Securing Sustainable Dendromass Production with Poplar Plantations in European Rural Areas

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Deliverable

D1.2 Findings from Environmental Impact Assessment (EIA)

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1.0		Christoph Leibing	Final version

List of Abbreviations

Abbreviation	Denotation
D4EU	Dendromass4Europe
WP	Work package
SRC	Short rotation coppice – fast-growing poplar tree plantation
EDO	Environmental district office

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1 Role and relevance of the deliverable within the project

The aim of this deliverable is to describe the process and results of the environmental impact assessment and to address the identified concerns voiced by the responsible environmental authority (environmental district office) in the light of our findings from the biodiversity monitoring and genetic impact analysis.

The increasing amount of data on biodiversity can give us a basis for successful implementation of SRC to the Slovak landscape in accordance with the management plan of the responsible environmental authority, thus minimizing the risk of negative environmental impact. The communication activities (WP6, task 6.2) connected to the preliminary findings regarding biodiversity could improve the acceptance of SRC in Slovakia in the future, which could increase the success of the land search activities (WP1, task 1.1), that are the backbone of this project.

2 Responsibilities

IKEA Industry Slovakia s.r.o. is responsible for submitting the requests for the consent of the responsible environmental body (environmental district office) for the establishment of SRC on a given locality.

DAPHNE Institute of Applied Ecology is responsible for gathering and evaluation of data needed for the assessment of impact of SRC localities on biodiversity.

Technische Universitaet Dresden is responsible for the investigation of the potential future genetic impact of the established SRC on the indigenous European black poplar populations (*Populus nigra* L.)

3 Task, problem definition and research objectives

As stated in the grant agreement "Fast-growing trees are planted in monocultures on agricultural land and have obvious advantages over native plants in competing for light, nutrient, and water resources. Therefore, large-scale tree plantations have led to increasing concerns regarding their adverse effects on biodiversity."

The legal framework of SRC in Slovakia is well defined, therefore there is an inherent control mechanism of the responsible environmental body in place. The monitoring of biodiversity in the preselected representative species groups (plants, birds, amphibians, butterflies and beetles) gives us a deeper insight to the dynamics of the SRC therefore it is an indispensable tool to evaluate the long term environmental impact of the established plantations. Furthermore, these results facilitate the implementation of additional steps that can increase the biodiversity on these plantations, or provide valuable habitat for the detected species.

As one of the concerns of the environmental body during the pre-alignment was the potential future impact of the established SRC on indigenous European black poplar populations (*Populus nigra* L.), this issue had to be also addressed within the framework of this task. However, this topic is subject to separate investigations by the project partner TU Dresden, Molecular Tree Physiology Group (TUD-MTPG). These results will be reported more detailed in a separate deliverable D1.3 (project Month

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M49). Here in D1.2, the scope of that investigation, and the methodology will be presented only with preliminary results.

The communication activities connected to these findings can also improve the perception of the general public (WP6, task 6.2), and resolve the uncertainties, resulting in formulation of a well-informed public opinion.

4 Theoretical background, scope and limitations

The § 18a(2) of Law No. 220/2004 Coll., on protection and exploitation of the agricultural land, defines the necessary proceedings of filling an application for registration in the Register of Areas of Short Rotation Plantations, that is maintained by the District Office, Land and Forest Department. From the beginning of the project until 1.8.2019 the responsible environmental body was issuing a statement regarding the effect of the establishment of the plantations in a given locality.

Positive statement was issued only in the case if no adverse effect - associated with the establishment of SRC - on native species was uncovered. Land and Forest Department, had to consider the statement of the environmental body during the aforementioned registration process. According to the novel of the Act No. 543/2002 Coll., on nature and landscape protection, the legal authority of the responsible environmental body has increased, as Land and Forest Department has to receive the consent of the environmental body on planting non indigenous species to register the given land in the Register of Areas of Short Rotation Plantations. In case of parcels of interest that are situated in Natura 2000 network, a "pre-assessment" is necessary according to § 28 of the Act No. 543/2002 Coll., on nature and landscape protection, whether the proposed project is the subject of environmental impact assessment according to § 18 (1) (g) of the Act No. 24/2006 Coll., on environmental impact assessment and on amendments to certain acts.

The aforementioned legal framework, serves as a good basis of evaluation of the establishment of SRC, and dedicates great decision power on the responsible authorities. This fact serves as a guarantee, that SRC is established only on localities, where it can't have a net negative effect on nature conservation. However due to the finite capacity of the environmental body it is important, that scientific data on the effect of SRC is provided, therefore facilitating a well-informed fact based decision making. Undeniably, the most important factor to understand the impact of SRC on nature conservation is the biodiversity that had been monitored in the framework of this project on species level, as it was considered the most effective approach whilst addressing a dynamic ecosystem.

5 Research design and methods

Inventory monitoring tries to get information on overall status of biodiversity on 74 SRC localities in season 2018 and 84 SRC localities in season 2019 (Map 1) by recording the presence of all vascular plant species passing through a transect over the whole area of every SRC locality. On the same transect the recordings of presence of specimens of additional species groups; mainly mammals, reptiles, insects and molluscs are collected.

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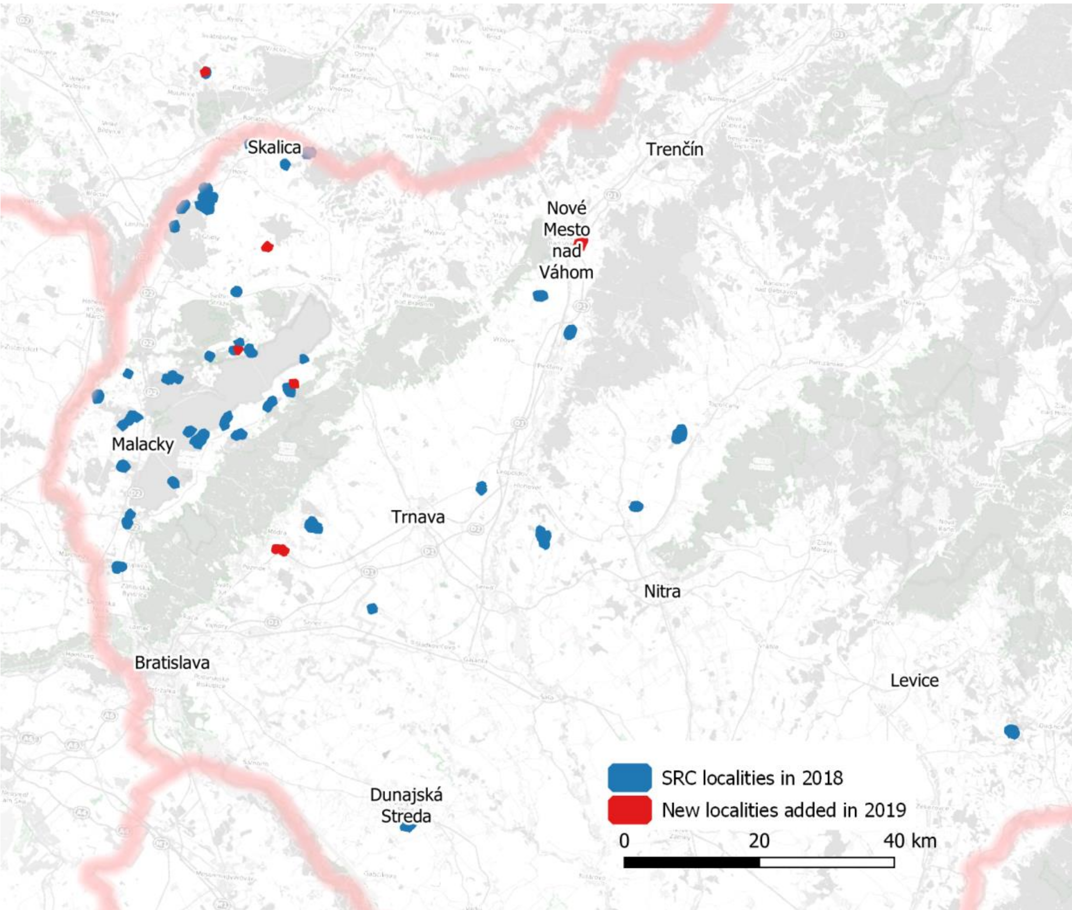

Map 1: SRC localities of inventory monitoring

The main task of reference monitoring is to find out the differences between SRC localities and their surrounding reference biotopes, leading to assessing the status of biodiversity in the area. It is done for 4 animal species groups: birds, amphibians, butterflies and beetles. For each animal species group, the representative SRC localities were selected on the base of occurrence of neighboring biotopes, type of biotope before plantation and suitability for particular species group. For each selected SRC locality there was defined at least one transect within the area of SRC and at least one transect for area of neighboring biotope as reference or control sample.

Considering the fact, that in conventional agriculture there is almost annual change of crop species, we chose to divide the plots into 3 different land use categories, arable land, grassland, and grass on arable land (where the registered land use type was arable land, but the established vegetation was similar to grassland). The results obtained from the biodiversity monitoring were analyzed in the context of previous land use. A work manual was created for the request for statement/consent from the responsible environmental body, prior to the establishment of the SRC.

For investigating the potential impact of the SRC poplars of native *P. nigra* genepools, TUD-MTPG carries out winter sampling and genetic investigation of existing rejuvenation and selected representative old poplar trees in Slovak Republic in the target region of the project D4EU. Generally, genetic admixture of natural stands (e.g. pollination of native *P. nigra* female trees with non-native seeds or pollen from planted introduced or hybrid pollen donors) requires the capability to flower. As the D4EU SRC poplars are too young (immature, juvenile) and they will be harvested after 4-6 years (= before maturation), we expect no potential for impact on native genepools by the present D4EU SRC plantations

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(see schema in **Fehler! Verweisquelle konnte nicht gefunden werden.**). However, any future risk potential for early maturation in later rotations, if any, should be carefully considered.

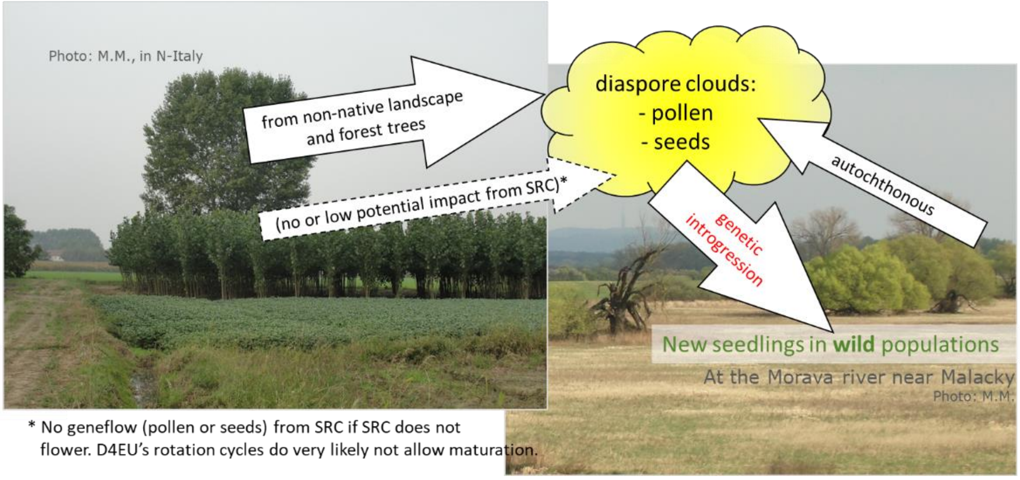

Figure 1: Current diaspore clouds in Europe are based on pollen or seed contribution by fertile trees, like from old-growth genetically 'pure' black poplars (valuable, but rare) but to a large extent also by planted old-growth hybrid poplars or poplar cultivars. – Not so from the D4EU plantations, as they are harvested as early as after 4 to 6 years and will very likely not flower before.

For investigating the potential impact of the SRC poplars on the native *P. nigra* genepools, TUD-MTPG carries out winter sampling and genetic investigation on existing rejuvenation and on selected, representative old poplar trees in Slovak Republic in the target region of the project D4EU. Because the present SRC poplar plantations do not flower (and because this is not likely to occur in the future due to short rotation cycles), the present-time situation of the genetic introgression by old existing trees from landscape, forest and ornamental poplar trees is therefore investigated. All samplings and genetic fingerprinting are done in accordance with legal regulations, like the Convention on Biodiversity, Nagoya Protocol on the Access Benefit Sharing (ABS) that has to be considered for international exchange of wild genetic resources. The TUD-MTPG received permission by the Slovak Ministry for Environmental Affairs to carry out the investigations, while ensuring that no other national regulation is affected.

The results of TUD-MTPG's investigations allow predictions about the impact potential and, if any, about the potential intensity. The following questions will be answered:

1. Very likely, genetic introgression has been existing already for a long time in West Slovakia and the Danube inland delta (see for instance Benetka et al. 2002) - How intensive is genetic admixture, as measured on the existing surviving rejuvenation in the project's target region?
2. As a reference how genetically "pure" the sampled genepool of poplars is, a collection from Germany are compared with the Slovak samples. – Is the potential for genetic introgression bigger or smaller in West Slovakia and where are the differences?
3. The seeds stemming from poplars in West Slovakia form the basis for new poplar generations. But the seeds themselves, germinating seedlings and young trees are subject to natural selection. Do seeds exist from both the native *P. nigra* and the existing poplar hybrids? Can something be said about differences in germination success and about survival of young trees?

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In addition, expert interviews (e.g. with the D4EU's external advisory Dr. Jan Weger, VUKOZ Dept. Phytoenergy, Czech Republic; Dr. Bartko, Lesy SR, Slovak Republic) as well as scientific literature evaluation is done, to collect region-specific knowledge for the assessment of potential impact on *P. nigra* genepools. The most important questions to experts are:

1. Is unexpected early flowering (i.e. maturation within 4 or 6 years of tree life) possible in the first rotation of SRC poplars? Is there scientific evidence that it has occurred somewhere in the EU, and what might be the conditions?
2. Is there scientific evidence for earlier tree maturation in the second or higher rotation cycle on an SRC when the stools are older, but the trees are only 3 or 4 years old?

6 Results

6.1 Biodiversity monitoring

The table show overview of collected data from the last two seasons.

Table 1: Overview of collected data from the last two seasons

Inventory mapping	localities		visits		transects km		species records		species	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
plant species records	74	84	76	87	114	126	2492	3141	310	341
animal species records	74	81	76	87	101	0*	715	280	102	42
Re-monitoring	**localities**		**visits**		**transects km**		**species records**		**species**	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
birds	14	15	26	32	99	145	966	1010	100	95
amphibians	8	9	34	111	86	148	157	284	9	8
beetles	8	9	32	36	27	30	978	1326	202	228
butterflies	8	9	54	62	28	31	380	431	37	41

The data overview of different groups of species are discussed in their dedicated section. The detailed data and findings of the biodiversity monitoring were submitted in WP1 D1.6 in month 34.

6.1.1 Birds (Aves)

In total 109 different bird species were recorded in the frame of two seasons. 40 of them were from SRC localities, 70 from control transect biotopes and 103 species were found in surrounding biotopes. 84% of the monitored area was converted to SRC from arable land. Higher number of species in areas out of SRC localities was recorded, as these areas covered different types of biotopes (fields, shrubs, grasslands and ruderal vegetation). Although the positive effect of SRC can be noticed in the fact, that the recorded number of bird species was in some cases higher in SRC localities than in control arable fields (demonstrated in Deliverable 1.6 in figure 6.1.1.), the most important added value of SRC in comparison with arable land is that it provides a more suitable nesting area for birds. This is clearly

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demonstrated in Deliverable 1.6, Figure 6.1.3, where in comparison with various habitats (shrubs, ruderal vegetation, grassland, and arable land) a similar value of diversity of probable and proved nesting bird species is present in both control and SRC localities. Probable and proved nesting activities were observed for 7 bird species (*Alauda arvensis, Carduelis cannabina, Lanius collurio, Lullula arborea, Motacilla flava, Passer montanus* and *Phasianus colchicus*).

6.1.2 Amphibians (Amphibia)

From 8 amphibian species that can possibly occur in the given region 6 were recorded on SRC localities: ***Bufo bufo, Bufo viridis, Hyla arborea, Pelobates fuscus***, *Pelophylax esculentus*, ***Rana dalmatina***. All of them are species of national importance, and in the case of four species (bold), are of European importance. The preliminary results of biodiversity monitoring (WP1, D1.6) showed, that the presence of amphibian species in SRC localities dominates over their reference samples. This can indicate a positive effect on the biodiversity and the abundance of amphibians on the established SRC, as the occurred land conversion was in this case also dominantly from arable land as shown in Figure 2, where amphibians can inhabit only the occasionally formed depressions that are destroyed once the land is sowed.

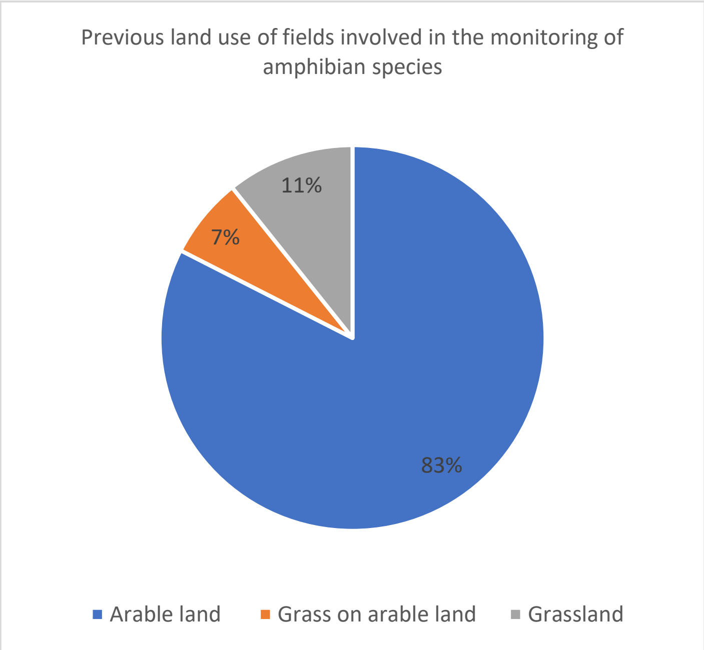

Figure 2: Previous land use of fields involved in the monitoring of amphibian species

6.1.3 Beetles (Coleoptera) and Butterflies (Lepidoptera)

During the period of monitoring 41 butterfly species were observed. 39 of them were also recorded in SRC localities, and 3 species were exclusive for SRC localities. From the recorded 228 species of beetles in total, 207 were observed also in SRC localities and 21 species only in SRC localities. Most of the butterflies can go through their life cycle within the SRC localities. It means that their feeding plants occur in these areas and all their life cycle stages can be observed. One of these observed species is ***Lycaena dispar***, a species of European importance.

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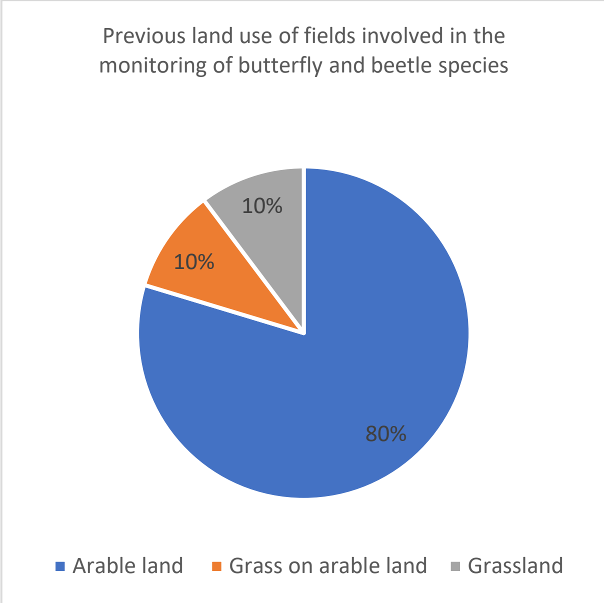

Figure 3: Previous land use of fields involved in the monitoring of butterfly and beetle species

According to the findings on the monitored SRC localities, we can say, that SRC is a more suitable biotope for butterfly and beetle species than arable land, but less suitable than natural biotopes like grassland or shrubs (however, in one locality there were higher numbers of beetles for the SRC locality, than in the neighboring degraded grassland). This fact is not surprising, due to the intensive use of insecticides in conventional agriculture (see example in Table 2).

Table 2: Example of chemicals used for the treatment of spring canola (online 1)

Compound	Effect	Frequency of application
clopyralid + picloram	herbicide	1
quizalofop-P-ethyl	graminicide	1
tebuconazole	fungicide	1-2
chlorpyrifos	insecticide	1-2
pyretroid	insecticide	1-2
thiacloprid	insecticide	1
diquat	desicant	1

The forming dense canopy of old poplar trees can suppress the undergrowth in the future, therefore might have a negative impact on the availability of feeding plants of butterflies, as it was administered on one monitored locality already.

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6.1.4 Plants

The total number of identified plant species in all SRC localities in the last two seasons was 406. All species were divided into groups according to their affinity to natural, semi-natural and artificial biotopes. 6 main groups of plants were identified: invasive, neophyte, ruderal, endangered, protected and other (Medvecká et al. 2012).

The Venn diagram in Figure 4 shows numbers and combinations of different plant species groups.

15 invasive, 30 neophytes and 106 ruderal plant species are considered as plants (114 in total) of artificial biotopes.

31 endangered, with 5 nationally protected plant species, and the 261 "other" species (292 in total) are representatives of natural and semi-natural biotopes.

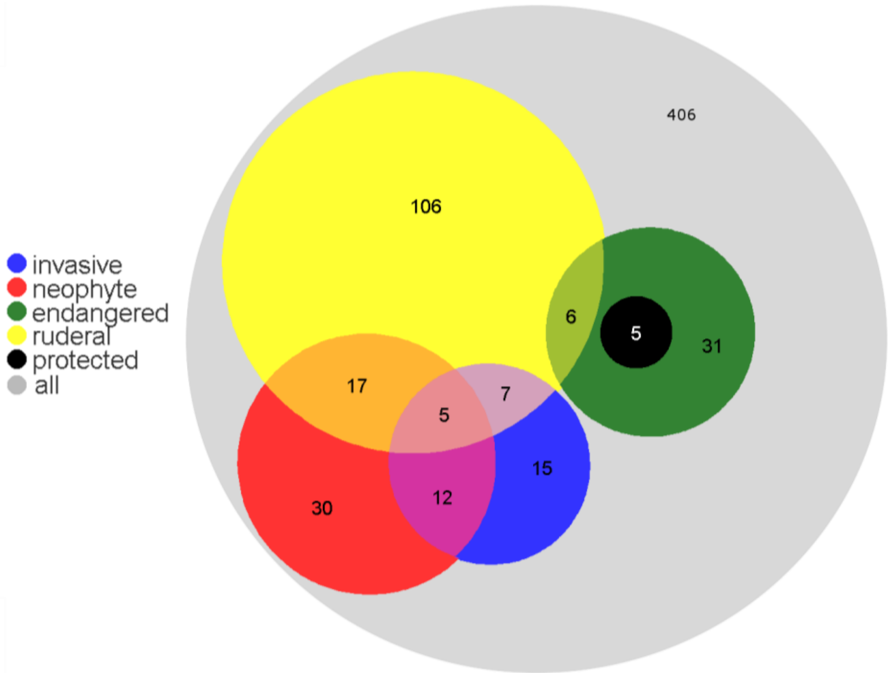

Figure 4: Numbers of plant species in different species groups and their combinations

The percentage of the abundance of different plant species from both seasons from all SRC localities is shown in Figure 5.

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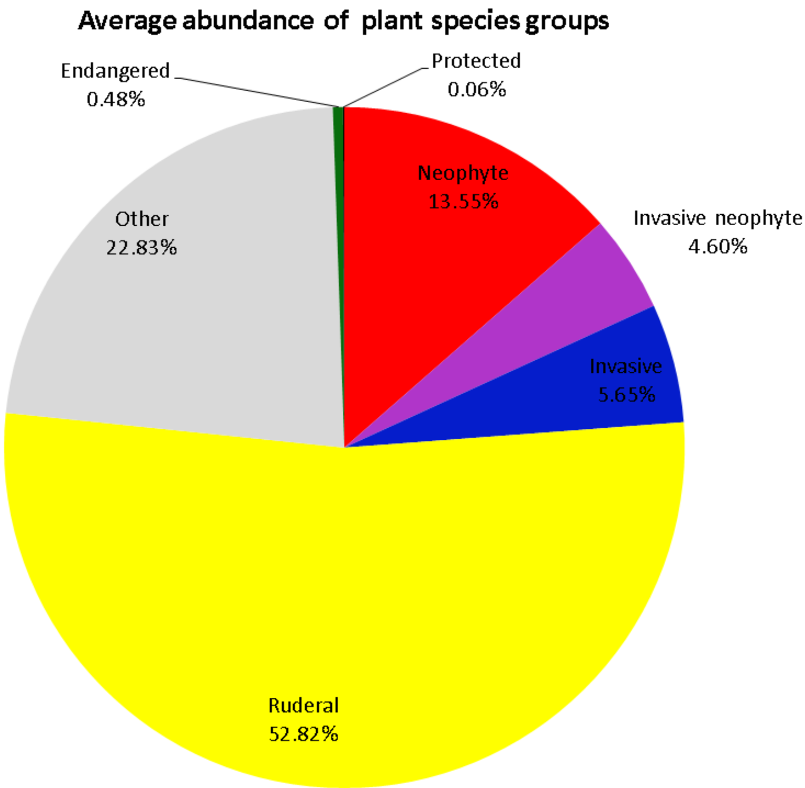

Figure 5: Abundance of plant species types in season 2018 and 2019

Endangered species were found in 46 SRC localities. Important occurrence of two endangered archeophyte species *Ranunculus arvensis* and *Aphanes arvensis* in three SRC localities was recorded. Probably the disturbances made by disking as a main management measure of the SRC localities is suitable for these species. Also psamophytic plant species like *Spergula morisonii* or *Teesdalia nudicabulis* are profiting from such type of management. Data from next years of monitoring can explain if these species can survive vegetation seasons with dense canopy of poplar trees.

It is visible in Figures 4 and 5 that although the number of "artificial" plant species (114) is much lower than the number of "natural" plant species (292) the overall average abundance is in favor of "artificial" species, thus making the SRC localities a place for living of rare species, but also a place for possible colonization of invasive neophytes.

6.2 Previous land use evaluation

When addressing the comparison of biodiversity on the established SRC, it is important to present the findings in the appropriate context. In the lowland parts of Slovakia, where these poplar plantations are established, it is mainly the context of intensively used agricultural land.

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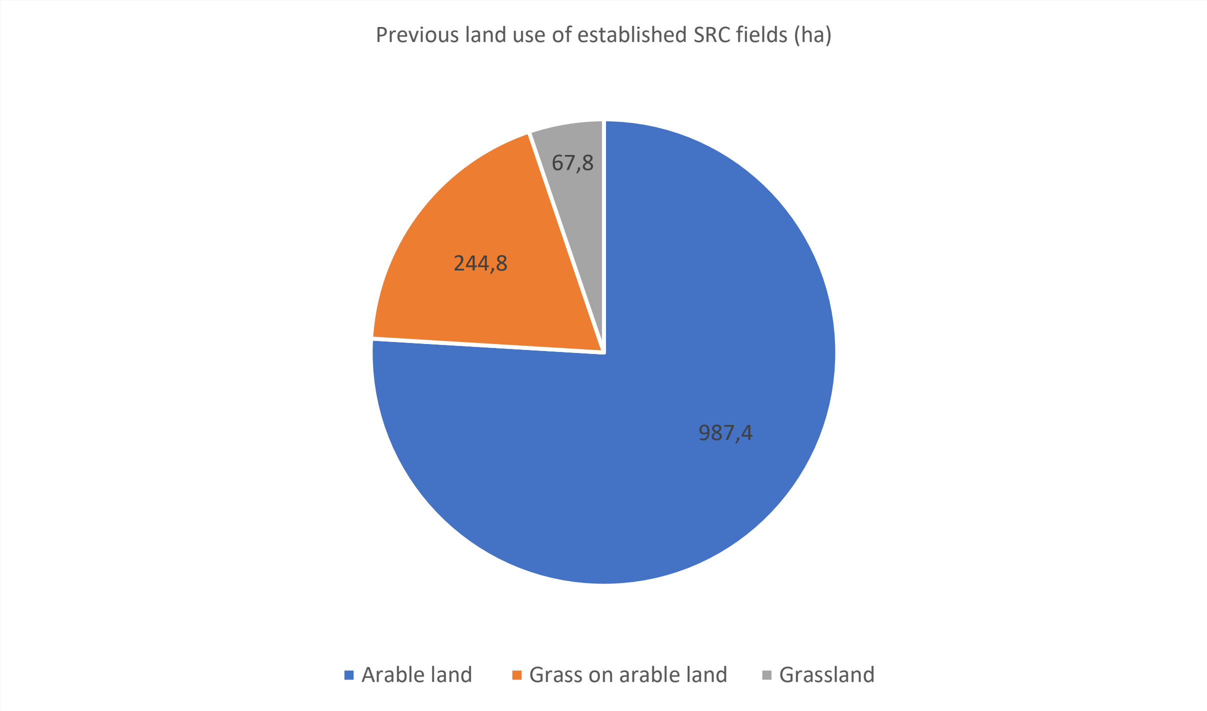

Figure 6: Previous land use types of established SRC localities (size in hectares)

When reflecting to the net amount of established plantations, more than ¾ of the the SRC area were established on arable land, thus intensive agricultural land use was changed to SRC. The frequency of replacement of grasslands with SRC was 5%, whilst 19% of grass on arable land, was converted to SRC.

6.3 Genetic analysis of present European black poplar genepools (*Populus nigra*) in the project region

6.3.1 Sampling of wild poplar rejuvenation and genetic investigations in gravel mines

TUD-MTPG does not sample in protected areas, as the natural rejuvenation of *P. nigra* is rather scarce all over Europe. The successful wild rejuvenation requires (i) flowering mature trees where female and male trees must be compatible and must flower at the same time, (ii) the trees must shed viable seeds, (iii) a habitat is needed that allows successful settling of the flying seeds and their successful germination, and (iii) the biotic and abiotic conditions must allow survival. Normally, *P. nigra* finds its ecological niche for germination only on open mineral soils with water access and soil aeration. Which are sand banks or gravel planes along rivers after a flood, for instance. There must be not too much fine material (loam or clay), and there must be no strong competition with weeds or other germinating tree species (in many areas of Europe, willows dominate over the poplars in rejuvenation after river floods, but *P. nigra* is often not competitive enough). There are non-natural ecosystems that are an anthropogenic “copy” of natural germination habitats for poplars and willows. That are gravel mines with water bodies, stone pits, moist construction sites, industrial or rubble fields with well-watered gravel or sand. All other habitats are normally not suitable for *P. nigra* germination. TUD-MTPG samples mainly in gravel mines on the edges of the water bodies (Figure 7).

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Figure 7: Situation at an actively operated gravel mine close to the river Morava (March), on Austrian territory. Trees in the background (left hand side) show rather typical black poplar-like habitus (approx. 10 or 20 years old). Right: poplar (likely P. deltoides and/or P. nigra - hybrids) and willow rejuvenation on gravel in a mine near Kopčany, in the background an old planted hybrid poplar forest.

Generally, we assume that the potential area for *P. nigra* rejuvenation in natural habitats is rather small, as the rivers do not have their full floodplain dynamics anymore. However, the Slovak situation, also in gravel mines, is much better for *P. nigra* than for instance in the Central and North German areas, where there are no old pure *P. nigra*. In West Slovakia, many old floodplain forests with many genetically pure *P.nigra* can be found. However, a direct comparison of quantities is not possible because of unequal sample numbers and unequal sampling conditions in the regions.

Figure 8: Positions of areas with gravel mines or other not protected artificial biotopes where Populus rejuvenation was sampled. Black squares in Germany = FastWOOD sample. Black squares (2018) and orange squares (2019, 2020) in Slovakia and Austria: D4EU sample.

Much poplar rejuvenation was found in gravel mines along the rivers Vah and Morava as well as in the Danube inland delta and at the Danube itself, but not so much along the Nitra river plain or at sampling positions close to the Malé Karpaty (e.g. near Modra).

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In all project years, the sampled cuttings were transported to Germany, potted and grown, and investigated for their leaf shape in the following growing season. Material for DNA extraction and genetic fingerprints was sampled from buds of the cutting material or later from the fresh summer leaves of potted plants. A subset of DNA markers on chloroplast and nuclear DNA is used for the multilocus fingerprinting (not finished). First results and parts of the methodology have been published (Meyer et al. 2018), see also Figure 9.

Figure 9: Poplar collection in the experimental garden of the TUD-MTPG (left). Middle: Leaf shape assessment with three leaf shapes (upper three) that indicate a *P. nigra* or *P.×canadensis* background and the other three indicating hybrid background (the lower leaf shape is that of mature *P. alba*). Right: Chloroplast DNA-Marker *trnDT* results for sampled and reference trees, allowing the distinction of the species assignment for the chloroplast.

As a first insight from leaf shapes, it was observed that there is relatively large amount of individuals that have black poplar-like leaf shapes, but also these with clearly *P. deltoides* – like leaf shapes. The genetic laboratory tests are currently going on, and the respective microsatellite locus subsets are currently compiled for an optimum multilocus-fingerprint. However, the comparison of leaf chloroplast DNA-marker loci between German and Slovak poplar samples shows that the sampled Slovak rejuvenation contains clearly less foreign material (Figure 100).

	Main leaf phenotype	FastWOOD collection: Number of individuals with *trnDT*-genotype			Total	D4EU collection (2018/19): Number of individuals with *trnDT*-genotype			Total
		ni. < 900 bp	bals. 1000-1100 bp	delt. > 1100 bp		ni. < 900 bp	bals. 1000-1100 bp	delt. > 1100 bp	
Aigeiros-like	quadrangular	10	-	1	11	81	1	4	86
	triangular	11	1	2	14	1	-	-	1
	triangular-oval	57	2	10	69	10	2	1	13
Tacamahaca-like	oval	-	1	1	2	-	-	-	-
	ovate	1	30	2	33	-	-	-	-
	ovate-lanceolate	1	10	1	12	-	-	-	-
	elliptic	-	2	-	2	-	-	-	-
	Total	80	46	17	143	92	3	5	100

Figure 10: Results of DNA-marker investigation on the chloroplast DNA (marker locus *trnDT*, cf. (Heinze 1998)). The investigated rejuvenation of poplars from Slovakia showed only black poplar-like (*Aigeiros*-like) leaf shapes that indicate a background in *P. nigra*, *P. deltoides* or their hybrids (*P. × canadensis*). Only for 8 out of 100 sampled young trees we have clear evidence that they are hybrids of *P. nigra* with foreign species. The other 92 will be investigated with microsatellite markers for detecting additional non-native hybrids. In the German sample, there are also many individuals with balsam poplar leaf shapes (all non-native) and many more hybrids in the total poplar rejuvenation collective.

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With the preliminary results, it can be confirmed what is known from international literature. The effective *P. nigra* populations in West Slovakia are still large and their flowering-related population-genetic processes seem to be relatively unaffected as compared, for instance, with the sparse populations along regulated rivers in North Germany. It is known that *P. nigra* prefers pure pollen from the own species. Therefore, it can be concluded that the Slovak populations have higher robustness against foreign pollen than populations in Germany, where there are less pollination partners. This might be an effect of a much higher population density of mature black poplars (*P.nigra*) along the rivers Vah, Morava, Maly Dunaj (Little Danube) and Danube. Old hybrids are present in SK (e.g. *P.×canadensis* as well as balsam poplars), but their pollen seems to play a less important role due to better availability of *P. nigra* pollen (according to our preliminary interpretation).

6.3.2 Seed sampling, literature and expert knowledge

It cannot be excluded entirely, that the poplar clones used in D4EU could become mature in future SRC rotations. However, this is not likely. At his point in time, TUD-MTPG has not found information or scientific reports about early maturation or about any severe impact in other regions of Europe due to early maturation of non-native poplars. Only in Argentina, a four-year old pure cottonwood (*P. deltoides*) was reported to flower in the first rotation. However, there is no pure *P. deltoides* clone used in D4EU, and there is no one known that is available in the EU. One interviewed expert for the genus *Populus* who is working in the field of poplar breeding and forest tree nursery management in Slovakia claimed that there has been no observation of development of vital seeds on female hybrid *P.×canadensis* clones in the landscapes of West Slovakia and in the Danube Inland Delta. On the other hand, scientific literature about the introgression into the *P.nigra* genepools often refers to the assumption that *P.×canadensis* hybrids are clearly able to develop pollen (that may result in backcrossing with the native black poplar *P.nigra*) or seeds. However, some publications do not provide a clearly measured quantification of that introgression.

A precise prediction for the present main operational clones used in the project (e.g. AF16 or AF18) is hence not possible, particularly because their sex is not yet known to the TUD-MTPG. The Checklist for Cultivars of *Populus* L. (poplar) of the International *Populus* Cultivar Registration Authority (FAO – International Poplar Commission, 2016) does not contain information about the sexes. We do not know, if the present main operational clones are able to flower at all when becoming aged trees, though this must be assumed here. Also, we were not able to receive information about positions of old trees of AF16 or AF18 that flower somewhere in the EU to get seeds from there for checking their vitality. Likely, there are no flowering trees, as the clones are normally planted in SRC and these trees are cut off before maturation

It is important to emphasize again, that both the protected genetically 'pure' black poplars as well as old hybrid poplar stands are present along the rivers Morava and Vah which are major tributaries of the Danube, thus forming a present mature-tree mixed population (or species complex) where hybridization might potentially occur. Opposed to these old poplars, there is very low risk of hybridization of the established SRC, as the planted poplars are harvested before reaching flowering (see above). However, if the AF16 or AF18 trees growing in the present planted SRC plantations would become mature at all, they (only potentially) can shed either pollen or seeds, depending on their sex. According to Benetka et. al. (2002, p. 118) "…introgression of *P. × canadensis* into the gene pool of black poplar is not likely to exceed 10%".

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Also it is shown in literature that black poplar is preferentially pollinated by its own pollen (Bialozyt et al. 2012; Ziegenhagen et al. 2008). It can be assumed that genetic admixture (introgression) is significantly influenced by seedlings from female *P.×canadensis* (Dode) Guinier plants pollinated by *Populus nigra* L. pollen (Benetka et. al., 2002). It is known from publicly available descriptions of the clones by the breeder (Alasia New Clones, Savigliano, Itlay) that AF16 is derived from a cross between *P. deltoides* 186-98 (Illinois USA) × *P. nigra* 621-93 (Italy) and AF18 from *P. deltoides* 62-98 (Iowa USA) × *P. nigra* 365-94 (Italy). Therefore, it should be assumed that AF clones belonging to the *P.×canadensis* clones (like AF2; AF16; AF18; AF24) would show life cycles that are comparable to these of well-known *P.×canadensis* clones in the region of South / Central Europe. Therefore, the TUD-MTPG selected four big old *P. nigra* trees as well as four trees showing *P.×canadensis* character in West Slovakia in 2019 because of their rich fruit development. In May 2019, scions with green fruits were cut and transported to Germany. The scions were incubated in a climate chamber to allow fruit and seed development. The seeds were prepared (fluff was removed with sieves and compressed air). It was very easy to obtain seeds from *P.nigra*. *P.×canadensis* scions carried much less and very thin seeds.

All seeds were immediately sown in a tree nursery. In the year 2020, the seedlings were potted and the TUD-MTPG will carry out genetic and phenotypic investigations in the summer seasons when leaves will be fully developed. As illustrated in Figure 11, there have been seed lots that germinated vital (mainly *P. nigra*) while other seed lots showed very poor germination. Final leave shape and genetic fingerprint investigations will be done later this year.

Figure 11: Different poplar seedling nursery trays after sowing in 2019. Each nursery tray represents one of the seed samples obtained from 4 Slovak pure *P. nigra* and 4 *P. × canadensis* old trees.

6.4 Learnings from the requested statements

As mentioned in chapter 4, the environmental body had to issue a statement, and after the novelization of the law give consent for planting non indigenous species. This means, that every established plantation had to go through a process of requesting statement from the environmental body. According to the experience of the land developers a work manual had been established.

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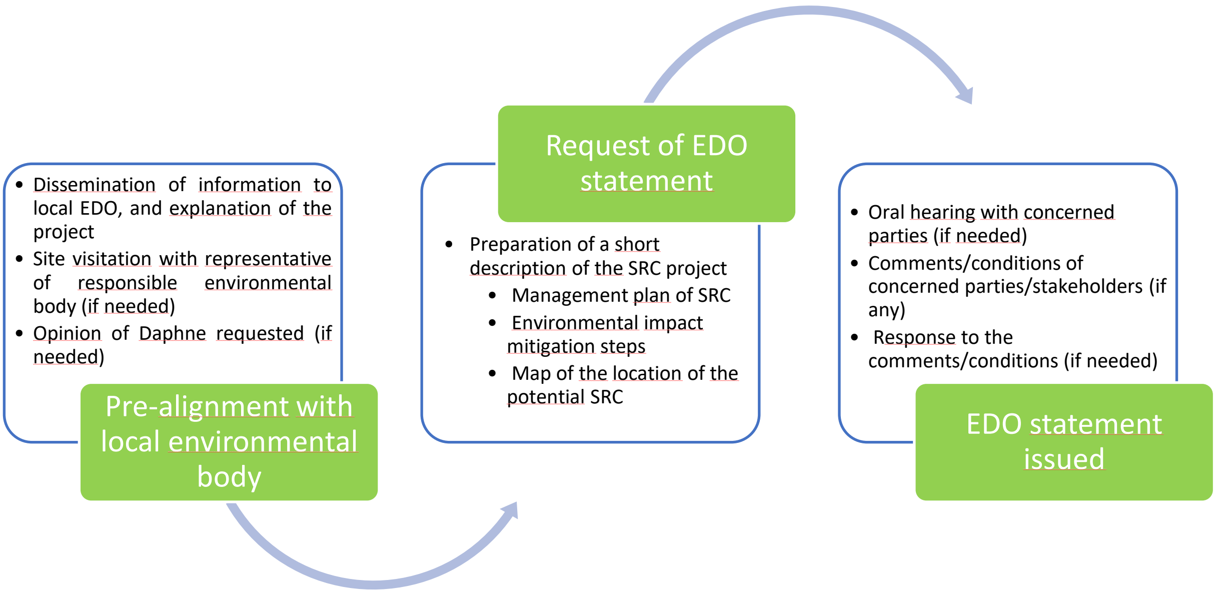

Figure 12: Work manual for the request for statement/consent from the responsible environmental body (EDO – environmental district office)

With this design, we were not only able to adjust our project according to the recommendation based on the findings from the biodiversity monitoring, and their previous experience, but we were also able to identify and address the concerns of the responsible environmental body.

Table 3: Categorization of concerns voiced by the environmental body

Concern	Category
Negative impact on the neighboring nature conservation site	Locality
Negative impact on the neighboring floodplain (the primary habitat of Populus nigra L.)	Locality
Alterations in the division and function of the landscape (fragmenting open fields, that are important for several bird species)	Locality
Fragmentation of the landscape and negative impact on wild game migration as a cause of fence establishment	Management
Emergence of invasive species on the field (Ambrosia artemisiifolia L., Solidago canadensis L.)	Management
Possibility of unsuccessful recultivation, and re-emergence of hybrid poplar	Management
Expansion of non-indigenous hybrid poplar species	Species characteristics
Hybridization potential with indigenous Populus nigra L.	Species characteristics

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7 Risks, monitoring and evaluation

Main risk is the negative attitude towards planting non-indigenous species, and the competition of SRC with food production. However, this risk can be partially compensated by the recognition of the role of SRC in climate change mitigation, and soil erosion protection, as well as focusing the land search activities on marginal land.

Concrete examples of implementation of the concerns of environmental body and Daphne:

Recommendation: Preserving or creation of natural micro-biotopes like water ponds or shrubs or tree solitaires within the area of SRC locality increases biodiversity by expanding of natural shelters for species which also use biotope of SRC area.

Reaction: Small valuable land depressions can be left open to a certain extent. Already implemented in the case of projects in 2020. Approx. 0.2 ha of periodically waterlogged depression left for self-development after the agreement with local environmental body.

Shrubs and solitaire trees are maintained on the localities, with consideration to the migration tracks of wild game.

Recommendation: Reducing of frequency and depth of disking can preserve many amphibian, reptile, butterfly and dragonfly species. At least avoiding the disking in the period of reproduction and developing of amphibian species can increase their population. Alternate disking by mulching can be less destructive.

Reaction: The disking is timed, with consideration to the amphibians (late spring, early summer). Disking schedule is communicated with Daphne, and their remarks are implemented.

Case: Minimally two families of Northern lapwing (*Vanellus vanellus*) recorded on field near Modra village.

Recommendation: Postpone disking.

Reaction: Disking postponed by 2 weeks until the younglings will be capable of flight.

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Map 2: Identified nesting locality of Northern lapwing (*Vanellus vanellus*) on established SRC in Modra

Recommendation: Placing new SRC localities near natural biotopes like brooks, wetlands, grasslands

Reaction: The feasibility of this recommendation is limited by other parameters within the scope of land search (ownership structure, soil feasibility, etc.), however, there are existing examples of such established SRC.

Recommendation: Making mosaic of trees by the age in area of SRC locality can increase its biodiversity.

Reaction: Planting native species on field edges that will be omitted at harvesting and remain the entire duration of the project is under consideration, as this could also serve as risk mitigation of introgression.

Recommendation: Mulching/disking in appropriate time can eliminate spreading of invasive species.

Reaction: The rows in between the poplar trees are disked according to the occurring weed pressure.

Other activities that can have positive effect on biodiversity:

The headlands and the borders of the plantations can be sown with flower strips, therefore serving as insecticide-free pasture for pollinators.

Proper geodetic marking of the area, ensures that the parcels are used only to the valid legal extent.

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Figure 13: Continuous additional plowing of previous land user decreased the area of a valuable habitat on field edge (exceedance of real parcel borders marked with red)

8 Deviations and next steps

In the next two seasons the inventory mapping and expertise monitoring will continue on all SRC localities from previous seasons and also new localities will be added.

Data from all seasons will be used for evaluation of biodiversity in SRC localities and surrounding biotopes and also for analyses of differences among SRC localities like regression analyses of biodiversity, tree canopy density and soil types. Evaluated data will be a baseline for assessment of impact of SRC localities on biodiversity and this will lead to recommendations for preserving or increasing of their biodiversity value.

The request for the consent of environmental body will be continuously submitted prior to the establishment of the plantation, and the potential concerns will be addressed, and considered.

The research on the potential future impact of the established SRC on indigenous European black poplar (*Populus nigra* L.) populations will be continued, and the results will be submitted in D1.3 report in month 49.

By communicating the preliminary results on our webpage, as well as in our dissemination videos, we would like to raise attention on the possible environmental impact of SRC in the context of previous land use.

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9 Conclusion

This deliverable is addressing the concerns voiced by the environment body, and provides the preliminary data on the potential environmental impact of establishing SRC in Slovakia. The value of the gained data is emphasized by the intensively discussed topic of the effect of intensive agriculture on the decreasing biodiversity that is mainly caused by intensive insecticide and pesticide use.

According to these preliminary results after two monitoring seasons and their evaluation we can assess impact of SRC localities on biodiversity.

Positive Impact on biodiversity:

- SRC localities established on the place of arable fields become an important refugium for different species groups and increases the biodiversity value of the area.
- Micro-localities of tree rows, which are not disked, can offer suitable shelters for plant and animal species within the SRC locality.
- SRC localities with one or two years old trees are good for most of the animal species groups. For bird species the "memory of the site" is used.
- Disking disturbances can be suitable for psamophytic and one-year species. Some of them are rare – *Aphanes arvensis*, *Ranunculus arvensis*, *Spergula morisonii*, *Teesdalia nudicaulis*.
- If some micro-localities of natural biotopes like tree solitaire or water ponds are present in SRC locality, its biodiversity value is higher.

Negative Impact on biodiversity:

- SRC localities established on the place of grasslands, wetlands decreases the original biodiversity value.
- SRC localities of 4 years old trees with dense canopy are sterile almost for all groups except beetles from *Carabidae* and some bird species.
- Open soil areas, after disking, offer pre-conditions for spreading of invasive species
- Disking of the SRC areas destroys reproduction micro-localities of amphibian species and very often kills adult reptile and amphibian species. Disking very often destroys plants which are a source of feeding for many butterfly species.

When interpreting these findings in the light of previous land use, they are indicating a positive land use change. In most of the cases SRC was found as a more valuable habitat than arable land. The help of intensive communication and cooperation with Daphne can lead to protection of biodiversity to the maximum possible extent, whilst still maintaining the economic feasibility of the project.

The preliminary results of genetic *P. nigra* investigations allow the conclusion that there is currently no potential risk for impact by the D4EU plantations on the genepool of the European black poplar (*Populus nigra*) in West Slovakia, as the trees do not flower, and as they will be harvested before maturity (4- 6 years harvesting cycles).

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Genetic admixture with non-native poplar genomes from the SRC plantations can only occur if the SRC trees would become mature and would flower in the future. No evidence (for or against) has been found yet for the 2nd or higher SRC rotation cycles that such SRC trees can become mature much earlier than normally. An unexpected early maturation and flowering should not be totally excluded. But even in case of flowering, a final successful establishment would require successful seed development, successful germination in the wild, and seedling survival.

Even if all conditions would be fulfilled, the impact would likely not be important. First, literature provides an estimation of (max.) 10 % of introgression from *P.×canadensis* into black poplar rejuvenation (Benetka et al. 2002). Secondly, the present own results show that old native *P. nigra* and old (non-native) *P.×canadensis* stands have been growing neighbored for many decades in West Slovakia. However, the present-day Slovak poplar rejuvenation is still rather unaffected by introgression, as compared with the German reference rejuvenation sample. Hence, it can be assumed that the natural availability of *P. nigra* pollen in Slovakia is higher for flowering female *P. nigra* than in many parts of Germany. Likely, this is one reason why the barrier against pollination with non-native pollen is functioning well in West Slovakia. Further investigations will be done and presented in a separate deliverable D1.3 (M49).

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