Large-scale restoration of Estonian alvar grasslands: impact on biodiversity and ecosystem services

Final report of the Action D.1. Biodiversity monitoring for project LIFE to Alvars (LIFE13NAT/EE/000082)

University of Tartu

Institute of Ecology and Earth Sciences

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Status and trends of grasslands in Estonia

Aveliina Helm

Estonian semi-natural grasslands

Adverse land-use changes, resulting in loss and degradation of habitats and simplification of landscapes, are among the major threats to biodiversity (Newbold *et al.* 2015; Elbakidze *et al.* 2018), and have substantial negative impact on ecosystem functioning and provision of ecosystem services that are fundamental to human well-being (Cardinale *et al.* 2012). Ecological restoration is a vital tool to fight the ongoing biodiversity crisis, ensure sustainable provision of ecosystem services and mitigate climate change (IPBES 2018). It enables to improve condition of degraded ecosystems, re-create habitat for characteristic and threatened species, restore ecosystem functions and services, and re-establish functionally connected networks of habitats. Restoration is vital for meeting the goals set in the Convention on Biological Diversity Aichi targets 5 and 11 (Convention of Biological Diversity 2010) and in many national biodiversity strategies, including that of Estonia (Keskkonnaministeerium 2012).

European grasslands have a long history of biodiversity-friendly agricultural land-use (Pärtel *et al.* 2007a). They are among the most diverse ecosystems on Earth and a habitat for substantial proportion of species in Europe (Pärtel *et al.* 2005; Maccherini & Santi 2012; Dengler *et al.* 2014). Biota of European grasslands has an ancient origin in Europe, being linked with the open and semi-open habitats created by wild herbivores roaming in Pleistocene landscapes in Europe (Hejcman *et al.* 2013). After the Last Glacial Period, the development and persistence of many grassland habitats is dependent on management by humans (grazing, mowing, pollarding, removal of shrubs etc.), thus the term 'semi-natural communities' is the most common for characterizing such habitats. In Estonia, also term 'heritage ecosystems' is in use for emphasizing the importance of long (often thousands of years) and biodiversity-friendly impact our ancestors have had on such habitats. Semi-natural grasslands are dependent on moderate management (grazing and mowing) and their long-term persistence is highly threatened by changes in land-use (Pärtel *et al.* 2005). Intensification of agriculture that has resulted in conversion of grassland into arable land in more fertile regions, while less fertile areas were abandoned, leading to subsequent overgrowing with shrubs and trees, or afforested (Pärtel *et al.* 1998; Bakker & Berendse 1999; Helm *et al.* 2006; Krauss *et al.* 2010; Dengler *et al.* 2014).

In Estonia, semi-natural grassland habitats were historically widespread, covering ca 1.8 million hectares in 1930s (1/3 of country's area) (Kukk & Kull 1997). Since 1950s, intensification of agriculture and abandonment of traditional land-use practices has resulted in severe loss of area of historical grasslands. By 2019, the area of grasslands has declined by 93%, with ca 127 000 hectares remained and less than 35 000 ha suitably managed by grazing or mowing (Estonian Environmental Registry database for semi-natural habitats) **(Figure 1)**.

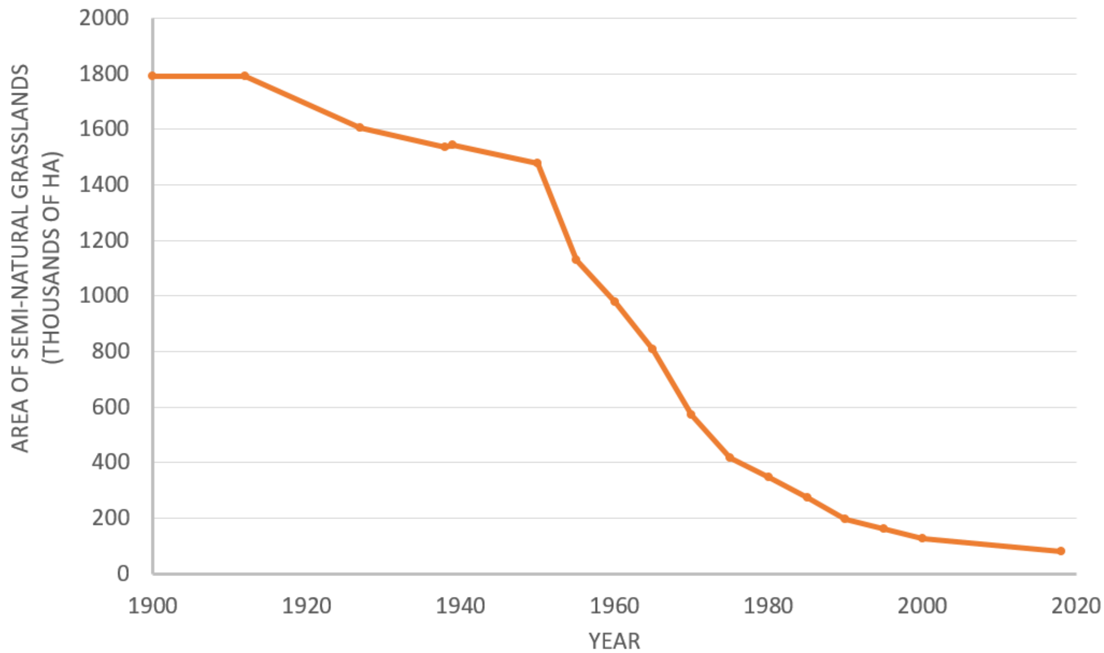

Figure 1. *Decline of area of semi-natural grassland habitats in Estonia since 1900. Data from Kukk & Kull 1997 and Estonian Environmental Registry database for semi-natural habitats.*

Alvar grasslands

Alvars are shallow-soiled (usually less than 20 cm) calcareous grassland communities on Ordovician or Silurian limestone bedrock (Rosén & Sjögren 1988; Pärtel *et al.* 1999). Alvars have limited distribution in the world − these grasslands grasslands occur exclusively in Sweden and Estonia (Sweden mainland, islands of Öland, Gotland, the Estonian islands of Saaremaa, Hiiumaa, Muhu, coastal parts of the Estonian mainland (Rosén 1982; Pärtel et al. 1999) **(Figure 2)**. One third of alvar grasslands in Europe occur in Estonia. Due to their high conservation value they belong to priority habitat type in European Union's Habitats Directive (6280* *Nordic alvar and precambrian calcareous flatrocks*). Most of Estonian alvars are among semi-natural communities and they have developed over past centuries and millennia as a result of moderate grazing. With the cessation of grazing, alvars overgrow with shrubs and trees, resulting in considerable declines in biodiversity (Pärtel *et al.* 1999). During the past century, cessation of grazing has led to extensive loss of alvar grassland area due to consequent overgrowing with shrubs and trees. Area of alvars in Estonia decreased from ca 50 000 ha in the 1930s to only 2 500 hectares of suitably managed grasslands in 2014. In 2019, there is in total ~17 199 ha of alvar grasslands remained in Estonia, out of which 6 100 ha has habitat condition estimate high or average. In total, 5 500 ha of alvar grasslands are grazed in 2019 - a two-fold increase from 2014 **(Figure 3, 4, 5)**.

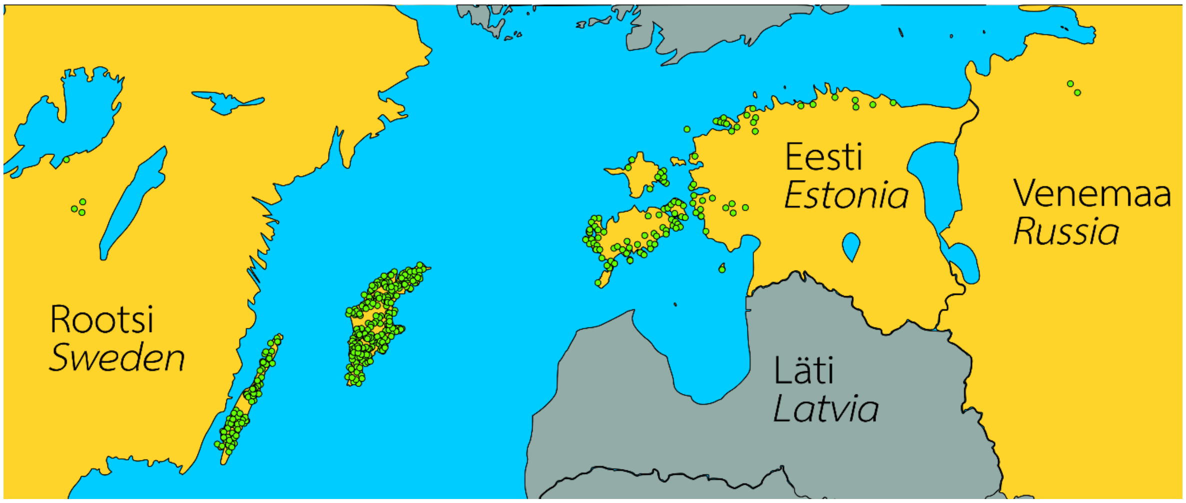

Figure 2. *Distribution of alvar grasslands in Europe. One third of all alvars in Europe are situated in Estonia.*

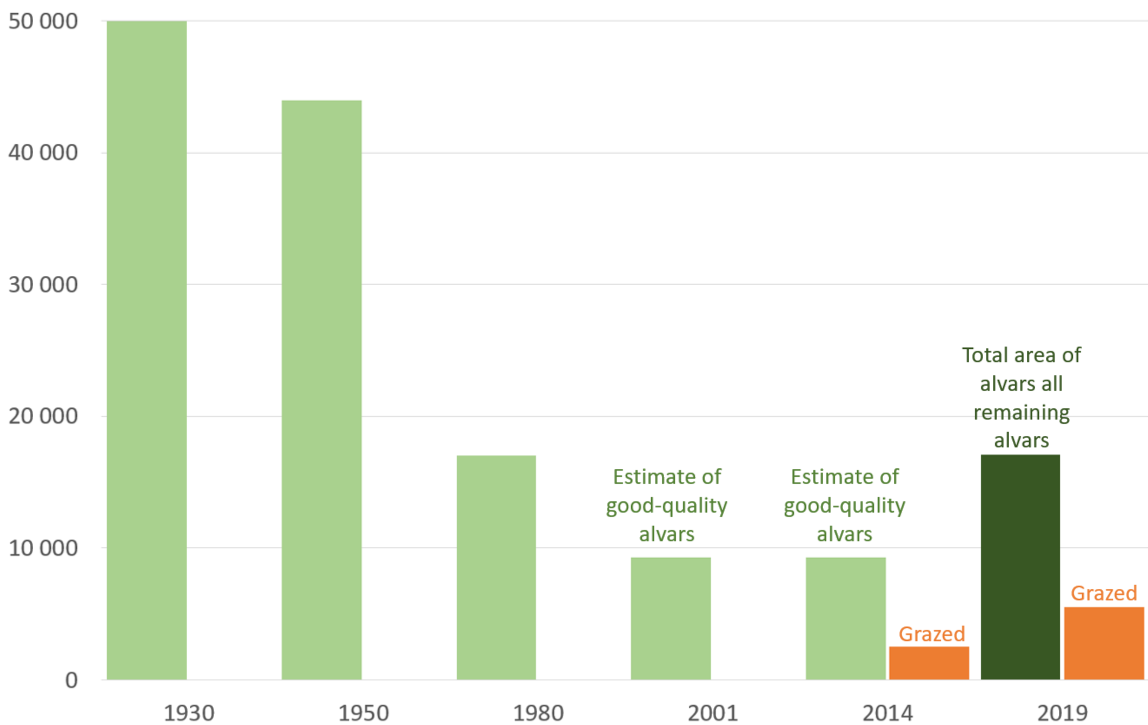

Figure 3. *Dynamics of area of alvar grasslands in Estonia since 1900 according to different sources (Laasimer 1965; Kukk & Sammul 2006; Helm 2009). For 2001 and 2014, estimate of good-quality alvar grassland (with habitat condition estimate high or average) area is provided. For 2019, total area of all remaining alvar grasslands is given according to combined information from Estonian Environmental Registry database for semi-natural habitats and inventory of Estonian Seminatural Community Conservation Association.*

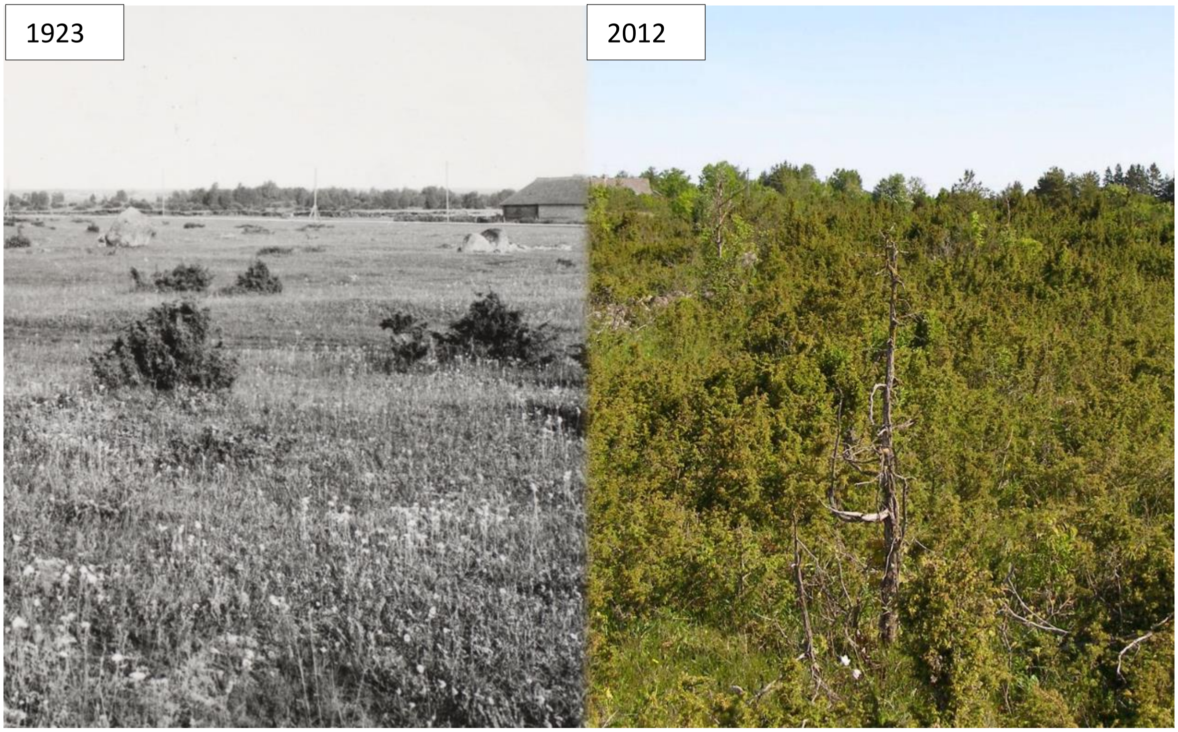

Figure 4. *Alvar grassland in Haavakannu, Northern Estonia in 1923 (left) and in 2012 (right). Cessation of traditional management (grazing) leads to shrub (mostly Juniperus communis) encroachment. Photo on the left: ERM: Fk 1523:2499 Haavakannu lood. Kuusalu khk. G. Vilbaste, 1922. Photo on the right: Aveliina Helm.*

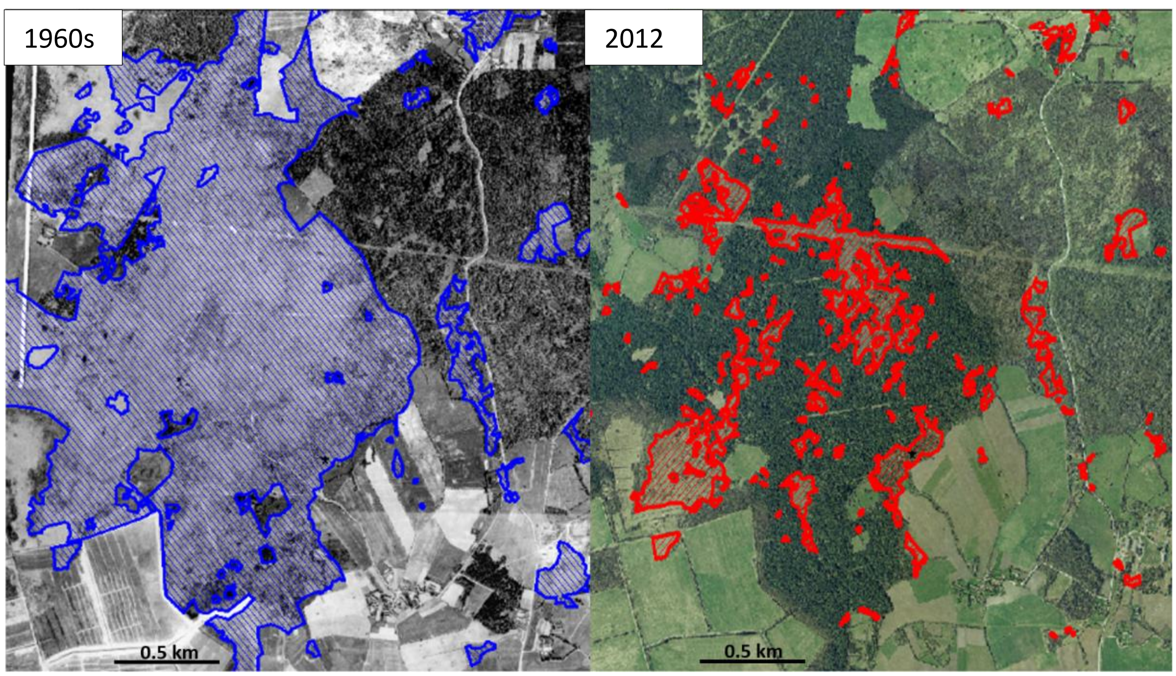

Figure 5. *Example of alvar overgrowing with shrubs and resulting decline of open grassland areas. Open area (shown in blue) in 1960s has declined to small isolated fragments of open habitat patches by 2012 (shown in red). Alvar grassland in Väike-Rahula, Saaremaa. Photo: Estonian Land Board.*

Biodiversity of alvar grasslands

Alvar grasslands are characterized by low herbaceous vegetation and sparsely distributed juniper (*Juniperus communis*) shrubs. In favorable condition, alvar grasslands have shrub cover of ca 30-40%, in some subtypes the shrub layer can be completely missing. Traditionally managed open alvar grasslands are characterized by very high biodiversity (van der Maarel 1988; Sjögren 1988; Pärtel et al. 1996). For vascular plants, maximum numbers of species have reached to 21 species per 10x10 cm plot, 49 species per 1x1 m plot and up to 160 species in community level (Pärtel et al. 1999). Altogether 268 Estonian plant species have been shown to be characteristic to alvars (Pärtel *et al.* 2007b). Alvars are also extremely rich in butterflies and other invertebrates. In Estonia, 68 species of butterflies have detected from alvars and in average one good-quality grassland site has shown to harbour ~30 butterfly species (Sang et al. 2010). Declining area and increasing isolation of habitat patches had put number of habitat specialist species under severe threat, as extinction debt of characteristic plant and butterfly species had been detected in those grasslands (Helm *et al.* 2006; Krauss *et al.* 2010; Sang *et al.* 2010). Helm et al. (2006) demonstrated that alvar grasslands on western Estonian islands have lost on average 70% of their original area between 1950s and 2000 and without restoration will face the loss of 40% of their current plant species number by the time extinction debt is paid (Helm et al. 2006).

Typical alvar grassland plant species include *Acinos arvensis, Anemone sylvestris, Artemisia rupestris, Asperula tinctoria, Astragalus danicus* (mostly in Muhu island and eastern part of Saaremaa), *Campanula rotundifolia, Carex ornithopoda, Galium verum, Helianthemum nummularium, Helictotrichon pratense, Linum catharticum, Potentilla tabernaemontani, Thymus serpyllum, Veronica spicata etc*. Alvars with very shallow soil and with limestone openings (*Festucetum* habitat type sensu Pärtel et al. 1999) are often dominated by *Festuca ovina, Sedum album, Sedum acre* and *Allium schoenoprasum*. Temporarily wet alvars from *Molinietum* habitat type are more productive and dominated by *Molinia caerulea, Sesleria caerulea* and can harbor variety of orchids. Most widespread alvar habitat type in Estonia is *Avenetum*-type, characterized by soil deeper than 5 cm but still less than 20 cm. This type is most species rich, harboring most of aforementioned species, but at the same time this type is also most susceptible to overgrowing with shrubs, due to relatively deeper soil and more mesic conditions compared to other types **(Figure 6)**.

Many of typical species on alvars have their main distribution in the south- eastern European steppe region (e.g. *Anemone sylvestris, Artemisia rupestris, Asperula tinctoria* and *Astragalus danicus*). Alvars in Northern Estonia harbor some very rare species from arctic-alpine group, including *Cerastium alpinum, Poa alpina* and (more widespread) *Potentilla crantzii*. Threatened and protected plant species on alvar grasslands also include at least 15 orchid species (*Ophrys insectifera, Orchis militaris, Orchis mascula, Orchis morio, Orchis ustulata, Gymnadenia conopsea, Herminium monorchis, Listera ovata, Coeloglossum viride, Cypripedium calceolus, Dactylorhiza incarnata, Dactylorhiza sambucina, Epipactis atrorubens, Platanthera bifolia, Platanthera chlorantha*) and number of other species protected in Estonia such as *Anthyllis coccinea, Asplenium ruta-muraria, Asplenium trichomanes, Cardamine hirsuta, Cerastium alpinum, Cotoneaster niger, Draba muralis, Geranium lucidum, Hornungia petraea, Malus sylvestris, Onobrychis arenaria, Oxytropis pilosa, Potentilla fruticosa, Pulsatilla pratensis, Saxifraga adscendens, Scabiosa columbaria, Tetragonolobus maritimus, Vincetoxicum hirundinaria.*

Alvar grasslands also harbor EU Habitats Directive Annex II species, for example *Cypripedium calceolus, Sisymbrium supinum*, *Thesium ebracteatum*, *Pulsatilla patens, Tortella rigens, Encalypta* *mutica.* The Bern Convention Appendix I includes following plant species occurring on alvar grasslands: *Botrychium multifidum, Botrychium matricariifolium, Sisymbrium supinum.* Species listed in Annex I of the EU Birds Directive depending on alvar grasslands either for nesting or feeding include *Sylvia nisoria* (red-backed shrike), *Lanius collurio* (barred warbler) and *Pluvialis apricaria* (Eurasian golden plover).

Our detailed survey on alvar grasslands before restoration activities also revealed that alvars are excellent habitats for many species groups whose presence on alvars were previously unknown (summarized in Helm 2017). For example, alvar grasslands harbor exceptionally high diversity of mycorrhizal fungi, altogether we detected 146 virtual taxa of arbuscular mycorrhizal (AM) fungi which is 41% of the total richness of AM fungi in the world. Alvar grasslands are also home for 154 ground-dwelling spider species, including 6 species that have not previously detected in Estonia (Meriste 2017).

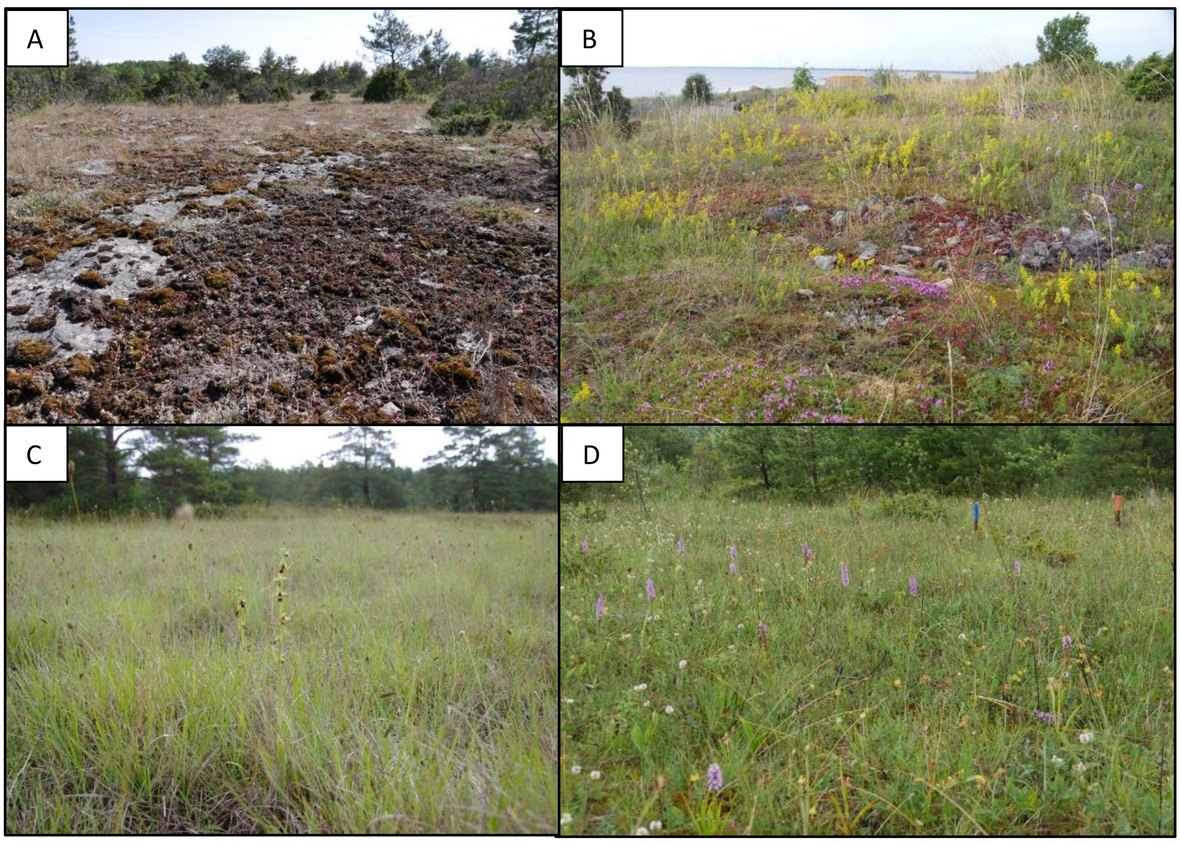

Figure 6. *Examples of different types of alvar grasslands. A) Festucetum-type alvar with very shallow soil and limestone outcrop openings in Atla-Eeriksaare region. B) Festucetum-type alvar in Lõu (Saaremaa, Sõrve). C) Molinietum-type temporarily wet alvar with Sesleria caerulea and Ophrys insectifera in Aruküla (Hiiumaa). D) Avenetum-type alvar grassland in Hanila. Photos: Aveliina Helm*

LIFE to Alvars - restoration of Estonian alvar grasslands

Large-scale calcareous grassland (alvar) restoration project "Life to Alvars" (LIFE13 NAT/EE/000082) was carried out in Estonia between 2014-2019 with the aim to restore 2500 ha valuable alvar grasslands. With the project, the area of managed sites for these valuable grasslands were doubled. Project activities included clearing of alvar grasslands from excessive shrubs and trees, re-introduction of traditional grazing and raising the awareness of alvar grassland values among local people. Ecological necessity of the restoration was urgent due to rapid overgrowing of remaining habitat areas and the threat of characteristic species extinctions.

Selection of project sites for LIFE to Alvars project

Site selection in this project was based on landscape approach, as advocated in many recent publications (Helm 2015, Prach *et al.* 2015; Aavik & Helm 2017). Selection was based on expert group discussions and on prior knowledge on historical distribution of alvar grasslands in Estonia. Project aimed to restore well-connected and large grassland areas in regions where alvar grasslands had been historically abundant, but where current high-quality areas were still present in the landscape. As found in number of studies (see overview in Aavik & Helm 2017), dispersal of species is relatively limited in space and in order to achieve rapid recovery of restored sites, it was made sure that every restored site was a) situated in the historical grassland area and in a region where alvar grasslands were abundant and b) good quality patches had still remained in the immediate surroundings or within restored areas. **Figure 7** demonstrates the rationale behind selection of restoration sites, where site 1 with larger historical grassland area and higher amount of remained habitat was included to the restoration, whereas site 2 with less historical area and very little remaining area was excluded.

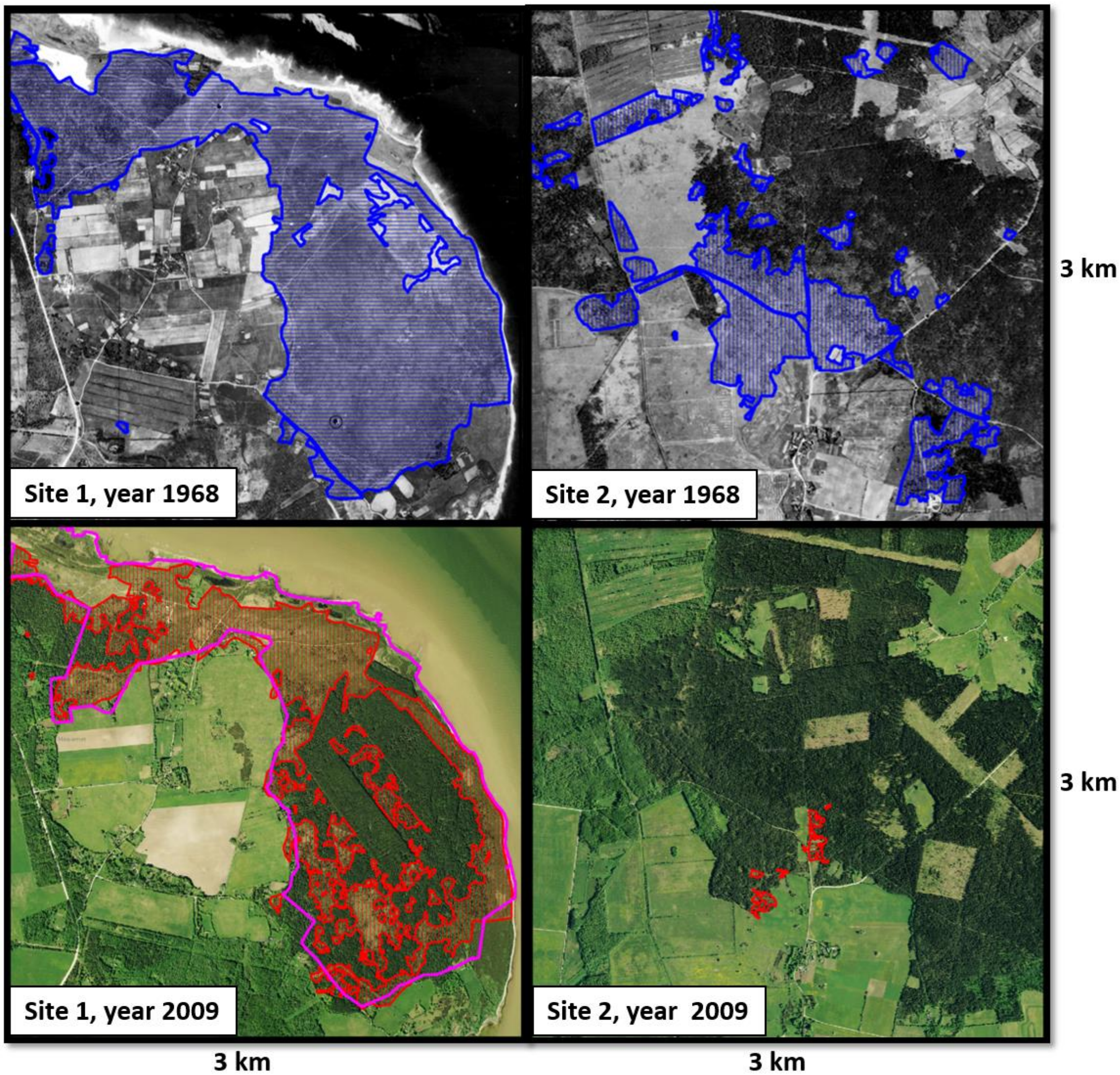

Figure 7. *Selection of project sites. Site 1 (left panel) has still relatively large amount of grassland fragments left nowadays (lower picture, grasslands are marked with red striping although most are overgrown with lower shrubs) compared to year 1968 (upper picture, grassland borders blue), making target species colonisation to restored areas likely. Site 2 (right panel) has only small grassland fragments left in the landscape, making the successful recovery of historical grassland more time-consuming and restoration more laborious. This site was not included to the project LIFE to Alvars. All pictures depict landscape of 3x3 km. Figure originates from Helm 2015.*

Biodiversity monitoring of LIFE to Alvars project

Study design and sampling methods

Biodiversity monitoring and assessment of restoration success was carried out according to the proposed and approved methods in the literature (de Bello *et al.* 2010).

To warrant the data quality for statistical analysis, monitoring was carried out on 35 grassland study regions, including both LIFE to Alvars restoration areas (covering all 25 project regions) as well as reference areas that were not restored **(Figure 8)**.

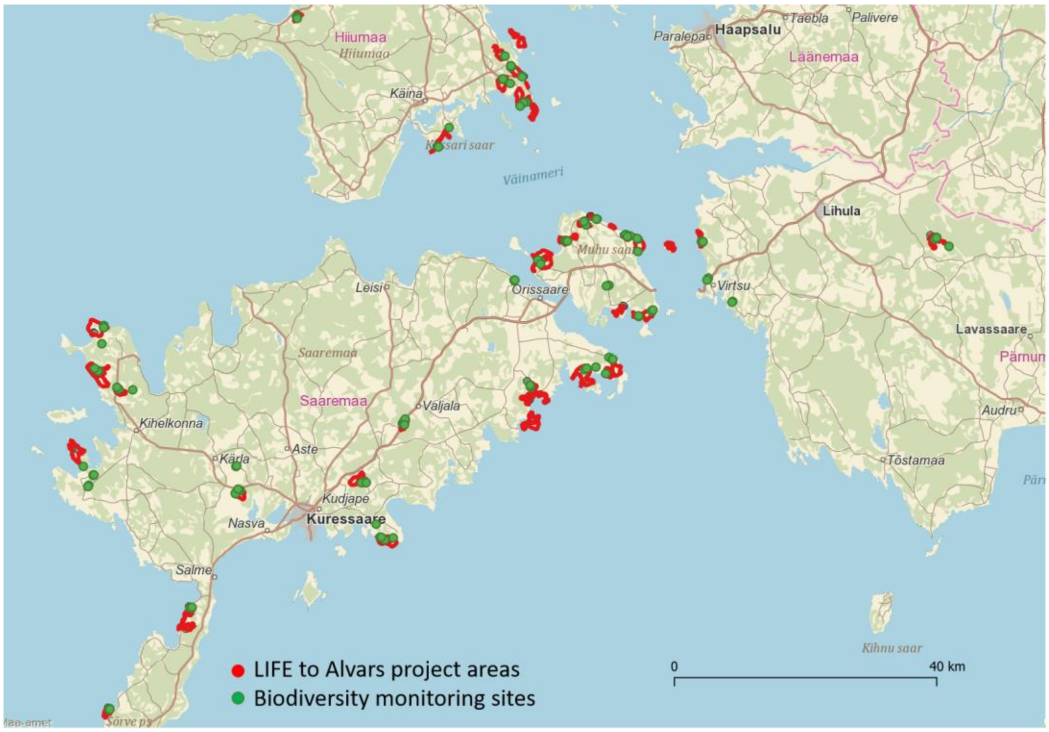

Figure 8. *Locations of 35 monitoring regions with altogether 138 monitoring sites. With red, LIFE to Alvars project areas are depicted, green dots indicate biodiversity monitoring sites. Sites that are located outside LIFE to Alvars project areas represent reference regions where no restoration occurs and that will allow to compare biodiversity dynamics between restored and unrestored regions in follow-up monitoring.*

On each study region, we had 4 study sites where thorough investigation was carried out, representing different different successional stages of alvar grasslands: a) 'open alvar' – good-quality alvar grasslands characterised by short herb layer and moderate (up to 30-40%) shrub cover with mostly juniper (*Juniperus communis*); b) 'overgrown alvar' - previously open alvar grasslands that have been overgrown with dense (<60% cover) juniper shrubs (*Juniperus communis*); and c) 'afforested alvar' - previously open alvar grasslands that have been afforested with Scots pine (*Pinus sylvestris*) in 1970-80s **(Figure 9)**. On each studied region, we also had a d) control site, representing good-quality alvar near to other study sites located in the same region, but outside restoration area **(Figure 9)**. Control sites were established to represent the restoration target and observe possible changes that are not related to restoration. Most of the control sites were not managed, thus it is expected that in future, their species richness starts to decline. On each observation site, plots with permanent marking were established, allowing to make direct before-after restoration comparisons and enabling to return for follow-up monitoring events. Altogether 138 sites were thoroughly surveyed.

To estimate the effect of restoration on biodiversity, number of indicators were described prior and after the restoration activities: biotic indicators, management and disturbance indicators and environmental indicators. Assessed biotic indicators included diversity vascular plants and butterflies, management and disturbance indicators included the information about management (grazing, other potential disturbance impacts) and environmental indicators included data about soil nutrient content (organic matter, soil phosphorus content), soil pH, soil depth and soil moisture and light. On each site, shrub and tree cover, height and other relevant information related to habitat condition was recorded (see the survey sheet "LOCATION" in the Appendix).

In addition, monitoring of vascular plants occurred on 2 sites (Neeme and Kurese) where seed-sowing occurred (see MTÜ Elurikas Eesti 2018) and paragraph **Monitoring of seed sowing**.

During the project period, fieldworks were carried out prior to the restoration (during 2014-2015) and following the restoration (2018-2019). To ensure adequate temporal replication, University of Tartu will also make efforts to pursue collection of information on long-term impacts of restoration also after the project period and will sample the restoration also in coming years. Re-surveys will be carried out by research groups in University of Tartu. The same set of people are preferred to work during all re-surveys, however, all additional participants will receive training prior to the fieldworks, minimizing observer bias.

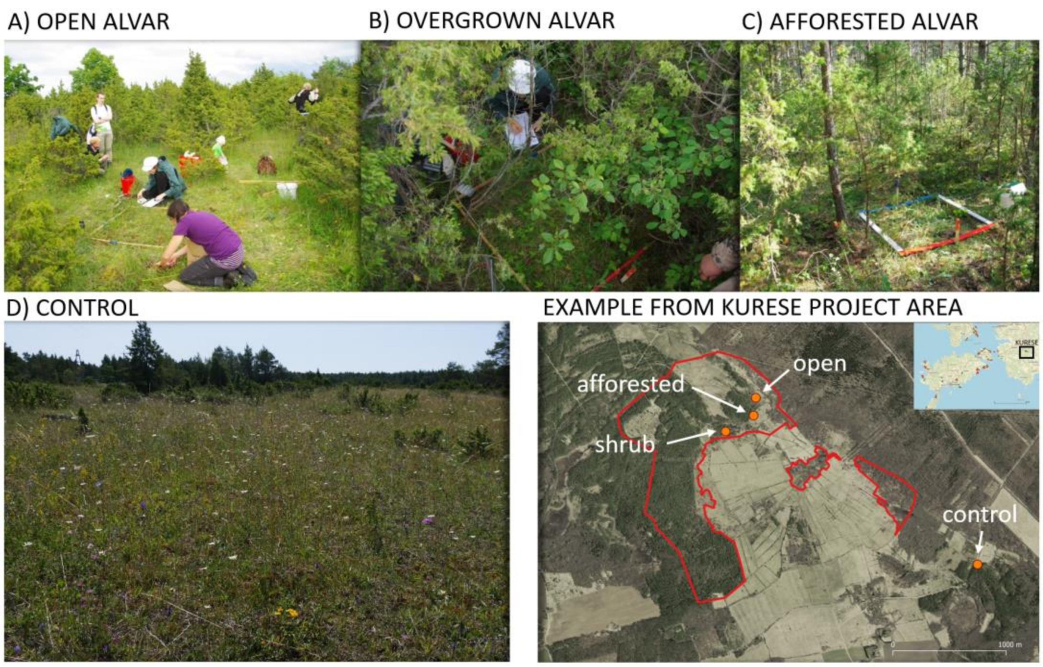

Figure 9. *Successional stages of monitoring sites before restoration: a) open alvar, b) overgrown alvar, c) afforested alvar that were subject to restoration and c) control site, representing good-quality alvar grassland that is not restored and is located outside restoration area. On the bottom-right figure, the example of how monitoring sites were located in restoration areas. Red lines depict borders of LIFE to Alvars project area 'Kurese', whereas dots indicate monitoring sites.*

Impact of alvar grassland restoration on vascular plant diversity

Vascular plant monitoring methods

Permanent plots were established on each monitoring site, where vascular plant species lists for standard botanical quadrat plot (1x1 meter) and surrounding area of 10-meter radius was compiled. On each site, species richness and abundance estimates (percentage cover) of all occurring vascular were described prior and after restoration on 138 sites belonging to different successional stages (see the description above). Before restoration, sites were visited in 2014 and 2015 in July. After restoration, all sites were visited in July 2019.

Vascular plant monitoring results

Prior restoration, in 2014-2015, altogether 281 herbaceous and 35 woody vascular plants were recorded from study sites. The highest species richness was detected on control sites (i.e. good-quality sites that were not a subject for restoration): in average 63 (±10.9 SD; minimum 44, maximum 79) species for 10-meter radius observation plot. In open grasslands, average species richness was 48 (±11.5; min 27, max 72) species, in overgrown areas 50 (±9.9; min 28, max 68) species and in afforested areas in average 39 (±15.3; min 7, max 64) species **(Figure 10)**. Average species richness per 1x1 m plots was 25 (±6.2; min 9, max 38) species for control sites, 23 (±6.6; min 12, max 40) species for open grasslands, 18 (±7.3; min 5, max 35) for overgrown sites and 11 (±7.3; min 1, max 26) for afforested grasslands. Results show that in larger scale (10-meter radius), shrub-covered overgrown areas are still relatively species rich as the monitoring plot covered quite large and heterogeneous area with some open patches remained between shrubs. However, in smaller scale, the vascular plant diversity is already declining and in afforested areas it is already very small.

After restoration, in 2019, altogether 299 herbaceous and 35 woody vascular plants were recorded from study sites. High species richness was again detected on control sites (i.e. good-quality sites that were not a subject for restoration): in average we found 61 (±10.3; min 38, max 77) species for 10-meter radius observation plot with no significant changes from 2014-2015 (paired t-test, $P>0.05$). In open grasslands, average species richness had increased from 48 species to 54 (±9.6; min 35, max 78) species. Species richness of overgrown areas had significantly increased from 50 species before restoration to 61 (±14.0; min 29, max 94) species (paired t-test, $P<0.05$). In afforested areas, the highest increase in species richness had occurred, as average values of 39 species per observation plot before restoration had increased to 68 (±14.5; min 22, max 86) species **(Figure 10)**. Average species richness per 1x1 m plots was 23 species for open alvars, 24 for previously overgrown sites, 20 for previously afforested grasslands and 25 species for control sites. Analysis show that both on larger scale (10-meter radius plot) and in smaller scale (1x1 meter plot), species richness had significantly increased in previously overgrown and afforested areas as a result of restoration.

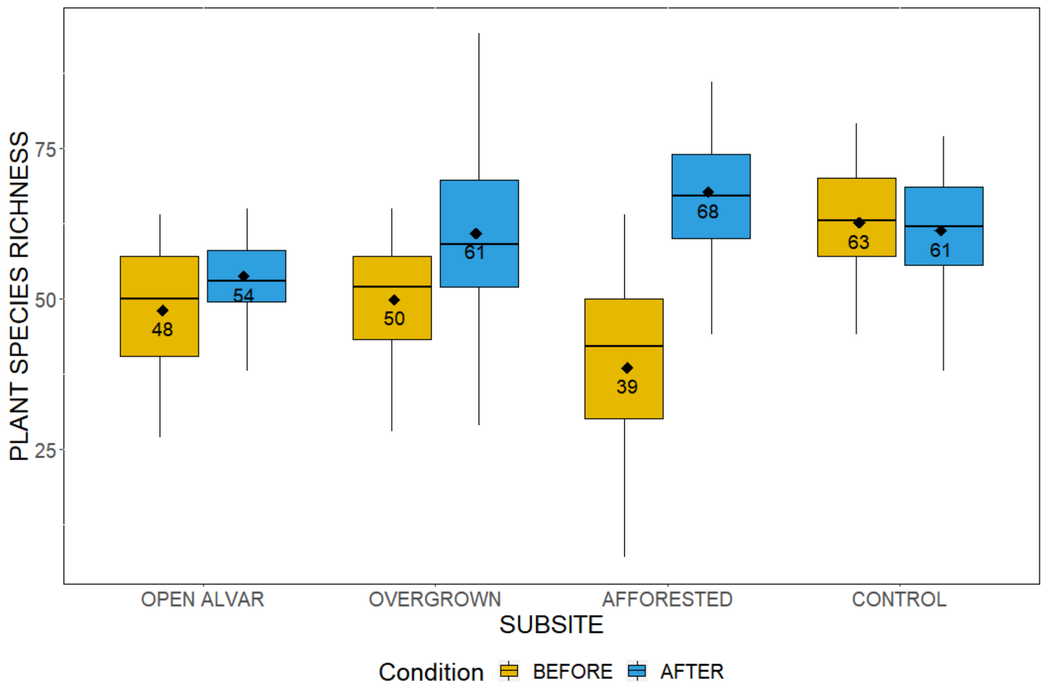

Figure 10. *Vascular plant richness per 10-meter radius observation plot in study sites covering different successional stages of alvar grasslands (open alvar, overgrown, afforested, control) before and after restoration. Control represents good-quality grasslands that are not a subject of restoration, but are not also currently managed, thus in a threat of subsequent decline of species richness. Diamond and number inside the box depict mean values, band inside the box indicates median, box indicates quartiles, whiskers minimum and maximum values.*

Most common herbaceous plant species over all habitat types prior restoration were *Filipendula vulgaris, Galium boreale, Helictotrichon pratense, Pimpinella saxifraga, Briza media, Asperula tinctoria* and *Centaurea jacea*. Number of species characteristic to open grasslands had increased their occurrence in the monitored sites following restoration. Following restoration, all abovementioned common species had increased their overall occurrence. However, species that most notably increased their overall occurrence after restoration were *Galium verum, Campanula rotundifolia, Festuca ovina, Achillea millefolium, Poa angustifolia, Poa compressa, Campanula persicifolia, Arenaria serpyllifolia, Acinos arvensis, Fragaria viridis, Cirsium vulgare, Agrostis vinealis, Arabis hirsuta, Cirsium arvense.* All of the species were found in more than 20 additional sites compared to their occurrence before restoration.

In **Table 1**, 50 most common herbaceous species for each successional stage (open, overgrown, afforested) after restoration are presented, together with the estimate of their overall occurrence (percentage of sites where they were found out of all monitored sites before and after restoration). Most notably, in previously afforested sites, *Arenaria serpyllifolia, Linum catharticum, Cerastium fontanum, Campanula rotundifolia, Galium verum, Hypericum perforatum* and *Leucanthemum vulgare* had markedly increased their occurrence. In addition to those characteristic alvar grassland species, also some ruderal species and species benefitting from more disturbed conditions had increased their occurrence, especially in previously afforested sites, for example *Cirsium arvense, Cirsium vulgare* and *Geum urbanum.* As in most sites only 1-3 years has passed since restoration activities, it is expected that these species will decrease in abundance in next years.

Surprisingly, species richness of characteristic alvar grassland plant species increased very quickly following restoration in previously overgrown and afforested areas **(Figure 11)**, indicating that many characteristic grassland species had recolonized the sites already 1-3 years after restoration (sampling was carried out in 2019, most of the areas were restored between 2016-2018). This can be due to two processes that can also have simultaneous impact: dispersal and seed bank. Dispersal from nearby good-quality grasslands is very likely as restoration sites were selected so that they would be in the vicinity of good-quality grasslands (see above **Selection of project sites for LIFE to Alvars project**). Following restoration, large grazing areas were formed, resulting in high likelihood of seed dispersal by grazing animals between restored and good-quality habitat. However, also seed bank may play important role. It has been shown in alvar grasslands in Estonia that overgrown areas have large and species-rich persistent soil seed banks consisting typical grassland species even 50 years following abandonment (Kalamees *et al.* 2012). Typical dry grassland species that have been found to form persistent and long-term seed banks in Estonian alvar grasslands include the same species we detected strong increases: *Acinos arvensis, Arenaria serpyllifolia, Arabis hirsuta, Campanula persicifolia, Campanula rotundifolia, Cerastium fontanum, Galium verum, Helictotrichon pratense, Hypericum perforatum etc.* (Kalamees *et al.* 2012). It is likely that the soil seed bank has persisted even in afforested sites, although they have been abandoned more than 60 years. Use of heavy machinery during restoration seem to have "kicked off" the germination of seeds from the seed bank. Both in overgrown and in afforested sites, there was dense layer of juniper and pine needles, hindering the ability of seeds to germinate. During restoration, the dense layer of litter was disturbed and mixed with underlying soil, likely resulting in better germination conditions for seeds.

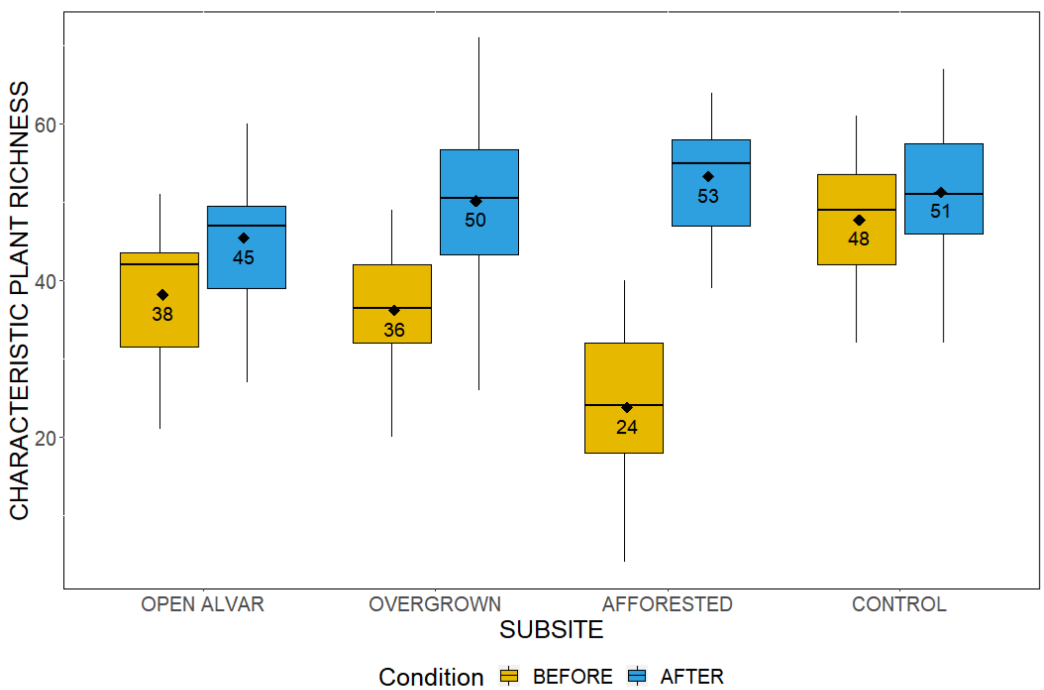

Figure 11. *Richness of characteristic plant species per 10-meter radius observation plot in study sites covering different successional stages of alvar grasslands (open alvar, overgrown, afforested, control) before and after restoration. Diamond and number inside the box depict mean values, band inside the box indicates median, box indicates quartiles, whiskers minimum and maximum values.*

Table 1. *Most common herbaceous plant species after restoration in successional type and their comparable occurrence before restoration. Species are ranked according to the percentage of their overall occurrence in particular type after restoration i.e. percentage of sites where they were found out of all monitored sites in given type (open, overgrown, afforested).*

		OPEN ALVAR	OPEN ALVAR			OVERGROWN	OVERGROWN			AFFORESTED	AFFORESTED
	Species	BEFORE	AFTER		**Species**	BEFORE	AFTER		**Species**	BEFORE	AFTER
		%	%			%	%			%	%
1	*Galium verum*	86	100		*Asperula tinctoria*	78	92		*Fragaria vesca*	89	97
2	*Filipendula vulgaris*	94	97		*Fragaria vesca*	78	92		*Galium boreale*	82	97
3	*Achillea millefolium*	83	97		*Galium boreale*	81	92		*Filipendula vulgaris*	82	94
4	*Festuca ovina*	86	92		*Galium verum*	81	92		*Festuca ovina*	61	88
5	*Sesleria caerulea*	75	92		*Briza media*	81	89		*Festuca rubra*	55	88
6	*Anthyllis vulneraria*	89	89		*Filipendula vulgaris*	92	89		*Veronica officinalis*	53	82
7	*Centaurea jacea*	89	89		*Achillea millefolium*	75	86		*Galium verum*	34	79
8	*Galium boreale*	81	89		*Cirsium acaule*	64	86		*Plantago lanceolata*	45	79
9	*Helictotrichon pratense*	92	86		*Pimpinella saxifraga*	75	86		*Asperula tinctoria*	50	76
10	*Pimpinella saxifraga*	86	86		*Centaurea jacea*	75	84		*Campanula rotundifolia*	42	76
11	*Linum catharticum*	89	83		*Helictotrichon pratense*	86	84		*Hypericum perforatum*	34	74
12	*Briza media*	83	83		*Sesleria caerulea*	72	84		*Leucanthemum vulgare*	34	74
13	*Thymus serpyllum*	89	81		*Festuca ovina*	67	81		*Rubus caesius*	42	74
14	*Asperula tinctoria*	86	78		*Anthyllis vulneraria*	42	76		*Carex flacca*	58	71
15	*Leucanthemum vulgare*	72	78		*Festuca rubra*	83	76		*Prunella vulgaris*	47	71
16	*Antennaria dioica*	81	72		*Leucanthemum vulgare*	64	76		*Centaurea jacea*	39	68
17	*Pilosella officinarum*	78	72		*Medicago lupulina*	69	76		*Cerastium fontanum*	8	68
18	*Hypericum perforatum*	58	69		*Campanula rotundifolia*	50	70		*Medicago lupulina*	34	68
19	*Campanula rotundifolia*	50	69		*Hypericum perforatum*	72	70		*Achillea millefolium*	29	65
20	*Inula salicina*	72	67		*Plantago media*	56	70		*Cirsium vulgare*	8	65
21	*Solidago virgaurea*	72	67		*Thymus serpyllum*	56	70		*Briza media*	45	62
22	*Plantago lanceolata*	61	67		*Plantago lanceolata*	64	68		*Cirsium acaule*	47	62
23	*Carex flacca*	78	64		*Poa angustifolia*	44	68		*Dactylis glomerata*	34	62
24	*Plantago media*	64	64		*Campanula persicifolia*	36	65		*Pimpinella saxifraga*	58	62
25	*Cirsium acaule*	61	61		*Carex flacca*	56	65		*Sesleria caerulea*	47	62
26	*Lotus corniculatus*	58	61		*Solidago virgaurea*	72	65		*Helictotrichon pratense*	45	59
27	*Medicago lupulina*	61	58		*Antennaria dioica*	47	62		*Plantago media*	32	59
28	*Poa compressa*	28	58		*Dactylis glomerata*	64	62		*Primula veris*	50	59
29	*Festuca rubra*	58	56		*Arabis hirsuta*	42	59		*Convallaria majalis*	63	56
30	*Arabis hirsuta*	31	56		*Inula salicina*	53	59		*Anthyllis vulneraria*	21	53
31	*Carex caryophyllea*	67	53		*Pilosella officinarum*	44	59		*Poa angustifolia*	24	53
32	*Trifolium montanum*	67	53		*Polygala amarella*	47	59		*Ranunculus polyanthemo*	50	53
33	*Acinos arvensis*	25	53		*Prunella vulgaris*	36	59		*Solidago virgaurea*	55	53
34	*Potentilla tabernaemontani*	67	50		*Veronica officinalis*	42	59		*Galium album*	18	50
35	*Carlina vulgaris*	50	47		*Linum catharticum*	67	57		*Lotus corniculatus*	21	50
36	*Primula veris*	44	47		*Primula veris*	47	57		*Pilosella officinarum*	29	50
37	*Agrostis stolonifera*	39	47		*Trifolium montanum*	44	57		*Trifolium pratense*	29	50
38	*Cerastium fontanum*	39	47		*Trifolium pratense*	11	57		*Campanula persicifolia*	18	47
39	*Fragaria vesca*	36	47		*Arenaria serpyllifolia*	11	54		*Carex caryophyllea*	3	47
40	*Trifolium pratense*	36	47		*Carex caryophyllea*	44	54		*Carex ornithopoda*	29	47
41	*Arenaria serpyllifolia*	25	47		*Carlina vulgaris*	31	54		*Cirsium arvense*	0	47
42	*Veronica spicata*	42	44		*Convallaria majalis*	47	51		*Geum urbanum*	3	47
43	*Dactylis glomerata*	36	44		*Rubus caesius*	42	51		*Linum catharticum*	18	47
44	*Convallaria majalis*	33	44		*Senecio jacobaea*	42	51		*Poa compressa*	11	47
45	*Senecio jacobaea*	33	44		*Cerastium fontanum*	33	46		*Senecio jacobaea*	11	47
46	*Prunella vulgaris*	47	42		*Galium album*	39	46		*Trifolium montanum*	21	47
47	*Sedum acre*	39	42		*Lotus corniculatus*	31	43		*Agrimonia eupatoria*	29	44
48	*Poa angustifolia*	19	42		*Poa compressa*	22	43		*Alchemilla vulgaris*	13	44
49	*Agrostis vinealis*	8	42		*Vicia cracca*	28	43		*Arenaria serpyllifolia*	0	44
50	*Artemisia campestris*	33	36		*Viola rupestris*	44	43		*Ranunculus acris*	37	44

Protected and/or red-listed species found from study sites prior restoration included following species: *Anacamptis pyramidalis, Asplenium trichomanes, Botrychium lunaria, Cypripedium calceolus, Dactylorhiza baltica, Dactylorhiza fuchsii, Dactylorhiza incarnata, Epipactis atrorubens, Epipactis helleborine, Gymnadenia conopsea, Ophrys insectifera, Scabiosa columbaria.* Following restoration, several protected species had decreased in overall occurrence, most notably *Listera ovata* and *Ophrys insectifera* whose occurrence in observed sites had declined 23% and 13%, respectively. However, as they had also declined in control sites without any restoration impact, the reason for their decline might also be extremely dry summer of 2018 that had visible impacts on vegetation in sampling year 2019.

Impact of alvar grassland restoration on butterfly diversity

Anu Tiitsaar

Butterfly monitoring methods

Butterfly diversity and abundance was measured before and after restoration using standardised transect counts. Each site was visited three times over the season to cover phenological aspects of different butterfly species. Before restoration, sites were visited over two years 2015 and 2016 (3-14 June in 2015, 15-26 July and 5-9 August in 2016) and after restoration all sites were visited three times over the summer 2019 (4-9 June, 4-12 July and 2-8 August). Method before and after restoration were kept constant with the same observer at both times (Anu Tiitsaar, PhD). Weather conditions and general approach followed standardized Pollard walk (Pollard, 1977) but transect were limited to 5 minutes per treatment (open grassland, shrub-covered grassland, afforested grassland and control) so that about 250 m long transect route included respective plant treatment plot. All observed butterfly individuals were counted and identified to species level whenever possible, except the *Leptidea sinapis/reali* and *Plebejus idas/argus* species pair, which cannot be unambiguously separated in field conditions. These together with some fast flying individuals (i.e *Argynnis* sp) remained at genus level.

Butterfly monitoring results

Over the two sampling periods (before and after restoration) total number of butterflies encountered was 1146 individuals from 54 species. Average number of individuals per site before restoration was 7.0 (ranging from 0-29) and 8.4 (0-33) after restoration. Average number of species per site before restoration was 3.4 (ranging from 0-15) and 4.0 (0-13) after restoration. Control sites had similar species and individual counts in both sampling periods indicating the effect of sampling year to be low. Most importantly, there was a clear positive effect of restoration in former shrub-covered and afforested sites where more than threefold increase in both number of individuals as well as species was detected **(Figure 12)**. Expectedly, the effect of restoration was the lowest at open habitat sites - there was small decrease in number of species and individuals after restoration. The reason behind this could be as simple as normal variation between years as well as potential (likely temporary) decrease in habitat quality for butterflies due to bush removal and soil disturbance. As around half of the restored sites were restored less than 3 years (between 2017 and 2019) prior sampling, butterfly abundance and richness is likely to increase in further.

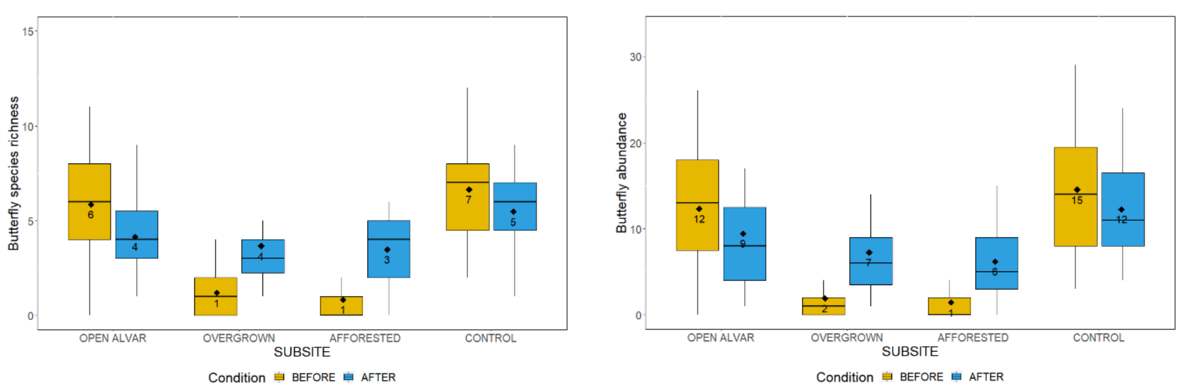

Figure 12. *Richness (left figure) and abundance (right figure) of butterflies before and after restoration. Diamond and number inside the box depict mean values, band inside the box indicates median, box indicates quartiles, whiskers minimum and maximum values.*

Impact of alvar grassland restoration on environmental conditions

The impact of restoration on soil conditions was assessed using different indicators: content of soil organic matter (SOM), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and soil depth (cm). Soil samples were collected in June-July both in 2015 (prior restoration) and in 2019 (after restoration). On each site, five 10 cm soil core samples were taken by a soil borer (steel cylinder with a diameter of 5.5 cm) roughly around 10 cm from plant community monitoring quadrat. To get the average soil depth of the sites, 10 measurements were taken around the plant community monitoring quadrat in 10 m radius. On selected sites, soil moisture and light availability was measured to track the changes in environmental conditions.

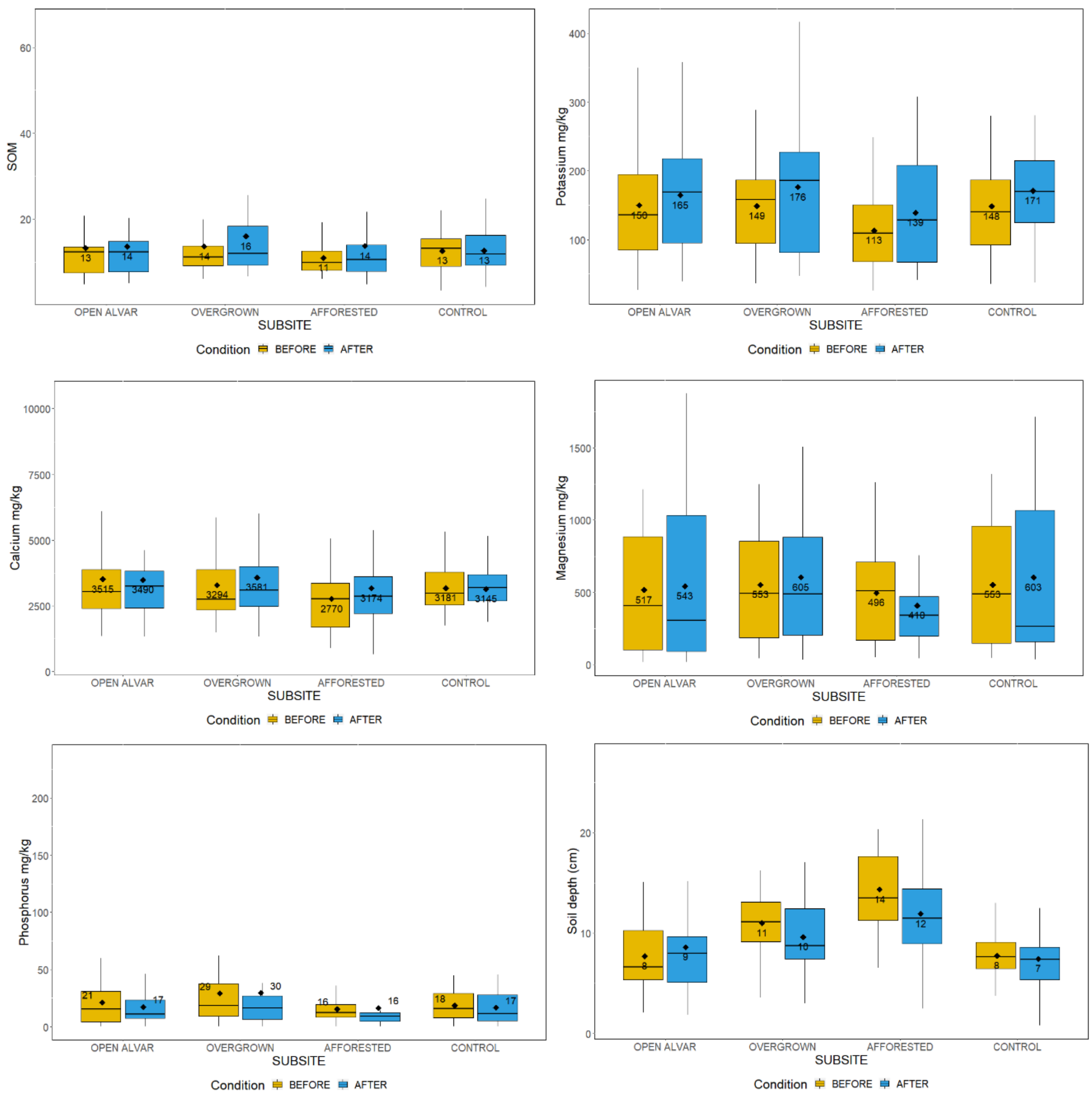

Figure 13. *Change in soil variables before and after restoration. Information about soil organic matter content, potassium, calcium, magnesium, phosphorus content and soil depth is given for alvar grasslands in different successional stages. Diamond and number inside the box depict mean values, band inside the box indicates median, box indicates quartiles, whiskers minimum and maximum values.*

Overall, there were little impact of restoration activities on soil conditions **(Figure 13)**. Soil organic carbon content remained the same after restoration, with the exception of slight increase in overgrown sites following restoration (from 13.6% in average to 16%). Soil parameters respond very slowly to any impacts, thus it is expected that if any changes occur due to restoration and subsequent management, the measurable impacts of it will be delayed. Information about soil and other environmental variables were used to quantify impact of restoration on soil quality and soil carbon storage (see the next paragraph) and were linked to biotic indicators.

Impact of alvar grassland restoration on the supply of ecosystem services

Elisabeth Prangel

Ecosystem service assessment methods

We selected pollination, climate regulation (soil carbon storage), soil quality maintenance, cultural benefits (conservation value, recreational value) and biodiversity as our observed ecosystem services. The selection was based on their importance and data availability – all data used was collected connected to the project LIFE to Alvars or by researchers from University of Tartu using the same restoration areas to collect additional data (bumblebee survey). To evaluate the potential of alvars to provide previously named ecosystem services and functions, we used different indicators that have been described to be directly connected to the provision of these services (table 2). To be able to compare and asses the services described by indicators with different units we used a novel approach to ecosystem services assessment. We standardized (z.score) all indicator values using scale function in R (R Core Team 2018) and attained a relative score of the ecosystem service per subsite in relation to the average of the given service over all subsites (expressed as 0). After data standardization the effect of overgrowing was analyzed using ANOVA mixed models in R.

Table 2. *Indicators used for assessing the provision of ecosystem services by alvar grasslands in their different successional stages.*

Services/functions	Indicators
Regulating	
1. Pollination	Bumblebee and butterfly abundance and species richness; Entomophilous vascular plant species richness and cover; Nectar resources estimation; Floral resources (abundance of flowering plants)
2. Soil carbon storage	Soil organic carbon content (SOC %)
3. Soil quality maintenance	Soil organic matter (SOM %); Soil phosphorus content (P mg/kg); Soil pH and depth;

Cultural benefits	
1. Recreational and cultural heritage value	Attraction potential: orchid species richness and number on flowering plants; cultural benefits evaluation survey;
2. Diversity conservation value	Abundance of endangered (RDB) or nationally scarce species
Supporting	
1. Biodiversity as a service itself	Overall number of species of all observed taxa

Ecosystem service assessment results

Open alvar grasslands have the highest capacity to deliver and maintain several observed ecosystem services and functions at the same time. There were significant differences in biodiversity and provision of pollination and cultural benefits before and after restoration in previously overgrown and afforested sites **(Figure 14)**. Provision of pollination and cultural benefits were significantly higher in restored habitat compared to their previously overgrown and afforested conditions. Biodiversity had the same trends. After restoration, previously open alvar sites had no significant differences in pollination and cultural benefits compared to before restoration state. Restoration did not have a significant effect on soil carbon storage and soil quality in different successional stages, indicating that both overgrown and afforested areas as well as open areas are equally good in providing these vital services.

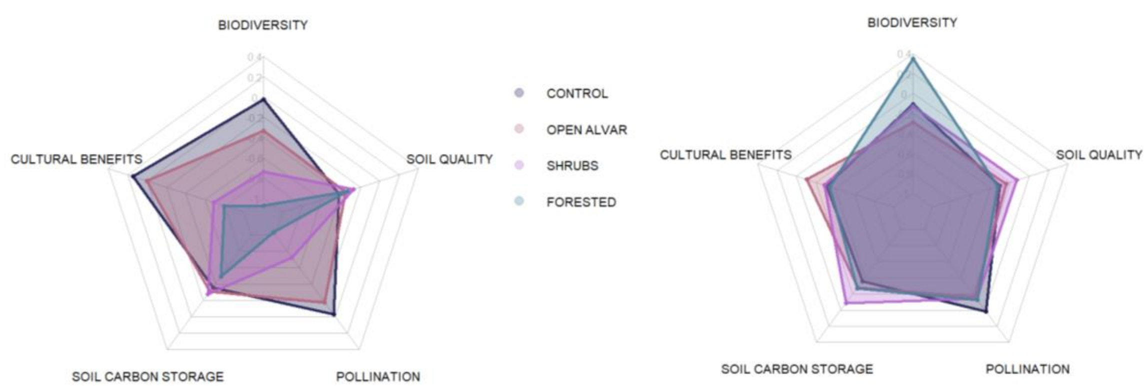

Figure 14. *Comparison of provision of ecosystem services before (left graph) and after (right graph) restoration in different successional stages of alvar grasslands. The larger the value, the higher the provision of particular service.*

Monitoring of seed sowing

As a part of the LIFE to Alvars project, two different seed distribution methods were used to introduce characteristic species to previously heavily overgrown alvar grassland sites. From the 25 project sites, Neeme site in north-western Saaremaa island and Kurese site in Estonian mainland where chosen as test areas of seed distributing. Details about seed sowing are presented in the report "LIFE to Alvars Action C.4. Restoration of habitat through seed sowing" (MTÜ Elurikas Eesti 2018). Seeds were sown on 2017 to Neeme site and 2018 to Kurese site and two methods were used for spreading seeds: green hay spreading and sowing of brush harvested seeds

Methods of monitoring seed sowing

For monitoring seed sowing effectiveness, we established permanent plots on both seed sowing sites describe vegetation composition and structure before and after sowing. Established permanent plots covered both methods of seed introduction as well as control plots. Randomly placed 5 permanent 1x1 meter plots were established in each following area in both sites: 1) restoration area where green hay spreading was applied, 2) restoration area where brush harvested seed sowing was applied, 3) restoration area without seed sowing, and 4) non-restored area without seed sowing. Example of how permanent plots are located in Neeme site is given in **Figure 15**.

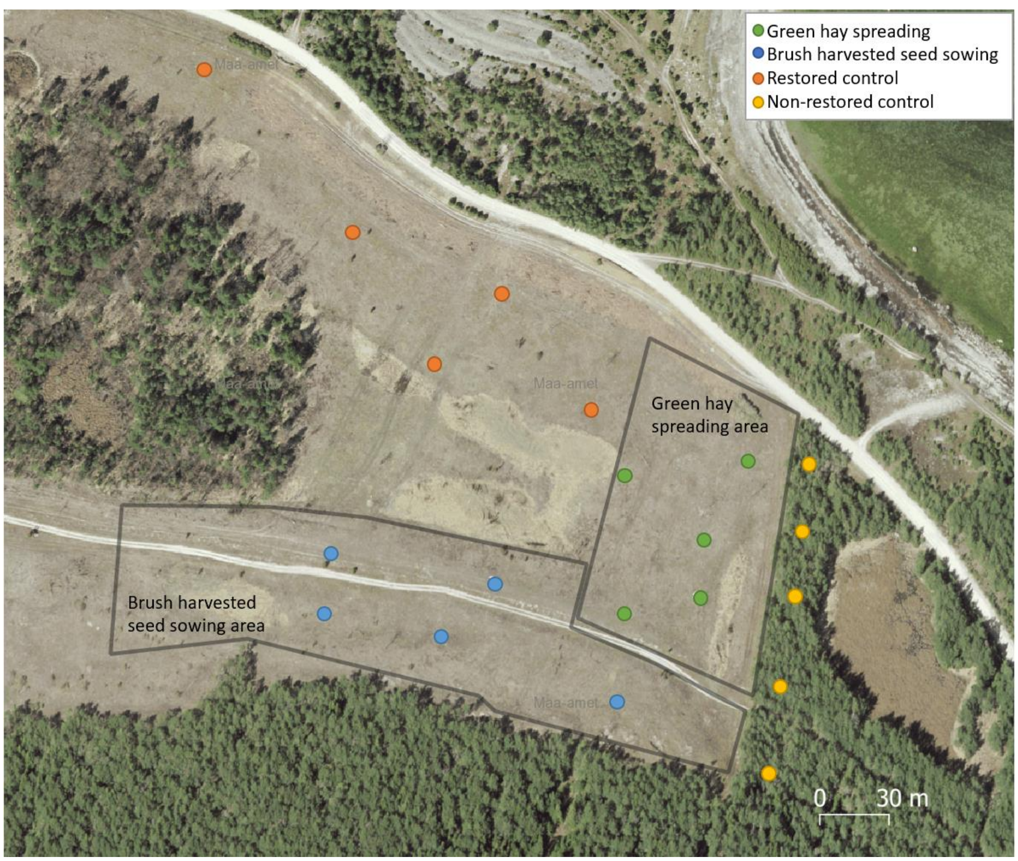

Figure 15. Location of permanent observation plots of seed-sowing activities in LIFE to Alvars Neeme project site.

On each permanent observation plot, plant species richness and abundance (percentage cover) were described in 1x1 m scale. Around the observation plot in 10-meter radius, presence of all species were recorded.
Monitoring was carried out prior seed sowing and after seed sowing. In Neeme project site, seeds were sown on 2017, and prior-sowing monitoring was carried out on the same year during the seed sowing. In Kurese, seeds were sown on 2018 and prior-sowing monitoring was also carried out on the same year. Follow-up monitoring occurred in August 2019, i.e. 2 years after seed sowing in Neeme site and 1 year after seed sowing in Kurese site.

Results of monitoring seed sowing

In average, plant species richness had increased on all restored sites, including restored control sites without seed addition. Richness of unrestored control sites had remained the same between two sampling periods **(Figure 16)**. There was no significant difference in species richness between restored sites where seeds had been applied and where they had not been applied (One-way ANOVA, Tukey post-hoc test, P>0.05). At the same time, restored plots where seeds were sown (by using both methods) had higher gain of species than plots where no seeds were sown (6.3±3.11(SD) vs 3.5±4.2 in average, respectively). However, as relatively little time (1-2 years) has passed since the seeds were sown, it is expected that the differences will be more pronounced in coming years.

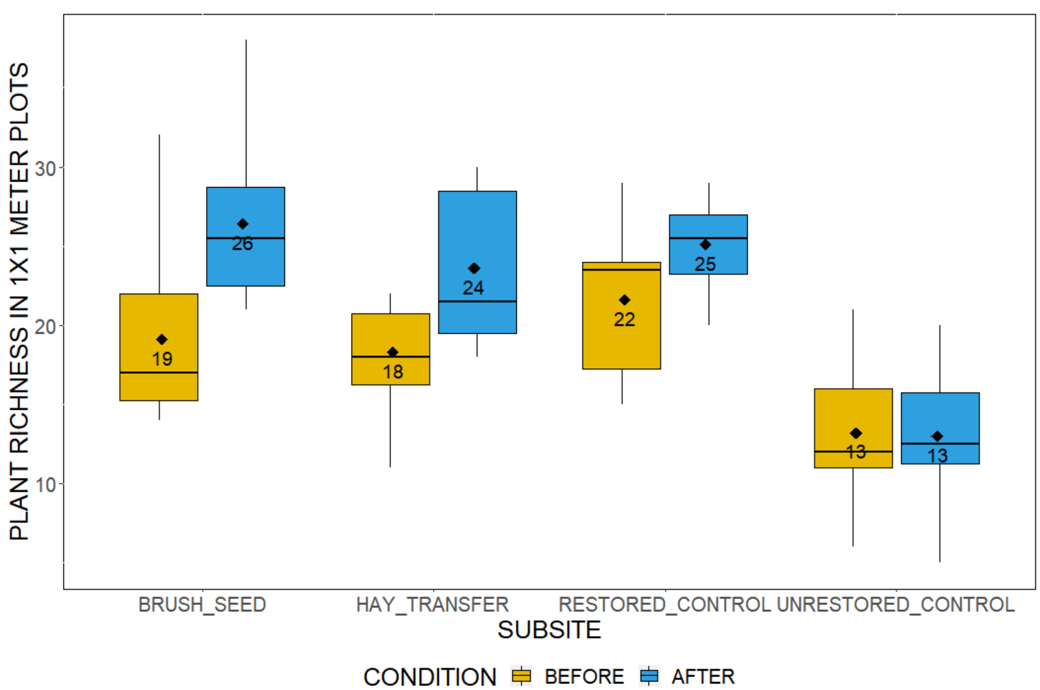

Figure 16. *Plant species richness in 1x1 meter observation plots before and after restoration. Plots covered areas where brush-harvested seeds were sown, where green hay transfer was applied and restored and unrestored areas where no seed addition occurred. Diamond and number inside the box depict mean values, band inside the box indicates median, box indicates quartiles, whiskers minimum and maximum values.*

Species that had considerably gained coverage in seed-sown plots (irrespective of sowing method) were *Asperula tinctoria, Daucus carota, Galium verum* and *Solidago virgaurea.* The monitoring of seed sowing activities will continue in 2020 and 2022, as too little time has passed since the activities and it is yet too early to make any conclusions about the effectiveness of the seed sowing.

Dissemination

Biodiversity monitoring methods and results have been presented in local and international media, in international conferences and in public presentations to Estonian audience. In coming years, the scientific publications about the biodiversity monitoring will be published. Until now, following dissemination activities have occurred:

Scientific publications

Helm, A. (2015). Habitat restoration requires landscape-scale planning. Applied Vegetation Science, 18 (2), 177−178.

Aavik, T. & Helm, A. (2017). Restoration of plant species and genetic diversity depends on landscape-scale dispersal. *Restoration Ecology*, *26:S92-S102.*

Popular science publications and book sections

Helm, A. (2018) Eesti loopealsete ökosüsteem ehk kes seal elab ja kuidas neile kinnikasvamine mõjub? XLIV Teoreetilise Bioloogia kevadkool, Schola Biotheoretica „Ökosüsteemsus“, lk 127-136. https://kevadkool.elus.ee/?do=files&sid=46 (accessed on 05.08.2019).

Prangel, E. (2018) Poollooduslikud rohumaad ja ökosüsteemiteenused. XLIV Teoreetilise Bioloogia kevadkool, Schola Biotheoretica „Ökosüsteemsus“, lk 137-144. https://kevadkool.elus.ee/?do=files&sid=46 (accessed on 05.08.2019).

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Helm, A., Prangel, E. (2018). Time-delayed dynamics of ecosystem services in disappearing grasslands. Ecosystem Service Partnership 2018 Europe regional conference. Ecosystem services in a changing world : moving from theory to practice. 15-19.10.2018, San Sebastian, Spain.

Helm, A. (2018). Eesti niidud – kellele ja milleks (Estonian grasslands – for whom and why?) 13th Estonian Ecology Conference, 20.04.2018, Tartu (in Estonian).

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Appendix

Survey sheet for site-level assessment of environmental conditions. Sheet was filled for all study sites both before (in 2014-2015) and after restoration (2019).

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LIFE to Alvars plant biodiversity survey

FORM "LOCATION" GENERAL DESCRIPTION OF THE PLOT SURROUNDINGS (10 M RADIUS AROUND THE PLOT)

1. SITE NAME and ABBREVIATION		
2. PLOT NR	3. PLOT TYPE: A - open grassland B - under junipers C- under pine. Other:...........	4. DATE, TIME
5. SURVEYERS		
6. PLOT COORDINATES (LAT, LONG, deg. decimals)		

7. Habitat type: 1 - Festucetum, 2 - Avenetum, 3a – wet alvar with Molinia, 3b – wet alvar with Sesleria
8. Covered with forest ....% of circle area; dense shrubbery ....% circle area; open alvar.......... % circle area
9. Type of bedrock: 0 – cannot estimate, 1– monolithic, 2 – shingle
10. Herb layer. Herb layer coverage: 1 – sparse (with bare soil inbetween), 2 – dense.
10.1. Bare rock ......% of area; bare soil ....% of area.
10.2 Herb layer height (except flower stalks): 0 – low (<9 cm), 1 – medium (ca 10-20 cm), 2 – high (20-40), 3 – very high
10.3. Presence of litter/needles: 0 – no litter, 1 – little litter (not hindering plant establishment), 2 – dense litter.
Comment on litter (made out of dead grasses, dead herbs, pine needles, juniper needles etc)..
10.4. Changes in herb layer (estimates of anything untypical): weeds, alien species, Brachypodium pinnatum
....: 1 – sparse,uniform 2 – patchy, 3 – dense, uniform; covers ....../......./....... % area
....: 1 – sparse,uniform 2 – patchy, 3 – dense, uniform; covers ....../......./....... % area
Comment on herb layer...
....
11. Shrubs: 0 – lacking, 1 – characteristic to open grassland (ca 0.3), 2 – extensive with coverage of ....%

Shrub species (with coverage above 0.05)	average height (m)	largest height (m)	coverage (0–1)	Shrub species (with coverage above 0.05)	average height (m)	largest height (m)	coverage (0–1)
1.				**3.**			
2.				**4.**			

12. Trees: 0 - lacking, 1 – tree species formula/list of species.. ....
12.1. Tree canopy coverage (0 – 1) ...., height (m) average: .... max:..................
12.2. Age of dominant trees: 1 – young, 2 – average, 3 – old, 4 – variable. Estimate ca .... years
13 Human influence. 13.1. Mowing: 0 – likely never mown, 1 – ended > 4 y ago, 2 – mown 1–3 y. ago, 4 – currently mown. Comment:...
13.2. Grazing: 0 – likely never grazed; ended > 10 y ago, 1 – ended 4–10 y ago; 2 – grazed 1–3 y ago; 3 – currently grazed; X – cannot estimate;
13.3. Grazing pressure: 1 – suitable pressure, 2 – overgrazed; 5 – too weak pressure. Comment.........................
....13.4. Who is grazing (species)? Estimated amount on site...
13.5. Other effects (underline): buildings, stone fences, trampling, road, power lines, old quarry, active quarry, pollution, littering, fertilization, woodcutting, shrubcutting, recent restoration activities, soil disturbance, presence of fertilized fields nearby (estimate the intensity of the effect) ....
....
14. Soil depth, cm (10 measurements). NB: Take also soil sample to paper bag (5 spoonfuls from random places)

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.

15. Habitat **quality/representability** as an open alvar grassland: A – very good, B – good, C – average, D – low
16. Description of plot location / Map/sketch of significant features / other comments on vegetation