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Legumes in natural post-fire successions of forest meadows and pastures in Northern Bulgaria

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Abstract: We have witnessed a drastic increase in global fires in recent years that are strongly linked to climate change. On the other hand, the prescribed fires are applied in agricultural practice; however, their effectiveness is controversial. It is important to study the effects of both types of fires – wild and prescribed to predict their impact on biota. In this study, four different locations in Central Norther Bulgaria that recovered after the fire were studied concerning the biodiversity and abundance of the first colonizer species. Two generations from the locations were studied and demonstrated that the legume plants are dominant. The abundant species and their morphological and physiological characteristics are described. The annual species like *Bituminaria bituminosa*, *Vicia striata*, and *Medicago minima* propagated with seeds and perennial rhizome *Lathyrus pratensis* and *Trifolium medium* show the best potential to resprout after the fire events. Presented results will help responsible authorities of our county to take adequate actions for burned areas recovery. The directions for future studies are highlighted.

Keywords: post-fire successions, legumes.

Introduction

According to a number of studies, the negative environmental impact of fires has increased sharply over the last decade. In Southern Europe, the fire frequency exceeds a higher level at which ecosystems can self-repair (Boer et al. 2020; Popović et al. 2021). In Bulgaria, for the period 2015-2019, the burned forest areas are approximately 20,000 ha. By assessing the environmental consequences, Tsakov et al. (2020) indicate that tree and grass vegetation in large anti-erosion areas has been destroyed, thus limiting their hydrological function. The same study presents data on disturbed biological balance in the affected forests.

Fires also cause significant changes in the structure and dynamics of forest meadows and pastures. The study of the species composition and structure of grass communities after the fire is important for clarifying the functioning of burned meadow ecosystems and, accordingly, for planning activities for their restoration. In this regard, it is necessary to assess the role of individual groups of grasses, including legumes, in self-healing processes. In a number of studies, species of the Fabaceae family have been identified as the first colonizers of burned forest and meadow areas (Arianoutsou & Thanos 1996; Morgan & Williams 2015; Carbone 2017; Carbone & Aguilar 2021). Due to their root structure and the process of symbiotic nitrogen fixation, these species have a significant contribution to improving edaphic conditions, limiting erosion processes, and restoring biodiversity after the fire (Morgan 1999).

Biological nitrogen fixation from legumes undergrowth is considered a long-term source of nitrogen balance in forest ecosystems exposed to unfrequent fires (Tierney et al. 2019; Hendricks et al. 2019). In Bulgaria, many legumes have a practically specific role as a self-healing, high-quality fodder resource after a fire. In this relation, in many cases, the forest fires are caused intentionally to increase legume abundance.

The mechanisms for self-restoration and the role of individual grass species and communities are specific to different ecological geographical areas. First of all, it is important to determine the relationship of species diversity with the specific climatic and edaphic conditions in the fire area and the season and power of the fire. Also of interest is the biology of self-regenerating species (life form, reproductive system, seed germination), as well as specific morphological, biological, and ecological characteristics related to self-establishment in burned ecosystems, for example, hard-coated seeds, open reproductive system, rhizomes system for reproduction; high moisture of the reproductive stems during seeds maturation, etc. Fires in many parts of our country are an important environmental factor and, accordingly, there is the intraspecific adaptation and genetic variability associated with it, which should also be analyzed.

The aim of this study is to establish the species composition and distribution of legumes in the pioneer phytocenoses of burned forest meadows and pastures in Northern Bulgaria and to create a basis for further studies of self-healing processes in burned meadow ecosystems in the following aspects:

• symbiotic activity of legume components in post-fire grass communities and changes in soil indicators and trophic relationships caused by specific species under specific environmental conditions;

• adaptive intraspecific genetic variability related to self-recovery of legume populations in burnt grasslands;

• flowering and interaction with the legume species pollinators in the post-fire successions and their influence on the restoration of the insects typical for the habitat and respectively for the restoration of the biotope;

• identification of legume species with potential for use in restorative sowing programs of burnt meadows and pastures.

Materials and Methods

The participation of the legumes in the pioneer phytocenoses of burned meadows and pastures was studied in the first or second vegetation after burning. The study was conducted in the hilly and foothill part of Central Northern Bulgaria, in the Lovech region, where in 2019, the frequency and scale of forest fires were the highest in the country (Report No. 19 Forest Fires in Europe, Middle East and North Africa 2019). The surveyed burned grassland regions are located at different altitudes. The geographical location, fire season, topographic and edaphic conditions are the following:

1. Location 1 - Forest pasture, an area of about 13 ha, burned in autumn 2019. It is located on the territory of Suhindol town. The altitude is 191 m, and the soil is leached chernozem with a neutral reaction.
2. Location 2 - Pre-mountain forest meadow, having an area of about 30 ha, and burned in autumn 2020. It is located on the territory of Brestovo village (Lovech municipality). The altitude is 495 m. The soil is acidic brown forest soil.
3. Location 3 - Foothill meadow, an area of about 3 ha, burned in autumn 2019. It is located on the territory of the Slivek village (Lovech municipality). The altitude is 260 m. The soil is acidic gray forest soil. The area is very sloping and eroded.
4. Location 4 - Pre-mountain forest meadow, the area is about 10 ha, burned in autumn 2019. The grassland is located in the area of Krapets Dam, at 520 m above sea level. The soil is acidic gray forest soil.

The plant plot was selected at each burned grass location, homogeneous in terms of microrelief, species composition, and structure. A transect was delineated on each of the selected plant plots. Beginning of the vegetation 2021, which was the first or second vegetation after the fire, phytocenotic observations were conducted in April, May and June. The species forming the group of legumes in the post-fire grass association were determined. An approximate relative estimate of the abundance of each identified legume species has been made. Basic morphological and physiological features were reported for the dominant species. Nomenclature of vascular plants follows Delipavlov et al. (2003).

Results

Legumes predominated in grassland in all studied burned areas (Tab 1).

In Location 1 (Loc1), the second vegetation after the fire, legumes were represented by ten species from five genera. The genus *Vicia* was represented by five species, the genus *Medicago* - by two species, and *Bituminaria*, *Melilotus,* and *Coronilla* were represented by one species each. The greatest abundance was observed for the annual seed propagating species *Bituminaria bituminosa* (Fig. 1a-d), *Medicago minima* (Fig. 1g), *Vicia striata* (Fig. 1e), *Vicia narbonensis* (Fig. 1h), and *Melilotus oficinalis* (Fig. 1f).

In Location 2 (Loc2), representing the first vegetation after the fire, four species of different genera were found. They were very high and equivalently abundant, forming grassland with a mosaic structure. The perennial species *Lathyrus pratensis* and *Trifolium medium* (Fig. 2d, 2e), propagating through seeds and rhizomes, and *Lotus corniculatus*, which reproduces only with seeds, formed compact stands (Fig. 2c). The seed-spreading short-lived species *Melilotus oficinalis* was found in smaller areas.

Fig. 1 Legume species with the greatest abundance in Loc1 (forest pasture, burned in autum 2019, territory Suhindol town, 191 m a.s.l., soil type - leached chernozem). a) *Bituminaria bituminosa* (April 2021); b) *B. bituminosa* (May 2021); c) *B. bituminosa* (June 2021); d) *B. bituminosa* (June 2021); e) *Vicia striata*, *Medicago minima* (May 2021); f) *Melilotus officinalis* (May 2021); g) *Medicago minima* (June 2021); h) *Vicia narbonensis* (May 2021); i) *Coronilla varia* (June 2021).

In Location 3 (Loc3) - xerophytic grass on a sloping and eroded area, representing the second vegetation after the fire, three annual species of legumes were observed from two genera. The vegetation cover of the locality consisted entirely of *Medicago minima* stand (Fig. 3a, 3b). Two established species of vetch - *Vicia villosa* and *Vicia striata* formed stands around the burnt bushes. Single plants of the perennial species *Coronila varia* were observed.

Fig. 2 Legume species with the greatest abundance in Loc2 (Pre-mountain forest meadow, burned in the autumn of 2020, the territory of Brestovo village, municipality Lovech, 495 m a.s.l., brown forest soils). a) *Lathyrus pratensis* (May 2021); b) *L. pratensis* (June 2021); c) *Lotus corniculatus* (June 2021); d) *Trifolium medium* (May 2021); e) *T. medium* (June 2021); f) *Melilotus officinalis* (April 2021).

Fig. 3 Legume species with the greatest abundance in Loc3 (Pre-mountain meadow, burned in the autumn of 2019, terrotory of Slivek village, municipality Lovech, 260 m a.s.l., gray forest soils).

The most leguminous herb species (twelve in total) were found in Location 4 (Loc4) - mesophytic forest meadow, representing the second vegetation after the fire. Most species (seven) belonged to the genus *Trifolium*. Four other genera, *Coronilla*, *Lathyrus*, *Vicia,* and *Ononis*, were represented by one species. Perennial rhizome species *Coronilla varia* (Fig. 4a) and *Trifolium medium* (Fig. 4b) were the most abundant. Two other species of clover - *Trifolium patens* (annual species) (Fig. 4d) and *Trifolium montanum* (perennial species) (Fig. 4e) have a relatively high presence in the grassland. *Lathyrus niger* (Fig. 4f) was also abundant, forming stands near burnt trees.

Discussion

Leguminous grasses predominate in the phytocenoses in the first and second vegetation after the fire in all observed areas. The established species composition can be related in the first place to the conditions of the habitats - moisture supply and edaphic factors. Following this, high species richness was observed in location 1, where the leached chernozem affords high fertility soil. Concerning location 4, high species richness is related to high air and soil moisture and the rich seed bank of *Trifolium* species. On the other hand, the annual, seed-propagating species were presented with majority under the drier conditions (Loc1), or on eroded soils with low soil water holding capacity (Loc3). It was published that annual legumes have a high level of flowering time plasticity and a great quantity of indehiscent pod containing hard-coated seeds (Pozo & Aronson 2000; Abdelguerfi & Marrakchi 2000, cited by Chebouti et al. 2015).

The growth of a large number of species of the genus *Vicia* can be explained by their large seeds, which according to Hill & Auld (2020), is associated with better

Fig. 4 Legume species with the greatest abundance in Loc4 (Foothill forest meadow, burned in the autumn 2019, 520 m a.s.l., gray forest soils). a) *Coronilla varia* (June 2021); b) *Trifolium medium* (June 2021); c) *Trifolium patens* and *Coronilla varia* (June 2021); d) *T. patens* (June 2021); e) *T. montanum* (June 2021); f) *Lathyrus niger* (June 2021).

preservation of germination after exposure to high soil temperatures and relatively stable residual seed bank after burning. According to our earlier results, the absolute seed mass (m1000) of the identified *Vicia* species is 34.7, 55.0, and 112 g for *Vicia striata*, *V. villosa* and *V. narbonensis*, respectively (Stoyanov et al. 2018). *Medicago minima* is a species with a small seed size (m1000 = 1.4 and 1.65 g according to Kabtni et al. 2020), but its presence in both locations (Loc1 and Loc3) is high. Most probably, the shape and hardness of the pods of this species protect the seeds from the effects of high temperatures. The species *M. mimima* is annual wild alfalfa highly abundant in the burned areas, although three other *Medicago* species, *M. arabica*, *M. polymorpha* and *M*. *lupulina,* were typical for Location 1.

Medicago minima has a wide edaphic and climatic adaptation (Chebouti et al. 2015). According to the same authors, the ecological adaptation of *M. minima* is associated with secondary metabolites in the species (Kabtni et al. 2020). It would be interesting to find whether the rection of the species to the conditions in the burned areas is modified through the secondary metabolites.

When the burned areas have moister soils, the perennial rhizome species *Lathyrus pratensis* (Loc2) and *Trifolium medium* (Loc 2 and Loc 4) grow on larger part of the post-fire successions areas. These species, also a perennial and, in general rhizome plant species, possess a great abundance in mesophytic and xerophytic burned grasslands. It can be considered that the open system of reproduction (seed and vegetatively - through rhizomes) is an important biological characteristic associated with self-healing ability after a fire. The species are typical for the habitats, but their high participation in grassland is increased greatly due to the burning of the areas.

The contribution of legumes to the restoration of ecosystems is based primarily on symbiotic nitrogen fixation, through which they affect the content of nitrogen and soil organic matter. The amount of nitrogen fixed symbiotically by grassland legumes is between 15-680 kg N ha^{-1} yr^{-1}, depending on the species, bacterial strain, and ecological conditions (Spehn et al. 2002).

In this regard, it is important to determine what is the symbiotic activity of identified legumes, the nitrogen/crude protein content in their biomass, and what changes in soil parameters cause particular species under specific environmental conditions, and as a consequence, how trophic relationships are affected in the ecosystem. When legumes represent a large part of the biomass formed in the burned area, they affect the soil characteristics directly and indirectly by their decomposition after the end of the growing season. According to Hendricks et al. (2019), the leaf tissues of legumes developed in the burned forest area have high concentrations of N, low concentrations of lignin, and a correspondingly low ratio of lignin: N (average 6.7 ± 0.9), which is indicative of tissues that are characterized by rapid loss of weight and N in the mineralization processes. The species we have found with a high abundance in the burned areas have a very high crude protein content. For example, for *Melilotus officinalis,* the amount of crude protein (CP) is 11.8 %; for *M. minima* – 19.86 %, for *Vicia striata* – 22.3 %, for *Vicia villosa* – 22.1 %, and *Lotus corniculatus*

Tab. 1 Legume species established in the monitored burned locations. Loc 1* - burned location 1, 2, 3, 4. The most abundant species are in bold.

Loc1*	Loc2	Loc3	Loc4
Bituminaria bituminosa	***Lathyrus pratensis***	***Medicago minima***	***Coronilla varia***
Medicago minima	***Lotus corniculatus***	*Vicia vilossa*	***Trifolium medium***
Vicia striata	***Trifolium medium***	*Vicia striata*	***Trifolium patens***
Melilotus oficinalis	*Melilotus oficinalis*	*Coronila varia*	***Latyrus niger***
Vicia narbonensis			***Trifolium montanum***
Coronilla varia			*Trifolium arvense*
Vicia grandiflora			*Vicia grandiflora*
Vicia vilossa			*Trifolium palidum*
Vicia gracca			*Trifolium striatum*
Medicago falcata			*Trifolium incarnatum*
			Vicia vilossa
			Ononis hircina

Tab. 2 Comparison between absolute seed mass (m1000s) of *Trifolium* species obtained in unburned and burned meadow

Unburned meadow	Burned meadow
Species/m1000s	
Trifolium pannonicum - 2.8-3.2 g	*Trifolium medium* - 0.85 g
Trifolium pratense -1.8 g	*Trifolium patens* - 0.4 g
Trifolium incarnatum - 3.1-4.1 g	*Trifolium montanum* - 1.6 g

– 19.6 % respectively (Chourkova 2013; Bozhanska et al. 2015, 2016; Ilieva & Naydenova 2016; Bozhanska 2020; Naydenova et al. 2022).

Kohmann et al. (2018) demonstrated that biomass is decomposed faster and higher level of N was released when legumes were included in the grass pasture system (Jaramillo et al. 2021). The lignin content is also important because when its value is high, like in C4 plants, the level of decomposition is low. The lower decomposition rate results from the fact that N components are attached to the lignin components, which consequently declines the level of microbial available N, and finally, the level of decomposition is low (Jaramillo et al. 2021). Accordig to our previously published data, acid-detergent lignin (ADL) content is 6.26 % for *M. minima*, 3.1 % for *M. officinalis* and 8.7 % for *L. corniculatus* (Chourkova 2013; Bozhanska et al. 2015; Bozhanska 2020; Naydenova et al. 2022).

The main chemical composition of *Bituminaria bituminosa* (Arabian pea) - the species that dominates in burned location 1, was not studied for Bulgarian accessions. According to Pecetti et al. (2007) the content of CP ranged from 9.4 to 16.1 % in the samples collected from Italy versus 17–20 % in the Spanish material (Viano et al. 2000 cited by Pecetti et al. 2007). The authors also reported a high concentration of water-soluble sugars, considering about 35 % from the dry matter. *Bituminaria bituminosa* could be defined as an atypical pioneer species for Northern Bulgaria. Given its ecological requirements, the species will be the subject of future interest as an indicator for changes in meadow ecosystems due to warming and drought conditions.

Flowering plants are present in great abundance from the first to several years after the wildfire (Keeley et al. 1981; Safford & Harrison 2004). Plants respond to the fire using several species-specific mechanisms related to increased germination, growth, and flowering, but consistent with the rescpective ecosystems and biogeographical regions (Keeley & Keeley 1982; Wrobleski & Kauffman 2003; Lybbert et al. 2017). This is confirmed by our results concerning Location 4 - mesophytic foothill meadow. In the study conducted during the period 2017-2019 on unburned grasslands in the same area, eleven species of clover were identified, with greater participation of *Trifolium pannonicum*, *T. pratense*, *T. incarnatum,* and *T. campestre* (Naydenova & Bozhanska 2020). After the fire, these species were absent or had little presence like *T. incarnatum*. Other species represent the genus *Trifolium* - *Trifolium medium*, *Trifolium patens* and *Trifolium montanum*, which have greater flowering rate and abundance in the grassland after the fire compared to the unburned meadow. Additionally, by comparison of *Trifolium* species representatives from the same area before and after the fire, based on the parameter m1000 seed, it was found that species formed small seeds, with a high percentage of hardiness dominated (Stoyanov et al. 2018) (Tab. 2).

According to Carbone & Aguilar (2021), the interactions between the flowering plant and pollinators and the soil properties are significantly damaged. On the other side, the interactions could be increased after a variety of fire histories but only when the area is burned with low or medium severity (Ponisio et al. 2016). Brown et. al. (2017) described that plants respond to the fire by mediating the flowering characteristics related to potential bud location, seasonal changes in bud exposure, and bud damage response. These particular traits have to be monitored in future studies for the species observed in the locations described here. Many species recorded during our post-fire successions studies produce quality nectar for an extended period. For example the flowering period of *Vicia striata* and *Coronilla varia* is from May till July, and for *Melilotus officinalis* – from May till June (Stoyanov et al. 2018). Of interest is how their attractivity for the pollinators helps restore the typical habitat insect community and, accordingly, restore the biotope.

The increased soil acidity in the soils affected by a fire on the territory of our country is presented in many current studies (Bogdanov 2013, 2014). High soil acidity is an important limiting factor for the establishment of legumes and nitrogen fixation levels. In this sense, assessing the species diversity in the post-fire succession is crucial for targeted analysis of soil changes after the fire. According to our findings, it could be supposed that species predominating on locations 2, 3 and 4 could have better ability for nitrogen fixation under acidic soil conditions. Ferguson et al. (2013) studies described the potential of *Bradyrhizobium* and *Rhyzobioum* species tolerance to different leels of pH. The author cited several publications which underlined that *Bradyrhizobium* sp. are more acid-tolerant than most *Rhyzobium* sp. Additionally, some strains of *R. tropici* and *R. loti* are highly acid-tolerant. It was published by Reeve et al. (2014) that the saprophytic competence to acidic soil is a requirement for strains used for inoculation of *Medicago* species like *M. murex*, *M.*

sphaerocarpus, and *M. polymorpha,* which are recommended and used for sowing programs in acidic areas with pH below 5.5. Additional studies will describe the acidic tolerance of the species presented in the studied burned areas.

Conclusion

This study describes the detailed monitoring of plant vegetation related with biodiversity and abundance in four distinct burned locations Northern Bulgaria. Results demonstrated that after the fire, the first colonizers of the monitored location belonged to the legumes herbs. The highly presented species are closely related to the edaphic conditions and moister supply of the investigated areas. In the dry localities with low capacity of the soil to save moisture, the annual species propagated with seeds like *Bituminaria bituminosa*, *Vicia striata* and *Medicago minima* are highly abundant. Perennial species with rhizome systems for reproduction like *Lathyrus pratensis* and *Trifolium medium* represent the majority of the plant cover if the soil capacity to keep moisture is higher at the locality. The seed coat hardiness and small seed size are two of the main characteristics that give a bigger chance for the species to survive and reproduce after the fire. The presence of legume herbs in the self-recovered meadows studied is the source for grazing with high protein content and low lignin content in plant matter.The last characteristic is related to the rate of the mineralization process. The legume species have a long-lasting flowering period, produce high-quality nectar, and are attractive to pollinators. Using the legumes species with a high capacity to recover after the fire (wild or intentional) in the sowing activities is an effective way to recover the burned localities and the natural relationship between the flowering plants and pollinators. Additional studies for nitrogen fixation capacity, phenotypic features concerning flowering and buds positions, seed coat structure, and hardiness will help to understand more for the phenomenon of legumes wild grasses to recover after fire events.

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