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RESEARCH ARTICLE

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Managing polyploid complexity in grassland restoration: Cytotype differentiation and implications for seed zones

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Abstract

1. Regional seed sources are increasingly used in ecological restoration. Polyploid complexes, i.e. taxa comprising multiple ploidy levels, are often part of regional seed mixtures, where ploidy levels are often not distinguished. However, mixing ploidy levels may foster their hybridisation, severely reduce offspring fitness and disrupt existing patterns of the geographic distribution of individual ploidy levels. Furthermore, ploidy levels may represent distinct genetic lineages. Yet, the exact geographic distribution of the ploidy levels and their genetic differentiation are rarely known.
2. Here, we focus on six polyploid complexes commonly used for the restoration of species-rich grasslands. We present high-resolution, national-scale geographic distributions of their ploidy levels, test their association with environmental gradients and quantify genetic differentiation between ploidy levels.
3. Ploidy levels within polyploid complexes were more differentiated than seed zones within individual ploidy levels. The abundance, spatial distribution and levels of sympatry versus parapatry of ploidy levels varied widely among polyploid complexes. Nevertheless, the spatial distribution of ploidy levels was always associated with environmental gradients. Mixed-ploidy populations were generally rare. *Campanula rotundifolia*, *Euphorbia cyparissias*, and *Pimpinella saxifraga* showed regional parapatry of ploidy levels, whereas *Achillea millefolium* agg., *Knautia arvensis*, and *Leucanthemum vulgare* agg. showed rather sympatric distribution patterns. Diploid *K. arvensis* was very rare and potentially non-native to Germany.
4. Using these datasets as case studies, we present a management decision framework for polyploid complexes in seed zone-based grassland restoration,

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requiring only cytotype distribution data that can be acquired at reasonable cost. The framework recommends four different per-zone management options based on data availability, cytotype presence, distribution, and frequency.

5. *Synthesis and applications*. Our decision framework enables seed producers and managers to take consistent action on the challenge of polyploid complexes in restoration. Different ploidy levels should not be mixed to avoid fitness losses. Depending on abundance and distribution, either the dominant ploidy level should be prioritised, or multiple ploidy levels need to be managed independently at the seed zone level.

KEYWORDS
conservation genomics, ecological restoration, genetic differentiation, intraspecific ploidy variation, mixed-ploidy, polyploid complex, polyploidy, RAD-seq, seed sourcing, seed transfer zones

1 | INTRODUCTION

Genetic diversity buffers populations from environmental changes (Jump et al., 2009) and can maximise the success of ecological restoration (Engelhardt et al., 2014; McKay et al., 2005). In plants, species complexes comprising different ploidy levels can contribute substantially to genetic diversity (Trávníček et al., 2012). Roughly, 12%–16% of plant species occur in multiple ploidy levels (Wood et al., 2009), and the proportion may be even higher in species commonly used for grassland restoration (Kramer et al., 2018). Grasslands cover 40% of the global land surface (Bardgett et al., 2021), yet, 49% show signs of degradation (Gang et al., 2014). Thus, knowing the geographic distribution and genetic differentiation of ploidy levels is particularly relevant for grassland management.

Polyploid species complexes complicate management and restoration because their ploidy levels ('cytotypes' hereafter) can diverge in their geographic distribution and ecological niches (Decanter et al., 2020). Cytotypes can either spatially coexist (sympatry) or be spatially segregated in either adjacent (parapatry) or disconnected ranges (allopatry). Sympatry and parapatry can be scale-dependent; for example, regionally co-occurring cytotypes can be segregated locally. When cytotypes exhibit distinct geographic distributions along environmental gradients, this suggests ecological differentiation. Furthermore, there is often a reproductive barrier immediately following whole-genome duplication as interploidy crosses lead to offspring with reduced viability (e.g., triploid block) and fertility (Ramsey & Schemske, 1998), which can be regarded as a form of outbreeding depression. Over time, cytotypes can diverge genetically, representing distinct genetic lineages (Kolář et al., 2017). Morphologically differentiated cytotypes are often recognised as different species, whereas undifferentiated cytotypes often remain taxonomically unresolved (Kolář et al., 2017). Nevertheless, cytotypes are separate units with unique conservational values (Trávníček et al., 2012). Yet, the occurrence, geographic distribution, and genetic differentiation of cytotypes are rarely known (Liu et al., 2022; Wallace et al., 2017), nor is it understood to what extent environmental gradients shape the geographic distributions.

This knowledge gap becomes particularly problematic in restoration because there is a risk of fitness losses and distortion of natural distribution patterns when cytotypes are not accounted for in seed provenancing. Fitness losses can be severe when populations of different cytotypes are inadvertently mixed during propagation and subsequent restoration (Burton & Husband, 2000; Wallace et al., 2017). This risk is prevalent throughout the seed transfer process in restoration, where cytotypes can come into contact at multiple stages (Delaney & Baack, 2011; Kramer et al., 2015). Despite occasional naturally occurring cytotype coexistence (Kolář et al., 2017, and references therein) and the principal possibility of among-cytotype hybridisation (Abbott et al., 1992; Suarez-Gonzalez et al., 2018), the mixing of cytotypes with unknown reproductive compatibilities, ecological niches, and geographic distributions is therefore generally discouraged in restoration (Kramer et al., 2018; McKay et al., 2005; Wallace et al., 2017).

Seed transfer zones ('seed zones' hereafter) and regional seeds are increasingly common approaches in a toolbox of seed provenancing strategies for restoration (De Vitis et al., 2017), including local and climate-adjusted provenancing (Bucharova et al., 2019). Seed zones are defined as 'geographical regions within which individuals […] of native species can be transferred with no detrimental effects on population mean fitness' (Hufford & Mazer, 2003, p. 147). Another objective of seed zones is to maintain spatial genetic variation (Van der Mijnsbrugge et al., 2010), and thereby, regional adaptation (Knapp & Rice, 1994; Miller et al., 2011). Regional seeds are sourced within a seed zone and then agriculturally propagated before being used in restoration projects within the same seed zone (Kiehl et al., 2014). Generalised seed zones that are applicable across species are often based on environmental patterns (e.g., Bower et al., 2014; Cevallos et al., 2020; Rivière et al., 2022) and exist in multiple European countries (e.g., NO, CH, CZ, FR, GB, DE, AU; De Vitis et al., 2017). Although generalised seed zones rarely reflect the exact patterns of genetic

variation (Durka et al., 2025; Kramer et al., 2015), genetic differentiation in plants often displays isolation-by-distance and isolation-by-environment (Michalski & Durka, 2012). Therefore, seeds from within a seed zone are typically more genetically similar to local natural populations than seeds from different seed zones, making them reasonable conservation units that balance genetic diversity and adaptation (Bucharova et al., 2019).

Due to the lack of cytotype data and limited guidelines for managing polyploid complexes, ploidy variation is typically not considered in seed zone-based restoration. Production lines are preferably collected from multiple source populations per seed zone before cultivation in dedicated agricultural fields (Bucharova et al., 2019). However, when source populations harbour different cytotypes, mixed-ploidy populations in production fields can yield reduced seed sets. Furthermore, hybridisation among different polyploid cytotypes may be possible due to lower reproductive barriers (Brown et al., 2024; Šemberová et al., 2023). Introgression of the resulting new cytotypes could then further distort natural patterns of genetic differentiation, which is in conflict with the aim of seed zones. Subsequent application of such seeds can undermine restoration success through fitness reductions, while also distorting natural distribution patterns of cytotypes.

In this study, we assess cytotype distributions and develop management recommendations for six polyploid species complexes, using Germany's regional seed zone system for grassland species as an example (Bucharova et al., 2019). Among the species for which certified, regional seeds are commercially available, at least 27% harbour multiple cytotypes (Durka et al., 2025). For most of these species, little is known about cytotype abundance, geographical distribution, and their genetic differentiation. Although generic guidelines for sourcing, propagation, and application of regional seeds are available (Kiehl et al., 2014), specific guidelines for polyploid complexes are lacking. Using samples from six polyploid complexes widely used in grassland restoration, collected across Germany, we determined ploidy levels using flow cytometry and assessed genetic differentiation by SNP genotyping. Specifically, we addressed the following hypotheses:

1. Genetic variation between cytotypes is higher than genetic variation between seed zones within cytotypes.
2. Environmental gradients shape the geographical distribution of cytotypes.

Based on our findings, we present a decision framework to manage ploidy variation in a generalised regional seed zone system.

2 | MATERIALS AND METHODS

2.1 | Seed zones, study taxa and sampling

The German seed zone system encompasses 22 generalised seed zones applicable across all common grassland species (Figure 1; Bucharova et al., 2019; ErMiV, 2011). Zones are largely based on established ecoregions (Meynen & Schmithüsen, 1953–1962). We used six perennial herbs that are polyploid complexes as study taxa (Table 1). They are widespread in Europe and common in Germany, yet their cytotype frequencies and distributions within and across seed zones are largely unknown: *Achillea millefolium* agg. (Asteraceae, here encompassing *A. millefolium* L., and both the closely related *A. collina* (Becker ex Wirtg.) Heimerl and *A. pratensis* Saukel & R. Länger (Guo et al., 2005)), *Campanula rotundifolia* L. s.str. (Campanulaceae), *Euphorbia cyparissias* L. (Euphorbiaceae), *Knautia arvensis* (L.) Coult. s.str. (Caprifoliaceae), *Leucanthemum vulgare* agg. (Asteraceae, here encompassing *L. vulgare* Lam. s.str., *L. ircutianum* DC.), and *Pimpinella saxifraga* L. (Apiaceae).

We used the datasets of these polyploid complexes from the RegioDiv project (for methodological details, see Durka et al., 2025). In short, voluntary collectors took leaf samples from sites all across Germany from 2020 to 2024. The sites were chosen according to fixed criteria to ensure they were as native as possible. In order to assess local, site-level sympatry, we added more individuals per sampling site (resulting in 1–3 individuals per site, averaging 1.5, cf. Table 2) to those reported by Durka et al. (2025).

2.2 | Ploidy assessment by flow cytometry

We determined the ploidy level of varying proportions of individuals per study taxon using flow cytometry on silica gel-dried leaf material (Table 2), except for *E. cyparissias* samples, for which the measurements were taken from Pungaršek and Frajman (2024). For some study taxa, reference chromosome counts for each ploidy level are available (Kolář et al., 2009; Oberprieler et al., 2011, 2022; Rauchová, 2007; Vidic et al., 2009). We used a general two-step protocol with Otto buffers with slight modifications (Table S1 for details). However, not all samples could be analysed by flow cytometry. According to preliminary analysis, all ploidy levels were genetically differentiated; therefore, we predicted ploidy levels for all samples using both flow cytometry results and genetic analyses in linear discriminant analysis (see below, Figure S1).

On the seed zone level, we qualitatively assessed the spatial distribution of cytotypes (sympatry, parapatry, or allopatry). We refer to parapatry in a strictly geographical sense, defined by adjacent (though sometimes partially overlapping) ranges of cytotypes, without assumptions about gene flow or phylogeography (cf. Bull, 1991). We defined cytotypes as locally sympatric if more than one cytotype was found per sampling site, considering only sites with more than one sample analysed (Table 2). At the seed zone level, we defined <25%, >25%, and >75% as ad hoc thresholds to delimit rare, major, and predominant cytotypes, respectively, using only one individual per site to avoid sampling bias.

2.3 | Genotyping

We used the ddRAD sequence data from Durka et al. (2025) and included additional samples (Table 2). We applied the same

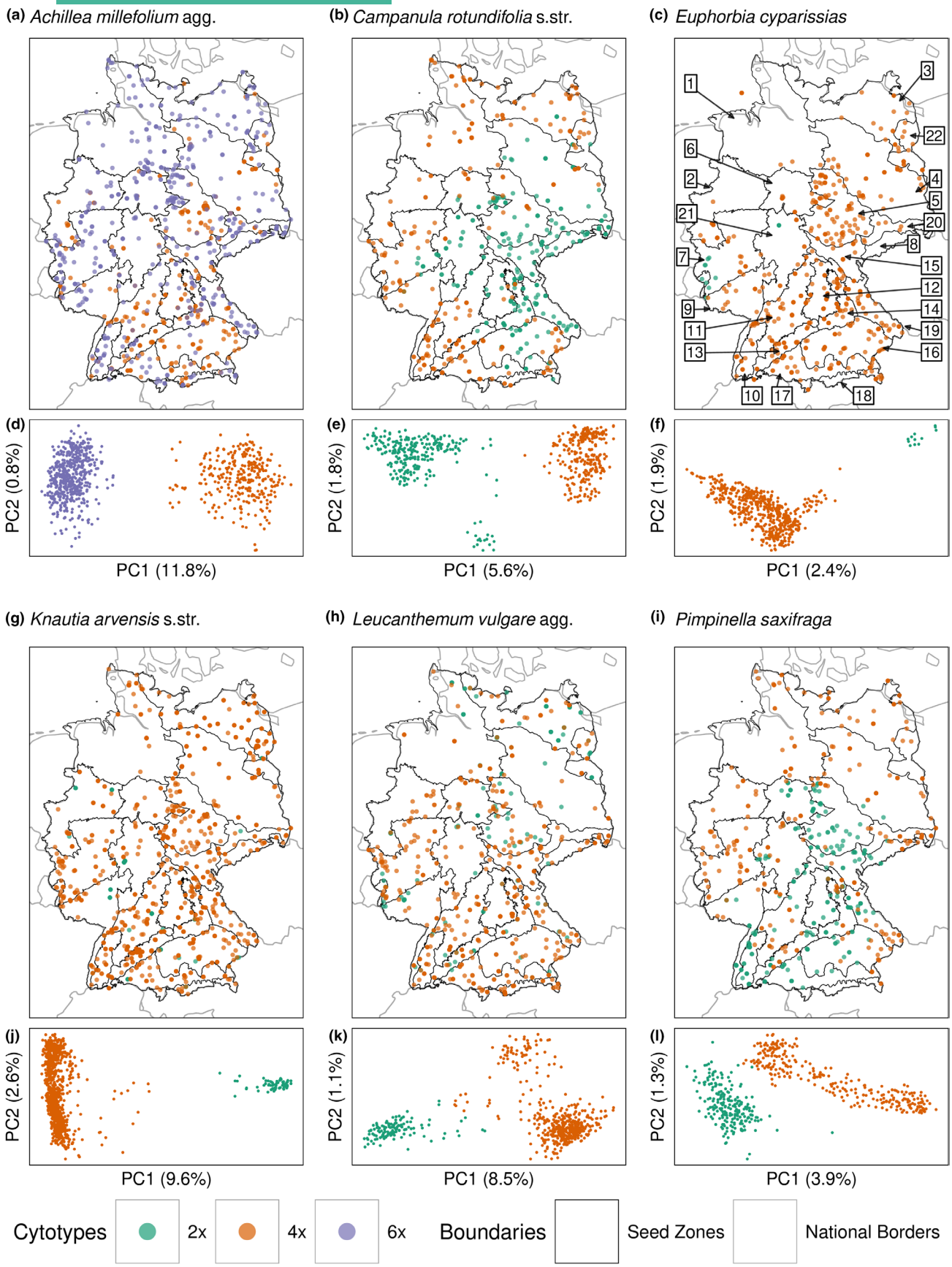

FIGURE 1 Geographical distribution (a–c, g–i) and genetic relationships among samples and their cytotypes (d–f, j–l) found in the six studied polyploid complexes. Panel (c) identifies the 22 seed zones in Germany (Bucharova et al., 2019). The percentages on the PCA axes (d–f, j–l) are the proportions of explained variance.

TABLE 1 The polyploid complexes and cytotypes in this study with their distribution in Europe and Germany.

Polyploid complex	Family	Cytotypes in this study	European distribution	Distribution in Germany	Key references
Achillea millefolium agg.	Asteraceae	$2n=4x=36$ (*A. collina* and *A. pratensis*) $2n=6x=54$ (*A. millefolium* s.str.)	Widespread in mesic grasslands	Common in mesic grasslands	Ehrendorfer (1953) Ehrendorfer and Guo (2006) Guo et al. (2005)
Campanula rotundifolia s.str.	Campanulaceae	$2n=2x=34$ $2n=4x=68$	Both cytotypes present	2*x*, 4*x*	Hegi (2008) Paule et al. (2017) Rauchová (2007) Šemberová et al. (2023) Wilson et al. (2020)
Euphorbia cyparissias	Euphorbiaceae	$2n=2x=20$ $2n=4x=40$	2*x*: S & W Europe 4*x*: Central & N Europe	Both cytotypes present	Pungaršek and Frajman (2024)
Knautia arvensis s.str.	Dipsacaceae	$2n=2x=20$ (ssp. *pannonica*) $2n=4x=40$ (ssp. *arvensis*)	2*x*: SE Europe 4*x*: Central and NW Europe	Both cytotypes present	Ehrendorfer (1962) Kolář et al. (2009) Rešetnik et al. (2014)
Leucanthemum vulgare agg.	Asteraceae	$2n=2x=18$ (*L. vulgare*) $2n=4x=36$ (*L. ircutianum*)	Both species widespread	Both species common and widespread *L. vulgare*: dry calcareous grasslands *L. ircutianum*: mesic, nutrient-rich habitats	Heywood (1976) Marchi (1982) Oberprieler et al. (2011) Oberprieler et al. (2022) Vogt (2024)
Pimpinella saxifraga	Apiaceae	$2n=2x=18, 20$ $2n=4x=40$	Most of Europe (except parts of Mediterranean)	Common	Jawarneh (2021) Mozolová (2007)

TABLE 2 Overview of descriptive statistics for the study taxa.

Taxon	# Samples	# FCM	# Sites	# SNPs	% Missing genotypes	Ploidy levels	Local sympatry	$\Phi_{ST,ploidy}$	$\rho_{ST,SZ,ploidy1}$	$\rho_{ST,SZ,ploidy2}$
Achillea millefolium agg., *A. millefolium* s.str., *A. collina/pratensis*	798	43	605	4083	12.7	4*x*, 6*x*	15 of 134 (11.2%)	0.0969	0.0445	0.0267
Campanula rotundifolia s.str.	542	432	363	3957	9.2	2*x*, 4*x*	2 of 110 (1.8%)	0.0526	0.0634	0.0662
Euphorbia cyparissias	533	34	373	5521	6.7	2*x*, 4*x*	0 of 93 (0%)	0.1201	–	0.058
Knautia arvensis s.str.	968	269	540	5522	6.1	2*x*, 4*x*	6 of 237 (2.5%)	0.2312	–	0.0539
Leucanthemum vulgare agg., *L. vulgare*, *L. ircutianum*	578	346	396	2415	12.2	2*x*, 4*x*	12 of 112 (10.7%)	0.0857	0.0466	0.0381
Pimpinella saxifraga	488	281	331	2873	9.5	2*x*, 4*x*	4 of 99 (4%)	0.0245	0.0473	0.0858

Note: # Samples: Number of individuals in the datasets after filtering. # FCM: Number of individuals for which the ploidy level has been measured using flow cytometry. # Sites: The number of sites that the samples are from. # SNPs: Number of SNP loci retained after filtering. % Missing genotypes: Percentage missing genotypes. Ploidy levels: Ploidy levels considered in this study. Local sympatry: Number of mixed-ploidy sites and proportion of sites for which more than one sample is available. $\Phi_{ST,ploidy}$: Genetic differentiation as Φ between ploidy levels, calculated with a simple AMOVA. $\rho_{ST,SZ,ploidy1}$, $\rho_{ST,SZ,ploidy2}$: Genetic differentiation measured as ρ among seed zones within the first and second ploidy level in the 'Ploidy levels' column, respectively, calculated with one simple AMOVA per cytotype subset with seed zone grouping.

genotyping and filtering procedure (e.g., biallelic SNPs, minor allele frequency 0.05 and thinning to one SNP per contig) to the resulting data set. Reference samples of closely related species were used to exclude putative misidentified samples (see appendix A.1.6.2 in Durka et al., 2025 for the principle). For subsequent analyses, we used read count data for reference and alternative alleles from the vcf files (Figure S2), filtering out sample × locus combinations with less than eight reads and subsequently whole loci with a call rate <40%. All following analyses were performed in R 4.3.1 (R Core Team, 2023) except where explicitly noted.

Cytotype-aware genotyping was then performed using the 'updog' package (Gerard et al., 2018), an implementation of empirical Bayesian approaches, with default parameters. We first predicted the cytotype of the individuals lacking flow cytometry measurements (64% of all individuals, ranging from 20% in *C. rotundifolia* s.str. to 95% in *A. millefolium* agg., Table 2) using linear discriminant analysis (Venables & Ripley, 2002). As predictors, we used axes from a principal component analysis (PCA, NIPALS algorithm; Stacklies et al., 2007) summarising individual SNP read count frequencies, with flow cytometry results serving as training data. Model accuracies were evaluated across successive sets of principal components, starting with the first axis alone and extending to the first 20 axes. The most accurate model was then selected, which used between one (*A. millefolium* agg.) and 11 (*L. vulgare* agg.) axes. The cytotype predictions together with read count frequencies then served as an input to 'updog', which calculated cytotype-aware genotypes, that is, diploid, tetraploid, or hexaploid genotypes, respectively. These genotypes were used for downstream analyses.

2.4 | Data analysis

We visualised genetic relationships of individuals by performing PCA on allelic frequency data based on the cytotype-aware genotypes. For each study taxon, we analysed overall differentiation between cytotypes by a simple analysis of molecular variance (AMOVA), and cytotype-specific differentiation among seed zones by two further AMOVAs. To test the first hypothesis, an additional, hierarchical AMOVA was performed with seed zones nested in cytotypes, resulting in a total of four AMOVAs per taxon. We followed Meirmans et al. (2018) and Meirmans and Liu (2018), who demonstrated that differentiation between cytotypes is best quantified by classical Φ-statistics (referred to as *F*-statistics by Meirmans), whereas differentiation among populations within cytotypes is best captured by ρ (Ronfort et al., 1998), and report them accordingly. Note that ρ values are not directly comparable with Φ values, though they remain comparable among themselves (Meirmans et al., 2018). While ρ-statistics are based on a matrix of squared Euclidean distances between individuals, Φ-statistics (which are analogues to Wright's *F*) also incorporate within-individual variance (Excoffier et al., 1992). For the latter, we first imputed missing genotypes based on seed zone-specific allele frequencies. For each locus and seed zone nested in cytotype, allele frequencies were estimated from observed

data with a Beta(0.5, 0.5) prior. If an entire seed zone had only missing data at a locus, frequencies were estimated from individuals of the same cytotype across all seed zones; otherwise from the global sample. Missing genotypes for each individual were then imputed by rounding the expected dosage, while respecting individual ploidy levels. Genotypes were then split into cytotype-specific numbers of pseudo-haplotypes using the 'make_haplotypes' function of 'poppr' (Kamvar et al., 2014), with which we conducted AMOVAs using a distance matrix between all pairs of haplotypes. All calculations were performed using the AMOVA implementation of the ade4 package (Dray & Dufour, 2007) with 499 permutations to obtain significance values.

We tested the second hypothesis using one generalised linear model per taxon. Two initial PCAs were performed, one for climate and one for soil. For each PCA, site-specific data for all sites studied in Durka et al. (2025) were extracted from WorldClim2 (Fick & Hijmans, 2017) and SoilGrids 2.0 (Poggio et al., 2021), respectively. In both analyses, the first three components had eigenvalues >1 and were thus retained, covering 86% and 84% of total variation, respectively. For the generalised linear models, we used a binary cytotype variable as the response, and the six retained components as predictors. We assessed significance for each model by an analysis of variance comparing the main effects model against a null model.

To visualise the differentiation between cytotypes alongside their distribution across the seed zones, we constructed phylogenetic networks. We calculated Nei's distances from the cytotype-aware genotypes using the StAMPP package (Pembleton et al., 2013), exported the matrices in NEXUS format to SplitsTree 6 (Huson & Bryant, 2024), where we constructed the network using the distance-based neighbour-net method (Bryant & Moulton, 2004).

2.5 | Recommendations for the seed zone-based management of polyploid complexes

We developed a decision framework for the seed zone-based management of polyploid complexes to avoid (1) fitness losses in the production of regional seeds as well as in the restored population and (2) distortion of existing patterns of geographic cytotype distribution. The framework does not apply when there is evidence that cytotypes have similar distributions and do interbreed frequently with no fitness losses. We developed the framework assuming that cytotype distribution data alone enable decisions that promote the goals of seed zones, that is, preserving natural distribution patterns and promoting favourable restoration outcomes. The framework gives management recommendations based on both expert knowledge and empirical criteria. The first criterion is the sufficiency of the available cytotype distribution data to apply the subsequent criteria. We suggest a target sampling site density of one site per 1000 km^2 as a benchmark for data sufficiency, though expert knowledge may establish sufficiency at lower densities (see Discussion). Beyond data sufficiency, the framework's empirical criteria are presence, spatial segregation, and frequency of cytotypes. For cytotype frequency, we used ad hoc thresholds to delimit rare, major, and predominant cytotypes, respectively (see above). We applied the framework to all six study taxa across the 22 German seed zones.

3 | RESULTS

Our final datasets contained between 488 and 968 (average: 651) samples and between 2415 and 5522 (average: 4062) SNPs (Table 2) per study taxon. All six taxa comprised two ploidy levels: diploids and tetraploids, except for *A. millefolium* agg., which consisted of tetraploids and hexaploids (Höfner, Kolář, et al., 2025). In all datasets, the cytotypes represented distinct genetic clusters in the principal component analysis (Figure 1) and neighbour-net analyses (Figure S3). Genetic differentiation between cytotypes ranged from $\Phi_{ST}=0.025$ in *P. saxifraga* to $\Phi_{ST}=0.231$ in *K. arvensis* (Table 2).

In all six taxa, except *P. saxifraga*, hierarchical AMOVA showed that genetic differentiation among cytotypes was substantially higher than among seed zones within cytotypes (Table 3; Table S2), corroborating the first hypothesis. Both levels of differentiation were significant across all six taxa. The differentiation among seed zones within cytotypes ranged between $\rho_{ST}=0.0267$ and $\rho_{ST}=0.0858$ across the study taxa (Table 2). We discarded the ρ_{ST} values for diploids in *E. cyparissias* and *K. arvensis* because of the low number of individuals (10 and 28, respectively). There was no consistent pattern across the six taxa, with the lower ploidy level either less (*P. saxifraga*), equally (*C. rotundifolia*), or more differentiated (*A. millefolium* agg., *L. vulgare* agg.) among seed zones.

Generalised linear models testing for the effects of climate and soil as predictors for cytotype identity were significant across all six taxa (Table 4), corroborating our hypothesis that the geographic distribution of cytotypes is driven by environmental gradients. The study taxa differed in how many individual predictors were significant (Table S3). Temperature seasonality and soil texture were significant more often than other climate and soil components.

In each study taxon, the two cytotypes occurred in varying abundances and geographical distributions. In *C. rotundifolia* and *P. saxifraga*, both cytotypes were roughly equally abundant (49% diploids and 51% tetraploids in *C. rotundifolia*, 45% diploids and 55% tetraploids in *P. saxifraga*) and occurred in relative parapatry (Figures 1 and 2). Parapatry was more pronounced in *C. rotundifolia*, with diploids restricted to Central and Eastern Germany and tetraploids dominating in the rest of the country. In *P. saxifraga*, diploid individuals dominated in Central and Southern Germany, while tetraploids were more frequent in the rest of Germany. In *L. vulgare* agg. and *A. millefolium* agg., the cytotypes occurred in regional sympatry. However, there were distinct geographic prevalences: Diploid *L. vulgare* s.str. (21%) was more frequent in parts of Northern Germany, while tetraploid *L. ircutianum* (79%) was predominant in parts of Southern Germany. Similarly, hexaploid *A. millefolium* s.str. was more common overall (64%) but appeared to be rather rare in Southern and Eastern Germany, where tetraploid *A. collina/pratensis* (36%) was dominant. Lastly, the data sets of *E. cyparissias* and *K. arvensis*

TABLE 3 Hierarchical AMOVA of seed zones (SZ) nested in ploidy, calculated using the R-package poppr (Kamvar et al., 2014) with the ade4 method (Dray & Dufour, 2007).

Taxon	Level	df	σ^2	Var. (%)	Φ	p
Achillea millefolium agg.	Betw. ploidy	1	37.8	9.6	0.096	0.002
	Betw. SZ within ploidy	41	4.8	1.2	0.013	0.002
Campanula rotundifolia s. str.	Betw. ploidy	1	24.5	5	0.050	0.002
	Betw. SZ within ploidy	36	14.6	3	0.032	0.002
Euphorbia cyparissias	Betw. ploidy	1	75.7	11.7	0.117	0.002
	Betw. SZ within ploidy	22	13.3	2.1	0.023	0.002
Knautia arvensis s.str.	Betw. ploidy	1	157	23	0.230	0.002
	Betw. SZ within ploidy	31	11.3	1.7	0.022	0.002
Leucanthemum vulgare agg.	Betw. ploidy	1	24.6	8.5	0.085	0.002
	Betw. SZ within ploidy	39	4.1	1.4	0.016	0.002
Pimpinella saxifraga	Betw. ploidy	1	7.4	2.2	0.022	0.002
	Betw. SZ within ploidy	37	10.5	3.2	0.032	0.002

Note: df: Degrees of freedom. σ^2: Variance component. Var. (%): Percentage explained variation. Φ: Measure of genetic differentiation, analogue to Wright's *F* (Excoffier et al., 1992). *p*: Significance value obtained from permutation test with 499 iterations. Only the levels 'Betw. ploidy' and 'Betw. SZ within ploidy' are shown, see Table S2 for all hierarchical levels.

TABLE 4 Generalised linear models testing the relationship between environmental gradients and cytotype distribution.

Taxon	df	χ^2	p	Nagelkerke's R^2
Achillea millefolium agg.	6	277.0	<0.001	0.405
Campanula rotundifolia s.str.	6	142.9	<0.001	0.313
Euphorbia cyparissias	6	37.2	<0.001	0.349
Knautia arvensis s.str.	6	44.0	<0.001	0.119
Leucanthemum vulgare agg.	6	47.8	<0.001	0.125
Pimpinella saxifraga	6	108.3	<0.001	0.269

Note: For each taxon, there are the same six predictors, that is, scores of three principal components for both climate and soil. df: Degrees of freedom. χ^2: This statistic represents the difference in deviance between the fitted model and a null model, indicating how much the predictors improve the model's fit. *p*: The likelihood of observing a chi-squared (χ^2) statistic as extreme or more extreme if the null hypothesis (Environmental gradients do not shape cytotype distribution) was true. Nagelkerke's R^2 is a pseudo R^2 value often used in logistic regression and generalised linear models to estimate the proportion of variance explained by the model.

both consisted mostly of tetraploids (98% and 94%, respectively). Diploids of *E. cyparissias* showed a parapatric distribution, restricted to the central west of Germany and one additional site further east. Diploids of *K. arvensis* occurred in isolated locations, mostly along the rivers Rhine and Danube in Southern Germany.

Geographical distributions of cytotypes were partly parapatric, yet, seed zone boundaries did not reflect the contact zones between cytotypes on a larger scale in any of the six taxa. This results in many seed zones containing more than one cytotype (Table 5; Figure 2). The seed zones capture only some of the differentiation among and within cytotypes, which can also be seen in the fragmentation of the seed zone branches in the corresponding phylogenetic networks (Figure S3).

Local sympatry, that is, the co-occurrence of more than one cytotype at the same site, was found in all six taxa except *E. cyparissias* (Figure S4). However, the frequency of local sympatry was low, reaching 11% in *A. millefolium* agg. and *L. vulgare* agg., while being smaller than 5% in the remaining three taxa.

4 | DISCUSSION

For six polyploid complexes commonly used in grassland restoration, we provide the most comprehensive spatial datasets of ploidy variation at a nationwide scale to date. Together with genetic differentiation data, these datasets allow us to evaluate how ploidy

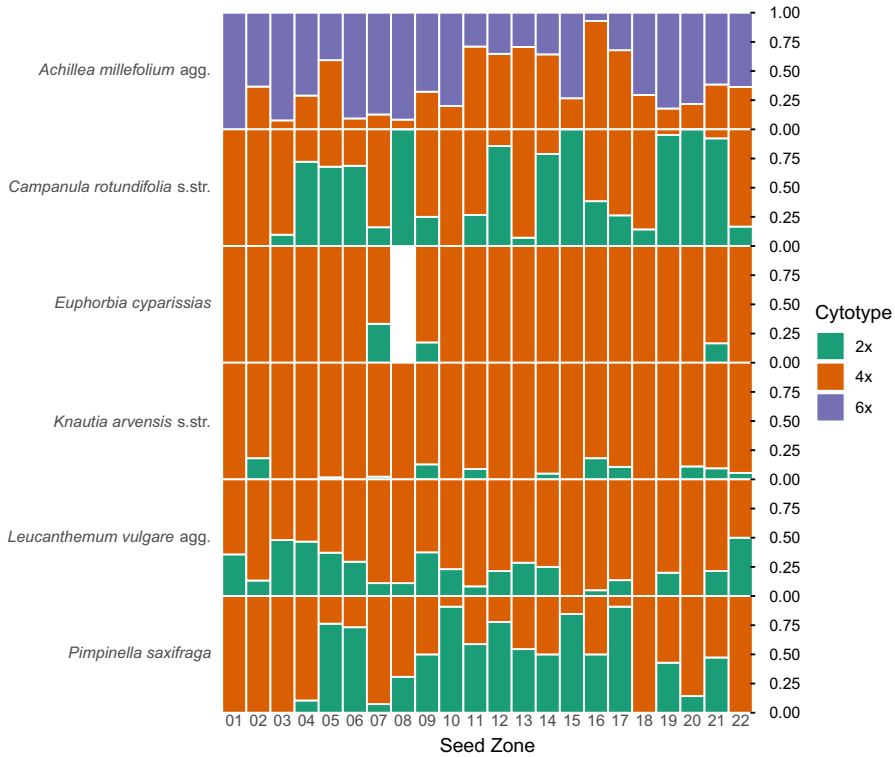

FIGURE 2 Ratios of observed cytotypes per taxon and seed zone.

differentiation relates to existing restoration practice. We show that genetic differentiation is higher between cytotypes than genetic differentiation between seed zones within cytotypes, which is in line with previous findings (Kaulfuß & Reisch, 2019; Kolář et al., 2012). Moreover, environmental gradients are significantly associated with cytotype distributions, highlighting the ecological differentiation between ploidy levels. Using our results, we developed a decision framework which can be broadly applied for addressing ploidy variation in seed zone-based restoration projects.

4.1 | Cytotype differentiation

Cytotypes within polyploid complexes showed significant genetic differentiation across all six study taxa, which corresponds to previous results (Oberprieler et al., 2011; Sutherland & Galloway, 2021). The strongest differentiation between cytotypes was observed in *K. arvensis* ($\Phi_{ST}=0.231$, cf. Kaulfuß & Reisch, 2019), while the weakest was found in *P. saxifraga* ($\Phi_{ST}=0.025$). Although absolute levels of genetic differentiation obtained by our approach may appear to be low, it is important to acknowledge that values of genetic differentiation vary with the marker type (Ai et al., 2014). They are lower with SNPs than with markers that are selected for high variation, such as microsatellites (Li et al., 2019) or AFLPs (Durka et al., 2017).

Significant differentiation between cytotypes and the potential for significant fitness losses due to interploidy hybridisation warrant the segregation of cytotypes in restoration, particularly when their abundance, distribution, and compatibility are unknown (Gibson et al., 2017; Richardson et al., 2023). This is especially true when the genetic differentiation between cytotypes is higher than between seed zones within cytotypes, as was the case with all study taxa except *P. saxifraga*. The differentiation of seed zones within cytotypes was significant and differed between cytotypes. These idiosyncratic differences may be due to the fact that the cytotypes were not distributed equally across seed zones or due to actual differences in gene flow-drift dynamics. Taken together, these observations suggest a hierarchical and asymmetric genetic structure relevant for seed zone-based restoration.

We acknowledge that there are cases where hybridisation between cytotypes and thereby gene flow may convey adaptation (Brown et al., 2024). In fact, potentially adaptive gene flow at contact zones is not eliminated when, as proposed here, cytotypes are segregated in seed zone-based restoration. In contrast, ignoring cytotypes in the production of regional seeds, where multiple source populations are often mixed, differs qualitatively and in scale from the spatially limited gene flow that occurs naturally at contact zones. As outlined in the introduction, such mixing risks reduced seed yields, fitness losses in cultivation and restoration, and introgression of novel cytotypes. Since a reliable seed yield is essential for the economic viability of seed producers (Mainz & Wieden, 2018), cytotype segregation in seed zone-based restoration is reasonable both ecologically and economically. The framework we develop here is designed for application by seed producers. As long as the risks described above are not assessed reliably, we therefore recommend the segregation of cytotypes in restoration.

In *Leucanthemum vulgare* agg., discordance between empirical ploidy and ploidy prediction was observed in 25 (7%) out of the 346 individuals for which cytometry data were available (Figure S1). While true tetraploids in the genetic cluster of diploids may be explained by autotetraploidisation, true diploids in the genetic cluster of tetraploids would be more difficult to explain. They would require either

TABLE 5 Individual cytotype counts and proposed management actions (M, see Figure 3) for six polyploid complexes frequently used in grassland restoration within the 22 German seed transfer zones.

	Achillea millefolium agg.		*Campanula rotundifolia* s.str.		*Euphorbia cyparissias*		*Knautia arvensis* s.str.		*Leucanthemum vulgare* agg.		*Pimpinella saxifraga*	
Zone	4x:6x	M	2x:4x	M	2x:4x	M	2x:4x	M	2x:4x	M	2x:4x	M
01	0:46	M1: 6x[a]	0:20	M1: 4x[a]	0:4	M1: 4x[a]	0:18	M1: 4x[a]	5:9	M4	0:15	M1: 4x[a]
02	11:19	M2: 4x, 6x	0:8	M1: 4x[a]	0:9	M1: 4x[a]	4:18	M1: 4x[a]	2:13	M1: 4x[a]	0:14	M1: 4x[a]
03	3:36	M1: 6x	3:28	M1: 4x	0:13	M1: 4x[a]	0:34	M1: 4x	12:13	M2: 2x, 4x[a]	0:18	M1: 4x[a]
04	11:27	M4	13:5	M4	0:27	M1: 4x[a]	0:23	M1: 4x[a]	7:8	M4	2:17	M1: 4x[a]
05	35:24	M2: 4x, 6x	17:8	M2: 2x, 4x	0:62	M1: 4x	1:55	M1: 4x	13:22	M2: 2x, 4x	29:9	M1: 2x
06	3:29	M1: 6x	11:5	M2: 2x, 4x	0:12	M1: 4x[a]	0:16	M1: 4x	5:12	M2: 2x, 4x	11:4	M2: 2x, 4x
07	6:41	M1: 6x	5:26	M1: 4x	5:10	M3: 2x, 4x[a]	1:42	M1: 4x	5:40	M1: 4x	2:25	M1: 4x
08	1:11	M1: 6x	12:0	M1: 2x	0:0	M4	0:9	M1: 4x	1:8	M1: 4x	4:9	M2: 2x, 4x
09	10:21	M2: 4x, 6x	3:9	M3: 2x, 4x[a]	4:19	M3: 2x, 4x[a]	4:27	M1: 4x	9:15	M2: 2x, 4x	8:8	M3: 2x, 4x[a]
10	3:12	M1: 6x	0:14	M1: 4x	0:9	M1: 4x	0:14	M1: 4x	3:10	M1: 4x	10:1	M1: 2x
11	22:9	M2: 4x, 6x	4:11	M3: 2x, 4x[a]	0:30	M1: 4x	4:41	M1: 4x	2:22	M1: 4x	10:7	M4
12	11:6	M2: 4x, 6x	12:2	M1: 2x	0:19	M1: 4x	0:19	M1: 4x	3:11	M1: 4x	7:2	M4
13	12:5	M2: 4x, 6x	1:13	M1: 4x	0:16	M1: 4x	0:30	M1: 4x	4:10	M2: 2x, 4x	6:5	M2: 2x, 4x
14	18:10	M2: 4x, 6x	15:4	M1: 2x	0:20	M1: 4x	1:19	M1: 4x	2:6	M1: 4x	6:6	M2: 2x, 4x
15	4:11	M2: 4x, 6x	12:0	M1: 2x	0:7	M1: 4x[a]	0:18	M1: 4x	0:12	M1: 4x	11:2	M1: 2x
16	26:2	M1: 4x	10:16	M2: 2x, 4x	0:30	M1: 4x	6:27	M1: 4x	1:19	M1: 4x	10:10	M2: 2x, 4x
17	19:9	M2: 4x, 6x	5:14	M2: 2x, 4x	0:26	M1: 4x	3:25	M1: 4x	3:19	M1: 4x	10:1	M1: 2x[a]
18	5:12	M2: 4x, 6x	1:6	M1: 4x	0:10	M1: 4x	0:8	M1: 4x	0:17	M1: 4x	0:1	M4
19	5:23	M1: 6x	20:1	M1: 2x	0:21	M1: 4x	0:25	M1: 4x	4:16	M1: 4x	9:12	M2: 2x, 4x
20	5:18	M1: 6x	10:0	M1: 2x	0:6	M1: 4x[a]	1:8	M1: 4x	0:9	M1: 4x	1:6	M1: 4x
21	5:8	M4	12:1	M1: 2x[a]	1:5	M1: 4x[a]	2:19	M1: 4x	3:11	M1: 4x[a]	9:10	M4
22	4:7	M2: 4x, 6x	1:5	M4	0:8	M1: 4x	1:17	M1: 4x	5:5	M2: 2x, 4x	0:4	M1: 4x[a]
∑	219:386		167:196		10:363		28:512		89:307		145:186	

[a]Expert knowledge indicated data sufficiency despite a sampling density below the target of one site per 1000 km^2.

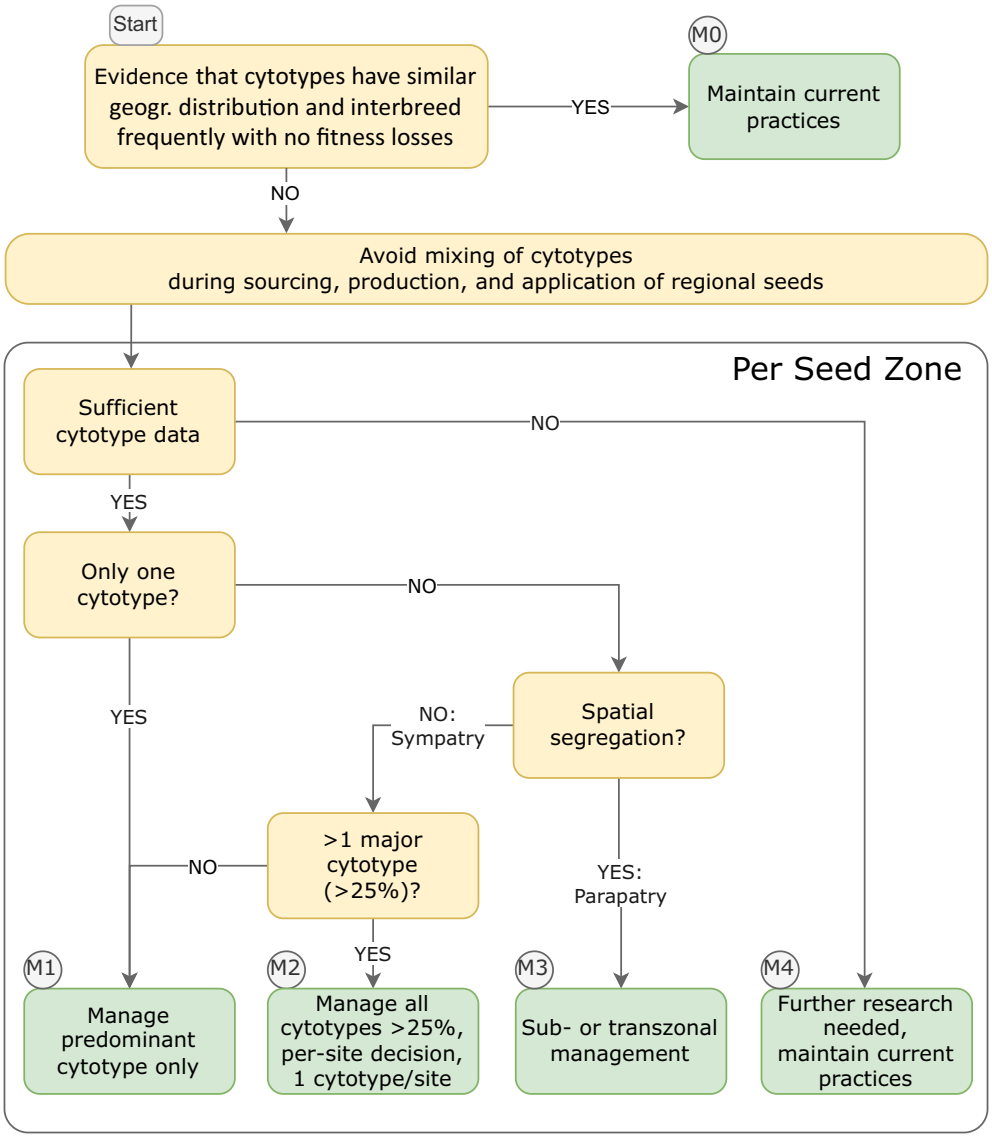

FIGURE 3 Decision framework for the seed zone-based management of polyploid complexes, featuring criteria (yellow) that lead to management recommendations (M, green). To provide practical guidance, we defined ad hoc thresholds of >25% and >75% to delimit major and predominant cytotypes, respectively.

backcrossing of triploid hybrids with diploids (Kolář et al., 2017) resulting in diploids genetically close to tetraploids. Although we observed no triploids, some individuals occupied intermediate genetic positions. These, however, lacked reliable ploidy data. Thus, the discordance between empirical and predicted ploidy could not be fully resolved. Given our low site-level sampling sizes, a more localised and comprehensive data set would be necessary for a quantitative assessment and mechanistic understanding of gene flow between cytotypes.

4.2 | Geographical patterns of cytotype distribution

Seed zones aim to protect natural distribution patterns, which requires an understanding of the patterns themselves. The geographic distribution of cytotypes is shaped by environmental gradients in all study taxa, highlighting the ecological differentiation of the cytotypes. Expectedly, taxa with more pronounced parapatric cytotype distributions (e.g., *C. rotundifolia*) yielded more significant environmental predictors, consistent with greater ecological differentiation between cytotypes. In general, ecological differentiation of cytotypes warrants their separate treatment in seed zone-based restoration to avoid maladaptation and adaptational dilution of existing populations. While the generalised linear mixed models could theoretically predict cytotype occurrences from environmental data, we refrained from this approach. Cytotype distributions likely result from a complex interplay of evolutionary and phylogeographic processes beyond mere environmental information, for example, in *E. cyparissias* and *C. rotundifolia*. We therefore relied on the empirical patterns.

At the national scale, the study taxa exemplify that cytotype distributions in polyploid complexes in general can range from sharp parapatry to predominantly sympatric occurrence. All study taxa

exhibited predominantly monoploid populations at the site level despite the variation in broader patterns. This reflects earlier findings that cytotype parapatry tends to be diffuse (Kolář et al., 2009) and is probably scale-dependent (Duchoslav et al., 2020).

Clear parapatry was observed in *C. rotundifolia* and *E. cyparissias*. Diploid *C. rotundifolia* occurred in large parts of Eastern Germany, whereas tetraploids occupied the rest of Germany. Their contact zone corresponds with two main genetic clades of *C. rotundifolia* s.str.: diploids and tetraploids in Eastern Europe and tetraploids and hexaploids in Western Europe and North America (Sutherland et al., 2018; Wilson et al., 2020). The dominance of diploids in the east of Germany continues throughout much of the Czech Republic (Šemberová et al., 2023), representing the largest continuous diploid range of *C. rotundifolia* s.str. known up to date. Diploid *E. cyparissias* was confined to a small region in western Germany. This region of diploids represents the north-eastern edge of a larger western European area of diploids extending southward to the Pyrenees and northward into the Netherlands (Pungaršek & Frajman, 2024). Tetraploids were the common cytotype across the rest of Germany, which extends into Scandinavia and most of Eastern Europe. This pattern supports the hypothesis that polyploidisation facilitated postglacial recolonisation in *E. cyparissias* (Pungaršek & Frajman, 2024), and similar patterns have been found in other species (Brochmann et al., 2004; Parisod et al., 2010; Zhao & Zhang, 2024). The parapatric patterns of cytotypes in both *C. rotundifolia* and *E. cyparissias* could represent secondary contact resulting from postglacial recolonisation routes into Central Europe from the west and east (Taberlet et al., 1998).

The patterns of parapatry were more complex in *P. saxifraga* and *A. millefolium* agg. Diploid *P. saxifraga* occupied much of Central and Southern Germany, whereas tetraploids dominated the rest of the country, corroborating the findings of Hunkeler and Favarger (1967). The area of diploid *P. saxifraga* continues eastward into the Bohemian Massif (Mozolová, 2007). In *A. millefolium* agg., the pattern of parapatry was more diffuse. Tetraploid *Achillea collina/pratensis* was surprisingly common and widespread, which is in line with recent morphology-based observations (Frank, 2011). Tetraploids predominated in Central Southern Germany and Central Germany, while the rest of Germany was mostly occupied by hexaploid *A. millefolium* s.str. with tetraploids interspersed.

A rather sympatric distribution of the cytotypes of *L. vulgare* agg. was observed, which aligns with previous findings on the European scale (Stutz et al., 2018). Generally, tetraploid *L. ircutianum* was more common than diploid *L. vulgare* s.str., which tended to occur in loose regional aggregations. Whether differences between cytotypes in phenotypic traits or ecological niches (Vogt, 2024) contribute to this pattern remains unknown.

The predominance of monoploid populations at individual sites, even within taxa exhibiting regional sympatry, carries direct implications for restoration practice. Mixed-ploidy populations were rare across all taxa, which is consistent with earlier findings where local sympatry ranges from absent to common across polyploid complexes (Gibson et al., 2017). The rarity of natural mixing, especially in the core zones of the lower ploidy cytotypes, suggests secondary contact rather than recurrent polyploidisation as the primary mechanism of cytotype coexistence (Petit et al., 1999). As a result, cytotype mixing in restoration sowings would create demographic conditions rarely observed in natural systems, with uncertain consequences for fitness and establishment. The distribution of *K. arvensis* agg. exemplifies these concerns. Diploids were rare (7%) and geographically isolated in our sampling, yet occurred in three of eight regions in commercial seed mixtures (Durka et al., 2017). A study from one of these regions reported tetraploids in all semi-natural meadows, while diploids were restricted to meadows that had been restored using regional seed (Kaulfuß & Reisch, 2019), suggesting that diploid *K. arvensis* might be non-native to Germany. In that case, the regional seed mixtures that were used did not accurately reflect natural cytotype distribution. The example of *K. arvensis* agg. illustrates the need for a cytotype-based decision framework that translates the diversity of cytotype distribution patterns into management recommendations.

4.3 | Implications of polyploid complexes for seed zone-based restoration: A management decision framework

The existence of polyploid species complexes has different consequences for different provenancing strategies. Here, we focus on seed zone-based restoration, where the sourcing, production, and application of seeds are carried out at the seed zone level. We found that the distribution of cytotypes rarely aligned with the seed zone borders (cf. Kramer et al., 2015). One possible solution is a redesign of the zones to fit the structure we found. However, such a redesign presently involves prohibitive hurdles: Legally, the current seed zones are generalised, that is, they apply to all grassland species that meet certain criteria (Prasse et al., 2010), and thus species-specific rules are impossible to implement. Furthermore, a change in seed zones would be potentially detrimental to the established producers of native seed and would take years to adapt (Mainz & Wieden, 2018), while the availability of regional seeds is currently one of the main limiting factors of grassland restoration (Pedrini & Dixon, 2020). However, adjustments of the seed zones are increasingly discussed in the context of climate change (Fremout et al., 2021; Marinoni et al., 2021), a concern addressed elsewhere (Höfner, Bucharova, et al., 2025). Despite limitations of the German seed zone system, the generalised scope of the zones, their unparalleled geographic detail, and the well-established regional seed industry remain major strengths. Moreover, the zones do cover a substantial part of the existing genetic variation across taxa (Table 2; Table S2; Durka et al., 2025; Höfner, Bucharova, et al., 2025). Therefore, our decision framework focuses on the management of cytotypes at the level of existing seed zones.

We recommend ploidy management strategies 'M0' to 'M4' per taxon-seed zone combination (Table 5) and based on three key criteria (Figure 3). The first criterion is the sufficiency of available cytotype distribution data, which is a prerequisite to reliably evaluate the

subsequent criteria (see Methods and below). The second criterion is the geographic distribution of cytotypes within and around the seed zone, where we differentiate between sympatry and parapatry of cytotypes. Lastly, the third criterion is the presence of cytotypes within a seed zone and, if more than one cytotype is present, their relative abundances.

All management recommendations aim to be ecologically conservative with limited data. When there is evidence that the geographic distribution of cytotypes is identical, and hybridisation is common and without negative fitness consequences, as may be the case for some higher-level interploid hybrids (Brown et al., 2024; Sutherland & Galloway, 2021), the framework is not applicable (M0 in Figure 3 and see below).

The first, simplest management recommendation, 'M1: Manage predominant cytotype only', applies in two cases: A given seed zone contains exclusively one cytotype (e.g., tetraploid *C. rotundifolia* in zone 10, Figure 1b), or multiple cytotypes occur in regional sympatry, but one of them is predominant, that is, exhibits a frequency of >75% (e.g., tetraploid *L. ircutianum* in zone 17, Figure 1h). We acknowledge that this limitation to one cytotype bears the risk of promoting minority cytotype exclusion, where a rare but naturally occurring cytotype in stable mixed-ploidy populations (e.g., Čertner et al., 2017) may be driven to extinction. However, maintaining separate production lines for low-frequency cytotypes may require disproportionate effort for seed producers, as these rarer cytotypes should be used sparingly precisely because of their low frequency. Moreover, as discussed above, mixing ploidies in seed production may risk fitness declines (Wallace et al., 2017). We therefore recommend managing only the predominant cytotype exceeding a frequency of 75%, accepting the trade-off between practical feasibility and the ecological risk of minority cytotype exclusion. When cytotypes are more evenly distributed (i.e., multiple cytotypes exceed 25% frequency), this trade-off shifts, warranting a different approach.

The second management recommendation, 'M2: Manage all cytotypes >25%', applies when there are multiple major cytotypes (each with relative abundance >25%) and their populations occur in sympatry (e.g., diploid *L. vulgare* s.str. and tetraploid *L. ircutianum* in zone 05, Table 2). We recommend maintaining all major cytotypes separately in cultivation and using only one cytotype per restoration site (cf. Kramer et al., 2018; Wallace et al., 2017). When multiple cytotypes are cultivated, it is essential that they are kept separate at all times to avoid outbreeding depression. Moreover, cytotypes can also have different environmental requirements (Decanter et al., 2020), as suggested by the association of environmental gradients with cytotype distribution which we observed, warranting differential use in restoration. For example, *L. ircutianum* prefers richer and fresher meadows, while *L. vulgare* s.str. may predominate on drier, nutrient-poor grasslands (Oberprieler et al., 2011). Presently, only tetraploid *L. ircutianum* is used by the most important producers of regional seed in Germany (Rieger Hoffmann, 2024; Saaten Zeller, 2024). For the seed zones where the diploids are a major cytotype as well, we therefore recommend extending the supply to *L. vulgare* s.str. to be used for restoration on drier sites.

The third management recommendation, 'M3: Sub- or transzonal management', applies when a seed zone contains multiple major cytotypes (>25%) and these occur in clear regional parapatry. In this case, we recommend splitting the seed zone for this taxon into subzones corresponding to the cytotypes, e.g., for *P. saxifraga* splitting zone 08 into a diploid west and a tetraploid east (Figure 1i). Accordingly, only one cytotype should be sourced, cultivated, and applied in each of these subzones. Managing cytotypes at the subzonal level may require disproportionate effort in small zones or prove logistically unfeasible (cf. Mainz & Wieden, 2018), in which case transzonal management across adjacent seed zones could offer a pragmatic alternative. For example, diploids of *P. saxifraga* in the west and tetraploids in the east of zone 08 could be managed together with zones 15 and 20, respectively.

The fourth management recommendation, 'M4: Further research needed; maintain current practices', applies to all polyploid complexes where there are insufficient data to evaluate the abundance ratio and geographic distribution of cytotypes. We suggest a target sampling site density of one site per 1000 km^2 (corresponding to between five sites in zone 08 and 49 sites in zone 01) as a benchmark, though expert knowledge may establish sufficiency at lower densities based on phylogeography, ecology, and environmental context. The sufficiency of available cytotype distribution data depends on the geographical context. For example, while sampling site density was low for *P. saxifraga* in seed zones 01, it can be reasonably assumed from the broader distribution pattern that tetraploids are the predominant cytotype in that zone. Cytotype distribution data are often sparse, even for complexes widely used in restoration, such as *Poa pratensis* L., *Centaurea scabiosa* L., *Galium mollugo* agg., *Galium verum* L., *Vicia cracca* L., and the six polyploid complexes addressed in this study. Maintaining or changing current practices is a trade-off between on one hand risking fitness losses and distortion of natural distribution patterns, and on the other hand unwarranted exclusion or segregation of cytotypes. Overall, in around two-thirds (68%) of the taxon-seed zone cases, one dominant cytotype could be identified (M1), ensuring feasible management. In one-quarter of the cases, our recommendation is to manage two cytotypes in parallel, in most cases requiring a per-site decision due to regional sympatry (21%, M2), but sometimes allowing for sub- or transzonal management when clear contact zones were identified (4%, M3). In 8% of the cases, no recommendation could be given (M4), warranting further research.

4.4 | Importance of cytotype distribution data

The necessity of cytotype distribution data is illustrated by the case of *K. arvensis* mentioned above: without systematic ploidy assessment, seed producers inadvertently introduced non-native cytotypes into restoration projects, precisely the outcome this framework aims to prevent. Still, our recommendation to segregate cytotypes in some cases may appear overly conservative, given that gene flow between cytotypes can increase genetic diversity, thus

conveying adaptive potential under certain conditions (Broadhurst et al., 2008; Brown et al., 2024). Natural selection will ultimately favour the better adapted cytotypes, which suggests that active management is unnecessary. However, the fitness consequences during this intervening period can be economically detrimental to seed producers and potentially catastrophic for restored populations already struggling to establish (Freitag et al., 2021; Török et al., 2021). Furthermore, the natural selection argument conflates natural gene flow at contact zones with the temporally and spatially disparate, zone-wide mixing that occurs when collecting from disparate source populations.

By managing cytotypes within existing seed zones, the framework maintains both regional adaptation and adaptive potential via diversity, while addressing cytotype-specific incompatibility, which is highlighted by rare local cytotype sympatry in our data. The seed zones were designed for common, widespread grassland species where fine-scale local adaptation can be expected to play a relatively minor role compared with regional adaptation (Macel et al., 2007). Moreover, many restoration sites lack sufficient locally adapted donor material in their immediate vicinity that could be harvested without compromising vulnerable source populations (Broadhurst et al., 2008; Bucharova et al., 2025; McKay et al., 2005). Importantly, cytotypes can still hybridise at natural contact zones. Gene flow is not eliminated even when cytotypes are managed in spatial separation during propagation. Furthermore, the M0 exception (see above) explicitly allows for maintaining current practices when hybridisation is known to be frequent and beneficial. By operating within established seed zone infrastructure while adding cytotype-aware decisions, this framework enhances rather than restricts current restoration practice.

The implementation of this framework requires large-scale cytotype distribution data. Flow cytometry offers an affordable method to obtain such data. First, for a given region, species exhibiting intraspecific cytotype variation need to be identified. Second, large-scale cytotype distribution patterns need to be described. The required sampling density, and thus the associated costs, depend on the complexity and scale of cytotype distribution patterns. However, individual restoration practitioners cannot be expected to conduct such cytotyping campaigns themselves. Rather, they should be funded by the responsible environmental agencies. Third, seed producers would then have to verify the cytotype of their accessions to apply the framework we suggest here.

Beyond mere geographical distribution of cytotypes, future research should examine how cytotype-specific ecological differences (e.g., stomatal characteristics, niche width, colonisation potential) might influence environmental adaptation (Tossi et al., 2022; Van de Peer et al., 2021), potentially informing cytotype selection for restoration under changing climatic conditions (Chen et al., 2022). Our decision framework enables practitioners to make informed cytotype management decisions with feasible data requirements, improving restoration outcomes while working within existing seed zone infrastructure.

AUTHOR CONTRIBUTIONS

Walter Durka, Stefan G. Michalski, and Johannes Höfner conceived and designed the research; Walter Durka, Stefan G. Michalski, Johannes Höfner, Christina M. Müller, Christoph Oberprieler, Kristýna Šemberová, and Alena Voltrová performed the experiments; Johannes Höfner, Walter Durka, and Stefan G. Michalski analysed the data; Johannes Höfner, Walter Durka, Stefan G. Michalski, Anna Bucharova, Filip Kolář, Christina M. Müller, Christoph Oberprieler, Kristýna Šemberová, and Alena Voltrová wrote and edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

Anna Bucharova is an Associate Editor of *Journal of Applied Ecology*, but took no part in the peer review and decision-making processes for this article.

DATA AVAILABILITY STATEMENT

Demultiplexed individual raw sequence data are available at ENA (https://www.ebi.ac.uk/ena). Sample data including ploidy data and ENA accession numbers can be found on Zenodo (https://doi.org/10.5281/zenodo.17396516; Höfner, Kolář, et al., 2025).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Flow cytometry details.

Table S2: Hierarchical AMOVA of seed zones (SZ) nested in ploidy, calculated using the R-package poppr (Kamvar et al., 2014) with the ade4 method (Dray & Dufour, 2007).

Table S3: Predictor estimates and significance from generalised linear models testing the first hypothesis with a binary cytotype vector as response; three climate and three soil principal components as predictors.

Figure S1: Principal Components of raw read counts (cf. Figure S2) with individuals coloured according to flow cytometry measurements (left) and ploidy prediction by linear discriminant analysis (right).

Figure S2: The workflow from raw reads to the visualisation and two different statistics of genetic differentiation.

Figure S3: Phylogenetic networks of each study polyploid complex showing seed zone (last edge of branch) and the predicted ploidy level (tip) of each individual.

Figure S4: Local, site-level sympatry of cytotypes in the six study taxa: Counts of different ploidy levels at each site with more than one sample.

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