Final Project Report

1. Contestant profile

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▪ Contestant occupation:	Master's students
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▪ Number of people in your team:	4

2. Project overview

Title:	Creating novel communities to increase biodiversity in Aru-Lõuna limestone quarry
Contest: (Research/Community)	Research
Quarry name:	Aru-Lõuna limestone quarry

Abstract

We are facing a strong decline in species richness due to the loss of habitats through human activities and it is important to find solutions for stopping it. One of the most diverse habitats in the world are grasslands growing on calcareous soils, but their management has become less profitable and they are often cultivated or abandoned. Disappearance of these habitats affects different organism groups including pollinators. Today's farming methods, such as the use of pesticides and herbicides, do not support species that are connected with farmlands and grasslands.

Main purpose of our project is to show how limestone quarries can be used for making an alternative habitat for grassland species. We can benefit from artificial landscapes that remain after mining, as calcareous bedrock in limestone quarries creates conditions suitable for the development of diverse grasslands.

We showed how creating novel communities will increase the biodiversity in Aru-Lõuna limestone quarry by using the help of mycorrhizal fungi. Our experiment indicated that adding soil to mined surface affects vegetation development and the growth of calciphilous meadow species positively. Our results demonstrate that limestone quarries can serve as valuable habitats for various species.

Final report

INTRODUCTION
Grasslands and biodiversity

Habitat loss and fragmentation are known reasons for today's declining biodiversity all over the world. The increasing demand for land has led to the loss of natural habitats and spatial connectivity between them. Grasslands are one of the important habitats, which have lost a remarkable area due to human activities, causing a decline in the abundance of grassland species, including pollinators. The decline is also caused by modern agriculture, where it is common to use pesticides and herbicides, which in addition to pests, affect useful insects and organisms who depend on them (Stoate *et al.*, 2001). Research shows, that insect and pollinator populations are declining globally (Potts *et al.*, 2010). Recent study from Germany revealed that within the past 27 years, flying insect biomass has declined by over 75% (Hallmann *et al.*, 2017). Insects have a central role in the functioning of ecosystem. They provide pollination, which is vital for natural plant communities and for agricultural crops (Potts *et al.*, 2010). The productivity of crops is directly linked to the existence of pollinators and because of that, mankind depends on that ecosystem service which is offered by insects (Potts *et al.*, 2010). Besides pollination, insects have other important roles in ecosystem processes: they are food source for higher trophic levels and they participate in nutrient cycling (Yang & Gratton, 2014). As the presence of insects is essential for the functioning of life, it is important to preserve habitats and species richness.

Grasslands and meadows are important habitats for insects. Grasslands growing on calcareous soils are one of the most diverse ecosystems in the world and flowering plants growing there offer a suitable habitat for pollinators (van Swaay, 2002). Usually quarries are forested after closing but planted forests could take centuries before they start supporting biodiversity (Humphrey, 2005). Therefore, limestone quarries could be used as creating semi-natural calcareous grasslands with thin soil, called alvars, which are characterized by high species richness of plants (Pärtel *et al*., 1999). Alvar area in Estonia has decreased about 70% with the last 50 years (Helm *et al.*, 2005). Suitable habitats for grassland species are lacking and abandoned limestone quarry areas would offer alternative habitats for these calciphilous plant species and therefore support other taxa dependent on them, for example insects and birds.

To aid in the formation of grassland and meadow communities, it can be considered to use the help of mycorrhizal fungi. After mining activity has ended, degraded landscapes remain. These landscapes, together with their biodiversity show very slow rates of recovery. During the mining of these areas, the topsoil is removed and stockpiled. Research has shown (Miller & Jastrow, 1992), that this soil loses much of its biota during storage. This could be a problem when mining stops as vegetation recovery also depends on soil microorganisms associated to them. One very important organism group to consider is mycorrhizal fungi.

Symbiotic relationship between mycorrhizal fungi and plants is called mycorrhiza. Fungi are dependent on the plant for organic C and with the help of fungi, plant gets nutrients from the soil. Among different types of mycorrhiza, arbuscular mycorrhiza (AM) is the most common mycorrhizal type - over 80% of terrestrial plants form this type of associations. Fungi who form AM, are from phylum *Glomeromycota*. Arbuscular mycorrhiza is relevant to all plant phyla and it is considered to be the most ancient mycorrhizal type (Smith & Read, 2008). AM fungi (AMF) play important role in the life of plants – external hyphae absorb non-mobile nutrients phosphorus (P), zinc (Zn), copper (Cu) from soil and also facilitate water and nitrogen (N) uptake. Hyphae form external mycelium, which extends the reach of plant roots. Nutrients are translocated over long distances in mycelium and finally transferred to the plant roots. Also, hyphae are able to penetrate soil pores, which are inaccessible to roots (Smith & Read, 2008). Mycorrhizal fungi can promote plant growth and seedling establishment (van der Heijden & Horton, 2009) and AM plants grown in AM hyphae rich soil often have higher drought resistance (Smith & Read, 2008). Therefore, these fungi could be useful in the harsh conditions of depleted quarries to restore species rich vegetation cover.

Description of the quarry

Aru-Lõuna limestone quarry is located in Estonia, Lääne-Viru county, Sõmeru parish, about 5 km from Kunda city. Cement production for Kunda cement factory started in 1960-s and in 1992 AS Kunda Nordic Cement was founded, which is a part of HeidelbergCement Group. If the Aru-Lõuna quarry would not have been made, there would be a wet forest (Eesti Geoloogiakeskus, 2012). Currently the mining activity takes place below the groundwater level and 310 l/s is pumped into river Toolse on average (Eesti Geoloogiakeskus, 2012).

Aru-Lõuna quarry has already high species richness - from plant inventories 269 species have identified. This shows that calcareous baserock creates suitable conditions for species rich communities, so the creation of grasslands are prefered rather than forests.

METHODS
Mychorrhiza

To create species rich plant community (in our case alvar-like grassland) with using the help of AM fungi, it is recommended to make an inoculum. Studies show that using inoculum enriched with AMF has a positive effect on development of plant communities by supporting plant growth and species richness in field conditions (Torrez *et al.*, 2016; Neuenkamp *et al.*, 2018). Adding mycorrhizal inoculum could be especially useful in degraded sites, where topsoil has removed, because the formation of mycorrhizal communities takes time (Torrez *et al.*, 2016; Wubs *et al.*, 2016) and reapplied topsoils have reduced AMF populations, which could affect plant community development (Miller & Jastrow, 1992). Soil inoculum is often made using the trap culture methodology, where a small amount of soil from diverse plant communities is collected and diluted with an inert substrate. Then mycorrhizal plants are sown on this substrate. Consequently, the AM fungal community in the substrate will form mycorrhiza with the plants and proliferate, resulting in a high abundance of AM fungi from a small amount of natural soil. In this way, diverse grassland communities are minimally harmed, but suitable conditions for the development of similar community are made. In this way, the development of wanted diverse plant community is hypothetically easier and more successful.

We planned and conducted an experiment in Aru-Lõuna limestone quarry, to test if adding soil to surface that had been already mined supports the formation of meadow community. At first, we wanted to test how adding inoculum enriched with AMF from alvar grasslands to mined surface promotes development of alvar grassland community. Due to complications, we couldn't get the soil from species-rich alvar grasslands in the right time and had to change our perspectives and aim. Instead of creating alvar grassland community we decided to examine, how adding soil supports vegetation development and growth. For that we used soil from grassland in service area, where plant diversity isn't as high as in alvar grasslands. Because the number of fungi is smaller in that soil, it was needed to take higher amount of soil. If we had made an inoculum, we would have had to take 4-5 times less soil. To test grassland species growth and promote the development of diverse plant community, it was needed to sow seeds which were bought from NPO Elurikas Eesti. They have collected seeds from alvar grasslands in Estonia, which have high plant diversity (Annex 1).

For the experimental patches we chose a higher place in the quarry, because currently the mining activity takes place below the groundwater level and after the mining activity stops and water pumping ends, the created patches would stay above the water. Vegetation had already developed on the chosen area, but freshly mined ground is essential for this experiment to test the development of vegetation. Because of that, Kunda Nordic team created conditions to the patches similar to the ground that remains after mining (Annex 2). Four patches (Figure 1), each 300m^2 in size, were made: control patch (0), patch with added soil and seeds (1), with added seeds (2) and with added soil (3). Control patch is not manipulated and shows natural succession. The amount of soil was 1 litre per m^2 and seeds 6 g per m^2. Fieldwork took place at the end of May 2018.

We put up a hypothesis that adding soil and seed mix to mined surface affects vegetation development and formation of the meadow community positively.

Figure 1. 0 - control patch, 1 - patch with added seeds and soil, 2 - patch with added seeds, 3 - patch with added soil.

Geographic Information Systems

To model our plan for reclamation of the quarry, we operated with geographic information systems (GIS). We used MapInfo Discover 16 to make our biological reclamation plan (Figure 4). This was made with available Lidar elevation data and data collected during the fieldworks. As MapInfo also offers the possibility to display surfaces in 3D, we created two 3D models. One model was constructed for more detailed observation of the island and the other one was made to give an overview of the whole quarry. We gained lots of experience using MapInfo tools and making 3D models. We believe that 3D modeling is an innovative method of quarry reclamation for giving an extensive overview of the quarry area in future as showed in biological and technological plans.

RESULTS

Patches were made on 16th of May 2018 and last check on them was on 7th of September 2018. Four months after making experimental patches and sowing the seeds, there were visible changes. Vegetation cover was more extensive on patches 1 and 2 compared to others (Figure 3). Observation showed that some species growing on patches 1 and 2 were absent from patches 0 and 3, for example brown knapweed (*Centaurea jacea*), corn chamomile (*Anthemis arvensis*), ribwort plantain (*Plantago lanceolata*). *Trifolium* cover was visibly larger on patch 1 compared to other patches. We found one species, hop trefoil (*Trifolium campestre*), which came with the seeds, it is common in western islands of Estonia, but not in mainland. While observing species we also saw diverse insect fauna, including butterfly, bumblebee (*Bombus*) and honey bee (*Apis*) species. Our patch had also been visited by European hare (*Lepus europaeus*).

First visit to patches was on 7th of June. It hadn't rained since sowing the seeds and vegetation growth was minimal (Annex 3). Our hopes weren't high for getting outstanding results but other visit in September showed that our experiment has positive results.

Diverse grassland communities take some years to fully develop. Then it is recommended to do species inventory and biomass weightings to identify precise differences between the control patch and manipulated patches.


Figure 2. Experimental patches after adding soil and sowing seeds.


Figure 3. View to experimental patches 1 and 0.

Biological plan

When mining activity ends, the quarry fills with water forming a lake. As a result, numerous islands are going to form. We chose the best locations for the habitats considering the landscape matrix. On our biological plan (Figure 4) the NW, NE and E part of the service area is covered with grassland. We located future grasslands so that they would relate to existing fields and meadows, which act as moving corridors, since pollinators move across open landscapes. Also, connectivity between grasslands on the eastern and north-eastern part is necessary for the dispersal of species and preservation of vital populations. This leads us to a narrower forest area on the eastern part. On the northern part of the quarry is a lower area. After mining activity ends and water level rises, the formation of wetland is expected there. Creating of grassland is not as advisable in this area. Otherwise, it requires too much soil and resources to build a higher surface. Therefore, we left this area for the development of wetland. Wetlands and calcareous fens are important habitats for protected species. For example, protected orchids, such as the *Epipactis palustris* and the *Gymnodenia conopsea*, as well as the rarest *Ophrys insectifera* and the *Liparis loeselii*, inhabit these areas (Rammul, Niitlaan, Reinsalu, & Keerberg, 2017).

Other areas surrounding the lake are covered with forest, as Aru-Lõuna quarry is already surrounded with 40-year-old mixed coniferous forest (Eesti Geoloogiakeskus, 2012).

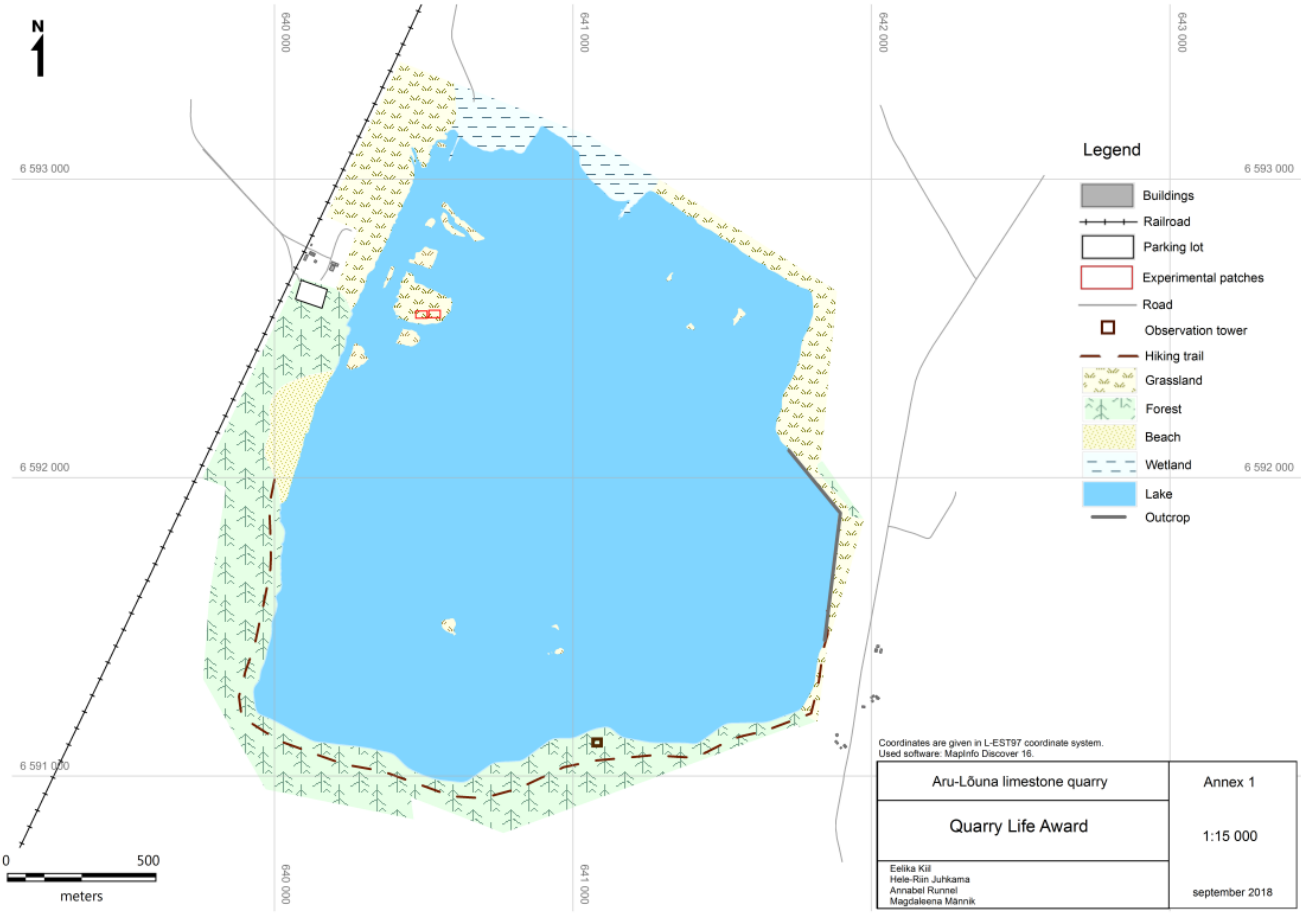

Figure 4. Biological plan

3D models

To give more accurate visualization of the quarry area, we created two 3D models. The first model displays the island where our experimental patches are located. On Figure 5 the island is viewed from the lake from NE side of the quarry. Figure 6 gives an overview of the whole quarry after reclamation. As it can be seen, SW and southern side of the quarry will be covered with a forest and on the northern and eastern side will be grasslands.

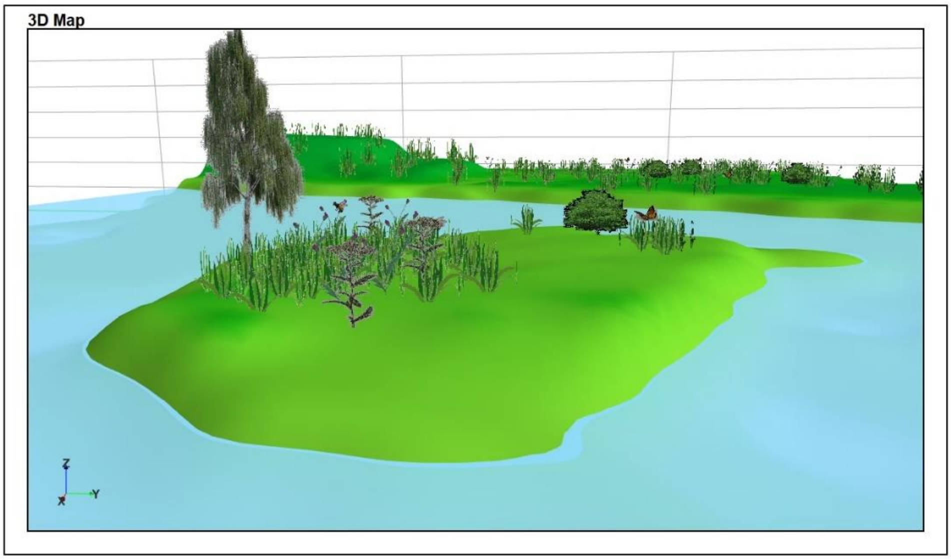

Figure 5. The view of island from the lake.

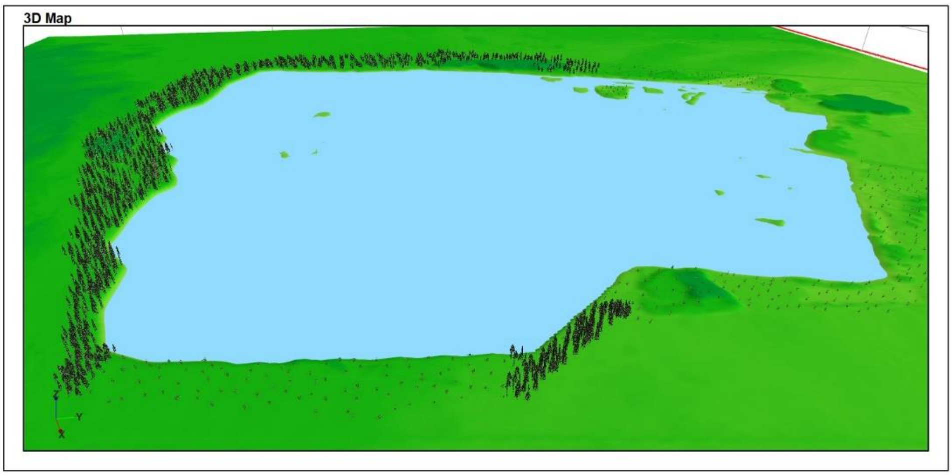

Figure 6. The view of the quarry after reclamation from east.

DISCUSSION

As can be seen from our results, the experiment was successful. There is a visible difference between the patches (Figure 3), so we can say that adding seeds and only a small amount of soil with microorganisms contributes to vegetation growth. After the creation of experimental patches, the weather conditions were not favourable for seedling establishment. Rainfall was minimal and high temperatures prevailed through summer. Despite the harsh conditions, there was a clear difference in vegetation growth between manipulated patches and a control patch (Figure 3). We can say from the results that adding soil to mined surface enhances vegetation development. There is also a chance that microorganisms from added soil helped the seeds to sprout in these weather conditions, as it is known that AM fungi help the plants to survive drought (Smith & Read, 2008). Vegetation contained multiple flowering plants, which were occupied by different pollinators, honey bees, butterflies and bumblebees. With this small amount of time our experimental patches developed enough to give pollinators and insects a feeding ground.

The clearest difference in vegetation growth and species richness was seen between patches where we had added soil and seeds (Figure 3) and only seeds (Annex 4). Adding seeds promoted to higher species richness, otherwise it would take longer for meadow species to arrive and establish the ground. If the idea of reclamation is to create a diverse grassland community to the quarry area, then based on our results it could be more effective when seeds from diverse community are added with soil. It must be pointed out that in our experiment we used soil from service area, which biota is not as rich as in soil from continuously grown semi-natural grassland. When recultivating with purpose to create as diverse meadow community as possible, it is recommended to use mycorrhizal inoculum made with soil from the same or similar community where the seeds are originated as it contains AMF which usually grow with those plant species (Torrez *et al.*, 2016).

Our results show that limestone quarries are suitable place for the formation of species-rich grassland communities and offer an alternative habitat for meadow species, who are suffering from the loss of habitats. Nowadays, it is common to recultivate quarries by afforesting them, but it takes centuries for planted forests to offer suitable habitats for forest species (Fritz *et al.*, 2008) and therefore supporting biodiversity. Efforts related with forest ecosystems could be instead directed towards making managed forests attractive for forest species, as they are usually lacking important features for preserving biodiversity (Humphrey, 2005). Calcareous grasslands on the other hand start to support pollinators as soon as vegetation has developed, as also seen in our experiment. Meadows with their flowering plants attract many insects whose presence in turn draws animals and birds who prey on them.

When planning quarry reclamation and creating new habitats to quarries, it is highly recommended to take their spatial distribution into account. For the dispersal of species and the preservation of populations it is important that creatable habitats will not remain spatially isolated and fragmented from similar habitats surrounding them (Fahrig, 2003). Landscape-scale planning is required and when possible, new grasslands must be made there, where they are connected with existing moving corridors, as we showed in our biological plan.

It must be remembered that calcareous grasslands are semi-natural communities (Wallis De Vries *et al.*, 2002), they need to be managed by mowing or grazing, to prevent them from overgrowing with trees and shrubs. It is a good opportunity to offer these areas for local farmers to keep their animals. Presence of pollinators is also important for local farmers, because crop productivity in connected with pollinator abundance (Potts *et al*., 2010).

Instructions for Kunda Nordic Cement and HeidelbergCement Group

As the seeds what we used in our experiment are from Estonian alvar grasslands, it may be possible to use our patches as a source for seeds in the future when recultivating Aru-Lõuna quarry and for further reclamation of other limestone quarries. For that, experimental patches need to be managed. To prevent area from overgrowing, it must be mowed annually, preferably at the end of July or in August.

When recultivating and using mycorrhizal inoculum, 0.2 - 0.25 litre of soil per 1 m^2 is needed. It is important to consult with experts. In our case, seeds were bought from NPO Elurikas Eesti and 1 kg of seeds costs 60 euros.

As mentioned, it is possible to start collecting seeds from our experimental patches, which would make the project cost less.

Our vision for future

After the mining activity, the quarry will be approximately with an area of 3.17 km^2 and 20 m deep. The surrounding area is flat and covered with a forest. Aru-Lõuna is undoubtedly very large artificial landscape element (will be located on the 16th position among the lakes of Estonia). The largest value could consider to be at least 35 million cubic meters of fresh water (Eesti Geoloogiakeskus, 2012). Before the opening of the Aru-Lõuna limestone quarry, the groundwater level was around 48 m a.s.l. Despite the mining, a significant reduction of groundwater levels of 3-5 m reaches only about 1-kilometer distance from the boundary of the quarry (Eesti Geoloogiakeskus, 2012). Due to the opening of a new quarry next to Aru-Lõuna, we have taken into account that the pumping will continue and the new groundwater level maximum chosen for our model was 45 m a.s.l. It is important to highlight that before the groundwater pumping stops in Aru-Lõuna, the trees and shrubs that will be under the water should be removed (preferably with the soil), as the vegetative matter begins to decay and thereby worsens the quality of water and pose a risk to water biota and people (Kukk and Kull, 2016).

Water bodies promote the diversity of the environment (including small water bodies). In their presence, a varied landscape of water, plants, shrubs and trees can be shaped. Plant vitality is enhanced by biodiversity. The flora in the lower parts of the water provides conditions for feeding, breeding and shelter for amphibians, dragonflies and birds. An archipelago will form in the water which is located far enough in the water and it could be perfect for water birds. Also, shallower areas in the northern part of the quarry are there to promote amphibian spread. Most of the freshwater species can populate the water bodies with a slope of approximately 1:10. For the wintering animals it is important to prevent water from freezing to the bottom, so the depth of the water body should be at least 1 to 1.5 meters deep, but this is not a problem in our case (Rammul, Niitlaan, Reinsalu, & Keerberg, 2017). Aru-Lõuna quarry relates to river Toolse and this also ensures the distribution of fish.

In our vision, we also focus on providing added value to our quarry. As the water body is one of the largest of the area, it is a recreational destination. Because of that, we suggest creating a designated beach area. In our model of the quarry, overall average slope is 1:2, but when creating a beach, the slope has to be at least 1:5 (Rammul, Niitlaan, Reinsalu, & Keerberg, 2017). Today's people can sometimes be withdrawn from nature and extra value could be added by creating a study and trekking trail to this region, in order to raise awareness of the disappearing of habitats and species loss. After recreation, this quarry could be considered as perfect place to study nature sciences. A sight platform can also be built on the hiking trail, where you can see the entire quarry area and the surrounding landscapes.

Our vision also covers the creation of an artificial outcrop to see limestones from Uhaku to Aseri stage. With this, we could introduce the geological development of the area, the limestone in the area (its usage) and the mining activities that took place. Outcrop must be cleaned of loose stones, so it could be accessible to people. Also, it must be boarded from the top by fences, bushes or bigger rocks to prevent people and animals from falling into water.

CONCLUSION

In the light of habitat loss, our project gives an overview on how novel grassland communities increase biodiversity in Aru-Lõuna limestone quarry with using the help of mycorrhizal fungi. The presence of grasslands gives shelter to insects and other species characteristic to these habitats. Welfare of pollinators should be everyone's concern, as they have a central role in the functioning of ecosystems.

We conducted an experiment in Aru-Lõuna quarry to test how adding soil and seeds to mined surface supports vegetation development and growth. Results showed that the vegetation cover was clearly more extensive when soil with seeds from diverse alvar grasslands was added. This shows that soil organisms contribute to vegetation growth and adding seeds promotes emerging of high species richness.

To give an overview of the quarry area we constructed two 3D models and a biological plan using geographic information systems (GIS). 3D models show us the whole quarry area as well as more detailed observation of the island. The area of the quarry will be approximately 3.17 km^2 and with depth 20 m. When pumping stops, a lake forms in the quarry. In addition to creating habitats for different species, there will also be a recreational area with beach, hiking trail, sight platform and outcrop. On our biological plan we chose the best distribution for the habitats considering the landscape matrix, connectivity and elevation data to support the dispersal of species and preservation of vital populations.

Our project indicates that quarry areas can support biodiversity and attract species after recultivation. According to our results, we can see that limestone quarries have suitable conditions for creating semi-natural calcareous grasslands, which are one of the most diverse plant communities in the world.

Project tags	
Project focus: ☐Beyond quarry borders ☒Biodiversity management ☐Cooperation programmes ☐Connecting with local communities ☒Education and Raising awareness ☐Invasive species ☐Landscape management ☐Pollination ☒Rehabilitation & habitat research ☐Scientific research ☐Soil management ☒Species research ☐Student class project ☐Urban ecology ☒Water management Flora: ☒Trees & shrubs ☐Ferns ☒Flowering plants ☒Fungi ☐Mosses and liverworts Fauna: ☒Amphibians ☒Birds ☒Insects ☒Fish ☒Mammals ☒Reptiles ☐Other invertebrates ☐Other insects ☐Other species	Habitat: ☒Artificial / cultivated land ☐Cave ☐Coastal ☒Grassland ☐Human settlement ☐Open areas of rocky grounds ☒Recreational areas ☒Sandy and rocky habitat ☐Screes ☐Shrub & groves ☐Soil ☐Wander biotopes ☒Water bodies (flowing, standing) ☒Wetland ☒Woodland Stakeholders: ☐Authorities ☒Local community ☒NGOs ☐Schools ☒Universities

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