

# Effects of grassland management on plants and invertebrates in Transylvania, Romania: A threat to local biodiversity hotspots

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## **GENERAL INTRODUCTION**

Quantifying and conserving biodiversity is now recognized as more important than ever.

During the last decades methods in agriculture have changed profoundly in Europe. Increased pressure for higher yields and the rising price of labor led to significant habitat alterations in Western Europe. As consequence, the traditional ways of grassland cultivation, which have been in use for hundreds of years, and which resulted in a high diversity of plants and invertebrates in nutrient-poor, semi natural grasslands (Zoller et al. 1986; Baur et al. 1996; Wilmanns 1998) were either treated more intensively or, in many cases, abandoned. Large areas of semi-natural grassland were lost due to intensified use (increased stocking rate and/or use of artificial fertilizers) or abandonment (Erhardt 1985a; Groombridge 1992; Antognoli et al. 1995). Detrimental effects of fertilization on the species diversity of plants and invertebrates are well-documented (Rosenzweig 1995; Ellenberg 1996). In contrast, studies regarding effects of grassland abandonment on biodiversity are scarce. On abandoned grasslands a secondary succession takes place, leading in most places to the local climax vegetation, i. e. forest.

Nutrient-poor grasslands are considered regional biodiversity hotspots and therefore of high conservation value (Blab & Kudrna 1982; Bignal & McCracken 1996; Sanchez-Zapata et al. 2003). As human-made habitats they harbor numerous species whose primordial habitats (floodplains, peatlands and rocky outcrops) have been vastly destroyed (Baur et. al. 1997, 2004). However, semi-natural grasslands are fragile because their maintenance depends on traditional farming techniques.

Romania's evolutionary history, biogeographical position and isolation of Transylvania and the Carpathian mountains are reflected in a high level of species diversity and endemic and sub-endemic species (ICAS 1996; Ioras 2003). Nowadays, these regions must be considered as hotspots of European biodiversity. Despite their high conservation value many of these grasslands are threatened by non-sustainable agriculture or abandonment. Now an additional threat arises from the changing agricultural policies due to the upcoming Romania's EU accession. In order to enrich the EU and to ensure the persistence of Romania's great variety of precious natural habitats the Natura 2000 network has to be established in Romania. Thus, precious habitats have to be proposed to the European Commission to be as Sites of Community Importance (pSCI). Due to limitations in financial and human resources and lack of scientific information, the common tendency is to declare as pSCI only existing protected areas, neglecting a huge part of the precious semi-natural habitats (WWF 2003). These habitats (that include steppe-like grasslands in Transylvania) are fragile because their maintenance depends on traditional farming techniques, and they would disappear within short time after abandonment or intensification. Conservation efforts should also consider the isolation of these habitats by improving the connectedness between remnants of steppe-like grassland. Thus, there is an urgent need for the development of adequate techniques to restore and conserve these grasslands.

The present thesis attempts to assess biodiversity in traditionally cultivated grasslands and to compare it with that of intensified and abandoned grasslands in Romania in three different regions of Transylvania: lowland steppe-like grasslands, the subalpine region and the alpine region of the southern Carpathians. In order to give a more accurate picture of the diversity in these regions we investigated not only one taxonomic group, but four: vascular plants, terrestrial gastropods and diurnal and nocturnal Lepidoptera. Another aim of this study is to foresee future development of the investigated grasslands as a consequence of political change and suggest management strategies and programs to prevent the loss of precious semi-natural habitats. This provided some results, which have conservation, social, political and educational implications.

The **first chapter** investigates species richness, species abundance, proportion of open-land, endemic and threatened vascular plants, gastropods, and diurnal and nocturnal Lepidoptera in six different vegetation types all originating from steppe-like grasslands in Transylvania, Romania. The steppe-like grasslands of Transylvania and their seral stages harbor a variety of plant and insect species that colonized this habitat from continental steppes (in Russia) during postglacial warm periods (Rakosy & Kovacs 2001; C.C., unpublished data). The subsequent isolation during colder periods resulted in several endemic species such as the plants *Astragalus peterfii* Jav. and *Salvia transsilvanica* Schur and the butterfly *Pseudophilotes bavius hungaricus* Drandt (Rakosy & Laszloffy 1997; Rakosy 1999; Rakosy & Kovacs 2001). Investigated vegetation types included extensively grazed pastures (initial stage), three seral stages of succession (early stage of abandoned grassland, abandoned grassland with shrubs, and climax forest), and two human-made grassland alterations, namely abandoned vineyards and *Pinus* plantations. We found a high plant and invertebrate species richness in the investigated steppe-like grasslands and their seral stages of abandonment. In climax forests, the final stage of natural grassland succession, diversity of all groups of organisms examined was significantly reduced. Furthermore, many of the rare and threatened grassland species of plants and Lepidoptera were replaced by common mesophilous species because of the reduced light. The abandonment of the extensively used vineyards created a valuable habitat for plants and invertebrates. *Pinus* plantations (a recent grassland alteration) have changed habitat quality and will have a devastating effect on the unique, indigenous diversity of these steppe-like grasslands as soon as the canopy closes. The four taxonomic groups differed in their response to the abandonment of steppe-like grassland, except that species richness of plants and diurnal Lepidoptera were positively correlated. Endemic species were found in all vegetation types except climax forests and *Pinus* plantations. All Transylvanian endemics and the majority of threatened species found were open-land species and open land species decreased with successional age in plants and gastropods. All investigated vegetation types harbored threatened (Red List) species.

The **second chapter** analyses the effects of abandonment and man-made habitat alterations of steppe-like grasslands only on diurnal and nocturnal Lepidoptera communities considering the xerophilous character of



the typical species of these habitats and their Red List status. Like the species richness and diversity of diurnal Lepidoptera the number of xerophilous and Red List species peaked in later successional stages with bushes and in abandoned vineyards and was low in forests and *Pinus* plantations. In contrast xerophilous and Red List species of moths showed their highest richness in early successional stages. Species richness, diversity, xerophilous and Red List species were not correlated between diurnal and nocturnal Lepidoptera. The observed species reactions confirm that diurnal Lepidoptera react strongly to environmental change and hence are good indicators of habitat change. The Renkonen classification showed that regional factors are more important than local factors for the recorded moth community.

**Chapter three** analyses the effects of land abandonment on plants, diurnal and nocturnal Lepidoptera and gastropods in subalpine semi-natural grasslands in Transylvania. Investigated vegetation types were extensive hay meadows (initial stage) and three seral stages of succession (early stage of abandoned hay meadow, naturally growing birch forest, and mature forest). The four taxonomic groups differed again in their response to the abandonment. Each stage of succession harbored the maximum species richness for one taxonomic group: extensive hay meadows for vascular plants, abandoned hay meadows for diurnal Lepidoptera, birch forest for nocturnal Lepidoptera and mature forests for gastropods. The number of characteristic open-land species decreased with successional age. The successional stages did not differ in the proportion of red-listed plant and diurnal Lepidoptera species. However, the proportion of nocturnal Lepidoptera species increased with successional age. Successional stages did not differ in number of plant species endemic to the Carpathians and Eastern Europe.

The **fourth chapter** describes the effect of intensified grazing on plants and gastropods in the alpine grasslands in two areas of the Southern Carpathians (Bucegi - calcareous - and Fagaras - silicious - Mountains). Lepidoptera species could not be investigated due to the improper weather conditions. Alpine grasslands in the Southern Carpathians harbor an extraordinary high diversity of plants and invertebrates, including Carpathic endemics. In the Southern Carpathians, sheep grazing has been reported since the beginning of the sixteenth century (Barbulescu and Motca, 1983). The sheep herds have always been large in this area, forcing the animals to graze also in adjacent forests, which were clear-cut to extend the pastures in the 19th century (Coldea, 2003). More recently, the size of the sheep herds increased further, as a result of the altered socio-economical situation since 1989, forcing the sheep to graze also on steep slopes, which are local hotspots for biodiversity. In Bucegi grazed sites had a lower plant and gastropod species richness than ungrazed sites. Grazed and ungrazed sites did not differ in the proportions of plant and gastropod species endemic to the Carpathians and to Eastern Europe. The total cover of Carpathic endemic plants was lower in grazed than in ungrazed sites. The abundance of the Carpathic endemic gastropod *Chondrula venerabilis* was reduced in grazed sites. In Fagaras grazed and ungrazed sites did not differ in species richness, abundance and proportion of endemic plant and gastropod species.

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## Chapter 1

### ***Alterations of Steppe-Like Grasslands in Eastern Europe: a Threat to Regional Biodiversity Hotspots***

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# Alterations of Steppe-Like Grasslands in Eastern Europe: a Threat to Regional Biodiversity Hotspots

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**Abstract:** *Recent changes in agriculture (intensification or abandonment) have resulted in a critical reduction of steppe-like grasslands in Eastern Europe. These grasslands harbor an extraordinarily high diversity of plants and invertebrates, including endemics, and are considered refugia for numerous threatened open-land species. We examined species richness, and abundance, proportion of open-land, endemic and threatened vascular plants, gastropods, and diurnal and nocturnal Lepidoptera in six different vegetation types all originating from steppe-like grasslands in Transylvania, Romania. Vegetation types included extensively grazed pastures (initial stage), three seral stages of succession (early stage of abandoned grassland, abandoned grassland with shrubs, and mature forest), and two human-made grassland alterations, namely abandoned vineyards and Pinus plantations. A total of 852 species (291 vascular plants, 24 gastropods, 129 diurnal and 408 nocturnal Lepidoptera) were found in the 22 study sites. The four taxonomic groups differed in their response to the abandonment of steppe-like grassland, except that species richness of plants and diurnal Lepidoptera were positively correlated. The complementarity of species composition increased with successional age in all taxonomic groups examined. The number of characteristic open-land species decreased with successional age in plants and gastropods. All investigated vegetation types harbored threatened (red-listed) species. Endemic species were found in all vegetation types except mature forests and Pinus plantations. All Transylvanian endemics and the majority of threatened species found were open-land species. Extensively cultivated vineyards, which have been abandoned for two to three decades, also maintained high plant and invertebrate diversities, comparable to those of the corresponding stages of grassland succession. In contrast, Pinus plantations (a recent grassland alteration) have changed habitat quality and will have a devastating effect on the unique, indigenous diversity of these steppe-like grasslands as soon as the canopy closes. To prevent losses of characteristic species, we suggest a rotational grassland management program that maintains different seral stages. Succession to mature forest and additional Pinus plantations should be prevented.*

**Key Words:** diurnal Lepidoptera, nocturnal Lepidoptera, endemic species, extinction, gastropods, habitat alteration, red-data species, succession, vascular plants

Alteraciones de Pastizales Similares a Estepas en Europa Oriental: una Amenaza para Sitios de Importancia para la Biodiversidad Regionales

**Resumen:** *Cambios recientes en la agricultura (intensificación o abandono) han resultado en la reducción crítica de pastizales similares a estepas en Europa Oriental. Estos pastizales albergan una diversidad extraordinariamente alta de plantas y animales, incluyendo endémicos, y son considerados refugios para numerosas*

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especies amenazadas. Examinamos la riqueza y abundancia de especies, la proporción de plantas vasculares, gasterópodos y Lepidoptera diurnos y nocturnos amenazados y endémicos en seis tipos de vegetación diferentes pero originados a partir de pastizales similares a estepas en Transilvania, Rumania. Los tipos de vegetación incluyeron pastizales apacentados extensivamente (etapa inicial), tres etapas serales de sucesión (etapa temprana de pastizal abandonado, pastizal abandonado con arbustos y bosque maduro) y dos alteraciones humanas de pastizales, particularmente viñedos abandonados y plantaciones de *Pinus*. Encontramos un total de 852 especies (291 plantas vasculares, 24 gasterópodos, 129 Lepidoptera diurnos y 408 nocturnos) en los 22 sitios de estudio. Los cuatro grupos taxonómicos difirieron en su respuesta al abandono del pastizal, excepto en que la riqueza de especies de plantas y de Lepidoptera diurnos estaban correlacionado positivamente. La complementariedad de la composición de especies incrementó con la edad sucesional en todos los grupos taxonómicos examinados. El número de especies de plantas y gasterópodos características de zonas abiertas decreció con la edad sucesional. Todos los tipos de vegetación investigados albergaron especies amenazadas (en listas rojas). Encontramos especies endémicas en todos los tipos de vegetación excepto en bosques maduros y plantaciones de *Pinus*. Todas las endémicas de Transilvania y la mayoría de las especies amenazadas fueron especies de zonas abiertas. Los viñedos cultivados extensivamente, que han estado abandonados durante dos o tres décadas, también mantuvieron una alta diversidad de plantas e invertebrados, comparable a la de las correspondientes etapas de sucesión de los pastizales. En contraste, las plantaciones de *Pinus* (una alteración reciente de pastizales) han cambiado la calidad del hábitat y tendrán un efecto devastador sobre la diversidad nativa, única, de estos pastizales tan pronto se cierre el dosel. Para prevenir pérdidas de especies características, sugerimos un programa de manejo rotatorio de pastizales que mantenga diferentes etapas serales. También se deben evitar las plantaciones de *Pinus* adicionales y la sucesión hacia bosque maduro.

**Palabras Clave:** alteración de hábitat, especies con datos rojos, especies endémicas, gasterópodos, Lepidoptera diurnos, Lepidoptera nocturnos, plantas vasculares, sucesión

## Introduction

In Europe deforestation of huge areas created land for agriculture since Roman times. Traditional ways of grassland cultivation, which have been in use for hundreds of years, resulted in a high diversity of plants and invertebrates in nutrient-poor, seminatural grasslands (Zoller et al. 1986; Baur et al. 1996; Wilmanns 1998). During the twentieth century increasing pressure for higher yields and the rising price of labor led to significant habitat alterations in Western Europe. Large areas of seminatural grassland were lost because of intensified use (increased stocking rate and/or use of artificial fertilizers) or abandonment (Erhardt 1985a; Groombridge 1992; Antognoli et al. 1995). Detrimental effects of fertilization on the species diversity of plants and invertebrates are well documented (Rosenzweig 1995; Ellenberg 1996). In contrast, studies regarding effects of grassland abandonment on biodiversity are scarce.

Dry, nutrient-poor grasslands are considered regional biodiversity hotspots and therefore of high conservation value (Blab & Kudrna 1982; Bignal & McCracken 1996; Sánchez-Zapata et al. 2003). As human-made habitats they harbor numerous species whose primordial habitats (floodplains, peatlands, and rocky outcrops) have been largely destroyed (Baur et al. 1997, 2004). Seminatural grasslands, however, are fragile because their maintenance depends on traditional farming techniques.

Romania has an extraordinarily high level of species diversity, 228 endemic and subendemic species (ICAS 1996;

Ioras 2003). Reasons for the high biodiversity include several biogeographical regions in Romania, the high habitat diversity, and the locally low intensity of agricultural land use. Extensive grazing was the dominant method of grassland management over large areas of Romania for many centuries. In recent years, grasslands have been either fertilized or abandoned, mainly as a result of the altered socioeconomic situation since 1989. Of particular concern are the Transylvanian steppe-like grasslands that harbor a variety of plant and insect species which colonized this habitat from continental steppes (in Russia) during post-glacial warm periods (Rakosy & Kovacs 2001; C.C., unpublished data). The subsequent isolation during colder periods resulted in several endemic species such as the plants *Astragalus peterfii* Jav. and *Salvia transsilvanica* Schur and the butterfly *Pseudophilotes bavius hungaricus* (Rakosy & Laszloffy 1997; Rakosy 1999; Rakosy & Kovacs 2001).

Despite the high conservation value of the Transylvanian steppe-like grasslands (Rakosy & Kovacs 2001), effects of their abandonment on biodiversity have not been examined. We compared diversity and abundance of vascular plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera in traditionally grazed steppe-like grasslands with those in natural stages of abandoned grassland in Transylvania. We also investigated the diversity and abundance of the four taxonomic groups in prominent habitats altered by human activity, abandoned vineyards and *Pinus* plantations. In the past two decades, large areas of the seminatural grasslands were afforested with



*Pinus nigra* Arnold, which presumably threatens specialized open-land species, the target group of our study.

The high species diversity of grasslands has been explained by the intermediate disturbance hypothesis (Wilson & Tilman 2002; Li et al. 2004; Shea et al. 2004). According to this hypothesis species diversity decreases with grassland abandonment. Surveys carried out in Western Europe confirmed this decrease in plant diversity of abandoned seminatural grasslands (Bischof 1981; Tasser & Tappeiner 2002; Dullinger et al. 2003). Similarly, studies focusing on threatened butterfly species in Britain show that grassland abandonment is the major cause of the butterfly decline (Thomas 1991). In investigations of entire butterfly communities, however, Erhardt (1985a, 1985b) and Balmer and Erhardt (2000) found that several threatened species benefit from advanced stages of abandonment. These contrasting results require further examination.

For practical reasons, conservation evaluations of grasslands are mostly based on a single taxonomic group, in many cases vascular plants (Usher 1986; Plachter 1991). The use of one taxonomic group for the assessment of biodiversity, however, implicitly assumes that the diversities of indicator and target groups are positively correlated (Oliver & Beattie 1996). Correlations between the diver-

sities of Lepidoptera and vascular plants seem to support this approach (Erhardt & Thomas 1991), although significant deviations from this expectation have been reported (e.g., Erhardt 1985a, 1985b). Our data allow a test of the assumption that succession-related diversity changes in different taxonomic groups covary.

We addressed the following questions: (1) Do different successional stages of grassland abandonment differ in species number, species abundance, and community structure of plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera? (2) How do the human-made habitat alterations (abandoned vineyards and *Pinus* plantations) affect these communities? (3) Do different successional stages differ in the number of threatened (red-listed) and endemic species? (4) To what extent is the response to habitat changes of a particular group an indicator for the other taxonomic groups?

## Methods

### Study Sites

We assessed plant and invertebrate diversities in 22 areas (Table 1) surrounding four Transylvanian villages (Caianu,

**Table 1.** Characteristics of the study sites in the Transylvanian steppe-like hills.

Habitat type	Study site <sup>a</sup>	Land use	Total area (ha)	Isolation <sup>b</sup> (km)	Soil pH
Extensively grazed pasture	V	extensively grazed by sheep	1	6	6.5
	S	extensively grazed by cattle	1	1	6.0
	S	formerly extensively grazed by cattle, nowadays partly grazed by sheep and partly mown	2	1	6.0
	C	extensively grazed by sheep	3	1	6.0
	C	extensively (each second or third year) grazed by sheep	10	1	6.5
Abandoned grassland	V	abandoned for 6–8 years	59	10	6.5
	V	abandoned for 6–8 years	59	10	6.5
	S	no regular management (mowing and grazing) for 70 years, abandoned for 3–5 years	2	4	6.5
	S	abandoned for 10–15 years	0.8	4	6.5
	K	extensively grazed by cattle and sheep during past 70 years, abandoned for 5–10 years	0.5	10	6.5
Abandoned grassland with shrubs	V	abandoned for 15 years	1	0.3	6.5
	S	abandoned for 30 years	5	0.3	6.5
	C	abandoned for 10–15 years	0.8	0.3	6.5
Mature forest	V	50- to 80-year-old wood	100	8	5.0
	V	50- to 80-year-old wood	100	8	5.0
	S	50- to 100-year-old wood	80	4	4.5
Abandoned vineyard	S	abandoned for 30 years	50	20	6.5
	S	abandoned for 30 years	50	20	6.0
	S	abandoned for 30 years	50	20	6.0
<i>Pinus</i> plantation	V	30 years old, little ground vegetation	20	7	6.0
	C	15 years old; strips of <i>Pinus</i> separated by planted <i>Ligustrum</i> rows	8	7	6.0
	C	15 years old	8	7	6.0

<sup>a</sup>Key: C, Caianu; K, Fanate; S, Suatu; V, Viisoara.

<sup>b</sup>Minimum distance to the nearest locality of the same vegetation type.

Fanate, Suatu, and Viisoara) in Romania ( $46^{\circ}33' - 46^{\circ}48'$  N,  $23^{\circ}36' - 23^{\circ}57'$  E, elevations between 270 and 400 m asl). The villages were situated 3–40 km apart. Within a village, the study sites were separated by 0.2–3 km. All sites faced south-southeast, south, or south-southwest and had similar slopes. Total annual rainfall in Cluj-Napoca (10–30 km from the field sites) ranged from 383 to 1401 mm (mean 674 mm), with an annual mean temperature of  $9.3^{\circ}\text{C}$  (data from 1997–2003; C.C., unpublished data).

We considered the following vegetation types: extensively grazed pasture (E,  $n = 5$  study sites), early stage of abandoned grassland (G,  $n = 5$ ), later stage of abandoned grassland with shrubs (B,  $n = 3$ ), mature forest (W,  $n = 3$ ), abandoned vineyard (V,  $n = 3$ ), and *Pinus* plantation (A,  $n = 3$ ). The first four habitat types represent seral stages of natural grassland succession. Extensive grazing means a low stocking rate of sheep or cattle for periods of 5–10 days one to three times per year. Abandoned vineyards represent a successional stage of former land-use practice. On these slopes grapes were cultivated in an extensive way: soil was hoed but not tilled and no fertilizer was used. *Pinus* plantations represent a recent habitat alteration. The plantations are not thinned and no fertilizer is used.

### Surveys

From spring to fall 2001, we surveyed diversity and abundance of vascular plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera in a  $50 \times 50$  m plot at each site. The positions of the plots were chosen so that the distance to the next different habitat was  $> 25$  m (in one case 10 m). Plots were surveyed for plants four times: spring, early summer, late summer, and fall. At each site (except for the mature forests and the *Pinus* plantations) we established a permanent subplot of  $10 \times 10$  m and used the Braun-Blanquet (1964) method to determine the abundance of all plant species (including woody plants) within the subplot. To complete the plant list for each site, we walked in a zigzag line over the entire study site and recorded all new species observed. In the mature forest and *Pinus* plantation, we again used the Braun-Blanquet method to record plants present in permanent plots of  $100 \times 100$  m. An increased sampling area proved essential for recording plant diversity in forests (Ellenberg 1996). The sampling effort was the same, however, at all investigation sites. Plant identification followed Tuttin et al. (1964–1980) and Ciocarlan (2000).

We used two methods to assess species richness and relative abundance of terrestrial gastropods. First, two people visually searched for living snails and empty shells in each  $50 \times 50$  m plot for 40 minutes between 22 May and 14 June 2001. Second, we collected five soil samples (total 2 L), including dead plant material, at randomly chosen spots in each  $50 \times 50$  m plot. Soil and litter samples were put through a sieve with a 1-mm mesh and then ex-

amined under a binocular microscope. Gastropod shells were sorted out of the samples and identified according to Grossu (1983).

We used a transect method (Douwes 1976; Hall 1981) to survey each plot for diurnal Lepidoptera. Surveys were conducted once every second week between 21 May and 9 September 2001. We walked plots in a serpentine pattern, 5 m wide. In this way the entire area was covered once by the butterfly net. Transects were surveyed only under good weather conditions (sunny, temperature  $\geq 18^{\circ}\text{C}$ , and wind speed  $\leq 16$  km/h [Beaufort scale 3]) between 900 and 1700 hours. We recorded all species of Rhopalocera, Hesperidae, and Zygaenidae. For Bombyces, Sphinges, Noctuidae, and Geometridae, we recorded all day-active species and all other species easily surveyed with the transect method. Multiple counting of the same individuals could not be completely avoided. This error may not affect our interpretation, however, because we compared the plots rather than species; thus, the error would be the same for each plot. The sequence in which transects were surveyed was alternated to avoid systematic effects of time of day.

We surveyed nocturnal Lepidoptera between 21 May and 12 October 2001. Moths were collected using a light trap (8 W UV tubes fixed to the top of black buckets covered by white funnels and fed by a 12 V accumulator) once a month in each plot. We did not survey on nights with a full moon. In their flight toward the light moths strike the transparent baffles that surround the lamp and fall through the funnel into the bucket (Rakosy 1999). Light traps were placed in the same place on each sampling occasion and were left on from dusk to dawn. We identified and released specimens in the morning. Identification of Lepidoptera followed Tolman and Lewington (1997) for butterflies and Forster and Wohlfahrt (1981), Rakosy (1996), and de Freina and Witt (1987, 1990, 2001) for moths. Problematic species were collected and identified by specialists through examination of genitals.

### Environmental Variables

We defined total area as the size of a homogeneous vegetation type surrounded by other habitat types (e.g., the area of an abandoned vineyard surrounded by grassland). We used maps to measure total area. Study sites were part of the total area. As a measure of isolation we determined the minimum distance to the nearest equal habitat type with maps (scale 1:50,000). In each plot we collected six soil samples at randomly chosen places. We mixed the samples to determine the soil pH (Hellige method, AVM Analyseverfahren, Freiburg, Germany).

### Data Analyses

In all analyses we considered the study sites the unit of investigation. We applied one-way analysis of variance (ANOVA) to examine whether different successional

grassland stages differed in species richness, abundance, and diversity of the four taxonomic groups (StatView 5.0, Abacus Concepts 1998). We used Scheffé's post hoc test to compare pairs of successional stages. Species richness, abundance, and diversity of organisms in abandoned vineyards and *Pinus* plantations were compared with the initial stage (extensively grazed pasture) and with the most similar vegetation type of natural succession (abandoned grassland with shrubs and mature forest, respectively) with unpaired *t* tests (log-transformed values).

To examine changes in species composition between seral stages of succession and between extensively grazed pastures and human-made grassland alterations, we performed an analysis of complementarity (Colwell & Codrington 1994) for the entire species list of each taxonomic group. The value of the complementarity index varies from 0 (species lists of two vegetation types are identical) to 1 (no species shared). To analyze the similarity of the species composition and abundance of the different vegetation types, we performed a correspondence analysis for each taxonomic group with JMP (SAS Institute 1995).

At each site the age of the successional stage was determined. In mature forests, however, only the age of the trees was known, indicating a minimum age for the forests. Therefore, we used Spearman rank correlations to examine whether environmental variables (total area, isolation, soil pH, and successional age) were intercorrelated and whether they influenced species richness, abundance, and diversity and the proportion of open-land and threatened and endemic species in the investigated taxonomic groups.

To examine possible associations between the species' habitat specificity and the vegetation type in which they were found, we assigned all plants and invertebrates to one of the following categories: open-land, forest, or ubiquitous species. Detailed information on the species' habitat specificity was obtained from Tutin et al. (1964–1980), Ellenberg (1996), and Ciocarlan (2000) for plants, Grossu (1983) for gastropods, Forster and Wohlfahrt (1981) and Blab and Kudrna (1982) for diurnal Lepidoptera, and Rakosy (1996) and de Freina and Witt (1987, 1999, 2001) for nocturnal Lepidoptera. Because generalized linear models with binomial error distributions (e.g., number of open-land species as dependent variable and total number of species as binomial total) gave similar results as ANOVAs with ratios transformed to the arc-sine square root, we used the ANOVAs to examine whether successional stages differed in their proportion of open-land species. The same ANOVA model was used to examine differences in the proportions of red-listed and endemic species. Unpaired *t* tests were used to evaluate whether abandoned vineyards and *Pinus* plantations differed in proportions of open-land, endangered

species, and endemic plant species (all arcsine-square root-transformed) from the corresponding stages of the natural grassland succession.

In Romania, red lists exist for plants (Boscaiu et al. 1994) and diurnal and nocturnal Lepidoptera (Rakosy et al. 2003) but not for terrestrial gastropods. We considered critically endangered, endangered, and vulnerable species as threatened.

To examine whether vegetation types differed in their proportion of endemic plants, we assigned each species to one of the following categories: Transylvanian endemic (occurring exclusively in northwestern Romania), Eastern European endemic (Balcanic, Pontic, Carpathic, and/or Dacic distributions), widespread (naturally occurring in large areas of one or more continents), or adventive (introduced alien). Similar information was not available for the three invertebrate groups.

We used Pearson's correlations to examine possible correlations between species richness (log-transformed) and proportions of open-land and red-listed species (both transformed to arc-sine square root) among the taxonomic groups investigated.

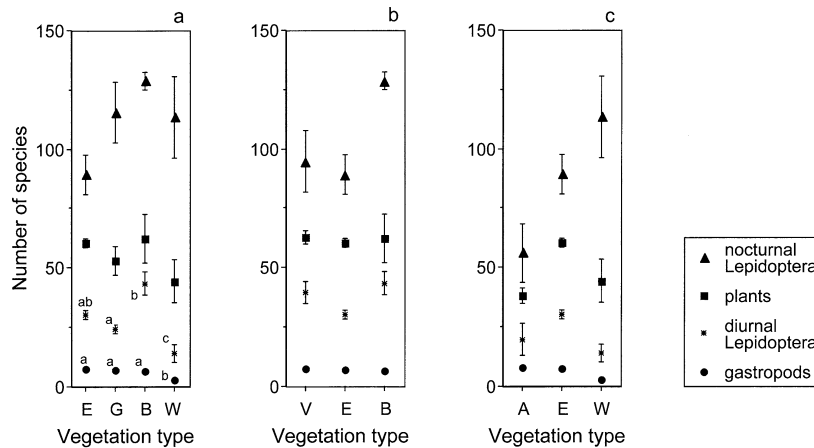
## Results

### Species Richness

We recorded 291 plant species in the study sites. In the four successional stages, mean plant species richness varied from 44.3 to 62.3 (Fig. 1a). Differences in plant species richness were not significant, however, because of the large within-vegetation type variation ( $F_{3,12} = 1.57$ ,  $p = 0.25$ ). For human-made grassland alterations, plant species richness in *Pinus* plantations was lower than in extensive pastures (the initial stage of succession;  $t_6 = 6.23$ ,  $p = 0.0008$ ) but did not differ from that of mature forests ( $t_4 = 0.60$ ,  $p = 0.58$ ). Plant species richness of abandoned vineyards did not differ from those of extensive pastures and abandoned grasslands with shrubs (in both cases,  $p > 0.5$ ; Figs. 1b-c).

Twenty-four gastropod species were recorded. The ANOVA indicated significant differences in species richness among the four successional stages ( $F_{3,12} = 6.68$ ,  $p = 0.0067$ ; Fig. 1a). Species richness was lower in the mature forest than in the preceding successional stages. Similarly, gastropod species richness was lower in the mature forest than in the *Pinus* plantation ( $t_4 = 2.85$ ,  $p = 0.047$ ; Fig. 1c). Abandoned vineyards, however, did not differ in gastropod species richness from the successional stages they were compared with (in both cases,  $p > 0.4$ ; Figs. 1b-c).

In all, 129 diurnal Lepidoptera species were recorded. The ANOVA revealed significant differences in species richness among the four successional stages ( $F_{3,12} =$



**Figure 1.** Species richness (mean  $\pm$  1 SE) of four groups of organisms in (a) different stages of grassland succession (ANOVA: vascular plants,  $F_{3,12} = 1.57$ ,  $p = 0.25$ ; terrestrial gastropods  $F_{3,12} = 6.68$ ,  $p = 0.0067$ ; diurnal Lepidoptera,  $F_{3,12} = 13.94$ ,  $p = 0.0003$ ; nocturnal Lepidoptera,  $F_{3,12} = 2.13$ ,  $p = 0.15$ ), (b) abandoned vineyards, and (c) *Pinus* plantations. Key: E, extensively grazed pasture; G, abandoned grassland; B, abandoned grassland with shrubs; W, mature forest; V, abandoned vineyard; A, *Pinus* plantation. In (a) different letters (a, b, c) indicate differences among successional stages (Scheffé's post hoc test,  $p < 0.05$ ). Vineyards (b) and *Pinus* plantations (c) were compared pairwise with two stages of natural succession (V with both E and B and A with both E and W) with unpaired *t* tests. In some groups of organisms the standard error is smaller than the symbol printed.

13.94,  $p = 0.0003$ ; Fig. 1a). Species richness was lower in mature forests than in extensive pastures and abandoned grasslands with shrubs. Abandoned vineyards and *Pinus* plantations did not differ in diurnal Lepidoptera species richness from the successional stages with which they were compared (in all cases,  $p > 0.09$ ; Figs. 1b-c).

A total of 408 nocturnal Lepidoptera species was recorded. Because of the huge variation within vegetation types, the four successional stages did not differ significantly in species richness ( $F_{3,12} = 2.13$ ,  $p = 0.15$ ; Fig. 1a). No difference was found between nocturnal Lepidoptera species richness in abandoned vineyards and those of extensive pastures and abandoned grassland with shrubs (in both cases  $p > 0.09$ ; Fig. 1b). Nocturnal Lepidoptera species richness in *Pinus* plantations, however, tended to be lower than those of extensive pastures ( $t_6 = 2.37$ ,  $p = 0.056$ ) and mature forests ( $t_4 = 2.65$ ,  $p = 0.057$ ; Fig. 1c).

#### Abundance of Invertebrates

A total of 2910 gastropod individuals was recorded. The four successional stages differed in gastropod abundance (Table 2). Gastropod abundance was significantly lower in the mature forest than in the other successional stages. Gastropod abundance of the abandoned vineyards did not differ from those of extensive pastures and abandoned grasslands with shrubs. Gastropod abundance was higher, however, in *Pinus* plantations than in mature forests.

We recorded 4565 diurnal Lepidoptera. There were significant differences among successional stages (Table 2). Diurnal Lepidoptera abundance was highest in abandoned grasslands with shrubs. In the mature forests, diurnal Lepidoptera abundance was lower than in the other successional stages. Abandoned vineyards and *Pinus* plantations did not differ in diurnal Lepidoptera abundance from the successional stages with which they were compared.

In all, 8963 individuals of nocturnal Lepidoptera were recorded. Nocturnal Lepidoptera abundance did not differ among successional stages (Table 2). Moth abundance in *Pinus* plantations, however, was lower than in extensive pastures and mature forests (Table 2).

#### Diversity

Plant diversity (Shannon-Wiener index) did not differ among the four successional stages ( $F_{3,12} = 1.37$ ,  $p = 0.30$ ). Furthermore, plant diversity did not differ among abandoned vineyards, extensive pastures, and abandoned grasslands with shrubs (unpaired *t* test, in both comparisons  $p > 0.13$ ). In contrast, *Pinus* plantations had lower plant diversity than extensive pastures ( $p = 0.0488$ ). The different successional stages did not differ in gastropod diversity ( $F_{3,12} = 2.73$ ,  $p = 0.09$ ). Gastropod diversity was lower, however, in abandoned vineyards than in extensive pastures ( $p = 0.0067$ ). Diurnal Lepidoptera diversity differed among the four successional stages ( $F_{3,12}$

**Table 2.** Number of individuals (mean  $\pm$  SE) of terrestrial gastropods and diurnal and nocturnal Lepidoptera recorded in the four stages of grassland succession and grassland altered by humans.\*

Vegetation type	Gastropods	Diurnal Lepidoptera	Nocturnal Lepidoptera
Grassland succession			
extensively grazed pasture (E) ( $n = 5$ )	132.4 $\pm$ 33.0a	211.6 $\pm$ 34.5a	408.0 $\pm$ 57.3
abandoned grassland (G) ( $n = 5$ )	192.2 $\pm$ 62.4a	172.8 $\pm$ 31.2a	549.4 $\pm$ 82.4
abandoned grassland with shrubs (B) ( $n = 3$ )	108.3 $\pm$ 66.8a	370.7 $\pm$ 69.7b	474.7 $\pm$ 42.2
mature forest (W) ( $n = 3$ )	3.7 $\pm$ 0.7b	55.0 $\pm$ 8.7c	360.7 $\pm$ 47.8
ANOVA, $F_{3,12}$	22.93	15.19	1.37
$p$	<0.0001	0.0002	0.30
Human-made grassland alterations			
abandoned vineyard (V) ( $n = 3$ )	220.7 $\pm$ 23.7	331.0 $\pm$ 44.6	414.3 $\pm$ 96.1
<i>Pinus</i> plantation (A) ( $n = 3$ )	96.3 $\pm$ 12.2	124.3 $\pm$ 47.4	142.3 $\pm$ 38.3
$p$ unpaired $t$ test			
V vs. E	0.14	0.09	0.99
V vs. B	0.15	0.69	0.52
A vs. E	0.64	0.16	0.012
A vs. W	<0.0001	0.31	0.044

\*Different letters (a, b, and c) in a column indicate significant differences (Scheffé's post hoc test:  $p < 0.05$ ) among successional stages.

= 10.78,  $p = 0.0010$ ). Mature forests had a lower diversity than abandoned grasslands with shrubs. Abandoned vineyards and *Pinus* plantations did not differ in diurnal Lepidoptera diversity from the successional stages they were compared with. Nocturnal Lepidoptera diversity differed among successional stages ( $F_{3,12} = 7.27$ ,  $p = 0.0049$ ). Moth diversity of extensive pastures was significantly lower than that of mature forests ( $p = 0.0162$ ). Furthermore, nocturnal Lepidoptera diversity of abandoned vineyards was lower than that of abandoned grasslands with shrubs ( $p = 0.0151$ ). Similarly, moth diversity of *Pinus* plantations was lower than that of mature forests ( $p = 0.0216$ ).

### Changes in Species Composition

As expected, the complementarity of species composition increased with successional stage in all taxonomic groups examined (Table 3), indicating a decreasing simi-

larity between seral stages of succession. Comparing the four taxonomic groups, complementarity was highest in vascular plants, followed by diurnal Lepidoptera and nocturnal Lepidoptera. In gastropods a similar complementarity was found among the first three stages of succession and was followed by a significant increase in complementarity in mature forests (Table 3).

Mean complementarity between abandoned vineyards and extensive pastures was similar to the comparison results among extensive pastures, abandoned grasslands, and abandoned grasslands with bushes (Table 3). Mean complementarity between *Pinus* plantations and extensive pastures was higher but did not reach the values of any comparisons with mature forests.

The results of the correspondence analysis confirmed the dissimilarity of mature forests. In all four taxonomic groups, the composition and abundance of species found in mature forests resulted in a distant and distinct cluster, whereas those of the other vegetation types formed a second, unstructured cluster (data not shown).

**Table 3.** Complementarity of species lists between vegetation types for each taxonomic group.<sup>a</sup>

Comparison <sup>b</sup>	Plants	Gastropods	Diurnal Lepidoptera	Nocturnal Lepidoptera	Mean $\pm$ SE
E-G	0.53	0.31	0.48	0.43	0.44 $\pm$ 0.05
E-B	0.62	0.44	0.52	0.41	0.50 $\pm$ 0.05
E-W	0.96	0.89	0.87	0.71	0.86 $\pm$ 0.05
G-B	0.63	0.38	0.53	0.40	0.49 $\pm$ 0.12
G-W	0.97	0.95	0.79	0.73	0.86 $\pm$ 0.06
B-W	0.94	0.81	0.86	0.67	0.82 $\pm$ 0.06
E-V	0.48	0.47	0.48	0.46	0.47 $\pm$ 0.005
E-A	0.62	0.38	0.62	0.66	0.57 $\pm$ 0.06

<sup>a</sup> Complementarity values range from 0 (identical species lists) to 1 (no species shared).

<sup>b</sup> Key: E, extensively grazed pasture; G, abandoned grassland; B, abandoned grassland with shrubs; W, mature forest; V, abandoned vineyard; A, *Pinus* plantation.

### Site Characteristics

The three environmental variables measured in the six vegetation types (Table 1) were significantly intercorrelated. The total area of the sites was positively correlated with their degree of isolation (Spearman rank correlation,  $r_s = 0.55$ ,  $n = 22$ ,  $p = 0.012$ ). Soil pH was negatively correlated with total area ( $r_s = -0.51$ ,  $n = 22$ ,  $p = 0.019$ ) because of the accumulating leaf litter in habitats with bushes and in mature forests, which represented the largest areas in our study. Leaf litter leads to a decrease in soil pH. Consequently, abundance of shelled gastropods decreased with decreasing soil pH ( $r_s = 0.50$ ,  $n = 22$ ,  $p = 0.023$ ). Species richness and abundance of diurnal Lepidoptera decreased with increasing area of the investigation sites ( $r_s = -0.44$ ,  $n = 22$ ,  $p = 0.044$  and  $r_s = -0.44$ ,  $n = 22$ ,  $p = 0.043$ ), mainly because of the large area of the species-poor mature forests. Abundance of nocturnal Lepidoptera decreased with decreasing soil pH ( $r_s = 0.55$ ,  $n = 22$ ,  $p = 0.011$ ). Plant species richness was not correlated with any of the environmental variables measured. Considering exclusively the four successional stages, site area was not correlated with successional age ( $r_s = 0.34$ ,  $n = 16$ ,  $p = 0.18$ ).

### Habitat Specificity

The four successional stages differed in the proportion of open-land species in three of the four taxonomic groups (plants:  $F_{3,12} = 23.08$ ,  $p < 0.0001$ ; gastropods:  $F_{3,12} = 24.25$ ,  $p < 0.0001$ ; nocturnal Lepidoptera:  $F_{3,12} = 52.02$ ,  $p < 0.0001$ ; diurnal Lepidoptera:  $F_{3,12} = 0.90$ ,  $p = 0.47$ ; Fig. 2). In plants and gastropods, the number of open-land species decreased with successional age (plants:  $r_s = -0.72$ ,  $n = 16$ ,  $p = 0.0055$ ; gastropods:  $r_s = -0.58$ ,  $n = 16$ ,  $p = 0.026$ ). *Pinus* plantations contained higher proportions of open-land species of plants ( $t_4 = 4.38$ ,  $p = 0.012$ ), gastropods ( $t_4 = 18.92$ ,  $p < 0.0001$ ), and noc-

turnal Lepidoptera ( $t_4 = 14.76$ ,  $p < 0.0001$ ) than mature forests (Fig. 2). *Pinus* plantations also had lower proportions of open-land species of plants ( $t_6 = 3.25$ ,  $p = 0.018$ ) and nocturnal Lepidoptera ( $t_6 = 4.73$ ,  $p = 0.003$ ) than extensive pastures. Abandoned vineyards did not differ from extensive pastures and abandoned grasslands with shrubs in the proportions of open-land species in any of the investigated taxonomic groups (Fig. 2; in all cases  $p > 0.09$ ).

### Red-Listed Species

The four successional stages differed in the proportion of threatened species (total of species considered as critically endangered, endangered, or vulnerable) in two of three taxonomic groups (no red list exists for gastropods; plants:  $F_{3,12} = 5.52$ ,  $p = 0.0129$ ; nocturnal Lepidoptera:  $F_{3,12} = 6.65$ ,  $p = 0.0068$ ; diurnal Lepidoptera:  $F_{3,12} = 0.94$ ,  $p = 0.45$ ; Table 4). No correlation between the proportion of threatened species and successional age could be found, however, in the groups examined (in all cases  $p > 0.23$ ). *Pinus* plantations and abandoned vineyards—the two human-made habitat alterations—did not differ in the proportion of red-listed species from the successional stages with which they were compared (Table 4). This was true for all three groups (in all cases  $p > 0.20$ ).

In plants, all seven threatened species were open-land species. In diurnal Lepidoptera, 11 (73.3%) of the 15 threatened species were open-land species, whereas the other 4 (26.7%) were forest species. In nocturnal Lepidoptera, 26 (81.3%) of the 32 threatened species were open-land species, 5 (15.6%) were forest species, and 1 (3.1%) was ubiquitous.

Critically endangered diurnal Lepidoptera species were recorded in all vegetation types. In nocturnal Lepidoptera, critically endangered species were recorded in only

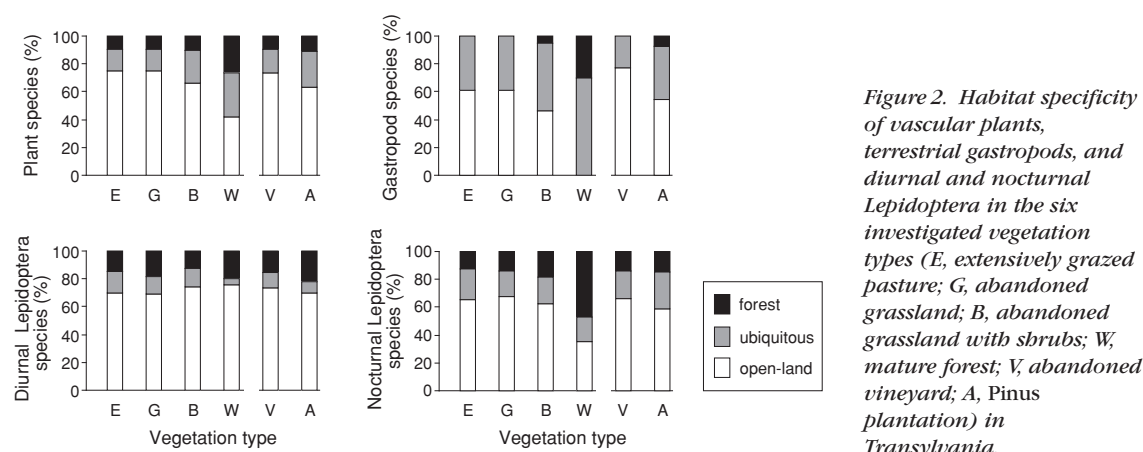


Figure 2. Habitat specificity of vascular plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera in the six investigated vegetation types (E, extensively grazed pasture; G, abandoned grassland; B, abandoned grassland with shrubs; W, mature forest; V, abandoned vineyard; A, *Pinus* plantation) in Transylvania.

**Table 4.** Number (mean  $\pm$  SE) of threatened species of vascular plants and diurnal and nocturnal Lepidoptera recorded in different vegetation types.

Vegetation type	Threat level*	Vascular plants (%)	Diurnal Lepidoptera (%)	Nocturnal Lepidoptera (%)
Extensively grazed pasture ( <i>n</i> = 5)	TRE	0.6 $\pm$ 0.4 (1.0)	5.0 $\pm$ 0.6 (16.4)	3.4 $\pm$ 1.2 (3.5)
	NT	0.4 $\pm$ 0.2 (0.7)	8.2 $\pm$ 1.0 (26.7)	9.8 $\pm$ 2.6 (10.4)
	LC	59.0 $\pm$ 1.6 (98.4)	17.0 $\pm$ 0.7 (56.8)	76.0 $\pm$ 5.8 (86.1)
Abandoned grassland ( <i>n</i> = 5)	TRE	1.8 $\pm$ 0.4 (3.4)	3.2 $\pm$ 0.6 (12.8)	9.4 $\pm$ 1.5 (8.0)
	NT	1.2 $\pm$ 0.2 (2.3)	6.2 $\pm$ 0.7 (25.7)	15.6 $\pm$ 2.1 (13.4)
	LC	50.2 $\pm$ 5.6 (94.2)	14.8 $\pm$ 1.2 (61.5)	90.4 $\pm$ 9.5 (78.6)
Abandoned grassland with shrubs ( <i>n</i> = 3)	TRE	0.7 $\pm$ 0.7 (0.9)	7.7 $\pm$ 0.9 (17.8)	6.0 $\pm$ 1.5 (4.7)
	NT	1.0 $\pm$ 0.6 (1.4)	10.7 $\pm$ 2.3 (24.0)	16.7 $\pm$ 2.3 (13.0)
	LC	59.7 $\pm$ 8.5 (97.7)	25.0 $\pm$ 2.1 (58.1)	106.0 $\pm$ 4.2 (82.3)
Mature forest ( <i>n</i> = 3)	TRE	0 $\pm$ 0 (0.0)	2.7 $\pm$ 0.3 (21.4)	1.3 $\pm$ 0.7 (1.0)
	NT	0.3 $\pm$ 0.3 (0.5)	3.3 $\pm$ 1.8 (20.6)	16.0 $\pm$ 3.5 (14.0)
	LC	44.0 $\pm$ 8.5 (99.5)	8.0 $\pm$ 2.0 (57.9)	96.0 $\pm$ 13.8 (84.9)
Abandoned vineyard ( <i>n</i> = 3)	TRE	1.3 $\pm$ 0.9 (2.1)	6.0 $\pm$ 0.6 (15.8)	4.3 $\pm$ 1.2 (4.4)
	NT	1.0 $\pm$ 0.6 (1.5)	9.0 $\pm$ 1.7 (22.6)	13.7 $\pm$ 2.0 (14.5)
	LC	60.3 $\pm$ 2.2 (96.4)	24.3 $\pm$ 3.8 (61.6)	76.7 $\pm$ 10.3 (81.1)
<i>Pinus</i> plantation ( <i>n</i> = 3)	TRE	0.7 $\pm$ 0.7 (1.6)	2.7 $\pm$ 0.9 (14.3)	1.3 $\pm$ 0.7 (2.1)
	NT	0 $\pm$ 0 (0.0)	5.0 $\pm$ 2.6 (18.7)	5.0 $\pm$ 4.0 (6.8)
	LC	37.3 $\pm$ 2.7 (98.4)	12.0 $\pm$ 3.5 (67.0)	49.7 $\pm$ 7.8 (91.1)

\*Key: TRE, threatened (red-listed categories critically endangered, endangered, and vulnerable); NT, nearly threatened; LC, least concern.

three vegetation types (extensively grazed pasture, abandoned grassland and mature forest), whereas no critically endangered plant species was found in any vegetation type.

#### Endemism

Four species endemic to Transylvania were recorded: three plant species (*A. peterfii*, *Onosoma pseudoarenaria* Schur, *S. transsylvanica*) and one moth (*Conisania poelli ostrogovici*). Grassland successional stages differed in their proportion of endemic plant species (total Transylvanian and Eastern European endemics;  $F_{3,12} = 9.94$ ,  $p = 0.0014$ ; Table 5). The proportion of endemic species, however, was not correlated with successional age ( $p = 0.18$ ). *Pinus* plantations had a higher proportion of endemic plant species than mature forests ( $t_4 = 5.19$ ,  $p = 0.0066$ ), but did not differ from extensively

grazed pastures ( $t_6 = 2.05$ ,  $p = 0.086$ ). Abandoned vineyards did not differ in their proportion of endemic plant species from the successional stage with which they were compared (in both cases  $p > 0.32$ ).

All three plant species endemic to Transylvania were open-land species. Thirty-three (89.2%) of the 37 Eastern European endemic plants we found were open-land species, 3 (8.1%) were forest species, and 1 (2.7%) was ubiquitous.

The moth endemic to Transylvania (*C. poelli ostrogovici*) was recorded in five study sites: one abandoned grassland, two abandoned grasslands with shrubs, and two abandoned vineyards. The occurrence of a single endemic moth species did not allow for statistical analyses. We found only two adventive plant species (*Prunus tenella* Batsch and *Fragaria viridis* Duchnese), which seem to play a minor role in the investigated vegetation types.

**Table 5.** Number (mean  $\pm$  SE) and percentage of endemic and widespread vascular plant species recorded in different vegetation types.

Vegetation type	Transylvania endemic (%)	Eastern Europe endemic (%)	Widespread (%)	Adventive (%)
Extensively grazed pasture ( <i>n</i> = 5)	0.2 $\pm$ 0.2 (0.3)	8.0 $\pm$ 0.9 (13.3)	50.8 $\pm$ 1.2 (84.8)	1.0 $\pm$ 0.0 (1.7)
Abandoned grassland ( <i>n</i> = 5)	0.4 $\pm$ 0.2 (0.9)	7.2 $\pm$ 1.1 (13.3)	44.6 $\pm$ 4.7 (84.0)	1.0 $\pm$ 0.3 (1.8)
Abandoned grassland with shrubs ( <i>n</i> = 3)	0.7 $\pm$ 0.7 (0.9)	8.7 $\pm$ 2.0 (13.9)	51.0 $\pm$ 7.6 (83.4)	1.0 $\pm$ 0.0 (1.7)
Mature forest ( <i>n</i> = 3)	0 $\pm$ 0 (0.0)	2.7 $\pm$ 0.9 (5.8)	41.7 $\pm$ 8.2 (94.2)	0 $\pm$ 0 (0.0)
Abandoned vineyard ( <i>n</i> = 3)	1.0 $\pm$ 0.6 (1.5)	8.7 $\pm$ 0.3 (13.9)	52.0 $\pm$ 2.5 (83.0)	1.0 $\pm$ 0.0 (1.6)
<i>Pinus</i> plantation ( <i>n</i> = 3)	0.3 $\pm$ 0.3 (0.8)	6.3 $\pm$ 0.7 (16.6)	30.3 $\pm$ 2.0 (80.1)	1.0 $\pm$ 0.6 (2.5)

### Correlations between Taxonomic Groups

Plant species richness was positively correlated with species richness of diurnal Lepidoptera ( $r = 0.59$ ,  $n = 22$ ,  $p = 0.0033$ ). No other correlations among the species richness of the investigated taxonomic groups were found.

The proportions of open-land species among plants, gastropods, and nocturnal Lepidoptera were highly intercorrelated (plant-gastropod:  $r = 0.81$ ,  $n = 22$ ,  $p < 0.0001$ ; plant-nocturnal Lepidoptera:  $r = 0.87$ ,  $n = 22$ ,  $p < 0.0001$ ; gastropod-nocturnal Lepidoptera:  $r = 0.89$ ,  $n = 22$ ,  $p < 0.0001$ ). The proportion of open-land diurnal Lepidoptera, however, was not correlated with that of any other group.

The proportion of threatened (sum of the red-listed categories critically endangered, endangered, and vulnerable) plant species was positively correlated with that of nocturnal Lepidoptera ( $r = 0.58$ ,  $n = 22$ ,  $p < 0.0038$ ), indicating that red-listed plant species are good indicators for threatened moths. No similar correlations were found between plants and diurnal Lepidoptera or diurnal and nocturnal Lepidoptera. The proportions of threatened plant and diurnal Lepidoptera species were not correlated with their own species richness (plant:  $r = 0.22$ ,  $n = 22$ ,  $p = 0.32$ ; diurnal Lepidoptera:  $r = -0.05$ ,  $n = 22$ ,  $p = 0.82$ ). In contrast, the proportion of threatened nocturnal Lepidoptera increased with increasing moth species richness ( $r = 0.53$ ,  $n = 22$ ,  $p = 0.0097$ ).

## Discussion

### Diversity in Seral Stages of Grassland Abandonment

Our results confirm the high plant and invertebrate species richness in the investigated steppe-like grasslands and their seral stages of abandonment. Both the extensively grazed grasslands and the successional stages investigated harbored unique plant and invertebrate communities. In contrast to previous studies on grassland succession in Central Europe (Bischof 1984; Zoller et al. 1984, Tasser & Tappeiner 2002), we found no decline in plant species richness in early successional stages. This result could be explained by a delayed succession due to the steppe-like character (dry soil, high insolation) of the examined grasslands and/or small-scale mosaic, abiotic conditions. The latter is correlated with the structural diversity of the habitat, which usually increases with successional age (Ellenberg 1996). A high structural diversity in turn favors the diversity of invertebrates. This might be the most important factor for the slight increase in species richness of diurnal and nocturnal Lepidoptera found in successional stages. For instance, the butterfly *Satyrrium acaciae* F. is a vulnerable species that was most abundant in abandoned grasslands with shrubs. This result confirms

findings from other studies that show that certain rare and threatened butterfly species benefit from grassland abandonment (Erhardt 1985a, 1985b; Thomas 1991; Balmer & Erhardt 2000).

Our results are consistent with the intermediate disturbance hypothesis in that plant and invertebrate diversities were indeed high in the investigated steppe-like grasslands. Lack of disturbance in abandoned successional stages, however, did not result in the expected decline in species diversity because of the reasons mentioned above.

Extensive cultivation of grapes was for many decades a widespread alternative of grassland use in Transylvania. In contrast to the present-day vineyards, which in most cases are intensively managed, the former extensive use of vineyards allowed for a diverse ground flora. Our results show that the abandonment of these vineyards created a valuable habitat for plants and invertebrates. In fact, abandoned vineyards did not differ in species richness and abundance in the four taxonomic groups from extensive pastures and abandoned grasslands with shrubs.

### Diversity in Mature Forests and Pine Plantations

In mature forests, the final stage of natural grassland succession, diversity of all groups of organisms examined was significantly reduced. Furthermore, many of the rare and threatened grassland species of plants and Lepidoptera were replaced by common mesophilous species because of the reduced light. The accumulated leaf litter in mature forests caused an increased soil acidity, which was followed by a shift in the composition of the ground vegetation. Only a limited number of snails can cope with the calcium carbonate deficiency of these soils. This resulted in a significantly reduced gastropod diversity in mature forests.

In the past decades, *Pinus* plantations were considered a more profitable land use than extensive grazing of the steppe-like grasslands in Romania. For these afforestations, *P. nigra*, a non-native species adapted to dry conditions, was used. In these plantations habitat quality for grassland species decreases with increasing tree age. Young plantations still contain strips of open grassland between the saplings. Growing trees increasingly shade these strips, which eventually disappear completely. Older *Pinus* plantations with closed canopies prohibit the growth of an herbaceous ground layer. The *Pinus* plantations we examined were 15–30 years old and still contained open, 2-m-wide strips of ground vegetation. This may explain the high level of diversity found in the investigated taxonomic groups. This diversity, however, is bound to decline in the near future for reasons cited above.

Because site area was not correlated with plant species richness, the larger sampling plots used in mature forests and *Pinus* plantations did not bias our results.



### Site Characteristics

The response of animal taxa to landscape variables is important for conservation planning. Landscape characteristics are significant predictors of species presence and abundance for several vertebrate taxa but not for the majority of invertebrates (reviewed in Mazerolle & Villard 1999). For invertebrates the structure of the habitat (composition, height, and structure of vegetation) might be important. Depending on the species-specific mobility and habitat requirement, the home range of invertebrate species can be very small (e.g., one or a few square meters in tiny gastropods; Baur & Baur 1988) or extend over a large area (e.g., several hectares in butterflies; Erhardt 1985b). Furthermore, it is often problematic to distinguish between the scale of a local patch and that of the landscape, especially when both are influenced by ongoing habitat alterations (Fahrig 2003). In our study, landscape-scale characteristics such as habitat area and minimum distance to the nearest equal habitat type had little effect on the species richness and abundance of the investigated invertebrate groups, whereas effects of patch scale variable (i.e., habitat characteristics, including the type of grassland management, abandonment, and afforestation) were pronounced. This result is consistent with other studies on invertebrates (Mazerolle & Villard 1999).

### Habitat Specificity, Red-Listed Species, and Endemism

Extensively grazed grasslands serve as refuges for numerous open-land species, which are of primary concern in Romania (Ioras 2003). In our study, the proportion of open-land species in plants and gastropods decreased with successional age, suggesting that succession has a detrimental effect on these groups.

A significant number of endangered and true endemic plant and invertebrate species had their abundance maxima in only one of the investigated open habitats. For example, the plant *O. pseudoarenaria* (a Transylvanian endemic and red-listed species) and the terrestrial gastropod *Vitrea transsylvanica* were most abundant in extensive pastures. Early stages of abandoned grassland were most favorable for the plants *S. transsylvanica* and *A. peterfii* (both Transylvanian endemic and red-listed species) and the lycaenid butterfly *Plebeius pylaon* (a red-listed species). In the successional stage with shrubs a few Lepidoptera species showed their maximum abundance, such as *Perconia strigilaria* and *Scotopteryx ignorata* (both red-listed species). Intriguingly, abandoned vineyards were also favored by some Lepidoptera such as the endangered Transylvanian endemic *C. poelli ostrogovici*.

### Correlations between Taxonomic Groups

The species richness of plants was positively correlated with that of diurnal Lepidoptera. We found no further

correlations among the species richness of the investigated taxonomic groups. Hawkins and Porter (2003) also reported a positive correlation between plant and butterfly diversity. They concluded, however, that plant diversity does not directly influence butterfly diversity but they both respond to similar environmental conditions. An attempt to correlate the diversities of vascular plants, butterflies, grasshoppers, gastropods, and carabid beetles showed that a single taxonomic group was a poor indicator for species diversity of other groups in nutrient-poor, dry grasslands in Switzerland (Baur et al. 1996; Niemelä & Baur 1998). Similarly, Lawton et al. (1998) found that none of the eight animal groups studied served as a good indicator taxon for changes in the species richness of other groups in a tropical forest. Thus in many cases one taxonomic group is a poor indicator for the overall diversity (Baur et al. 1996; Niemelä & Baur 1998). In contrast to these findings, butterflies are realistic and practical indicators of species change and decline in plants, other insects, and birds because of their rapid response to habitat change (Thomas et al. 2004). Regarding threatened species, we found that the proportions of red-listed species in plants and nocturnal Lepidoptera were correlated. No further correlations, however, were significant. Thus our study advocates a shopping-basket approach to the measurement of diversity and to conservation evaluation (i.e., measuring species richness and abundance of several taxonomic groups instead of just one [Launer & Murphy 1994; Oliver & Beattie 1996]).

### Implications for Conservation and Management

Our results emphasize the high conservation value of Transylvanian steppe-like grasslands and their seral stages of abandonment. These habitats harbor true endemics and a number of species that have dramatically declined in Europe and thus are highly threatened (e.g., the butterfly *Chazara briseis*). Consequently, steppe-like grasslands are also relevant as a potential source for the recolonization of restored grasslands in Eastern and Central Europe. Without appropriate management, however, these seminatural grasslands will convert to mature forest.

Each of the four taxonomic groups reacted differently to grassland abandonment and all investigated vegetation types contained threatened species. To maintain this extraordinary diversity, a mosaic consisting of extensively grazed, steppe-like grassland and the grassland's seral stages of succession should be preserved. This could be achieved by rotational management of the different successional stages. A similar approach has been proposed for nutrient-poor, seminatural grasslands in Central Europe (Duffey et al. 1974; Morris & Thomas 1991; Erhardt 1995). This approach would require a specific management plan to prevent losses of unique species, which happened in earlier conservation attempts in Western Europe (Thomas 1984, 1991).

Another important concern is the high degree of isolation of the remaining steppe-like grasslands. Dispersal of animals that do not fly, such as terrestrial gastropods, is often restricted. The agricultural landscape in Transylvania is increasingly dominated by intensively cultivated fields and fragmented by roads. These unsuitable habitats and linear traffic constructions act as barriers for nonflying animals (Baur & Baur 1990; Wirth et al. 1999). Conservation efforts should therefore also consider the isolation of these habitats by improving the connectedness between remnants of steppe-like grassland.

*Pinus* plantations represent a recent grassland alteration in Transylvania. The canopy of the investigated plantations was not yet closed, which allowed some open-land species to survive. As soon as the canopy of the growing trees closes, however, the unique plant and invertebrate species of the grassland will disappear (van Wesenbeeck et al. 2003). Therefore, new plantations should not be planted. Where possible, existing plantations should be cut and the original grassland restored. We hope our study not only increases knowledge of the uniqueness of the steppe-like grasslands in Transylvania but also raises the awareness of the responsible authorities to this uniqueness.

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## **Chapter 2**

# ***Steppe-Like Grasslands and their Abandonment in Romania: Highly diverse Butterfly and Moth Communities at Risk***

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## ***Steppe-Like Grasslands and their Abandonment in Romania: Highly Diverse Butterfly and Moth Communities at Risk***

### **Abstract:**

Extensively used grasslands have long been recognized as important habitats for maintaining biodiversity in agricultural areas in Europe. However, the role of abandonment of these grasslands on biodiversity is still less clear. Here, we investigated effects of abandonment and man-made habitat alterations of steppe-like grasslands on diurnal and nocturnal Lepidoptera communities in Transylvania, Romania. Steppe-like grasslands in Romania are of particular concern, since they are relic habitats harboring a high diversity of plants and invertebrates, including xerophilous specialists. Red List species and even true endemics.

In particular, we studied extensive pastures, three stages of abandonment (early successional stages, late successional stages with shrubs and mature forests) as well as abandoned vineyards and *Pinus* afforestations (man-made habitat alterations).

We recorded a total of 105 diurnal and 407 nocturnal Lepidoptera species in the 22 study sites. Species richness and diversity (Shannon-Wiener index) of diurnal Lepidoptera were high in extensive pastures and early successional stages, peaked in later successional stages with bushes and in abandoned vineyards and were low in forests and *Pinus* afforestations. This pattern was also reflected in xerophilous and Red List species, target groups of our study. In contrast to diurnal Lepidoptera, diversity of nocturnal Lepidoptera was also high in forests. Species richness, diversity, xerophilous and Red List species were not correlated between diurnal and nocturnal Lepidoptera.

Detrended correspondence analyses (DCA) and cluster analyses using Renkonen distances confirmed the observed patterns, but revealed in addition the significance of regional effects in the different vegetation types.

For conservation purposes, the best strategy would be to preserve a mosaic of all successional stages. This could be achieved with rotational management. Particularly relevant are later successional stages with bushes and abandoned vineyards. These habitats do not require intensive management, but further succession to forests must be prevented.

*Pinus* afforestations should be prevented wherever possible, and already planted *Pinus* trees removed to restore natural successional stages, as most species still present in *Pinus* afforestations will disappear when the canopy of these afforestations closes.

**Key words:** habitat alteration, succession, diversity, xerophilous species, Red List species, diurnal and nocturnal Lepidoptera

## Introduction

In Europe traditional ways of grassland cultivation have been in use for hundreds of years, and have resulted in a high diversity of plants and invertebrates, particularly so in nutrient-poor, semi-natural grasslands (Zoller et al. 1986; Baur et al. 1996; Wilmanns 1998). Dry, nutrient-poor grasslands are generally considered the grassland biotope with the highest conservation value and key habitats for maintaining biodiversity in agricultural areas in Europe (Blab & Kudrna 1982; Bignal & McCracken 1996; Poschlod and WallisDeVries 2002; Sanchez-Zapata et al. 2003). As man-made habitats, created by deforestations since Roman times, they harbor numerous species whose primordial habitats (floodplains, peatlands, and rocky outcrops) have been vastly destroyed (Zoller 1954; Erhardt 1985a,b; Baur et al. 1997, 2004). However, semi-natural grasslands are fragile habitats because their maintenance depends on traditional farming techniques.

Until 50 years ago, most of this grassland had been managed in a similar manner. However, since the Second World War there has been an industrialization of agriculture. Increasing pressure for higher yields and the rising price of labor led to significant habitat alterations, first in the richer Western European nations, and later also in poorer countries when they joined the EEC. This socio-economic change is now also spreading through Eastern Europe.

As a result, large areas of semi-natural grassland were lost due to intensified use (increased stocking rate and/or application of artificial fertilizers) or abandonment (Erhardt 1985a; Groombridge 1992; Antognoli et al. 1995). Whereas detrimental effects of fertilization on the species diversity of plants and invertebrates are well documented (e.g. Rosenzweig 1995; Ellenberg 1996), studies regarding effects of grassland abandonment on biodiversity are still scarce, and those that exist are contradictory. Surveys carried out in Western Europe showed a decrease in plant diversity of abandoned semi-natural grasslands (Bischof 1981; Tasser & Tappeiner 2002; Dullinger et al. 2003). Similarly, studies focusing on threatened butterfly species in Britain indicated that grassland abandonment is the major cause of the butterfly decline (Thomas 1991). However, in investigations of entire butterfly communities, Erhardt (1985a, b) and Balmer and Erhardt (2000) found that several threatened species benefit from advanced stages of abandonment. Hence there is an urgent need for information on how important these habitats are for biodiversity,

Romania has an extraordinarily high level of species diversity with a total of 228 endemic and sub-endemic species (ICAS 1996; Ioras 2003). Reasons for this high biodiversity include the various biogeographical regions in Romania, the high habitat diversity and the locally low intensity of agricultural land use. However, the altered socio-economic situation since 1989 has caused a decrease of extensively grazed or mown semi-natural grasslands, due to intensification and/or abandonment as in Western European countries. This trend has even been accelerated at present, as Romania is due to enter the EEC, and continuing economic pressure accompanying the current development will result in further reduction of the traditional agricultural practices still used in remote areas. Of particular concern are the Transylvanian steppe-like grasslands that harbor a variety of plant and insect species that colonized this habitat from continental



steppes (in Russia) during postglacial warm periods (Rakosy & Kovacs 2001; C.C., unpublished data). The subsequent isolation during colder periods resulted in several endemic species such as the plants *Astragalus peterfii* Jav. and *Salvia transsilvanica* Schur and the butterfly *Pseudophilotes bavivus hungahcus* Drandt (Rakosy & Laszloffy 1997; Rakosy 1999; Rakosy & Kovacs 2001).

Despite the high conservation value of Transsylvanian steppe-like grasslands (Rakosy & Kovacs 2001), effects of their abandonment on biodiversity have not been examined until recently (Cremene et al. 2005). Here we compare the butterfly and moth faunas of 22 sites in four different successional stages, from extensively grazed, steppe-like pastures to woodland. We included also habitats altered by human activity, i. e. abandoned vineyards and *Pinus* plantations. In the past two decades, large areas of semi-natural grasslands were afforested with *Pinus nigra* Arnold, which presumably threatens specialized xerophilous steppe species, a target group of our study.

Diurnal Lepidoptera are a well established group of bioindicators as they react quickly to environmental change (e. g. Erhardt 1985a,b; Thomas et al. 2004). Correlations between the diversities of Lepidoptera, birds and vascular plants support this approach (Erhardt & Thomas 1991, Swengel and Swengel 1999), although significant deviations from this expectation have also been reported (e.g. Erhardt 1985a, b) and other studies showed that one taxonomic group is a poor indicator for overall diversity (Baur et al. 1996, Niemelä and Baur 1998, Lawton et al. 1998). Nevertheless, diurnal Lepidoptera, and butterflies in particular, are a charismatic group, which makes the results usable for public discussion and political action. In contrast, much less is known about moth communities.

Thus, our aim was to investigate effects of abandonment and man-made habitat alterations on butterfly and moth communities in steppe-like grasslands in Romania. In particular, we addressed the following questions: (1) Do different successional stages differ in species richness and diversity of butterflies and moths? (2) Do different stages support distinct butterfly and moth communities? (3) How do man-made habitat alterations (abandoned vineyards and *Pinus* plantations) affect these communities? (4) How are characteristic xerophilous and Red List species affected by abandonment and man-made habitat alterations?

## Methods

### Study Sites

We assessed butterfly and moth diversities in 22 areas (Table 1) surrounding four Transylvanian villages (Caianu, Fanate, Suatu and Viisoara) in Romania (46° 33' - 46° 48' N, 23° 36' - 23° 57' E, elevations between 270 and 400 m asl). The villages were situated 3-40 km apart. Within a village, the study sites were separated by 0.2-3 km. All sites faced south southeast, south, or south southwest and had similar slopes. Total annual rainfall in Cluj-Napoca (10-30 km apart the field sites) ranged from 383 to 1401 mm (mean 674 mm) with an annual mean temperature of 9.3° C (data from 1997-2003; C.C., unpublished data).

The following vegetation types were considered: extensively grazed pasture (E,  $n = 5$  study sites), early stage of abandoned grassland (G,  $n = 5$ ), later stage of abandoned grassland with shrubs (B,  $n = 3$ ), climax forest (W,  $n = 3$ ), abandoned vineyard (V,  $n = 3$ ), and *Pinus* afforestation (A,  $n = 3$ ). The first four habitat types represent seral stages of natural grassland succession. Extensive grazing means a low stocking rate of sheep or cattle for periods of 5-10 days one to three times per year. Abandoned vineyards represent a successional stage of former land-use practice. On these slopes grapes were cultivated in an extensive way; the soil was hoed, but not tilled and no fertilizer was used. *Pinus* afforestations represent a recent habitat alteration. The afforestations are not thinned and no fertilizer is used. At each site the age of the successional stage was determined. However, in climax forests only the age of the trees was known, indicating a minimum age for the forests.

## Surveys

From spring to fall 2001, we surveyed diversity and abundance of diurnal and nocturnal Lepidoptera in a 50 x 50 m plot at each site. The positions of the plots were chosen so that the distance between habitats exceeded 25 m (in one case 10 m).

We used a transect method (Douwes 1976; Hall 1981) to survey each plot for diurnal Lepidoptera. Surveys were conducted once every second week between 21 May and 9 September 2001. We walked plots in a serpentine pattern, 5-m wide. In this way the entire area was covered once by the butterfly net. Transects were surveyed only under good weather conditions (sunny, temperature  $> 18^{\circ}$  C and wind-speed  $< 16$  km/h (Beaufort scale 3)) between 900 and 1700 hours. We recorded all species of Rhopalocera, Hesperidae, and Zygaenidae. For Bombyces, Sphingae, Noctuidae and Geometridae, we recorded all day-active species and all other species easily surveyed with the transect method. Multiple counting of the same individuals could not be completely avoided. This error will not affect our interpretations, because we compared the plots rather than species; thus the error would be the same for each plot. The sequence in which transects were surveyed was alternated to avoid systematic effects of time of day.

We surveyed nocturnal Lepidoptera between 21 May and 12 October 2001. Moths were collected using a light trap (8 W UV-tubes fixed to the top of black buckets covered by white funnels and fed by a 12V accumulator) once a month in each plot. We did not survey on nights with a full moon. In their flight toward the light moths strike the transparent baffles that surround the lamp and fall through the funnel into the bucket (Rakosy 1999). Light traps were placed in the same place on each sampling occasion and were left on from dusk to dawn. We identified and released specimens in the morning. Identification of Lepidoptera followed Tolman & Lewington (1997) for butterflies, Forster & Wohlfahrt (1981), Rakosy (1996), and de Freina & Witt (1987, 1990, 2001) for moths. Problematic species were collected and identified by specialists through examination of genitals.

## Data Analyses

In all analyses we considered the study sites as the unit of investigation. Species richness (overall xerophilous species, Red List species) as well as species diversity (using the Shannon-Wiener index; Magurran 2003) was compared between habitat types using one-way ANOVAs and Fisher's PLSD post hoc tests with the program StatView (Abacus Concepts 1998). We compared pairs of successional stages. Abandoned vineyards and *Pinus* afforestations were compared with the initial stage (extensively grazed pasture) and with the most similar vegetation type of natural succession (abandoned grassland with shrubs and climax forest, respectively).

To investigate differences in species composition and abundance between the different vegetation types, we further analyzed the data by classification and ordination methods. We performed a detrended correspondence analysis (DCA) separately for diurnal and nocturnal Lepidoptera. Prior to ordination, the data were ln-transformed. Species that were less frequent than the median frequency were down-weighted in proportion to their frequencies (Eilertsen et al. 1990). DCA was performed using CANOCO version 4.5 (ter Braak and Smilauer, 2002).

To confirm and refine the results obtained by DCA, we performed cluster analyses by calculating pairwise Renkonen distances (Renkonen 1938; Krebs 1999) between all sites and drawing the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) trees (Sneath & Sokal 1973).

In a last step, we compared the lists of species abundances qualitatively to investigate the effects of succession on single species or groups of species. For each species we calculated the average abundance per vegetation type. Differences of abundance of single species in different habitat types were assessed with t-Tests on  $\log(x+1)$  transformed data. Differences were classified either as very strong (shown as '«' or '»', equivalent to  $p < 0.01$ ), strong (shown as '<' or '>', equivalent to  $p < 0.05$ ), weak (shown as '≤' or '≥', equivalent to  $p < 0.10$ ) or no difference (equivalent to  $p > 0.10$ ). This comparison is descriptive. We assigned all species, except for those recorded only once, to the vegetation types of most frequent occurrence, and defined them as 'characteristic' for these vegetation types.

Target groups of our study were xerophilous and Red List species. Species were characterized as xerophilous according to Forster & Wohlfahrt (1981), Blab & Kudrna (1982) and Rakosy & Laszloffy (1997) for diurnal Lepidoptera, and Rakosy (1996), Rakosy & Laszloffy (1997) and de Freina & Witt (1987, 1999, 2001) for nocturnal Lepidoptera. In Romania, Red Lists exist for diurnal and nocturnal Lepidoptera (Rakosy et al. 2003). In our analyses, we summarized all Red List categories (critically endangered, endangered, vulnerable and nearly threatened species).

We used Spearman Rank correlations to examine possible correlations between diurnal and nocturnal Lepidoptera and between species richness, xerophilous and Red List species.

## Results

### Species Richness and Diversity

A total of 4531 diurnal and 8963 nocturnal Lepidoptera belonging to 105 and 407 species respectively, was recorded during the study period.

Species richness of diurnal Lepidoptera differed significantly between the investigated habitats (Table 2,3). Differences between all successional stages were significant except for grazed pastures (E) and early stages of abandonment (G). Interestingly, later successional stages with bushes (B) had the highest species richness. Abandoned vineyards (V) were almost as rich in species as these stages, and tended to harbor more species than extensive pastures. In contrast, species richness in *Pinus* afforestations (A) was significantly lower than in extensive pastures and did not differ from climax forests (W). Diversity (Shannon-Wiener index) showed a similar pattern as species richness (Table 2,3), except that the difference between grazed meadows and successional stages with bushes was not significant.

The pattern for nocturnal Lepidoptera was clearly different from and not correlated with that observed for diurnal Lepidoptera (Spearman rank correlation  $r_s = 0.024$ ,  $n = 22$ ,  $P = 0.91$ ; Table 2,3). We detected a lower number of significant differences in the species richness of moths between the different successional stages, and they concerned different comparisons. For instance, grazed meadows tended to be less species rich than early successional stages, species richness in abandoned vineyards also tended to be lower than in successional stages with bushes and, interestingly, *Pinus* afforestations harbored significantly less moth species than forests. These differences are even more pronounced in the diversity (Shannon-Wiener index) of the investigated communities. Note that species richness and diversity of moths again peaked in later successional stages with bushes.

### Xerophilous and Red List Species

The number of diurnal xerophilous Lepidoptera species was correlated with the number of other, non-xerophilous species in the different vegetation types ( $r_s = 0.832$ ,  $n = 22$ ,  $P < 0.001$ ; Table 2,3). It peaked in abandoned vineyards and was lowest in forests (Table 2,3). It was also high in later successional stages with bushes and again low in *Pinus* afforestations. Interestingly, the pattern of xerophilous moth species was not correlated with that of diurnal Lepidoptera ( $r_s = 0.164$ ,  $n = 22$ ,  $P = 0.45$ ; Table 2,3). However, also in moths the number of xerophilous species tended to correlate with the number of the other recorded moth species ( $r_s = 0.361$ ,  $n = 22$ ,  $P = 0.098$ ; Table 2,3). Nocturnal xerophilous species peaked in early successional stages.

The number of diurnal Red List species was also correlated with the number of other recorded species ( $r_s = 0.739$ ,  $n = 22$ ,  $P < 0.001$ ; Table 2,3), and this correlation was also observed for moths ( $r_s = 0.810$ ,  $n = 22$ ,  $P < 0.001$ ; Table 2,3). Again the number of diurnal and nocturnal Red List species was not correlated ( $r_s = 0.098$ ,  $n = 22$ ,  $P = 0.65$ ).

## Community Resemblance

In the detrended correspondence analysis (DCA, Fig 1a) of diurnal Lepidoptera, the first axis (Eigenvalue = 0.302) explained 22.2% of the variance, axes 1 and 2 (Eigenvalue = 0.091) together explained 28.9% of the variance. Four clusters can be distinguished in the plot. The first cluster (lower right) comprises the investigated forests, the second (middle to upper left) the *Pinus* afforestations, the third (upper left) later successional stages with bushes, and the fourth (lower left) extensive pastures, early successional stages and abandoned vineyards. For nocturnal Lepidoptera, the first axis (Eigenvalue = 0.348) of the DCA explained 21.1% of the variance, and axes 1 and 2 (Eigenvalue = 0.109) together 27.7% (Fig. 1b). Regarding succession, nocturnal Lepidoptera separated into three groups only, i. e. forests (right), *Pinus* afforestations (middle) and the remaining habitats (left). However, within this last group, there was a tendency for clustering according to locality. Thus, the sites investigated at Suatu clearly clustered together (upper left), whereas study sites of Caianu and Viisoara were less clearly separated (lower left).

The dendrogram of diurnal Lepidoptera produced by the cluster analysis using Renkonen distances (Fig. 2) clearly separated the different habitat types, except for abandoned vineyards and extensive pastures. In contrast, the dendrogram of nocturnal Lepidoptera (Fig. 3) revealed a pattern clearly distinct from that of diurnal Lepidoptera. Whereas forests and *Pinus* afforestations formed each again a distinct group, all other investigated sites clustered in the first place according to geographic area, i. e. the villages Viisoara, Suatu and Caianu. Within Viisoara and Caianu there was then a clear separation according to vegetation type. This was somewhat less clear for Suatu, where only the later successional stage with bushes was clearly separated from the other investigated sites (extensive pastures, early successional stages and abandoned vineyards).

## Species Reactions

Each vegetation type was characterized by the maximum abundance of several diurnal Lepidoptera species (Table 4,5). Even forests and *Pinus* afforestations were characterized by a few peaking species. However, successional stages with bushes and abandoned vineyards were clearly the habitats in which most species had their maximum abundance, whereas in extensive pastures and early successional stages the number of peaking species was lower. Xerophilous and Red List species again paralleled this pattern. Note the high number of Red List species among the species which were only recorded once and hence not assigned to a particular habitat.

A complete comparable list for moths (407 spp.), as given for diurnal Lepidoptera (Table 4), would exceed the scope of this paper (a list is available on request from the authors). The number of characteristic species peaking in the different investigated habitats parallels species richness of moths, and so does the number of peaking xerophilous and Red List species (Table 5).

## Discussion

### Species Richness and Diversity

Species richness and diversity of diurnal Lepidoptera were similar in extensive pastures and early successional stages, and peaked in later successional stages with bushes, a similar finding as in a comparable study in the Swiss Jura mountains (Balmer and Erhardt 2000). Interestingly, abandoned vineyards were also highly diverse in diurnal Lepidoptera, whereas diversities in *Pinus* afforestations and climax forests were low.

Nocturnal Lepidoptera showed less differences in species richness and diversity, but also peaked in later successional stages, and were least diverse in *Pinus* afforestations. In contrast to diurnal Lepidoptera, moths were also highly diverse in forests. This pattern was likely caused by the many moth species feeding on trees and shrubs in their larval stage, in contrast to butterflies, whose larvae rather feed on herbs.

Although species richness and diversity of diurnal and nocturnal Lepidoptera were not correlated, our study shows that for both groups later successional stages with shrubs and abandoned vineyards are the most diverse and hence the most valuable habitat types. These results also confirm earlier studies on butterfly communities (Balmer and Erhardt 2000, Erhardt 1985), but contradict the famous findings from Britain, where abandonment of extensively cultivated grassland was and still is a major cause for the strong decline in butterflies (Thomas 1991).

### Xerophilous and Red List Species

The pattern observed for species richness and diversity was also reflected by xerophilous and Red List species, target groups of our study. As most recorded xerophilous species are also on the Red List (diurnal Lepidoptera: 84%, nocturnal Lepidoptera: 68%; Rakosy & Laszloffy 1997), patterns found for xerophilous species are bound to be paralleled by patterns of Red List species, although xerophilous species account for lower fractions of all recorded Red List species (diurnal Lepidoptera: 38%, nocturnal Lepidoptera: 16.5%; Rakosy et al. 2003). The only major deviance between xerophilous and Red List species concerned nocturnal Lepidoptera in forests, where Red List species paralleled total species richness, whereas xerophilous species in forests are naturally rare. Note that xerophilous as well as Red List species of diurnal Lepidoptera did again peak in later successional stages and in abandoned vineyards. These peaks were also recorded for moths, but interestingly, xerophilous as well as Red List species of moths showed their highest richness in early successional stages, emphasizing also the significance of this successional stage for conservation.

### Communities

Both detrended correspondence analyses (DCA) and classification after Renkonen revealed comparable patterns. Regarding the investigated

vegetation types, they showed better resolutions for diurnal than for nocturnal Lepidoptera. However, forests and *Pinus* afforestations remained well separated in both groups.

In contrast to diurnal Lepidoptera, the Renkonen classification of nocturnal Lepidoptera showed another important feature, as it clustered successional stages and abandoned vineyards according to locality in the first place, whereas the successional pattern appeared less clearly and only within each locality. This shows the importance of regional as opposed to local factors for the recorded moth communities.

## Species Level

A high proportion of the recorded Lepidoptera species (> 75% for both, diurnal and nocturnal Lepidoptera) had a specific abundance maximum in one particular vegetation type, and was hence characteristic for this vegetation type. All vegetation types, including even *Pinus* afforestations, harbored characteristic species peaking in abundance (Table 4,5). Although the number of characteristic diurnal Lepidoptera species was rather low in extensive pastures and early successional stages, these habitats contained some species of high conservation value in Western European countries (e. g. *Pyrgus carthami*, *Glaucopsyche atexis*) as well as in Romania (e. g. *Pseudophilotes baton*, *Penthopthera morio*, *Colias chrysotheme*; Rakosy et al. 2003).

The number of characteristic nocturnal Lepidoptera species was particularly high in early successional stages, a parallel to xerophilous and Red List species.

Altogether, the pattern of species characteristic for particular vegetation types (including characteristic xerophilous and Red List species) confirms the high value of steppe-like grasslands and their successional stages, including abandoned vineyards. Furthermore, many of these characteristic species may require a specific successional stage for their long term survival.

As there were relatively few generalists, the observed species reactions confirm that diurnal Lepidoptera react strongly to environmental change and hence are good indicators of habitat change. This certainly applies also to moths. They are, however, harder to monitor, yet may be better indicators for forests. This rather unexplored area may be more relevant in the future, since forests, have become intensively managed in many areas during the last decades, despite a lesser demand for fire wood.

A particularly relevant example for habitat specificity is the Lycaenid *Pseudophilotes bavius hungaricus* (Rakosy 1999), endemic to steppe-like habitats of Romania. Although we missed it in our regular transects due to its early flight period, additional surveys showed its occurrence in early successional stages and abandoned vineyards near the village Suatu. In these sites, its larval host, *Salvia transsilvanica*, also occurs. *Pseudophilotes bavius hungaricus* is highly specialized to and dependent on steppe-like habitats, as is its larval host plant. It therefore needs special attention and care.

Another example for a species requiring special attention is the Hesperiid *Muschampia cribrellum*. This species is typical for the steppe-like grasslands of Transylvania (Rakosy and Goia 1997), which represent the westernmost limit of its distribution. It is critically endangered in Romania, the present populations being relicts and highly isolated (Rakosy et al 2001, Rakosy et al. 2003). This

species had its maximum occurrence in abandoned vineyards, where also its xerophilous larval hosts, *Potentilla cinerea* and *Potentilla recta*, occurred. A similar example to *Muschampia cribrellum*, but of lesser concern for Romania, is *Chazara briseis*, a highly threatened species in Western Europe.

## Conservation Implications

Our study confirms the high conservation value of Transylvanian steppe-like grasslands and their successional stages, including abandoned vineyards. These habitats are all host to many specific, xerophilous and Red List species and a number of species that have dramatically declined and are thus highly threatened in Europe (e. g. *Chazara briseis*). Consequently, the investigated habitat types are also relevant as potential recolonization sources for areas, where these threatened species have already been lost.

Our study also shows that later successional stages with bushes and abandoned vineyards are particularly important habitats for diurnal Lepidoptera as well as for moths, and confirms earlier investigations which have emphasized the importance of later successional stages for conservation (Erhardt 1985, Balmer and Erhardt 2000). This seemingly contradicts findings from Britain, where abandonment of grasslands has been identified as a major threat to many endangered butterfly species and has even led to the extinction of *Macutinea arion* (Thomas 1998). However, the situation in Britain is special, as many (most) of the threatened butterfly species occur at the northern border of their range in Britain, and hence abandonment critically deteriorates the microclimatic conditions required for these species, whereas further south the increase of structural diversity and the lack of disturbance in abandoned grasslands actually favors many endangered species (Balmer and Erhardt 2000; this study).

As expected, forests were species poor in diurnal Lepidoptera, and particularly also in xerophilous species. However, they also harbored a high number of moths, including Red List species. As forests are not particularly threatened at present, they are of lesser concern for conservation. This may, however, change in the future and may cause additional conservation problems.

Altogether, our study confirms earlier conclusions regarding conservation strategies. The best strategy would be to preserve a mosaic of all successional stages, including abandoned vineyards. This could be achieved with a rotational management, as has also previously been suggested for other regions (Duffey et al. 1974; Morris & Thomas 1991; Erhardt 1995). Particularly relevant are later successional stages with bushes and abandoned vineyards with their terraces and little stone walls, structures which are particularly valuable for xerophilous species. These habitats do not require intensive management. However, further succession to forests must of course be prevented, as otherwise all specific species, particularly also xerophilous and Red List species, would be lost.

In contrast, *Pinus* afforestations should be prevented wherever possible. Diversity in *Pinus* afforestations is already low, and most of the recorded species were found in the open corridors between the growing trees. As the trees grow older, these corridors will disappear and the diversity of these plantations will further decrease. Hence, where possible, already planted *Pinus* trees should be removed to restore natural, later successional stages or possibly even extensive pastures.

The Renkonen plot of moths (Fig. 3) revealed a further important, but often



neglected aspect for conservation, i. e. regionality. Even though the investigated areas were not situated all that far from each other (3-40 km), their moth communities were clearly distinct. Also in the plots for diurnal Lepidoptera, study sites of the different investigated areas tended to cluster together (Figs. 1a, 2). This shows that regional aspects even on a smaller geographical scale must not be neglected for conservation purposes.

We hope this study alerts authorities responsible for the persistence of these unique habitats, particularly under the present socio-economic conditions, as Romania is about to join the EEC.

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**Table 1. Characteristics of the study sites in the Transylvanian steppe-like hills.**

Habitat type	Study site <sup>a</sup>	Land use	Code
Extensively grazed pasture (E)	V	Extensively grazed by sheep	EV1
	S	Extensively grazed by cattle	ES2
	S	Formerly extensively grazed by cattle, nowadays partly grazed by sheep and partly mown	ES3
	C	Extensively grazed by sheep	EC4
	C	Extensively (each second or third year) grazed by sheep	EC5
Abandoned grassland (G)	V	Abandoned for 6-8 years	GV1
	V	Abandoned for 6-8 years	GV2
	S	No regular management (mowing and grazing) for 70 years, abandoned for 3-5 years	GS3
	S	Abandoned for 10-15 years	GS4
	K	Extensively grazed by cattle and sheep during past 70 years, abandoned for 5-10 years	GK5
Abandoned grassland with shrubs (B)	V	Abandoned for 15 years	BV1
	S	Abandoned for 30 years	BS2
	S	Abandoned for 10-15 years	BC3
Climax forest (W)	V	50-80 year-old wood	WV1
	V	50-80 year-old wood	WV2
	S	50-100 year-old wood	WS3
Abandoned vineyard (V)	S	Abandoned for 30 years	VS1
	S	Abandoned for 30 years	VS2
	S	Abandoned for 30 years	VS3
<i>Pinus</i> afforestation (A)	V	30 year-old, little ground vegetation	AV1
	C	15 year-old; strips of <i>Pinus</i> separated by planted <i>Ligustrum</i> rows	AC2
	C	15 year-old	AC3

<sup>a</sup> C = Caianu, K = Fanate, S = Suatu, V = Viisoara

Table 2. Means ( $\pm$  SE) of species richness, diversity (Shannon-Wiener index), number of xerophilous and Red List species of diurnal (Di.L.) and nocturnal Lepidoptera (Noc.L.) in the different investigated habitats (E, extensively grazed pasture; G, abandoned grassland; B, abandoned grassland with shrubs; W, climax forest; V, abandoned vineyard; A, *Pinus* plantation)

	Number of species $\pm$ SE			Diversity $\pm$ SE			Number of xerophilous species $\pm$ SE			Number of Red-List species $\pm$ SE		
	Di.L.	Noc.L.	Di.L.	Noc.L.	Di.L.	Noc.L.	Di.L.	Noc.L.	Di.L.	Noc.L.	Di.L.	Noc.L.
E (n=5)	29.2 $\pm$ 1.5	89.2 $\pm$ 8.6	2.55 $\pm$ 0.12	3.82 $\pm$ 0.10	3.2 $\pm$ 0.4	8.0 $\pm$ 1.8	7.8 $\pm$ 0.7	13.2 $\pm$ 3.7				
G (n=5)	22.8 $\pm$ 1.6	115.4 $\pm$ 12.9	2.42 $\pm$ 0.08	4.17 $\pm$ 0.09	2.0 $\pm$ 0.9	15.6 $\pm$ 1.4	6.2 $\pm$ 1.0	25.0 $\pm$ 3.5				
B (n=3)	40.3 $\pm$ 5.0	128.3 $\pm$ 3.5	2.94 $\pm$ 0.13	4.38 $\pm$ 0.06	5.3 $\pm$ 1.3	11.0 $\pm$ 1.5	13.3 $\pm$ 2.0	22.7 $\pm$ 0.9				
W (n=3)	11.0 $\pm$ 2.9	113.3 $\pm$ 17.4	1.56 $\pm$ 0.10	4.40 $\pm$ 0.15	1.0 $\pm$ 0.6	2.0 $\pm$ 0.6	3.3 $\pm$ 1.5	17.3 $\pm$ 3.9				
V (n=3)	37.7 $\pm$ 4.4	94.3 $\pm$ 12.8	2.72 $\pm$ 0.10	3.92 $\pm$ 0.10	6.0 $\pm$ 0.6	10.7 $\pm$ 2.4	13.3 $\pm$ 1.2	18.0 $\pm$ 3.2				
A (n=3)	18.7 $\pm$ 6.9	56.0 $\pm$ 12.2	1.99 $\pm$ 0.48	3.59 $\pm$ 0.17	1.7 $\pm$ 0.9	4.0 $\pm$ 1.2	4.3 $\pm$ 1.2	6.3 $\pm$ 4.5				

Table 3. Probability values of analyses of variance (ANOVA) and post-hoc comparisons of species richness, diversity (Shannon-Wiener index), xerophilous and Red List species of diurnal (Di.L.) and nocturnal (Noc.L.) Lepidoptera communities in the investigated habitats (E, extensively grazed pasture; G, abandoned grassland; B, abandoned grassland with shrubs; W, climax forest; V, abandoned vineyard; A, *Pinus* plantation).

	Overall ANOVA		Post-hoc comparisons											
			Pairs of successional stages						Pairs of habitat alterations					
			E,G 1,2	E,B 1,3	E,W 1,4	G,B 2,3	G,W 2,4	B,W 3,4	V,E	V,B	A,E	A,W		
Species richness Di.L.	F 8.956	P 0.0003	0.146	0.035	0.002	0.002	0.027	<0.001	0.099	0.628	0.044	0.175		
Species richness Noc.L.	3.971	0.0156	0.092	0.034	0.172	0.445	0.904	0.438	0.765	0.091	0.067	0.008		
Diversity Di.L.	6.222	0.0022	0.572	0.157	0.002	0.065	0.004	<0.001	0.542	0.452	0.044	0.161		
Diversity Noc.L.	7.665	0.0008	0.017	0.002	0.002	0.200	0.156	0.900	0.508	0.018	0.167	<0.001		
Xerophilous species Di.L.	5.512	0.0039	0.237	0.077	0.069	0.009	0.388	0.003	0.025	0.604	0.193	0.604		
Xerophilous species Noc.L.	8.966	0.0003	0.002	0.220	0.021	0.068	<0.001	0.004	0.274	0.901	0.108	0.458		
Red List species Di.L.	11.101	<0.0001	0.287	0.005	0.017	<0.001	0.107	<0.001	0.005	0.999	0.055	0.601		
Red List species Noc.L.	3.336	0.0297	0.018	0.085	0.434	0.656	0.156	0.368	0.365	0.429	0.201	0.074		

Table 4. List of all diurnal Lepidoptera species recorded during the study period arranged by the vegetation type in which they were most frequently recorded.

Family	Species	N	Red List Romania	Mean abundance							
				Extensive pasture (E)	Abandoned grassland (G)	Abandoned grassland with bushes (B)	Climax forest (W)	Abandoned vineyard (V)	comparisons E-V B-V (A)	comparisons E-A W-A (A)	
Species with maximum abundance in E											
Hesperiidae	<i>Erynnis tages</i>	11	LC	1.6>	0.0	0.3	0.0	0.3	0.3	0.3	
	<i>Pyrgus carthami</i>	9	LC	1.4≥	0.0	0.0	0.0	0.7	0.0	0.0	
Pieridae	<i>Colias crocea</i>	59	LC	4.6	3.0	3.3	0.0	3.0	0.7	0.7	
Lycanidae	<i>Polyommatus icarus</i>	21	LC	1.8	1.6	0.7	0.0	0.7	0.0	0.0	
	<i>Plebeius argus</i>	572	LC	56.0>	11.2<	52.7>	0.0	15.7	>	10.3	≥
	<i>Arctia agestis</i>	3	LC	0.6	0.0	0.0	0.0	0.0	0.0	0.0	
	<i>Pseudophilotes baton</i> x	2	NT	0.2	0.2	0.0	0.0	0.0	0.0	0.0	
	<i>Glaucopsyche alexis</i>	3	LC	0.4	0.0	0.0	0.0	0.3	0.0	0.0	
Nymphalidae	<i>Vanessa cardui</i>	20	LC	3.0	0.6	0.7	0.0	0.0	0.0	0.0	
Lymantriidae	<i>Penthophera morio</i>	6	NT	1.2	0.0	0.0	0.0	0.0	0.0	0.0	
Species with maximum abundance in G											
Hesperiidae	<i>Hesperia comma</i>	2	LC	0.0	0.4	0.0	0.0	0.0	0.0	0.0	
Pieridae	<i>Colias chrysotheme</i> x	3	VU	0.2	0.4	0.0	0.0	0.0	0.0	0.0	
Nymphalidae	<i>Araschnia levana</i>	2	LC	0.0	0.4	0.0	0.0	0.0	0.0	0.0	
Zygaenidae	<i>Zygaena purpuralis</i>	49	LC	3.6	3.8	1.7>	0.0	2.3		0.0	≥
Species with maximum abundance in B											
Hesperiidae	<i>Ochlodes venatus faunus</i>	2	LC	0.0	0.2	0.3	0.0	0.0	0.0	0.0	
Papilionidae	<i>Papilio machaon</i>	9	NT	0.2	0.6	1.0	0.0	0.3		0.3	
Pieridae	<i>Leptidea sinapis</i>	113	LC	3.0	3.8<	12.7>	0.3	9.3	≤	4.0	
	<i>Colias alfaccariensis/hyale</i>	115	NT	8.2>	3.6	9.7>	0.0	7.3		1.7	>
	<i>Aporia crataegi</i>	111	LC	7.6	5.8	8.7>	0.3	4.3		1.3	>
Lycanidae	<i>Satyrium acaciae</i>	119	VU	1.0	0.2<	33.7>	0.0	0.3	»	3.7	
	<i>Satyrium spini</i>	6	NT	0.0	0.0	1.7	0.0	0.3		0.0	



<i>Plebeius idas</i>	5	NT	0.4	0.0<	1.0	0.0	0.0	0.0	0.0
<i>Polyommatus bellargus</i>	13	LC	1.0≥	0.0<	1.3	0.0	0.0	1.3	0.0
<i>Cupido argiades</i>	3	LC	0.0	0.0	0.7	0.0	0.0	0.3	0.0
<i>Polyommatus coridon</i>	2	LC	0.0	0.0	0.3	0.0	0.0	0.3	0.0
<i>Nymphalis urticae</i>	2	LC	0.0	0.0<	0.7	0.0	0.0	0.0	0.0
<i>Brenthis daphne</i>	7	VU	0.0	0.6	1.3	0.0	0.0	0.0	0.0
<i>Boloria dia</i>	35	LC	0.6	1.2	4.7	0.0	0.0	2.7	1.3
<i>Melitaea cinxia</i>	27	LC	1.2	1.8	2.7	0.0	0.0	0.7	0.7
<i>Melitaea athalia</i>	27	LC	1.0	1.0	3.7	0.0	0.0	2.0	0.0
<i>Melitaea britannica</i>	28	LC	1.4	0.0≤	5.0	0.0	0.0	1.7	0.3
<i>Melitaea phoebe</i>	2	NT	0.0	0.0	0.7	0.0	0.0	0.0	0.0
<i>Minois dryas</i>	223	LC	3.6	12.6	23.7»	0.0	0.0	2.7	21.0
<i>Mantia jurina</i>	241	LC	8.6	6.8	22.0	7.3	14.0	7.0	11.3
<i>Coenonympha glycerion</i>	106	LC	4.4	2.0	15.3	0.0	0.0	7.0	2.3
<i>Coenonympha arcania</i>	3	LC	0.0	0.0	0.7	0.0	0.0	0.3	0.0
<i>Procris pruni</i> x	3	NT	0.0	0.0<	1.0	0.0	0.0	0.0	0.0
<i>Zygaena carniolica</i>	113	NT	3.4	8.8	10.7≥	0.0	0.0	6.3	0.3
<i>Macroglossum stellatarum</i>	7	LC	0.2	0.4	0.7	0.0	0.0	0.7	0.0
<i>Siona lineata</i>	30	LC	1.2	1.2	3.0	0.0	0.0	2.7	0.3
<i>Dyscia conspersaria</i>	3	NT	0.2	0.0	0.3	0.0	0.0	0.3	0.0
<i>Idaea ochrata</i> x	91	NT	1.4	4.6	9.7	0.7	0.7	8.7	1.3
<i>Perconia strigillaria</i>	12	EN	0.0	0.2≤	2.0	0.3	0.3	1.3	0.0
<i>Idaea rufaria</i> x	3	NT	0.0	0.0	0.7	0.0	0.0	0.0	0.3
<i>Scotopteryx ignorata</i> x	1	EN	0.0	0.0	0.3	0.0	0.0	0.0	0.0
<i>Cataclysmes rigata</i>	3	LC	0.0	0.0<	1.0	0.0	0.0	0.0	0.0
<i>Minoa murinata</i>	2	NT	0.0	0.0<	0.7	0.0	0.0	0.0	0.0
<i>Helionota glarearia</i>	2	LC	0.0	0.0	0.3	0.0	0.0	0.0	0.3
<i>Acontia lucida</i> x	4	LC	0.0	0.0≤	1.3	0.0	0.0	0.0	0.0
<i>Tyta luctuosa</i>	6	LC	0.4	0.0<	0.7	0.0	0.0	0.7	0.0
<i>Emmelia irabealis</i>	24	LC	1.6>	0.0<	2.7	0.0	0.0	2.3	0.3
<i>Euclidia glyphica</i>	9	NT	0.4	0.0<	1.7	0.0	0.0	0.7	0.0
<i>Heliothis maritima</i> x	1	LC	0.0	0.0	0.3	0.0	0.0	0.0	0.0
<i>Eriogaster lanestris</i> x	1	VU	0.0	0.0	0.3	0.0	0.0	0.0	0.0

Species with maximum abundance in W											
Riodinidae	<i>Hamearis lucina</i>	2	LC	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0
Nymphalidae	<i>Vanessa atalanta</i>	18	LC	0.2	0.6	1.7	2.3	0.0	2.3	0.0	>
	<i>Argynnis paphia</i>	5	LC	0.0	0.0	0.0	1.3	0.3	1.3	0.0	0.0
	<i>Pararge aegeria</i>	28	LC	0.0	0.2	0.0	0.0	0.0	9.0	0.0	>
Geometridae	<i>Camptogramma bilineata</i>	21	NT	0.2	0.2	1.0	2.3	1.3	2.3	1.7	0.0
Notodontidae	<i>Notodonta tritophus</i>	2	LC	0.0	0.0	0.0	0.3	0.3	0.3	0.0	0.0
Species with maximum abundance in V											
Hesperiidae	<i>Muschampia cribrellum</i> x	14	CR	0.2	0.8	1.3	0.0	1.7	0.0	0.0	0.0
	<i>Muschampia tessellum</i> x	3	CR	0.2	0.0	0.0	0.0	0.7	0.0	0.0	0.0
Papilionidae	<i>Iphiclydes podalirius</i>	24	NT	1.0	0.8	2.0	0.0	3.0	0.0	0.0	0.0
Pieridae	<i>Pieris rapae/napi</i>	696	LC	18.8<	34.2	35.0	21.0	74.0	<	<	13.7
	<i>Pontia daplidice</i>	52	LC	1.6	2.0	2.7	0.0	8.3	<	<	0.3
Lycaenidae	<i>Callophrys rubi</i>	12	LC	0.2	0.4	1.3	0.0	1.7	0.0	0.0	0.0
	<i>Plebeius pylaon</i> x	55	EN	2.4	3.4	3.7	0.0	5.0	0.0	0.0	>
	<i>Polyommatus thersites</i>	16	NT	0.2	1.0	0.0	0.0	3.3	<	<	0.0
	<i>Cupido osiris</i> x	3	VU	0.2	0.0	0.0	0.0	0.7	0.0	0.0	0.0
Nymphalidae	<i>Everes decolorata</i> x	1	VU	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
	<i>Brenthis hecate</i>	2	VU	0.0	0.0	0.0	0.0	0.7	<	<	0.0
	<i>Melanargia galathea</i>	668	LC	30.2	37.8	43.0»	0.7	54.0	0.0	11.7	0.0
	<i>Chazara briseis</i> x	246	NT	15.2	4.4	6.0	0.3	43.0	<	<	0.0
	<i>Coenonympha pamphilus</i>	90	LC	3.6	2.0	6.7»	0.0	13.3	<	<	0.7
	<i>Melitaea aurelia</i>	55	LC	3.2	2.8	3.3	0.0	4.3	0.0	0.7	0.7
Geometridae	<i>Autographa gamma</i>	97	LC	6.6	4.0	4.7	1.0	8.3	0.0	0.7	0.7
	<i>Scopula incanata</i> x	1	NT	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
	<i>Scopula virgulata</i> x	4	NT	0.0	0.0	0.0	0.0	0.7	0.0	0.7	0.7
	<i>Rhodostrophia vibicaria</i> x	1	LC	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
	<i>Lythria purpuraria</i> x	1	NT	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
Arctiidae	<i>Amata phegea</i>	2	LC	0.2	0.0	0.0	0.0	0.3	0.0	0.0	0.0
Species with maximum abundance in A											
Pieridae	<i>Pieris brassicae</i>	7	LC	0.4	0.0	0.0	0.0	0.0	0.0	1.7	0.7
	<i>Argynnis aglaja</i>	2	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
Geometridae	<i>Pseudopanthera macularia</i>	106	LC	0.0	0.2<	5.7	1.0	1.3	1.0	27.0	<

## Single records except xerophilous species, not assigned

Hesperidae	<i>Charcharodus alceae</i>	1	LC	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Papilionidae	<i>Parnassius mnemosyne</i>	1	NT	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lycenidae	<i>Polyommatus daphnis</i>	1	LC	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Plebeius argyrognomon</i>	1	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0
Nymphalidae	<i>Argynnis niobe</i>	1	LC	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Limenitis populi</i>	1	VU	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
	<i>Lopinga achine</i>	1	VU	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
	<i>Nymphalis c-album</i>	1	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Geometridae	<i>Hemistola chrysoprasaria</i>	1	NT	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Scotopteryx bipunctaria</i>	1	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
	<i>Thalera fimbrialis</i>	1	NT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Scopula ornata</i>	1	LC	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Enatarga atomaria</i>	1	LC	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Idaea antiquaria (macilentaria)</i>	1	NT	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Idaea degeneraria</i>	1	NT	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
	<i>Idaea aureolaria</i>	1	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Aretiidae	<i>Diacrisia sannio</i>	1	NT	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	<i>Dysauxes punctata</i>	1	EN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Lymantriidae	<i>Dicallomera fascelina</i>	1	LC	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Noctuidae	<i>Agrotis ipsilon</i>	1	LC	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
	<i>Herminia tarsicrinalis</i>	1	NT	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0

x = xerophilous species

Table 5. Total numbers of diurnal (Di.L.) and nocturnal (Noc.L.) Lepidoptera species, xerophilous and Red List species with maximum abundance in the investigated habitats, summary table (E, extensively grazed pasture; G, abandoned grassland; B, abandoned grassland with shrubs; W, climax forest; V, abandoned vineyard; A, *Pinus* plantation).

	Total number of species		Xerophilous species <sup>a)</sup>		Red List species	
	Di.L. (%)	Noc.L. (%)	Di.L. (%)	Noc.L. (%)	Di.L. (%)	Noc.L. (%)
E	10 (9.5)	31 (7.6)	1 (5.3)	3 (8.8)	2 (4.8)	8 (5.8)
G	4 (3.8)	85 (20.9)	1 (5.3)	17 (50.0)	1 (2.4)	30 (21.6)
B	40 (38.1)	83 (20.4)	7 (36.8)	7 (20.6)	16 (38.1)	23 (16.5)
W	6 (5.7)	88 (21.6)	0	0	1 (2.4)	22 (15.8)
V	21 (20.0)	28 (6.9)	10 (52.6)	6 (17.6)	12 (28.6)	8 (5.8)
A	3 (2.9)	12 (2.9)	0	1 (2.9)	0	3 (2.2)
Not assigned (single records)	21 (20.0)	80 (19.7)	0	0	10 (23.8)	45 (32.4)
<b>Total</b>	<b>105</b>	<b>407</b>	<b>19</b>	<b>34</b>	<b>42</b>	<b>139</b>

<sup>a)</sup> For xerophilous species also single records were assigned.

### **Legends to the figures**

Figure 1. Ordination (DCA) diagram of the Lepidoptera communities of the investigated sites in Transylvania, Romania. Site codes are defined in Table 1,

Figure 2. Dendrogram of cluster analysts (UPGMA) using Renkonen distances for the diurnal Lepidoptera communities of the investigated sites in Transylvania, Romania. Site codes are defined in Table 1.

Figure 3. Dendrogram of cluster analysis (UPGMA) using Renkonen distances for the nocturnal Lepidoptera communities of the investigated sites in Transylvania, Romania. Site codes are defined in Table 1.



Fig. 1a. Diurnal Lepidoptera

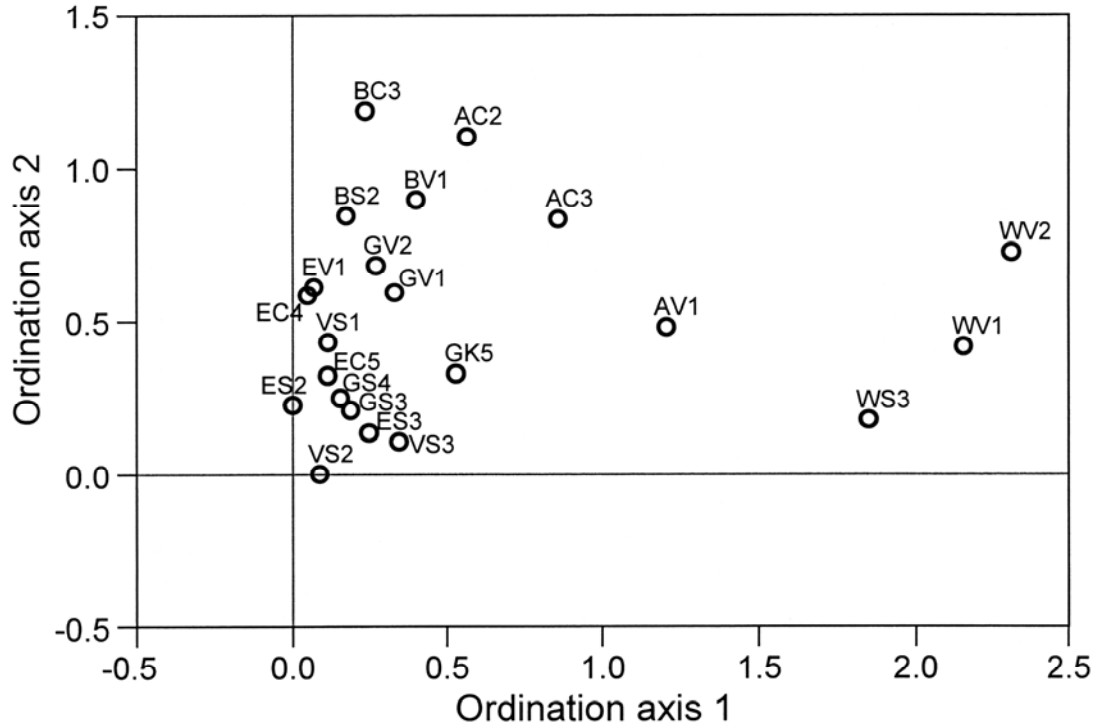


Fig. 1b. Nocturnal Lepidoptera

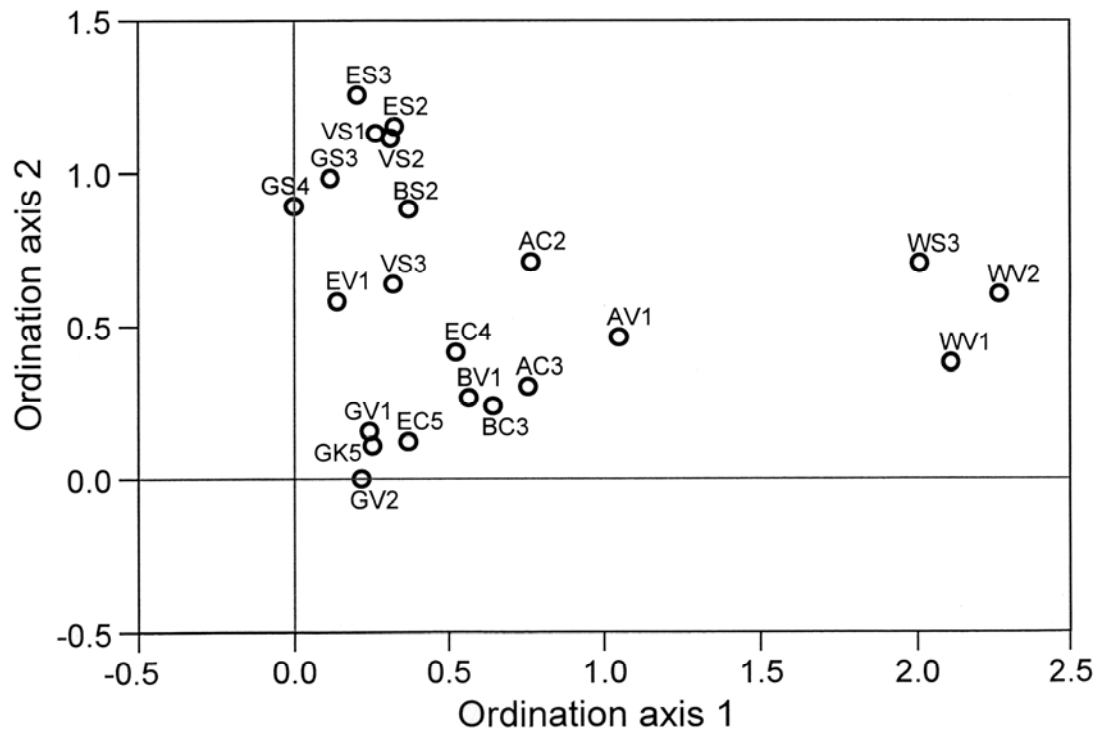


Fig. 2. Diurnal Lepidoptera

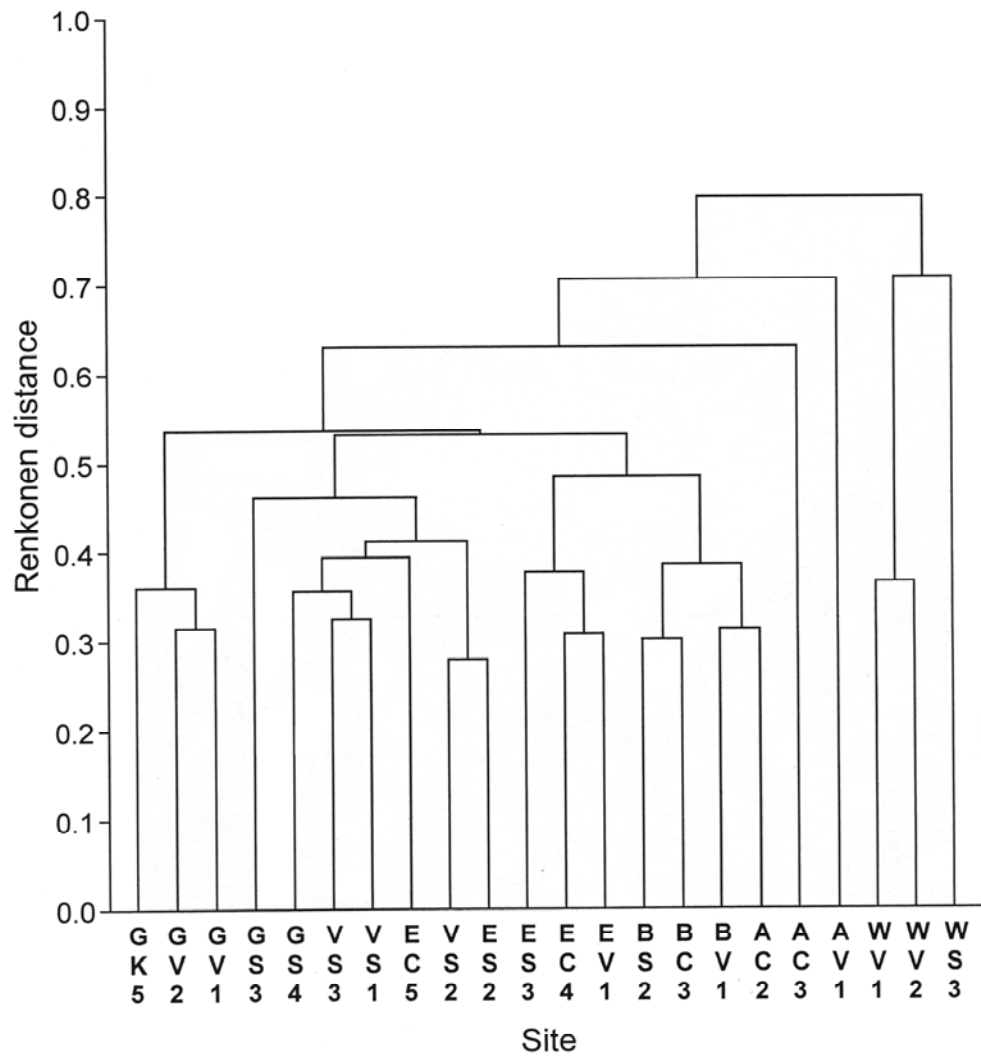
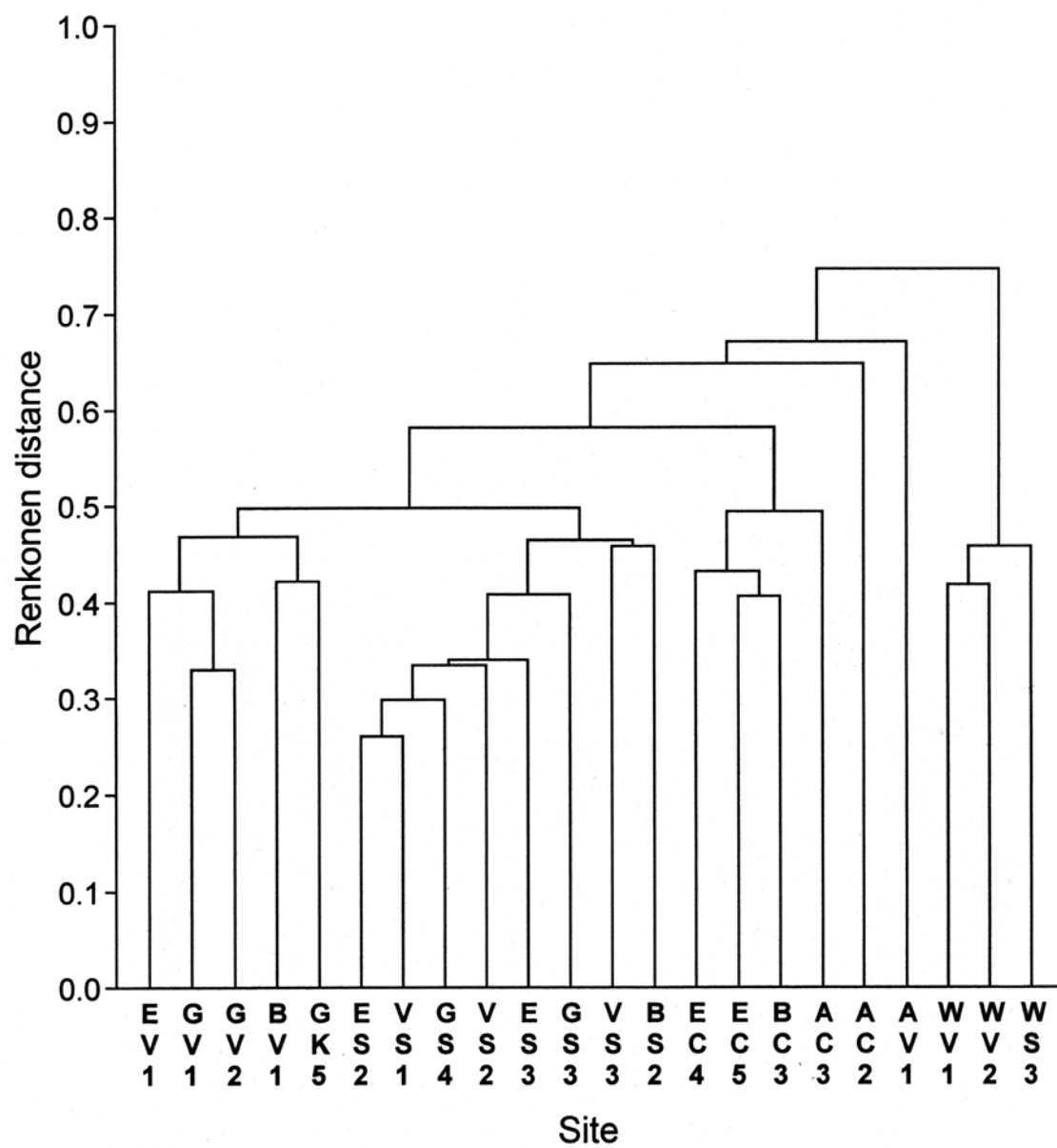




Fig. 3. Nocturnal Lepidoptera





**Chapter 3**

***Effects of abandonment of subalpine hay meadows on  
plant and invertebrate diversity in Transylvania,  
Romania***

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## Effects of abandonment of subalpine hay meadows on plant and invertebrate diversity in Transylvania, Romania

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### ABSTRACT

Recent changes in agriculture (intensification or abandonment) have resulted in a critical reduction of semi-natural grasslands in Eastern Europe. Subalpine semi-natural grasslands in Transylvania, Romania, harbour a high diversity of plants and invertebrates, including endemics, and are considered refugia for numerous threatened open-land species. We investigated effects of land abandonment by examining species richness, species abundance, proportion of open-land, endemic and threatened vascular plants, gastropods, and diurnal and nocturnal Lepidoptera in extensive hay meadows (initial stage), and three seral stages of succession (early stage of abandoned hay meadow, naturally growing birch forest, and mature forest) in the mountainous region of Baisoara in Transylvania. A total of 626 species (225 vascular plants, 16 gastropods, 68 diurnal and 317 nocturnal Lepidoptera) were found in the 16 study sites (four replicates per successional stage). The four taxonomic groups differed in their response to the abandonment of hay meadows. Each stage of succession harboured the maximum species richness for one taxonomic group: extensive hay meadows for vascular plants, abandoned hay meadows for diurnal Lepidoptera, birch forests for nocturnal Lepidoptera, and mature forests for gastropods. In all four taxonomic groups the complementarity of species composition increased with successional age, whereas the number of characteristic open-land species decreased with successional age. The four successional stages did not differ in proportion of red-listed plant and diurnal Lepidoptera species. In nocturnal Lepidoptera, however, the proportion of red-listed species increased with successional age. Furthermore, successional stages did not differ in number of plant species endemic to the Carpathians and Eastern Europe. Our results indicate the high conservation value of all stages of subalpine grassland succession for the indigenous biodiversity of Transylvania. To prevent losses of characteristic species, we suggest a rotational grassland management program that maintains different successional stages.

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## 1. Introduction

Maintaining biodiversity in agricultural areas is one of the major challenges for biodiversity conservation in Europe (Bignal and McCracken, 1996; van Diggelen et al., 2005). Traditional rural biotopes, particularly semi-natural grasslands, have been recognised as key habitats for maintaining biodiversity in European agricultural areas (Poschold and Wallis-DeVries, 2002). As human-made habitats, dry, nutrient-poor grasslands harbour numerous species whose primordial habitats (floodplains, peatlands, and rocky outcrops) have been vastly destroyed (Baur et al., 1997, 2004). However, semi-natural grasslands are fragile because their maintenance depends on traditional farming techniques. During the twentieth century, increasing pressure for higher yields and the rising price of labour led to significant habitat alterations in Western Europe (Poschold et al., 2005). Large areas of semi-natural grassland were lost due to intensified use (increased stocking rate and/or use of artificial fertilizers) or abandonment (Erhardt, 1985a; Groombridge, 1992; Baur et al., 2002; Strijker, 2005).

In mountainous areas of Central and Mediterranean Europe, abandonment of semi-natural grassland is a growing threat to biodiversity (Fischer and Wipf, 2002; Labaune and Magnin, 2002; Tasser and Tappeiner, 2002; Dullinger et al., 2003). Similar information is not available for montane and subalpine grasslands in Eastern Europe. In the present study, we investigated effects of land abandonment on the biodiversity of subalpine hay meadows in Transylvania, Romania. We assessed the species richness and species abundance of vascular plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera in extensive hay meadows and three seral stages of succession. Romania has an extraordinarily high level of species diversity with a total of 228 endemic and sub-endemic species (ICAS, 1996; Ioras, 2003). Reasons for the high biodiversity include several biogeographical regions in Romania, the high habitat diversity and the locally low intensity of agricultural land use. Extensive grazing and mowing were the dominant methods of grassland management in the Transylvanian mountains for many centuries (Reif et al., 2003; Rusdea et al., 2005). In recent years, however, semi-natural grasslands have been increasingly abandoned, mainly because of depopulation of remote mountain valleys.

The high species diversity of grasslands has been explained by the intermediate disturbance hypothesis (Wilson and Tilman, 2002; Li et al., 2004; Shea et al., 2004). According to this hypothesis, species diversity decreases with grassland abandonment. Surveys carried out in Western Europe confirmed this decrease in plant diversity of abandoned semi-natural grasslands (Bischof, 1981; Tasser and Tappeiner, 2002; Dullinger et al., 2003; Pykälä et al., 2005). Similarly, studies focusing on threatened butterfly species in Britain show that grassland abandonment is the major cause of the butterfly decline (Thomas, 1991). However, in investigations of entire butterfly communities, Erhardt (1985a,b) and Balmer and Erhardt (2000) found that several threatened species benefit from advanced stages of abandonment. These contrasting results require further examination.

For practical reasons, conservation evaluations of grasslands are mostly based on a single taxonomic group, in many

cases vascular plants (Usher, 1986; Plachter, 1991). However, the use of one taxonomic group for the assessment of biodiversity implicitly assumes that the diversities of indicator and target groups are positively correlated (Oliver and Beattie, 1996). Correlations between the diversities of vascular plants and Lepidoptera seem to support this approach (Erhardt and Thomas, 1991), although significant deviations from this expectation have been reported (e.g. Erhardt, 1985a,b). Our data allow a test of the assumption that succession-related diversity changes in different taxonomic groups covary. Moreover, our findings provide basic information to identify and implement the best strategy of biodiversity conservation in the threatened hay meadows in the Transylvanian mountains.

We addressed the following questions: (1) Do different successional stages of subalpine grassland abandonment differ in species number, species abundance and community structure of plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera? (2) Do different successional stages differ in the number of endemic and threatened (red-listed) species? (3) To what extent is the response to habitat changes of a particular group an indicator for the other taxonomic groups?

## 2. Materials and methods

### 2.1. Study sites

We assessed plant and invertebrate diversities in 16 study sites (Table 1) in the surroundings of the Transylvanian village of Muntele Baisoara in Romania (45°33'N, 23°28'E) at elevations between 1130 and 1250 m a.s.l. (Fig. 1). Total annual rainfall in Muntele Baisoara (at 1361 m a.s.l.) averages 848 mm, with an annual mean temperature of 4.8 °C (Rusdea et al., 2005). We considered the following vegetation types: extensive hay meadow (M,  $n = 4$  study sites), early stage of abandoned hay meadow (G,  $n = 4$ ), naturally growing birch forest on meadows abandoned for 20–50 years (B,  $n = 4$ ) and mature forest (W,  $n = 4$ ). The four habitat types represent seral stages of natural grassland succession in mountainous areas of Romania (Rusdea et al., 2005). Grassland abandonment is a relatively recent phenomenon in Transylvania, becoming significant in the past decades. Hence, grassland succession could not yet reach its final stage, i.e. mature forest. We therefore decided to include existing mature forest as final successional stage in our study. This forest is original and has not been logged for at least two centuries. We expected that forest species will colonize the developing woods originating from abandoned grasslands, as a network of mature forest still exists in the Baisoara valley.

The design of the survey consisted of four blocks each containing one replicate of the four successional stages (Fig. 1). We chose study sites of similar elevation, exposure, inclination and area. Two study sites of each successional stage were situated on the N- to NW-facing slope and two on the S- to SE-facing slope of the valley (symmetrical design). Distances between study sites of the same successional stage averaged 1310 m (range 590–2120 m) for hay meadows, 1540 m (360–2320 m) for abandoned hay meadows, 1690 m (340–2550 m) for birch forests, and 1900 m (390–3460 m) for mature forests (Fig. 1). The underlying bedrock of this area consists of meso-

**Table 1 – Characteristics of the study sites in the mountainous region of Baisoara in Transylvania, Romania**

Successional stage	Study site number	Land use	Total area (ha)	Elevation (m a.s.l.)	Exposure	Inclination (°)
Extensive hay meadow	1	Mown once a year	4	1160	N-NW	24.0
	2	Mown once a year	1	1130	NW	20.5
	3	Mown once a year	1.5	1180	SE	20.0
	4	Mown once a year	1	1220	SE	15.5
Abandoned hay meadow	5	Abandoned for 3–5 years, partly grazed by cattle	2	1140	N-NW	12.5
	6	Abandoned for 5–8 years	10	1170	NW	19.0
	7	Abandoned for 3–5 years	1.5	1180	SE	18.5
	8	Abandoned for 3–5 years, irregularly mown	1	1230	SE	15.0
Naturally growing birch forest	9	30–35 years old	2	1140	NW	13.0
	10	20 years old	1.5	1160	W-NW	19.5
	11	40–50 years old	2	1180	S-SE	17.5
	12	20–25 years old	1.5	1250	E-SE	9.5
Mature forest	13	50–100-year-old wood	3.5	1210	N-NW	29.5
	14	50–100-year-old wood	2	1130	W-NW	24.5
	15	50–80-year-old wood	4.5	1140	SE	24.0
	16	50–80-year-old wood	3	1160	E-SE	20.5

zoic sedimentary succession and palaeozoic chlorite schists. Soils of all sites were acidic.

## 2.2. Surveys

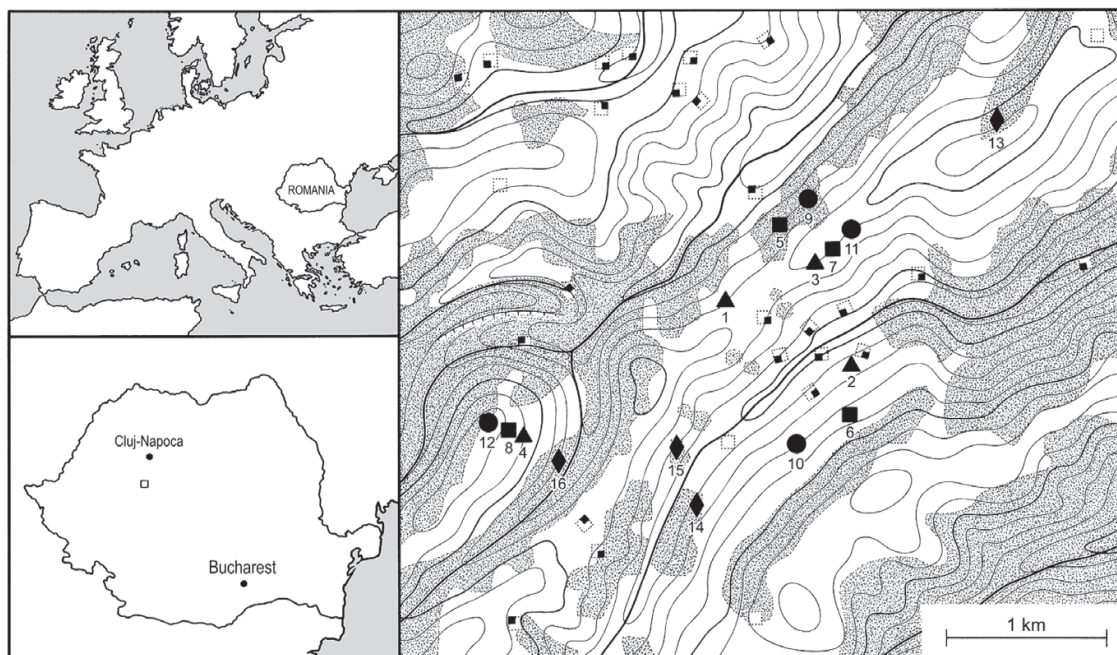
From May to September 2002, we surveyed diversity and abundance of vascular plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera in a 50 × 50 m plot at each site. The positions of the plots were chosen so that the distance to the next different habitat was >50 m. Plots were surveyed for plants four times: spring, early summer, late summer, and autumn. At each site (except for the naturally growing birch forests and mature forests) we established a permanent subplot of 10 × 10 m and used the Braun-Blanquet (1964) method to determine the abundance of all plant species (including woody plants) within the subplot. To complete the plant list for each site, we walked in a zig-zag line over the entire study site and recorded all new species observed. In the naturally growing birch forest and mature forest, we again used the Braun-Blanquet method to record plants present in permanent plots of 100 × 100 m. An increased sampling area proved to be essential for recording plant diversity in forests (Ellenberg, 1996). However, the sampling effort was the same at all investigation sites. Plant identification followed Tutin et al. (1964–1980) and Ciocarlan (2000).

We used two methods to assess species richness and relative abundance of terrestrial gastropods (Oggier et al., 1998). First, two people visually searched for living snails and empty shells in each 50 × 50 m plot for 40 min between 14 and 30 June 2002. Second, we collected five soil samples (total 2l), including dead plant material, at randomly chosen spots in each 50 × 50 m plot. Soil and litter samples were put through a sieve with a 1-mm mesh and then examined under a binocular microscope. Gastropod shells were sorted out of the samples and identified according to Grossu (1981, 1983).

We used a transect method (Douwes, 1976; Hall, 1981) to survey each plot for diurnal Lepidoptera. Surveys were conducted once every second week between 15 May and 6 September 2002. We walked plots in a serpentine pattern, 5-m wide. In this way the entire area was covered once by the butterfly net. Transects were surveyed only under good weather conditions (sunny, temperature  $\geq 18$  °C and wind-speed  $\leq 16$  km/h (Beaufort scale 3)) between 1000 and 1700 h. We recorded all species of Rhopalocera, Hesperidae, and Zygaenidae. For Bombyces, Sphingae, Noctuidae and Geometridae, we recorded all day-active species and all other species easily surveyed with the transect method. Multiple counting of the same individuals could not be completely avoided. However, this error may not affect our interpretations because we compared the plots rather than species; thus the error would be the same for each plot. The sequence in which transects were surveyed was alternated to avoid systematic effects of time of day.

We surveyed nocturnal Macrolepidoptera between 1 May and 30 September 2002. Moths were collected using a light trap (8 W UV-tubes fixed to the top of black buckets covered by white funnels and fed by a 12 V accumulator) once a month in each plot. We did not survey on nights with full moon. In their flight toward the light moths strike the transparent baffles that surround the lamp and fall through the funnel into the bucket (Rakosy, 1999). Light traps were placed in the same place on each sampling occasion and were left on from dusk to dawn. We identified and released specimens in the morning. Identification of Lepidoptera followed Tolman and Lewington (1997) for butterflies, Forster and Wohlfahrt (1981), Rakosy (1996), and De Freina and Witt (1987, 1990, 2001) for moths. Problematic species were collected and identified by specialists through examination of genitals.

Species lists of all four groups of organism can be obtained from the authors.



**Fig. 1** – Maps showing Romania (top left), the location of the study area (open square) in Transylvania (bottom left), and the locations of the study sites in the Baisoara valley. Triangles refer to extensive hay meadows, squares to abandoned hay meadows, dots to naturally growing birch forests, and diamonds to mature forests. Numbers indicate the replicates (see Table 1). Contour interval is 20 m and shaded areas indicate mature forest. Tiny quadrats refer to small farms with wooden fences (dotted quadrats).

### 2.3. Site characteristics

We defined the total area as the size of a homogeneous vegetation type surrounded by other habitat types (e.g., the area of a naturally growing birch forest surrounded by grassland). We used maps to measure total area and to assess the average elevation of the study sites which were part of the total area. In each study site, we collected six soil samples at randomly chosen places. We mixed the samples to determine the soil pH (Hellige method, AVM Analyseverfahren, Freiburg, Germany). At the same points we measured the inclination of the slope using a trigonometrical method (six measurements per study site).

In all study sites examined, soil pH was 4.5, indicating acid soils. The study sites of the four successional stages did not differ in area (ANOVA,  $F_{3,12} = 0.98$ ,  $P = 0.44$ ) and elevation ( $F_{3,12} = 0.26$ ,  $P = 0.86$ ; Table 1). Furthermore, the study sites did not differ in exposure (multisample version of the Watson–Williams test,  $P > 0.9$ ; Batschelet, 1981). However, the study sites differed in inclination (hay meadows:  $20.0 \pm 1.7^\circ$  (mean  $\pm$  SE); abandoned hay meadows:  $16.3 \pm 1.5^\circ$ ; birch forest:  $14.9 \pm 2.2^\circ$ ; mature forest:  $24.6 \pm 1.9^\circ$ ;  $F_{3,12} = 4.49$ ,  $P = 0.0248$ ; Table 1). The four site variables successional age, inclination, elevation, and total site area were not intercorrelated (Spearman rank correlation, in all cases  $P > 0.09$ ), except that site area tended to be positively correlated with successional

age ( $r_s = 0.46$ ,  $n = 16$ ,  $P = 0.0729$ ), mainly due to the large size of mature forests.

### 2.4. Data analyses

In all analyses we considered the study sites as the unit of investigation. We applied oneway analysis of variance (ANOVA) to examine whether different successional grassland stages differed in species richness and abundance of the four taxonomic groups (StatView 5.0; Abacus Concepts, 1998). We used Scheffé's post hoc test to compare pairs of successional stages.

As our sampling effort was the same for all study sites, the species richness and abundance found in each group represent true differences among successional stages. However, one could argue that differences in number of individuals affect the number of species recorded (Gotelli and Colwell, 2001). We therefore calculated individual-based rarefaction curves according to Krebs (1998) for diurnal and nocturnal Lepidoptera (for plants no individual-based data were collected; in gastropods the number of individuals found was too low for rarefaction). To assess the variation around the expected number of species, 100 random samples were drawn from the pooled replicates within each successional stage.

To examine changes in species composition between extensive hay meadows and seral stages of succession, we



performed an analysis of complementarity (Colwell and Coddington, 1994) for the entire species list of each taxonomic group. The value of the complementarity index varies from 0 (species lists of two vegetation types are identical) to 1 (no species shared). To examine whether species composition of a particular study site was affected by the species pool of neighbouring habitats, we calculated complementarity indices for all pairwise comparisons of successional stages separately. If the focal community is influenced by individuals from the neighbouring habitat, then the complementarity index is reduced. This effect depends on the mobility of the organisms and should decrease with increasing distance. We tested this idea for diurnal and nocturnal Lepidoptera by relating complementarity indices to the geographical distance between pairwise combinations of study sites. Because plants and terrestrial gastropods are rather sedentary organisms, we did not expect any exchange of individuals between neighbouring sites in these groups.

We used detrended correspondence analysis (DCA) to examine whether the 16 study sites – based on their structures of the plant and invertebrate communities – can be assigned to the four successional stages (Hill and Gauch, 1980). DCA was performed separately for each group of organism. However, because of the small numbers of gastropod species and individuals, DCA could not be performed in this group. For the DCA, the vegetation cover-abundance code adapted from Braun-Blanquet (1964) was applied using the following weights: 0.1 for 'R', 0.5 for '+', 3 for the category 1, 7.5 for the category 2, 15 for the category 3, 25 for the category 4, and 40 for the category 5 (see Buschmann et al., 2005). Prior to ordination, the data were log-transformed. Species that were less frequent than the median frequency were down-weighted in proportion to their frequencies (Eilertsen et al., 1990). DCA was performed using CANOCO version 4.5 (ter Braak and Smilauer, 2002).

At each site the age of the successional stage was determined. In mature forests, however, only the age of the trees was known, indicating a minimum age for the forests. Therefore, we used Spearman rank correlation to examine whether successional age was correlated with species richness, species abundance, and the proportions of open-land and threatened and endemic species in the investigated taxonomic groups.

To examine possible associations between the species' habitat specificity and the successional stages in which they were found, we assigned all plants and invertebrates to one of the following categories: open-land, forest or ubiquitous species. Detailed information on the species' habitat specificity was obtained from Tutin et al. (1964–1980), Ellenberg (1996), and Ciocarlan (2000) for plants, Grossu (1981, 1983) for gastropods, Forster and Wohlfahrt (1981) and Blab and Kudrna (1982) for diurnal Lepidoptera, and Rakosy (1996) and De Freina and Witt (1987, 1990, 2001) for nocturnal Lepidoptera. Because generalized linear models with binomial error distributions (e.g. number of open-land species as dependent variable and total number of species as binomial total) gave similar results as ANOVAs with transformed (arcsine square root) ratios, we used the ANOVAs to examine whether successional stages differed in their proportion of

open-land species. The same ANOVA model was used to examine differences in the proportions of red-listed and endemic species.

In Romania, red lists exist for plants (Boscaiu et al., 1994) and diurnal and nocturnal Lepidoptera (Rakosy et al., 2003) but not for terrestrial gastropods. In the analyses, we considered all red-listed species (the categories critically endangered, endangered, vulnerable, and nearly threatened).

To examine whether vegetation types differed in their proportion of endemic plants, we assigned each species to one of the following categories: Transylvanian endemic (occurring exclusively in northwestern Romania), Eastern European endemic (Balcanic, Pontic, Carpathic, and/or Dacic distributions), widespread (naturally occurring in large areas of one or more continents), or adventive (introduced alien). Similar information was not available for the three invertebrate groups.

We used Pearson's correlations to examine possible correlations between species richness (log-transformed) and proportions of open-land and red-listed species (both arcsine-square root-transformed) among the taxonomic groups investigated.

### 3. Results

#### 3.1. Species richness

A total of 225 plant species were recorded in the study sites. In the four successional stages, mean plant species richness varied from 41.8 (birch forest) to 52.3 (hay meadows; Table 2). However, differences in plant species richness were not significant because of the large within-successional stage variation. Plant species richness was not correlated with successional age of the study site. Sixteen gastropod species were recorded. The four successional stages did not differ in gastropod species richness (Table 2).

In all, 68 diurnal Lepidoptera species were recorded. The ANOVA revealed significant differences in species richness among the four successional stages (Table 2). Species richness was lower in mature forests than in abandoned hay meadows (Scheffés post-hoc test,  $P = 0.021$ ). In addition, rarefaction showed that abandoned hay meadows had a higher number of diurnal Lepidoptera species compared to hay meadows (Fig. 2). Species richness of diurnal Lepidoptera decreased with successional age ( $r_s = -0.59$ ,  $n = 16$ ,  $P = 0.0220$ ).

A total of 317 nocturnal Lepidoptera species was recorded. The four successional stages differed significantly in species richness (Table 2), which ranged from 86.3 in hay meadows to 142.5 in naturally growing birch forests. Species richness of nocturnal Lepidoptera increased with successional age ( $r_s = 0.67$ ,  $n = 16$ ,  $P = 0.0097$ ). Rarefaction curves for nocturnal Lepidoptera showed that naturally growing birch forests had the highest and hay meadows the lowest number of species. Judged by the variation in the samples (95% confidence intervals in Fig. 2), these differences were only found with more than 400 individuals per sample. In contrast to diurnal Lepidoptera, which did not show any differences in the number of species between the two different forest habitats, species numbers of

nocturnal Lepidoptera were clearly higher in naturally growing birch compared to mature forests.

Considering all taxonomic groups examined, each seral stage of grassland succession harboured the maximum species richness for one particular group: extensive hay meadows for vascular plants, abandoned hay meadows for diurnal Lepidoptera, birch forests for nocturnal Lepidoptera, and mature forests for gastropods.

### 3.2. Abundance of invertebrates

In all, 71 gastropod individuals were found. The four successional stages did not differ in gastropod abundance (Table 3). We recorded 1298 diurnal Lepidoptera. There were significant differences in the number of diurnal Lepidoptera observed in the different successional stages (Table 3). Diurnal Lepidoptera abundance was highest in hay meadows and abandoned hay meadows. In mature forests, however, diurnal Lepidoptera abundance was lower than in the two meadow types. Species abundance of diurnal Lepidoptera decreased with successional age ( $r_s = -0.72$ ,  $n = 16$ ,  $P = 0.0056$ ) and decreased with increasing inclination ( $r_s = -0.52$ ,  $n = 16$ ,  $P = 0.0424$ ).

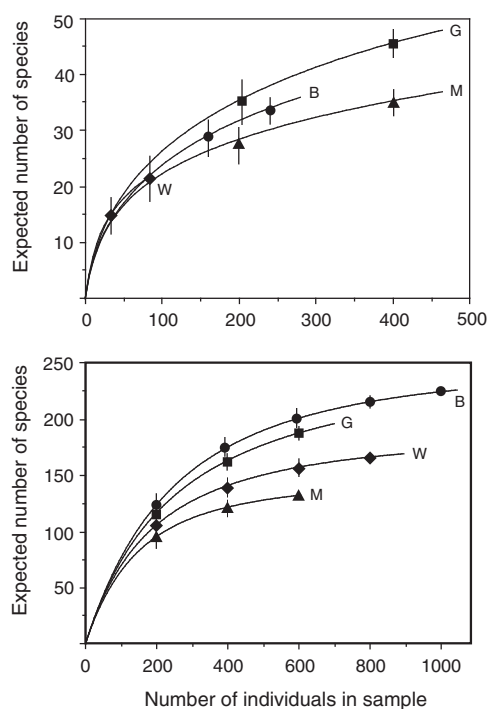
In all, 3255 individuals of nocturnal Lepidoptera were recorded. Nocturnal Lepidoptera abundance differed among successional stages (Table 3). It was highest in birch forests, followed by mature forests and abandoned hay meadows. Species abundance of nocturnal Lepidoptera increased with successional age ( $r_s = 0.77$ ,  $n = 16$ ,  $P = 0.0028$ ).

Considering the three groups of invertebrates examined, different stages of grassland succession had a maximum abundance for one organism group.

### 3.3. Changes in species composition

As expected the complementarity of species composition increased with successional age in all taxonomic groups examined (Table 4), indicating a decreasing similarity between seral stages of succession. Comparing the four taxonomic groups, complementarity was highest in gastropods, followed by diurnal Lepidoptera and nocturnal Lepidoptera.

In diurnal Lepidoptera species composition of hay meadows was influenced by the species pool of neighbouring abandoned meadows in two of four blocks (study site comparisons



**Fig. 2** – Rarefaction curves for diurnal (top panel) and nocturnal (bottom panel) Lepidoptera in extensive hay meadows (M, triangles), abandoned hay meadows (G, squares), naturally growing birch forests (B, dots), and mature forests (W, diamonds). The solid lines give the expected number of species in samples of the number of individuals given on the horizontal axes. Data points are medians ( $\pm 95\%$  confidence intervals) of 100 random samples drawn from the observed data. Note that error bars for some points have been slightly moved to enhance clarity.

3 vs. 7 and 4 vs. 8, which were 130 m and 100 m apart; Fig. 1). This was not the case in the other two comparisons, in which the hay meadow and the abandoned meadow were 310 m and further apart. Moreover, in the other comparisons of succes-

**Table 2** – Species richness (mean  $\pm$  SE) of vascular plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera recorded in the four stages of grassland succession

Group of organism	Successional stage <sup>a</sup>				ANOVA	
	Hay meadow (M)	Abandoned hay meadow (G)	Naturally growing birch forest (B)	Mature forest (W)	$F_{3,12}$	P
Vascular plants	52.3 $\pm$ 4.2	49.8 $\pm$ 5.5	41.8 $\pm$ 6.8	46.3 $\pm$ 6.0	0.68	0.58
Gastropods	1.3 $\pm$ 0.9	1.3 $\pm$ 1.3	1.3 $\pm$ 0.6	3.0 $\pm$ 0.7	1.28	0.33
Diurnal Lepidoptera	19.0 $\pm$ 2.6	24.3 $\pm$ 1.2	16.0 $\pm$ 3.9	9.3 $\pm$ 1.2	5.18	0.0158
Nocturnal Lepidoptera	86.3 $\pm$ 1.7	102.5 $\pm$ 7.9	142.5 $\pm$ 6.4	117.8 $\pm$ 5.9	16.05	0.0002

The following pairwise comparisons revealed significant differences (Scheffé's post hoc test:  $P < 0.05$ ) between successional stages: G–W in diurnal Lepidoptera; M–B, M–W, and G–B in nocturnal Lepidoptera. a  $n = 4$  for each successional stage.

**Table 3 – Number of individuals (mean ± SE) of terrestrial gastropods, and diurnal and nocturnal Lepidoptera recorded in the four stages of grassland succession**

Group of organism	Successional stage <sup>a</sup>				ANOVA	
	Hay meadow (M)	Abandoned hay meadow (G)	Naturally growing birch forest (B)	Mature forest (W)	F <sub>3,12</sub>	P
Gastropods	3.5 ± 3.2	7.8 ± 7.8	1.8 ± 0.8	4.8 ± 1.8	0.45	0.73
Diurnal Lepidoptera	116.5 ± 20.6	116.3 ± 1.7	70.0 ± 30.8	21.8 ± 3.2	10.82	0.0010
Nocturnal Lepidoptera	152.3 ± 7.5	175.5 ± 12.6	261.8 ± 8.9	224.3 ± 14.0	18.17	<0.0001

The following pairwise comparisons revealed significant differences (Scheffé's post hoc test:  $P < 0.05$ ) between successional stages: M–W and G–W in diurnal Lepidoptera; M–B, M–W, and G–B in nocturnal Lepidoptera.  
<sup>a</sup>  $n = 4$  for each successional stage.

**Table 4 – Complementarity of species lists between successional stages for each taxonomic group<sup>a</sup>**

Comparison <sup>b</sup>	Plants	Gastropods	Diurnal Lepidoptera	Nocturnal Lepidoptera	Mean ± SE
M–G	0.42	0.50	0.45	0.47	0.46 ± 0.02
M–B	0.57	1.00	0.57	0.73	0.72 ± 0.10
M–W	0.90	1.00	0.77	0.85	0.88 ± 0.05
G–B	0.58	0.88	0.55	0.50	0.63 ± 0.09
G–W	0.89	0.93	0.75	0.71	0.82 ± 0.05
B–W	0.74	0.83	0.59	0.40	0.64 ± 0.09

<sup>a</sup> Complementarity values range from 0 (identical species lists) to 1 (no species shared).

<sup>b</sup> M, extensive hay meadow; G, abandoned hay meadow; B, naturally growing birch forest; W, mature forest.

sional stages no such effect was found. In nocturnal Lepidoptera, species composition was not affected by neighbouring habitats, as complementarity indices did not increase with geographical distance in any pairwise comparison of successional stages.

In plants, the DCA ordination revealed a separation of the study sites into three groups (Fig. 3a). The first axis (Eigenvalue = 0.814) explained 21.8% of the variance in species data (together with the second axis 30.1%), and showed that hay meadows and abandoned hay meadows (together with one site of naturally growing birch forest) clustered to a single group, whereas the remaining three birch forest sites and the mature forest sites each clustered to distinct groups.

In diurnal Lepidoptera, the DCA ordination also revealed a separation in groups according to the successional stages (Fig. 3b). The first axis (Eigenvalue = 0.401) explained 20.9% of the variance in species data (together with the second axis 29.0%). Study sites in hay meadows and those in abandoned hay meadows clustered together with two sites of naturally growing birch forest (both on S- and E-exposed slopes). The study sites in mature forest formed another distinct cluster with the two birch forest sites on N- to W-exposed slopes in its proximity.

In nocturnal Lepidoptera, the DCA ordination revealed a separation of the study sites into groups which corresponded to the seral stages of succession (Fig. 3c). The first axis (Eigenvalue = 0.556) explained 33.3% of the variance in species data (together with the second axis 40.2%). Thus, in all three groups of organism, the first axis of the DCA separated the study sites on the basis of the variation in species composition according to the successional gradient.

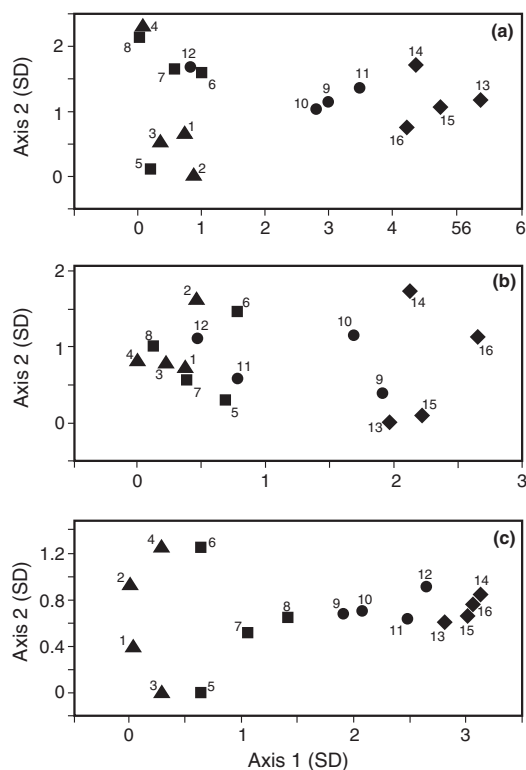
### 3.4. Habitat specificity

The four successional stages differed in the proportion of open-land species in all four taxonomic groups (plants:  $F_{3,12} = 44.15$ ,  $P < 0.0001$ ; gastropods:  $F_{3,6} = 15.04$ ,  $P = 0.0034$ ; diurnal Lepidoptera:  $F_{3,12} = 3.90$ ,  $P = 0.0372$ ; nocturnal Lepidoptera:  $F_{3,12} = 51.94$ ,  $P < 0.0001$ ; Fig. 4). In plants, gastropods and nocturnal Lepidoptera, the proportion of open-land species decreased with successional age (plants:  $r_s = -0.93$ ,  $n = 16$ ,  $P = 0.0003$ ; gastropods:  $r_s = -0.85$ ,  $n = 10$ ,  $P = 0.0113$ ; nocturnal Lepidoptera:  $r_s = -0.95$ ,  $n = 16$ ,  $P = 0.0002$ ; Fig. 4). In diurnal Lepidoptera, the number of open-land species tended to decrease with successional age ( $r_s = -0.46$ ,  $n = 16$ ,  $P = 0.0737$ ).

### 3.5. Red-listed species

The four successional stages differed in the proportion of threatened nocturnal Lepidoptera species (total of species considered as critically endangered, endangered, vulnerable or nearly threatened) but not in those of plants and diurnal Lepidoptera (no red list exists for gastropods; nocturnal Lepidoptera:  $F_{3,12} = 7.95$ ,  $P = 0.0035$ ; plants:  $F_{3,12} = 0.97$ ,  $P = 0.44$ ; diurnal Lepidoptera:  $F_{3,12} = 0.39$ ,  $P = 0.76$ ; Table 5). The proportion of threatened nocturnal Lepidoptera species was positively correlated with successional age ( $r_s = 0.78$ ,  $n = 16$ ,  $P = 0.0026$ ). No correlation between the proportion of threatened species and successional age could be found in plants ( $P = 0.11$ ) and diurnal Lepidoptera ( $P = 0.61$ ).

In plants, one (regionally) endangered species (*Abies alba*) and one vulnerable species (*Arnica montana*) were recorded. The former is a forest species, the latter an open-land species.



**Fig. 3 – Ordination diagrams based on detrended correspondence analysis of: (a) plants, (b) diurnal Lepidoptera, and (c) nocturnal Lepidoptera communities in extensive hay meadows (triangles) and three seral stages of succession (squares for abandoned hay meadows, dots for naturally growing birch forests, diamonds for mature forests) in the Transylvanian mountains, Romania, displaying the major variation in species composition. Numbers indicate the study sites (see Table 1).**

Among the diurnal Lepidoptera, two vulnerable species (*Cupido osiris*, *Jordanita notata*) were found, both are open-land species. Among the nocturnal Lepidoptera, one critically endangered species (*Ourapteryx sambucaria*, a forest species) and eight vulnerable species (*Acyla flavicornis*, *Chersotis cuprea*, *Isturgia roraria*, *Itame brunneata*, *Lithomoia solidaginis*, *Ochlopleura musiva*, *Perapherapteryx sexalata*, and *Trichiura crataegi*) were recorded, six of them belonging to open-land and two to forest species. Considering all three taxonomic groups, 69% of the critically endangered, endangered and vulnerable species were open-land species and 31% were forest species.

Individuals of the critically endangered moth *O. sambucaria* were recorded in one naturally growing birch forest and three mature forests.

### 3.6. Endemism

Three of the 225 plant species recorded (1.3%) were endemic to the Carpathians: *Campanula polymorpha*, *Silene dubia* and

*Symphytum cordatum*. Further 12 plant species (5.3%) were endemic to Eastern Europe. One of the 16 gastropod species found (6.3%) was endemic to the Carpathians: *Vestia turgida*. Among the diurnal and nocturnal Lepidoptera recorded in this study, no Carpathian and Eastern European endemics occurred.

Successional grassland stages did not differ in the number of endemic plant species (total Carpathians and Eastern European endemics: hay meadows: 2.8 species, the other stages of succession each 1.3 species;  $F_{3,12} = 1.04$ ,  $P = 0.41$ ). One (33.3%) of the three plant species endemic to the Carpathians was an open-land species, the other two (66.7%) were forest species. The open-land species *C. polymorpha* was only found in two extensive hay meadows, whereas one of the forest species was recorded in a single birch forest and the other in a mature forest. Nine (75.0%) of the 12 Eastern European endemic plants found in our study were open-land species, the remaining three (25.0%) were forest species. The snail endemic to the Carpathians (*V. turgida*), a forest species, was found in one mature forest area.

Interestingly, we did not record any adventive plant species in the 16 study sites examined.

### 3.7. Correlations between taxonomic groups

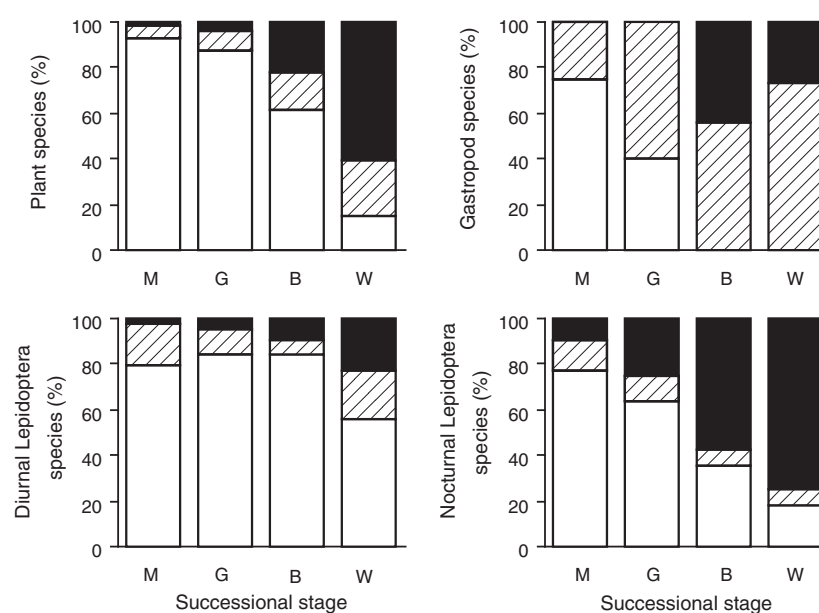
No correlations among the species richness of the investigated taxonomic groups were found. However, the proportions of open-land species among plants, gastropods and nocturnal Lepidoptera were highly intercorrelated (plant-gastropod:  $r = 0.70$ ,  $n = 10$ ,  $P = 0.0225$ ; plant-nocturnal Lepidoptera:  $r = 0.86$ ,  $n = 16$ ,  $P < 0.0001$ ; gastropod-nocturnal Lepidoptera:  $r = 0.84$ ,  $n = 10$ ,  $P < 0.0012$ ). In contrast, the proportion of open-land species in diurnal Lepidoptera was not correlated with that of any other group.

We found no correlations among the proportions of threatened (sum of the red-list categories critically endangered, endangered, vulnerable, and nearly threatened) species of the taxonomic groups examined. This indicates that red-listed species of any taxonomic group cannot be used as indicators for threatened species in other groups. The proportions of threatened plants and nocturnal Lepidoptera increased with increasing plant and moth species richness, respectively (plants:  $r = 0.59$ ,  $n = 16$ ,  $P = 0.0136$ ; nocturnal Lepidoptera:  $r = 0.54$ ,  $n = 16$ ,  $P = 0.0312$ ). In contrast, the proportion of threatened diurnal Lepidoptera species was not correlated with its own species richness ( $r = -0.34$ ,  $n = 16$ ,  $p = 0.20$ ).

## 4. Discussion

### 4.1. Diversity in hay meadows and seral stages of succession

The results of our study confirm the high plant and invertebrate species richness in the investigated extensive hay meadows and their seral stages of abandonment. Each successional stage contained characteristic plant and invertebrate species. Furthermore, each successional stage harboured the maximum species richness for one particular taxonomic group. In contrast to previous studies on grassland succession in central and northern Europe (Bischof, 1984;



**Fig. 4** – Habitat specificity of vascular plants, terrestrial gastropods, and diurnal and nocturnal Lepidoptera in the four investigated successional stages (M, extensive hay meadow; G, abandoned hay meadow; B, naturally growing birch forests; W, mature forest) in Transylvanian mountains. White areas of bars refer to open-land species, hatched areas to ubiquitous species, and black areas to forest species. For each successional stage mean values of four replicates are shown.

Table 5 – Number (mean $\pm$ SE) and percentage (in parentheses) of red-listed species of vascular plants, and diurnal and nocturnal Lepidoptera recorded in different successional stages				
Successional stage <sup>a</sup>	Threat level <sup>b</sup>	Vascular plants	Diurnal Lepidoptera <sup>c</sup>	Nocturnal Lepidoptera
Extensive hay meadow	TRE	0.5 $\pm$ 0.3 (0.9)	0.5 $\pm$ 0.3 (2.7)	1.8 $\pm$ 0.3 (2.0)
	NT	1.5 $\pm$ 0.3 (2.8)	2.0 $\pm$ 0.7 (10.2)	6.8 $\pm$ 0.9 (7.8)
	LC	50.3 $\pm$ 3.8 (96.3)	16.5 $\pm$ 2.3 (87.2)	77.8 $\pm$ 1.0 (90.2)
Abandoned hay meadow	TRE	0.5 $\pm$ 0.3 (1.0)	0.8 $\pm$ 0.3 (3.2)	2.3 $\pm$ 0.5 (2.3)
	NT	0.8 $\pm$ 0.5 (1.5)	3.3 $\pm$ 0.9 (13.1)	11.5 $\pm$ 2.2 (11.2)
	LC	48.5 $\pm$ 5.6 (97.4)	20.3 $\pm$ 1.1 (83.7)	88.8 $\pm$ 7.6 (86.4)
Naturally growing birch forest	TRE	0.3 $\pm$ 0.3 (0.4)	0 $\pm$ 0 (0)	4.5 $\pm$ 0.6 (3.1)
	NT	0.5 $\pm$ 0.3 (1.0)	1.8 $\pm$ 0.5 (12.9)	19.5 $\pm$ 1.0 (13.8)
	LC	41.0 $\pm$ 6.4 (98.6)	13.5 $\pm$ 3.7 (82.9)	118.5 $\pm$ 5.8 (83.1)
Mature forest	TRE	0.3 $\pm$ 0.3 (0.4)	0 $\pm$ 0 (0)	3.5 $\pm$ 0.3 (3.0)
	NT	0.8 $\pm$ 0.3 (1.5)	1.5 $\pm$ 0.3 (18.4)	18.3 $\pm$ 0.3 (15.6)
	LC	45.3 $\pm$ 5.7 (98.1)	7.5 $\pm$ 1.5 (78.8)	96.0 $\pm$ 6.2 (81.3)

a For each vegetation type n = 4.  
b TRE, threatened (red list categories critically endangered, endangered, and vulnerable); NT, nearly threatened; LC, least concern.  
c Two species: data deficiency.

Zoller et al., 1984; Tasser and Tappeiner, 2002; Pykälä et al., 2005), we found no decline in plant species richness in early successional stages. Similar results were reported from abandoned steppe-like grasslands in Romania (Cremene et al., 2005). The high plant species diversity in abandoned hay meadows could be explained by a delayed succession and/or small-scale mosaic of abiotic conditions. The latter is correlated with the structural diversity of the habitat, which

usually increases with successional age (Ellenberg, 1996; Morris, 2000). A high structural diversity in turn favours the diversity of invertebrates. This might be the most important factor for the significant increase in species richness of nocturnal Lepidoptera with successional age. For instance, we recorded the noctuid moth *Chersotis cuprea* (a vulnerable species) most frequently in naturally growing birch forests. This confirms other studies that showed that certain rare

and threatened butterfly species benefit from grassland abandonment (Erhardt, 1985a,b; Thomas, 1991; Balmer and Erhardt, 2000; Cremene et al., 2005). The low species richness of gastropods can be explained by the acidic soils of the study sites examined (Wäreborn, 1969).

In mature forests, the final stage of natural grassland succession, diversity of the examined groups of organisms was not reduced, except for diurnal Lepidoptera. This finding contradicts the results of other studies (e.g. Bischof, 1984; Ellenberg, 1996). A possible explanation for this discrepancy is that we investigated original forests which have not been logged for several decades, whereas forests examined in other studies frequently were of more recent origin, and in some cases isolated, and hence less diverse. Furthermore, successional changes in species richness differ depending on the taxonomic group examined. For instance, nocturnal Lepidoptera have rarely been considered in successional studies. Gastropod communities tend to be species richer in forests than in open habitats (Wäreborn, 1969), which is not the case in butterflies.

Most interestingly, diurnal and nocturnal Lepidoptera reacted in opposite directions to progressive succession. Species richness and abundance of diurnal Lepidoptera declined with successional age, while those of nocturnal Lepidoptera increased. The increase in structural diversity with successional age may favour nocturnal Lepidoptera (see above), but the successive decrease of open habitat negatively affects diurnal Lepidoptera. The majority of European butterfly species prefers open habitats (Blab and Kudrna, 1982).

Our results only partly support the intermediate disturbance hypothesis. Although plant and invertebrate diversities were high in the investigated hay meadows, the lack of disturbance in abandoned successional stages did not result in the expected decline in species diversity.

The complementarity analysis showed that species composition in all four taxonomic groups gradually changed with successional age. Furthermore, neighbouring habitats did not affect the species composition of a particular successional stage in diurnal and nocturnal Lepidoptera, except for diurnal Lepidoptera in two closely situated hay meadows and abandoned hay meadows. This supports the finding that the majority of diurnal Lepidoptera are habitat-specific and rather sedentary.

Detrended correspondence analysis, which considers both species presence and abundance, confirmed the changes in species composition according to the successional gradient. Interestingly, the ordination diagrams of these analyses revealed no separation of site exposure, except for diurnal Lepidoptera in naturally growing birch forests (Fig. 3b; butterfly communities at the sites 11 and 12 on S- to E-facing slopes were similar to those of open habitats). This indicates that exposure had only a neglectable effect on species composition compared to successional age.

Extensively cultivated grasslands such as the investigated hay meadows serve as refuges for several open-land species, which are of primary concern in Romania (Ioras, 2003). In our study, the proportion of open-land species of all investigated taxonomic groups decreased with successional age, suggesting that succession has a detrimental effect on open-land species. The number of red-listed species of plants and diurnal

Lepidoptera, however, was not correlated with successional age, a parallel finding to a study on plants in semi-natural grasslands in Finland (Pykälä et al., 2005). On the other hand, the number of red-listed nocturnal Lepidoptera increased with successional age in our study. Thus, open-land species of all taxonomic groups and red-listed nocturnal Lepidoptera responded in opposite directions to grassland abandonment.

The number of recorded endemic species was generally low in all four taxonomic groups, and was not correlated with successional age. Most important were the plant species endemic to the Carpathians, *Campanula polymorpha*, recorded in two hay meadows, *Silene dubia*, found in one natural birch forest, and *Symphytum cordatum*, recorded in one mature wood. *Vestia turgida*, a land snail endemic to the Carpathians, occurs in moist woodland, under logs and ground litter (Sulikowska-Drozd, 2005), as recorded in our study. Although locally common at few places, this snail is endangered in Poland and other parts of the Carpathian mountains (Wiktor and Riedel, 1992).

#### 4.2. Correlations between taxonomic groups

We found no correlation between the species richness of the investigated taxonomic groups. Similar findings were obtained in previous studies. For example, an attempt to correlate the diversities of vascular plants, butterflies, grasshoppers, gastropods and carabid beetles showed that a single taxonomic group was a poor indicator for the species diversity of other groups in nutrient-poor, dry calcareous grasslands in Switzerland (Baur et al., 1996; Niemelä and Baur, 1998). Moreover, Lawton et al. (1998) found that none of the eight animal groups studied served as a good indicator taxon for changes in the species richness of other groups in a tropical forest. Thus, it appears that one taxonomic group is a poor indicator for the overall diversity (Baur et al., 1996; Niemelä and Baur, 1998). In contrast to these findings and the present results, plant and butterfly diversities were positively correlated in other studies (Erhardt, 1985a,b; Hawkins and Porter, 2003; Cremene et al., 2005). Butterflies have also been found to be realistic and practical indicators of species change and decline in plants, other insects and birds due to their rapid response to habitat change (Thomas et al., 2004). In our study, plant species richness did not differ among successional stages, whereas diurnal Lepidoptera species richness decreased with succession. This may explain why plant and butterfly species richness were not correlated. Thus, our study advocates a shopping-basket-approach to the measurement of diversity and to conservation evaluation of hay meadows – i.e., measuring species richness and abundance of several taxonomic groups instead of just one (Launer and Murphy, 1994; Oliver and Beattie, 1996).

#### 4.3. Implications for conservation and management

Our results show the high conservation value of subalpine hay meadows and their seral stages of abandonment in Transylvania. However, without an appropriate management these hay meadows and their successional stages will convert into mature forest. We suggest a rotational management of hay meadows and the different successional stages before

succession has reached the stage of mature forest. This could be achieved by supporting traditional hay making and maintaining seral stages with subsidies from governmental and/or private organisations. A similar approach has been proposed for nutrient-poor semi-natural grasslands in Central Europe (Duffey et al., 1974; Morris and Thomas, 1991; Erhardt, 1995; Dzwonko and Loster, 1998; Waldhardt and Otte, 2003). This approach requires a specific management plan to prevent the loss of unique species, which happened in earlier conservation attempts in western Europe (Thomas, 1984, 1991). For instance, our study provided the first confirmed record of the burnet moth *Jordanita notata* for Romania. This species was, however, restricted to south-facing hay meadows and hay meadows abandoned for only 3–5 years. Once lost, it may be difficult to reestablish rare target species such as *J. notata* in our study area. In southern Finland, for example, rare grassland species did not respond to resumed grazing although restoration of abandoned grassland by cattle grazing was promising (Pykälä, 2003). We hope our study not only increases knowledge of the uniqueness of extensive hay meadows and their successional stages in Transylvania, but also raises the awareness of the responsible authorities of this uniqueness.

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**Chapter 4**

***Intensified Grazing Affects Endemic Plant and  
Gastropod Diversity in Alpine Grasslands of the  
Southern Carpathians, Romania***

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## **Intensified Grazing Affects Endemic Plant and Gastropod Diversity in Alpine Grasslands of the Southern Carpathians, Romania**

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### **Abstract**

Alpine grasslands in the Southern Carpathians, Romania, harbor an extraordinarily high diversity of plants and invertebrates, including Carpathic endemics. In the past decades, intensive sheep grazing caused a dramatical decrease in biodiversity and led even to eroded soils at many places in the Carpathians. Because of limited food resources, sheep are increasingly forced to graze on steep slopes, which were formerly not grazed by livestock and are considered as local biodiversity hotspots. We examined species richness, abundance and number of endemic vascular plants and terrestrial gastropods on steep slopes which were either grazed by sheep or ungrazed by livestock in two areas of the Southern Carpathians. On calcareous soils in the Bucegi mountains, a total of 177 vascular plant and 19 gastropod species were recorded. Twelve plant species (6.8%) and 4 gastropod species (21.1%) were endemic to the Carpathians. Grazed sites had lower plant and gastropod species richness than ungrazed sites. Furthermore, grazed sites tended to harbor fewer plant and gastropod species endemic to the Carpathians than ungrazed sites. On acid soils in the Fagaras mountains, a total of 96 vascular plant and 9 gastropod species were found. In this mountain area, however, grazed and ungrazed sites did not differ in species richness, abundance and number of endemic plant and gastropod species. Our findings confirm the high biodiversity of grasslands on steep slopes in the Southern Carpathians and caution against increasing grazing pressure in these refuges for relic plants and gastropods as well as for other invertebrates.

### **Introduction**

Alpine grasslands are unique habitats for a variety of plant and invertebrate species (Körner, 1999; Nagy et al., 2003). Seasonal pastoral activities have been practised for many centuries on accessible areas of these natural grasslands (Messerli and Yves, 1997). To increase grazing areas, semi-natural grasslands have been created in mountain areas below the tree line by forest logging (Ellenberg, 1996; Coldea, 2003). Although vascular plant species richness of various natural and semi-natural grasslands in general increases under moderate grazing (Collins et al., 1998; Sternberg et al., 2000), effects of grazing on biodiversity vary considerably among ecosystems and among different taxa (Niemelä and Baur, 1998; Pärt and Söderström, 1999; Balmer and Erhardt, 2000; Cremene et al., 2005). Moreover, patterns of biodiversity and their driving processes

obviously vary with spatial and temporal scale (Crawley and Harral, 2001; Dullinger et al., 2003). Pasture management should therefore be adjusted to the local conditions to identify and implement the best strategy of biodiversity conservation.

In contrast to the general trend of grassland abandonment in remote areas, land use is increasingly intensified in easily accessible mountain areas (Tasser and Tappeiner, 2002). Transhumant shepherding is the traditional use of subalpine and alpine grasslands in the Southern Carpathians, Romania. For example, historical records document sheep grazing in the Bucegi mountains since the beginning of the sixteenth century (Barbulescu and Motca, 1983). In these mountains, the sheep herds have always been large, forcing the animals to graze also in adjacent forests, which were clearcut to extend the pastures in the 19th century (Coldea, 2003). More recently, the size of the sheep herds increased further as a result of the altered socio-economic situation since 1989. Detrimental effects of overgrazing and trampling on plant diversity and vegetation structure, and eroded soils have been reported on the plateau of the Bucegi mountains (Barbulescu and Motca, 1983; Coldea, 2003). As a result, grazing pressure increased on so far extensively used, adjacent steep mountain slopes. This is of particular concern as the Southern Carpathians harbor a high number of endemic and relic plant and invertebrate species (ICAS, 1996; Ioras, 2003).

The aim of the present study was to examine effects of intensified grazing on these steep slopes, so far only occasionally grazed by sheep and wildlife (chamois). As indicators of biodiversity we used vascular plants and terrestrial gastropods, which both have been shown to be sensitive indicators of habitat change (Ellenberg, 1996; Labaune and Magnin, 2002). We assessed the species diversity and species abundance of vascular plants and terrestrial gastropods in both intensively grazed sites and in sites only occasionally grazed by chamois in two areas in the Southern Carpathians, Romania. In particular, we addressed the following questions: (1) How are plant and gastropod diversity and abundance affected by intensified sheep grazing on steep slopes? (2) How do endemic and threatened (red-listed) species respond to intensive grazing? (3) Are responses to grazing correlated between plants and gastropods?

## **Materials and Methods**

### *STUDY AREAS AND SAMPLING*

The effect of intensified pastoral management on the alpine plant and gastropod diversity was examined in two areas in the Southern Carpathians in Romania: in the

Bucegi mountains (45° 24' N, 25° 31' E) 30 km SSW of Brasov and in the Fagaras mountains (45° 37' N, 24° 37' E) 40 km SE of Sibiu. The two mountain areas are 80 km apart from each other and differ in the underlying bedrock: the investigated slopes of the Bucegi mountains consist of limestone, while the sites in the Fagaras mountains are situated on silicious bedrock.

The Bucegi mountains are built of Mesozoic sedimentary rocks (mainly limestones, sandstones and conglomerates), which are exposed to erosion since middle Tertiary. This resulted in complex tectonic and morphological structures, with numerous small valleys and ridges on steep slopes. In the Bucegi mountains, the first protected areas were established in 1943 ([www.bucegipark.ro](http://www.bucegipark.ro)). The protected areas were several times enlarged, resulting in the Nature Park Bucegi with a total area of 326.6 km<sup>2</sup>, including 13 nature reserves (ca. 90 km<sup>2</sup>) in 2000. A significant part of the nature reserves is located on the steep mountain slopes, while the gently inclined slopes on the plateau, which are heavily overgrazed at many places and contain huge areas of eroded soil, are not protected. Our study was conducted on natural alpine grasslands at the border of the Abruptul Prahovean Nature Reserve (Table 1).

The Nature Park Bucegi contains approximately 30% of the total plant diversity in Romania. The steep slopes of the Bucegi mountains harbor a most interesting vegetation with numerous Carpathic endemic and glacial relic plants (Coldea and Cristea, 1998). At places with calcareous bedrock, the gastropod fauna is rich with several species endemic to the Carpathians (Grossu, 1981, 1983).

The Fagaras mountains are a traditional area for Romanian shepherds, who have been practising transhumant pastoralism for many centuries (Puscaru-Soroceanu et al., 1981). Our study sites were located on crystalline bedrock with brown acid soils in subalpine, man-made grasslands in the valley north of the Balea Nature Reserve. The flora of this valley is characteristic for the alpine region of the Southern Carpathians and includes several Carpathic endemic species (Puscaru-Soroceanu et al., 1981; Barbulescu and Motca, 1983). The Balea Nature Reserve is considered as relatively species-poor in invertebrates ([www.ipmsb.ro/rezervnat.htm#balea](http://www.ipmsb.ro/rezervnat.htm#balea)). However, some Carpathic endemic invertebrates can still be found in this area, e.g. the land snail *Arianta aethiops aethiops* (Baur et al., 2000).

In July 2003, we surveyed the diversity and abundance of vascular plants and terrestrial gastropods on steep slopes at five sites intensively grazed by sheep (hereafter grazed sites) and at five sites only occasionally grazed by chamois (hereafter ungrazed



sites) in the Bucegi mountains (Table 1). Grazed study sites were parts of pastures repeatedly visited by large sheep herds (> 500 individuals). Ungrazed study sites were situated on the same slopes but separated from grazed sites either by rock walls or stands of *Pinus mugo*. Each study site measured approximately 20 x 30 m. Similarly, we examined four grazed and four ungrazed sites in the Fagaras mountains (Table 1). Grazed and ungrazed sites were situated 110 - 2600 m apart in the Bucegi mountains and 120 - 1530 m in the Fagaras mountains.

For plants we compiled a species list of grasses and forbs for each site. In addition to presence/absence, we also recorded the abundance of each species in a subplot of 10 x 10 m using the Braun-Blanquet method (1964). Walking in a zigzag line over the entire study site (20 x 30 m) the list of plant species was completed by recording all new species observed. Plant identification followed Tutin (1964-1980) and Ciocarlan (2000).

Species richness and relative abundance of terrestrial gastropods were assessed by visually searching for living snails and slugs and for empty shells. Five people searched in each 20 x 30 m plot for a total of 120 minutes. Fieldwork was only conducted under conditions favorable for gastropod activity (moist vegetation and high air humidity). Identification of gastropods followed Grossu (1981, 1983).

At each study site, the following ecological variables were measured: elevation (in meters above sea level, extracted from topographical maps; Bucegi mountains, scale 1 : 53 000; Fagaras mountains, scale 1 : 60 000), exposure (degrees from south using a compass), inclination (average of six measurements), soil pH (average of six soil samples using the Hellige method; AVM Analyseverfahren, Freiburg, Germany), two measures of vegetation height (average height of the forb and the grass layer, both based on six measurements), and the percentage area covered by rock and scree (estimated to the nearest 10%).

In the Bucegi mountains, grazed and ungrazed study sites did not differ in elevation, soil pH and area covered by rocks and scree (Table 1; t-tests, in all comparisons,  $P > 0.35$ ).

However, grazed sites faced slightly more to north-east, whereas ungrazed sites tended to face to south-east (Mardia-Watson-Wheeler-test,  $P = 0.05$ ). Furthermore, grazed sites occurred on slightly flatter slopes than ungrazed sites ( $33.8^\circ \pm 3.8^\circ$  (SD) vs  $39.2^\circ \pm 1.9^\circ$ ;  $t_8 = 2.94$ ,  $P = 0.0188$ ). As expected, the vegetation was shorter at grazed sites than at ungrazed ones (forb height:  $7.0 \pm 2.7$  cm vs  $23.0 \pm 2.7$  cm,  $t_8 = 9.24$ ,  $P <$

0.0001; grass height:  $18.0 \pm 4.5$  cm vs  $30.0 \pm 0$  cm,  $t_8 = 6.00$ ,  $P = 0.0003$ ).

In the Fagaras mountains, grazed and ungrazed sites did not differ in exposure, elevation, inclination, soil pH and area covered by rocks and scree (Table 1; t-tests, in all comparisons,  $P > 0.14$ ). Again, the vegetation was shorter at grazed than at ungrazed sites (forb height:  $6.8 \pm 2.4$  cm vs  $18.8 \pm 8.5$  cm,  $t_6 = 2.71$ ,  $P = 0.0352$ ; grass height:  $22.5 \pm 5.0$  vs  $37.5 \pm 9.6$  cm,  $t_6 = 2.78$ ,  $P = 0.0321$ ).

### DATA ANALYSES

The StatView program package (SAS Institute, 1998) was used for statistical analyses. Means  $\pm$  1 SD are given unless otherwise stated. In all analyses we considered the study sites as the unit of investigation. As calcareous and silicious soils are known to harbor a different flora and fauna, we analysed data from the Bucegi and Fagaras mountains separately. Data on species richness, number of gastropod individuals, and number of endemic and red-listed species were log-transformed.

Because the grazed and ungrazed sites examined in the Bucegi mountains slightly differed in exposure, we used analyses of covariance (ANCOVA, type III model) with grazing/no grazing as factor and exposure as covariate to examine possible differences in species richness, number of gastropod individuals, and number of endemic and red-listed species. Similarly, data from the Bucegi mountains were analysed using the same ANCOVA-model with inclination as covariate. However, in no case inclination had a significant influence on the examined variables. Therefore, these analyses are not presented. In the Fagaras mountains, the grazed and ungrazed sites examined differed neither in exposure nor in inclination. For this area we used unpaired t-tests to examine possible differences between grazed and ungrazed sites in species richness, number of gastropod individuals, and number of endemic and red-listed species.

We used detrended correspondence analysis (DCA) to examine changes in plant and gastropod communities between grazed and ungrazed study sites (Hill and Gauch, 1980). DCA was performed separately for both groups of organisms and both mountain areas. For the DCA, the vegetation cover-abundance code adapted from Braun-Blanquet (1964) was applied using the following weights: 0.1 for 'R', 0.5 for '+', 3 for the category 1, 7.5 for the category 2, 15 for the category 3, 25 for the category 4, and 40 for the category 5 (see Buschmann et al., 2005). Prior to ordination, the data were log-transformed. Species that were less frequent than the median frequency were down-weighted in proportion to their frequencies (Eilertsen et al., 1990). DCA was performed

using CANOCO version 4.5; ter Braak and Smilauer, 2002). In plants, comparisons of species richness and number of endemic and red-listed species are based on the species list of each study site (20 x 30 m), whereas comparisons of communities are based on abundance data of 10 x 10 m subplots.

To examine whether grazed and ungrazed sites differed in the number of endemic plants or gastropods, we assigned each species to one of the following categories: Romanian Carpathian endemic (occurring exclusively in the Carpathians of Romania), Eastern European endemic (Balcanic, Pontic, Carpathic and/or Dacic distributions) and widespread (naturally occurring in large areas of one or more continents). Total cover of plants endemic to the Carpathians was calculated for each site as the sum of the cover values of all Carpathic endemic plants (see above). In Romania, a red list exists for plants (Boscaiu et al., 1994) but not for terrestrial gastropods.

We used Pearson's correlations to examine possible correlations between species richness and numbers of endemic and red-listed species within and between plants and gastropods. For data of the Bucegi mountains, we calculated partial correlations, keeping exposure constant.

## Results

### *PLANT AND GASTROPOD SPECIES RICHNESS*

A total of 177 plant species was recorded at the study sites of the Bucegi mountains. Grazed sites had a lower plant species richness ( $57.8 \pm 10.9$ ; mean  $\pm$  SD) than ungrazed sites ( $64.2 \pm 8.6$ ; ANCOVA:  $F_{1,6} = 6.44$ ,  $P = 0.0443$ ). The exposure of the sites did not affect the number of plant species ( $P = 0.51$ ), but there was a tendency for an interaction between grazing and exposure ( $P = 0.0573$ ). Nineteen different gastropod species were recorded at the study sites of the Bucegi mountains. Grazed sites harbored fewer gastropod species ( $5.4 \pm 3.5$ ) than ungrazed sites ( $8.4 \pm 2.3$ ; ANCOVA:  $F_{1,6} = 6.00$ ,  $P = 0.0498$ ). Again, site exposure did not influence the number of gastropod species ( $P = 0.29$ ), and there was a tendency for an interaction between grazing and exposure ( $P = 0.0573$ ).

In all, 96 plant species were found in the study sites on silicious bedrock in the Fagaras mountains. Grazed ( $29.3 \pm 6.4$ ) and ungrazed ( $30.8 \pm 4.6$ ) sites did not differ in plant species richness ( $t_6 = 0.46$ ,  $P = 0.66$ ). At the same sites, a total of 9 gastropod species was recorded. Grazed sites ( $3.3 \pm 2.2$ ) and ungrazed sites ( $3.8 \pm 1.3$ ) did not differ in gastropod species richness ( $t_6 = 0.68$ ,  $P = 0.52$ ).

*COMMUNITY STRUCTURE*

In plants growing in the Bucegi mountains, the DCA ordination revealed a separation of grazed and ungrazed study sites, except that one grazed site (#10) was positioned among ungrazed sites (Fig. 1a). The first axis (Eigenvalue = 0.379) explained 25.1% of the variance in species data (together with the second axis 37.7%). In plants of the Fagaras mountains, grazed and ungrazed sites were clearly separated (Fig. 1b). The first axis (Eigenvalue = 0.683) explained 28.4% of the variance in plant species data (together with the second axis 39.6%). Similarly, in terrestrial gastropods living in the Bucegi mountains, the DCA ordination revealed a clear separation of grazed and ungrazed study sites (Fig. 1c). The first axis (Eigenvalue = 0.273) explained 27.7% of the variance in species data (together with the second axis 35.7%). In gastropods of the Fagaras mountains, however, there was no separation between grazed and ungrazed sites (Fig. 1d). The first axis (Eigenvalue = 0.534) explained 40.0% of the variance in gastropod species data (together with the second axis 59.1%).

*ABUNDANCE OF GASTROPODS*

A total of 1611 gastropod individuals were recorded (1496 in the Bucegi mountains and 115 in the Fagaras mountains). In the Bucegi mountains, on average  $114.6 \pm 62.9$  individuals were found in grazed sites and  $184.6 \pm 47.6$  individuals in ungrazed sites. However, due to the large within-treatment variation in number of individuals the difference in gastropod abundance was not significant (ANCOVA:  $F_{1,6} = 1.16$ ,  $P = 0.32$ ). The exposure of the site did not influence the number of gastropod individuals recorded ( $P = 0.80$ ) and there was no interaction between grazing and exposure ( $P = 0.52$ ). Similarly, grazed and ungrazed sites in the Fagaras mountains did not differ in the number of gastropod individuals ( $15.8 \pm 4.0$  vs.  $13.0 \pm 9.8$ ;  $t_6 = 0.92$ ,  $P = 0.39$ ).

In the Bucegi mountains, the number of recorded gastropod individuals increased with increasing grass height (partial correlation, exposure kept constant:  $r = 0.82$ ,  $n = 10$ ,  $P = 0.0086$ ). No similar relationship between number of gastropods recorded and vegetation height was found in the Fagaras mountains ( $r = -0.17$ ,  $n = 8$ ,  $P = 0.70$ ).

The two mountain areas differed also in the proportion of slugs (gastropods without shell) found. In the Bucegi mountains, only 14 (0.9%) out of the 1496 individuals recorded were slugs, whereas in the Fagaras mountains 88 (76.5%) of the 115 gastropod individuals were slugs (chi square = 1028.8,  $df = 1$ ,  $P < 0.0001$ ).

## ENDEMISM

Twelve (6.8%) of the 177 plant species recorded in the Bucegi mountains were endemic to the Carpathians (*Cerastium transsilvanicum*, *Dianthus gelidus*, *Dianthus tenuifolius*, *Gypsophila petraea*, *Hypericum richeri* ssp. *transsilvanicum*, *Koeleria macrantha* ssp. *transsilvanica*, *Onobrychis montana* ssp. *transsilvanica*, *Oxytropis carpatica*, *Phyteuma wagneri*, *Saxifraga demissa*, *Thlaspi dacicum* and *Thymus pulcherrimus*) and another 20 species (11.3%) were endemic to Eastern Europe. The number of plant species endemic to the Carpathians tended to be lower in grazed sites ( $3.0 \pm 2.6$ ) than in ungrazed sites ( $4.0 \pm 1.4$ ; ANCOVA:  $F_{1,6} = 5.18$ ,  $P = 0.0632$ ). The number of Carpathic endemic plant species was not affected by the exposure of the sites ( $P = 0.21$ ), but there was a significant interaction between grazing and exposure ( $P = 0.0494$ ), indicating that the number of endemic plant species in grazed areas was differentially affected by exposure. Furthermore, the total cover of Carpathic endemic plants was lower in grazed ( $1.0 \pm 1.5\%$ ) than in ungrazed sites ( $3.9 \pm 3.8\%$ ; ANCOVA:  $F_{1,6} = 10.03$ ,  $P = 0.0194$ ). The exposure of the sites did not influence the total cover of endemic plants ( $P = 0.89$ ), but there was a tendency for an interaction between grazing and exposure ( $F_{1,6} = 5.94$ ,  $P = 0.0506$ ).

Two (2.1 %) of the 96 plant species recorded in the Fagaras mountains were endemic to the Carpathians (*Hypericum richeri* ssp. *transsilvanicum* and *Thymus pulcherrimus*) and another 9 species (9.4%) were endemic to Eastern Europe. Grazed and ungrazed sites did not differ in the number of Carpathic endemic plant species ( $1.3 \pm 1.0$  vs  $0.5 \pm 0.6$ ;  $t_6 = 1.15$ ,  $P = 0.29$ ). Neither did the grassland types differ in total cover of plants endemic to the Carpathians (grazed:  $0.2 \pm 0.3\%$ , ungrazed:  $0.8 \pm 1.5\%$ ;  $t_6 = 0.11$ ,  $P = 0.92$ ).

Four (21.1%) of the 19 gastropod species recorded in the Bucegi mountains were endemic to the Carpathians (*Alopija canescens*, *Alopija livida*, *Chondrula venerabilis* and *Faustina faustina*) and one further species (*Oxychilus inopinatus*) was endemic to Eastern Europe. The number of gastropod species endemic to the Carpathians was marginally significantly lower in grazed than in ungrazed sites ( $2.2 \pm 0.8$  vs.  $3.2 \pm 0.4$ ; ANCOVA:  $F_{1,6} = 5.94$ ,  $P = 0.0507$ ). Site exposure did not influence the number of endemic gastropod species ( $P = 0.10$ ), but there was a significant interaction between grazing and exposure ( $F_{1,6} = 6.43$ ,  $P = 0.0443$ ), indicating that the number of endemic gastropod species in grazed areas was differentially affected by exposure. Considering the number of gastropod individuals, on average  $96.4 \pm 63.1$  individuals endemic to the

Carpathians were found in grazed sites and  $134.0 \pm 22.6$  in ungrazed sites. However, because of the large within-treatment variation in number of endemic individuals, this difference was not significant (ANCOVA:  $F_{1,6} = 0.10$ ,  $P = 0.76$ ). Furthermore, there was no effect of site exposure on the number of endemic gastropod individuals ( $P = 0.64$ ) nor was there any interaction ( $P = 0.77$ ). Considering single species, the Carpathic endemics *A. livida* and *F. faustina* occurred in most of the sites examined. The abundance of both species did not differ between grazed and ungrazed sites (ANCOVA,  $P = 0.71$  and  $P = 0.92$ , respectively). However, the abundance of another Carpathic endemic, *C. venerabilis* was affected by sheep grazing:  $5.4 \pm 7.8$  individuals were found in grazed sites and  $70.0 \pm 39.1$  individuals in ungrazed sites (ANCOVA:  $F_{1,6} = 10.54$ ,  $P = 0.0175$ ). The abundance of *C. venerabilis* was not significantly influenced by the exposure of the site (ANCOVA:  $P = 0.07$ ), but there was an interaction between grazing and exposure ( $F_{1,6} = 7.09$ ,  $P = 0.0374$ ). *C. venerabilis* occurred in all 5 ungrazed sites and in 2 of the 5 grazed sites. The other 2 endemics (*A. canescens* and *O. inopinatus*) were only found at a single site each.

None of the 9 gastropod species found in the study sites of the Fagaras mountains was endemic to the Carpathians.

#### RED-LISTED SPECIES

In the Bucegi mountains, 4 vulnerable and 19 nearly threatened (rare) plant species were recorded in the sites examined (no critically endangered or endangered species occurred in these sites). Grazed and ungrazed sites did not differ in the number of red-listed plant species (total of species considered as vulnerable or nearly threatened; grazed sites  $10.4 \pm 2.4\%$ , ungrazed sites  $8.2 \pm 3.3\%$ ; ANCOVA:  $F_{1,6} = 3.86$ ,  $P = 0.10$ ). The exposure of the sites did not influence the number of red-listed species ( $P = 0.78$ ), and there was no interaction between grazing and exposure ( $P = 0.09$ ).

In the Fagaras mountains, one vulnerable and 4 nearly threatened plant species were found in the study sites. Grazed and ungrazed sites did not differ in the number of red-listed plant species ( $2.0 \pm 1.6$  vs  $1.8 \pm 1.0$ ;  $t_6 = 0.04$   $P = 0.97$ ).

#### CORRELATIONS BETWEEN PLANTS AND GASTROPODS

Plant species richness was positively correlated with gastropod species richness in the Bucegi mountains (partial correlation, keeping exposure constant,  $r = 0.67$ ,  $n = 10$ ,  $P = 0.0480$ ), but not in the Fagaras mountains ( $r = -0.48$ ,  $n = 10$ ,  $P = 0.25$ ). In the Bucegi

mountains, plant species richness was also positively correlated with the number of plants endemic to the Carpathians (partial correlation  $r = 0.91$ ,  $n = 10$ ,  $P = 0.0003$ ) and the number of Carpathic endemic gastropods (partial correlation  $r = 0.87$ ,  $n = 10$ ,  $P = 0.0044$ ). Gastropod species richness itself was correlated with the number of gastropods endemic to the Carpathians (partial correlation  $r = 0.85$ ,  $n = 10$ ,  $P = 0.0067$ ). However, the number of red-listed plant species was neither correlated with plant species richness nor with the number of endemic plants or gastropods. Furthermore, none of these variables were intercorrelated in the Fagaras mountains.

## Discussion

Over the past centuries, pastoralism has taken up much of the grasslands in the Southern Carpathians (Barbulescu and Motca, 1983). Due to the high grazing pressure, the grassland area was stepwise enlarged by clear-cutting forests and areas covered by shrubs and bushes (Puscaru-Soroceanu et al., 1981). However, the problem of overgrazing by too large sheep herds grazing in relatively small areas remained. The huge number of sheep reduced plant diversity, and subsequently, the soil became eroded, particularly on flatter parts of the mountains (Coldea, 2003). As a consequence, the originally wide-spread, species-rich grasslands can today only be found on the steeper slopes, which until recently were less intensively or not at all grazed by livestock. However, since 1989, the altered socioeconomic conditions in Romania favor a further increase in the size of sheep herds, which still have to graze on the same mountain pastures. As a result, grazing pressure extended into these unique, species-rich grassland remnants on steep slopes.

Our study shows that in the Bucegi mountains species richness of plants and terrestrial gastropods is reduced in grasslands on steep, intensively grazed slopes compared with sites only occasionally grazed by wildlife. Management by grazing is known to alter the botanical composition and structure of grassland vegetation (Morris, 2000). Intermediate levels of disturbance are assumed to increase plant species richness and reduce the dominance of competitive species (Curry, 1994). However, effects on gastropod communities are less well studied. Grazing may influence gastropods indirectly by altering the amount and quality of the food supply and by changing the microclimate, or directly by trampling the snails' shells (Martin and Sommer, 2004). The latter is of particular importance for gastropods, because on steep slopes grazing

sheep frequently move pieces of stone which results in crushed snails (Baur et al., 2000). Our results indicate that the extent of disturbance by grazing is too high to maintain the former species-rich plant and gastropod communities in the Bucegi mountains.

The effects of intensive sheep grazing on the plant and gastropod communities of acid soils in the Fagaras mountains were less pronounced. Areas with silicious bedrock are generally less species-rich in plants and particularly in gastropods than areas with calcareous soils (Ellenberg, 1996; Wäreborn, 1970, 1992; Waldén, 1981). Hence, in all these areas an increased grazing pressure might not so strongly affect species richness. However, in the present study, species composition and abundance in plants were altered in a characteristic and pronounced way in the Fagaras mountains (Fig. 1b), suggesting that intensive sheep grazing affects the vegetation composition also on silicious bedrock.

On acid soils only a limited number of gastropods can cope with the calcium carbonate deficiency. Slugs (gastropods without shell) can better live under such conditions. This may explain the high proportion of slugs in the Fagaras mountains. However, gastropod abundance and species richness remained low, preventing significant differences between grazed and ungrazed sites. Furthermore, slugs are presumably less sensitive to livestock trampling than shelled gastropods (Martin and Sommer, 2004).

Both investigated mountain areas harbored species endemic to the Carpathians, but they were more numerous in the calcareous grasslands of the Bucegi mountains than in the Fagaras mountains. In the Bucegi mountains there was a tendency for grazed sites to harbor fewer endemic plants and gastropods than ungrazed sites. Furthermore, the total cover of Carpathic endemic plants was reduced on grazed slopes in this mountain area. Different endemic plant and gastropod species reacted differently to grazing pressure. For example, the Carpathic endemic gastropods *Chondrula venerabilis* is a specialized grassland species, which showed a reduced abundance in grazed sites. By contrast, the Carpathic endemics *Alopija livida* and *Faustina faustina*, two gastropod species feeding on algae and lichens, occur mainly on vertical rock surfaces and thus are less exposed to trampling by grazing livestock. Hence, the abundance of both *A. livida* and *F. faustina* did not differ between grazed and ungrazed sites.

Plant species richness was positively correlated with that of terrestrial gastropods in the Bucegi mountains. In steppe-like grasslands and their seral stages of succession in



Transylvania, Romania, no correlation between plant and gastropod species richness was found (Cremene et al., 2005). Similarly, the species richness of vascular plants was a poor indicator for species richness of gastropods in nutrient-poor, dry calcareous grasslands in Switzerland (Baur et al., 1996; Niemelä and Baur, 1998). In the present study, plant diversity may not directly influence gastropod diversity, but both may respond to similar environmental conditions.

#### *IMPLICATIONS FOR CONSERVATION AND MANAGEMENT*

Our study shows that in the Bucegi mountains plant and gastropod diversity and abundance are significantly reduced by sheep grazing on formerly ungrazed, steep slopes. This is of particular concern because the plateau of the Bucegi mountains is already heavily overgrazed, which resulted in the local extinction of numerous indigenous plant species (Barbulescu and Motca, 1983; Coldea, 2003). The grasslands investigated in our study belong to the last remaining refuges for several endemic and relic plant and gastropod species. If overgrazing by sheep should further extend into these particularly valuable grassland remnants, their diverse flora and fauna would be at risk. Thus, an appropriate management should aim to protect these last refuges on steep slopes from overgrazing. Most of these valuable grasslands are part of the 13 nature reserves of the Nature Park Bucegi ([www.bucegipark.ro](http://www.bucegipark.ro)). The findings of our study indicate that there is an urgent need to implement the protection aims of the existing nature reserve. Furthermore, the restoration of overgrazed grasslands should be promoted, even if this may require decades. At present, the situation in the Fagaras mountains appears to be less critical, but should also be observed with attention.

Our study confirms the high biodiversity value of grasslands on steep slopes, not only for endemic and relic plant and gastropod species, but also for more widespread species in the Carpathian mountains. It also shows the detrimental effects of intensified sheep grazing on these so far unthreatened grasslands, which were only occasionally grazed by wildlife. Other taxonomic groups such as butterflies and moths may also suffer under the increasing grazing pressure. For example, populations of the moth *Grammia quenseli*, critically endangered in Romania, and a number of other arctic-alpine moth species, declined extremely during the last decades in the Bucegi mountains, and might even become extinct without conservation measures (Rakosy et al., 2003; Dinca, 2005).

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TABLE 1. Characteristics of the sites surveyed in the Bucegi and Fagaras mountains, Southern Carpathians, Romania.

Mountain area	Site	Grassland management*)	Elevation (m a.s.l.)	Exposure	Inclination (°)	Soil pH	Forb / grass height (cm)	Rock cover (%)
Bucegi	1	U	2220	E-ESE	38	6.5	25 / 30	10
	2	U	2080	S-SE	37	6.0	20 / 30	20
	3	U	2120	S-SSE	40	7.0	20 / 30	20
	4	U	2040	E-SE	42	6.5	25 / 30	20
	5	U	1910	E-ESE	39	6.5	25 / 30	25
	6	G	2070	NE	31	6.5	5 / 20	10
	7	G	2120	NNE	32	6.5	5 / 10	15
	8	G	2100	ENE	34	6.5	10 / 20	20
	9	G	2070	NNE	32	6.5	5 / 20	15
	10	G	1940	NE	40	5.5	10 / 20	20
Fagaras	1	U	1700	W	29	4.0	20 / 50	1
	2	U	1750	W-WSW	35	4.0	30 / 40	15
	3	U	1750	W	24	4.5	15 / 30	15
	4	U	1820	WNW	35	4.5	10 / 30	5
	5	G	1620	WSW	27	4.5	10 / 30	5
	6	G	1650	W	30	4.0	7 / 20	5
	7	G	1700	W	22	4.0	5 / 20	15
	8	G	1780	WSW	20	4.0	5 / 20	10

\*) U, ungrazed; G, grazed

Figure

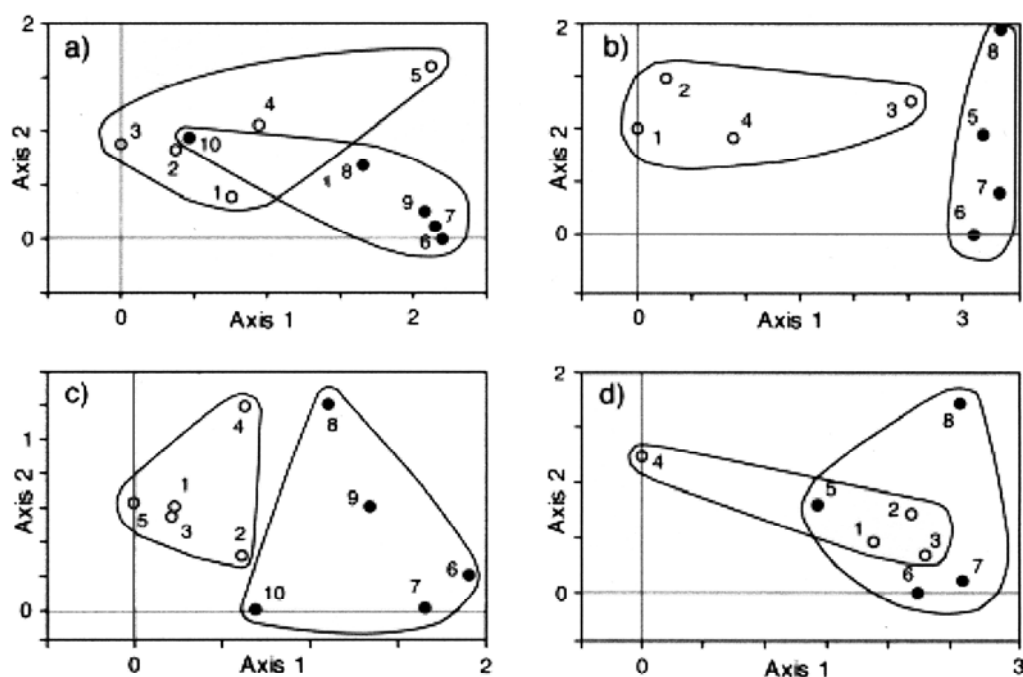


FIGURE 1. Ordination diagram based on detrended correspondence analysis of plants and terrestrial gastropods in grazed and ungrazed grasslands in the Southern Carpathians, Romania, displaying the major variation in species composition: (a) plants in the Bucegi mountains, (b) plants in the Fagaras mountains, (c) terrestrial gastropods in the Bucegi mountains, and (d) terrestrial gastropods in the Fagaras mountains. Open dots indicate ungrazed sites and full dots grazed sites. Numbers refer to the study sites (see Table 1).





## **Summary and General Conclusion**

Nutrient-poor grasslands are considered regional biodiversity hotspots and therefore of high conservation value (Blab & Kudrna 1982; Bignal & McCracken 1996; Sánchez-Zapata et al. 2003). Nutrient-poor grasslands in Transylvania and the Carpathians, Romania, harbor a variety of endemic and sub-endemic species of plants and invertebrates which are threatened by non-sustainable agriculture or abandonment.

The aim of this thesis was to investigate biodiversity and species compositions in traditionally cultivated grasslands and to compare it with that of intensified and abandoned grasslands in Romania in three different regions of Transylvania: lowland steppe-like grasslands, the subalpine region and the alpine region of the southern Carpathians. In order to give a more accurate picture of the diversity in these regions we investigated not only one taxonomic group, but four: vascular plants, terrestrial gastropods and diurnal and nocturnal Lepidoptera. Another aim of this study was to foresee future development of the investigated grasslands as a consequence of political change, and to suggest management strategies and programs to prevent the loss of precious semi-natural habitats.

**Chapter one** describes the species richness, species abundance, proportion of open-land, endemic and threatened vascular plants, gastropods, and diurnal and nocturnal Lepidoptera in six different vegetation types (extensively used pastures and their early and late successional stage of abandonment, climax forest and two man-made habitat alterations, abandoned vineyards and *Pinus* plantations) all originating from steppe-like grasslands in Transylvania, Romania.

We found a high plant and invertebrate species richness in the investigated steppe-like grasslands and their seral stages of abandonment. In climax forests, the final stage of natural grassland succession, diversity of all groups of organisms examined was significantly reduced. Furthermore, many of the rare and threatened grassland species of plants and Lepidoptera were replaced by common mesophilous species because of the reduced light. The abandonment of the extensively used vineyards created a valuable habitat for plants and invertebrates. *Pinus* plantations (a recent grassland alteration) have changed habitat quality and will have a devastating effect on the unique, indigenous diversity of these steppe-like grasslands as soon as the canopy closes. Effects of patch scale variable (i.e., habitat characteristics, including the type of grassland management, abandonment, and afforestation) were pronounced on the species richness and abundance of the investigated invertebrate groups. The proportion of open-land plant and gastropod species, that are of primary concern in Romania (Ioras 2003) decreased with successional age. All investigated vegetation types harbored threatened species. Endemic species were found in all vegetation types except mature forests and *Pinus* plantations. The four taxonomic groups differed in their response to the abandonment of steppe like grassland, except that species richness of plants and diurnal Lepidoptera were positively correlated. This confirms the study of Hawkins and Porter (2003). They concluded however, that plant diversity does not directly influence butterfly diversity but that both groups of organisms respond to similar environmental conditions.

These results emphasize the high conservation value of Transylvanian steppe-like grasslands and their seral stages of abandonment. Since each of the four taxonomic groups reacted differently to grassland abandonment, a mosaic consisting of extensively grazed areas and the grassland's seral stages of succession should be preserved.

The **second chapter** analyses the effects of abandonment and man-made habitat alterations of steppe-like grasslands only on diurnal and nocturnal Lepidoptera communities considering the xerophilous character of the typical species of these habitats and their Red List status.

Like the species richness and diversity of diurnal Lepidoptera the number of xerophilous and Red List species peaked in later successional stages with bushes and in abandoned vineyards and was low in forests and *Pinus* plantations. In contrast, xerophilous and Red List species of moths showed their highest richness in early successional stages, emphasizing also the significance of this successional stage for conservation. Species richness, diversity, xerophilous and Red List species were not correlated between diurnal and nocturnal Lepidoptera. Correspondence analyses (DCA) and classifications after Renkonen revealed for both diurnal and nocturnal Lepidoptera a clear separation between forests, *Pinus* plantations and the rest of the vegetation types. In contrast to diurnal Lepidoptera, classification of nocturnal Lepidoptera showed another important feature, as it clustered successional stages and abandoned vineyards according to locality in the first place, emphasizing the importance of regionally. The observed species reactions confirm that diurnal Lepidoptera react strongly to environmental change and hence are good indicators of habitat change. Several specific diurnal Lepidoptera like *Pseudophilotes bavius hungaricus*, endemic to Transylvania's steppe-like grasslands, *Muschampia cribrellum* and *M. tesselum*, typical xerothermophilous species, would require special conservation attention, since they occur just in highly isolated, relic populations. The investigated habitat types are also relevant as potential recolonization sources for areas where some species (e.g. *Chazara briseis*) are highly threatened and have dramatically declined.

The response of plants, gastropods and diurnal and nocturnal Lepidoptera to abandonment of hay meadows in subalpine semi-natural grasslands in Transylvania was different too (**chapter three**). Each stage of succession harbored the maximum species richness for one taxonomic group: extensive hay meadows for vascular plants, abandoned hay meadows for diurnal Lepidoptera, birch forest for nocturnal Lepidoptera and mature forests for gastropods. Similar to the results from the steppe-like grasslands, no decline in plant species richness in early successional stages was recorded, due to a delayed succession and/or a small scale mosaic of abiotic conditions. A high structural diversity found in later successional stages favors the diversity of invertebrates. The number of red listed plant and diurnal Lepidoptera species was not correlated with successional age. Similar results were found in a study on plants in semi-natural grasslands in Finland (Pykälä et al. 2005). In contrast to open-land species, the number of red-listed nocturnal Lepidoptera species increased with successional age. This study showed too that one taxonomic group is a poor indicator for the overall diversity (Baur et al, 1996; Niemelä and Baur 1998). These results show the high conservation value of

subalpine hay meadows and their seral stages of abandonment in Transylvania.

The **fourth chapter** describes the effect of intensified grazing on plants and gastropods in the alpine grasslands in two areas of the Southern Carpathians (Bucegi, calcareous, and Fagaras, silicious mountains). Alpine grasslands in the Southern Carpathians harbor an extraordinary high diversity of plants and invertebrates, including Carpathic endemics, which are now threatened by the high grazing pressure.

Our study showed that plant and gastropod species richness are reduced in grazed sites compared with sites only occasionally grazed by wildlife in the Bucegi mountains. The effects of intensive sheep grazing on plant and gastropod communities were less pronounced on the acid soils of the Fagaras mountains. This is due to the fact that plants and gastropods are generally less species rich on acidic soil. However the species composition and abundance in plants were altered in a particular way, suggesting that intensive sheep grazing affects the vegetation composition also on silicious bedrock. Grazed and ungrazed sites did not differ in the proportion of endemic plants and gastropods, but total cover of Carpathic endemic plants was reduced in grazed sites in the Bucegi mountains. This might be explained by the fact that different endemic plant and gastropod species react differently to grazing pressure.

Our study confirms the high biodiversity of alpine grasslands and shows the detrimental effects of intensified sheep grazing.

### **General conclusion**

The present thesis documents the high biodiversity of semi-natural grasslands and their successional stages of abandonment at different altitudes: lowland steppe-like grasslands, subalpine mown meadows and alpine pastures in Transylvania, Romania. Not only early but also late successional stages seem to be of high conservation value due to the high species diversity found and their composition in open-land, endemic, xerophilous and red-listed species. Climax forests are less diverse and harbor less endemic and threatened species.

Investigated taxonomic groups differed in their responses to the abandonment of semi-natural grasslands in lowland steppe-like grasslands and subalpine meadows. Thus one taxonomic group is a poor indicator for the overall diversity.

The abandonment of formerly extensively used vineyards resulted in a highly precious habitat, with high diversity and many threatened, endemic and red-listed species.

The *Pinus* plantations have changed the habitat quality and will have a devastating effect on the unique and indigenous diversity of steppe-like grasslands as soon as the canopy closes.

This thesis also shows that the intensification of sheep grazing is a major threat to diversity and to endemic and threatened species of plants and gastropods in alpine pastures in Fagaras and Bucegi mountains.



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