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Cross-Continental Ecological Drivers Behind Trait Clines in the Forest Grass *Milium effusum*

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ABSTRACT

Aim: Widespread species encounter a range of variable climates that can lead to intraspecific trait clines. Such clines can be the result of phenotypic plasticity, genetic differences, or both. Although latitude often explains a large part of trait variation, it is crucial to investigate the underlying environmental variables to understand current and future trait responses. Cross-continental comparisons of species that are native on multiple continents provide a rarely used approach that can help identify the environmental drivers of intraspecific trait clines.

Location: Europe and North America.

Time Period: 2021–2023.

Major Taxa Studied: *Milium effusum* L. (Poaceae).

Methods: To quantify the influence of environmental gradients on functional traits across geographical regions that vary in climate, we sampled *M. effusum* seeds from 23 European and 14 North American populations and transplanted them in a common garden. We measured 10 vegetative, reproductive, and phenological traits. We used 30-year averages of 19 bioclimatic variables,

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while accounting for the latitudinal and elevational position of the population origins, to compare the trait-environment relationships between continents.

Results: Our results showed that European populations occupy a broader climatic range than North American populations. Differences between continents were found in most of the traits as well as in the multivariate trait space. The traits were affected more by bioclimatic variables than by latitude or elevation. While flowering, leaf thickness, specific leaf area, and reproductive height showed parallel clines to the environment between continents, vegetative height and biomass showed contrasting clines.

Main Conclusions: Environmental influences from population origins revealed parallel clines between the continents for functional traits, suggesting shared selective pressures, while contrasting clines for plant size indicated different evolutionary trajectories, potential bottlenecks, or interactions with unknown ecological factors. This study highlights the complex interplay of genetic, environmental, and evolutionary factors in shaping phenotypic variation in native species across continents.

1 | Introduction

Macroclimate varies widely within continents and is correlated with geography, which is reflected in worldwide vegetation zones (Takhtajan 1986; Liu et al. 2023). Alexander von Humboldt (1845) recognised that climate, especially temperature and precipitation, is critical in explaining vegetation zones within and across regions. In his work, he highlighted the similarities in plant and vegetation types found in similar climates worldwide. Charles Darwin (1859) expanded on this by introducing the mechanisms of natural selection and adaptation, explaining how species' climatic niches evolve and how geographical barriers contribute to the diversification of species. In modern times, their insights are still applicable for investigating global patterns of plant distribution and have been complemented by an array of new insights, including the importance of species-specific responses to environmental variables (Santamaría et al. 2003).

Widespread species encounter a wide range of climatic conditions and environmental gradients which may lead to intraspecific clines in traits across a species' geographical range. These clines (i.e., measurable gradients in phenotypic traits) can be the result of phenotypic plasticity, neutral non-adaptive evolution via processes like genetic drift, or adaptive evolution by means of natural selection (McGoey et al. 2020). When plants from populations across broad geographical or environmental gradients are grown under common conditions, genetically based intraspecific trait clines may be revealed, which can suggest adaptive evolution driven by the bioclimatic conditions or other factors in the environment of origin (Keller et al. 2009). Moreover, by comparing such intraspecific trait-environment relationships between distant populations, it is possible to test whether similar selection pressures from comparable environments have led to the parallel evolution of similar phenotypes, which could provide evidence for the adaptive nature of these clines. An additional possibility could also be alternative evolutionary pathways resulting in the same adaptive outcomes visualised as parallel clines. Divergent clines could indicate either a unique set of evolutionary adaptations in response to a unique set of environmental conditions despite broad-scale climatic similarities, or neutral evolutionary processes (Keller et al. 2009).

Many studies investigating native versus introduced population patterns across continents (Europe, North America, Asia) have focused on geographical ranges and used factors such as latitude and elevation that successfully explained trait variation (Alexander et al. 2009; Dlugosch and Parker 2008; Li and Feng 2009; Maron et al. 2004). However, latitude and elevation are only proxies of

those environmental variables that ultimately constitute the selective forces responsible for shaping the observed trait variation. To foster our understanding of the mechanisms leading to spatial patterns in trait variation, we should identify the potential selective factors, particularly those related to temperature and precipitation, which form important selective forces influencing phenotypic divergence and driving rapid trait adaptation (Siepielski et al. 2017).

Species with broad geographical distributions, especially those with range disjunctions across regions or continents, provide unique opportunities to test for parallel adaptive evolution. To date, most studies of intraspecific clines across continents have focused on recently introduced exotic or invasive species, comparing patterns of evolutionary change along similar gradients between their native and non-native ranges (Montague et al. 2008; McGoey et al. 2020; Ollivier et al. 2020; Reeb et al. 2024). Patterns of local adaptation (McGoey et al. 2020) and parallel clines (Montague et al. 2008) are frequently highlighted in measured traits compared between native and introduced populations, suggesting rapid adaptation to environmental gradients associated with latitude and elevation, such as temperature and precipitation (McGoey et al. 2020; Ollivier et al. 2020). Evidence also suggests that these clines often result from selective pressures as opposed to neutral processes, with some studies even showing repeated or parallel evolutionary changes following introduction (Liao et al. 2020). Although these examples based on recent introductions of exotic species are valuable for documenting rapid or short-term evolutionary processes, genetic bottlenecks during introduction and colonisation may strongly influence and even inhibit evolutionary processes or outcomes in the newly colonised range (Liao et al. 2020). As such, cross-continental comparisons of species that are native to both continents would therefore constitute a better test for parallel evolution of trait clines in response to similar environmental gradients.

An ideal study system to explore long-term parallel evolution of clines is *Milium effusum* L. (wood millet), a widespread grass species, native to two continents: Eurasia and North America. The species is classified as a member of the Circumpolar Boreo-Temperate floristic element, a group of plant species with native ranges at mid- to higher latitudes spanning portions of North America and Eurasia (Preston and Hill 1997). Phylogenetic data suggest the *Milium* lineage diverged from related grasses ~16 million years ago, with the extant crown group species potentially beginning to diverge ~10–14 million years ago, likely in south-central Eurasia (Soreng et al. 2022), where all six recognised *Milium* species occur today. *Milium effusum* is the most

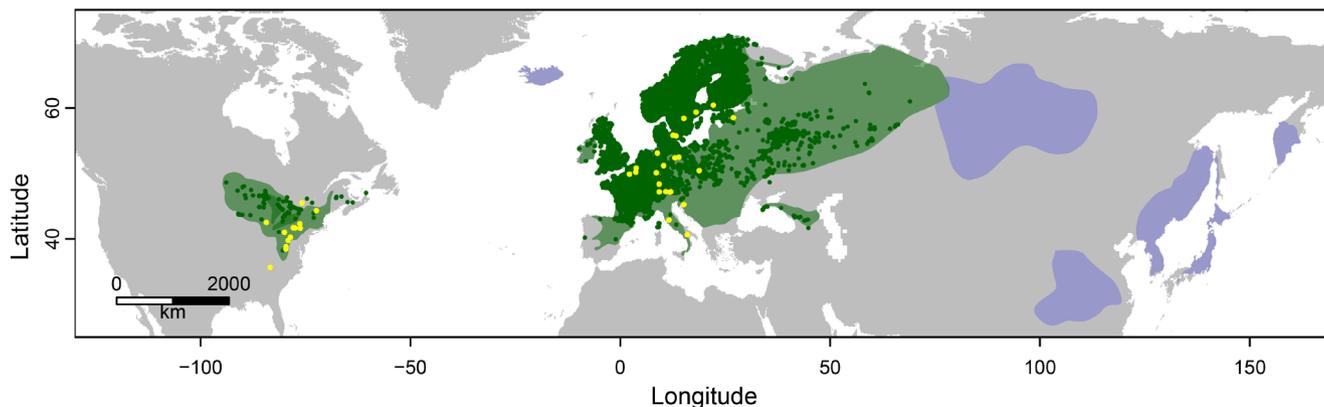


FIGURE 1 | Range map of *Milium effusum*. Dark green points are filtered observations of the species during the years 1900–2022 obtained from GBIF (GBIF 2024). Dark green polygons represent the native range, while blue polygons represent the range where the species is naturalised. The 37 sampled populations are indicated using yellow dots (14 across eastern North America and 23 across Europe).

widespread of these species and is unique in having a native distribution extending to North America. The long-distance dispersal event(s) that took *M. effusum* to North America have not been dated, but appears to be quite ancient, with sufficient time for North American plants to show subtle morphological differences (formally *M. effusum* var. *cisatlanticum*; Flora of North America 2025).

Prior research has shown the presence of latitudinal clines in European populations of *M. effusum*, with decreasing performance in individuals transplanted southwards from their site of origin (De Frenne et al. 2011). Here, we expand the geographical scope to consider *M. effusum* across both its European and North American range areas. Specifically, we (1) use environmental variables related to temperature and precipitation from the North American and European distribution ranges of *M. effusum* to investigate climatic niche overlap between the two continents, (2) compare trait clines in *M. effusum* from populations sampled on the two continents using a common garden experiment, and (3) relate trait data from a common garden to environmental gradients spanned by the source populations. We ask the following questions: (a) Do the North American and European populations occupy similar climatic niches? (b) Do North American and European plants differ from each other in phenotypic and phenological traits? (c) Do the sampled North American and European populations show genetically based clines in vegetative, reproductive, and phenological traits related to climatic gradients? And if so, do they show similar phenotypic clines in relation to comparable environmental gradients, suggesting parallel evolution?

2 | Methods

2.1 | Study Species

Milium effusum (Poaceae) is a forest understorey grass, reaching heights of 50–150 cm, and flowering during June and July depending on its latitudinal and elevational location across its geographical range (De Frenne et al. 2017). Seeds have no particular dispersal adaptations, but dispersal over longer distances

occurs occasionally by means of epi- and endozoochory (von Oheimb et al. 2005). Stems, as well as the flat, smooth, hairless leaf blades and sheaths, ascend from decumbent bases. Resprouting through short stolons plays a role in perennation and in the case of *M. effusum*, which is clonal to a certain degree, vegetative growth can be viewed as a fitness proxy, since larger trait averages (i.e., greater vegetative proliferation) will typically be beneficial (Møller et al. 2024). *Milium effusum* is a shade-tolerant species, growing mostly in temperate deciduous woodlands, but can also occur in meadows, along railways and roads, and in rocky habitats (De Frenne et al. 2017). *Milium effusum* is native to large parts of the Northern Hemisphere (Figure 1), including most of Europe (subsp. *effusum* and *alpicola*), parts of North America (subsp. *cisatlanticum*) (De Frenne et al. 2017; Flora of North America 2025), and in parts of Asia (Figure 1). Furthermore, both di- ($2n=14$) and tetraploid ($2n=28$) populations occur in Europe (Bennett and Thomas 1991) while ploidy levels are unknown for North American populations.

To map the distribution area of *M. effusum* (Figure 1), we used existing distribution range maps based on expert judgement available for Eurasia (Figure S1A) and North America (Figure S1B). Using QGIS (QGIS.org 2025), we overlaid the distribution range maps with a world map before manually drawing polygons (Figure 1). Furthermore, we downloaded occurrence records from GBIF (www.gbif.org) on 16th of July 2024 (GBIF 2024). We filtered them by removing records lacking coordinate information and those originating from freshwater habitats. We only kept records if they had an existing institution code, were made between the years 1900–2022, had coordinate uncertainty less than or equal to 21,000 m based on an arbitrary decision, and presented the current occurrence status. Furthermore, we only incorporated one record per species per location as well as one record per 21 km² grid cell to match the filtered coordinate uncertainty.

2.2 | Experimental Design

In 2021, seeds of *M. effusum* were collected from 23 European and 14 North American populations across substantial portions of the species range in both regions (Figure 1 and Table S1).

Within each population, seeds were collected from 10 to 15 randomly selected individuals (hereafter referred to as seed families). There was no strict minimum distance between sampled plants, but an effort was made to cover the entire area of the populations. Seeds were collected by clipping the whole inflorescence of an individual when seeds at the top of the inflorescence were mature (i.e., turning brown and dry and falling out of the glumes easily).

In November 2021, we randomly selected five to eight seed families (i.e., accessions) for the common garden experiment from the 10–15 sampled individuals (see Table S1 for exact numbers). For each seed family, we created five replicates by sowing 25 seeds of that seed family into five pots with five seeds per 1.5L pot (“Typ T, Struktur 1B” potting soil, Hawita, Vechta, Germany). We placed the pots in a common garden in Frankfurt am Main, Germany (50.1697222°, 8.6320833°). The pots were positioned in a shade house under 45% shading cloth, which simulated a forest overstorey. We watered regularly and whenever needed in order to maintain moist soil conditions typical of natural *M. effusum* habitat.

2.3 | Trait Measurements

Seedling emergence was recorded three times per week after sowing, from December 2021 to March 2022. As plants reached reproductive maturity in their second growing season in Spring 2023, flowering start (i.e., when stamens were visible) was recorded three times per week throughout April and May. Once flowering reached its peak (i.e., more than 50% of the individuals were flowering), we measured vegetative traits in June 2023. In each pot, we counted the number of ramets (i.e., a potentially independent individual derived by vegetative reproduction from a single parent plant) as well as the proportion of flowering ramets based on the five sowed seeds. Furthermore, we measured both the vegetative (longest leaf) and reproductive (longest inflorescent ramet) height. Moreover, three leaves were randomly chosen per pot and cut off to measure the chlorophyll content inferred through a SPAD meter (Konica Minolta Handheld Portable Spad-502plus Chlorophyll Meter), which produces relative values that are proportional to the amount of chlorophyll in the leaf based on the relative proportions of red and near infrared radiation reflected. In the same three leaves, we measured leaf thickness (Mitutoyo IP65 Coolant Proof Micrometre) individually for each leaf and total fresh weight for all three leaves combined to the nearest 0.001 g (Sartorius CPA225D-0CE balance). We also measured the total leaf area (‘Easy Leaf Area’ app, version 2.0) of all three leaves combined. After the fresh biomass of the leaves was weighed, it was dried for 48 h at 60°C before we determined the dry weight. From the measurements mentioned above, we calculated the average specific leaf area (SLA; leaf area/dry mass) and the average leaf dry matter content (LDMC; dry mass/fresh mass) of the three leaves to use as indicators for plant resource use. Lastly, in mid-July 2023, the entire aboveground biomass per pot was harvested 1 cm above the soil surface, left to dry at room temperature for 1 week before being dried for 48 h at 60°C and weighed to a precision of 0.001 g (RADWAG PS600.R2 balance). Two complete locations (Swabian Alb in Germany and Great Smoky Mountains in North Carolina, USA) had to be excluded from the dataset due to

germination failure, most likely because immature seeds were sampled or damaged during shipping.

2.4 | Statistical Analyses

First, 19 bioclimatic variables (Table S2) were extracted from the WorldClim database (Fick and Hijmans 2017) for all European and North American seed source population sites using the ‘worldclim_global’ command from the ‘geodata’ package (Hijmans et al. 2024) and setting the spatial resolution to 2.5-arcminutes. We used this climatic data in the form of 30-year averages (1970–2000) to test for associations between climate and patterns of functional trait variation among populations and continents that might be the result of clinal adaptation along key environmental gradients. Specifically, to describe key aspects of climatic variation among seed source sites and between the European and North American ranges of *M. effusum*, we performed a principal component analysis (PCA) using the ‘prcomp’ command from the ‘factoextra’ package (Kassambara and Mundt 2017) and applied a varimax factorial rotation to improve the identification of the main bioclimatic variables (log-scaled) along the first two principal component axes (PC1 and PC2, Figure S2). A factorial rotation sacrifices some of the variance in the PCA in order to have a simpler and more interpretable structure. We extracted PC1 and PC2 to use the scores of all the population sites in subsequent analyses. The top five bioclimatic variable loadings of PC1 were precipitation of driest month, mean temperature of wettest quarter, precipitation seasonality, mean temperature of driest quarter, and precipitation of wettest month (Figure S3A), while the top five bioclimatic variable loadings of PC2 were mean diurnal range, precipitation of coldest quarter, mean temperature of warmest quarter, mean temperature of coldest month, and precipitation of warmest quarter (Figure S3B).

Second, we visualised the climatic space for the European and North American continents as a whole (i.e., background conditions) and for the entire geographical range of *M. effusum* based on the extracted GBIF occurrences. To do so, we obtained shapefiles of North America and Europe and created a set of 10,000 evenly distributed points on each of the two continents (Figure S3) using the command ‘st_sample’ from the package ‘sf’ (Pebesma 2018). Moreover, we displayed the niche overlap between the geographical ranges of *M. effusum* on the two continents based on PC1 and PC2 by adding density curve histograms for each continent based on the GBIF occurrence data. Lastly, we highlighted the 37 sampled populations. To quantify the environmental niche overlap of *M. effusum* between its North American and European ranges, we calculated Schoener’s *D* metric (Schoener 1970):

$$D = 1 - \frac{1}{2}(\sum |d1 - d2|) \quad (1)$$

with *d1* denoting the density distributions of PC1 and PC2 based on all occurrence points (GBIF + sampled populations) documented for the European range of *M. effusum* and *d2* denoting the density distribution of PC1 and PC2 based on all occurrence points (GBIF + sampled populations) documented for the North American range (Equation (1)). Schoener’s *D* metric ranges from 0 to 1, with 0 indicating no overlap and completely different niche distributions, and 1 indicating complete niche overlap and

identical niche distributions. While Schoener's D metric takes the density of the occurrences into account, we are also interested in the pure extent of the occurrence points on each continent and their overlap. To quantify the climatic space on each continent occupied by *M. effusum*, we calculated the proportion of the available PCA space (PC1 and PC2) that was occupied by the occurrence points on each continent after rounding their PC values to the nearest decimal point. We then calculated the proportion of the European occurrence points overlapping with North American, and *vice versa*.

Third, we performed a PCA across all 10 measured ecological and functional traits (germination start, flowering start, vegetative height, reproductive height, proportion of reproductive ramets, LDMC, SLA, SPAD, leaf thickness, and above-ground biomass) to investigate the multivariate trait space covered by *M. effusum* plants grown from the 37 European and North American seed source populations. Each of the 10 measured traits was individually log-transformed, scaled for appropriate comparison, and factorially rotated. Furthermore, 95% confidence ellipses were calculated separately for Europe and North America. We applied a perMANOVA to test for continental differences of the multivariate trait space using the 'adonis2' command from the 'vegan' package (Dixon 2003).

Lastly, to investigate phenotypic clines in relation to environmental gradients represented by PC1 and PC2 scores of the 37 seed source population sites, we performed 10 separate (one per trait variable used as separate response variables) linear mixed-effects models (LMMs). Fixed effects in the model formula included PC1, PC2, continent, and their two-way interaction terms, while accounting for the original latitudinal and elevational position of the sampled populations as covariates in the fixed effects. The inclusion of two-way interaction terms between PCs and continent allowed us to account for continent-specific responses to the same climatic variables, where a significant interaction between a given PC axis and continent may reflect different evolutionary pressures for the focal PC axis. We included seed family nested within population origin as a random intercept term. We used the 'lmer' command from the 'lme4' package (Bates et al. 2009) assuming a Gaussian family, followed by an ANOVA on the fitted models, using the 'Anova' command from base R. Residual distributions and heteroscedasticity of all models were investigated visually via q-q plots and histograms.

All data analyses and visualisations were conducted using R (R Core Team 2023) and R Studio (version 2024.12.1).

3 | Results

3.1 | Geographical Range and Niche Overlap

The bioclimatic PCA indicated that PC1 captured variation primarily associated with wetter and warmer environments, while PC2 represented gradients associated with greater daily temperature range, as well as warmer and wetter seasons. Comparing the climatic niche of *M. effusum* between Europe and North America, we estimated a Schoener's D metric score of 0.429. European occurrences were predominantly associated with mid to low PC2 values, corresponding to conditions with smaller mean diurnal

ranges, higher precipitation during the coldest quarter, and greater seasonal temperatures (i.e., warmer summers and colder winters). Furthermore, European populations also occupied a distinct niche space at the intersection of high PC1 and high PC2 scores (Figure 2). In contrast, North American populations were uniquely distributed along low PC1 and high PC2 scores (Figure 2). These conditions are primarily shaped by reduced precipitation in the driest month, moderate temperatures in the wettest quarter, and elevated precipitation seasonality. The environmental niche occupied, as displayed by PC1 and PC2, by North American populations appeared to be notably narrower compared to that of European populations, which exhibited broader climatic occurrences. More specifically, the climatic space inhabited by *M. effusum* in North America was 15.7% out of the total climatic space available across that continent, whereas the distribution range of *M. effusum* in Europe fills 73.5% of the entire climatic space available in Europe (Figure 2 and Figure S4). In North America there are several combinations of climatic conditions, primarily dry and cold continental conditions (Mid and West North America), not occupied by *M. effusum*, whereas in Europe, it is only the dry and hot combination of climatic conditions (i.e., Mediterranean climate) where *M. effusum* does not occur (Figure 1 and Figure 2; Figure S4). Lastly, while the European environmental niche overlapped with 74.8% of the North American niche, the North American niche only matched with 22.0% of the European niche.

3.2 | Continental Phenotypic Differences

The trait PCA exposed significant continental differences in the multivariate trait space (perMANOVA: $R^2 = 0.167$, $F_{1,757} = 151.51$, $p = 0.001$). PC1 explained 20.1% of the observed variation and was mainly characterised by the proportion of reproductive ramets and flowering start (Figure 3 and Figure S4a). PC2 explained 16.0% of the observed variation and was mainly characterised by reproductive height and leaf thickness (Figure 3 and Figure S4b). Furthermore, PC3 also explained a higher proportion of the observed variation (14.1%) and was mainly characterised by germination start and SPAD (Figure S4c). Lastly, PC4 explained 12.1% of the observed variation and was mainly characterised by LDMC (Figure S4d). Individuals originating from Europe both germinated (Figure 4a) and flowered (Figure 4b) significantly earlier (approximately 30 days and 3 days, respectively) compared to individuals from North America (Table 1). LDMC, vegetative height, biomass and the proportion of reproductive ramets were significantly larger in European individuals (Figure 4f,g,i,j and Table 1), while leaf thickness, SLA, and reproductive height were significantly greater in North American individuals (Figure 4c,e,h and Table 1). There was no significant continental difference for SPAD (Figure 4d and Table 1).

3.3 | Environmental Influences on Cross-Continental Clines

Flowering start was significantly associated with bioclimatic PC1, latitude, and elevation (Table 2) showing that individuals originating from wetter and warmer environments, higher latitudes, and lower elevations in both continents flowered earlier (Figure 5a-c). Leaf thickness showed a negative correlation with bioclimatic PC1 and PC2 (Table 2) with overall thinner

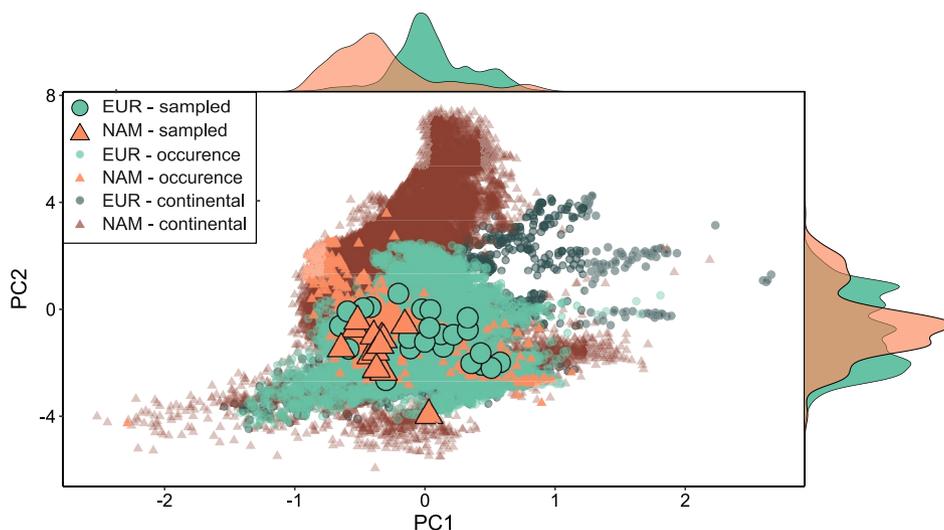


FIGURE 2 | Visualisation of the climatic space available in North America and Europe captured by principal components PC1 (related to a wetter and warmer environment) and PC2 (related to greater daily temperature swings, as well as warmer and wetter seasons), based on a principal component analysis (PCA) of 10,000 random background points per continent, on top of which are overlaid GBIF occurrences of *Milium effusum* in Europe (EUR; bright green circles) and North America (NAM; bright orange triangles) as well as the sampled populations which are depicted by larger symbols. To better illustrate overlap within the climatic space, marginal plots were added to show the distribution of PC1 and PC2 for all of the occurrences extracted from GBIF (GBIF 2024).

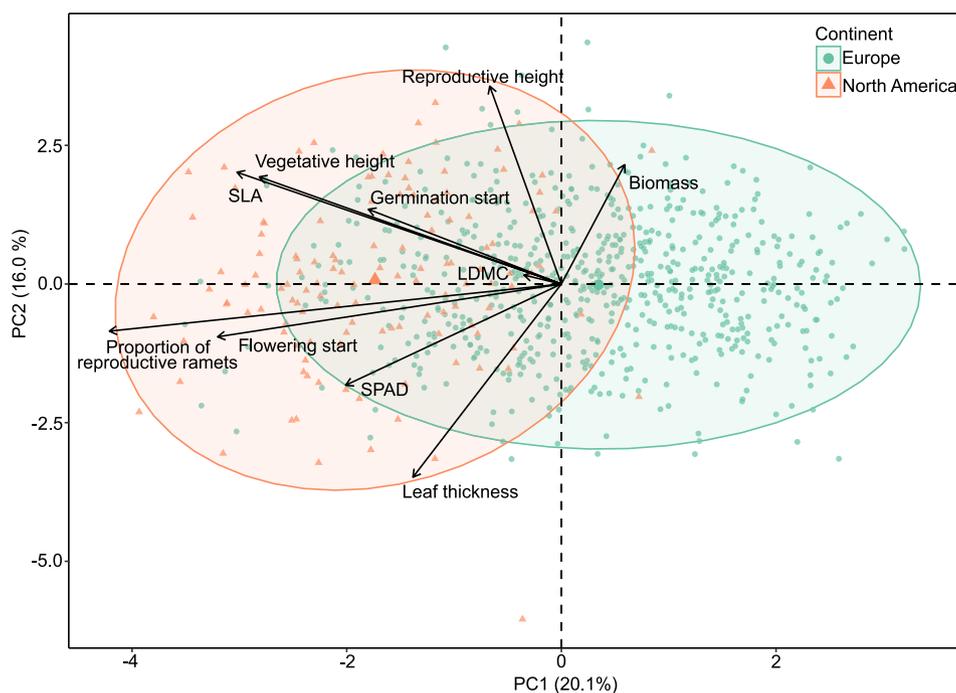


FIGURE 3 | Scatterplot of the principal component analysis (PCA) with factorial rotation to help visualise the multivariate trait space for European (green dots, $N=653$ plants) and North American (orange triangles, $N=142$ plants) individuals of *Milium effusum* grown in a common garden. The encircled area indicates the 95% confidence ellipses for the European (green) and North American (orange) individuals. SPAD is a measure of the chlorophyll content, SLA denotes the specific leaf area, LDMC denotes the leaf dry matter content.

leaves in individuals originating from populations exposed to wetter and warmer climates (Figure 5d), as well as greater daily temperature swings, warmer seasons, and wetter winters and summers (Figure 5e). SLA showed a positive correlation with bioclimatic PC2 (Table 2 and Figure 5f). The proportion of reproductive ramets showed a positive correlation with latitude (Table 2) with a higher amount of reproductive structures

in individuals originating from northern latitudes (Figure 5g). Vegetative height showed a positive correlation with bioclimatic PC1 while interacting with continent (Table 2), leading to lower vegetative height in North American individuals originating from wetter and warmer environments, while the vegetative height of European individuals differed little (Figure 5h). Reproductive height showed a significant negative correlation

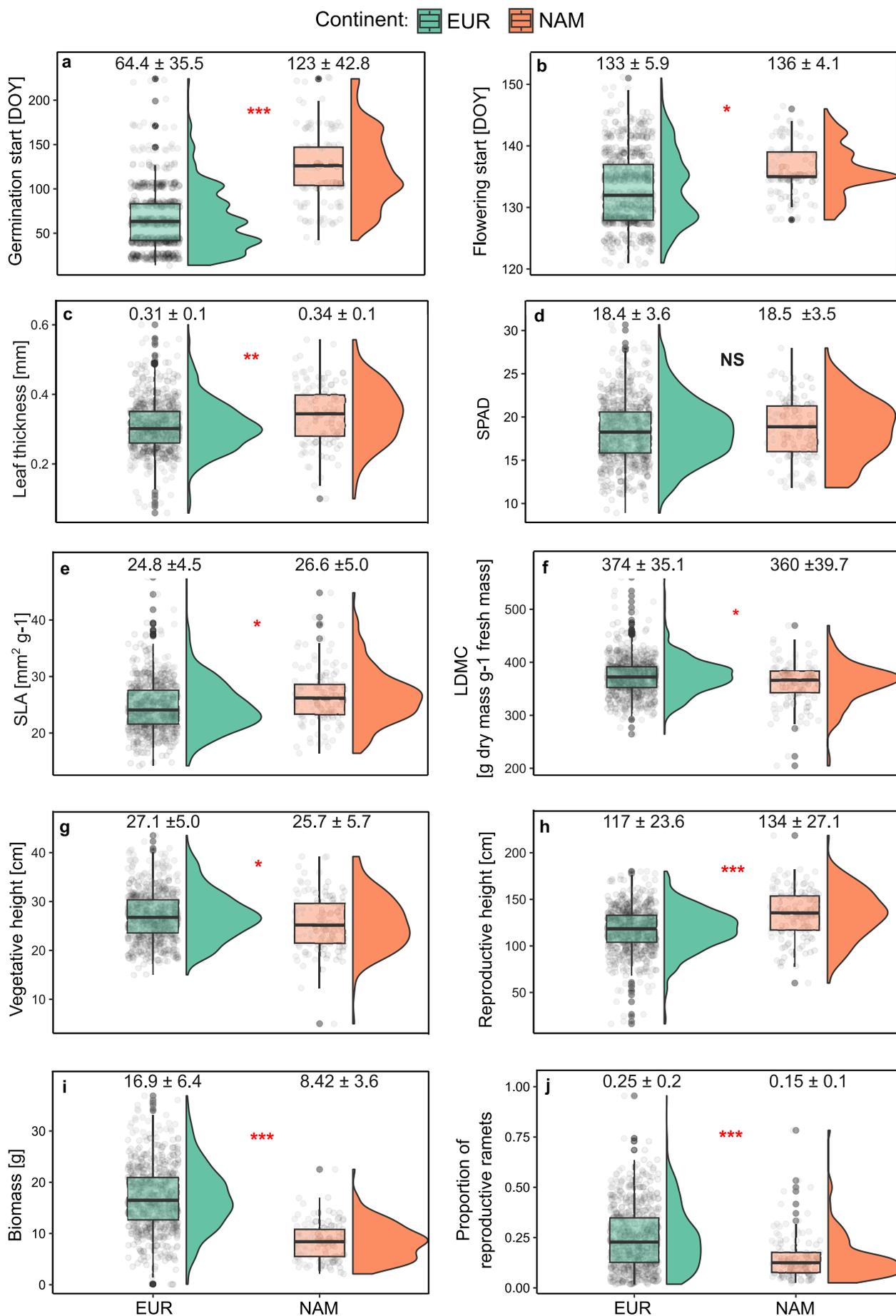


FIGURE 4 | Legend on next page.

FIGURE 4 | Functional traits of *Milium effusum* from Europe and North America measured in a common garden: (a) germination start; (b) flowering start; (c) leaf thickness; (d) SPAD; (e) SLA; (f) LDMC; (g) vegetative height; (h) reproductive height; (i) biomass; and (j) proportion of reproductive ramets for Europe (EUR: Green, $N=653$ plants) and North America (NAM: Orange, $N=142$ plants). Jittered data points and boxplots are displayed on the left side and horizontal half violin plots on the right side. Mean trait values \pm the standard deviations are given above each set of boxplots and half violins. SPAD is a measure of the chlorophyll content, SLA denotes the specific leaf area, LDMC denotes the leaf dry matter content. Non-significant differences are indicated by NS and significant P-values are indicated by * (<0.05), ** (<0.01) and *** (<0.001) based on Table 1.

TABLE 1 | Continental effect on functional traits of *Milium effusum* measured in a common garden.

Response variable	χ^2	<i>p</i>	Comparison
Germination start	34.253	<0.001	EUR<NAM
Flowering start	4.983	0.026	EUR<NAM
Leaf thickness	8.023	0.0046	EUR<NAM
SPAD ^a	0.150	0.699	NS
SLA ^b	6.400	0.011	EUR<NAM
LDMC ^c	4.237	0.040	EUR>NAM
Vegetative height	4.237	0.040	EUR>NAM
Reproductive height	13.996	<0.001	EUR<NAM
Biomass	46.485	<0.001	EUR>NAM
PRR ^d	17.095	<0.001	EUR>NAM

Note: The measured traits are response variables, while continent is the explanatory variable ($N=795$ plants). Population is set as a random intercept term. All denominator degrees of freedom were 1. Chi-square and *p*-values are given for all models. Significant *p*-values are indicated in bold. The direction of the (trait) comparison is provided.

^aSPAD is a measure of the chlorophyll content.

^bSLA denotes the specific leaf area.

^cLDMC denotes the leaf dry matter content.

^dPRR is the proportion of reproductive ramets.

with PC1 (Table 2), as individuals from cooler, less seasonal, and drier conditions grew taller reproductive ramets and inflorescence (Figure 5i). Lastly, biomass was significantly correlated with PC1, PC2, continent, the interaction between PC1 and continent, and latitude (Table 2). More specifically, European individuals originating from warmer and wetter climates had greater biomass, while North American individuals produced more biomass when originating from cooler, less seasonal, and drier conditions (Figure 5j). However, individuals from more climatically stable environments from both continents also produced more biomass (Figure 5k), and individuals from both continents produced greater biomass with increasing latitude (Figure 5l). No associations of PC1, PC2, or latitude were found on germination start, SPAD, or LDMC.

4 | Discussion

We report distinct continental differences in the geographical range and multivariate trait space of *M. effusum* between Europe and North America. Trait expressions also differed between both continents and were influenced more strongly by environmental variables than by latitude or elevation, with some effects varying depending on the continent of origin. While

traits describing functional behaviour, such as flowering start, leaf thickness, SLA, proportion of reproductive ramets, and reproductive height showed parallel trait clines to the environment between continents (environment main effects), traits of plant size, specifically vegetative height and biomass, showed contrasting clines (continent-environment interaction). These findings underpin the complex interplay of genetic, environmental, and evolutionary factors in shaping phenotypic variation across continents.

4.1 | Geographical Range and Niche Overlap

The majority of the geographical range of *M. effusum* lies in Western Eurasia, where the climate is characterised by high precipitation and moderate temperature seasonality; in contrast, eastern North American populations occur in continental areas with higher variation in temperature, precipitation, and seasonality. Though we identified a moderate climatic overlap between the two continents, large parts of the available niche space suggested by the European range remain uncolonized in North America, suggesting additional climatic tolerances in European populations not shared with their North American counterparts or perhaps limited range filling caused by restricted dispersal or physiological constraints. In addition, some bioclimatic niche space that underpins the extensive distribution of *M. effusum* in Europe is lacking in North America. More specifically, based on the Köppen-Geiger global climate classification, temperate oceanic and hot-summer Mediterranean climate is not available for *M. effusum* to the same degree in North America as it is in Europe. In North America, these climatic zones are geographically limited. Taken together, the environmental conditions that support the widespread occurrence of *M. effusum* in Europe are less prevalent or accessible in North America, restricting the species' suitable niche space. Another possible explanation could be that while it is known that *M. effusum* has two ploidy levels present in Europe (Bennett and Thomas 1991), only one ploidy might be present in North America. It is well known that species have larger geographical ranges when multiple levels of ploidy are present, and that polyploids often have larger distributions than their diploid ancestors (Kolář et al. 2017). Future studies should focus on uncovering ploidy levels of the included study populations as this might play a crucial role. Lastly, it could also be argued that the North American populations have gone through a bottleneck, and perhaps have lost some important genetic variation, limiting the species' ability to respond to novel environmental conditions (Young et al. 1996) or that simply the unoccupied niche space areas primarily located in the West Coast/Pacific NW might be inaccessible for colonisation from eastern North America due to a vast dispersal barrier across large expanses of non-forest habitats.

TABLE 2 | Effects of environmental variation among populations and of continent of origin on functional traits of *Milium effusum* measured in a common garden.

Response variable	PC1			PC2			Continent			PC1: Continent			PC2: Continent			Latitude			Elevation		
	PE	X ²	p	PE	X ²	p	PE	X ²	p	PE	X ²	p	PE	X ²	p	PE	X ²	p	PE	X ²	p
Germination start	↓	0.544	0.461	↓	1.487	0.223	↑	18.759	<0.001	↑	1.250	0.264	↑	0.054	0.816	↑	3.431	0.064	↑	1.118	0.290
Flowering start	↓	8.205	0.004	↓	1.739	0.187	↑	10.427	0.001	↓	0.096	0.756	↓	0.210	0.647	↑	6.040	0.014	↑	9.362	0.002
Leaf thickness	↓	11.024	< 0.001	↓	5.192	0.023	↑	2.428	0.119	↑	0.040	0.841	↑	2.158	0.142	↑	0.647	0.421	↓	0.035	0.851
SPAD ^a	↑	0.862	0.353	↓	0.203	0.653	↑	3.401	0.065	↑	0.000	0.990	↑	0.027	0.869	↑	1.834	0.176	↑	1.103	0.294
SLA ^b	↑	2.636	0.104	↑	7.766	0.005	↓	0.078	0.780	↓	0.155	0.694	↓	0.816	0.366	↓	3.017	0.082	↓	0.759	0.384
LDMC ^c	↑	0.009	0.926	↓	2.535	0.111	↓	0.861	0.353	↓	0.579	0.447	↓	4.436	0.035	↑	1.167	0.280	↑	0.001	0.981
Vegetative height	↓	2.327	0.127	↓	0.085	0.771	↓	3.619	0.057	↓	6.353	0.012	↑	0.029	0.864	↓	0.472	0.492	↑	0.073	0.787
Reproductive height	↓	10.144	0.001	↓	1.845	0.174	↑	2.634	0.105	↓	2.231	0.135	↑	2.790	0.095	↑	1.314	0.252	↑	0.462	0.497
Biomass	↑	9.489	0.002	↑	9.867	0.002	↓	31.544	< 0.001	↓	4.260	0.039	↓	0.438	0.508	↓	5.858	0.016	↓	0.002	0.964
PRR ^d	↑	2.158	0.142	↑	0.350	0.554	↓	11.377	< 0.001	↑	0.002	0.965	↓	1.406	0.236	↓	5.073	0.024	↓	1.748	0.186

Note: The measured traits are the response variables in distinct linear mixed-effect models (LMMs), while principal components (PC1, PC2), latitude, elevation, continent as well as interactions between PC1 and PC2 with continent are used as explanatory variables. Seed family nested within population was included as a random intercept term. The sample size (N) is 795 plants. All denominator degrees of freedom were 1. The direction of the interaction parameter estimates (PE) are given with North America used as the reference for factors; upwards arrows (↑) indicate positive estimates and downwards arrows (↓) indicate negative estimates. X² and p-values are given for all models. Significant p-values are indicated in bold.

^aSPAD is a measure of the chlorophyll content.

^bSLA denotes the specific leaf area.

^cLDMC denotes the leaf dry matter content.

^dPRR is the proportion of reproductive ramets.

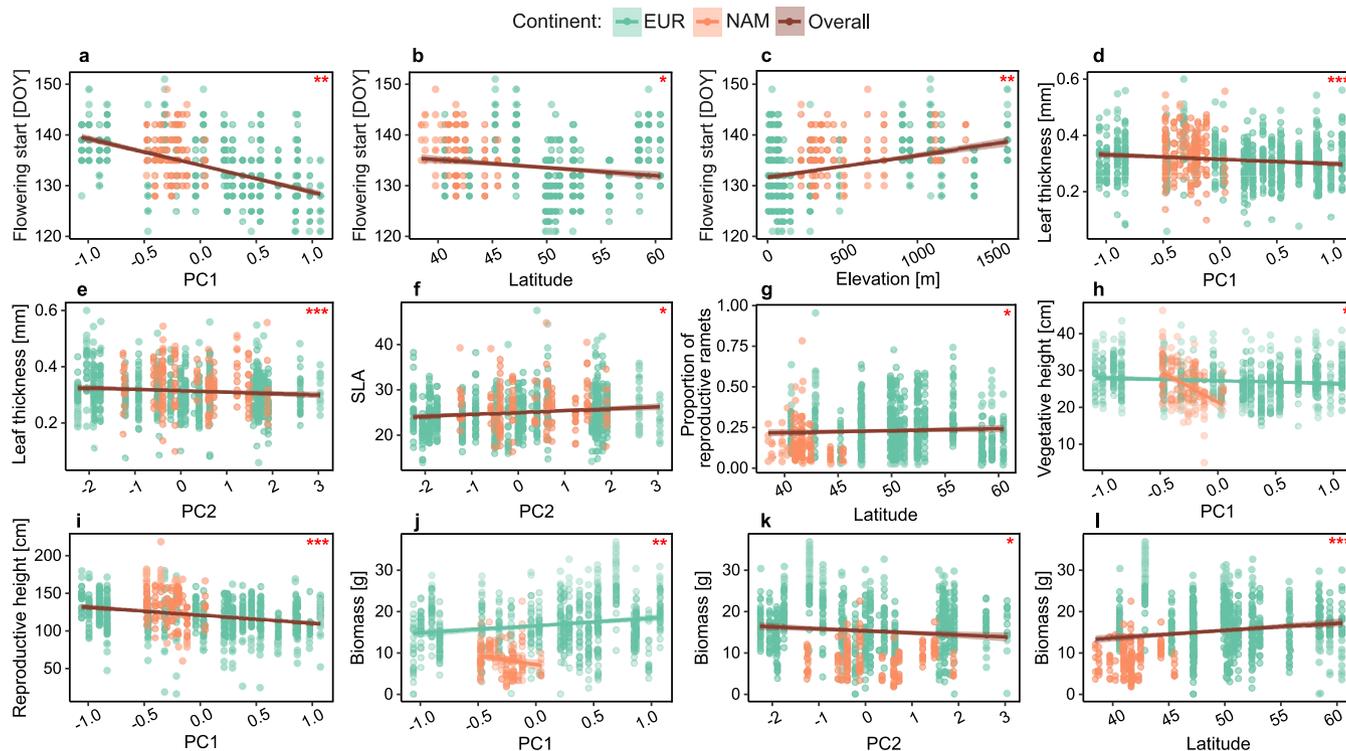


FIGURE 5 | Significant relationships between the extracted scores of the first principal component of the bioclimatic PCA (PC1: Related to precipitation of driest month, mean temperature of wettest quarter, and precipitation seasonality), the second (PC2: Related to mean diurnal range, precipitation of coldest quarter, and mean temperature of warmest quarter), and latitude with functional traits of *Milium effusum* from Europe (EUR: Green, $N = 653$ plants) and North America (NAM: Orange, $N = 142$ plants) measured in a common garden. Relationships are based on results from linear mixed-effect models (LMMs) presented in Table 2 for: (a) flowering start and PC1; (b) flowering start and latitude; (c) flowering start and elevation; (d) leaf thickness and PC1; (e) leaf thickness and PC2; (f) SLA and PC2; (g) proportion of reproductive ramets and latitude; (h) vegetative height and PC1; (i) reproductive height and PC1; (j) biomass and PC1; (k) biomass and PC2; and (l) biomass and latitude. SLA denotes the specific leaf area. Significant P-values are indicated by * (< 0.05), ** (< 0.01) and *** (< 0.001) based on Table 2.

4.2 | Continental Phenotypic Differences

We showed pronounced continental differences in the multivariate trait space. Reproductive allocation, traits linked to growth strategies, and structural investments determined the trait differentiation among continents. European populations germinated and flowered significantly earlier compared to North American populations. Similarly, a recent study of six herbaceous species naturalised in North America found earlier flowering in populations in Europe compared to North America (Reeb et al. 2024). Several of the studied European populations originated from higher latitudes than what was available from North America. When individuals originating from more northern latitudes and typically colder locations are transplanted to a common garden located further south, germination might be triggered earlier compared to other populations with different temperature requirements, leading to the observed earlier germination and subsequent flowering (Møller et al. 2023).

Leaf thickness, SLA, and LDMC differed significantly between the two continents. Individuals from North America had thicker leaves, which can be associated with major physiological processes such as photosynthesis, water use efficiency, and net productivity (Aneja et al. 2024). Furthermore, the high SLA combined with low LDMC in North American individuals suggests a priority for rapid biomass production, whereas the low SLA and

high LDMC in European individuals could indicate a priority for nutrient conservation (Garnier et al. 2001). Ultimately, this points towards North American individuals potentially being more lenient in adopting a competitive strategy, while European individuals are more stress tolerant (Grime 1977), as a potential result of different biotic and competitive environments occurring on the continents.

For *M. effusum*, biomass, vegetative height, and proportion of reproductive ramets can be viewed as fitness proxies, and greater trait means in European individuals could therefore indicate better adaptation or adaptive plastic responses to the environmental conditions experienced in the common garden in Europe compared to the North American individuals experiencing a more novel climatic environment. The nutrient-conserving strategy (low SLA, high LDMC) would allow European individuals to utilise resources over time and accumulate more total biomass and greater investment into reproductive structures, leading to more ramets reaching the reproductive stage (Poorter and De Jong 1999). In contrast, North American individuals' greater reproductive height might reflect their prioritisation of pollen and seed dispersal or other benefits linked to reproductive success, associated with either a high-resource strategy (Poorter and De Jong 1999) or an escape strategy (Franks et al. 2007). Furthermore, differences in ploidy levels could also cause the observed continental differences if we assume that North

American individuals are of single ploidy, while in Europe both di- and tetraploids occur (Bennett and Thomas 1991), stressing the importance of establishing ploidy levels in future studies.

Finally, it is important to note that as this experiment was carried out in a common garden, we can assume that the observed phenotypic differences we found among individuals from different populations and between continents are genetically based (Turesson 1922). In our case, maternal effects are most likely negligible, as most measurements were only taken in the second growing season, after individuals had gone through marcescence. However, some of these observed phenotypic differences might also be driven by the presumed difference in population size (Ellstrand and Elam 1993). Smaller populations from North America (Table S1) would be more prone to potential genetic risks as a result of genetic drift, inbreeding, or limited gene flow (Ellstrand and Elam 1993), potentially contributing to the observed differences. Furthermore, the wider geographical range of *M. effusum* in Europe can provide European populations with greater phenotypic variation due to the broader range and variety of encountered environmental conditions (Friedman et al. 2019). Future studies should try to acquire phylogenetic data, which would allow us to estimate the timing of the disjunction between European and North American populations of *M. effusum*.

4.3 | Environmental Influences on Cross-Continental Clines

Bioclimatic variables accounted for the majority of the observed trait-environment relationships, explaining 9 out of 12 significant relationships, while latitude and elevation contributed to only two and one relationships, respectively. Vegetative height and biomass were both found to be significantly affected by the interaction between bioclimatic PC1 and continent, showing that whereas European individuals grew taller and bigger when originating from warmer and wetter climate, North American individuals were smaller in size. Here, contrasting clines could imply different evolutionary outcomes under the same selective pressure, as indicated in the niche overlap of PC1.

The majority of the significant clinal trait patterns shared the same direction between continents (flowering start, leaf thickness, SLA, reproductive height, and biomass) and these similar slopes between continents suggest that the same environmental variable might have caused evolution towards the same clines on both continents (Yang et al. 2021). Strong latitudinal and environmental clines in vegetative traits have been found in previous studies comparing native and introduced/invasive populations (Maron et al. 2004; van Boheemen et al. 2019; van Kleunen and Fischer 2008; Yang et al. 2021), indicating rapid evolution towards similar clines in both phenological and growth-related traits between continents (Maron et al. 2004). Ultimately, our study species *M. effusum* might have independently evolved similar clines in response to the environmental conditions on two different continents. A crucial next step to study cross-continental patterns in *M. effusum* would be to conduct a reciprocal transplant to further study local adaptation including in situ trait measurements. This would also allow for

greater consideration when choosing and sampling the study populations. Selection of sites should focus on covering whole gradient ranges of the bioclimatic variables that were found to be important for the genetically based phenotypic variation in the traits measured within this study.

5 | Conclusions

In summary, this study highlights significant continental differences in the multivariate trait space of *M. effusum* and the effect of bioclimatic variables from the population origin. The limited distribution of *M. effusum* in North America, despite suitable climatic conditions, raises questions about historical dispersal trajectories (maybe even pre-Glacial) and potential bottlenecks. Furthermore, environmental influences from population origins revealed parallel clines suggesting convergent or parallel evolution in response to similar selective pressures in Europe and North America. Interestingly, evidence of contrasting clines between Europe and North America for some traits might suggest divergent evolutionary solutions to similar abiotic factors or the overriding influence of other ecological factors not considered here, such as regional differences in diversity and competitive environments. Future research focusing on ploidy levels will be crucial for understanding the evolutionary dynamics of *M. effusum* and its ecological strategies in different biogeographical contexts. Overall, this study highlights the complex interplay of genetic, environmental, and evolutionary factors in shaping phenotypic variation in a widespread cross-continental forest species.

Author Contributions

C.M., H.K. and M.S. conducted the experiment and took the measurements. C.M. drafted the first version of the manuscript and led the writing process. C.M., P.D.F., M.S., H.K., M.M.-S. and J.F.S. conducted the statistical analyses. C.M., P.D.F., J.M.H., J.B., J.B., H.H.B., J.C., G.D., M.D., B.F., J.H., P.-O.H., B.L.I., A.K., J.L., J.L., M.M.-S., A.M., A.O., B.W. and F.S. sampled the seeds. All co-authors provided comments on the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data and R code used to conduct the analyses will be made available in Dryad upon acceptance of the manuscript: <https://doi.org/10.5061/dryad.n8pk0p36k>. Reviewer link: http://datadryad.org/share/LINK_NOT_FOR_PUBLICATION/jbqAR210MMOXvM7GWqY_tJaG5J-4bvWePN5WvuG7ww4.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Distribution maps providing the basis for the range polygons used in Figure 1. **Figure S2:** PCA results for the 19 bioclimatic variables. **Figure S3:** Contributions of the bioclimatic variables to PC1 and PC2. **Figure S4:** Contributions of the measured traits to PC1 and PC2 (Figure 3 PCA). **Table S1:** List with information on the location of the sampled populations. **Table S2:** List of the 19 bioclimatic variables derived from the WorldClim database.