Original Research Article

Climate-driven in-situ trait variation in an annual ruderal grass across Europe

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Running title:

Climate-driven in-situ trait variation in a ruderal grass

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Abstract

Background and Aims: Plant functional traits link environmental conditions to plant performance and adaptation. Growing evidence suggests that intraspecific trait variation can be as important as differences between species, yet large intraspecific studies of in-situ variation remain rare. While most studies have focused on plant morphological traits, the concentrations of elemental nutrients in seeds have received much less attention so far.

Methods: We conducted a large-scale in-situ study of the widespread annual ruderal grass *Hordeum murinum*. We sampled 2070 individuals from 207 populations across a large part of its native range in Europe and North Africa. We measured seed ripening phenology and growth-related traits in-situ and analyzed concentrations of elemental nutrients in the seeds.

Key results: We found that *Hordeum murinum* grew larger, produced seeds later, and had heavier seeds in colder and wetter regions. Plants growing in denser vegetation were taller and produced heavier seeds but formed fewer spikes. Concentrations of elemental nutrients in the seed generally declined with seed weight and were primarily driven by climatic variables, whereas soil conditions had only minor effects on plant traits and seed nutrients. Population identity explained a substantial proportion of trait variation, indicating a possible genetic component.

Conclusions: Our findings provide a comprehensive view of how *Hordeum murinum* responds to environmental gradients across its European distribution. Climatic variables, particularly temperature, are key drivers of reproductive timing and concentrations of elemental nutrients in the seed, whereas local environmental conditions, such as biotic pressures, are more critical for growth-related traits. Together, these patterns indicate that *Hordeum murinum* modulates its growth and reproductive investment along environmental gradients, balancing phenology, stress tolerance, and limited competitive capacity.

Keywords: climate, environment, grasses, *Hordeum murinum*, in-situ sampling, intraspecific trait variability, ruderals, seed elemental nutrients

Introduction

Plant functional traits are key adaptations to optimize plant performance depending on environmental conditions (Bruelheide et al., 2018). While most studies have focused on comparing mean functional traits across species or entire communities (Moles et al., 2009), substantial evidence documents considerable intraspecific variation in plant functional traits (Albert, 2015; De Frenne et al., 2013). This variation reflects functional differences within and among plant populations in response to local environmental conditions (Klein-Raufhake et al., 2022), and its magnitude can be similar to variation observed between species (Tautenhahn et al., 2019). Heritable intraspecific trait variation is typically evaluated in common garden experiments (Leiblein-Wild & Tackenberg, 2014), but trait measurements in natural environments are essential for an ecologically meaningful understanding of how individuals perform under such conditions (Helsen et al., 2017).

Key life-history and morphological traits, such as reproductive phenology, plant height, specific leaf area, and seed weight, directly influence plant survival and reproductive success (Adler et al., 2014; Farris & Lechowicz, 1990). Variation in these traits reflects local abiotic and biotic conditions, including water availability, soil nutrients, pH, and intensity of competition (Andrade et al., 2014; Helsen et al., 2017; Lechowicz & Blais, 1988). Life-history and morphological traits also vary along large-scale climatic gradients in temperature and precipitation (Leiblein-Wild & Tackenberg, 2014; Lemke et al., 2015). Annual plants growing in warmer and drier climates often flower earlier than their conspecifics growing in colder and wetter climates, which allows them to complete their life cycles before environmental conditions get too dry and hot during summer (Evans et al., 2005). Plants in drier regions are generally shorter and produce fewer spikes than conspecifics in wetter regions, a pattern commonly attributed to adaptation to water limitation and thermal stress (Nour et al., 2024). Based on a meta-analysis, De Frenne et al. (2013) found that plants from low-latitude populations tend to produce heavier seeds than conspecific individuals from high-latitude populations, although the direction and magnitude of latitude effects differ considerably among species. Nevertheless, studies of intraspecific variability in life-history and morphological traits are still limited, particularly in sufficiently high spatial resolution across large geographic and climatic gradients.

An important but often overlooked set of plant traits is the concentration of elemental nutrients in the seeds. They are essential for seedling establishment and early survival because seedlings initially rely entirely on internal nutrient reserves before root systems are sufficiently developed for external acquisition (Slot et al., 2013). Like morphological plant traits, seed nutrient concentration is affected by the environment. Nutrient-rich soils generally promote higher seed nutrient concentrations, as greater soil nutrient availability increases the amount of nutrients

available for seed filling (Long et al., 2025). Climatic factors such as temperature and water availability can further modulate seed nutrient concentrations, partly through their effects on nutrient uptake and the duration of seed filling (Sehgal et al., 2018). Seed nutrient concentrations also vary along environmental gradients, with direction of these relationships being species-specific (Etienne et al., 2018). Most of our understanding of how the environment affects variation in seed nutrients comes from crop species, and there is a notable gap regarding the intraspecific variability in the concentration of elemental nutrients in seeds in wild plants (see De Frenne et al., 2011; Li et al., 2023; Wang et al., 2025; Wu et al., 2024).

Plant functional traits are not independent, but often covary within species (Sandel et al., 2016). For example, larger plants often produce larger seeds (Guo et al., 2010; He et al., 2023; Hendrix & Sun, 1989). Concentrations of seed elemental nutrients tend to decrease with greater seed weight (Uauy et al., 2006), probably because larger seeds contain a higher proportion of carbohydrates or lipids and thus, lower concentrations of elemental nutrients (Wang et al., 2016). However, empirical data examining intraspecific covariation between seed nutrient concentration and morphological traits in populations of wild species growing in their characteristic habitats remains scarce (De Frenne et al., 2011; Henery & Westoby, 2001).

In-situ intraspecific trait variation in wild plants is particularly interesting in species with broad geographic distributions. These species encounter a wide range of environmental conditions, which is reflected in a large variation in life-history traits (Lemke et al., 2015). Such variation can be related to, for example, climatic, edaphic, or land use gradients. However, such gradients often covary across large scales, which makes the identification of the drivers of intraspecific variability based on a small number of populations difficult (De Frenne et al., 2013; Helsen et al., 2017). Disentangling the effects of individual environmental factors on intraspecific variation

requires data collected from many populations across diverse environments, at high spatial resolution and on a large scale, but such data is rare so far.

To fill this gap, we focused on in-situ trait-environmental association of a wild grass, *Hordeum murinum* L. (mouse barley, wall barley), a relative of cultivated barley (*H. vulgare* L.). This species is native to Europe and adjacent regions, widespread, and commonly occurs in human-disturbed habitats (Davison, 1977). We measured seed ripening phenology and growth-related traits of 2070 plants from 207 populations distributed from North Africa to southern Scandinavia. For a subset of 940 plants from 196 populations, we additionally quantified the concentration of elemental nutrients in the seeds. We related these traits to climatic variables, local competition intensity, and soil conditions.

Specifically, we tested three main hypotheses: (i) Seed ripening phenology, growth-related traits, and concentrations of elemental nutrients in the seeds covary with each other. Specifically, we expect that growth-related traits will negatively correlate with seed ripening phenology, because plants that grow longer and larger invest in reproduction only later. Growth-related traits will also be negatively correlated with concentrations of elemental nutrients in the seed, because larger plants produce larger seeds that contain more carbohydrates and thus lower concentrations of elemental nutrients through dilution effect. (ii) Seed ripening phenology and growth-related traits will vary mainly along climatic gradients. Specifically, we expect that plants in warmer and drier areas will be smaller and reproduce earlier because they prioritize reproduction over growth in spring to avoid heat and drought stress in summer. Plants in colder and wetter areas can grow longer, larger, and reproduce later. (iii) Concentrations of elemental nutrients in the seeds will be mainly associated with local conditions, particularly soil quality, because plants growing in nutrient-rich soils have access to more nutrients to invest in the seeds.

Materials and methods

Study species

Hordeum murinum L. is a winter annual grass native to the Mediterranean region, most of Europe, Central Asia, Western Himalayas, Macaronesia, and the Azores. It has been introduced by humans to Australia, North America, and many other parts of the world, where it has become invasive (Fleet & Gill, 2010; Jacobsen & Bothmer, 1995). Across most of its range, *H. murinum* typically grows in regularly disturbed open habitats such as fallow land, roadsides, urban green spaces (e.g., median strips), riverbanks, vineyards, agricultural field margins, and only rarely in meadows (Davison, 1977).

Hordeum murinum typically flowers between early spring and late summer, depending on the region. Flowers are predominantly self-pollinating, and seeds are dispersed through animals or humans when spikelets adhere to animal fur or human clothing. Seeds typically germinate from late summer or early autumn through winter, plants overwinter in vegetative stage and start flowering in spring (Davison, 1977; Jacobsen & Bothmer, 1995; Mizianty, 2006).

Hordeum murinum is a part of an aggregate taxon *Hordeum murinum* agg. that comprises three subspecies differing by cytotypes: diploid (2n = 2x = 14, subspecies *glaucum* (Steud.) Tzvelev), tetraploid (2n = 4x = 28, subsp. *murinum*, or *H. murinum* s. str.), and hexaploid (2n = 6x = 42, subsp. *leporinum* (Link) (Cuadrado et al., 2013). This study focuses on the tetraploid cytotype, *H. murinum* subsp. *murinum*, across Europe and the Mediterranean region (Figure 1). In the southern part of this region, below latitude 45°, *H. murinum* also occurs as diploid (Jacobsen &

Bothmer, 1995), but the exact distribution of the two cytotypes is unclear. As we could not differentiate the subspecies in the field based on morphology, we determined the cytotype of the sampled populations via flow cytometry (see Appendix). Tetraploid populations were detected throughout the entire sampled range, whereas both diploids and tetraploid populations occurred in mainland Spain, Algeria, and Morocco, some populations contained both ploidy levels (Figure S1). As we focus on tetraploids here, we excluded all diploid and mixed populations from the main analyses. Differences between diploids and tetraploids populations are not addressed here, but corresponding data are provided in the Appendix to document cytotypic variation within the sampled dataset (Figure S1, S2, S3, Table S1).

Plant material and in-situ trait scoring

We scored and sampled 242 natural populations of *H. murinum* across Europe, of which 207 populations were identified as exclusively tetraploid and included in the main analyses (Figure 1). Most populations were sampled during the seed ripening period in summer 2023, while six populations were sampled in 2022 and four in 2024. Depending on geographic location, the seed ripening period ranged between March and August. We defined a population as a site with the presence of at least ten individual plants. We kept at least 1 km distance between two neighboring populations, with a median nearest-neighbor distance of approximately 18 km, to increase the likelihood that the populations are genetically differentiated.

Within each population, we randomly sampled 10 plants spaced at least 5 m apart. For each plant, we measured plant height, defined as the length of the longest tiller excluding the spike, because ripe spikes tended to disintegrate as soon as we touched the plant. We then recorded the number

of tillers bearing spikes (both unripe and ripe) and harvested one spike per plant with at least 50 % visibly ripe seeds. In the laboratory, we extracted all seeds from the harvested spikes, counted and weighed them, and stored them in a fridge. As the spikes rapidly disintegrate when ripe, we rarely collected a whole spike. We thus did not use the number of seeds per spike in further analysis, but rather seed weight standardized to thousand-seed weight.

We used the seed collection date as a proxy for seed ripening phenology. We mostly sampled the seeds when we observed that the first spikes in a population are yellow, which indicates ripe seeds. In some cases, later collection was unavoidable, however, plants typically retain ripe seeds for approximately two to three weeks before complete seed shattering. This introduces only limited uncertainty at the population level relative to inter-populational differences, which spanned approximately six months.

For six of the 207 tetraploid populations, all spikes were empty, without seeds; however, seed ripening phenology, growth-related traits, and environmental measurements were still available. For two populations, we obtained ripe spikes and soil samples (see below), but measurements of growth-related traits and local environmental variables were missing. These populations were retained where possible for analyses not requiring the missing variables.

Environmental variables

To characterize local environmental conditions, we focused on the intensity of competition and soil characteristics. We visually estimated vegetation cover and the cover of impervious surfaces (e.g., asphalt, cobblestone, or concrete) within a 1 m² area surrounding each sampled plant. We

also collected soil samples from beneath each sampled plant and pooled at the population level. Soil samples were air-dried at room temperature and stored under refrigerated conditions upon arrival at the laboratory.

We obtained climatic variables from the CHELSA database (Karger et al., 2021), which provides climate data for the period 2000-2020 at a ca. 1 km² (30 arcsec) spatial resolution. We initially extracted all ten bioclimatic variables that were available at the 30 arcsec resolution. Because these variables were highly collinear ($r \geq |0.5|$, Figure S4), we retained only mean annual temperature (°C), temperature seasonality (°C, standard deviation of the monthly mean temperatures), and annual precipitation (mm) for subsequent analyses, because they allow intuitive interpretation and were the least correlated with other variables.

To measure the pH of the soil, we added 25 mL of 0.01 M $CaCl_2$ solution to a subsample of 10 g of the pooled soil samples, followed by shaking for one hour. After the suspension had settled, we measured the pH of the supernatant using a calibrated pH electrode (HANNA instruments, pH 211 Microprocessor pH Meter).

To quantify plant-available inorganic nitrogen, phosphorus, and sulphur as a proxy for readily available soil nutrients in the soil, we analyzed soil concentrations of nitrate, phosphate, and sulphate. Specifically, 100 mg of soil was extracted in 300 µL of deionized water for 6 h at 30 °C. After 15 min centrifugation at 4 °C at a maximum speed of 12 000 g, the supernatant was transferred into HPLC vials with inlets. Inorganic anions were quantified using a Dionex ICS-1100 ion chromatography system equipped with a Dionex IonPac AS22 RFIC (4 x 250 mm) analytical column (Thermo Scientific, Darmstadt, Germany) Dietzen et al., 2020). A 4.5 mM $NaCO_3$/1.4 mM $NaHCO_3$ solution was used as a running buffer. Standard curves were generated

using the external standard solutions of 0.05, 0.1, 0.2, and 0.5 mM KNO_3, K_2HPO_4, K_2SO_4, and KCl.

Concentration of elemental nutrients in the seed

We used inductively coupled plasma - mass spectrometry (ICP-MS) to determine the elemental concentrations of nutrients in the seed, specifically macronutrients Ca, K, Mg, P, and S, and micronutrients Cu, Fe, Mn, Mo, Ni, and Zn (Almario et al., 2017). Two to three seeds per individual (8 to 20 mg) were dried at 60 ºC overnight and homogenized to a fine powder. The seed material was digested with 500 µL of 67% (w/w) HNO_3 in 15 mL polypropylene Falcon tubes overnight at room temperature. Subsequently, loosely closed tubes containing the samples were heated in a 95 °C water bath until the liquid became completely clear (approximately 30 min). After cooling to room temperature for 10–15 min, the samples were placed on ice and diluted with 4.5 mL of deionized water. The samples were centrifuged at 2,000 × g for 30 min at 4 °C, and the supernatants were transferred into new tubes for analysis. The elemental concentrations were determined using an Agilent 7700 ICP-MS (Agilent Technologies) (Almario et al., 2017). The selected method allows quantification of many elemental nutrients except nitrogen, because it involves digesting the material in HNO_3. We acknowledge that nitrogen is important for plant performance, and the absence of data on this element is a limitation of this study. Future studies combining elemental analysis with nitrogen quantification methods would provide a more complete picture of seed nutrient composition and its ecological relevance.

To reduce analytical costs while maintaining broad geographic coverage, we analyzed the concentration of nutrients in the seeds for only a subset of six randomly selected plants per

population and restricted sampling to populations located at least 10 km apart. This design aimed to capture large-scale spatial variation in seed nutrient concentrations without compromising the assessment of fine-scale variability. In total, we analyzed the concentrations of elemental nutrients in the seed for 940 individuals from 196 populations.

Statistical analysis

First, we explored the strength and direction of covariation among seed ripening onset, growth-related traits (height, spike number, and seed weight), and concentrations of elemental nutrients in the seed. We performed Pearson's correlation analyses and visualized significant correlations using a correlation matrix generated with the *Hmisc* package (Harrell Jr, 2025). Because seed ripening onset was available only at the population level, we used the population means for correlations involving this trait. Correlations among other traits were calculated at the individual plant level. All correlation tests were corrected for multiple testing using Benjamini-Hochberg correction (Benjamini & Hochberg, 1995).

We applied the same approach to examine correlations among environmental predictors, including the selected climatic variables, vegetation cover, impervious surface cover, and soil properties. For this analysis, we used population-level averages for all predictors as most environmental variables were measured at the population level, except vegetation cover and impervious surface cover. Vegetation cover and impervious surface cover were strongly negatively correlated ($r = -0.62$), which adversely affected model stability. We therefore retained only vegetation cover for subsequent analyses.

Second, we assessed whether populations differ in terms of growth-related traits and concentrations of elemental nutrients in the seed. To do this, we ran separate linear models for each parameter, using population identity as a categorical predictor (Table S2). Seed ripening onset could not be included in this analysis because it was available only at the population level.

Third, we tested how the onset of seed ripening, growth-related traits, and concentrations of elemental nutrients in the seed relate to climate and environmental factors. Explanatory variables were three climatic predictors (mean annual temperature, temperature seasonality, and annual precipitation) and five local environmental variables (vegetation cover, soil pH, and bioavailable soil nutrients: nitrate, phosphate, and sulphate).

The onset of seed ripening was available only at the population level. We thus related the onset of seed ripening to population-level averages of the environmental variables in a linear model. To test for spatial autocorrelation, we applied the function *moran.test*, *spdep* package (Bivand, 2022) on the model and found significant spatial autocorrelation in the ordinary least-squares model residuals (Moran's $I = 0.12$, $p < 0.001$). Therefore, we refitted the model using a spatial lag regression, *spatialreg* package (Bivand et al., 2021), which substantially reduced spatial autocorrelation in the residuals (Moran's $I = 0.04$, $p = 0.08$). Because spatial regression models do not provide conventional R^2 values, we quantified model fit using a pseudo-R^2 statistic, calculated as one minus the ratio of the residual variance to the total variance of the response variable (Veall & Zimmermann, 1996). To assess the relative contribution of individual predictors, we compared changes in AIC after sequentially removing each predictor from the full model (Burnham & Anderson, 2002). The resulting ΔAIC values were standardized to percentages to express the relative contribution of each predictor to overall model fit.

Growth-related traits and concentrations of elemental nutrients in the seed were available at the level of individual plants. We thus built fourteen linear mixed models (using the *lme4* package (Bates et al., 2015)), one model each for the three plant growth-related traits and the 11 investigated seed nutrients as respective response variables. As fixed explanatory variables, we used the three climatic and five local environmental predictors. Population identity was included as a random effect to account for the non-independence of plants within each population. To test spatial autocorrelation, we plotted the residuals from each of the 14 linear mixed models on a map, with point size representing residual magnitude and color indicating whether the residuals were negative or positive. We did not find discernible spatial patterns that would indicate autocorrelation of the residuals and retained the original models (Dale & Fortin, 2014). To determine the proportion of variation explained by the models (fixed, random, and whole model R^2 value), we used the *rsq* package (Zhang, 2016). The relative importance of individual fixed-effects predictors was assessed using the *partR2()* function from the *partR2* package (Stoffel et al., 2021).

To meet model assumptions, we log- or square root-transformed the response variables where necessary (Tables S4, S5, and S6) and evaluated the model fit through residual diagnostics (Zuur et al., 2010). All data analyses were conducted using R version 4.3.1 (R Core Team 2023) within the RStudio environment.

Results

The onset of seed ripening, an aspect of plant reproductive phenology, was positively correlated with the average number of spikes per plant and negatively correlated with concentrations of

elemental nutrients in the seeds (Figure 2). In other words, populations that ripened seeds later tended to produce more spikes per plant, whereas their seeds had lower concentrations of elemental nutrients. Growth-related traits (plant height, seed weight, and spike number) were positively correlated with each other, with the strongest relationship between plant height and seed weight (r = 0.60) (Figure 2). Similarly, ~70% of the pairwise correlations among seed nutrient concentrations were positive. The strongest positive correlation was between P and Mg (r = 0.65). Seed ripening onset and growth-related traits were generally negatively correlated with elemental nutrient concentrations in the seeds, particularly Ca, K, Mg, P, Ni, and Zn. The strongest negative correlations were between seed ripening onset and K (r = -0.49), and between seed weight and Ca and K (both r = -0.44). Molybdenum (Mo) deviated from this pattern, showing a positive correlation with seed ripening onset (r = 0.31), seed weight, and spike number (both r = 0.09). Pairwise correlations between environmental predictors (climate, ground cover, soil properties), even when significant, were weak (in most cases $r < 0.3$; Figure 3). The only exceptions were correlations between vegetation cover and cover of impervious surface (r = -0.62), and between mean annual temperature and temperature seasonality (r = -0.44; Figure 3).

All three growth-related traits were significantly differentiated among populations, with population identity explaining 37-38 % of the variation when we fitted population identity as a single predictor (Table S2). Seed nutrient concentrations were also significantly differentiated among populations, with the highest differentiation observed for Cu ($R^2 = 0.57$) and the lowest for Ni ($R^2 = 0.24$). In models including environmental predictors as fixed variables and population identity as random factor, population identity (marginal R2) still explained more variability than the environmental predictors in both growth-related traits and seed nutrient concentrations (pie charts in Figures 4 and 5, Table S2, S3, S4). Population differentiation in seed

ripening onset could not be assessed because this trait was represented by a single value per population.

Seed ripening phenology was primarily related to climate and, to a lesser extent, soil nitrate. Seed ripening occurred earlier in populations in warmer regions and in regions with lower temperature seasonality. Together, these two climatic variables explained approximately 88 % of the variation in seed collection timing among populations. Seed ripening occurred slightly later in nitrate-rich soils, although this variable explained only a small proportion of variation ($R^2 = 0.04$).

Growth-related traits were related to local vegetation cover and climate. Plant height and seed weight were primarily affected by vegetation cover, annual precipitation, and mean annual temperature, with increased vegetation cover, higher annual precipitation, and lower mean annual temperature leading to taller plants and heavier seeds (Figure 4). The number of spikes was most affected by mean annual temperature and vegetation cover, with number of spikes per plant being higher in colder climates, and when plants were surrounded by less vegetation.

The concentrations of elemental nutrients in seeds (Ca, K, Mg, P, S, Mn, Mo, Ni, and Zn) were most affected by the mean annual temperature. In general, warmer climates were associated with higher elemental concentrations, except for Mo, which showed an opposite response (Figure 5). Effects of temperature seasonality and annual precipitation varied among nutrients, for example, K and Cu decreased with increasing seasonality, whereas Mg and Ni increased with temperature seasonality. Local predictors also showed element-specific effects. For example, seed Fe and Mn concentrations significantly decreased with soil pH, while Mo increased with soil pH. Nitrate had a positive effect on the concentration of Mg, P, and Ni in seeds. Other soil properties, such as phosphate and sulfate levels, had mostly no effect on the seed elemental nutrient concentration,

except for soil sulfate, which had a positive effect on seed Cu. Vegetation cover was negatively associated with Ca, K, Zn, Cu, and Ni, although the variance explained by vegetation cover was low (Figure 5).

Discussion

Understanding how plants respond to environmental change requires integrative analyses linking plant functional traits with local environmental conditions and broad-scale climatic gradients (Moran et al., 2016). Here, we present a comprehensive, continental-scale analysis of intraspecific trait variation in a wild plant species spanning approximately 4500 km. The dataset combines in-situ seed ripening phenology and growth-related traits of 2070 individual plants across 207 populations, complemented by concentrations of elemental nutrients in the seeds for a subset of 940 individuals of *Hordeum murinum*. To our knowledge, this dataset represents one of the most extensive collections of in-situ data on seed elemental nutrient concentrations in a wild plant species across such a broad geographic gradient. This unique dataset allowed us to determine that variations in reproductive phenology, growth-related traits, and seed nutrient concentrations are not driven by the same environmental factors.

Our results reveal clear ecological patterns: *H. murinum* in colder and wetter regions grew larger, produced seeds later, and had heavier seeds. Plants growing in denser vegetation were also taller and produced heavier seeds but formed fewer spikes, highlighting the importance of local biotic context for growth-related traits. Seed elemental concentrations tended to decline with increasing seed weight and were primarily associated with climatic variables, whereas soil properties generally showed weak associations with both growth-related traits and concentrations of

elemental nutrients in the seed. Importantly, population identity explained a substantial proportion of variation in plant traits and concentrations of elemental nutrients in the seed beyond the effects of measured environmental predictors, suggesting the presence of persistent population-level differences.

Plant phenology and growth-related *traits*

Plants in colder regions ripened seeds later in the season. Specifically, seed collection dates, used as a proxy for seed ripening phenology, ranged from March in Mediterranean regions to August in northern Central Europe. Temperature effects on plant phenology are widespread across plant species (Boyko et al., 2023), particularly in annuals, which often complete their life cycle earlier in spring in warm and dry regions to avoid summer drought and heat stress (Evans et al., 2005). Our results are therefore in line with these general patterns.

Plants were generally larger in colder and wetter regions. Specifically, plant height, seed weight, and number of spikes were all positively intercorrelated and decreased with mean annual temperature, and increased with mean annual precipitation (significant for the first two traits). A similar increase in plant height in colder areas has been reported by De Frenne et al. (2011), who attribute this to the longer photoperiod during the growth season in northern (colder, longer photoperiod during growing season) versus southern (warmer, shorter photoperiod) parts of Europe. In the case of *H. murinum*, plants in the warmer and drier regions in the south senesce in spring to avoid losses through drought and heat stress, which means they experience a relatively short growing period and grow during winter when the photoperiod is relatively short. On the other hand, plants in the colder and wetter regions can prolong their growth into late spring when

the photoperiod is long, accumulate more biomass and consequently more carbohydrates in the seeds (Dupont & Altenbach, 2003).

Plant height and seed weight also increased in denser vegetation (Figure 4). Plants commonly grow taller in denser vegetation to escape competition for light (Moles et al., 2009), and plant size is positively genetically correlated with seed weight in barley (He et al., 2023). Dense vegetation also slightly reduced the number of spikes produced per plant, probably because of increased competition, with plants having to invest more in elongation growth to escape competition for light, rather than investing in reproduction. Plants in denser vegetation thus invested their carbohydrates in fewer seeds, leading to increased individual seed weight (Salisbury, 1942). It is also possible that denser vegetation implies more resources that can be invested in the seed, but vegetation cover correlated neither with precipitation as a proxy of water availability, nor with nutrient availability in the soil, except for sulphate, but even this relationship was weak.

Available soil nutrients and soil pH had no significant effect on the growth-related traits, and only a limited effect on plant phenology. This is surprising given the ample literature documenting the effect of nutrient addition on traits like height or number of flowers (Andrade et al., 2014; Barker & Pilbeam, 2015; Lechowicz & Blais, 1988). However, increased soil nutrient availability may promote the growth of co-occurring species, thereby intensifying competitive pressure and potentially negating any specific growth benefits to individual plants (Tan et al., 2025). Furthermore, some nutrients, particularly nitrate and sulphate, are highly mobile in soil and prone to leaching, and concentrations of these nutrients in soil at the time of sampling possibly do not reflect the availability of these nutrients during the mother plant growth and seed development (Bünemann & Condron, 2007; Cameron et al., 2013). Another possible reason may lie in our

sampling design: we used one bulk soil sample per population. If the soil conditions of individual plants within the site strongly varied, we did not capture this variation, which may have reduced our ability to detect the effect of soil on plant performance. On the other hand, our results correspond to recent analyses of global intraspecific trait variability in grasses, which have found that soil characteristics are far less important than climate (Griffin-Nolan et al., 2025).

Concentrations of elemental nutrients in the seeds

Our dataset, comprising seeds from almost 1000 plants chemically analyzed for concentrations of eleven elemental nutrients across a broad geographic gradient, provides a rare perspective on intraspecific variation in seed nutrient concentrations. To our knowledge, no comparable dataset exists that captures intraspecific seed nutrient variation at this spatial and ecological scale. Such breadth allows us to address patterns that remain largely unexplored, as previous studies have rarely examined seed nutrient composition across extensive geographic ranges within a wild species (but see De Frenne et al., 2011; Wang et al., 2025; Wu et al., 2024 for smaller-scale studies).

Seed nutrients were generally positively intercorrelated. The strongest correlation was between seed P and Mg ($r = 0.65$, Figure 2), likely because their uptake and transport are partly coordinated (Weih et al., 2021), and because most seed P is stored as phytic acid, which binds cations including Mg (Wu et al., 2009). Generally, nutrient loading into seeds is often co-regulated during seed development (Himelblau & Amasino, 2001).

We found negative correlations between some seed nutrients, particularly Ca, K, and Ni, and the growth-related traits, particularly seed weight. In other words, larger seeds had lower concentrations of these elements. Similar trade-offs between seed weight and protein content

have been reported in cereals (Uauy et al., 2006) and the model species *Arabidopsis thaliana* (Chardon et al., 2014). This relationship has been linked to the accumulation of carbohydrates in the endosperm, specifically, larger seeds have more carbohydrates and thus lower concentrations of elemental nutrients (Sehgal et al., 2018).

The concentration of most elemental nutrients in seeds increased with mean annual temperature. This relationship was partially driven by decreasing seed weight towards warmer regions, particularly for seed elemental nutrients that negatively correlated with seed weight (Ca, K, and Ni, Figure S5). Plants from warmer areas had higher concentrations of these nutrients, likely because they had lower relative concentrations of carbohydrates. However, concentrations of nutrients that were unrelated to seed weight also increased with mean annual temperature (Mg, P, S, Mn, Ni, and Zn). Positive effect of mean annual temperature on nutrient concentrations aligns with findings of a recent study on two grass species along a latitudinal gradient in China (Wang et al., 2025), and with a study on European forest herb (De Frenne et al., 2011).

Soil characteristics had only a limited effect on nutrient concentration in the seeds. The strongest effect had soil pH, which negatively affected the concentration of Fe and Mn and positively affected Mo. The negative effects of pH on Fe and Mn are not surprising because the bioavailability of Fe and Mn is higher in acidic environments (Ramzani et al., 2016; Sims, 1986). Similarly, Mo is more available in alkalic soils (Rana et al., 2025). Surprisingly, soil available nutrients, specifically nitrate, phosphate, and sulphate, did not affect the concentration of the respective elements in the seeds, and had, in general, only a very small effect on seed concentration of other elemental nutrients. This contrasts with ample literature on crops showing that soil nutrient availability is crucial for nutrient concentrations in the seeds (Joy et al., 2015; H. Marschner, 2012; Wortmann et al., 2018). As mentioned above, one possible explanation is the

high mobility of nitrate and sulphate in the soil (Bünemann & Condron, 2007; Cameron et al., 2013). Another reason might be the pooling of soil per population, which prevented us from capturing fine-scale mosaics in soil characteristics experienced by individual plants. However, our results are in line with the few existing studies on wild species that show that seed nutrient concentration across large geographic gradients is highly variable and only weakly correlated with soil properties (Wang et al., 2025; Wu et al., 2024).

Population differentiation

Although many environmental predictors were significantly associated with growth-related traits and concentrations of elemental nutrients in the seeds, they explained only 4.9-12.4 % and 3.3-22 % of the variation, respectively. Much more variation (32-34 % and 20-45 %, respectively) was explained by population identity, independent of environmental factors. In other words, plants growing in one population had more similar traits than predicted by environmental conditions.

One possible explanation is that our environmental variables did not fully capture site-specific conditions experienced by individual plants. However, the most important environmental drivers of plant traits across large geographic scales are associated with climate (Lemke et al., 2015) and are thus unlikely to stand behind the large proportion of variability explained solely by the site identity. More likely, the similarity of plants growing at one site is caused by genetic factors. *H. murinum* is predominantly self-pollinating, and thus, most plants at one site are likely to be close relatives with identical or nearly identical genetic backgrounds (Volis et al., 2010). Possibly, also the variability associated with environmental predictors, particularly climate, might be at least partially genetically underpinned, because climatic gradients might covary with genetic

differentiation (Leiblein-Wild & Tackenberg, 2014). However, as the present data were measured in-situ, it is impossible to disentangle the contribution of genetic variation and direct response to environment (plasticity) to variation in plant traits. This will require further research, including growing plants in a common environment, optimally in combination with molecular genetic analysis.

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Conflict of interest statement

The authors declare that there are no conflicts of interest associated with this study.

Authors' contribution

AB and MK conceived the idea, AB, HV, TH, and MK designed the study, HV analyzed the data and wrote the first draft, all authors collected data, edited the manuscript, and approved the submission.

Data availability statement

The data supporting the findings of this study are not publicly available at the time of submission but will be deposited in an appropriate public repository (e.g. Zenodo) upon acceptance of the manuscript.

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References

Adler, P. B., Salguero-Gómez, R., Compagnoni, A., Hsu, J. S., Ray-Mukherjee, J., Mbeau-Ache, C., & Franco, M. (2014). Functional traits explain variation in plant life history strategies. *Proceedings of the National Academy of Sciences*, *111*(2), 740–745. https://doi.org/10.1073/pnas.1315179111

Albert, C. H. (2015). Intraspecific trait variability matters. *Journal of Vegetation Science*, *26*(1), 7–8. https://doi.org/10.1111/jvs.12240

Almario, J., Jeena, G., Wunder, J., Langen, G., Zuccaro, A., Coupland, G., & Bucher, M. (2017). Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition. *Proceedings of the National Academy of Sciences*, *114*(44). https://doi.org/10.1073/pnas.1710455114

Andrade, B. O., Overbeck, G. E., Pilger, G. E., Hermann, J.-M., Conradi, T., Boldrini, I. I., & Kollmann, J. (2014). Intraspecific trait variation and allocation strategies of calcareous grassland species: Results from a restoration experiment. *Basic and Applied Ecology*, *15*(7), 590–598. https://doi.org/10.1016/j.baae.2014.08.007

Barker, A. V., & Pilbeam, D. J. (2015). *Handbook of Plant Nutrition*. CRC Press.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bivand, R. (2022). R Packages for Analyzing Spatial Data: A Comparative Case Study with Areal Data. *Geographical Analysis*, *54*(3), 488–518. https://doi.org/10.1111/gean.12319

Bivand, R., Millo, G., & Piras, G. (2021). A Review of Software for Spatial Econometrics in R. *Mathematics*, *9*(11), 1276. https://doi.org/10.3390/math9111276

Boyko, J. D., Hagen, E. R., Beaulieu, J. M., & Vasconcelos, T. (2023). The evolutionary responses of life-history strategies to climatic variability in flowering plants. *New Phytologist*, *240*(4), 1587–1600. https://doi.org/10.1111/nph.18971

Bruelheide, H., Dengler, J., Purschke, O., Lenoir, J., Jiménez-Alfaro, B., Hennekens, S. M., Botta-Dukát, Z., Chytrý, M., Field, R., Jansen, F., Kattge, J., Pillar, V. D., Schrodt, F., Mahecha, M. D., Peet, R. K., Sandel, B., van Bodegom, P., Altman, J., Alvarez-Dávila, E., … Jandt, U. (2018). Global trait–environment relationships of plant communities. *Nature Ecology & Evolution*, *2*(12), 1906–1917. https://doi.org/10.1038/s41559-018-0699-8

Bünemann, E. K., & Condron, L. M. (2007). Phosphorus and Sulphur Cycling in Terrestrial Ecosystems. In P. Marschner & Z. Rengel (Hrsg.), *Nutrient Cycling in Terrestrial Ecosystems* (Bd. 10, S. 65–92). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-68027-7_3

Burnham, K. P., & Anderson, D. R. (Hrsg.). (2002). Advanced Issues and Deeper Insights. In *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* (S. 267–351). Springer. https://doi.org/10.1007/978-0-387-22456-5_6

Cameron, K. c., Di, H. j., & Moir, J. l. (2013). Nitrogen losses from the soil/plant system: A review. *Annals of Applied Biology*, *162*(2), 145–173. https://doi.org/10.1111/aab.12014

Chardon, F., Jasinski, S., Durandet, M., Lécureuil, A., Soulay, F., Bedu, M., Guerche, P., & Masclaux-Daubresse, C. (2014). QTL meta-analysis in Arabidopsis reveals an interaction between leaf

senescence and resource allocation to seeds. *Journal of Experimental Botany*, *65*(14), 3949–3962. https://doi.org/10.1093/jxb/eru125

Cuadrado, Á., Carmona, A., Jouve, N., & Ouzounis, C. A. (2013). *Chromosomal Characterization of the Three Subgenomes in the Polyploids of Hordeum murinum L.: New Insight into the Evolution of This Complex*. https://doi.org/10.1371/journal.pone.0081385

Dale, M. R. T., & Fortin, M.-J. (2014). *Spatial Analysis: A Guide For Ecologists*. Cambridge University Press.

Davison, A. W. (1977). The Ecology of Hordeum murinum L.: III. Some Effects of Adverse Climate. *Journal of Ecology*, *65*(2), 523–530. https://doi.org/10.2307/2259498

De Frenne, P., Graae, B. J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., De Kort, H., De Schrijver, A., Diekmann, M., Eriksson, O., Gruwez, R., Hermy, M., Lenoir, J., Plue, J., Coomes, D. A., & Verheyen, K. (2013). Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology*, *101*(3), 784–795. https://doi.org/10.1111/1365-2745.12074

De Frenne, P., Kolb, A., Graae, B. J., Decocq, G., Baltora, S., De Schrijver, A., Brunet, J., Chabrerie, O., Cousins, S. a. O., Dhondt, R., Diekmann, M., Gruwez, R., Heinken, T., Hermy, M., Liira, J., Saguez, R., Shevtsova, A., Baskin, C. C., & Verheyen, K. (2011). A latitudinal gradient in seed nutrients of the forest herb Anemone nemorosa. *Plant Biology*, *13*(3), 493–501. https://doi.org/10.1111/j.1438-8677.2010.00404.x

Dupont, F. M., & Altenbach, S. B. (2003). Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. *Journal of Cereal Science*, *38*(2), 133–146. https://doi.org/10.1016/S0733-5210(03)00030-4

Etienne, P., Diquelou, S., Prudent, M., Salon, C., Maillard, A., & Ourry, A. (2018). Macro and Micronutrient Storage in Plants and Their Remobilization When Facing Scarcity: The Case of Drought. *Agriculture*, *8*(1), 14. https://doi.org/10.3390/agriculture8010014

Evans, M. E. K., Hearn, D. J., Hahn, W. J., Spangle, J. M., & Venable, D. L. (2005). Climate and life-history evolution in evening primroses (Oenothera, Onagraceae): A phylogenetic comparative analysis. *Evolution; International Journal of Organic Evolution*, *59*(9), 1914–1927.

Farris, M. A., & Lechowicz, M. J. (1990). Functional Interactions among Traits that Determine Reproductive Success in a Native Annual Plant. *Ecology*, *71*(2), 548–557. https://doi.org/10.2307/1940308

Fleet, B., & Gill, G. (2010). *Barley grass, an emerging weed threat in southern Australian cropping systems* (S. 5) [15th Agronomy Conference].

Griffin-Nolan, R. J., Vorontsova, M. S., & Sandel, B. (2025). Predicting global intraspecific trait variation of grasses. *Ecography*, *2025*(8), e07627. https://doi.org/10.1002/ecog.07627

Guo, H., Mazer, S. J., & Du, G. (2010). Geographic variation in seed mass within and among nine species of Pedicularis (Orobanchaceae): Effects of elevation, plant size and seed number per fruit. *Journal of Ecology*, *98*(5), 1232–1242. https://doi.org/10.1111/j.1365-2745.2010.01688.x

Harrell Jr, F. E. (2025). *Hmisc: Harrell Miscellaneous. R Package Version 4.2-0* (S. 5.2-2) [Dataset]. https://doi.org/10.32614/CRAN.package.Hmisc

He, T., Angessa, T. T., & Li, C. (2023). Pleiotropy Structures Plant Height and Seed Weight Scaling in Barley despite Long History of Domestication and Breeding Selection. *Plant Phenomics*, *5*, 0015. https://doi.org/10.34133/plantphenomics.0015

Helsen, K., Acharya, K. P., Brunet, J., Cousins, S. A. O., Decocq, G., Hermy, M., Kolb, A., Lemke, I. H., Lenoir, J., Plue, J., Verheyen, K., De Frenne, P., & Graae, B. J. (2017). Biotic and abiotic drivers of intraspecific trait variation within plant populations of three herbaceous plant species along a latitudinal gradient. *BMC Ecology*, *17*(1), 38. https://doi.org/10.1186/s12898-017-0151-y

Hendrix, S. D., & Sun, I.-F. (1989). Inter-and intraspecific variation in seed mass in seven species of umbellifer. *New Phytologist*, *112*(3), 445–451. https://doi.org/10.1111/j.1469-8137.1989.tb00336.x

Henery, M. L., & Westoby, M. (2001). Seed mass and seed nutrient content as predictors of seed output variation between species. *Oikos*, *92*(3), 479–490. https://doi.org/10.1034/j.1600-0706.2001.920309.x

Himelblau, E., & Amasino, R. M. (2001). Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. *Journal of Plant Physiology*, *158*(10), 1317–1323. https://doi.org/10.1078/0176-1617-00608

Jacobsen, N., & Bothmer, R. von. (1995). *Taxonomy in the Hordeum murinum complex (Poaceae)*. Nordic Journal of Botany. https://doi.org/10.1111/j.1756-1051.1995.tb00177.x

Joy, E. J. M., Broadley, M. R., Young, S. D., Black, C. R., Chilimba, A. D. C., Ander, E. L., Barlow, T. S., & Watts, M. J. (2015). Soil type influences crop mineral composition in Malawi. *Science of The Total Environment*, *505*, 587–595. https://doi.org/10.1016/j.scitotenv.2014.10.038

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., Zimmermann, N. E., Linder, H. P., & Kessler, M. (2021). *Climatologies at high resolution for the earth's land surface areasCHELSA V2.1 (current)* (Version 2.1, S. 2.1 KB) [Geotiff]. EnviDat. https://doi.org/10.16904/ENVIDAT.228.V2.1

Klein-Raufhake, T., Höfner, J., Hölzel, N., Knorr, K.-H., Lampei, C., Mudrák, O., & Bucharova, A. (2022). Nitrogen limitation reduces the performance of target plant species in restored meadows. *Restoration Ecology*, *30*(7), e13608. https://doi.org/10.1111/rec.13608

Lechowicz, M. J., & Blais, P. A. (1988). Assessing the contributions of multiple interacting traits to plant reproductive success: Environmental dependence. *Journal of Evolutionary Biology*, *1*(3), 255–273. https://doi.org/10.1046/j.1420-9101.1998.1030255.x

Leiblein-Wild, M. C., & Tackenberg, O. (2014). *Phenotypic variation of 38 European Ambrosia artemisiifolia populations measured in a common garden experiment*. https://doi.org/10.1007/s10530-014-0644-y

Lemke, I. H., Kolb, A., Graae, B. J., De Frenne, P., Acharya, K. P., Blandino, C., Brunet, J., Chabrerie, O., Cousins, S. A. O., Decocq, G., Heinken, T., Hermy, M., Liira, J., Schmucki, R., Shevtsova, A.,

Verheyen, K., & Diekmann, M. (2015). Patterns of phenotypic trait variation in two temperate forest herbs along a broad climatic gradient. *Plant Ecology*, *216*(11), 1523–1536. https://doi.org/10.1007/s11258-015-0534-0

Li, J., Tian, D., Yu, K., Guo, H., Zhang, R., Wang, J., Zhou, Q., & Niu, S. (2023). Seed nutrient is more stable than leaf in response to changing multiple resources in an alpine meadow. *Ecological Processes*, *12*(1), 43. https://doi.org/10.1186/s13717-023-00454-9

Long, J., Gao, X., & Miao, Y. (2025). Effects of environmental factors on the phenotypic traits and seed element accumulation of wild Elymus nutans in Tibet. *Scientific Reports*, *15*(1), 1838. https://doi.org/10.1038/s41598-025-85415-2

Marschner, H. (2012). *Marschner's Mineral Nutrition of Higher Plants*. Academic Press.

Mizianty, M. (2006). Variability and structure of natural populations of Hordeum murinum L. based on morphology. *Plant Systematics and Evolution*, *261*, 139–150. https://doi.org/10.1007/s00606-006-0437-6

Moles, A. T., Warton, D. I., Warman, L., Swenson, N. G., Laffan, S. W., Zanne, A. E., Pitman, A., Hemmings, F. A., & Leishman, M. R. (2009). Global patterns in plant height. *Journal of Ecology*, *97*(5), 923–932. https://doi.org/10.1111/j.1365-2745.2009.01526.x

Moran, E. V., Hartig, F., & Bell, D. M. (2016). Intraspecific trait variation across scales: Implications for understanding global change responses. *Global Change Biology*, *22*(1), 137–150. https://doi.org/10.1111/gcb.13000

Nour, M. M., Aljabi, H. R., AL-Huqail, A. A., Horneburg, B., Mohammed, A. E., & Alotaibi, M. O. (2024). Drought responses and adaptation in plants differing in life-form. *Frontiers in Ecology and Evolution*, *12*. https://doi.org/10.3389/fevo.2024.1452427

Ramzani, P. M. A., Khalid, M., Naveed, M., Ahmad, R., & Shahid, M. (2016). Iron biofortification of wheat grains through integrated use of organic and chemical fertilizers in pH affected calcareous soil. *Plant Physiology and Biochemistry*, *104*, 284–293. https://doi.org/10.1016/j.plaphy.2016.04.053

Rana, M., Sankhyan, N. K., Thakur, P., Babal, B., Anjali, Sharma, S., Kumari, S., & Kumar, P. (2025). Molybdenum in soil-plant system: Bioavailability, dynamics and implications for sustainable crop production. *Discover Soil*, *2*(1), 89. https://doi.org/10.1007/s44378-025-00118-4

Salisbury, S. E. J. (1942). *The Reproductive Capacity of Plants: Studies in Quantitative Biology*. G. Bell.

Sandel, B., Monnet, A.-C., & Vorontsova, M. (2016). Multidimensional structure of grass functional traits among species and assemblages. *Journal of Vegetation Science*, *27*(5), 1047–1060. https://doi.org/10.1111/jvs.12422

Sehgal, A., Sita, K., Siddique, K. H. M., Kumar, R., Bhogireddy, S., Varshney, R. K., HanumanthaRao, B., Nair, R. M., Prasad, P. V. V., & Nayyar, H. (2018). Drought or/and Heat-Stress Effects on Seed Filling in Food Crops: Impacts on Functional Biochemistry, Seed Yields, and Nutritional Quality. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01705

Sims, J. T. (1986). Soil pH Effects on the Distribution and Plant Availability of Manganese, Copper, and Zinc. *Soil Science Society of America Journal*, *50*(2), 367–373. https://doi.org/10.2136/sssaj1986.03615995005000020023x

Slot, M., Palow, D. T., & Kitajima, K. (2013). Seed reserve dependency of Leucaena leucocephala seedling growth for nitrogen and phosphorus. *Functional Plant Biology: FPB*, *40*(3), 244–250. https://doi.org/10.1071/FP12255

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2021). partR2: Partitioning R^2 in generalized linear mixed models. *PeerJ*, *9*, e11414. https://doi.org/10.7717/peerj.11414

Tan, T., Xia, H., He, C., Wei, Y., Liu, X., Zhang, Z., He, J., & Jiang, L. (2025). Trait-Mediated Competition for Light Underpins Plant Diversity Loss Under Eutrophication. *Global Change Biology*, *31*(9), e70521. https://doi.org/10.1111/gcb.70521

Tautenhahn, S., Grün-Wenzel, C., Jung, M., Higgins, S., & Römermann, C. (2019). On the relevance of intraspecific trait variability—A synthesis of 56 dry grassland sites across Europe. *Flora*, *254*, 161–172. https://doi.org/10.1016/j.flora.2019.03.002

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., & Dubcovsky, J. (2006). A NAC Gene Regulating Senescence Improves Grain Protein, Zinc, and Iron Content in Wheat. *Science*, *314*(5803), 1298–1301. https://doi.org/10.1126/science.1133649

Veall, M. R., & Zimmermann, K. F. (1996). Pseudo-R2 Measures for Some Common Limited Dependent Variable Models. *Journal of Economic Surveys*, *10*(3), 241–259. https://doi.org/10.1111/j.1467-6419.1996.tb00013.x

Volis, S., Shulgina, I., Zaretsky, M., & Koren, O. (2010). Epistasis in natural populations of a predominantly selfing plant. *Heredity*, *106*, 300–309. https://doi.org/10.1038/hdy.2010.79

Wang, H.-Y., Huang, J., Zhang, L., Qiu, G., Bu, R., Tang, K. W., Marrs, R. H., & Tong, C. (2025). Comparison of seed traits between an invasive plant and its native competitor along a latitudinal gradient. *Oecologia*, *207*(3), 49. https://doi.org/10.1007/s00442-025-05688-2

Wang, M. L., Grusak, M. A., Chen, C. Y., Tonnis, B., Barkley, N. A., Evans, S., Pinnow, D., Davis, J., Phillips, R. D., Holbrook, C. C., & Pederson, G. A. (2016). Seed Protein Percentage and Mineral Concentration Variability and Their Correlation with Other Seed Quality Traits in the U.S. Peanut Mini-Core Collection. *Peanut Science*, *43*(2), 119–125. https://doi.org/10.3146/PS156-15.1

Wortmann, C. S., Dicko, M. K., Maman, N., Senkoro, C. J., & Tarfa, B. D. (2018). Fertilizer Application Effects on Grain and Storage Root Nutrient Concentration. *Agronomy Journal*, *110*(6), 2619–2625. https://doi.org/10.2134/agronj2018.04.0274

Wu, H., Meng, H., Jiang, M., & Wei, X. (2024). Intraspecific variation of multi-elements in seeds of Euptelea pleiospermum and its association with soils, climate, and leaf elements. *Journal of Plant Ecology*, *17*(6), rtae090. https://doi.org/10.1093/jpe/rtae090

Wu, P., Tian, J.-C., Walker, C. E. (Chuck), & Wang, F.-C. (2009). Determination of phytic acid in cereals – a brief review. *International Journal of Food Science and Technology*, *44*(9), 1671–1676. https://doi.org/10.1111/j.1365-2621.2009.01991.x

Zhang, D. (2016). *rsq: R-Squared and Related Measures* (S. 2.7) [Dataset]. https://doi.org/10.32614/CRAN.package.rsq

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems: *Data exploration*. *Methods in Ecology and Evolution*, *1*(1), 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

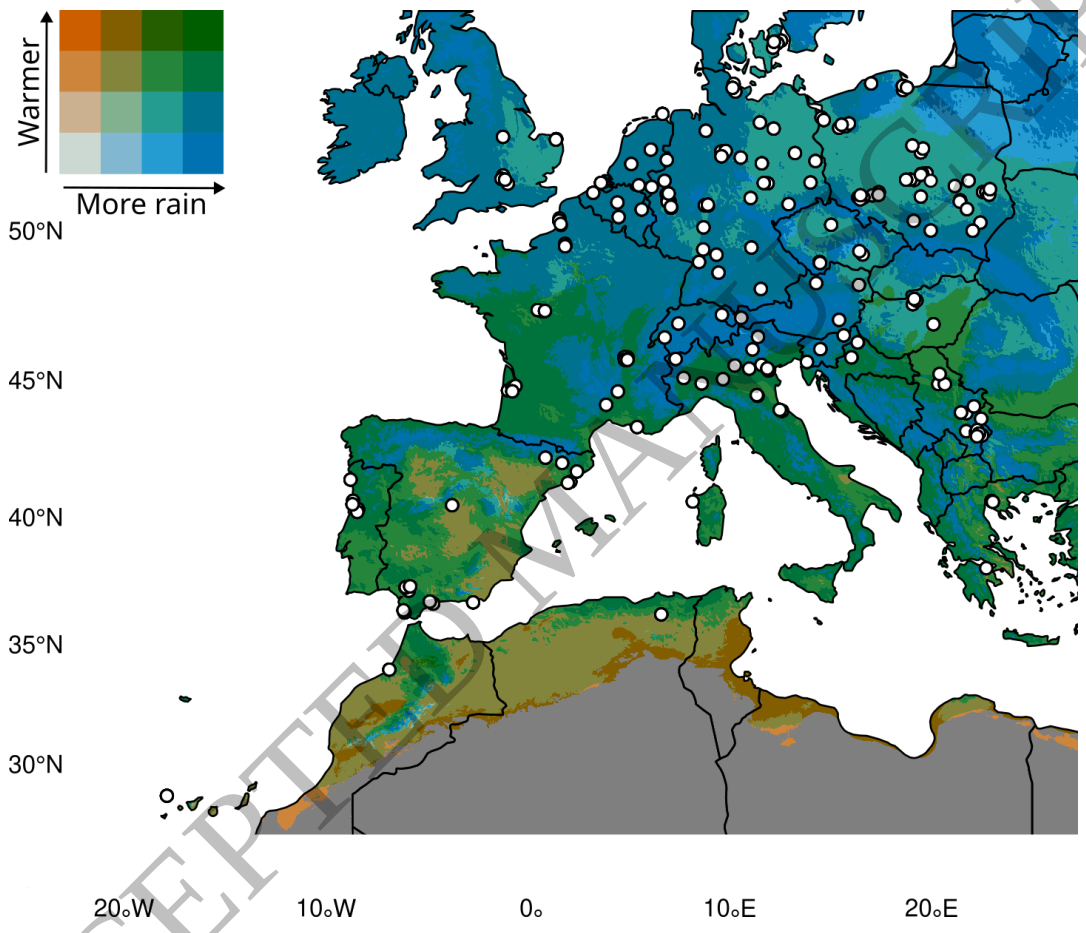

Figure 1| Climate map of the in-situ sampled populations of *Hordeum murinum* across Europe and North Africa. Color shading shows the average climate from the years 2000-2020 of mean annual temperature and annual precipitation. Only tetraploid populations are shown (See Figure S1 for diploids and mixed populations).

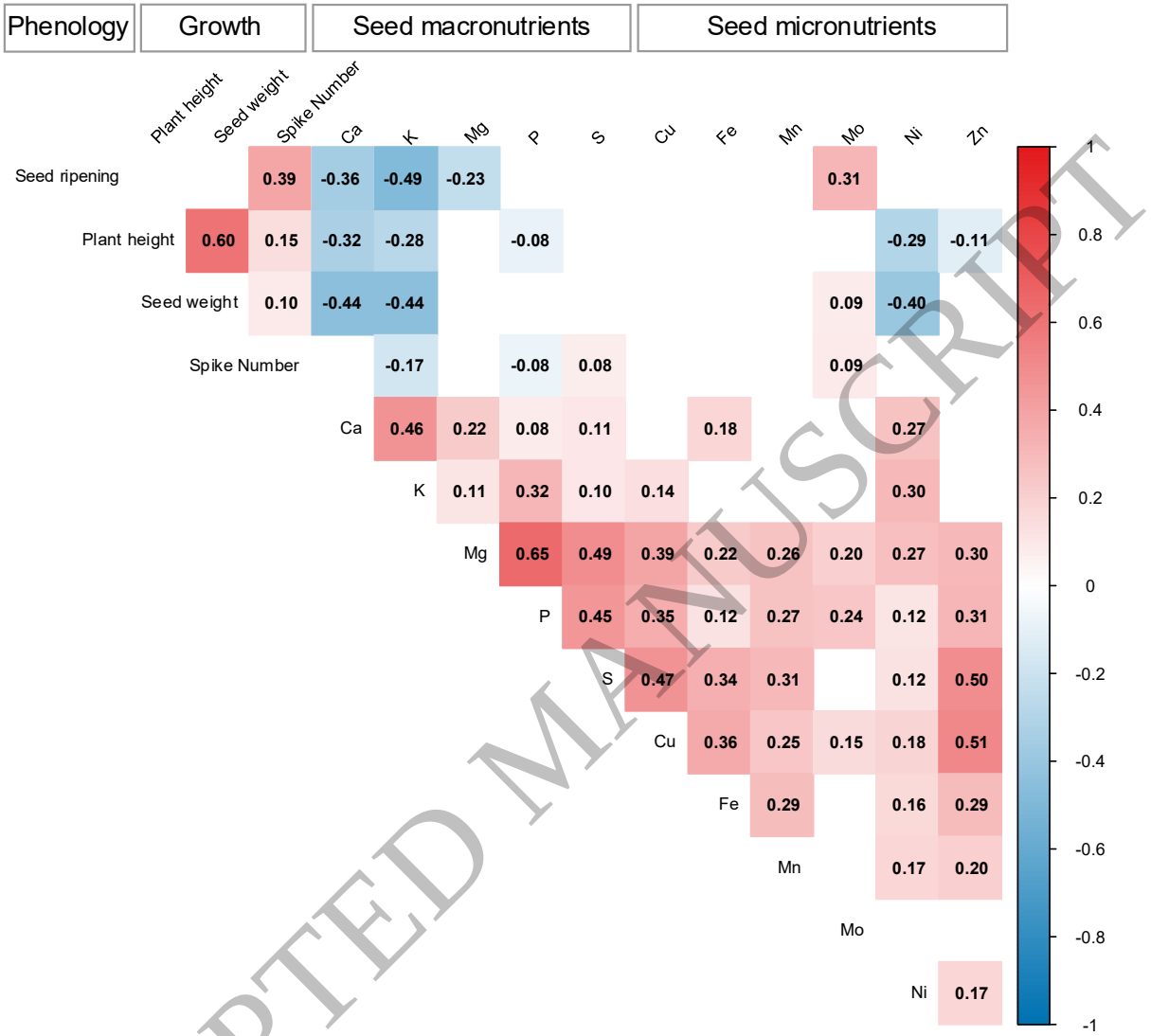

Figure 2| Pearson's correlation coefficient between seed ripening phenology, growth-related traits, and seed nutrient concentrations (macro- and micronutrients). The number and color intensity in the cells indicate the strength and direction of the individual correlation. Empty cells indicate non-significant relations ($p > 0.05$). The significance threshold was adjusted for multiple testing using Benjamini-Hochberg correction.

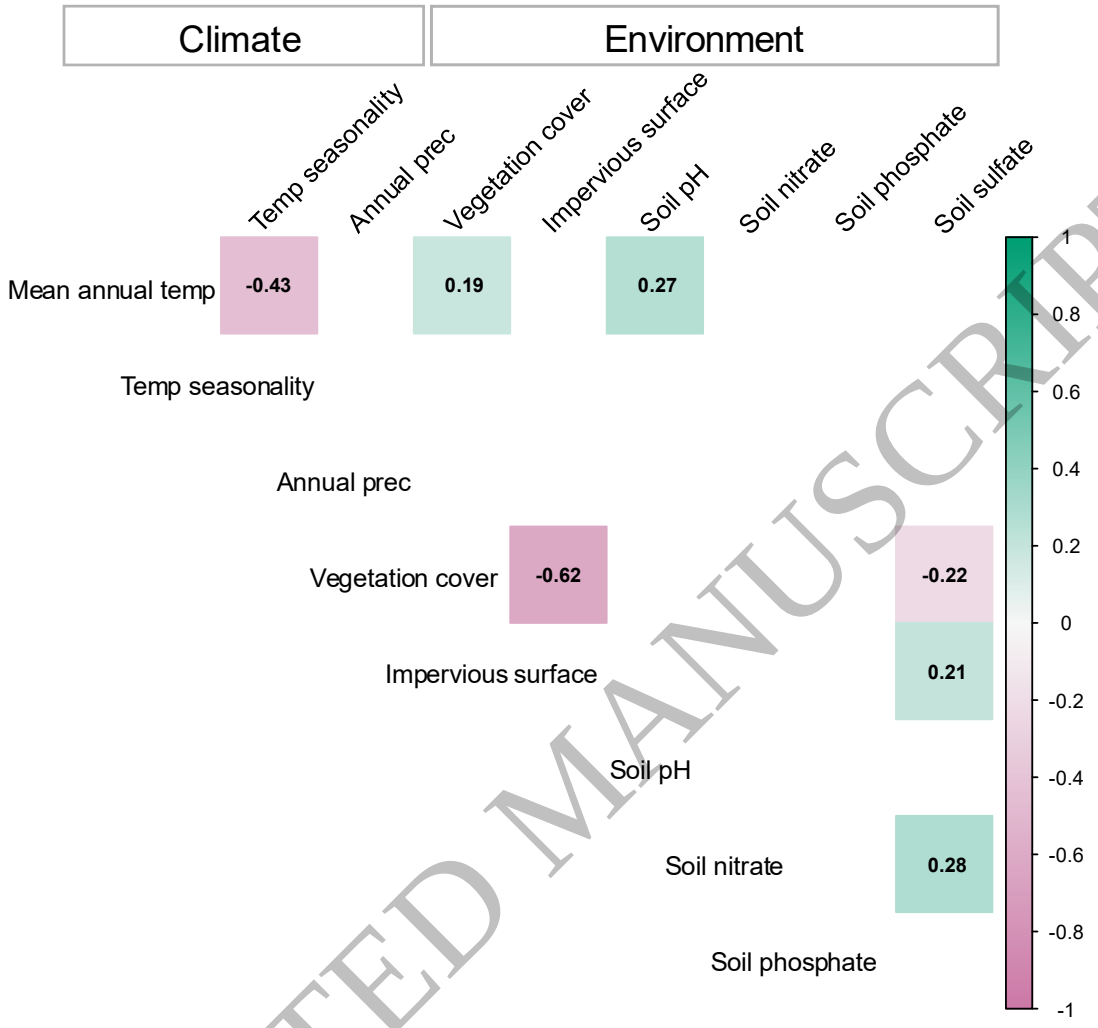

Figure 3| Pearson's correlation coefficient between all predictors. Note that most predictors were available at population level only, and vegetation and impervious surface were averaged across all plants within each population. The number and color intensity in the cells indicate the strength and direction of the individual correlation. Empty cells indicate non-significant relations ($p > 0.05$). The significance threshold was adjusted for multiple testing using Benjamini-Hochberg correction.

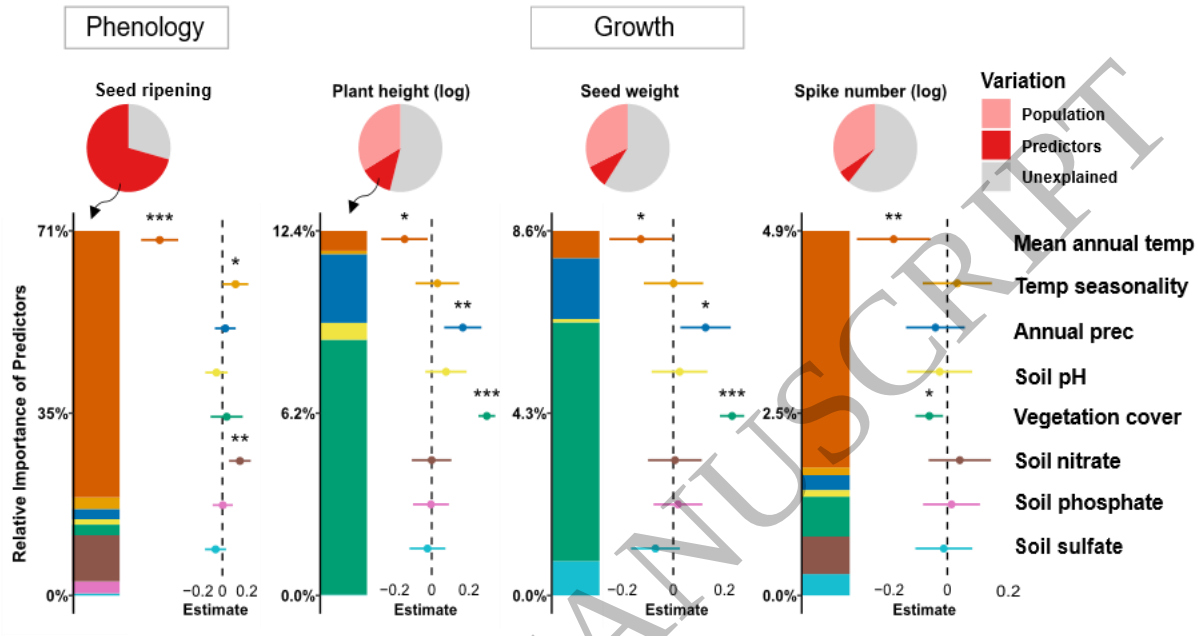

Figure 4| Intraspecific variation in plant phenology and growth-related traits. Results of a spatial lag model and a linear mixed model, respectively, testing for the effect of climate and environment. Each plot contains three main components: (1) Pie charts show the R^2 partitioning of each model split into population (variance explained by the random effect, light red), predictors (variance explained by the fixed effects, dark red), and unexplained variance (grey). (2) Bar plots show the estimated relative importance of the environmental predictors (proportion of explained variance of each predictor). (3) Forest plots exhibit effect size estimates (points) with confidence intervals (error bars). Asterisks indicate the significance levels of each predictor (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Predictors were scaled before the analysis. Some response variables were log-transformed before analysis (as indicated in the variable name).

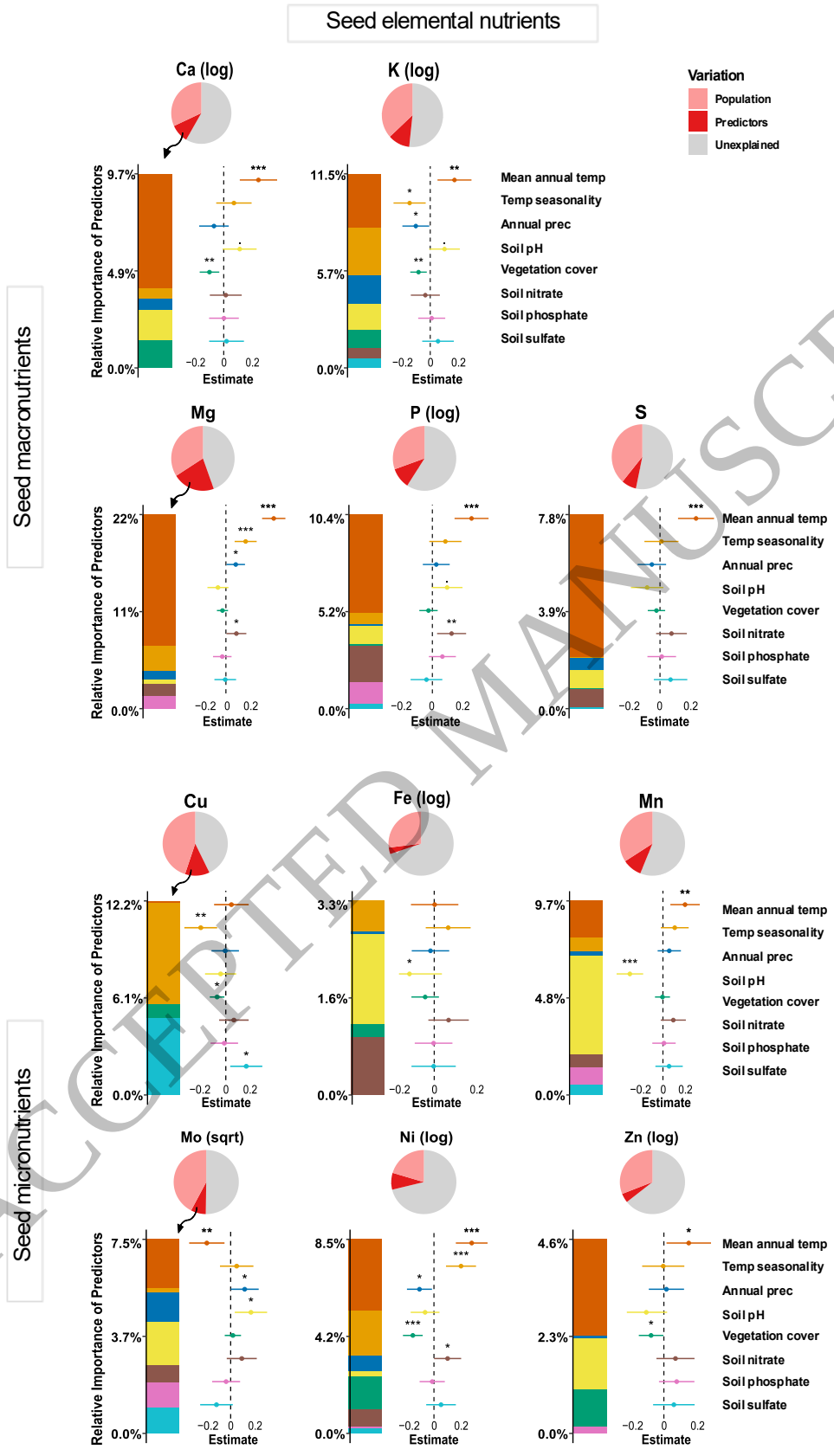

Figure 5| Intraspecific variation in concentrations of elemental nutrients in the seed, results of linear mixed model testing for the effect of climate and environment. Each plot contains three main components: (1) Pie charts show the R^2 partitioning of each model split into population (explained variance by the random effect, light red), predictors (explained variance by the fixed effects, dark red), and unexplained variance (grey). (2) Bar plots show the estimated relative importance of the environmental predictors (proportion of explained variance of each predictor). (3) Forest plots exhibit effect size estimates (points) with confidence intervals (error bars). Asterisks indicate the significance levels of each predictor (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Predictors were scaled before the analysis. Some response variables were log-transformed before analysis (as indicated in the variable name).

Figure legend

Figure 1: Climate map of the in-situ sampled populations of *Hordeum murinum* across Europe and North Africa.

Figure 2: Pearson's correlation matrix between seed ripening phenology, growth-related traits, and seed nutrient concentrations

Figure 3: Pearson's correlation matrix between all predictors.

Figure 4: Intraspecific variation in plant phenology and growth-related traits, results of spatial lag model and linear mixed model testing for the effect of climate and environment.

Figure 5: Intraspecific variation in concentrations of elemental nutrients in the seed, results of linear mixed model testing for the effect of climate and environment.