations and origin of artificial stands (Arbez and Miller 1971; Arbez et al. 1974; Afzal-Rafii et al., 1996; Bojovic et al., 2005; Afzal-Rafii and Dodd 2007). Main protocols were made using terpenes and, only in the last years, a suite of chloroplast DNA microsatellites (Afzal-Rafii and Dodd 2007). In some cases, geographic variation was detected and in insular populations (such as Sicily or Corsica) were unique due to long isolation supporting the hypothesis that they represent the most original form of Black pine (Afzal-Rafii et al., 1996). Bojovic et al. (2005) used a similar approach to study genetic variability between *Pinus nigra* populations in south-western Europe. In such case, the analysis demonstrated that Corsican Black pine was widely used in southern part of France and Spain. On the other side, very few genetic analysis were made on Italian's populations and many of them were about *Pinus nigra* ssp. *laricio* from Calabria and Sicily (Bonavita et al., 2013).

Concerning management aspects of Black pine forests, very few populations are managed for timber production, especially in Italy and in the Mediterranean area. Across Europe, forest management is strictly connected to nature of populations and the connection between gaps and natural regeneration is a well know issue (Muscolo et al., 2007; Mercurio et al., 2009; Tiscar et al., 2011). In natural areas (such as MaPPs) silvicultural treatments are aimed to conserve the richness and to improve the stability of the populations (Hermanin, 1980; Dida et al., 2002; Isajev et al., 2004; Ciancio et al., 2006; Climent et al., 2006, Gugliotta et al., 2006). In planted forests it is oriented to accelerate successions' dynamics, enforcing restoration of of autochthonous broadleaves following the natural spatial distribution and regeneration and localized treatments (Nocentini, 1995; Ciancio et al., 2004; Ordóñez et al., 2005; Nocentini and Puletti 2009; Tiscar and Linares 2011). However, in many Black pine plantations, no thinning activities were made during the time. Many stands (especially in private lands) have been abandoned and are concluding their ecological function to prepare soils for more exigent species. In such conditions, management planners need to find the most economic solution to solve the problem, which is, in general, a strong thinning activity (30% - 40% of the total basal area) and the exploitation of trees for bioenergy.

2.2 The natural MaPP of Abruzzo

The Italian peninsula is a well known hotspot of genetic diversity due to its morphological conformation and geographic position (Petit et al., 2003). The target species of these study is the Black pine of Villetta Barrea a variety of *nigra* subspecies (*Pinus nigra* Arnold ssp. *nigra* var. *italica* Hochst), naturally distributed only in Abruzzo on approximately 400 hectares. However, during the years and similar to any other Italian region, many plantations were artificially made also in Abruzzo, mixing different subspecies and provenances introduced for soil protection of bare lands abandoned by agriculture.

Concerning needles size and morphological traits, which were often demonstrated to be strictly connected to the genetic structure of populations (Giacobbe, 1933, 1937; Gellini, 1968; Blasi et al., 2005; Bruschi et al., 2006), Black pine of Villetta Barrea is intermediate between the two Italian subspecies (*nigra* and *laricio)*. Concerning both leaves and total height it is generally smaller than others but is more drought-resistant especially for total annual precipitations. About soils preferences, despite the geographical distance, it is more similar to Austrian pine than to Calabrian preferring calcareous soils. A summary of morphological traits is reported in Table 1.

Table 1: Main features of Italian Black pines (from (Gellini and Grossoni, 2003)

	ssp. *laricio*	ssp. *nigra* var. *italica*	ssp. *nigra*
Max height (range)	40-50 m	15-20 m	25-50 m
Stem characters	slender	never slender	rarely slender
Leaves	8-16-20 cm not much rigid clear green	5-7-10 cm rigid dark green	8-14 cm very rigid dark green
Native areas	Sicily and Calabria	Abruzzo	Friuli-Venezia-Giulia
Soil	acidic	calcareous	calcareous
Annual precip.	1400-1800 mm	1400 mm	1700-2300 mm
Summer prec.	80-120 mm	100-130 mm	300-400 mm

Since the first part of XX century, several studies were made on these particular variety to describe and classify it properly. Giacobbe (1933; 1937) was the first highlighting differences with northern provenances and ecological traits. Gellini (1968) with a morphological comparison demonstrated the particularity and singularity of the provenance. Later, other studies were conducted on Black pines of Villetta Barrea's population and surroundings areas and mainly with dendrochronology approaches (Hermanin, 1980; Schweingruber 1985; Biondi and Visani, 1993). Anyway, again, general aims were to detect differences among populations, to describe natural dynamics and to search for the natural and autochthonous area of the variety in Abruzzo. Recently, a taxonomic and genetic study on some populations into the National Park of Majella in the Municipality of Fara San Martino (Bruschi et al., 2006) reported the presence of a very old population, morphologically not significantly different from trees of *italica* variety sampled from the area of Camosciara into the natural Park of Abruzzo Lazio and Molise.

The studied Black pine's population is composed by two different parts (Figure 5).

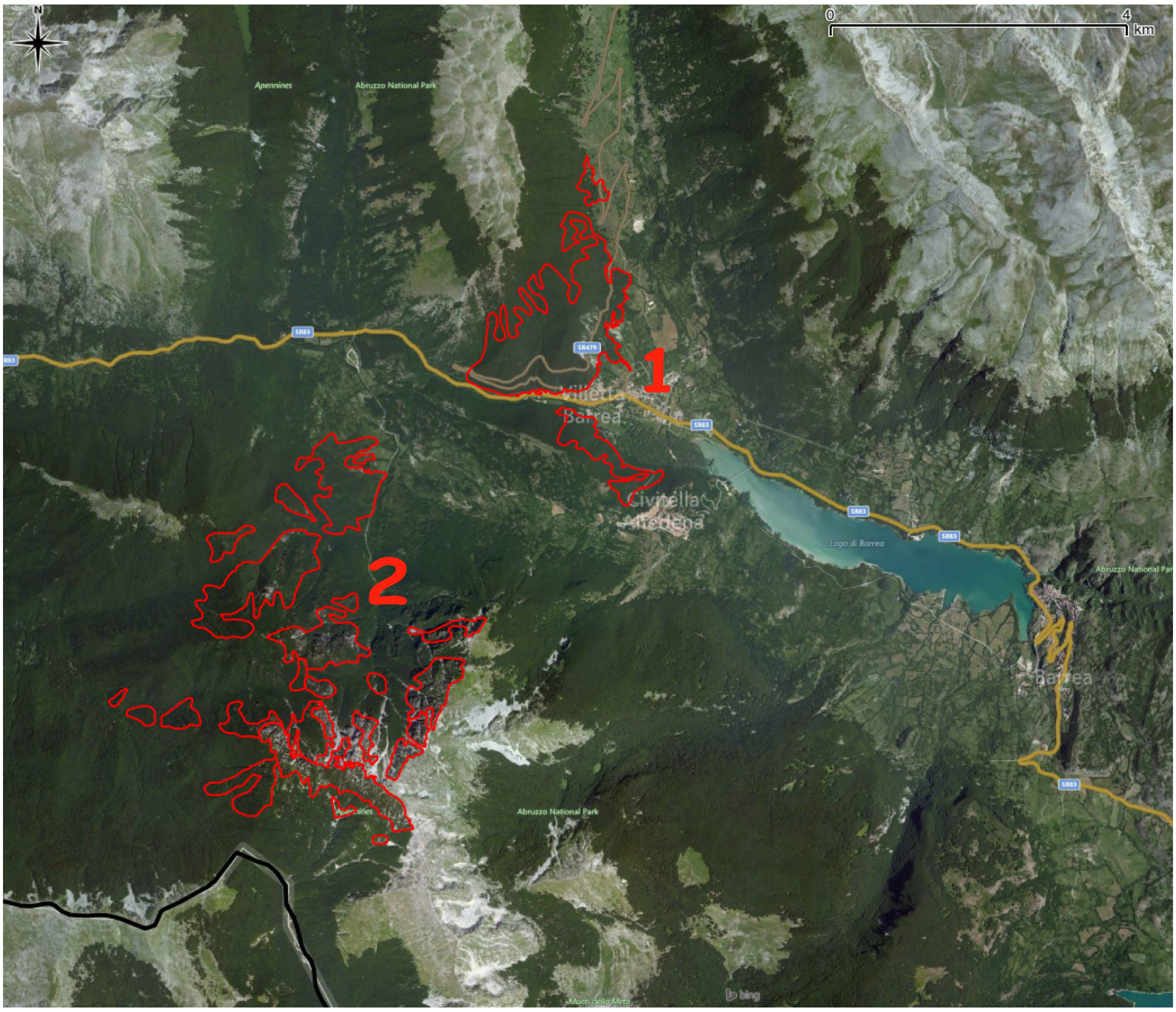


Figure 5: The MaPP of Villetta Barrea with the seed stand (1) and the Camosciara area (2)

The first one is a seed stand registered in the Forest Basic Materials Register of Abruzzo Region (code ABR04[1]) extended 105 hectares and located near the village of Villetta

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[1] In Italy the management of Forest Reproductive Materials (see 1999/105/CE) has been demanded to Regional Administrations since 2003.

Barrea (41.7768 N, 13.9374 E - Figure 6) and even if 21 registered seed stands for *Pinus nigra* spp. are distributed across Italy (16 concerning ssp *laricio* in Calabria), this is the only one for the *italica* variety of *nigra* subspecies.

Figure 6: Winter picture of the seed stand ABR04 (green part)

The remaining part is an older population, located in to the Camosciara wood and included within a protected area. This is a forest included into the Natural Park of Abruzzo Lazio and Molise and some zones can be classified as an "altitudinally-marginal forest population" due to small, old and deformed tress growing on rocks around 1700-2000 metres a.s.l (Figure 7). However gene-flows among these different parts of the whole natural population are abundant and continuous and, in the end, those different parts can be treated and considered as a unique MaPP.

Figure 7: Higher part of the altitudinally MaPP

2.3 Geographical and forest environment of Abruzzo Region

The research has been conducted at regional scale, covering the whole Abruzzo territory. Abruzzo is a wide geographic and administrative region in central peninsular Italy on the eastern Adriatic side. It is about 10,700 km^2 and is a mainly mountainous (65%) and hilly (34%) region, with only a small narrow coastal plain (1%). The climate is highly variable, being strongly influenced on one side by the presence of the Adriatic sea and the Balkans dominant winds and, on the other by the Apennines, with mountains among the highest in the whole chain ("Gran Sasso d'Italia" reach 2,912 m a.s.l.). Due to this orographic structure, the climate of the coastal part is typically Mediterranean, with hot and dry summers and maximum precipitations in autumn whereas the interior part has a more continental climate with hot summer and snowy winters.

According to the last national forest inventory (INFC 2005, Gasparini and Tabacchi, 2011), forests in Abruzzo cover approximately 4,500 km^2 of the region (42%). The main forest categories are pure beech (30% of the total forest area) and oak forests (about 20%), mainly represented by Turkey and Downy oaks. Conifers represent only 7% of the total, mainly spread by reforestation activities. Abruzzo is hots of a quantity of Balkan an Alpine endemic *taxa* very relevant for their rarity in Italy and released in post-glacial refugia. Among the, forest species are silver birch (*Betula pendula*) generally growing above abandoned pastures, mugo pine (*Pinus mugo* Turra) and some populations of *Abies alba* in the south of the region. Black pine forests cover in Abruzzo an area of 19,185 ha which 2,896 hectares only are classified as native pure stands of *Pinus nigra* J.F. Arnold ssp. *nigra* var. *italica* whereas the remaining 16,289 ha are mixed or planted with Black pines from Calabria (*Pinus nigra* J.F. Arnold ssp. *laricio* Maire), Austrian Pine (*Pinus nigra* J.F. Arnold ssp. *nigra* var. *nigra*) and Bosnian Pine (*Pinus heldreichii* H.Christ. 1863).

3. Materials and methods

3.1 Climate data and marginality analysis at European level

As reported in introduction (chapter 1.1) it is relatively difficult to detect and define the status of MaPPs on the ground. The *geographical distance* as thresholds is a quite simple method but it is useful for geographical marginality only and does not give any information about local climate conditions where populations are growing neither genetics. On the other side, *ecological distances* (e.g. multivariate distances based on landscape heterogeneity) may highlight ecologically-marginal populations but are dependent to climatic data reliability, availability and abundance. In addition, when statistical analysis are forced to consider not-correlated variables, such as regressive models or evaluation of climatic heterogeneity (Metzger et al., 2013) all ecological information are not easy to be considered together with their interactions.

When the ecological niche of forest species and relationships between organisms and climate have to be investigated, it is very important to use reliable data. In addition, relationships between different climate factors have to be carefully considered (Braunish et al., 2013). To achieve these goals, several climatic and bioclimatic indices were calculated and proposed since the first years of the XX century, in order to study climatic variability and the dynamics of forest's envelopes. For instance, the most famous classification in Italy was created by Aldo Pavari in 1916, as an adaptation for the Italian environment of the scheme proposed by Mayr in 1906 and finally reviewed by De Philippis (1937). At European level, other classifications were created. Among them the De Martonne's aridity index (1927), the Emberger's pluviotermic quotient (1930) and the Rivas-Martinez indices (2009) are considered the most efficients.

Due to the wide spreading of web knowledge and data storage tools, many climatic datasets are freely available on the web (temperature, rainfall, climatic indices, etc.) both at national or global scale (e.g. PRISM, ClimateNA, ClimateEU, ClimateSA, and many others). One of the most important climate database is WorldClim (Hijmans et al., 2005), which is widely used in research activities (4,986 citations on Google Scholar at the present time). This is a Global climate database with a spatial resolution is 30-arc second (approximately 1 Km at the equator) even if other coarser resolution are available (2.5 - 5 - 10 minutes). Available data for the whole world are:

- minimum, maximum and mean temperature;
- total annual precipitation;
- 19 bioclimatic variables, calculated with previous data (Table 2).

WordlClim precipitation maps were created extracting data from about 47,500 locations, mean temperature records derived from 24,542 stations and minimum and maximum temperatures from 14,835. The averaged period is 1950-2000 and data were interpolated with the thin-plate smoothing spline algorithm (Hijmans et al., 2005). However, bioclimatic variables are the most used and complete dataset and are derived from monthly temperature and rainfall values in order to generate more biologically meaningful variables. The bioclimatic variables represent annual trends (e.g., mean annual temperature, annual precipitation) seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters). In addition, WorldClim provides predictions for two future temporal thresholds (2050 and 2080) for each of the four different Representative Concentrations Pathways scenarios (Figure 3) as a result of the work of many working groups from every part of the world.

Table 2: list of available bioclimatic data from www.worldclim.org

Label	*Variable*	*Scaling Factor*	*Units*
BIO1	Annual Mean Temperature	10	Degrees Celsius
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	10	Degrees Celsius
BIO3	Isothermality (BIO2/BIO7)	100	Dimensionless
BIO4	Temperature Seasonality (Standard Deviation)	100	Degrees Celsius
BIO5	Max Temperature of Warmest Month	10	Degrees Celsius
BIO6	Min Temperature of Coldest Month	10	Degrees Celsius
BIO7	Temperature Annual Range (BIO5-BIO6)	10	Degrees Celsius
BIO8	Mean Temperature of Wettest Quarter	10	Degrees Celsius
BIO9	Mean Temperature of Driest Quarter	10	Degrees Celsius
BIO10	Mean Temperature of Warmest Quarter	10	Degrees Celsius
BIO11	Mean Temperature of Coldest Quarter	10	Degrees Celsius
BIO12	Annual Precipitation	1	Milimeters
BIO13	Precipitation of Wettest Month	1	Milimeters
BIO14	Precipitation of Driest Month	1	Milimeters
BIO15	Precipitation Seasonality (Coefficient of Variation)	100	Fraction
BIO16	Precipitation of Wettest Quarter	1	Milimeters
BIO17	Precipitati on of Driest Quarter	1	Milimeters
BIO18	Precipitation of Warmest Quarter	1	Milimeters
BIO19	Precipitation of Coldest Quarter	1	Milimeters

In this background and in this study, due to the need to analyse the MaPP of Abruzzo and to compare it with the other populations of the Black pine across Europe, two different approaches were used and combined. The first was the evaluation of the geographical distance combined with ecological features. Using the EUFORGEN distribution map as baseline (www.euforgen.org) and the bioclimatic dataset from WorldClim, the "eco-geographical" distance between the target area (the MaPP) and the whole range of the species in Europe was checked applying the Mahalanobis distance method (Mahalanobis

1936), The Mahalanobis distance is a multi-dimensional generalization of measuring how many standard deviations away a specific location is from the mean of another location or group of them. This distance is zero if means are the same whereas it grows as the first moves away. Mahalanobis distance is not affected by units and scales, and takes into account the correlations of the data set (Camussi et al., 1991). In our case, geographical distance was measured in metres and climate data were obtained from WorldClim raster maps with 30 seconds of arc resolution. To avoid redundancy on the following analysis, high-correlated variables (Pearson's coefficient higher than 1.0) were detected and removed from the database, similar to most recent studies (Metzger et al, 2013).

The second step was an ecological analysis which was performed using only the climatic information and considering *Pinus nigra*'s range as a domain. To characterise Black pine subspecies growing sites, the polygonal shapefile of the distribution map was rasterized with a spatial resolution of I km (the same of Worldclim maps) and divided into six parts, corresponding to the six subspecies of *Pinus nigra*. For each of them, climate data were extracted into GIS environment and a Principal Component Analysis (PCA) with correlation matrix (due to different scales between variables) was used to analyse ecological data and compress the information in to few not-correlated variables. The first two Principal Component were then used to create a new "XY plot" where each location (= pixel with an area of 1 km^2) was projected, detecting differences between subspecies. In such new environment the "ecological position" of each *nigra* subspecies and of the population of Villetta Barrea were investigated.

3.2 Climate data at Regional level: the interpolation of local data

As any other globally-interpolated data, WorldClim database has its pros and cons. Even if it is widely appreciate for the Global coverage and 1 Km of spatial resolution, it is demonstrated that accuracy on some regions (e.g. the Mediterranean area or mountain regions) is quite low (Gonzalo, 2010). As reported by Hijmans et al. (2005) in the conclusion section of their published paper, WorldClim data is not adequate for very detailed analysis and local studies. Reasons lay on the low representativeness of mountainous environment (very few meteorological stations were located at high elevations). So, in many European cases and local studies, some problems could arise due to the morphology of Mediterranean basin or fragmentation of the target species.

When a more detailed dataset is required, statistical downscaling (Jones and Thornton, 2013) and interpolation of climatic data obtained by local monitoring network are the only possible ways. Downscaling methods are generally based on regressions (singular, polynomial or splines) or on statistical relationships between climate data and widely-measured ancillary data (e.g. elevation) and are often used to improve prediction at national level of Global Circulation Models (GCMs). On the other side, interpolation methods are very common in climatology and connected studies but those represent a quite complex and time-consuming way (Kurtzman and Kadmon 1999; Yang et al., 2004; Attorre and others 2007; Blasi et al., 2007; Ferrari and others 2007; Hofstra et al., 2008; Huixia and others 2011; Brunetti et al., 2013). In both cases, to increase spatial resolution and to obtain reliable surfaces, representativeness of meteorological network and statistical relationships between climate and widely measured variables (elevation, distance from sea, slope, aspect, etc.) are compulsory (Bhowmik and Costa, 2014).

Concerning the study case and available data (Worldclim maps), a new climate dataset was developed for this area interpolating regional monitoring network data to tackle problems connected to global climate data in Mediterranean area and Abruzzo Region. Abruzzo has a relatively abundant and well-distributed meteorological network with 137 stations (approximately 1/100 km^2) for rainfall observation and, among the 137 stations, 57 for temperature (approximately 1/200 km^2). The distribution is geographically-homogeneous (Figure 8) and quite similar to other Italian regions (e.g. Lazio; Attorre et al., 2007). Anyway, a known critical issue of this database was that, due to orographic conformation of Abruzzo, the spatial environment, especially high elevations, was not properly represented (Table 3). Meteorological data are stored by the Regional Agro-meteorological Centre of the Region and freely distributed under request.

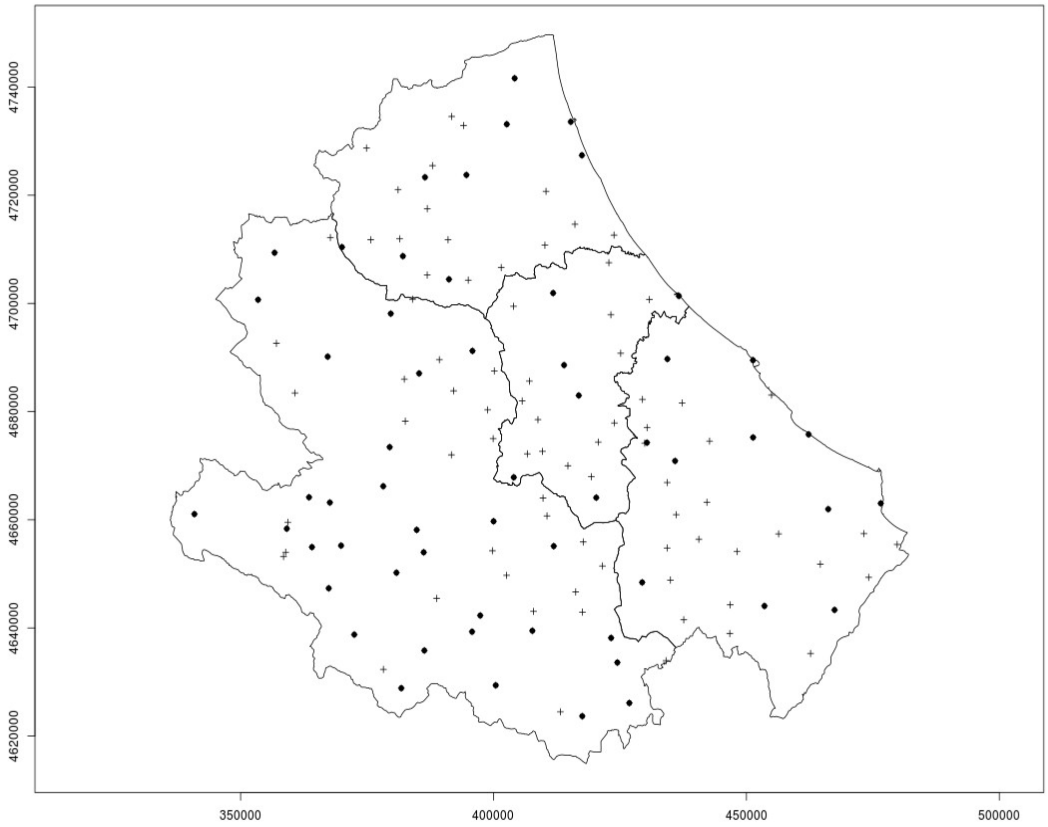

Figure 8: Abruzzo's meteorological network; dots represent pluviotermine sensors whereas crosses are pluviometric-only. Black lines represents administrative limits

In this study, minimum, mean and maximum annual temperature were calculated averaging a survey period of 40-50 years, depending on the lifespan of each meteorological station. The same was done for precipitation data, using a mean value of annual and monthly precipitation. In both cases, climatic data were geo-referenced in WGS84 UTM 33 North reference system (code EPSG 32633).

Table 3: Range covered by Abruzzo's meteorological networks (percentage)

Network	*ELEV (m)*	*SLO (%)*	*ASP (degrees)*	*DSEA (m)*	*GSR (W/m^2)*
Temperature	0 - 1531 (52%)	0 - 197 (85%)	0 - 360 (100%)	0 - 99942 (95%)	558 - 2382 (98%)
Precipitation	0 - 1967 (67%)	0 - 197 (85%)	0 - 360 (100%)	0 - 99942 (95%)	558 - 2382 (98%)

Tested algorithms

Literature is full of examples of parametric and nonparametric interpolation methods. Most of them enclose different algorithms of distance weighting (Kurtzman and Kadmon 1999), single and multiple linear regressions (Maselli 2001; Blasi et al., 2007; Hofstra et al., 2008; Brunetti et al., 2013), nearest neighbour algorithms (Yang et al., 2004), spline functions (Sboarina 2001; Hofierka et al., 2002) and geostatistical methods like kriging and cokriging in their forms (Cressie 1991; Ashraf et al., 1997; Attorre et al., 2007; Brunetti et al., 2013). Anyway, it is very hard to select the best interpolation method. Quality of interpolation mainly depends on nature of data, statistical relationships between climatic variables and physiographic parameters (elevation, slope, aspect and distance from sea), study scale and required spatial resolution (Caruso and Quarta 1998; Kurtzman and Kadmon 1999; Wong et al., 2004; Yang et al., 2004; Attorre et al., 2007; Blasi et al., 2007; Hofstra et al., 2008; Huixia et al., 2011). As a consequence, comparative studies applied to study cases are very frequent in literature (Caruso and Quarta 1998; Kurtzman and Kadmon 1999; Rea and Eccel 2004; Wong et al., 2004; Yang et al., 2004; Attorre et al., 2007; Ferrari et al., 2007; Huixia et al., 2011; Brunetti et al., 2013).

To perform interpolation of climate data in Abruzzo, following the available literature and especially those concerning the Italian peninsula (Attorre et al., 2007; Brunetti et al., 2013), nine of the most commonly-used or important algorithms were compared with the same dataset. The aim was to obtain climate maps with 100 meters of spatial resolution. Algorithms were grouped in two big families, parametric (with the subsections geostatistical methods) and nonparametric. More in detail:

- *Multivariate Linear Regression* (MLR) was used as an example of general ordinary least squares estimators;
- *Inverse Distance Weighted* (IDW) and k-*nearest neighbor* (K-NN) were detected as quick and easy deterministic methods;
- *Regularized Spline with Tension* (RST) was considered as the main method of GRASS GIS software often used in many situation (Sboarina 2001; Yilmaz and Tolunay 2012);
- *kriging* (Ordinary, OK; Universal, UK; and Regression, RK) and *Cokriging* (Ordinary, OcoK; and Universal; UcoK) were used as most powerful and well referenced parametric and geo-statistics methods.

Comparisons were performed calculating four bioclimatic indices and the corresponding climatic factors used to generate them. The four indices were calculated with the following formulas for each meteorological station where both temperature and precipitation were available (57 cases only).

1) De Martonne Aridity index (1927):

$$DMAi=\left(\frac{MAP}{MAT+10}+\frac{12*DMP}{DMT+10}\right)/2$$

where:

MAP = Mean Annual Precipitation

DMP = Driest month Mean Precipitation

MAT = Mean Annual Temperature

DMT = Driest month Mean Temperature

2) Emberger Pluviotermic quotient (1930):

$$EMPq=\frac{(100*MAP)}{(HMTx^{2}-CMTm^{2})}$$

where:

MAP = Mean Annual Precipitation

HMTx = Hottest month Maximum Temperature

CMTm = Coldest month Minimum Temperature

3) Rivas-Martinez Continentality index (2009):

$$RMCi=HMT^{2}-CMT^{2}$$

where:

HMT = Hottest Month mean Temperature

CMT = Coldest Month mean Temperature

4) Rivas-Martinez Termic index (2009):

$$RMTi=1*(MAT+CMTx+CMTm)$$

where:

MAT = Mean Annual Temperature

CMTx = Coldest month Maximum Temperature

CMTm = Coldest month Minimum Temperature

Five physiographic parameters were used as co-variables for the computation in the analysis, adding them to Latitude (LAT) and Longitude (LON). From the Digital Elevation Model (DEM) provided by the Regional Cartographic Office with a spatial resolution of 10 meters, a new DEM was obtained re-sampling the first one to a coarser resolution of 100 meters. After the re-sampling procedure, Elevation (ELEV), Aspect (ASP), Slope (SLO), Distance from sea (DSEA) and Global Solar Radiation (GSR) were calculated for each meteorological station through GRASS GIS modules (GRASS Development Team 2014).

Brief description of tested algorithms

M*ultivariate Linear Regression* (MLR) is a quite simple parametric method which uses mathematical relationships between the parameter to be interpolated and one or more variables known in the whole territory. It is well known that in case of climatic data and especially for temperature, elevation is the main driving factor and this assumption has been sometimes used to interpolate temperatures with regressive methods or to remove the trends from the data (Ashraf et al., 1997; Rea and Eccel 2004; Maracchi et al., 2005; Attorre et al., 2007). For the whole Italian territory, Blasi et al., (2007), for example, used a weighted regression method with elevation to interpolate temperature data, weighting the nearest meteorological station for each pixel at a geometrical resolution of 250 meters. Multivariate techniques are also commonly used (Maselli, 2001) including slope, aspect or distance from the sea as independent variables. In our case, for each parameter to be calculated (temperature, precipitation or bioclimatic index), a stepwise regression (backward type) based on the Akaike's information criterion (AIC, Akaike H, 1974) was performed to select best predictors and estimate regression coefficients.

Inverse Distance Weighted (IDW) is a deterministic and nonparametric method for local interpolation where values at unknown points are calculated as the weighted average of the values available at the known points (meteorological stations in climatology). Weights are calculated in relation to the (geographical) distance from the point to be interpolated and the nearest known points. Typical problems of this method are that maximum and minimum values are always known points of the dataset and distance between points does not consider differences in elevation. For that reasons, with IDW algorithm, temperatures on relieves are very difficult to be estimated and, in such cases, to avoid spatial drifts and when correlation between data to be interpolate and a co-variable is high enough, a detrended-IDW (D-IDW) can give better results (Ashraf et al., 1997; Rea and Eccel 2004; Attorre et al., 2007). The interpolation process through the IDW, in this study, was considered as an easy and fast-to-use interpolation method.

K-Nearest Neighbour (K-NN) algorithm (Cover and Hart 1967) is a nonparametric method for classification and is also one of the simplest of all machine learning algorithms. It is based on multidimensional proximity between objects and, similar to IDW, the unknown object is classified by a majority vote of its neighbours. The main difference between IDW and K-NN is that, with the second algorithm, proximity between observations is evaluated not in a geographical sense but in a new multidimensional space. In such case, new coordinates are two or more variables which are available for the whole area and highly-correlated with the data to be estimated. Even if K-NN have been widely used in forestry to predict forest variables such as volumes, forest indices or forest ecotypes (Mäkelä and Pekkarinen 2004; Hector et al., 2001; Chirici et al., 2012) in some cases it was also performed to predict climatic scenarios (Yates et al., 2003; Sharif et al., 2007). Anyway, even if the application of the K-NN in interpolation of climatic data is limited, it may be successful for dense measurement networks.

Regularized Spline with Tension (RST, Mitas and Mitasova 1999; Neteler et al., 2008) is a nonparametric interpolation method which belongs to numerical analysis. Unlike polynomial interpolation, RST works dividing the interval of analysis in more sub-intervals and using *Tension*, *smoothing*, *segmax* (i.e. maximum number of points in the segment) and *npmin* (i.e. minimum number of points used for interpolation) as parameters to modulate the interpolation process. These parameters describe how far from the real values the interpolated surface will be and optimal parameters are tuned-up through a cross-validation procedure, typically leave-one-out (Yilmaz et al., 2012). High parameters force the surface shape (especially smoothing) while with low parameters the surface is more regular. For further information see Neteler et al., (2008). Splines functions were used very often to interpolate climate data due to its speed and usability. WorldClim database (Hijmans et al., 2005) is the most famous example and was created fitting a second-order thin plate spline using latitude, longitude, and elevation as independent variables.

Kriging is a parametric and geostatistical method for interpolation introduced by D. G. Krige in 1984 (Krige, 1984). Kriging is usually known as "*The gaussian process*" and is believed to be the Best Linear Unbiased Method (Nalder and Wein 1998; Li and Heap 2008). The variogram (or semivariogram depending on statistical packages and software) analysis is the core of kriging algorithm and is an evaluation of spatial autocorrelation among observational points, as function of the distance between them. The (semi)variogram *model* is the main option to be set and most used are *Linear*, *Exponential*, *Circular*, *Spherical* and *Gaussian*. Parameters of each (semi)variogram model are:, *range*, *sill* and *nugget*. The *range* is the distance value at which the model begins to become flat (end of autocorrelation), *sill* is the value of (semi)variance at the range value and *nugget* is the value of (semi)variance at zero distance. In the complex, many different types of kriging are available, which can work differently with the same dataset. Main differences refer to statistical assumptions and construction of the (semi)variogram. Considering our dataset and literature, three different types were considered: *Ordinary Kriging* (OK), *Universal Kriging* (UK) and *Regression Kriging* (RK). With OK the spatial variation of the variable that has to be interpolated is assumed to be statistically homogeneous in the whole area of study. In addition, the mean is not known but is determined during the interpolation. In UK instead, the spatial variation (and the mean) is not statistically homogeneous but is driven by an external drift which is added to the Kriging model to improve the prediction. For this reason, UK is alternatively called "Kriging with external Drift" and very often used in climatology, giving best results when correlation with co-variables is high enough (Attorre et al., 2008). Concerning RK, many attention must be paid about the theory of this method, due to many confusion in literature and among researchers. "*Regression kriging*" is a term that has been used to define many different types of methods. Antonic et al., (2001) for example, defined RK as "*Kriging after detrending*" where the trend function and estimated residuals are modelled separately. However this is the same definition of UK and results are exactly the same. In other cases, RK was used to define an ensemble method merging a regressive interpolation of data and the ordinary kriging of regression residuals (Odeh et al., 1995; Attorre et al., 2007; Tveito et al., 2008) In our study, RK has been calculated following the second form and considering it as a variant of OK.

Cokriging is an extension of Kriging and follows the same statistical assumption (normalized data, random distribution of points, analysis of autocorrelation) but deep differences exist between them which rely on internal functions and complexity of calculations. In this case, only *ordinary* and *universal* cokriging are available. Cokriging works with a multivariate (semi)variogram (the co-(semi)variogram), uses cross-covariance functions and is a method that needs more time for computation and more efforts in modelling steps. It is proved to be very powerful when secondary information is abundant and easy to sample and, in meteorology, it is often used combined with radar data

(Schuurmans 2007) and when there is a high number of co-variables all over the area of study, strongly correlated with the climatic data. For interpolation in Abruzzo region, in this study, both *Ordinary cokriging* (OcoK) and *Universal cokriging* (UcoK) were tested.

Data analysis and comparison method

A statistical analysis of available data is very important to ensure the quality of the interpolation process and to improve the accuracy of results (Huixia et al., 2011). Not-normally distributed data (in case of parametric methods), errors in data storage and outliers are the most common issues that must be detected and solved.

After a dataset-screening and before any other statistical analysis, the normality of distribution was tested for all variables (temperature, precipitations and indices) with the Kolmogorov-Smirnov normality test (Dallal and Wilkinson 1986) and followed by a data normalization when necessary. In case of negative values of temperatures (e.g. the minimum temperatures of the coldest month - CMTm), a transformation from Celsius to Kelvin degrees was preferred. Secondly, a screening for detection of statistical trends in data structure was made, performing a multivariate linear regressions between each climatic variables or indices and all physiographic parameters.

After data check, each interpolation method was set-up properly. For MLR each climatic factor or bioclimatic indices had its own regression model as output of a stepwise regression of backward type. For OK, UK, RK, OcoK and UcoK, the(semi)variogram model (generally an Exponential-class or Spherical model) and the optimal values of *range*, *sill nugget* were selected through the analysis of scatterplots and (co)variograms. For K-NN, the optimal number of neighbours and the kernel were calculated automatically and concerning IDW and RST, default parameters were used. IDW's power was set equal to 1 with n = 12 whereas for RST tension=100, smoothing=0.2, segmax = half number of observations, segmin = total number of observations available were used.

To compare models and in order to asses differences and rank them, a cross-validation procedure was performed with a leave-one-out (LOO-CV) approach (Pichard et al., 1984) due to small number of observations. Three indices were used to assess accuracy of prediction and calculated with the following formulas:

$$\mathrm{RMSE}=\sqrt{\frac{\sum_{i=0}^{n=57}(predicted-observed)^2}{n}} \qquad \mathrm{MAE}=\frac{\sum_{i=0}^{n=57}|predicted-observed|}{n}$$

$$\mathrm{RMSE\%}=\sqrt{\frac{\sum_{i=0}^{n=57}\frac{(predicted-observed)^2}{observed}}{n}}$$

Root Mean Square Error (RMSE) was used to assess algorithms' accuracy as a simple and efficient parameter, easy to measure and widely used in literature (Attorre et al., 2007; Blasi et al., 2007; Cencetti et al., 2007). Absolute Mean Error (AME) and percentage RMSE (RMSE%) were calculated to add more information and to compare results with different scales (Celsius degrees for temperatures, millimetre for precipitation, pure numbers for indices). RMSE% was also used to perform a nonparametric ANOVA through the Kruskal-Wallis rank sum test (Kruskal and Wallis 1952) to rank all performances and compare them also with WorldClim's results in Abruzzo region.

All acronyms of tested algorithms, bioclimatic indices, climatic factors and physiographic parameters are reported in Table 4.

Table 4: List of acronyms

GROUP	*Name*	*Acronym*
Interpolation methods	Linear Regression	REG
	Inverse Distance Weighted	IDW
	K Nearest Neighbors	K-NN
	Regularized Spline with Tension	RST
	Ordinary Kriging	OK
	Universal Kriging	UK
	Regression Kriging	RK
	Ordinary Cokriging	OcoK
	Universal Cokriging	UcoK
Physiographic parameters	Latitude	LAT
	Longitude	LON
	Elevation	ELEV
	Slope	SLO
	Aspect	ASP
	Distance from sea	DSEA
	Global Solar Radiation	GSR
Climate variables	Mean Annual Precipitation	MAP
	Driest Month mean Precipitation	DMP
	Mean Annual Temperature	MAT
	Driest Month mean Temperature	DMT
	Hottest Month mean Temperature	HMT
	Hottest Month maximum Temperature	HMTx
	Coldest Month mean Temperature	CMT
	Coldest Month minimum Temperature	CMTm
	Coldest Month maximum Temperature	CMTx
	De Martonne Aridity index	DMAi
	Emberger Pluviotermic quotient	EMPq
	Rivas-Martinez Continentality index	RMCi
	Rivas-Martinez Termic index	RMTi

3.3 Structure of the seed stand (in space and time)

As reported before, the MaPP of Villetta Barrea can be divided in two different parts (chapter 2.2). While the Camosciara areas are covered by strict management rules and no silvicolture is allowed due to the institution of the National Park, the seed stand is currently managed by the municipality of Villetta Barrea. Considering the aim of this study (relationships between Black pine of Villetta Barrea in its natural range, silvicolture and climate), the dendrometric measurements and the dendrochronological analysis were performed only on the managed part of the MaPP. More in particular:

- dendrometric measurements were used to characterize the seed stand and to describe the spatial structure of that part of the MaPP;
- tree-rings analysis was used to detect climate growth trends between the species and the local climate to describe the temporal structure of the population and to select most important driving factors for further analysis (Ecological Modelling).

The seed stand covers an area of about 105 hectares and, in the last management plan of the Municipality of Villetta Barrea (approved for the time period 2002-2011), it was divided into five management units (Figure 9). To achieve our goals, 5 circular plots with 20 meters of radius were distributed across the managed area. Plots' position were initially chosen following a random but stratified criteria (one for each management units) to describe the population variability and to investigate the most important areas for seed production. However, after a first survey on the field, two management units (n.17 and n.20) were not investigated due to small extension and similarity to others (n.17 was equal to n.18 and only divided by the street) and species abundance (n.20 was mainly occupied by beech). Consequently, 2 of the 5 circular plots were re-assigned to the two main management units (18 and 21). Each plot was localised on the ground with GPS and sampled trees were numbered to make areas permanent for further analysis, future surveys or future projects.

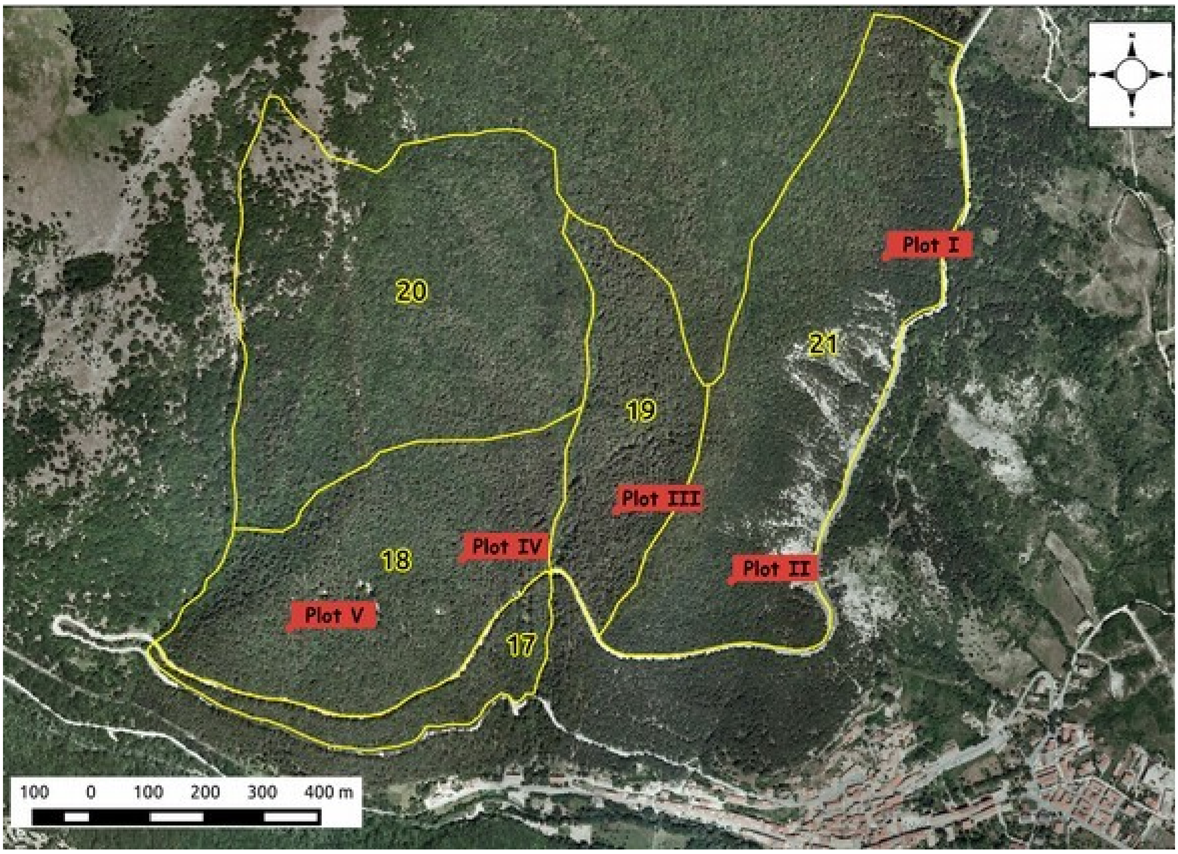

Figure 9: Spatial distribution of the five survey plots

Dendrometric measurements

Diameter at breast height (DBH) of all trees in each plot were measured and 10-15 representative heights were sampled to build hypsometric curve. Volumes were calculated with ForIT package of R Cran software (Puletti et al., 2014) which is the implementation of the biomass and volume models carried out by Gasparini and Tabacchi (2011) and Tabacchi et al. (2011) during the 2nd Italian National Forest Inventory.

The dendrometric data were used to determine the forest's structure in relation to physiographic parameters of the site and the past management. To assess differences between plots, an ANOVA on DBH was performed. Each plot was considered as a different treatment while differences between them concerning slope and aspects were included into the error's variance. Main features of the 5 plots are summarized in Table 5.

Table 5: Main characters of plots

Plot	*Elevation*	*Slope*	*Aspect*	*Manag. unit*
I	1210 m	70%	Est	21
II	1180 m	45%	South	21
III	1200 m	50%	West	19
IV	1170 m	10%	South-Est	18
V	1180 m	60%	South	18

Dendrochronology

Tree-rigs analysis is becoming a quite common step approaching MaPPs (Amodei et al., 2012, Mazza et al., 2013) due to the possibility to investigate past dynamics and to detect correlation between species and climate. Anyway, some principles must be carefully considered and followed to perform an optimal analysis.

The technique is based on the “Principle of aggregate tree-rings” (Cook, 1987) which is the most accepted in dendrochronology and considers a tree-ring series as a linear aggregate of several unobserved sub-series as a consequence of various interactions. This principle states that any individual tree-growth series can be "decomposed" into an aggregate of environmental factors both "endogenous" and "exogenous" affecting the patterns of tree growth over time. The terms "endogenous" and "exogenous" are used to differentiate forest disturbances that were developed by the forest trees themselves (endogenous) from disturbances that arise from processes independent of the forest (exogenous). For instance, gap-phase stand dynamics are endogenous processes in the forest while an insect attack or thinning activities are exogenous processes.

The aggregate series in a fixed moment " t " can be expressed with the following formula:

$$R_t = A_t + C_t + D1_t + D2_t + E_t$$

where:

- R_t is the observed ring-width series;
- A_t is the age-size related trend in ring-width;
- C_t is the climatically-related environmental signal;
- $D1_t$ is the disturbance pulse caused by a local *endogenous* disturbance;
- $D2_t$ is the disturbance pulse caused by a stand-wide *exogenous* disturbance;
- E_t is the largely unexplained year-to-year variability not related to the other signals.

A_t, C_t, and E_t are assumed to be continuously present in R_t while $D1_t$ and $D2_t$ may or may not be present depending on the intervention of a disturbance has occurred at a considered time or not. When the aim is to study the relationships between growth (R_t) and climate ($C_{t,}$) the other factors should be minimized and consequently, a correct sampling of trees is very important.

Following the structure of the formula, the data collection and the subsequent pre-analysis on the seed stand of Villetta Barrea were made in order to minimize the effects of A, D1 and D2 and maximise C. After dendrometric measurements, 5-7 cores for each plot (depending on abundance of suitable trees) were extracted with a 5-mm diameter increment borer at breast height on the stem of healthy dominant and straight trees to maximize the effect of "C". Later in laboratory, each series was prepared on a wooden support, measured with LINTAB6® and cross-dated to remove error due to missing rings or human mistakes. After that, each tree-ring series was standardized separately with a double detrending procedure. The negative exponential curve was used to remove trends due to the increasing tree circumference due to age (A) whereas low-frequency variance and other disturbances (D1 and D2) were removed using a spline function (Cook, 1981) with a 50% frequency response (cut-off) of 10 years to emphasize higher inter-annual frequency variance (Cook, 1981; Biondi and Visani, 1993; Amodei et al., 2012).

Before classical climatic correlation analysis, to complete the information derived from ANOVA on DBH, also not-standardized and standardized tree ring series were used to asses differences between plots (on the common growing period only). The analysis was performed to check dissimilarities and to verify if plots would be grouped on the same way of DBHs.

After that, the influence of climate on tree-ring growth was investigated using mean correlation function (CF) and moving correlation functions (MCF) based on Pearson's correlation coefficients (Fritts, 1976). CF and MCF are based on the same assumptions but they use different principles and time-period. While CF is used to consider the whole growing period of trees, MCF uses a smaller period of time (generally 20-30-40-50 years depending on available data and species sensitivity), repeated for the entire interval. In this case, a 30 year window was used to avoid biases induced by extreme events and to retain the influence of mid-frequency climate variations. In both cases (CF and MCF) monthly climate variables were sequenced from October of the previous year (t-1) to September of the year of growth (t) and results of correlation functions were tested for significance using the 95% percentile range method after a bootstrap process with 1000 replications.

Concerning historical climate data used to estimate the degree of correlation between tree rings series and climate, data from a meteorological station near the MaPP of Villetta

Barrea and located in Barrea village (41.7570 N, 13.9919 E, approx. 1,000 metres a.s.l.) were initially obtained. However, the data series were very discontinuous, especially concerning precipitation data. To solve this problem, a complete dataset was derived from ClimateEU database, an unpublished software for interpolation of climate data from PRISM database (Daly et al. - http://www.ualberta.ca/~ahamann/data/climateeu.html) with monthly precipitations and temperatures from 1901 to 2009. ClimateEU data were compared with local data calculating the fitness of ClimateEU which was tested with a regression method. In addition, Mann-Kendall nonparametric test was applied to assess the presence of climatic trends which could influence the analysis (Brunetti et al., 2006).

3.4 Ecological Modelling

The identification of potentially-suitable areas for forest species in view of the *Global Change* effects is becoming mandatory to support the management of forest genetic resources (Willis et al., 2009; Wang et al., 2012; Isaac-Renton et al., 2014; Porfirio et al., 2014; Yu et al., 2014). To apply climate change prediction in forestry activities, many different subjects and researchers' skills are involved. On one side, climatology is fundamental to forecast future scenarios (Giannakopoulos et al., 2005, 2009; IPCC, 2013; Bellucci et al., 2013). On the other side, ecology and biology skills are basic to forecast true impacts on ecosystems and species distributions. In addition, a matter of interest is also the interaction of biotic versus abiotic factors at margins of the natural range (Guisan and Zimmermann, 2000) and especially for planted/introduced species. In such background, prediction of future impacts on forest ecosystems are a mixture of climatic prediction and adaptability of the species that must be considered in a holistic view and tackled under many different aspects (Pearson and Dawson, 2003; Austin, 2007; Trivedi et al., 2008; Sinclair et al., 2010).

Ecological Niche Models (ENM) and *Species Distribution Models* (SDM) are well known methods used to link the ecological niche/distribution of forest species with a set of environmental variables, that can be used as predictors in a modelling procedure (Elith and Leathwick, 2009). However, even if, in both cases, the aim is to predict the environmental suitability for a species (i.e. survival or performance) and they are generally used as synonyms, literature is full of discussions about that issue due to the real possibility to perform ENMs properly (Guisan and Thuiller, 2005; Elith and Leathwick, 2009; McInerny and Etienne, 2012; Warren, 2012; McInerny and Etienne, 2013; Warren, 2013). The kernel of the discussion is about the real possibility to perform ENMs which would require a full knowledge of ecological dynamics that are impossible to include into a mathematical model. For instance, inter- and intra-specific competition, seed dispersal, biological connection between the target forest species and animals and many other issues should be incorporated in into SDM to obtain areal ENM. In few words, to perform a real ENM researchers should be able to define and model the real and the potential niche of a species.

- In ENM the core of the computation is the ecological niche and predictors are used to define and compare it with the background ecological environment and competitors;
- In SDM ecological niche concept is supplied by specie's geographical distribution and, in such case, it is supposed that the natural range explicate all the ecological power of the species.

In addition, both can also be used to incorporate future climate predictions and, in such case, future distribution of species can be forecast. That become a very interesting way to study and predict climate change effects on populations (Willis et al., 2009; Sinclair et al., 2010; Attorre et al., 2011; Cheaib et al., 2012; Pellat et al., 2012; Wang et al., 2012; Forester et al., 2013; Isaac-Renton et al., 2014).

Tested algorithms

In this study and as many other cases, the SDM approach was used due to amount of information available and the biased geographical distribution of Black pine in Abruzzo (see chapter 2.2). A huge number of different algorithms for SDMs were performed in literature, often compared each other to assess algorithms' power and suitability for different nature of data and ecological zones (Stockwell and Peterson, 2002; Zaniewski et al., 2002; Pearson et al., 2003; Elith et al., 2006). Similar to interpolation of climatic data, also performances of SDMs are strictly connected to data accuracy and reliability. Autocorrelation between presence points, collinearity between predictors, biased distribution and information of species occurrence (presence, absence and pseudo-absences) are only few of the most common problem in Ecological Modelling. In this work, through a SDM approach, four algorithms were compared:

- *Generalized Linear Model* (GLM)
- *Surface Range Envelop* (SRE, also known as BIOCLIM)
- *Multivariate Adaptive Regression Splines* (MARS)
- *Random Forest* (RF)

Each method is widely used in literature, each with its pros and cons. In this study, the four methods were selected to study differences between regression methods (GLM and MARS) and classification methods (SRE and RF). All methods were implemented in *biomod2* package (Thuiller et al., 2014) which was adopted to perform the spatial analysis.

GLM is generally known as “*Logit Model*” it is used for binomial regression (1, 0) and it is widely available in statistical packages (Bedia et al., 2011) and the optimal regression formula is generally calculated through a stepwise procedure, using AIC criteria. A purpose of this algorithm in this study was to compare specific algorithms for species distribution modelling with a general and simple regressive method.

SRE, generally known as BIOCLIM, is a simple and fast algorithm (very few computational-time is required for modelling) considered as example of profile-technique. It is a presence-only algorithm (Beaumont and Hughes, 2002; Zaniewski et al., 2002; Tsoar et al., 2007) and computes the similarity of a location by comparing the values of environmental variables at any location to a percentile distribution of the training sites. The closer to the median, the more suitable the location is. Anyway tails of the distribution are not distinguished, so the 10th percentile is treated as equivalent to 90th percentile (Hijmans et al., 2013). The algorithm has been often used for species distribution modelling for its ready-to-use quality and is the classic “*climate-envelope-model*” even if, as reported in Elith et al. (2006) it generally does not perform as good as some other modelling methods, especially predicting climate change effects (Hijmans and Graham, 2006).

MARS (Friedman, 1991) is a powerful nonparametric tool, mainly used for data mining. It is an adaptive procedure and similar to GLM it is based on regressive methods and well suited for high-dimensional problems (i.e, a large number of inputs). It can be considered as a generalization of stepwise linear regression or a modification of the CART method (Hasie et al., 2008). The main feature of MARS is that the algorithm works sub-setting the dataset in different subsections which are modelled separately and connected at the end of computation.

RF regression-model algorithm (Breimann, 2001) belongs to the machine-learning group and derives from Classification and Regression Trees. In this case, the regression is built using predictors to classify object which are sampled randomly through a bootstrap procedure. The number of randomly-sampled predictors is, in general, the square root of the total number for classification and one-third for regression. Tree nodes are created using the randomly-sampled predictors (generally climatic variables or bioclimatic indices as our case) that had the smallest classification error and, for each step, RF created a different regression tree, splitting data into groups, the "bagged sample" and the "out-of-bag sample". The first is used to create the tree and the second is used to calculate the classification error (OOB-error). After a specific number of trees (*ntree* parameter with is generally set to 500) the "forest" is created and the computation ends.

Presence/Absence data

As reported before, SDMs works with species occurrence. Even if "*presence*" and "*absence*" concepts are quite easy to understand, they must be carefully considered before modelling. Indeed at the present time, due to human history and activities on forest ecosystems, a quantity of the current geographical distribution of the target species was/is influenced by human activities. Forest management for timber production, reforestation on poor and vulnerable soils or on abandoned lands, etc... have strongly modified the distribution of forest species especially in Europe as well as in most of the world. As a result, in some cases such as *Pinus nigra* spp., the present distribution, which could be used as "presence points" in an Ecological Modelling activities, may not represent the real ecological niche of the species. In such context, planted forests, for example, could be considered in two different ways: i) "presence" if the target of the study is to analyse the suitable envelope of the species, ii) "absence" if only the natural distribution should be investigated. Consequently, and similar to presence points, absence data could be considered “true-absence” only when referred to natural distribution and when absence data identify a real and completely unsuitable zone for the specie (for soil, climate and ecological conditions). In addition, absence points are generally much more abundant than presence and, when the proportions of presences and absences are not equal (or not equally weighted), SDMs prediction can be asymmetric, deviating towards the extreme that has got a greater number of cases (Real et al., 2006). To solve that problems, presence and absences must be carefully analysed and considered. However, despite definitions, which brought researchers to define ENM only a theoretical model (McInerny GJ and Etienne 2012; McInerny GJ and Etienne 2013), a widely adopted solution is to use all the information available and to consider presence as “true presence” and absence as as “pseudo-absence”.

Black pine of Villetta Barrea is a very interesting and significant species for Ecological modelling due to its current distribution. The species has been widely used for afforestation activities and was planted much far away from ts natural range. However a lot of artificial stands are well adapted and well growing and this is something that must be considered and included into the modelling steps. For this reasons, in this study, the concepts of “*potential-presence*” and “*pseudo-absence*” were adopted, using the entire current distribution of the species in Abruzzo. Presence data were extracted from Abruzzo forest eco-types map (Corona et al., 2009) considering as presence all polygons of the “*Natural stand of Villetta Barrea pine*” and any other population of Abruzzo included into the “A*fforestation in mountainous areas*” category. However, SDMs work with coordinates of presence points as input data and with a simple extraction algorithm, polygonal data were converted in coordinates, considering the centroid of each rasters' cell covered (in total or in part) by a polygon belonging to the first or the second category. As a result, 10,047 presence points in WGS84 UTM 33N reference system were obtained. On the other side, the same was done with pseudo-absences data (444,143 locations) using all the others polygons. To solve the problem of prevalence, from the full dataset (10,047 presences and 444,143 absences) an equal number of presence and absence points were randomly extracted weighting them equally during the computation (Barbet-Massin et al., 2012). As a result, five different datasets (PArepI, PArepII, ParepIII, ParepIV, PArepV) with the same total number of points (20,094), the same presence data but different pseudo-absences were created.

Climate data preparation, future scenarios, model scores and evaluation methods

To model the present or future distribution of species with SDMs, it is very important to respect some statistical and mathematical assumptions. Collinearity between predictors and autocorrelation between presence/absence data are the most relevant issues that must be analysed and removed. To achieve that, presence points were carefully controlled with geostatistical methods, whereas the 19 bioclimatic layers plus the soil map were tested for collinearity (Montgomery and Peck, 1982).

To model the current distribution, climate layers were obtained interpolating climatic regional data whereas to predict the future distribution in Abruzzo two future scenarios (ABR1 and ABR2) were developed at regional scale from them. Following Giannakopoulos et al. (2005) predictions for the Mediterranean area, present climatic layers were modified as reported in Table 6. On the other side, considering that predictions were calculated for a small time period (from 2030 to 2060) the soil map was kept as stable. The two new dataset were developed for Abruzzo territory only, with the same spatial resolution of current climate data (100 metres).

Table 6: Climatic scenario for Abruzzo

SEASON	**ABR1**		**ABR2**	
	Temperature	Precipitation	Temperature	Precipitation
Spring	+1°C	-25%	+2°C	-45%
Summer	+2°C	-40%	+4°C	-60%
Autumn	+1°C	stable	+2°C	stable
Winter	+1°C	-25%	+2°C	-45%

Accuracy of the four algorithms were assessed using a bootstrapping procedure with 30 runs for each dataset (Stockwell and Peterson 2002; Wisz et al., 2008; Liu et al., 2011). During the computation, the importance of each predictors was estimated as an average over the 30 runs. At the same time, to rank algorithms' performances the *Area Under Receiver Operating Characteristic Curve* (AUC; Phillips et al., 2006; Liu et al., 2011) and *True Skill Statistics* (TSS; Allouche et al., 2006) were calculated. AUC and TSS were calculated with a split-sample approach (Van Houwelingen and Le Cressie, 1990), dividing each dataset in “training sites” and “test sites” with a 80% - 20% splitting size (Boslaugh and Watters, 2008; Melini, 2013) for both presence and pseudo-absence points. AUC and TSS are both indicators of goodness of prediction and while the first varies between 1 and 0 the second ranges between -1 and 1. TSS corresponds to the sum of sensitivity and specificity-minus-one and has the additional advantage of being fully independent from the species prevalence and the size of the validation dataset. Results of evaluation processes were used to perform a nonparametric ANOVA on TSS values considering models as treatments and the five Presence/Absence datasets as Blocks.

After model comparison, to avoid lack of information and biases during the random extraction, predictions of models with TSS higher than 0.7 (Araùjo et al., 2005; Forester et al., 2013) were used to calculate an ensemble model. The whole procedure is graphically-reported in Figure 10.

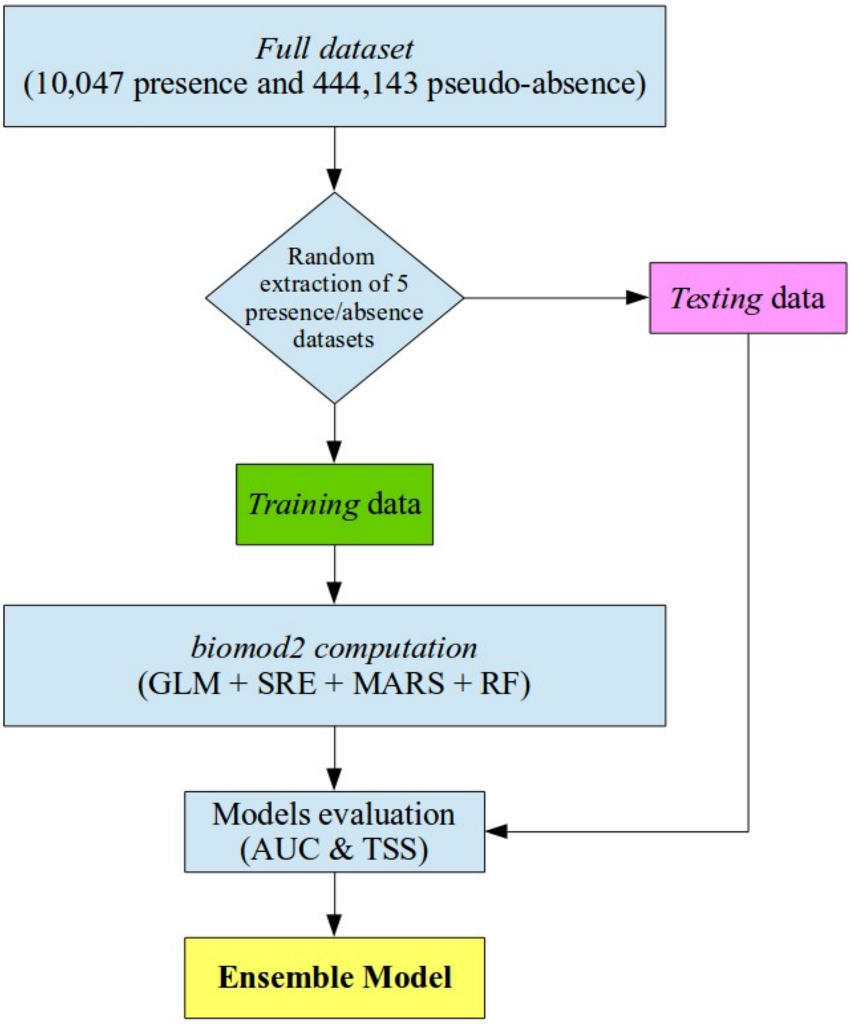

Figure 10: Computational model for building the ensemble SDM

With the ensemble model, three different suitability maps were obtained, one for each scenario (ABR0, AB1, ABR2). To calculate the potential suitable area for Black pine in Abruzzo, maps were transformed rescaling pixel values between 1 (perfect possibility to survive) and 0 (not suitable for the species). The binary transformation was carried out using the threshold which maximize TSS, a method known to improve the accuracy of prediction (Jimenez-Valverde and Lobo, 2007; Barbet-Massin et al., 2012). Pixels (1 pixel = 1 ha) with a value equal or higher than a specific threshold (0.7) were counted to estimate the potential suitable area for each scenario. The same procedure was performed for elevation values computing general statistics (minimum, median, mean, mode and maximum) to check a possible rising at higher elevation of the suitable envelope. The ROC-Optimized Probability Threshold (ROC-OPT) was not used due to its known tendency to overestimate the true occurrence of species with low prevalence (Orallo et al, 2012).

3.5 Softwares, packages and desktop environment

Most of the work has been carried out with open-source software, freely available and multi-platform. QGIS 2.6 (QGIS Development Team 2014) was used for map visualization, analysis and management whereas GRASS GIS 6.4.4 and R 3.1.1 (R Core Team 2014) with eight added packages were used for statistical and geostatistical analysis:

- *biomod2* (Thuiller et al., 2014) for SDM tools;
- *bootRes* (Zang and Biondi, 2012) for climatic correlation;
- *dismo* (Hijmans et al., 2013) for SDM tools;
- *dplR* (Bunn 2008; Bunn 2010; Bunn 2014) for tree-rings series analysis;
- *gstat* (Pebesma 2004) for geostatistical analysis and data interpolation;
- *raster* (Hijmans 2014) for managing raster data and interpolation;
- *rgdal* (Bivand et al., 2014) for managing vector data in R;
- *usdm* (Naimi, 2013) for data analysis.

4. Results

4.1 Marginality detection

Results of the estimation of the Mahalanobis distance are reported in Figures 11 and 12 as density-plot and QQ-plot. The analysis detected a relatively long distance between the MaPP site and the remaining zones of the *Pinus nigra* range. The density function showed a bivariate distribution mainly around 10 and 15 values and QQ-plot confirmed an overall high value distance. Elevation was the main driving force and as long as it increased the difference increased too.

Concerning PCA plots (Figure 13) only *mauretanica* and *dalmatica* subspecies were totally enclosed into the "ecological cloud" of *Pinus nigra* spp. (black points), which probably mean that they did not include any population located at margins of the ecological distribution. The distributions of the others four subspecies were instead much wider and partially overlapped. In that case, some ecological features could be probably shared.

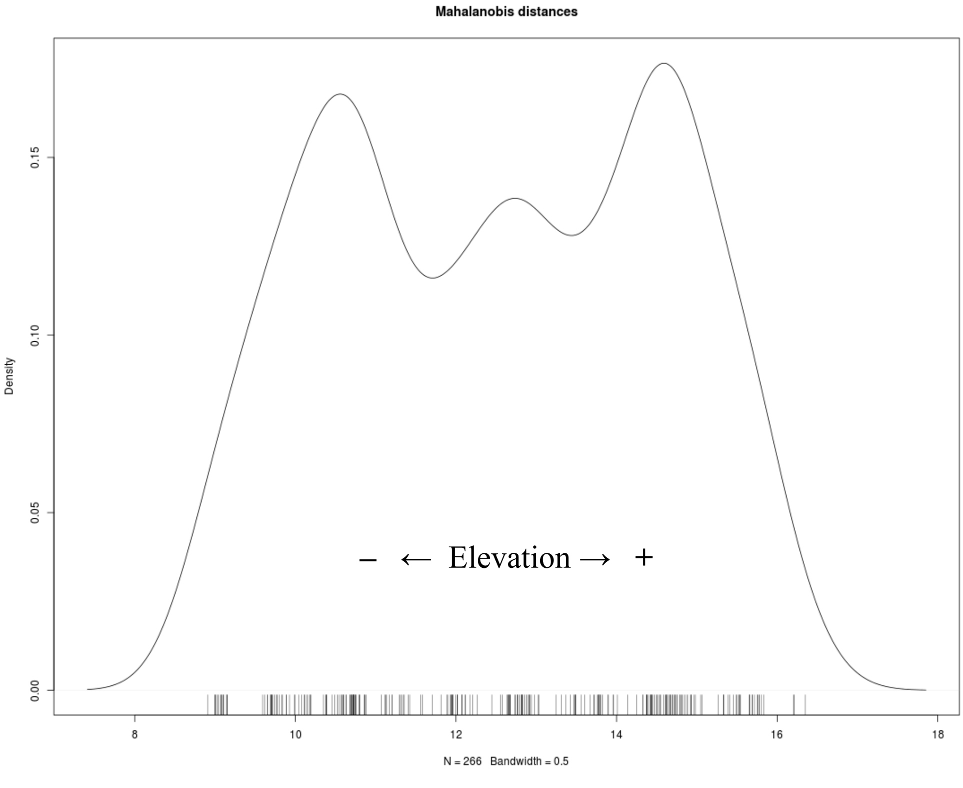

Figure 11: Mahalanobist distance between MaPP area and Pinus nigra range in Europe.
X axis = distance; Y axis = density/frequency. Higher values corresponds to higher elevations

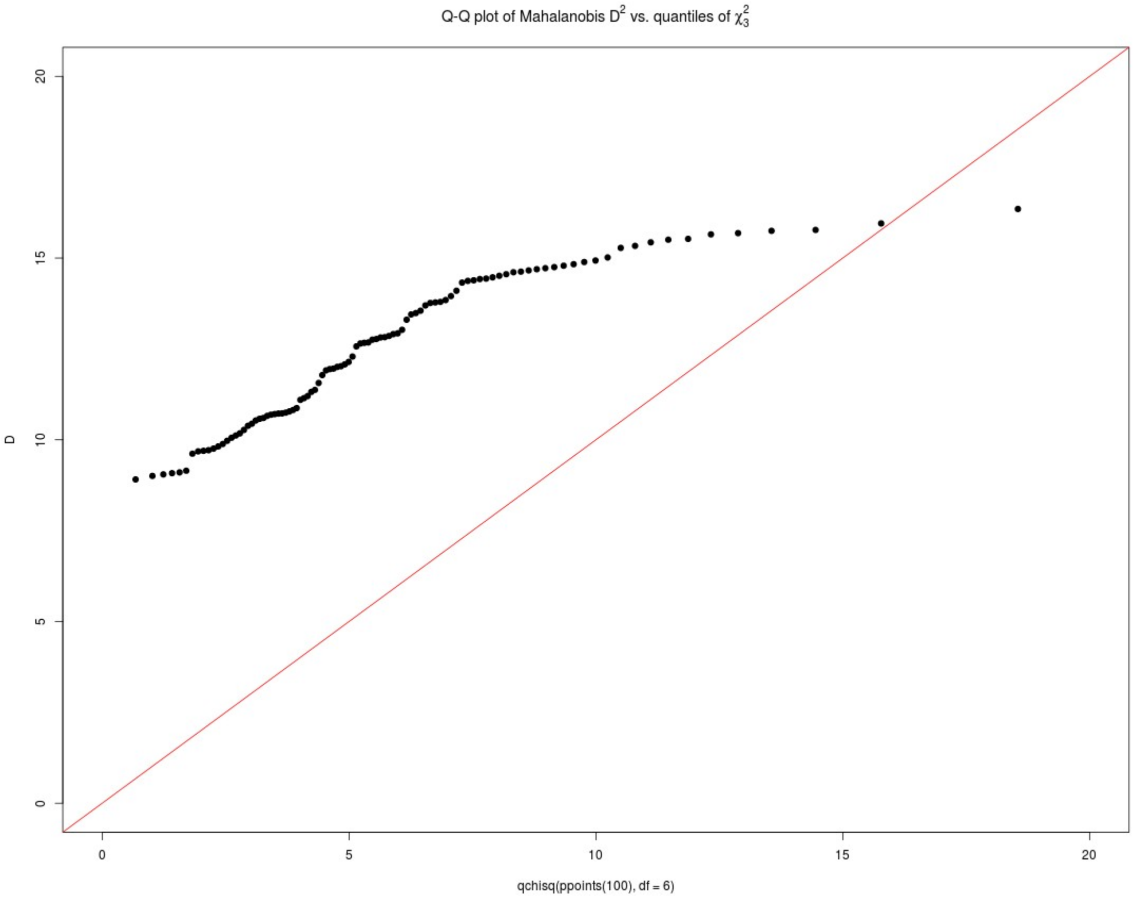

Figure 12: QQ-plot of mahalanobis distance. Black dots are the position of pixels of MaPP area while red line is the y=x equation

A more detailed plot on *nigra* subspecies and the population of Villetta Barrea is projected in Figure 14, showing that the MaPP of Abruzzo (yellow points) is totally enclosed into the *nigra* "environment" (red cloud).

Concerning Italian environment and the distribution of Balck pine spp. (only *nigra* and *laricio* subspecies) Figure 15 reports Walter and Lieth diagrams for the main Seed stands across Italy. The diagrams demonstrated the differences between southern locations from Calabria (Sila and Aspromonte) and northern locations (Cles, Prato allo Stelvio, Resia and Carso Triestino). Calabrian provenances present a typical diagram of "Mediterranean regions" whereas northern provenances are adapted to colder climate without winter rainfalls. Villetta Barrea (red outline) is located between those two extremes, without higher winter rainfalls but with a small aridity period.

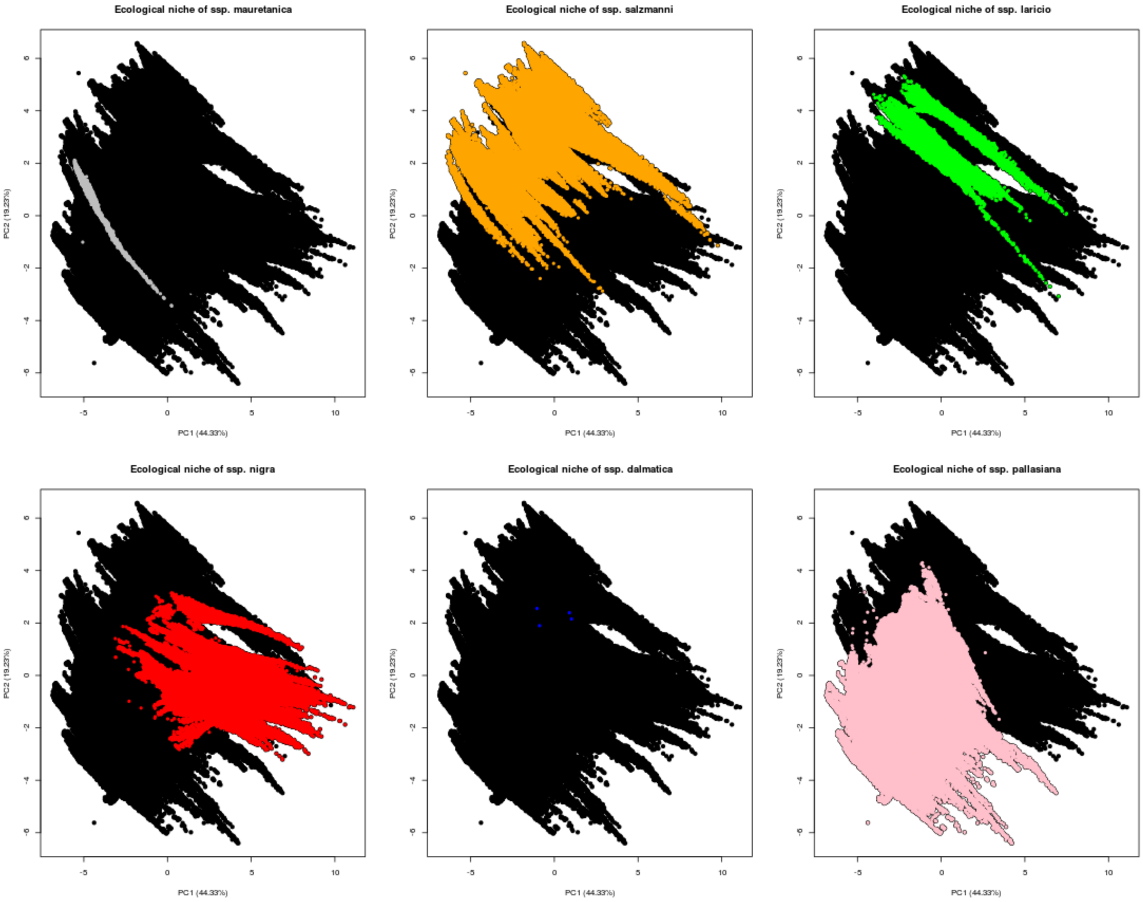

Figure 13: Principal Components coordinates plots. Each subspecies (colored dots) is projected against the global distribution (black dots)

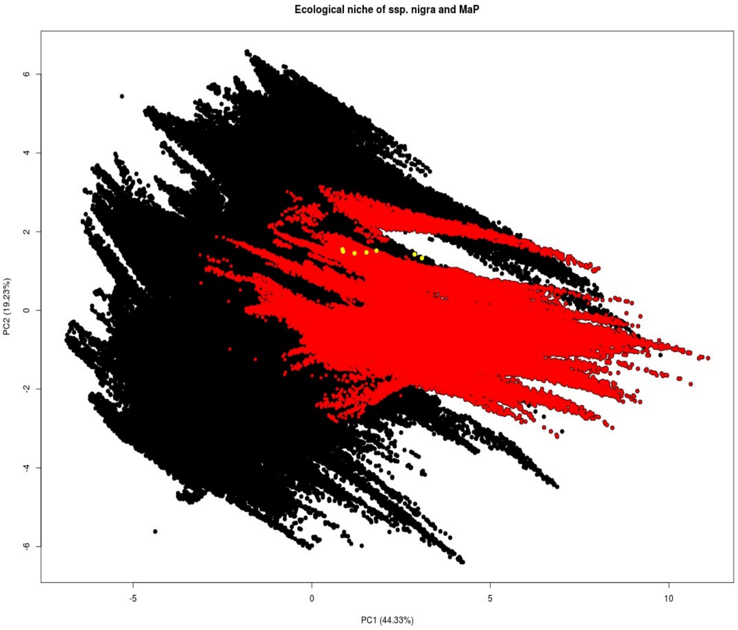

Figure 14: Principal Components coordinates plots for nigra subspecies (red) and MaPP of Abruzzo(yellow).

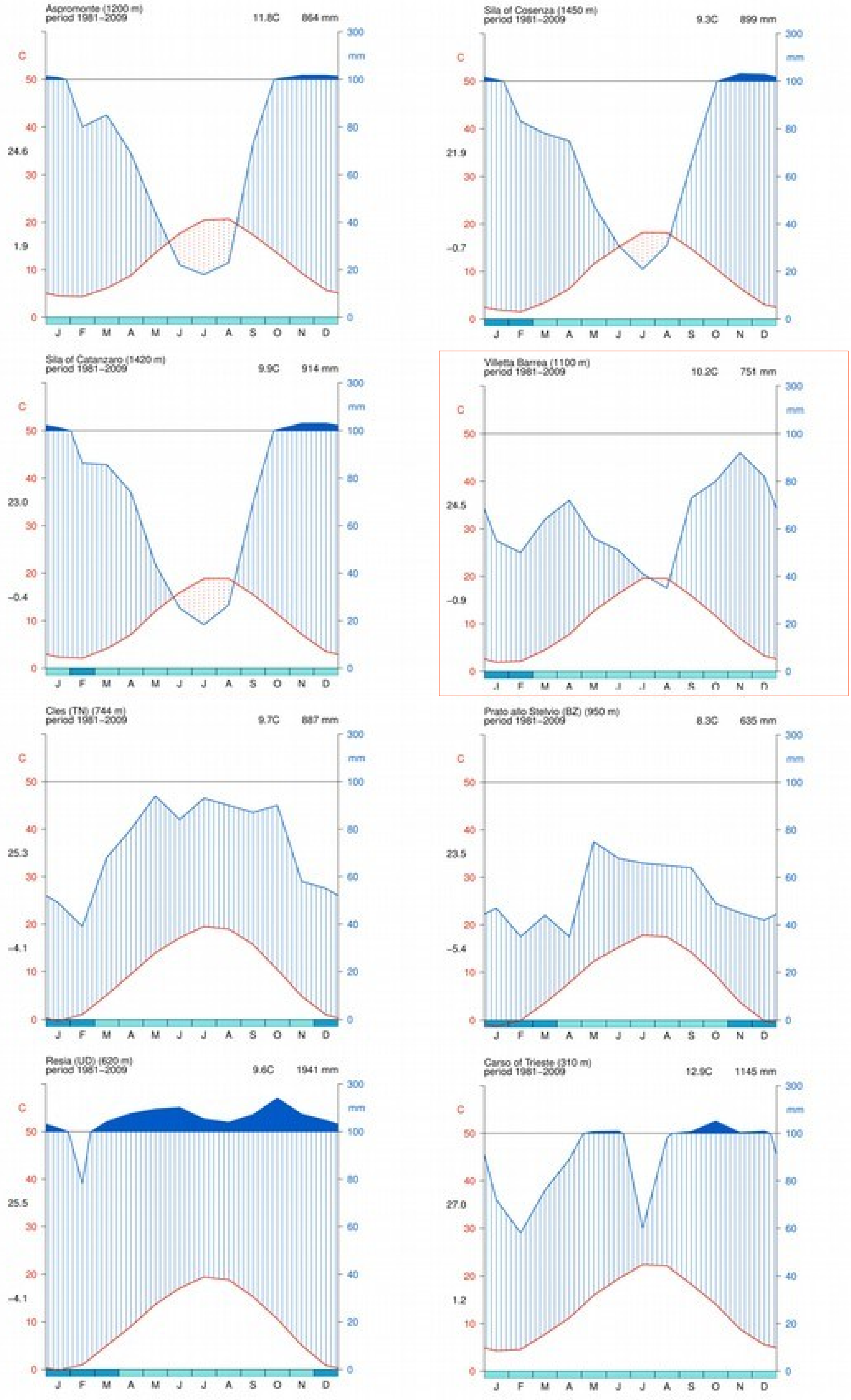

Figure 15: Walter and Lieth diagrams for the main Seed stands of Pinus nigra spp. Across Italy. Villetta Barrea is outlined with red colour.

4.2 Interpolation of local climatic data

The preparatory analysis on climate data detected that, in many cases, data were not normally distributed (Table 7) and not strongly correlated with physiographic parameters (Table 8). Pearson correlation coefficients were relatively low, varying between 0.62 (EMPq ~ ELEV) and 0.24 (DMP ~ SLO) and only 14 couples on 65 were statistically significant. The regression-analysis' results are reported in Table 9. Adjusted R^2 ranged between 0.15 of the temperature of the coldest month (CMT) and 0.41 of Emberger Pluviotermic Quotient (EMPq).

Table 7: Kolmogorov-Smirnov normality test for climatic variables and indices (α 0.05)

Data	D value	p-value
MAP	0.0973	0.0029
Transformed MAP	0.0577	0.3210
DMP	0.0917	0.0067
Transformed DMP	0.0604	0.2542
MAT	0.1150	0.0582
Transformed MAT	0.1049	0.1228
CMT	0.1365	0.0099
Transformed CMT	0.1100	0.0987
DMT	0.1184	0.0453
Transformed DMT	0.1114	0.0755
HMT	0.1012	0.1552
CMTm	0.1436	0.0051
Transformed CMTm	0.1140	0.0654
CMTx	0.1260	0.0247
Transformed CMTx	0.1131	0.0666
HMTx	0.1160	0.0539
DMAi	0.0959	0.2141
EMPq	0.1220	0.0340
Transformed EMPq	0.0970	0.2012
RMCi	0.1064	0.1115
RMTi	0.1308	0.0164
Transformed RMTi	0.0959	0.2148

Table 8: Correlation Matrix (Spearman) between climatic data and physiographic parameters. In bold are p-value < 0.005 (n=57)

	MAP	*DMP*	*MAT*	*CMT*	*DMT*	*HMT*	*CMTm*	*CMTx*	*HMTx*	*DMAi*	*EMPq*	*RMCi*	*RMTi*
ELEV	**0.45**	0.18	-0.26	-0.23	-0.31	-0.30	-0.19	-0.26	**-0.43**	**0.55**	**0.62**	**-0.41**	-0.24
SLO	**0.34**	**0.24**	0.04	0.12	0.01	0.01	0.23	-0.02	-0.25	0.26	0.33	-0.34	0.08
ASP	-0.11	-0.08	0.36	0.36	**0.38**	**0.38**	**0.40**	0.29	0.26	-0.37	-0.36	0.23	**0.37**
DSEA	**0.36**	-0.15	-0.32	-0.33	**-0.38**	-0.37	**-0.40**	-0.19	-0.22	0.32	0.34	-0.21	-0.33
GSR	0.02	-0.08	-0.03	-0.05	-0.08	-0.06	-0.05	-0.02	-0.04	0.02	0.05	-0.11	-0.04

Table 9: Regression models analysis. For each variables, significance of parameters are reported with the following legend: p<0.1 (.), p<0.05 (*), p<0.01 (**), p<0.001 (***) not significant (ns), not used (-)

Variable	*R^2/ adj R^2*	*Interc.*	*LAT*	*LON*	*ELEV*	*SLO*	*ASP*	*DSEA*	*GSR*
MAP	0.22 / 0.20	***	-	-	*	*	-	ns	-
DMP	0.18 / 0.16	***	-	-	***	-	ns	***	-
MAT	0.20 / 0.17	***	-	-	-	-	*	.	-
DMT	0.22 / 0.19	***	-	-	-	-	*	*	-
HMT	0.22 / 0.19	***	-	-	-	-	*	*	-
HMTx	0.20 / 0.17	***	-	-	**	-	ns	-	-
CMT	0.18 / 0.15	**	-	-	-	-	*	.	-
CMTm	0.24 / 0.20	ns	-	-	-	ns	.	*	-
CMTx	0.14 / 0.11	***	-	-	ns	-	.	-	-
DMAi	0.33 / 0.29	***	-	-	**	-	*	-	ns
EMPq	0.43 / 0.41	**	-	-	***	-	-	-	*
RMCi	0.17 / 0.14	***	-	-	ns	.	ns	-	-
RMTi	0.17 / 0.14	***	-	-	ns	-	*	-	-

Concerning interpolation processes, RMSE, AME and RMSE% from each interpolation method for all analysed climatic factors and indices are reported in Table 10, showing a very wide range of errors. RMSE for temperature varied between 2.08°C of RK for CMTx and 2.87°C with K-NN for CMTm whereas for MAP was between 142.1 mm of UK and 230.2 mm for RK and for DMP was between 5.47 mm of OK and 10.04 mm with RST. Minimum values of RMSE% were found interpolating RMCi with UcoK (3.8%) and maximum with interpolation of for CMTm with K-NN (295.3% of).

Table 10: Cross-validation results for climatic factors and indices. Best results are reported in bold

	ALGORITHMS									
	IDW	*RST*	*MLRG*	*K-NN*	*OK*	*RK*	*UK*	*OcoK*	*UcoK*	
	166.78	181.90	174.1	197.62	144.4	230.2	**142.1**	150.0	149.1	RMSE
MAP	130.95	103.34	149.55	147.43	102.5	176.8	**96.8**	102.0	102.8	MAE
	17.7%	17.1%	20.5%	19.2%	13.4%	22.3%	**12.8%**	13.3%	14.8%	RMSE%
	7.28	6.17	10.04	7.95	**5.47**	5.56	5.65	6.03	5.83	RMSE
DMP	5.95	4.48	7.81	6.04	**4.22**	4.22	4.14	4.40	4.29	MAE
	18.1%	20.1%	22.3%	24.1%	**12.3%**	12.3%	12.1%	12.6%	12.4%	RMSE%
	2.39	2.40	2.43	2.47	2.38	2.46	2.40	**2.32**	2.33	RMSE
MAT	1.85	1.83	1.97	1.91	1.90	2.04	1.91	**1.82**	1.83	MAE
	22.7%	21.7%	23.1%	24.1%	23.1%	23.1%	23.4%	**21.9%**	22.0%	RMSE%
	2.21	2.34	2.32	2.43	2.33	**2.17**	2.33	2.22	2.23	RMSE
CMT	1.74	1.77	1.82	1.95	1.80	**1.69**	1.78	1.71	1.73	MAE
	50.0%	255.8%	53.0%	57.2%	54.8%	**50.4%**	53.0%	53.0%	54.0%	RMSE%
	2.49	2.63	2.52	2.62	2.57	**2.49**	2.63	2.51	2.52	RMSE
DMT	2.03	2.06	2.04	2.08	2.06	**2.04**	2.08	2.00	2.00	MAE
	12.9%	13.3%	13.1%	13.4%	13.4%	**12.9%**	13.6%	13.0%	13.1%	RMSE%
	2.65	2.61	2.51	2.61	2.54	**2.46**	2.60	2.48	2.49	RMSE
HMT	2.00	2.03	2.01	2.07	2.04	**2.01**	2.06	1.98	1.97	MAE
	13.7%	13.1%	12.9%	13.9%	13.2%	**12.7%**	13.4%	12.8%	12.9%	RMSE%
	2.46	2.53	2.74	2.87	2.47	**2.42**	2.58	2.47	2.50	RMSE
CMTm	1.96	1.96	2.17	2.34	1.91	**1.90**	1.99	1.93	1.97	MAE
	130.2%	291.6%	281.9%	295.3%	145.0%	**138.5%**	125.1%	103.8%	149.7%	RMSE%
	2.14	2.31	2.14	2.17	2.17	**2.08**	2.18	2.09	2.13	RMSE
CMTx	1.67	1.79	1.66	1.68	1.76	**1.65**	1.74	1.65	1.68	MAE
	43.5%	36.1%	39.3%	45.1%	44.9%	**40.9%**	41.7%	41.1%	39.7%	RMSE%
	2.22	2.36	2.20	2.14	2.27	2.21	2.25	**2.12**	2.17	RMSE
HMTx	1.79	1.89	1.76	1.61	1.80	1.79	1.76	**1.67**	1.71	MAE
	8.7%	9.0%	8.7%	8.8%	8.9%	8.7%	8.8%	**8.4%**	8.5%	RMSE%
	6.46	6.13	6.87	6.59	6.11	5.98	**5.79**	5.97	6.13	RMSE
DMAi	5.00	4.60	5.23	5.13	4.72	4.68	**4.39**	4.64	4.70	MAE
	21.4%	23.6%	22.7%	22.0%	21.2%	20.7%	**19.1%**	19.1%	19.8%	RMSE%
	36.01	45.24	90.84	37.16	38.27	84.83	43.71	**35.60**	37.28	RMSE
EMPq	29.08	29.09	38.04	27.57	28.44	35.13	28.25	**26.31**	27.32	MAE
	28.3%	31.5%	62.2%	28.8%	25.5%	58.0%	29.9%	**23.9%**	26.0%	RMSE%
	0.67	0.67	0.66	0.65	0.67	0.67	0.65	0.65	**0.65**	RMSE
RMCi	0.52	0.53	0.49	0.50	0.53	0.52	0.49	0.50	**0.48**	MAE
	3.9%	3.8%	3.8%	3.8%	3.8%	3.8%	3.8%	3.8%	**3.8%**	RMSE%
	70.07	70.96	73.56	72.96	70.92	69.56	70.96	**68.80**	70.09	RMSE
RMTi	53.29	53.99	56.49	57.87	56.39	55.49	53.92	**52.58**	52.92	MAE
	65.3%	55.2%	56.3%	58.2%	62.8%	58.2%	56.3%	**56.5%**	57.0%	RMSE%

Kruskal-Wallis rank sum test on RMSE% showed a chi-square value of 1.7932 with 8 degrees of freedom and a p-value of 0.9867, assessing no difference between RMSE%. Boxplots of errors are reported in Figure 16.

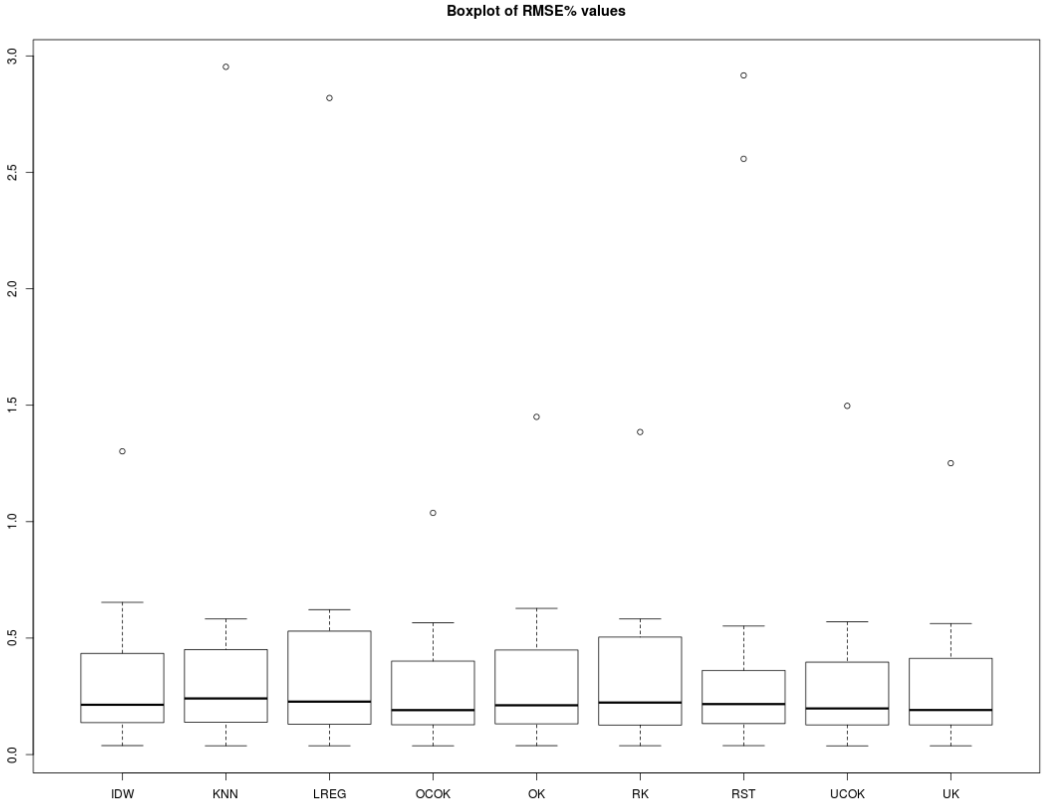

Figure 16: Boxplots of RMSE% for the nine interpolation methods. Interpolation methods are in alphabetic order and, as evident, many outliers are present

The comparison between WorldClim database and interpolated data is reported in Table 11 demonstrating the improvement of interpolation of local data. WorldClim showed higher errors up to 90.4% in case of annual precipitation.

Table 11: WordClim data vs local interpolated data on bioclimatic variable number 12 (mean annual precipitation) and 1 (mean annual temperature)

Variables	*Data or Method*	*RMSE*	*MAE*	*RMSE%*
MAP (bio12)	*WorldClim*	845.1 mm	816.7 mm	90.4 %
	UK of local data	142.1 mm	96.8 mm	12.8 %
MAT (bio1)	*WorldClim*	2.87 °C	2.01 °C	24.5 %
	OcoK of local data	2.32 °C	1.82 °C	21.9 %

4.3 Structure and growing trends of the seed stand

Dendrometric data were collected during the 2012 growing season and are summarized in Table 12 and Figures 17 and 18. Mean DBH ranged between 22.8 cm of plot III and 28.3 cm of plot and extreme cases were represented by plots number III and IV. Highest mean height (19.5 m), maximum trees number (1178 tr/ha), maximum basal area per hectare (60.2 m^2) and maximum volume per hectare (574.5 m^3) were measured in plot IV. On the other hand, plot III represents the area with lowest trees (mean height was 11.8 m), smaller basal area (39.6 m^2) and volume per hectare (259.7 m^3) and highest variation of diameters (64%). In addition, plots III and IV were also characterized respectively by the oldest and the youngest dominant trees. Hypsometric curves are compared in Figures 18. Plots I, II and III had a very similar curve with the same slope but different values. the tallest trees were measured in plot IV.

More in general, the structure of the population was very variable especially concerning the mean height and volumes.

Table 12: summary of dendrometric values for each plot. M_dbh = mean diameter at breast height (cm); M_ht = mean height (m); Tr/ha = trees per hectare; G/ha_P = basal area of Pinus trees per hectare (m^2); Vol/ha_P = volume of Pinus trees per hectare (m^3); G/ha_B = broadleaves' basal area per hectare (m^2); Age = range of sampled dominant trees (years).

PLOT (M.unit)	M_dbh	CV_dbh	M_ht	Tr/ha	G/ha_P	Vol/ha_P	G/ha_B	Age
I (21)	24.5	42%	13.9	1138	46.6	321.0	1.3	124-179
II (21)	26.4	56%	14.3	923	50.6	372.5	0.2	104-145
III (19)	22.8	64%	11.8	1082	39.6	259.7	0.7	143-184
IV (18)	28.0	35%	19.5	1178	60.2	574.5	0.8	80-99
V (18)	28.3	41%	16.52	724	45.7	266.0	2.0	104-118

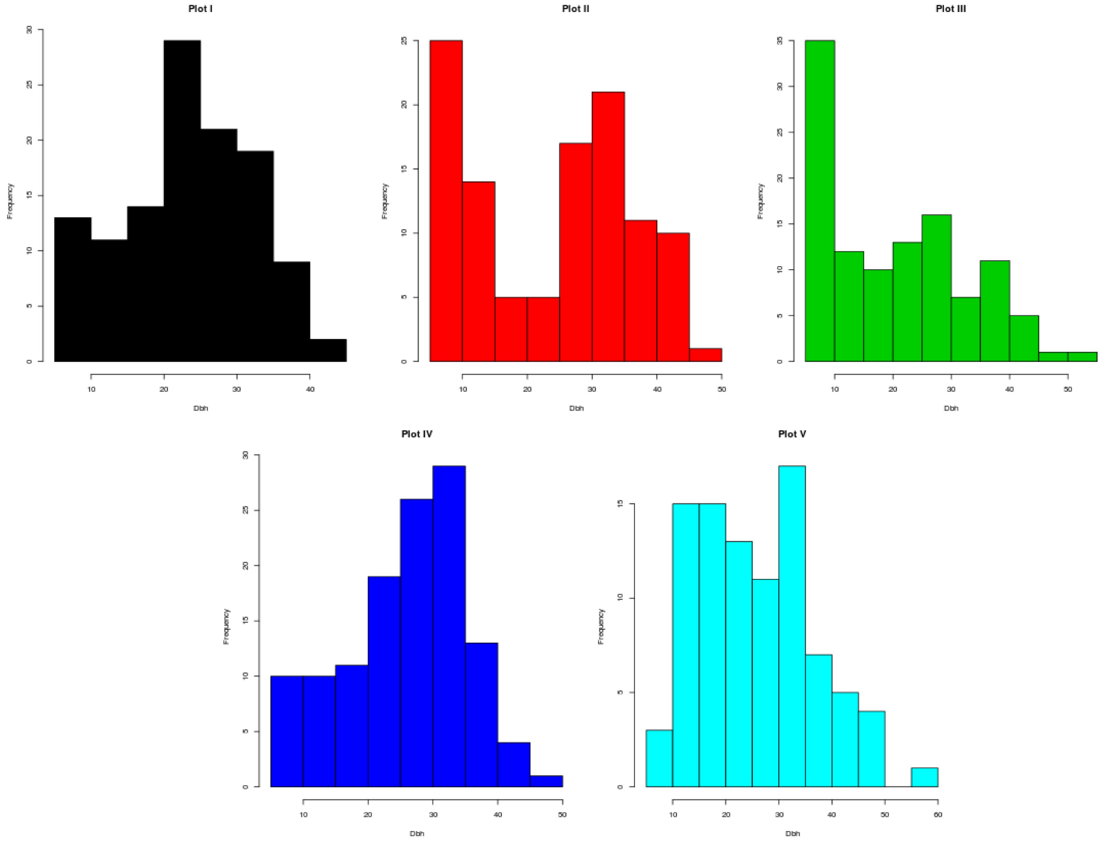

Figure 17: Dbh frequecy

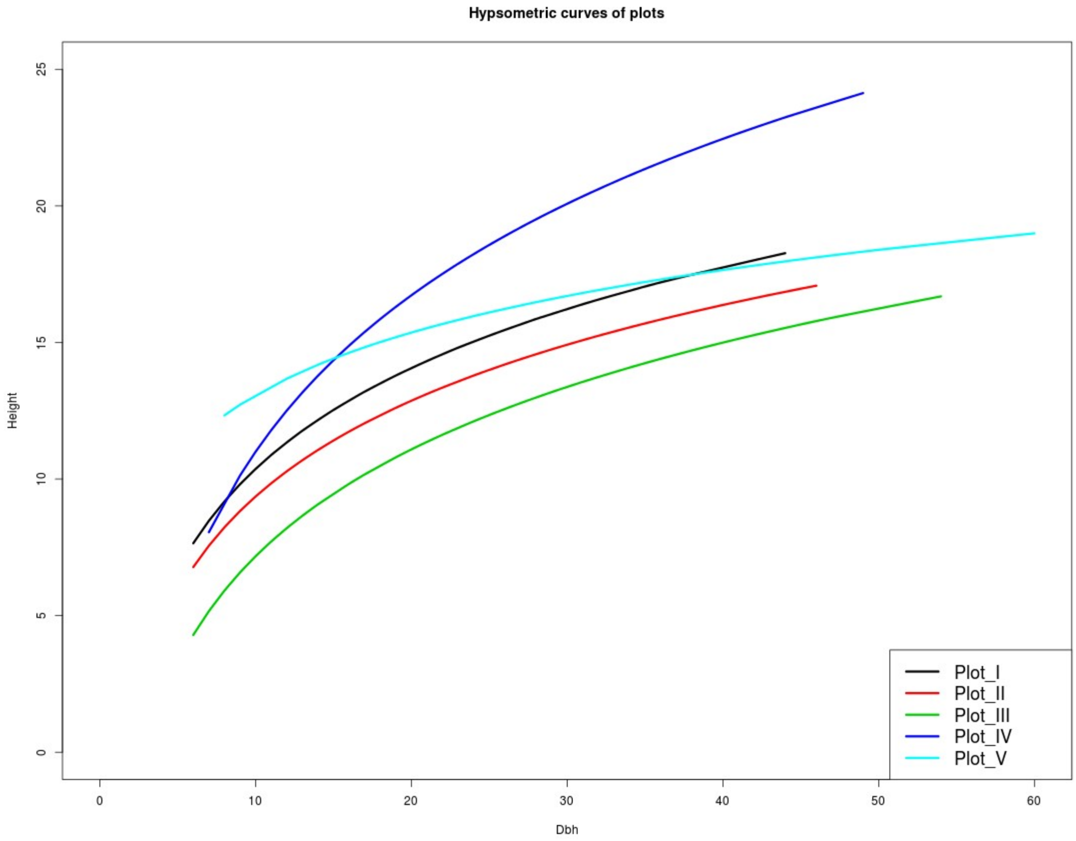

Figure 18: Hypsometric curves

The ANOVA on DBH confirmed high variability and, as expected, grouped the plots following exactly the structure of the management plan (Table 13 and Figure 19).

Table 13: Results of Kruskal-Wallis Rank Sum Test and relative post-hoc test (Wilcoxon Rank Sum and Signed Rank Tests)

Statistical Mean DBH	*Plot (management unit)*	*Group*
26.43089	IV (18)	A
26.28571	V (18)	A
23.02586	II (21)	B
22.62903	I (21)	B
19.18852	III (19)	C

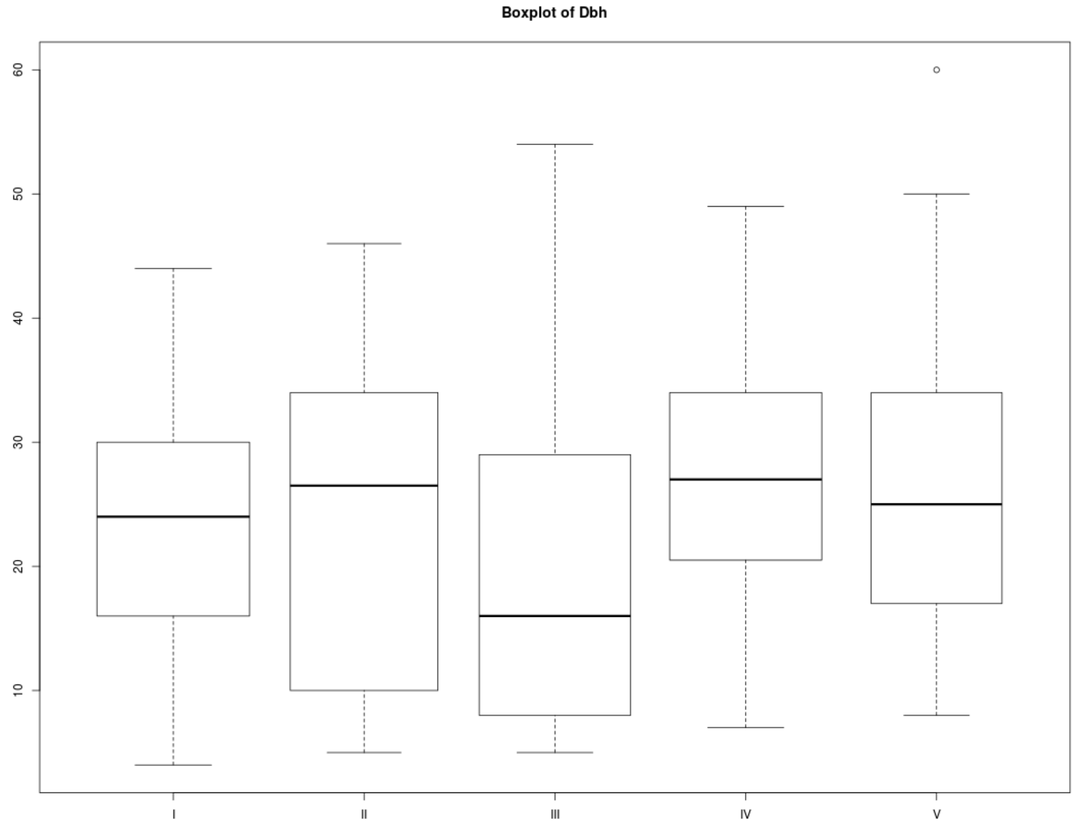

Figure 19: Boxplot of DBHs

The dendrochronological sampling was made during the following year (2013) before the beginning of the growing season. As for ANOVA on DBH, also the analysis on raw tree rings series showed statistically significant differences among plots. However plots were grouped differently but, as expected, following the age of sampled trees (Table 14 and Figure 20). On the other hand, ANOVA on detrended (=standardized) chronologies on the common growing period (1933-2012) did not demonstrate the same differences and mean increment were not significantly different (Figure 21). In such condition, the mean standardized chronology of the whole population could be calculated as a simple mean of all the detrended series.

Table 14: Results of Kruskal-Wallis Rank Sum Test and relative post-hoc t*est (Wilcoxon Rank Sum and Signed Rank Tests) on raw chronologies*

Statistical Mean ringwidth	Plot (management unit)	Group
1.515 mm	IV (18)	A
1.137 mm	V (18)	B
0.973 mm	II (21)	B
0.744 mm	III (19)	C
0.710 mm	I (21)	C

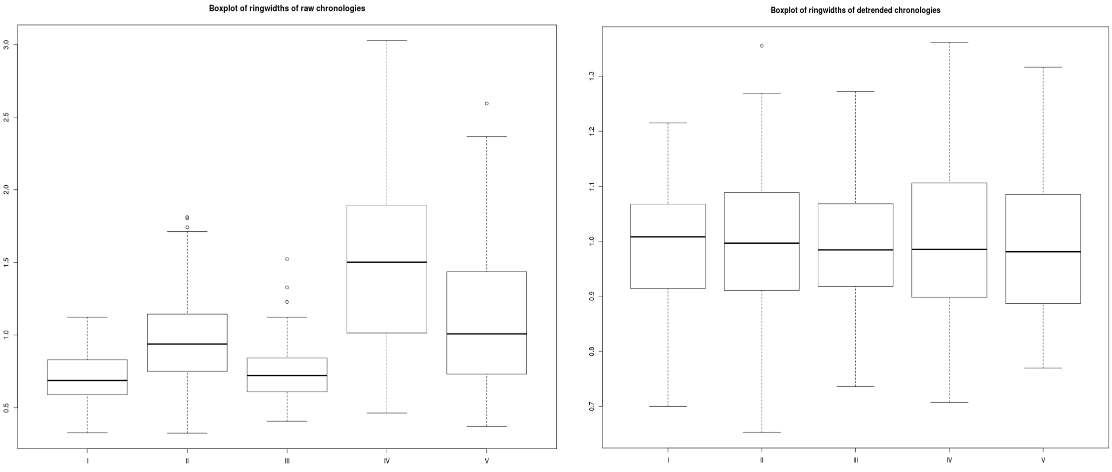

Figure 20 & 21: Boxplots of ring-width of the 5 mean raw chronologies and 5 mean detrended chronologies on the common growing period (1914-2012)

In Table 15 the main statistics about tree rings series, plots chronologies and the mean chronology of the population are reported. Ages of dominant trees ranged between 80 and 184 years with a mean annual increment between 0.627 mm (Plot IV Tree n.1 - P4T1) and 1.967 mm (Plot V Tree n.1 - P5T1). Sensitivity was generally low whereas the first order autocorrelation was quite high emphasizing the high inter-annual correlation between tree-ring widths. Anyway, calculating first-order autocorrelation coefficients on standardized indices, absolute values ranged between 0.11 and 0.22 which means that only a small amount of low-frequency year-to-year variation was not corrected with the standardization procedure.

Table 15: Main statistics of tree rings series. M = mean increment; SD = standar deviation; SK = Skewness; S1 & S2 = sensitivity of first and second order; G = Gini coefficient; AR = Autocorrelation; ARS = Autocorrelation after standardization.
Values are expressed in millimetres and calculated on the whole length of the cores

Series	**First**	**Last**	**Years**	**M**	**SD**	**SK**	**S1**	**S2**	**G**	**AR**	**ARS**
P1T1	1834	2012	179	0.896	0.281	0.924	0.160	0.162	0.172	0.751	-0.119
P1T2	1889	2012	124	1.016	0.340	0.767	0.192	0.188	0.185	0.679	0.041
P1T3	1868	2012	145	0.887	1.011	3.045	0.262	0.217	0.478	0.858	0.062
P1T4	1870	2012	143	1.022	0.747	4.240	0.210	0.210	0.298	0.688	0.091
P1T5	1867	2012	146	1.146	0.737	1.975	0.188	0.179	0.307	0.921	-0.077
P1T6	1864	2011	148	0.954	1.212	3.144	0.179	0.166	0.502	0.913	0.066
P1T7	1869	2012	144	1.054	0.720	2.585	0.184	0.178	0.304	0.874	0.144
M-P1	*1834*	*2012*	*179*	*1.080*	*0.847*	*2.287*	*0.123*	*0.127*	*0.287*	*0.926*	*0.142*
P2T1	1868	2012	145	0.989	1.256	2.648	0.254	0.209	0.538	0.911	-0.150
P2T2	1909	2012	104	1.341	0.572	0.752	0.233	0.229	0.232	0.728	0.101
P2T3	1873	2012	140	1.108	0.983	2.129	0.270	0.240	0.418	0.911	0.187
P2T4	1898	2012	115	1.241	0.659	0.453	0.184	0.176	0.299	0.883	0.126
P2T5	1886	2012	127	1.053	1.159	1.959	0.321	0.239	0.508	0.913	-0.046
P2T6	1892	2012	121	1.515	0.770	1.258	0.250	0.232	0.269	0.801	0.032
M-P2	*1868*	*2012*	*145*	*1.137*	*1.493*	*1.163*	*0.156*	*0.157*	*0.359*	*0.899*	*0.100*
P3T1	1870	2012	143	0.790	0.262	0.587	0.206	0.207	0.183	0.666	-0.177
P3T2	1858	2012	155	0.857	0.289	0.574	0.221	0.222	0.189	0.601	-0.114
P3T3	1837	2012	176	0.896	0.638	1.239	0.255	0.239	0.375	0.840	-0.013
P3T4	1829	2012	184	0.885	0.608	2.255	0.230	0.205	0.319	0.892	-0.095
P3T5	1829	2012	184	1.203	0.911	2.085	0.239	0.254	0.359	0.851	0.073
M-P3	*1829*	*2012*	*184*	*1.036*	*0.679*	*2.692*	*0.147*	*0.151*	*0.279*	*0.925*	*0.067*
P4T1	1914	2012	99	1.672	0.627	0.581	0.232	0.223	0.208	0.719	-0.056
P4T2	1933	2012	80	1.941	0.756	-0.033	0.206	0.203	0.218	0.718	-0.166
P4T3	1925	2012	88	1.659	0.843	1.747	0.194	0.174	0.258	0.791	-0.098
P4T4	1927	2012	86	1.631	1.344	1.161	0.264	0.227	0.427	0.888	-0.004
P4T5	1922	2012	91	1.718	1.519	1.684	0.282	0.247	0.437	0.822	0.205
P4T6	1918	2012	95	1.390	0.898	1.665	0.251	0.257	0.324	0.790	0.127
M-P4	*1914*	*2012*	*99*	*1.745*	*0.785*	*0.698*	*0.165*	*0.162*	*0.249*	*0.872*	*0.060*
P5T1	1907	2012	106	1.967	1.479	1.322	0.222	0.203	0.390	0.855	-0.030
P5T2	1908	2012	105	1.154	0.861	1.753	0.241	0.246	0.375	0.751	0.029
P5T3	1905	2012	108	1.758	1.072	1.375	0.276	0.258	0.321	0.766	-0.067
P5T4	1909	2012	104	1.512	0.926	0.920	0.183	0.190	0.334	0.891	0.070
P5T5	1895	2012	118	1.841	1.618	2.081	0.226	0.196	0.417	0.843	0.022
M-P5	*1895*	*2012*	*118*	*1.984*	*1.62*	*1.916*	*0.156*	*0.157*	*0.402*	*0.852*	*0.078*
chrono	**1829**	**2012**	**184**	**1.578**	**0.823**	**1.165**	**0.141**	**0.146**	**0.278**	**0.873**	**0.081**

Before climatic correlations, ClimateEU data were carefully checked and compared local data for the common time-period available (1955-2010). Regressions were always highly significant with a very high adj-R^2 for temperature but not for precipitations which due to many missing values (mainly for and storage errors) in Barrea's database. However, in every cases, slope of functions showed a very high p-level, which means that both the ClimateEU's and Barrea's trends were statistically comparable (Table 16).

Table 16: Results of regressions analysis based on the linear formula $y = mx + q$. Significance of parameters are reported with the following legend: $p<0.1$ (.), $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***)

Variable	*Adj R^2*	*Intercept (q)*	*Slope (m)*
Minimum temperature	0.927	-0.655***	0.918***
Maximum temperature	0.977	2.617***	0.955***
Annual precipitation	0.369	15.705**	1.143***

The Mann-Kendall test assessed the absence of climate trends and an increasing linear trend in annual maximum and minimum temperatures was clearly observed with +0.009°C/year for minimum and +0.004°C/year for maximum. However particular trends were detected in the periods 1900-1940, 1941-1975 and 1976-2010. In the first period, minimum temperatures, compared with the maximum ones, were detected as growing more rapidly (+0.015°C/year versus +0.006°C/year). In the central period minimum temperatures remained quite stable while maximums had a sensible decrease as long as 1960 and up to the same values of first years of 1900. In the third period, temperature continued to rise with very similar mean values (+0.029°C/year for minimum temperatures and +0.026°C/year for maximum).

Concerning precipitations, the same periods and trends were analysed, even if very low adjusted-R^2 (0.07 – 0.04) demonstrated a lack of statistical relationships. Anyway, a general decrease between 1901 and 2010, even if relatively low and about -1.02 mm/year, was undeniable (Figure 22, third plot with green line)

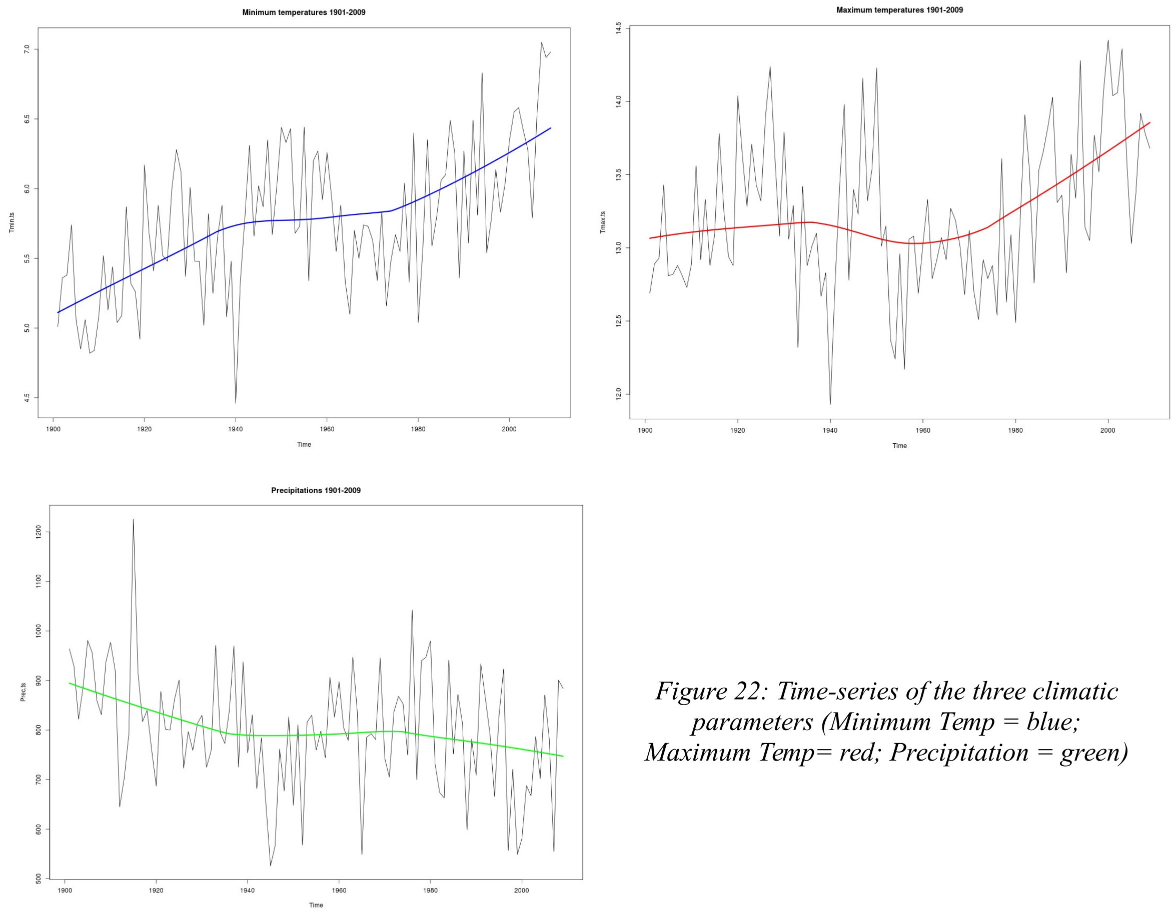

Figure 22: Time-series of the three climatic parameters (Minimum Temp = blue; Maximum Temp= red; Precipitation = green)

Concerning correlation between the species growth and climate, CF and MCF showed different trends and results. With CF a significant and positive correlation was found with minimum temperatures of previous December, current February and March and negative in July and September. The maximum temperatures of the same winter months were correlated on the same way (positive) but the negative correlation was detected only in May of the growing season. Concerning precipitation, growth was significant and negatively correlated only with the events of the previous December (Table 17).

On the other side, the analysis with MCF (Figure 23) underlined many periods with significant relationships between radial growth and climate and while some were roughly temporally stable, others appeared unstable throughout the period. Main correlations with temperatures were generally confirmed, even if not continuous, across the whole time-series. More evident and continuous correlations were detected with minimum temperatures of current February and March but only as long as 1950. Further, only minimum temperatures of the current March were significant but only between 1975 and 1982. Other positive correlations were present and more continuous in the considered period and concerning maximum temperatures of the same months. In the end, concerning the last decades, significant correlations were found with October (minimum) December and September (maximum) only.

Concerning precipitations very low and fragmented events were detected, especially in the last years (1973-2010) and regarding May and June of the growing period (t).

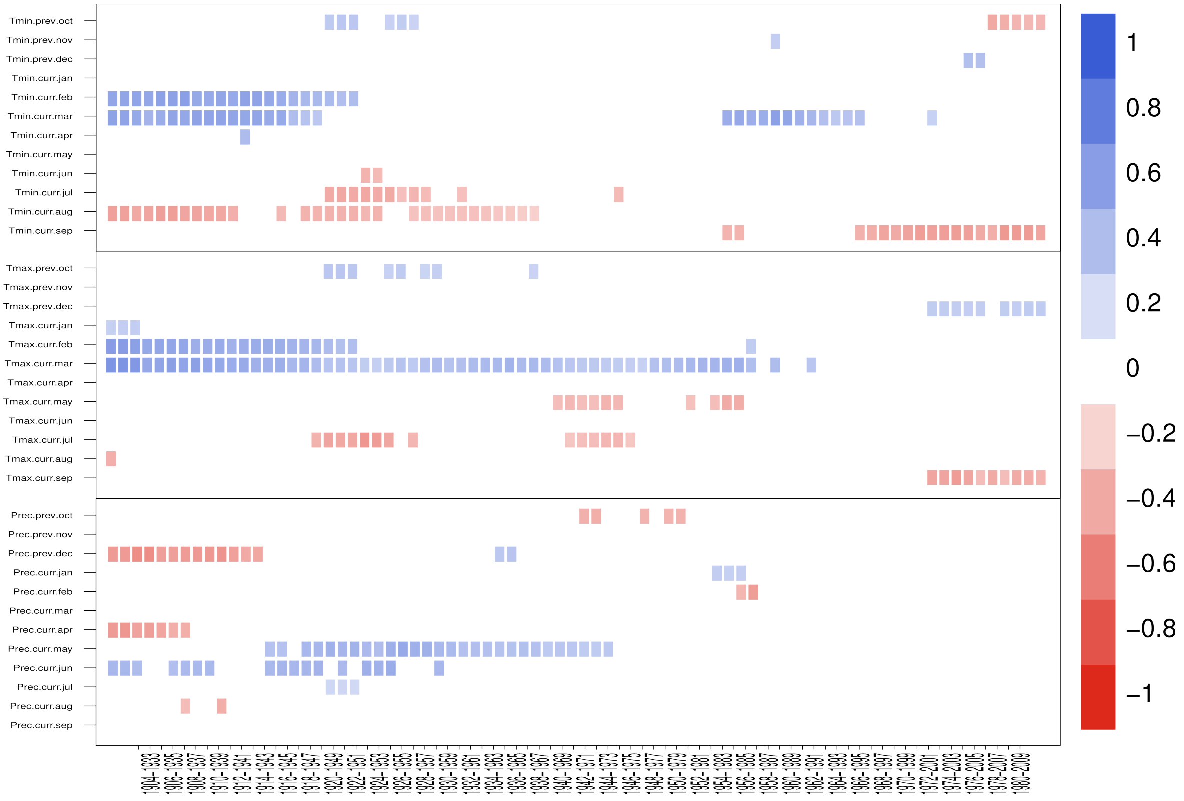

Figure 23: MCF plot. Only significant correlations are showed following the legend on the right part of the plot

Table 17: Results of mean CF between mean detrended chronology and climate variables
CI = Confidence Interval

Variables	*Coef*	*Significant*	*CI.lower*	*CI.upper*
Tmin.prev.oct	-0.0688	FALSE	-0.2196	0.0956
Tmin.prev.nov	0.0985	FALSE	-0.0695	0.2600
Tmin.prev.dec	**0.2313**	**TRUE (+)**	**0.0347**	**0.4132**
Tmin.curr.jan	0.0670	FALSE	-0.1036	0.2601
Tmin.curr.feb	**0.2223**	**TRUE (+)**	**0.0325**	**0.3900**
Tmin.curr.mar	**0.2660**	**TRUE (+)**	**0.1042**	**0.4197**
Tmin.curr.apr	0.0488	FALSE	-0.1261	0.2198
Tmin.curr.may	-0.1062	FALSE	-0.2838	0.0781
Tmin.curr.jun	-0.1227	FALSE	-0.2648	0.0248
Tmin.curr.jul	**-0.1861**	**TRUE (-)**	**-0.3446**	**-0.0255**
Tmin.curr.aug	-0.1444	FALSE	-0.3156	0.0302
Tmin.curr.sep	**-0.2209**	**TRUE (-)**	**-0.3747**	**-0.0562**
Tmax.prev.oct	0.0410	FALSE	-0.1386	0.2226
Tmax.prev.nov	0.1196	FALSE	-0.0755	0.3018
Tmax.prev.dec	**0.2088**	**TRUE (+)**	**0.0360**	**0.3798**
Tmax.curr.jan	0.0270	FALSE	-0.1670	0.2141
Tmax.curr.feb	**0.2250**	**TRUE (+)**	**0.0486**	**0.3811**
Tmax.curr.mar	**0.2989**	**TRUE (+)**	**0.1454**	**0.4287**
Tmax.curr.apr	0.0088	FALSE	-0.1475	0.1875
Tmax.curr.may	**-0.1712**	**TRUE (-)**	**-0.3319**	**-0.0019**
Tmax.curr.jun	-0.0904	FALSE	-0.2853	0.1016
Tmax.curr.jul	-0.1361	FALSE	-0.3187	0.0572
Tmax.curr.aug	-0.1272	FALSE	-0.3114	0.0617
Tmax.curr.sep	-0.1864	FALSE	-0.3583	0.0072
Prec.prev.oct	**-0.1830**	**TRUE (-)**	**-0.3532**	**-0.0082**
Prec.prev.nov	-0.0227	FALSE	-0.2215	0.1678
Prec.prev.dec	0.0958	FALSE	-0.0967	0.2558
Prec.curr.jan	0.1304	FALSE	-0.0222	0.2931
Prec.curr.feb	-0.1115	FALSE	-0.2909	0.0767
Prec.curr.mar	-0.0417	FALSE	-0.2236	0.1457
Prec.curr.apr	0.0407	FALSE	-0.1401	0.2099
Prec.curr.may	0.1269	FALSE	-0.0426	0.2758
Prec.curr.jun	0.2643	FALSE	0.0671	0.4326
Prec.curr.jul	-0.0428	FALSE	-0.2146	0.1332
Prec.curr.aug	-0.0641	FALSE	-0.2360	0.0919
Prec.curr.sep	-0.0241	FALSE	-0.2384	0.1695

4.4 Future scenarios for Black pine in Abruzzo

Mean AUC and TSS values of 30 bootstrap runs and of full models for each dataset are reported in Table 18, whereas global means and standard deviations are reported in Table 19. In every cases RF performed better than other three algorithms, with the highest AUC and TSS and the smallest standard deviation. On the other side, SRE was the worst method especially concerning TSS, which is also a more sensible index of goodness of prediction than AUC (Allouche et al., 2006). With ANOVA only this high difference between SRE and RF was detected as statistically significant (p-value < 0.05). Boxplots of Friedman test on TSS are shown in Figure 24.

Table 18: Mean AUC and TSS of 30 bootstrap trials of partial (80% - 20%) and full models

Model	PArep	AUC	TSS	AUC_full	TSS_full
MLR	I	0.953	0.782	0.953	0.782
SRE	I	0.813	0.626	0.813	0.626
MARS	I	0.978	0.863	0.978	0.865
RF	I	0.999	0.983	1.000	1.000
MLR	II	0.954	0.784	0.955	0.784
SRE	II	0.811	0.622	0.812	0.624
MARS	II	0.975	0.849	0.979	0.865
RF	II	1.000	0.983	1.000	1.000
MLR	III	0.955	0.787	0.955	0.787
SRE	III	0.816	0.632	0.815	0.629
MARS	III	0.978	0.865	0.977	0.864
RF	III	0.999	0.985	1.000	1.000
MLR	IV	0.955	0.786	0.955	0.786
SRE	IV	0.813	0.626	0.813	0.626
MARS	IV	0.979	0.865	0.979	0.862
RF	IV	0.999	0.984	1.000	1.000
MLR	V	0.954	0.782	0.954	0.782
SRE	V	0.810	0.621	0.811	0.623
MARS	V	0.977	0.857	0.980	0.865
RF	V	0.999	0.984	1.000	1.000

Table 19: Global mean and standard deviation of AUC and TSS values of partial (80% - 20%) and full models

Model	*ROC*	*TSS*	*ROC_full*	*TSS_full*
MLR	0.954	0.784	0.954	0.784
	0.0009	*0.0023*	*0.0009*	*0.0023*
SRE	0.813	0.625	0.813	0.626
	0.0022	*0.0043*	*0.0015*	*0.0023*
MARS	0.977	0.860	0.979	0.864
	0.0014	*0.0068*	*0.0011*	*0.0013*
RF	0.999	0.984	1.000	1.000
	0.0001	*0.0008*	*0.0000*	*0.0000*

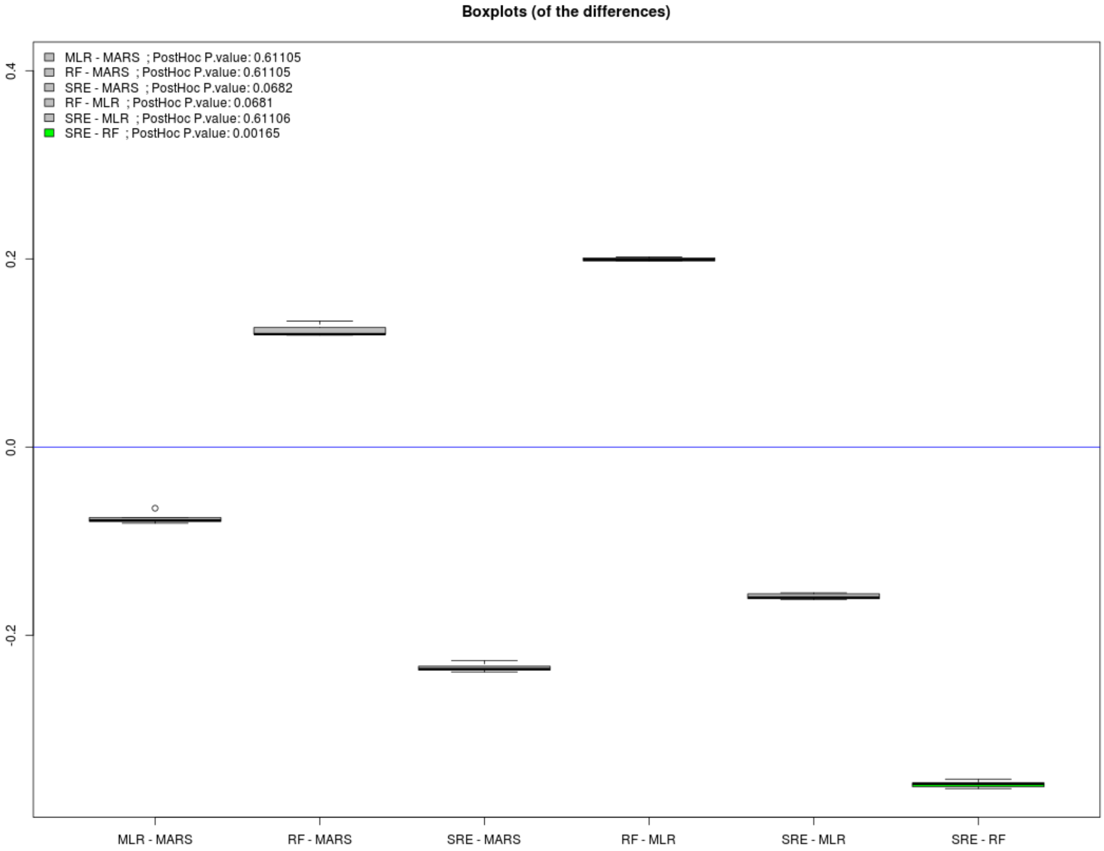

Figure 24: Friedman test on TSS of models. The only significant difference was detected between SRE and RF

Concerning predictors, the importance of each variable is reported in Table 20 with *bio5* (Max Temperature of Warmest Month) that was detected as the more important variable for all models. Climatic data were much more important than soil data, especially in MARS model where they were not useful at all.

Table 20: Variables importance (full models only)

Variables	*GLM*	*SRE*	*MARS*	*RF*
bio4	0.040	0.107	0.091	0.115
bio5	**0.829**	**0.512**	**0.943**	**0.495**
bio7	0.004	0.160	0.121	0.191
bio14	0.055	0.176	0.109	0.134
bio19	0.040	0.080	0.052	0.174
Ecopedology	0.155	0.254	0.000	0.108

The ensemble model was then created to forecast future distribution of Black pine in Abruzzo, as a weighted mean of prediction of full models with TSS > 0.7. Tables 21 and 22 report values related to potential suitable area and elevation limits for the three modelled scenarios. In *ABR0* scenario the ensemble model calculated 229,991 hectares of potentially-suitable area (pixels values > 0.7), much higher than the present distribution which is 19,185 ha. In *ABR1* and *ABR2* this estimated area decreased very strongly and respectively of -72.1% and -96.5%. According to this prediction, also elevation of suitable envelope was predicted to change. Minimum elevation of Black pine's populations in Abruzzo was predicted to increase from 342 metres a.s.l. of the present potential distribution to 1,530 metres of the second scenario (+347.4%). Mean elevation shifted of approximately +700 metres and maximum elevation reached 2,431 metres (+18.4%).

Table 21: Potential distribution in hectares for model and scenario (cells value > 0.7)

EM_ABR0	*EM_ABR1*	*EM_ABR2*
229,991 ha	64,107 ha (-72.1%)	2,244 ha (-96.5%)

Table 22: Elevation variation in metres (and percentage referring to ABR0) for model and scenario (cell value > 0.7)

Model	Minimum	Median	Mean	Mode	Maximum
EM_ABR0	342	1,265	1,259	1,272	2,054
EM_ABR1	392 (+14.6%)	1,638 (+29.5%)	1,634 (+29.8%)	1,657 (+30.3%)	2,488 (+21.1%)
EM_ABR2	1,530 (+347.4%)	2,010 (+58.9%)	1,979 (+57.2%)	2,096 (+64.8%)	2,431 (+18.4%)

Finally, Figures 25 and 26 represent a graphical comparisons between the current (*ABR0*) and the worst (*ABR2*) modelled scenarios in the surrounding area of Villetta Barrea. Green colours (dark green and clear green) correspond to "potentially-suitable area" whereas red colours (yellow, orange and red) were used for the not suitable lands. The model correctly predicted the current natural distribution in *ABR0*, which was drawn as black polygons (Figure 25). However in *ABR2* the situation was completely changed and current distribution was not predicted to be suitable anymore. In this last scenario, three different zones were selected as suitable by the model on the mountains surrounding the Sangro river. The first one was on the higher parts of the Camosciara area ("Monte Capraro", "Monte Petroso" and "Monte Tartaro") whereas other two were on the opposite side of the valley on "Monte Greco", "Monte Marsicano" and "Monte della Corte".

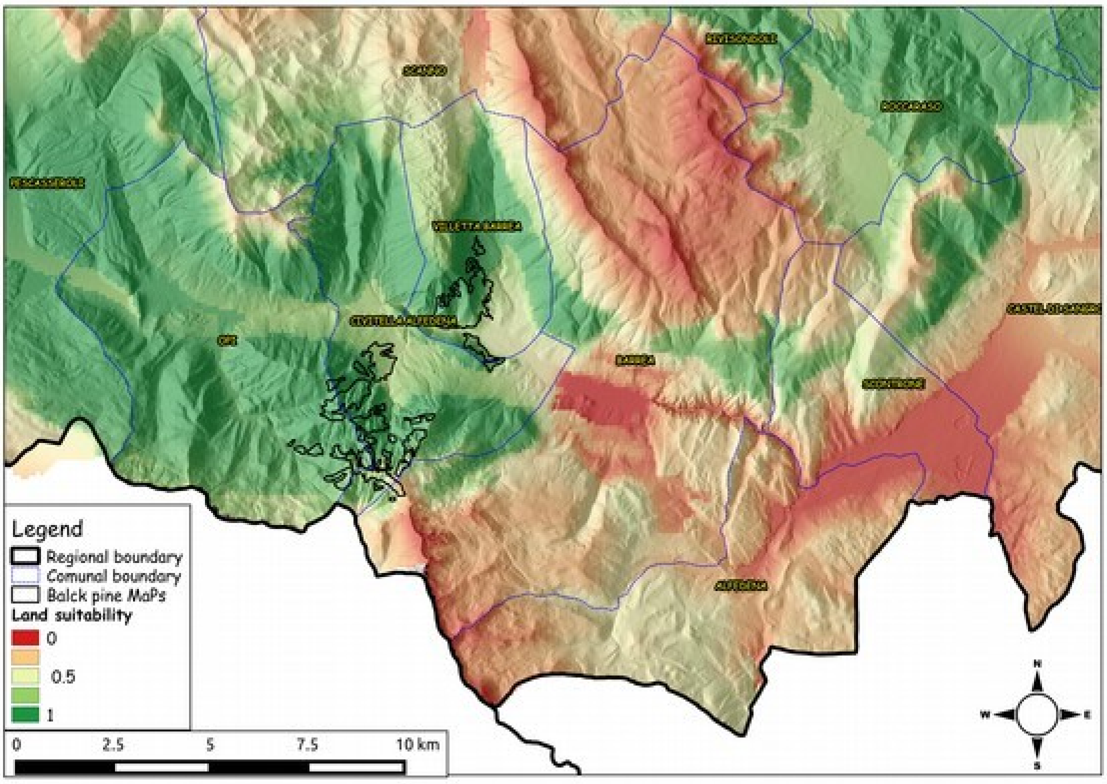

Figure 25: ABR0 scenario. Green colours correspond to areas where the species can survive at the present time whereas red and yellow are not suitable for the species

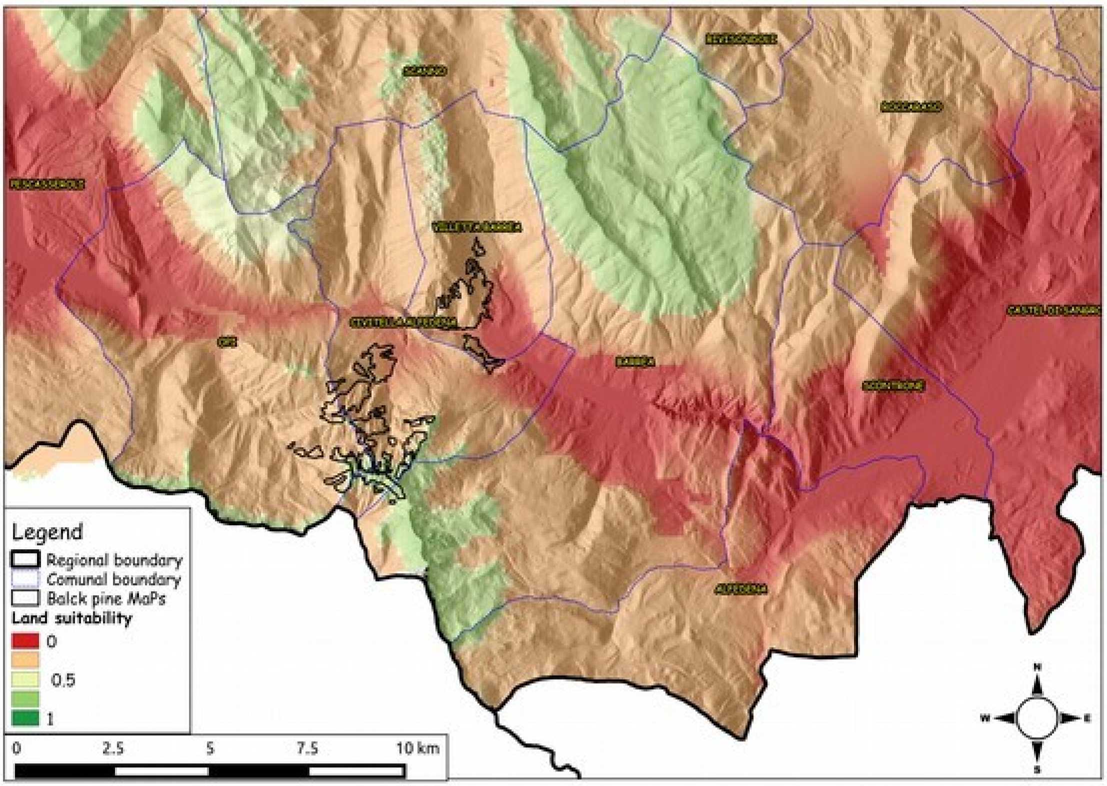

Figure 26: ABR2 scenario. Green colours correspond to areas where the species could probably could move to survive in future whereas red and yellow areas are not suitable for the species

5. Discussion

5.1 Ecologically-marginal population or not?

The marginality analysis showed different results and, apparently, the Mahalanobis distance and PCA plots seemed to be discordant. On one side the Mahalanobis distance detected a considerable difference between the ecological environment of the MaPP of Abruzzo and the ecological feature of Black pine subspecies across Europe. On the other hand, the PCA showed that the MaPP was apparently well included into the "Black pine ecological environment". Anyway, in both cases, some additional issues must be considered and, as the main aspect, the characteristics of climate data used for this analysis. As mentioned before, WorldClim's spatial resolution is 1 km which is probably good at European scale and continental zones but not for Mediterranean area neither for Abruzzo. Many studies suggested WorldClim dataset as a good reference map but preferred interpolation at local level especially in mountainous zones (Hijmans et al., 2005). Concerning that, other dataset were available for these study, for example ClimateEU software. This is an unpublished software package for the European environment and equivalent to ClimateWNA, which was created for Western North America (Wang et al., 2012; Hamann et al., 2013). In both cases the databases were generated with the Parameter-elevation Regressions on Independent Slopes Model (PRISM - Daly et al., 2008). The software can be used to query climate data at any location of the entire European Union countries (from 34.26° to 71.24° of Latitude and from -10.74° to 44.24° of Longitude) and to generate gridded climate surfaces in Albers Equal Area projection. Anyway, climate data are often very similar to WorldClim, the interpolation method and the spatial resolution is coarser (4 km) and, in conclusion, WorldClim remains, in any cases, the most detailed and used global reference layer. That's why, even in this study, WorldClim database was preferred. Another problem of many Global climate data is the interpolation method which influenced also our results. In Mahalanobis analysis elevation was detected as the main driving force to discriminate differences. Elevation is well known to be a very important variable which influence climate variations but it is not the only one. Even distance from sea, slope, aspect, solar radiation and soil's nature must be considered in models as predictors of environmental variability. In WorldClim database (and ClimateEU too) elevation was the only physiographic parameters (Hijmans et al., 2005). As a consequence, elevation as main driving force for variability was an expected result.

As a second issue, the use of PCA 2D plot mus be carefully weighted. In our case, the first and the second components explained only the 60% (approximately) of the total variance which means that a huge amount of variation is not considered, probably hiding many other features. More simply, it is very likely that two observations which seem to be very close each other in a 2D plot would be very far away in a 3D plot and in a multivariate environment.

Concerning genetic data, the knowledge about the population of Villetta Barrea is quite scarce. As reported in others sections, many investigations were conducted in Meidterranean area but most of them considered only populations from Calabria, Sicily, Corsica and Northern part of Italy (Alps). However, recent studies have enforced the correlation between morphological traits and genetic structures of the Black pine's populations (Bruschi et al., 2006; Rafii and Dodd, 2007) and it was evident that the population of Abruzzo is quite different from the others (see Table 1).

Finally, summarizing the collected results, available genetic information and the quality of performed analysis, the MaPP of Villetta Barrea partially match some requirements that were indicated by Hampe and Petit (2005). The isolation from the core and the possibility to find very old trees, as long as 500 years old in Camosciara area (Biondi and Visani, 1996), suggested the possibility to classify it as a "*rear-edge, isolated and Altitudinally-MaPP*". Anyway, genetic aspects such as heterozigosity, within-population diversity and *Gst* should be provided with further specific analysis to classify the population properly, comparing it with other natural stands from Italy and/or Europe (maybe from Calabria, Sicily, Corsica, Trentino South-Tyrol and Friuli-Venezia-Giulia).

5.2 Interpolation of climatic data at local level

Analysing the bare results (RMSE) of the spatial interpolation at regional level, bad results seemed to be obtained. The comparison demonstrate that none of the nine algorithms was suitable for the interpolation of all variables at the same time, RMSE for climatic factors were often quite high (around 2°C) and no significant differences were detected between models. In addition results were worse than others cases of study performed in Italy (Attorre et al., 2007; Blasi et al., 2007). Reasons rely on fact that Abruzzo, despite the relatively small geographic extension, has a very particular orographic conformation, determining a high climatic variability without possibility to determine a specific external drift. Abruzzo's geographic position (Mediterranean region and eastern side of Italy) and the altitudinal ranges (from sea level to 2912 metres) nearby the coast which represent a barrier to continental winds and precipitations from central Europe and the Balkans (Di Lena et al., 2013) enforce the complexity of interpolation. An additive problem that increased difficulties of interpolation in Abruzzo is the distribution of meteorological stations. Several studies demonstrated the importance of the amount of data for interpolation (Sluiter, 2009) but very few discussed about their distribution (Wong et al., 2004; Bhowmik and Costa, 2014). As explained before (see Methods chapter), Abruzzo has a good network (1 station on every 100 squared km for precipitation and on 200 for temperatures) but it is not representative of higher elevation and consequently, more complex and time-consuming methods (as OcoK and UcoK) didn't performed properly in mountain zones and at higher elevations. As reported in Cencetti et al., (2007), this demonstrates that model's complexity and prediction's accuracy are not always connected to each other.

Anyway, there are many other features that must be considered and discussed. First of all, even if very slight, we can say that interpolating temperatures and precipitations with geostatistical parametric methods (especially RK and OcoK) gave best performances. RK and OcoK were generally the best algorithms for temperature interpolation (5 cases on 7 for RK and 2 on 7 for OcoK) whereas UK and UcoK performed well for MAP and OK with DMP data. Also concerning indices, best results were obtained with UK (for DMAi), OcoK (for EMPq and RMTi) and UcoK (for RMCi). In addition, even if ANOVA did not demonstrate statistical difference between models, parametric methods always performed better than nonparametric. Parametric methods, despite of all compulsory requirements like normality of data, analysis autocorrelation, etc. use more information and geostatistical relationships between available points (Rea and Eccel 2004, Cencetti et al., 2007). In addition, geostatistical methods provide also variance of prediction, which describes the spatial quality of the results helping researchers in understanding the results.

Secondly, it is indubitable that errors of predictions were quite higher than similar studies at regional or national scale. For instance, not very far from Abruzzo, but on the opposite side of Apennines (the region of Lazio), Attorre et al., (2007) interpolated similar climate factors and indices with lower RMSE. Anyway reasons rely on statistical relationships between climatic factors and physiographic parameters. In Lazio very high correlation were detected and, in these cases geostatistical methods such as UK (kriging with external drift) as the best interpolation method. Anyway, in some cases such as our case of study, the aim of the comparison is to solve the problem of low spatial resolution of global data. In this case, if we compare the interpolated data with WorldClim data (Table 22) the improvement is evident. RMSE of WorldClim maps, calculated as Root Mean Squared Error of differences between the value of each meteorological station and the correspondent raster cell of WorldClim layers, is around 2.8 °C higher than RMSE derived from local interpolation.

As additional issue, interpolation of climate indices must be discussed. The study demonstrated also that the interpolation of DMAi, EMPq RMCi and RMTi reduced both computational time and RMSE% (Table 10). This happened because mathematical operation between climatic factors worked as data transformation which increased the relationships between dependent and independent variables. Anyway, correlations remained low and even regressions had small, R-squared (Table 7). Nevertheless, available data must be considered and statistically weighted before decision. When more rainfalls data than temperatures data (and *vice versa*) are available (as our study case) the loss of information in case of interpolation of bioclimatic indices must be considered. In fact, in our case, to calculate indices we used only 57 points, the same as for temperature but less than the precipitation dataset. In such cases a sensible loss of information must be considered and, even if with lower errors, in such case the decision was not to use interpolated indices.

So, with the necessity of a high spatial resolution maps in a heterogeneous and complex area, representativeness of climatic data is the main driving factor to obtain reliable prediction. If statistical relationships between climatic factors and physiographic parameters is missing, it is impossible to use most complex and powerful algorithms (such as Universal cokriging) and comparison become the only way to work. Consequently, interpolation of climatic and bioclimatic indices is very time-consuming and must be studied very carefully. Often temperature and precipitation data are provided by different meteorological networks and can have different consistency. The more accurate (and detailed) will be interpolated layers, the more reliable could be predictions of climate change effects. MaPPs are a fragile ecosystems located at margins of species distribution where Global change will be more dangerous for forest populations, with loss of adaptive genetic diversity (Csaba et al., 2009).

5.3 Seed stand structure and interactions with climatic factors

Results stressed that the seed stand was structured as a pure Black pine forest where broadleaves were very rare and more present where local conditions (such as ditches or more fresh expositions) allowed them to be more competitive. As overall, concerning the vertical structure, the population had two well separated levels with the Black pine as dominant species. Beech and ash were mainly present as stumps due to selective cutting in the past years and animals effects. Anyway in many areas, especially where beech was not able to compete, such as southern exposures or thinner soils, Black pine occupied both levels.

Dendrometric data collected in 2012 did not reported any significant difference between the present time and the situation before the management plan's approval. In fact no management activities were conducted during the last years, as reported into the economic book of the management plan.

Data from plots and ANOVA on DBH demonstrated that the structure of the forest was influenced by the (past) forest management but also that mean increment was mainly influenced by age, fertility and ecological dynamics. As a consequence, three different structures were dominating the population:

- **Adult sections (management unit 18 - plots 4 and 5)**: parts of the seed stand which can be assimilated to even-aged populations with big diameters and small variation between them. In these parts, volumes were mainly related to the total number of trees and variable from 280 m^3/ha up to 570 m^3/ha. Maximum age of dominant trees varied between 80 and 120, small diameters were very rare and mainly referred to young broadleaves. Maximum heights of trees were around 21 metres.

- **Old sections (management unit 21 - plots 1 and 2)**: trees were smaller than the previous group, mainly due to the lower fertility. Trees number was less variable but coefficient of variation of DBH was higher. Volumes were quite homogeneous around 350 m^3/ha but ages of bigger trees were very different, from 104 to 179 years. In this case, structure was assimilated to uneven-aged populations.

- **Very old sections (management unit 19 - plot 3)**: mean DBH and height were the smallest of the whole area. Density was very high, mainly due to high number of small trees and coefficient of variation was high too, indicating a very variable composition. Volumes were the lowest, around 250 m^3/ha and both vertical levels were populated by Black pine. Also these part could be assimilated to and uneven-aged populations, were the Black pine was able to regenerate naturally. In this area the oldest (dominant) trees were found, from 143 to 184 years old.

Concerning seed production, it was very good in every part. Abundant amount of cones were stored on the branches each year the population was surveyed (2012-2013-2014) and concerning health of tree no dizziness were registered. Only in some cases and some areas, trees had deep injuries due to past activities of resin extraction (Figure 27).

Figure 27: An adult pine with the consequences of past resin exploitation

Concerning climate-growth relationships, dendrochronology demonstrated that, despite different mean increment between the zones, which was mainly connected with the age of trees, the entire population had reacted at the same way. In this case, as expected, forest (past) management didn't play a key role on that. As reported in many other studies on Black pine spp. sensitivity to high summer temperatures was confirmed (Génova and Fernández 1999; Martin-Benito et al. 2010; Amodei et al., 2012). On the other hand, no correlation with precipitations effects was detected and reasons probably rely on two main causes. On one side there is the autoecology of Black pine of Villetta Barrea which is a little bit more tolerant drought stresses than others subspecies (Gellini and Grossoni, 2003). On the other side, it is known that soils' nature and local ecological conditions can enforce or reduce water availability during different seasons as well as amount snow on winter. It was demonstrated that growth response and climate should be considered according to soil substrate characteristics and that pines on quartzite bedrock were much more sensitive to precipitations than similar populations on dolomite and calcareous bedrock (Génova and Martinez-Morillas 2002). In calcareous soils the effect of chemical alteration of calcareous may offer the opportunity to pine roots to explore deeper levels of the soil (Amodei et al., 2012).

The positive correlation between radial growth and late-winter temperatures (February and March) in CF results was also mentioned by Linares and Tiscar (2010) on Salzmann pine in southern Spain. In these case, trees may open buds earlier and, consequently, increase the length of the growing season. These effect is very likely to happen in Villetta Barrea, where water availability was demonstrate not to be a problem. Results from MCF analysis dove the attention on few months with very unstable correlation and without statistical significance in the last decades. Months that were detected as main driving forces with CF, proved to be relevant only for past events such as temperatures of February and March. On the opposite, some factors that were not relevant with CF become important with MCF analysis and especially in the last decades, such as minimum temperatures of the previous October and current September. In this case connection is probably linked to cold stresses in which are likely to happen in late September at high elevation. High temperatures in the late summer can induce a longer growing season which can suddenly stop when an extreme event occurs. In this case the moving windows approach of MCF demonstrated to be a very good tool to study adaptability to climate change of forest species. Anyway, the genetic provenance must also be considered as a possible driver for variability.

5.4 Ensemble modelling and future scenarios for the MaPP in Abruzzo

Despite many recent works used RF as unique (or unique-based) algorithm to predict present and future distribution of forest species (Wang et al., 2012; Henderson et al., 2014; Isaac-Renton et al., 2014), even regression-based models (GLM and MARS) performed quite well in this study. However, as expected, RF was the model that showed higher TSS and AUC and contribute more consistently in projections whereas SRE confirmed its poor ability in SDM applications and its unsuitability to predict climate change effects (Hijmans and Graham, 2006). Anyway, the use of a group of algorithms, runs and datasets with *biomod2* ensemble weighting method, can consistently contribute to improve RF prediction which is often affected by random extraction of trees and variables. On the other side, the possible overfitting of some models (such as GLM and MARS) can be reduced by RF. In these context, ensemble model of *biomod2* package can correct some biases in calculations, making the prediction more stable than the classical packages of the various algorithms (*randomForest* - Liaw and Wiener, 2002; *earth* - Milborrow 2014; *stats* - R Core Team, 2014).

Concerning TSS values for selecting models, many works used 0.7 as a useful threshold (Araùjo et al., 2005; Forester et al., 2013). In our case, the same level was used and, analysing ANOVA results, it is evident that the only one model that was statistically different from RF was also the only one that had TSS values under 0.7. So, in this case,we could add that RF could be even considered as a “decision-maker” algorithm confirming its supremacy to other methods.

Analysing changes in species distribution and elevation movement, models reported a very high variation of suitable area. Anyway, the first aspect that must be considered is why the suitable area in ABR0 was so overestimated than the real distribution (+2,000%!!!). SDMs rely only on occupied ecological niche, compared to the environmental variability. On the opposite, forest ecosystems are a dynamic and complex system with a mixture of species which interact and compete for natural resources and where, the modelled species can prevail or not for ecological dynamics (illness, slope, aspect, nature of soils, seed dispersal, etc.) or human-connected reasons (thinning activities, timber production, etc...). Anyway, this is a quite common issue happening in ecological modelling activities and due to impossibility of including in model inter-specific competition. In addition, we must also consider that Black pine is a very plastic species which can grow in a very wide spectrum of areas. As a consequence, it has been widely planted in the past across the region and even in areas where it couldn't grow naturally due to competition between species (Beech and Oaks above the others) or spatial distance between natural areas and planted zones. However this is a know aspects, due to the concept of "potential suitable area" that was used in this study as a measurement of the complete interaction between the climate and the species in Abruzzo.

Concerning future distribution, no one of present part of the MaPP is forecast to be stable in ABR2 scenario and the predicted loss of suitable area was very close to total extinction (-95%). Abruzzo's topographic morphology was predicted to play a key role in conservation of the MaPP. In addition, the model suggested that, in some cases, trees at higher elevation in Camosciara area, that are not able to develop cones at the moment, will probably become reproductive due to warming climate. In this case, those trees may allow the species to colonize new territories at higher elevations, increasing migration capacity of Black pine of Villetta Barrea. In this context, that "new" source of seeds will be a huge advantage versus competitors such as the beech. Beech and/or Oaks are frequently mixed with Black pine in many zones across Abruzzo, Italy and Europe competing for the natural resources. However those species are not able to migrate at higher elevation without the help of animals. In addition future-suitable lands are actually bare and inhospitable for trees species and, maybe, in this scenario, Black pine will probably be more adapt to colonize those new environments.

6. Conclusions and future development of the research

In this work a case study with a very important forest species for Mediterranean area was presented. The Mediterranean regions harbour most of the European genetic variability and many micro-habitats occur in this area that can supply suitable conditions for populations of many forest species. However some gained information and results must be summarized and enforced:

- The MaPP of Villetta Barrea is a "*rear-edge and altitudinally-marginal forest population*" but no reliable information were added concerning ecological traits or genetics in this study. Field data (not reported in this work) confirmed that the population of the Camosciara area is composed by old and small trees living in the higher parts of mountains but, even if not sampled, we can assume that these trees had reacted in the same way of the ones sampled in this study from the seed stand.
- Global climate data are not accurate enough for local studies. The Mahalanobis distance and PCA detected a very high environmental variability but analysis were too less reliable due to nature of WorldClim data and spatial resolution. Errors of WorldClim in Abruzzo were higher than those derived from regional monitoring network and interpolation at regional level was preferred. Errors were lower and spatial resolution was increased. In addition, most importantly in the context of rear-edges populations, bioclimate models often rely on climate data derived from global circulation models and ignore that regional-scale climate changes can be buffered locally by topographic heterogeneity (Hampe and Petit, 2005).
- Past forest management and local conditions (fertility of soil, aspect, slope) influenced forest structure but tree-rings analysis showed that the correlation with

climate factors was not affected by that. In addition, this work demonstrated that influence of climate factors is no longer active and that the species is probably adapting to a new changing environment. This will probably play a key role in future and is a issue that must be carefully considered during ecological modelling activities and management planning.

- SDMs are a very powerful tool to forecast future scenarios. However, to use SDMs' properly and not as a simple exercise (especially when they are used to forecast future scenarios for a target species), they should never be considered as a way to predict the future dynamics, but as a method to understand what is more likely to happened in the future. In fact, nobody knows exactly how fast the changes in temperature and rainfall will be, and how different species will adapt to future environments. It should be also considered that different biotic and abiotic factors are involved (Pearson and Dawson, 2003).
- A specific conservation strategy for the species should be considered in order to observe future development and manage the FGR properly. Even if models are generally very pessimistic predicting a huge loss of suitable area in very few decades, Black pine (and forest species in general) seems to own the ability to react to future climate scenarios and to have already the possibility to migrate to areas that will probably be suitable and used as "*refugia*". It is also true that future climate developments are likely to be faster than the migration-ability of the species (Csaba et al., 2009) and there are a lot of unknown aspects of forest populations that need to be deeper investigated. Biological, genetic and ecological skills are requested to contribute to these studies, to validate models and to combine different approaches. Hiwever, species-specific analysis like dendrochronology has proved to be a very useful tool to study the phenotypic plasticity of the species.

In conclusion, many other questions and issues must be developed to cover all possible studies connected to MaPPs. For instance, genetic information about species and their local adaptation must be carefully considered. Comparing the MaPP of Villetta Barrea with other populations and subspecies (mainly *nigra* and *laricio*) to assess if it is a case of genetic adaptation or an example of phenotypic plasticity should be encouraged. At the same time, ecological modelling with multiple species should be added. In such case, if we introduce the SDM of a competitor of Black pine (e.g. Beech) the potential area would probably decrease. In addition, a subsequent modelling of different provenances should be performed in order to consider local adaptation of forest populations (Isaac-Renton et al., 2014). Information about growth rate (e.g. site index) would be very useful to weight presence points differently and to transform the SDM output from "*survival map*" to "*productivity map*".

Adaptation of species is then an evident and complicated issue that must be included into Ecological modelling. The MaPP, especially concerning the Camosciara area, is one of the oldest forest populations living at the rear-edge of European Black pine distribution and it could play a key role in understanding the future development of the whole species. Due to the small size of the natural group, a specific conservation strategy of this MaPP should be developed. Seed orchards, dynamic *ex-situ* conservation and assisted migration, added to an *in-situ* adaptive management could be very useful to manage such a valuable genetic material in view of the effects of a rapidly changing climate.

7. Scientific acknowledgements and connected projects

This study has been carried out as part of the ABRFORGEN project “*Implementation of forest nursery chain and organization of a modern management of forest genetic resources in Abruzzo*“ a partnership between CRA-SEL and Abruzzo Region, the COST Action FP 1202 “*Strengthening conservation: a key issue for adaptation of marginal/peripheral populations of forest trees to climate change in Europe (MaP-FGR)*“ and the EUFGIS project.

Special thanks are due to:

- Dr. Fulvio Ducci and Dr. Cristiano Castaldi from Forest Genetic Resources Laboratory of CRA-SEL and Prof. Susanna Nocentini from GESAAF Department of University of Florence for the funding and the scientific support;
- Dr. Daniela Di Silvestro and Dr. Francesco Contu form the Agricultural Policy and Rural Development and Management of Abruzzo Region as partners of the ABRFORGEN project;
- Dr. Bruno Di Lena and Dr. Fabio Antenucci from Policy and Rural Development and Management of Abruzzo Region for the elaboration of climatic data;
- Dr. Valeria Gallucci, Dr. Alma Piermattei and Prof. Carlo Urbinati from Università Politecnica delle Marche, Prof. Luigi Hermanin from GESAAF Department of University of Florence and Dr. Paolo Merlini from Forest Genetic Resources Laboratory of CRA-SEL for the help in dendrochronology;
- Dr. Duncan Ray (Forest Research of Scotland), Dr. Vincent Badeau (INRA-Nancy) and all the other Components of the WP8 of TREES4FUTURE project for the meaningful help with climate data and SDMs;

- Prof. Jùlian Gonzalo from the University of Valladolid, leader of WG1 of the Cost Action FP102 and Dr. Ugo Chiavetta from Forest Geomatics Laboratory of CRA-SEL for the help with SDMs and spatial interpolation of climatic data;
- Prof. Robert J. Hijmans from the Department of Environmental Science and Policy of the University of California for the help with *raster* package;
- Dr. Wilfred Thuiller from Laboratoire d'Ecologie Alpine, CNRS, France for the help with *biomod2* package;
- The GFOSS and the *r-sig-geo* communities (R Special Interest Group on using Geographical data and Mapping).

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