

Habitat extremity promotes the survival of rapidly declining orchid *Neotinea ustulata* in Poland

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Abstract: Grasslands are global hotspots of biodiversity, but in many regions the abandonment of traditional management has led to secondary succession and the disappearance of specialized grassland species. An example of such a species is the burnt-tip orchid *Neotinea ustulata* (syn. *Orchis ustulata*), formerly known from ~130 sites across Poland, which has experienced a rapid population decline. However, the species and the reasons for its disappearance have not yet been the subject of comprehensive ecological research in Poland. Therefore, in the present study we sampled Polish localities of *N. ustulata* outside the mountainous areas where its presence has been reported in the 21st century and collected both population and ecological data. Besides describing the current distribution and ecological niche of the species in Poland, we aimed to identify environmental conditions at both local (vegetation and soil) and regional (landscape and climate) scales that favour the survival of the most viable populations of *N. ustulata*. Of the 24 localities confirmed in the 21st century, we found no individuals of *N. ustulata* in eight during our survey in the spring and summer of 2022, while further six populations were much smaller than in previous inventories. One population, located in an active quarry, could not be surveyed. Only nine populations of *N. ustulata* were found to be relatively stable. Compared to extinct and declining populations, stable populations of *N. ustulata* occupied unshaded xeric grasslands on shallow soils in areas with a more arid climate. Our study suggests that, due to ineffective or non-existent conservation efforts, habitat extremity appears to play a crucial role in the continued survival of this species in Poland.

Keywords: burnt-tip orchid, conservation, endangered species, grassland, *Neotinea ustulata*, *Orchidaceae*, orchid, Poland, population

Introduction

In central Europe, most grasslands are ecosystems maintained by grazing, mowing or burning and undergo secondary succession following land-use abandonment (Hejman et al. 2013, Dengler et al. 2020). Semi-natural, extensively managed grasslands are habitats for many animal species, particularly arthropods (e.g. Leśniewska et al. 2015, Markl et al. 2022), as well as sun-loving reptiles, such as snakes and lizards, birds and small mammals (Barańska & Jermaczek 2009, Dengler et al. 2020). These grasslands are also

rich in fungi (Łuszczyński et al. 2022). European dry and mesic grasslands rank among the most species-rich ecosystems in the world in terms of plant diversity at small spatial scales (Klimeš et al. 2001, Merunková et al. 2012, Wilson et al. 2012). They also serve as important reservoirs of genetic diversity, hosting regionally endemic genetic lineages of plants and animals (Kirschner et al. 2020, Klichowska et al. 2020). Because of their high biodiversity value, many grassland types are designated as Natura 2000 habitats (Barańska & Jermaczek 2009).

Despite being biodiversity hotspots (Dengler et al. 2020), European grassland ecosystems are highly threatened (Habel et al. 2013). Due to political and economic factors, up to 90% of grasslands with a high natural value were lost across many European regions during the 20th century (Waldén 2018). Since the 1950s, people from rural areas have been migrating to industrialized urban areas, leading to widespread abandonment of managed semi-natural habitats (Carboni et al. 2015). This has triggered secondary succession, which can profoundly alter plant species composition (Dzwonko & Loster 2008) and reduce the abundance of endangered species (Podgórska & Łazarski 2021). Additionally, many valuable xerothermic grasslands have been destroyed through ploughing, afforestation or the exploitation of natural resources, such as limestone (Hanczaruk & Bąba 2020). In contrast to forests, grasslands are often overlooked and undervalued due to their open structure (Lyons et al. 2023). Certain charismatic grassland species can help elevate public awareness and promote grassland-focused conservation (Wilczek & Chromy 2012). Orchids are especially important in this regard, as most European orchid species grow in grasslands (Delforge 2006) and often garner special attention from the public due to their aesthetic appeal.

Threatened plant species are often habitat specialists adapted to specific conditions and frequently exhibit an S-strategy (Grime 1973, Fujita et al. 2014). Habitat extremity can promote the persistence of such specialist species in habitats prone to secondary succession. It can interfere with anthropogenic disturbance in both negative (Kozub et al. 2019) and positive ways (Hájek et al. 2020). Although dry grasslands in the nemoral zone are predominantly non-natural communities that depend on human-induced disturbance, their development is also strongly controlled by environmental stressors, such as water and nutrient deficits (Dengler et al. 2020), and exacerbated by more extreme mezo- and microclimates and poorly developed soils. Many dry grassland species are well adapted to such stress and frequent disturbance but they are poor competitors for light (Grime 1973).

One of the orchid species that can be considered a grassland specialist is the burnt-tip orchid *Neotinea ustulata* (Tali 2004, Tali et al. 2004). Although, from a European perspective, *N. ustulata* is classified as Least Concern (LC) (Bilz et al. 2011), it is considered endangered (EN) in Poland (Bernacki et al. 2014, Kaźmierczakowa et al. 2016), critically endangered in the Czech Republic (Grulich 2012) and Lithuania (Rašomavičius 2021), highly threatened in Germany (Metzing et al. 2018), endangered in Slovakia (Eliáš et al. 2014), and “disappearing” in Ukraine (Didukh 2009) and Belarus (Kachanovsky et al. 2015). Historically, this species was known from nearly 130 localities in Poland, ~ 90 of which were located outside mountainous areas. Most of these populations no longer exist, and recent observations suggest a continued rapid decline of the species in Poland (Bernacki et al. 2014). However, the current status of extant populations of *N. ustulata* and the relationship between its ecological requirements and decline in Poland have not

been thoroughly investigated. Therefore, the main objectives of this work were (i) to verify all localities of *N. ustulata* in Poland outside mountainous areas in Poland where the species has been recorded since 2000 and describe their current status; (ii) to assess population trends at these localities; (iii) and to compare local and regional habitat conditions among stable, declining and extinct populations in order to better understand the ecological requirements of the species, and help to maintain its populations in the future.

Material and methods

The study species

Neotinea ustulata (L.) R. M. Bateman, Pridgeon et M. W. Chase (syn. *Orchis ustulata* L.) is a member of the orchid family (*Orchidaceae*). It is a relatively small (usually not exceeding 30 cm in height during flowering) bulbous perennial. The burnt-tip orchid often enters dormancy in late summer and autumn, producing a wintergreen bluish leaf rosette. The species is widely distributed across Europe (except its northern parts) with isolated populations in the Caucasus, the Urals, and western Siberia (Tali et al. 2004). In Poland, it was historically recorded throughout the country (Bernacki et al. 2014, Fig. 1) but in recent decades, its presence has only been confirmed in the Lesser Polish Upland (Podsiedlik & Bednorz 2011, Łazarski 2016, Łazarski 2019, Łazarski & Przemyski 2019; Fig. 1) and in the mountain ranges of the Carpathians and Sudetes. However, all mountain populations are currently represented by a few or single individuals (Krukowski 2002, Mirek & Piękoś-Mirkowa 2008, Frey & Tybur 2012, Kozłowska-Kozak et al. 2014, Oklejewicz et al. 2015, Binkiewicz 2016).

The species grows primarily in grasslands but also occurs in early successional forests, inhabiting dry to fresh soils, often formed on carbonate-rich bedrock (Tali et al. 2004). It is classified as a European temperate species (Preston & Hill 1997), preferring moderately warm summers and relatively cold winters (Tali et al. 2004). The species is primarily pollinated by males of various tachinid fly species (Martel et al. 2021a, b, Paulus 2022).

Some disagreement exists regarding the classification of this species, as it has been divided into two subspecies or varieties (used interchangeably in literature) – *N. ustulata* subsp. *ustulata*, which flowers in May, and *N. ustulata* subsp. *aestivalis*, which flowers in June–July and is taller than the former subspecies (Tali & Kull 2001, Haraštová-Sobotková et al. 2005). Flowering time and plant height are not the only differences; the two taxa also differ in floral morphology, the composition of low- or semi-volatile compounds, number of flowers, fruit set, pollinator guilds, and the efficiency of pollinaria removal (Martel et al. 2021a). Both subspecies occur in Poland (Bernacki et al. 2014).

Reports of contemporary locations of the species

We collected all available archival material on the distribution of the species in Poland. In the present study, we included only those populations that were revisited and confirmed between 2000 and the year preceding the fieldwork, based on literature and personal communication. Mountain ranges were excluded, as precise resampling of populations in those areas was often impossible due to a lack of accurate coordinates or locality

Table 1. Number of *Neotinea ustulata* individuals in each locality. Abbreviations: SOAI – the sum of all individuals; SOAFS – the sum of all flowering shoots; SS – single shoots; C – clusters; V – vegetative individuals; G – generative individuals

Locality	Number of individuals from older sources (data from 2000–2021)			Individuals found in the study in 2022						Trend category
	Łazarski (2016)	(Fiedor & Kucharzyk 2015, unpublished data)	Others	SOAI	SOAFS	SS	C	V	G	
<i>Neotinea ustulata</i> subsp. <i>ustulata</i>										
Berbersówka	1	5	unconfirmed (Podsiedlik & Bednorz 2011)	1	1	1	0	0	1	decline
Brzeziny	–	–	several dozen (authors obser- vations from the year 2020)	–	–	–	–	–	–	–
Garb Pińczowski	–	421	544 individuals (Ciosek & Bzdón 2000)	430	638	316	109	5	425	stable
Grabówki	71	102	–	140	168	114	26	0	140	stable
Chęciny (ul. Długosza)	–	15	–	3	5	1	2	0	3	decline
Kików	–	102	–	26	35	20	2	4	22	decline
Kowala (Trzuskawica)	–	9	single individual (Podsiedlik & Bednorz 2011)	0	0	0	0	0	0	extinct
Kowala Mała	4	–	–	0	0	0	0	0	0	extinct
Kowala School	–	–	observed (Łazarski 2019)	0	0	0	0	0	0	extinct
Lipowica	5	–	–	6	5	5	0	1	5	stable
Podpolichno	0	0	single individual (Podsiedlik & Bednorz 2011)	0	0	0	0	0	0	extinct
Rzepka	~50 shoots	28	observed (Podsiedlik & Bednorz 2011)	31	54	20	11	0	31	stable
Panieńska Górka	90	138+48	–	130	160	105	21	4	126	stable
Sosnówka	11+6	43	9 individuals (Podsiedlik & Bednorz 2011)	3	5	1	1	1	2	decline
Stawiany	–	1,638	–	1,384	1,478	1,197	122	65	1,319	stable
Szydłów	–	–	several dozen between 2012–2021 (A. Hendler pers. comm.)	50	66	35	12	3	47	stable
Zamkowa	1	21	observed (Podsiedlik & Bednorz 2011)	3	3	3	0	0	3	decline
Zegzela	72	–	–	49	63	41	7	1	48	decline
Zelejowa	0	–	single individual (Podsiedlik & Bednorz 2011), 6 individuals (seen by authors last time in 2004)	0	0	0	0	0	0	extinct
Sterczów-Ścianka	–	–	2 individuals (seen by authors in 2003)	0	0	0	0	0	0	extinct
<i>Neotinea ustulata</i> subsp. <i>aestivalis</i>										
Babia Góra	–	–	single individual (observed by authors in 2021)	0	0	0	0	0	0	extinct
Dalnia	0	–	single individual, last seen around the year 2000 (A. Hendler pers. comm.)	0	0	0	0	0	0	extinct
Grabina	0	–	single individual observed by authors in 2021	2	2	2	0	0	2	stable
Murawy Dobromierskie	–	–	18 individuals (Łazarski & Przemyski 2019)	20	21	19	1	0	20	stable

descriptions, as well as uncertainties in the flowering periods of both *N. ustulata* subspecies at varying altitudes (Table 1). We assumed that populations mentioned in older literature but not confirmed in the 21st century have been extinct for more than 20 years, given the numerous unsuccessful attempts by amateur and professional botanists to relocate them in recent decades (e.g. Łazarski 2016). The likelihood of the species reappearing at these localities is considered very low due to substantial landscape changes, including grassland disappearance caused by succession, afforestation, ploughing, limestone mining, or urbanization.

Field work

We visited the selected localities during the flowering period of both the typical and late-flowering varieties of the species (first half of May, late June, and early July of 2022). Of the preselected localities, we were unable to access one site in Brzeziny (Table 1), located in an active quarry, where our request for access permission was denied. At all other sites, we counted and mapped (using a Garmin GPS device) all individuals of *N. ustulata* in each population. We also distinguished between flowering and non-flowering individuals, recording the number of flowering shoots in the latter.

We selected sites that best represented the vegetation composition and habitat conditions of each population. For extinct populations, selection was based on available historical data (including GPS coordinates, where available) and the last known location where the species had been observed. The number of sites was determined by the size and fragmentation of the populations. For small populations, typically occupying very small areas (tens of square metres), we selected the site with the highest concentration of individuals. For larger and fragmented populations, which occupied variable habitats, we selected additional representative sites, two for populations with up to 100 individuals and three for those with more than 100 individuals. Sites were marked with a GPS receiver and a buried magnet to enable precise revisiting.

During the latter half of June and the first half of July 2022, at all 33 preselected and precisely relocated sites (using a Garmin GPS device and a magnetometer), we sampled vegetation and local environmental conditions following a slightly modified version of the standardized methodology described by Dengler et al. (2016). Within each 10 m² relevé, we estimated the total vegetation cover, as well as the cover of tree, shrub, herb, and cryptogam layers, the cover of bare ground, and the percentage cover of every vascular plant species (Supplementary Table S1). In addition, we measured or assessed various local environmental parameters: slope aspect, slope inclination, vegetation height, soil depth, maximum microrelief (defined as the highest perpendicular distance between the ground and a vertically placed metal rod), litter thickness (mean of five ruler-based measurements), and the cover of litter and dead wood. We also estimated the cover of major soil fractions – stones and rocks (diameter > 63 mm), gravel (2–63 mm), and fine soil (< 2 mm) – under the vegetation and litter layer (with total fractions summing to 100%). Soil depth and vegetation height were measured at five randomly selected points within each 10 m² relevé. For these measurements, we used a 1-metre-long metal rod with a diameter of 1 cm. Vegetation height was measured by dropping a flat plastic disc from a height of 1 metre onto the vegetation at five random locations and recording the distance from the ground to where the disc landed. Mixed soil samples were collected from five points within each relevé at a depth of 5–10 cm.

Each 10 m² plot served as the centre of a 100 m² area within which we assessed the combined cover of tree and shrub layers. Land use was recorded at each site based on visible signs of human activity (mowing, grazing) or the absence thereof (abandonment). In most managed sites, land management took place in the previous year or during winter. Only one site (Zegzela) was mown between the orchid count in early May and the plot sampling in mid-July. This could have affected only the measurements of vegetation height and its standard deviation, and is not expected to have had a significant influence on the results.

Laboratory analyses of soil samples

Soil samples were air-dried at room temperature immediately after field collection. We then measured soil pH (in water), electrical conductivity (EC, in a 1:2.5 solution), and organic matter content (as loss on ignition, 1.5 h at 550 °C). Coarse soil texture classes were determined by sieving: soil skeleton (diameter > 2 mm), very coarse sand (1–2 mm), coarse sand (0.5–1.0 mm), medium sand (0.25–0.50 mm), fine sand (0.10–0.25 mm), and very fine sand (0.05–0.10 mm). To quantify the content of fine fractions, i.e. coarse silt (0.02–0.05 mm) and fine silt and clay (< 0.02 mm), we applied the Casagrande hydrometric method, modified by Prószyński (Mocek et al. 2000). The classification of soil fractions followed the Polish Standard (1998).

Total nitrogen (TN) was determined using the dry combustion method with a Thermo Scientific Flash 2000 analyser. Available phosphorus (P-PO₄³⁻, mg/kg) was measured using the Olsen method with a SAN++ Skalar continuous flow analyser (molybdate method). Potassium (K) was quantified using a Cantraa700 flame atomic absorption spectrometer following sample digestion in nitric acid (Berghof Speedwave 4).

Data preparation

The taxonomic nomenclature of the species in the relevés was standardized according to the Euro+Med (2006+) PlantBase. The relevés were digitized using TURBOVEG (Hennekens & Schaminée 2001).

The heat-load index was calculated based on slope aspect and inclination. It was assumed that slopes with a 225° aspect receive the highest diurnal heat load (Parker 1988). Accordingly, the heat-load index attains a maximum (positive) value on steep southwest-facing slopes, a minimum (negative) value on steep northeast-facing slopes, and a value of zero on flat terrain. Using the plot coordinates, we retrieved elevation data from a digital elevation model (DEM) with a vertical resolution of 0.15 m and a horizontal resolution of 1 m (available at <https://mapy.geoportal.gov.pl>). Additionally, we extracted nine bioclimatic variables from the CHELSA database (Karger et al. 2017, 2018, Brun et al. 2022) at a spatial resolution of ~1 km: annual mean temperature, diurnal temperature range, annual temperature range, total annual precipitation, precipitation in the wettest month, precipitation in the driest month, precipitation seasonality, mean monthly climate moisture index, and vapour pressure deficit.

Potential habitat area for *N. ustulata* was considered a landscape-level variable in our study. We mapped and calculated the area of each grassland patch using up-to-date orthophoto maps available through Geoportal (<https://mapy.geoportal.gov.pl>). All GIS analyses were performed in QGIS 3.16.0 (QGIS Development Team 2023).

To complement our directly measured environmental variables with vegetation-derived data that may better reflect long-term ecological conditions, we calculated mean ecological indicator values (EIVs) based on the composition of vascular plant species. For this purpose, we used the Ecological Indicator Values for Europe (EIVE) (Dengler et al. 2023). EIVE employs uniform, continuous quasi-metric scales ranging from 0 to 10 for five ecological niche dimensions: soil moisture (M_EIVE), soil reaction (R_EIVE), soil nitrogen (N_EIVE), light (L_EIVE), and temperature (T_EIVE). Calculations were performed using community-weighted means, with species weighted by their percentage cover.

To assess the conditions necessary to maintain stable populations of *N. ustulata*, we categorized the study sites into three population trend groups based on a comparison of the most recent historical data with our current inventory (see Table 1): 0 – extinct, referring to populations where no individuals were found; 1 – declining, for populations that showed a decrease of 20% or more in the number of individuals since the previous assessment; and 2 – stable, for populations that maintained a relatively consistent size (a decrease of less than 20% or an increase in the number of individuals).

Data analysis

All analyses were performed using R (R Core Team 2023). To analyse species composition data, we used Detrended Correspondence Analysis (DCA), implemented in the *vegan* package (Oksanen et al. 2022). Prior to DCA, species cover values were square-root transformed to reduce the influence of dominant species. Scores from the first four axes were added to subsequent analyses, denoted as DCA1, DCA2, DCA3, and DCA4. To quantify the variation captured by each axis, we calculated total inertia and eigenvalues using Correspondence Analysis (CA).

In total, we considered a set of 53 habitat parameters (for an overview, see Supplementary Table S2; for the correlation matrix, see Supplementary Table S3). To identify the variables most important in distinguishing among the three *N. ustulata* population trend categories, we applied the Boruta feature selection algorithm, which uses a Random Forest approach (implemented in the R package ‘Boruta’; Kursa & Rudnicki 2010). Due to the small number of observations, we could not apply more complex models such as regression analysis.

To test for significant differences in habitat parameters between populations with different trends, we used the Kruskal–Wallis test followed by a post-hoc Dunn’s test. The choice of a non-parametric statistical test was necessary due to the unequal number of observations within population trend categories. To test the relationship between land-use abandonment and population trend, we used the Fisher’s exact test, with abandonment treated as a binary factor variable (0–1). Below, in the Results and Discussion, only the habitat parameters that were indicated as important by the Boruta algorithm (see Supplementary Fig. S1) and with significant results in the Kruskal–Wallis test (see Supplementary Table S4) are presented. Other results are presented in Supplementary Fig. S2.

Results

The species was observed between 2000 and 2021 by various authors in 24 localities, all situated in the Lesser Poland Upland (Table 1; Fig. 1). In 2022, during visits to 23 of these sites, we confirmed the presence of the species in 15 localities: two of *Neotinea ustulata* subsp. *aestivalis* and 13 of *Neotinea ustulata* subsp. *ustulata*. The species was not found in eight localities, and these populations were therefore classified as extinct (Table 1). Six populations were smaller than reported in earlier inventories. Only nine populations of *N. ustulata* exhibited a relatively stable or slightly increased number of individuals compared to previous studies. Of the existing populations, four comprised more than 100 individuals, five contained 20–100 individuals, and six harboured 1–19 individuals (Table 1). In total, across all 23 surveyed localities, we recorded 2,278 individuals of the species.

The DCA ordination revealed dissimilarities in the plant species composition between plots representing different trend categories of *N. ustulata* populations (Fig. 2). Within stable populations, the dominant species were typical for dry grasslands (*Festuco-Brometea*), e.g. *Briza media*, *Festuca stricta* subsp. *sulcata*, *Prunella grandiflora*, and *Thesium linophyllum*. In contrast, the vegetation at sites with extinct populations was dominated by competitive clonal grasses such as *Poa angustifolia* and *Brachypodium pinnatum*; species typical for fringe communities (*Trifolio-Geranietea*) such as *Geranium sanguineum* or *Melampyrum nemorosum*, or shrub and forest species including *Prunus spinosa*, *Pinus sylvestris*, and *Rubus* spp. (Fig. 2).

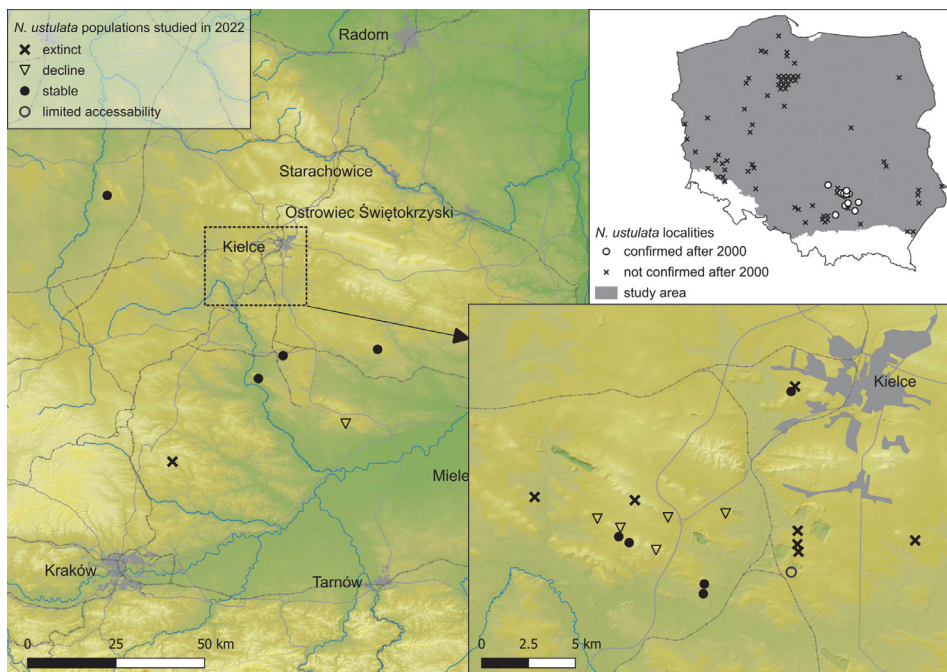


Fig. 1. Location of the study area and all studied populations of *Neotinea ustulata* with the assigned population trends.

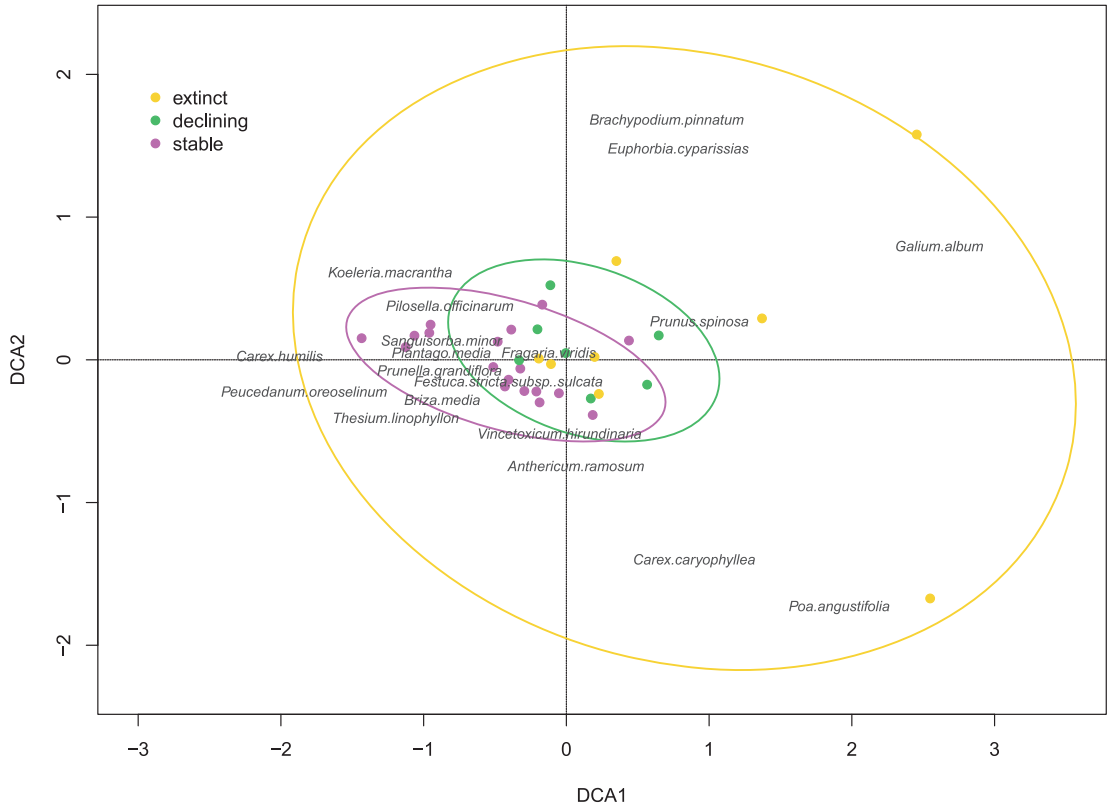


Fig. 2. Detrended Correspondence Analysis (DCA) ordination of plant species composition in plots in different trend categories of *Neotinea ustulata* populations. Species cover values were transformed with a square root. The yellow ellipse means extinct populations, the populations with a decline in the number of individuals of *N. ustulata* are within the green ellipse, and the purple ellipse shows stable populations. Dominant species names are shown in gray. The first and the second axes explain 11.0% and 8.4% of variability, respectively.

The Boruta algorithm identified 10 out of the 53 studied variables as important for distinguishing among the three population trend categories. These variables, listed in decreasing order of importance, were: mean soil depth, litter cover, soil nitrogen (N_EIVE), herb layer cover, standard deviation of soil depth, soil reaction (R_EIVE), area of surrounding grasslands, mean vegetation height, mean monthly climate moisture index, and presence of trees and shrubs in the surroundings (Supplementary Fig. S1).

All of the above-mentioned variables differed significantly among the population trend categories. Among the climatic variables, the mean monthly climate moisture index was significantly lower at sites with stable populations than at those with extinct populations. Grasslands with extinct and declining populations had smaller areas than those with stable populations (Fig. 3).

Sites with stable populations were characterized by significantly lower mean soil depth and soil depth standard deviation than the other two population trend categories (Fig. 3). Vegetation at sites with stable populations had lower herb cover and mean height compared to sites with declining and extinct populations. Stable populations occurred in

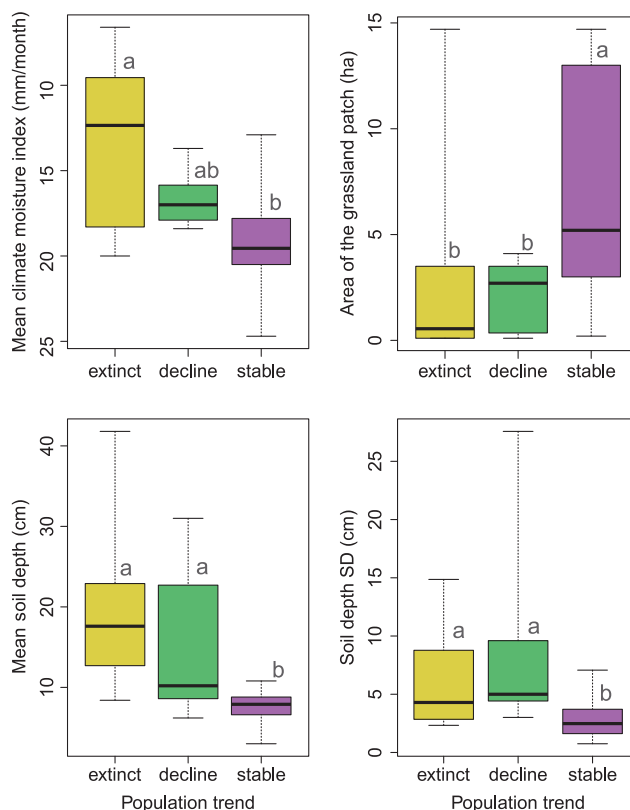


Fig. 3. Climate, landscape, and soil variables significantly differentiated among sites with different population trends of *Neotinea ustulata*: extinct (n = 8), decline (n = 7), and stable (n = 18). Compact letter display (CLD) obtained via Dunn's test ($P < 0.05$) shown in gray above boxplots.

vegetation characterized by lower mean ecological indicator values for nitrogen than those at sites with declining or extinct populations.

The mean ecological indicator value for soil reaction was significantly lower at sites with extinct populations than at sites in the other two trend categories. The percentage cover of trees and shrubs in the surroundings (in 100 m² plots), as well as litter cover, were significantly lower at sites with stable populations than at sites with declining and extinct populations (Fig. 4).

Five sites were grazed, and eight sites were mown. Land-use abandonment was observed at 44% of sites with stable populations, 86% of sites with declining populations, and 75% of sites with extinct populations. However, the Fisher's test did not confirm significant differences in the occurrence of land-use abandonment among the three trend categories ($P = 0.12$).

Among the variables not selected by the Boruta algorithm as important, 10 still differed significantly between the population trend categories (see Supplementary Fig. S2). These included precipitation in the wettest month, maximum microrelief, gravel cover, skeleton content, fine sand content, shrub cover, standard deviation of vegetation height, litter thickness, soil moisture (M_EIVE), and position along the first DCA axis. The

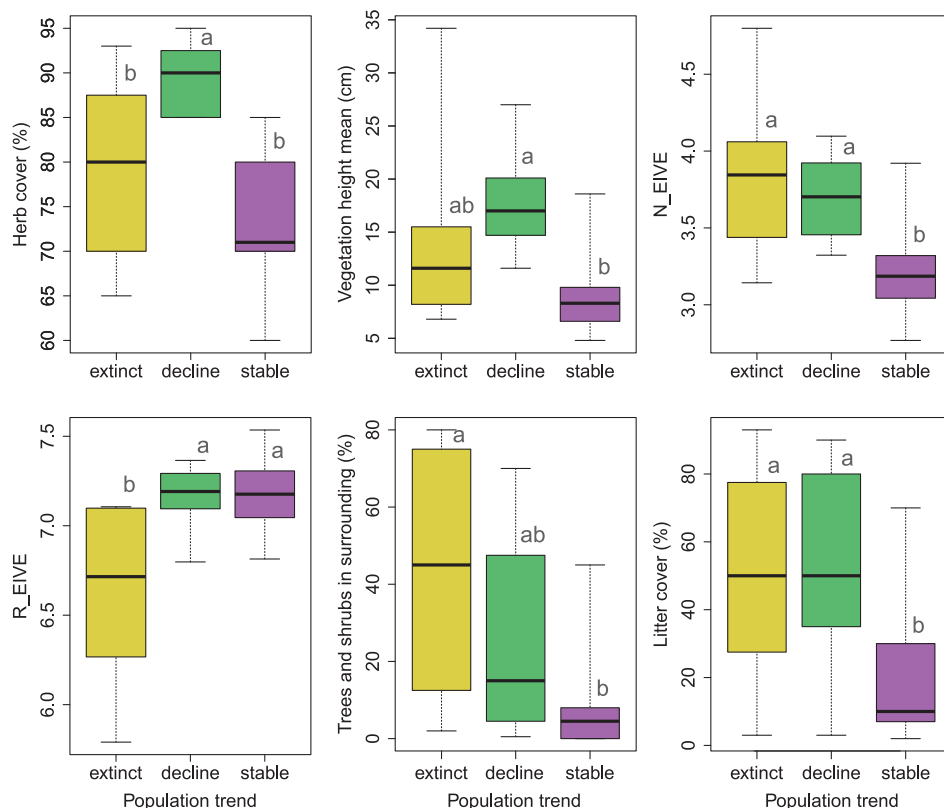


Fig. 4. Vegetation characteristics, mean ecological indicator values (EIVE: N – soil nitrogen, R – soil reaction), and variables related to abandonment and secondary succession significantly differentiated among sites with different population trends of *Neotinea ustulata*: extinct (n = 8), decline (n = 7), and stable (n = 18). Compact letter display (CLD) obtained via Dunn's test ($P < 0.05$) shown in gray above boxplots. Please note that vegetation height measures relate only to the herb layer.

remaining studied variables were neither identified as important by the Boruta algorithm nor showed significant differences between the population trend categories (Supplementary Fig. S2).

Discussion

The current state of Neotinea ustulata populations in Poland

Since our study was based on revisiting populations confirmed between 2000 and 2021, finding just over half of them in 2022 is cause for concern. However, our findings also demonstrate that *N. ustulata* can reappear after several years of absence, as observed at the Grabina locality. This raises the question of whether populations classified as extinct are truly lost. In some cases, individuals recorded as recently as the previous year were not found. It has been hypothesized that due to high grassland fragmentation and advancing forest succession, *N. ustulata* may undergo periods of dormancy, persisting underground

while awaiting improved conditions (Shefferson & Tali 2007). On the other hand, the species experiences higher mortality during dormancy than when above ground (Shefferson & Tali 2007). This suggests that *N. ustulata* may either perish due to critically low population sizes and deteriorating habitat quality (Kazlauskas et al. 2022), or survive through dormancy, albeit with high risk.

Approximately 60% of all individuals recorded in our study were located at a single site, Stawiany. Although the situation in Poland is not as critical as in Lithuania, where ~98% of all individuals are concentrated in just two populations (Kazlauskas et al. 2022), it remains highly concerning. If anything were to happen to that one big population harbouring more than half of the species' individuals in the country, *N. ustulata* could potentially vanish from Poland, as it occurs only in very low numbers in mountainous regions (e.g. Kozłowska-Kozak et al. 2014, Binkiewicz 2016).

Distinguishing population trends was somewhat problematic because a single individual can produce multiple flowering shoots. In this study, each individual was marked, and the number of shoots was counted (Table 1). However, other researchers often do not define precisely what they are counting, which may lead to an overestimation of individual numbers. In our case, most historical data were collected by the coauthors using a consistent definition of an individual. It is also important to note that grouping populations based solely on size, rather than trend, could yield very different results. For example, some abundant populations are in decline (e.g. Garb Pińczowski), while some small populations are stable or increasing (e.g. Lipowica).

There is an ongoing debate regarding the level of differentiation between the two subspecies of *N. ustulata* from phenological, morphological, genetic, and ecological perspectives (Haraštová-Sobotková et al. 2005, Tali et al. 2006, Trávníček et al. 2021). Although we did not conduct quantitative measurements, our study revealed visible differences in morphology and flowering time. *Neotinea ustulata* subsp. *ustulata* flowers in May, is smaller, has larger and wider leaves, forms a basal leaf rosette, and possesses a larger flower head (Fig. 5A). In contrast, *N. ustulata* subsp. *aestivalis* flowers in July, has smaller leaves emerging from the stalk, lacks a main leaf rosette, and exhibits a slimmer shoot and smaller flower head (Fig. 5B).

In our study, only two populations of *N. ustulata* subsp. *aestivalis* were identified, which precluded a formal test of ecological niche differentiation between the subspecies. However, in both sites where *N. ustulata* subsp. *aestivalis* occurred, the herb layer was relatively tall and typical of fringe communities of the class *Trifolio-Geranietea*. In Austria, this subspecies has also been reported, though rarely, from wet grasslands alongside *Epipactis palustris* and *Gentiana pneumonanthe* (Paulus 2022). Although the sites with *N. ustulata* subsp. *aestivalis* in our study were not as wet as those described in the above-mentioned study, our observations support the general trend that *N. ustulata* subsp. *aestivalis* tends to occur in more moist and shaded environments than *N. ustulata* subsp. *ustulata*.

What factors support populations of Neotinea ustulata?

Climate, landscape, and topography

Our results indicate that populations of *N. ustulata* located in areas with higher climatic water deficits exhibit better survival. This finding contrasts with the general distribution

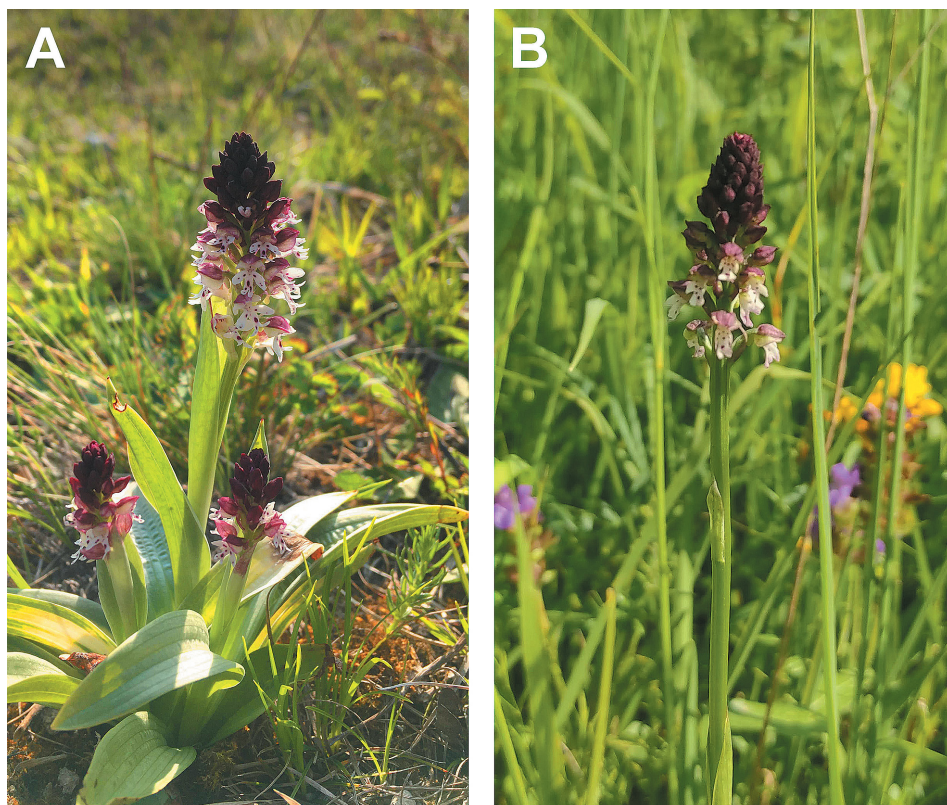


Fig. 5. General appearance of the two *Neotinea ustulata* subspecies present in the study area. (A) *N. ustulata* subsp. *ustulata*, (B) *N. ustulata* subsp. *aestivalis*. Photos by A. Adamska.

of the species, which also inhabits regions with more humid climates than our study area (e.g. England, the Alps) (Tali et al. 2004). These results may suggest that the realized niche of the species, in terms of climatic conditions, is narrower at the local scale than the fundamental niche across its broader range. A climatic water deficit may slow successional processes, such as the encroachment of trees (Erdős et al. 2018), and reduce the primary productivity of herbaceous vegetation (He et al. 2023), which can be particularly important for the persistence of *N. ustulata* in unmanaged sites common across the study area.

This finding is additionally supported by the fact that the species appears to be indifferent to the heat-load index. In most cases, it is found on south-, south-east- or south-west-facing slopes; however, the largest population we recorded, in Stawiany, is situated on north-west-, west- and north-east-facing slopes. The biology of the species also suggests that it is probably not very drought-resistant (Tali et al. 2004). Thus, its affinity with a more xeric macroclimate is likely not related to its microclimatic requirements, but rather to the overall better habitat quality in drier regions. This may imply that if competition for light is sufficiently low due to other factors (such as appropriate management or shallow soils), the species could potentially have a more widespread geographical distribution in Poland.

The affinity of stable *N. ustulata* populations with larger habitat patches likely stems from the higher habitat quality in these areas or a greater probability of suitable conditions being present. Larger patches may experience reduced edge effect (less shading from sites, drier and more wind-exposed sites, microclimate) (Laurance 1991). Some competitive herbaceous species, such as *Brachypodium pinnatum*, are rhizomatous and can quickly spread into early-successional grasslands when the species is already well established nearby, particularly in slightly moister places such as forest edges (Bąba et al. 2012).

Neotinea ustulata may also be among the first species to go extinct during contraction of habitat patches as habitat specialists are generally more vulnerable to habitat loss (Dembicz et al. 2021). Many of the small grassland patches examined in this study are merely remnants of previously larger habitats. These trends are consistent with island biogeography theory, which suggests that larger habitat patches can support more species (Lindgren & Cousins 2017, Dembicz et al. 2021).

Soil

The soil analysis showed that stable populations grow on very shallow (less than 10 cm deep) soils overlying bedrock, in contrast to extinct populations, which occupied deeper soils (Fig. 3). Shallow and rocky soils act as a stressor, reducing competition from vascular plants and slowing the encroachment of trees and shrubs (Zobel & Kont 1992).

The affinity for shallow soils could explain the survival of many *N. ustulata* populations in the Chęciny region, whose geology is unique within the Polish Uplands, otherwise dominated by Mesozoic and Cenozoic sedimentary rocks. This region contains large areas of Palaeozoic limestones and dolomites (Urban & Wróblewski 2004). The hardness of these rocks and their resistance to weathering result in frequent rock surface exposure or coverage by only very thin layers of soil, i.e. conditions similar to those of northern European alvars, which also develop on Palaeozoic carbonates (Pärtel et al. 1999) and support populations of *N. ustulata* (Tali et al. 2004).

Vegetation characteristics

The Detrended Correspondence Analysis based on the vegetation composition of all studied sites (Fig. 2) suggests that the vegetation of stable populations shares more similarities than that of extinct ones. On the one hand, sites representing stable and declining populations mostly contained plant species associated with dry grasslands (class *Festuco-Brometea*) (Mucina et al. 2016). This implies that the realized niche of the species in non-mountainous Poland is currently quite narrow and confined to typical dry grasslands. On the other hand, the vegetation of sites where the species has gone extinct represents a broader range of communities, including places with visible successional changes toward fringe and shrubby vegetation, as well as sites dominated by rhizomatous, competitive grasses such as *Brachypodium pinnatum* or *Poa angustifolia*, which are known to negatively affect grassland species richness (Barańska & Jermaczek 2009, Bąba et al. 2012). Most of these sites lack vegetation records from the period when the species was still present. Nonetheless, it is likely that their current vegetation structure has been shaped by the same processes that led to the species' extinction, rather than resembling the conditions that once supported its persistence.

Stable populations of *N. ustulata* were found in areas with lower herb layer cover and vegetation height compared to the declining populations. A lower cover and height of the herb layer may indicate a high level of environmental stress, but it can also result from regular disturbance related to land use, such as mowing or grazing. Limited competition for light is crucial for *N. ustulata*, as the rosettes of this small, light-demanding species can be shaded by more competitive, taller plants, particularly during the flowering and fruiting periods. Increasing competition from woody plants may have contributed to the lower herb height and ground cover in sites with extinct populations. Therefore, it is not surprising that we did not observe any significant differences between sites with stable and extinct populations.

We found that grasslands developed under more nutrient-poor, alkaline, and drier conditions (see Supplementary Fig. S2), with higher levels of environmental stress as indicated by EIVE, were more favourable for species survival. Interestingly, this was not confirmed by direct measurements (soil nitrogen, phosphorus, soil pH, and heat-load index), although it was an expected result. Vegetation may serve as a more reliable indicator of long-term environmental conditions than precise point measurements (Wamelink et al. 2002). In the case of key nutrients such as nitrogen and phosphorus, their bioavailability depends on soil moisture. Therefore, in water-deficient environments, laboratory results may not accurately reflect the actual availability of these nutrients to plants (Hooper & Johnson 1999, Mariotte et al. 2020).

Neotinea ustulata, like other orchids, depends on mycorrhizal associations, which supply it with nutrients, including phosphorus (Davis et al. 2022). This gives the species a competitive advantage in calcium-rich, high-pH soils, where phosphorous may be unavailable to most plants (Vitousek et al. 2010). The absence of significant differences in light (L_EIVE) and temperature (T_EIVE) values (see Supplementary Fig. S2) may result from the presence of pioneer shrubs and trees often found at sites with declining or extinct populations, as species from these groups typically have high indicator values for both light and temperature.

Land use abandonment

Structural features of vegetation related to secondary succession, i.e. the encroachment of trees and shrubs, accumulation of litter, and shading of the wintergreen rosettes, clearly negatively impact the studied populations of *N. ustulata*, as has been demonstrated for other grassland specialists (Diekmann et al. 2014). Therefore, it was surprising to observe no significant negative impact of land-use abandonment on the survival of the focal species. This finding may result from the small number of managed sites in our dataset. However, it may also suggest that extreme habitat conditions can maintain early successional characteristics even in abandoned dry grasslands, thereby allowing the species to persist for many years without human intervention.

Major threats and perspectives

The main threats to *N. ustulata* populations are habitat loss and degradation. It appears that the species in Poland is following a trajectory of decline similar to that observed for heliophilous and dry grassland orchid species in north-western and northern Europe (Kull & Hutchings 2006, Vogt-Schilb et al. 2015). Our study suggests that the niche

occupied by the species in Poland, outside mountainous areas, has been severely restricted due to habitat loss. Grasslands situated in less extreme locations with deeper soils have either disappeared or undergone changes so profound that they can no longer support populations of the burnt-tip orchid, even though the species itself would tolerate more mesic conditions if competition for light were reduced through appropriate management. Our hypothesis regarding niche contraction (see Scheele et al. 2017) is further supported by historical records from the study region, which indicate that the species once occurred in mesic meadows (Żukowski 1976) and even at the edges of alkaline fens (Lisowski et al. 1968).

Management practices that could mitigate negative changes in this species' habitat are seldom applied nowadays. In Poland, grazing animals on dry grasslands is unprofitable because the habitat provides forage of lower quality (Grygierzec et al. 2019). As a result, many dry grasslands, even those protected within nature reserves, landscape parks, and Natura 2000 sites, are undergoing secondary succession. Additionally, many of the remaining abandoned grasslands are being afforested, converted into orchards and farmland, sold for construction, or used as illegal dumpsites.

Stone quarrying also poses a serious threat to *N. ustulata* populations and their habitat, as limestone and dolomite are extracted from several active quarries in the region, some located just dozens of metres from recently lost populations. While old, abandoned quarries can serve as refugia for threatened biodiversity (Řehounková et al. 2011), modern industrial-scale quarrying – typical of the region – should be regarded as a significant threat to the species. Another widespread issue in the area is unregulated off-road vehicle use, which can reduce the number of dry grassland species by facilitating the spread of highly competitive plants such as *Arrhenatherum elatius* on bare soil exposed by tyre tracks (Żywiczka & Pławska-Olejniczak 2011). This anthropogenic impact, combined with abandonment and the dumping of organic waste, promotes the invasion of grasslands by non-native species (Czortek et al. 2023). Such trends were observed in Sosnówka, where *Echinops sphaerocephalus* was recorded, and in Panieńska Górka, where *Solidago canadensis* was found. Invasive species pose a threat not only to *N. ustulata* but also to dry grasslands more broadly, as they reduce species richness, diversity, and evenness (Hejda et al. 2009, Adamska et al. 2023).

Finally, *N. ustulata* may also be accidentally destroyed by tourists, as some populations are located near trails and recreational infrastructure. The aesthetic appeal of the species raises further concern, as individuals may be dug up and illegally sold, an issue suspected in some well-known populations. Unfortunately, there is often little that can be done to prevent such practices in Poland due to the lack of evidence required to establish liability, particularly in the absence of reliable data collected through systematic monitoring.

Few orchid species in Poland are regularly assessed as part of a national monitoring programme, which represents a crucial first step in any effective conservation plan. While scientists can conduct such monitoring, components like individual counts could be supported by citizen science, as many orchid enthusiasts visit these sites annually (Phillips et al. 2020). This is also true for *N. ustulata* in Poland, where much of the recent population data has been collected by amateur botanists. However, for a species as critically endangered as this one, systematic and standardized monitoring of all remaining populations should be established.

However, all of the above activities would not halt the loss of *N. ustulata* populations if effective conservation methods are not applied to its habitat, i.e. dry grasslands. Although most of the non-mountainous populations confirmed in Poland after 2000 were located in Natura 2000 sites, where the habitat “6210 Semi-natural dry grasslands and scrubland on calcareous substrates (*Festuco-Brometalia*)” is protected, this has not prevented the disappearance or degradation of the species and its habitat, as demonstrated in the present study. Moreover, two of the three populations located in nature reserves (Murawy Dobromierskie, Zelejowa, and Sterczów-Ściana) are now extinct. Several factors may account for this, including land ownership issues, the establishment of inappropriate conservation targets, and insufficient funding to support long-term habitat management and the institutions responsible for its implementation. These shortcomings must be addressed if the species and its habitat are to survive in Poland.

Conclusions

Our study confirmed an ongoing decline in both the extent of occurrence and population size of *Neotinea ustulata* in non-mountainous areas of Poland. Approximately one-third of the known populations have likely been lost in just over two decades. Currently, the largest population harbours around 60% of all individuals recorded in this study.

The species' occurrence has become restricted to grasslands experiencing the highest levels of environmental stress and the lowest levels of competition for light. These habitats are characterized by large patches dominated by typical dry grassland species, the absence of tall competing plants, low cover of shrubs and trees, shallow soils with abundant rock fragments, and a dry macroclimate. Unlike many other orchid species, *N. ustulata* has proven to be a true grassland specialist, undergoing rapid decline when competition for light increases. An action plan for the conservation of this species in Poland is urgently needed. As part of this plan, regular monitoring of *N. ustulata* populations should be established. To maintain viable populations, further habitat loss and degradation must be halted through effective conservation measures. Where habitat patches have been lost or severely degraded, restoration actions should be considered.

Supplementary materials

Fig. S1. Results of the Boruta feature selection algorithm.

Fig. S2. Overview of the environmental variables for different categories of population trends of *Neotinea ustulata* rejected by the Boruta feature selection algorithm.

Table S1. Phytosociological relevés.

Table S2. Overview and summary statistics of the environmental variables.

Table S3. Correlation matrix.

Table S4. Results of the Kruskal–Wallis test.

Supplementary materials are available at <https://www.preslia.cz>

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Extrémní stanoviště podporují přežívání v Polsku rychle ubývajících orchidejí *Neotinea ustulata*

Travnaté porosty představují celosvětová ohniska biodiverzity, avšak v mnoha regionech trpí v důsledku opuštění tradičního hospodaření a následné sukcese, což vede k vymizení specializovaných druhů vázaných na tyto biotopy. Příkladem takového druhu je vstavač osmahlý (*Neotinea ustulata*, syn. *Orchis ustulata*), který byl dříve znám z přibližně 130 lokalit po celém Polsku, ale jeho populace v průběhu let rychle ubývaly. Přesto však tento druh a důvody jeho rychlého vymizení dosud nebyly v Polsku předmětem komplexního ekologického výzkumu. V této studii jsme proto zkoumali, kde byl *N. ustulata* v 21. století zaznamenán mimo horské oblasti, a shromáždili jsme údaje o populacích a jejich ekologických podmínkách. Kromě popisu současného rozšíření a ekologické niky tohoto druhu v Polsku jsme se snažili rozlišit environmentální podmínky na lokální (vegetace a půda) i regionální úrovni (krajina a klima), které podporují přežití nejživotaschopnějších populací *N. ustulata*. Ze 24 lokalit potvrzených ve 21. století jsme během našeho terénního průzkumu na jaře a v létě 2022 nenašli žádné jedince *N. ustulata* na osmi z nich, zatímco dalších šest populací bylo výrazně menších než při předchozích inventarizacích. Jedna populace, nacházející se v aktivním lomu, nebyla sledována. Pouze devět populací *N. ustulata* lze označit za relativně stabilní. Ve srovnání s vyhynulými a ustupujícími populacemi rostly stabilní populace *N. ustulata* v nezastíněných suchých trávnicích na mělkých půdách v oblastech se sušším klimatem. Naše studie naznačuje, že vzhledem k neefektivní nebo zcela chybějící ochraně je to právě extrémnost stanoviště, co zajišťuje přežití tohoto cenného druhu v Polsku.

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