Received: 30 January 2024 | Accepted: 2 January 2025

DOI: 10.1111/1365-2435.70003

Functional Ecology

RESEARCH ARTICLE

Phenology-Trait Relationships Across Different Scales and Organisational Levels

Drivers of phenological transitions in the seedling life stage

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Funding information
German Research Foundation, Grant/Award Number: DFG-FZT 118 and 202548816; Directorate for Biological Sciences, Grant/Award Number: DBI #1907214

Handling Editor: Giulia Ghedini

Abstract

1. Plant functional ecology research has primarily focused on juvenile and adult plants even though regeneration from seed can be the most consequential life-history bottleneck with cascading influence on later stages of growth and reproduction. Understandings of relationships among phenology, morphology and growth-related functional traits have improved our knowledge of plant life-history strategies and adaptive responses to changing climate. However, whether relationships among phenological and morpho-physiological traits exist during plant regeneration is unknown. We also lack understanding of the relative importance of these relationships compared with those of regeneration phenology with other factors like plant phylogeny, geographic location and whether a species is native or non-native to the location.
2. To better understand these gaps in knowledge, we evaluated three phenological traits (days to germination, first and third true leaves) and six morpho-physiological traits (seed mass, relative growth rate, root elongation rate, root: shoot ratio, specific leaf area and seedling C:N) associated with regeneration for 131 forb species from six globally distributed grasslands.
3. Morpho-physiological traits showed several significant correlations with phenological traits. Boosted regression trees revealed that their relative importance in predicting phenological traits varied among the three phenological stages (34%–51%). Interestingly, the relative importance of morpho-physiological traits on the phenological stages was comparable to that of phylogeny (36%–46%). In general, species with faster phenologies produced seedlings that grew faster. The influence of geographic location on phenological traits was strongest at germination

(29%) and decreased (8%–15%) at later phenological stages. Native versus non-native origin had little to no impact (0%–2%) on regeneration phenology.
4. Strong relationships between days to germination and geographic location indicate signatures of local adaptation in the earliest life stages. Similar morpho-physiological trait values between native and non-native forbs imply that trait matching may be essential for non-native establishment. While associations between phenological and morpho-physiological traits during regeneration have not been previously recognized, our results suggest that these are complex and variable across plant regeneration. Better understanding of these associations and their variation across plant life stages may help capture species shifts with ongoing climate change and be used to develop novel approaches to seed-based restoration.

KEYWORDS
biogeography, forb, germination, grassland, invasive plants, phenology, plant recruitment, seedling functional traits

1 | INTRODUCTION

Plant life cycles involve a series of developmental stages, including germination, seedling establishment, flowering and fruiting. The timing, or phenology, of these stages influences plant survival and has over time generated species-level phenological variation. Recent research (Bucher & Römermann, 2021; Liu et al., 2021) suggests that phenological characteristics, or traits, are closely linked to other functional traits related to morphology, growth or physiology (morpho-physiological traits hereafter). Smaller herbaceous species, for example, tend to leaf out, flower, set fruit and senesce earlier than taller more productive plant species independent of environmental context (Sporbert et al., 2022). Like phenological traits at later life stages, morpho-physiological traits associated with plant regeneration from seed should also influence phenology leading to trait similarity among species. However, the drivers of phenology during the very earliest stages of seedling growth have yet to be evaluated. This lack of knowledge is unfortunate since regeneration from seed can be the most consequential bottleneck in a plant's life cycle (Eriksson & Ehrlén, 2008; James et al., 2011) with cascading influence on later stages of plant growth and reproduction (Donohue, 2002, 2005; Galloway, 2001). Moreover, seeds, seedlings and adult plants face distinct selective pressures, so morpho-physiological traits and trait correlations may change as plants mature (Mason et al., 2013; Razzaque & Juenger, 2022). For instance, recent findings show a positive link between seed mass and seedling growth (Larson et al., 2021), which conflicts with the well supported negative association between seed mass and juvenile or adult growth (Gibert et al., 2016; Larson et al., 2016; Marañon & Grubb, 1993). Climate-driven shifts in plant regeneration phenology may also impede community restoration efforts (James et al., 2019). Thus, a better understanding of phenological and morpho-physiological trait relationships during plant regeneration could help quantify species-specific recruitment niches and seed-based restoration efforts (Al-Gharaibeh et al., 2024; Larson et al., 2023).

Plant regeneration is a multi-step process initiated by seed production and dispersal that is influenced by seed traits like seed mass (Hierro et al., 2020; Moles & Westoby, 2006; Saatkamp et al., 2019; Thompson et al., 1998; Wu et al., 2023). The timing of germination determines the environmental conditions that young seedlings will experience and thus the likelihood of seedling transitions to juvenile and adult life stages (Hierro et al., 2009). Early germination can yield competitive advantages earlier and later in life while late germination can increase seedling survival and hence plant reproduction and survival (Gioria et al., 2018; Godoy & Levine, 2014; Rathcke & Lacey, 1985). Because of these close connections between regeneration phenology and fitness, regeneration phenology should be as phylogenetically conserved as phenology at later life stages (Barak et al., 2018; Seglias et al., 2018; Wang et al., 2009).

During the first stages of plant regeneration from seed, environmental filters may limit plant functional trait variation in a manner that results in sets of shared traits among species (Keddy, 1992; Kraft et al., 2015). Thus, in addition to phylogenetic signals, we might expect to see strong geographic signals on regeneration phenology. Recent efforts have distinguished the relative importance of morpho-physiological traits, phylogeny and geographic-level environmental variation on species phenology for juvenile and adult plants (Rauschkolb et al., 2024; Sporbert et al., 2022) but whether regeneration phenology will follow the same trend is unknown.

It is also unclear to what extent local conditions affect the similarity of phenological strategies between native and non-native species at the regeneration life stage (Lemoine et al., 2016). Differences in morpho-physiological traits could allow non-native species to avoid direct competition with native species during regeneration (Abrams, 1983; Gioria et al., 2018; Godoy & Levine, 2014; MacArthur & Levins, 1967). Non-native species may also evolve to

exhibit similar traits to those of natives (Callaway et al., 2022), which could be particularly important if linked to successful establishment.

Here, we seek to better understand relationships between phenological and morpho-physiological traits related to plant regeneration from seed and to assess their impact relative to plant phylogeny, geographic location and native versus non-native origin (Figure 1a). We focus on three phenological traits related to regeneration (days to germination, days to first true leaf, days to third leaf) and six morpho-physiological traits (seed mass, relative growth rate, root elongation rate, root–shoot ratio, seedling carbon to nitrogen ratio, specific leaf area; see Table 1 for morpho-physiological traits and their anticipated relationship to phenology). All traits were measured on 131 forb species distributed across six grassland sites globally (16–24 species per grassland; Figure 1b; Tables S1–S3). Our first objective (OBJ 1) was to examine relationships among phenological and morpho-physiological traits related to regeneration. Second, (OBJ 2) we assessed the importance of these relationships by evaluating the relative influence of morpho-physiological traits, phylogeny, geographic location and native versus non-native origin in predicting phenological traits. As reported for functional trait-phenology associations from adult life stages (Sporbert et al., 2022), we expect to find that phylogeny will more strongly impact phenological traits than morpho-physiological traits or geography. Furthermore, the influence of morpho-physiological traits on phenological traits should exceed that of geography (Sporbert et al., 2022). We anticipate that our results will improve our ability to forecast regeneration phenology, which has important implications for processes like community assembly and seed-based restoration, and to improve our understanding of the stages of invasion by successful non-native species.

2 | MATERIALS AND METHODS

2.1 | Study system and species

We examined phenological and morpho-physiological traits of 131 forb species from grasslands in Argentina, Germany, New Zealand, Serbia and the United States (California and Montana; Figure 1b; Tables S1 and S2).

Geographic locations reflected a range of abiotic and biotic environmental conditions found in semi-arid grasslands (Table S3). From each geographic location, we selected 16–24 study species (all forbs), with half of the species in each system classified as native and half non-native (i.e. originally introduced from another location). Acknowledging broad spatio-environmental variation is important because the outcome of native versus non-native comparisons in biotic interactions can strongly vary along spatio-environmental gradients (Shah et al., 2014; Sheng et al., 2022), and thus, sampling from disparate geographic locations is a necessary approach to avoid unrepresentative results coming from a particular environmental context (Lucas et al., 2024). We collected or purchased seed from family pairs of native and non-native species within each geographic

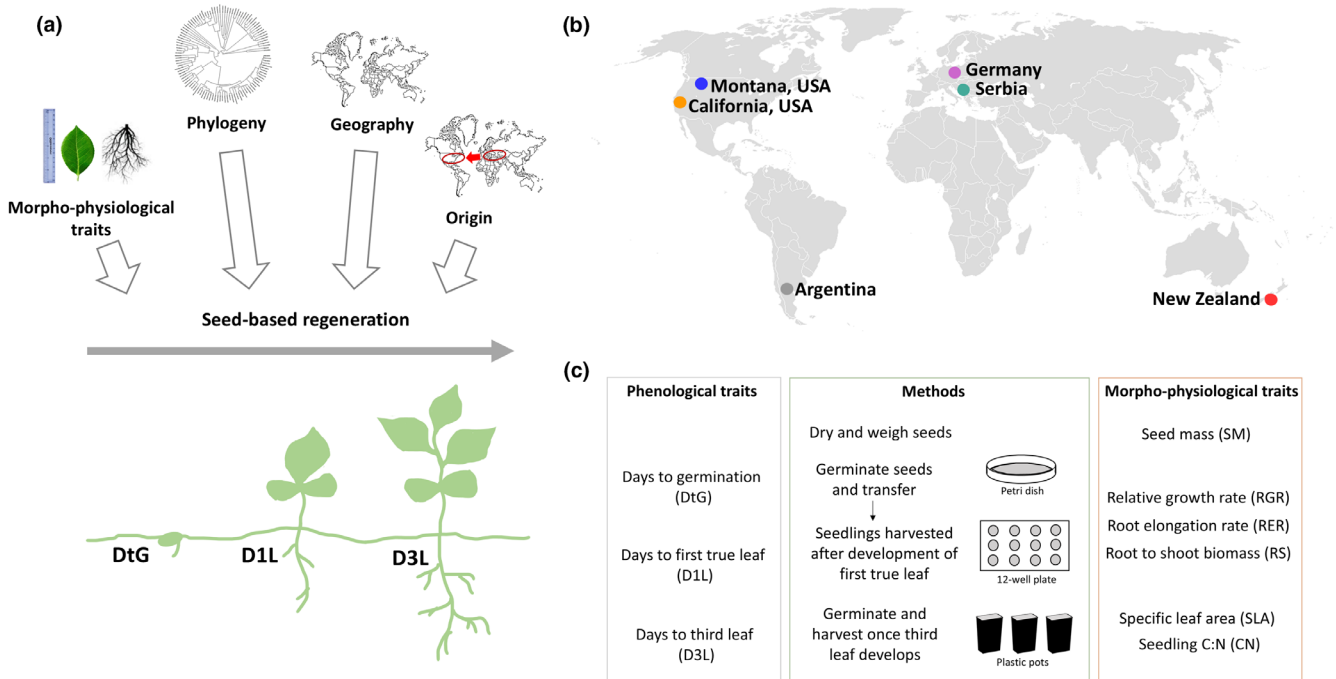

FIGURE 1 Conceptual approach used here to evaluate drivers of plant phenology during regeneration from seed. (a) Potential drivers of regeneration phenology include morpho-physiological traits, shared evolutionary history (i.e. phylogeny), environmental variation (i.e. geography) and native or non-native origin (i.e. origin) with the relative impact of each factor potentially being variable among three different stages of regeneration (days to germination (DtG), days to first leaf (D1L) or days to third leaf (D3L)). (b) Location of our six study grasslands and (c) conceptual figure describing the methods used to obtain the three phenological and six morpho-physiological traits considered in this study.

TABLE 1 Functional traits considered in this study (i.e. traits related to morphology, growth or physiology), their predicted ecological function and known or presumed links with regeneration phenology, when possible.

Trait	Predicted ecological function	Potential link to regeneration phenology
Seed mass	Germination (Baskin & Baskin, 1998) and seedling survival (Westoby et al., 2002; but see Moles & Westoby, 2004)	Smaller seeds are associated with faster germination and faster seedling development (Baskin & Baskin, 1998; Grime et al., 1981; Shipley et al., 1989; but see Barak et al., 2018)
Relative growth rate	Competitive ability (Huston & Smith, 1987) and seedling survival (Kitajima, 1994; Sack & Grubb, 2001)	Rapid growing seedlings germinated faster (Larson et al., 2016)
Root elongation rate	Resource acquisition (Leck et al., 2008), drought tolerance (Harrison & LaForgia, 2019) and competitive ability (Aoyama et al., 2023)	Slower germinating species quickly produce a large initial root biomass while faster germinating species maintain faster rates of root growth throughout establishment (Larson et al., 2016)
Root:shoot ratio	Drought tolerance (Harrison & LaForgia, 2019) and competitive ability (Rowe & Leger, 2011)	Higher root:shoot ratios have been associated with later (Yang et al., 2015) and earlier (Souza & Fagundes, 2014) germination
Specific leaf area	Productivity (Westoby et al., 2002; Wright et al., 2004), competitive ability (Pillay & Ward, 2014; Reichmann et al., 2016) and drought tolerance (Poorter et al., 1990)	Seedlings of species with higher specific leaf area were associated with faster germination (Larson et al., 2016)
Seedling C:N	Photosynthetic capacity (DeJong & Doyle, 1985), productivity and biomass allocation (Elser et al., 2010; Luo et al., 2017) and drought tolerance (Poorter et al., 1990)	In adults, species with higher C:N ratios are associated with earlier initial growth, earlier flowering and later leaf senescence (Bucher et al., 2018; Bucher & Römermann, 2020; Sporbert et al., 2022)

location, where possible. There were 14 instances (out of 41 attempts) where this was not possible because only native or non-native representative species occurred in these geographic locations (Table S4). Species were selected that are generally abundant within a geographic location and that may increase the taxonomic diversity in our sample. This meant that in some cases, the same species was sampled within its native and non-native range (Table S2) to reflect our interest in parsing apart patterns of adaptation among co-occurring species with different or similar evolutionary histories. This is important because differences in regeneration traits can be more pronounced between native and non-native populations of one species than between different species (Kožić et al., 2024).

Species life-history strategies (annual, biennial and perennial) within each geographic location reflected the dominant life-history strategy of forbs in that location (Table S2; annual-48 species, biennial-5 species or perennial-78 species). This sampling design meant that we were not set up to accurately parse apart life-history strategy impacts on phenological traits. To ensure life-history strategy was not a confounding factor, we included it initially in our models, but it was not significant in any of our models and thus excluded from our final analyses.

Seeds for our study species were field harvested or purchased locally with field harvested seeds for each species derived from at least 50 individuals per population (Table S2). No permits were necessary for field collections. Note that there was no difference in the phenological traits of field harvested versus purchased seeds (days to germination: $F_{1, 129}=0.11$; $p=0.738$; days to first true leaf: $F_{1, 129}=0.07$, $p=0.796$; days to third leaf: $F_{1, 129}=0.31$, $p=0.577$).

2.2 | Regeneration traits

We measured three phenological traits (days to germination, days to first true leaf, days to third leaf) and six morpho-physiological (seed mass, relative growth rate, root elongation rate, root–shoot ratio, seedling carbon to nitrogen ratio, specific leaf area) related to early regeneration for each species (see Table 1 for morpho-physiological traits and their anticipated relationships to phenology, Figure 1c).

To determine *seed mass*, we dried at least 15 seeds of each species at 60°C for 48 h and weighed each individually. We placed at least 20 seeds in three Petri dishes lined with filter paper (Whatman #1) for each species. Petri dishes were placed into growth chambers set

to a diurnal setting of 12 h of high light at 24°C and 12 h of darkness at 13°C. Seeds were watered as needed and Petri dishes were randomized daily within the growth chamber. We monitored germination of seeds and growth of seedlings daily at the same time. Seed germination (presence of a protruding radicle) was recorded for individual seeds and used to determine the average *days to germination* (number of days since starting seeds till germination) for each species.

The first 12 seeds to germinate per species were moved into the individual wells of a 12-well plate on the day they germinated. Each well was lined with one piece of filter paper (Whatman #1). As seedlings were being transferred into well plates, we measured their root length (mm) under a dissecting microscope. Seedlings continued to be monitored daily until the first true leaf (first leaf after cotyledon) was visible under a microscope. The date at which each seedling produced its first true leaf was recorded and used to determine the *days to first true leaf* (number of days from germination to true leaf). At this time point, we remeasured root length, harvested the seedlings and dried them at 60°C for 48 h prior to weighing their root and shoot masses. *Root elongation rate* was calculated as root growth (difference in root length between germination and first true leaf)/number of days between germination and first true leaf (Larson et al., 2016). With the biomass data, we calculated the *root to shoot ratio* (root: shoot ratio). The *relative growth rate* was calculated as whole seedling mass/number of days between germination and first true leaf. Recording these morpho-physiological traits on the day when the first true leaf appeared allowed us to standardize comparisons across species. We chose this phenological time point because it reflects a critical developmental stage that roughly marks the transition from heterotrophy to autotrophy (Steeves & Sussex, 1989).

Further traits were obtained from another trial from seedlings grown in pots (Figure 1c). We scattered approx. 100 seeds of each species onto the surface of pots filled with potting soil, recorded this date and placed pots into a growth chamber (Argentina, Serbia, Germany, New Zealand) or greenhouse (California, Montana; conditions were standardized across both settings: 12 h of high light at 24°C and 12 h of darkness/lower light at 13°C). A recent opinion paper discussed the importance of measuring seedling traits under standardized conditions (Winkler et al., 2024), and we thus recognize that the introduction of potential variation between our two experimental settings was not ideal. However, we think that our approach worked well and was a necessary solution to the variation in access among institutions. In the future, we aim to collect continuous measurements of temperature within each experimental setting to more precisely measure phenology as growing degree days and allow greater standardization across studies. Seedlings were harvested on the day that they produced their third true leaf to determine the *days to third leaf* (difference between the date of sowing and the date seedlings produced a third leaf). We then randomly selected 10 seedlings and scanned the first true leaf of each seedling on a flatbed scanner (Epson V33) and used these photographs to measure leaf area in ImageJ (Rueden et al., 2017). Leaves were subsequently dried at 60°C for 48 h and weighed. For each leaf, *specific leaf area* was calculated by dividing leaf area by leaf mass. All remaining seedlings were bulked, dried at 60°C for 48 h, ground and then analysed for percent carbon and nitrogen with an elemental analyser (Eurovector, Pavia, Italy) to estimate *seedling carbon:nitrogen ratio* (seedling C:N).

2.2.1 | Replication statement

	Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
OBJ 1	Functional trait (nine functional traits)	Species (131 species)	Replication of phenological and morpho-physiological trait measurements per species was 10.67 on average. See Table 2 for more details
OBJ 2	Functional trait (nine functional traits)	Phylogeny (12 eigenvectors); geographic location (six locations); plant origin (native or non-native)	Phenological and morpho-physiological traits were measured on 16–24 species from each of six geographic locations and/ or 131 species were used to calculate each of 12 phylogenetic eigenvectors

2.3 | Data analysis

All analyses were performed in R version 4.2.1 (R Core Team, 2022). For all analyses, we used mean trait values to represent each species. All trait values other than seedling C:N and specific leaf area were log-transformed prior to analyses. We employed principal components analysis (PCA) to summarize and visualize associations among the phenological and morpho-physiological traits using the R package *FACTOEXTRA* (Kassambara & Mundt, 2020; OBJ 1). We also used Pearson correlation to assess the strength and direction to which our three phenological traits and six morpho-physiological traits were associated (OBJ 1).

To assess the relative importance of morpho-physiological traits, phylogeny, geographic location and plant origin for predicting phenological traits (OBJ 2; Figure 1a), we used Boosted Regression Tree (BRT; Elith et al., 2008) models. BRTs are suitable for analysing large data sets with numerous different predictor variables because this method is relatively insensitive to co-linearity and missing values in the predictor variables are handled with minimal loss of information by using surrogates (Bianchini & Morrissey, 2020; Elith et al., 2008). For the BRT models, we used each respective phenological trait as a dependent variable (three BRT models). Predictor variables included the mean morpho-physiological trait values, phylogenetic eigenvectors, geographic location and plant origin. For phylogeny, we followed the phylogenetic eigenvector regression by Diniz-Filho et al. (1998) that has been applied in several studies (Bianchini & Morrissey, 2020; Pistón et al., 2019; Rauschkolb et al., 2024; Sporbert et al., 2022). First, we created a phylogenetic tree of the 131 species using the R package *V. PHYLOMAKER* (Jin & Qian, 2019).

TABLE 2 Phenological and morpho-physiological traits included in this study (Trait) with trait abbreviations (Abbrev), units of measurement (Unit), minimum (Min), median, mean and maximum (Max) trait value for all species (131 species), and number of individuals per species (*N* rep).

Trait	Abbrev	Unit	Min	Median	Mean	Max	*N* rep
Phenological traits							
Days to germination	DtG	day	1.00	3.17	4.58	15.7	12
Days to first true leaf	D1L	day	3.50	7.27	8.55	28.9	12
Days to third leaf	D3L	day	19.0	42.0	42.2	66.0	10
Morpho-physiological traits							
Seed mass	SM	mg	0.01	0.51	1.46	23.8	15
Relative growth rate	RGR	mg/day	0.00	0.09	0.33	1.12	12
Root elongation rate	RER	mm/day	0.02	0.98	1.73	8.46	12
Root:shoot ratio	RSR	—	0.04	0.26	0.31	0.97	12
Specific leaf area	SLA	mm^2/mg	2.12	47.1	49.9	118	10
Seedling C:N	CN	—	4.94	19.3	19.6	41.2	1

Next, a pairwise distance matrix was computed from the phylogenetic tree (Figure S3) and the eigenvectors from this distance matrix were extracted with a principal coordinate analysis, that is eigenvector loadings on the axes, using the *APE* package (Paradis & Schliep, 2019). Following Bianchini and Morrissey (2020), phylogenetic eigenvectors represent the phylogenetic relationships among species and control for phylogenetic autocorrelation if a sufficiently high number of eigenvectors is included in the analysis. Therefore, the first 12 of a total of 108 eigenvectors were included as covariates in our BRT models since they explained 90% of the phylogenetic structure in the distance matrix.

We fitted BRT models using the R package *GBM* (Greenwell et al., 2020), with a Gaussian error distribution and a fraction of training data (bag fraction) of 0.5, a tree complexity of 1, a learning rate of 0.01 and a tolerance of 0.01. Model performance was assessed with cross-validation. Partial dependency plots were used to display the response between each predictor variable and the respective phenological response variable, independent of all other predictors included. We then quantified the relative importance (%) for all explanatory variables for predicting phenological traits, that is (i) the six morpho-physiological traits, (ii) the 12 phylogenetic eigenvectors, (iii) geographic location and (iv) plant origin.

3 | RESULTS

3.1 | Relationships between phenological and morpho-physiological traits

We found weak, but significant, positive correlations among all three phenological traits (Figure S2). Species that germinated faster also matured faster to the first and third true leaf ($r=0.33$, $p<0.001$; $r=0.29$, $p<0.01$, respectively; Figure S2). Relationships between phenological and morpho-physiological traits differed for each phenological trait and varied from significantly negative to significantly positive to non-significant (Figure 2; Figure S2). Days to germination was not significantly correlated with any morpho-physiological trait. Days to first true leaf was negatively correlated with relative growth rate ($r=-0.28$, $p<0.01$) and specific leaf area ($r=-0.2$, $p<0.05$) and positively correlated with root: shoot ratio ($r=0.27$, $p<0.01$). Similarly, days to third leaf was negatively correlated with relative growth rate ($r=-0.29$, $p<0.01$) and root elongation rate ($r=-0.19$, $p<0.05$), and positively correlated with seedling C:N ($r=0.28$, $p<0.01$; Figure 2).

Our PCA of phenological and morpho-physiological traits shows that species from different geographic locations and from native and non-native origins overlap greatly in space. The first three PC axes explained 62.3% of the variance among phenological and morpho-physiological traits related to regeneration (Figure 3; Figure S1; Table S5). PC1 (26.4% of the variation) had negative loadings of days to third leaf and positive loadings of relative growth rate representing a spectrum of slow to fast seedling maturation and growth. PC2 (18.0% of the variation) had negative loadings of days to germination and seed mass while root elongation rate and root:shoot ratio were positively loaded, suggesting that fast germinating smaller seeded species allocated more biomass to their faster growing roots (Figure 3; Table S5). For PC3 (16.9% of the variation), all three phenological traits (days to germination, days to first true leaf, days to third leaf) were positively associated with root elongation rate and seedling C:N ratio (Figure S1; Table S5).

3.2 | Relative importance of phenological trait predictors

Our three BRT models revealed moderate to good cross-validation (cv = 0.35–0.61, Figure 4; Figures S4–S6). Of the overall variation in

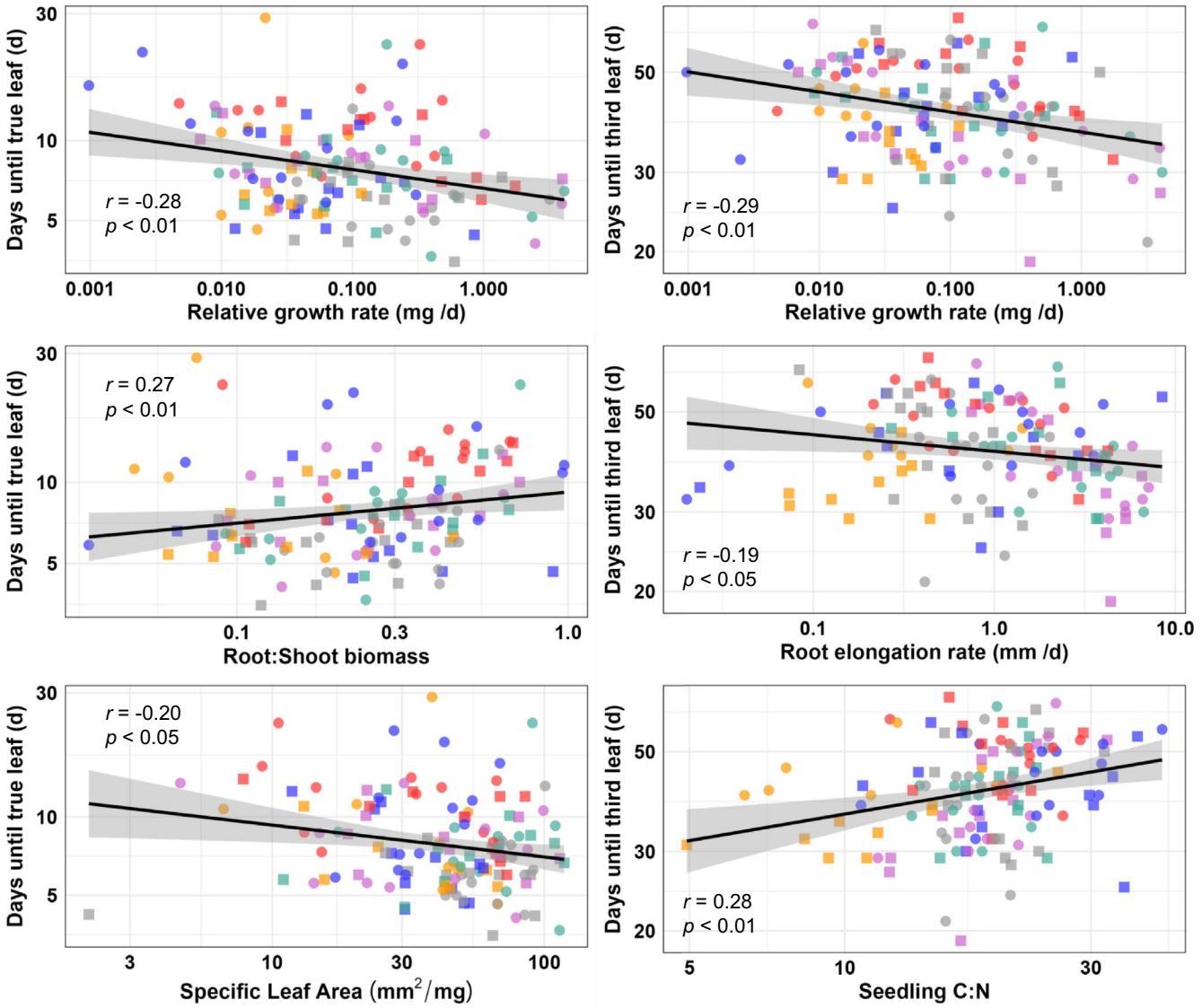

FIGURE 2 Bivariate associations among phenological and morpho-physiological traits for 131 forb species from six geographic locations (Argentina = grey, Serbia = green, California (USA) = orange, Germany = purple, Montana (USA) = blue, New Zealand = red). Each point represents the mean value for a single species. All trait variables other than C:N and SLA were $\log_e$-scaled for plotting. Only significant associations are shown (see Figure S2 for non-significant associations; Table 2 for trait means). Non-native species are shown with circles and native with squares.

the respective BRT models, morpho-physiological traits explained 34.4%, 37.1% and 51.4% of the variation for days to germination, days to first true leaf and days to third leaf, respectively (Figure 4). Faster germinating species had faster seedling root elongation rates (relative importance: 7.70%) and smaller seed mass (8.60%; Figure 4; Figure S4). The BRT also showed that the first true leaf appeared earlier in species with higher seedling relative growth rates (10.3%) and higher specific leaf area (9.40%; Figure 4; Figure S5). Similarly, days to third leaf was shorter in species with higher seedling relative growth rates (14.2%) and lower seedling C:N ratio (12.1%; Figure 4; Figure S6). Phylogeny explained 36.2% of the variation for days to germination, 45.8% of the variation for days to first true leaf and 40% of the variation for days to third leaf, which was overall comparable to the impact of morpho-physiological traits (Figure 4). For all BRT models, the influence of morpho-physiological traits and phylogeny on phenological traits exceeded that of geographic location and plant origin (Figure 4). Geographic location also decreased in its explanatory power of phenological traits as seedlings matured, with geographic location explaining 28.8% of the overall variation in BRT models for days to germination, but only 15.4% of the variation for days to first true leaf and 7.5% of the variation for days to third leaf. The stronger impact of geographic location on the two earlier phenological traits appeared to be mainly driven by species from two locations. In particular, species from Montana (USA) and Argentina germinated and grew their true leaves faster than species from other locations (Figure S5). The influence of plant origin on phenological traits was negligible (0%–1.6%).

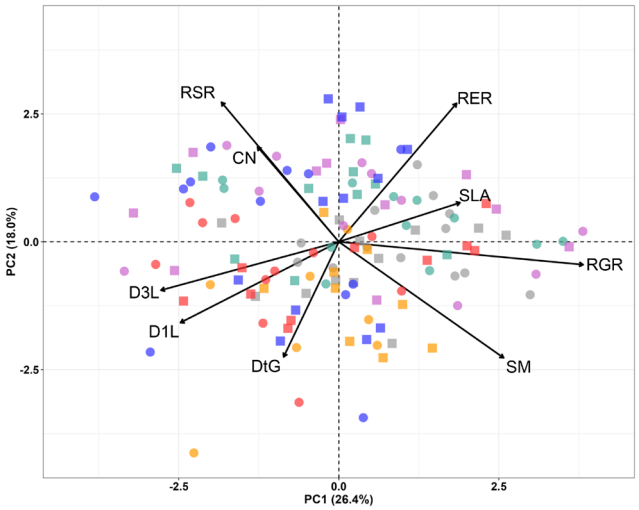

FIGURE 3 Principal components analysis (PCA) representing the multivariate space of three phenological and six morpho-physiological traits related to regeneration. Traits were measured on 131 forb species from six geographic locations (Argentina = grey, Serbia = green, California (USA) = orange, Germany = purple, Montana (USA) = blue, New Zealand = red). The first and second principal components (PC1 and PC2) accounted for 26.4% and 18.0% of the total variation, respectively. PC1 was associated with fast-growing (RGR) and fast-maturing (D3L) seedlings. PC2 was associated with smaller seeded (SM) species that germinated faster (DtG) and produced seedlings with more below than aboveground biomass (RSR) and fast-growing roots (RER). Overlapping symbols are present when species shared trait associations. Further trait abbreviations are given in Figure 1c, PC loadings are given in Table S5. Non-native species are shown with circles and native with squares.

4 | DISCUSSION

Plant functional traits, phylogeny, geographic location and plant origin have been shown to independently and interactively influence the timing of plant phenological stages in juvenile and adult plants (Ettinger et al., 2018; Sporbert et al., 2022; Wolkovich & Cleland, 2014). Work presented here demonstrates that these factors are also linked to key phenological stages involved in plant regeneration from seed. Our research makes a first attempt to connect multiple successive stages of regeneration phenology with non-phenological traits related to morphology, growth and physiology, relativize the effects of morpho-physiological traits with that of other potentially impactful factors, and place this new understanding into the broader context of adult plant phenology and functional ecology.

4.1 | Associations between phenological and morpho-physiological traits

Our finding that the three phenological traits associated with regeneration were highly positively correlated with one another suggests that selection consistently differentiates between fast versus slow phenological traits at the earliest life stage. Demonstrated connections between the timing of germination and the timing of flowering (Burgess et al., 2007; Galloway, 2001) suggest that associations between early and later phenological stages are likely. However, given that the direction of adult plant phenological trait associations vary from positive to negative (Sporbert et al., 2022), retaining a fast or slow phenology across plant development is unlikely. Such versatility may be required for survival across years with fluctuating environmental variability.

With respect to correlations between phenological and morpho-physiological traits, we found that faster germination tended to occur in smaller seeded species, which also allocated more biomass to their faster growing roots; a finding supported by previous research (Baskin & Baskin, 1998; Souza & Fagundes, 2014; Yang & Midmore, 2005). Species with smaller seeds typically produce more seeds that disperse more easily. This trait combination may benefit colonization by species with narrow environmental windows for establishment (Turnbull et al., 2004). Species that produced first true leaves earlier in our study also grew more rapidly and produced bigger, thinner leaves. In adult plants, such trait associations have been associated with increased competitive ability (Huston & Smith, 1987; Reichmann et al., 2016). However, our finding that fast-maturing seedlings (days to third leaf) that grew more rapidly had higher tissue nitrogen content does not align with expectations based on adult phenological and morpho-physiological trait associations (Bucher et al., 2018; Sporbert et al., 2022). Resource economic spectra for adult plants are often centred about the importance of traits and trait associations for resource capture and growth (Reich, 2014; Shipley, 2006). In contrast, germination often occurs during more favourable conditions meaning that trait associations during regeneration may have more to do with environmental cues (Donohue et al., 2010) or seed-based resources (Kidson & Westoby, 2000).

4.2 | Relative importance of morpho-physiological traits, phylogeny, geographic location and plant origin on phenological traits

Morpho-physiological traits varied in the direction and strength of their influence on each phenological trait, suggesting that, as with later phenological stages (Huang et al., 2018), species may tightly align their strategies for phenological and morpho-physiological traits to maximize their chances of survival throughout regeneration. Of the three phenological traits considered here, morpho-physiological traits had the most pronounced influence on days to third leaf, the latest of our investigated phenological stages. This pattern might be expected, given that by the time a seedling has a third true leaf, the whole plant system is more actively contributing to growth. Our finding coincides with those in adult plants showing that morpho-physiological traits had less impact on early phenology, such as leaf out, than on the later phenological stages, such as flowering or fruiting (Sporbert et al., 2022). However, we

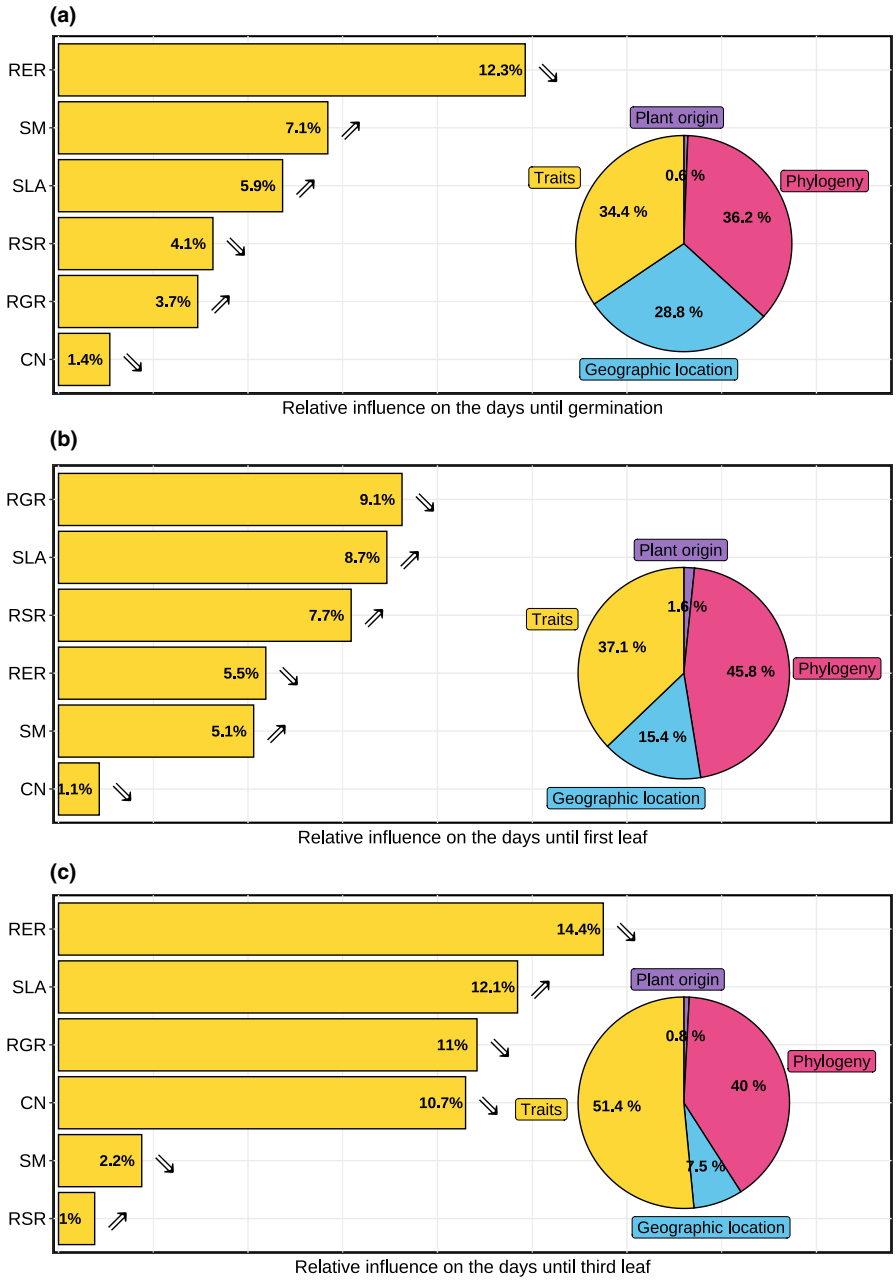

FIGURE 4 Barplots showing the relative importance of six morpho-physiological traits on predicting three phenological traits related to regeneration, deduced from boosted regression trees (BRTs). BRT models were fitted for (a) days until germination (cross-validation correlation (cv) = 0.35), (b) days until first true leaf (cv = 0.61) and (c) days until third leaf (cv = 0.42). Arrows at the end of bars convey the direction of each morpho-physiological trait's influence on phenology (positive or negative). Pie charts summarize the overall contributions of the variables grouped by morpho-physiological traits (yellow), phylogeny (i.e. first 12 axes of phylogenetic eigenvectors, magenta), geographic location (sky blue) and plant origin (purple). For trait abbreviations, see Table 2.

found that the overall relative importance of seedling morpho-physiological traits, compared with that of other factors, was higher than reported for adult plants (Sporbert et al., 2022). It is also important to note that while frequently a focus of trait research, seed mass was never the most influential morpho-physiological trait in explaining seedling phenological traits. Instead, to best understand seedling phenology, the morpho-physiological traits of seedlings were more important, and thus should be considered more broadly in functional ecology research (Larson & Funk, 2016; Winkler et al., 2024).

Given the strong influence of natural selection on seedling development (D'Aguillo & Donohue, 2023; Donohue et al., 2010), we expected to see a similar or stronger impact of phylogeny on phenology in seedlings compared with adult plants. However, we found that the relative impact of phylogeny compared with morpho-physiological traits on phenological traits in seedlings was generally weaker than reported for adult plants (Rauschkolb et al., 2024; Sporbert et al., 2022). This may be because natural selection also promotes specialization in phenological and morpho-physiological traits (Evans & Cabin, 1995; Ravigné et al., 2009). Specialization in phenology is especially important during seedling regeneration, where synchronizing germination and growth with favourable environmental conditions is crucial for survival. The role of phenological and morpho-physiological traits in shaping natural selection in the wild is still unclear and requires field studies to assess how phenological shifts affect seedling fitness (see D'Aguillo & Donohue, 2023). From an applied perspective, the equal importance of phylogeny and morpho-physiological traits in

early seedling stages suggests that considering phylogenetic relationships in restoration efforts could improve trait-based strategies focused on seed and seedling function (Larson et al., 2023; Larson & Funk, 2016).

While geographic location generally had a lower influence than phylogeny and morpho-physiological traits on regeneration phenology, the phenological trait for which it had the strongest explanatory power was days to germination. This supports previous suggestions that environmental factors may be particularly influential during regeneration from seed where they would be expected to limit trait variation within a geographic location in a manner that results in shared traits among co-occurring species (Keddy, 1992; Kraft et al., 2015). For adult plants, environmental factors associated with geographic location are known to have strong impacts on phenology at later life stages (Rauschkolb et al., 2024; Sporbert et al., 2022) and greater clarity in future work may be gained by evaluating the effects of individual environmental factors, such as climate and soil characteristic of the studied sites.

Our finding that plant origin had very little impact on phenological traits contrasts with research explaining the success of non-native plants by differences in phenological and morpho-physiological traits as compared to native plants (Harrison & LaForgia, 2019; Mathakutha et al., 2019; Molina-Montenegro et al., 2012; but see Zettlemoyer et al., 2022). A potential explanation for this discrepancy is that even though plant origin, as a category, is often used to distinguish plant evolutionary histories and functional traits, by accounting more directly for morpho-physiological traits and phylogeny in the BRTs, it became less useful in describing variation in seedling phenological traits. In support of prior research, similar phenological and morpho-physiological trait values for native and non-native forbs across and within geographic location also suggests that functional trait alignment may be essential for non-native seed-based establishment (Lortie & Hierro, 2022; but see Strauss et al., 2006). However, our results could also be explained by the fact that we used family pairs of native and non-native species meaning that unrelated non-native species may use different morpho-physiological traits or trait combinations and successfully establish. Finally, trait plasticity, which was not considered here, can also provide advantages to non-native plants by improving their ability to adapt to new climates and take advantage of favourable but unpredictable environmental conditions (Wainwright & Cleland, 2013; Nagy et al., 2024; but see Palacio-López & Gianoli, 2011), and should be considered in future comparisons.

4.3 | Future perspectives and connections with adult plant phenology and functional ecology

The characterization of seedling versus juvenile or adult functional traits is a relatively new field of ecology, and much work is still needed in comparing trait values across methodologies. Here, we relied on methods commonly used in seedling trait ecology (reviewed in Winkler et al., 2024), but further progress is needed in developing, validating and testing new methods. For example, to standardize trait values across species, we measured traits at distinct developmental stages so that species-specific differences in growth rate would not bias our results, as might be the case if traits were measured after a certain number of days after germination. Our results may have been different if we had considered another developmental stage or different functional group, such as grasses or shrubs. Similarly, variations in seed source (purchased or wild collected; Pizza et al., 2021) and seed storage (De Vitis et al., 2020) may have caused functional changes in the seedlings. We therefore hope that more attention will be paid to better understanding differences in seedling phenological and morpho-physiological traits in the future.

Close linkages of phenological and morpho-physiological trait associations at very early stages of regeneration means that natural selection should continually modify plant phenological responses in a manner that matches the habitat requirements of successive life stages (D'Aguillo & Donohue, 2023). Although the direction and strength of such linkages may vary (CaraDonna et al., 2014; Hoyle et al., 2015; Sporbert et al., 2022), better understanding of trait associations across the plant life cycle would greatly improve our ability to predict plant phenological shifts with changing climate and the implications of such changes on regeneration-oriented processes, such as seed-based restoration.

AUTHOR CONTRIBUTIONS

Mandy L. Slate and Christoph Rosche designed the study with input from Isabell Hensen. Mandy L. Slate, Loralee Larios, Christoph Rosche, Dávid U. Nagy, Jose L. Hierro and Lauren Waller contributed plant material. Mandy L. Slate, Christoph Rosche, Dávid U. Nagy and Felicitas Wolf collected the trait data. Mandy L. Slate, Maria Sporbert and Christoph Rosche performed the analyses and produced the graphs. Mandy L. Slate wrote the first draft of the manuscript. All authors contributed substantially to the subsequent revisions.

ACKNOWLEDGEMENTS

M.L.S. is grateful to Ray Callaway, Doug Emlen, Eve-Lyn Hinkley, Brett Melbourne, Ben Sullivan, the University of Montana, and the University of Colorado, Boulder, for equipment and greenhouse access. Ashley Braae, Natalie Hyde, Natalie Pelton and Bing Huo helped with data collection through support from the Undergraduate Research Opportunities Program at the University of Colorado, Boulder and MPG Ranch. We also thank Simon Dobernecker, Wladimir Gelfgat and Katrin Kittlaus for their invaluable efforts in the laboratory. M.L.S. is also grateful for support provided by an NSF Postdoctoral Fellowship in Biology (DBI #1907214). M.S., I.H., L.K. and C.R. acknowledge the support of iDiv funded by the German Research Foundation (DFG–FZT 118, 202548816). We are grateful for the comments from reviewers and editors, which improved this manuscript considerably.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.573n5tbjg (Slate et al., 2025).

STATEMENT ON INCLUSION

Our study brings together authors from a number of different countries. All authors were engaged early on with the research and played key roles in interpreting and synthesizing our results.

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SUPPORTING INFORMATION

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

Figure S1. Principal Components Analysis of nine regeneration traits.

Figure S2. Pearson's correlation matrix of correlation coefficients.

Figure S3. Phylogenetic tree of the 131 study species.

Figure S4. Partial dependency plots from BRTs for days until germination.

Figure S5. Partial dependency plots from BRTs for days until first true leaf.

Figure S6. Partial dependency plots from BRTs for days until third leaf.

Table S1. Study species by geographic location.

Table S2. Study species by family, origin, life-history strategy, geographic location and seed source.

Table S3. Characteristics of the six geographic locations.

Table S4. Number of phylogenetically paired families per geographic location.

Table S5. Factor loadings for all traits on first three principal components.

Table S6. Pagel's lambda statistic for phenological and morpho-physiological traits.

How to cite this article: Slate, M. L., Sporbert, M., Hensen, I., Hierro, J. L., Korell, L., Larios, L., Nagy, D. U., Pearson, D. E., Waller, L., Wolf, F., & Rosche, C. (2025). Drivers of phenological transitions in the seedling life stage. *Functional Ecology*, *00*, 1–14. https://doi.org/10.1111/1365-2435.70003