1   Biodiversity-rich European grasslands: ancient, forgotten ecosystems

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4

5   **Abstract**

6   Worldwide reforestation has been recommended as a landscape restoration strategy to
7   mitigate climate change in areas where the climate can sustain forest. This approach may
8   threaten grassland ecosystems of unique biodiversity as such policies are based on the false
9   assumption that most grasslands are man-made. Here, we use multiple lines of evidence
10   (palaeoecological, pedological, phylogenetic, palaeontological) from Central Eastern Europe
11   and show that various types of grasslands have persisted in this area throughout postglacial
12   i.e. the past 11,700 years. A warm and dry climate, frequent fires, herbivore pressure, and
13   early Neolithic settlements kept forests open until widespread forest clearance beginning
14   4000-3000 years ago. Closed forest cover has been the exception for the past two million
15   years. This long-term persistence has likely contributed to the high biodiversity of these
16   grasslands. Consequently, we call for a more cautious prioritisation of the protection of what
17   may be erroneously considered natural, i.e. forests, by many environmental specialists and
18   managers. Instead we provide a new framework for a better understanding of the evolution
19   and persistence of different grassland types and their biodiversity, so that grasslands can be
20   better understood, valued and conserved.

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22   Keywords: ancient grasslands, anthropogenic disturbance, climate change mitigation, fire,
23   fossil records, herbivores
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26   **1. Introduction**
27   The World Resources Institute (WRI) Atlas of Forest and Landscape Restoration was
28   designed to identify opportunities for landscape restoration worldwide, an initiative supported
29   by several international organisations concerned with land degradation, climate change
30   mitigation and biodiversity loss (WRI, 2015). It contrasts the potential extent of tree cover
31   based on climate conditions with the current distribution of forest globally. This map identifies
32   23 million km^2 of land worldwide suitable for tree planting, mostly being currently open
33   landscapes with grassland (http://www.wri.org/applications/maps/flr-atlas). However,
34   grassland experts oppose the offsetting of agricultural deforestation through the afforestation
35   of grassy ecosystems arguing that this approach ignores the unique biodiversity, cultural
36   significance and important ecosystem services provided by this ecosystem (Willis et al.,
37   2008; Parr et al., 2014; Veldman et al., 2015a,b; Bond et al., 2016; Joshi et al., 2018). Their
38   imperative is to map "old-growth" grasslands, where tree cover is naturally sparse and where
39   such afforestation would be detrimental. Another important debate is how to provide
40   strategies to reduce the impact of the ongoing abandonment of high biodiversity grasslands,
41   therefore preventing succession towards low biodiversity secondary shrub and forest
42   communities (Biró et al., 2010; Valkó et al., 2018a).
43   Here we go further and challenge the perception that treeless areas in temperate regions,
44   where the current climate would permit forest development, have all previously been forested
45   and therefore grasslands and open canopy woodlands are secondary habitat types in these
46   regions. This perception fails to consider the vital role of natural disturbances such as fire or

herbivores (Bond and Keeley, 2005). We illustrate this by analysing evidence from Central Eastern Europe. The choice of this region is based on the following arguments: i) it hosts one of the largest tracts of grasslands in Europe crucial for maintaining biodiversity in European agricultural landscapes (Wilson et al., 2012); ii) has one of the highest small-scale species diversities in the world (Dengler et al., 2014; Turtureanu et al., 2014; Chytrý et al., 2015); and iii) is a transitional, complex region between closed forest and steppe biomes (Bohn et al., 2003) and hence contains grasslands of diverse origin and history. Yet, despite these features, grasslands are rarely highlighted as biodiversity hotspots. This is because the key characteristics and ecological processes important for this classification, such as biodiversity intactness and a lack of human disturbance, cannot be readily applied to them (Mittermeier et al., 2011). Our goal is to better define grassland types based on their origin, age and the drivers of their formation and maintenance so that grasslands can be better understood, valued and conserved.

2. A new framework for defining European grassland types

Bohn et al. (2003) provided a geobotanical expert assessment, which maps Europe's potential natural vegetation (PNV) i.e. the vegetation cover that would exist today in the absence of human activity. It defines most areas that are currently covered by grasslands or open woodlands in Central Eastern Europe as dominated by deciduous broadleaved forest or mixed coniferous and broadleaved forest (Fig. 1). Open, or at least partly open vegetation types, are only recognised in the lowlands of the Carpathian Basin. Fossil records show that grasslands and open canopy woodlands covered extensive areas in Central Eastern Europe during the Pleistocene (i.e., the past 2 million years) when cold and dry climate conditions prevailed (Kuneš et al., 2008; Ellenberg and Leuschner, 2010; Feurdean et al., 2014; Magyari et al., 2014). Warmer climate conditions during the Holocene (i.e. the last 11,700 years) then greatly reduced the potential distribution and/or extension of grasslands (Birks and Willis, 2008). An especially critical period for grassland persistence was the mid Holocene period (9000-4500 cal yr BP), when moister climatic conditions triggered forest expansion (Roberts et al., 2018). Identification of warm/moist stage refugia for grasslands (i.e. locations where they persisted) during the mid Holocene is therefore of crucial importance for understanding ancient grasslands.

It is widely accepted that natural grasslands growing on rocky skeletal and other poor soils with a permanent or seasonal moisture deficit, i.e. outcrops, steeper slopes, gravel riverbanks, salt and sandy soils the so-called primary grasslands have survived continuously in small pockets throughout the Holocene in their current locations (Lang, 1994; Poschlod and WallisDeVries, 2002). Currently, the extent of these grassland types is limited, apart from grasslands growing on salt and dry sandy soils, which are more common in the Carpathian Basin (Molnár and Borhidi 2003; Deák et al., 2014). It is therefore highly improbable that these small, isolated grassland areas were the only refugia of the extraordinarily rich grassland flora of so-called "semi-natural" grasslands. The high genetic diversity of some grassland plant species in this region (Turtureanu et al., 2014) and the remarkable species richness and endemic plant and animal species typical for grasslands (Chytrý et al., 2015) suggest a wider extent of primary grasslands during the Holocene.

In contrast to the primary grasslands, open canopy woodlands and grasslands currently found in areas where climate and soils would allow forest growth, and which are only extensively managed (i.e., no artificial fertiliser and pesticide application) are considered to be semi-natural (Pärtel et al., 2005; Leuschner and Ellenberg, 2017). Here, we challenge the view that most of these grasslands have replaced formerly naturally occurring forests within recent centuries or millennia. We present multiple lines of evidence (palaeobotanical, pedological, phylogenetic, palaeontological) from five countries in Central Eastern Europe, a region with some of the highest-biodiversity grasslands of the world. Firstly, we review published direct records of past grassland occurrence (pollen, plant macrofossils, charcoal)

99 from both natural (lakes, bogs) and archaeological archives, alongside other indirect fossil
100 (pedological, zoological) and recent genetic evidence from Central Eastern Europe.
101 Secondly, we review the characteristics of the environmental and disturbance factors
102 (climate, fire, herbivores and human impact) during the Holocene in this region. Finally, we
103 evaluate whether continuous grassland presence was possible under the Holocene climatic
104 conditions, fire and grazing regimes, and increasing anthropogenic impacts.
105 Based on their age and the drivers of their formation, we set out a new framework for three
106 types of grasslands in Central Eastern Europe: 1) Primary, natural grasslands on skeletal
107 and other poor soils, which have existed throughout the Holocene until the present; 2)
108 Primary, ancient grasslands on deeper soils, maintained by climate and disturbances during
109 the early Holocene and then predominantly by disturbances until the present; and 3) Semi-
110 natural grasslands, extensively managed grasslands, formed and maintained by
111 anthropogenic disturbances during the late Holocene (Table 1). We argue for the recognition
112 of the importance of previously overlooked ancient grasslands that have persisted throughout
113 the Holocene, maintained by natural and later also by anthropogenic disturbances.

114
115 **3. Multi-proxy evidence for grassland persistence during the Holocene**
116 **3.1 Fossil plant evidence**
117 Pollen and palaeobotanical records from natural archives (lakes and peatbogs) in currently
118 grassland-rich areas in Central Eastern Europe indicate the prevalence of a more open
119 landscape between 11,700 and 9000 cal yr BP and the maximum extent of forest cover
120 between 9000 and 4500 cal yr BP (Figs.1, 2; Table 2; Fig.S1). While these studies show a
121 reduction in grassland cover, especially of xerothermic and floodplain grasslands during the
122 mid Holocene, there is no evidence of their widespread disappearance. Rather, there is
123 strong support for their persistence, given the concurrent presence of many grasslands, i.e.
124 *Adonis* spp.*, Artemisia* spp.*, Centaurea* spp.*, Festuca rubra*, *Festuca* spp.*, Filipendula* spp.*,*
125 *Helianthemum* spp.*, Potentilla erecta, Potentilla* spp.*, Sanguisorba* spp.*, Trifolium* spp.,
126 *Thymus* spp.*,* and light-demanding tree and shrub taxa during this period (Fig. 1; Table 2).
127 Archaeobotanical reports from Hungary and Poland suggest an even greater proportion of
128 heliophilous taxa growing locally than pollen records indicate (Fig. 1; Table 2). Remains of
129 grassland species including steppe elements, i.e. *Asperula cynanchica*, *Phleum pratense,*
130 *Plantago media, Stipa pennata, Stipa* sp.*, Silene vulgaris, Teucrium chamaedrys*, have been
131 reported from archaeobotanical records of early Neolithic sites from Germany (Fig. 1; Table
132 2) and Czech Republic (Archaeobotanical Database of the Czech Republic;
133 http://www.arup.cas.cz). Calcareous grasslands have also been identified in the Neolithic
134 lakeshore sites in the northern foothill of the Alps (Fig. 1; Table 2). The species found are
135 typical of closed xerophilous and mesophilous grasslands at sites where trees would have
136 been able to grow under the climatic conditions of the mid Holocene. Taken together, fossil
137 plant evidence from natural archives and archaeological sources suggest that grasslands
138 existed locally before the start of the Neolithic and therefore before marked human impacts.
139 These findings also demonstrate grassland persistence throughout the mid Holocene,
140 although archaeological records show the occurrence of higher grassland diversity than that
141 found in pollen records.

142
143 **3.2. Zoological evidence**
144 Indirect evidence for the persistence of open, or partly open landscapes throughout the mid
145 Holocene comes from the palaeontological remains of animal species restricted to extensive,
146 open habitats (Fig. 1). Results from the Carpathian Basin (Hungary and Romania) show that
147 several species typical of steppic environments e.g. *Asinus hydruntinus* (European Wild
148 Ass), *Equus ferus* subsp. *gmelini* (Eastern European Wild Horse), *Microtus gregalis* (Narrow
149 Headed Vole), *Ochotona pusilla* (Steppe Pika), *Otis tarda* (Great Bustard) and *Vipera ursinii*
150 subsp. *rakosiensis* (Meadow Adder) were abundant during the early Holocene (Németh et

al., 2017). They became discontinuously present from 8000 cal yr BP and several of these species disappeared between 5000 and 4000 cal yr BP at a time of increased anthropogenic pressure, but in a grassland landscape. Fossil malacological records in currently grassland-rich landscapes reveal a similar picture (Fig. 1); a continuous Holocene presence of strictly open habitat molluscs (*Chondrula tridens, Helicopsis striata,* and *Vallonia pulchella*) unable to survive in closed forests (Ložek, 2005; Horsák et al., 2009; Moskal-del Hoyo et al., 2018). Overall, while there is evidence that many open habitat mollusc species contracted their range during mid Holocene forest expansion, there is also robust support for their local long-term persistence, and consequently also for the continuity of grassy ecosystems.

3.3 Phylogeographic evidence

Phylogeographic analysis represents a further source of data facilitating the interpretation of the distribution of past grasslands. A pattern of genetic diversity decline from core populations in southern Siberia towards the range periphery of smaller populations in western Europe has been confirmed for several grassland plant species including *Adonis vernalis* (Hirsch et al., 2015), *Iris aphylla* (Wroblewska 2008), *Stipa capillata* (Wagner et al., 2011) and *Stipa pennata* (Wagner et al., 2012). These studies have also revealed a surprisingly low genetic differentiation between central and peripheral intermediate populations, or a complete lack of private alleles among peripheral populations (e.g. Wagner et al. 2011; Hirsch et al., 2015), which may reflect the absence of any long-standing isolation of these populations. This implies that these species must have had a more continuous past distribution in Central and Eastern Europe allowing gene flow and interbreeding. Rapid progress in the field of DNA analysis, especially environmental DNA, may shed further light on the origin and past range distribution of grasslands (Thomsen and Willerslev, 2015)

3.4 Pedological evidence

A further line of evidence used in the interpretation of former vegetation distributions comes from soil types (IUSS WRB 2006). In Central Eastern Europe, dark soils (chernozems) from steppe and forest steppe zones are considered to have developed before the spread of forests and to have persisted under open or semi-open vegetation (Pokorný et al., 2015). Others, however, view these soils as having survived under forest development (Eckmeier et al., 2007). A palaeo-pedological analysis from the Transylvanian Basin (Romania) shows the occurrence of islands of dark soils of Pleistocene age (20,000-14,000 cal yr BP), which, in drier areas, persisted until the present (Pendea et al., 2002) suggesting grassland persistence throughout the Holocene. However, in other areas of the Transylvanian Basin, dark soils were overlain by Luvisols, typical of nemoral forests, about 5000 cal yr BP (Timar et al., 2010) when wetter climatic conditions prevailed, whilst the current vegetation is predominantly grassland. Grassland occurrence on soils typically favouring forests may be explained by the prevalence of open woodlands throughout the Holocene, allowing the long-term persistence of dark soil, rather than the post-deforestation formation of this soil type. Forest soils occur extremely rarely on chernozems developed on loess substrates in the Hungarian Plain therefore suggesting the long-term existence of steppe grasslands (Máté 1957, Molnár 2010). Indeed, the continuous dominance of grasslands from the Late Pleistocene on loess deposits in the southern Carpathian Basin has been recently demonstrated on the basis of n-alkane biomarkers (Marković et al., 2018). Thus, pedological evidence from chernozems, including those developed on loess, shows that chernozems existence under open or semi-open vegetation.

4. Drivers of grassland persistence during the Holocene
4.1 Climate conditions
Proxy-based and climate simulations indicate warmer-than-present summer temperatures,

lower precipitation and soil moisture, and greater seasonality in the early Holocene (11700-9000 cal yr BP) in Central and Eastern Europe at the time of maximum grassland extent (Feurdean et al., 2013; 2014; Heiri et al., 2014). Palaeoclimatological records show a decline in temperatures in this region from approximately 9000 to 4500 cal yr BP (Heiri et al., 2015; Tóth et al., 2015; Hajkova et al., 2016). Climate simulations are consistent with this pattern of mid Holocene cooling, but also marked precipitation and soil moisture increases in the mid latitudes in Europe (Feurdean et al., 2013). Palaeoecological reconstructions show that significant forest expansion occurred in response to cool and moist conditions in Central Eastern Europe (Magyari et al., 2010; Feurdean et al., 2015; Kuneš et al., 2015; Novenko et al., 2016; Pokorný et al. 2015; Jamrichová et al., 2017; Moskal-del Hoyo et al., 2018). Therefore, both proxy and modelled palaeoclimatic and palaeoecological evidence from the lowlands of Central Eastern Europe clearly show that grasslands were most extensive during the warm and dry climatic conditions, with prolonged droughts, of the early Holocene (11,700-9000 cal yr BP) and became restricted under wetter conditions during the mid Holocene (9000-4500 cal yr BP; Fig. 2). This illustrates the stronger competitive advantage of grasslands over trees when resources are limited i.e., lower moisture availability and prolonged droughts.

4.2 Fire

The role of fire as one of the main drivers of the rise in grassy ecosystem during the Miocene has been been advocated (Osborne and Behling, 2006; Strömberg, 2011) and confirmed by fossil records from C_4 dominated grasslands in Africa (Hoetzel et al., 2013). This is not surprising as dominant grassland species have fine fuels with rapid curing and fast regrowth rates as well as perennating buds near or below the soil surface; adaptations that ensure regeneration after disturbances that damage the above-ground parts of the plant (He and Lamont, 2018). Thus, whilst grass and herbs can withstand frequent fire, this shift in fire regime may have harmed previously dominant tree species adapted to infrequent fire (He and Lamont, 2018). Although, short-term, field-based burning experiments in Hungary found conflicting results about the effect of fire on grassland biodiversity (Valkó et al., 2014, 2018b), controlled laboratory and small-scale field experiments examining the effect of fire on seeds found a predominantly negative effect of fire on seed germination in grassland species, however, some positive effects (Fabaceae) also emerged (Ruprecht et al., 2013; 2015). Disturbances by fire have recently been considered essential for increased grassland competitive advantage over trees during the Holocene in Central Eastern Europe (Magyari et al., 2010; Feurdean et al., 2015). For example, a positive effect of frequent fires on the competitive advantage of grasses over trees and, by this means, on the extent of grassland in Transylvania, Romania, has been inferred from sedimentary charcoal particles and pollen (Feurdean et al., 2013). On the contrary, the decline in fire frequency during the mid Holocene has been shown to be detrimental for grassland extent. The significance of global fire activity in grassy biomes during the early and late Holocene, based on charcoal datasets, has recently been emphasised by Leys et al (2018). Taken together, neo- and palaeoecogical evidence indicate that fire may have had a more important role in the shifts between forest and grassland, and in grassland maintenance, than previously thought. Exploring to what extent temperate grasslands are associated with frequent fires and which grassland species/communities are most resilient or benefit mostly from fire could be useful for the enhancement of management practices, i.e., preventing excessive dominance by competitor grass species as well as the succession towards shrublands and forests.

4.3 Herbivores

Large herbivorous mammals influence the physiology and growth of plants and are considered ecological keystones in maintaining tree–grass coexistence (Crawley, 1983;

Sankaran et al., 2005). Released from megaherbivore pressure and with a change in climate at the Pleistocene-Holocene boundary, European lowlands witnessed substantial forest regeneration and a reduction in vegetation openness (Vera, 2000; Svenning, 2002). Megaherbivore extinction may also have had a cascading effect on the population size and diversity of small mammals dependent on vegetation openness and indirectly on the fire regime (Gill et al., 2014). However, comparatively, little attention has been given to the effect of the declining population size or extinction of wild herbivores, or the subsequent role of livestock, on landscape structure during the mid to late Holocene. We know from palaeontological and archaeozoological records in the Carpathian Basin that several large herbivores i.e., *Alces alces* (Eurasian Elk)*, Bison bonasus* (European Bison)*, Dama dama* (Eurasian Fallow Deer), *Equus ferus* subsp. *gmelini* (Wild Horse) and *Equus hemionus* (Asiatic Wild Ass) became discontinuously present from the mid Holocene, i.e. 8000 cal yr BP and that many became extinct by 4000 cal yr BP (Németh et al., 2017; Bejenaru et al., 2018). In contrast, livestock numbers increased from 6500 cal yr BP (Schumacher et al., 2016). Domestic livestock could prevent forest encroachment in the absence or with a low density of wild herbivores. Domestic animals can replace wild herbivores as dispersal agents (Bruun and Fritzbøger, 2002; Cosyns et al., 2005), however, their movement is limited by agricultural practices. Combined fossil records of fauna, pollen and coprophilous fungi that reproduce exclusively on animal dung (*Sporormiella* spp.*, Sordaria* spp.*, Podospora* spp.) can provide means of assessing the effects of herbivores on grassland dynamics and also the timing of the shift in influence from grazing by wild herbivores to livestock grazing (Gill et al., 2009). Such records are still scant in Europe, but the existing studies generally show the increasing effects of grazing by domestic livestock from 5000 cal yr BP (Schumacher et al., 2016). Understanding the responses of grasslands to different grazing animals (body size, grazing intensity and height, foraging strategy and forage selectivity) will be essential in the development of future grassland management strategies as various forms of livestock grazing have been proposed to simulate the effects of grazing and browsing by wild herbivores (Poschlod and WallisDeVries, 2002; Bakker et al., 2004; Tóth et al., 2016; Poschlod, 2017).

4.4. Early human impact counteracted the encroachment of forest onto primary grasslands

As the increase in forest cover from 8000 cal yr BP coincided with the spread of Neolithic culture across South Eastern Europe (Bogaard et al., 2004; Kreuz 2008), a critical question in respect to grassland extent is whether anthropogenic impacts could have counteracted the climate-driven development of a closed forest (Pokorný et al., 2015). Archaeological datasets from this part of Europe indicate that Neolithic settlements tended to be established in open landscapes and that field sizes were small (Moskal-del Hoyo et al., 2013; Chapman, 2017; Marinova and Ntinou, 2017). As people first settled in naturally open landscapes, this tendency could explain the apparent lack of major deforestation at this time in the pollen records from Central Eastern Europe (Fig. 2). Fire activity was naturally high during the early Holocene (Magyari et al., 2010; Feurdean et al., 2013), and humans may have taken advantage of wildfires to extend their agro-pastoral activities into freshly burned habitats. In agreement with Pokorný et al. (2015) we hypothesise that early anthropogenic land management may have slowed, or partially arrested, the development of closed forest favoured by the wetter climatic conditions of the mid Holocene at locations with low biomass productivity, contributing to the maintenance of landscape openness.

4.5. Semi-natural grasslands replacing forests: when and how?

Individual pollen records, as well as large-scale quantitative vegetation reconstructions from Central Eastern Europe, show that the level of anthropogenic impact on forest remained low

until about 6000-5000 cal yr BP (Magyari et al., 2010; Feurdean et al., 2015; Kuneš et al., 2015; Jamrichová et al., 2017; Fig. 2). Modelled vegetation and land use (arable and pasture cover) changes across Europe suggest that open areas expanded gradually from previously cleared forest after ca. 6000 cal yr BP (Kaplan et al., 2017). A noticeable increase in the abundance and richness of grassland along with the decline in total forest cover but increase for *Quercus,* a tree taxon typical for woodland and woody pasture, in Central Eastern Europe occurred from 4700-3500 cal yr BP onwards (Jamrichová et al., 2017). This demonstrates a growing anthropogenic role in the extension of grasslands and the formation of open woodlands. These grasslands belong to the so-called semi-natural grasslands that developed from forests and are maintained by land management (Pärtel et al., 2007; Pereira et al., 2017). Technological advances in agriculture and the expansion of urban centres and farms from the Late Bronze Age and Iron Age (3500 cal yr BP) have led to both an extension and intensification of the land use in Central Europe (Poschold, 2015; Rösch et al., 2016). It is therefore not surprising that from this time onwards, the richness and extent of grassland has been found to correlate closely with prehistoric settlement density and land management (Poschlod and WallisDeVries, 2002; Pärtel et al., 2005; Hajkova et al., 2011; Hejcman et al., 2013; Poschlod, 2017). Later on i.e. from the 15th to 20th centuries, grassland expansion is strongly linked to sheep flock migration. Livestock acted as dispersal vectors and their mobility may be one of the reasons that ancient and older semi-natural grasslands may have similar species diversity (Poschlod and WallisDeVries, 2002; Molnár et al. 2012; Poschlod, 2017). The sowing of hayseed and mowing may also have promoted grassland expansion in many parts of Europe (Babai and Molnár, 2014). In summary, semi-natural grasslands expanded into formerly forested sites and have subsequently been maintained by a variety of land management practices including grazing, burning and mowing.

5. A new framework for Holocene grassland persistence; conservation consequences
We provide a new framework distinguishing three types of biodiversity-rich grasslands in Central Eastern Europe. These are: primary grasslands on skeletal and other poor soils (primary grasslands I), ancient grasslands maintained by natural and anthropogenic disturbances (primary grasslands II), and semi-natural grasslands developed as a result of human activities replacing forests (Fig. 3; Table 1). We have identified the reasons for the continuous presence of primary grasslands during the Holocene including both natural i.e. climate conditions and soils (primary I), climate and disturbance factors i.e. fire and grazing (primary grasslands II). Neolithic people may have first settled in naturally open areas such as grasslands or grassland-woodland mosaics arresting the development of a full forest cover when the climate became wetter (mid Holocene), indirectly favouring the preservation and expansion of grasslands. The intensification of human impact from 4700-3500 cal yr BP onwards subsequently lead to considerable extension of semi-natural grassland on formerly forested sites. A succession from grassland to forest after the cessation of land management (e.g. grazing or burning) is not necessarily a proof against the primary or ancient aspect of grasslands, but may indicate the lack of disturbances. Livestock grazing in the late Holocene has replaced ancient grazing by megaherbivores prevailing until the early Holocene and that by large herbivores throughout the mid Holocene.

Our findings also reveal misconceptions about the origin of Central Eastern European grasslands and open canopy woodlands. The concept of a previously continuous, closed forest in extant grassland-rich landscapes where climatic conditions are favourable for forest fails to hold true, as grasslands are likely to have been continuously present throughout the Holocene. The long-term persistence of grasslands at these locations is probably an important reason for one of the highest small-scale species richness, many endemic, worldwide in these habitats. Such species-rich plant communities can require millennia to develop and only well-connected grassland patches can support genetically diverse plant

populations. These findings challenge the commonly held view that conservation activities should primarily focus on the protection of forests in many areas of Central Eastern Europe. Anthropogenic impacts tend to focus on forest clearance, but this perspective paper highlights that conservationists and land managers need to carefully consider that, in many cases, it is not primary forests that hold the highest biodiversity. Further, human-made, or managed environments, such as extensively managed grasslands, are long-term landscape features, contain unique plant and animal communities, and provide important ecosystems services. Our findings support the recent wider acceptance of the notion that people and nature should not be separated in the societal discourse of environmental science (Mace 2014). Finally, we advocate the need for a more detailed understanding of the role of disturbances in grassland-forest dynamics, to avoid the overly simplistic assumption that sparse tree cover is evidence of past deforestation. Fossil records provide such data and the routine incorporation of palaeoecological investigations into environmental management is a key step in developing science-based evidence for the conservation of the biodiversity of grasslands. Thus, our regional case study supports the advocacy of Willis et al. (2010), Barnosky et al. (2017) and Whitlock et al. (2018) for merging palaeobiology and conservation biology as well as an appreciation of the dynamic history of species and ecosystems, including the role of humans.

Figures captions

Figure 1. Location of the study area in Europe (A) and the distribution of the main vegetation types in Central Eastern Europe based on the potential natural vegetation map of Europe (B; Bohn et al., 2003). Colour symbols show location of various types of fossil records extracted from literature (Table 2 and S1) indicating either continuous grassland presence throughout the Holocene or during the afforestation phases of the mid Holocene (9000-4000 cal yr BP). These fossil records reveal that grasslands were continuously present throughout the Holocene in places where potential natural vegetation has been assumed to be forest.

Figure 2. Pollen based reconstruction of forest (green) versus open land cover (yellow) from Central Eastern Europe during the Holocene using the pseudobiomisation method (Fyfe et al., 2015). Cumulative land cover record was constructed by spatially aggregating 96 pollen records extracted from the Pangaea Database and distributed across the region shown in Fig.1 and Fig. S1. Forest cover includes both broadleaf and conifer trees, whereas open land cover includes pastures/natural grasslands, and arable/disturbed land. Geological and archaeological periods as well as the predominance of each grassland type throughout the Holocene are also highlighted. Trends in simulated growing season temperature and precipitation for Lake Stiucii, Romania after Feurdean et al. (2015).

Figure 3. The effect of climate, soils and disturbances by fire, herbivores and humans on the three types of grasslands and forest. Blue line denote a positive effect, red line a negative effect and grey both effects.

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661

662 **Supplementary Material**

663

664 **Figure S1** Location of sites extracted from the European Pollen Database (EPD) and used to
665 construct Figure 2.

666 **Table S1.** Location of sites extracted from the literature and used to construct Figure 1.

667

Table 1. Species-rich grassland types

| Grassland type	Characteristics
Primary grassland I	Natural grasslands on skeletal and other poor soils with moisture deficit
Primary grassland II	Ancient grasslands formed and maintained mainly by climate conditions and natural fires, herbivores and, later, also influenced by anthropogenic disturbances
Semi-natural grassland	Secondary grasslands formed and maintained by anthropogenic disturbances (deforestation, livestock grazing, cultivation, use of fire) in areas suitable for forests during the late Holocene

685 Table 2. Fossil pollen, plant macrofossil and charcoal evidence for the presence of various types of grasslands during the early and mid Holocene. Note:
686 on the basis of pollen analysis differentiation between primary grassland and semi-natural grassland is not always possible.
687

Location	Taxa	Period	References
Romania	*Artemisia, Aster*-type, Caryophyllaceae undiff., Compositae Cichorioideae, *Centaurea,* Chenopodiaceae, *Helianthemum, Filipendula, Potentila,* Poaceae, *Plantago lanceolata, Plantago major/P. media, Rumex acetosa, Thalictrum, Chenopodium, Suaeda maritina, Festuca, Lycopus, Thymus,* Leguminosae undiff.	Holocene	Feurdean et al., 2015
Hungary	*Artemisia, Achillea, Ajuga, Adonis, Astragalus, Allium, Aster*-type, Poaceae, *Centaurea, Filipendula, Festuca, Euphorbia,* Caryophyllaceae undiff., *Gagea,* Chenopodiaceae, Compositae Cichorioideae, *Dianthus*-type, *Genista, Helianthemum, Hypericum, Inula,* Leguminosae undiff., *Lotus*-type, *Plantago lanceolata, Plantago major / P. media, Potentilla, Trifolium, Rumex acetosa, Teucrium, Thymus, Thalictrum, Verbascum, Sanguisorba*	Holocene	Magyari et al., 2010
Hungary	*Chenopodium album, Echinochloa crus-galli, Fallopia convolvulus Galium spurium, Melandrium album, Plantago lanceolata, Polycneum arvense, Polygonum aviculare, Polygonum mite, Polygonyum minus, Rumex acetosa, Setaria pumila, Setaria viridis, Bromus sp., Chenopodium sp., Galium, Ononis sp., Polygonum sp., Rumex sp., Stipa sp., Trifolium sp., Vicia sp.,* Fabaceae, Poaceae, Polygonaceae, Caryophyllaceae	Neolithic	Moskal del Hoyo et al., 2018
Poland (S)	*Artemisia, Stipa* sp., *Knautia arvensis, Hypericum, Plantago media, P. lanceolata, Filipendula, Aster-type,* Compositae Cichorioideae, Poaceae, Chenopodiaceae	Mid Holocene	Moskal del Hoyo et al., 2018
Czech Republic	*Artemisia, Achillea, Ajuga, Astragalus, Filipendula,* Chenopodiaceae, *Centaurea,* Caryophyllaceae undiff., Compositae Cichorioideae, *Genista, Filipendula, Helianthemum, Hypericum, Inula*-type, *Plantago lanceolata, P. media,* Poaceae, *Potentilla-type, Ranunculus acris*-type, Rubiaceae, *Rumex acetosa, Teucrium, Thymus, Thalictrum, Verbascum*	Holocene	Kuneš et al., 2015 Jamrichova et al., 2017 Hajeck et al., 2016

	Country	Species	Period	Reference
719	**Austria**	Poa pratensis/trivialis, Festuca ovina/rubra, Brachypodium pinnatum,	Neolithic	Körber-Grohne 1990
720		Anthoxanthum odoratum, Stipa pennata		
721				
722	**Germany (S)**	Alchemilla vulgaris, Asperula cynanchica, Bupleurum falcatum, Carex muricata,	Neolithic	Kreuz et al., 2005, 2008
723		Centaurea, Daucus carota, Euphrasia, Phleum pratense, Poa annua, Polygonum aviculare,		Kreuz and Schäfer, 2011
724		Stipa sp., Stipa pennata, Rumex acetosella, Stellaria graminea, Trifolium campestre,		
725		Galium cf. verum, Galium molugo, Urtica dioica, Teucrium chamaedrys, Chenopodium spp.,		
726		Veronica arvensis, Plantago media		
727				
728	**Germany (NW)**	Achillea ptarmica, Angelica sylvestris, Anthriscus sylvestris, Artemisia cf.campestris,	Preboreal	Knörzer 1996
729		Crepis biennis, Chaerophyllum hirsutum, Dianthus spp., Empetrum nigrum,	& Boreal	
730		Euphorbia cyparissias, Festuca cf.pratensis, Festuca rubra, Filipendula ulmaria, Galium mollugo,		
731		Heracleum sphondylium, Hypochaeris radicata, Juncus cf. effusus, Knautia arvensis,		
732		Lysimachia vulgaris, Laserpitium prutenicum, Poa pratensis / angustifolia,		
733		Polemonium coeruleum, Scabiosa columbaria, Plantago lanceolata,		
734		Potentilla tabernaemontani, Ranunculus acris, Rumex acetosa, Rumex tenuifolius,		
735		Sanguisorba officinalis, Scabiosa aff.columbaria, Silene vulgaris, Thalictrum flavum,		
736		Valeriana officinalis, Valeriana procurrens, Viola canina		
737	**Germany (NW)**	Achillea millefolium, Leontodon autumnalis, Agrostis tenuis, Melandrium rubrum,	Neolithic	Knörzer 1996
738		Phleum nodosum, Poa cf.pratensis, Artemisia cf.campestris, Poa cf.trivialis,		
739		Polygonum bistorta, Potentilla argentea, Carex ovalis, Prunella vulgaris,		
740		Centaurea cf.nigra, Ranunculus repens, Cerastium cf.semidecandrum, Rumex tenuifolius,		
741		Chrysanthemum leucanthemum, Deschampsia caespitosa, Selinum carvifolium,		
742		Festuca rubra, Silaum silaus, Heracleum sphondylium, Stachys recta, Hieracium pilosella,		
743		Trifolium arvense, Hypericum cf.maculatum, Trifolium dubium, Hypericum tetrapterum,		
744		Valerianella dentata, Juncus bufonius / conglomeratus, Veronica cf.arvensis,		
745		Trifolium repens, Juncus cf.effusus, Viola tricolor		
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Highlights

- We prove a new framework for understanding long-term dynamics of grasslands
- This framework facilitates the identification of ancient grasslands worldwide
- Evidence of the long-term grassland continuity in potential forest areas
- Grassland continuity explains the high current species richness of this ecosystem
- Better classification highlights the importance of this overlooked environment
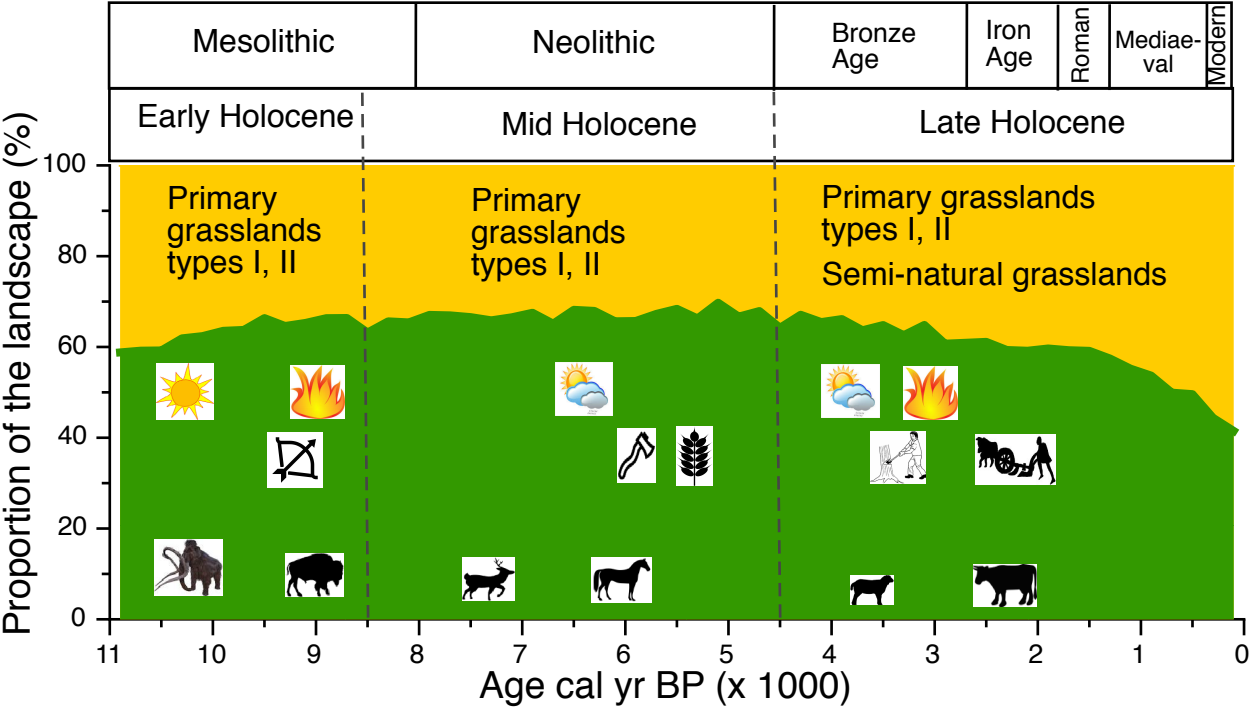
Biodiversity-rich European grasslands: ancient, forgotten ecosystems

Feurdean, Angelica[1], Ruprecht Eszter[2], Molnar Zsolt[3], Hutchinson Simon M[4], Hickler Thomas[1,5]

[1]Senckenberg Biodiversity and Climate Research Centre (BiK-F), Frankfurt am Main, Germany; angelica.feurdean@senckenberg.de;
[2]Hungarian Department of Biology and Ecology, Babeș-Bolyai University, Cluj-Napoca, Romania, Republicii 42, Cluj-Napoca, RO-400015, Romania; eszter.ruprecht@ubbcluj.ro
[3]MTA Centre for Ecological Research, Institute of Ecology and Botany, Vácrátót, Hungary H-2163; molnar.zsolt@okologia.mta.hu
[4]School of Environment and Life Science, University of Salford, Salford, Greater Manchester, UK, M5 4WT UK: s.m.hutchinson@salford.ac.uk
[5]Department of Physical Geography, Goethe University, Frankfurt am Main, Germany, Altenhöferallee 1, 60438; Thomas.Hickler@senckenberg.

*Corresponding author. Senckenberg Biodiversity and Climate Research Centre (BiK-F), Senckenberganlage, 25, 60325, Frankfurt am Main, Germany, angelica.feurdean@senckenberg.de; angelica.feurdean@gmail.com;Tel: Phone +49-06975-421870

Conflict of Interest
There is no conflict of interest with any other people or organizations.

1   Biodiversity-rich European grasslands: ancient, forgotten ecosystems

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5   **Abstract**

6   Worldwide reforestation has been recommended as a landscape restoration strategy to
7   mitigate climate change in areas where the climate can sustain forest. This approach may
8   threaten grassland ecosystems of unique biodiversity as such policies are based on a false
9   assumption that most grasslands are man-made. Here, we use multiple lines of evidence
10   (palaeoecological, pedological, phylogenetic, palaeontological) from Central Eastern Europe
11   and show that various types of grasslands have persisted in this area throughout the
12   postglacial i.e., the past 11,700 years. A warm and dry climate, frequent fires and herbivore
13   pressure, and early Neolithic settlements kept forests open until widespread forest clearance
14   from 4000-3000 years ago. Closed forest cover has been the exception for about two million
15   years. This long-term grasslands persistence is probably important for the high biodiversity of
16   grasslands. Consequently, we call for a more caution prioritisation of the protection of what
17   may be erroneously considered natural, i.e. forests, by many environmental specialists and
18   managers. Instead we provide a new framework for a better understanding of the evolution
19   and persistence of different grassland types and their biodiversity, so that grasslands can be
20   better understood, valued and conserved.
21

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23   Keywords: fossil records, ancient grasslands, fire, herbivores, anthropogenic disturbance,
24   climate change mitigation
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26

27   **1. Introduction**
28   The World Resources Institute (WRI) Atlas of Forest and Landscape Restoration was
29   designed to identify opportunities for landscape restoration worldwide, an initiative supported
30   by several international organisations concerned with land degradation, climate change
31   mitigation and biodiversity loss (WRI, 2015). It contrasts the potential extent of tree cover
32   based on climate conditions with the current distribution of forest globally. This map identifies
33   23 million km^2 of land worldwide suitable for tree planting, mostly being currently open
34   landscapes with grassland (Fig. SI1). However, grassland experts oppose the offsetting of
35   agricultural deforestation through the afforestation of grassy ecosystems arguing that this
36   approach ignores the unique biodiversity, cultural significance and important ecosystem
37   services provided by this ecosysthem (Willis et al., 2008; Parr et al., 2014; Veldman et al.,
38   2015a,b; Bond et al., 2016). Their imperative is to map "old-growth" grasslands, where tree
39   cover is naturally sparse and where such afforestation would be detrimental. Another
40   important debate is how to provide strategies to reduce the impact of the ongoing
41   abandonment of high biodiversity grasslands, therefore hampering succession towards low
42   biodiversity secondary shrub and forest communities (Biró et al., 2010; Valkó et al., 2018a).
43   Here we go further and challenge the perception that treeless areas in temperate regions,
44   where the current climate would permit forest development, have all previously been forested
45   and therefore grasslands and open canopy woodlands are secondary habitat types in these
46   regions. This perception fails to consider the vital role of natural disturbances such as fire or
47   herbivores (Bond and Keeley, 2005). We illustrate this by analysing evidence from Central

48   Eastern Europe. The choice of this region is based on the following arguments: i) it hosts one
49   of the largest tracts of grasslands in Europe crucial for maintaining biodiversity in European
50   agricultural landscapes (Wilson et al., 2012); ii) has one of the highest small-scale species
51   diversities in the world (Dengler et al., 2014; Turtureanu et al., 2014; Chytrý et al., 2015); and
52   iii) is a transitional, complex region between closed forest and steppe biomes (Bohn et al.,
53   2003) and hence contains grasslands of diverse origin and history. Yet, despite these
54   features, grasslands are rarely highlighted as biodiversity hotspots. This is because the key
55   characteristics and ecological processes important for this classification, such as biodiversity
56   intactness and a lack of human disturbance, cannot be readily applied to them (Mittermeier
57   et al., 2011). Our goal is to better define grassland types based on their origin, age and the
58   drivers of their formation and maintenance so that grasslands can be better understood,
59   valued and conserved.
60
61   **2. A new framework for defining European grassland types**
62   Bohn et al. (2003) provided a geobotanical expert assessment, which maps Europe's
63   potential natural vegetation (PNV) i.e., the vegetation cover that would exist today in the
64   absence of human activity. It defines most areas that are currently covered by grasslands or
65   open woodlands in Central Eastern Europe as dominated by deciduous broadleaved forest
66   or mixed coniferous and broadleaved forest (Fig. 1). Open, or at least partly open vegetation
67   types, are only recognised in the lowlands of the Carpathian Basin. Fossil records show that
68   grasslands and open canopy woodlands covered extensive areas in Central Eastern Europe
69   during the Pleistocene (i.e., the past 2 million years) when cold and dry climate conditions
70   prevailed (Kuneš et al., 2008; Ellenberg and Leuschner, 2010; Feurdean et al., 2014;
71   Magyari et al., 2014). Warmer climate conditions during the Holocene (i.e., the last 11,700
72   years) then greatly reduced the potential distribution and/or extension of grasslands (Birks
73   and Willis, 2008). An especially critical period for grassland persistence was the mid
74   Holocene period (9000-4500 cal yr BP), when moister climatic conditions triggered forest
75   expansion (Roberts et al., 2018). Identification of warm/moist stage refugia for grasslands
76   (i.e., locations where they persisted) during the mid Holocene is therefore of crucial
77   importance for understanding ancient grasslands.
78   It is widely accepted that natural grasslands growing on rocky skeletal and other poor soils
79   with a permanent or seasonal moisture deficit i.e., outcrops, steeper slopes, gravel
80   riverbanks, salt and sandy soils the so-called primary grasslands have survived continuously
81   in small pockets throughout the Holocene in their current locations (Lang, 1994; Poschlod
82   and WallisDeVries, 2002). Currently, the extent of these grassland types is limited, apart
83   from grasslands growing on salt and dry sandy soils, which are more common in the
84   Carpathian Basin (Molnár and Borhidi 2003; Deák et al., 2014). It is therefore highly
85   improbable that these small, isolated grassland areas were the only refugia of the
86   extraordinarily rich grassland flora of so-called "semi-natural" grasslands. The high genetic
87   diversity of some grassland plant species in this region (Turtureanu et al., 2014) and the
88   remarkable species richness and endemic plant and animal species typical for grasslands
89   (Chytrý et al., 2015) suggest a wider extent of primary grasslands during the Holocene.
90   In contrast to the primary grasslands, open canopy woodlands and grasslands currently
91   found in areas where climate and soils would allow forest growth, and which are only
92   extensively managed (i.e., no artificial fertiliser and pesticide application) are considered to
93   be semi-natural (Pärtel et al., 2005; Leuschner and Ellenberg, 2017). Here, we challenge the
94   view that most of these grasslands have replaced formerly naturally occurring forests within
95   recent centuries or millennia. We present multiple lines of evidence (palaeobotanical,
96   pedological, phylogenetic, palaeontological) from five countries in Central Eastern Europe, a
97   region with some of the highest-biodiversity grasslands of the world. Firstly, we review
98   published direct records of past grassland occurrence (pollen, plant macrofossils, charcoal)
99   from both natural (lakes, bogs) and archaeological archives, alongside other indirect fossil

100 (pedological, zoological) and recent genetic evidence from Central Eastern Europe.
101 Secondly, we review the characteristics of the environmental and disturbance factors
102 (climate, fire, herbivores and human impact) during the Holocene in this region. Finally, we
103 evaluate whether continuous grassland presence was possible under the Holocene climatic
104 conditions, fire and grazing regimes, and increasing anthropogenic impacts.
105 Based on their age and the drivers of their formation, we set out a new framework for three
106 types of grasslands in Central Eastern Europe: 1) Primary, natural grasslands on skeletal
107 and other poor soils, which have existed throughout the Holocene until the present; 2)
108 Primary, ancient grasslands on deeper soils, maintained by climate and disturbances during
109 the early Holocene and then predominantly by disturbances until the present; and 3) Semi-
110 natural grasslands, extensively managed grasslands, formed and maintained by
111 anthropogenic disturbances during the late Holocene (Table 1). We argue for the recognition
112 of the importance of previously overlooked ancient grasslands that have persisted throughout
113 the Holocene, maintained by natural and later also by anthropogenic disturbances.
114

115 **3. Multi-proxy evidence for grassland persistence during the Holocene**
116 **3.1 Fossil plant evidence**
117 Pollen and palaeobotanical records from natural archives (lakes and peatbogs) in currently
118 grassland-rich areas in Central Eastern Europe indicate the prevalence of a more open
119 landscape between 11,700 and 9000 cal yr BP and the maximum extent of forest cover
120 between 9000 and 4500 cal yr BP (Figs.1, 2; Table 2). While these studies show a reduction
121 in grassland cover, especially of xerothermic and floodplain grasslands during the mid
122 Holocene, there is no evidence of their widespread disappearance. Rather, there is strong
123 support for their persistence, given the concurrent presence of many grasslands and light-
124 demanding tree and shrub taxa i.e., *Artemisia* spp.*, Thymus* spp.*, Festuca rubra*, *Festuca*
125 spp.*, Sanguisorba* spp.*, Adonis* spp.*, Helianthemum* spp.*, Potentilla erecta*, *Potentilla* spp.*,
126 *Centaurea* spp.*, Filipendula* spp.*, Trifolium* spp. during this period (Fig. 1; Table 2).
127 Archaeobotanical reports from Hungary and Poland suggest an even greater proportion of
128 heliophilous taxa growing locally than pollen records indicate (Fig. 1; Table 2). Remains of
129 grassland species including steppe elements i.e., *Stipa pennata, Stipa* sp.*, Phleum pratense*,
130 *Teucrium chamaedrys, Asperula cynanchica*, *Plantago media*, *Silene vulgaris*, have been
131 reported from archaeobotanical records of early Neolithic sites from Germany (Fig. 1; Table
132 2) and Czech Republic (Archaeobotanical Database of the Czech Republic;
133 http://www.arup.cas.cz). Calcareous grasslands have also been identified in the Neolithic
134 lakeshore sites in the northern foothill of the Alps (Fig. 1; Table 2). The species found are
135 typical of closed xerophilous and mesophilous grasslands at sites where trees would have
136 been able to grow under the climatic conditions of the mid Holocene. Taken together, fossil
137 plant evidence from natural archives and archaeological sources suggest that grasslands
138 existed locally before the start of the Neolithic and therefore before marked human impacts.
139 These findings also demonstrate grassland persistence throughout the mid Holocene,
140 although archaeological records show the occurrence of higher grassland diversity than that
141 found in pollen records.
142

143 **3.2. Zoological evidence**
144 Indirect evidence for the persistence of open, or partly open landscapes throughout the mid
145 Holocene comes from the palaeontological remains of animal species restricted to extensive,
146 open habitats (Fig. 1). Results from the Carpathian Basin (Hungary and Romania) show that
147 several species typical of steppic environments e.g., *Ochotona pusilla* (Steppe Pika),
148 *Microtus gregalis* (Narrow Headed Vole), *Equus ferus* subsp. *gmelini* (Eastern European
149 Wild Horse), *Asinus hydruntinus* (European Wild Ass), *Otis tarda* (Great Bustard) and *Vipera*
150 *ursinii* subsp. *rakosiensis* (Meadow Adder) were abundant during the early Holocene
151 (Németh et al., 2017). They became discontinuously present from 8000 cal yr BP and

several of these species disappeared between 5000 and 4000 cal yr BP at a time of increased anthropogenic pressure, but in a grassland landscape. Fossil malacological records in currently grassland-rich landscapes reveal a similar picture (Fig. 1); a continuous Holocene presence of strictly open habitat molluscs (*Chondrula tridens, Vallonia pulchella,* and *Helicopsis striata*) unable to survive in closed forests (Ložek, 2005; Horsák et al., 2009; Moskal-del Hoyo et al., 2018). Overall, while there is evidence that many open habitat mollusc species contracted their range during mid Holocene forest expansion, there is also robust support for their local long-term persistence, and consequently also for the continuity of grassy ecosystems.

3.3 Phylogeographic evidence

Phylogeographic analysis represents a further source of data facilitating the interpretation of the distribution of past grasslands. A pattern of genetic diversity decline from core populations in southern Siberia towards the range periphery of smaller populations in western Europe has been confirmed for several grassland plant species including *Adonis vernalis* (Hirsch et al., 2015), *Stipa pennata* (Wagner et al., 2012), *Iris aphylla* (Wroblewska 2008) and *Stipa capillata* (Wagner et al., 2011). These studies have also revealed a surprisingly low genetic differentiation between central and peripheral intermediate populations, or a complete lack of private alleles among peripheral populations (e.g. Wagner et al. 2011; Hirsch et al., 2015), which may reflect the absence of any long-standing isolation of these populations. This implies that these species must have had a more continuous past distribution in Central and Eastern Europe allowing gene flow and interbreeding. Rapid progress in the field of DNA analysis, especially environmental DNA, may shed further light on the origin and past range distribution of grasslands (Thomsen and Willerslev, 2015)

3.4 Pedological evidence

A further line of evidence used in the interpretation of former vegetation distributions comes from soil types (IUSS WRB 2006). In Central Eastern Europe, dark soils (chernozems) from steppe and forest steppe zones are considered to have developed before the spread of forests and to have persisted under open or semi-open vegetation (Pokorný et al., 2015). Others, however, view these soils as having survived under forest development (Eckmeier et al., 2007). A palaeo-pedological analysis from the Transylvanian Basin (Romania) shows the occurrence of islands of dark soils of Pleistocene age (20,000-14,000 cal yr BP), which, in drier areas, persisted until the present (Pendea et al., 2002) suggesting grassland persistence throughout the Holocene. However, in other areas of the Transylvanian Basin, dark soils were overlain by Luvisols, typical of nemoral forests, about 5000 cal yr BP (Timar et al., 2010) when wetter climatic conditions prevailed, whilst the current vegetation is predominantly grassland. Grassland occurrence on soils typically favouring forests may be explained by the prevalence of open woodlands throughout the Holocene, allowing the long-term persistence of dark soil, rather than the post deforestation formation of this soil type. Forest soils occur extremely rarely on chernozems developed on loess substrates in the Hungarian Plain therefore suggesting the long-term existence of steppe grasslands (Máté 1957, Molnár 2010). Indeed, the continuous dominance of grasslands from the Late Pleistocene on loess deposits in the southern Carpathian Basin has been demonstrated on the basis of n-alkane biomarkers (Marković et al., 2018). Thus, pedological evidence from chernozems, including those developed on loess, shows that chernozems existence under open or semi-open vegetation.

4. Drivers of grassland persistence during the Holocene
4.1 Climate conditions
Proxy-based and climate simulations indicate warmer-than-present summer temperatures,

203 lower precipitation and soil moisture, and greater seasonality in the early Holocene (11700-
204 9000 cal yr BP) in Central and Eastern Europe at the time of maximum grassland extent
205 (Feurdean et al., 2013; 2014; Heiri et al., 2014). Palaeoclimatological records show a decline
206 in temperatures in this region from approximately 9000 to 4500 cal yr BP (Heiri et al., 2015;
207 Tóth et al., 2015; Hajkova et al., 2016). Climate simulations are consistent with this pattern of
208 mid Holocene cooling, but also marked precipitation and soil moisture increases in the mid
209 latitudes in Europe (Feurdean et al., 2013). Palaeoecological reconstructions show that
210 significant forest expansion occurred in response to cool and moist conditions in Central
211 Eastern Europe (Magyari et al., 2010; Feurdean et al., 2015; Kuneš et al., 2015; Novenko et
212 al., 2016; Pokorný et al. 2015; Jamrichová et al., 2017; Moskal-del Hoyo et al., 2018).
213 Therefore, both proxy and modelled palaeoclimatic and palaeoecological evidence from the
214 lowlands of Central Eastern Europe clearly show that grasslands were most extensive
215 (during the warm and dry climatic conditions, with prolonged droughts, of the early Holocene
216 (11,700-9000 cal yr BP) and became restricted under wetter conditions during the mid
217 Holocene (9000-4500 cal yr BP). This illustrates the stronger competitive advantage of
218 grasslands over trees when resources are limited i.e., lower moisture availability and
219 prolonged droughts.
220
221 **4.2 Fire**
222 The role of fire as one of the main drivers of the rise in grassy ecosystem during the Miocene
223 has been been advocated (Osborne and Behling, 2006; Strömberg, 2011) and confirmed by
224 fossil records from C_4 dominated grasslands in Africa (Hoetzel et al., 2013). This is not
225 surprising as dominant grassland species have fine fuels with rapid curing and fast regrowth
226 rates as well as perennating buds near or below the soil surface; adaptations that ensure
227 regeneration after disturbances that damage the above-ground parts of the plant (He and
228 Lamont, 2018). Thus, whilst grass and herbs can withstand frequent fire, this shift in fire
229 regime may have harmed previously dominant tree species adapted to infrequent fire (He
230 and Lamont, 2018). Although, short-term, field-based burning experiments in Hungary found
231 conflicting results about the effect of fire on grassland biodiversity (Valkó et al., 2014, 2018b),
232 controlled laboratory and small-scale field experiments examining the effect of fire on seeds
233 found a predominantly negative effect of fire on seed germination in grassland species,
234 however, some positive effects (mainly from Fabaceae family) also emerged (Ruprecht et al.,
235 2013; 2015). Disturbances by fire have recently been considered essential for increased
236 grassland competition over trees during the Holocene in Central Eastern Europe (Magyari et
237 al., 2010; Feurdean et al., 2015). For example, a positive effect of frequent fires on the
238 competitive advantage of grasses over trees and, by this means, on the extent of grassland
239 in Transylvania, Romania, has been inferred from sedimentary charcoal particles and pollen
240 (Feurdean et al., 2013). On the contrary, the decline in fire frequency during the mid
241 Holocene has been shown to be detrimental for grassland extension (Fig. 3). Taken together,
242 neo- and palaeoecogical evidence indicate that fire may have had a more important role in
243 the shifts between forest and grassland, and in grassland maintenance, than previously
244 thought. Exploring to what extent temperate grasslands are associated with frequent fires
245 and which grassland species/communities are most resilient or benefit mostly from fire could
246 be useful for the enhancement of management practices, i.e., preventing excessive
247 dominance by competitor grass species as well as the succession towards shrublands and
248 forests.
249
250 **4.3 Herbivores**
251 Large herbivorous mammals influence the physiology and growth of plants and are
252 considered ecological keystones in maintaining tree–grass coexistence (Crawley, 1983;
253 Sankaran et al., 2005). Released from megaherbivore pressure and with a change in climate
254 at the Pleistocene-Holocene boundary, European lowlands witnessed substantial forest

regeneration and a reduction in vegetation openness (Vera, 2000; Svenning, 2002). Megaherbivore extinction may also have had a cascading effect on the population size and diversity of small mammals dependent on vegetation openness and indirectly on the fire regime (Gill et al., 2014). However, comparatively, little attention has been given to the effect of the declining population size or extinction of wild herbivores, or the subsequent role of livestock, on landscape structure during the mid to late Holocene. We know from palaeontological and archaeozoological records in the Carpathian Basin that several large herbivores i.e., *Bison bonasus* (European Bison)*, Alces alces* (Eurasian Elk)*, Equus ferus* subsp. *gmelini* (Wild Horse)*, Equus hemionus* (Asiatic Wild Ass) and *Dama dama* (Eurasian Fallow Deer) became discontinuously present from the mid Holocene, i.e., 8000 cal yr BP and that many became extinct by 4000 cal yr BP (Németh et al., 2017; Bejenaru et al., 2018). In contrast, livestock numbers increased from 6500 cal yr BP (Schumacher et al., 2016). Domestic livestock could prevent forest encroachment in the absence or with a low density of wild herbivores. Domestic animals can act as dispersal vectors for plant propagules (Bruun and Fritzbøger, 2002; Cosyns et al., 2005) and thereby increase the connectivity of grassland patches in a heterogeneous landscape. Combined fossil records of fauna, pollen and coprophilous fungi that reproduce exclusively on animal dung (*Sporormiella* spp.*, Sordaria* spp.*, Podospora* spp.) can provide means of assessing the effects of herbivores on grassland dynamics (Fig. 3) and also the timing of the shift in influence from grazing by wild herbivores to livestock grazing (Gill et al., 2009). Such records are still scant in Europe, but the existing studies generally show the increasing effects of grazing by domestic livestock from 5000 cal yr BP (Schumacher et al., 2016). Understanding the responses of grasslands to different grazing animals (body size, grazing intensity and height, foraging strategy and forage selectivity) will be essential in the development of future grassland management strategies as various forms of livestock grazing have been proposed to simulate the effects of grazing and browsing by wild herbivores (Poschlod and WallisDeVries, 2002; Bakker et al., 2004; Tóth et al., 2016; Poschlod, 2017).

4.4. Early human impact counteracted the encroachment of forest onto primary grasslands

As the increase in forest cover from 8000 cal yr BP coincided with the spread of Neolithic culture across South Eastern Europe (Bogaard et al., 2004; Kreuz 2008), a critical question in respect to grassland extent is whether anthropogenic impacts could have counteracted the climate-driven development of a closed forest (Pokorný et al., 2015). Archaeological datasets from this part of Europe indicate that Neolithic settlements tended to be established in open landscapes and that field sizes were small (Moskal-del Hoyo et al., 2013; Chapman, 2017; Marinova and Ntinou, 2017). As people first settled in naturally open landscapes, this tendency could explain the apparent lack of major deforestation at this time in the pollen records from Central Eastern Europe (Fig. 2). Fire activity was naturally high during the early Holocene (Magyari et al., 2010; Feurdean et al., 2013), and humans may have taken advantage of wildfires to extend their agro-pastoral activities into freshly burned habitats. In agreement with Pokorný et al. (2015) we hypothesise that early anthropogenic land management may have slowed, or partially arrested, the development of closed forest favoured by the wetter climatic conditions of the mid Holocene at locations with low biomass productivity, contributing to the maintenance of landscape openness.

4.5. Semi-natural grasslands replacing forests: when and how?

Individual pollen records, as well as large-scale quantitative vegetation reconstructions from Central Eastern Europe, show that the level of anthropogenic impact on forest remained low until about 6000-5000 cal yr BP (Magyari et al., 2010; Feurdean et al., 2015; Kuneš et al., 2015; Jamrichová et al., 2017; Fig. 2). Modelled vegetation and land use (arable and pasture cover) changes across Europe suggest that open areas expanded gradually from previously

cleared forest after ca. 6000 cal yr BP (Kaplan et al., 2017). A noticeable increase in the abundance and richness of grassland along with the decline in total forest cover but increase for *Quercus,* a tree taxon typical for woodland and woody pasture, in Central Eastern Europe occurred from 4700-3500 cal yr BP onwards (Jamrichová et al., 2017). This demonstrates a growing anthropogenic role in the extension of grasslands and the formation of open woodlands. These grasslands belong to the so-called semi-natural grasslands that developed from forests and are maintained by land management (Pärtel et al., 2007; Pereira et al., 2017). Technological advances in agriculture and the expansion of urban centres and farms from the Late Bronze Age and Iron Age (3500 cal yr BP) have led to both an extension and intensification of the land use in Central Europe (Poschold, 2015; Rösch et al., 2016). It is therefore not surprising that from this time onwards, the richness and extent of grassland has been found to correlate closely with prehistoric settlement density and land management (Poschlod and WallisDeVries, 2002; Pärtel et al., 2005; Hajkova et al., 2011; Hejcman et al., 2013; Poschlod, 2017). Later on i.e., from the 15th to 20th centuries, grassland expansion is strongly linked to sheep flock migration. Livestock acted as dispersal vectors and their mobility may be one of the reasons that ancient and older semi-natural grasslands may have similar species diversity (Poschlod and WallisDeVries, 2002; Molnár et al. 2012; Poschlod, 2017). The sowing of hayseed and mowing may also have promoted grassland expansion in many parts of Europe (Babai and Molnár, 2014). In summary, semi-natural grasslands expanded into formerly forested sites and have subsequently been maintained by a variety of land management practices including grazing, burning and mowing.

5. Anew framework for Holocene grassland persistence; conservation consequences
We provide a new framework distinguishing three types of biodiversity-rich grasslands in Central Eastern Europe: small-scale primary grasslands on skeletal and other poor soils (primary grasslands I); ancient grasslands that were not forested because of natural and anthropogenic disturbances (primary grasslands II), and semi-natural grasslands developed as a result of human activities replacing forests (Fig. 4; Table 1). We have identified the reasons for the continuous presence of primary grasslands during the Holocene including both natural i.e., climate conditions and soils (primary I), climate and disturbance factors e.g., fire and grazing (primary grasslands II). Neolithic people may have first settled in naturally open areas such as grasslands or grassland-woodland mosaics arresting the development of a full forest cover when the climate became wetter (mid Holocene), indirectly favouring the preservation and expansion of grasslands. The intensification of human impact from 4700-3500 cal yr BP onwards subsequently lead to considerable extension of semi-natural grassland on formerly forested sites. A succession from grassland to forest after the cessation of land management (e.g. grazing or burning) is not necessarily a proof against the primary or ancient aspect of grasslands, but may indicate the lack of disturbances. Livestock grazing in the late Holocene has replaced ancient grazing by megaherbivores prevailing until the early Holocene and that by large herbivores throughout the mid Holocene.

Our findings also reveal misconceptions about the origin of Central Eastern European grasslands and open canopy woodlands. The concept of a previously continuous, closed forest in extant grassland-rich landscapes where climatic conditions are favourable for forest fails to hold true, as grasslands are likely to have been continuously present throughout the Holocene. The long-term persistence of grasslands at these locations is probably an important reason for one of the highest small-scale species richness, many endemic, worldwide in these habitats. Such species-rich plant communities can require millennia to develop and only well-connected grassland patches can support genetically diverse plant populations. These findings challenge the commonly held view that conservation activities should primarily focus on the protection of forests in many areas of Central Eastern Europe.

357   Anthropogenic impacts tend to focus on forest clearance, but this perspective paper
358   highlights that conservationists and land managers need to carefully consider that, in many
359   cases, it is not primary forests that hold the highest biodiversity. Further, human-made, or
360   managed environments, such as extensively managed grasslands, are long-term landscape
361   features, contain unique plant and animal communities, and provide important ecosystems
362   services. Our findings support the recent wider acceptance of the notion that people and
363   nature should not be separated in the societal discourse of environmental science (Mace
364   2014). Finally, we advocate the need for a more detailed understanding of the role of
365   disturbances in grassland-forest dynamics, to avoid the overly simplistic assumption that
366   sparse tree cover is evidence of past deforestation. Fossil records provide such data and the
367   routine incorporation of palaeoecological investigations into environmental management is a
368   key step in developing science-based evidence for the conservation of the biodiversity of
369   grasslands. Thus, our regional case study supports the advocacy of Willis et al. (2010),
370   Barnosky et al. (2017) and Whitlock et al. (2018) for merging palaeobiology and conservation
371   biology as well as an appreciation of the dynamic history of species and ecosystems,
372   including the role of humans.
373

Figures captions

Figure 1. Location of the study area in Europe (A) and the distribution of the main vegetation types in Central Eastern Europe based on the potential natural vegetation map of Europe (B; Bohn et al., 2003). Colour symbols show location of various types of fossil records extracted from literature (Table S1 and 2) indicating either continuous grassland presence throughout the Holocene or during the afforestation phases of the mid Holocene (9000-4000 cal yr BP). These fossil records reveal that grasslands were continuously present throughout the Holocene in places where potential natural vegetation has been assumed to be forest.

Figure 2. Pollen based reconstruction of forest (green) versus open land cover (yellow) from Central Eastern Europe during the Holocene using the pseudobiomisation method (Fyfe et al., 2015). Cumulative land cover record was constructed by spatially aggregating 96 pollen records extracted from the Pangaea Database and distributed across the region shown in Fig.1 and Fig. S2. Forest cover includes both broadleaf and conifer trees, whereas open land cover includes pastures/natural grasslands, and arable/disturbed land. Geological and archaeological periods as well as the predominance of each grassland type throughout the Holocene are also highlighted. Trends in simulated growing season temperature and precipitation after Feurdean et al. (2015).

Figure 3. Reconstruction of tree, grassland and arable land cover from sedimentary pollen, fire frequency from sedimentary charcoal and herbivore numbers based on sedimentary coprophilous fungi from a grassland-rich landscape in Transylvania, Romania during the Holocene (Feurdean et al., 2015). This multi-proxy approach ilustrates the role of fire and wild herbivores on vegetation openess during the early Holocene and the role of human impact (deforetation, burning, livestock grazing) during the late Holocene. Note that the icons only tenetativly ilustrate the evolution of grazing type, burning and cultivation during the Holocene.

Figure 4. The effect of climate, soils and disturbances by fire, herbivores and humans on the three types of grasslands and forest. Blue line denote a positive effect, red line a negative effect and grey both effects.

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Supplementary Material

Figure SI1. The World Resources Institute (WRI) Atlas of Forest and Landscape Restoration, which identified areas currently occupied by open woody landscapes with grasslands that could be suitable for various degrees of reforestation. Mosaic restoration means that forests are embedded in a mosaic of land uses, whereas remote restoration

681 refers to the restoration of degraded forest in remote areas, mainly in the northern boreal
682 zone.

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685 **Figure S2** Location of sites extracted from the European Pollen Database (EPD) and used to
686 construct Figure 2.

687 **Table S1.** Location of sites extracted from the literature and used to construct Figure 1.

688

Table 1. Species-rich grassland types

Grassland type	Characteristics
Primary grassland I	Natural grasslands on skeletal and other poor soils with moisture deficit
Primary grassland II	Ancient grasslands formed and maintained mainly by climate conditions and natural fires, herbivores and, later, also influenced by anthropogenic disturbances
Semi-natural grassland	Secondary grasslands formed and maintained by anthropogenic disturbances (deforestation, livestock grazing, cultivation, use of fire) in areas suitable for forests during the late Holocene

Table 2. Fossil pollen, plant macrofossil and charcoal evidence for the presence of various types of grasslands during the early and mid Holocene. Note: on the basis of pollen analysis differentiation between primary grassland and semi-natural grassland is not always possible.

Location	Taxa	Period	References
Romania	*Artemisia, Aster-type,* Caryophyllaceae undiff., Compositae Cichorioideae, *Centaurea,* Chenopodiaceae, *Helianthemum, Filipendula, Potentila,* Poaceae, *Plantago lanceolata, Plantago major/P. media, Rumex acetosa, Thalictrum, Chenopodium, Suaeda maritina, Festuca, Lycopus, Thymus,* Leguminosae undiff.	Holocene	Feurdean et al., 2015
Hungary	*Artemisia, Achillea, Ajuga, Adonis, Astragalus, Allium, Aster-*type, Poaceae, *Centaurea, Filipendula, Festuca, Euphorbia,* Caryophyllaceae undiff., *Gagea,* Chenopodiaceae, Compositae Cichorioideae, *Dianthus-type, Genista, Helianthemum, Hypericum, Inula,* Leguminosae undiff., *Lotus-*type, *Plantago lanceolata, Plantago major / P. media, Potentilla, Trifolium, Rumex acetosa, Teucrium, Thymus, Thalictrum, Verbascum, Sanguisorba*	Holocene	Magyari et al., 2010
Hungary	*Chenopodium album, Echinochloa crus-galli, Fallopia convolvulus Galium spurium, Melandrium album, Plantago lanceolata, Polycneum arvense, Polygonum aviculare, Polygonum mite, Polygonyum minus, Rumex acetosa, Setaria pumila, Setaria viridis, Bromus sp., Chenopodium sp., Galium, Ononis sp., Polygonum sp., Rumex sp., Stipa sp., Trifolium sp., Vicia sp.,* Fabaceae, Poaceae, Polygonaceae, Caryophyllaceae	Neolithic	Moskal del Hoyo et al., 2018
Poland (S)	*Artemisia, Stipa* sp., *Knautia arvensis, Hypericum, Plantago media, P. lanceolata, Filipendula, Aster-type,* Compositae Cichorioideae, Poaceae, Chenopodiaceae	Mid Holocene	Moskal del Hoyo et al., 2018
Czech Republic	*Artemisia, Achillea, Ajuga, Astragalus, Filipendula,* Chenopodiaceae, *Centaurea,* Caryophyllaceae undiff., Compositae Cichorioideae, *Genista, Filipendula, Helianthemum, Hypericum, Inula-*type, *Plantago lanceolata, P. media,* Poaceae, *Potentilla-*type, *Ranunculus acris-*type, Rubiaceae, *Rumex acetosa, Teucrium, Thymus, Thalictrum, Verbascum*	Holocene	Kuneš et al., 2015 Jamrichova et al., 2017 Hajeck et al., 2016

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740	**Austria**	*Poa pratensis/trivialis, Festuca ovina/rubra, Brachypodium pinnatum,*	Neolithic	Körber-Grohne 1990
741		*Anthoxanthum odoratum, Stipa pennata*		
742				
743	**Germany (S)**	*Alchemilla vulgaris, Asperula cynanchica, Bupleurum falcatum, Carex muricata,*	Neolithic	Kreuz et al., 2005, 2008
744		*Centaurea, Daucus carota, Euphrasia, Phleum pratense, Poa annua, Polygonum aviculare,*		Kreuz and Schäfer, 2011
745		*Stipa* sp*., Stipa pennata, Rumex acetosella, Stellaria graminea, Trifolium campestre,*		
746		*Galium cf. verum, Galium molugo, Urtica dioica, Teucrium chamaedrys, Chenopodium spp.,*		
747		*Veronica arvensis,Plantago media*		
748				
749	**Germany (NW)**	*Achillea ptarmica, Angelica sylvestris, Anthriscus sylvestris, Artemisia cf.campestris,*	Preboreal	Knörzer 1996
750		*Crepis biennis, Chaerophyllum hirsutum, Dianthus spp., Empetrum nigrum,*	& Boreal	
751		*Euphorbia cyparissias, Festuca cf.pratensis, Festuca rubra, Filipendula ulmaria, Galium mollugo,*		
752		*Heracleum sphondylium, Hypochaeris radicata, Juncus cf. effusus, Knautia arvensis,*		
753		*Lysimachia vulgaris, Laserpitium prutenicum, Poa pratensis / angustifolia,*		
754		*Polemonium coeruleum, Scabiosa columbaria, Plantago lanceolata,*		
755		*Potentilla tabernaemontani, Ranunculus acris, Rumex acetosa, Rumex tenuifolius,*		
756		*Sanguisorba officinalis, Scabiosa aff.columbaria, Silene vulgaris, Thalictrum flavum,*		
757		*Valeriana officinalis, Valeriana procurrens, Viola canina*		
758	**Germany (NW)**	*Achillea millefolium, Leontodon autumnalis, Agrostis tenuis, Melandrium rubrum,*	Neolithic	Knörzer 1996
759		*Phleum nodosum, Poa cf.pratensis, Artemisia cf.campestris, Poa cf.trivialis,*		
760		*Polygonum bistorta, Potentilla argentea, Carex ovalis, Prunella vulgaris,*		
761		*Centaurea cf.nigra, Ranunculus repens, Cerastium cf.semidecandrum, Rumex tenuifolius,*		
762		*Chrysanthemum leucanthemum, Deschampsia caespitosa, Selinum carvifolium,*		
763		*Festuca rubra, Silaum silaus, Heracleum sphondylium, Stachys recta, Hieracium pilosella,*		
764		*Trifolium arvense, Hypericum cf.maculatum, Trifolium dubium, Hypericum tetrapterum,*		
765		*Valerianella dentata, Juncus bufonius / conglomeratus, Veronica cf.arvensis,*		
766		*Trifolium repens, Juncus cf.effusus, Viola tricolor*		
767				
768				
769				
770				

Vegetation types

- Steppe
- Forest-steppe
- Termophilous mixed deciduous broadleaf forests
- Mesophytic broadleaf deciduous and mixed broadleaved conifer forests
- Other (mostly wet and alpine) vegetation types

Sites

- Pollen
- Plant macrofossils
- Soils
- Molluscs
- Multi-proxy

Mesolithic	Neolithic	Bronze Age	Iron Age	Roman	Mediae-val	Modern

Early Holocene	Mid Holocene	Late Holocene

Proportion of the landscape (%)

Primary grasslands types I, II

Primary grasslands types I, II

Primary grasslands types I, II

Semi-natural grasslands

Growing season T (C°)

Growing season PP (mm)

Age cal yr BP (x 1000)

Skeletal soils — Primary grasslands I (on skeletal soils) — Fire

Primary grasslands II (on potential forest area) — Herbivores

Precipitation

Semi-natural grasslands (originate from forest) — Human impact

Temperature

Forest

Sites

- 🔺 Pollen
- 🔶 Plant macrofossils
- ⬠ Soils
- 🟪 Molluscs
- 🔴 Multi-proxy
- ⊙ EPD sites

Vegetation types

- Steppe
- Forest-steppe
- Termophilous mixed deciduous broadleaf forests
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- Other (mostly wet and alpine) vegetation types

0 50 100   200 Km