



Unraveling the roles of various ecological factors in seedling recruitment to facilitate plant regeneration

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ABSTRACT

Unraveling the mechanisms causing restricted regeneration and limited distribution range has become a core issue for biodiversity conservation. Anthropogenic disturbances are changing a variety of ecological factors, but how their relative importance varies across multiple regeneration stages is still unclear even in a single species. In this study, we focused on *Metasequoia glyptostroboides*, a relict species suffering a severely restricted regeneration in the wild, and performed controlled experiments using 54,600 seeds to investigate seedling emergence and growth in both its natural and adjacent unoccupied habitats to disentangle the roles of seed availability, interspecific competition and herbivore/omnivore attack across early regeneration stages. Our results showed that these factors mainly exerted their predominant effects at initial regeneration stages. Compared with herbivore/omnivore attack, seed availability and interspecific competition were the crucial factors regulating seedling emergence. The first-year seedling survival rate was only significantly influenced by interspecific competition, and the survival of second-year seedlings was independent of all these factors. Moreover, we detected similar impacts of these factors in the adjacent unoccupied habitats to those in the natural habitats, indicating their roles in limiting population expansion. Our findings provide some suggestions to assist the natural regeneration and range expansion of *M. glyptostroboides*, and our experiment design can be applied in the assessment of endangerment causes for other endangered plants.

1. Introduction

Drastic loss of plant species and the consequent degradation of ecosystems are occurring worldwide, largely as the response to anthropogenic disturbances like land use changes and fragmentation (Pimm et al., 2014; Suding et al., 2015). Besides the direct destruction of plant populations, human impacts also threaten the persistence of plant species through restricting their natural regeneration (Stevens-Rumann et al., 2018; Hewitt et al., 2019; Wang et al., 2020). Such reduced success of regeneration is particularly devastating for endangered plants, because they usually have extremely small population sizes and narrow distribution ranges (Gómez-Aparicio et al., 2005; Liu et al., 2020). Therefore, revealing the underlying mechanisms of restricted regeneration has become a core issue for biodiversity conservation and

ecosystem restoration (Hens et al., 2017; Gardner et al., 2019).

Restricted regeneration can be caused by a variety of ecological factors, of which insufficient seed availability, strong inter-specific competition and serious herbivore/omnivore attack are generally considered to be crucial (Nathan and Muller-Landau, 2000; Münzbergová and Herben, 2005; Brandt and Seabloom, 2012; Wang et al., 2019a). However, to date the relative roles of these factors have been long debated even in a single species (Poulsen et al., 2007; Norgauer and Newbery, 2010). This is mainly because they often impose their major effects at different regeneration stages but most extant studies only focused on regeneration at a particular or very few stages (Nathan and Muller-Landau, 2000; Burkart et al., 2010).

Insufficient seed availability due to low seed rain density and limited seed dispersal can critically suppress seed germination and seedling

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emergence (Clark et al., 1998; Geertsema et al., 2002; Münzbergová and Herben, 2005). Though seed availability is associated with the following density-dependent processes regulating seedling mortality, its impacts are likely to be less important at seedling growth stages, because interspecific competition with herbs and shrubs, and herbivore/omnivore attack are expected to be the dominant effects on seedling survival (and they are also crucial in seedling emergence) (Gómez-Aparicio et al., 2005; Burkart et al., 2010; Brandt and Seabloom, 2012; Carlson et al., 2020). However, these two factors may play minor roles at later regeneration stages, as saplings/young trees have become competitive and less vulnerable to herbivore/omnivore attack (Nathan and Muller-Landau, 2000; Collin et al., 2018; Wang et al., 2020). Therefore, we hypothesize that these three factors predominantly release their influences at the initial regeneration stages.

Moreover, facilitating population expansion into adjacent unoccupied habitats will greatly relieve the risk of local extinction in the disturbed areas and is an important part of the *in situ* conservation for endangered plants (Tang et al., 2018; Seahra et al., 2019). The limiting ecological factors in a plant's natural habitats, for example, limited seed availability, interspecific competition and herbivore/omnivore attack may also be effective during its colonization to new habitats (McCormick and Jacquemyn, 2014; Alexander et al., 2015). However, researches so far have rarely evaluated the relative roles of these factors in population expansion.

Therefore, for a better understanding of how regeneration and population expansion are restricted in plants, it is necessary to carry out investigations across multiple regeneration stages to examine the effects of various ecological factors and design a life stage-specific management for restoration and conservation programs. In this study, we used the endangered plant *Metasequoia glyptostroboides*, which suffers seriously restricted regeneration in its wild populations (Tang et al., 2011), as a case study to check the potential factors leading to the regeneration difficulty at early regeneration stages.

Metasequoia glyptostroboides is widely considered to be a "living fossil" and a flagship species for biodiversity conservation in China (Li et al., 2005; Tang et al., 2011). This species is the only extant species of genus *Metasequoia* (Cupressaceae) that was once widely distributed over the northern hemisphere, and abundant fossilized remains have been found across both Old and New Worlds (Lepage et al., 2005; Li et al., 2012). *M. glyptostroboides* was considered to have become extinct for several million years from early Pleistocene until the rediscovery of living trees in central China in 1946 (Ma, 2003; Lepage et al., 2005). Given its narrow distribution range (c. 800 km²) and small population size (c. 5500 wild individuals), it has been listed as an endangered species of IUCN (Farjon, 2013). It was also listed as one of the most evolutionarily distinct and global endangered of all the gymnosperm species (Forest et al., 2018), and as a wild plant with extremely small populations in China (Ren et al., 2012).

Although it has now been successfully introduced to approximate 60 countries, wild *M. glyptostroboides* populations are declining mainly due to severely restricted regeneration (Bartholomew et al., 1983; Tang et al., 2011). Many mature individuals can produce thousands of seeds per year, but heavy seed harvest for plantation has been conducted in the past decades, and thus seed availability is severely restricted (Wang et al., 2005). Furthermore, land-use change like agriculture exploitation and medicinal plant cultivation has been intensified recently, leading to dense herbs and shrubs and more exotic phytophagous insects (Personal observation by Y.-Y.L. and X.-Y.C.), which may prevent seedling emergence and impact seedling survival of *M. glyptostroboides*. However, whether these disturbances have significantly influenced natural regeneration of *M. glyptostroboides* and what is the relative importance of seed availability, interspecific competition and herbivore attack changes at different regeneration stages need to be evaluated to pinpoint the major bottlenecks in the conservation of *M. glyptostroboides*. Low seed viability is not likely to be a main limiting factor because the germination rate of *M. glyptostroboides* seeds can reach up to about 30% (Li et al.,

2012), with amounts of seedlings in its native habitats in the last mid-century (Chu and Cooper, 1950). In addition, while climate changes are a dominant factor limiting its distribution range, little effects of recent climate change in the central area of its natural distribution range have been found on its population regeneration (Fan et al., 2020).

Here, we focused on wild *M. glyptostroboides* populations and tracked the seed/seedling fates across seedling emergence and growth stages (Fig. 1a), to examine the effects of seed availability, interspecific competition and herbivore attack in both its natural and nearby unoccupied habitats. Specifically, we address the following two questions: (i) which ecological factors predominantly restrict regeneration at different stages? and (ii) what are the main factors preventing population expansion into adjacent unoccupied habitats? Based on our findings, we proposed some suggestions for *in situ* recovery of wild *M. glyptostroboides* populations, which are also likely to be applied in the conservation of other endangered plants.

2. Materials and methods

2.1. Study species and study area

Metasequoia glyptostroboides (Cupressaceae) is a wind-pollinated and wind-dispersed monoecious conifer. *M. glyptostroboides* regenerates via seeds in wild populations. Its seeds, inside megasporangiate cones, are thought to be non-dormant in soil. They mature in November and germinate in the following spring. The regeneration of wild *M. glyptostroboides* populations is seriously restricted, as almost all naturally growing trees are adults, and natural seedlings and saplings were rarely found in recent forty years (Tang et al., 2011). However, in the population survey conducted shortly after the rediscovery of *M. glyptostroboides* in 1946, abundant seedlings (c. 20% of the total wild individuals) were recorded (Chu and Cooper, 1950).

Most (c. 99%) wild *M. glyptostroboides* individuals are narrowly distributed within a mountainous area covering approximately 800 km² in Lichuan County, Hubei Province, China (E 108.48°–108.79°, N 29.97°–30.43°) (Hu, 1980). This region has an average annual rainfall of 1319 mm, cool summer with average temperature in the warmest month of 22.6 °C in August and warm winter with average temperature in the coldest month of 1.9 °C in January (Tang et al., 2011). The local soil is yellow-brown, with pH values ranging from 6.01 to 6.19 and organic content ranging from 2.9% to 7.4% in surface soil (Tang et al., 2011). Wild individuals of *M. glyptostroboides* were mainly found within an altitude range of 800–1400 m in the valleys, usually with creeks running through (Chu and Cooper, 1950), reflecting its preference for humid environments.

The vegetation type in the natural habitats of *M. glyptostroboides* is a deciduous and evergreen broadleaved mixed forest, with *Cunninghamia lanceolata*, *Liquidambar formosana*, *Castanea henryi*, *C. seguinii*, and *Cryptomeria fortunei* being the common tree species. Though *M. glyptostroboides* is among the locally dominant trees in the canopy (usually with heights > 20 m), usually with large seed production on a mature tree, heavy seed harvest for plantation in nursery gardens across all its natural habitats in recent decades is thought to limit its seed availability (Wang et al., 2005).

The understory plant community mainly comprises shrubs (e.g., *Camellia japonica*, *Hydrangea strigosa*, *Cyclosorus gongylodes*, and *Spiraea* sp.) and a variety of herbs (e.g., *Elatostema involucreatum*, *Galium odoratum*, *Viola vaginata*, *Houttuynia cordata*, and *Polygonum hydropiper*). The medicinal plant *Coptis chinensis* had been widely planted in both the natural and the adjacent unoccupied habitats of *M. glyptostroboides* over the last few decades. After *C. chinensis* was harvested, subsequent thriving ground vegetation and thick leaf litters prevent seeds of *M. glyptostroboides* from approaching the soil surface and cause strengthened interspecific competition during seedling growth, further restricting seed availability and threatening seedling emergence and survival (Vann et al., 2004; Li et al., 2012). Seeds and seedlings of

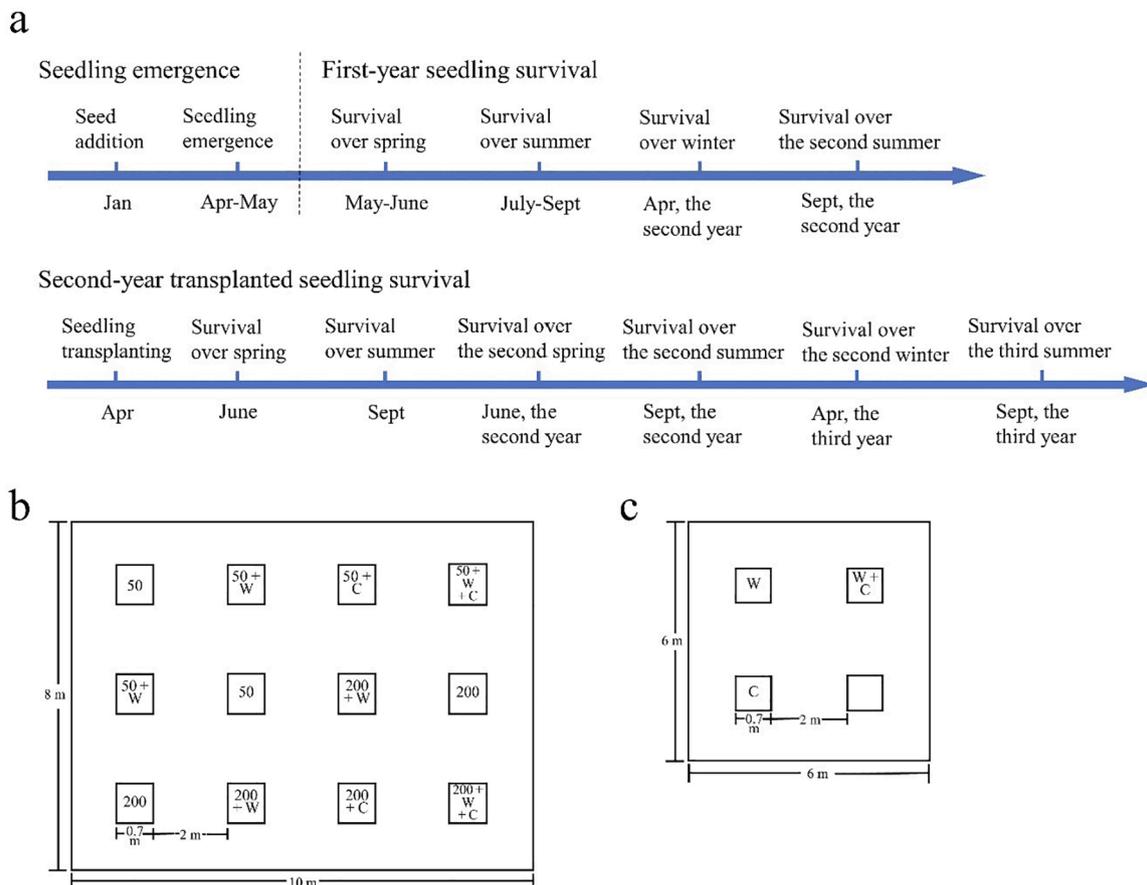


Fig. 1. Study design across different stages of *Metasequoia glyptostroboides* regeneration (a-c). (a) Observation schedules for each seed and seedling survival experiment. Note that in the experiment of first-year seedling survival conducted in 2013, an additional observation was conducted in the middle of each season (a total of seven observations). (b) Experiment design for seedling emergence and survival of emerged seedlings. We showed the placement of plots in each block and the treatment(s) conducted in each plot. (c) Experiment design for the survival of second-year transplanted seedlings. The placement of plots in each block and the treatment(s) conducted in each plot were represented. “50”: 50 additional seeds, “200”: 200 additional seeds, W: weeding, C: covering meshes.

M. glyptostroboides were not preferred by herbivores, with only larvae of two moths being recorded to feed on the leaves and roots of seedlings (Liu et al., 1978).

The typical adjacent unoccupied habitats are located on mountain slopes and ridges, where the vegetation and the physical conditions like pH value and organic content in surface soil are similar to the natural habitats of *M. glyptostroboides*. These suggest the potential of wild *M. glyptostroboides* populations to expand into these unoccupied habitats.

2.2. Controlled experiments for seedling emergence and first-year seedling survival

In the central area of natural distribution range of *M. glyptostroboides*, we performed controlled experiments to investigate seedling emergence and seedling survival in six study sites containing the typical natural habitats where wild *M. glyptostroboides* is a dominant tree species (Table S1), to evaluate the effects of ecological factors on regeneration success (Fig. 1a). The experiments were first conducted from 2013 to 2014 and were repeated from 2014 to 2015. Relatively stable climate conditions were recorded during our study period, with the annual precipitation varying from 1221 mm to 1461 mm and the average temperature in the coldest (January) and the hottest (August) month ranging from 5.3 °C to 7.5 °C and from 26.2 °C to 28.3 °C, respectively (<http://cdc.cma.gov.cn>).

(1) Seedling emergence. Plump *M. glyptostroboides* seeds were collected in autumn 2012 from about 50 wild adults in six study sites,

and then these collected seeds were mixed. In each study site we divided the habitats into two types, i.e., natural habitats of *M. glyptostroboides* and unoccupied habitats adjacent to the natural habitats (at least 100 m apart from the nearest edge of natural habitats). We set up one or two blocks in each habitat type in each site, with a total of 18 blocks across the six study sites (Fig. 1b; Table S1), except in two sites (WJW and YJ) without blocks in unoccupied habitats due to lack of typical habitats. Each block covered an area of 100 m², and 12 plots (each occupied a square area of 0.5 m²) were set up with a minimum interval of 2 m in every block.

To test the impact of limited seed availability, in early January 2013 we placed 50 or 200 *M. glyptostroboides* seeds in each plot as additional seeds (i.e., 50-add and 200-add plots) (Fig. 1b). The numbers of added seeds were determined based on the number of seeds we collected. Each block contained six 50-add and six 200-add plots. In total, 1500 seeds were placed in each block, with 27,000 seeds being used in this experiment. To evaluate the influence of interspecific competition with ground vegetation, we removed herbaceous weeds (weeding, including both the above- and below-ground parts of weeds) and leaf litters in half of the plots in each block (Fig. 1b). In addition, we tested the effect of herbivores/omnivores attack by covering half of the plots with polyester fabric meshes with 1 mm² holes in each block (Fig. 1b). Therefore, in each habitat type, our experiment design comprised a total of three factors (seed availability, interspecific competition, and herbivore/omnivore attack) that may impact seedling emergence. After seed placement, we marked and recorded each emerged seedling every week from early April to late May, to calculate the number of emerged

seedlings per plot.

Meanwhile, we set up four additional plots where no seeds were added (Fig. 1b) in each block located in forest interior (a total 28 non-add plots across all study sites except WJW and YJ due to limitation in habitat area), to estimate the contribution of local soil seed bank to regeneration of *M. glyptostrobooides*. The seedling emergence experiment was repeated in spring 2014, we set up 28 blocks in eight study sites using a total of 27,600 seeds (Table S1).

(2) First-year seedling survival experiments. To test the effects of the three factors on seedling survival, we tracked each emerged seedling in all plots during the period from May 2013 to October 2014. During this period, we conducted seven observations to check whether an emerged seedling was dead or alive (Fig. 1a). During the period from May 2014 to September 2015, we carried out four times of observations to track the growth status of seedlings emerged in the seedling emergence experiment in 2014 (Fig. 1a). Our observations roughly covered the growing season of *M. glyptostrobooides* (from spring to early autumn, because it is a deciduous species and defoliation usually starts in the middle of October).

2.3. Survival of transplanted second-year seedlings

To assess the effects of interspecific competition (i.e., weeding treatment) and herbivore/omnivore attack (i.e., treatment of covering meshes) on the late seedling stage of *M. glyptostrobooides* in each habitat type, we carried out the experiment measuring the survival of transplanted second-year seedlings. In October 2012, we collected and mixed seeds from 20 wild *M. glyptostrobooides* trees, and all emerged seedlings were cultivated in the soil from the natural habitats in a local nursery for one year. Then, a total of 1200 second-year seedlings were transplanted into four study sites in April 2014 (Table S1), and the mean height of transplanted seedlings was 17.55 cm (standard error, SE 0.13 cm) and did not vary significantly between different treatments in each factor (Linear mixed models: $P > 0.05$ in all comparisons) (Y.-Y. Li, unpublished data). In each study site, we set up one and two blocks in the natural and unoccupied habitats, respectively. Each block comprised four plots with the minimum interval of 2 m between any two plots, and 25 transplanted seedlings were planted into each plot. We weeded and covered the plants with meshes in half of the plots (Fig. 1c). From April 2014 to August 2016, we checked the survival of transplanted second-year seedlings six times, which covered the growing seasons in these three years (Fig. 1a).

2.4. Data analyses

To reveal the factors limiting the regeneration and expansion of *M. glyptostrobooides* populations, we analyzed data from different regeneration stages, including seedling emergence, seedling survival, and survival of transplanted second-year seedlings. All statistical analyses were carried out using R version 3.6.0 (R Development core team 2019).

For each experiment period, we first tested the effects of four factors (habitat type, seed availability, interspecific competition and herbivore/omnivore attack) as well as their interactions on the number of emerged seedlings per plot using generalized linear mixed models (GLMMs), implemented with R package *lme4* version 1.1–21 (Bates et al., 2015). In GLMMs, we assumed a negative binomial distribution of residuals and setting blocks nested in study sites as the random effect. We implemented stepwise model simplification approach from the highest interaction (i.e., the four-way interaction) using likelihood ratio tests (LRTs), and this process would be terminated when a higher interaction term was significant (e.g., we could not delete any two-way interactions if a three-way interaction was significant). The effect size of a factor or an interaction was represented by the absolute value of regression coefficient in the most parsimonious model.

We then assessed the effects of the four factors (habitat type, seed availability, interspecific competition and herbivore/omnivore attack)

and their interactions on the survival rates of first-year seedlings across different observations in each experiment period, using survival analysis in R package *coxme* version 2.2–14 (Therneau, 2018) and setting plots nested in blocks and blocks nested in study sites as the random effect. We simplified models following the same approach mentioned above. The absolute values of regression coefficients (i.e., LN (hazard ratio)) in the most parsimonious model were used to represent the effect sizes of remaining factors/interactions.

For the transplanted second-year seedlings, we evaluated the impacts of three factors (habitat type, interspecific competition and herbivore/omnivore attack) and their interaction on the survival rates across various observations, using survival analysis in R package *coxme* by setting plots nested in blocks and blocks nested in study sites as the random effect. We performed stepwise model simplification and evaluated the effect sizes of remaining factors and interactions in the most parsimonious model.

3. Results

3.1. Seedling emergence

During both experiment periods, only 49 emerged *M. glyptostrobooides* seedlings (25 in 2013 and 24 in 2014) were observed in the non-add plots, and these seedlings were found in 46% (13 of 28) and 25% (8 of 32) of the non-add plots in 2013 and 2014, respectively, indicating that local seed bank could hardly support the natural regeneration of *M. glyptostrobooides*. We therefore only included the 50-add and 200-add plots in the analyses.

In total, we found 2990, with a mean of 13.8 (standard error, SE 1.1) seedlings per plot, and 7592, with a mean of 35.1 (SE 2.6) seedlings per plot, emerged seedlings across all study sites in the seedling emergence experiments conducted in 2013 and 2014, respectively. No significant interactions between/among different factors were detected in both experiments (Table S2), showing that all the effects of four factors were reciprocally independent. The number of emerged seedlings per plot was significantly higher in the unoccupied habitats than in natural habitats in 2014 ($P = 0.011$), and there was no significant difference between two habitat types in 2013 ($P = 0.077$) (Table S2; Fig. 2a & b). In both experiments, the 200-add plots contained significantly more emerged seedlings than the 50-add plots (both $P < 0.001$), and there was a significantly higher number of emerged seedlings in the weeded plots than the non-weeded plots (both $P < 0.001$), but a significant effect of herbivore/omnivore attack was only found in 2014 ($P = 0.003$) (Table S2; Fig. 2a & b). Although the effect sizes of seed availability and interspecific competition varied across different experiment periods, these factors had much stronger impacts than herbivore/omnivore attack (Fig. 2c).

3.2. First-year seedling survival

The overall survival rate of first-year seedlings at the end of experiments were 10.0% (299 of 2990 emerged seedlings) and 6.11% (464 of 7592 emerged seedlings), during the two experiment periods (2013–2014 and 2014–2015). The effects of four factors were reciprocally independent because there were no significant interactions among/between them (Table S3). We found similar survival rate of first-year seedlings in both habitat types during the experiment period 2013–2014 ($P = 0.084$), while this survival rate in the natural habitats was significantly lower than that in the unoccupied habitats across all observations in the experiment period 2014–2015 ($P = 0.012$) (Table S3; Fig. 3a & b). During both experiment periods, interspecific competition was the only single factor significantly influencing the survival rate of seedlings across all observations (both $P < 0.001$) (Table S3; Fig. 3c & d and Fig. S1). Moreover, the effect size of interspecific competition was far larger than seed availability and herbivore/omnivore attack (Fig. 3e).

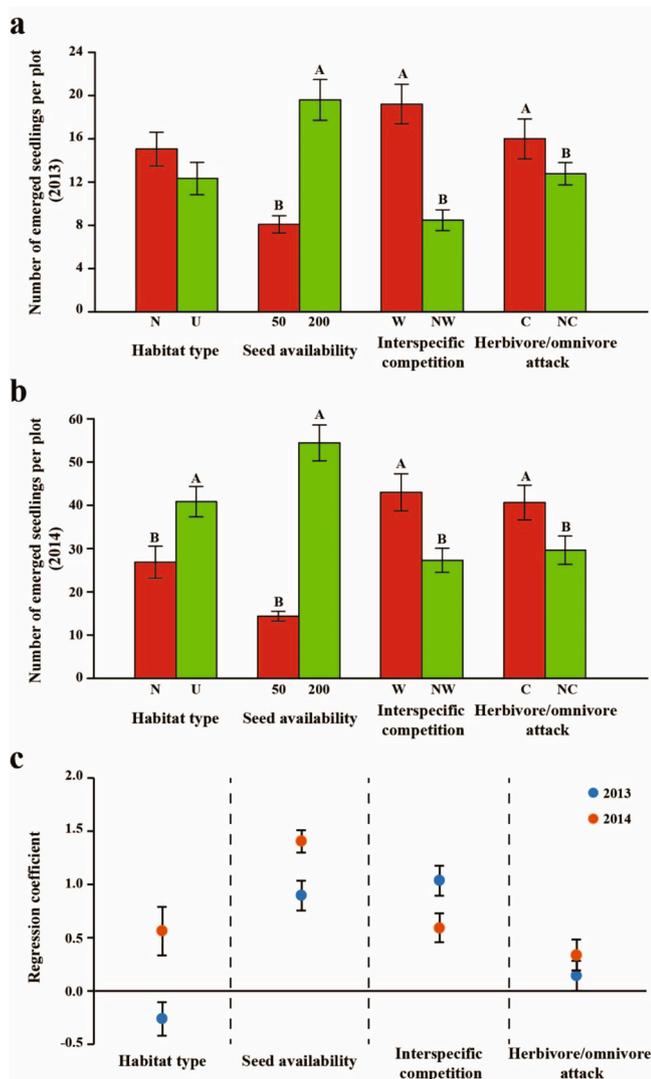


Fig. 2. Comparisons of different levels within each factor (mean \pm SE) (a–b) and effect sizes (the absolute values of regression coefficients in GLMMs) of different factors on the number of emerged seedlings in the two experiment periods (2013–2014 and 2014–2015) (c). Based on the results from GLMMs, values labeled with “A” were significantly higher than those labeled with “B” (Table S2). N: natural habitats, U: unoccupied habitats adjacent to natural habitats, “50”: 50 additional seeds, “200”: 200 additional seeds, W: weeding, NW: non-weeding, C: covering meshes, and NC: not covering.

3.3. Survival of transplanted second-year seedlings

The overall survival rate of transplanted second-year seedlings was 14.8% with 177 of 1200 seedlings surviving at the end of the experiment period 2014–2016. During this period, we failed to detect any significant impacts of habitat type, interspecific competition, herbivore/omnivore attack and their interactions on the survival rates of transplanted second-year seedlings (Table S4; Fig. S2).

4. Discussion

Insufficient seed availability, strong inter-specific competition and serious herbivore/omnivore attack are widely considered to be the primary factors threatening plant regeneration and leading to their endangerment (Geertsema et al., 2002; Truffaut et al., 2017; Collin et al., 2018; Davis and Gedalof, 2018; Hewitt et al., 2019; Carlson et al., 2020). However, their relative roles across different regeneration stages are still unclear. Here, we found that the relative importance of these ecological

factors varied across different regeneration stages of *M. glyptostroboides* and that their effects were predominantly released at the initial regeneration stages, consistent with our hypothesis. Compared with herbivore/omnivore attack, seed availability and interspecific competition exerted far stronger impacts on seedling emergence, but only interspecific competition significantly contributed to the survival of first-year seedlings. However, none of these factors significantly affected the survival of second-year seedlings. Intriguingly, seedling density in the adjacent unoccupied habitats was not lower than that in the natural habitats. This suggests that severely limited seed dispersal caused the failure of colonization in these habitats in accordance with some previous studies (Geertsema et al., 2002; Hewitt et al., 2019), and that seedlings suffered from the similar influences of our studied factors to those in the natural habitats due to no significant interactions between habitat type and these factors (Tables S2–S4).

Consistent with previous studies (Núñez-Ávila et al., 2013; Seidl et al., 2016), recent human activities predominantly contribute to the formation of the two crucial limiting factors (i.e., insufficient seed availability and drastic interspecific competition) in our study area. During the recent few decades, almost all seeds from the productive wild trees were harvested to be cultivated in the nurseries for the need of urban greening and horticulture, leading to seriously limited seed availability. Dense herbaceous and litter layers can create a strong barrier limiting the light seeds to approach the soil (Mallik, 2003; Standish et al., 2007), and thus the dense ground vegetation and thick leaf litter after harvesting the medicinal plant *C. chinensis* might further strengthen the restriction of seed availability. More importantly, through allelopathic effect and nutrition competition, dense herbs can suppress the seed germination and the seedling growth of *M. glyptostroboides* (Vann et al., 2004). However, herbivore/omnivore attack only imposed significant effects on the seedling emergence in one experiment period and played a minor role at each studied regeneration stage. This is probably because thin and pale color seeds of *M. glyptostroboides* are less attractive to frugivores and its seedlings may own high level of resistance (e.g., producing a mass of secondary metabolites) like some other gymnosperms, e.g., *Ginkgo biloba* (Guan et al., 2016).

At later stages of seedling growth, instead of the three factors, seedling survival is likely to be regulated by intraspecific competition (e.g., Janzen-Connell effects), pathogen infestation and abiotic factors (Comita et al., 2014; Hens et al., 2017; Gill et al., 2018; Wang et al., 2019a). In addition, seedling/sapling mortality may also be the result of decreased fitness as threatened plants usually have low population densities and therefore suffer high level of inbreeding (Li et al., 2012; Santos et al., 2018; Hamabata et al., 2019). Nevertheless, sufficient emerged seedlings and high seedling survival rate at early stages form the foundation of successful regeneration and stable population structure (Gardner et al., 2019; Wang et al., 2020).

Based on our findings, we propose a human-assisted natural regeneration strategy to facilitate the long-term persistence of wild *M. glyptostroboides* populations. This method includes one human intervention: removing the dense herbaceous layer and litters – just before seed mature season, i.e., September and October – to allow the naturally fallen seeds to approach the soil. Seed harvest should be stopped to promote seed availability. The removal of the herbaceous layer also decreases the impact of interspecific competition and enhances seedling emergence and survival. Using this method, except for the removal of the herbaceous layer, no human assistance is needed, and we expect that *M. glyptostroboides* populations can regenerate naturally. Furthermore, population expansion of *M. glyptostroboides* into the adjacent unoccupied habitats will greatly benefit *in situ* conservation. Our results showed that *M. glyptostroboides* population could expand into the unoccupied habitats adjacent to natural habitats once seed dispersal barrier was removed and that population regeneration suffered from the same limiting factors operating in its natural habitats, indicating the potential of applying our human-assisted natural regeneration strategy

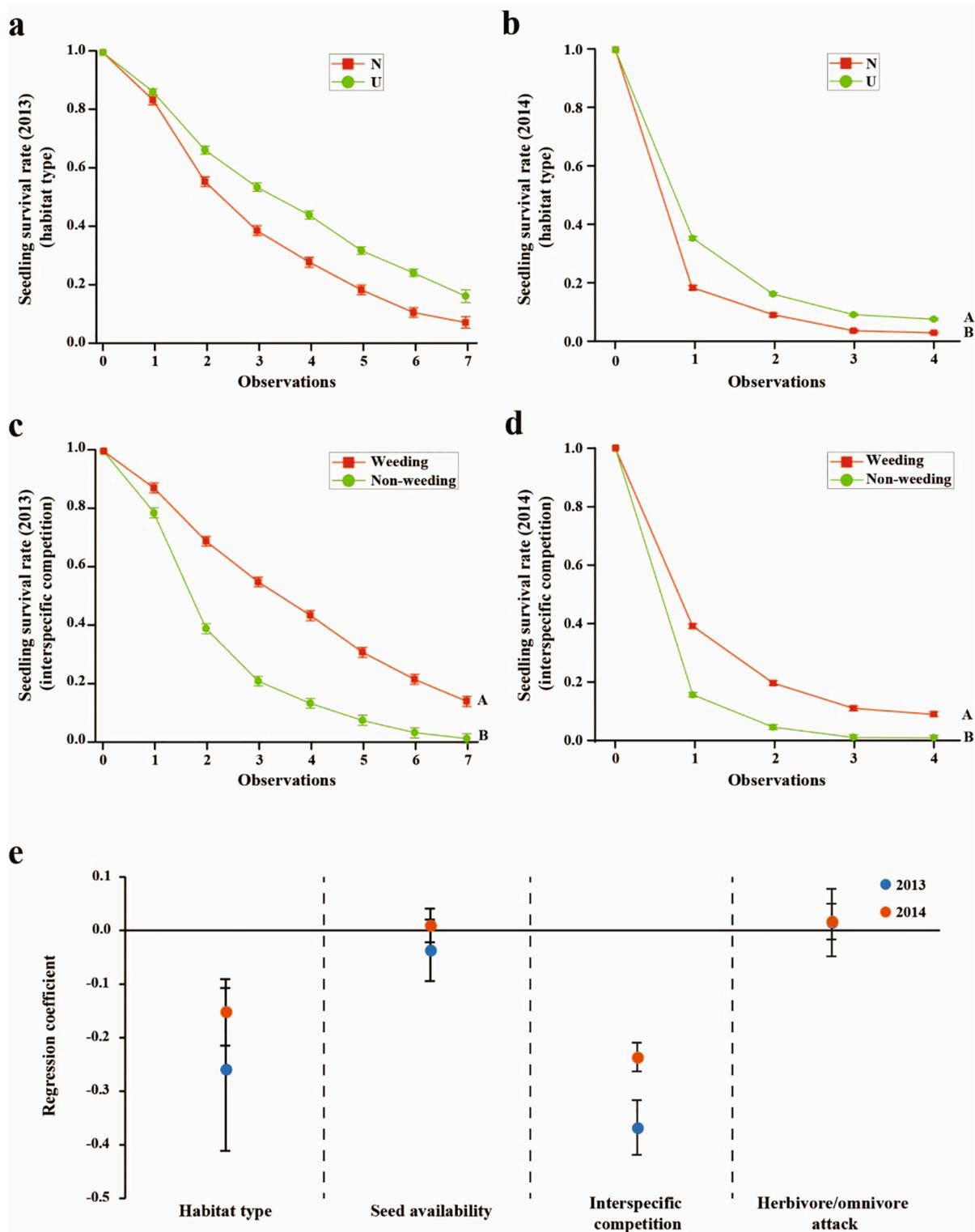


Fig. 3. Effects of habitat type and interspecific competition on the survival rates (mean \pm SE) of first-year seedlings across multiple observations in the experiment periods from 2013 to 2014 and from 2014 to 2015 (a-d) and effect sizes (the absolute values of regression coefficients in survival analysis) of different factors in different experiment periods (e). N: natural habitats, U: unoccupied habitats adjacent to natural habitats. The survival rate curve labeled with "A" had a significantly lower hazard ratio than that labeled with "B" (Table S3). W: weeding, and NW: non-weeding.

to the expanded populations. Nevertheless, for further improving our conservation strategy, it is of great necessity to carry out additional experiments considering the effects of other potential limiting factors (e.g., variation in soil water content and temperature) as well as their interactions with the interspecific competition.

Restricted regeneration contributes crucially to the drastic

acceleration in loss of plant diversity (Butchart et al., 2010; Reiter et al., 2016; Tang et al. 2018; Peters et al., 2019). However, it is still unclear how plants adapt or maladapt the rapid alterations in biotic and abiotic environments, i.e., eco-evolutionary dynamics (Zupping-Dingley et al., 2014; Wang et al., 2019b). Our study involving multiple phases of regeneration provides new insights into establishing links between

population decline and ongoing human disturbance by delineating the spatiotemporal changes in the effects of each limiting factor, and will greatly help improve the conservation strategies of other endangered plants and forest managements.

Data statement

The data presented in this manuscript are available from the Dryad Digital Repository (<https://doi.org/10.5061/dryad.kwh70rz0c>).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2021.119219>.

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