RST – Scientific report regarding the implementation of the "***REASONING* – Drought resilience and ecological value of planted Douglas fir, Norway spruce, and Silver fir conifers in the face of climate change**" project (PN-III-P1-1.1-TE-2019-1099; Financing Contract for Project Execution TE 75 / 2020)

FINAL REPORT (15/09/2020 - 14/09/2022)

I. FORESEEN AND ACCOMPLISHED OBJECTIVES:

The **main objective** of the REASONING project was to conduct ecological and genetic studies on mixed Douglas fir (non-native to Europe), Norway spruce (native to cool and mountainous regions of Europe) and silver fir (native to Romania) plantations from Romania. This main objective was based on the fact that, although the economic importance of Douglas fir seems to be very well established (i.e., timber), we still lack important knowledge on its growth performance and drought resilience in the face of climate change. Additionally, although there are several studies comparing Douglas fir and Norway spruce plantations, little is known about Douglas fir and silver fir plantations, although this latter species is native to Europe, common in Central and Eastern Europe, needs very similar to Douglas fir climatic condition and it is also valued for its timber quality [1, 2].

Based on the foreseen main objective of the REASONING project, three mixed Douglas fir, Norway spruce and silver fir plantations were identified (**Act. 1.1 and 1.1.1 from the Stage I of the Implementation plan**) and sampled in the SW of Romania: Ana Lugojana, Rusca Montana and Otelu Rosu (**Fig. 1**; Ana Lugojana, Rusca Montana and Otelu Rosu Forest Districts). These three plantations are all located in the Poiana Rusca Mountains.

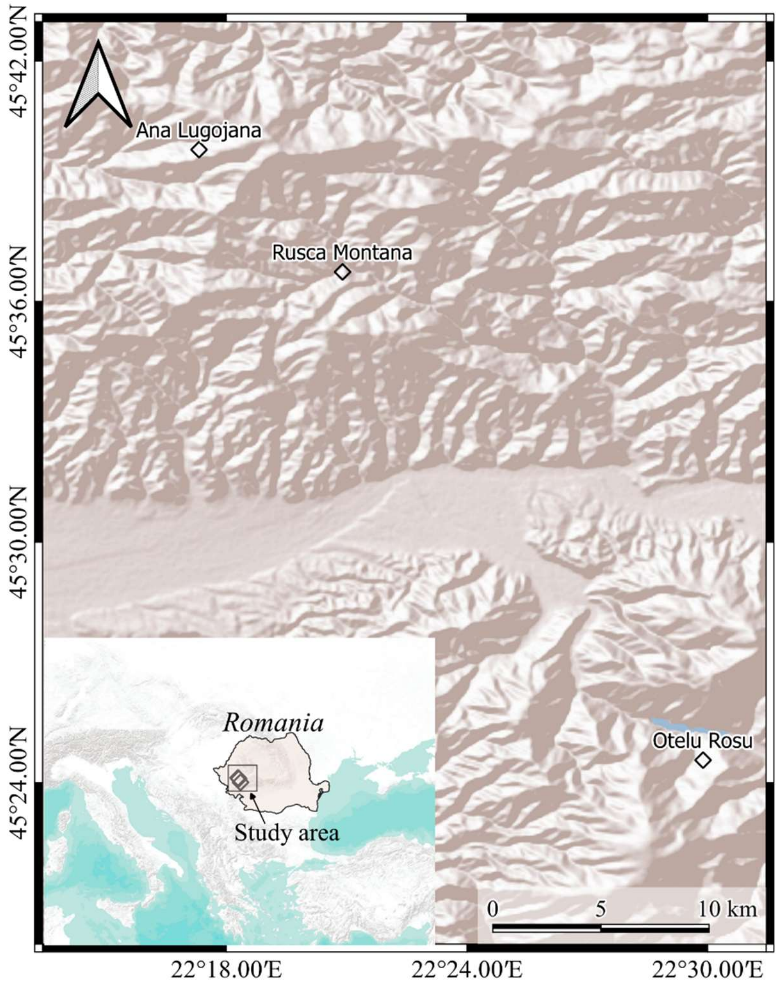

Fig. 1 The location of the three study sites (Ana Lugojana, Rusca Montana and Otelu Rosu) in the SW of Romania (Poiana Rusca Mountains).

The three mixed Douglas fir, Norway spruce and silver fir plantations have been further used to accomplish the **specific objectives** of the REASONING project:

(**1**) **Study the historical growth rates (i.e., tree rings)** of the Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites. In order to accomplish this foreseen objective, the members of the research team performed a field campaign in 2020 (**Act. 1.2 from the Stage I of the Implementation plan**). During this field campaign, the sampling scheme presented in the original project proposal was followed and, at each of the three study sites, 25 adult trees for each of the three planted conifer species were randomly selected and sampled (**Act. 1.2.1 from the Stage I of the Implementation plan**). Sampling consisted in extracting, from each tree, two wood cores using increment borers with an inner diameter of 5 mm (Haglöf, Sweden). In total, 450 wood cores were manually extracted from 225 trees (i.e., 25 trees x 3 species x 3 study sites; **Table 1**). Additionally, the diameter at breast height (DBH) and height of all the sampled trees, along with their geolocalization, were also measured and registered (**Table 1**).

Following the field campaign, the extracted wood cores were prepared based on standard dendrochronological procedures and the tree rings were measured (i.e., to the nearest 0.01 mm) using the CDendro and CooRecorder software (Cybis Elektronik & Data, Saltsjöbaden, Sweden; **Act. 1.3 and 1.3.1 from the Stage I of the Implementation plan; Act. 2.1 and 2.1.1 from the Stage II of the Implementation plan**). The statistical accuracy of the crossdating process (i.e., potential dating errors; quality of the tree ring measurements) was repeatedly checked using the COFECHA program [3]. In total, 215 trees (74 of Douglas fir, 70 of Norway spruce and 71 of silver fir; **Table 1**) were accurately crossdated and included in the final **growth database**. The obtained growth database was further used to conduct dendrochronological and dendroecological analyses (**Act. 2.3 and 2.3.1 from the Stage II of the Implementation plan**) in order to compare the growth patterns of the three species (i.e., performance) and to evaluate their drought resistance/tolerance/resilience to past severe drought events (i.e., tree-rings faithfully register the climatic conditions to which the trees are being exposed to during their life). In order to do so, along with the growth database, the Standardized Precipitation-Evapotranspiration Index (SPEI) was also obtained, processed and used [4, 5].

The results of the objective (1) are detailed in the II. RESULTS AND INDICATORS section below and will be published in a specialized journal: Petrea Ș., Petritan I.C., Gazol A., Curiel Yuste J., Hereş A.-M., *in prep*. (Act. 3.2 and 3.2.1 from the Stage III of the Implementation plan).

(**2**). **Study the genetics** of the Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites. To accomplish this foreseen objective, during the field campaign conducted in 2020 (**Act. 1.2 from the Stage I of the Implementation plan**), needles and cambium were also collected from exactly the same 225 Douglas fir, Norway spruce and silver fir trees from Ana Lugojana, Rusca Montana and Otelu Rosu study sites (**Act. 1.2.1 from the Stage I of the Implementation plan**). The genetics sampling scheme followed the one presented in the original project proposal. The genetics sampling was performed using either a telescopic scissor, needed to reach the needles situated up in the crown of the trees, either a very resistant metallic cylinder, needed to extract cambium. Following the field campaign, the needles and cambium samples were used to extract DNA (**Act. 1.3 and 1.3.1 from the Stage I of the Implementation plan; Act. 2.1 and 2.1.1 from the Stage II of the Implementation plan**). For this, 60-70 mg of biological tissue (i.e., needles, cambium) were first introduced in a 2 ml cylinder together with 2 tungsten balls and milled (30 Hz) for 2-3 minutes using the Tissue Lyser Roche MM400. DNA extraction was then performed following the CTAB protocol that consists of several steps. First step: in order to break the cellular membranes, 1000 µl 2 x CTAB Extraction Buffer (containing CTAB, PVP, and β-mercaptoethanol) and 10-15 µl of Proteinase K, were added to the milled samples that were then introduced into a Thermomix for 30 minutes at 65°C and 550 rotations / minute. Second step: in order to separate and denature some specific proteins, 200 µl of Wet Chloroform were added, and then, in order to eliminate residuals of CTAB and to purify the extracted DNA, 600 µl of Isopropanol (-20 °C) and 1000 µl of Wash Buffer (i.e., 50 µl ammonium acetate + 40 ml ethanol 76% + 10 ml of H2O) were added. Finally, to the resulted solution, 50 µl of Elution Buffer were added. Following DNA extraction, its quality and concentration were tested using a Nanodrop 8000 spectrophotometer. To do so, 1µl of the DNA stock solution was used. The concentrations resulted to be high enough, but the quality of the DNA, estimated considering the 260/280 ratio for which the optimal values range between 1.8 and 2, was in some cases low due to impurities. Thus, depending on the DNA quantity and quality, we needed to make different dilutions (i.e., 1:10 - 1:30) in order to obtain a concentration of approximatively 20 – 30 ng/µl. The obtained **genetics database** was further used to conduct analyses (**<u>Act. 2.3 and 2.3.1 from the Stage II of the Implementation plan</u>**) in order to identify the putative geographic origin of the seeds that have been used to plant Douglas fir, Norway spruce, and Silver fir and to further evaluate the genetic diversity of the selected mixed plantations, information that would allow us to better understand local adaptation, and the fate of these conifer plantations considering climate change. Due to the huge volume of work regarding the genetics database, only the Douglas fir samples have been statistically analysed and finalized from this point of view so far. The Norway spruce and silver fir samples are currently under ongoing statistically analyses treatment (in prep.).

The results of the objective (2) are detailed in the II. RESULTS AND INDICATORS section below and will be published in a specialized journal: Curtu Al., Ciocîrlan E., Şofletea N., Petritan C., Petrea Ş., Hereş A.-M., Assessing genetic diversity and structure of Douglas-fir (*Pseudotsuga menziesii*) plantations in Romania based on nuclear and chloroplast microsatellite polymorphisms, *submitted* to Annals of Forest Research (<u>Act. 3.2 and 3.2.1 from the Stage III of the Implementation plan</u>).

(**3**). **Study the understorey and competition** of the Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites. To accomplish this foreseen objective, the members of the research team conducted a second field campaign in 2021 (**<u>Act. 2.2 and 2.2.1 from the Stage II of the Implementation plan</u>**). During this field campaign, all 225 Douglas fir, Norway spruce and silver fir trees, from which wood cores were extracted and needles and cambium samples were collected in 2020, were revisited at the Ana Lugojana, Rusca Montana and Otelu Rosu study sites. Thanks to the fact that during the 2020 field campaign all 225 trees had been geolocalized, they were easy to be found again in 2021. This sampling scheme followed the one presented in the original project proposal and it was performed following the methodology described by Curiel Yuste et al. 2019 [6], i.e., a circle of 5 m diameter was established around each of the 225 selected trees and a detailed understory and competition survey was carried out within this circle. Specifically, all seedlings were counted and identified at the species level. At the same time, all the dominant competing trees were also identified at the species level and their diameters and distances to the 225 trees (i.e., the reference trees) were measured. The **understorey database** was further used to estimate the regeneration capacity of the three studied conifer species (Douglas fir, Norway spruce and silver fir) and of other tree and shrubs species. The **competition database** was further used to estimate the competing pressure to which the 225 sampled Douglas fir, Norway spruce and silver fir trees are exposed to at the Ana Lugojana, Rusca Montana and Otelu Rosu study sites (**Act. 3.1 and 3.1.1 from the Stage III of the Implementation plan**).

The results of the objective (3) are detailed in the II. RESULTS AND INDICATORS section below and will be published in a specialized journal: Petrea Ș., Petritan I.C., Gazol A., Curiel Yuste J., Hereş A.-M., *in prep*. (Act. 3.2 and 3.2.1 from the Stage III of the Implementation plan).

(4). **Evaluate the ecosystem services** of the Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites. To accomplish this foreseen objective, we collaborated with dr. Stefano Balbi and his PhD student Alba Márquez Torres from the k.LAB (BC3 - Basque Centre for Climate Change; Spain) who used the ARIES (ARtificial Intelligence for Ecosystem Services) software to run ecosystem services analyses (**Act. 3.1 and 3.1.1 from the Stage III of the Implementation plan**). This collaboration was established and presented in the original project proposal where dr. Stefano Balbi and his PhD student Alba Márquez Torres were included as external collaborators. Their ecosystem services analyses were based on the **growth, understorey and competition databases** that were presented above (i.e., specific objectives (1) and (3)) and on the geolocalization of the 225 Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites.

The results of the objective (4) are detailed in the II. RESULTS AND INDICATORS section below and will be included in an article in a specialized journal (Act. 3.2 and 3.2.1 from the Stage III of the Implementation plan).

II. RESULTS AND INDICATORS:

The foreseen objectives of the REASONING project have been accomplished according to the original project proposal and the implementation plan.

The results of the REASONING project are detailed below based on the specific objectives that were presented in the **I. FORESEEN AND ACCOMPLISHED OBJECTIVES** section.

(**1**) **Historical growth rates**

The obtained growth database contained raw tree ring widths (TRW). In order to obtain a better estimate of the overall tree growth and to remove the low-frequency fluctuations determined by the increasing stem size and tree age over time, the measured TRW series were transformed into basal area increment (BAI), using the dplR R package [7, 8]. Specifically, to obtain BAI, the "bai.out" function was used. As BAI datasets started only in 1980 for the Norway spruce trees from Ana Lugojana and Rusca Montana study sites (**Table 1**), a common study period (i.e., 1980-2020) was used for all three conifer species and study sites to run further analyses. Specifically, BAI and SPEI data were used to perform different statistical analyses.

Table 1. Main characteristics of the sampled Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Study site	**Ana Lugojana**			**Otelu Rosu**			**Rusca Montana**		
Species	**silver fir**	**Douglas fir**	**Norway spruce**	**silver fir**	**Douglas fir**	**Norway spruce**	**silver fir**	**Douglas fir**	**Norway spruce**
Initial no. of trees	25	25	25	25	25	25	25	25	25
Final no. of trees	23	25	24	24	24	22	24	25	24
Period	1966 - 2020	1979 - 2020	1980 - 2020	1972 - 2020	1971 - 2020	1974 - 2020	1975 - 2020	1975 - 2020	1980 - 2020
DBH (cm)	34.61	36.51	34.26	36.36	36.87	36.56	35.39	38.64	37.42
Height (m)	24.02	26.74	25.68	27.28	30.53	29.82	26.98	29.59	27.27

Period, the period of time covered by the sampled trees; *DBH*, diameter at breast height; *Height*, the height of the trees.

According to the obtained results, the growth patterns (i.e., performance) of the Douglas fir, Norway spruce and silver fir trees from Ana Lugojana and Rusca Montana study sites showed statistically significant positive trends (**Fig. 2**). Instead, at the Otelu Rosu study site, the growth patterns of the three studied conifer species differed between them. Specifically, silver fir trees showed a statistically significant positive growth trend, Norway spruce trees showed a statistically significant negative growth trend, while Douglas fir trees showed no growth trend (**Fig. 2**). Overall, these results show that silver fir, a native to Romania species, performs well no matter the study site. Instead, although at the Ana Lugojana and Rusca Montana study sites, Douglas fir and Norway spruce seem to perform well, at the Otelu Rosu study site, their growth seems to be limited. The

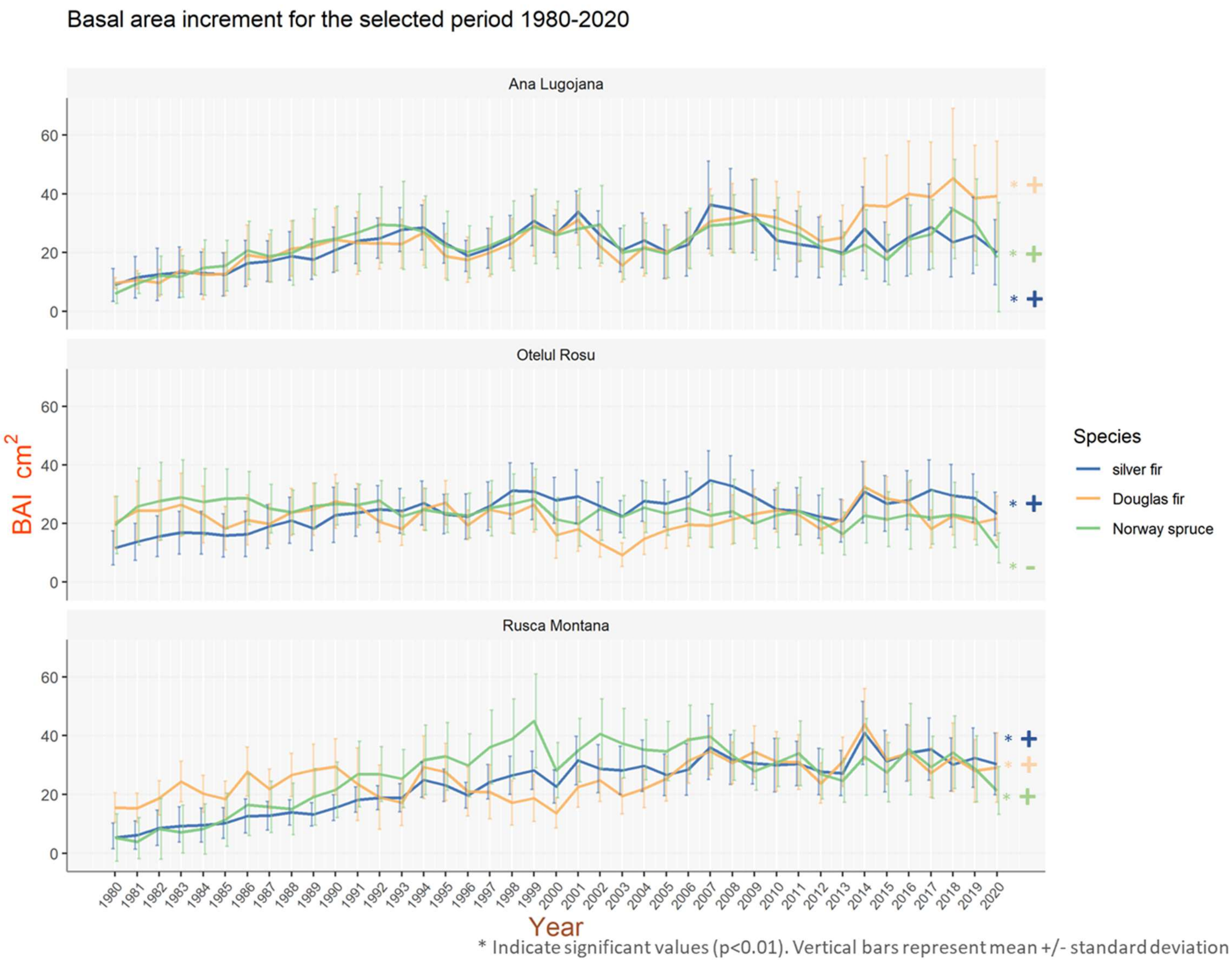

Fig. 2 Growth trends (BAI) of the Douglas fir, Norway spruce and silver fir trees from Ana Lugojana, Rusca Montana and Otelu Rosu study sites

possible underlying factors for their growth limitation need to be further investigated, i.e., soil or microclimatic conditions, among others, might be involved.

In order to evaluate the growth responses of the Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites to severe drought events, the resistance (capacity to overcome drought events), recovery (capacity to recover after drought events), resilience (capacity to grow similar to pre-drought conditions) and relative resilience (resilience that takes into account the damage that occurred during the drought event) indices were calculated [9]. First of all, several severe drought events (i.e., 1983, 1994, 2000 and 2011) were identified between 1980 and 2020, at the three study sites, using the Standardized Precipitation-Evapotranspiration Index (SPEI) [4, 5] index. Then, three years of growth before and after these severe drought events were also considered. The results of the statistical analyses (i.e., generalized least squares models followed by least-square means based on Tukey HSD tests [10, 11]) performed with these datasets showed that there are no significant differences between the Douglas fir, Norway spruce and silver fir trees from Ana Lugojana, Rusca Montana and Otelu Rosu study sites in terms of resistance, recovery, resilience and relative resilience to severe drought events (**Fig. 3**). In other words, all three species responded very similarly when they faced severe drought events, with Douglas fir not showing any advantage in this regard comparing with the other two native to Romania (silver fir) and native to Europe (Norway spruce) species.

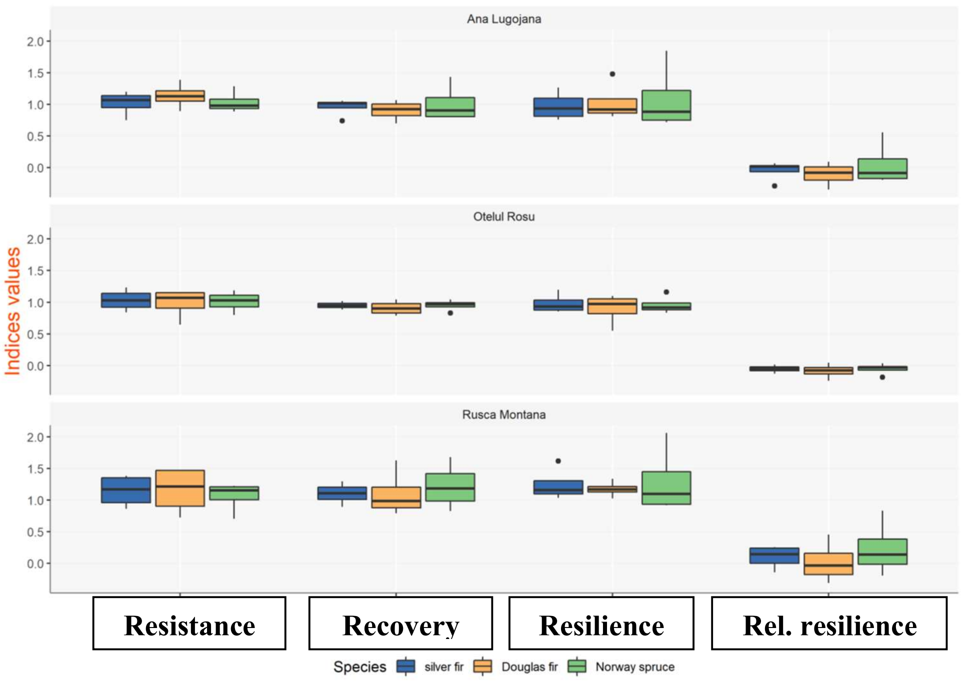

Fig. 3 Results of the generalized least squares models followed by least-square means based on Tukey HSD tests showing no statistically significant differences in terms of resistance, recovery, resilience and relative resilience between the Douglas fir, Norway spruce and silver fir trees from Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Indicators:

All the results showed in this section have been presented to two international conferences by means of two oral presentations:

- **29 October 2021**, Petrea Ş., Petritan I.C., Hereş A.-M., "Mixed silver fir, Douglas fir and Norway spruce plantations in the SW of Romania – growth and components of tree resilience to severe droughts". 5th Edition of the International Conference "Integrated Management of Environmental Resources"; Suceava, Romania; oral presentation;
- **24-27 May 2022**, Petrea Ş., Petritan I.C., Gazol A., Curiel Yuste J., Hereş A.-M., "Drought resilience and stability of coexisting planted silver fir, Norway spruce and Douglas fir trees in the face of climate change". TRACE2022 "Tree-Rings in Archaeology, Climatology and Ecology"; Erlangen, Germany; oral presentation.

Additionally, the results showed in this section will be included in an article that is currently in preparation: **Petrea Ș., Petritan I.C., Gazol A., Curiel Yuste J., Hereş A.-M., *in prep*.**

(**2**). **Genetics**

The obtained genetics database was split considering the three studied conifer species. Specifically, so far, the Douglas fir genetics database has been statistically treated in order to estimate this species genetic diversity and to identify possible historical causes that might explain the genetic structure and variation of the studied Douglas fir populations (Ana Lugojana, Rusca Montana and Otelu Rosu). Data analyses included different statistical approaches (UPGMA, unweighted pair group method with arithmetic mean; DAPC, Discriminant Analysis of Principal Components; AMOVA, analysis of molecular variance and pairwise FST) and specific software (Micro-Checker [12]; GenAlEx v.6.51b2 [13]; Haplotype Analysis 1.05 [14]; Treeview [15]; ARLEQUIN [16]). Furthermore, the Median-Joining method [17] and the Qgis 3.22 software were used to construct the cpSSR haplotypes of the sampled Douglas fir trees and to map their genetic structure and distribution, respectively. Finally, in order to evaluate the genetic structure of the populations, the Bayesian clustering method (Structure software v.2.3.4) [18] was used. The final K value (potential genetic clusters) was assessed based on log likelihoods (ln Pr(X|k)) and the Δk method [19] considering the STRUCTURE HARVESTER v.0.6.94 [18, 19, 20].

Regarding the nuclear SSR markers, the obtained results showed that mean number of alleles per locus (Na) varied between 14.500 (Otelu Rosu) and 15.700 (Rusca Montana) (**Table 2**). The genetic diversity was high, while the observed heterozygosity varied between 0.724 (Otelu Rosu) and 0.806 (Rusca Montana) (**Table 2**). As regarding the inbreeding coefficient, only the Douglas fir population from Otelu Rosu showed a > 0.1 value. Overall, the obtained results suggest very minor differences among the three Douglas fir populations from Ana Lugojana, Rusca Montana and Otelu Rosu study sites (**Table 2**). These results are further supported by the UPGMA analyses, based on Nei's genetic distance, and by the DAPC analyses that altogether showed no differentiation between the three Douglas fir populations.

Table 2. The variation of the genetic diversity indices, based on nuclear microsatellite loci, of the Douglas fir populations from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Study site	**Ana Lugojana**	**Otelu Rosu**	**Rusca Montana**
Species	**Douglas fir**	**Douglas fir**	**Douglas fir**
Na	15.200	14.500	15.700
He	0.870	0.880	0.876
Ho	0.777	0.724	0.806
F	0.096	0.168	0.077

Na, number of alleles; *He*, expected heterozygosity; *Ho*, observed heterozygosity; *F*, inbreeding coefficient.

Regarding the genetic variation, assessed by cpSSR markers, the obtained results showed that the number of haplotypes detected for each population varied between 3 (Ana Lugojana) and 5 (Otelu Rosu), the effective number of haplotypes varied between 2.462 (Ana Lugojana) and 4.000 (Otelu Rosu), the haplotype richness varied between 1.607 (Ana Lugojana) and 2.750 (Otelu Rosu), the haplotype diversity varied between 0.679 (Ana Lugojana) and 0.857 (Otelu Rosu), while the mean genetic distance between trees varied between 0.655 (Ana Lugojana) and 12.179 (Rusca Montana) (**Table 3**).

Table 3. The haplotype variation, based on chloroplast microsatellite loci, of the Douglas fir populations from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Study site	**Ana Lugojana**	**Otelu Rosu**	**Rusca Montana**
Species	**Douglas fir**	**Douglas fir**	**Douglas fir**
A	3	5	4
Ne	2.462	4.000	2.909
Rh	1.607	2.750	2.143
He	0.679	0.857	0.750
D^2sh	0.655	1.750	12.179

A, number of haplotypes detected for each population; *Ne*, effective number of haplotypes; *Rh*, haplotype richness; *He*, haplotype diversity; D^2sh, mean genetic distance between trees.

The obtained results regarding the genetic structure of the populations, based on the Bayesian clustering method (Structure software v.2.3.4) [18], showed a most likely value of 2 for the K number. Additionally, the results obtained based on the structure analyses of the nuclear SSR markers and the cpSSR markers (**Fig. 4**), showed an undifferentiated genetic structure by population.

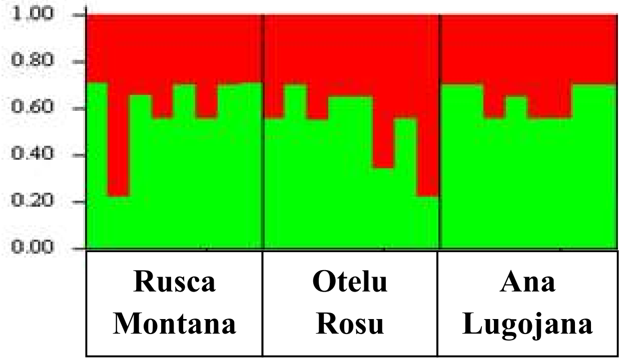

Fig. 4 Results of the structure analyses of the cpSSR markers, estimated by K = 2, of the Douglas fir populations from Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Indicators:

All the results showed in this section will be included in an article that is currently submitted for publication to a specialized journal: **Curtu Al., Ciocîrlan E., Şofletea N., Petritan C., Petrea Ş., Hereş A.-M., Assessing genetic diversity and structure of Douglas-fir (*Pseudotsuga menziesii*) plantations in Romania based on nuclear and chloroplast microsatellite polymorphisms, *submitted* to Annals of Forest Research.**

(**3**). **Understorey and competition**

The understorey and competition databases were used to calculate the Shannon alpha-diversity [21] and the Hegyi competition [22] indices, separately for each of the 225 sampled Douglas fir, Norway spruce and silver fir reference trees. To calculate the Shannon alpha-diversity index, the "diversity" function available from the vegan R package [23] was used. To calculate the Hegyi competition index, the "pairwise" function available from the Siplab R package [24] was used. Both the calculated Shannon alpha-diversity and the Hegyi competition indices were extended back in time, i.e., by considering them as being constant, up until the year 2005. In order to take this decision, the Forest Management Plans of the Forest Districts Ana Lugojana, Otelu Rosu and Rusca Montana, where all the information regarding these plantations is available, were consulted. According to these management plans, no sanitation cleaning and/or thinning have been performed within the three plantations in the last 15 years (i.e., 2020-2005). In order to further confirm this information, segmented or broken-line models were fitted between TRW data and time by using the "segmented" function available from the segmented R package [25, 26] and applying the Davies test. The results of these analyses showed no significant breakpoints from 2005 onwards. These results confirm thus the information available from the management plans. Otherwise significant breakpoints would have been found due to competition release.

In order to look for within study sites differences, i.e., in terms of the Shannon alpha-diversity and the Hegyi competition indices, between Douglas fir, Norway spruce and silver fir trees, ANOVA (followed by a Tukey's Honest Significant Difference (HSD) post hoc test) or Kruskal-Wallis (followed by a pairwise Wilcoxon post hoc test with a Bonferroni correction) analyses were performed, depending on the normality of the data.

The obtained results showed that, within study sites, there are no significant differences between the Douglas fir, Norway spruce and silver fir trees in terms of the Shannon alpha-diversity and the Hegyi competition indices (**Table 4**; **Fig. 5**). In order words, all three species show a very similar alpha-diversity (seedlings with a DBH < 10 cm and a height > 10 cm and all the shrubs) and undergo the same competition pressure from their neighbours. Further analyses will nevertheless deepen into the obtained results and see if there are significant differences between the three study sites and if the Hegyi competition index might possibly also explain, along with other factors, the growth rates of the three studied conifer species from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites.

Table 4. Within study sites differences, i.e., in terms of the Shannon alpha-diversity index, between Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Study site	**Ana Lugojana**			**Otelu Rosu**			**Rusca Montana**		
Species	**silver fir**	**Douglas fir**	**Norway spruce**	**silver fir**	**Douglas fir**	**Norway spruce**	**silver fir**	**Douglas fir**	**Norway spruce**
Shannon alpha-diversity index	0.75[a] (0.34)	0.62[a] (0.42)	0.65[a] (0.44)	0.91[a] (0.31)	0.99[a] (0.38)	0.95[a] (0.25)	0.62[a] (0.27)	0.62[a] (0.33)	0.60[a] (0.30)

Numbers within parentheses represent standard deviations. Lowercase "a" letters indicate the lack of within study sites significant differences between Douglas fir, Norway spruce and silver fir trees.

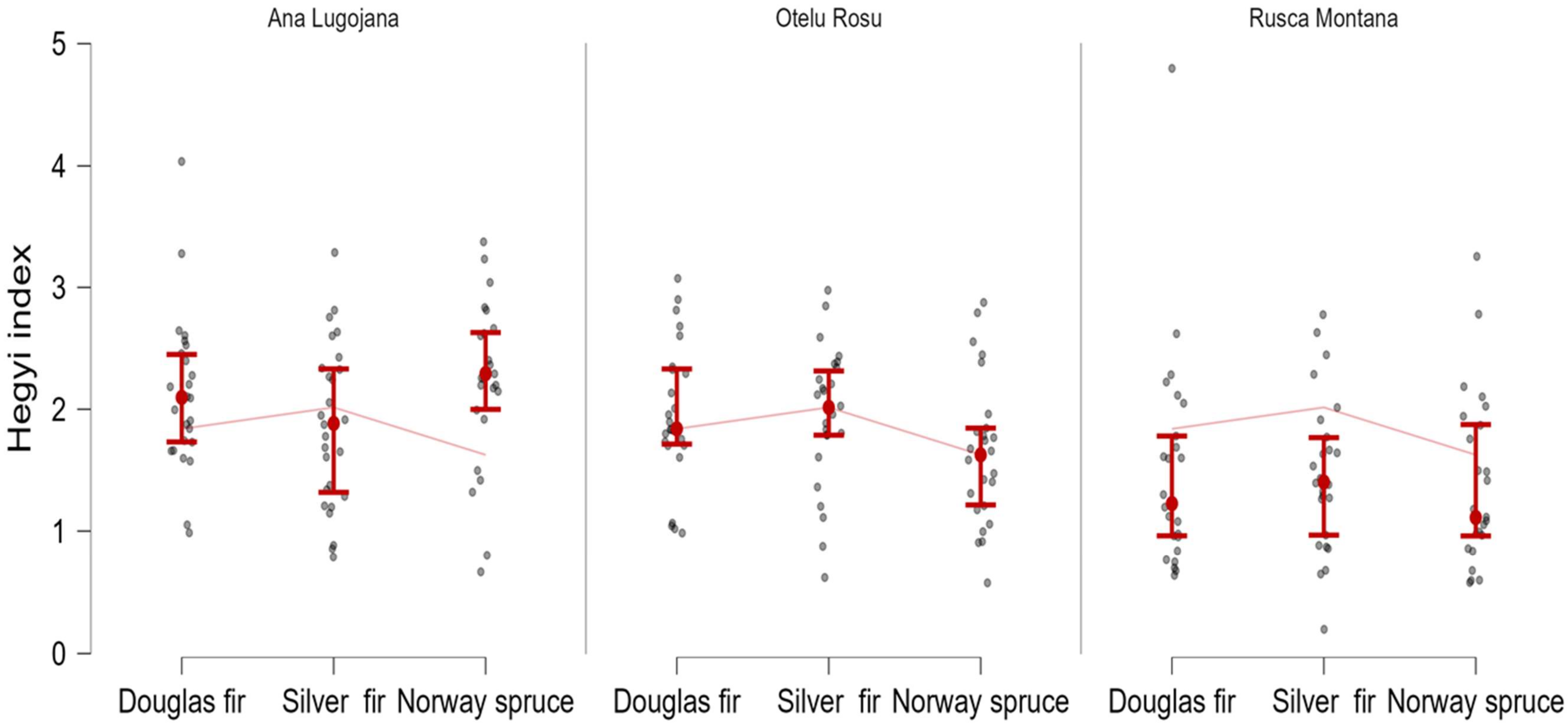

Fig. 5 The Hegyi index of the Douglas fir, Norway spruce and silver fir trees from Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Indicators:

All the results showed in this section have been presented in an international conference:

- **24-27 May 2022**, Petrea Ş., Petritan I.C., Gazol A., Curiel Yuste J., Hereş A.-M., "Drought resilience and stability of coexisting planted silver fir, Norway spruce and Douglas fir trees in the face of climate change". TRACE2022 “Tree-Rings in Archaeology, Climatology and Ecology”; Erlangen, Germany; oral presentation.

Additionally, the results showed in this section will be included in an article that is currently in preparation: **Petrea Ş., Petritan I.C., Gazol A., Curiel Yuste J., Hereş A.-M., *in prep*.**

(**4**). **Ecosystem services**

The growth, understorey and competition databases, along with the geolocalization of the 225 sampled trees, have been used to do an evaluation of the ecosystem services that the three Douglas fir, Norway spruce and silver fir plantations from the Ana Lugojana, Rusca Montana and Otelu Rosu might provide to nature and the human society (i.e., carbon sequestration / storage, recreation, etc.). To do so, dr. Stefano Balbi and his PhD student Alba Márquez Torres from the k.LAB (BC3 - Basque Centre for Climate Change; Spain) have introduced the three above mentioned databases into the ARIES (ARtificial Intelligence for Ecosystem Services) software (https://aries.integratedmodelling.org/).

The results obtained so far in this regard have shown the following:

i). According to the IPCC tier 1 model, the Otelu Rosu study site (**Fig. 6, lower right corners**) appears to be the one that has most potential in terms of vegetation carbon sequestration / storage capacity. Nevertheless, it needs to be highlighted the fact that, according to the same model, the growth data, along with the Shannon and Hegyi indices, indicate that several trees (mainly Norway spruce) from the Ana Lugojana and Rusca Montana study sites have actually the higher potential in terms of vegetation carbon sequestration / storage capacity (**Table 5**). The IPCC tier’s models have been described for both emissions factors and activity data. Specifically, the IPCC tier 1 model, used in these analyses, represents the basic model, and is frequently utilizing IPCC-recommended country-level defaults.

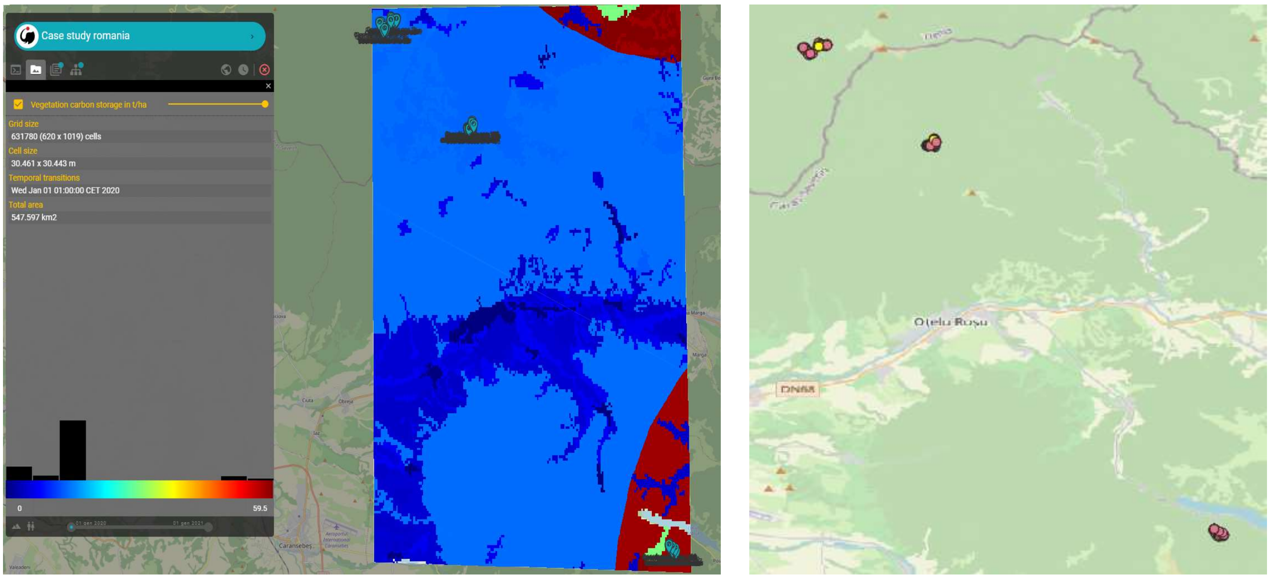

Fig. 6 Vegetation carbon sequestration / storage capacity, based on the growth data, the Shannon and the Hegyi indices, of the Douglas fir, Norway spruce and silver fir trees from Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Table 5. The trees (mainly Norway spruce one), located at the Ana Lugojana and Rusca Montana study sites, that have a higher potential in terms of vegetation carbon sequestration / storage capacity

Species	Tree no.	Study site	Growth	Shannon index	Hegyi index
Norway spruce	L36	Ana Lugojana	6.72	0.00	0.66
Norway spruce	L58	Ana Lugojana	6.49	0.00	0.80
Norway spruce	M45	Rusca Montana	5.90	0.36	0.58
Douglas fir	L9	Ana Lugojana	5.89	0.24	1.87
Norway spruce	M9	Rusca Montana	5.80	0.28	0.67
Norway spruce	M27	Rusca Montana	5.59	0.91	1.18
silver fir	M55	Rusca Montana	5.40	0.60	0.86
Douglas fir	L30	Ana Lugojana	5.32	1.20	1.73
Norway spruce	M1	Rusca Montana	5.24	0.33	0.60
Douglas fir	L13	Ana Lugojana	5.18	1.07	1.99

ii). When overlapping with the CORINE land cover layer [27], the obtained results showed that the Ana Lugojana and Rusca Montana study sites seem to offer more habitat for pollinators. Nevertheless, it needs to be highlighted the fact that these results are not related with the presence of the studied Douglas fir, Norway spruce and silver fir species but with a higher presence of broadleaf species within these two study sites (**Fig. 7a**). Despite these apparently positive results, no pollinated crops could be identified near these study sites (**Fig. 7b**).

a b

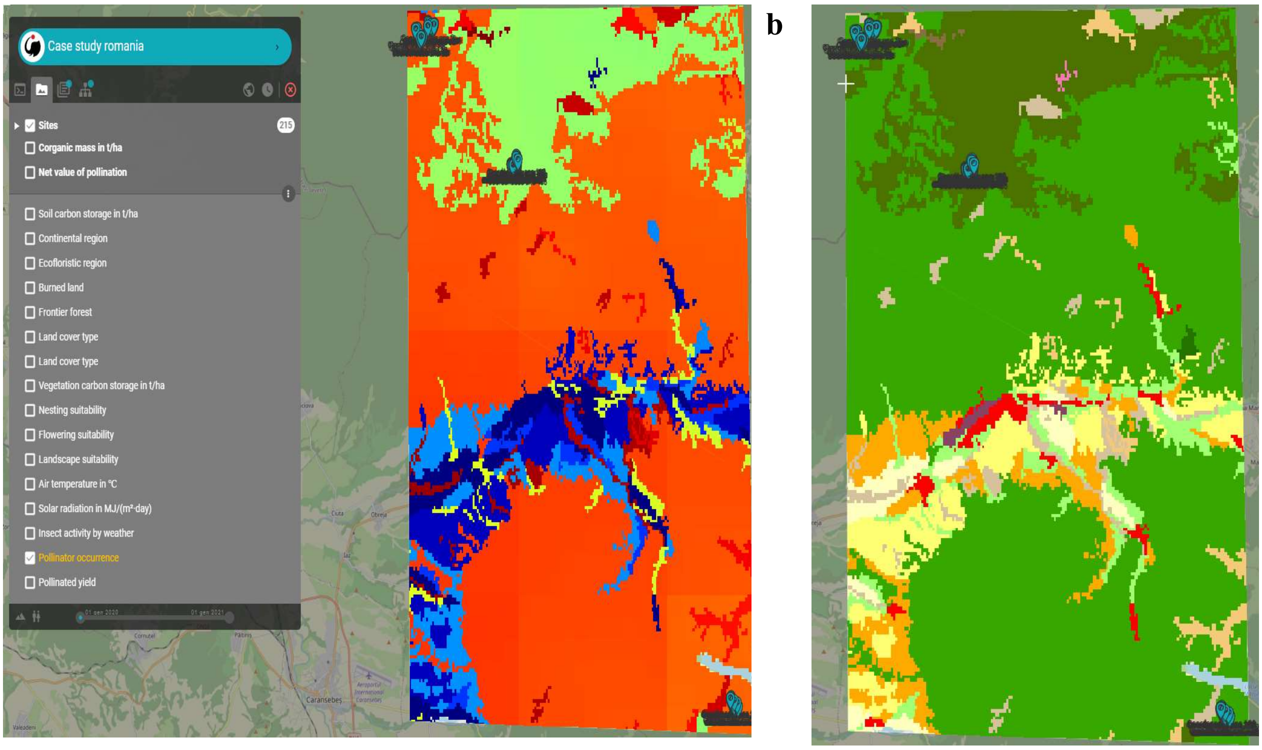

Fig. 7 The potential to offer habitat for pollinators of the Douglas fir, Norway spruce and silver fir mixed plantations from Ana Lugojana, Rusca Montana and Otelu Rosu study sites

iii). The obtained results showed that the recreation possibilities of the Otelu Rosu study site are theoretically better than the ones from the Ana Lugojana and Rusca Montana study sites. This theoretical recreation value of the Otelu Rosu study sites is explained by the fact that this study site is more accessible than the other two ones. Indeed, the accessibility encountered by the model can be corroborated by the members of the research team that have visited the three study sites for the 2020 and 2021 field campaigns. Another interesting result, regarding the recreational possibilities of the three study sites, has been obtained for the Rusca Montana study site for which a greater theoretical supply was found. This result is related with the fact that, based on the CORINE land cover layer [27], people might have a slightly greater desirability for mixed forest compositions rather than for monocultures (**Fig. 8**).

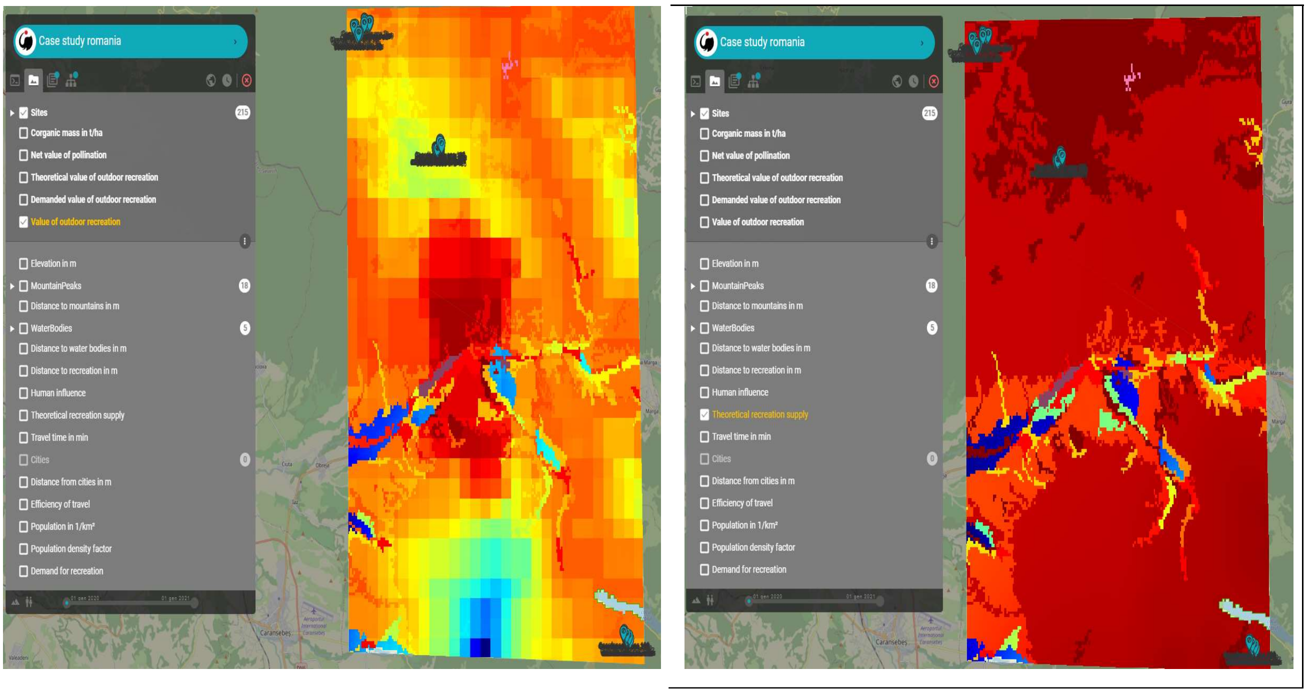

Fig. 8 The theoretical recreation value of the Douglas fir, Norway spruce and silver fir mixed plantations from Ana Lugojana, Rusca Montana and Otelu Rosu study sites

Indicators:

All the results showed in this section will be included in an article in a specialized journal once they will be finished and completed with soil and biomass models that are currently being built and validated.

III. IMPACT:

All the foreseen objectives of the REASONING project have been accomplished between 15/09/2020 and 14/09/2022, as detailed in the I. FORESEEN AND ACCOMPLISHED OBJECTIVES and II. RESULTS AND INDICATORS sections above.

To conclude, the results obtained from the REASONING project have shown that the Douglas fir, Norway spruce and silver fir trees from the Ana Lugojana and Rusca Montana study sites present positive growth trends. Instead, at the Otelu Rosu study site, only the silver fir trees present a positive growth trend. Additionally, no advantage (i.e., in terms of resistance, recovery, resilience, relative resilience) was found for the Douglas fir trees comparing with the Norway spruce and silver fir trees when facing severe drought events. Regarding the Douglas fir, the genetic analyses performed with this species have shown that there is no differentiation between the three Douglas fir populations from Ana Lugojana, Rusca Montana and Otelu Rosu study sites. Furthermore, the obtained results regarding the genetic structure of the populations, based on the structure analyses of the nuclear SSR markers and the cpSSR markers, showed an undifferentiated genetic structure by population, a common pattern among conifer species. The understorey and competition data have further showed that there are no significant differences between the three conifer species when considering the Shannon alpha-diversity and the Hegyi competition indices. Finally, the ecosystem services provided by the three mixed plantations of Douglas fir, Norway spruce and silver fir at the Ana Lugojana, Rusca Montana and Otelu Rosu study sites, do not seem to provide particular benefits to nature and the human society. Although some theoretical benefits have been highlighted in the II. RESULTS AND INDICATORS section above, these ecosystem services need to be regarded and interpreted with caution as they have a lot of *nuances* that need to be considered before any strong conclusion could be drawn regarding them.

More severe and frequent drought events are already not just an upcoming future climate change scenario, but a nowadays reality [28] affecting forest ecosystems at all levels (i.e., structure, functions, and ecosystem services) all over the world [29, 30]. In this context, human society and forests need to adjust and adapt together in order to face such challenges. Specifically, we need forests of the future to continue to provide key ecosystem services and satisfy at the same time the needs of the human society in a sustainable way, and for this to happen we need well planned forestry measures. The REASONING project has brought novel scientific data for Romania. To the best of our knowledge, previous to this project, there have been no publications combining ecological and genetic data on the Douglas fir plantations that have been established in Romania. The results of the REASONING project complete thus the available information that we currently have in Europe on this non-native species by adding an important piece to this species plantations' puzzle. Additionally, by studying mixed planted stands of Douglas fir, Norway spruce and silver fir, the REASONING project has filled in an ecological and genetic knowledge gap not only in Romania but also in Europe as, although several studies have been published on mixed stands of Douglas fir and Norway spruce, the scientific literatures lacks data on mixed stands of Douglas fir with silver fir [1]. The outcome of the REASONING project will therefore be of high interest for both the scientific community and stakeholders (i.e., society, foresters) as all three conifer species play important ecological and economic functions. The strength of the REASONING project was defined by its interdisciplinarity as it combined dendrochronology, dendroecology, genetics, understorey and competition surveys and models as main techniques of investigation. It gathered thus a team of experts in all these fields of investigation that combined their knowledge to obtain, as complete as possible, important results on the mixed Douglas fir, Norway spruce and Silver fir plantations from Ana Lugojana, Rusca Montana and Otelu Rosu study sites. The innovative and interdisciplinary approach of the REASONING project has allowed us to shade light on the ecological and genetic patterns that underlie processes in mixed conifer plantations on one hand,

and to make estimations on the ecosystem services they might provide on the other hand. The obtained results have been disseminated at international conferences (Petrea et al., 2021; Petrea et al., 2022) and will be published and highlighted in specialized journals (Curtu et al., *submitted*; Petrea et al., *in prep*.). At a smaller scale, the REASONING project has definitely contributed to the dendrochronological, genetic and ecological studies developed in Romania. The international team gathered for the project (**the four members of the research team [Ana-Maria Hereş, Elena Ciocîrlan, Ştefan Petrea and Ion Catalin Petritan] and the external collaborators [Alexandru Lucian Curtu, Jorge Curiel Yuste, Antonio Gazol Burgos, Stefano Balbi and Alba Márquez Torres]**) has also tighten its established collaboration, further triggering optimal conditions for a continued research network around the topic of the climate change impact on forests ecosystems, a hot topic worldwide. Additionally, the junior researchers that have also participated in the REASONING project have benefited from the expertise of the other senior members of the research team, forming themselves and strengthening and enlarging their knowledge and scientific skills.

All the information regarding the REASONING project, its implementation and its results, may be also found on the following webpage: https://silvic.unitbv.ro/ro/cercetare/525-proiect-reasoning.html

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