AAGE V. JENSEN CHARITY FOUNDATION

PONT | PRESPA OHRID NATURE TRUST

MANAGEMENT

OF **WET MEADOWS** IN **PRESPA**

stakeholder engagement and fulfilling knowledge gaps regarding their **ecology** and **biodiversity**

MACEDONIAN ECOLOGICAL SOCIETY

DECEMBER, 2023

All results presented in this report refer to the component Wetlands, waterbirds & climate within the frame of the project **The Prespa Project - Biodiversity Conservation in Transboundary Prespa** implemented in North Macedonia, funded by Prespa Ohrid Nature Trust. All activities were conducted in the timeframe of 36 months (2021-2023).

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CONTENT

Abbreviations

PONT Prespa Ohrid Nature Trust
NGO Non-Governmental Organisation
NP National Park
MES Macedonian Ecological Society

1 Introduction

Wet meadows are critical ecosystems characterized by their high water table and saturated soil conditions. These habitats support diverse plant and animal life (Poschlod and Biewer 2005), providing a range of essential ecosystem services, such as water filtration, flood control, and carbon sequestration (Cebrián-Piqueras et al. 2017). They play a crucial role in maintaining water quality, regulating water flow (Hefting et al. 2013), and mitigating the impacts of climate change (Potschin and Haines-Young 2016). Wet meadows provide a diverse range of ecological niches, support a variety of wildlife, including migratory birds, amphibians, waterfowl, and numerous plant species. Conservation and sustainable management of wet meadows ensures the provision of vital ecosystem services, benefiting both the local communities and the associated diversity of plant and animal species.

Wet meadows in North Macedonia are mainly distributed in the lowlands. However, intensive agriculture in most of the flat lowland areas contributed to significant degradation of these habitats. In this regards, among the largest preserved areas are located in Prespa region, along the Prespa Lake shoreline. In this region, they have been traditionally used by the local communities, which contributes to their dependency on human activities. On the other hand, in the past several decades the majority of them are impacted by several human-induced stresses such as pollution, drainage and conversion to arable land.

Wet meadows are important, integral part of the diverse Prespa landscape. Therefore, they have received special attention in the previous phase of activities whereas the main outcomes were summarized in the report *Estimation of biomass and recommendation for management of wet meadows in Prespa* (Nikolov et al. 2020). According to this report, the following management recommendations were given:

Removal of overgrowth in selected meadows in order to prevent succession
Stimulation of summer mowing practices and usage of biomass for fodder
Monitoring the changes of biodiversity
Monitoring for the changes in nutrient levels

1.1 Conceptual framework

As continuation of previous work, we have developed a conceptual framework for the period of 2021-2023 (Figure 4). As recommended in the previous report (Nikolov et al. 2020), our main aim was piloting management activities as a measure for successful conservation of these habitats and their biodiversity. However, we wanted to choose the right methodological approach which will promote sustainable management on one hand, but also will provide benefit for the local communities. Even though management was in our main focus, we needed to analyse this process from two perspectives 1) stakeholder's needs, opinions and attitudes and 2) scientific arguments that support this type of management. In this regard, these two aspects needed to be incorporated in our conceptual framework (Figure 1).

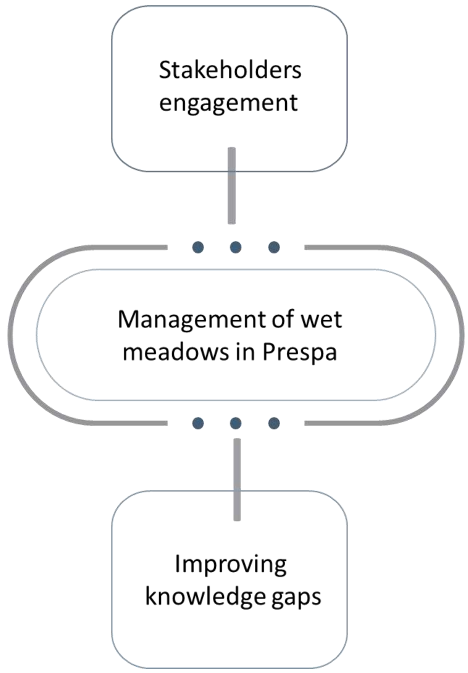


Figure 1 Part of the conceptual framework development

Stakeholder engagement plan was developed and was mainly based on intensive communication and interaction with all stakeholder groups, especially the local stakeholders. The aim of these activities was to primarily network stakeholders through providing common points within the prizma of management (Figure 2). This intended to increase their cooperation, but was also important for future sustainability of these type of management.

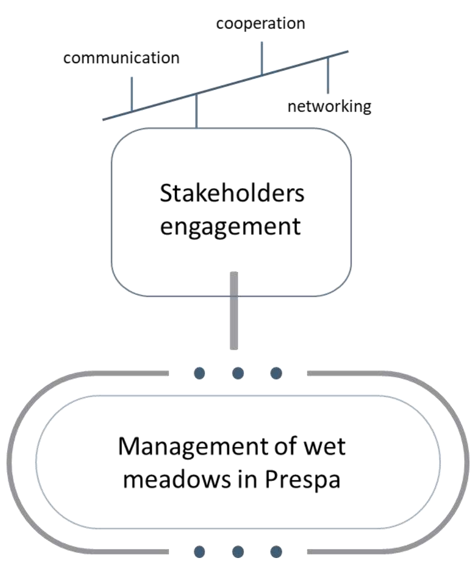


Figure 2 Part of the conceptual framework development

Human influences on wet meadows in Prespa are obvious, but not explored in-depth. To fill this knowledge gap, we have conducted several ecological studies in order to provide science based evidence for better management practices. Alongside, these studies provided insight into the threats and intended to give overview of the overall condition of the meadows along the shoreline. Another perspective was assessment of the impact of the management practices on biodiversity. This was planned to be done by few monitoring activities that needed to also increase the knowledge of wet meadows biodiversity. Among the most important monitoring activities was establishment of a better baseline for vegetation monitoring with high resolution digitalisation of all habitat types. This was accompanied by an inventory of wet meadows (Figure 3).

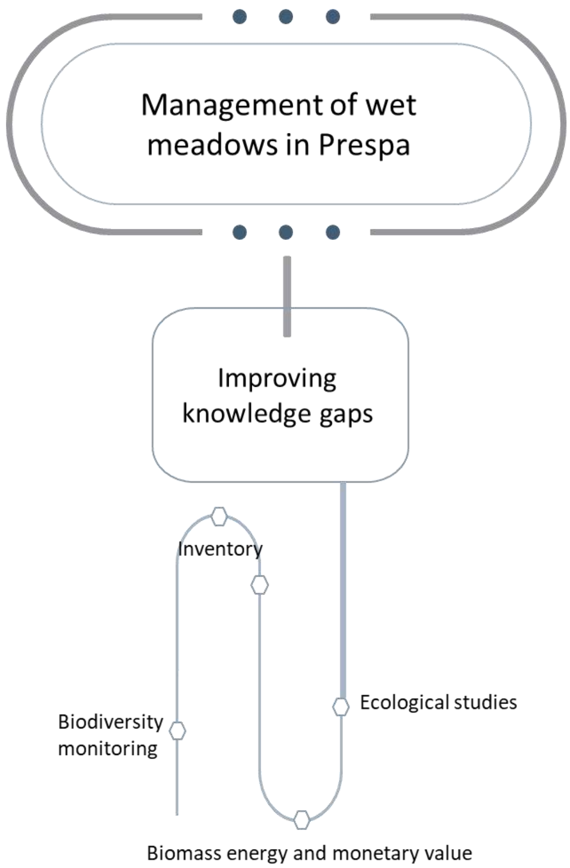


Figure 3 Part of the conceptual framework development

Prespa watershed is positioned between three countries. Habitats do not recognise country borders, therefore we wanted to assess their connectivity in a larger scale. To do so, we have extended the scope for this activity. This assessment is of high significance for future, integrative coordination, management and conservation actions.

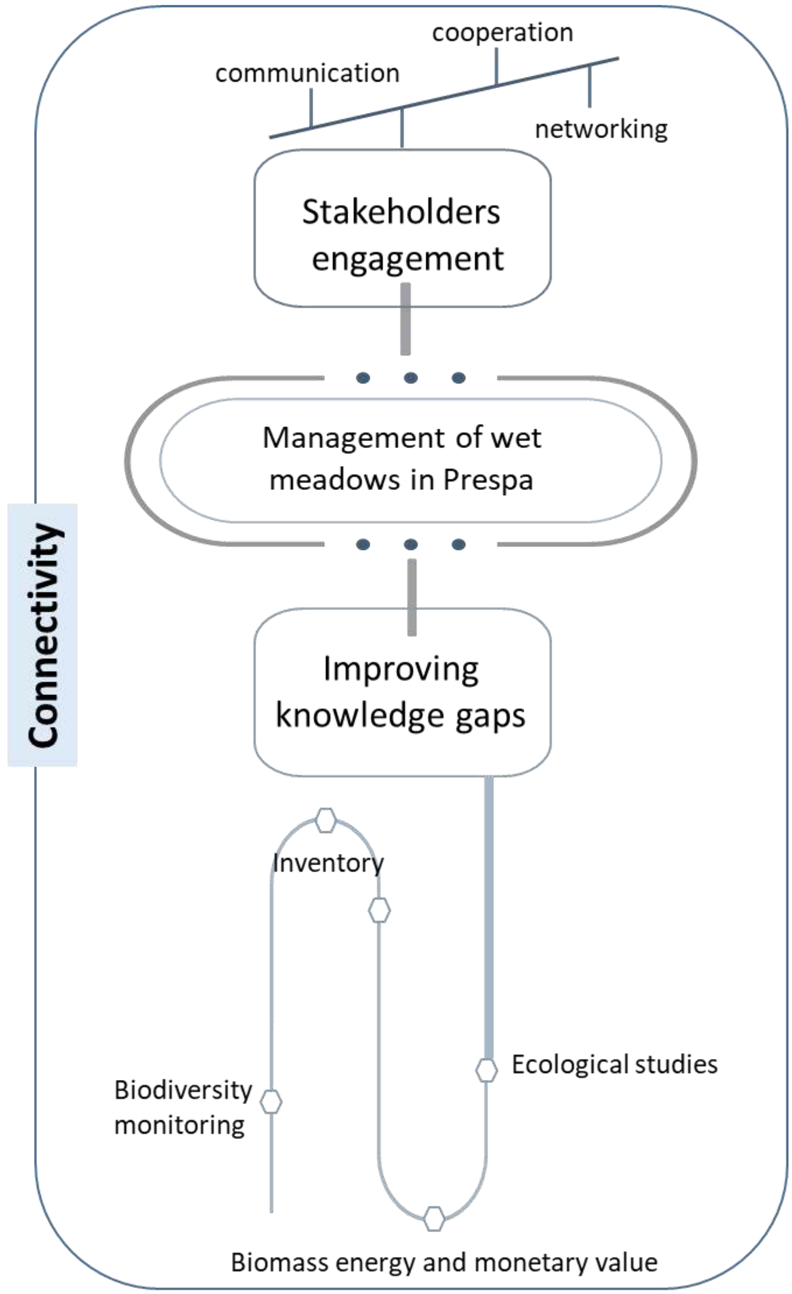


Figure 4 Conceptual framework for research and management activities on wet meadows in Prespa

This conceptual framework offers more holistic approach towards conservation of wet meadows in Prespa. With previous research it was concluded that management of wet meadows is an urgent required action. This was possible for the meadows distributed in Ezerani Nature Park, however it was necessary to develop a management approach. The developed approach gives equal priority to generation of much needed scientific arguments as well as direct involvement of local stakeholders.

2 Spatial distribution of wet meadows in Prespa

Spatial information regarding habitat types in Prespa is crucial for a holistic view of the overall condition. Wet meadows are part of the habitat mosaicsm in Prespa, therefore we wanted to assess their position in the landscape. Additionally, we wanted to test methodology that offers high resolution map, which opens perspectives for replication and comparability. These aspects are the premisis for sucesful future habitat monitoring activities.

2.1 Methodology

Drone recording activities along the Prespa Lake shoreline started in 2021. An evaluation was conducted in March and April of 2021 to see whether this activity could be completed. A framework was developed with the requirements that needed to be met. The following minimal requirements were defined:

- Sunny period for better reflection of the colours of the vegetation,
- No winds stronger than 3 m/s, in order to manage the drone with higher stability,
- To keep the time period from 10:00 to 14:00 as reference due to the height of the sun, i.e. avoiding problems that would appear from the shadows of tall trees.
- To select appropriate optimal distance from two adjacent take-off points, in order to optimize coverage and safe locations for taking off and landing the drone.

We established that the second half of June and the first part of July was the optimum period for fieldwork after examining the weather forecasts and field conditions.

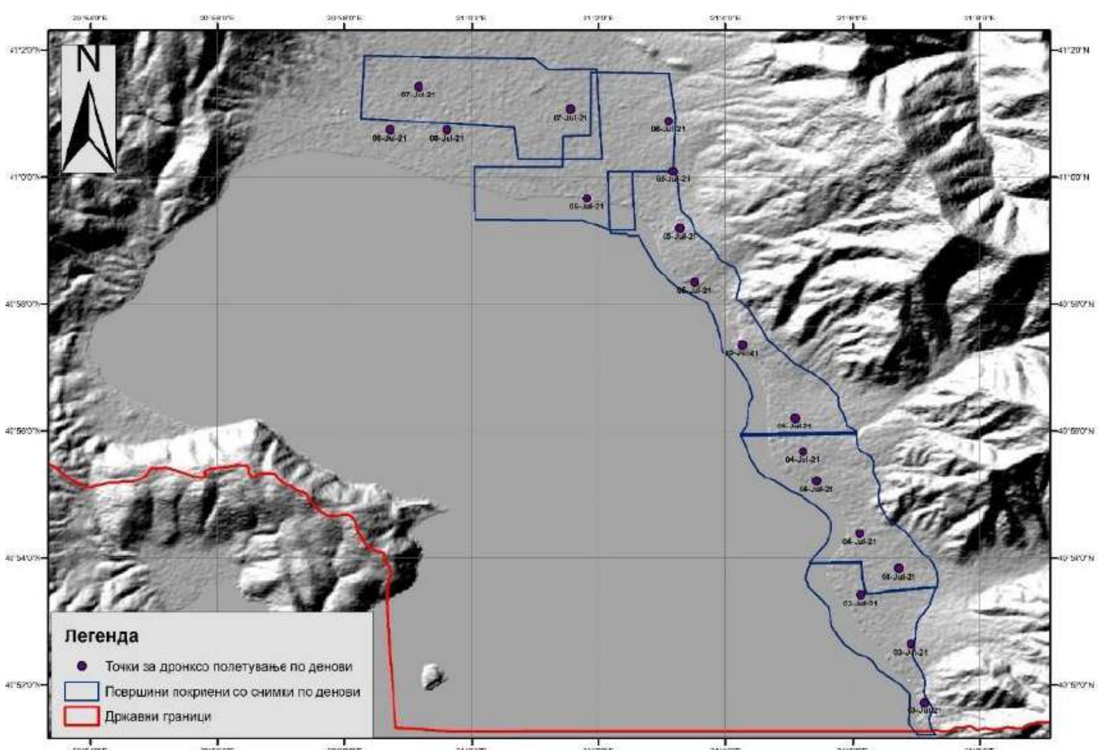


Figure 5 Map of Prespa Basin (North Macedonia) with drone take-off points and surface area covered with areal photos

During the field work, additional field data on vegetation was collected. The conducted field work resulted with about 10,000 image files and panoramic videos collected along the shoreline (Figure 5).

After the field work activities, data processing was conducted. This process was devided in four phases. In the first phase, a selection of low-quality images was made. With this process about 2500 files were deleted from the database. At the end of the data processing, a database of 7,500 files in .jpg format was created, which served as a resource in the production of the ortho-photographic model.

In the second phase, the database of images was placed in the Pix4Dmatic software for processing and creating an orthophotogrammetric model of the recorded area. The orthophoto model represents a mosaic of all images obtained as a result of the first operational phase.

The third phase covered additional data processing that included adjustments based on the local conditions, changes and needs for additions, improvement of gaps etc. This phase was neccesery so to determine a methodology for implementation of the digitization process of the habitat types. During this phase, it was determined that in order to start the digitalization activity, we will need additional data sources such as Google Earth ESRI (Imagery), spatial information of the habitat map produced in 2018 (Fotiadis et al. 2018). During the analysis, observations were made regarding the major changes caused by the lowering of the water level of the lake, certain natural successive changes and changes caused as a result of some anthropogenic activities.

The fourth phase was the digitization (delineation) of the habitat types in Prespa. The delineation was done in consultation with the habitat map of 2018 which was used as the main indicator for determining habitat types but also as baseline for comparison of the occurred changes.In 2018, the delineation was made on the basis of Google Earth ESRI (Imagery), which means less visibility in comparison to the drone images taken in 2021. Due to the differences in the methodological approach, we concluded that comparability of the data in this 4-year period was difficult.

2.2 Results and Discussion

Table 1 presents the surface areas of the different habitat types along Prespa shoreline. If we exclude the surface of the Lake from the total analysed area, which in the table is represented with the categories *Plankton communities of mesotrophic standing waters* and *Natural eutrophic lakes with Magnopotamion or Hydrocharition*, then the area for the terrestrial habitats is represented by 4055, 28 ha. In relation to that, we can observe that within the area of interest, **wet meadows are represented by 11.3%**, reedbeds by 21.5%, forests by 17.7% and the dry steppic areas by 6.3%. The rest of the habitats are more or less habitats affected by anthropogenic activities.

If we analyze the mosaicism and distribution of wey meadows in Prespa, we can conclude that 72% of the meadows belong to the Calamagrostis epigeios comm. 20.2% are the habitat types Helleno-Moesian riverine and humid Trifolium meadows, while 7.8% are Beds of large *Carex* spp. The Mediterranean tall humid herb grasslands of the Molinio-Holoschoenion are represented with very small percentage.

Table 1 Surface area of different habitat types along Prespa shorline

Habitat type	Area (ha)
Mediterranean tall humid herb grasslands of the Molinio-Holoschoenion	0.039
Water courses of plain to montane levels with the Ranunculion fluitantis and Callitricho-Batrachion*	0.802
Alluvial forests with Alnus glutinosa and Fraxinus excelsior*	101.057
Beds of large Carex spp.	35.68
Calamagrostis epigeios comm.	329.19
Early-stage broadleaved deciduous plantations	12.2
Gravel with very sparse or no vegetation	31.27
Helleno-Moesian riverine and humid Trifolium meadows	92.49
Intensively-farmed crops interspersed with strips of natural and/or semi-natural vegetation	84.38
Natural eutrophic lakes with Magnopotamion or Hydrocharition - type vegetation	485.22
Pannonic sand steppes	223,.9
Phragmites australis beds and Typha beds	587.09
Phragmites australis beds normally without free-standing water & Typha beds normally without free-standing water*	277.67
Plankton communities of mesotrophic standing waters	14824.94
Residential buildings of villages and urban peripheries	86.23
Rural commercial units	63.33
Salix alba and Populus alba galleries	604.91
Scattered residential buildings	0.08
Scirpus lacustris beds normally without free-standing water	7.80
Small-scale intensive unmixed crops (<1ha)	1264.09
Small-scale market gardens and horticulture, including allotments	248.1
Unknown	5.28
Total	19365.44

The monitoring of habitat's condition is planned to be conducted in every four years, in order to enable adequate comparability of the images. The areal model provided high resolution data presents a very useful tool that can help in assessing trends and observing changes, especially in regards of habitat transformations promoted by wet meadow's management activities.

Additional field records are needed for the Mediterranean tall humid herb grasslands of the Molinio-Holoschoenion habitat type since there is uncertainty in the dataset from 2018. With further research increasment of their surface areas. It should be mentioned that in the creation of the final map, the ground truth data from Melovski and Fotiadis were taken as the main resource for habitat identification. Due to the timespan in line with the dynamic changes of the overall environmental conditions in the region, this information should certainly be updated with additional field data that will significantelly improve the quality of the map.

3 Management of wet meadows in Prespa

Wet meadows are considered threatened biodiversity hotspots (Lepš 2014; Zedler and Kercher 2005). Over many years, humans have maintained these ecosystems through frequent and regular grazing and mowing, which is also accurate for the Prespa region. A variety of environmental conditions and the conventional management approach facilitate the coexistence of several species. However, because conventional maintenance is no longer profitable, a lot of meadows are being abandoned these days (de Bello et al. 2010; Doležal et al. 2011). Abandoning traditional practices in combination with increasing climatic changes leads to a succession process in Prespa that contributes for the quick development of an alder-dominated bushy vegetation as well as expansion of reedbeds. Thus, as has previously reported (Smith et al. 2009), wet woodlands are progressively replacing wet meadows.

On the other hand, wet meadows in Prespa are decreasing in size mainly to land transformation to orchards. The unsustainable irrigation practices additionally increase the succession rate. However, one of the biggest threats in Prespa basin is the uncontrollable fertilisation and nutrient load from organic waste. In this context, herbaceous communities in wet meadows are crucial to assimilation of nutrients (Kuusemets et al. 2000). By eliminating aboveground biomass, the practice of mowing meadows during the peak of biomass production, allows removal of part of the assimilated nutrients (Maron and Jefferies 2001).

Having all of the above in consideration, in the first phase we have conducted a biomass estimation analyses (Nikolov et al. 2020). During this three year cycle, we piloted this management activity, by also involving local stakeholders.

3.1 Methodological approach

It is very important to highlight that our management approach tended to be holistic. This means that it takes in consideration not only the conservation needs, but also involvement of local stakeholders (see more in Chapter 7), thus enhancing the social benefits of management activities. In this regard, selection of meadows and mowing methods was in coordination with Municipality of Resen, as manager of Ezerani protected area, alongside with local stakeholders providing traditional knowledge.

Three main criteria were developed for selection of meadows for management activities (Figure 6). The selection process itself was supported by desktop analysis as well as field visit to potential locations. Moreover, the final decision was in coordination with Ezerani Nature Park management as well as the opinion and suggestions of the locals. Accessibility was also one of the priorities.

Figure 6 Main criteria for selection of wet meadows for management activities

In almost all cases, mechanization was used for mowing activities in the Prespa region according to the survey conducted in 2019 (Nikolov et al. 2020). Therefore, taking in consideration the local mowing practices, light mechanization - tractor with mower on it (Figure 7) was the chosen mowing method for our activities. The initial mowing dynamics was planned to be once per year with rotating system each year. This means that one selected meadow should not be mowed two years in a row in order to decrease intensive pressure of the ecosystem as well as to provide time for regeneration of local biodiversity.

Figure 7 Mowing of a wet meadow with tractor with mower on it by our local stakeholder ©Marija Chobanova

The hay was turned over and gathered into bales for quicker drying after the mowing was complete. The hay was moved to baling once it has been determined to be sufficiently dry. Once more, baling was done with machinery—a baler that is affixed to a tractor (Figure 8).

Following the hay bales' removal from the meadows, a predetermined number of them are chosen randomly and weighed on a scale to ascertain the average weight of the bales (Figure 9). The total quantity of relatively dry biomass mowed is then computed by extrapolating the bale's weight and the number of bales generated.

Lastly, hay was received by local farmers who showed interest in its further use. They were responsible for its transportation.

Figure 8 Balling with a baler affixed to a tractor ©Lazar Nikolov

Figure 9 Weighting the bales on a scale ©Lazar Nikolov

3.2 Management activities

All managed wet meadows belong to the zones for active management and sustainable use of Ezerani Nature Park, in accordance with our stated criteria regarding the management of wet meadows (Figure 6). In regards to the habitat types, all selected meadows mainly belong the *Calamagrostis epigejos* communities. They occupy vast areas in the pilot sites and this species is known for its competitive nature. Therefore, meadows dominated by *Calamagrostis epigejos* are often managed by mowing in order to enhance plant species diversity (Házi et al. 2011).

Figure 10 presents the location of all managed meadows and the time dynamics of their management. In 2020, small meadow was mowed, as a test site (it is not included in the further statistics). The same plot with additional surface area was mowed again in 2022. What is important to note is that these habitats can be quite dynamic even in short periods of time such as three year cycle. Therefore, all management activities should always take in consideration local changes which also means that changes in the management plan can take place. In this regard, measurement of additional parameters, especially climatic, is highly recommended. Due to higher water levels and increased rainfall in 2023, we have decided that mowing of some sites can be done annually. Favourable hydrological condition in 2023 provided high biomass even to already mowed surface areas.

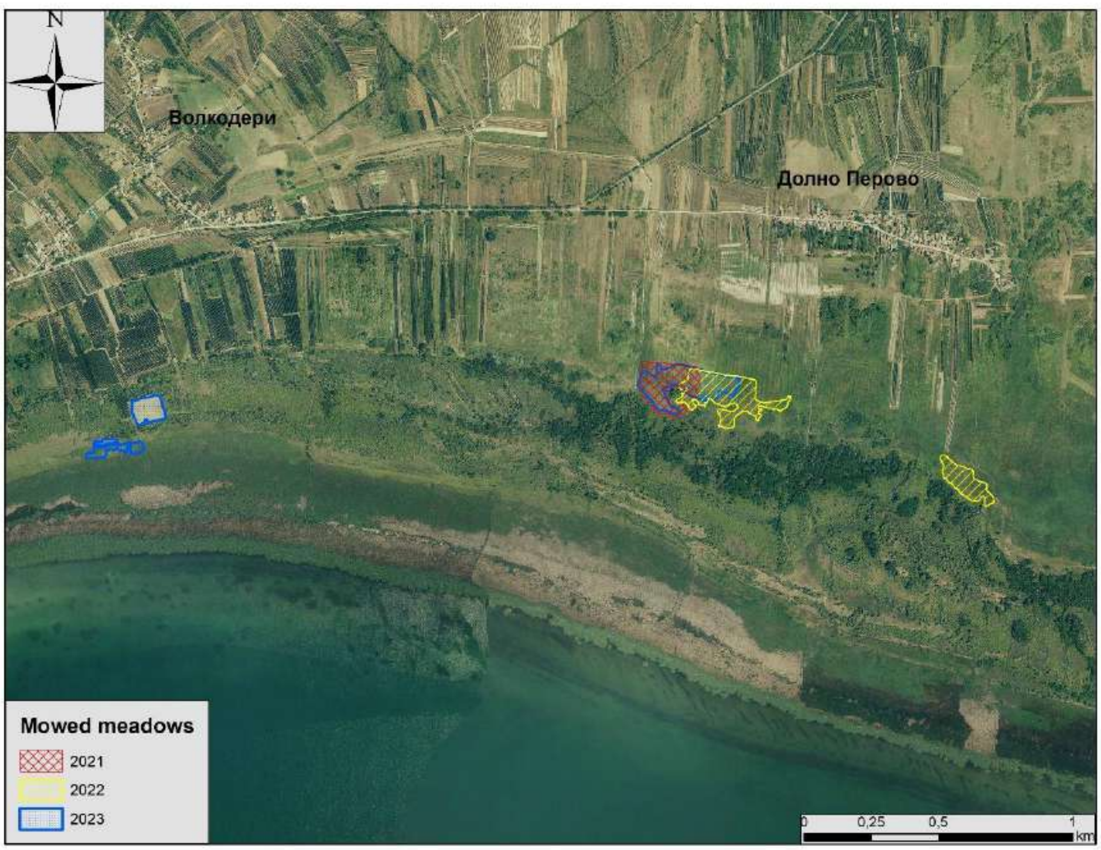


Figure 10 Managed wet meadows in Ezerani Nature Park

Summary of the outcomes from the three years of wet meadow management is provided in Table 2. From this, it is evident that three hectares of surface area covered by wet meadows were mowed in 2021. A total of 600 hay bales, weighing an average of 12.87 kg were produced. Consequently, 7722 kg of biomass have been harvested and removed from the system. As of 2022, there were six hectares of mowed meadows spread over two different plots. The increase of three hectares surface area contributes to production of 1400 bales of hay where one bale weighs 14.16 kg on average. In total, 19500 kg of biomass had been mowed.

Table 2 Overview of the main results from the three year cycle of active management

Year	Area [ha]	Average bale weight [kg]	Number of bales	Total biomass [kg]
2021	3	12,87	600	7722
2022	6	14,16	1400	19500
2023	6	15,4	2000	31736
Total	**15**	**42,43**	**4000**	**58958**

There is a very small overlap in the area of mowed meadows in 2021 and 2022. The surface area mowed in 2023 was again six hectares. It partly overlaps the surface area mowed in 2022 and completely overlaps the surface area mowed in 2021. Even though our initial plan was to have a two-year rotation system, due to the observed biomass production in the area mowed in 2022, we have decided to partly repeat the mowing there as well. The explanation for the higher biomass growth can be the high precipitation rate observed in early 2023. Another reason can also be the already reduced biomass from the mowing of the previous year which in combination with more favorable climatic conditions potentially provided better growth conditions. These assumptions were also confirmed with our calculations and biomass measurements (see comparison between results in Chapter 4.1 and Chapter 5.2. We have produced more hay (2000 bales, weighing an average of 15.4 kilogram) in comparison with year 2022, even though the mowed surface area is the same in both years. In total, 31 736 kg, or 31t, of biomass was produced.

Wet meadows in Prespa are/were traditionally mowed even with twice per year dynamics (information obtained through communication with locals). Yet, according to the latest data from the conducted survey in 2020 (Nikolov et al. 2020), locals in Prespa who mow their meadows, do it annually. Our rotation approach was mainly initiated with consideration of biodiversity. Still, what we didn't consider a lot was the scale factor. From our current experience in this perspective, we believe that if managed sites are within a larger surface area of a meadow habitat, disturbances or negative impact on biodiversity are mitigated. In this regard Tälle et al. (2018) analysed 29 relevant studies where mowing once per year, i.e. the traditional mowing frequency was compared to mowing every fifth, third or second year in order to assess the effects of different mowing frequencies on biodiversity of flora and fauna. They conclude that more frequent mowing generally had a more positive effect, but differences between frequencies were small. Additionally, studies suggest that long term annual mowing practices should be in place in order to improve wet meadow's quality in regards of their vegetation structure (Moog et al. 2002; Köhler et al. 2005; Hansson and Fogelfors 2000). Unmanaged wet meadows are very quickly degradable, while changes in plant species composition with management are slower in comparison with dry grasslands (Galvánek and Lepš 2009).

As a summary, fifteen hectares of wet meadows were mowed over a three-year period. A total of 58958kg, or 58.9t, of dry plant material was removed from the meadows, yielding about 4000 bales of hay.

4 Ecological studies in wet meadows in Prespa

This chapter elaborates on the conducted ecological studies on the wet meadows in Prespa. The aim of these studies was to improve the knowledge regarding wet meadow's ecology so that it can support future activities, especially management. Additionally, prior to starting any restoration activity, there is always a need for ecological data. Therefore, we consider these results valuable in this regard. Also, some of the analysis intended to quantify threats such as eutrophication and pollution, so the potentially addressed measures can be targeted. Having all of this in consideration, we have developed an ecosystem approach, where we have analysed ecological features of the biotic and abiotic component of wet meadows (Figure 11).

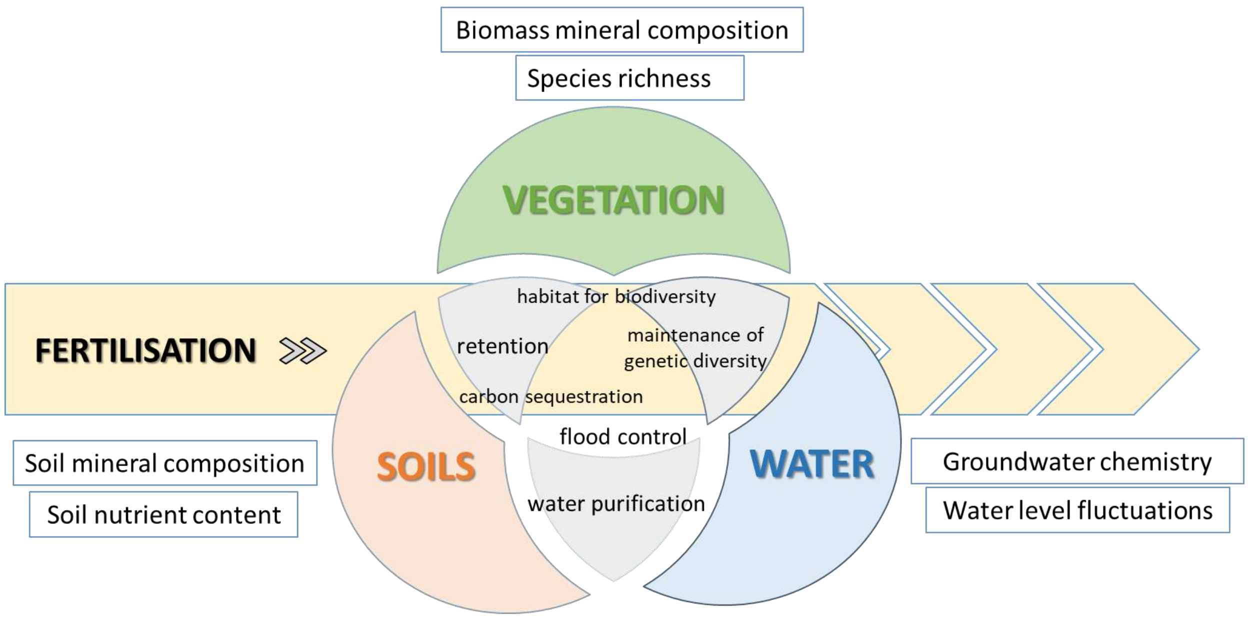


Figure 11 Objectives in our studies of wet meadow ecosystems in Prespa

Wet meadows in Prespa are temporary flooded ecosystems. To understand their role in the complex Prespa landscape we tried to explore the interactions between the three main components: vegetation, soil and water. This approach is of particular importance in wet meadows since the specifics of all three components are crucially important for their specific processes and functions such as water purification, flood control, retention, carbon sequestration etc. This means that if we want to enhance the supply of these ecosystem services, we need to assess the condition of the ecosystem, since the capacity of the ecosystem to provide ecosystem services is directly connected to its condition. Moreover, in landscapes with high anthropogenic pressures, this data is even more valuable. Prespa is such landscape, therefore we have also conducted a small scale fertilization study.

These studies should emphasize the role of the wet meadows in the Prespa basin, especially in regards to retention of nutrients. With this, they support the tested management activities that intend to reduce this nutrient load that also reaches lake waters. Besides accumulation in the biomass, the retention capacity is particularly important in the soil. Once minerals and nutrients (unfortunately we did not test pesticides) reach the meadows through groundwater, the water fluctuations allow retention to some of these materials.

4.1 Species richness, biomass and soils chemical composition

In the previous phase of research (Nikolov et al. 2020), we have reported biomass results and soils physical characteristics. Due to time limitations, we did not report the results on soils' and biomass' chemical composition. However, these analysis have been conducted and are elaborated in addition.

4.1.1 Methodology

Soil and plant material were collected from eight wet meadows along Prespa Lake at average altitude of 850 m. (Table 3). The field research was carried out in the period 14-16.06.2019 and 05-07.08.2019.

Table 3 Overview of the studied wet meadows

Locality code (based on the settlement the meadow refers to)	Toponym	X-coordinate	Y-coordinate	Altitude	Community (according to Fotiadis et al. 2018)
Nakolec 1	Sredno Pole	21.095607	40.892396	847	D5.21 - Beds of large Carex spp.
Nakolec 2	Leska	21.111960	40.889236	857	Calamagrostis epigeios comm.
Dolno Perovo 1	Solejnca	20.972946	41.011197	851	Calamagrostis epigeios comm.
Dolno Perovo 2	Perovski	20.999301	41.013274	852	D5.21 Beds of large sedges
Asamati	Ruzhin	21.040126	40.998642	851	E3.31 Trifolium meadows
Grnchari	Lokche	21.037445	41.008410	853	Calamagrostis epigeios comm.
Stenje 1	Blato (east)	20.920562	40.933417	848	E3.31 Trifolium meadows
Stenje 2	Blato (west)	20.912296	40.933483	850	D5.21 Beds of large sedges

Plant material was collected via line transects in June and August. Five samples within transect were taken, using square frames with 0, 0625 m^2 surface area. Where possible, plants were directly cut on site, while some were processed afterwards. Plant species were identified and all individuals from each species were counted. The floristic diversity was calculated according to the Shannon diversity index.

One soil profile was excavated in each wet meadow. Soil typification was assessed according to MASIS - Macedonian soil information system (http://www.maksoil.ukim.mk/masis/). Material was collected from different depths and kept in properly labelled bags.

The mineral composition (K, Ca, Mg, Na, Fe, Mn, Zn and Cu) was determined by atomic absorption spectroscopy Agilent 55A and Agilent 240Z (Allen 1989). Content of phosphorus was determined according to the spectrophotometric method based on reduction of phosphomolybdenic acid (Fiske and Subbarow 1925), while for the content of total organic nitrogen, the Micro Kjeldahl method (Bradstreet 1954) was applied. All soil chemical analyses were conducted on 2 mm fractions of absolute dry samples.

4.1.2 Results and Discussion

Three transects (Dolno Perovo 1, Dolno Perovo 2 and Grnchari) have diversity index lower than 1, while only one transect (Stenje 1) has higher than two (Figure 12).

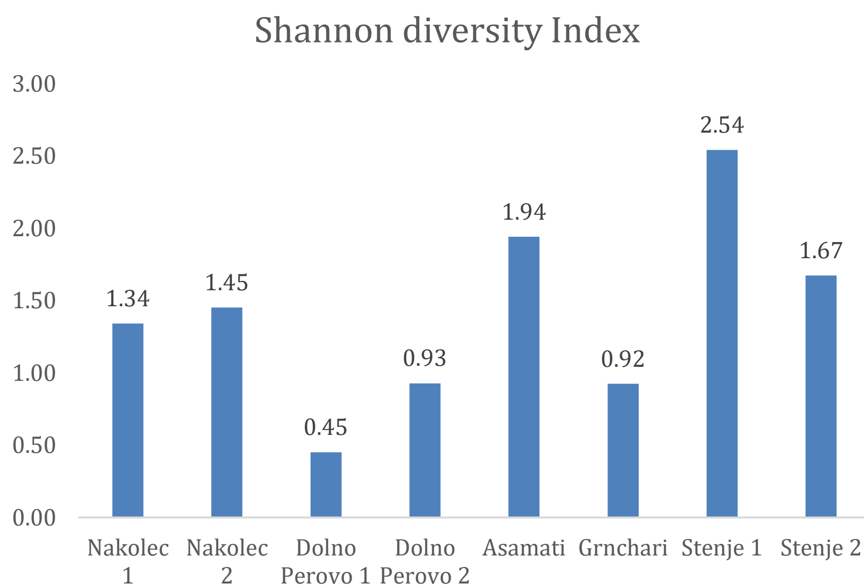


Figure 12 Shannon diversity index of eight wet meadows in Prespa

The mineral content of each plant species in all transects was analysed including the following elements: P, N, K, Ca, Mg, Na, Fe, Mn, Zn and Cu (Annex 12.1). The average content of different elements within the biomass of each transect is presented in Table 4 and Table 5.

Table 4 Average values of the mineral content in the Prespa wet meadow's biomass per square meter measured in June

Locality Code	N [g/m²]	P [g/m²]	K [g/m²]	Ca [g/m²]	Mg [g/m²]	Na [g/m²]	Mn [g/m²]	Fe [g/m²]	Zn [mg/m²]	Cu [mg/m²]
Nakolec 1	6.379	0.577	3.344	1.388	0.231	0.052	0.036	0.013	-0.598	2.021
Nakolec 2	3.188	0.372	3.473	1.880	0.264	0.047	0.010	0.043	4.494	2.808
Dolno Perovo 1	19.526	1.574	13.416	2.250	0.547	0.118	0.049	0.149	27.235	6.408
Dolno Perovo 2	8.354	0.961	7.344	1.803	0.355	0.056	0.050	0.016	11.357	3.468
Asamati	2.611	0.236	1.484	2.272	0.279	0.023	0.024	0.034	6.265	0.874
Grnchari	17.018	2.337	11.283	3.176	0.859	0.309	0.079	0.081	9.401	4.607
Stenje 1	0.528	0.034	0.292	0.381	0.035	0.015	0.002	0.003	0.914	0.212
Stenje 2	1.535	0.137	0.878	0.494	0.102	0.050	0.010	0.028	3.929	0.862

Table 5 Average values of the mineral content in the Prespa wet meadow's biomass per square meter measured in August

Locality Code	N [g/m²]	P [g/m²]	K [g/m²]	Mg [g/m²]	Na [g/m²]	Mn [g/m²]	Fe [g/m²]	Zn [mg/m²]	Cu [mg/m²]
Nakolec 1	1.862	0.211	1.302	0.137	0.013	0.011	0.008	0.739	0.806
Nakolec 2	1.889	0.223	1.449	0.250	0.014	0.018	0.030	2.204	1.523
Dolno Perovo 1	1.162	0.095	0.625	0.089	0.006	0.004	0.006	2.420	0.705
Dolno Perovo 2	3.545	0.571	4.099	0.195	0.018	0.018	0.007	5.984	1.564
Grnchari	2.131	0.208	1.068	0.114	0.005	0.014	0.018	-0.683	0.894
Stenje 1	0.668	0.048	0.344	0.072	0.054	0.006	0.003	0.789	0.329
Stenje 2	2.447	0.178	1.892	0.301	0.088	0.008	0.043	5.188	1.530

According to the average mineral content in the biomass, elements can be presented in the following order: **N > K > Ca > P > Mg > Na > Mn > Fe > Zn > Cu**.

Soil samples were divided according to depth and rearranged according to soil horizons classification. The water capacity ranged from 15.24% to 59.5% in the analysed soil profiles. Soil bulk density was the lowest in the top soil layers (Table 6). According to MASIS, we have identified three soil types: fluvisol (Nakolec 1 and Dolno Perovo 1), Mollic Fluvisol (Stenje 1) and Gleysol (Nakolec 2, Dolno Perovo 2, Asamati, Grnchari and Stenje 2).

The average content of phosphorus in each transect varies from 0.02 % to 0.2 % (Figure 13). The average content in the A horizons is higher than in the deeper, Gso/Gr horizons.

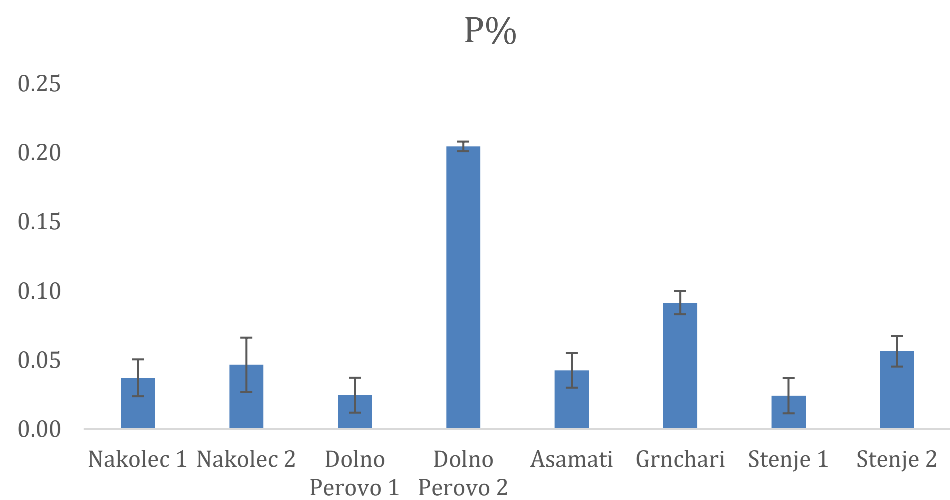


Figure 13 Average phosphorus content in soils of eight wet meadows along Prespa Lake with standard errors

Table 6 Soil samples, their physical characteristics (water retention capacity and bulk density) and mineral content

Locality	Soil horizon	Horizon depth [cm]	Soil type	Water retention capacity (%)	Bulk density (g/cm^3)	K (%)	Ca (%)	Mg (%)	Na (%)	Fe (%)	Mn (%)	Zn (mg/kg)	Cu (mg/kg)
Nakolec 1	A	(0-6)	fluvisol	43.66	0.876	0.523	0.041	0.298	0.030	1.280	0.080	42.773	8.186
	IIC	(6-30)		39.38	1.296	0.351	0.031	0.300	0.021	1.043	0.051	41.690	7.018
	IIIC	(30 +)		19.66	1.362	0.175	0.011	0.320	0.037	0.935	0.039	39.992	4.071
Nakolec 2	A	(0-10)	Gleysol	38.91	0.433	0.858	0.013	0.515	0.056	2.525	0.030	92.202	29.829
	Gso	(10-38)		42.81	1.112	1.040	0.013	0.511	0.073	2.108	0.031	80.738	23.493
	Gr	(38+)		41.10	1.440	0.855	0.018	0.442	0.070	1.918	0.033	61.885	17.620
Dolno Perovo 1	A	(0-9)	fluvisol	37.89	1.068	0.336	0.003	0.164	0.031	0.951	0.041	59.275	6.053
	IIC	(9-23)		15.24	1.313	0.342	0.003	0.156	0.039	0.791	0.014	58.064	8.091
	IIIC	(23+)		34.61	0.790	0.530	0.004	0.225	0.042	1.209	0.018	50.960	13.928
Dolno Perovo 2	A	(0-9)	Gleysol	32.28	1.012	0.865	0.017	0.408	0.057	1.478	0.086	120.184	32.803
	AC	(21+)		31.81	1.040	0.920	0.020	0.357	0.054	1.830	0.084	107.442	31.670
Asamati	A	(0-9)	Gleysol	45.75	1.026	0.774	0.011	0.382	0.044	2.215	0.067	89.840	21.147
	Gso	(21+)		37.95	1.267	0.917	0.017	0.523	0.034	3.040	0.127	82.801	26.169
Grnchari	A	(0-8)	Gleysol	45.38	0.914	0.624	0.232	0.875	0.037	3.435	0.081	99.608	22.188
	Gso	(8-22)		41.97	1.561	0.549	0.291	0.854	0.036	3.740	0.079	122.556	18.979
Stenje 1	A	(0-12)	Mollic Fluvisol	35.13	1.140	0.196	0.004	0.136	0.026	0.850	0.019	60.229	5.321
	AC	(24+)		29.18	1.328	0.140	0.002	0.093	0.015	0.519	0.007	43.345	3.697
Stenje 2	A	(0-5)	Gleysol	59.50	0.881	0.669	0.004	0.335	0.051	2.704	0.068	103.656	23.877
	Gso	(5-38)		49.01	1.137	0.264	0.002	0.349	0.055	3.836	0.045	106.983	23.963
	Gr	(38+)		48.05	1.256	0.296	0.005	0.399	0.057	0.903	0.226	127.118	28.303

The average content of total organic nitrogen in each transect varies from 0.17 % to 0.43 % (Figure 14). The average content in the A horizons is higher than in the deeper, Gso/Gr horizons.

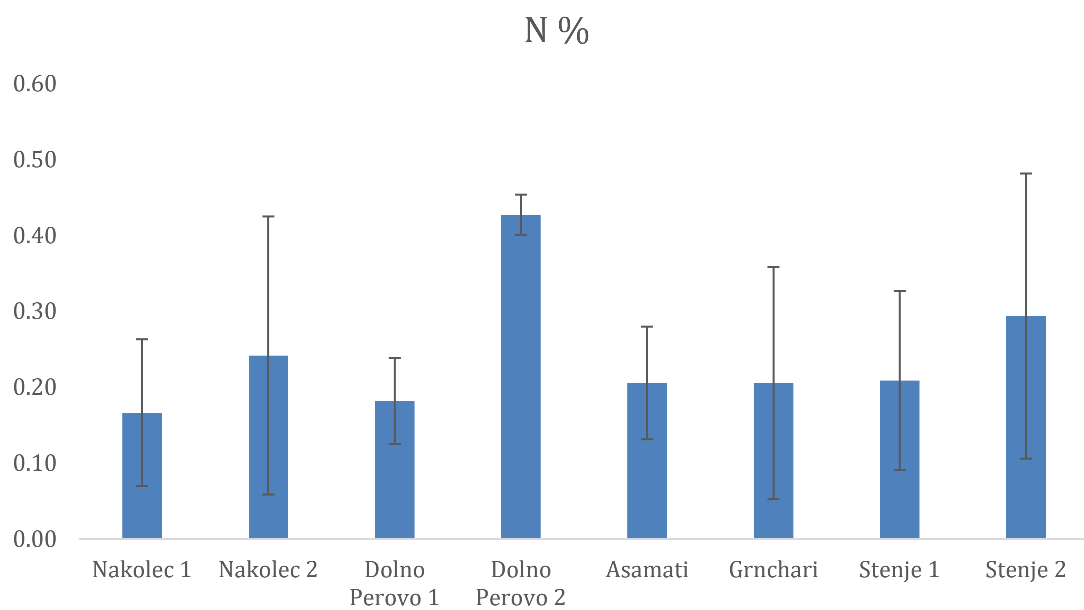


Figure 14 Average total organic nitrogen content in soils of eight wet meadows along Prespa Lake with standard errors

According to the total mineral content, elements can be presented in the following order: **Fe** > **K** > **Mg** > **N** > **Mn** > **P** > **Na** > **Ca** > **Zn** > **Cu**.

4.2 Groundwater chemistry and water level fluctuations

Wet meadows in Prespa are temporary flooded ecosystems. Having in consideration that ongoing succession was observed, one important component that needed attention was the water. Therefore, we wanted to focus on groundwater chemistry and its fluctuations in order to understand the dynamics in the changes of the water regime. We believe that these result can put light in potential rewetting activities. Also, annual fluctuations provide information for timing of management activities such as mowing.

4.2.1 Methodology

Water sampling was done by installation of transects of piezometers at three different sites. All piezometers are hand-made from plastic pipes with section of holes (covered with fine screen in order to stop sediments) at specific length, to collect water from particular depth in the soil. The pipes are extended above the ground surface, above the depth of surface water flooding. 10 cm below the ground surface and to the top of the pipe above the ground, the pipe does not have any holes in order collection of surface water to be avoided (*Figure 15*).

Figure 15 Piezometers and driller for their positioning in the soil ©Marija Chobanova

The pipes were placed in an excavated hole and sealed with the excavated soil material. Finally, a cap was put on top of the pipe with small hole in it so it would eliminate potential vacuum that can influence the water level measurements. In one of the pipes at each site, a data-logger (HOBO Water Level U20L-04) was installed in order to automatically measure water levels on an hour intervals. Water level data were read-out with portable shuttle HOBO Onset U-DTW-1.Collected water was kept in a field refrigerator, transported to the laboratory for plants' ecology at the Institute of Biology in Skopje and was further analyzed for its chemical composition. At each site, pH and electrical conductivity were measured during the field work with portable pH and electrical conductivity meter.

Chemical analyses were performed on the collected material, which included primarily two preparatory procedures: fine filtration for the determination of nitrates, nitrites and ammonia nitrogen, and the other involved combustion of the samples. 300 – 350 ml of the water was filtered through a Wattman filter paper. Due to different colloidal coloration, we used laboratory activate carbon, stirred intensively, and then filtered the sample. The goal was to obtain a clear and colorless filtrate. The filtrate was used for compound analysis using spectrophotometric methods. Ammonium nitrogen was determined by a method based on the reaction between ammonium compounds and HgCl2 in the presence of NaOH, developing dimercurium ammonoxyiodide with a characteristic yellow to orange color. The principle of the reaction of brucine-sulfuric acid with nitrates was applied for the spectrophotometric determination of nitrates at a wavelength of 400 nm, while for the determination of nitrites a spectrophotometric method (520 nm) was used, which is based on the reaction of nitrites with sulfanilic acid and α-naphthylamine. First, standards with different concentrations were prepared. A stock solution of known concentration was added to each standard in a series of dilutions, and then the necessary reagents. Minerals (K, Ca, Mg, Na, Fe, Mn and Zn) were determined by atomic absorption spectroscopy on an Agilent 55 (Allen, 1989) with precombustion in an Ethos Lean microwave oven (method: EPA3015A). The content of total soluble particles, inorganic soluble particles and total organic matter in the dry residue was determined by evaporation and combustion of the dry residue in a mouflon oven (Schulte et al. 1991).

Basic calculations for all parameters were made in the Microsoft Excel program. Correlation analyses were performed in R software. Water level analysis were done in HOBOware software.

4.2.2 Results and Discussion

Installation of piezometer transects was done in September 2021. Three wet meadows were selected in Ezerani Nature Park: one in Asamati and two at the locality Solejnca, near the village Dolno Perovo (marked as Perovo 1 & Perovo 2). In all three localities four piezometers in different soil depths were installed in order to collect water from different soil horizons. Surface water was also collected. Water sampling was done when there was sufficient water in the piezometers in those localities that were flooded. This was mainly done in early spring months in 2022 and 2023 (Table 7). However, it can be concluded that the locality Perovo 2 was rarely flooded enough for sufficient quantity of water necessary for the chemical analysis. Most of the field visits shown very little or not at all water in all of the piezometers, especially the more shallow ones.

Table 7 Sampling dynamics of groundwater from three selected wet meadows in Prespa

Locality	Comment	Depth [cm]	Date	CODE
Asamati	unmanaged	20 - 40	24.02.2022	A3
Asamati	unmanaged	80	24.02.2022	ANV1
Asamati	unmanaged	80	24.02.2022	ANV2
Perovo 2	mowed in 2021	80	24.02.2022	PKNV1
Perovo 1	unmanaged	0 - 10	24.02.2022	PN1
Perovo 1	unmanaged	10-20	24.02.2022	PN2
Perovo 1	unmanaged	20 - 40	24.02.2022	PN3
Perovo 1	unmanaged	80	24.02.2022	PNNV1
Perovo 1	unmanaged	surface	24.02.2022	PNP
Snow	/	snow	24.02.2022	PS
Asamati	unmanaged	surface	31.03.2022	AP

Locality	Comment	Depth [cm]	Date	CODE
Asamati	unmanaged	80	31.03.2022	ANV1
Asamati	unmanaged	0-10	31.03.2022	A1
Asamati	unmanaged	10-20	31.03.2022	A2
Asamati	unmanaged	20-40	31.03.2022	A3
Asamati	unmanaged	80	31.03.2022	ANV2
Perovo 2	mowed in 2021	80	31.03.2022	PNV1
Perovo 2	mowed in 2021	20-40	31.03.2022	P3
Perovo 1	unmanaged	surface	31.03.2022	PNP
Perovo 1	unmanaged	0-10	31.03.2022	PN1
Perovo 1	unmanaged	10-20	31.03.2022	PN2
Perovo 1	unmanaged	20-40	31.03.2022	PN3
Perovo 1	unmanaged	80	31.03.2022	PNNV1
Asamati	unmanaged	surface	13.05.2022	AP
Asamati	unmanaged	80	13.05.2022	ANV1
Asamati	unmanaged	0-10	13.05.2022	A1
Asamati	unmanaged	10-20	13.05.2022	A2
Asamati	unmanaged	20-40	13.05.2022	A3
Asamati	unmanaged	80	13.05.2022	ANV2
Perovo 2	mowed in 2021	80	13.05.2022	PNV1
Perovo 1	unmanaged	10-20	13.05.2022	PN2
Perovo 1	unmanaged	20-40	13.05.2022	PN3
Perovo 1	unmanaged	80	13.05.2022	PNNV1
Perovo 1	unmanaged	0-10	09.03.2023	PN1
Perovo 1	unmanaged	10-20	09.03.2023	PN2
Perovo 1	unmanaged	20-40	09.03.2023	PN3
Perovo 1	unmanaged	80	09.03.2023	PNNV1
Perovo 1	unmanaged	80	09.03.2023	PNNV2
Perovo 2	mowed in 2021	80	23.04.2023	PNV1
Perovo 1	unmanaged	0-10	23.04.2023	PN1
Perovo 1	unmanaged	10-20	23.04.2023	PN2
Perovo 1	unmanaged	20-40	23.04.2023	PN3
Perovo 1	unmanaged	80	23.04.2023	PNNV1
Perovo 1	unmanaged	80	23.04.2023	PNNV2
Perovo 1	unmanaged	surface	23.04.2023	PNP

The measured total suspended solids, inorganic suspended solids, as well as the total organic matter from the suspended particles in the analyzed samples show expected trend in the wet meadow in Asamati, while Perovo 1 shows the opposite trend. Perovo 2 has higher average values for TSS and ISS in the piezometer for water levels (Figure 16).

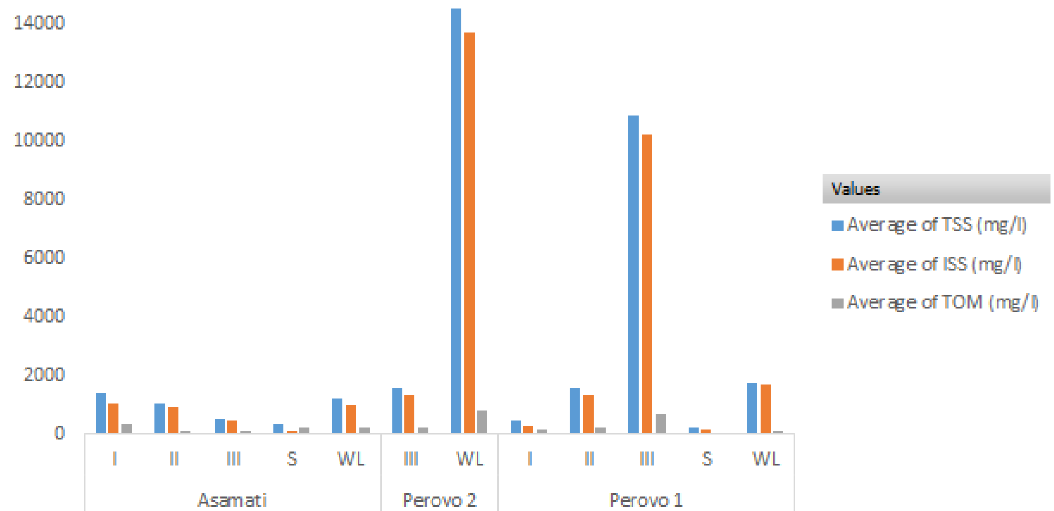


Figure 16 Total suspended solids (TSS), inorganic suspended solids (ISS) and total organic matter (TOM) in the different piezometers from three wet meadows in Ezerani Nature Park

In terms of different forms of nitrogen, ammonium nitrogen, nitrates and nitrites were measured. It can be observed that the concentration of NO_3^- is much higher than the concentration of NO_2^- and NH_4^+ (Figure 17).

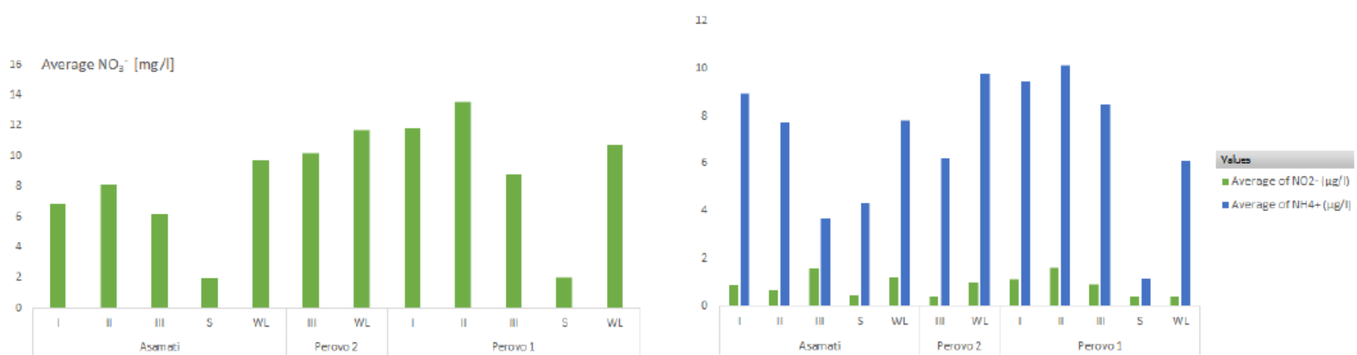


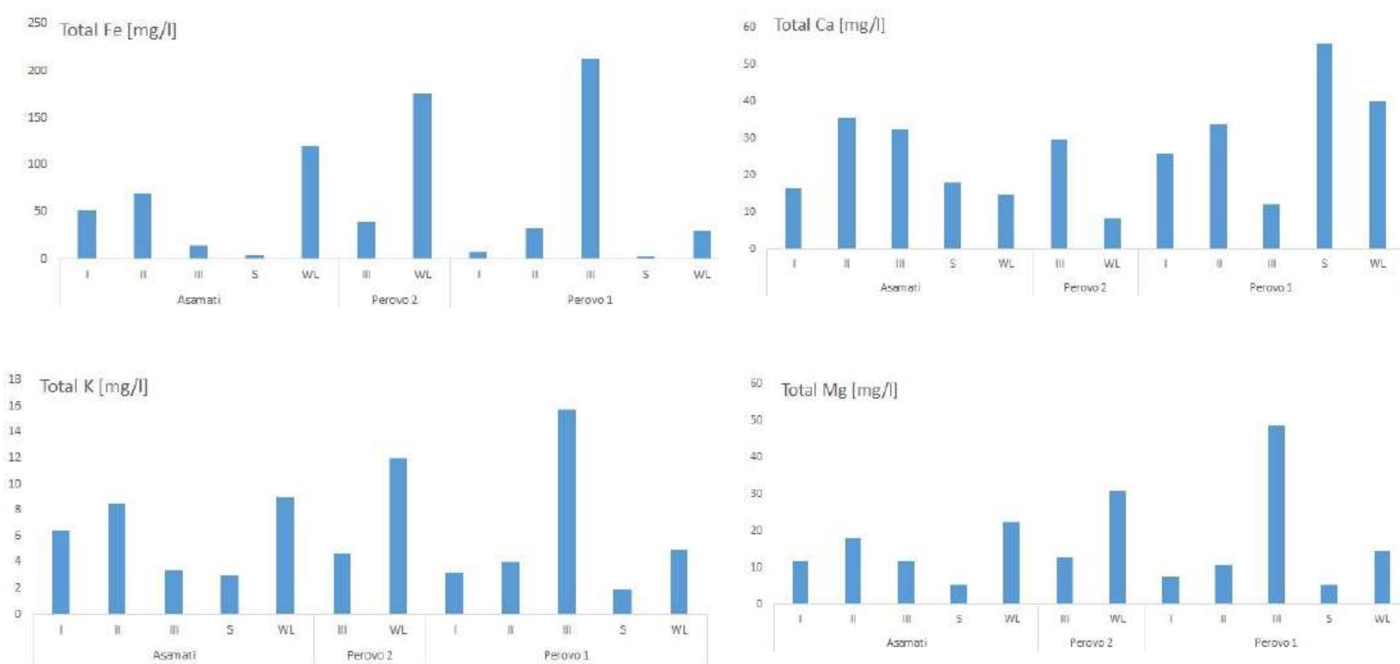
Figure 17 NO_2^-, NO_3^- and NH_4^+ in the different piezometers from three wet meadows in Ezerani Nature Park

In all analysed samples, the mineral composition of the waters was determined for the following elements: Mn, Mg, Fe, Na, K and Ca. These elements were determined as total elements in the waters where as the average contents for each locality is presented in Table 8. Fe has the highest value of all elements with concentration few times higher in comparison with the other elements. However, the order of values for all elements presented in Table 8 is similar for all three localities. Interestedly, Annex 12.1 shows that the pH measurements in April 2023 have significantly higher values, which can mean that alkaline groundwater increases the pH reaction of the wet meadows.

Table 8 Total mineral content, average temperature, pH and conductivity in the groundwater from three selected wet meadows in Prespa

Locality	Mineral content	Temperature ˚C	pH	Conductivity
Asamati	Fe < Ca < Mg < K < Na < Mn < Zn	10.9	7.1	176.2
Perovo 1	Fe < Ca < Mg < K < Na < Mn < Zn	9.4	7.5	214.8
Perovo 2	Fe < Mg < Ca < K < Na < Mn < Zn	10.6	7.4	257.6

Figure 18 presents the analysed total elements in groundwater and surface water in the three analysed wet meadows. It gives a comparative overview, but also an overview of the measured elements by depth gradient (in each piezometer). From the result we can again conclude that there is no significant difference between all three sites. The concentration of Mn is somewhat higher at the Asamati locality in comparison to the other two. Also, for all elements (besides Ca in Perovo 1) the surface water has lower values in comparison to the groundwater. An analysis was also made in terms of seasonal dynamics, during which no drastic peaks were determined in any of the elements.



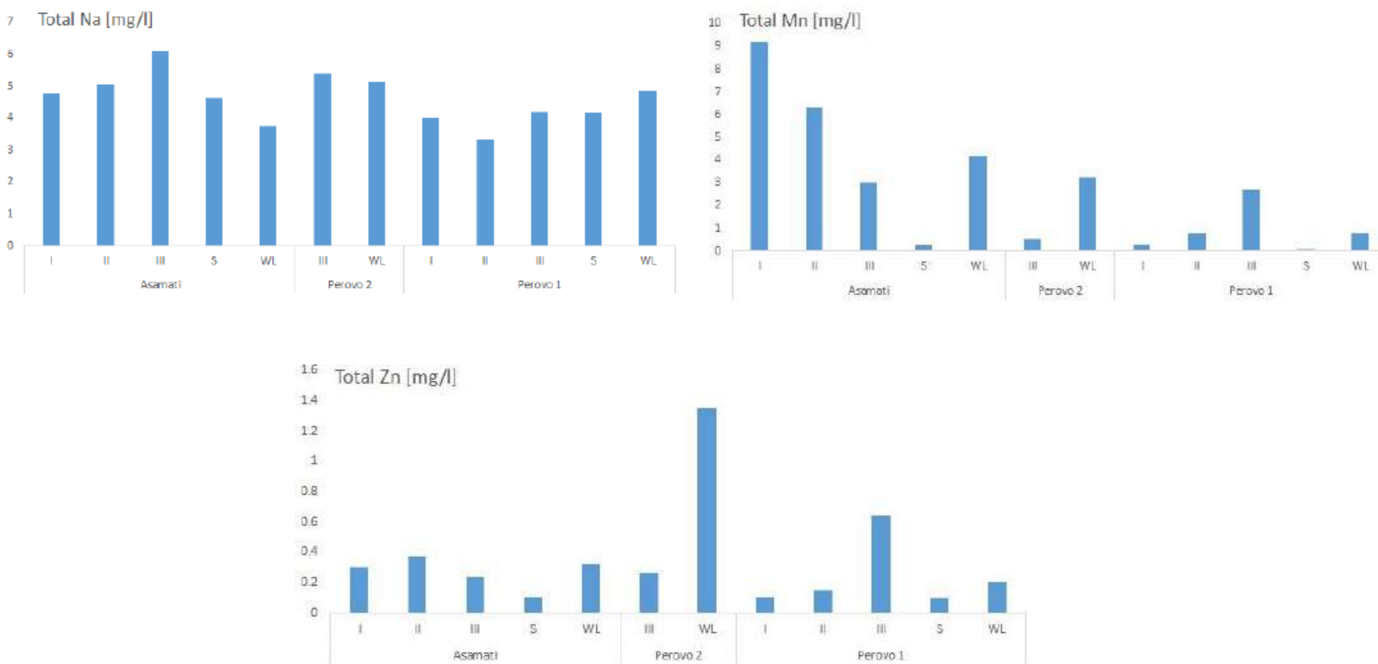


Figure 18 Average values of the total content of K, Ca, Mg, Mn, Zn, Fe and Na in the different piezometers from three wet meadows in Ezerani Nature Park

Having in consideration that there were no significant differences between the localities, Figure 20 presents the average values for the measured parameters in the different depths from all three localities. Increasing trend with depth can be observed for K and Mg, while Mn, Na and Zn have more uniform concentrations.

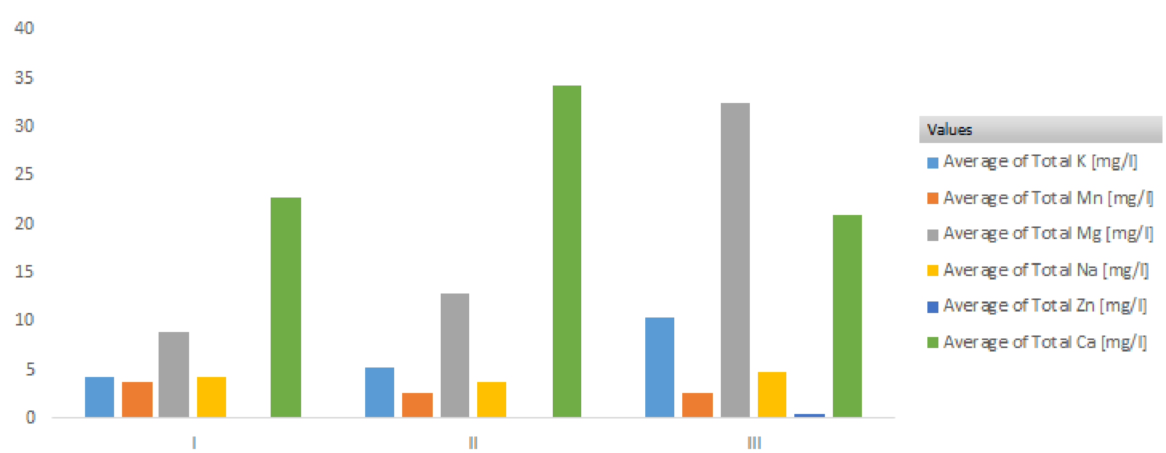


Figure 19 Average values of the total content of K, Ca, Mg, Mn, Zn and Na in the different piezometers (depths) from all three wet meadows in Ezerani Nature Park

The iron on the other hand, as an element in highest concentration shows linear increasing trend with depth, while the surface water has much lower content. The concentration in the piezometer for water level measurements is understandably high since there is mixture of the water from all depths (Figure 20). Further elaboration regarding these results is given in Chapter 4.4.

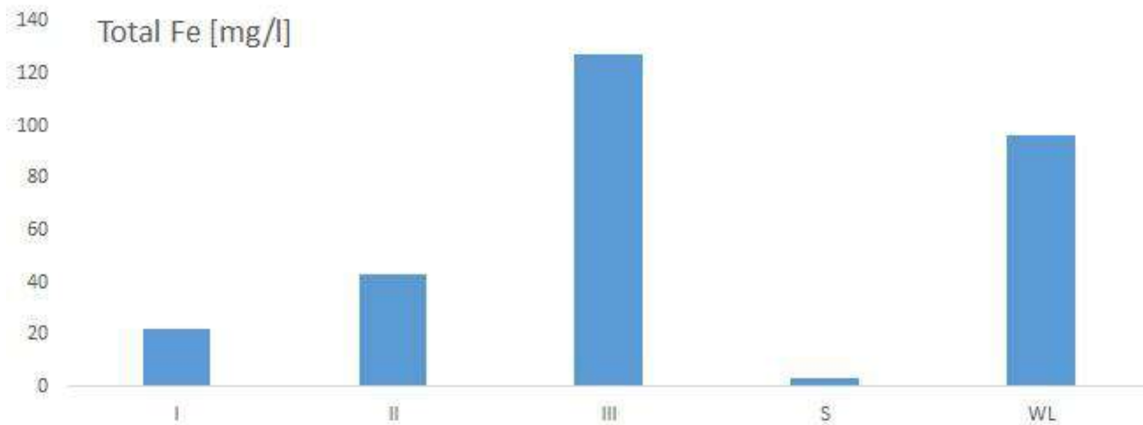


Figure 20 Average values of the total content of Fe in the different piezometers (depths) from all three wet meadows in Ezerani Nature Park

Water levels

In parallel with the chemical analysis, we followed the water levels in the same three wet meadows. Namely, in the deepest well, data logger was placed that took measurements on an hour basis (Figure 21). This type of methodology was tested for the first time in Prespa (as far as our knowledge).

Figure 21 Transect of piezometers in a wet meadow at Asamati Locality in Ezerani Nature Park ©Marija Chobanova

The acquired outcome provides an initial assessment of the hydrological status of the three investigated wet meadows. Generally, discernible variations in water levels over time underscore the necessity for continuous measurements. For instance, during the initial phase post-installation, elevated water tables are evident across all three sites during the late winter months. However, the Asamati locality exhibits a dissimilar trend in the corresponding period of the subsequent year (2023), as corroborated by our field observations. Despite adhering to overarching expectations concerning annual fluctuations, instances such as this illuminate not only annual disparities but also spatial distinctions. Conversely, the Perovo 1 locality manifests a comparable annual trend with flooding, while Perovo 2 indicates a minimal amplitude of water fluctuations, predominantly portraying arid conditions for a significant duration, even though measured concurrently and in close proximity with Perovo 1 locality. This underscores the imperative for further investigations to elucidate the factors influencing water tables and accounting for these observed differences. The main limiting factor for obtaining better results was the depth of our wells. The depth of the deepest piezometers where the data loggers were placed, ranged from 75-80 cm in the ground.

Our analysis reveals that groundwater levels in the wet meadows likely extend much deeper, especially during the summer period. Consequently, atmospheric measurements by the data loggers, functioning as barometers occurred when water levels were beneath the maximum depth, introducing interference in the final results as the loggers do not register dry conditions. Nonetheless, comparing these measurements with barometric pressure data from the dedicated logger for barometric measurements validates the observed field conditions, namely, a dearth of water in the wells. Initially assuming better groundwater saturation in these meadows, our results suggest otherwise, necessitating the installation of substantially deeper piezometers to comprehensively grasp water fluctuations along a depth gradient. With multi-year monitoring, comparison will be enabled, as well as identification of critical points in the water level. For these reasons, starting this type of monitoring is a very significant first step towards defining and monitoring the overall hydrological condition of the wet meadows.

The data from the data loggers covers the period from November 2021 until April 2023. During this period, the data loggers have collected 12 387 samples for measurements of water temperature and water levels. Due to the limit from the depth of the wells, we cannot accurately calculate the exact extent of the fluctuations, however, Figure 22 presents the trends in all three localities. Asamati locality shows increasing trend of the water levels exceeding to the surface of the ground, where the highest water table was recorded between January and March 2022. However, after this period, the water tables decrease significantly and maintain below detection limit, with insignificant, small fluctuations in the upcoming late winter and early spring months. Perovo 1 on the other hand is the locality with best water saturation from all three localities. It shows dry conditions during the period of June 2022 until January 2023 which indicates that not only summer, but also autumn and winter periods the water table is deeper than 80 cm. However, significant increase is detected in the late winter and early spring months in 2023 which follows similar trend though with more fluctuations to the same period in 2022. Perovo 2 locality is the driest wet meadow with very small amplitude of water fluctuations and mostly dry conditions. Small peaks are detected in April 2022 and March 2023.

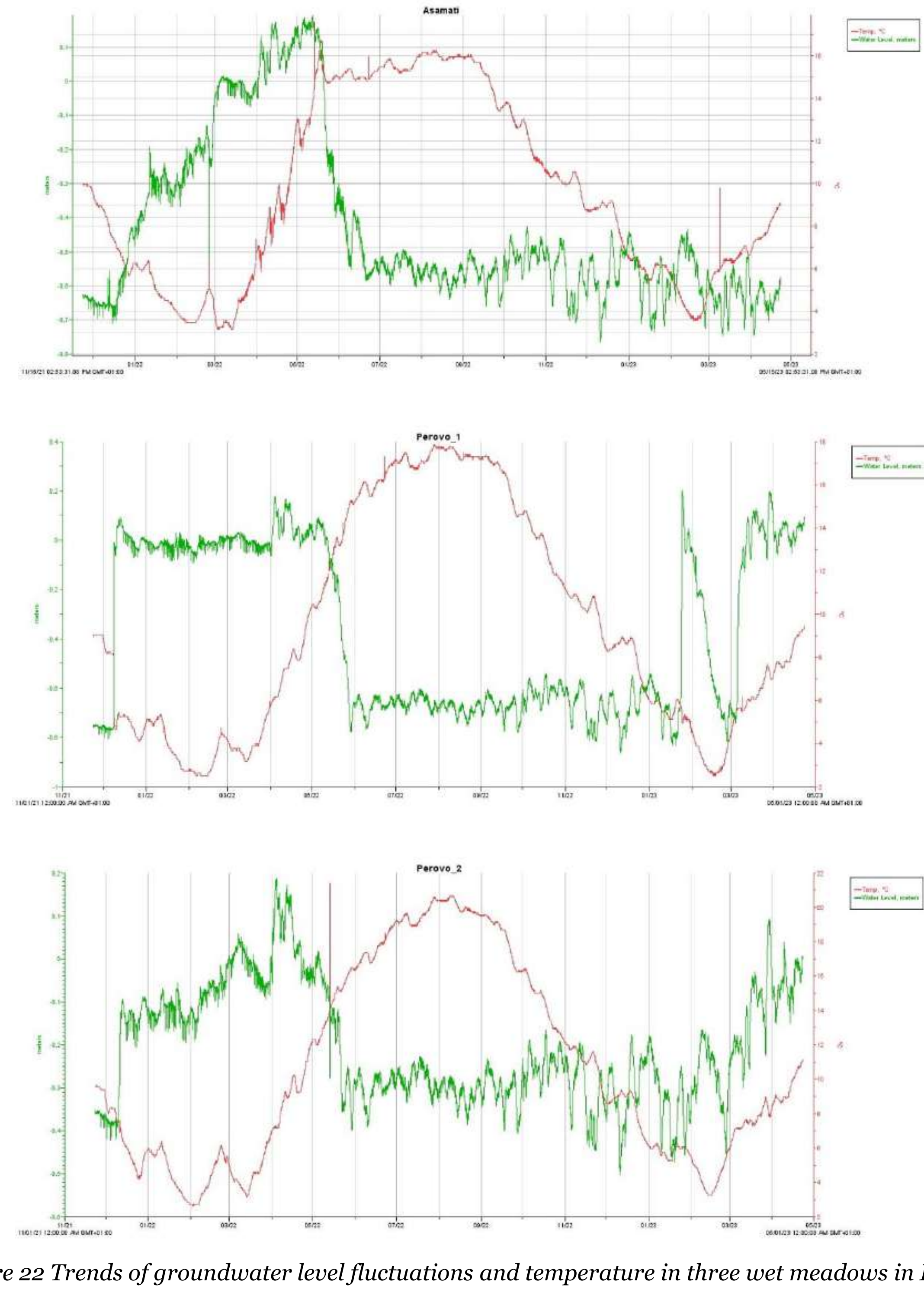


Figure 22 Trends of groundwater level fluctuations and temperature in three wet meadows in Ezerani Nature Park

4.3 Fertilisation experiment

Unsustainable agricultural activities are responsible for habitat loss and destruction. Wet meadows in Prespa are particularly affected from the intensive agricultural activities in the region. Many of the wet meadows have been converted into new apple orchards and this trend is continuing every year. According to the data from the socio-economic analysis published in the study on Ezerani (Smith et al. 2009), it is pointed that there is low awareness among the population about the importance of wetlands which are part of the Ezerani Nature Park. Although agricultural threats received attention in Prespa mainly through different projects, there is no direct study of their influence on wet meadows. Therefore, we have decided to conduct a small scale fertilization experiment[1] in one wet meadow in Ezerani Nature Park. For this study we set two main objectives (Figure 23).

Assessment of fertilisation intensity

Assessment of the soil retention capacity

Figure 23 Objectives for our fertilisation experiment

These objectives put frame to our conceptual thinking and designed our experiment. Firstly, we aimed to assess the quantity of fertilisers applied in Prespa (mainly on agricultural land e.g. orchards). Then, we used this information for our experimental set up (explained in methods) to eventually test the soil retention capacity.

4.3.1 Methodology

Survey

Anonymous face-to-face field surveying was conducted in order to gain insight into the intensity of fertilisation and the type of fertilizers used in Prespa. The survey questionnaire was made according to the needs for setting up the experiment. The interviews were conducted in several villages in Prespa region. The target group of this survey was owners of orchards near Prespa Lake. The original questionnaire is presented in Annex 12.2.

Experimental design

With aim to provide data on pollution with artificial fertilizers, we designed experimental setup for an in situ experiment. We used plastic lysimeters with a depth of 15 cm and surface area of 0.15 m^2. To place the plastic lysimeters, holes were dug in the soil of one wet meadow with a size suitable for the plastic lysimeter. The topsoil that was removed from the hole along with the above ground vegetation was returned intaced inside each of the plastic lysimeters. Holes were opened at the bottom of the plastic lysimeters and plastic funnels were attached to the underside of the lysimeters, covering all the openings at the bottom of the lysimeter. The funnel with its

[1] The survey and soil analysis were funded by the young ecologists program Dr. Ljupcho Melovski in a form of small grant coordinated by Dragana Pelikudovska Trajchevska in 2022. These, and the results from the water analysis are now part of two batchelor thesis (Пеликудовска 2023; Дочовска 2023).

narrow end was connected to a plastic bottle in which the water was collected. Two layers of mesh were placed above the holes on the lysimeters in order to prevent soil sediment and living organisms from entering the plastic bottles. These bottles collected precipitation water that percolated through the vegetation and soil (Figure 24).

Figure 24 Design of the lysimeters ©Dragana Pelikudovska Trajchevska

On May 23, 2022, a total of 18 lysimeters (in further text plots) were installed in a wet meadow located in the protected area Ezerani. The experiment simulated two scenarios 1) fertilised and 2) non- fertilized meadows (plots). Additionally, both scenarios had different management settings a) not managed, b) mowed and c) burnt. The mowing was done by cutting the aboveground biomass in the plot with scissors, while the burnt management was done by burning the aboveground biomass. So, in total, there were six different combinations and each combination was represented with three plots. All plots were appropriately marked on the field. Three plots were set in one actively fertilized orchard bellow the irrigation system through which fertilisers are applied (Figure 25).

Figure 25 Labeled plots with different designs and treatment scenario in a wet meadow in Ezerani Nature Park (above) and plot placed in apple orchard (below) ©Marija Chobanova

The placed plots were left untreated for a month, therefore analysis were done before the fertilization treatment and after the fertilization treatment. After a month of settling, NPK fertilizer was applied to nine of the experimental plots with quantity corresponding to the usual application of fertilisors by locals – data derived from the survey analysis. The remaining nine plots were left to simulate the natural condition of wet meadows with different management settings. Before applying the fertilizer, on the same day, soil and water samples were collected for chemical analysis. The second collection of soil material was done on August 19, 2022 while water samples were collected in October (due to lack of rain). Water sampling was further done in March 2023 without previous additional fertilization.

Laboratory soil analysis

The collected soil samples from the field were dried at room air temperature and then sieved through a 2 mm sieve. The sieved soils were further dried in a dryer at a temperature of 105˚C to absolute dry mass. In this way, the samples were prepared for the further chemical analyses.

Soil organic matter was determined using dry combustion at a temperature of 550˚C for 2 hours (Schulte et al. 1991). For pH determination, 10g of the air-dry soil was measured and mixed with 25 ml of distilled water with pH of 6.7. The solution was mixed for 5 minutes and left to settle for about 45 minutes. The pH was then measured with a laboratory pH meter. The classification of soils based on their pH reaction was made according to Filipovski (1984). The Kjeldahl method was used to determine the total organic nitrogen (Bradstreet 1954). The sample was combusted with H_2SO_4 and K_2SO_4 in the presence of selenium. The solutions prepared in this way were combusted in a sand bath at a temperature ranging from 180˚C to 250˚C until discoloration or ash white, light green color. After the complete combustion, distillation of ammonia nitrogen was done according to the Kjeldahl semi-micro method, where HCl was used as an acceptor, and titration was done with NaOH. Determination of readily available phosphorus (P_2O_5) and potassium (K_2O) were determined according to the AL method of Egner, Riehm and Domingo (Oreshkin 1980). The soil sample is extracted with AL extraction solution within 4 hours at a temperature of 20°C. Phosphorus content was determined colorimetrically in the obtained extract, and potassium content was determined with flame photometry. Organic carbon in the soil was determined according to the Kotsman method (Bogdanović et al. 1966) based on the oxidation of organic carbon with a 0.1 N solution of $KMnO_4$ added in excess. Titration of the excess was done with 0,1 N $H_2C_2O_4$. For mineral content and heavy metals analysis (K, Ca, Mg, Na, Fe, Mn, Zn, Pb, Ni, Cd и Cu), samples were combusted in a sand bath at a temperature ranging from 120˚C to 180˚C and then were determined by atomic absorption spectroscopy on Agilent 55 and Agilent 240Z (Allen, 1989).

Laboratory water analysis

1L water samples were collected in clean plastic bottles filled to the top with water (without air). From the collected water from the field, about 300 ml of the water was filtered through Vatman filter paper - blue band. Since the waters were intensively colloidal, one teaspoonful of laboratorial activate carbon was added to all samples prior to filtration. With this, all samples were prepared for further chemical analysis.

A spectrophotometric method based on the reaction of nitrites with sulfanilic acid and α-naphthylamine was used to determine nitrites in water. For the determination of nitrates, the principle of the method is based on the reaction of brucine-sulfuric acid with nitrates, resulting in a yellow coloration. The intensity of the yellow coloring is directly proportional to the amount of nitrates. These analysis were done on the filtered water samples.

Unfiltered samples were combusted in Ethos Lean microwave. We followed the EPA 3015A protocol where 45ml water sample is mixed with 5ml of nitric acid and combusted for an hour on temperature up to 200 ˚C. These samples were than used for determination of minerals and heavy metals (K, Ca, Mg, Na, Fe, Mn, Zn, и Cu) by atomic absorption spectroscopy on Agilent 55 and Agilent 240Z (Allen, 1989).

4.3.2 Results and Discussion

The conducted survey intended to collect data regarding the usual fertilizer application in apple orchards in Prespa in terms of quantity but also type of fertilisor. We than used this information to set our experimental design in the wet meadow. Even though the survey results refer to fertilisers used in orchards, we are aware of the tendency of local community in Prespa to overfertilise everything, even wet meadows. Even tough wet meadows in Prespa are generally not fertilized (Nikolov et al. 2020) still, 8% from the 25% which are, are fertilized with organic fertilisors while 17% with artificial fertilizers. On the other side, regardless of the place of application, intensive nutrient load reaches all ecosystems in the landscape. Therefore, we wanted to test the changes that will take place in wet meadow's soils and precipitation water, by simulating high intensive fertilization.

In total, 39 people who own apple orchards were surveyed. All respondents were male, aged between 22 and 74 years whereas the group between 40 and 50 years of age occurs with the highest frequency. The respondents were from ten Prespa villages: Asamati, Grnchari, Dolno Perovo, Drmeni, Ezerani, Nakolec, Podmochani, Rajca, Slivnica and Stenje. Respondents with high school education dominated (55%) while most of the respondents are full-time employed (18 or 46%). Only 7 respondents are self-employed farmers. Regarding the question of occupation, even 54% did not answer the question. In the rest of the answers, somewhat more are employed in the private sector, and less in public administration. Apple production is a basic livelihood for only 5 respondents (13%). Another 12 (31%) partially depend on apple production. Most of the respondents (77%) own orchards with an area between 1 and 3 ha. Only one respondent has orchards less than 1 ha. To perform the work in the orchards, most of the respondents hire seasonal workers (72%), and seven respondents (8%) do all the work themselves.

All respondents confirmed that they use fertilisors for their apple orchards. All respondents use NPK fertilisers with different ratios of the elements. The most frequently (23%) used ratio (alone or in combination with others) is 8:16:24. Finally, most of the respondents (39%) use from 400 - 600 kg/ha, and even 15% use from 800 - 1000 kg/ha. Most of the respondents (92%) expressed that they apply fertilisers one to three times per year, mostly (44%) in spring. 59% apply the fertilizer by hand or combined (41%) with machinery. In the year 2022 when this survey was conducted, there was an increase of the price of all fertilisors. Therefore, we asked our respondents if that would influence to the quantity and frequency of application. Majority of the respondents expressed that they would not apply fertilisors at all, while only 3 people expressed that the price would not have any effect on their usual practices (Table 9).

Table 9 Answers regarding the question if the increased price would influence the quantity and frequesncy of application of fertilisors

Answers	Number of respondents
Yes, both quantity and frequency will be reduced	7
Yes, the frequency will be reduced	3
Yes, the quantities will be reduced	2
Yes, I will not use fertilisors at all	24
No, the price will not have any affect	3

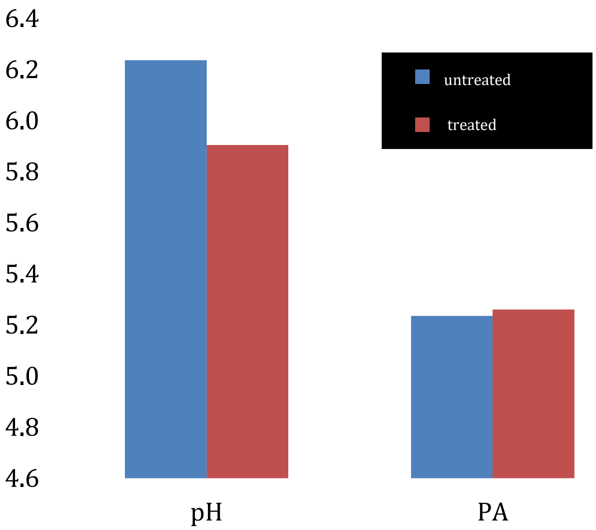
Even though relatively small number of respondents, having observed the situation on the field we believe that these responds reflect the real situation. Trpevski et al.(2000) present fertilization techniques in apple fields in Prespa. They also present application of 500-700kg/ha with fertilisor ratio of 4:7:28. We should emphasize that in the past 20 years in the Prespa region, numerous projects were implemented in the direction of introducing "organic" agriculture with the use of less artificial fertilizers and pesticides. It is obvious that the intensity of using artificial fertilizers has not changed at all, while the composition of the used fertilizers is changing, probably depending on their availability on the market. For comparison, in the countries of the European Union, about 80 kg/ha of artificial fertilizers are used in fruit production (European Commission 2001). Although this data refers to different types of orchards (apples, pears, peaches, but also lemons, oranges and olives), it can be concluded that the use of artificial fertilizers in the Prespa region is far above the European average.

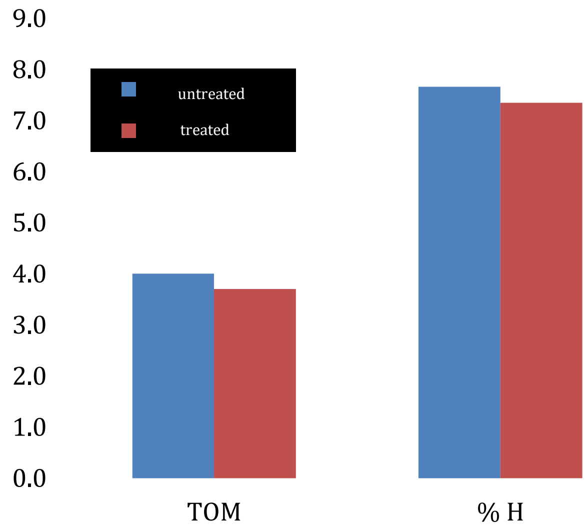
Firstly, we analysed the chemical composition of the soils before the fertilization treatment. These values presents us the initial soil conditions. The average values of the measured parameters are presented in Table 10. Acidity (pH) varies between 5.94 and 7.14 with an average value of 6.12. Total organic nitrogen (N) has maximum values of 0.305% and lowest values of 0.056%, with an average value of 0.19%. The highest values for phosphorus of 3.595g/100g are and the lowest values are 0.921g/100g. The highest value for potassium (K_2O) is 13.68 g/100g. Calcium (Ca) in relation to potassium (K) has a higher average value of 1964.24. Regarding heavy metals, manganese shows the highest average values with 5.19 $mg \cdot kg^{-1}$, and copper shows the lowest average value with 0.36 $mg \cdot kg^{-1}$.

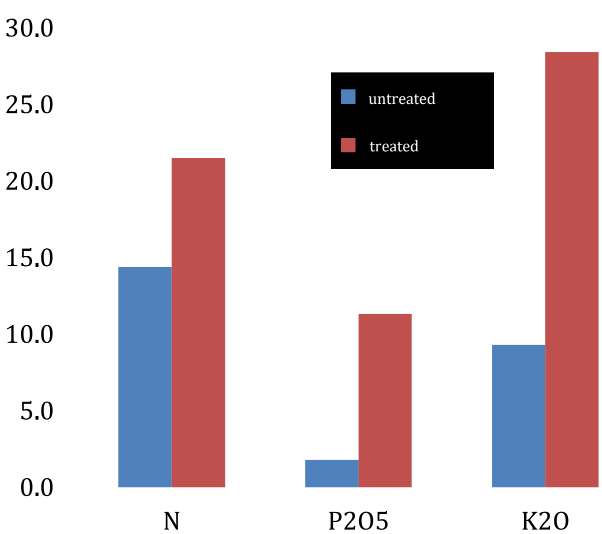
Table 10 Average values of different parameters measured in soils before fertilization treatment

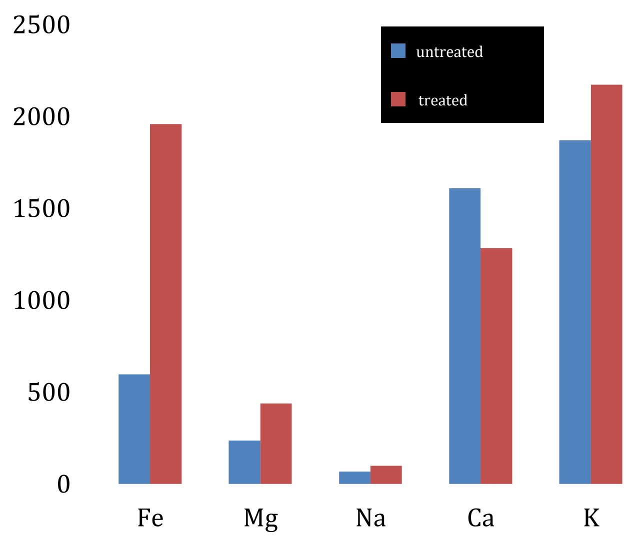
Parameters	pH	PA	TOM	H	N	P_2O_5	K_2O	Fe	Mg	Na	Ca	Mn	K	Zn	Cu	Pb	Cd	Ni
			%			[g/100g]		[$mg \cdot kg^{-1}$]										
Average value	6.12	5.3	4.1	7.8	0.2	2.2	10.3	151.9	61.8	72.8	1964.2	5.2	1780.3	2.5	0.4	3.4	0.4	3.2
StDev	0.2	0.2	0.3	0.3	0.1	0.9	3.1	1.8	0.8	1.6	7.0	0.4	6.2	0.2	0.1	0.3	0.1	0.2

The obtained results for the quantities of applied fertiliser per ha were than recalculated to the surface area of our plots. 10g NPK fertilisor was applied to nine plots that were assigned to be the treatment plots: 8% N (total nitrogen), 16% P (as P_2O_5) and 24% K (as K_2O) which is equivalent to around 700 kg/ha. Samples were again collected one month after the fertilization treatment. Figure 26 shows the differences between the mean values of the analysed parameters between unfertilized and fertilized plots. Differences, especially between the values for pH, N, Mn, P_2O_5 and K_2O are noticeable, for which we determined that there is a statistically significant difference (t-test, $p<0.05$). For all of these parameters (with the exception of pH), there is an increase in their concentrations, which is a direct consequence of the application of artificial fertilizer. A similar trend can be observed for the following elements: Fe, Mg, Na, Zn, Cu, Pb and Ni, but in these cases we did not find statistically significant differences, probably due to the heterogeneity of the results and the relatively limited dataset. Changes are not observed for potential acidity (PA), TOM and percentage of humus. Of the elements, the only reverse trend is observed for Ca, which is understandable, due to its greater solubility in acidic soils, but this conclusion should be confirmed by further research.









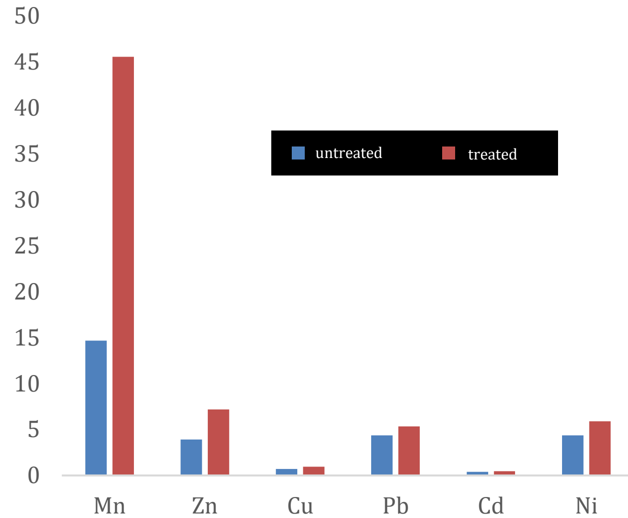


Figure 26 Average values of all analysed parameters and comparison between the treated and untreated plots

General overview of the differences between the average values of the results between the different types of management are presented in Table 11. Extreme differences were not observed while the small dataset limited us for conducting further statistical analysis. Additionally, Table 11 presents the measurements from the plots in the apple orchards.

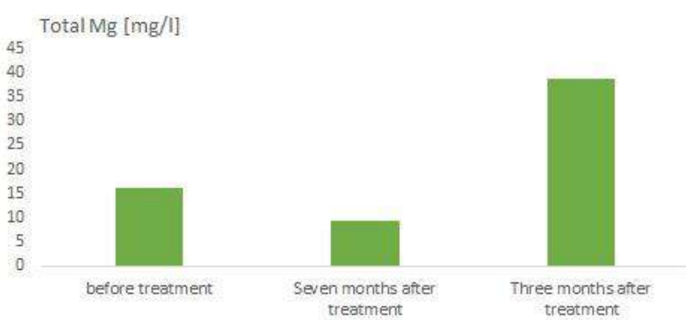
Table 11 Average values of measured parameters (N in %, all others in mg/kg) within the different scenarios shown in different management scenarios

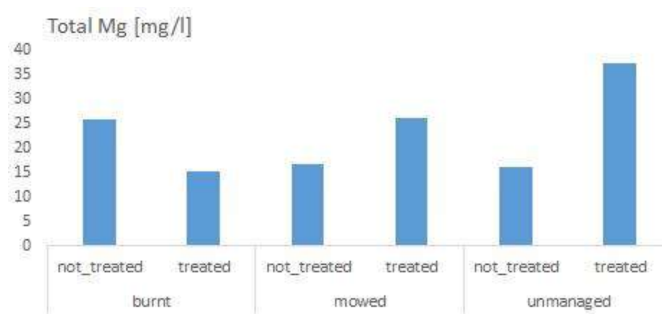
Plots	N	Mn	Fe	Mg	Na	Ca	K	Zn	Cu	Pb	Cd	Ni
Meadow												
treated	**0.19**	**45.52**	**1958.19**	**437.22**	**97.95**	**1283.53**	**2172.34**	**7.16**	**0.94**	**5.34**	**0.47**	**5.90**
burnt	0.17	7.98	445.42	144.00	118.34	2328.56	2770.68	2.02	0.46	4.92	0.34	4.01
mowed	0.17	55.64	1938.79	296.69	82.56	979.30	2118.69	6.77	1.17	5.80	0.41	6.99
unmanaged	0.19	66.55	3462.93	1168.66	90.94	565.43	1543.42	12.74	1.24	5.75	0.38	7.98
Before burning	0.24	51.91	1985.61	139.52	99.97	1260.84	2256.56	7.13	0.88	4.87	0.73	4.64
untreated	**0.18**	**24.62**	**1044.95**	**341.48**	**67.76**	**1477.18**	**1848.72**	**4.63**	**1.13**	**4.59**	**0.43**	**4.82**
burnt	0.12	17.95	327.76	660.41	56.55	1309.84	1804.02	4.33	0.80	4.34	0.34	4.95
mowed	0.16	27.19	1605.24	100.54	66.71	1316.92	2082.97	5.86	1.17	6.02	0.45	5.82
unmanaged	0.16	6.28	147.63	57.79	85.24	1499.15	1509.60	2.02	0.39	2.51	0.18	3.06
Apple orchard												
treated	**0.20**	**98.90**	**4202.05**	**705.34**	**112.15**	**2800.41**	**6173.01**	**12.59**	**3.81**	**5.40**	**0.34**	**7.18**
Apple orchard	0.20	98.90	4202.05	705.34	112.15	2800.41	6173.01	12.59	3.81	5.40	0.34	7.18
untreated (by us)	**0.32**	**42.51**	**1183.83**	**542.50**	**97.59**	**4794.30**	**6834.82**	**5.62**	**1.44**	**4.00**	**0.65**	**3.75**
Young apple orchard	0.29	37.89	1153.66	497.62	109.37	4580.59	7466.86	5.37	1.32	3.72	0.63	3.66
Old apple orchard	0.34	47.11	1214.00	587.36	85.81	5007.99	6202.76	5.86	1.55	4.27	0.65	3.84

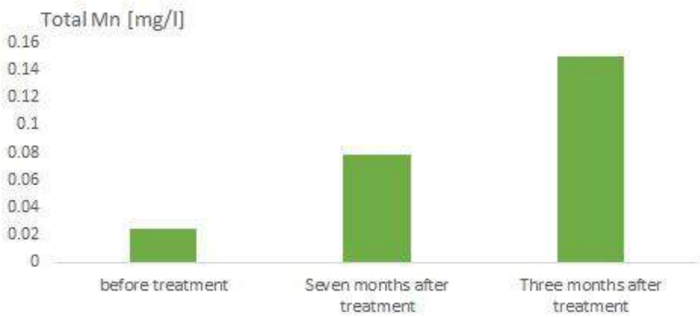
Our results show that the application of artificial fertilizers changes the chemical composition of the soils. Also, the reduction of the pH reaction in the soils where artificial fertilizer has been applied can be observed. According to the data shown in the graphs above, an increase in nitrogen (N) and available forms of phosphorus (P_2O_5) and potassium (K_2O) can be ascertained. In particular, the concentration of potassium has increased, considering that in the applied fertilizer NPK 8:16:24 where potassium is represented with the highest percentage. According to the obtained results, we can say that wet meadows participate in the retention of nutrients from the application of artificial fertilizers. Many countries have implemented wetland restoration projects to reduce nitrogen loads in surface and groundwater (Hoffmann and Baattrup-Pedersen 2007). Several researchers from different European countries have studied the effectiveness of different techniques (Grootjans et al. 2002). According to Bakker & Olff (1995) management with traditional mowing management is essential for conservation and maintenance of wet meadows.

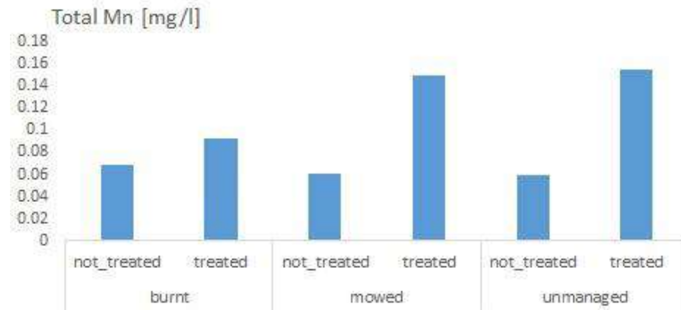
It is obvious that the content of heavy metals is higher in the fertilized plots compared to the non-treated ones. In particular, an increased content of manganese (Mn) is observed, as well as zinc (Zn), lead (Pb) and nickel (Ni). Moreover, the content of cadmium (Cd) and copper (Cu) does not differ much between the soil from fertilized and unfertilized plots. The availability of heavy metals in the soil depends on the pH reaction of the soil, so according to Filipovski (2003), the increase in the concentration of heavy metals in the soil is amplified by an increased in acidity. The obtained results lead us to believe that the increased availability of heavy metals may be a consequence of soil acidification from the applied artificial fertilizer.

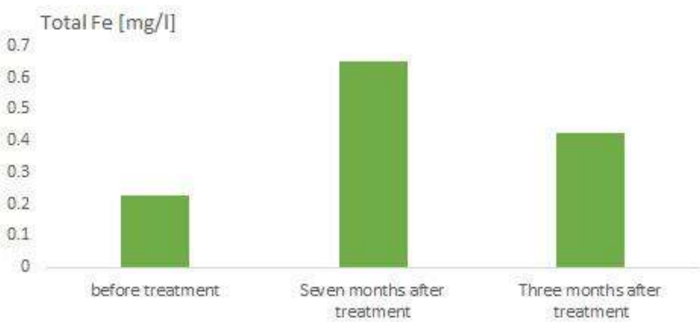
In our attempts to further test soil retention and fertilisor's influence on wet meadows, we have also conduced water analysis. Rainwater that enters the plots and percolates through wet meadow's vegetation and soils was collected thanks to our experimental design (see more in Chapter 4.3.1). In total, 51 water samples were collected in June 2022, October 2022 and March 2023. With our analysis we wanted to assess the chemical composition before and after the fertilization treatment, as well as to detect differences within different management practices (Figure 27).

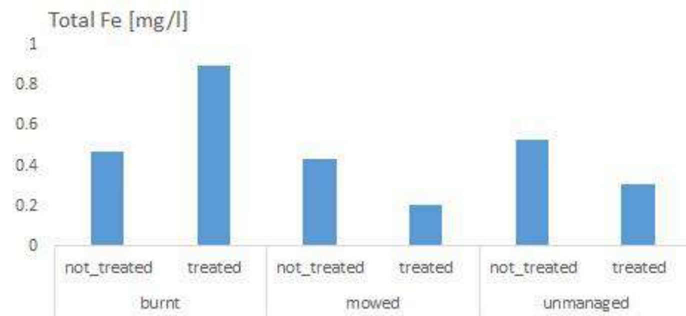


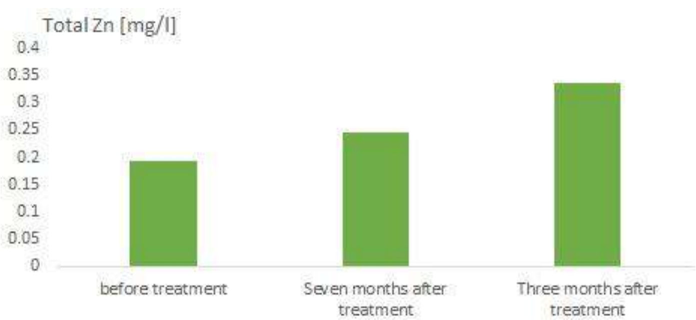


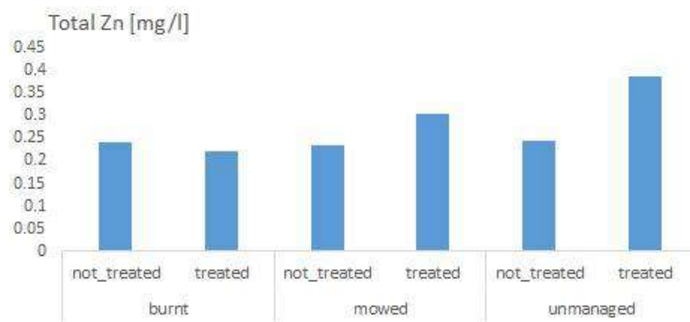

Total Mn [mg/l]
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
before treatment
Seven months after treatment
Three months after treatment

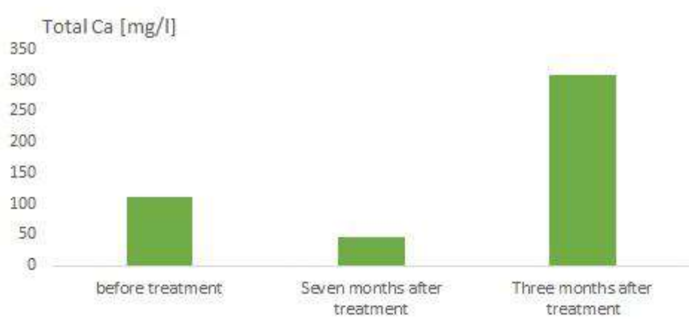

Total Mn [mg/l]
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
not_treated
treated
not_treated
treated
not_treated
treated
burnt
mowed
unmanaged

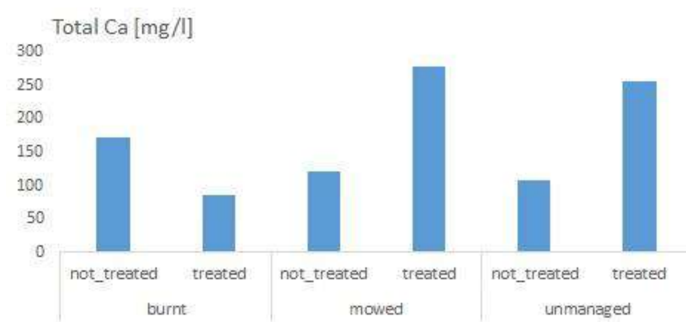

Total Fe [mg/l]
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
before treatment
Seven months after treatment
Three months after treatment

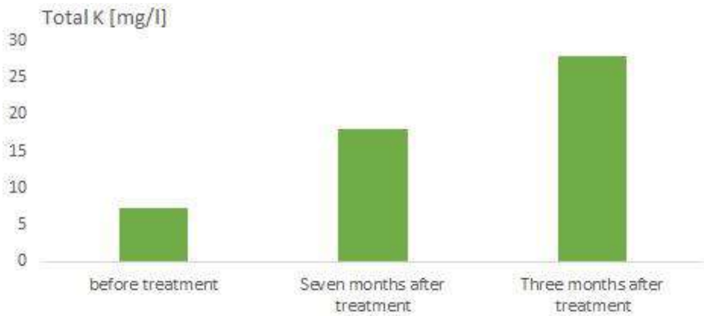

Total Fe [mg/l]
1
0.8
0.6
0.4
0.2
0
not_treated
treated
not_treated
treated
not_treated
treated
burnt
mowed
unmanaged


Total Zn [mg/l]
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
before treatment
Seven months after treatment
Three months after treatment


Total Zn [mg/l]
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
not_treated
treated
not_treated
treated
not_treated
treated
burnt
mowed
unmanaged

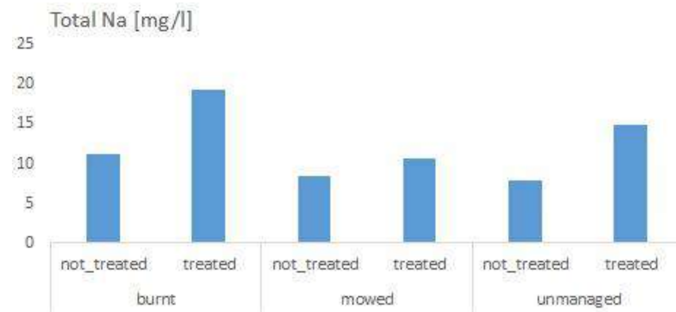

Total Ca [mg/l]
350
300
250
200
150
100
50
0
before treatment
Seven months after treatment
Three months after treatment

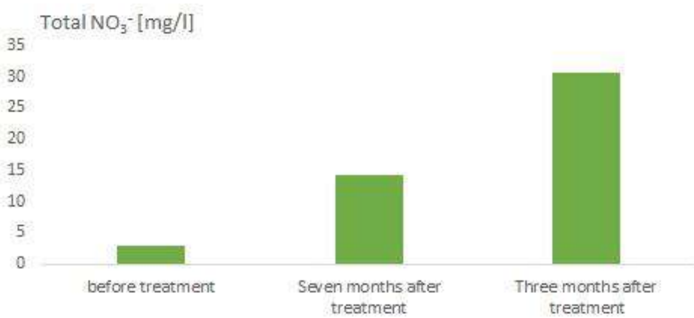

Total Ca [mg/l]
300
250
200
150
100
50
0
not_treated
treated
not_treated
treated
not_treated
treated
burnt
mowed
unmanaged


Total K [mg/l]
30
25
20
15
10
5
0
before treatment
Seven months after treatment
Three months after treatment

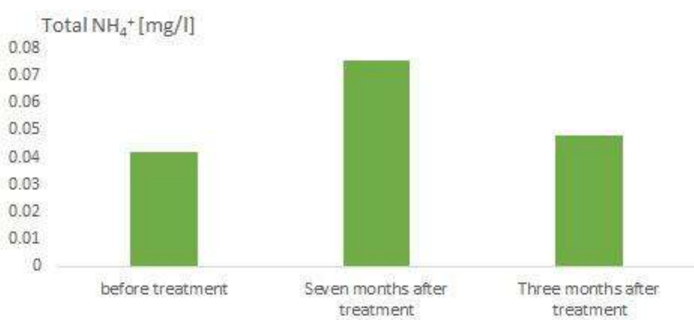

Total K [mg/l]
45
40
35
30
25
20
15
10
5
0
not_treated
treated
not_treated
treated
not_treated
treated
burnt
mowed
unmanaged


Total Na [mg/l]
16
14
12
10
8
6
4
2
0
before treatment
Seven months after treatment
Three months after treatment


Total Na [mg/l]
25
20
15
10
5
0
not_treated
treated
not_treated
treated
not_treated
treated
burnt
mowed
unmanaged


Total NO_3^- [mg/l]
35
30
25
20
15
10
5
0
before treatment
Seven months after treatment
Three months after treatment


Total NO_3^- [mg/l]
35
30
25
20
15
10
5
0
not_treated
treated
not_treated
treated
not_treated
treated
burnt
mowed
unmanaged


Total NH_4^+ [mg/l]
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
before treatment
Seven months after treatment
Three months after treatment

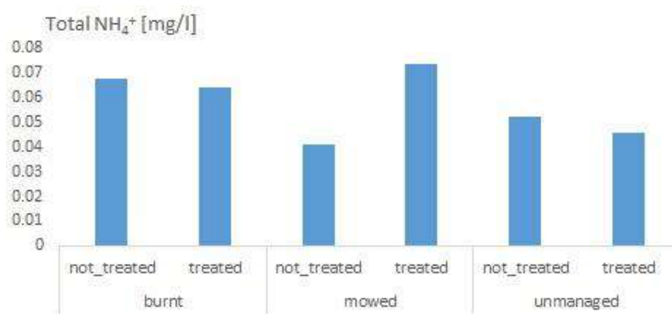

Total NH_4^+ [mg/l]
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
not_treated
treated
not_treated
treated
not_treated
treated
burnt
mowed
unmanaged

Figure 27 Average values for total Mn, Mg, K, Ca, Na, Zn, Fe, NO_2^-, NO_3^- and NH_4^+ content in soil water in wet meadow in Prespa presented in different time intervals and in different management scenarios

According to the soil water analysis results shown above, differences can be observed in mowed and unmanaged plots before fertilization treatment. Thus, for some of the elements such as Mn, Fe, NH_4^+ NO_2, much higher values can be observed in the non-mowed plots compared to the mowed ones. On the other hand, no significant differences were observed between mowed and unmanaged plots for the other measured parameters. The application of artificial fertilizers changes the chemical composition of the waters. It is obvious that the content of K, NO_2^-, NO_3^-, Na is higher in mowed, unmanaged and burnt plots compared to non-fertilized ones. From the results, it was established that there is a difference in the chemical composition of soil water in some elements between fertilized and mowed plots and fertilized unmanaged plots, so the concentration of NH_4^+, NO_2^-, NO_3^-, K and Ca is higher in mowed treated compared to unmanaged treated plots. While the concentration of sodium (Na), manganese (Mn), zinc (Zn) iron (Fe) and magnesium (Mg) show higher values in unmanaged treated plots compared to mown treated. Wet meadows in Prespa are often target to fires that affect the chemical composition of the waters. From the obtained results, we can determine that the fires have a negative effect on the soil water and this can be clearly seen in the following parameters: Fe, K, Na, NO_2^-, NO_3^-, which have higher values in the burned treated plots compared to non-treated ones. According to the results of the soil analyses carried out in the wet meadow, higher concentrations of manganese (Mn) are observed in the treated plots, while in the soil water analyses we observe lower concentrations of Mn. A similar trend is observed in the results of iron (Fe) analyses, higher concentrations in soil samples compared to soil water samples. These results indicate that wet meadows participate in the retention of some nutrients and heavy metals applied through artificial fertilizers. A reverse trend can be observed in the results for calcium. The soil analyses of the treated plots show lower concentration of calcium, compared to the analysis of the soil water from the treated plots.

4.4 Connecting the dots: threats, pressures and overall condition

Upon amalgamating the derived outcomes, our studies indicate that the wet meadows within the Prespa region manifest heterogeneity as ecosystems, engaging in interactions with contiguous ecosystem types in the landscape. In consideration of their ecological attributes, they can be characterized as wetlands (Figure 28) experiencing seasonal water saturation, predominantly during the late winter and early spring months.

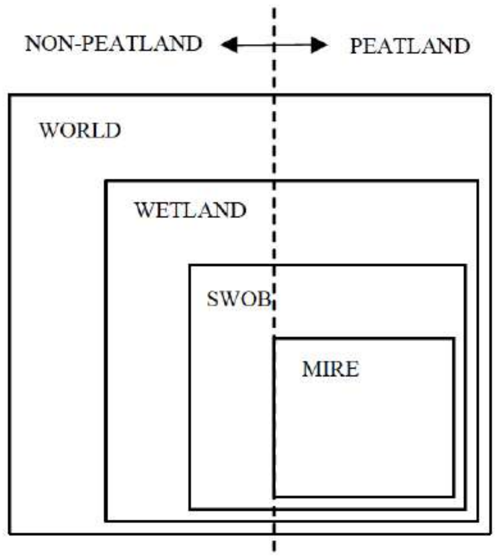


Figure 28 Definition and classification of peatlands according to Joosten et al. (2017)

Their classification as wetlands aligns with the examined plant communities. However, discernible shifts in vegetation structure unequivocally signal an ongoing succession necessitating management interventions (Figure 29). Some of these alterations occur rapidly, driven by the competitive nature of certain plant species. In accordance with local knowledge, numerous meadows traditionally mowed have undergone conversion to agricultural land or transitioned into habitats dominated by reedbeds.

The deterioration of wet meadows in the Prespa region results from a confluence of factors, including climate change, alongside anthropogenic influences such as external eutrophication, land conversion, and drainage. It is imperative to underscore that a comprehensive understanding of diverse processes is pivotal in formulating conservation measures.

Figure 29 Wet meadow overgrowth with shrubs and dominance of Calamagrostis epigeios in Prespa ©Marija Chobanova

In context of succession, external eutrophication is one important factor that needs attention. In Prespa, this is done by intensive fertilisation of agricultural land, but also with direct disposal of organic waste on the wet meadows, mainly apples (Figure 30). This massively happens in almost all ecosystems along the shoreline in very big quantities (tonnes), especially as a protest by the local farmers when the purchase price of the apples is low.

Figure 30 Apples undergoing decomposition deposited in a wet meadow in Prespa ©Marija Chobanova

The initial impetus for undertaking various ecological analyses was to evaluate the role of wet meadows in the accrual and retention of nutrients and minerals. In assessing the accumulation rate, we have examined the chemical composition of the biomass. Considering the mowed biomass, one can compute the quantities of nutrients and minerals extracted through the mowing process. For the calculation of the extracted quantities, the measured content in absolute dry biomass was aligned with air dried biomass and then calculated for the air dried mowed biomass (Table 12). The mowed biomass corresponds to 15 ha surface area, however annual biomass variations should be taken in consideration as pointed earlier (see more in Chapter 3.2). Therefore, we cannot exactly express the extracted quantities per ha area, nevertheless we can use the values of the three year cycle as indicative values. In this regard, we have extracted 55 kg N per ha, 43 kg K per ha and 5.3 kg P per ha. As a reminder, the analysis from the survey for application of fertilisors (on agricultural land), resulted in 700 kg NPK per ha (Chapter 4.3.2).

Table 12 Calculation of extracted quantities of different chemical elements with mowing

Calculation formula		**Chemical elements**								
		Mn	Fe	Mg	Ca	K	P	N	Cu	Zn
% E in absolute dry biomass	$\% E = \frac{mk * p}{100}$ E = chemical element mk = average of absolute dry mass of all plant species in Perovo 1 p = average of the measured content (in %) in absolute dry biomass of the given element E	0.0025	0.0022	0.0305	0.1631	0.3078	0.0381	0.3923	0.0002	0.0007
Kg E in air dry biomass	$kg\ E = \frac{mt * 1000}{\%E}$ E = chemical element mt = average of air dry mass of all plant species in Perovo 1 $\%E$ = % of element in absolute dry biomass of the given element E	0.0902	0.0776	1.0847	5.8039	10.95	1.3574	13.95	0.0072	0.0256
Kg E in total mowed biomass	$kg\ Et = \frac{mb * kg\ E}{1000}$ E = chemical element mb = kg of total mowed biomass (air dried) from three years (58958 kg) kg E = kg of element in air dry biomass of the given element E	**5.3191**	**4.5753**	**63.9524**	**342.19**	**645.66**	**80.032**	**822.97**	**0.4219**	**1.5096**
% E in air dried mowed biomass	$\% Et = \frac{kg\ Et}{mb * 100}$ E = chemical element mb = kg of total mowed biomass (air dried) from three years (58958 kg) kg Et = kg of element E in mb	**0.0090**	**0.0078**	**0.1085**	**0.5804**	**1.0951**	**0.1357**	**1.3959**	**0.0007**	**0.0026**

The majority of plant species inhabiting wet meadows undergo an annual cycle, with their biomass contributing to soil degradation processes. The swift degradation rate, attributed to the low water tables and ensuing oxidation conditions, results in the release of nutrients and minerals into the soil. Subsequently, some of these elements are leached by rainwater or groundwater and conveyed to the Lake.

Besides with biomass removal, the retention capacity of the wet meadows can be increased with improving the hydrological conditions. High water tables were observed in only short period of the year, which we believe does not reflect the former hydrological regime. Namely, near by the study area in Perovo locality there is a big ditch that alters the water levels. Additionally, all over the region there are many wells that pump significant amount of water for irrigation purposes (Figure 31).

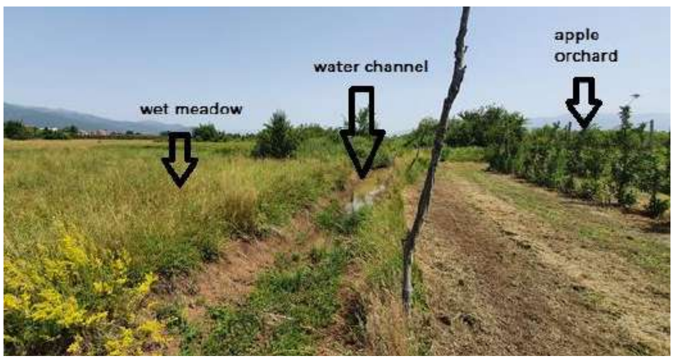
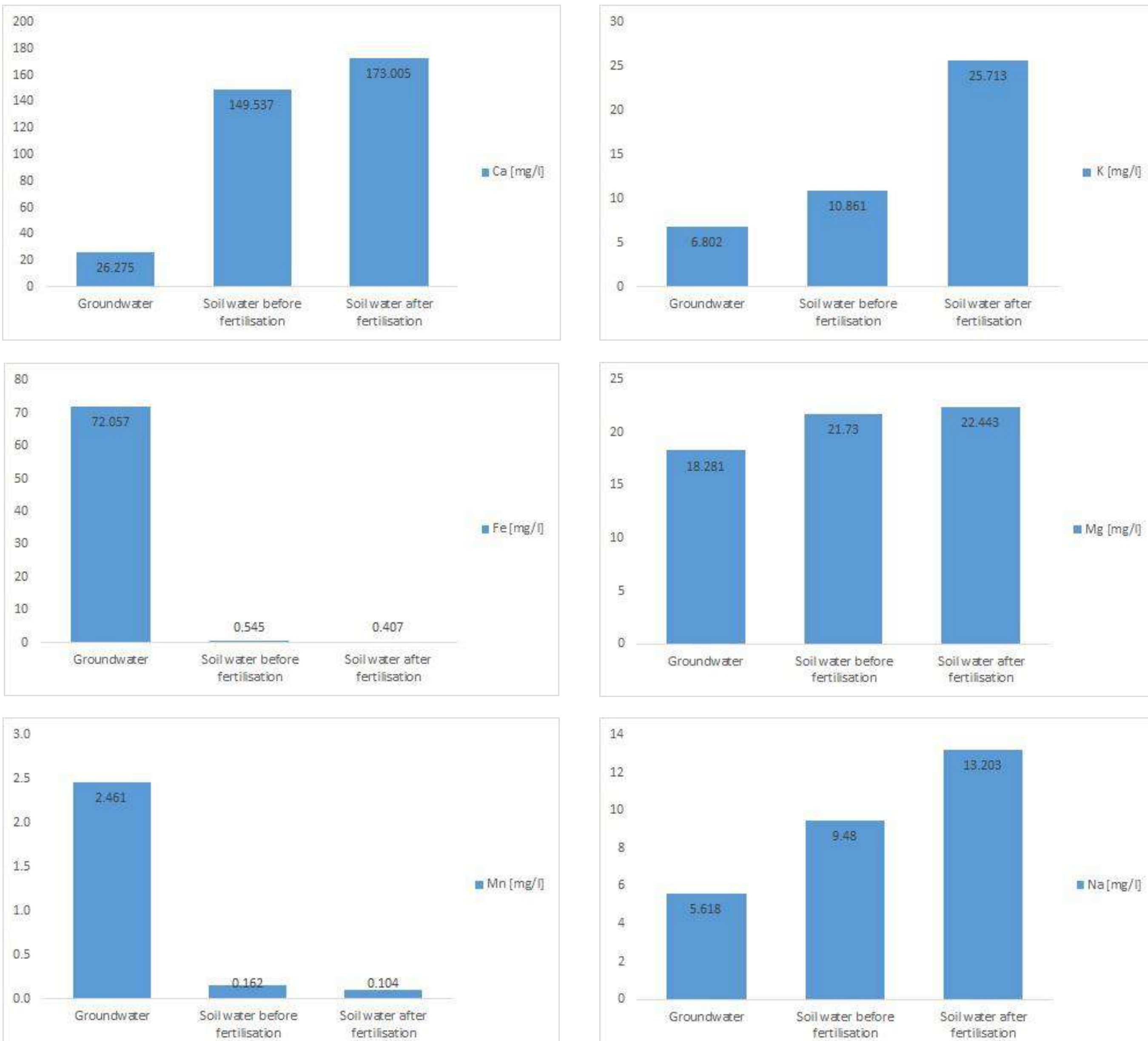


Figure 31 Man-made ditch (left) and water extraction from Stenjsko Blato with water pump for irrigation purposes (right) ©Marija Chobanova

Unfortunately, there is no exact information regarding their quantity or the quantity of water extraction. Lastly, drastic water level changes are observed in the lake water, which is likely provoked by decrease in water quantities on a Basin level. All of these factors collectively contribute to alterations in the hydrological conditions of wet meadows, exerting adverse effects on their ecological integrity. With diminished water tables and an accelerated degradation rate, the accumulation of dead organic matter is markedly limited. The humus content in the soil at the Perovo locality stands at 7.8% (Table 10), a comparatively low value when juxtaposed with authentic peatland soils (Trencheva 2021). Moreover, the humus horizon (A) in the soil is relatively shallow (Table 6), signifying a reduced accrual of organic matter. Despite the latent potential, the existing capacity for carbon sequestration in the soil is notably modest. Considering the negative trajectory of hydrological conditions in recent years, it is conceivable that degradation surpasses accumulation, thereby transforming these ecosystems from carbon sinks into carbon sources. Further investigation is warranted to assess the decomposition rate (for e.g. litter bags method), with a particular focus on elucidating the principal endogenous and exogenous factors influencing the humification or mineralization of organic substances. The water table assumes critical importance in peatland development as it regulates species composition by inducing anoxia (lack of oxygen) at depth, thereby impeding decomposers and facilitating peat accumulation.

The conducted analysis permits a straightforward juxtaposition between groundwater chemistry and the chemical composition of soil water. Notably, with the exception of manganese (Mn) and iron (Fe), groundwater manifests lower concentrations of almost all elements. Moreover, discernible evidence of fertilizer influence is apparent, as evidenced by elevated concentrations of calcium (Ca), potassium (K), sodium (Na), nitrite (NO_2^-), and ammonium

(NH_4^+) (Figure 32). These compounds traverse the hydrological network and reach the lake water, where their dynamics in element cycling vary contingent upon numerous factors inherent to the lake as a water body. Consequently, this form of pollution, particularly nutrient load, amplifies the eutrophication rate in the lake. However, our studies do not precisely quantify the exact input, necessitating further comparison with lake water analyses. Nevertheless, this simple experiment serves to quantify the pollution of soil water resulting from the application of the most prevalent quantity and type of fertiliser in the region.



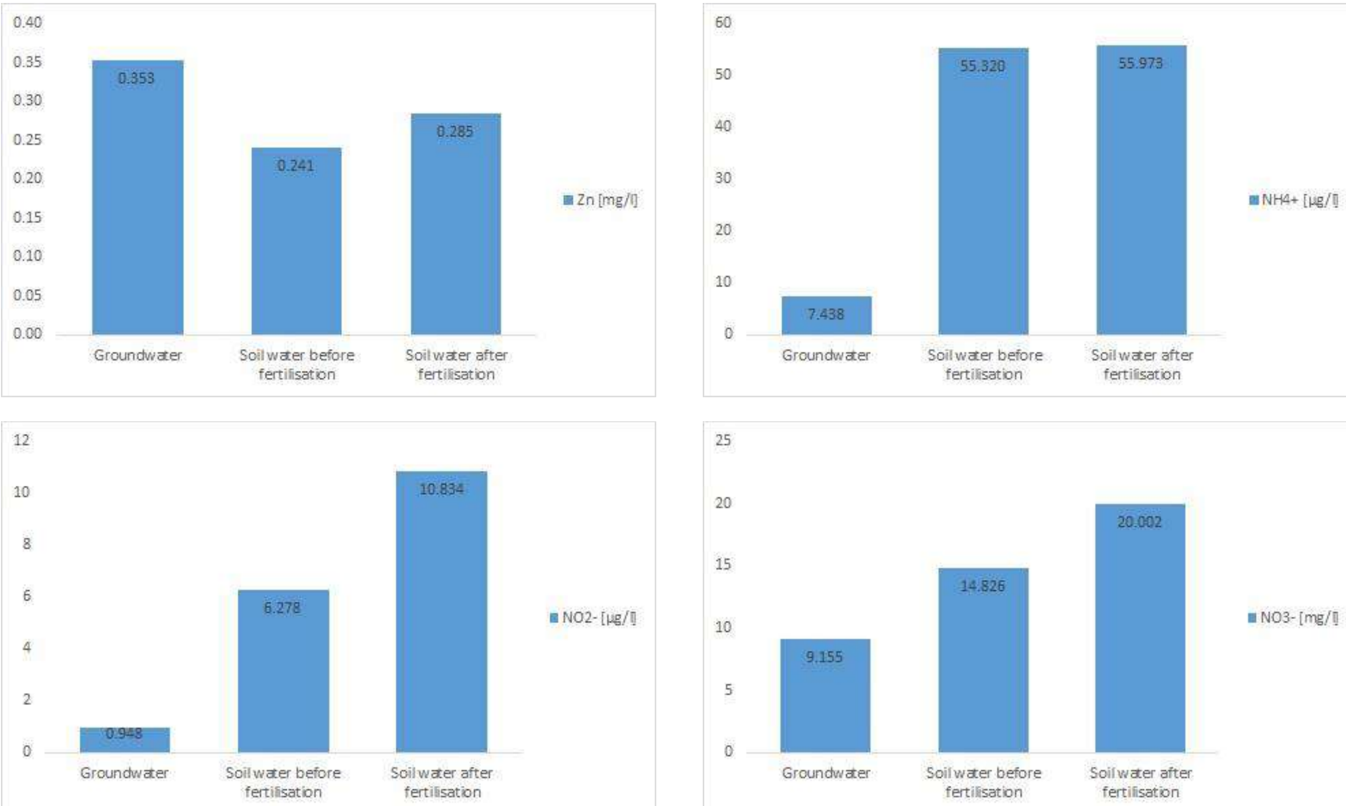


Figure 32 Groundwater and soil water (before and after fertilisation) chemical composition

It is worth emphasising the significantly higher concentrations of Fe and Mn in the groundwater. Namely, these concentrations exceed the European Union (EEA 2020) and Macedonian (Службен весник на Република Македонија 1999) reference values. Considering the extensive utilization of groundwater for irrigation in the region, it is imperative to undertake supplementary analyses to corroborate the aforementioned findings. Anticipatedly, higher concentrations of the investigated metals are expected in groundwater as opposed to soil water, given that precipitation water inherently exhibits minimal concentrations of these elements. As precipitation water percolates through the soil, these elements typically form robust complexes with soil particles. Cations such as calcium (Ca), sodium (Na), and potassium (K) readily dissolve, consequently being present in soil water samples. In contrast, groundwater traverses the bedrock, and its chemical composition should ostensibly reflect the geological attributes of the area. According to Stafilov et al. (2022), distribution of chemical elements in Prespa's soil aligns with the region's geological characteristics, with localized elevated concentrations attributed to lithogenic sources. While Stafilov et al. assert the absence of indications of anthropogenic soil pollution, heightened concentrations of iron (Fe) and manganese (Mn) in groundwater could also arise from unsustainable agricultural practices promoting the reduction of Fe-Mn oxides (Zhai et al. 2021). Additional analyses are warranted to further validate these assertions. High concentrations of Fe^{2+} and Mn^{2+} (as mainly present form in groundwater) are toxic to many plant species (El-Jaoual and Cox 1998; Saaltink et al. 2017).

When Fe is oxidised, e.g. low water tables, it builds complexes with phosphorus, which decreases the solubility of the phosphates. In this way, P as the fundamentally important nutrient becomes unavailable for plants. Further studies should be made in order to assess the nutrient limitations as a result of fluctuating water levels in the wet meadows in Prespa. The effect of Fe pump load through its reduction with high water tables can be one of the reason for higher production – more phosphates available means better production resulting in higher biomass. Thus, future management should definitely include rewetting or changes in the hydrological regime, in order to complement the removal of nutrients and minerals with mowing activities. However, assumptions regarding phosphorous availability in Prespa have to be made with caution without further research. This is due to the high nitrate leaching from the agricultural lands which according to Smolders et al. (2010) provokes phosphate eutrophication in groundwater fed wetlands.

In relation to water levels, another parameter that requires more attention is the already mentioned NO_3^-. In almost anoxic conditions (with high water tables), NO_3^- reduction begins even before complete removal of O_2. The denitrification process chemically transforms the nitrates to a neutral substance (N_2) and is highly effective. According to Pieterse et al. (2005) on only few meters from heavily fertilised cornfield (600kg N ha^{-1} yr^{-1}), hardly any N is present in peat water. This illustrates the important role of wet meadows (with good hydrological conditions) in terms of water purification.

Ultimately, one of the noteworthy outcomes pertains to alterations in soil pH consequent to the application of fertilizers. Soil pH is a critical determinant of nutrient availability due to its impact on the solubility of soil cations and the absorptive capacity of plant roots for nutrients (Sharpley 1991). Generally, a reduction in soil pH augments the accessibility of cations, particularly essential micronutrients such as iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) (Lutz et al. 1972). The sustained decline in pH can result in heightened mobility of highly competitive iron (Fe) and aluminum (Al), forming insoluble complexes with phosphates. Conversely, our investigations have revealed high groundwater pH values, contributing to an elevation in soil pH. This phenomenon is substantiated by concentrations of base cations (e.g., calcium and magnesium), which function as neutralizing agents for soil acids. Nevertheless, the accumulation of peat, especially under conditions of persistent high water tables, diminishes the impact of groundwater. Consequently, further research is imperative to elucidate the precise mechanisms and the influence of fertilizers on soil pH, thereby enhancing our ability to anticipate patterns and inform optimal management strategies.

5 Assessment of biomass energy and monetary value

In order to create even stronger bond between the management activities and local stakeholders, we have conducted an assessment of the energetic and monetary value of the produced biomass by implementing mowing practices. The necessity of this assessment arise by the simple question: *Why have people abounded this traditional practice*? This is a rather complex issue that has no simple answer. However, one reappearing point was *do locals have use of the hay*? This point was particularly important for us, since the main aim was to remove the biomass from the system. In order to do so, we had to think of potential beneficiaries of this biomass. Since our approach was to reconnect local communities with the wet meadows, other alternatives such as composting, selling or similar, were not an option. Having in consideration that stakeholder engagement was essential, with this assessment we wanted to generate even more science-based arguments that can be used in further communication with stakeholders or give additional ideas regarding “biomass waste” management.

This assessment attains for several aspects (Figure 33). In ecological terms, the chemical composition of the plant biomass provides information regarding their role in the ecosystems in terms of nutrient cycling. This information is also important regarding their influence on surrounding ecosystems as well. Generating this type of data provides scientific records regarding “ecological management” of the meadows that can be helpful for planning restoration or conservation activities, but also land use in general.

Wet meadows in Prespa have connection with farmers not only through mowing but also through grazing activities. Therefore, the knowledge of the nutritional value of the meadow’s biomass is essential. For herbivores, meadows and pastures represent the most cost efficient source of nutrients. So, it is critical to understand the nutrient status in order to maintain optimal animal health and productivity. In animal husbandry, the acquired nutritional value data are useful for creating balanced diets and improving breeding technics.

The local and regional economies may be significantly impacted with assessing the monetary worth of meadow’s biomass. For instance, pastures can provide fodder for cattle, which has an immediate impact on pastoralists' revenue. Ethical distribution of economic gains and sustainable resource management depend on an understanding of this monetary worth.

Understanding the plants composition can support attempts to preserve and safeguard biodiversity of meadows. Prioritizing conservation efforts might be aided by knowing which plant species are rare or endangered. The wet meadows also contribute to carbon sequestration, an important aspect of mitigating climate change. The capacity of the land to store carbon and the possible effects of changes in land use on greenhouse gas emissions can both be revealed by chemical composition analysis.

Information regarding the structure of the meadows can help landowners and government organizations make sustainable decisions about conservation and land management. Planning for land use, such as whether to develop or preserve grassland areas, can also be influenced by this type of assessments.

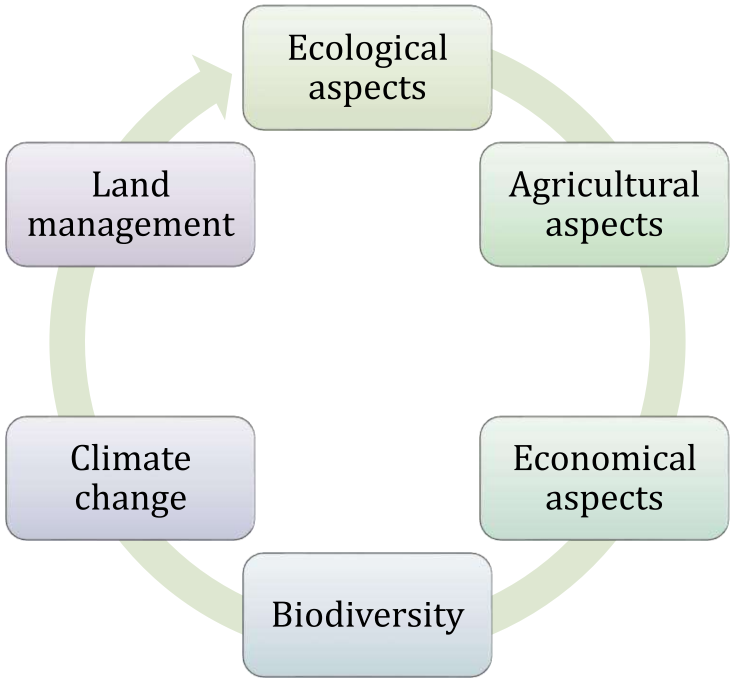


Figure 33 Tackled aspects that are connected with biomass energy and monetary assessment

In general, understanding the chemical composition, nutritional content and monetary value of the meadows biomass is important for agricultural, ecological, and policy reasons. It is crucial to biodiversity conservation, sustainable land management and the general health of both local communities and natural ecosystems.

5.1 Methodology

The collection of the plant material was performed according to (Edvan et al. 2016). In June 2023, average samples of 28 points were taken. For each mean sample, individual samples were taken from at least 16 microsites, with an average distance of 12–15 steps. The weight of the individual sample was 50-70 g. The total plant weight was reduced by using the squaring method (Edvan et al. 2016). The weight of the obtained medium sample was 300-350 g. The measurement was done on a field scale with an accuracy of 1 g. The collected samples were packed in plastic vacuum bags, from which the air was squeezed out, to preserve the original moisture. All samples were appropriately marked.

In three locations, plant material was harvested according to the method by Edvan et al. (2016). A quadratic frame was randomly placed on the microsite. The quadratic frame is made of aluminum profiles and it has dimensions of 1.30 x 1.30 m. Its surface area is 1.69 m^2. Using a motorized mower, the entire amount that was inside the frame was harvested. Measurements of the obtained biomass were carried out on a field scale with an accuracy of 1 g. These results were used in calculating the average aboveground biomass for defined plots. On the same day, samples for nutrient analysis were transported to the Faculty of Agricultural and Food Sciences in Skopje, to the feed analysis laboratory.

The same procedure was repeated in August 2023. In the second collection of plant biomass, average samples of 14 points were taken. The production was also measured from three locations by again using the quadratic frame method.

5.1.1 Sample reduction prior laboratory chemical analysis

The reduction of the samples was performed according to the ISO 6498 standard for the preparation of animal feed samples. The samples were first dried for 24 hours at a temperature of 60˚C, to determine their original-gross moisture content. In this condition, the particle size and weight of the samples were reduced.

As a next step of reduction, we reduced the samples particle size by first reducing them to laboratory-test samples, from which test samples (additional cutting and grinding) were taken to determine the physico-chemical parameters. In this way all samples were homogenized and prepared for further analysis.

The reduction of the sample weight was done by alternative shovelling. This is a very simple technique that has the following advantages:

1) Can be implemented in the lab or during fieldwork
2) Does not require extra equipment (for ex. Separator)
3) Has minimal requirements for maintenance and decontamination
4) It generates all number of separations
5) It has very small error of sample separation

The sample was split into two equal subsamples, one part containing the odd individual samples and the other subsample containing the even individual samples. The same was repeated until obtaining a laboratory-test sample with a mass of 100 g. The test samples were taken from this sample for the analysis of the predicted physico-chemical parameters.

5.1.2 Laboratory analysis

All samples are analyzed in officially accredited feed analysis laboratory at the Faculty of agriculture and food sciences in Skopje. A conventional Weende chemical analysis was performed on each sample by examining:

- moisture (drying at 105 °C),
- crude proteins (determination of nitrogen by wet mineralization and calculation of crude proteins),
- crude fats (determination of residue after extraction with an organic solvent),
- raw fiber (undissolved residue after treatment with dilute acid and base),
- ash (combustion at 550 °C).

With this classical chemical analysis method that dates back to 1860, data on the content of moisture, crude proteins, crude fats, crude fiber and ash are obtained. This method dates back to 1860. The value of NFE (nitrogen-free extractive substances) is obtained by calculation and represents a fraction of easily digestible carbohydrates. However, this method also has certain disadvantages. It is based on the assumption that NFE is the most digestible fraction, and that crude fiber is the least digestible fraction. However, in the NFE fraction, hemicellulose, cellulose and lignin are extracted in a greater or lesser percentage, which certainly do not belong to the digestible fraction. Therefore, the Van Soest method (1991) is recommended as a complement to the Weende method. The Van Soest-detergent extraction method includes determinations of:

- the neutral detergent fiber-NDF (treatment with neutral detergent),
- acid detergent fiber-ADF (treatment with acid detergent),
- the acid detergent lignin-ADL (treatment with 72% sulfuric acid),
- proteins insoluble in neutral detergent - NDICP and
- proteins insoluble in acidic detergent - ADICP

According to the Van Soest method, the dry matter can be divided into two fractions (Van Soest et al. 1991). The first fraction represents cell content soluble in neutral detergent and is composed of proteins, carbohydrates, organic acids and pectins. This fraction, in the largest percentage is usable for animals. The second fraction is represented by cell walls, i.e. fibers insoluble in neutral detergent (NDF). This fraction consists of cellulose, hemicellulose and lignin. In terms of availability, this fraction shows significant variations between different forages, so it is treated with an acidic detergent and thus the fraction known as acidic detergent fibers (ADF) consisting of lignin and cellulose is distinguished. Treatment of the acid detergent fiber fraction with 72% sulfuric acid separates the lignin. The content of cellulose (ADF - lignin) and hemicellulose (NDF - ADF) is calculated, and non-structural carbohydrates can also be calculated (NSC = 100 - (SP,% + NDF,% + EE, %. + ash, %)). NDICP and ADICP are obtained when the crude proteins in NDF and in ADF are determined, which are bounded, but are important for calculating the energy (TDN) of the samples.

The expression of the results of the physico-chemical methods is on naturally dry (air dry) and absolute dry matter. The energy value is expressed in units of dry matter, because forages for ruminants vary widely in terms of dry matter content. This is because the norms for ruminants are calculated on the dry matter of the ration, and also the composition of the ration-meal is based on the nutrients expressed in dry matter.

Dry matter: It is expressed in %. It represents the plant biomass without the water content. Rations are composed on the basis of dry matter, but normally, ruminants are fed naturally (air) dry food.
Crude ash: The fraction gives an overview of the amount of macro and micro minerals in a forage.
Crude protein: the nitrogen in the forage is determined and then multiplied by a factor of 6.25 (on average, protein contains 16% nitrogen). This fraction includes both protein and non-protein (NPN) nitrogen. Such an example of non-protein nitrogen is the artificial fertilizer urea which contains 45%, while the protein equivalent (45 x 6.25) is 281% protein.
Crude fats: This nutrient is the most energy dense per unit mass. In addition to fatty acids, this fraction contains pigments, waxes, sterols and other substances, but digestible energy is provided only by fatty acids.
Crude fiber: this fraction consists of complex carbohydrates: cellulose and hemicellulose and the cyclic hydrocarbon compound-lignin. This fraction may also contain traces of encapsulated proteins and silicon in the lignocellulosic network.

NFE nitrogen-free extractive substances: this fraction is obtained indirectly, when 100 is subtracted from the sum of the values of: moisture, ash, protein, fat and crude fiber. As a rule, the NFE fraction includes starch, sugars, fructans, pectins, organic acids and pigments.
NDE: neutral detergent extract. It is the content of the plant cell soluble in a neutral detergent and consists of proteins, carbohydrates, organic acids and pectins. This fraction is mostly usable for animals.
NDF: neutral detergent fiber. This fraction is made up of plant cell walls, i.e. fibers insoluble in neutral detergent, and more precisely, they are cellulose, hemicellulose and lignin (NDF).
ADF: acid detergent fiber: This is the residue of the cell wall of plant cells, after it has been treated with an acid detergent. This fraction consists of cellulose and lignin (ADF)
ADL: The residue of the sample after treatment with an acid detergent and then treated with 72% sulfuric acid gives a new residue which is acid detergent lignin.
NSC: non-structural carbohydrates: this fraction is obtained indirectly. It is calculated when 100 is subtracted from the sum of: proteins, neutral detergent fiber, fat and ash.

As parameters for the calculation of the energy value of the samples the following are also used:

→ **NDICP**: neutral detergent insoluble crude proteins. Proteins are determined in the NDF, the value of which is then used in the energy value calculation formulas (NDICP).
→ **ADICP**: acid detergent insoluble crude proteins. Proteins are determined in ADF, the value of which is then used in the formulas for calculating energy value (ADICP).

This research was done through a descriptive-analytical method. For the purposes of the study, a literature review was first performed. This intended to summarize previous professional and scientific findings that focus on the influence of mowing on the changes in the chemical composition of the plant communities. Furthermore, the data from the collected and analyzed samples were processed statistically. The factors affecting the obtained results are described and statistically processed (Figure 34).

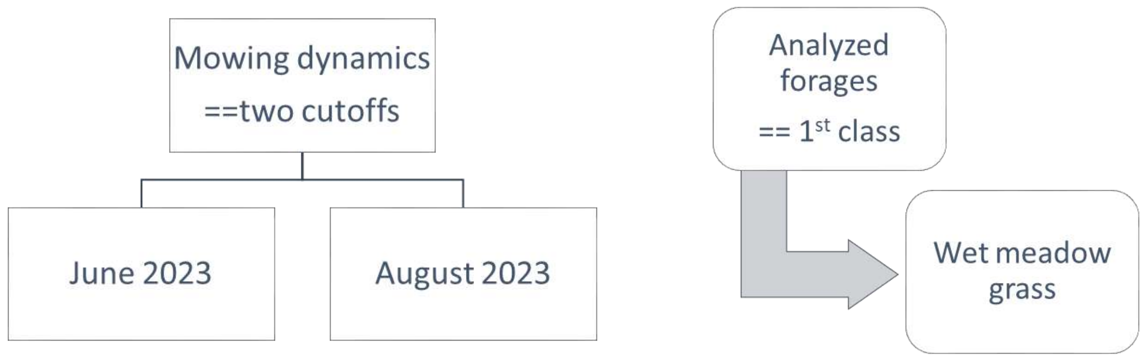


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Figure 34 Structural two-step flow

The variables that were a subject of our research are ten fractions from the performed chemical analysis:

- Moisture
- Ashes
- crude proteins
- crude fats
- crude fiber
- NDF
- ADF
- ADL
- NDICP
- ADICP

After the review of available published information, the relationship between the studied factors and variables was investigated. The impact of each of the factors is analyzed individually, according to the specific classes.

The variables were examined within the certain classes and the analyses of the collected samples were statistically presented, first of all through the basic statistical indicators:

- average
- minimum
- maximum
- standard deviation
- coefficient of variation

Then the effects of the harvesting (cutoff), as well as the deviation from literature were also subject to the analysis. All indicators are presented individually, by each cutoff.

Based on all analysed data, the research aims to provide conclusions and recommendations for future research and experiments, with the aim of obtaining more detailed information about the interaction between the examined factors and variables.

5.1.3 Calculations of the monetary value

The monetary value of the meadow hay was calculated using the method of calculating the monetary value of forages (Petersen 1932; Kilmer et al. 1994; Wilson et al. 2021). It is based on the quality and costs of two most common forages: corn grain (maize) and soybeans meals with 48% protein content in the dry matter. The determination is based on the individual price of the most important parameters for the nutritional value, namely crude proteins and energy (TDN-total digestible nutrients). Unit prices are also calculated. This method is very acceptable for practical application and it is currently the most commonly applied method.

5.2 Results and Discussion

In total, 42 samples collected from wet meadows in Ezerani Nature Park were analysed for their chemical composition. All analyses have been conducted in the officially accredited feed analysis laboratory at the Faculty of agriculture and food sciences in Skopje. The analysis of these samples provided an opportunity to compare and perceive changes in values within the two different cutoff samplings of material.

5.2.1 Influence of mowing on the quality of the biomass

In comparison between the first (June) and second (August) biomass collection, the average values of the analysed chemical parameters with the Weende method, we detected reduction of the protein content (9.66%) in the second cut. All average values are within the literature range values that meadow grasses meet. The values for the investigated parameters for both cutoffs in 2023 are presented in Table 13. Additionally, they are visually presented in Figure 35.

Table 13 Results from the Weende physico-chemical method

Parameters, average	June (28 samples)	August (14 samples)
dry matter, %	36.57	52.04
ash/CM, %	8.84	8.59
crude protein/CM, %	10.98	9.66
crude fat/CM, %	2.29	2.28
crude fiber/CM, %	32.81	32.76

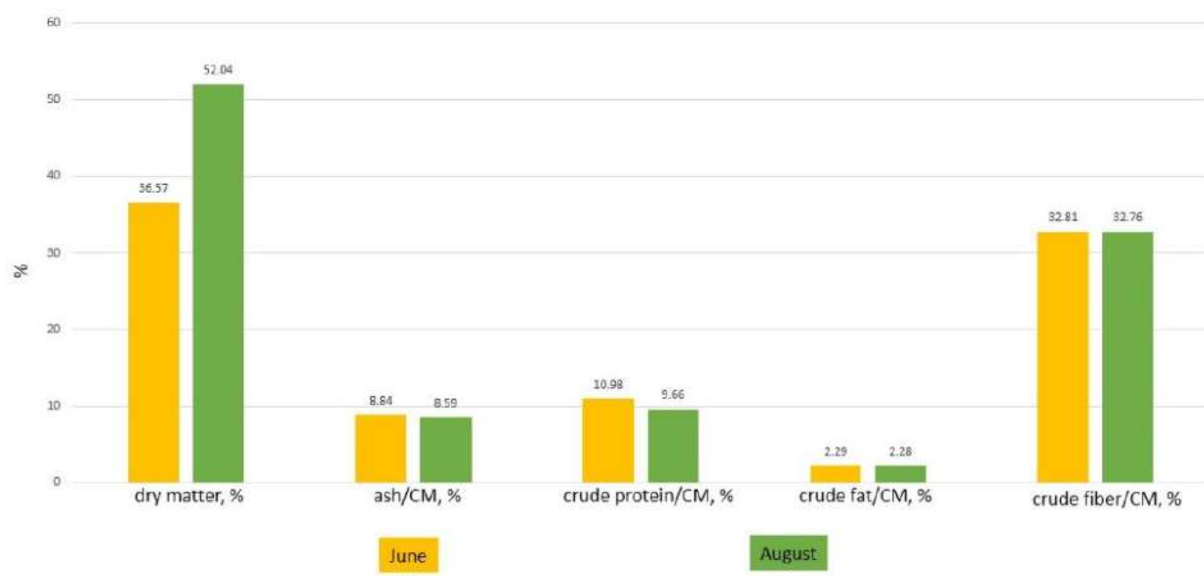

Figure 35 Average values of the analysed chemical parameters in both sampling periods

The average values of the physico-chemical parameters with the Van Soest method show increase of NDF and ADF (77.56 % and 42.12%) in the second cutoff (August). The average values are within literature data ranges for meadows. The results are presented both in Table 14 and Figure 36.

Table 14 Results from the Van Soest physico-chemical method

Parameters, average	June (28 samples)	August (14 samples)
NDF/CM, %	65.87	77.56
ADF/CM, %	38.05	42.12
ADL/CM, %	7.25	8.89
NDICP/CM, %	4.16	3.87
ADICP/CM, %	2.09	1.9

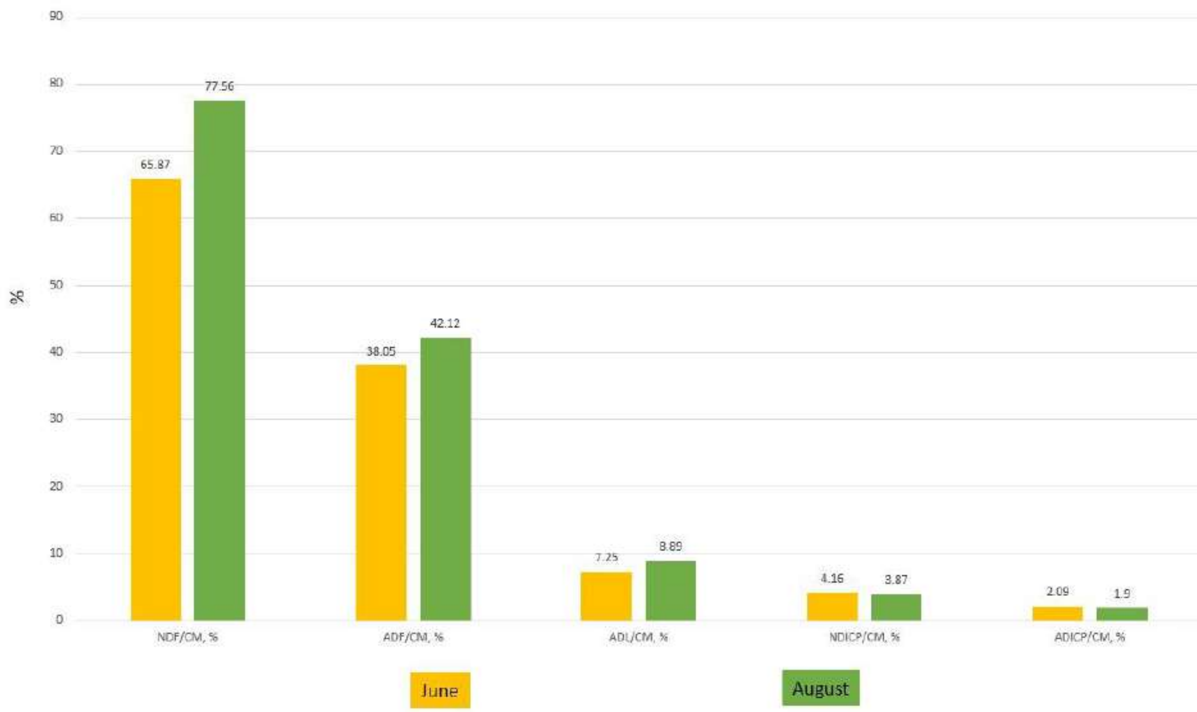

Figure 36 Average values of the analysed chemical parameters in both sampling periods

In summary, most of the parameters are relatively stable (ash, protein, fat, fiber, ADL, NDICP and ADICP).

The minimum and maximum values shown in Table 15(and visually represented in Figure 37) present the individual variations.

Table 15 Results from the Van Soest physico-chemical method

Parameters	June (28 samples)		August (14 samples)	
	Minimum	**Maximum**	**Minimum**	**Maximum**
dry matter, %	27.23	67.73	37.59	65.22
ash/CM, %	5.74	18.77	5.97	12.51
crude protein/CM, %	3.43	21.62	5.94	23.36
crude fat/CM, %	1.85	3.28	1.96	2.6
crude fiber/CM, %	22.89	35.49	28.5	34.11

Parameters	June (28 samples) Minimum	June (28 samples) Maximum	August (14 samples) Minimum	August (14 samples) Maximum
NDF/CM, %	36.35	72.12	64.88	82.72
ADF/CM, %	27.99	43.62	37.42	46.52
ADL/CM, %	4.7	9.46	6.76	11.53
NDICP/CM, %	1.31	8.14	2.36	9.3
ADICP/CM, %	0.64	4.04	1.15	4.65

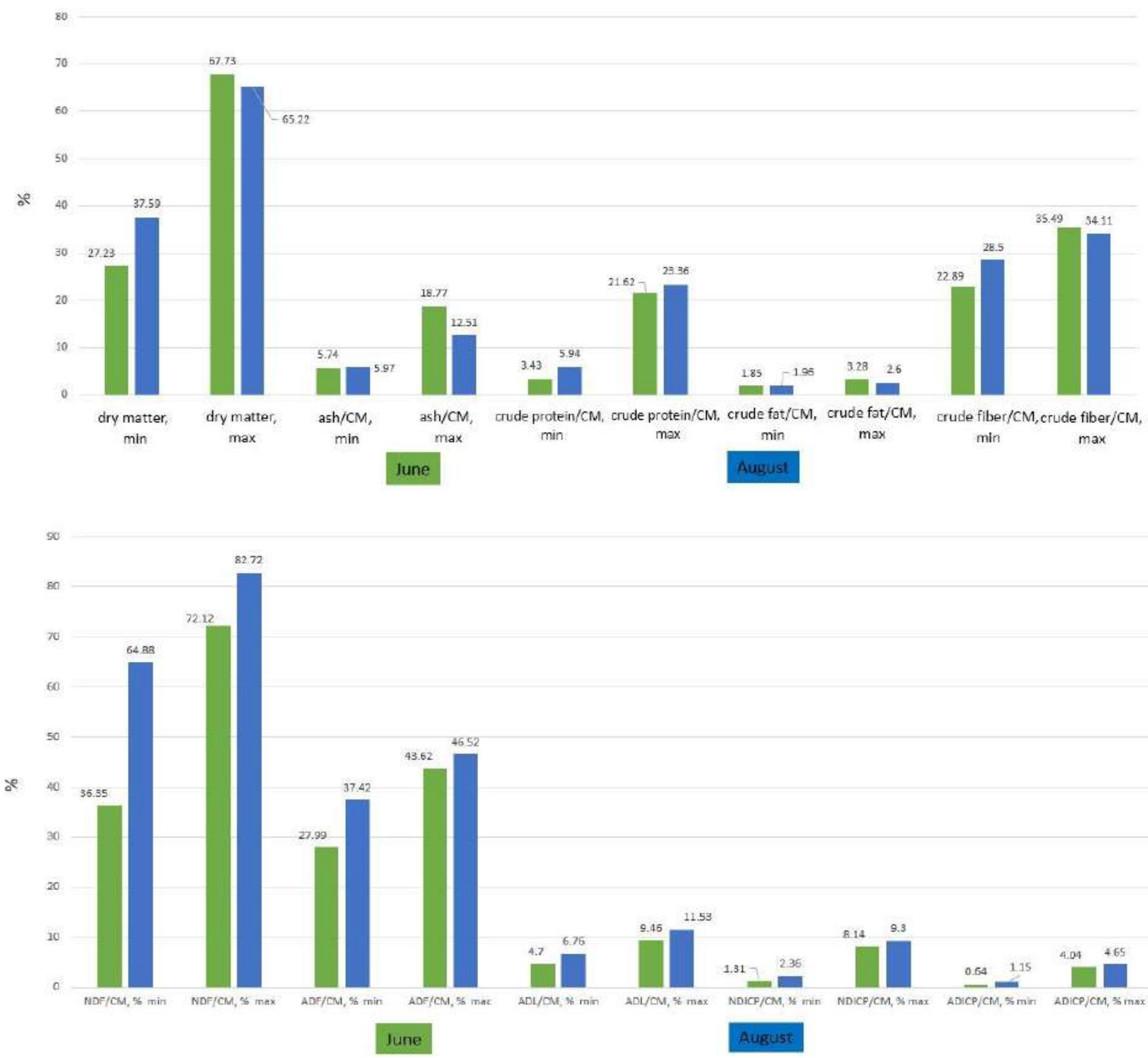

Figure 37 Minimum and maximum values of the analysed chemical parameters in both sampling periods

The largest absolute deviations in the parameters from the Weende analysis are recorded for proteins (3 - 23.36%), ash (5.74 - 18.77%) and crude fiber (22.89 - 35.49%). Other parameters have smaller deviations. In the results of the Van Soest analysis, it can be noted that the deviations are in a wide range for all parameters. Namely, the highest percentages are in ADICP (0.64 - 4.65%), NDICP (1.31 - 9.3'%), ADL (4.7 - 1.53%), NDF (36.35 - 82.72%) and ADF (27.99 – 46.52%).

Additionally, the standard deviation of the measured parameters was calculated, in order to determine the dispersion of the data in relation to the mean value. Most of the obtained values indicate a low standard deviation which means that the obtained results are grouped around the average. In the parameters of the Weende method, a higher standard deviation can be observed for the crude fiber from the first cutoff and for the proteins from the samples in the second cutoff (Table 16). A larger standard deviation was recorded for both the NDF results from the first and the second cutoff, as well as for the ADF in the first cutoff (Table 17).

Considering that the coefficient of variation is an indicator that is sensitive to extreme deviations in the sequence, possible high values would indicate some significant deviations in individual results in relation to the average value. The above-mentioned results-trends are also reflected in this parameter (18.99%-proteins, first cutoff, 11.02% - crude fiber in the first cutoff, 42.76 and 19.32% in NDF and 12.59% in ADF).

Table 16 Standard deviation and variation coefficient (Weende method)

Cutoff/Statistics		Ash	Proteins	Fats	Fibers
June	StDEV	2.56	2.62	0.37	3.32
	CV	6.58	6.86	0.14	11.02
August	StDEV	2.05	4.36	0.18	2.57
	CV	4.19	18.99	0.03	6.62

Table 17 Standard deviation and variation coefficient (Van Soest method)

Cutoff/Statistics		NDF	ADF	ADL	NDICP	ADICP
June	StDEV	6.54	3.55	1.05	0.98	0.49
	CV	42.76	12.59	1.11	0.96	0.24
August	StDEV	4.4	2.4	1.54	1.74	0.87
	CV	19.32	5.76	2.38	3.03	0.75

5.2.2 Monetary value assessment

The calculation of the monetary value is based on the energy value of the forage, the protein content and the price of kg forage. Following National Research Council (1989), calculations for the energy value of forages are derived using the following formulas:

$tdNFC = 0.98 \times (100 - (NDF_{CP} + CP + EE + Ash)) \times PAF$
$tdCPf = CP \times \exp(-1.2 \times (ADICP / CP))$
$tdEE = EE - 1$
$tdNDF = 0.75 \times (NDF_{CP} - ADL) \times (1 - (L / NDF_{CP})^{0.667})$
$TDN_{1X} (\%) = tdNFC + (tdEE \times 2.25) + tdNDF - 7$
$NE_{L\text{-}3X} (Mcal/kg) = 0.0245 \times TDN_{1X} - 0.12$

Table 19 presents the energy and monetary values of the two different cutoffs for the researched wet meadow. It can be noted that the average price is 8.29 den/kg hay.

Table 18 Energy and monetary value of the two cutoffs of meadow hay

Cutoff/Calculations	June		August	
	TDN/KG/SM	price, den/kg	TDN/KG/SM	price, den/kg
Average	33.44	**8.18**	26.77	**8.41**
Min	23.22	4.24	18.87	3.77
Max	53.95	11.91	41.01	9.96
StDEV, %	4.98	1.07	5.62	1.53
CV, %	24.76	1.15	31.61	2.34

5.2.3 Biomass analysis

In three locations during the first and second material sampling we have harvesting an area with the help of quadratic frame (see methods). The results regarding the calculated biomass are given in Table 19.

Table 19 Aboveground biomass calculated from fresh and dry matter

June	kg quadratic frame*	kg fresh matter/ha	kg dry matter/ha
Harvest one	1.53	9053.25	3310.78
Harvest one	1.35	7988.17	2921.27
Harvest one	1.77	10473.37	3830.11
Average		9171.60	3354.05
August	kg quadratic frame*	kg fresh matter/ha	kg dry matter/ha
Harvest one	0.427	2526.63	1264.83
Harvest one	0.71	4201.18	2103.11
Harvest one	0.454	2686.39	1344.81
Average		3138.07	1570.92
Average value per season		12309.66	4924.97

*The surface area of the quadratic frame is 1,69 m^2

The calculations for the total energy value of forages (NEL/MJ/kg SM) per hectare are:

Average energy value of 1 kg SM is 2.745 MJ
Total NEL/MJ/kg SM = 4925 kg x 2,745 MJ NEL/kg = 13.519 MJ/NEL/kg SM

The energy needs of cows with an average daily milk production of 20 liters are 110 NEL/MJ per day. This means that 30% of these needs can be met with the energy from this type of hay. That means approximately 35 NEL/MJ per day (about 12.75 kg of hay per day/cow. 365 days multiplied by 12 kg = 4,380 kg/cow/year). If the total energy value of one hectare (13,519 NEL/MJ) is divided by the annual need of one cow, the number of cows that can be fed with hay from Ezerani will be obtained.

13,519 : 4,380 = 3.09 cows/hectare

The energy needs of adult sheep are 6 NEL/MJ per day. This means that 70% of these needs can be met with the energy from this type of hay. That means approximately 4.2 NEL/MJ per day (about 1.5 kg hay per day/sheep. 365 days multiplied by 1.5 kg = 548 kg/sheep/year. If the total energy value of one hectare (13,519 NEL/MJ) is divided by the annual need of one sheep, the number of sheep that can be fed with hay from Ezerani is obtained.

13,519 : 548 = 24.7 sheep/hectare

The above data on the load on one hectare with the number of cows and sheep should be multiplied by the total area of wet meadows in Ezerani if we want to assess their full potentail in this regard.

The obtained results of the analyses indicate a similarity with the literature data for all the investigated parameters, when it comes to the grass communities from the Poaceae family. Results for grasses in Croatia for ash (7.5 – 18.8%), protein (7.4 -15.3%), fat (2.6 – 3.8%) and crude fiber (22.4 – 30 .1%) from the Britvec survey (Britvec et al. 2004) are similar to our averages of the investigated parameters.

Bovolenta et all (2008), investigated the chemical composition and energy value of 12 grass species from the Alp region. The values ranged from 7.1 -20.2% for crude proteins, 40.2 - 70.1% for NDF and 1.9 - 2.9 MJ NEL,/kg SM, the exception being 6.9 MJ NEL for white clover , which is a leguminous forage crop.

An extensive study in Brazil with data on ash, protein, fat, NDF, ADF and energy - TDN was done by Tontini et all (2021), Their results present ash with 7.6 – 12.1%; proteins 15.2 – 21.6; fat 2.4 – 4.2%; NDF 45.7 – 65.1%; ADF 27.7 37.3% and TDN 57.8 – 67.8%. These grasses have a higher percentage of protein and energy, and a lower percentage of NDF and ADF, but these are better quality grasses compared to those examined in our study.

Bayissa et all (2022),investigated the chemical composition of three species in Ethiopia. Limit values for dry matter, ash, NDF, ADF, fat and crude protein ranged as following: 89.64 – 94.06%; 6.71 - 12.56%; 61.84 – 74.48%; 45.20 – 52.99%; 0.87 – 1.86% and 8.46 – 12.90%.

Vitasović et all (2020), in grazed grassland from the island of Pag, Croatia, obtained results for the representation of some investigated parameters, namely crude proteins in the range of 8.3 – 18.1% NDF 78.9 – 79%; ADF 39.6 – 48.3%; ADL 8.6 – 11.2%.

Slightly higher values for crude protein (12.88 – 14.23%), NDF (54.23 – 62.10%) and ADF (29-78 – 33.78%) were obtained by Hazim Serkan Tenikecier and Ertan Ates (2018) in their study conducted in northern Bulgaria. This is due to different species and families, which by default have a higher percentage of crude protein and lower NDF and ADF values.

The obtained price for 1 kg of dry matter of meadow hay from Ezerani Nature Park (8.30 denars/kg) is in good correlation with the market price of meadow hay in North Macedonia in the period from January to November 2023 - 9.30 denars/kg of meadow hay .

6 Monitoring of biodiversity

The purpose of the monitoring efforts was to gather information on species associated to wet meadows. We concentrated on managed meadows to evaluate the possible impact of mowing activities on biodiversity. Monitoring guideline (Annex X) was created and discussed with the experts. Species are connected to wet meadows in altered ways and have different mobility. Some use them as their primary habitat, while others use them as sites for feeding or reproduction. As a result, the scope of the monitoring was expanded, and experts were required to develop specific monitoring plans for their target group/species.

The initial proposed monitoring sites (Figure 38) were presented to experts as well as the expected outcomes of the monitoring activities.

Figure 38 Proposed monitoring sites of wet meadows in Prespa

6.1 Amphibians

First data on amphibians along Prespa Lake's coastline, wet meadows included, spans from 1966. However, when analyzing habitats that go through intense temporal shifts, such as wet meadows, these data are often rendered unusable. Namely, they are not accompanied by exact geographic location (i.e. GPS), and their locations are rather descriptive (e.g. Dimovski (1981) only states the names of the closest populated place, or the authors of the "Draft Feasibility Study for Ezerani" (Smith et al. 2009) state for the European leaf frog: "Within Ezerani Nature Reserve, the species is most abundant in the marshes along the lake"). Unfortunately, this precludes any inferences on the possible physical shifts of wetlands based on changing distribution of these animals, particularly species known to be heavily dependent on wetlands during the reproductive phase such as amphibians. The only published contemporary sources of amphibian distribution in North Macedonia come from the National Red List of Threatened Amphibians (Sterijovski and Arsovski 2020).

During consultations on establishing regular monitoring of wet meadows in Prespa, amphibians were chosen as potential indicators of the impact of management practices, or lack thereof i.e., is there an effect of annual mowing of wet meadows on amphibian diversity or reproductive patterns? Amphibians were considered as suitable candidates as they are heavily dependent on water, particularly during the breeding phase or the larval stages of their complicated life cycles (tadpoles and gilled newt larvae). Namely, amphibians that can be found in Prespa require standing water to mate and deposit their eggs, and the larvae need water bodies until they mature and develop terrestrial breeding mechanisms (lungs). While Lake Prespa clearly offers a water body year-round, it is unfortunately unsuitable for most amphibians as it is too large and has too many potential predators. The heavily vegetated coastal lake reedbeds might offer suitable habitats, but they are almost impossible to monitor and are very different from the wet meadows that are of interest to this study. Ideally, most amphibians in Prespa require temporary water bodies, termed breeding pools or reproductive centers that dry out during the summer season and are therefore unsuitable for fish predators. Such temporary pools are abundant in Prespa during the spring season when general flooding of the wetlands occurs, especially in our defined wet meadows. Unfortunately, as previous data only establishes the presence or absence of species from the general region, detailed monitoring per selected wet meadow patches had to be established for the first time during this project, and all data for those areas are new.

6.1.1 Materials and methods

Animals were either searched for visually (frogs and toads), captured with bottle traps and aquatic funnel traps (newts), or their presence was ascertained by searching for vegetation where they had deposited their eggs (newt eggs are easily distinguishable to the species level in Prespa). As bottle traps proved to be quite inefficient and in fast fluctuating water bodies can even fill completely and drown trapped individuals, they were only employed for one season and further replaced by aquatic funnel traps. On the other hand, as trapping is performed overnight, it would be extremely time-consuming and labour-intensive to cover a vast area such as the wet meadows surrounding Prespa. Aquatic funnel traps can and should be used in the future once suitable localities are established for quantitative monitoring i.e., monitoring population sizes. Nevertheless, the vastness and general good connectivity of their reproductive centres in "Ezerani" Nature Park during the breeding season (spring) means that such endeavour would be next to impossible. *The only suitable candidate that should be taken into consideration for* *quantitative assessments in the future is the strictly protected, but highly threatened Stenje marsh in National Park "Galichica"*. Ultimately, during the final year, newts were monitored only qualitatively by searching for reproductive activity. This method is much more simple and less time-consuming than trapping as it only involves finding submerged vegetation in the shallows with folded leaves in which eggs are hidden (Figure 39). This way, much greater areas can be monitored and importantly, the methodology can be performed by people that have received very little training such as rangers and other protected area managers. This should be taken into account in the future, and potential monitoring personnel can eventually, and fairly simply perform such monitoring in a coordinated way across the basin.

Figure 39 (Left) Submerged vegetation with deposited Macedonian crested newt eggs in Ezerani nature park and (right) unfolded leaves with eggs from Macedonian crested newt (large, 3rd, 5th and 7th from the left) and Smooth newt (small – 1st, 2nd, 4th and 6th from left) ©Dragan Arsovski

6.1.2 Results and Discussion

Amphibian observations in wet meadows across coastal Prespa in North Macedonia from 2021-2023 fail to inform on the efficiency of wet meadow management, or lack thereof. This is mainly a result of the short time period and the sporadic nature of the observations (Table 20) that preclude any correlations and any potential consequent inferences.

Nevertheless, the obtained familiarities are invaluable as they can provide recommendations on future amphibian monitoring in Prespa – an endeavour that has never before been established in North Macedonia, let alone Prespa. Initially, some species are rather ubiquitous across the Macedonian side of the basin, and likely also on a transboundary scale, which makes them unsuitable indicator species: Marsh frog, Agile frog and European leaf frog. As a result, in 2023 they were only opportunistically monitored, and the lack of observations only expresses field effort, and not actual presence/absence. The Balkan spadefoot is an extremely elusive species and therefore also an unsuitable indicator species. Newts, on the other hand, have a slightly more "complicated ecology" than the frogs and toads that inhabit Prespa. Namely, they can only easily be encountered during the breeding season in spring, while during the other seasons they start their elusive terrestrial phase. Since during the breeding season, they are restricted to breeding centers, monitoring efforts in wet meadows can be concentrated in flooded parts of these habitats during springtime in Prespa making newts the most suitable indicator candidates. This report provides the first precise data on newt distribution in Prespa of North Macedonia, and opens room for additional more structured monitoring in the future. Consequently, as this is the first attempt at establishing a monitoring scheme for amphibians in the area, the following recommendations should sincerely be followed when constructing future monitoring schemes.

Table 20 Amphibian encounters in select wet meadows (see text) throughout Macedonian Prespa. Since temporary water bodies i.e. potential newt breeding pools shift distribution annually, GPS coordinates are given for the specific locality where monitoring/newt trapping was performed that year. Years in parentheses next to meadows indicate when they had been mowed (management). O – a trapping method was employed but no individuals were captured.

	Smooth newt	**Macedonian crested**	**Marsh frog**	**Agile frog**	**European leaf frog**	**Balkan sapdefoot**
			2021			
Nakolec	O	O	X		X	
Asamati	O	O	X	X	X	X
Ezerani	O	O	X	X	X	
Perovo (2020)	41.0035, 20.9948		X	X	X	
Stenje	40.9342, 20.9195	40.9340, 20.9198	X	X	X	
			2022			
Nakolec	40.8916, 21.1064	O	X	X	X	
Asamati	O	41.0015, 21.0363	X	X	X	X
Ezerani (2021)	O	O	X	X	X	
Perovo			X	X	X	
Stenje	40.9341, 20.9196	40.9341, 20.9196	X	X	X	
			2023			
Nakolec	40.8986, 21.0905		X			
Asamati		41.0013, 21.0153			X	
Ezerani (2022)		41.0309, 21.0175			X	
Perovo					X	
Stenje						

6.2 Butterflies

Butterflies play an important role as bioindicators thus present suitable group for monitoring of habitats. The reason behind this is their intricate life cycle and the need for good habitat condition.

Wetlands are very important ecosystems, on the other hand globally threatened (Davidson 2014). Wet habitats support the greatest range of endangered species, according to estimations of population trends of butterfly species in Europe and their function as bioindicators in different biotopes. Additionally, the populations of specialist species for wet habitats are in the greatest decline (-15%), right after specialists species for grasslands (-19%). Drainage is cited as the greatest threat, as it is an anthropogenic activity that immediately destroys wet ecosystems (van Swaay et al. 2006).

Schaider and Jakšić (1989) conducted the last comprehensive study regarding butterfly diversity and distribution in the country. According to their 10x10 km grid cell provided maps, the Prespa region in the Macedonian side should number 124 butterfly species, which is 60% of the total number of butterflies of Macedonia, and 25% of the European species. This list of butterfly species for the Prespa region is outdated and does not take into account the dynamic changes in the habitats, especially the ones close to the shore of Prespa Lake (Bozhinovska and Melovski 2020). The number is also relative, since it is not connected with information about the habitat type. Additional data is reported in Bozhinovska (2010) and more recently in Bozhinovska & Melovski (2020). In the previous phase of wet meadows research activities, butterflies were selected insect group for monitoring. The study reported 29 butterfly species from nine localities (Nikolov et al. 2020). The richest locality were Asamati and Nakolec. However, the presence of the Large Cooper was not confirmed even though it is previously recorded for Prespa. Therefore, recommendations were given for further research, especially targeting this wetlands flagship species (Boggs and Ehrlich 2008).

6.2.1 Target species

Lycaena dispar (L.) is a species from the Lycaenidae family with a wingspan of 25-40 mm. This species has sexual dimorphism. The anterior, upper wings of the males are fiery orange, with a thin black border and a typical black line. Females, on the other hand, have an extra spot in the middle and an array of dark black spots postmedia. At both genera, the underside of the upper wings is pale orange with very black accents and a broad grayish-blue band marginally. The underside of the hind wings is grayish-blue tint in basal area, with many black dots forming a row in postdiscal area and a broad pale orange band marginally. This species can be confused with several other species of the genus Lycaena. The larva feeds on different dock species (Rumex) (Tolman 1997), mainly: *Rumex hydrolapathum*, *Rumex crispus* and *Rumex aquaticus*. It first feeds on the underside of the leaves, making specific shapes as it avoids biting the upper epidermis. Because of this behavior, experienced researchers can easily identify it even when freshly hatched. Furthermore, as the larva grows in size, they also eat through the leaf, forming irregular holes. Because of shorter daylight in September, diapause is encouraged. Diapause is characterized by cessation of feeding, followed by migration to the base of the host plant. Depending on the climatic conditions, the resumption of feeding occurs between the second half of March and the beginning of May. There are two to three generations in the period from May to September. Adults fly from May to September, with each generation active from four to six weeks, while one individual has a lifespan of about 20 to 36 days. Population densities are usually very low (0.25-10 individuals per hectare), which often makes their detection difficult. Adults are very mobile and can migrate long distances in search of new territories. They are active during the day, and the males are territorial within a radius of 20 meters. The eggs are very specific, light gray, with six or seven star-shaped indentations, dorsoventrally slightly flattened, with a cavity in the middle and are about 0.6 mm in diameter (Strausz et al. 2012).

6.2.2 Material and methods

Butterfly fauna was studied in different wet meadow sites along the Prespa Lake shoreline. Our approach tested several methods. In 2021, one-time transect counting was conducted. The counting of individuals followed linear transect (Van Swaay et al. 2012) method. In 2022 unsystematic sampling was conducted during a three day field trip. However, in 2023 we have decided to focus on the Large Cooper, as a target species. Butterflies were registered via direct observation or when necessary, they were caught using entomological net and released after identification. For all specimens information on field were noted in Memento Database ver. 4.10.0. Specimens were identified according to Tolman (1997, 2008), Pamperis (1997), Lafranchis (2004) and Popović (2011). The nomenclature is according to de Jong (2014). Data are stored into Microsoft Excel database.

6.2.3 Results and Discussion

Results from field surveys show low number of species and individuals which is also in line with previous conclusions (Nikolov et al. 2020). In general, population studies should be done in order to assess trends. Therefore, these results fail to provide conclusions regarding the influence of mowing activities. Additionally, the time span of the survey is insufficient even for indicative conclusions. Ezerani locality refers to larger area including all managed meadows from 2020 and 2023.

In 2021 we have selected six wet meadows along the shoreline where we have been conducting our counting transects (Table 21). Field trips were conducted in May and July. Stenjsko Blato was not included in the locality sites during the field trip in May. In total, we registered 32 species. The most abundant species is *Coenonympha pamphilus* (Linnaeus 1758) with 19 registered individuals in total. Locality with largest number of species and highest abudance is Stenjsko Blato. However, all localities have generally low number of common species and individuals.

We managed to register the presence of the Large Cooper in Asamati. One adult individual was observed (Table 21).

Table 21 Butterfly species recorded on wet meadows with field trips conducted in 2021

Species	Ezerani	Stenjsko Blato	Nakolec 1	Nakolec 2	Asamati	Pretor	Total
	29.05.2021						
Coenonympha pamphilus (Linnaeus 1758)	5		1		5	3	14
Aricia agestis (Denis & Schiffermüller 1775)						1	1

Species	Ezerani	Stenjsko Blato	Nakolec 1	Nakolec 2	Asamati	Pretor	Total
Colias croceus (Fourcroy 1785)			1	2	1	1	5
Cupido minimus (Fuessly, 1775)				1			1
Iphiclides podalirius (Linnaeus 1758)				1			1
Lycaena phlaeas (Linnaeus 1761)						1	1
Polyommatus bellargus, (Rottemburg, 1775)			1				1
Melitaea phoebe (Denis & Schiffermüller 1775)	2		1				3
Pyrgus malvae (Linnaeus, 1758)	1						1
Plebejus (Plebejus) *argus* (Linnaeus 1758)			1	1			2
Polyommatus icarus (Rottemburg 1775)			1			4	5
Vanessa atalanta (Linnaeus 1758)				1			1
Pontia edusa (Fabricius 1777)				1			1
Pyrgus sidae (Esper 1784)				1			1
Total (14 species)	**3**	**0**	**6**	**7**	**2**	**5**	**38**
			03-04.07.2021				
Aricia agestis (Denis & Schiffermüller 1775)			1				1
Polyommatus icarus (Rottemburg 1775)		1	1				2
Coenonympha pamphilus (Linnaeus 1758)	1	1	1			2	5
Leptidea duponcheli (Linnaeus 1758)				1			1
Melitaea didyma (Esper, 1778)		1		1	1		3
Brenthis daphne (Bergsträsser 1780)				1		2	3
Lycaena dispar (Haworth 1802)					1		1
Colias croceus (Fourcroy 1785)	1		1			1	3
Celastrina argiolus (Linnaeus, 1758						2	2
Thymelicus sylvestris (Poda 1761)	1						1

Species	Ezerani	Stenjsko Blato	Nakolec 1	Nakolec 2	Asamati	Pretor	Total
Melanargia galathea (Linnaeus 1758)	1	1	1	1			4
Maniola jurtina (Linnaeus 1758)		4					4
Thymelicus lineola (Ochsenheimer 1808)		2			1		3
Leptidea sinapis (Linnaeus 1758)		2				1	3
Brintesia circe (Fabricius 1775)						2	2
Pontia edusa (Fabricius 1777)					1		1
Lycaena alciphron (Rottenburg 1775)	1						1
Aglais io (Linnaeus 1758)	1						1
Anthocharis cardamines (Linnaeus, 1758)	1						1
Nymphalis antiopa (Linnaeus, 1758)				1			1
Plebejus (Plebejus) *idas* (Linnaeus 1761)				1			1
Lycaena tityrus (Poda 1761)				1			1
Total (21 species)	**7**	**8**	**5**	**7**	**4**	**6**	**44**

In 2022, we visited four localities and detected 26 species. The wet meadow in Pretor was eliminated from our survey due to its small surface area and low number of observed species in 2021. Additionally, the locality Nakolec 1 was also eliminated since it had a lot of influence of pesticides from the surrounding orchards. The field survey was carried out on 13.05.2022 and 23-24.06.2022. We have reduced the number of visited localities and we focused only on those with highest density of butterflies. The presence of the Large Copper was not confirmed this year (Table 22).

Table 22 Butterfly species recorded on wet meadows with field trips conducted in 2022

Species/Locality	Ezerani	Stenjsko Blato	Nakolec	Asamati
Aglais io (Linnaeus 1758)	√		√	
Aporia crataegi (Linnaeus 1758)		√	√	
Argynnis (Argynnis) *pandora* ([Denis & Schiffermüller], 1775)			√	
Argynnis (Argynnis) *paphia* (Linnaeus 1758)		√		
Brintesia circe (Fabricius 1775)		√		
Callophrys rubi (Linnaeus, 1758)				√
Celastrina argiolus (Linnaeus, 1758)			√	
Coenonympha arcania (Linnaeus 1761)		√		
Coenonympha pamphilus (Linnaeus 1758)	√	√		√

Species/Locality	Ezerani	Stenjsko Blato	Nakolec	Asamati
Colias croceus (Fourcroy 1785)	√	√	√	√
Cupido minimus (Fuessly, 1775)		√		
Issoria lathonia (Linnaeus 1758)				√
Lycaena alciphron (Rottenburg 1775)			√	
Lycaena phlaeas (Linnaeus 1761)	√	√		√
Maniola jurtina (Linnaeus 1758)		√	√	
Melanargia galathea (Linnaeus 1758)	√	√	√	
Melitaea didyma (Esper, 1778)	√	√	√	
Plebejus (Plebejus) *argus* (Linnaeus 1758)	√	√	√	
Pieris rapae (Linnaeus 1758)		√		
Plebejus (Plebejus) *idas* (Linnaeus 1761)		√		
Polygonia c-album (Linnaeus 1758)			√	
Polyommatus icarus (Rottemburg 1775)	√	√	√	
Pontia edusa (Fabricius 1777)	√		√	√
Pyrgus malvae (Linnaeus, 1758)	√			
Thymelicus lineola (Ochsenheimer 1808)	√		√	
Vanessa atalanta (Linnaeus 1758)		√		
Vanessa cardui (Linnaeus 1758)	√	√	√	

In 2023 we focused on searching for eggs of the Large Copper. However, we had no luck to confirm its presence in Asamati again, or somewhere in or around the managed meadows. Nevertheless, the Large Cooper presence was confirmed in Stenjsko Blato with one male individual (Table 23). Additionally recorded species in Stenjsko Blato are presented in Table 23.

Table 23 Butterfly species recorded in Stenjsko Blato during field trip conducted in 2023, data provided by Dr. Dime Melovski

Species	Locality
Melitaea cinxia (Linnaeus 1758)	Stenjsko Blato
Coenonympha pamphilus (Linnaeus 1758)	Stenjsko Blato
Pieris brassicae (Linnaeus 1758)	Stenjsko Blato
Maniola jurtina (Linnaeus 1758)	Stenjsko Blato
Issoria lathonia (Linnaeus 1758)	Stenjsko Blato
Iphiclides podalirius (Linnaeus 1758)	Stenjsko Blato
Vanessa cardui (Linnaeus 1758)	Stenjsko Blato
Cyaniris semiargus (Rottemburg, 1775)	Stenjsko Blato
Lycaena tityrus (Poda 1761)	Stenjsko Blato
Lycaena dispar (Haworth 1802)	Stenjsko Blato
Pyrgus sidae (Esper 1784)	Stenjsko Blato
Plebejus (Plebejus) *argus* (Linnaeus 1758)	Stenjsko Blato

In total, 45 species were recording during the field surveys in the period of 2021-2023. It is important to notice that all visited sites did not have high abundance of butterfly species in terms of species but also individuals. This observation should be confirmed with more intensive sampling dynamics, especially throughout the vegetation season. Additionally, several factors should be analysed: weather condition, fires, influence of pesticides, fragmentation etc. Nevertheless, we managed to detect 16 additional species in comparison with the previous survey where in total 29 species were observed. With additional research more species can be expected. The presence of The Large Copper was of enormous importance regarding the structure of butterfly fauna in Prespa. This species is listed in Annexes II and IV of the Habitats Directive due to the narrowing of its distribution area. Despite the declining trend of populations in Northwest Europe, it is considered spreading in Central and Northeast Europe, so it has been removed from some red lists where it was previously included. In North Macedonia however, it is a strictly protected species and categorized as a vulnerable species. Additional efforts should be made for further research of this species in Prespa which is not an easy task, especially since their low population densities (of the adults). Due to this, we have attempted to apply the method of egg counting but with no success. Special attention should be addressed in order to identify its threats and to create an appropriate monitoring plan that will be in harmony with ongoing management activities. It is compulsory that this species should be taken in consideration for future management or restoration actions/plans.

6.3 Small mammals

One of the selected groups that aims to assess how the changes of the management of wet meadow (meadow mowing) influence the biocenosis in this ecosystem, were the small mammals.

There are not many published data on presence and distribution of small mammals along Prespa Lake's coastline (Krištufek and Petkovski 1989; Krystufek and Petkovski 1990; Petrov et al. 1992; Boshamer et al. 2006; Krystufek and Petkovski 2003). Most of the available data is without exact geographic location, locations being rather descriptive and referring to the closest populated place, as well as away from the coastline. Thus, the aim of the survey was to get preliminary (baseline) data on presence and diversity of small mammals within the area of the managed (mowed) wet meadows in Prespa, and afterwards to use this data as a basis to continue and optimize the monitoring.

6.3.1 Methodology

The survey was done at three different sites with wet meadows – Dolno Perovo (03-04 June 2021), Ezerani (27-28 July 2021) and Asamati (01-02 October 2021). We used the methods of livetraps and snap-traps (Figure 40) to collect data on small mammals. The traps were set at 2-5 meters distance from each other along random linear transects. On each transect, we set 10 live or snap-traps. At site Dolno Perovo we set 3 transects (one of the transects in a mowed meadow in 2020), at Ezerani - 3 transects and at Asamati - 5 transects. Traps for sampling, baited with salami, oats and apple, were set before dusk, and were checked the next day early in the mourning. Captured individuals were photographed and identified to the species level and released on site. All data (species, number of individuals, location etc.) were recorded into a local MES Memento database.

 

Figure 40 Livetrap model Longworth (left) and snap-trap used for sampling (right) ©Aleksandar Stojanov

6.3.2 Results and Discussion

The sampling resulted with four different species of small mammals captured with a total of 15 registrations: Lesser white-toothed shrew (*Crocidura suaveolens)* - 2 registrations, Yellow-necked field mouse (*Apodemus flavicolis)* - 10 registrations, Long-tailed field mouse (*Apodemus sylvaticus)* - 2 registrations and Common vole (*Microtus arvalis)* - 1 registration (Figure 41). The results of the sampling are presented in Table 24.

Table 24 Number of registrations for each captured species per site

Site	Number of registrations			
	Crocidura suaveolens	*Apodemus flavicolis*	*Apodemus sylvaticus*	*Microtus arvalis*
Dolno Perovo	1	4	1	
Ezerani		3		
Asamati	1	3	1	1

Crocidura suaveolens

Apodemus flavicolis

Apodemus sylvaticus

Microtus arvalis

Figure 41 Photographs of all four captured species ©Aleksandar Stojanov

The survey on small mammals in the area of the wet meadows conducted during 2021 was a baseline survey providing preliminary data on the presence and diversity of small mammals in wet meadows. However, it is early to conclude from these results if wet meadows management provides better conditions for higher diversity and populations of small mammals. Thus, the survey and monitoring of small mammals should continue in order to get more data and see the influence of managed wet meadows on small mammals. The survey should be extended to additional 1-2 representative sites with wet meadows.

7 Stakeholders engagement

Besides its natural values, cultural values add unique level of complexity to the diverse landscape of Prespa Region. Since this complexity is a product of people and nature relationship, any nature conservation activities without involvement of all local stakeholders cannot be sustainable in long term. This is particularly accurate for management activities of wet meadows as they have been traditionally managed by local communities. Hence, building stronger relations with the local stakeholders has been a key component of our work over the past three years.

Identifying our primary stakeholders was the first step in the stakeholder engagement process. The next step was to involve them directly in our management and research efforts with the goal of having them benefit from our project's outcomes. Farmers and administrators of protected areas made up the two largest categories of direct stakeholders. However, the outreach of our activities always tended to be broader, therefore our target was also raising awareness in the general public as well.

Farmers have been immediately involved in the management process activities, even in the early stages of planning. At first, we needed their cooperation as potential recipients of the mowed biomass, as they can use it mainly as fodder. However, their engagement was very crucial in the whole concept of this type of management due to the need of sustainability. The main goal was to connect the need for sustainable management practices in the protected areas and link it with locals who have technical capacities for mowing the meadows on one side and herdsman that can use the hay and benefit the open areas where they can graze their livestock on the other side. In this way, different groups of farmers had mutual benefit from the management activities in the protected area. Removal of the hay is as equally important process as the mowing of the wet meadows. Offering it to farmers intended to provide additional resource for their animals and potentially lower their costs.

Managers of protected areas Ezerani Nature Park and National Park Galichica have also been identified as important stakeholders. All wet meadows related activities mainly took place within the Ezerani Nature Park therefore rangers were involved in almost all steps (Figure 42). In this way, their capacities were significantly straightened but also all activities helped the management body in fulfilling targets from their management plan.

Finally, other stakeholders were also included in part of the activities with aim to promote, educate and raise awareness regarding wet meadows in Prespa.

Figure 42 Field work activities together with rangers from Ezerani Nature Park, ©Marija Chobanova

7.1 Networking and public awareness

Wet meadow management and research activities have led to intensification of the cooperation between stakeholders. This cooperation is fundamental for the integrative management approach that we wanted to achieve. It was straightened by continuous networking of all actors in order to identify the mutual objectives and methods for common actions.

During this three year period, our strategy was to involve stakeholders in practical work and network them in this way. The main reason was for them to feel part of the process so they can understand their role in the process, their potential contributions as well as responsibilities. This course was crucial for us to design a future official (formal) network of stakeholders. This network should define and achieve common goals for sustainable use and management of wet meadows in Prespa. It should represent a body with decision making power through participative process. Even though the similar logic is behind the public debates of the management plans of the operating protected areas, our experience shows that stakeholders usually do not take active participation or express initiatives through the stakeholder's groups of the protected areas. Reasons for this are most likely more different factors, especially the lack of understanding among stakeholders for their roles. It is also valuable that management plans refer to many aspects, so the stakeholder groups of the protected areas are sometimes broader. Moreover, our stakeholder analysis showed that the level of trust among the different stakeholder groups is low. Therefore, the initial steps for future establishment of a formal network is building this trust system by actively working on achieving goals from different perspectives that lead to a common, desirable outcome.

In our networking activities the main stakeholders were the local farmers, wet meadow owners, National Park Galichica, Municipality of Resen and experts from different fields. The positive cooperation between different stakeholders can be best described with two different examples. In the first case, we worked on straightening the communication and cooperation between the Municaplity of Resen, as a management body of Ezerani Nature Park and the local farmers. Once the meadows were mowed, one of the main tasks was to remove the hay from the mowed location. Since the beginning, we decided that we will offer this biomass to the local farmers. However, we wanted this process to be transparent and mainly led by the Municipality of Resen, so they can build the trust with one of the main stakeholder for this activity. Therefore, we helped the Municipality to prepare a plan for the distribution of the bales. According to this plan, the Municipality opened a call where farmers could express interest. In this way, six farmers expressed interest and received 100-400 bales of hay (Figure 43).

Figure 43 Hay distribution to local farmers ©Lazar Nikolov

The second example was the intensification of the cooperation between two protected areas gravitating in Prespa. In this case, our main goal was to establish synergies between National Park Galichica and Ezerani Nature Park through management activities of wet meadows. The produced hay from the wet meadows provided fodder for the wildlife (deer) repro center in NP Galichica. This biomass supported the repro center throughout the winter months in 2022 and 2023. In exchange, NP Galichica organized the transport of the hay from the meadows to the repro center. With this activity, Ezerani Nature Park provided 700 bales of hay and significantly reduced the costs of the repro center (Figure 44).

Figure 44 Group photo of hay exchange activities between Ezerani Nature Park and NP Galichica, 2022 ©MES

During the three-year project period, efforts were made to raise public awareness and educate the local population in Prespa, regarding the role of wet meadows in maintaining ecological balance in the Prespa ecosystem. In this regard, numerous meetings were organized with different stakeholders in the region, where some of the main objectives were discussed in details (Figure 45).

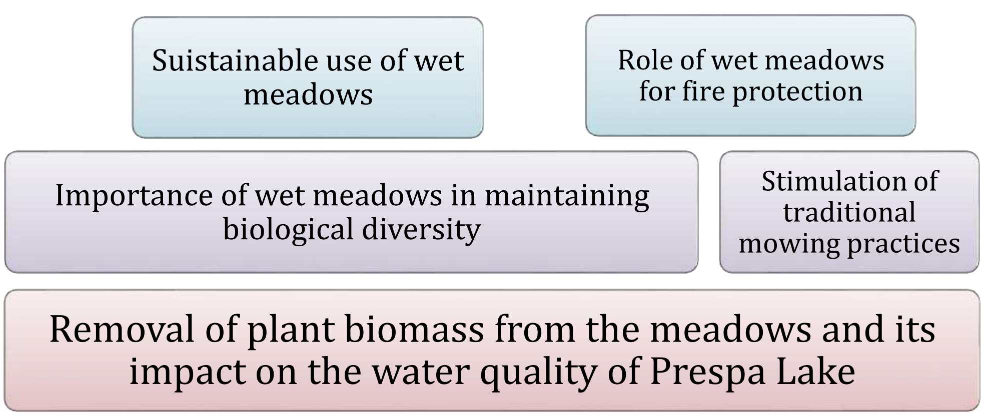

Figure 45 Discussion themes regarding Prespa wet meadows

Several promotion and education activities were also conducted. During the mowing period in 2021, an interview was recorded about the condition and significance of the wet meadows for the needs of documentary video for MES activities. In the same year, students from the Biology Students' Research Society from Skopje had the opportunity to participate in the field work activities and to gain knowledge regarding the entire mowing method. They volunteered to calculate the mowed area and count the produced bales. In 2022, biology students from the Institute of biology in Skopje were involved in field work activities. Before the mowing season, experiment was set up in order to assess the retention capacity of the soil in regards to organic fertilizers (see more in Chapter 4.3). Besides the field work, all students were introduced with the wet meadows as ecosystems, all ongoing conservation and monitoring activities (Figure 46). During the mowing season later that year volunteers from France were present during the field work activities and received full training in the management activities.

Figure 46 Field work with biology students in wet meadows in Prespa, 2022 ©Dragana Trajchevska

Besides volunteers and students outside Prespa region, we wanted to involve young people from the region as well. Therefore, during the same year, a visit to the wet meadows was organized with members of EKO Gerila, a youth NGO from Resen. The aim of the visit was to present the wet meadows as ecosystems, to discuss about their importance and the provisioning of ecosystem services, as well as to promote the process of wet meadow management (Figure 47). Additionally, we aimed to network the NGO with the rangers of the Ezerani Nature Park as well.

Figure 47 Field visit to wet meadows in Prespa with rangers from Ezerani Nature Park and EKO Gerila NGO, ©Dragana Trajchevska

In 2023, biology students from the Institute of biology in Skopje were again introduced with the wet meadows' activities during their practical field work in Prespa region (Figure 48). After the mowing season an interview was recorded for the needs of the documentary "Prespa - the thirsty lake".

Figure 48 Microclimate measurements conducted by biology students in a wet meadow 2023, ©Marija Chobanova

Dissemination of results especially in regards of the research work was further done by preparation of two bachelor thesis (Пеликудовска 2023; Дочовска 2023). One oral (Nikolov et al. 2022) and two poster presentations (Velevski 2022; Pelikudovska 2022) were completed at the 6th Congress of Ecologists in Ohrid, 2022. Additionally, scientific publications are in preparation.

As a final activity related with the stakeholders was the organization of a final event (Figure 49). The main goal with this event was to gather all relevant stakeholders together, as well as to validate everyone's contributions. The most important part of this event was the field visit to the managed wet meadows where the results of the three year conducted activities were presented. Very positive feedback was received from all participants including the major of Municipality of Resen.

Figure 49 Final event and networking of all relevant stakeholders 2023, ©MES

8 Inventory of wet meadows

Once we started with the planning activities in regards to wet meadow's management, monitoring and research, we have encountered big knowledge gap for basic information. Spatial information was also lacking, therefore we have decided to put efforts to collect and organise data so it will represent basis for all future activities. Our initial objectives were to collect data regarding ownership, management, concessions, cattle breeding, grazing activities, biodiversity etc. However, our main priority was to identify the ownership status since this parameter is crucial for management activities as owners of wet meadows are among the most important stakeholders. Additionally, the spatial distribution of the ownership was also valuable information for further planning activities.

Even though our main interest were the meadows themselves, all ecosystems in the Prespa landscape have very dynamic interactions. This is especially visible in the past few years, with the extreme fluctuations of the Prespa's Lake waters. Therefore, we have decide our scope regarding the ownership status to all habitats and ecosystems along Prespa Lake. In order to analyse the ownership status, we have requested access to the National Cadastre database. However, this database provides only overview of the data, while the spatial information was not available. Therefore, we have decided to digitalise all polygons from the Cadastre data base map, which was mainly manual digitalisation with visual interpretation of the physical borders of each polygon from the Cadastre database with the help of the Web Mapping Service (WMS) system. The digitalisation and data processing were done in ArcGIS 10.2.2.

In total, we have digitalised 11 490 cadastre units in the area of the habitat map (Figure 50).

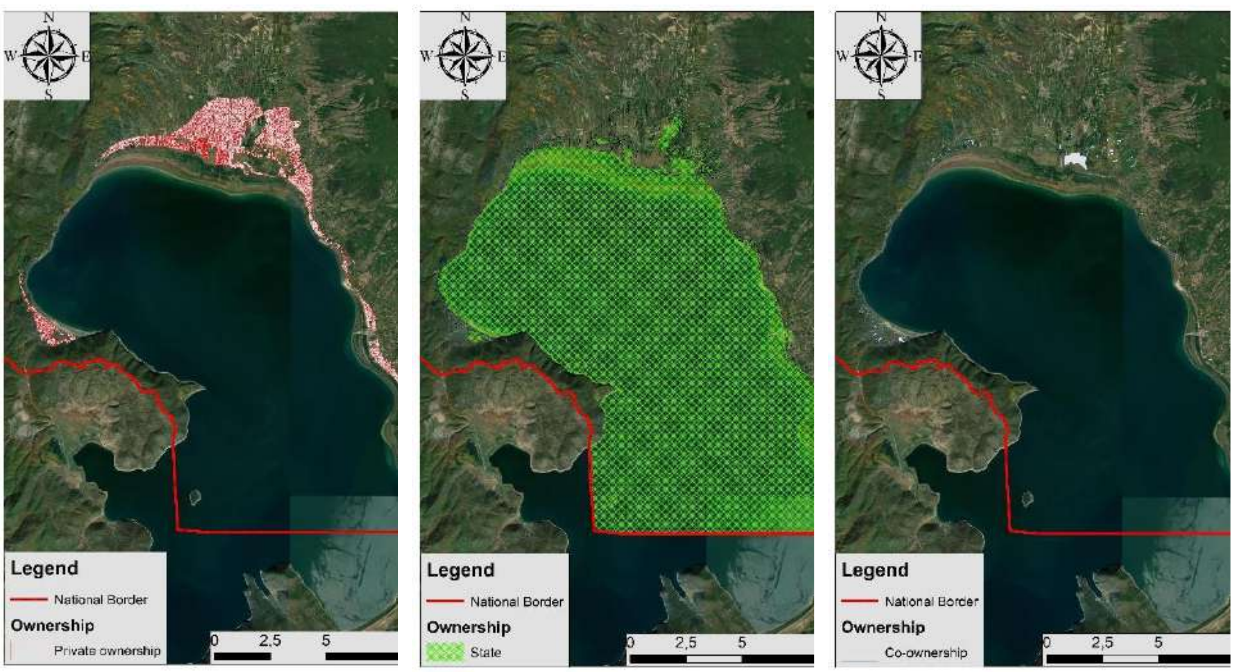

Figure 50 Digitalized polygons from the Cadastre data

The total surface area of all polygons is 19 403 ha. 17 490 ha are state owned land, 1825 ha are private land while 88.5 ha are mixed land (co-ownership). 0.2 ha are registered as land without known ownership. Figure 52 shows the percentage of the ownership status.

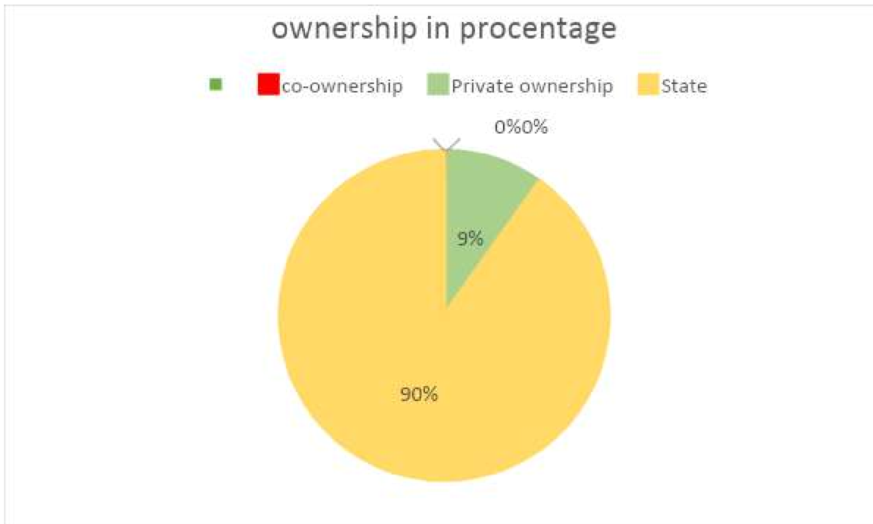

Figure 51 Ratio of land ownership

Most of MES activities in Prespa are conducted in terestial ecosystems. Due to this, we have excluded the lake water as an ecosystem type and did the ownership calculations again. According to the Cadastre data, the Prespa Lake is presented as one cadaster unit with surface area of 16.798 ha. From Figure 52 we can see that the ownership ratio is now drastically changed and that majority of the land is now privately owned.

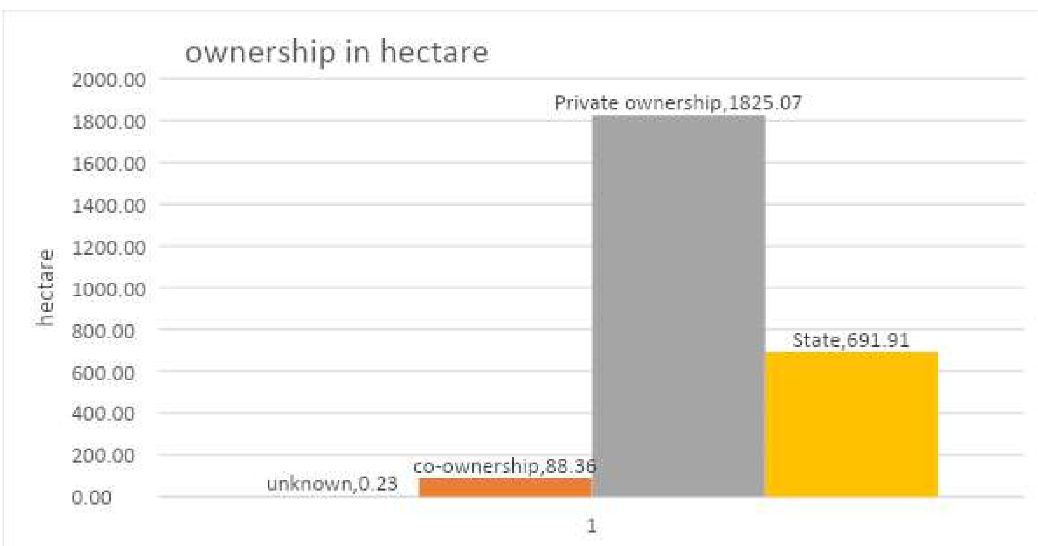

Figure 52 Land ownership without Prespa Lake water unit

Besides the general land use analysis, we have made analysis of the ownership status of the wet meadows (Table 25). Borders for wet meadow's area are extracted from the habitat map of Prespa (see more in Chapter 2).

Table 25 Ownership status of wet meadows

Habitat name (Fotiadis et al. 2018) and ownership	Sum of area_m²	hectare
Molinio-Holoschoenion	392,61	0,039
State	392,61	0,039
Carex spp.	356802,06	35,680
Co-ownership	16,5	0,002
Private ownership	284140,25	28,414
State	72645,31	7,265
Calamagrostis epigeios comm.	3291122,66	329,112
Co-ownership	41517,1	4,152
Private ownership	1452347,76	145,235
State	1797257,8	179,726
Trifolium meadows	924914,65	92,491
Co-ownership	43170,28	4,317
Private ownership	362968,6	36,297
State	518775,77	51,878
Total	**4573231,98**	**457,323**

This analysis shows that 238.9 ha of the total meadow area is state owned, while 209.9 ha is private ownership. Only 8.4 ha is co-ownership. Moreover, the private owners have 1810 polygons/cadaster units of wet meadows. Most of the private units are continuous areas. Ownership status of the wet meadows within the borders of Ezerani Nature Park is presented in Figure 53 where it can be noted that the majority of meadows are state owned.

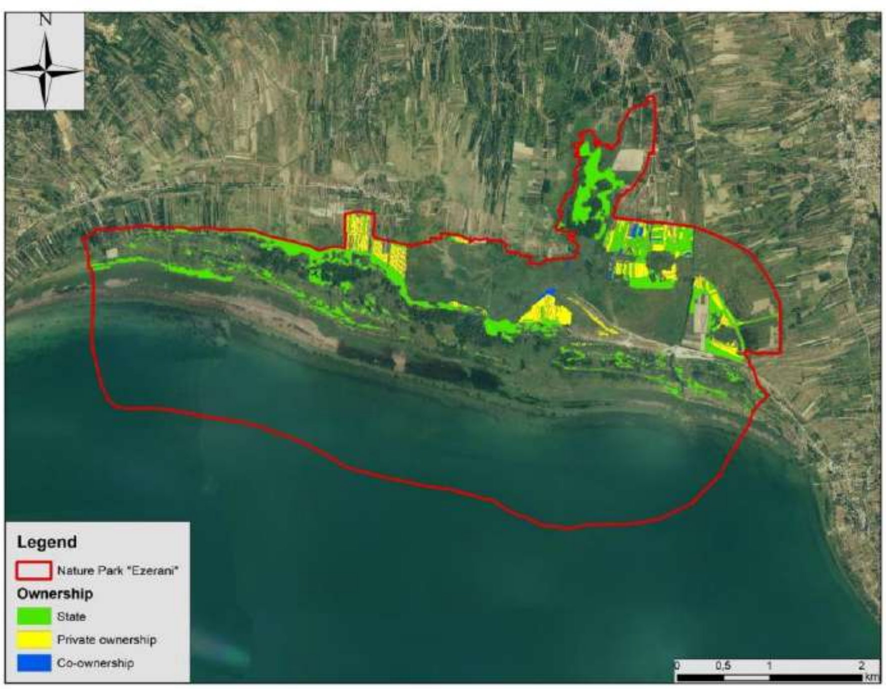

Figure 53 Ownership status of wet meadows within the borders of Ezerani Nature Park

9 Connectivity assessment of wet meadows on transboundary level

Sustaining the ecological integrity and promoting connectivity of wet meadows is vital for supporting biodiversity and sustaining ecosystem service provision (Krause et al. 2015). Fragmentation of these habitats due to land conversion, shift of land use practices, or infrastructure development impedes the functionality of wet meadows (Aavik et al. 2013). Sustainable land management practices (Kolos and Banaszuk 2013) and establishment of wildlife corridors is vital for safeguarding the connectivity (Churko et al. 2020) and ecological resilience of these wetland ecosystems and ensuring their long-term conservation.

In this regard, this chapter aims to assess connectivity of wet meadows in the Prespa Basin and identify critical corridors for facilitating species movement, using the Macedonian crested newt - *Triturus macedonicus* as a target species. This assessments should provide additional recommendations on potential management strategies for strengthening the links between isolated habitat patches of wet meadows and hence improve functionality of these wetland ecosystems.

9.1 Wet meadows in Prespa Basin

Wet meadows in Prespa basin cover a total area of ~290 ha, 40% of which in North Macedonia, 41% in Greece and 19% in Albania. Wet meadows cover 7% of total area of wetland habitats in Prespa basin that is less than 0.5% of total vegetation coverage in Prespa basin. With reference to Habitat Directive the wet meadows in the Prespa basin are characterised as *6420 Mediterranean tall humid herb grasslands of the Molinio-Holoschoenion* (Eunis reference: *E3: Seasonally wet and wet grasslands; E3.31 Helleno-Moesian riverine and humid Trifolium meadows* (Fotiadis et al. 2018). Wet meadows in Prespa basin have small area coverage and are often interspaced within and around the reed belt. In Prespa, this habitat boasts high species richness, with 38 plant taxa recorded in just 16 m^2. Wet meadows play an important role in maintaining wetland biodiversity and serves as a vital habitat for birds and amphibians.

The primary threat to this habitat arises from fluctuations in groundwater levels induced by the unsustainable use of water for irrigating intensely cultivated agricultural areas, particularly orchards. Groundwater levels are further impacted by the natural fluctuations of water reserve in Prespa basin. Accounting for the added effects of climate change i.e. rising temperatures and shifts in precipitation further contribute to water scarcity issues, posing a threat to the availability and sustainability of groundwater resources. Other important threat is succession due to abandonment of traditional mowing practices and land conversion with secondary threats from garbage disposal.

9.2 Target species

Triturus macedonicus (Karaman, 1922), commonly known as the Macedonian crested newt, is a species of newt endemic to the Balkan Peninsula, known for their prominent crests along their backs and tails. The Macedonian crested newt, is known to prefer specific habitats for breeding, foraging, and shelter. The species favors shallow wetlands such as ponds, marshes, and slow-moving streams with dense aquatic vegetation, including submerged and emergent plants, which provide cover and nesting sites. These habitats provide suitable conditions for breeding and larval development, offer protection from predators and are favorable for foraging. They typically inhabit shallow water bodies within wet meadows, where they feed on small invertebrates and contribute to the overall ecosystem balance (Mulkeen et al. 2017).

Being prone to population decline in response to environmental changes and fragmentation, amphibians are often targeted for conservation and management initiatives. Pond-breeding amphibians, in particular, meet substantial challenges due to prolonged droughts and extreme temperatures induced by climate change. These environmental shifts directly impact water availability, a critical element linked to their life cycle and overall survival. Functional connectivity is of utmost importance for preserving the structure, development, and long-term viability of spatially structured amphibian populations and meta-populations (Danelis et al. 2023).

Local isolation and loss of functional connectivity are crucial factors that affect the viability of pond-breeding amphibian populations and should be accounted for in the design and implementation of effective conservation actions.

With consideration of the ecological traits of the Macedonian crested newt, the importance of functional connectivity for sustaining viable breeding populations and data availability on species presence, the Macedonian crested newt was selected as a target species for assessing the functional connectivity of wet meadows in Prespa basin.

9.3 Methodology

Data preparation, data processing and mapping were carried in ArcGIS 10.2 software. Landscape connectivity analysis was assessed using Graphab 2.8 software (Foltête et al. 2021, 2012). The assessment was carried using modified vector data on wetland habitat type and distribution along Prespa Lake coastline produced by Fotiadis et al. (2018). Wetland habitat map was supplemented by available data on ecosystem types (Trencheva et al. 2020) and land cover[2] to produce a habitat map for the defined area of interest, the Prespa basin. All habitat types were then graded for their suitability to sustain breeding populations of newt (Figure 54) based on available literature data (ARG 2010) and expert knowledge for species habitat requirements. For the purpose of the assessment, wet meadows were defined as core patches, and all other habitat were assigned weighting factors with values ranging [0 - 100] (where 0 indicates the core areas, and 100 - most unfavourable habitat types), representing the accumulative resistance cost of species movement through each raster cell to the nearest habitat patch.

[2] Corine Land Cover, EEA (2018). https://doi.org/10.2909/71c95a07-e296-44fc-b22b-415f42acfdf0

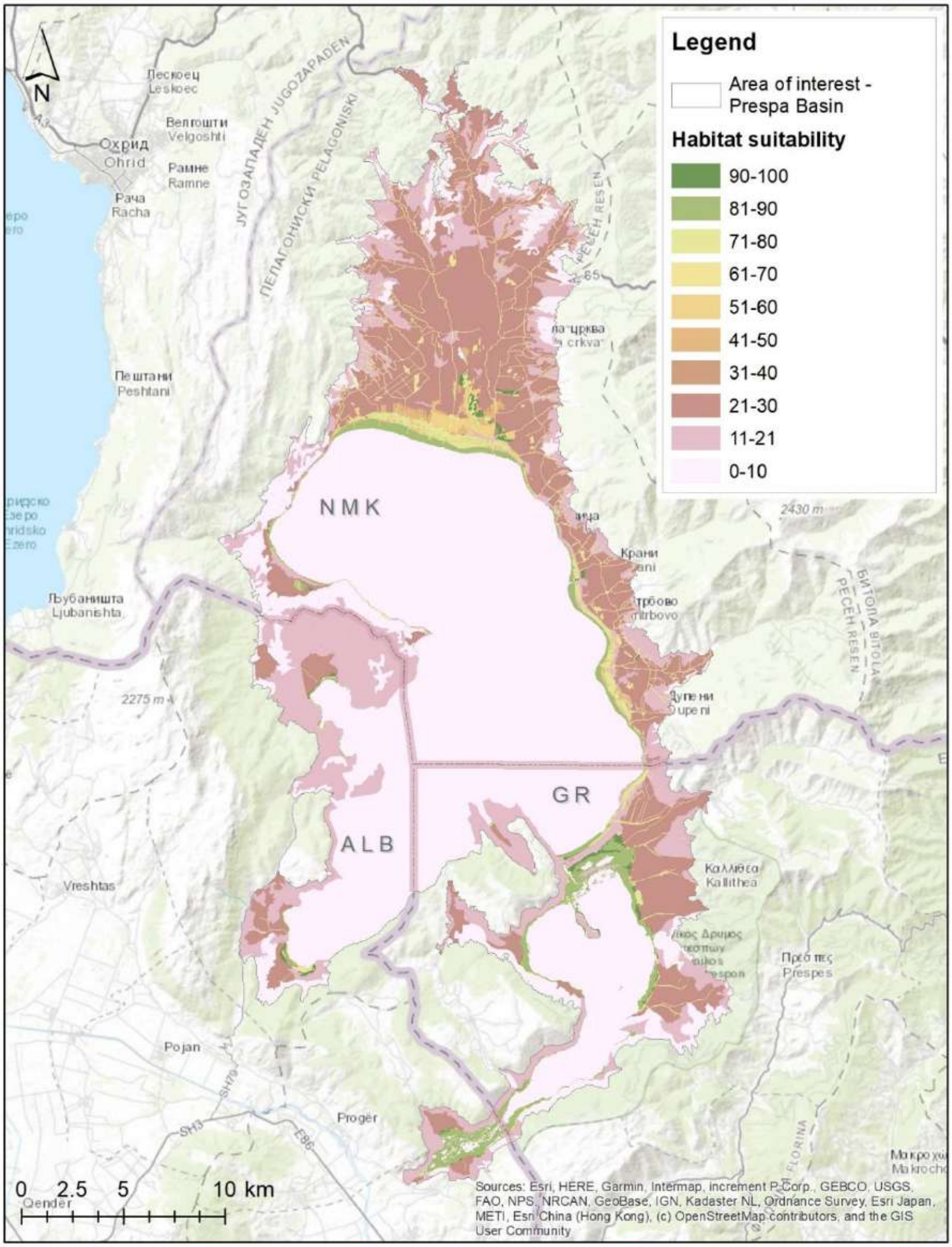

Figure 54 Map of habitat suitability for the Macedonian crested newt (Triturus macedonicus)

The habitat connectivity analyses were performed using 5x5 m rasterized habitat map, 5x5 m raster cell of suitable habitat taken as a minimum core area patch. With consideration to available literature data (Haubrock and Altrichter 2016), a distance of maximum 1000 m through unsuitable habitats was taken as a threshold distance that species can cross from one patch to another, not accounting for links crossing patches i.e. how often a patch lies on the shortest path between all pairs of patches in the graph. Links were saved as paths representing the actual route of the link between two patches identifying and delineating potential roots or pathways that facilitate the movement of the Macedonian crested newt between different habitat patches.

Functional connectivity was assessed using 'delta Probability of Connectivity' assessing the relative importance of each element (patch node/corridor link) by computing the rate of variation in the global metric induced by each addition/removal [resulting value 0-1].

Cumulative connectivity values of corridor links and habitat patches was then calculated as follows:

$$PVxy = AREA(PATCHxy)*dPC(PATCHy); \quad \sum PVx = PVx1+PVx2+\ldots+PVxn \qquad \text{where:}$$

"x" – area of interest; "PATCHy" - core patch "y"; "AREA(PATCHxy)" - area of the segment of "PATCHy" inside area of interest "x"; "dPC" - delta connectivity of probability value; "PVxy" - Patch segment connectivity value; "∑PVx" - cumulative connectivity value of all core patch segments in the area of interest "x"

$$CVxz = LENGTH(CORRIDORxz)*dPC(CORRIDORz)*REAL\_DIST(CORRIDORz)/COST\_DIST(CORRIDORz);$$

$$\sum CVx = CVx1+CVx2+\ldots+CVxn \qquad \text{where:}$$

"x" - area of interest; "CORRIDORz" - corridor "z"; "LENGTH(CORRIDORxz)" - length of the segment of "CORRIDORz" inside area of interest "x"; "dPC" - delta connectivity of probability value; "REAL_DIST(CORRIDORz)" - real distance of "CORRIDORz"; "COST_DIST(CORRIDORz)" - weighted distance of "CORRIDORz"; "CVxz" - Corridor segment connectivity value; "∑CVx" - cumulative connectivity value of all corridor segments in the area of interest "x"

In order to evaluate the likelihood of functional connectivity between habitat patches assuming most favorable conditions and identify potential corridors for functional use in the event of habitat restoration, the analysis was reiterated with a 40% reduction in weighting factors.

9.4 Wet meadows connectivity assessment output

Prespa basin sustains total of 234 patches of wet meadows that for the purpose of the analysis were considered as most suitable habitat for the Macedonian crested newt. However, even 48% of identified habitat patches are with area of less than 100 m^2, 21% have an area between 100 and 1000 m^2, 15% have an area between 1001 m^2 and 1 ha, 11% have an area between 1 and 10 ha and the area of only 4% of the habitat patches exceeds 10 ha. The extent of wet meadows in Macedonia closely approximates that of wet meadows in Greece, while the area of wet meadows in Albania is 50% lesser. Graphical representation of patch area/perimeter ratio relative to the patch number is provided on Figure 55.

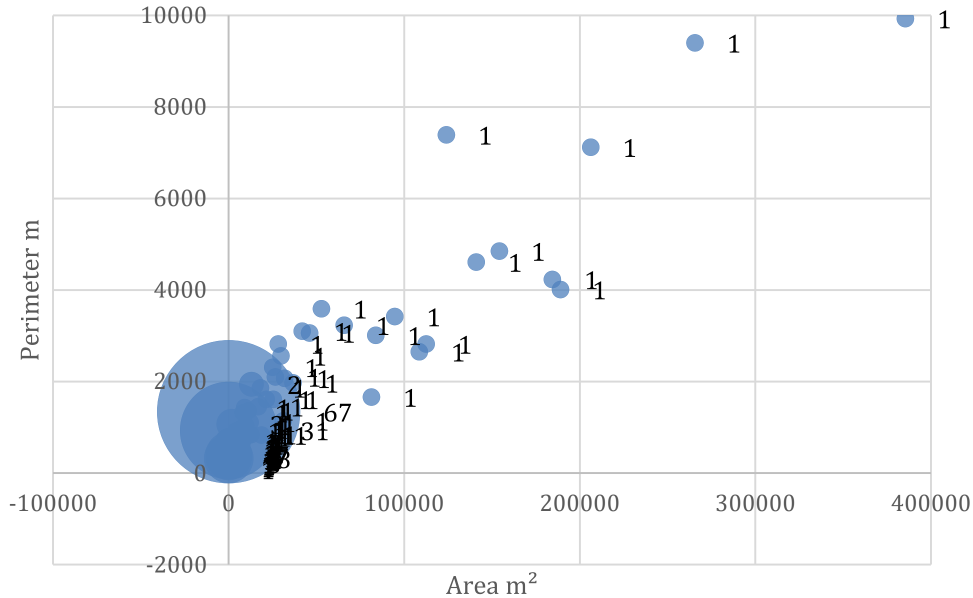

Figure 55 Plot of patch area/perimeter relative to the patch number indicated by bubble size

Highest connectivity of habitat patches in Prespa basin was noted for the habitat patches in the area between Great Prespa Lake and Small Prespa Lake (0.41), followed by the habitat patches 2 km SW of Plati in the same area (0.22). Lower connectivity (ranging between 0.07 to 0.12) was noted between the patches of wet meadows in the area beween Dolno Perovo and Asamati (south of Ezerani) in North Macedonia. Lowest connectivity between habitat patches was observed in Albania, highest in Kalamas (0.3) and Zaroshka (0.2). Highest corridor segment connectivity value was observed in the area of Opa and Mikrolimni in Greece and in in the area between Ramnik to Ruzhin, Ezerani in North Macedonia

Map of probability of connectivity is provided in Figure 56.

Corridor permeability, ranging from 1 to 2000, is indicative of the cumulative cost-weighted values assigned to raster cells that the target species must traverse. A higher cost corresponds to a lower likelihood of the species utilizing the corridor. The maximum path distance between patches approximates 800 m. Accounting for the complete true distance between habitat patches i.e. taking into consideration the path length within the habitat patch and intermediate patches, the maximum path length approximates 3 km.

Corridor permeability is a measure directly linked to the functionality of a corridor, aligning with the outcomes of habitat connectivity. In simpler terms, when corridor permeability is higher, the corridor is more functional, increasing the likelihood of species using it. This heightened functionality, in turn, enhances the overall functional connectivity between habitat patches. In essence, a higher corridor permeability not only signifies improved corridor functionality but also elevates the probability of species utilizing it, thereby fostering increased connectivity among habitat patches.

Map of functional corridors in Prespa basing is provided on Figure 57.

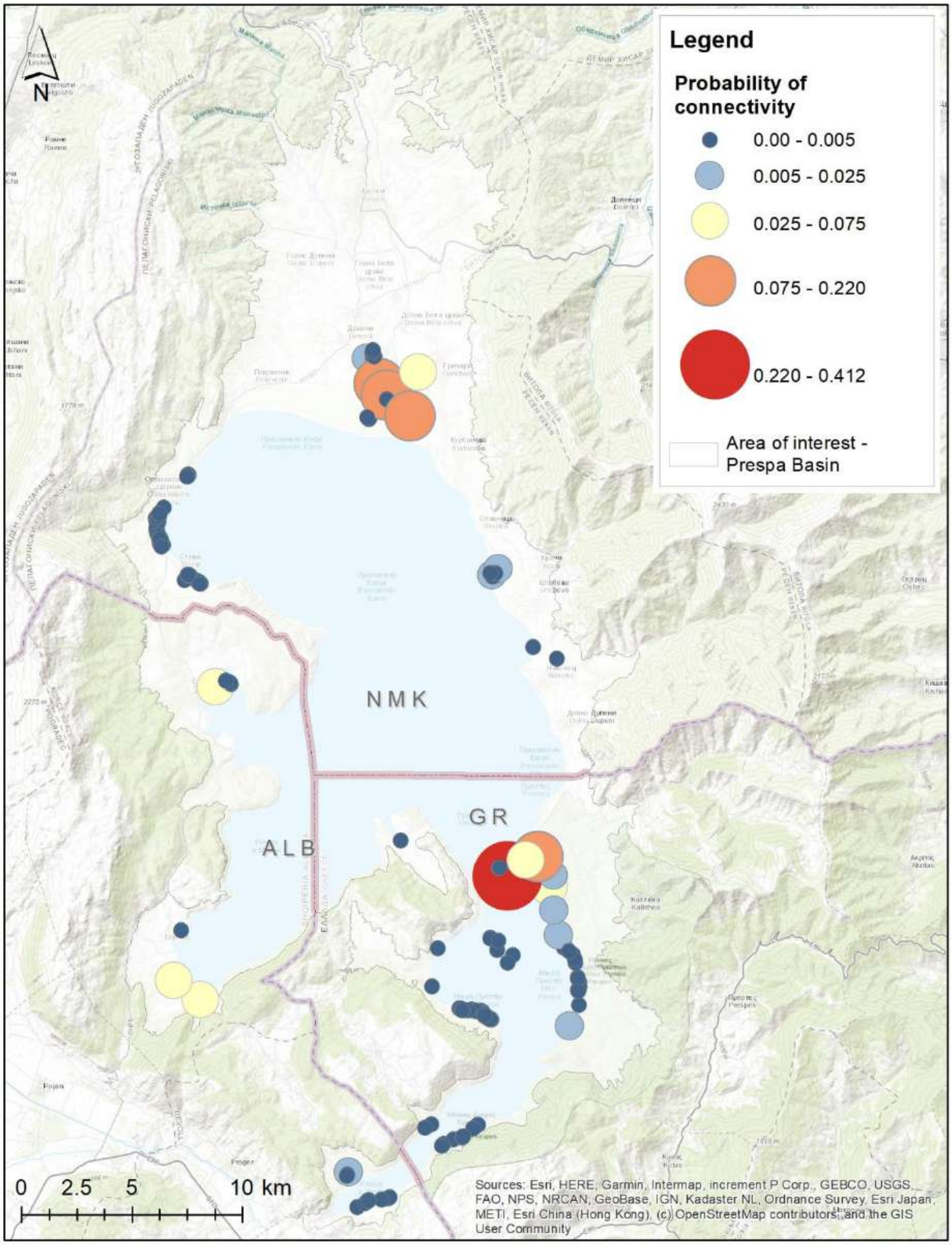

Figure 56 Habitat patch nodes based on probability of connectivity outputs in Prespa basin

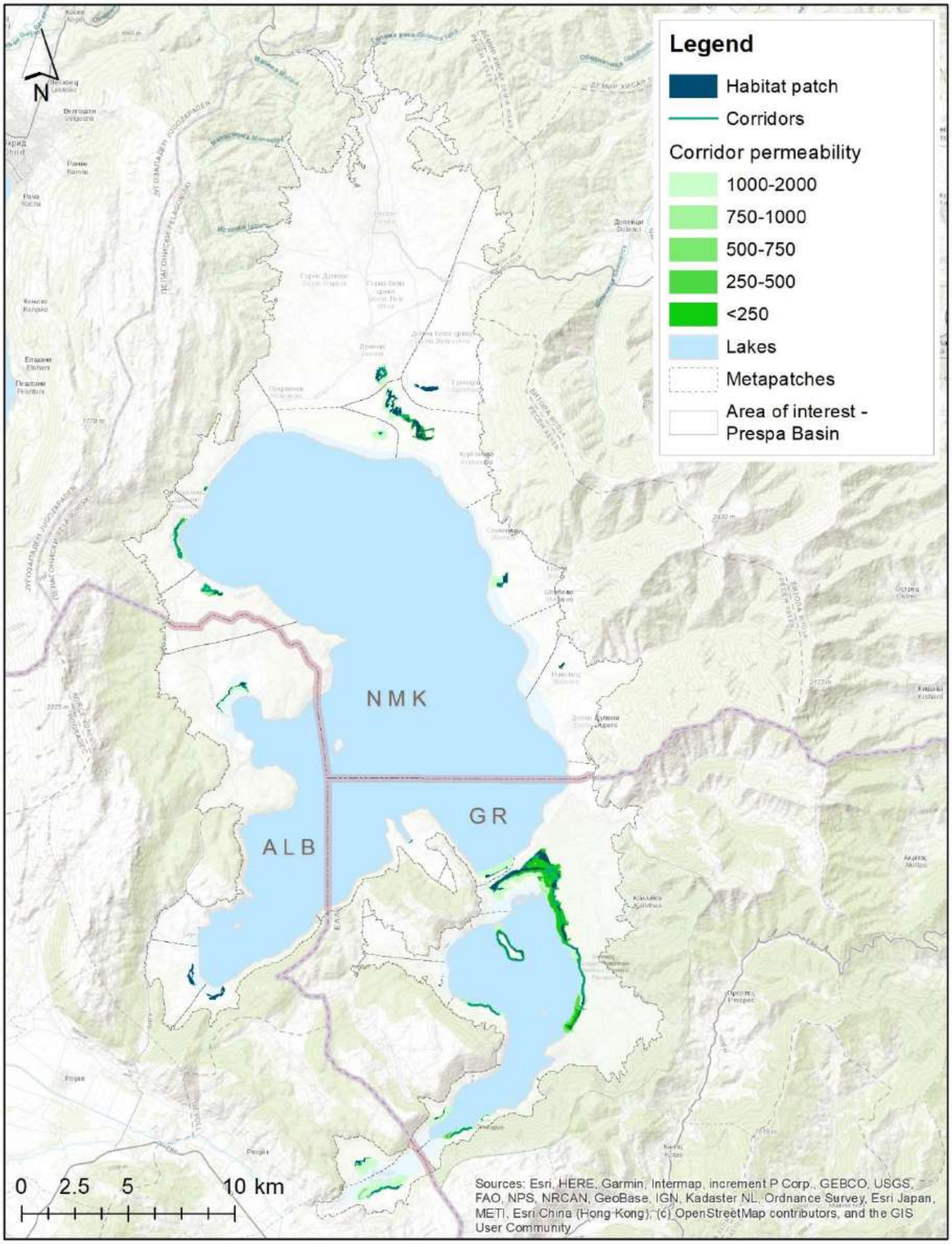

Figure 57 Corridor permeability in Prespa basin

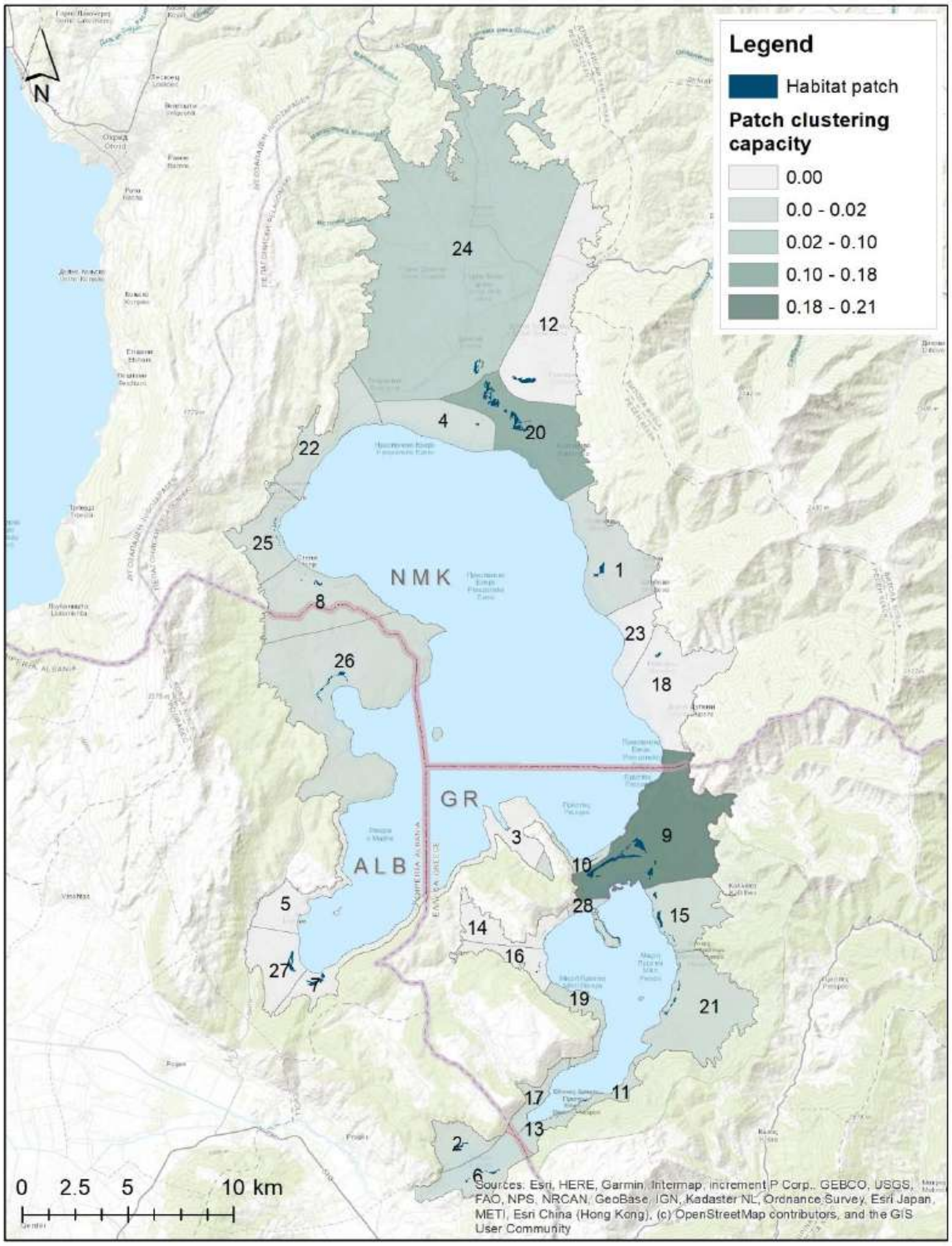

Figure 58 Patch clustering capacity indicative of meta-patch spatial arrangement

The probability of connectivity outputs have outlined 26 interconnected groups of habitat patches – meta-patches within Prespa basin (Figure 58). These meta-patches play a vital role in comprehending and augmenting habitat connectivity, which is intricately connected to the preservation of biodiversity and the facilitation of ecological processes. Meta-patches represent areas of interconnected and functional habitat patches, serving as a visual depiction of the spatial constraints influencing the movement of species, genetic exchange, and ecological interactions.

The cumulative patch value (∑PV) and cumulative corridor value (∑CV) presented in Table 26 serve as indicators of the comprehensive functional connectivity of wet meadows in the Prespa basin concerning the functional connectivity associated with each neighboring country.

Table 26 Cumulative corridor and suitable habitat patch value in Prespa basin

Country	Cumulative Patch Value (∑PV)	Cumulative Patch Value (∑PV) – normalized (% from maximum recorded ∑PV)	Cumulative Corridor Value (∑CV)	Cumulative Corridor Value (∑CV) – normalized (% from maximum recorded ∑CV)
ALB	1.4	4.6	0.01	0.2
GR	20.7	69.6	3.68	95.1
NMK	7.7	25.8	0.18	4.7
Total	29.8	100.0	3.87	100.0

When the analysis was reiterated with a 40% reduction in weighting factors, presuming most optimal conditions for species movement in order to assess the improved functional connectivity between habitat patches in the context of habitat restoration, the findings reveal a significant improvement in functional connectivity across all three countries, that is noteworthy in North Macedonia and Albania. Results show an increase of Cumulative Corridor Value of 51.4% in North Macedonia, 10.23% increase in Greece and 8.33% increase in Albania. Adequately it is assessed that the Cumulative Patch Value in North Macedonia would increase by 8.33%, by 7.13% in Albania and by 3.4% in Greece and the number of meta-patches was reduced by 27%.

Specifically, habitat restoration actions on the stretch from Kamnik to Korija, below village Ezerani in North Macedonia will secure connectivity between meta-patches number 24 and 20. Habitat restoration action in the area of Zaroshka in Albania will secure connectivity between meta-patches number 27 and 7 (Figure 59). Though habitat patch and corridor values for wet meadows in Greece were assessed as highest, habitat restoration and active management actions to increase habitat patch area and improve corridor functionality in the area of Pili will enhance connectivity between meta-patches 14, 16 and 19.

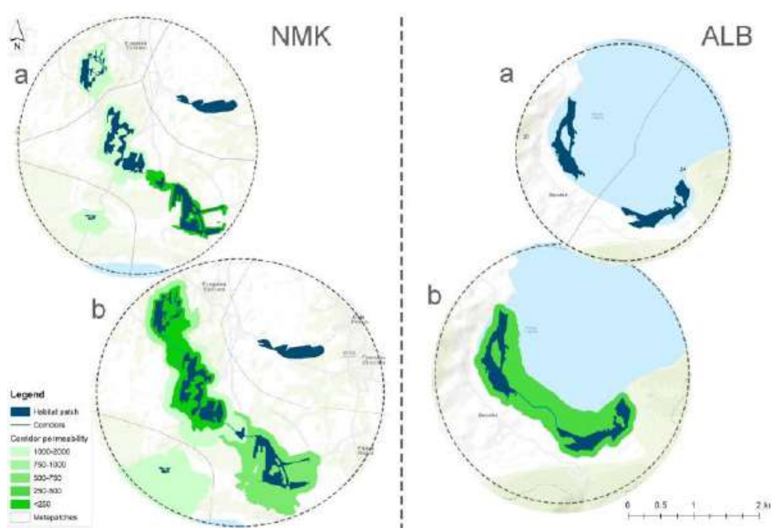

Figure 59 Preview of habitat patch connectivity a) extant condition and b) presuming most optimal conditions for species movement in the context of habitat restoration

10 Conclusions and recommendations

This last chapter of the report gives an overview of the key findings (Figure 60) and lesions learned from our conducted activities in the three year cycle (2021-2023). Moreover, it provides specific recommendations which should be taken in consideration for any future activities. We believe that these activities have provided solid overview of different aspects related to wet meadows in Prespa. Considering the diversity of all conducted activities, they could allow selection for future focus areas for more in-depth activities.

Figure 60 Key finding from our conducted activities

10.1 Is mowing the right and effective management practice?

Wet meadows in Prespa have ongoing successional changes. Besides the moisture factor, abandonment of mowing practices also contributes to succession since these habitats are human-depended. Management by mowing is practiced not only in protected areas, but also on private land, especially in EU member countries. This is evident from the fact that EU provides several financial schemes for management and restoration of semi-natural grasslands (Stoate et al. 2009). Eco-schemes are one of the instruments available in the toolbox of the Common Agricultural Policy (CAP) which ought to deliver on the European Green Deal ambitions. Yet, past and current economic compensations do not drastically improve meadow's recent status (Walden 2018). On the other hand, in this sense, farmers in North Macedonia as a non-EU member country should be motivated in alternative ways. Nevertheless, their involvement is of great significance due to conserving not only the natural but also cultural values of Prespa through conserving the traditional management practices.

Contrary to the above, even though we are revisiting mowing as a traditional practice in Prespa, it is also important to discuss changes in ecosystem conditions and demographics in the region, reduced grazing, extensive land use and increased threats. This by itself shows that reintroducing abandoned practices should fit new context conditions, and can therefore set challenges. Some of these challenges can be overcome with good planning that should rely on scientific and ground true data. This is why, in parallel with management activities, monitoring and research activities should continue in the future as well, so to support, guide and design the management process.

While farmers are one of the key stakeholders regarding management, we see higher responsibility for maintenance of ecological integrity and restoration efforts in protected areas. They should be the main driver off all management activities in order to support slow transitions between the dynamic habitat changes in Prespa, especially in respect to climate change. For example, Ezerani Nature Park was the direct beneficiary of all results obtained with our activities. Some of the benefits from the management activities are prevention of wet meadow's habitat loss, generated ecological knowledge, even easier interception of fires due to biomass clearings thus increasing accessibility. This only supports the necessity of addressing more serious attention to wet meadows in the future by the protected area.

Management actions should be planned on longer term, but also with annual changes. They should always take in consideration local changes thus the management plan should have flexibility. In this regard, measurement of additional parameters, especially climatic such as precipitation, is highly recommended.

Our experience showed that additional mowing equipment should be explored since in some sites we had difficulties to cut the biomass due to its density and toughness. This especially refers for potentially cutting reedbeds in the future for opening surface areas for habitat transformation. All practical experiences and recommendations from our Greek colleagues (Grillas et al. 2021) in this regards should be taken in consideration. On the other side, mowing was much easier on already mowed meadows before (the previous or two years before). Therefore, even in technical aspect, mowing continuation is also important.

Finally, management of wet meadows intends to lower the nutrient load in the system that through groundwater reaches the lake.

10.2 Do we know enough about wet meadow's ecology?

The straight forward answer is no. To generate ecological knowledge also takes time. Changes in time are especially accurate for such a dynamic landscape as Prespa Basin. However, our results have provided an insight in different aspects of wet meadow's ecology. Therefore, they can serve as good baseline for more in-depth analysis and additional hypothesis.

The obtained results should be connected with scientific data from the other ecosystems/habitat types in Prespa. This is especially refers for data regarding nutrients, minerals and pollutants. In this way we can get better understanding for the dynamics of different processes and fluxes can be quantified. In this context, more in-depth comparative analysis can be done for groundwater and surface water chemistry. This can provide an overview of the condition of the water in the Basin in different points in the landscape, but will also position the role of wet meadows in water retention and purification. Focused ecohydrological study in the future can result in a hydrological model for Prespa that can also be predictive. This type of model can provide different scenarios and can navigate different management strategies.

Additional studies should focus on different types of pollutants in terms of estimation of their influence on wet meadows. This especially refers to pesticides. On the other hand, we also recommend follow up studies that should put light on the effects of mowing practices. Monitoring of the changes of the plant communities as a consequence of the management should be conducted. With this data, adaptations in the mowing frequency or scale can be made. This types of studies are important for successful revitalisation of wet meadows, especially the most degraded ones, because it can provide information of the trends and rate of change in the given local context. If any additional management/restoration measures are applied in the future, vegetation changes (structure, but also nutrient and mineral content) should be closely followed.

Lastly, management of protected areas requires a landscape approach. We hope that these results reflect the ecological importance of wet meadows in Prespa for the whole Prespa Basin. Therefore, we suggest that they should have more prompt position in the management strategies, especially in regards of collecting more scientific data as well as monitoring of their biodiversity. This will require enhanced cooperation and communication between different stakeholders. Unsynchronised activities in regards of collection and availability of data were observed, which we believe hinders systematic and continuous knowledge gain. We recommend higher transparency and respect for data availability from all engaged entities in the future. In this regard, we really hope that these results will also be taken in consideration especially from the most relevant stakeholders such as Ezerani Nature Park, Galichica National Park, National Hydrometeorological Service and Hydrobiological Institute in Ohrid. For this, we have provided the full dataset (Annex 12.1) from all measurements in this report.

10.3 Does mowing reduce the biomass quality in terms of its chemical, nutritive and monetary value?

Our results from the physico-chemical analyses are in line with literature data for similar types of grasses. The protein and energy content meet the average needs of forage (meadow hay) in the daily meal of large and small ruminants. It is important to be noted that this is a first study of this kind for the wet meadows in Prespa. It provides valuable information regarding the nutrient content of the hay that can be used for alternative management activities e.g. grazing. Part of our produced hay was used by the repro center in National Park Galichica. Providing the data for the energy value of the hay was also important in order to make sure that the forage meets the needs of the animals there. This information also opens new perspectives for other economic activities as well. Potentially, forage from meadow hay can be commercially produced.

Having in consideration that the monetary calculation depends on the hay quality, it was interesting to see if mowing has an influence on the quality of hay. Our results show that there is no significant difference of the hay's quality before and after mowing activities.

The obtained price for 1 kg of dry matter of meadow hay from Ezerani Nature Park (8.30 denars/kg) is in good correlation with the market price of meadow hay in North Macedonia in the period from January to November 2023 - 9.30 denars/kg of meadow hay.

Further in-depth studies can be conducted regarding the chemical composition of certain species during two or more seasons (years), in certain phases of the vegetation, before grazing or before mowing and determination of the in-vitro digestibility of the biomass from the wet meadows. A study of the ethology of ruminant grazing is also possible, as there is a change in grazing behavior over time, which is related to the structural and chemical modification of these habitats. The grazing time of ruminant animals is strongly influenced by the structural (leaf: stem ratio and height) and chemical (ADF, TDN) characteristics of the plants. As such, management strategies can optimize legume and grass height and stimulate regrowth and abundance of leaves compared to stems and improve grazing animal performance.

10.4 Are amphibian, butterfly or mammalian species affected by management activities?

In summary, we can conclude that effects on biodiversity from management practices are not easy to establish. This is due to many reasons such as intensity of sampling and time limitation (three years is not sufficient time). However, no major direct negative impact had been observed as well. Nevertheless, monitoring activities should continue in order to reduce potential disturbances and provide insights for better planning of management activities.

In addition, recommendations for future work are given for each of the monitored groups.

Amphibian observations in wet meadows across coastal Prespa in North Macedonia from 2021-2023 fail to inform on the efficiency of wet meadow management, or lack thereof. This is mainly a result of the short time period and the sporadic nature of the observations that preclude any correlations and any potential consequent inferences. Both the Macedonian crested newt and the Smooth newt should be monitored in Prespa in the future. Monitoring needs to take place in spring, starting in April until June. Depending on the season, monitoring should start at least one week after the first flooding of wet meadows occurs, or after submerged vegetation appears. This gives enough time for newts to migrate to the breeding pools, court and females start laying eggs on vegetation. In consultation with locals, preferable rangers of protected areas, transects should be established, preferably down already well-known roads that cross by or through flooded areas – there is already a decently developed road network in Nature Park "Ezerani". Once transects are established they should be patrolled by at least one person and each place where eggs are found, noted.

During the past few years, butterfly sampling provides sporadic data for Prespa Region, with focus on wet meadows. This is mainly due to the frequency of sampling and lack of systematic approach. However, we notice increased trend in butterfly diversity, which is indicative and only confirms the conclusions that more intensive sampling will provide better overview. Nevertheless, we were not able to directly observe the effects of mowing practices. On the other side, we have confirmed the presence of the important species, the Large Cooper, which is listed on Annex II and Annex IV from the Habitats Directive. Given the low population density of this species, an appropriate monitoring solution is to monitor the species by counting its eggs. In this way, for the sites where it is monitored, not only the presence and occurrence is confirmed, but also the reproduction of the population. Females lay their eggs individually or in small groups on the leaves of the host plants. Larger clusters initiate oviposition of more than one female, or that one female has visited the same leaf several times. The eggs are located on the upper (sometimes on the lower) surface of the leaf of the plant with which the larva is later fed and hatched ten days after laying. The number of eggs that a female lays varies on a large scale and reaches from an average of 50 eggs to 300 eggs (as many as 400 in captive individuals). According to some authors, in favourable conditions they can produce up to 750 eggs. The eggs of this species have very characteristic shape, size and colour and their recognition is unmistakable (Figure 61). After hatching, the empty eggs (shells) remain on the leaf of the plant for several days or up to several weeks, as the larvae do not eat them.

Figure 61 Left: eggs of Lycaena dispar; Right: egg shells and one unhatched egg of Lycaena dispar (photo credis: (Strausz et al. 2012)

Habitat selection criteria for eggs monitoring of the Large Copper are based on finding suitable habitats where plant species from the genera *Rumex* are present. In ideal cases, database along with map of all places where these plants are observed in the wetland should be created. Afterwards, an objective selection of the potential habitats can be made, considering other parameters of the species ecology. In the selected habitat, the host plants are observed and depending on the entire area of the monitored habitat, the number of plant stems for eggs monitoring is determined (smaller area - more stems). Considering the surface areas of the selected points for monitoring it is recommended to include all plant stems of the genus *Rumex* within these areas. For the purpose of unified counting in different seasons, but also in order to monitor the host plants themselves (they can be destroyed and / or eaten), during the first counting a sketch / scheme of the location of the plant stems of the host plants that are monitored can be prepared.

During the monitoring, the following parameters are recorded in the field diary:

- number of eggs on one leaf (hatched and unhatched);
- number of eggs per whole plant;
- height above the ground and length of the leaves on which eggs are present.

Monitoring of the Large Copper with eggs counting method needs to be carried out two times per year, at the end of the flying season of the adults, from both generations. The field trips can be conducted at mid June – beginning of July for the first generation, and mid August – beginning of September for the second (Table 27).The monitoring dynamics can be modified and it should be annually adapted to weather conditions. On basis of the outcome from this monitoring, more concrete recommendations regarding mowing activities can be given.

Table 27 Months in which monitoring activities should be conducted (eggs counting)

Stage/Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Eggs						+	+	+	+			

The survey on small mammals in the area of the wet meadows conducted during 2021 was a baseline survey providing preliminary data on the presence and diversity of small mammals in wet meadows. However, it is early to conclude from these results if wet meadows management provides better conditions for higher diversity and populations of small mammals. Thus, the survey and monitoring of small mammals should continue in order to get more data and see the influence of managed wet meadows on small mammals. The survey should be extended to additional 1-2 representative sites with wet meadows.

10.5 Are stakeholders involved enough and how to engage them more in the future?

Close cooperation between stakeholders is also crucial for sustainability of management activities. This was best reflected through the networking of the two protected areas (Ezerani Nature Park and Galichica National Park). Enhancement of their cooperation in the future should be done by identification of common needs on all levels. On the ground activities that are conducted with mutual efforts are recognized as best practice. Additionally, this cooperation needs to be expanded with other protected areas as well both on national and transboundary level.

As explained earlier, wet meadows and the local community in Prespa are in close relation. Our approach for direct involvement of stakeholders especially in management activities aims to restore connections between ecosystem services supply and demand. Our activities have created a very solid fundament for establishment of a formal network of stakeholders. The fundaments are related to gained contacts especially with local farmers. Continuous communication and presence on the field significantly contributed for gaining their trust, which is a premises for functionality of potential, future network. This future network is important not only for the sustainability of management activities, but also for additional collaboration regarding eutrophication and pollution issues.

Raising awareness for wet meadows in Prespa should also motivate researchers, especially young scientists. More in-depth understanding of their role within the landscape can provide better and more specific recommendations for management. This refers not only to ecologists, but also social scientists.

Finally, more intensive communication and cooperation of stakeholders should be enforced on transboundary level. More efforts should be made to network especially the local communities, so they can have an opportunity to exchange or even initiate mutual actions.

10.6 How well connected are wet meadows on transboundary level, why is this important and how to improve connectivity?

Ongoing collaborative efforts for supported traditional management of wet meadows with local communities and stakeholders in North Macedonia have raised the awareness on the importance of sustaining the integrity of wet meadows and have provided tangible evidence on the benefits provided for both local communities and biodiversity. While advocating for and encouraging the continued application of consistent traditional management practices is crucial for maintaining a balanced ecological succession, incorporating restorative management practices at intermediate stages is essential. This approach ensures the sustained supportive role of wet meadows in preserving biodiversity and securing the provision of ecosystem services in the Prespa region.

To enhance connectivity between wet meadows and facilitate species dispersal, a comprehensive set of recommended actions and strategies focused on habitat restoration and management, can be explored. Among these, maintaining habitat integrity through vegetation management, including management and control of invasive plant species that may have a negative impact on wet meadows native vegetation, is essential for sustaining appropriate vegetation density and structure.

Establishing new and maintaining extant wildlife corridors that connect wet meadows, will secure unrestricted movement of species between habitats. Application of buffer zones along watercourses and between wet meadows can further safeguard and enhance connectivity, providing additional habitat for amphibians and facilitating their movement. Additional efforts to restore degraded wet meadows and sustain groundwater levels should consider creation or restoration of ponds and clearance of channels.

Efforts to enhance the integrity of wet meadows should include initiatives to raise awareness regarding the importance of sustainable utilization of groundwater for agricultural purposes, concurrently increasing community awareness of and resilience to climate change. This involves educating stakeholders and communities about responsible water management practices that balance agricultural needs with the preservation of wetland ecosystems, emphasizing the importance of adopting efficient irrigation techniques, implementing water-saving technologies, and promoting sustainable use of groundwater resources. Furthermore, addressing connectivity on a larger scale, requires recognizing the importance of regional cooperation for successful conservation outcomes whilst collaborating with local conservation organizations, government agencies, and research institutions to leverage resources and expertise is crucial for the effective implementation of conservation strategies. Finally it is recommended to establish long-term monitoring programs to assess target populations of species whose ecology is closely linked to wet meadows, as well as evaluating habitat conditions and connectivity over time in order to adapt and implement management strategies as needed.

Implementing a combination of these actions, tailored to the specific characteristics of the wet meadow habitats can significantly improve habitat connectivity and the overall conservation of these important ecosystems.

11 Literature

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12 Annexes

12.1 Supplementary material in Microsoft Excel format for all conducted measurements presented in Chapter 4

12.2 Original questioner (in Macedonian language) for the results presented in Chapter 4.3

Претставување: *Добар ден. Јас сум Драгана Пеликудовска, студент на Природно-математички факултет во Скопје. Во моментов ја работам мојата дипломска во која ја истражувам поврзаноста помеѓу овоштарниците и влажните ливади во Преспа, односно како вештачките ѓубрива кои се користат за наѓубрување на овоштарници влијаат врз влажните ливади. Во продолжение ќе ви поставам неколку прашања, а вашите одговори се целосно анонимни и ќе бидат искористени исклучиво за истражувачки цели.*

1. Дали поседувате овоштарник?

1. Да => оди на прашање D1
2. Не => крај на прашалникот

Демографија

D1. Пол

1. Машки
2. Женски

D2. Возраст (*да се запишат години*)

D3. Место на живеење

Општина ________________, населено место _________________.

D4. Кое ниво на образование имате завршено?

1. Незавршено основно образование
2. Основно образование
3. Средно образование
4. Вишо
5. Високо образование (универзитет)
6. Магистер на науки
7. Доктор на науки

D5. Работен статус:

1. Вработен со полно работно време. => оди на прашање D6
2. Вработен со скратено работно време, работи повремено. => оди на прашање D6
3. Самовработен (земјоделец, занаетчија)
4. Невработен, стечаец, технолошки вишок
5. Домаќинка
6. Студент / ученик
7. Пензионер

D6. Занимање:

1. Јавна администрација, здравство, образование, полиција

2. Вработен во приватен сектор

3. Сопственик на бизнис

4. Друго. запиши _________________

D7. Дали вашата егзистенција зависи од производство на јаболка?

1. Да
2. Не
3. Делумно

Прашања

2. Колкава површина на овоштарници поседувате?

1. помалку од 1 ха
2. 1-3 ха
3. 3-5 ха
4. 5-10 ха
5. повеќе од 10 ха

3. Како ги извршувате работите во овоштарникот:

1. Сите работи ги извршуваме во рамките на потесната фамилија
2. Најмуваме сезонски работници
3. Ги давам под наем

4. Дали овоштарниците ги прихранувате со вештачки ѓубрива?

1. Да => оди на прашање 5
2. Не => оди на прашање 11

5. Какви ѓубрива користите? (*да се запише името на ѓубривото и состав*

6. Колкава количина на ѓубриво користите? (*да се запише килограми на хектар*)

7. Колку пати во годината наѓубрувате?

1. 1-3 пати
2. 3-5 пати
3. 5-8 пати
4. Над 8 пати

8. Во кој период ги наѓубрувате овоштарниците? (*можни се повеќе одговори*)

1. Пролет
2. Лето
3. Есен
4. Зима
5. Друго. Запиши__________________

9. На кој начин ги аплицирате ѓубривата? (*можни се повеќе одговори*)

1. Рачно
2. Со механизација
3. Преку систем за наводнување

4. Друг начин. Запиши ________________

10. Дали зголемувањето на цената на вештачките ѓубрива оваа година ќе влијае на количината и честотата на наѓубрување?

1. Да, честотата на наѓубрување ќе се намали
2. Да, количината на наѓубрување ќе се намали
3. Да, воопшто нема да користам вештачки ѓубрива
4. Не, честотата и количината на наѓубрување ќе останат исти

11. Зошто не користите вештачки ѓубрива?

1. Немам потреба
2. Користам други продукти/ѓубрива
3. Премногу се скапи и не можам да си дозволам
4. Друго. Запиши _________________________

12. Дали би купувале и користеле органско ѓубриво доколку компостарата во Ресен работи со полн капацитет?

1. Да
2. Да, но би користел/а и вештачко ѓубриво
3. Не, сметам дека вештачкото ѓубриво е подобро

Забелешки: