FACULTY OF AGRONOMY
DEPARTMENT OF CROP SCIENCE

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Study on the Potential of Natural and Artificial Grasslands for CO_2 Sequestration

ABSTRACT

of a dissertation submitted for the award of the educational and scientific degree "Doctor"

Scientific specialty: "Forage Production and Grassland Management"

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Plovdiv
2026

The research was conducted during the period 2019–2021 at four sites: Plovdiv, Rozino, Beklemeto, and Devin.

The dissertation comprises 176 pages and contains 37 tables and 61 figures. The cited literature includes 288 sources, of which 10 are in Cyrillic and 278 are in Latin script.

The dissertation was discussed at an extended departmental council of the Department of Crop Science at the Faculty of Agronomy, Agricultural University – Plovdiv.

The defense of the dissertation will take place on 08.06.2026 at 11:00 hours at Lecture Hall 1 at the Agricultural University – Plovdiv before a Specialized Scientific Jury approved by Order of the Rector RD – 16-475 of 02.04.2026.

Reviewers:

Opinions:

The defense materials are available to interested parties in the library of the Agricultural University – Plovdiv, 12 Mendeleev Blvd.

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1. INTRODUCTION

Grassland plants fix CO_2 through the process of photosynthesis, whereby inorganic carbon is transformed into organic compounds. Part of the synthesized carbohydrates is incorporated into the structure of aboveground and belowground biomass (leaves, stems, roots), while another part enters the soil through root exudates, decomposing plant mass, and microbial transformation, forming stable forms of soil organic carbon. It is precisely the soil in grassland ecosystems that constitutes a significant long-term carbon reservoir, often accumulating larger quantities compared with aboveground biomass.

According to the latest data, grassland ecosystems (meadows and pastures) worldwide cover approximately 3.2 billion hectares, which represents about 70% of the total agricultural area (Ramankutty et al., 2008; Tubiello et al., 2013). These ecosystems perform an important function in the overall carbon balance, accumulating approximately 20% of the world's carbon stocks, predominantly in the form of soil organic carbon (Schlesinger, 1977; Conant, 2010).

In our country, grassland communities also occupy a substantial share of the agricultural land fund, with the area of meadows, pastures, and commons amounting to approximately 1.7 million hectares, or about 33.5% of the utilized agricultural land (Meshinev et al., 2005; Apostolova et al., 2006). This share highlights the potential of grassland ecosystems in the country, both in terms of maintaining biodiversity and ecosystems, and as a potential resource for carbon storage in connection with the sustainable management of agricultural landscapes.

The functionality of grasslands extends far beyond their role solely as a forage source. They are of major importance in protecting the soil against erosion, regulating the water regime, and contributing to biodiversity in grassland communities (Porqueddu and Roggero, 1994). Over the last decade, grassland ecosystems have also been regarded as an opportunity for capturing and storing CO_2, which is a major greenhouse gas and a factor with a significant effect on climate change.

Based on current data, it is clear that grasslands can be a powerful tool in the fight against climate change. Identifying the components that stimulate carbon uptake, as well as developing innovative management practices, are of key importance for increasing the capacity of these ecosystems to absorb CO_2. In the context of a changing climate, efforts to promote sustainable grassland management will be important for reducing emissions and mitigating the negative effects of global warming. This study aims to investigate the potential of natural and artificial grasslands for carbon capture, which may contribute to long-term ecological equilibrium.

2. AIM AND TASKS

The present dissertation aims to study the capacity of natural and artificial grasslands for carbon uptake. In order to achieve the stated aim, the following specific tasks were carried out:

The capacity for carbon uptake and storage in natural grasslands was studied.

The capacity for carbon uptake and storage in artificial grasslands was studied.

The relationship between climate, species composition, and carbon accumulation in plants and soil was established.

3. MATERIALS AND METHODS

3.1. Object of the study

The object of the study consists of four different sites characterized by specific features.

Site 1 – artificial grassland in the area of the experimental field of the Agricultural University – Plovdiv

Site 2 – natural grassland in the area of Rozino village, Plovdiv Province

Site 3 – natural grassland in the Beklemeto locality, in the region of the town of Troyan

Site 4 – natural grassland in the area of the town of Devin

3.2. Determination of species composition

The species composition of the grasslands was determined according to the Braun-Blanquet method. This method is a classical phytocoenological approach for studying and classifying vegetation by describing the composition and spatial distribution of plant species within a given sample plot (quadrat), the number of which depends on the type of vegetation and the area.

Table 1. Braun-Blanquet scale for abundance and cover

Value	**Meaning**
r	single plants, very low cover
+	few plants, cover <1%
1	cover 1–5%
2	cover 5–25%
3	cover 25–50%
4	cover 50–75%
5	cover >75%

The method uses a scale for assessing the abundance and cover of species, which allows a rapid visual evaluation of their share in the plant community (Braun-Blanquet, 1964).

3.3. Determination of soil organic carbon content

The determination of organic carbon was carried out in the accredited laboratory of the Agricultural University – Plovdiv, using a standardized method (BDS ISO 14235:2002).

3.4. Determination of basal cover of grasslands

Basal cover was determined using a grid quadrat (50 × 50 cm). In each site, four quadrats were placed at several locations in order to establish the density and uniformity of grass cover in the surveyed area. One square of the quadrat corresponds to 1% of the area. The areas not occupied by vegetation are summed (subjectively), and the percentage (A) occupied by these bare patches in the quadrat is determined. The total percentage of covered area is calculated using the formula X = (25 – A) × 4 (Yancheva Hr., 1994).

3.5. Monitoring the dynamics of CO_2 fluxes

To determine the activity of CO_2 uptake per unit area of the monitored sites, a chamber was used that allows measurement of CO_2 content within a limited volume.

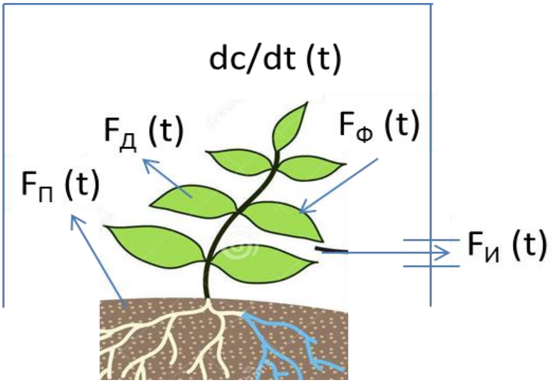

Figure 1. Schematic representation of CO_2 fluxes in the chamber, representing CO_2 dynamics (Fnet)

Legend: FП (t) is the diffuse efflux from the soil, FФ (t) is photosynthesis, FД (t) is aboveground plant respiration, FИ (t) is the leakage flux from the chamber. dc / dt (t) is the change in CO_2 concentration over time t in the upper part of the chamber (after Kutzbach et al., 2007).

3.6. Climatic data

In order to analyze agrometeorological conditions, daily air temperature and precipitation data for the period 1991–2020 were used. For this purpose, the Climate Data Store dataset from the Copernicus Program was used, a component for Earth observation of the European Union's space program, which observes the planet and its environment for the benefit of all European citizens. The information services offered by the Program are based on satellite Earth observations and in situ (non-space) data. The dataset is based on hourly ECMWF ERA5 surface-level data and is referred to as AgERA5. The variables provided in this dataset meet the input requirements of most agricultural and agroecological models (https://cds.climate.copernicus.eu/datasets/sis-agrometeorological-indicators?tab=overview).

3.7. Statistical analysis

The statistical processing of the experimental data was carried out by the method of multiple regression (Multiple Regression). This is a statistical method used to investigate the relationship between one dependent variable (result) and two or more independent variables (factors).

4. RESULTS AND DISCUSSION

4.1. Agrometeorological characterization of the meteorological conditions in the studied regions during the experimental period

4.1.1. Deviation of the mean monthly air temperature during the year of the experiment

The analysis of air temperature values during the years of the study shows positive deviations in all three winter months, with the exception of the values of the indicator in January 2019 in the area at a higher altitude (Fig. 2). The overall deviation in February reaches and exceeds 2°C. In March, except 2021, the deviations are also positive, which leads to an earlier start of the vegetation season than normal. During the remaining spring months—April and May—the deviations, with minor exceptions, are negative. In June, temperature fluctuations are within 1°C, and thereafter they increase and remain persistently above the climatic norm. The largest deviations (more than 3°C) are observed in November 2019. An exception is observed in September and October 2021, as well as during the summer months of 2021, when positive deviations reach and exceed 2°C.

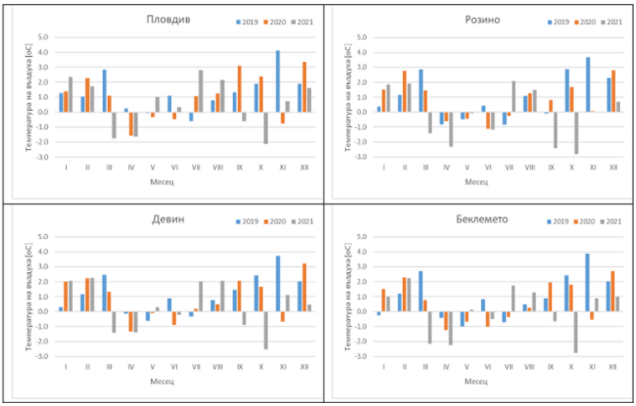

Figure 2. Deviation of the mean monthly air temperature during the experimental years relative to the reference period

Probability assessments define 2019 as a hot year (with the highest temperature sum) in the areas of Devin, Beklemeto, and Plovdiv, whereas in Rozino it was moderately warm. The year 2020 was close to normal in Devin, Beklemeto, and Rozino, whereas in Plovdiv it was characterized as moderately cold (Fig. 3). The year 2021 was cold in the Rozino area, while in the remaining sites it corresponded to the climatic norm.

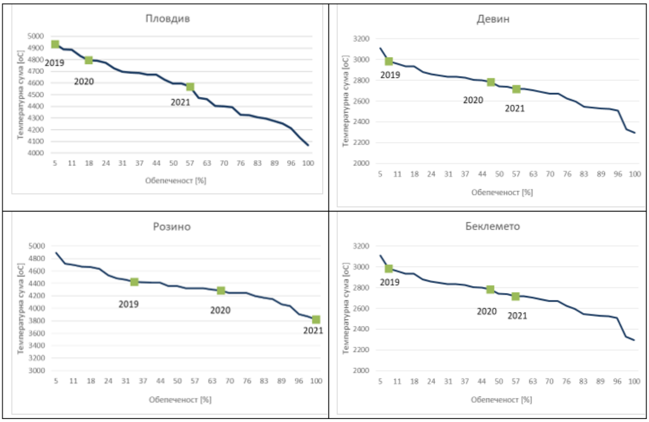

Figure 3. Characteristics of thermal conditions during the experimental years

4.1.2. Deviation of the mean monthly precipitation totals during the experimental years

Significant positive deviations in precipitation were observed in November 2019 at all stations, whereas the deviation in June occurred only in Plovdiv and Rozino. Negative deviations were observed in March and August at all sites (Fig. 4). In March 2020, significant positive deviations were recorded. Negative deviations were observed in September and November at all sites, and in Plovdiv and Rozino, also in July and August. The largest positive deviations were recorded in 2021, in January and October, with the exception of Beklemeto. Negative deviations during the summer were observed in Plovdiv.

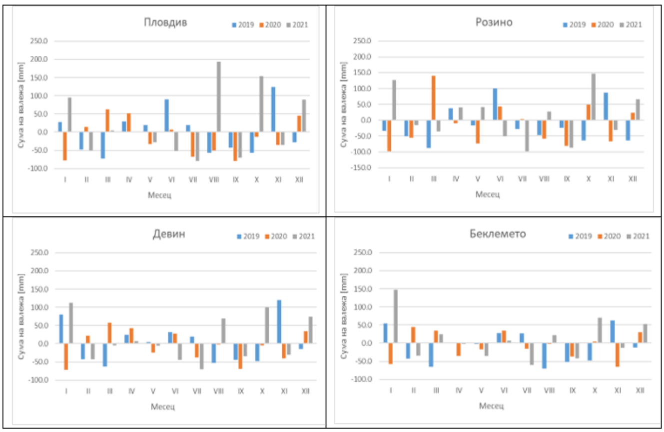

Figure 4. Deviation of the mean monthly precipitation totals during the experimental years relative to the reference period

The mean annual precipitation totals define 2019 as close to normal for the Beklemeto area and moderately humid in the remaining sites. The year 2020 was normal in the Rozino and Devin sites and moderately dry in Plovdiv and Beklemeto (Fig. 5). In Plovdiv, 2021 was normal, moderately dry in Rozino and Devin, and dry in Beklemeto.

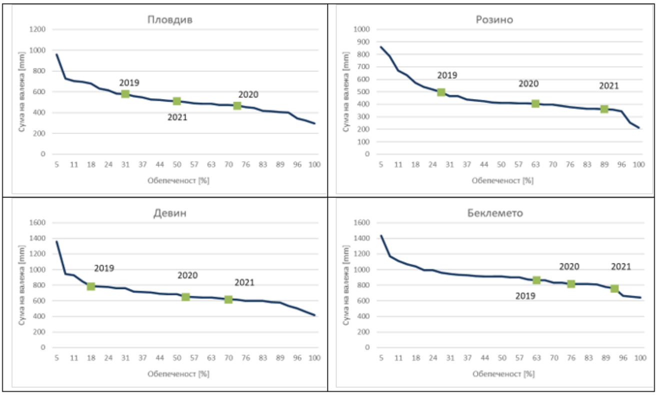

Figure 5. Characteristics of moisture conditions during the experimental years

4.1.3. Duration of the vegetation season

In 2019, the vegetation season in all considered regions was longer than normal by approximately 10 to 20 days. The extension of the period is due to the later termination of vegetation in autumn (Fig. 6). In 2020, the vegetation season was also longer than normal, while in 2021 it was close to normal in Devin and Beklemeto, and shorter in Rozino.

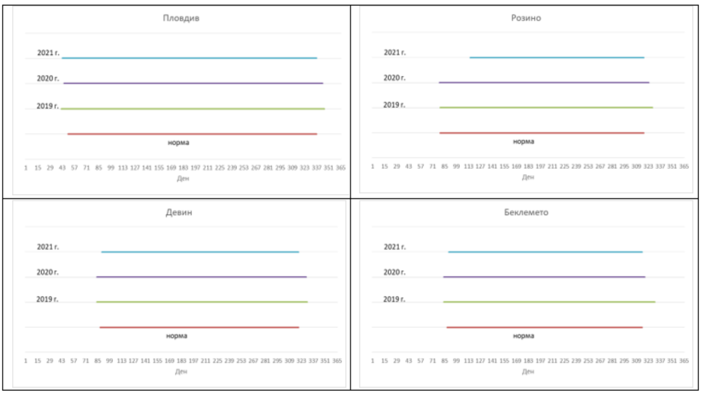

Figure 6. Duration of the vegetation season in the observed plots during the experimental years

The highest number of rainless periods in 2019 was recorded in Rozino—two periods in spring and one in summer. In 2020, again in Rozino, three periods were recorded, in late spring, summer, and early autumn. In 2021, prolonged summer and autumn droughts were recorded, with the exception of the Beklemeto area (Table 2).

Table 2. Duration of rainless periods during the experimental period

Location	2019	2019	2020	2020	2021	2021
Location	Duration (days)	Period	Duration (days)	Period	Duration (days)	Period
Plovdiv	33	18.08-19.09	16	5-20.05	23	4.07-25.08
Plovdiv	22	10.10-30.10	49	20.08-7.10	38	31.08-7.10
Rozino	26	14.03-8.04	18	4.05-21.05	47	3.07-18.08
Rozino	16	21.04-6.05	16	28.07-12.08	39	31.08-8.10
Rozino	47	4.08-19.09	34	5.09-8.10		
Devin	34	17.08-19.09	21	5-25.09	36	21.07-25.08
Beklemeto	38	17.08-23.09	21	5-25.09		

4.2. Study of species composition and basal cover of the grasslands

Site 1 – Plovdiv

The analysis of the data for the period 2019–2021 allows both the persistence of the initially introduced cultivated species and the degree of natural enrichment of the grassland with leguminous and forb components to be traced (Table 3).

Table 3. Description of species composition and basal cover of Site – Plovdiv

Site - Plovdiv	Site - Plovdiv	Site - Plovdiv	Site - Plovdiv
Year	**2019**	**2020**	**2021**
Total basal cover (%)	97	95	95
Average grass stand height (cm)	35	40	30
Number of species	9	15	15
Grasses			
1. Phleum pratense	3	2a	2a
2. Lolium perenne - BARRAGE	3	2a	2a
3. Festuca rubra rubra	2b	2a	2a
4. Lolium perenne – BARFLIP	2b	2a	2a
5. Poa pratensis	2b	1	1
Legumes			
6. Trifolium repens	+	+	+
7. Trifolium arvense		+	+
8. Medicago lupulina		+	+
Forbs			
9. Chondrilla juncea		r	r
10. Cichorium intybus	+	1	1
11. Convolvulus arvensis	+	2a	1
12. Galium verum		r	+
13. Hypochaeris radicata	+	2a	1
14. Linum bienne		+	+
15. Moenchia mantica	+	1	+

On the basis of the analysis, several clearly expressed trends in grassland development at the site can be distinguished.

1. Maintenance of high basal cover. Throughout the entire period, the grassland maintained a very high degree of cover—95–97%, with minimal presence of bare patches and high resistance to thinning. On the other hand, such cover limits erosion, reduces the risk of secondary weed infestation, and creates prerequisites for more efficient moisture use.

2. Increase in species diversity. The transition from 9 to 15 species is one of the most important characteristics of the observed period. An increase was observed both in leguminous vegetation and in forbs.
3. Stability of grass dominants.

Despite the entry of new species, grasses remained the leading group. This is a sign of good persistence of the initially established grass mixture and of successful adaptation of the cultivated species to local conditions.
4. Gradual increase in species diversity (Fig. 7). A transition towards a more diverse and more resilient structure under changing climatic conditions was observed.

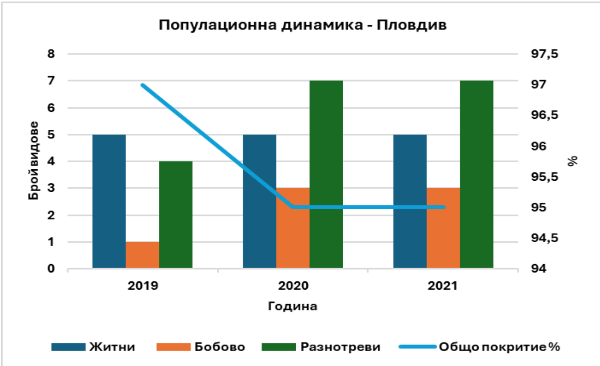

Figure 7. Dynamics of the main vegetation parameters

The obtained results allow the conclusion that the grassland at the site is characterized by high structural stability, good basal cover, and a pronounced tendency toward species enrichment. During the period 2019–2021, no degradation of the grassland area was observed; on the contrary, basal cover remained consistently high, grass species retained their role as the main component, legumes increased their participation, and the total number of species increased significantly and stabilized (Fig. 7). All this provides grounds for defining the site as a stable grassland with a favorable coenotic composition.

Site 2 – Rozino

The Rozino site is characterized by a grassland in which relatively high coenotic diversity of grasses, legumes, and forbs is observed. Data for the period 2019–2021 outline the trends in the development of the grass community, the stability of the main functional groups, and changes in the structural indicators (Table 4).

The analysis of the main structural indicators shows that the grassland at Site 2 (Rozino) is characterized by a relatively high, but dynamic, degree of basal cover (Table 4). In 2019, it was 87%, in 2020, it decreased to 85%, and in 2021, it increased to 90%. These fluctuations are not large, but they are indicative of ongoing internal coenotic changes and of a certain sensitivity of the grassland to environmental conditions. The decrease in basal cover in 2020 can be interpreted as the result of a temporary reduction in the density of some of the dominant components. Their subsequent increase in 2021 to 90% shows that the grassland not only recovered, but even improved its structure. This is a sign of good recovery capacity and relatively high ecological plasticity of the grass vegetation in this area.

Table 4. Description of species composition and basal cover of Site 2 – Rozino

Site 2 - Rozino	Site 2 - Rozino	Site 2 - Rozino	Site 2 - Rozino
Year	**2019**	**2020**	**2021**
Total basal cover (%)	87	85	90
Average grass stand height (cm)	40	30	45
Number of species	21	21	20
Grasses			
1. Agrostis capillaris	2a	3	2m
2. Anthoxanthum odoratum	1	+	1
3. Cynosurus echinatus		+	1
4. Danthonia alpina	+		
5. Festuca valesiaca	2a	+	1
6. Poa pratensis		+	2m
7. Nardus stricta	1	1	1
Legumes			
8. Chamaespartium sagittale	1	+	1
9. Genista depressa	+	+	1
10. Lotus corniculatus	+		+
11. Trifolium alpestre		1	+
Forbs			
12. Achillea millefolium	+	2a	2m
13. Asperula cynanchica	+	+	1
14. Centaurium erythraea		+	+
15. Galium verum	2a	+	1
16. Hieracium hoppeanum		+	
17. Linum bienne	+		
18. Plantago lanceolata	+		+
19. Potentilla argentea	+		+
20. Rumex acetosa	1	+	+
21. Rumex acetosella	+		
22. Scabiosa ochroleuca	1	+	+
23. Scleranthus perennis	+		
24. Silene frivaldskyana		+	
25. Silene roemeri	+	1	+
26. Stellaria graminea		+	
27. Teucrium chamaedrys	+	+	
28. Thymus jankae	+	1	+

During the study period, no sharp change in the number of species at the site was observed, and, together with the good basal cover, this confirms that no degradation processes were taking place during the analyzed period. Rather, a dynamic equilibrium was found, in which individual species changed their participation, but the grassland preserved its overall structure.

In Fig. 8, the data on basal cover and the ratio of plant groups in the grassland and their developmental trend are presented. The obtained results show that the grassland in the Rozino area is characterized by rich species diversity, relatively stable basal cover, and a clearly expressed participation of forbs. During the period 2019–2021, no degradation of the grass cover was established; rather, dynamic stabilization of the community was observed while preserving a more complex botanical structure.

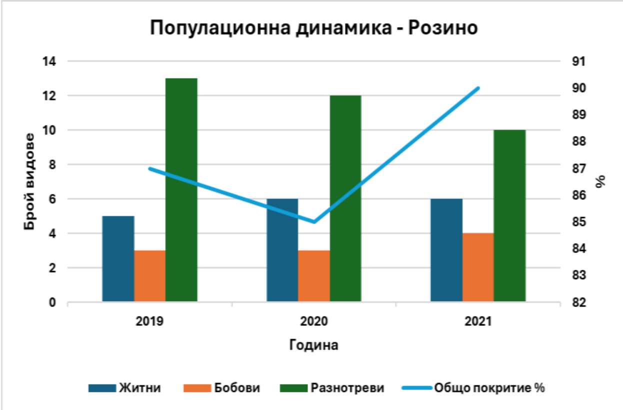

Figure 8. Dynamics of the main vegetation indicators

This characterizes the site as a valuable object for analysis with regard to productivity and the sustainable management of grassland coenoses.

Site 3 – Beklemeto

The Beklemeto area is characterized by a grassland with an exceptionally high degree of basal cover and rich species diversity. The data for the period 2019–2021 show a relatively stable participation of grasses and a rich set of leguminous and forb species, which gives the site a complex and ecologically stable structure (Table 5).

Table 5. Description of species composition and basal cover of Site 3 – Beklemeto

Site 3 - Beklemeto			
Year	2019	2020	2021
Total projective cover (%)	100	100	100
Average grass stand height (cm)	30	30	25
Number of species	32	32	33
Grasses			
1. Agrostis capillaris	2b	2a	+
2. Aira elegantissima	+	1	1
3. Anthoxanthum odoratum	+		+
4. Chrysopogon gryllus	2b	2a	2b
5. Festuca valesiaca	1	2a	2a
6. Koeleria macrantha		+	1
7. Phleum subulatum	+	1	+
8. Poa bulbosa	1	1	1
9.Sieglingia decumbens	+	+	+
10. Vulpia myurus	+	1	1
11.Deshampsia flexuosa	1	1	2b
Legumes			
12. Genista depressa	+		+
13. Lotus angustissimus		+	
14. Medicago lupulina	+		+
15. Trifolium arvense		+	+
16. Trifolium campestre		+	+
17. Lotus corniculatus	+	+	+
Acid grasses			
18. Luzula campestris	1	+	+
Forbs			
19.Achillea millefolium	+	1	+
20. Anthemis austriaca		+	+
21. Asperula cynanchica	+		
22. Chondrilla juncea	+	1	+
23. Dianthus armeria		+	+
24. Eryngium campestre	+	r	
25. Euphorbia cyparissias	+	1	1
26. Galium tenuissimum		+	
27. Galium verum	+		
28. Hieracium hoppeanum	2b	1	1
29. Hypericum olympicum	+	1	
31. Hypochaeris radicata	+		+
32. Jasione heldreichii	+		
33. Logfia arvensis		+	+
34. Petrorhagia prolifera		+	+
35. Pinus sp.	r		
36. Plantago lanceolata	+		+
37. Plantago subulata	+	2a	1
38. Rumex acetosella	+		
39. Sanguisorba minor		+	+
40. Scabiosa ochroleuca	+	1	1
41. Scleranthus perennis	+	1	1
42. Thymus jankae	1	1	1
43. Verbascum phlomoides	+	1	1

The analysis of data on species composition and grass cover at the site shows an exceptionally high and constant degree of basal cover—100% throughout the entire observation period (Fig. 9). Species diversity is very high and stable. There is a well-developed grass component with stable dominance, which provides a good structural basis for the grassland. Legumes show moderate but stable participation with a tendency toward enrichment.

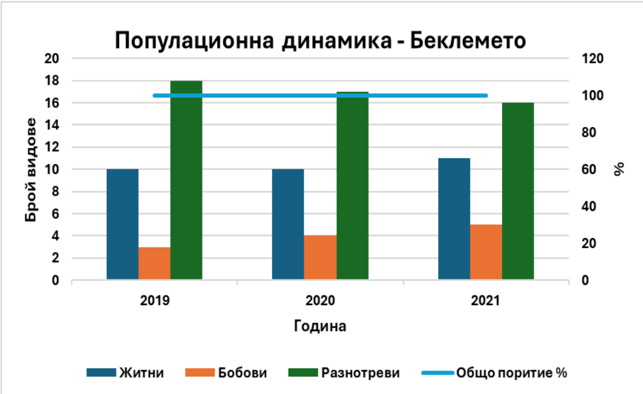

Figure 9. Dynamics of the main vegetation indicators

Site 4 – Devin

The site near Devin is characterized by a high degree of basal cover, good species diversity, and clearly expressed participation of the individual groups of grasses, legumes, and forbs (Table 6).

The analysis of the data shows that basal cover in 2019 was 95%, in 2020 it decreased to 90%, and in 2021 it again reached 95%. This is indicative of stable good grass cover throughout the study period. The average height of the grass stand varied within the range of 20–25 cm, with a slight increase in 2020 and a return in 2021 (Table 6). This indicates a relatively stable vertical structure of the grass cover. The number of species also varied, increasing from 18 in 2019 to 19 in 2020 and 2021, and likewise showing stability with respect to the observed indicator throughout the entire study period. Again, as in the other sites, participation of the three main coenotic groups was observed.

Table 6. Description of species composition and basal cover of Site 4 – Devin

Site 4 - Devin	**Site 4 - Devin**	**Site 4 - Devin**	**Site 4 - Devin**
Year	**2019**	**2020**	**2021**
Total basal cover (%)	95	90	95
Average grass stand height (cm)	20	25	20
Number of species	18	19	19
Grasses			
1.Agrostis capillaris	+	+	+
2.Anthoxanthum odoratum	1	1	+
3.Deschampsia caespitosa	+	1	+
4.Festuca pratensis	1	2b	1
5.Lolium perenne	+	+	+
6.Poa pratensis	2a	1	+
7.Nardus Stricta	1	1	+
Legumes			
8.Lotus corniculatus		+	+
9.Trifolium hybridum	1	+	+
10. Trifolium patens	3	1	+
11. Trifolium pratense	1	+	1
Forbs			
12. Achillea millefolium		+	+
13. Hypochaeris radicata	+	+	+
14. Mentha longifolia	+	+	+
15. Moenchia mantica	2a	2a	1
16. Myosotis scorpioides	+		
17. Plantago lanceolata	+	1	+
18. Prunella laciniata	+	+	+
19. Ranunculus acris	1	+	+
20. Rumex acetosa	+	+	+

Based on the performed analysis of species composition and grass cover at the site near Devin, the following summary can be made:

During the three-year period, the grassland showed good structural stability, maintaining a high degree of basal cover (Fig. 10). The number of species did not vary widely, which is an indicator of a good species balance in the grass cover. The participation of grass species was leading, and they dominated the structure of the grass community throughout the entire study period, regardless of fluctuations in the participation of some species. Legumes were less well represented, but had a clearly expressed presence and an important influence on the quality of the grassland. Annual intraspecific dynamics were observed with respect to the participation of the individual units. Forb species complemented the species diversity at the site.

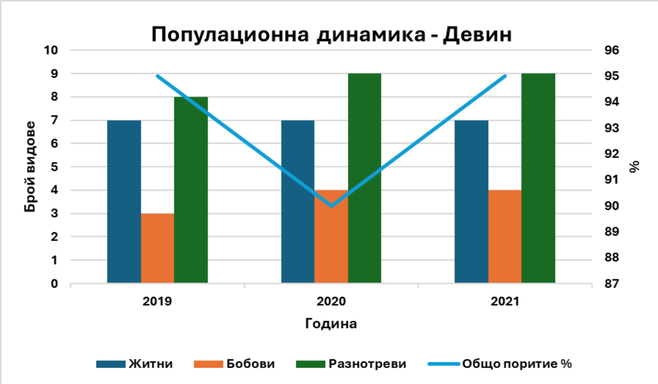

Figure 10. Dynamics of the main vegetation indicators

The comparative analysis carried out on the pastures in the four studied sites (Plovdiv, Rozino, Beklemeto, and Devin), with emphasis on the potential for carbon dioxide accumulation, shows that differences in species composition, basal cover, grass stand height, and observed species diversity are directly related not only to the ecological sustainability of the respective pasture communities, but also to their potential for carbon dioxide fixation and carbon accumulation.

One of the most important indicators related to carbon accumulation potential is the degree of vegetation cover on the soil surface. The better the basal cover and the denser the vegetation, the larger the photosynthetically active area and the more efficiently carbon is absorbed.

On this basis, considerable differences are observed among the four sites:

• Site 3 – Beklemeto stands out with 100% basal cover throughout the entire study period;
• Site 1 – Plovdiv maintains very high basal cover—95–97%;
• Site 4 – Devin is also characterized by high values of this indicator—90–95%;
• Site 2 – Rozino shows relatively lower values—85–90%.

As a result of the analysis of the potential possibility for CO_2 accumulation, the conclusion may be drawn that Beklemeto has the most favorable structural prerequisites for intensive photosynthetic activity, since the plant cover is continuous, without bare patches, and with potentially maximal utilization of the area. This contributes to intensive accumulation of aboveground biomass and roots, which is of great importance for the accumulation of organic carbon in the soil.

Species diversity is the second key factor determining the carbon potential of pasture phytocoenoses. Pastures with higher floristic heterogeneity usually use the ecological resources of the habitat more fully, as the different species occupy different ecological niches, have different root system depths, different growth rates, and different seasonal activity. This leads to fuller utilization of light, moisture, and nutrients and ultimately to higher overall biomass productivity.

The comparison among the sites shows clear differentiation according to this indicator:

- Beklemeto: 32–33 species
- Rozino: 20–21 species
- Devin: 18–19 species
- Plovdiv: 9–15 species

The highest species diversity was established at Site 3 – Beklemeto, which defines it as the richest phytocoenological community. This is particularly important from the point of view of carbon accumulation, since high biodiversity implies the presence of functional complementarity among the individual plant components. Some species form dense aboveground biomass, others develop a deeper or more branched root system, while still others have a longer period of active vegetation.

Rozino also stands out with high species richness, though to a lesser degree than Beklemeto. However, a large part of the species diversity there is due to various herbaceous species, which do not always have an equal contribution to the formation of productive aboveground mass. Therefore, the large number of species at this site is undoubtedly positive from an ecological point of view, but it does not automatically mean the highest capacity for carbon accumulation.

Devin occupies an intermediate position, combining relatively good species diversity with a more compact and balanced structure.

Plovdiv, although at the beginning of the study it had the smallest number of species, showed a clear tendency toward floristic enrichment. This is particularly important because it means that the carbon potential of the pastures is not static, but increases with the development of a more complex phytocoenotic organization.

Not only the number of species, but also the structure of the species composition by functional groups is of decisive importance for the capacity of grass communities to accumulate carbon dioxide. The accumulated quantities depend on the proportions among grasses, legumes, forbs, and, in some cases, acid grasses.

Grass species are the main structural and productive component of pastures. They constitute a substantial part of the aboveground biomass, form a well-developed root system, and provide a high degree of basal cover. Therefore, they are of primary importance for CO_2 accumulation and the subsequent build-up of organic carbon in the soil.

The grass group is best developed in Plovdiv, where the pastures were initially formed by a grass mixture of cultivated species with a strongly expressed dominant role. It is followed by the Beklemeto site, where grasses are abundant in terms of species composition, and the group is well differentiated. In the Devin area, grasses are a stable and structurally important element, and in Rozino, the group also has a stable presence, but the forb component is very strongly expressed.

Based on the analysis, Plovdiv and Beklemeto show the most favorable grass-component structure with maximum potential for carbon accumulation.

Leguminous crops are of particular importance for carbon accumulation, although they often do not dominate quantitatively. Their role is expressed mainly in the biological fixation of atmospheric nitrogen, improvement of nitrogen nutrition in pastures, improvement of the productivity of the accompanying grass species, improvement of the quality of plant biomass, and indirect support of the accumulation of organic matter in the soil. From this point of view, pastures in which the leguminous component is stably represented, without necessarily being quantitatively dominant, are particularly favorable.

From the point of view of the combined effect between grasses as a basis and the participation of legumes, Plovdiv has particularly good prerequisites for efficient CO_2 uptake, since irrigation conditions and the artificially created grass mixture probably support both productivity and the stability of the leguminous component.

Forbs do not have a unidirectional role with regard to carbon accumulation. On the one hand, they increase biodiversity, improve the structural complexity of pastures, and can support fuller use of ecological resources. On the other hand, excessive participation of plants from this group may reduce the relative share of highly productive grass species. From this point of view, Beklemeto and Rozino stand out with the strongest development of the forb component. Devin has a moderate participation in the group, whereas Plovdiv shows controlled and gradual inclusion of forbs without disturbing the grass-based foundation. Consequently, forbs contribute to the greatest extent to the ecological sustainability and species diversity of the grasslands and, indirectly, to their capacity to accumulate carbon in the long term.

The accumulation of carbon dioxide is inseparably linked with the accumulation of biomass. In pasture ecosystems, a significant part of the fixed carbon is stored not only in the aboveground plant mass, but also in the root system and in soil organic matter. Therefore, in assessing carbon potential, not only the height of the pasture vegetation should be taken into account, but also its density and functional structure.

According to this indicator, Beklemeto shows the most favorable combination of full cover, exceptionally high species diversity, and a stable grass component, which implies great potential for the formation of both aboveground and belowground biomass. Plovdiv stands out with very high basal cover and a clearly expressed productive basis formed by cultivated grass species, which implies a high intensity of carbon fixation. The site at Devin also has a good and balanced structure of grass cover, which gives it a stable, though probably more moderate, capacity for carbon accumulation. Rozino has good species diversity, but lower basal cover, which, combined with the strong participation of forbs, probably limits its potential for the studied indicator compared with the other sites.

It is important to emphasize that mountain and less disturbed grass communities, such as those in Beklemeto and to some extent in Devin, often accumulate a significant part of the carbon in the soil thanks to their persistent root mass and the relatively slow mineralization of organic matter. This increases their value as long-term carbon sinks.

4.3. Determination of carbon content in the soil

The summarized analysis of the results for the period 2019–2021 (Fig. 11) shows a clearly expressed seasonal dependence, with the highest values of organic carbon being established in spring and autumn, when climatic conditions are most favorable for photosynthetic activity and biomass accumulation.

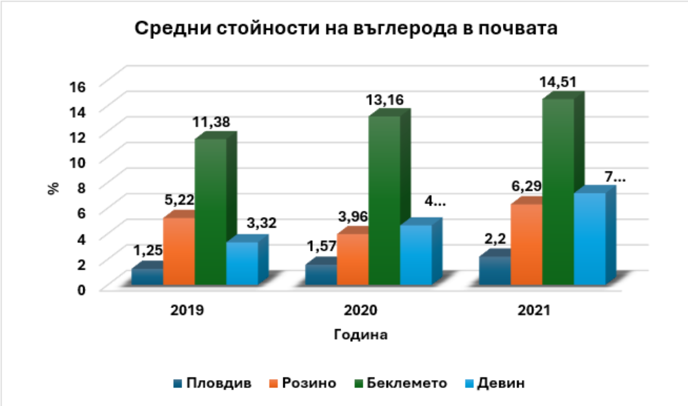

Figure 11. Values of soil organic carbon, 2019–2021

The highest carbon accumulation potential was established at the Beklemeto site, which is due to the specific soil-climatic conditions, the high content of organic matter, and the well-developed grass vegetation. The Devin and Rozino sites also demonstrate good potential, with Devin standing out for the stability of the indicators and Rozino for more pronounced dynamics, but with good recovery capacity.

4.4. Determination of CO_2 uptake activity per unit area

To trace the dynamics of CO_2 uptake over the years, the results for each year were averaged and are presented in Fig. 12. Variability in this indicator among the different sites is observed over the years, but 2021 stands out with the highest values in all sites compared with 2019 and 2020, while the lowest activity was recorded in 2020, except Beklemeto, where photosynthetic activity remained relatively high. Lowland grasslands show a high potential for carbon uptake, but they are strongly dependent on moisture and temperature; therefore, such strongly expressed seasonal fluctuations in photosynthetic activity are observed. This is confirmed by the results obtained at the Plovdiv site, where the highest average values were recorded in 2021 (3.559 mg $CO_2/m^2/s$), and the lowest in 2020 (2.611 mg $CO_2/m^2/s$). This again confirms what has been obtained by other authors, namely that climatic fluctuations have a serious impact on the above-mentioned processes (Austin et al., 2004; Liu et al., 2016; Merbold et al., 2009; Nielsen and Ball, 2015). In the Rozino site, a typical foothill area, stable photosynthetic activity and CO_2 uptake are observed, with close values between the years.

The highest values were also recorded in 2021 (2.171 mg $CO_2/m^2/s$), and the lowest in 2020 (1.761 mg $CO_2/m^2/s$). CO_2 uptake values are close and vary within a narrow range, with slight interannual fluctuations. At the Beklemeto site, the highest value was recorded in 2021 (4.302 mg $CO_2/m^2/s$), and the lowest (1.115 mg $CO_2/m^2/s$) in 2019.

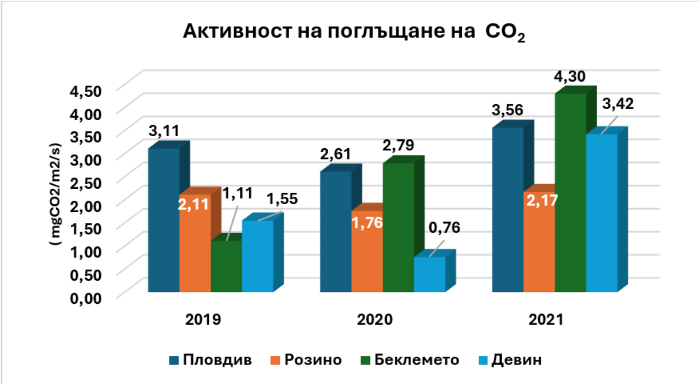

Figure 12. Mean values of CO_2 assimilated by grass vegetation

The results confirm that mountain grasslands are extremely sensitive to climatic conditions, but under optimal parameters, they reach maximum photosynthetic efficiency. At the Devin site, the highest values were again recorded in 2021 (3.422 mg $CO_2/m^2/s$), and the lowest (0.763 mg $CO_2/m^2/s$) in 2020.

The data from the three-year period show a strong dependence of the intensity of CO_2 uptake on meteorological conditions and altitude. The analysis reveals clearly expressed variations in CO_2 uptake among the sites (Fig. 12).

4.5. Determination of the diurnal variation of CO_2 fluxes

The diurnal changes in CO_2 fluxes during the three experimental years show both seasonal and annual fluctuations (Fig. 13). In 2019, a negative balance of CO_2 fluxes was recorded due to the higher values of uptake compared with those of CO_2 release from the vegetation at all sites. In 2020, due to the highly unfavorable climatic conditions, the opposite tendency was observed, and a positive balance of CO_2 fluxes was recorded. In 2021, a difference was observed among the sites. Plovdiv and Beklemeto showed a negative balance, while Rozino and Devin showed a positive one, since higher CO_2 release was recorded in them.

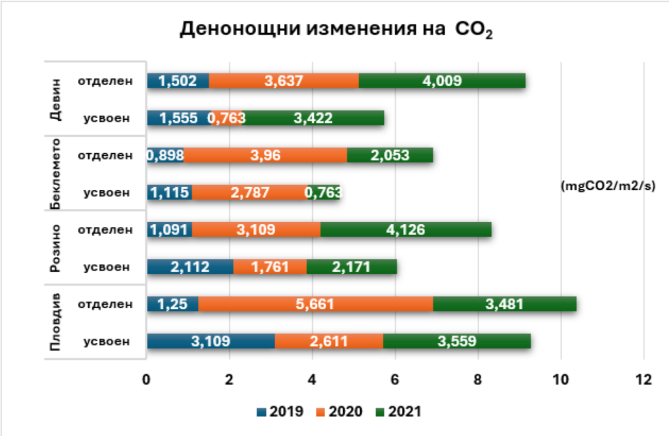

Figure 13. Mean annual diurnal changes in CO_2 fluxes from grasslands during the period 2019–2021

4.6. Determination of the diurnal fluctuations of soil respiration

The summarized mean annual data on the diurnal fluctuations in soil respiration in the four studied areas—Plovdiv, Rozino, Beklemeto, and Devin—for the period 2019–2021 are presented in Figure 14. The indicator soil respiration reflects the intensity of microbial and root activity, which strongly depend on climatic and ecological factors.

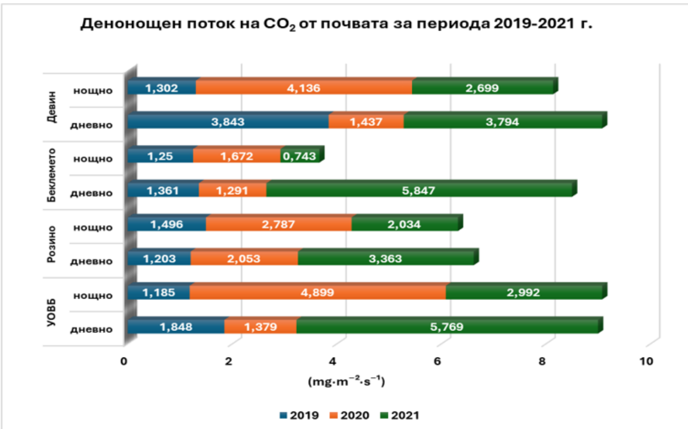

Figure 14. Diurnal CO_2 flux from the soil during the period 2019–2021

Over the years, a tendency toward increasing soil CO_2 emissions was observed. In 2019, the values of the indicator were relatively low and stable both during the day and at night, which indicates moderate and uniform biological activity of the soil. In the second year (2020), increased nocturnal respiration was recorded at all sites, especially in Plovdiv and Devin, where night values exceeded daytime values. In

2021, a sharp increase in daytime respiration was observed in Plovdiv and Beklemeto, which is an indicator of enhanced microbial activity and/or accelerated decomposition of organic matter, probably as a result of higher daytime temperatures. In contrast, night values decreased, which emphasizes the influence of temperature on respiratory processes. Rozino shows the most moderate dynamics, with a gradual increase in values over the years and a relatively small difference between day and night, which suggests stable microclimatic conditions. Devin stands out with the highest nocturnal activity in 2020, which may be due to the specific hydrothermal regime of the area.

In conclusion, it can be stated that soil respiration is a major indicator of biological activity. The obtained results show a clearly expressed seasonal and annual variability of soil respiration, with temperature, moisture, vegetation type, and organic matter content acting as key factors. This statement is fully confirmed by results obtained in a number of other studies showing that temperature and precipitation strongly influence soil respiration.

4.7. Study of the capacity for carbon uptake and storage in natural and artificial grasslands

The study covers the period 2019–2021 and includes four locations: Plovdiv, Rozino, Beklemeto, and Devin, arranged along a clearly expressed altitudinal gradient and representing different types of grasslands.

The results of the regression analyses prove that CO_2 uptake is highly sensitive to temperature, with a tendency toward a decrease in net uptake as temperature rises. Moisture exhibits a dual effect—stimulating at moderate levels and limiting under waterlogging. The data on soil organic carbon reveal clear spatial differentiation among the locations. The Beklemeto site records the highest and most stable values throughout the entire period, with the average annual concentrations of organic carbon increasing from 11.38% (2019) to 14.51% (2021). The Rozino and Devin sites are characterized by moderate values and a clearly expressed increasing trend, especially in 2021. The Plovdiv site demonstrates the lowest values, but also shows a gradual increase over the period.

These results clearly prove that geographical location, grassland type, and the manner of use exert a strong influence on long-term carbon storage in the soil.

4.8. Establishing the relationship between climate, species composition, and carbon accumulation in plants and soil

The obtained results clearly show that climatic factors, namely temperature and precipitation, are of decisive importance for the intensity of carbon exchange in the ecosystem. Temperature exerts a dual effect: on the one hand, it stimulates photosynthetic activity and the growth of grass vegetation up to optimal values, and on the other hand, high temperatures above the optimum intensify respiratory processes and the mineralization of organic matter. This is particularly pronounced in the low-mountain and lowland sites, where higher summer temperatures lead to lower efficiency of carbon accumulation in the soil.

Precipitation appears as the second key climatic factor whose influence is closely linked to the carbon cycle. In mountain areas, precipitation ensures stable soil moisture both for the good development of grass vegetation and for the stabilization of organic carbon. At the same time, the results indicate the presence of a threshold response, in which excessive precipitation does not lead to a proportional increase in carbon uptake; on the contrary, the process of CO_2 uptake and accumulation is inhibited.

The species composition of grass communities manifests itself not so much as a direct determining factor of the quantity of carbon assimilated or accumulated, but as a factor determining the resilience of the system to climatic fluctuations. Increased species and functional diversity, especially the participation of leguminous species, contributes to a more even uptake of carbon during the vegetation period and to more stable accumulation of organic matter in the soil.

This effect is clearly expressed at the Beklemeto site, where high species richness compensates for interannual differences in climatic conditions. Conversely, at the Plovdiv site, relatively low species diversity limits the ability of the ecosystem to mitigate extreme temperature and moisture conditions, which is reflected in lower soil organic carbon content.

Mountain areas are distinguished by a higher potential for long-term carbon storage, which may be explained by lower temperatures, slower mineralization, and more stable soil conditions. This corresponds to the results distinguishing ecosystems with high carbon reserves from those with high, but less stable, productivity.

The combination of climatic, biotic, and soil data shows that the carbon cycle in grassland ecosystems is the result of complex interactions among factors with different temporal and spatial dynamics. Climate sets the limit, vegetation determines the efficiency of the carbon flux, and soil accumulates it; this stability depends on the relationship among the individual factors.

In the context of climate change, these interactions acquire particular importance. The results of the present study underline the need for an integrated approach in the management of grassland ecosystems, aimed at maintaining plant diversity, optimizing use, and conserving soil carbon stocks, which is a key element of strategies for mitigating climate change.

4.9. Statistical processing of the results

For the statistical processing, data from the dynamics and activity of CO_2 uptake and soil respiration of grasslands during the period 2019–2021 were used. Climatic factors (temperature, moisture) and species composition were analyzed. The analyses aimed to show the relationship among the factors, as well as which of them had the greatest weight in CO_2 accumulation, photosynthetic activity, and species composition over the years. The results of the analyses make it possible to assess the resilience of grassland ecosystems under climate change conditions.

4.9.1. Regression model between factors X_1 – species composition and X_2 – temperature on CO_2 uptake

For the three years of the study, the regression models show a strong linear relationship between factors X_1 and X_2 (species composition and temperature) and the dependent variable Y (CO_2 uptake). The analysis of the individual regression coefficients proves that increasing species composition exerts a positive and statistically significant influence on CO_2 uptake. Temperature exerts a stronger negative influence on CO_2 uptake, and its increase leads to a reduction in uptake intensity and an increase in photorespiration (Fig. 15).

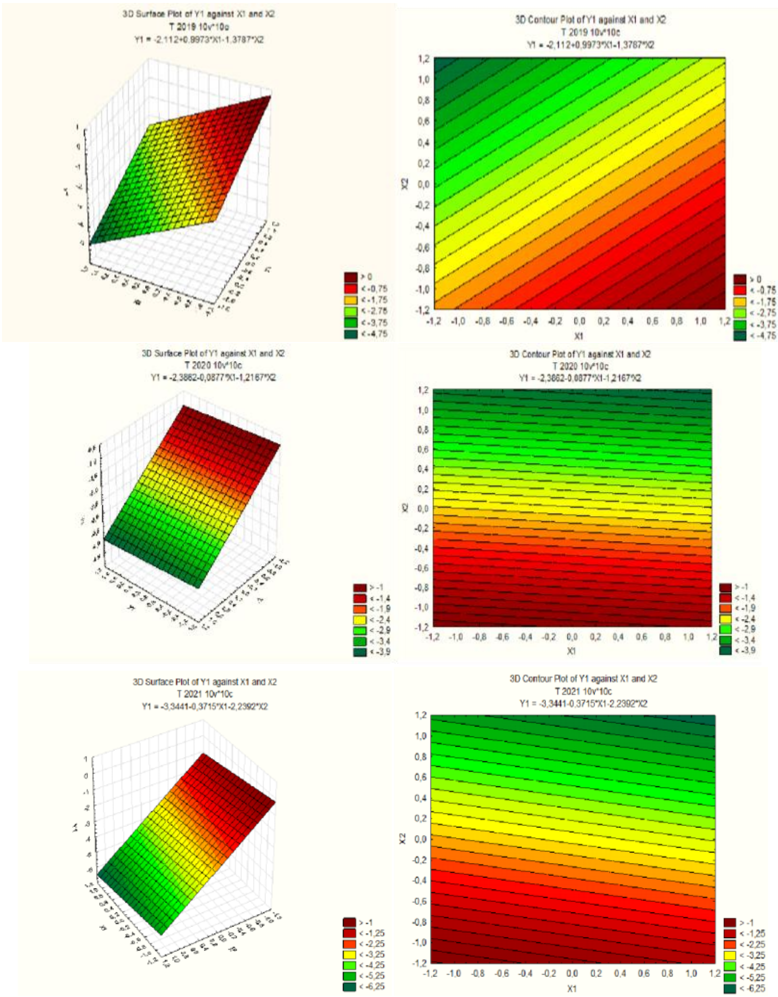

Figure 15. Three-dimensional and contour graphs reflecting the influence of species composition and temperature on CO_2 uptake for 2019 (a), 2020 (b), and 2021 (c)

4.9.2. Regression model between factors X_1 – species composition and X_2 – soil moisture on CO_2 uptake

For the study period (2019–2021), multiple linear regression models were developed in which the dependent variable Y—CO_2 uptake by the plant cover—was analyzed in relation to two main ecological factors: X_1—species composition and X_2—soil moisture. By applying regression analysis, the influence of these factors on the intensity of CO_2 uptake was evaluated, and the main relationships between them were established. The analysis proves that soil moisture (X_2) has a weak positive influence on CO_2 uptake, whereas species composition (X_1) exerts a weak negative influence on the dependent variable (Y) (Fig. 16).

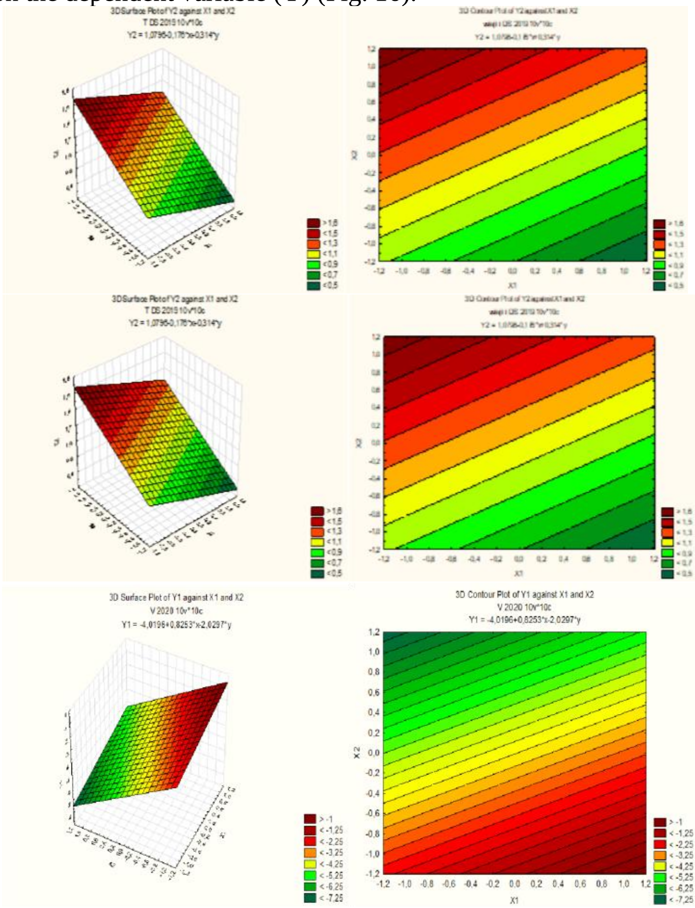

Figure 16. Three-dimensional and contour graphs reflecting the influence of species composition and moisture on CO_2 uptake for 2019 (a), 2020 (b), and 2021 (c)

4.9.3. Regression analysis between factors X_1 – soil moisture and X_2 – air temperature on the diurnal variation of CO_2 from grasslands

From the obtained data on the diurnal changes of CO_2 for the study period, it was established that factor X_1 has a greater negative influence on CO_2 flux during the light part of the day, as with increasing values, a decrease in CO_2 is observed. Air temperature (X_2) exerts a stronger positive influence on the variable. With increasing values of factor X_2, the CO_2 flux also increases, provided that the remaining factors remain constant.

The analysis proves that factors X_1 and X_2 exert different effects on variable Y during the different parts of the day (Fig. 17).

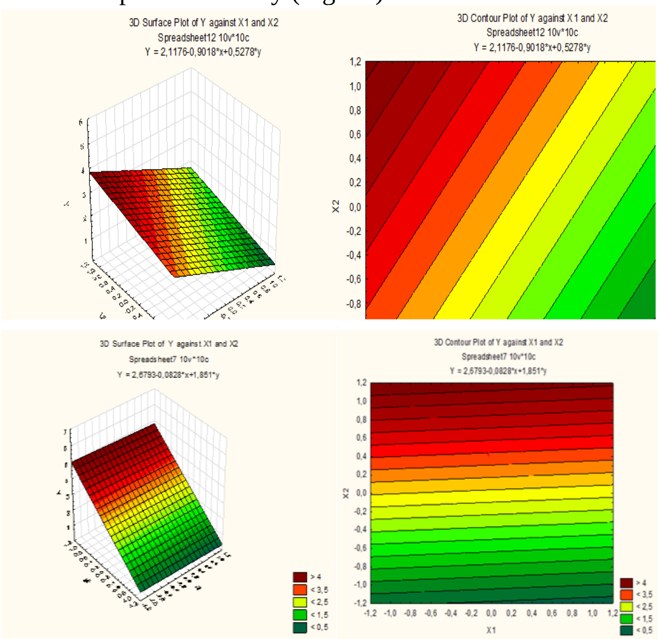

Figure 17. Three-dimensional and contour graphs reflecting the influence of soil moisture and air temperature on the diurnal variation of CO_2 from grasslands

4.9.4. Regression analysis between factors X_1 – soil moisture and X_2 – air temperature on soil respiration

The regression model shows the influence of the two factors on soil respiration during the day. Factor X_2 has the strongest positive influence on soil respiration during the day. As the values of X_2 increase, a clearly expressed increase in the values of Y is observed. Soil moisture exerts a weak negative influence on the dependent variable Y. This defines factor X_2 (air temperature) as dominant over soil respiration during the day (Fig. 18).

In conclusion, the analysis of the data from 2019–2021 proves that CO_2 uptake by grass vegetation is the result of dynamic interaction among climatic conditions, species composition, and geographical location. Whereas under moderate and favorable conditions, moisture stimulates the accumulation of the carbon balance, under extreme or unfavorable conditions, it causes the opposite effect.

From the summarized analysis of the data, the regression models, and the graphs, the dominant role of temperature and moisture as key climatic factors in CO_2 uptake by grass vegetation was established.

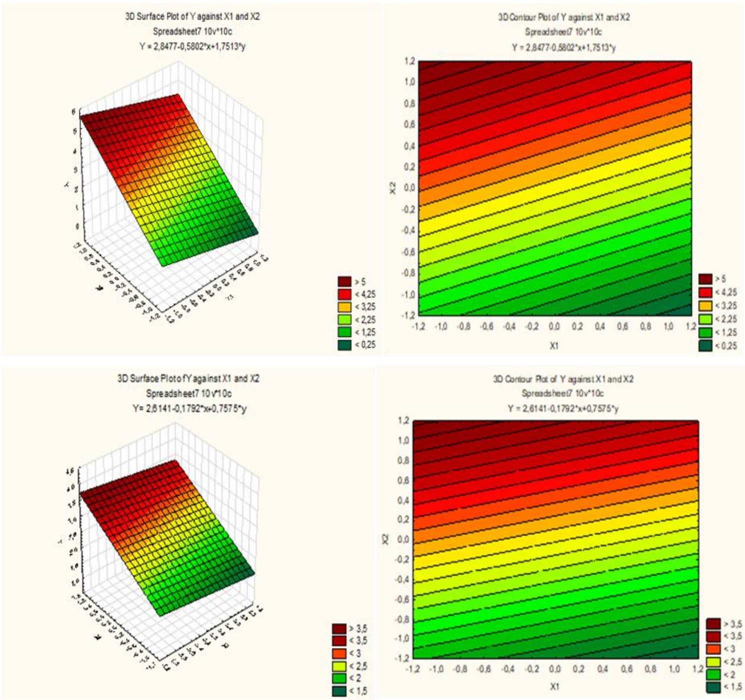

Figure 18. Three-dimensional and contour graphs reflecting the influence of soil moisture and air temperature on soil respiration

Within all regression analyses, the temperature factor manifests itself as a statistically significant indicator, and in most cases, its effect is negative. As temperature rises, CO_2 uptake decreases, which may be interpreted as a result of temperature stress (accelerated photorespiration and low photosynthetic activity). These results prove that temperature acts not only as a regulating factor, but also as a limiting factor, especially at values exceeding the optimum for the physiological processes of plants.

Soil moisture also shows a complex and nonlinear nature of influence compared with temperature. The data clearly outline two functional regimes:

Stimulating regime – at moderate moisture levels, an increase in CO_2 uptake is observed, associated with improved water balance and active gas exchange.

Stress regime – at very high and excessively low moisture values, the effect becomes negative.

The combined analysis shows that moisture cannot compensate for the negative effect of high temperature, and in certain cases even intensifies it. In scenarios with simultaneously high temperature and high moisture, the lowest values of grassland ecosystem performance are observed, which is clearly manifested in the regression surfaces as "depressed zones". This interaction underlines the presence of a climatic threshold beyond which grass communities shift from a regime of adaptation to a regime of stress.

Species composition does not manifest itself as a universally dominant predictor, but it has an important role. The data show that its influence is associated with the fact that, as the number of species increases, CO_2 uptake also increases. This is due to changes in species composition (a greater percentage of legumes), spatial location, and more favorable climatic conditions. However, at altitudes above 1600 m, CO_2 uptake begins to decrease. This is due to lower temperatures, slower plant metabolism, and a shorter vegetation period. This is clearly visualized in the regression surfaces, where it defines the "framework" of the process, but not its dynamics.

The analyses carried out prove that the limiting factors in CO_2 uptake are temperature and moisture, whereas the species composition of the vegetation stimulates uptake. This analysis underlines the high sensitivity of grass communities to climate change.

5. CONCLUSIONS

1. Agroclimatic conditions show a clearly expressed difference between lowland and mountain areas. In the lowland areas (Plovdiv and Rozino), mean annual precipitation is 638–654 mm, temperature sums reach 4350–4500°C, and the vegetation period is 286–288 days, whereas in the mountain areas (Beklemeto and Devin) precipitation is significantly higher (870–1020 mm), the vegetation period is shorter (about 232 days), and temperature sums are considerably lower (2727°C).
2. During the study period, positive temperature deviations up to and above 2–3°C were established, as well as uneven distribution of precipitation, which led to an extension of the vegetation season by 10–20 days and the formation of prolonged rainless periods, reaching up to 47–49 days in certain areas.
3. The analysis of climatic factors shows substantial spatial differentiation in the conditions for vegetation development, with the lowland areas being characterized by a higher risk of drought (summer precipitation minima and frequent dry periods), whereas the mountain areas are distinguished by a more favorable water regime, higher precipitation, and more stable ecological conditions.
4. A tendency toward increase and stabilization of species diversity was established, expressed in the development of more complex, resilient, and self-regulating semi-natural grasslands.

5. Grasses dominate in all sites and form the basis of the grass stand, legumes have a key role in nitrogen enrichment and productivity, and forbs contribute to biodiversity and ecological plasticity.
6. The highest ecological resilience, species diversity, and structural completeness were established at the Beklemeto site, whereas the remaining sites show balanced but less differentiated phytocoenoses.
7. The potential for CO_2 accumulation is directly dependent on basal cover, species diversity, and functional structure, being highest in grasslands with dense cover and a well-developed grass-legume component.
8. The content of organic carbon in the soil is highest in mountain areas. It shows seasonal dynamics determined by climatic conditions, with grassland coenoses demonstrating a high capacity for carbon accumulation and ecological resilience.
9. The content of soil organic carbon during the period 2019–2021 varied significantly by seasons and by sites. The highest values were observed in spring and autumn, with Beklemeto showing the highest accumulation potential, followed by Devin and Rozino, whereas limited accumulation was recorded at the Plovdiv site due to unfavorable climatic conditions.
10. High values of photosynthetic activity were established in May and October, and a significant decrease occurred in July. This clearly shows that CO_2 uptake depends to a great extent on temperature and soil moisture supply. The highest values of the indicator were recorded in 2021, and the lowest in 2020, which proves the strong influence of climatic conditions on the carbon balance.
11. The diurnal balance of CO_2 fluxes shows dominance of uptake during the light part of the day, whereas release predominates at night. Seasonal and local climatic factors determine the transitions between uptake and release, proving the complex interaction between the processes of photosynthesis and respiration.
12. Soil respiration is a key component of the carbon cycle, with clearly expressed diurnal and seasonal dynamics. The intensity of the process is strongly influenced by temperature, moisture, and organic matter content and, together with photosynthesis, determines the capacity of phytocoenoses to accumulate organic carbon.
13. The results of the regression analysis confirm the dominant role of meteorological conditions in determining the intensity of carbon uptake by plants. In contrast, carbon stocks in the soil are formed under the influence of both climatic and ecological conditions.
14. A clear difference was established between phytocoenoses with high instantaneous productivity and those with higher potential for long-term carbon storage. Mountain grass communities play the role of stable carbon reservoirs, whereas lowland and low-mountain systems are more sensitive to climatic changes.
15. Maintaining high plant diversity and sustainable management of grasslands are key conditions for optimizing the carbon balance and for increasing the adaptive capacity of phytocoenoses under climate change conditions.

6. SCIENTIFIC AND THEORETICAL CONTRIBUTIONS

1. A concept was developed for the spatial differentiation of agroclimatic conditions depending on altitude, and their influence on the duration of the vegetation period, temperature sums, and precipitation regime was demonstrated.
2. Theoretical understanding of the impact of climate change on grasslands was enriched by establishing trends toward rising temperatures, extension of the vegetation period, and increasing frequency of droughts.
3. Regularities were established in the spatial organization and structural-functional differentiation of grassland phytocoenoses depending on climatic factors.
4. The relationship between species diversity, the functional structure of plant communities, and their ecological resilience and self-regulation was demonstrated.
5. The role of the main functional plant groups (grasses, legumes, and forbs) in building productive and resilient grassland ecosystems was shown.
6. Theoretical knowledge of carbon-cycle processes in grassland coenoses was expanded through analysis of the relationship among photosynthesis, soil respiration, and carbon accumulation.
7. Relationships between climatic factors and the intensity of carbon exchange were established, and the leading role of temperature and moisture was demonstrated.
8. The distinction between grasslands with high instantaneous productivity and those with high potential for long-term carbon storage was substantiated.
9. The concept of mountain grass communities as stable carbon reservoirs in the context of global climate change was further developed.

7. SCIENTIFIC AND APPLIED CONTRIBUTIONS

1. A comprehensive agroclimatic assessment of areas at different altitudes was carried out, which can be used in planning and land-use management.
2. The drought risk in lowland areas was assessed, and zones with a more favorable water regime were identified, which has practical significance for the adaptive management of grasslands.
3. An approach was proposed for assessing the ecological resilience of grassland ecosystems based on species diversity, structural characteristics, and climatic conditions.
4. The potential of different grass communities for CO_2 accumulation was quantitatively evaluated, making it possible to use this information in the development of various measures for regulating climate change.
5. The seasonal dynamics of soil organic carbon were established, which can be used in the monitoring and assessment of soil fertility and carbon balance.
6. The influence of climatic factors on photosynthetic activity and soil respiration was demonstrated, which can be used in the development of sustainable ecological practices.
7. A regression model was developed for evaluating the influence of climatic and ecological factors on carbon exchange in grass communities.

8. Phytocoenoses with high potential for long-term carbon storage, suitable for various practices related to restoring the carbon balance, were identified.
9. The importance of maintaining high biodiversity and sustainable management of grasslands for optimizing the carbon balance was established.
10. The obtained results can be used in the development of strategies for adaptation to climate change and for the sustainable management of agroecosystems.