

Comparison of the geographic variation in seed traits of a native species (*Phragmites australis*) to an alien invasive species (*Spartina alterniflora*) in Chinese coastal marshes

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Abstract

Seeds are crucial for both maintaining existing plant populations and for colonizing new areas, and knowledge of traits helps us understand the colonization potential of successful species. One way to study seed-trait variation is to make comparisons along latitudinal gradients. Usually, however, only geographic variation in seed mass is studied systematically and knowledge of other traits is lacking, e.g. traits associated with seed production and nutritional status. Moreover, there have been few comparisons between native species and exotic invasives. Here, therefore, we compared morphological traits and nutrient concentrations of the seed of two competing species, a native species (*Phragmites australis*) and an alien one (*Spartina alterniflora*) along a 20°-latitudinal gradient of the Chinese coast and investigated which environmental factors were important in driving differences between these two contrasting species. Significant differences were found between the two species for nine of the 13 traits in their responses to latitude. Furthermore, temperature-mediated climate factors were the dominant variables influencing the latitudinal variation of seed traits in both species compared to soil properties. For the first time, our study shows the latitudinal variations in seed traits of the native *P. australis* and alien *S. alterniflora*. Our study predicts that the greater fecundity of high-latitude *S. alterniflora* populations poses a threat to *P. australis*, the native competitor.

Introduction

Seed development through to seedling emergence is one of the most pivotal phases in the life history of plants (Harper, 1977). Seeds are different from other plant organs because they can exist independently of the parent plant, and their traits vary greatly both within- (Liu et al., 2016; Marchelli and Gallo, 1999; Winn and Werner, 1987) and between-species (Guo et al., 2010; Moles et al., 2007; Murray et al., 2004; Soper Gorden et al., 2016). This has led to different species having distinct reproductive strategies as a way of encompassing diverse abiotic conditions (latitude, altitude, etc.). However, in contrast to the vegetative organs of the plant (e.g., leaves), ecologists have only recently begun investigating the large geographical patterns of plant reproductive organs (e.g., seed).

One way to study the relationship between plant traits and environmental factors is to assess changes in traits at the macro-geographic scale using either latitudinal or altitudinal gradients. This approach provides a natural testing ground for exploring the response of widespread living organisms to biological and environmental change (Körner 2007; De Frenne et al. 2013). We would expect the form and function, of both plants and their seeds to vary in the diverse environments found along such gradients indicating different resource acquisition strategies of plant species (Díaz et al. 2016). Therefore, there has been much interest in determining how latitudinal gradients affect seed mass, an important plant functional trait that plays a major role in reproductive success (Daco et al. 2021; Ulrich et al. 2023). However, conflicting results have been found when seed mass has been compared along altitudinal gradients with some studies showing a reduction in seed mass with increasing latitude (Donoso, 1979; Gallagher and Leishman, 2012; Marchelli and Gallo, 1999; Moles et al., 2007; Moles and Westoby, 2003) and others showing the opposite trend (Graae et al. 2009; Carón et al. 2014). Moreover, almost all studies of seed

traits along latitudinal gradients concentrate on seed size and mass, and much less is known about the effects on other seed traits, e.g., seed set, seed production, and seed nutrient concentration. To our knowledge, research on latitudinal gradients of seed nutrient concentrations is particularly scarce (De Frenne et al. 2010). Given the importance of seed nutrients for initial seedling growth, surprisingly little attention has been paid to seeds compared to plant leaves and fine roots (Hu et al. 2020; You et al. 2023). Meanwhile, these patterns observed in leaves and roots, which can be explained by the limitation of soil nutrients at higher latitudes (geochemical hypothesis) and by direct temperature effects on plant physiology (growth rate hypothesis) (Lovelock et al. 2007; Elser et al. 2000), may not be comparable to seed. This lack of knowledge may limit our understanding of resource acquisition strategies of plants across latitudes. Furthermore, it is also possible that within-species variations in the traits of species with a widespread distribution may be related to environmental factors that also vary along the geographic gradient, especially across different latitudes (Wright et al. 2017; Ren et al. 2020). Numerous studies have examined the association of seed traits with variation across environmental gradients (Murray et al. 2004; Soper Gorden et al. 2016; Wu et al. 2018). However, most previous studies focused only on climate-dependent abiotic factors without considering the effects of soil, and their interaction or the relative importance of climate and soil factors.

Knowledge about seeds and their traits along latitudinal gradients, which almost always reflect a climatic range, is of particular importance in investigating the threat to native ecosystems of invasive alien species (Guo et al., 2023; Soper Gorden et al., 2016; Wu et al., 2018). Invasive species have been identified as one of the problems associated with global warming, this is driven by globalization as well as climate change and is increasing in speed, scale, and damaging impact with little evidence that this impact will diminish (Seebens et al. 2017). The enhanced sexual reproductive capacity of some invasive species relative to native species has been shown to be an important factor in their success (van Kleunen et al. 2015). Seed mass and size are important indicators for assessing the invasive ability of many invasive plants, but the relationship between seed size and invasive ability can vary depending on species (Leishman et al. 2000), with some alien species relying on small seeds for long-distance dispersal and invasion (Moodley et al. 2013), and others relying on large seeds for survival in specific habitats (Burke and Grime, 1996). Therefore, to achieve an evidence-based approach for predicting potential harm caused by invasive species, the first step is to understand how species reproduce and the factors that affect reproductive success (Andersen et al. 2004). Here, we compare the seed traits of an invasive species relative to a native one on a latitudinal gradient along the coast of China.

The native species is *Phragmites australis*, a cosmopolitan plant growing mainly in wetlands. This species has high morphological diversity and plays an instrumental role in the maintenance of biodiversity and ecosystem function. It is often used as a model species, i.e. the native counterpart, for studies on biological invasions and responses to climate change (Ren et al. 2020; Liu et al. 2024). This makes it an excellent choice to compare with the non-native macrophyte *Spartina alterniflora* along the eastern coast of China, where it is encroaching extensively upon indigenous *P. australis* ecosystems (Strong and Ayres 2013). *S. alterniflora* is native to the Atlantic and Gulf Coasts of America and has become widely invasive subsequent to its introduction in 1979 (Meng et al. 2020). Both species are

distributed over a large latitudinal range and have a wide range of climatic habitats and ecological niches (Liu et al., 2016; Packer et al., 2017). Recent empirical studies have found latitudinal variations in morphology (Liu et al., 2022; Meyerson et al., 2016), growth (Liu et al. 2016; Ren et al. 2020), and phenology (Bastlová et al. 2006) of both species. However, knowledge of possible latitudinal variation in seed traits, vital for plant recruitment and population regeneration, is lacking.

Here, we compared the pattern of latitudinal variation influencing seed traits in both species, the native *P. australis*, versus the invasive alien *S. alterniflora*, an invasive alien) and assessed the impacts of climatic and soil factors that might influence them across a 20°-latitudinal transect along the coastal marshes of China. In doing so, we address two main questions: (1) Are there any variation differences in seed traits between the native and alien species along the coastal latitudinal gradient in China? (2) If so, which environmental factors are most important in influencing these variations? Furthermore, we hypothesized that: (i) there will be significant latitudinal patterns in most seed trait variables for at least one species; (ii) seed traits will exhibit parallel latitudinal clines in both species across the latitudinal gradient; and, (iii) climate variables will be the dominant factor driving the latitudinal pattern of different species, compared to soil ones. This study also provides new insights into the ecological mechanisms of latitudinal variation of the reproductive organ morphology in native and invasive species.

Material and methods

Study region

We collected seeds and soils from ten coastal marshes on a 2,100 km, 20°-latitudinal-range transect along the Chinese coast (Fig. 1). This transect covered tropical, sub-tropical and warm-temperate climatic zones; the range in mean annual temperatures was from 10.6 to 24.1°C and annual precipitation from 839 to 2023 mm (Table S1).

Climate variables

Climate data was acquired for each site from the NOAA—National Centers or Environmental Information database (<https://www.ncei.noaa.gov>). Data (four measures of temperature and four measures of precipitation) were abstracted from the meteorological station closest to each sample site (always < 50 km distance, Table S1).

Seed collection and sampling survey

All samples (seeds and soils) were collected during the growing season (September 2021 to January 2022) from sites that were large contiguous areas (> 50 km²) of either *P. australis*- or *S. alterniflora*-dominated salt marsh. At each site, we randomly selected five subsites that were located at > 50-m intervals. Then, we selected five 1 × 1 m quadrats that were separated by 10 m to collect seeds and measure plant densities. In each quadrat, we counted the number of adult plants and flowering culms to estimate individual and spikelet densities, and harvested seeds for seed mass assessment. As we found

large-scale unfilled seeds in the field (especially for *S. alterniflora*), we increased the number of subsites for seed set assessment to 10 for *P. australis* and 15 for *S. alterniflora*. We sampled five inflorescences of plants from each subsite located randomly at > 10 m intervals where we collected healthy and mature inflorescences that were free of herbivory. Thereafter, high within-individual variation in seed size was detected (Michaels et al., 1988), so we similarly increased the number of subsites for seed size assessment to 20 at each site. Fifty filled seeds from five apparently healthy individuals were collected randomly from each subsite for seed size traits assessment. Care was taken to ensure that the collected inflorescences had not been grazed by insects. Seeds were separated, air-dried and then stored at 4°C.

Within each quadrat, the pH, salinity and conductivity of soil-pore water were measured, and surface sediments were sampled (0–15 cm depth, 25 cm diameter, areas = 491 cm²). Samples were air-dried and ground to pass a 0.15 mm sieve before elemental physio-chemical properties analysis (Table S2).

Seed traits

Seed set, an indication of potential seed viability, was estimated by collecting inflorescences from each subsite. Seed set (*S*%) was then calculated as the number of filled seeds/the number of spikelets for each inflorescence (Ishii and Kadono, 2002). Assessment of the number of unfilled spikelets with non-viable seeds was done by touch. Seed production, (*SP*, number.m⁻²) was calculated as individual density × spikelet rate × average number of seeds per spikelet.

Thereafter, we used electronic digital calipers (Deli 91150, China) to measure the length (*L*), width (*W*) and thickness (*T*) of each seed to an accuracy of 0.01 mm (Fig. S1). *L* was measured on the longitudinal axis of the seed; *W* was the diameter of the largest cross-section perpendicular to the longitudinal axis and *T* was the straight-line distance from the third plane perpendicular to the seed width. From these data, three variables were derived (1) seed surface area ($S = L \times W$), (2) seed volume ($V = L \times W \times T$), (3) seed aspect ratio ($AR = L / W$) and (4) seed shape index, $SSI = (L + W) / 2T$ (Table S3). For seeds of both species, five sets of 100 filled seeds were weighed separately from each subsite to calculate mean seed mass (*M*).

A seed sample (ca. 3-5g) was selected from the first three subsites sampled (*n* = 3 per site), oven-dried at 65 °C, and milled in a ball mill (Jingling Tiss-24, China) and used to determine concentrations of carbon (C), nitrogen (N) and phosphorus (P). Seed trait data are presented in Tables S3 and S4 for morphological and nutrient variables respectively.

Chemical analyses

C, N and P concentrations were measured in both seeds and soils. C and N were determined using an elemental analyzer (Elementar Vario MAX, Germany), and P was measured using a flow injection auto-analyzer (Skalar San⁺⁺, Netherlands) after HClO₄-H₂SO₄ digestion (Hu et al. 2018). Soil C, N and P were denoted TC, TN and TP respectively.

Statistical analysis

Initially, the Generalized Additive Mixed Model (GAMM) was used to test whether each seed trait varied between the two species along the latitudinal gradient. We chose GAMM rather than other regression approaches due to the possibility of non-linear relationships between seed traits and latitude. GAMM is a semi-parametric and flexible regression technique that is not limited to linear relationships and statistical data distributions making it ideal for modeling the different between-treatment latitudinal responses detected here (Crawley, 2013). It is often used to predict "softened", smooth functional relationships between the response variable and explanatory variables (Pedersen et al. 2019), with the advantage of no predefined shapes for describing functional relationships. Thus, we used a GAMM approach to determine the general patterns of the relationships between the several seed variables with respect to latitude for both *P. australis* and *S. alterniflora*. To ensure the normality of the data, all seed morphological traits were $\log_{10}$ -transformed and proportional data were logit-transformed before analysis (Warton and Hui 2011). Each GAMM variable was modeled individually using the "gamm" function in the 'mgcv' package (Wood 2017); each model treated species (factor), latitude (vector) as fixed effects, and sampling subsites nested within latitude as random effects (Guo et al., 2023). Cubic regression smoothing functions and three knots were used to avoid over-fitting the data, while still allowing for unimodal or slightly more complex model fits (Wood 2011). We used the 'ggplot' package to draw the latitudinal clines between the response variables and latitude through the final model.

To answer question 2, we used multiple regression to assess the joint effects of climate and soil on the latitudinal variations of seed traits for each species separately. All environmental variables were $\log_{10}$ -transformed before analyses. First, we used a backward stepwise multiple regression to select the best-fit models with the smallest AIC value of each seed trait. Second, we divided the variables in the best-fit model into two categories: soil and climate. Then, linear mixed models were fitted using the 'lme4' package to evaluate the response of seed traits to both climate and soil variables as fixed effects and sampling subsites nested within latitude as a random effect (Bates et al. 2015). Hereafter, we used the 'glmm.hp' package to evaluate the relative importance of the two predictors as drivers of the variations of seed traits (Lai et al. 2022) and visualized the modeled results using the 'ggplot2' package (Wickham, 2016). All analyses here were performed in R Statistical Environment v4.1.1 (R Core Team 2020).

Results

Difference of seed traits between *P. australis* and *S. alterniflora*

There were significant differences between the species for 12 of the 13 trait variables measured, the exceptions were seed C concentration (Fig. 2; Table S5). For seed number traits, *P. australis* produced significantly more seeds than *S. alterniflora* ($p < 0.001$, Fig. 2a, b). However, *S. alterniflora* had significantly larger seeds than *P. australis* ($p < 0.001$, Fig. 2c-i; Table S5). For seed nutrient traits, the small-seeded *P. australis* contained higher N and P concentrations than the large-seeded *S. alterniflora* ($p < 0.01$, Fig. 2j,k; Table S5).

Seed trait variation along the latitudinal gradient

All variables apart from seed thickness and seed C concentration also showed a significant relationship with latitude for at least one of the species (at least $P < 0.05$, Fig. 2). Seed thickness and C concentration will not be discussed further. Seed set was lowest at low latitudes for both species, although *P. australis* had a greater seed set than *S. alterniflora* (Fig. 2a). However, the seed set for both species increased with latitude; *P. australis* peaking at ca. 32°N and *S. alterniflora* increasing consistently and converging with *P. australis* above 35°N (Fig. 2a). Seed production of *P. australis* was greater than *S. alterniflora* over the entire latitudinal range with both species increasing with increasing latitude (Fig. 2b).

S. alterniflora had larger seeds on all morphological traits measured (mass, length, width, thickness, surface area, volume, shape index, and aspect ratio) across the entire latitudinal range compared to *P. australis* (Table S5). *P. australis* showed a slight curvilinear relationship in all morphological traits (except seed aspect ratio) with a minimum between 30–35°N which contrasted with *S. alterniflora* which showed a reduction in all traits over the entire latitudinal range (Fig. 2e-i).

The concentrations of the seed nutritional variables showed completely different responses. Seed C concentrations did not show any significant difference between species or in response to latitude for either species (Table S5). Seed N concentrations were greater in *P. australis* than *S. alterniflora* and both declined with latitude, *S. alterniflora* reducing faster than *P. australis* (Fig. 2j). *P. australis* had a significantly greater seed P concentration than *S. alterniflora* ($P < 0.01$, Fig. 2k) with the concentration reducing with latitude for *P. australis* whereas *S. alterniflora* showed no significant relationship.

Relationships between seed traits and environmental factors

The selected models from the stepwise multiple regression for seed traits all produced highly-significant models with the exception of *S. alterniflora* seed shape index $R^2 = 0.09$; all other trait variables $R^2 > 0.50$, Table S6). More climate variables were included in the best-fit model relative to soil variables (Table S6). The LMMs explained 27% and 35% of variation in seed production and P concentration of *P. australis* respectively, as well as the variations of seed mass (19%), thickness (54%), volume (34%), and N concentration (16%) of *S. alterniflora* (Table S7). Since there were no significant patterns of latitudinal variation in seed thickness of *S. alterniflora* (Table S5), here we concentrate on seed mass, volume, and N concentration of *S. alterniflora*.

For *P. australis*, climate variables had the dominant effect, accounting for 62.13% of the explained variations in the latitudinal pattern of seed production, followed by soil variables explaining 37.87%. Specifically, the mean temperature of the warmest month and soil conductivity had significant negative associations with seed production, no significant effects were detected for the other variables (Fig. 3a; Table S8). Climate variables had the dominant influence in the variation of seed P concentration (87.39%), compared with soil variables (12.61%). Seed P concentration was associated positively with soil salinity, mean annual temperature (MAT), growing degree hours (GDH), mean precipitation of the

wettest month (MPWM) and relative humidity (RH), and associated negatively with soil C concentration, mean temperature of the coldest month (MTCM), mean annual precipitation (MAP) and mean temperature of the warmest month (MTWM) (Fig. 3b; Table S8).

For *S. alterniflora*, climate variables explained the largest part of the variation in both seed mass and volume (86.63%, 80.72%). Seed mass significantly decreased with increasing soil C concentration, salinity, MTWM, MTCM and MAP. Meanwhile, soil pH, MAT and GDH had significant positive effects (Fig. 4a; Table S8). Moreover, seed volume showed a significantly negative association with soil salinity, C concentration, MTCM and MPWM, and a significantly positive relationship with soil N concentration, GDH and MTWM (Fig. 4b; Table S8). In contrast to the above, soil variables explained the largest fraction of variation in seed N concentration (82.78%), followed by climate variables (17.22%). Seed N concentration significantly increased with increasing soil pH, MAT, GDH and MPDM, while decreasing with increasing MTCM, MAP and MTWM (Fig. 4c; Table S8).

Discussion

Latitudinal variation in seed traits has been shown to be a potential driver of the success of invasive alien species in new areas (Liu et al., 2016; Liu & Zhang, 2021). Most studies of seed traits have concentrated solely on the invasive species and here we include a comparison in the variation in seed traits of an exotic alien species (*S. alterniflora*) with a native species (*P. australis*) that the alien is likely to impact. We carried out this comparison on a 2,100-km 20°-transect along the whole coastal zone of China. Overall, our comparisons showed considerable differences in both morphological and chemical traits. Moreover, the latitudinal variations of seed traits in both species are more closely related to climate variables than to soil variables.

Non-parallel seed traits pattern along latitudinal gradients between the two species

There were differences between the two species for almost all traits measured, the exception was seed C concentration. Moreover, significant latitudinal patterns were observed in most trait variables for at least one species, which confirmed our hypothesis 1. Most studies on variation in seeds with latitude concentrate on seed mass and size and most of these studies show a decreasing trend in seed mass and size with latitude (Moles et al. 2007; Soper Gorden et al. 2016; Wu et al. 2018). Here, however, there were clear differences between species. *S. alterniflora* had heavier and bigger seeds and showed a decreasing response with latitude (Fig. 2e-g), whereas *P. australis* produced much lighter, smaller seeds and showed a curvilinear response across the latitudinal gradient with the lowest size at mid-latitudes (30-35°N) (Fig. 2f). This result is contrary to most previous inter-specific and intra-specific studies (Daco et al. 2021; Wu et al. 2018; Moles and Westoby 2003). Interestingly, both seed set and seed production by *P. australis* were much greater than *S. alterniflora* at low latitudes, and they both showed increasing clines with latitude (Fig. 2a, b). The few other studies that have investigated the latitudinal pattern of seed set have found conflicting results (Liu et al. 2016; Daco et al. 2021).

Previous studies have shown that differences in seed traits among plants reflect different reproductive responses, with larger seeds having a significant advantage in resource competition, being less dependent on the environment and being more adaptable (Manning et al. 2009). Smaller seeds have a greater dispersal capacity and can therefore potentially colonize occupy more habitats and over longer distances (Moles and Westoby 2006). Our results indicate that the alien plant *S. alterniflora* wins by “quality”, while the native plant *P. australis* wins by “quantity”.

In contrast to our hypothesis 2, latitudinal variation in seed traits was not consistent between the two species (Fig. 2). As non-parallel latitudinal patterns were detected, this suggested that the two species have different responses across the latitudinal gradient. One might predict that a successful colonizer would produce greater seed numbers, i.e., a greater propagule pressure, and hence an increased likelihood of establishment (Bufford and Hulme, 2021). However, large-seeded species may not always have an establishment advantage over the species that produce a large number of small seeds (Coomes and Grubb, 2003; Moles and Westoby, 2004). We know that *P. australis* is distributed mainly in the northern part of the Chinese coast and *S. alterniflora* dominates the mid-section (Gu et al. 2021) although there are some places where they are sympatric (Guo et al., 2023; Xu et al., 2020). We found that seed set in *P. australis* was greatest at mid-latitudes (Fig. 2a), while seed size was lowest at mid-latitudes (Fig. 2c-h). The intra-specific variation in *P. australis* seed along the latitudinal gradients may be due to a plastic response associated with reduced resource availability caused by competition with *S. alterniflora* (Minchinton et al. 2006). *P. australis* in mid-latitudes produced more, smaller seeds suggesting a higher fitness for reproduction and survival which might improve their ability to colonize under the invasive *S. alterniflora* (Belzile et al. 2010; Bufford and Hulme, 2021).

For *S. alterniflora*, the prolific seed set at high latitudes indicates that they produce a continuing invasive threat northwards with subsequent competition and possible displacement of the native species *P. australis* at higher latitudes (Liu et al., 2016; Zhang et al., 2020). At present, *P. australis* still dominates the northern coast of China, but continuous monitoring is needed to assess future colonization by *S. alterniflora*. This study is the first to our knowledge to compare latitudinal variation in seed numbers of invasive and native plants. In short, evidence is accumulating that the invasive speed of *S. alterniflora* in northern China is likely to be inexorable (Liu et al., 2016).

Less attention has been paid to latitudinal clines in seed nutrient concentrations. We found that both seed N and P concentrations were significantly greater in *P. australis* than in *S. alterniflora*. Seeds with high N concentration have more free amino acids, which are essential for seedling growth and development, and provide seedlings with a growth rate advantage (Ching and Rynd, 1978). P is a major component of the cell nucleus, protoplasm, and nucleoproteins in plants, and is essential for cell division and tissue development (Mulligan and Patrick, 1985). As seedlings derive all of their nutrients in the early growth stages (first 20 days or so) from internal resources, seeds with high N and P concentrations will have a competitive advantage during establishment (Lamont and Groom, 2013; Milberg and Lamont, 1997).

Our results also indicate that seeds of both species showed the same latitudinal variations in seed N concentration, that is, seed N concentration decreased with increasing latitude. This agrees with De Frenne et al. (2011) who showed that seed N concentration decreased with increasing latitude in *Anemone nemorosa*. Seedlings of plants in southern latitudes may have to cope with more environmental risks, such as higher herbivory pressure (Cronin et al., 2015; Guo et al., 2023) and summer drought (Drenovsky and Richards, 2005). Seedlings produced from seeds with higher N concentrations show better resistance to poor environmental conditions than those with lower N concentrations (Naegle et al. 2005).

Latitudinal gradients in seed traits mainly related to climate

Given that environmental variables vary along latitudinal gradients, factors such as climate (e.g. temperature and precipitation) and soil variables (e.g. soil salinity, pH, and nutrients) are often found to affect seed trait variations (Murray et al. 2004; Tautenhahn et al. 2008; Carón et al. 2014; Soper Gorden et al. 2016; Wu et al. 2018). However, most previous studies have investigated the effects of climate factors on seed trait variation and have not considered the relative importance of climate and soils (Murray et al. 2004; De Frenne et al. 2011; Soper Gorden et al. 2016; Wu et al. 2018). Our study considered the relative importance of the effects of climate and soil variables on the variation of seed traits in two plant species and the results are consistent with hypothesis 3. In general, latitudinal variations in seed traits of the two species were related primarily to climate factors, while soil factors were much less influential on seed traits.

For the exotic *S. alterniflora*, we found that temperature-dependent climate variables had a major association on both seed mass and size (volume), with temperature and growing degree hours having a stronger influence compared to precipitation, which is consistent with Murray et al. (2004) and Wu et al. (2018). We also found that mean annual temperature and growing degree hours influenced seed mass positively, perhaps because higher temperatures would enhance photosynthesis in plants, which could in turn increase the biomass available for the production of large seeds (Murray et al. 2004; Hovenden et al. 2008). Soil salinity and nutrients appeared as secondary factors influencing variations in seed mass and size, with smaller seeds at high salinity and larger seeds at high N concentration. This is consistent with many studies that have investigated the relationship between seed size and soil resources (Carmona et al. 2015; Dirks et al. 2017; Santini et al. 2017), and this pattern could be attributed to growth-allometry and size-asymmetric competition (DeMalach et al. 2019).

We also found that environmental characteristics had a greater impact on seed production than seed size in *P. australis*. In particular, seed production was mainly affected by climate variables, with less seed production in high mean temperatures of the warmest month. As the thermal optimum for photosynthesis is about 30 °C, periods of extremely high temperatures will likely reduce *P. australis* productivity (Srivastava et al. 2014), and this might explain why *P. australis* are mainly distributed in northern China. Furthermore, we found that temperature-dependent climate factors were the dominant factors in the variation of seed P concentration in *P. australis* across the latitudinal gradient. Specifically, high seed P concentration was correlated with high mean annual temperature and growing degree hours.

According to the growth rate hypothesis, rapidly growing plants need more P concentration for the synthesis of messenger RNA to achieve high growth rates (Elser et al. 2000). This result is consistent with the resource availability hypothesis that plants at low latitudes grow faster than those at high latitudes due to the resource limitations imposed by climate (Hahn and Maron 2016). Hence, organisms with high growth rates at high temperatures contain higher P concentrations. It is striking that soil properties play a crucial role in determining the latitudinal variation of seed N concentration in *S. alterniflora*, with higher seed N concentration at higher soil pHs (Fig. 4c). This suggests that our findings were also consistent with the geochemical hypothesis, which emphasizes the important role of soil nutrients in determining organism element concentrations (Lovelock et al. 2007). Compared to *S. alterniflora*, environmental factors had a negligible association with the seed traits of *P. australis*. Therefore, *S. alterniflora* may have a greater ability than *P. australis* to adapt to the rapidly-changing climate on a large geographical scale. However, the relationship between soil and climate variables and seed traits can be complicated and poorly understood, so further research is needed.

Conclusions

We found significant latitudinal variations in the seed traits and seed nutrient concentrations of both *P. australis* and *S. alterniflora* in the coastal tidal marshes of China, while there were dissimilarities in latitudinal patterns in seed traits between the two species. Since phenotypic variation can facilitate the adaptation of plant species to different environments, variation in seed traits may improve the adaptability of these species. We suggest that the invasive species *S. alterniflora* has greater adaptability to environmental variation compared to the sympatric native species *P. australis*, which may help its invasion of the Chinese entire coast.

On the other hand, our study quantifies the relative importance of temperature and soil effects on geographic patterns of seed traits in both species. We found that the observed variation in seed traits along environmental gradients in both species may highlight the significant impact of climate factors. Meanwhile, our study indicated that *S. alterniflora* has the potential to respond more positively to environmental changes than *P. australis*. As such, we predict that the invasion of *S. alterniflora* in the northern Chinese coastal zone is likely to continue in the future. Our study recommends that more strict and valid management measures should be implemented to prevent and control the massive invasion of *S. alterniflora* in northern China.

Declarations

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Figures

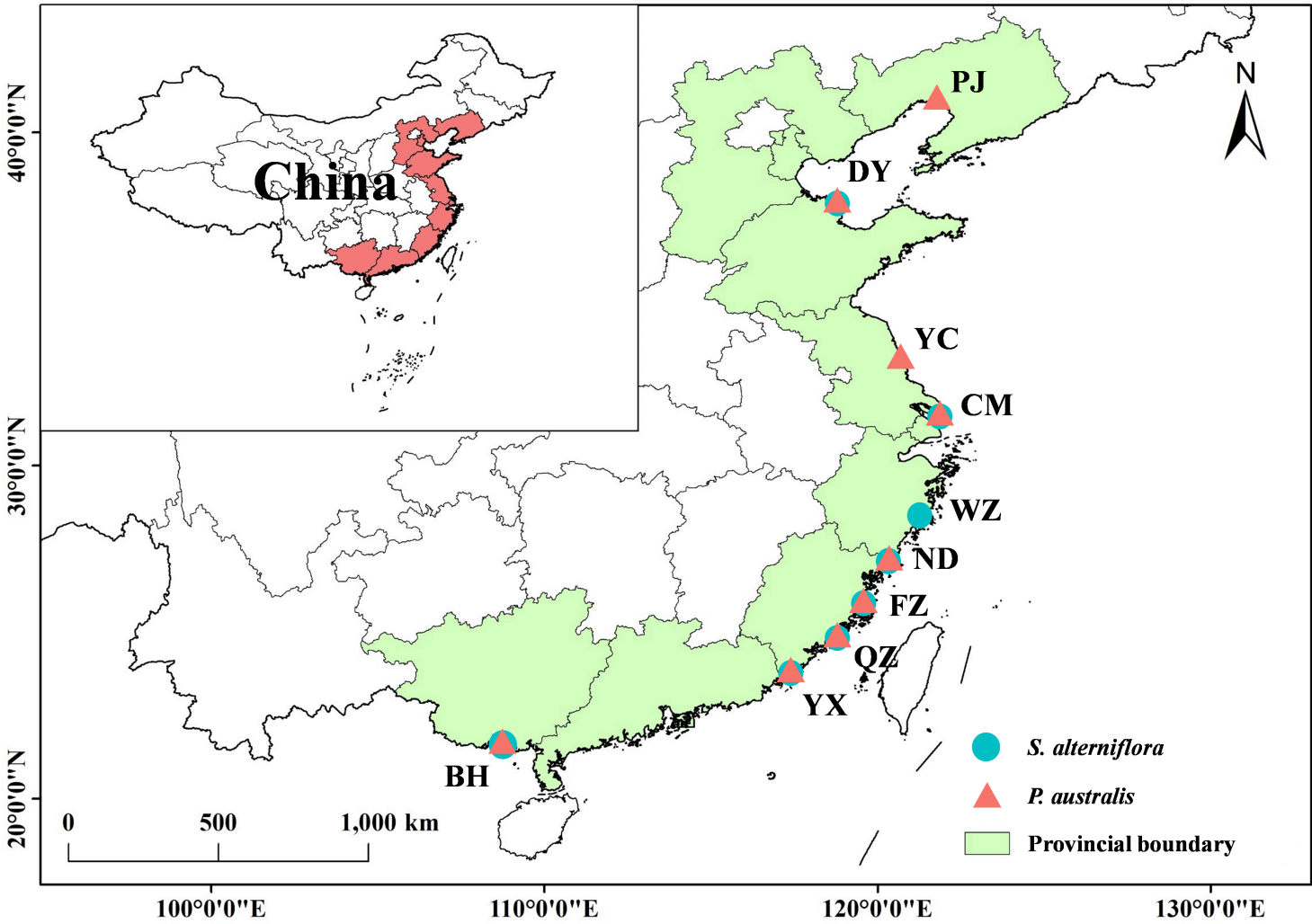
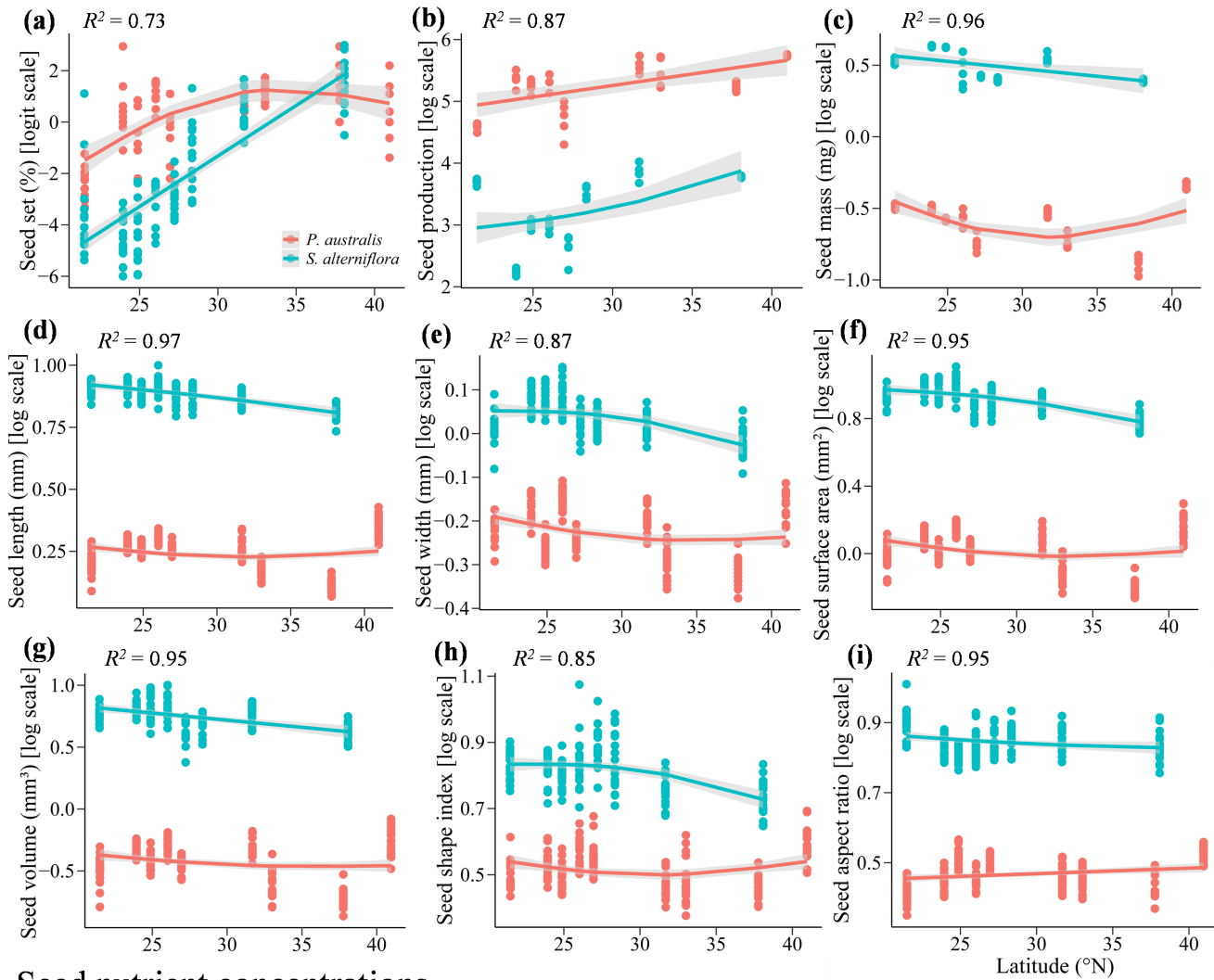


Figure 1

Locations of the sampling sites along the 20° (2100 km) latitudinal transect of tidal marshes along the coastal zone of China for collection of the *Phragmites australis* and *Spartina alterniflora* seeds and soils. Detailed information is provided in Table S1.

Seed morphological traits



Seed nutrient concentrations

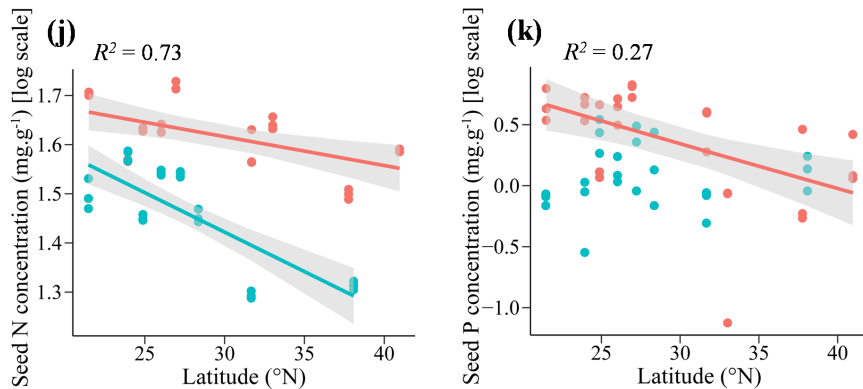


Figure 2

Modelled responses of 11 seed properties of *Spartina alterniflora* (SA, blue) and *Phragmites australis* (PA, red) on a 20°-latitudinal transect along the coastal zone of China. Each panel shows the fitted relationship (between seed traits and latitude for each species separately). We plotted the regression lines ($\pm 95\%$ CL) for each plant species; where no line is shown there was no significant relationship

detected. In each panel, R^2 -values is presented and $p < 0.05$ indicates a significant effect. Full statistical information is available in Table S5.

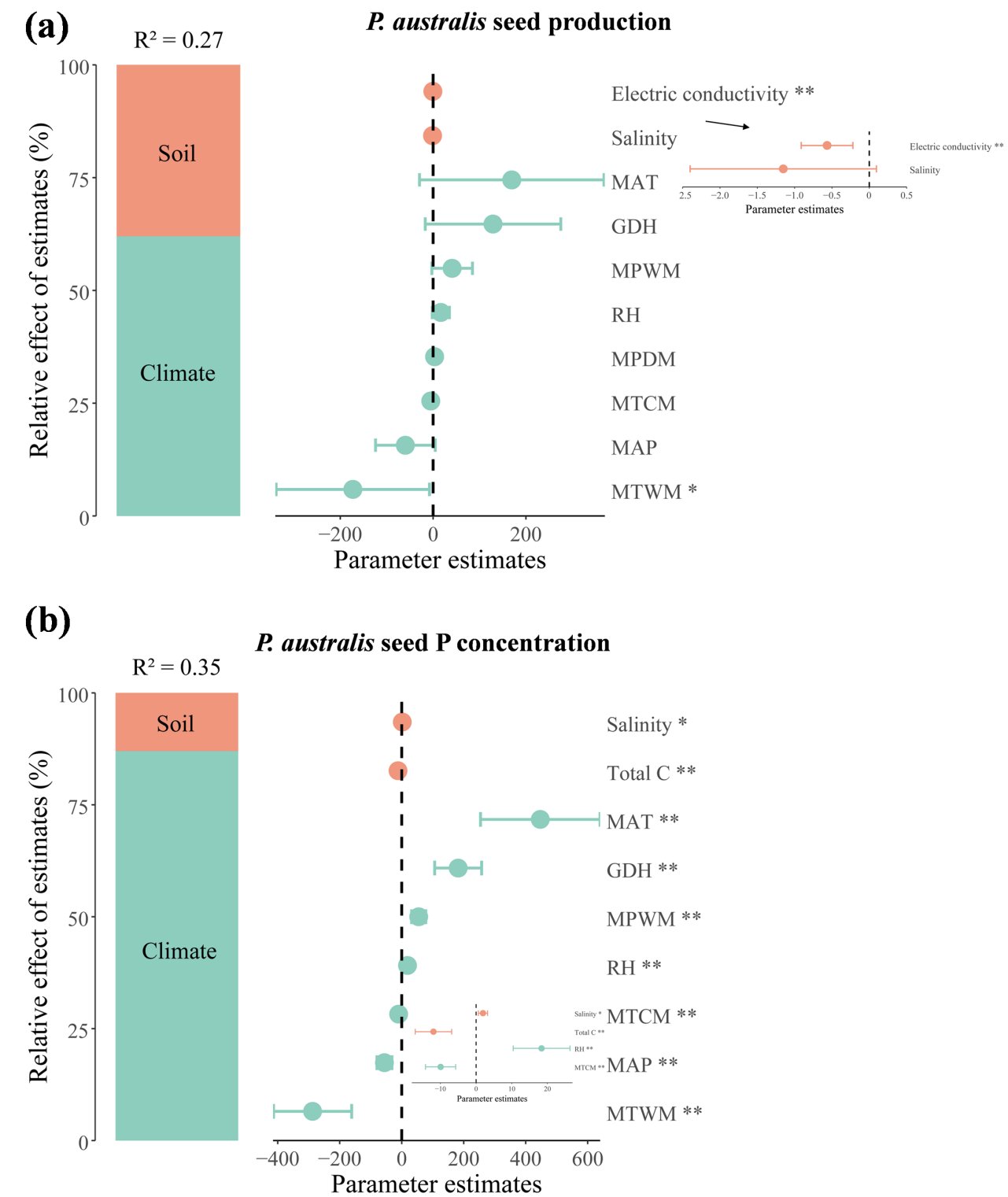


Figure 3

Relative contributions (left) and effect sizes (right) of environmental predictors on (a) seed production ($\log_{10}$ -transformed) and (b) seed P concentration ($\log_{10}$ -transformed) of *Phragmites australis* sampled from a 20°-latitudinal transect along the coastal zone of China. Full statistical information is available in

Table S8. Key to codes: Electric conductivity = soil electric conductivity; Salinity =soil salinity; Total C = total soil C concentration; MAT = mean annual temperature; GDH = growing degree hours; MPWM = mean precipitation of the wettest month; RH = relative humidity; MTCM = mean temperature of the coldest month; MAP = mean annual precipitation; MTWM = mean temperature of the warmest month.

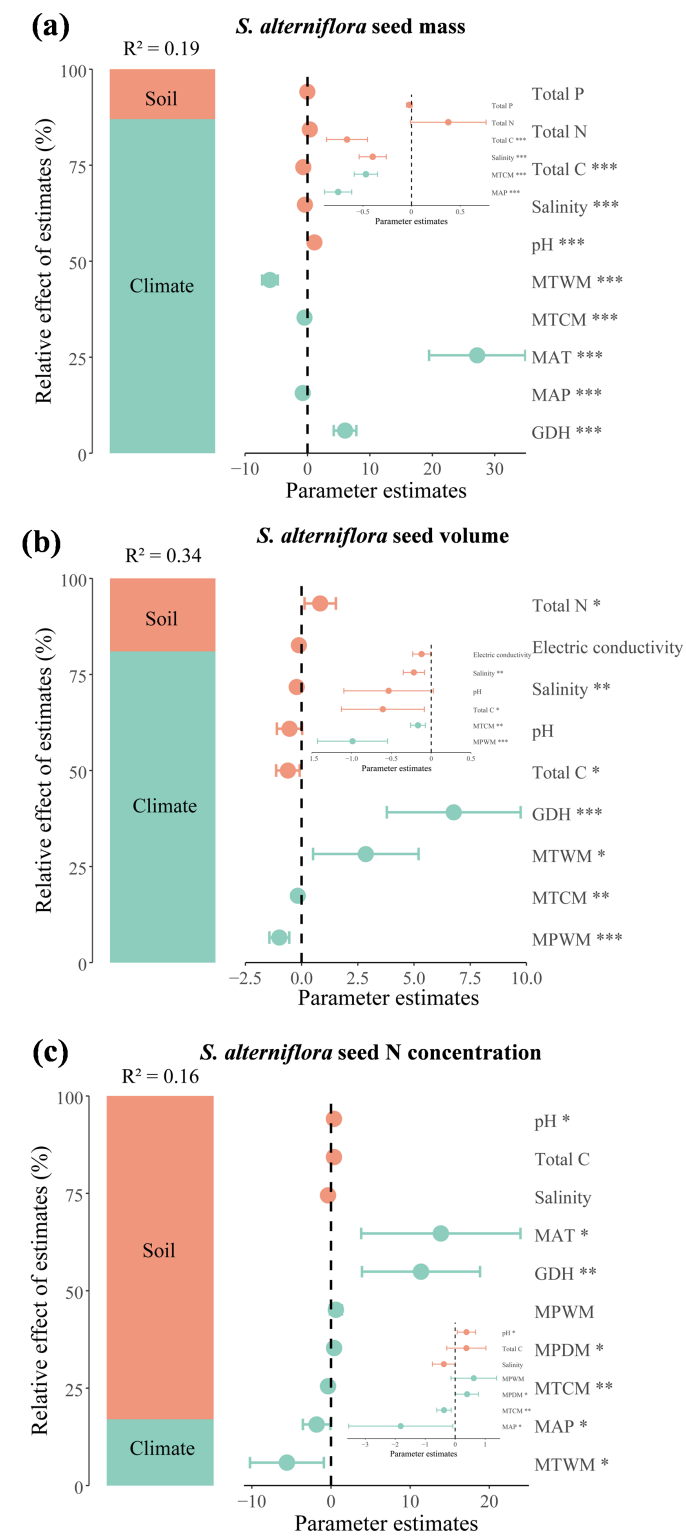


Figure 4

Relative effects of multiple predictors of (a) seed mass ($\log_{10}$ -transformed), (b) seed volume ($\log_{10}$ -transformed), and (c) seed N content ($\log_{10}$ -transformed) of *Spartina alterniflora* sampled from a 20°-latitudinal transect along the coastal zone of China. Full statistical information is available in Table S8. Abbreviations follow the legend in Fig. 3.

Supplementary Files

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