

RESEARCH ARTICLE

Nitrogen limitation reduces the performance of target plant species in restored meadows

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Restoring habitats degraded by intensive agriculture is challenging, and the resulting communities often have lower quality and host fewer species than reference ecosystems. To improve restoration outputs, we need to understand what limits both establishment and performance of target species in restored populations. In this study, we focused on grassland restoration with regional seeds and compared the performance of two target herbs, *Betonica officinalis* and *Centaurea jacea*, between restored and reference populations. We also measured plant functional traits and environmental characteristics to understand which parameters affect population performance. Individual plants of both species were smaller in restored populations, which indicates reduced performance. Leaves of plants from restored populations contained more $\delta^{15}\text{N}$ and, in *Betonica*, also less nitrogen and higher C:N ratio, which suggests that the performance of the target species may be limited by nitrogen. Nitrogen limitation of all restored communities was further corroborated by the low N:P ratio of bulk biomass. In *Centaurea*, we also recorded massive herbivory damage in restored populations, which likely further reduced this species' performance in restored meadows. In summary, target plants in restored populations showed lower performance than conspecifics in reference sites, probably due to nutrient imbalance (low nitrogen availability) and excessive herbivory damage.

Key words: functional traits, grassland, N:P ratio, population, regional seeds, restoration

Implications for Practice

- To improve the performance of target plant species, restoring the soil nutrient balance at the initial stage of a restoration project would be desirable. However, this is a difficult task.
- Target plant species in restored meadows suffered substantially from herbivory. Therefore, diversifying and increasing habitat structures in restored meadows could enhance the establishment of natural enemies and thus, counteract excessive herbivory damage.

Introduction

Land-use change and particularly the expansion of intensive agriculture are major causes of habitat degradation and drivers of biodiversity loss (Díaz et al. 2019). While habitat conservation is necessary to reduce biodiversity loss, reversing the negative trend requires active restoration of already degraded habitats (Leclère et al. 2020). To upscale restoration efforts, the UN recently declared the decade 2021–2030 as The Decade on Ecosystem Restoration (<http://www.decadeonrestoration.org>).

Restoration often takes place on former agricultural lands, but transforming these back to a former reference community is challenging, and success is uncertain (Fagan et al. 2008; Conrad & Tischew 2011). One important reason is that environmental conditions of ex-arable lands differ from those of natural habitats. For example, large-scale intensive agriculture cultivation tends to

reduce spatial heterogeneity, and tillage and fertilization commonly impact soil properties and water regimes (Mclauchlan 2007; Turley et al. 2020). As a result, ex-arable lands typically have increased pH levels, depleted soil organic matter (SOM), decreased soil microbial diversity, and altered nutrient cycles (Mclauchlan 2007; Turley et al. 2020).

Contrary to the intuitive assumption that formerly fertilized fields are enriched with nutrients, the reality is a bit more complicated. Ex-arable lands indeed show increased plant-available

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phosphorus and potassium contents but often lack nitrogen and other macronutrients (Walker et al. 2004; Mclauchlan 2007; Smits et al. 2008). This lack is a consequence of tillage degraded soil structure that interferes with natural nutrient cycling and enhances mineralization rates, resulting in rapid loss of mobile elements such as plant-available sulfur and nitrogen (Watzka et al. 2006; Mclauchlan 2007; Amelung et al. 2019). Moreover, fertilizer application stimulates processes like denitrification and nitrification, which discriminate against ^{15}N , leading to ^{15}N enrichment of the soil (Watzka et al. 2006). Yet, total nitrogen stocks are typically not refilled but virtually depleted in the long term, because surpluses and particularly the light ^{14}N get lost via leaching (rain washes out nitrate) and volatilization (conversion of ammonium to ammonia gas) (Watzka et al. 2006; Mclauchlan 2007). In contrast, phosphorus and potassium, supplemented by fertilization, accumulate in the soil because they bind to mineral and organic substances (Mclauchlan 2007; Amelung et al. 2019). Long-term effects of agricultural land use on soil calcium contents depend on the execution and extent of liming (Amelung et al. 2019) and on the alkalinity of bedrock. Tillage can bring basic substrates to the surface and increase pH. Consequently, postagricultural soils are substantially altered in comparison to natural ecosystems.

Changes in soil conditions caused by agriculture persist for decades, not only after abandonment but even after active restoration (Bizzari et al. 2015; Helm et al. 2019; Turley et al. 2020). This has implications for the restoration output because altered soil conditions shift nutrient limitations for individual plants (Schelfhout et al. 2015), reduce native plant establishment, and limit restoration success (Walker et al. 2004; Zirbel et al. 2017). To improve the outputs of postagriculture restoration projects, we need a mechanistic understanding of how these altered environmental conditions affect the establishment and performance of individual plant species.

A useful tool to study the link between environment and plant performance is an analysis of plant functional traits (Pérez-Harguindeguy et al. 2016; Bruelheide et al. 2018). Numerous studies have shown that community weighted means of plant functional traits can provide robust indicators for environmental conditions. For example, communities at dryer sites typically have higher proportions of structural tissue: they have smaller and thicker leaves, and their leaf biomass contains more total carbon, also reflected in a smaller specific leaf area (SLA; leaf area/leaf dry mass) (Chaves et al. 2002; Hodgson et al. 2011; Jung et al. 2014). Further, closing the stomata to minimize water loss leads to the enrichment of the heavy carbon ^{13}C isotope (i.e. higher values of $\delta^{13}\text{C}$) (Chaves et al. 2002; Klaus et al. 2016). Communities growing at nutrient-rich sites, especially rich in nitrogen, typically have higher leaf nitrogen content (LNC), lower C:N ratio in their leaf biomass, and they have softer and thinner leaves, resulting in higher SLA (Hodgson et al. 2011; Klaus et al. 2011; Pérez-Harguindeguy et al. 2016). Notably, higher nutrient availability following fertilization also increases water stress and subsequently $\delta^{13}\text{C}$ (Klaus et al. 2016). On the contrary, increased $\delta^{15}\text{N}$ in leaves, that is, the enhanced content of the heavy ^{15}N isotope, suggests nutrient limitation because plants would discriminate against

^{15}N if the nitrogen supply would exceed the plants' demand (Michener & Lajtha 2008). In other words, plant functional traits provide a mechanistic understanding of how the differences between restored and intact original habitats affect plants.

While most studies focused on trait differences between communities and between species, evidence emerges that substantial variation of plant functional traits also occurs within species (Wellstein et al. 2017; Tautenhahn et al. 2019, 2020). Within-species trait values also commonly correlate with the environment, mostly in a similar direction as at the community level (Jung et al. 2014; Yue et al. 2019, but see Dong et al. 2020). Comparison of life-history traits among populations of the same species growing at different sites thus could inform us about environmental conditions that shape population performance. This is especially important in the restoration context, where within-species trait differences may help to understand which environmental factors limit the performance of the target species at the restored sites.

To address this knowledge gap, we focused on the differences in plant functional traits between populations of two species growing both in ancient and in restored meadows in the White Carpathian Mountains, Czech Republic. The two herbs, *Centaurea jacea* and *Betonica officinalis*, are among the target species of restoration (Prach et al. 2013) and grow both at restored and ancient reference sites. Yet, they are more abundant at ancient ones (Prach et al. 2014, Bucharova, 2019 pers. obs.). To understand this discrepancy, we compared life-history traits of these two species between the two types of habitats. We also analyzed soil properties and the chemical composition of the community biomass to characterize the environmental conditions at the restored and the ancient sites and to understand which environmental factors shape the plants' performance.

We hypothesize that (1) past land use affects environmental factors and notably shifts the type of nutrient limitation for plants at restoration sites and that (2) the respective effects of land use on environmental properties are also reflected by differences in plant functional traits between populations, and that (3) plants in restored populations show a reduced performance.

Methods

Study System

The study took place in a protected landscape area in the White Carpathians Mountains, Czech Republic (Fig. 1). The low mountain range consists of cretaceous marly flysch layers with gleyic calcareous cambisols. The mean annual temperature is 9.3°C, and the mean annual precipitation is 627 mm (Jongepierová 2008). The ancient semi-natural meadows of the region are among the most species-rich grasslands on Earth (Wilson et al. 2012; Chytrý et al. 2015). During the second half of the twentieth century, many of these meadows were transformed into arable land, but since 2000 exists a program to restore ex-arable land back to grasslands. We selected five localities with pairs of ancient and restored meadows, 10 meadows in total. The restored meadows were created between 2001 and 2003 by sowing a regional seed mixture. The seeds originated

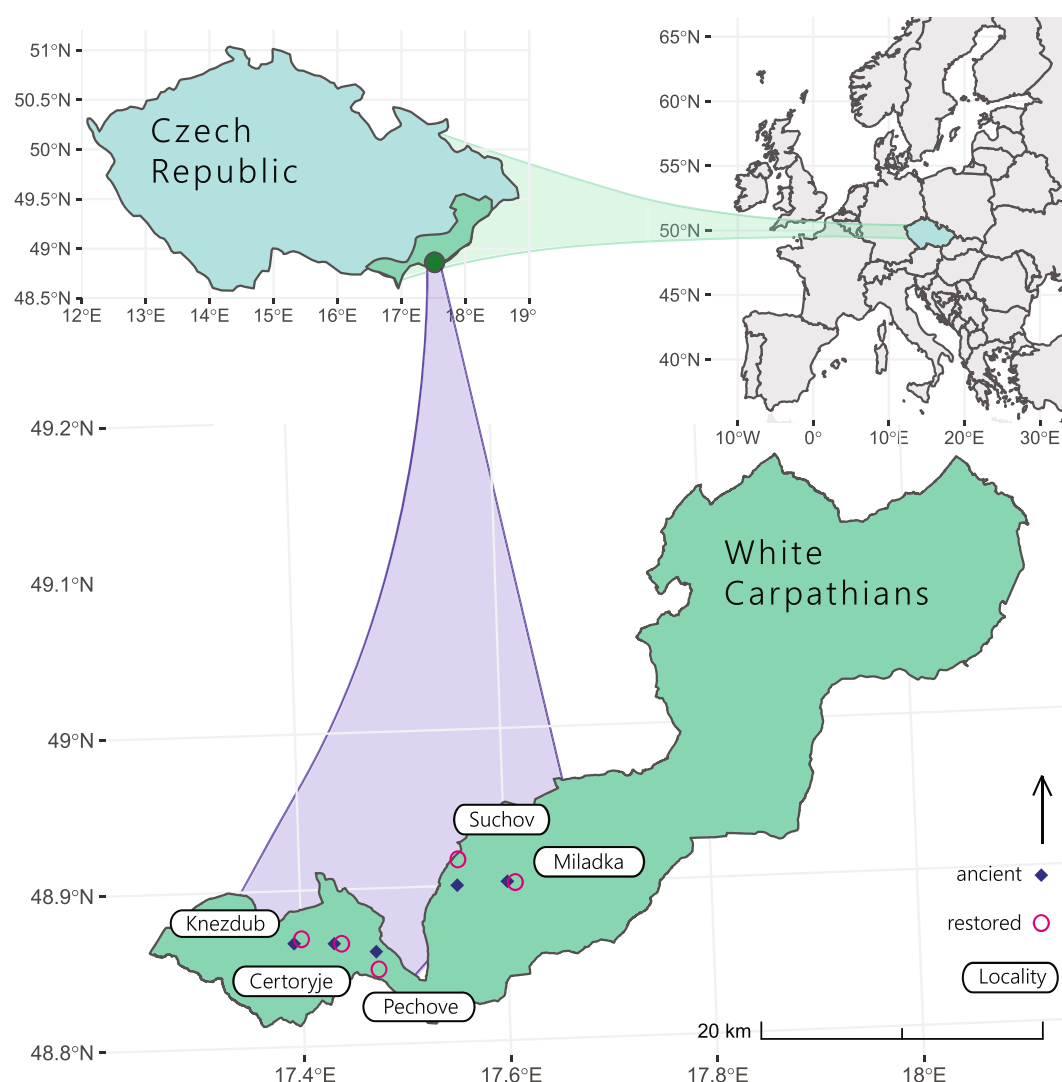


Figure 1. Location of the study.

from ancient meadows in the region. Grass seeds were brush-harvested and directly used, while forbs were hand-collected and propagated in seedbeds before sowing (Jongepierová et al. 2007; Jongepierová 2008). The restored meadows are nowadays dominated by sown species, especially grasses. Cover of spontaneously established species is less than 5% (Mitchley et al. 2012). On average, 66% of the sown target species established, but their abundance is still lower than in the reference communities (Prach et al. 2013). At the time of our study, restored sites showed lower species richness, lower vegetation cover, and greater dominance of grasses than ancient meadows (personal observation).

Environmental Characteristics

To characterize the environmental conditions of the meadows, we analyzed the chemical properties of soil and plant community biomass. The elemental composition of the community biomass integrates both temporal and spatial

variation of nutrient availability in the soil, and is an excellent proxy for the availability of particularly those nutrients, which undergo substantial variations throughout the vegetation period (e.g. nitrogen). Chemical properties of biomass thus better mirror the availability of many nutrients than a one-time measurement of soil nutrients. Moreover, the derived measures of nutrient ratios in biomass reflect the plants' actual nutritional status (Römhild 2011) and can be used to assess plant nutrient limitation (Koerselman & Meuleman 1996; Palpurina et al. 2019).

At each of the 10 meadows (5 restored, 5 ancient), we collected 7 biomass and 7 topsoil samples, in total 70 soil and 70 biomass samples. The community biomass was cut 2 to 3 cm above the ground from 0.1-m² quadrats, placed evenly across the meadow. Within a 1 m radius around each quadrat, we collected five 10-cm deep topsoil aliquots with a core sampler of 3 cm diameter and pooled them into one sample. Both biomass and soil samples were air-dried until transportation to the laboratory in Münster, Germany, for further analysis.

Soil samples were dried at 70°C, weighed, and ground with mortar and pestle to pass a 2 mm mesh. We extracted plant-available phosphorus (P) and potassium (K) in calcium-acetate-lactate following standard protocols (Blume et al. 2011), measured soluble phosphorus using a spectrophotometer (Cary 100 UV–Vis, Santa Clara, CA, U.S.A.) (Murphy & Riley 1962), and the amount of plant-available potassium with a flame

Table 1. Overview on central environmental traits and plant functional traits which were examined in this study.

Trait	Abbreviation	Ecological Function/ Indication
Environmental traits		
<i>Biomass composition</i>		
N (%)	N	Essential for plant metabolism
P (%)	P	Essential for plant metabolism
K (%)	K	Essential for plant metabolism
Ca (%)	Ca	Essential for plant metabolism
S (ppm)	S	Essential for plant metabolism
N:P ratio	N:P	Nutrient supply
<i>Soil properties</i>		
pH	pH	Soil acidity
Soil organic matter (%)	SOM	Soil fertility; water retention
P (mg/g)	P	Amount of plant-available phosphorus
K (mg/g)	K	Amount of plant-available potassium
Plant functional traits and their indications		
<i>Morphological traits</i>		
Plant height (cm)	Height	Competitive ability, fitness
Specific leaf area (cm ² /g)	SLA	Competitive ability, water stress, nutrient acquisition
<i>Leaf chemical traits (%)</i>		
Leaf nitrogen content (%)	LNC	Resource acquisition; competitive ability
Leaf carbon content (%)	LCC	Resource acquisition; competitive ability
C:N ratio	C:N	Resource acquisition; competitive ability
<i>Stable isotopes</i>		
Leaf $\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$	Water use efficiency, water stress
Leaf $\delta^{15}\text{N}$ (‰)	$\delta^{15}\text{N}$	Nitrogen source

photometer (PFP7/C, Jenway, Stone, U.K.). We measured the pH in a 0.01 M CaCl₂ suspension with a potentiometric glass electrode (c.f. DIN ISO 10390) and SOM by ignition loss using a muffle furnace (c.f. DIN 19684-3).

Biomass samples were dried at 70°C, weighed, and milled to a fine powder in a vibration mill (Micro Mill 400, Retsch, Haan, Germany). Concentrations of macronutrients (N, Ca, K) were determined through near-infrared spectroscopy (Spectra Star 2400 RTW, Unity Scientific, Milford, CT, U.S.A.) using calibration models described by Kleinebecker et al. (2011). To measure the concentration of phosphorus and sulfur in biomass, we used wavelength-dispersive X-ray fluorescence spectroscopy (WD-XRF; Primus ZSX II, Rigaku, Tokyo, Japan), calibrated with a set of 15 certified reference materials comprising plant and peat materials. We calculated the N:P ratio in aboveground bulk biomass to examine nutrient limitation.

Plant Functional Traits

We focused on two perennial species, *Centaurea jacea* (Asteraceae) and *Betonica officinalis* (Lamiaceae). Both species were target restoration species in the study region and are common in ancient and restored meadows, although with lower density in the latter (Prach et al. 2013). In May 2019, we collected 20 ± 2 random individuals per species and meadow, keeping a distance of at least five meters between them to avoid close relatives. We neither collected under trees nor in border zones to avoid shading and edge effects.

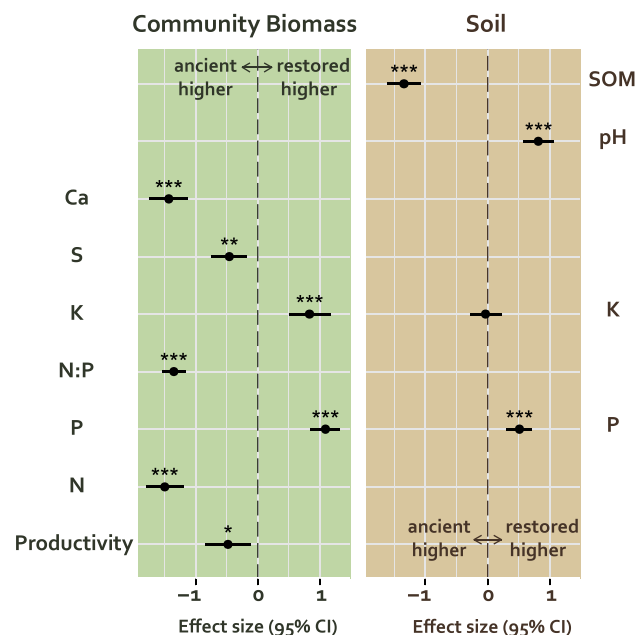


Figure 2. Effects of historical land use on soil and plant biomass nutrients. Model estimates (mean $\pm$ SE) for the effect of historical land use on soil properties ($n = 70$) and biomass composition ($n = 70$). Points represent the model effect estimate of the measured trait, solid lines reflect their confidence intervals, and the vertical dashed lines correspond to no effect of historical land use. SOM, soil organic matter. Levels of significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. Bulk biomass and soil traits of ancient and restored sites in White Carpathian Mountains, Czech Republic. Values are mean $\pm$ SE. Each value shows the mean of 35 samples, which were gathered from each five ancient or restored sites. SOM, soil organic matter.

Site	Ancient	Restored
Biomass		
Productivity (g)	24.7 $\pm$ 1.2	22.47 $\pm$ 1
N (%)	1.6 $\pm$ 0.05	1.24 $\pm$ 0.03
P (%)	0.13 $\pm$ 0.01	0.16 $\pm$ 0.01
N:P	13.7 $\pm$ 0.71	7.97 $\pm$ 0.17
K (%)	3.85 $\pm$ 0.05	4.15 $\pm$ 0.04
S (ppm)	1577.77 $\pm$ 39.79	1381 $\pm$ 29.38
Ca (%)	0.95 $\pm$ 0.03	0.72 $\pm$ 0.02
Soil		
pH (CaCl ₂)	5.62 $\pm$ 0.16	6.31 $\pm$ 0.14
pH (H ₂ O)	6.1 $\pm$ 0.16	6.85 $\pm$ 0.13
K (mg/g)	47.47 $\pm$ 4.26	38.17 $\pm$ 3.89
P (mg/g)	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00
SOM (%)	12.4 $\pm$ 0.26	10.98 $\pm$ 0.37

We measured morphological and chemical functional traits that reflect growth and resource usage (Table 1). The morphological traits were plant height and SLA. For SLA measurements, we scanned 3–10 fully developed leaves per plant, including their petioles, using a flat-bed scanner with a resolution of 600 dpi. We processed and analyzed the scans with FIJI (Schindelin et al. 2012), dried the scanned leaves for 48 hours at 70°C, and weighed them. *Centaurea* suffered from significant herbivory damage by beetle larvae present in substantial amounts on the plants at the time of study. We estimated herbivore damage visually as the proportion of the leaf eaten by herbivores averaged across the three youngest, fully developed

leaves. We also collected 20 larvae and reared them to obtain adults, which were later determined by an expert to be *Galeruca pomonae* and *G. tanacetii*.

We measured chemical leaf traits such as leaf carbon content (LCC) and LNC, their ratio (C:N), and the stable isotopic composition expressed in the standard δ -notation $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ (Table 1). Chemical leaf traits were measured in eight random plants per species and meadow using the same leaves as for SLA analysis. After grinding the dried leaves to a fine powder with mortar and pestle, samples were analyzed using an elemental analyzer (Eurovector EA3000, Milano, Italy) coupled with an isotope-ratio mass spectrometer (Nu Instruments Nu Horizon, Hekatech, Wegberg, Germany). Samples were measured against laboratory working standards: BBOT (2,5-Bis(5-tert-butyl-2-benzoxazolyl)thiophene; Hekatech, Wegberg, Germany) and ground birch leaves (IVA Analysetechnik e.k., Meerbusch, Germany). As certified international reference, we used caffeine for calibration (Coplen et al. 2006). To estimate the precision of the analyses, we ran internal laboratory working standards after every 10th sample.

Statistical Analysis

We performed all statistical analyses in R (R Development Core Team 2021, version 3.6.0). We tested for differences between ancient and restored sites regarding environmental and plant functional traits by analyzing each trait using a linear mixed-effects model and accounting for unequal variances of factor levels, using the nlme package (Pinheiro et al. 2018). In these models, the respective trait was the response variable, historical land use (restored/ancient), the fixed factor, and the locality a random factor. For *Centaurea*, the differences in SLA were

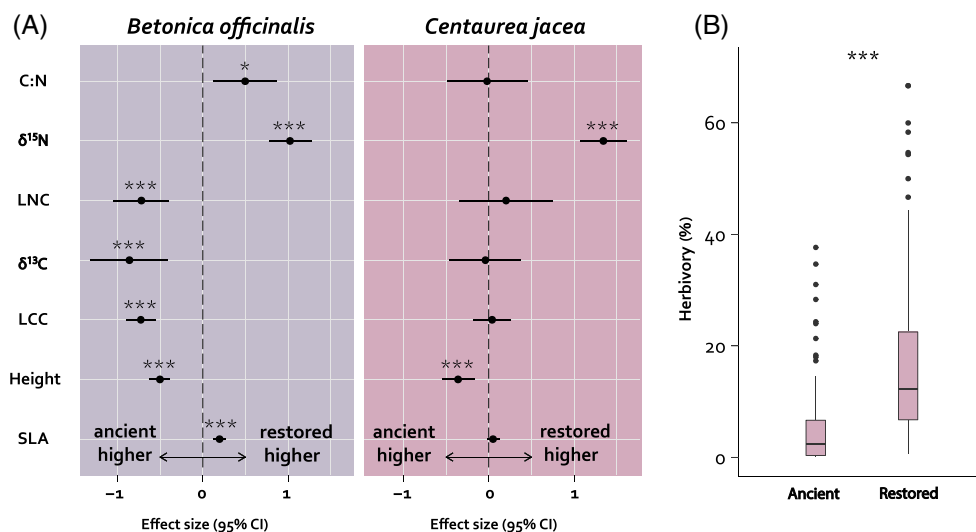


Figure 3. (A) Effects of historical land use on plant traits. Model estimates (mean $\pm$ SE) for the effect of historical land use on plant functional traits of both study species. Whole plant traits were measured on 20 $\pm$ 2 individuals per site and species: *Betonica officinalis* ($n = 189$) and *Centaurea jacea* ($n = 187$). Chemical traits were determined for eight plants per site ($n = 120$ per species). Points represent the effect estimate of the measured trait. Solid lines reflect their confidence intervals. The vertical dashed lines correspond to no effect of historical land use. LCC, leaf carbon content; LNC, leaf nitrogen content; SLA, specific leaf area. Levels of significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (B) Herbivory damage of *Centaurea jacea* leaves at ancient and restored meadows. For model results, see Table S2.

Table 3. Descriptive statistics of population differentiation between ancient and restored sites in plant functional traits of two grassland plants in White Carpathian Mountains, Czech Republic. Total values are given in mean ($\pm$ SE). LCC, leaf carbon content; LNC, leaf nitrogen content; SLA, specific leaf area.

Site	<i>Betonica officinalis</i>		<i>Centaurea jacea</i>	
	Ancient (n = 84)	Restored (n = 105)	Ancient (n = 105)	Restored (n = 82)
Height (cm)	18 $\pm$ 0.34	14.6 $\pm$ 0.34	26.2 $\pm$ 0.79	21.2 $\pm$ 0.54
SLA	172 $\pm$ 5.42	201 $\pm$ 3.78	164 $\pm$ 1.99	168 $\pm$ 3.59
LNC (%)	2.48 $\pm$ 0.06	2.33 $\pm$ 0.1	2.41 $\pm$ 0.08	2.52 $\pm$ 0.11
$\delta^{15}\text{N}$ (‰)	5.69 $\pm$ 0.33	8.52 $\pm$ 0.31	6.63 $\pm$ 0.22	9.63 $\pm$ 0.25
LCC (%)	43 $\pm$ 0.15	41.2 $\pm$ 0.17	41.3 $\pm$ 0.14	41.4 $\pm$ 0.18
$\delta^{13}\text{C}$ (‰)	-29.3 $\pm$ 0.13	-29.8 $\pm$ 0.14	-29.4 $\pm$ 0.14	-29.5 $\pm$ 0.1
C:N	17.7 $\pm$ 0.47	18.4 $\pm$ 0.61	17.3 $\pm$ 0.46	17.1 $\pm$ 0.58
Herbivory (%)	NA	NA	5.51 $\pm$ 0.75	17.8 $\pm$ 1.8

corrected for herbivory by including herbivory as a fixed effect in the model. All variables were scaled prior to the analysis. For graphical visualization, we used the ggplot2 package (Wickham 2016).

Results

Environmental Characteristics

Both soil and community biomass analysis indicated considerable differences between ancient and restored sites (Fig. 2). Soil from restored meadows contained less SOM but more soluble P and had a higher pH. Only soil potassium supply did not differ between ancient and restored meadows (Fig. 2). Restored meadows produced less biomass, and the community biomass consistently contained less calcium, sulfur, and nitrogen but more potassium and phosphorus (Fig. 2). The N:P ratio in community biomass was significantly higher in ancient meadows than in restored ones (Fig. 2). For absolute values, see Table 2; for exact figures for all traits and sites, see Figure S1, and for model results, see Table S1.

Plant Functional Traits

Both *Centaurea* and *Betonica* individuals differed in plant morphological traits between ancient and restored sites. Plants were significantly smaller in restored meadows in both species (Fig. 3A). *Betonica* had higher SLA in restored meadows, *Centaurea* not (Fig. 3A). In *Centaurea*, tissue damage due to herbivory was more than three times higher on restored meadows (Fig. 3B).

Plants from ancient and restored meadows differed in their chemical composition. Both species showed a higher $\delta^{15}\text{N}$ at restored sites (Fig. 3A). Furthermore, for *Betonica*, C:N was higher at these sites, whereas LCC, LNC, and values of $\delta^{13}\text{C}$ were higher in ancient meadows (Fig. 3A). For absolute values, see Table 3; for exact figures for all traits and sites, see Figure S2, and for model results, see Table S2.

Discussion

We investigated within-species differences in plant functional traits and performance, and their potential environmental

drivers. We found distinct differences between ancient and restored sites at all studied characteristics: soil chemistry, community biomass composition, morphological plant functional traits, and plant leaf chemistry. Plants in restored meadows were generally smaller than conspecific plants from ancient meadows, and functional traits indicated nitrogen limitation of plants in the restored populations.

Effect of Historical Land Use on Environmental Characteristics

Soil conditions at restored meadows profoundly differed from the soils at ancient sites. SOM was low, likely as a consequence of former tillage (McLauchlan 2007). Tillage also brought base-rich substrate from deeper soil layers to the surface, which increased soil pH. Past fertilization caused increased soil phosphorus availability in restored sites. This element typically accumulates in the soil because it strongly binds to mineral phases and organic matter. Therefore, soil phosphorus enrichment is a common agricultural legacy (McLauchlan 2007). In contrast, soil potassium contents did not differ between restored and ancient meadows, even though this element is also a common ingredient of agricultural NPK fertilizers. However, potassium is extracted in relatively large quantities by harvesting biomass and may also be leached from the topsoil (Weil & Brady 2017). Besides, we measured plant-available (= soluble and exchangeable) potassium only, but our method did not account for its fixed form: clay layers typically fix and retain excess potassium, which becomes proportionally released with sinking concentrations of soluble plant-available potassium, e.g. due to plant uptake (Amelung et al. 2019). It is, therefore, not surprising that the soils of restored meadows are typically not enriched with potassium (Smits et al. 2008; Parkhurst et al. 2021, but see Helm et al. 2019).

The macronutrient composition in community biomass largely reflected the soil conditions and revealed relative nitrogen limitation in the restored meadows. Specifically, the N:P ratio was lower in the biomass of restored meadows. The N:P ratio can inform us about nutrient limitations: According to the classification described by Palpurina et al. (2019), ratios between 10 and 15 indicate N:P colimitation, below 10 N-limitation, and above 15 P-limitation. The ancient meadows were predominantly colimited by both nitrogen and phosphorus (average N:P ratio of 14), a condition that allows the coexistence

of many plant species even under moderately productive conditions (Palpurina et al. 2019). In contrast, vegetation in the restored meadows had a surplus of phosphorus while being limited by nitrogen (average N:P ratio of 8), a condition that often results in less productive and less species-rich communities (Palpurina et al. 2019). Indeed, we found that restored meadows had decreased productivity compared to ancient ones, and the restored communities were also less species-rich (Prach et al. 2014). In ancient meadows, nitrogen is bound in organic matter and slowly mineralizes. In restored meadows, former tillage depleted SOM pools and thus, caused a loss of nitrogen from the system. Former fertilization did not elevate long-term soil nitrogen content because it is extracted in large quantities by harvest and may also be subject to leaching due to its high mobility (McLauchlan 2007).

Biomass from the restored meadows contained less calcium and sulfur, but interestingly more potassium. Since grasses retain less calcium than herbs (Thompson et al. 1997), lower calcium contents in the biomass of restored meadows are likely due to the dominance of grasses (personal observation). Sulfur retention largely depends on the accumulation of SOM (Knights et al. 2000), which was lower on restored sites. Finally, elevated potassium contents in biomass may be linked to past fertilization, as potassium is a common compound of mineral fertilizers (Smits et al. 2008). Plant roots take up 10–60% of potassium from the subsoil (Amelung et al. 2019), which might explain why we found higher potassium contents in biomass but not in soil samples, which we took from the topsoil at a depth of 10 cm only.

Effect of Historical Land Use on Plant Functional Traits

In both species, *Centaurea jacea* and *Betonica officinalis*, plant functional traits differed between ancient and restored sites. Plants at restored meadows were consistently smaller than plants at ancient meadows. This suggests reduced performance (Vellend 2005) and may be the reason why both species are less abundant in the restored meadows. As the low abundance of target species is a common problem of postagricultural grassland restoration (Fagan et al. 2008; Prach et al. 2013), it is critically important to understand which factors limit the performance of these species.

The reduced performance of the target species cannot be attributed to enhanced competition because the restored meadows were generally less productive, and the vegetation was less dense. Furthermore, the genetic diversity of these restored populations was at least as high as the one of populations growing at the ancient sites (Höfner et al. 2021). Thus, low genetic diversity and subsequent inbreeding also cannot explain their low performance. Alternative explanations are thus needed.

Plant functional traits in both species indicated low nitrogen availability at the restored sites, corresponding to the community biomass results. Moreover, leaves of both *Centaurea* and *Betonica* from restored meadows were comparatively enriched in ^{15}N . As plants preferably incorporate the lighter nitrogen isotope ^{14}N , the enrichment of ^{15}N may be a consequence of

long-term agricultural activities and input of fertilizer and concomitant losses of ^{14}N upon N turnover in the soil (Watzka et al. 2006). Other functional traits, specifically lower LNC and a higher C:N ratio in *Betonica*, further point towards nitrogen limitation in the restored meadows (Ågren 2004; Pérez-Harguindeguy et al. 2016). As nitrogen is an essential element for plant growth, such intraspecific differences in nitrogen-related traits suggest that the reduced performance of target herbs at restored meadows may be, at least partly, a sign of nutrient imbalance, primarily due to nitrogen limitation.

Betonica individuals from restored meadows suffered less from drought stress than individuals from ancient meadows, as indicated by their lower $\delta^{13}\text{C}$. Under dry conditions, plants close their stomata to minimize water loss, which reduces the discrimination against ^{13}C , leading to a relatively higher uptake of the heavier carbon isotope in their biomass (Michener & Lajtha 2008; Klaus et al. 2016; Yue et al. 2019). The higher $\delta^{13}\text{C}$ in plants from restored meadows surprised us because previous studies of postagricultural soils indicated reduced water holding capacity, partly due to the reduction of SOM (Brudvig et al. 2013; Bizzari et al. 2015). In our study system, local researchers and practitioners also expected the restored meadows to be dryer because they are less productive, and insect communities of the restored meadows have an overall more xerothermic character (Jongepierová et al. 2019). Nevertheless, the results of the ^{13}C analysis in biomass suggest the opposite. The drought stress on ancient meadows seems substantial, as the magnitude of the difference in $\delta^{13}\text{C}$ between restored and ancient meadows roughly corresponds to differences detected in controlled drought experiments (Peuke et al. 2006; Meyer-Grünefeldt et al. 2016). The reason for the reduced water stress at restored meadows can be twofold. First, since restored meadows are less productive, they lose less water through transpiration, which is the major way of evaporation in hot summers (Ma et al. 2020). Hence, there is less competition for water in restored meadows, individual plants suffer less from drought, and their tissue contains less heavy carbon ^{13}C (Ramírez et al. 2009). Second, we cannot exclude that tillage altered the soil towards a higher water holding capacity. In any case, water availability is higher at restored meadows and is thus unlikely to have caused the observed reduced plant performance.

The differences in plant functional traits between restored and ancient meadows were remarkably species-dependent. While *Betonica* populations differed in all recorded traits, *Centaurea* only in two out of seven. The difference between these species possibly lies in their root system. *Betonica* has shallow roots, which spread up to 60 cm deep in the topsoil (Kutschera & Lichtenegger 1992). The topsoil is the layer most affected by agricultural activities, and so are the traits of species that root here. In contrast, *Centaurea* has tap roots that can reach 2–3 m deep (Kutschera & Lichtenegger 1992), and can acquire water and nutrients also from deeper soil layers that are less affected by agriculture. Consequently, its functional traits less reflect the past land use. In any case, the two species belong to different families and differ in many characteristics. Therefore, their comparison cannot provide general information on which species might alter their phenotype in response to former land use and

which not. For this, it is necessary to study many more species (van Kleunen et al. 2014).

Differences Between Restored and Ancient Meadows in Herbivory

Centaurea jacea plants from restored meadows were three times more damaged by herbivores than plants from ancient meadows. For this species, herbivory thus could be the reason for reduced performance. The prevailing herbivores were larvae of *Galeruca pomonae* and *G. tanacetii*, common polyphagous beetles feeding preferably on species from the Asteraceae family (Biological Research Center 2021). The greater damage of plants from restored meadows may be connected to soil nutrients. Generalist herbivores tend to prefer *Centaurea* plants from nutrient-poor substrates (Kuglerová et al. 2019), and thus, they should prefer *Centaurea* from restored meadows. However, a more likely explanation is the dissimilar landscape structure. Ancient meadows are scattered with solitary trees and rich in edge structures, which provide shelter for predatory insects and birds (Dix et al. 1995). In contrast, restored meadows cover vast homogenous areas and lack habitat structure, providing fewer sheltering opportunities for predators that, therefore, may be less abundant here. Consequently, the herbivore community is probably less controlled and can thrive (Jončepierová et al. 2019).

Meadows restored at former arable land rarely reach the biodiversity levels of ancient reference sites. Here we show that additionally to reduced species richness, target species also show reduced performance. Soil properties and the analysis of plant functional traits revealed that plants at restored sites suffer from nutrient imbalance, and their performance is likely limited by nitrogen deficiency. Restoring the soil nutrient balance on ex-arable lands is feasible in the initial stage of restoration projects, e.g. via topsoil removal (Hölzel & Otte 2003) or phosphorus mining (Schelfhout et al. 2015), but becomes more difficult once the plant community is established. Nitrogen deficiency could be addressed by nitrogen addition (Walker et al. 2004). Yet, caution is necessary because the system is rich in phosphorus, and even modest nitrogen addition could cause overdominance of grasses and loss of rare species (Kirkham et al. 2014).

Another factor limiting plant performance of some target species may be excessive herbivory, likely because of lacking natural enemies. This problem could be addressed by increasing habitat structures at restored meadows, e.g. by planting solitary trees or hedges as habitats for predators and parasitoids, which in turn could control herbivore populations (Badenhausser et al. 2020).

In summary, it is challenging to restore species-rich meadows at postagricultural lands. Simple seed addition is essential but surely just one of the steps to establish target species populations similar to the ones at natural sites. The factors limiting the individual performance of target species can range from overdominance of certain species (Mathar et al. 2015) via nutrient imbalance to challenging biotic interactions. If the reestablishment of populations and communities similar to the original reference is the goal of restoration, a complex set of measures is

needed to support the establishment and persistence of the target species.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Descriptive statistics of environmental differences between ancient and restored sites in soil and community biomass.

Figure S2. Descriptive visualization of population differentiation between ancient and restored sites in plant functional traits of two grassland plant species.

Table S1. Summary of analysis of variance of univariate linear mixed models: results of all fitted nlme models.

Table S2. Summary of analysis of variance of univariate linear mixed models: results for all models.

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