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Evaluating potential benefits of restoring selected peatlands in Slovak Republic

Emphasis on carbon stocks and biodiversity

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1 Introduction

Peatlands are wetland ecosystems with water-saturated conditions that slow down decomposition, resulting in formation of organic matter called peat that consist of partly decomposed plants and litter. Thus, peatlands deliver ecosystem services such as carbon storage, but also carbon sequestration. Peat formation is a slow process, and yearly uptake of carbon dioxide (CO_2) is low. However, over thousands of years, the below ground peat layer can reach meters of depth and, thus, peatlands hold large amounts of carbon. Worldwide, they cover only about 3% of terrestrial land, but store about 30% of the global soil carbon (Gorham 1991). To reach the global climate goals, it is crucial to keep this carbon stock in the ground (Bossio et al. 2020).

Peatlands have been drained for peat extraction, to increase agricultural land and improve forestry, but is also lost to other land-use change purposes such as infrastructure and developmental projects (e.g. roads, industrial areas, wind-power plants). Disturbances in the hydrological balance in peatlands turn peatlands into sources of carbon, instead of sinks, as drying increase decomposition that results in large emissions of CO_2. About 12% of the world's peatlands are degraded with emissions corresponding to 4% of the global anthropogenic emissions (UNEP 2022). The peatland and histosol areas are relatively small in Slovak Republic compared to peatland rich countries of Europe, albeit that the total area is currently unclear with estimates varying between 420 ha (NIR 2022, Slovak Republic) to 6000 ha (Tanneberger et al. 2017). The current assumption is that the higher estimates were either an error, or that most organic soil has already disappeared (NIR 2022, Slovak Republic). Altogether 90% of all peatlands in Slovak republic has been estimated to be lost (Joosten & Clarke 2002). This estimation is in line with the status of most peatlands in Central Europe: pristine peatlands have practically disappeared, and degraded areas with near-natural or degraded status are in urgent need of restoration to avoid losing these key habitats.

To reverse the negative consequences of peatland degradation, hydrological restoration has emerged as an important action. Restoration actions have also been emphasized as one of the most potent measures for reducing the net greenhouse gas (GHG) emissions from 'Agriculture, forestry and other land use' – sector of the Intergovernmental Panel of Climate Change (Lee et al. 2023). Rewetting peatlands will significantly contribute to recover key ecosystem services at national and global levels, halting biodiversity loss, preserving soil carbon stocks and providing for nutrient retention. Peatland restoration may also play a role in water retention with implications to fire and flood control, in relevant areas. Degraded peatlands will, over time, be lost alongside most ecosystem services they provide which will have a significant and very negative impact on the environment (including climate), both directly and indirectly (Günther et al. 2020).

Species richness in peatlands varies with nutrient availability, and poor peatlands usually present a low number of species. However, since peatland species usually are specialists, many are rare and threatened because of habitat loss. About half of the intact peatlands in Europe have been lost and, in the EU countries, approximately 85% of the remaining peatlands are currently threatened (Peatland Atlas 2023). Therefore, restoring and conserving the remaining peatlands is an important contribution to safeguard the unique biodiversity they hold.

The project Ecohydrological Restoration of Peatlands in the Carpathians (ECORESP-C) is an EEA/Norway Grant project led by DAPHNE, an institute of applied ecology in the Slovak Republic, with NINA as partner. The project aimed at restoring hydrological conditions or/and applying restoration management at 12 peatland sites in the Slovak Republic to improve their conservation status and provision of ecosystem services. The project included a variety of peatland habitats such as bog woodland, transition mires and quaking bogs, alkaline fens, inland salt meadows, and raised bogs. It was carried out from April 2022 to April 2024.

As part of the project, we have calculated the below ground carbon stocks in the peatland habitats (sites) considered in this project. The calculations of carbon stock provide an estimate of the

potential amount of carbon that will be conserved due to restoration actions implemented during the project. Furthermore, we have used environmental DNA (eDNA) methods for assessing biodiversity at the sites. eDNA is a novel technique to assess species diversity. It is increasingly becoming a tool for monitoring biodiversity as it is efficient and allows for the detection of a wide range of species, including microorganisms, many of which would be missed with 'traditional' biodiversity monitoring methods. By analysing the eDNA samples collected before restoration, we provide a baseline of biodiversity to be used for monitoring biodiversity changes in the years to come after restoration. In this report, we summarize the findings of fungi biodiversity on the peatlands considered in this project. Fungi play a crucial role in peatland ecosystems by participating in processes such as nutrient cycling and organic matter decomposition. Hence, knowing their diversity and nutritional strategies provides insights on their ecological roles and interactions with other organisms. We expect that biodiversity will gradually change over time after the implementation of the restoration actions.

2 Methods

Twelve study sites were included in the project (Table 1), mainly in the northern part of Slovakia, but also one site in the west (Figure 1).

Table 1. Study sites in ECORESP-C.

ID	Site	Area	Wetland type
1	Bariny	18,7 ha	Alkaline fen
2	Boserpalské mláky	2,68 ha	Swamp forest
3	Hanšpíle	36 ha	Transitional fen and quaking mire
4	Havrania dolina	10,4 ha	Swamp forest
5	Klinské rašelinisko	15 ha	Bog and fen
6	Krivý kút	33,8 ha	Alkaline fen
7	Makoviská	95 ha	Swamp forest
8	Medzi bormi	10,3 ha	Transitional fen and quaking mire
9	Sivá Brada	20,3 ha	Inland salt marsh
10	Spišskoteplické slatiny	26,4 ha	Fen
11	Tisovnica	11,6 ha	Forest mire (ombrotrophic/bog)
12	Trstinné lúky	60 ha	Transitional fen and quaking mire

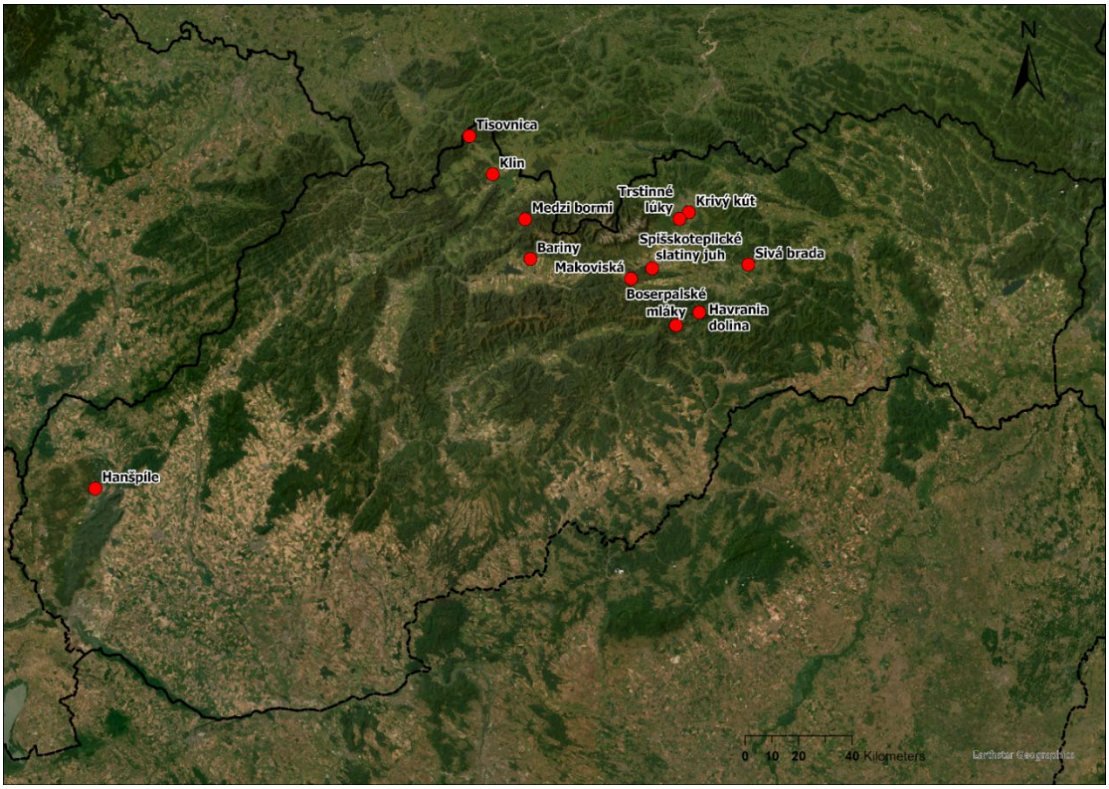

Figure 1. ECORESP-C study sites in Slovakia.

2.1 Carbon stocks

Carbon stocks in peat were estimated from all sites using the online tool CarbonViewer (https://carbonviewer.nina.no/, Cretois et al. 2023). It was developed in Norway to provide an easy-to-use app for land use managers for calculating carbon stocks in peatlands considered for land-use change, e.g. road construction. It allows for estimations of the total peat volume at a given site and then use peat properties (bulk density and carbon content in peat) to calculate the total carbon stock.

Multiple peat depth measurements are needed from a given peatland area to estimate a reliable peat volume (Kyrkjeeide et al. 2023). Thus, we measured peat depth with regular intervals at each site, ranging from 10 meters between measurements at small sites and 30 meters at large sites. Depths were taken using a peat probe (Hisco). The peat probe was inserted into the peat soil until it hit solid ground. Then, the total length of the peat probe in ground was recorded together with the coordinates of the measuring spot. An Excel table with coordinates and peat depth was prepared as an input file for the CarbonViewer tool. In addition, a polygon (e.g. shapefile) of the site is needed. This was made using the coordinates from peat depths measurements. We used the coordinates in ArcGis Pro to draw the polygon of each site. Some sites had more than one polygon, as they had more than one habitat type (e.g. fen and bog) or they had several smaller sites within a larger site. We used aerial photos to draw the boundary line of the polygons and included the area that had the same habitat type. Both input files, the shapefile with a polygon and the Excel file with the depths, were uploaded for each site in CarbonViewer for the calculation of the peat volumes.

Furthermore, soil samples were collected at three sites to provide soil property data for carbon stock calculations. Soil samples were taken from at least three spots at each site and at several depths where possible (Table 2). The site Klinské Rašelinisko (Klin) is a large bog that is drained in the upper layer, at least down to 50 cm. Spišskoteplické slatiny had shallow peat, and the first sample was taken from highly degraded peat. To provide data from fens, we sampled at Belianske lúke, a peatland that was restored about 20 years ago (Grootjans et al. 2012). The samples were taken by digging a hole in the peat and cutting out a block measuring 10x10x10 cm, which gives a litre of peat. At the deepest depth in Klin, we used a peat corer (Eijkelkamp) to extract the sample. Samples were measured for their bulk density (BD; weight of defined volume of dry sample) after drying them in 105C for 24hrs and for their organic matter content (loss of ignition; LOI), by combusting the samples in 350C for 6hrs.

Table 2. The overview shows sites where peat was sampled for peat properties analyses in the lab, the habitat, state of the site, and the depth where the sample were taken.

ID	Site	Habitat	State	Sample ID	Depth (cm)	Sample volume
5	Klinské rašelinisko	Bog	Drained	Klin_1_1	0-10	1 L
5	Klinské rašelinisko	Bog	Drained	Klin_1_2	10-20	1 L
5	Klinské rašelinisko	Bog	Drained	Klin_1_3	20-30	1 L
5	Klinské rašelinisko	Bog	Drained	Klin_2_1	0-10	1 L
5	Klinské rašelinisko	Bog	Drained	Klin_2_2	20-30	1 L
5	Klinské rašelinisko	Bog	Drained	Klin_2_3	40-50	1 L
5	Klinské rašelinisko	Bog	Drained	Klin_2_4	130-160	318 mL
5	Klinské rašelinisko	Bog	Drained	Klin_2_5	170-200	318 mL
5	Klinské rašelinisko	Bog	Drained	Klin_3_1	0-10	1 L
5	Klinské rašelinisko	Bog	Drained	Klin_3_2	20-30	1 L
5	Klinské rašelinisko	Bog	Drained	Klin_3_3	150-180	318 mL

5	Klinské rašelinisko	Bog	Drained	Klin_3_4	190-220	318 mL
10	Spišskoteplické slatiny	Fen	Drained	Spis_1_1	5-15	1 L
10	Spišskoteplické slatiny	Fen	Drained	Spis_2_1	5-15	1 L
10	Spišskoteplické slatiny	Fen	Drained	Spis_3_1	0-10	1 L
-	Belianske lúky	Fen	Restored	M78_1_1	0-10	1 L
-	Belianske lúky	Fen	Restored	M78_1_2	15-25	1 L
-	Belianske lúky	Fen	Restored	M78_1_3	40-50	1 L
-	Belianske lúky	Fen	Restored	SAR1_2_1	0-10	1 L
-	Belianske lúky	Fen	Restored	SAR1_2_2	20-30	1 L
-	Belianske lúky	Fen	Restored	SAR1_2_3	70-80	106 mL
-	Belianske lúky	Fen	Restored	SAR1_2_4	85-100	159 mL
-	Belianske lúky	Fen	Restored	R3_1_1	0-10	1 L

2.2 Biodiversity sampling and bioinformatics

Two types of samples, water and soil, were collected from eight of the sites included in ECORESP-C and Belianske lúky for eDNA analyses (Table 3).

Table 3. Overview of sites sampled for eDNA analyses, number of samples retrieved per type, volume of water filtered per filter (see methods section) and the main habitats sampled within each site.

ID	Site	Number of soil samples	Number of water samples	Litres per filter	Habitats sampled
1	Bariny	3	3	0.7 - 2L	Fen, Reed and Wetland
-	Belianske lúky	3	3	1.3 - 2.3 L	Natural fen, restored fen and rich fen
5	Klinské rašelinisko	2	3	0.5 - 1L	Bog and fen
7	Makoviská	3	3	1 -2L	Young forest peatland with and with no tree cover
8	Medzi bormi	2	2	1L	Bog and fen
10	Spišskoteplické slatiny	3	3	2 – 2.2L	Degraded fen and fen
11	Tisovnica	3	3	0.8 - 2L	Degraded bog and forested bog with pine or spruce
12	Trstinné lúky	2	2	1 - 2L	Restored fen and degraded forest peatland
	Negative samples		3		
	Total	**21**	**25**		

Samples within each site were collected in different habitats to maximize the biodiversity retrieved. Each soil sample was obtained by placing two 30-meter measuring tapes perpendicular to each other (**Figure 2**) to delineate two perpendicular transects. Four subsamples per transect (n = 8) were collected using a sterilized knife at 5 and 10 metres from the intersection, then pooled

together and homogenized by hand inside a plastic bag (**Figure 3**). A 100 mL subsample of soil was then transferred to a sterile plastic bag labelled with a unique ID for the site-sample. A total of 21 soil samples were kept as cold as possible during the field campaign, then transported to the NINAGEN laboratory facilities where they were stored at 4°C until further processing. Water samples were obtained from several small ponds per site using a peristaltic pump with sterile silicone tubbing, NatureMetrics GF 5.0/PES 0.8µm filters and 1L plastic buckets to collect water (**Figure 4**). A variable volume of water was filtered, depending on how fast the suspended particles clogged the filter. A total of 22 water samples and 3 negative samples (air pumped for 10 seconds) were taken. All the filters were then filled with 1.5 mL of ATL buffer, properly labelled and preserved at room temperature for further processing.

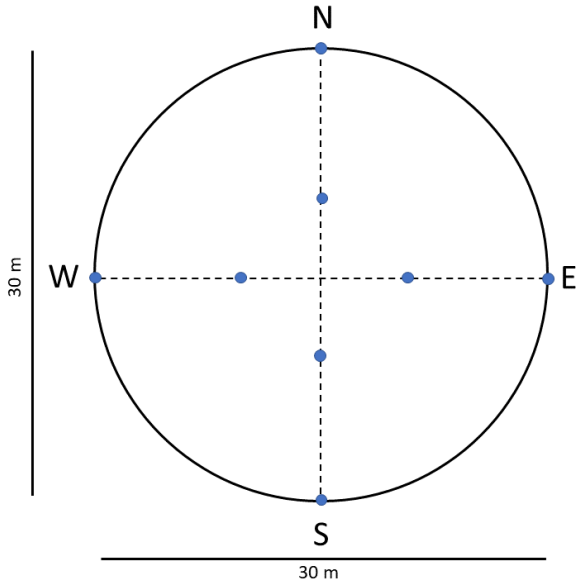

Figure 2. eDNA samples were taken along two transects at each site.

Figure 3. Soil samples for eDNA analyses were homogenized by hand and transferred to a sterile plastic bag.

Figure 4. Water samples were taken from open water bodies at the study sites.

DNA was extracted from soil samples using the FastDNA Spin Kit for Soil in 50ml tubes (MP Biomedicals). Up to 10 g of soil from each sample was transferred to a 50ml Garnet Lysing Matrix Tubes, and 9.8ml of Sodium Phosphate buffer and 1.2ml of MT buffer were added. The contents were homogenized on a Fastprep96 machine at 1600rpm for 40s. The tubes were then centrifuged for 15min at 2900rpm. After centrifugation, 1ml of supernatant was transferred to a 2ml Eppendorf tube with 250µl of Protein Precipitation Solution and mixed by hand for 10s before continuing with the FastDNA SPIN Kit for Soil for 2ml tubes according to the manufacturer's specifications. DNA was finally eluted in a 1.5ml eppendorf tube with 200µl AE buffer (Qiagen) at 55°C. DNA amplification by PCR used the primer combination fITS7/ITS4 (Ihrmark et al. 2012, White et al. 1990) to target the ITS2 region of fungi. The fITS7/ITS4 primers were modified to include overhang sequences allowing them to be used in a a two-step Illumina library contstruction protocol. The PCR conditions for the first PCR followed those specified in Ihrmark et al. 2012, and White et al. 1990. In the second PCR step, we dual-indexed Illumina-tailed amplicons, using IDT for Illumina DNA/RNA UD indexes (Illumina) under PCR conditions with a heated lid, 95 °C for 3 min, followed by a total of 8 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. We visualised the PCR products on a Tape Station (Agilent 4200, Agilent, Santa Clara, CA, USA) to check for amplification success and cleaned the amplicons using magnetic beads (MAGBIND TotalPure NGS, Omega Bio-Tek Inc., Norcross, GA, USA) after each PCR step. Finally, the indexed amplicons were normalized based on Tape Station concentrations and pooled into libraries for sequencing using the Illumina NovaSeq platform at the Norwegian Sequencing Centre (NSC) in Oslo. The sequences obtained from NSC were quality filtered and denoised using the dada2 package (Callahan et al., 2016) to obtain amplicon sequence variants (ASV). Then, to determine the taxonomic level for each ASV, we used the machine learning algorithm IDTAXA together with the UNITE database (Nilsson et al. 2018), and BLAST searches against GenBank. The ASVs were then assigned trophic information and nutritional modes based on the FUNguild database through the FUNguildR package in R (Nguyen et al. 2016).

2.3 Statistics

Total ASV richness per sample was estimated using abundance-based predictions of unobserved diversity derived from rarefaction curves using the iNEXT package in R (Hsieh et al.

2016). Variation in estimated ASV richness within and between sites was then visualized with boxplots.

Non-metric multidimensional scaling (NMDS) analysis was used to investigate variation in community composition (betadiversity) among the sites. The metaMDS function of the vegan package in R was used to analyse an abundance-based Bray-Curtis dissimilarity matrix assuming 3 dimensions.

To compare ASV richness between the restored fen and the other fen types while accounting for uneven sampling effort, we calculated the estimated shared species between the restored fen and each of the other fen types based on incidence data using the Chao2-shared estimate in the SpadeR R-package (Chao et al. 2016).

3 Results

3.1 Carbon stocks

Carbon stocks in peat were computed for 20 polygons at 12 sites. Overview of overview of area, volume of peat, mean depth, number of depth measurements and the carbon stock of each polygon is presented in Table 4. The total carbon stock of all study sites is 72 915 tons of C, which equals 267 598 tons of CO_2 or the emissions of 43 868 Slovakians in 2023 (Global Carbon Budget 2023).

Table 4. Overview of study sites, number of polygons drawn from each site and used in carbon calculations, number of peat depths measurements, the area at the site used for carbon calculations, the volume estimated from each site, and the total carbon stock calculated.

ID	Site	Polygons	Number of depth measurements	Area (m^2)	Volume (m^3)	Carbon stock (ton C)*
1	Bariny	2	106	42033	48127	2812
2	Boserpalské mláky	1	147	56203	16718	977
3	Hanšpíle	1	220	90033	91778	5363
4	Havrania dolina	1	67	32079	13705	801
5	Klinské rašelinisko	2	118	154496	332282	19419
6	Krivý kút	1	142	89369	98003	5727
7	Makoviská	3	97	165013	73877	4317
8	Medzi bormi	2	122	76858	53480	3126
9	Sivá brada	2	142	44178	36256	2119
10	Spišskoteplické slatiny juh	3	86	26683	9211	538
11	Tisovnica	1	176	140363	302459	17676
12	Trstinné lúky	1	273	255856	171802	10040
	Total		1696	1173164	1247698	72915

*Calculations are based on bulk density and organic matter content from samples of continental Europe given in Table 2 in Loisel et al. 2014.

The sites with the deepest peat layers and largest volumes and carbon stocks, are the two bog sites (Figure 5). There is a large variation in average depths at the sites (Figure 6), with the bog sites having the deepest peat. Klinské rašelinisko is a raised bog (Figure 7) with fen at the eastern margin. The depth here were measured down to 4,2 meters and the average were 2 meters. The carbon stock is estimated to 19 419 tonn C. At Tisovnica (Figure 8) is a forested bog with depths at 4,5 meters and an average depth of 2,2 meters (Figure 6).

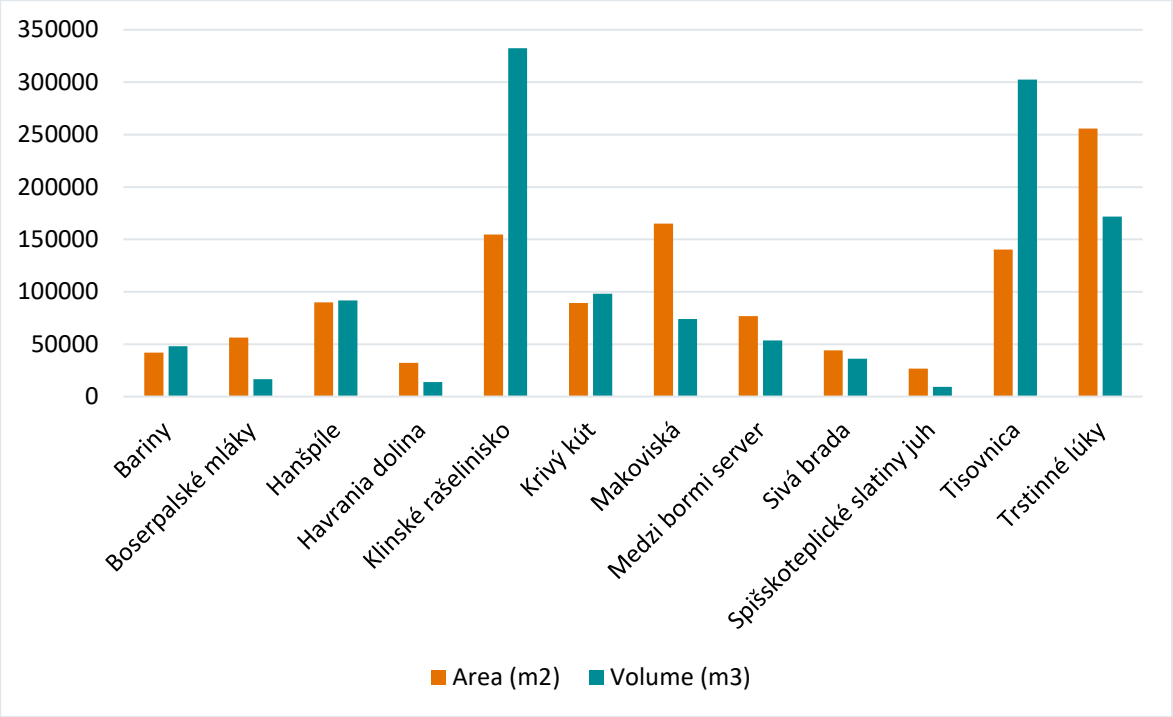

Figure 5. The area (m^2) and volume (m^3) of peat at at ECORESP-C study sites.

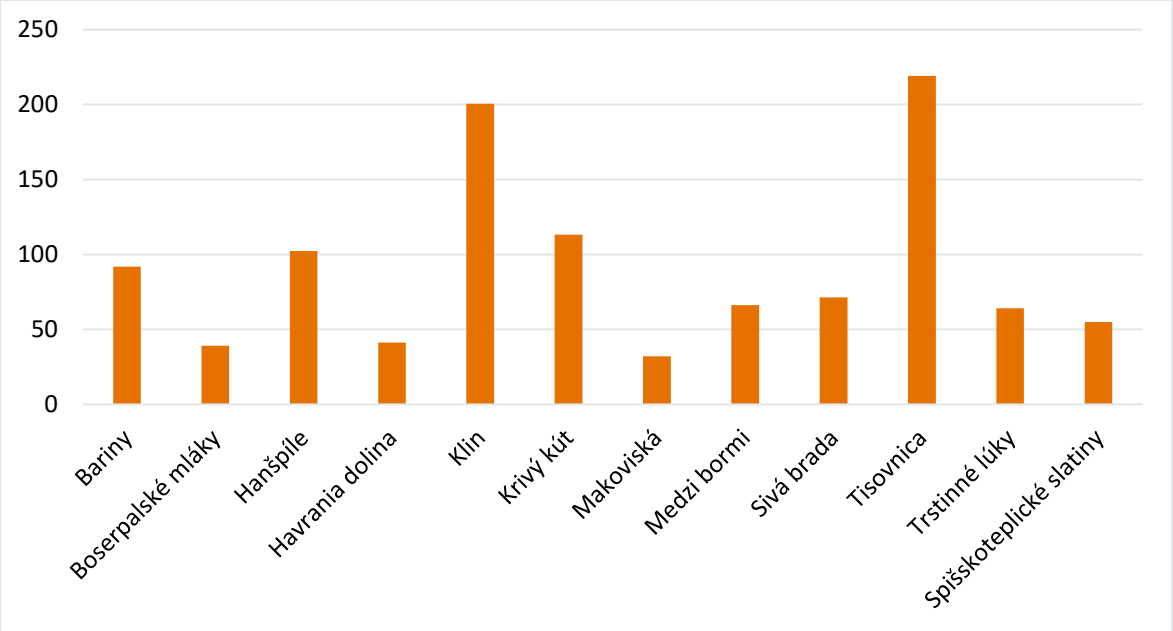

Figure 6. Average peat depths (cm) at ECORESP-C study sites.

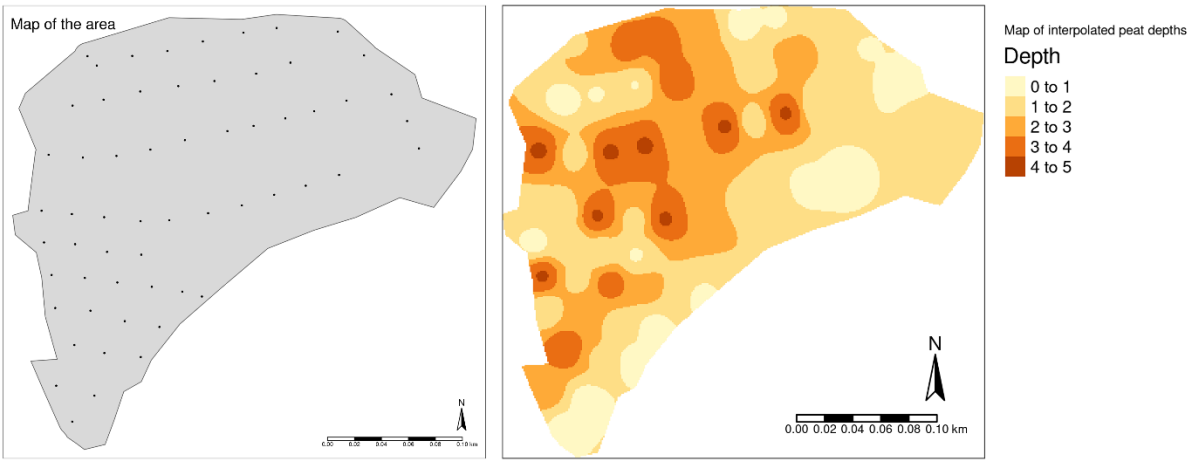

Figure 7. Map of the bog at Klinské rašelinisko (fen at the eastern margin not included in polygon) showing where peat depths measurements were taken (left) and the interpolated depth at the site (right) with darker color indicating deeper peat.

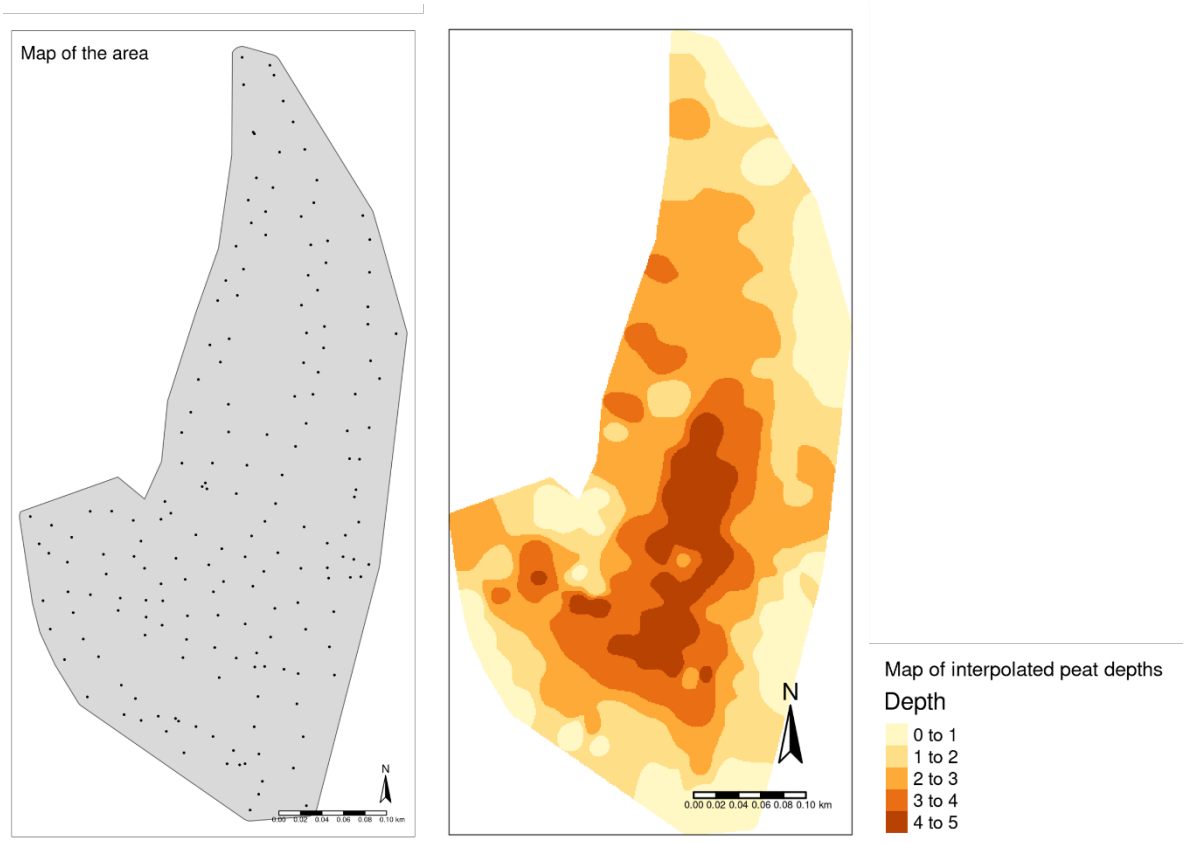

Figure 8. Map of the bog forest in Tisovnica showing measurement points for peat depth(left) and the interpolated depth at the site (right) with darker color indicating deeper peat.

Peat properties of samples from three sites (two study sites and one reference site) are shown in Table 5. The mean BD of peat samples collected were 0.137, 0.306, and 0.183 kg/dm3 at Klinské rašelinisko, Spišskoteplické slatiny and Beliansky lúky, respectively. The average LOI were 94.7 46.5 andand 75.5 % at Klinské rašelinisko, Spišskoteplické slatiny and Beliansky lúky, respectively. Spišskoteplické slatiny had the most degraded peat (sample Spis_1_1).

Using *in situ* data from ECORESP-C project for BD and LOI, results ~6-10% higher estimates for carbon stocks compared to those computed with default values from literature (Loisel et al. 2014).

Carbon stock of the bog at Klinské rašelinisko (the area shown in Figure 4) was 6719 tonn C using Loisel et al. (2014), but 7458 tonn C using the project's results (Table 5). Likewise, for Spišskoteplické slatiny, the carbon stock increases from 517 using Liosel et al. (2014) to 840 tonn C with project's results (Table 5).

Table 5. Overview of study sites site, sample IDs, volume of peat, bulk density, pH, Loss on ignition (LOI), gram of CaCO-C/cm^3, gram soil organic carbon per cm^3 and gram Soil organic carbon+TIC per cm^3 for all peat samples taken at two ECORESP-C study sites and at the re-stored site.

Site	Sample ID	Sample vol (L)	Bulk density (g/cm3)	pH (H_2O)	LOI 350 °C (%)	gCaCO3 -C/cm^3	gSOC/cm^3	gSOC+ TIC/cm^3
Klinské rašelin-isko	Klin_1_ 1	1	0.105	3.3	94.6		0.050	0.050
Klinské rašelin-isko	Klin_1_ 2	1	0.109	3.3	90.0		0.049	0.049
Klinské rašelin-isko	Klin_1_ 3	1	0.208	3.3	90.8		0.095	0.095
Klinské rašelin-isko	Klin_2_ 1	1	0.081	3.2	93.7		0.038	0.038
Klinské rašelin-isko	Klin_2_ 2	1	0.260	3.1	93.8		0.122	0.122
Klinské rašelin-isko	Klin_2_ 3	1	0.086	3.0	97.5		0.042	0.042
Klinské rašelin-isko	Klin_2_ 4	0.318	0.122	3.4	97.6		0.059	0.059
Klinské rašelin-isko	Klin_2_ 5	0.318	0.102	3.7	97.2		0.050	0.050
Klinské rašelin-isko	Klin_3_ 1	1	0.172	3.3	89.2		0.077	0.077
Klinské rašelin-isko	Klin_3_ 2	1	0.149	3.1	97.3		0.072	0.072
Klinské rašelin-isko	Klin_3_ 3	0.318	0.110	3.5	97.9		0.054	0.054
Klinské rašelin-isko	Klin_3_ 4	0.318	0.141	3.8	97.2		0.068	0.068

Spišskoteplické slatiny	Spis_1_1	1	0.290	6.6	65.5	0.002	0.094	0.096
Spišskoteplické slatiny	Spis_2_1	1	0.272	7.3	43.5	0.002	0.058	0.060
Spišskoteplické slatiny	Spis_3_1	1	0.356	7.8	30.6	0.003	0.053	0.056
Belianske lúky	M78_1_1	1	0.136	6.8	72.4	0.001	0.049	0.050
Belianske lúky	M78_1_2	1	0.165	5.7	82.0		0.068	0.068
Belianske lúky	M78_1_3	1	0.242	6.0	73.6		0.089	0.089
Belianske lúky	SAR1_2_1	1	0.148	7.3	74.3	0.001	0.054	0.055
Belianske lúky	SAR1_2_2	1	0.229	6.2	76.3		0.087	0.087
Belianske lúky	SAR1_2_3	0.106	0.231	6.3	75.6		0.087	0.087
Belianske lúky	SAR1_2_4	0.159	0.153	6.3	82.8		0.063	0.063
Belianske lúky	R3_1_1	1	0.157	6.0	66.7		0.053	0.053

3.2 Biodiversity – Fungi

The total dataset comprised 8,638,959 reads. After the bioinformatic processing (quality control and filtering, demultiplexing, denoising and taxonomic assignment), the final dataset consists of 5,052,148 reads distributed in 3,871 Amplicon Sequence Variants (ASVs) assigned to fungi taxa. Figure 9 shows the total number of ASV found in the different localities. The number of ASV present in Belianske Lúky, the restored fen, represents ~12% of the total ASVs in the dataset. Moreover, it shows between 43% and 49% less ASV richness than the three localities with the highest ASV diversity.

In this dataset, trophic mode and nutritional strategies could be assigned to only ~13% of the ASVs, with 3,376 ASVs remaining unassigned. However, the proportion of ASVs that can be successfully assigned is similar across localities (Figure 10), and such comparisons of nutritional strategies across localities are limited to only those ASVs for which trophic mode data is available (Figure 11).

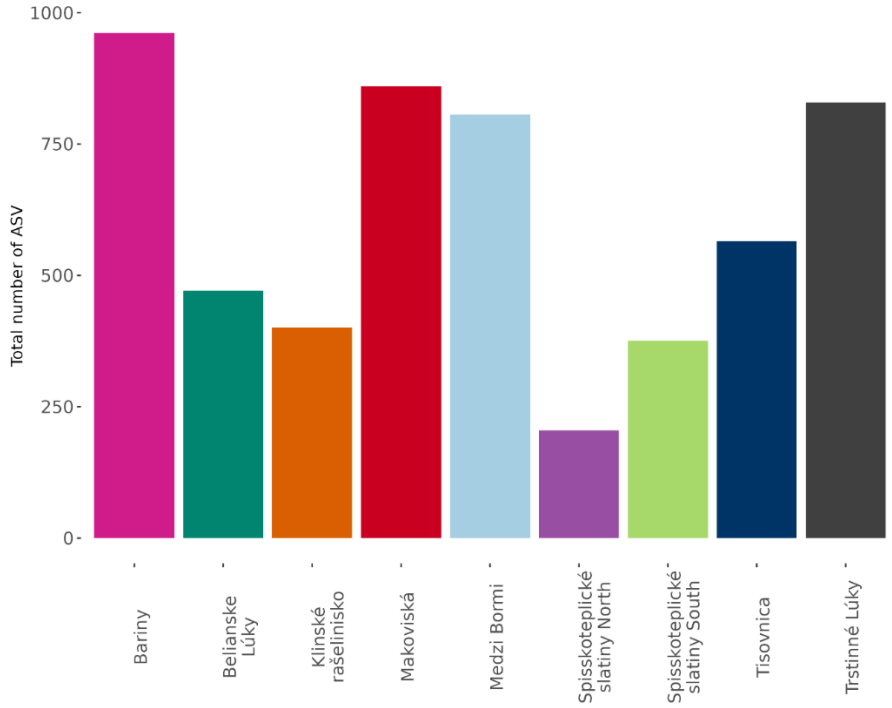

Figure 9. Bar plot showing the total number of ASV present at each peatland site.

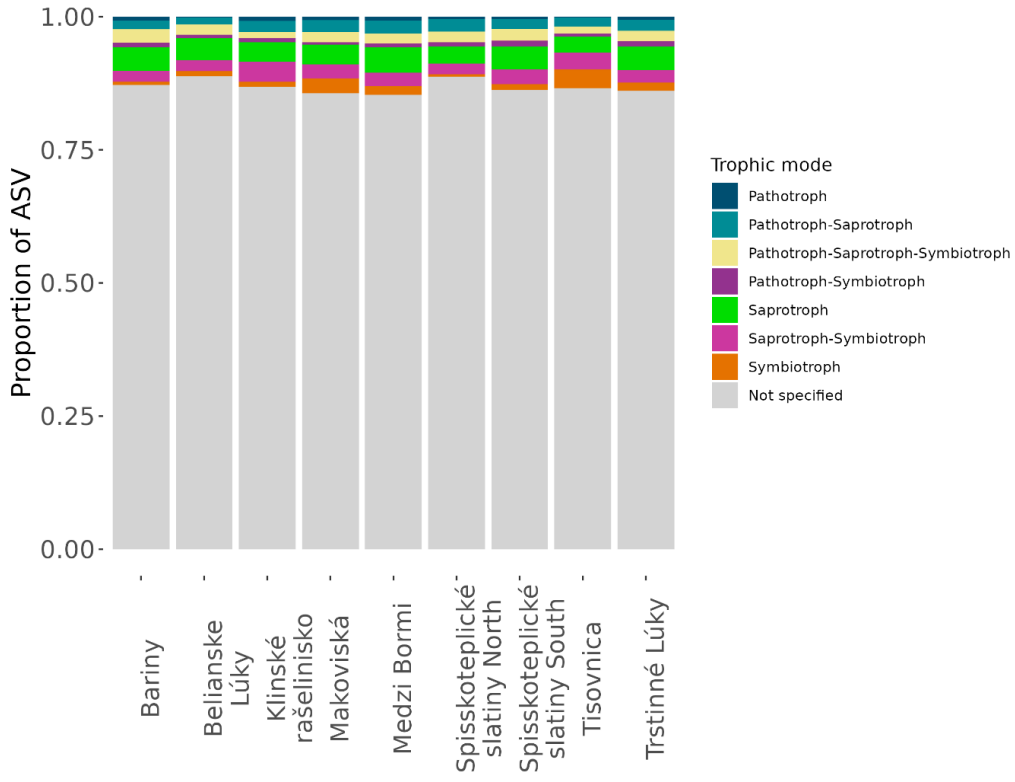

Figure 10. Bar plot depicting the proportion of ASV from seven trophic modes per peatland site, together with the proportion of ASV with no assignment for trophic mode on the reference database.

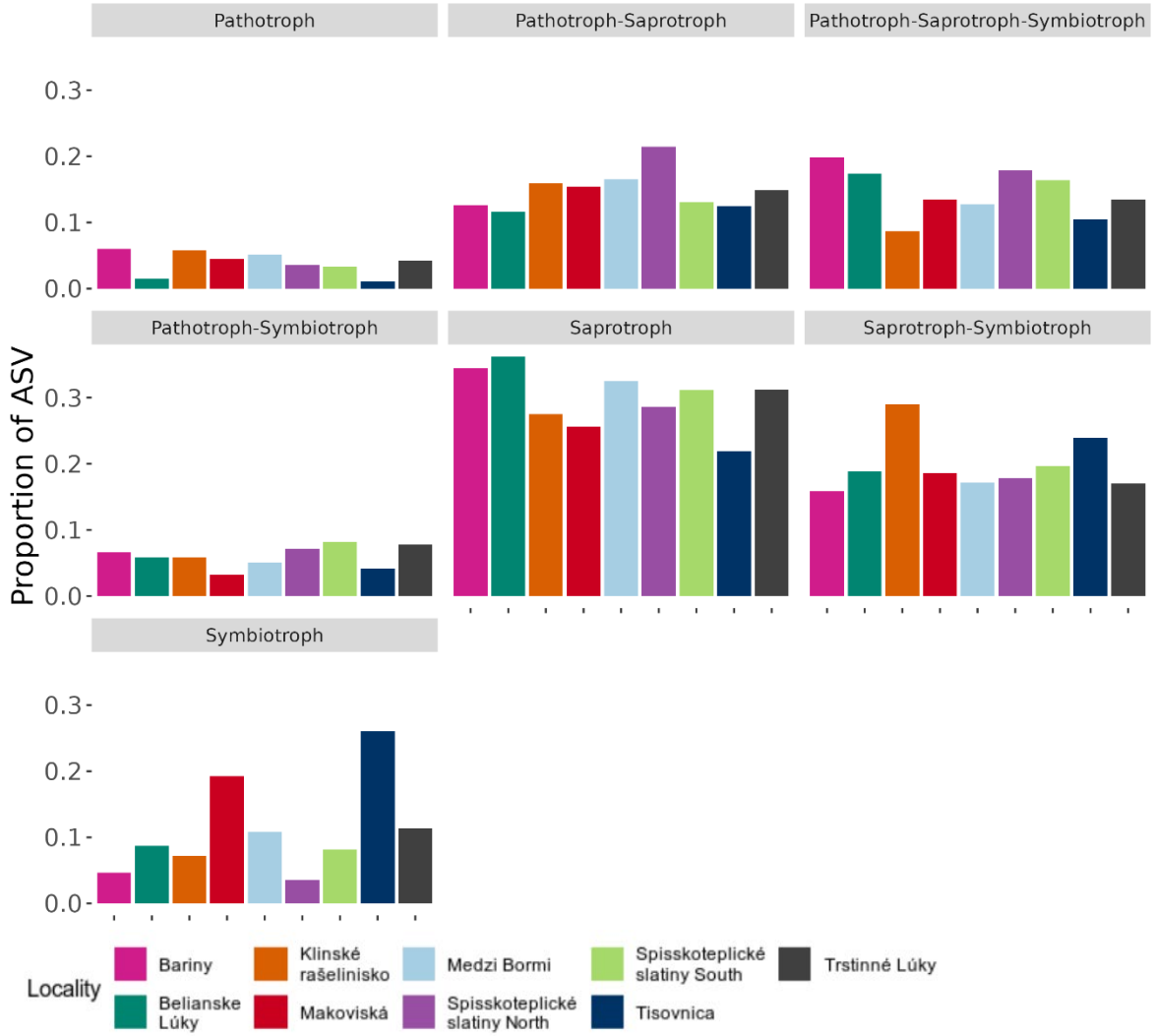

Figure 11. Bar plots showing the proportion of ASVs per peatland locality across seven trophic modes. Each panel represents a different trophic mode and each bar represents the proportion per locality.

The most predominant nutritional strategy for fungi across localities is saprotrophy, followed by the combination of saprotrophy and symbiotrophy. Tisovnica and Makoviská, with habitats classified mostly as forested bog and forest swamp, exhibit the largest proportion of symbiotroph ASV. Additionally, Klinské rašelinisko and Tisovnica, the two bogs in the dataset, have the largest proportion of saprotroph-symbiotroph ASV. The differential proportions in these three localities align with the localities included in cluster two in the nMDS (Figure 13).

Belianske Luky, the restored fen, has 230 unique ASVs, that is, ~49% of its ASVs are not found in other localities. However, only 20 of those ASVs have a trophic mode assigned in the reference database. That represents an insufficient number to elucidate if the ASVs that differentiate from the restored fen provide an important function in healthy fens.

NMDS analysis (Figure 12) shows variation in community composition within localities and only weak clustering by locality. This result is aligned with the sampling design and with the goal of the biodiversity assessment: to collect samples from different habitats within each locality to maximize the biodiversity captured.

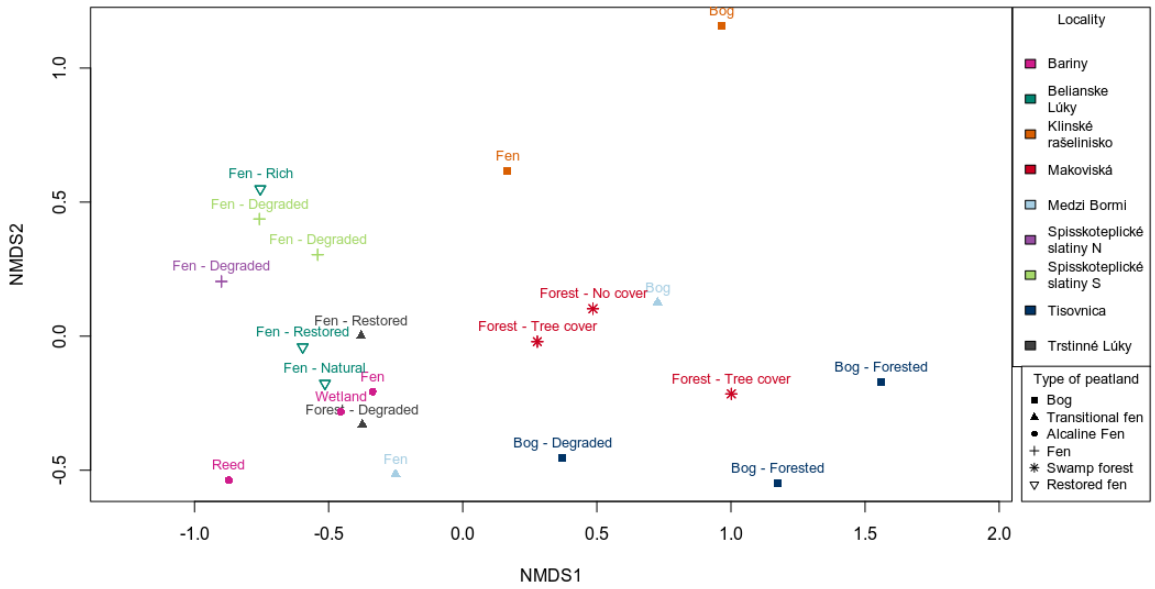

Figure 12. nMDS graph with the spatial ordination of each soil sample based on a dissimilarity matrix. Localities are in different colors and the six types of peatlands presented as shapes. The distance used to calculate the dissimilarity matrix was Bray-Curtis, the engine was isoMDS and k was 3. The stress value was 9.36.

Therefore, adding information on the habitats sampled within locality suggests the existence of two clusters in the spatial structure displayed. Figure 13 shows the area of the ellipses of the clusters calculated with a 95% confidence interval. One cluster comprises mostly the habitats classified as fen (in yellow), whereas the second cluster comprises mostly the habitats classified as bog and forest (in purple). It is worth noting that the sample obtained in a bog habitat inside Medzi Bormi, which is classified as a transitional (likely poor) fen as the predominant type, falls inside the second cluster. Nevertheless, in Klinské rašelinisko, classified as a bog, the sample obtained in a fen habitat at the edge of the bog, falls close yet outside the 95% confidence interval of the 'fen cluster'.

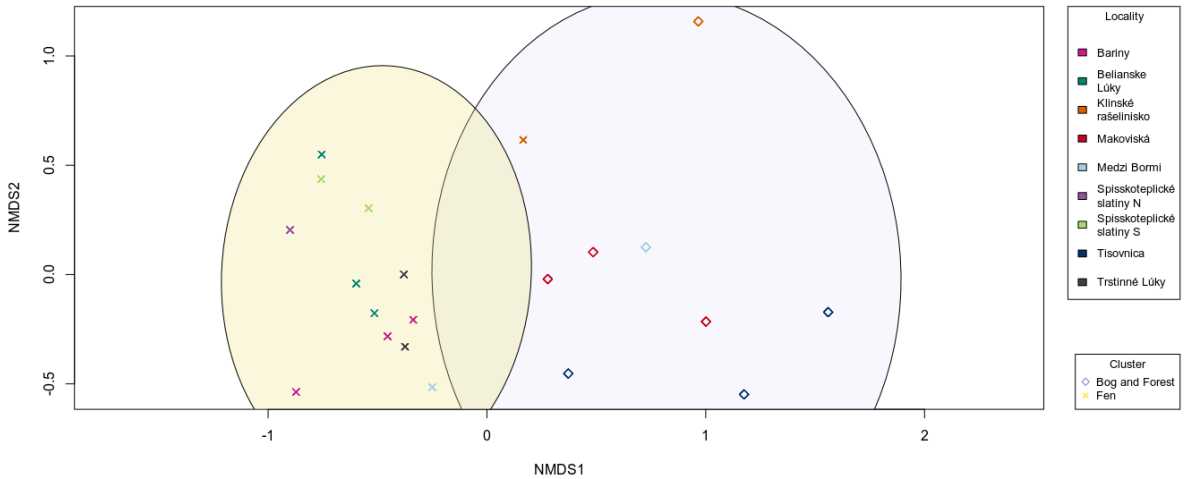

Figure 13. nMDS graph with the spatial ordination of each soil sample based on a dissimilarity matrix and two clusters calculated using a 95% confidence interval. The area of the clusters is shown in yellow (fen) and in purple (bog and forest). Localities are shown in different colors, whereas the shape of the points reflects the categories used to infer the clusters. The distance used to calculate the dissimilarity matrix was Bray-Curtis, the engine was isoMDS and k was 3. The stress value was 9.36.

The boxplot in Figure 14 illustrates the ASV richness estimates per sample within locality, integrating sampling effort, abundance data and predictions of unobserved diversity derived from rarefaction curves. The mean ASV richness per locality ranges between 250 in Spisskoteplické slatiny North and over 600 ASV in Medzi Bormi. When adding information about the type of peatland, transitional fens and the alkaline fen have the highest mean diversity values, followed closely by the swamp forest. Conversely, the degraded fens have 2.5 times less ASV diversity per sample, while the bog localities fall in an intermediate category, with mean values in the range of 300-350 species. The restored fen has a mean diversity of ~350 ASV, a value between the richest fens and the degraded fens. All sites show considerable variability in species richness per sample (variation of 50 or more species per sample). Notably, the range in species richness in the restored fen samples is larger than in other localities, with the richest sample hosting more than 8 times the ASV diversity of the poorest sample.

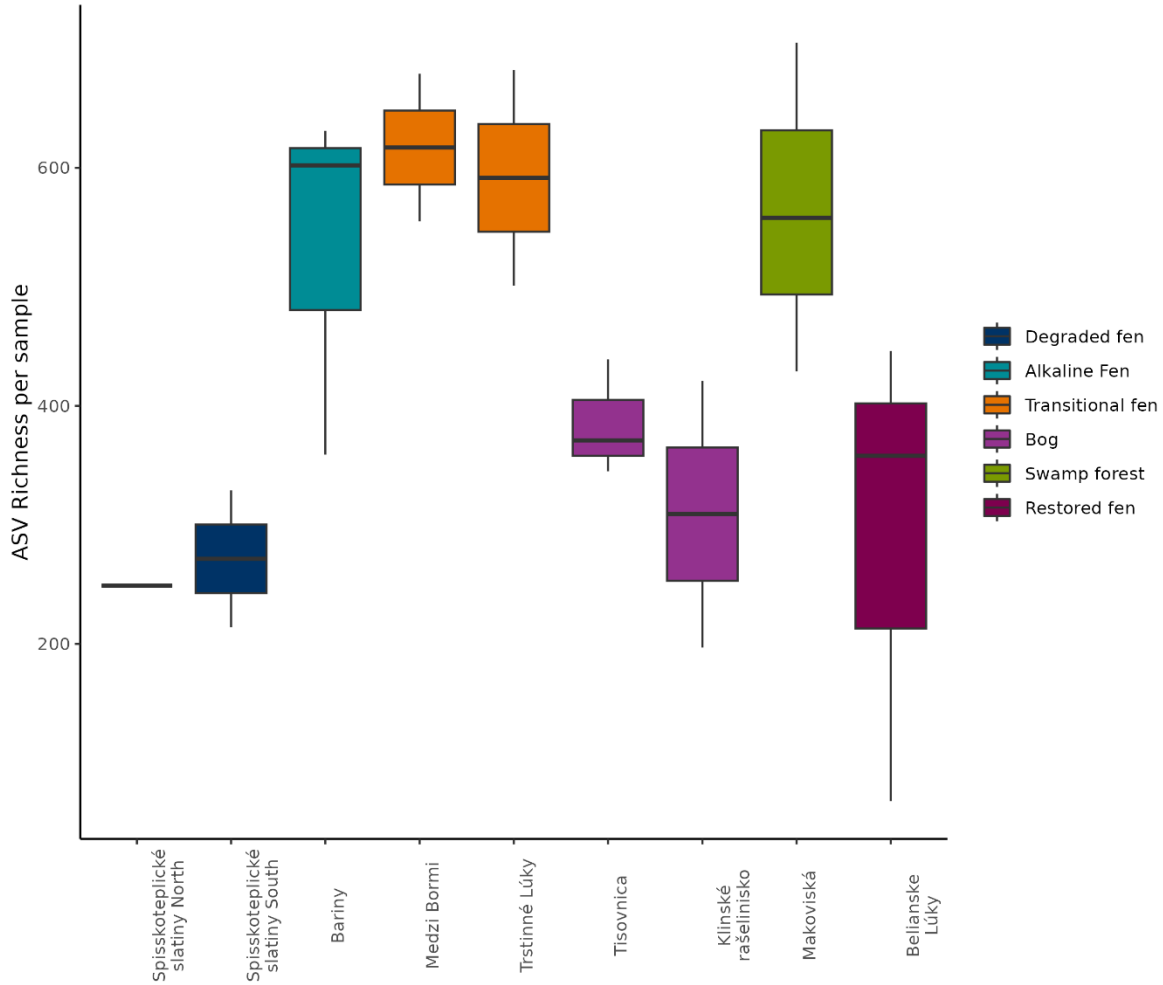

Figure 14. Boxplot showing the localities on the x axis and the estimated ASV richness per sample on the y axis. The colors in the boxes reflect the type of peatland.

Since the values for species richness between the distinct types of fens spread over a broad range, we calculated the number of shared ASV between the restored fen and the other three types. The restored fen has 96, 114, and 154 ASV in common with the degraded fens, the alkaline fen, and the transitional fens, respectively. To account for uneven sampling effort across the different fen types, we further calculated the estimated shared ASV using the Chao2-shared

estimate (Chao et al., 2016) (Figure 15). The restored fen shares on average ~262 ASV with the transitional fens, 55% of its diversity, and substantially fewer with the degraded and alkaline fens (Figure 15).

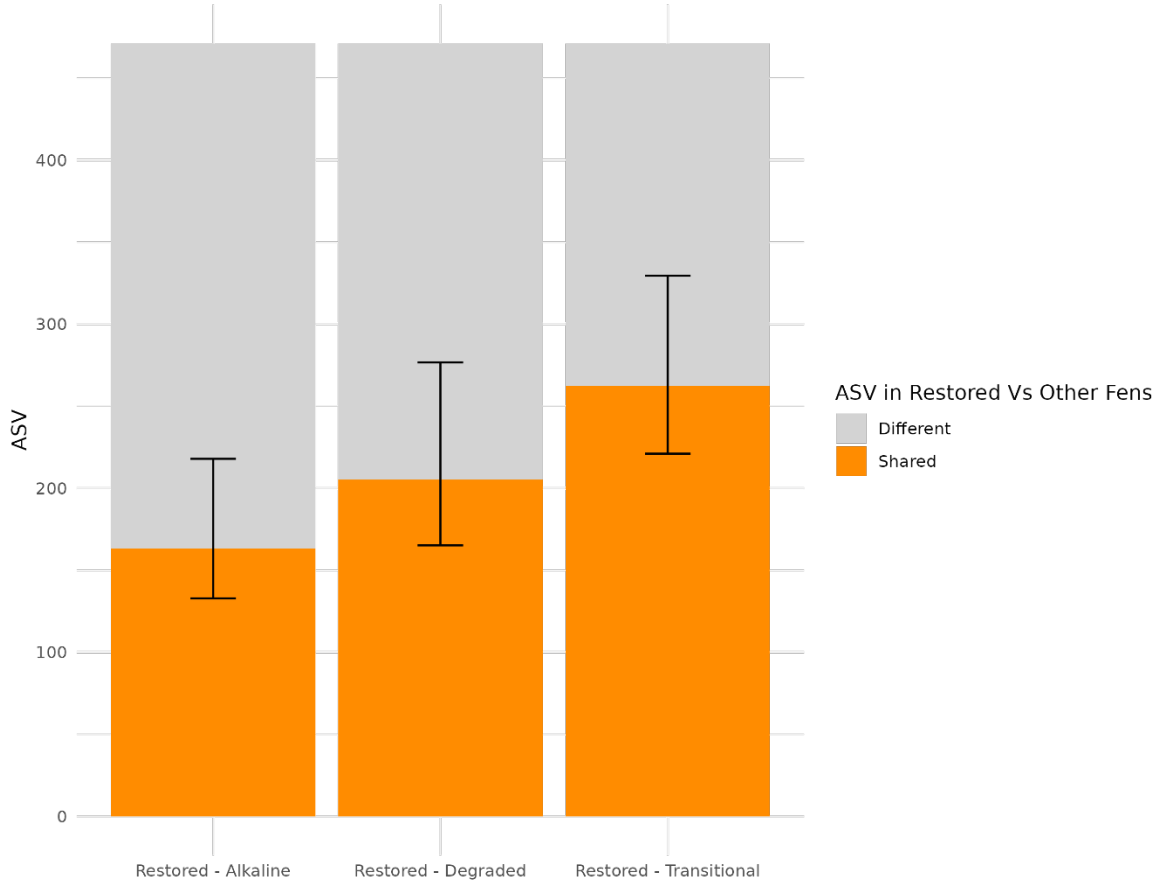

Figure 15. Bar plot showing the estimated number of shared and different ASV between the restored fen and the alkaline fen, the degraded fens and the transitional fens with a 95% confidence interval.

4 Conclusions

Carbon stocks in the ECORESP-C sites are highly variable, as expected. This is due to the large variation in habitats studied in the project, which included fens, bogs, swamp forests, etc. The two sites with the largest carbon stocks are the bog sites (Klinské rašelinisko and Tisovnica). This is not a surprise as the peatland habitats in these sites are ombrotrophic (bogs), i.e. they are rainwater fed and have low levels of nutrients. This is the last successional stage of a developing mire, and their peat depths tend to be very deep. Thus, the carbon stock per area unit becomes substantial. For Tisovnica, there is also a considerable carbon stock in the aboveground biomass as the bog is forested. Thus, the carbon stock calculated in this project should be considered a minimum. This is also the case for the swamp forest sites assessed, as we only included carbon stock in the soil.

We measured soil properties in samples taken at two study sites. Our samples from Klinské rašelinisko (bog) and Spišskoteplické slatiny (fen and degraded fen) are not representative for all study sites. Therefore, to estimate carbon stocks more accurately, we used soil property values from Loisel et al. 2014, as this dataset consists of significantly more sites than what was included in this project. However, using the soil properties collected *in situ* at the bog (Klinské rašelinisko) yields a higher carbon content than using the dataset in Loisel et al. (2014). Uncertainties in the estimates for global peatland carbon stocks is being debated, and our results highlight the need for *in situ* measurements to accurately determine the amount of carbon peatlands store (Nichols & Peteet 2019, Yu et al. 2021).

Due to their small area, peatlands in Slovak Republic do not contribute significantly to national GHG emissions. When calculating GHG emissions using IPCC default emission factors for the project's study sites as degraded (current status) and as restored (anticipated status), the reduction to GHG emissions remains negligible at national level. Restoring nutrient poor bogs will be, to a degree, climatically more beneficial than restoring fens. However, when calculating the annual losses of soil carbon using the same emission factors and subtracting those from now comparatively accurately determined carbon stocks, it is likely that most of the project's study sites are lost within a century, if not restored. With them, most ecosystem services provided by those habitats will also be lost. Restoring the peatlands will increase the lifetime of nutrient rich ecosystems significantly and will render the nutrient poor ecosystems in practice eternal. Even though the relative insignificance of peatlands in national GHG inventory of Slovak Republic, these habitats are unique and often threatened and should, thus, be prioritized for peatland restoration efforts based on other ecosystem services, e.g. as habitats for rare species and biodiversity.

Assessing the effectiveness of any biodiversity conservation intervention, including restoration actions, is critical to learn and improve our conservation policies. For a robust assessment of restoration actions, good data on the status of both biodiversity and the abiotic conditions of the sites subject to restoration prior the intervention is essential. Peatlands have particular biodiversity and biogeochemical features, key elements for the ecosystem services that they provide. These characteristics and ecosystem services are reduced (or even disappear) when the conditions of peatlands degrade due to negative anthropogenic impacts.

One of the most important biotic features of peatlands is the microbial biodiversity, including fungi. In ECORESP-C, we provide a baseline dataset on fungi biodiversity in degraded peatlands that will be very valuable in future assessments of the restoration interventions. It is worth noting that no environmental variables were recorded at the time of sampling and, therefore, it is not possible to measure their influence in explaining the results and patterns in our data.

The four sites corresponding to the less degraded fens and the swamp forest have the highest total number of ASV per site and per sample. In that regard, there is high variability in within-site ASV richness, as expected. Our sampling method was designed to maximise biodiversity

monitoring and thus, samples were taken from different habitats within a site; explaining the differences in ASV composition between samples from the same site. When looking at the estimated richness of fungi ASV per peatland type, the less degraded fens and the swamp forest bog have the highest diversity per sample, as expected (fens and swamp forests are expected to have higher diversity). The most degraded fens had considerably lower richness than the less degraded. Opposite to expectations, the restored fen (Belianske Luky) did not show the highest richness levels, but nevertheless higher than the most degraded ones. Possible explanations for finding half the diversity of ASV per sample in the restored site compared to peatlands that are pending to be restored are, the impact of unmeasured environmental factors (i.e. pH, nutrient availability, water fluctuations), e.i. that the restored site may be more homogenous than the degraded ones hosting only peatland specialists, or a genetic bottleneck induced by a severe degradation state prior to the restoration process that requires decades for genetic variability to increase.

Even though information on trophic mode and nutritional strategy was only available for 13% of the ASV, we were able to detect the relevance of saprotrophy and symbiotrophy across sites, two crucial ecosystem functions in peatlands. Saprotrophic fungi decompose dead organic matter and play a crucial role in decomposition of the organic matter that accumulates in the peat fraction from dead plant material. Symbiotroph fungi establish symbiotic relationships with organisms such as plants and trees, and are essential for plants in nutrient-poor environments.

Furthermore, the largest proportion of symbiotroph ASV was found in habitats classified as forested bog and forest swamp. Fungal communities in forested peatlands are predominantly symbiotrophic due to the establishment of ectomycorrhizal associations with plants and trees. This symbiotic interaction is especially valuable in particularly nutrient-poor environments, such as bogs, since fungi help plants enhance their nutrient acquisition. Hence, it also explains why Klinské rašelinisko and Tisovnica, the two bogs in the dataset, have the largest proportion of saprotroph-symbiotroph ASV.

The community composition of the soil samples was best represented by clusters of habitat type rather than of peatland site. Such grouping reflects the purpose of the sampling design, to maximize the biodiversity captured in every locality, but it also highlights the detection of underlying similarities in fungal biodiversity between peatland types.

As of 2023, the restored site has, on average, an estimated 55% of its ASV in common with fens characterized as transitional fens and quaking mires and over 40% in common with fens considered degraded. The status of Belianske Lúky after the restoration process undertaken ~20 years ago provides valuable baseline information to evaluate the restoration activities that are being performed in the seven peatlands studied here, and particularly of the fens.

The high number of ASVs in our dataset without information on their trophic mode and nutritional strategy, indicates that fungi barcode reference databases are still incomplete for many taxa that occur in the study sites. It also highlights the importance of having complete and accurate barcode reference libraries to reliably assign taxonomy in biodiversity assessments performed using eDNA metabarcoding techniques. As this relatively novel technique evolves and matures, it will be possible to routinely assign ASVs to described taxa to species level and, consequently, study biodiversity and species interactions in more detail. The dataset collected here will provide an important contribution to the mapping of the microbiome in Central Europe.

This data will be stored in a database at NINA and be available upon request and for comparison in a potential post-intervention study.

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