TRAIT-BASED PLANT COMMUNITY ASSEMBLY, ECOLOGICAL RESTORATION, AND THE BIOCONTROL OF INVASIVE EXOTIC PLANT SPECIES

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TRAIT-BASED PLANT COMMUNITY ASSEMBLY, ECOLOGICAL RESTORATION, AND THE BIOCONTROL OF INVASIVE EXOTIC PLANT SPECIES

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Table of Contents

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EDITORIAL
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Editorial: Trait-Based Plant Community Assembly, Ecological Restoration, and the Biocontrol of Invasive Exotic Plant Species

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Keywords: ecological restoration, functional trait, community assembly, ecosystem functioning, biocontrol, trait-based restoration

Editorial on the Research Topic

Trait-Based Plant Community Assembly, Ecological Restoration, and the Biocontrol of Invasive Exotic Plant Species

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Given trees' photosynthetic carbon capture ability, a large number of forest reforestation initiatives (such as the Bonn Challenge, the related AFR100, and the New York Declaration on Forests) have been established globally to limit global warming to 1.5°C by 2050 (The Bonn Challenge, 2011; UN Climate Summit, 2014). However, the current reforestation strategies do not appear to be highly effective in restoring the degraded tropical rainforests (due to rubber plantation) into late-successional tropical rainforests (Crouzeilles et al., 2016, 2017). Global reforestation strategies are divided into active (mono-planting or mixed-planting exotic, native, and several early-successional tree species, or economically important tree species), and passive (natural regeneration) reforestations (Lamb et al., 2005; Reid et al., 2018). The standard for selecting exotic, native, yet several early-successional or economically important tree species is based on their high survival and fast-growth rates under severely degraded conditions of forests that have large open areas (Martínez-Garza and Howe, 2003; Shimamoto et al., 2018). However, the arrival and establishment of late-successional species can easily be inhibited when these species thrive in the degraded forest (Mesquita et al., 2015; Shoo et al., 2016; Karina et al., 2021). This may explain why active reforestation usually fails to achieve the same ecological restoration success as natural regeneration (Crouzeilles et al., 2017). As such, it is very important that we develop effective and efficient methods for restoring them to natural or semi-natural conditions (Dobson et al., 1997; Hobbs and Harris, 2001). The first and most important step in this process is identifying species that are most appropriate and effective for restoration applications (Brown and Amacher, 1999; Jones, 2013; Fry et al., 2014). However, this step requires a comprehensive understanding of the ecological restoration theory, including information on species' interactions, successional processes, and resource-use patterns. Because these processes differ greatly across different ecosystems, this remains an enormous challenge. As this type of information is typically lacking, the selection of candidate species for restoration purposes are typically chosen using a trial-and-error method (Rosenthal, 2003; Padilla et al., 2009; Ostertag et al., 2015).

Plant functional traits, including both intra- and interspecific variations in morphological, physiological, and phenological characteristics, are fundamental to understanding plant adaptations and distributions. Previously, trait-based methods have been used to quantify plant community assembly and life history strategies. These methods have also been deemed an effective way of selecting candidate species for use in ecological restoration and the biocontrol of invasive exotic plant species. Trait-based plant community assembly can also provide guidance on how to use functional traits to perform ecological restoration. Trait-based life history strategies can not only reveal how invasive exotic plant species successfully invade the native ecosystem, but also facilitate the selection of native plant species to perform biocontrol. Despite the remarkable importance of functional traits in community assembly, ecological restoration, and biocontrol of invasive exotic plant species, the original questions have not yet been fully answered. Additionally, there is still a need for the development and successful application of a specific, step-by-step procedure to guide the use of functional traits for selecting species for ecological restoration and biocontrol of invasive exotic plant species. Therefore, we organized this Research Topic to understand: (1) how to use functional traits to perform investigative ecological restoration and biocontrol of invasive exotic plant species, and (2) trait-based community assembly and life history strategies during ecological restoration. The objective of this interdisciplinary Research Topic is to bring together the current research on trait-based community assembly and life history strategies during natural and artificial restoration, trait-based ecological restoration, and the biocontrol of invasive exotic plant species.

Till now our Research Topic has received 13 manuscripts and finally published 11 papers. These papers can be summarized in the following five main directions: (1) how reduced biodiversity caused by global change and biotic invasion affect biodiversity, (2) how to use functional traits to guide the protection of endangered plant species, (3) trait-based protocol for selecting appropriate species to facilitate reforestation and prevent biotic interaction, (4) trait-based protocol for using reforestation to recover mining-induced extremely degraded forest ecosystems, and (5) the influences of reforestation on ecosystem functioning and service and how to use functional traits to further improve the related reforestation project. Their details are described as below:

Global nitrogen eutrophication, which is disrupting the intimate plant-arbuscular mycorrhizal fungi (AMF) symbiosis, can alter the diversity and physiological functions of soil AMF greatly. However, shifts of beta diversity and the intrinsic patterns of AMF community dissimilarities in response to nitrogen addition remain unclear. Based on a 7-year nitrogen addition experiment in a Qinghai-Tibet Plateau alpine meadow, Lu et al. discovers the mechanism of arbuscular mycorrhizal fungi (AMF) diversity change along nitrogen addition gradient, by partitioning two aspects (Simpson and Nestedness) of the overall community beta diversity. They found that nitrogen addition not only decreased AMF richness, but the overall community beta diversity also showed different patterns along and within nitrogen addition treatment. AMF community overall beta diversity along the nitrogen addition gradients was induced by the increased nestedness dissimilarity, while the variation within treatments was explained by both increased Simpson and nestedness dissimilarities, which was correlated with plant and soil environmental dissimilarities. Eutrophication of aquatic ecosystems is a serious global issue. By measuring the possible effects of deer population on nitrate concentrations in a nearby stream in a forest ecosystem in Japan. Tsuboike et al. (2021) shows that the estimated community-level NRA inside the fence was 5.6 times higher than that of the open area and the difference was greatest early in the season. The authors concluded that degradation of the understory vegetation, especially of herbaceous plants, by the deer overpopulation was one of the reasons for the increased nitrate concentration in the stream.

By using functional traits, some useful conservation methods of two endangered plant species (*Horsfieldia hainanensis* and *Ottelia cordata*) and overgrazed meadows in Qinghai Tibetan Plateau have been uncovered. For example, by quantifying the photosynthetic traits (i.e., maximum photosynthesis rate, photosynthetic pigment indicators, leaf morphological traits, and leaf water content) of *H. hainanensis* to different intensity of light transmittance (7.3–44.41%), finds Wang et al. that *H. hainanensis* can have high potential to fulfill natural return, when letting it be exposed to 44.41% light transmittance. Similarly, by quantifying the relative importance of biological traits that were related to demographic traits (growth, survival, and reproduction) and water depth on population dynamic (abundance) of *O. cordata*, Shen et al. reveals that in the short term for avoiding the potential harm or even extinction of *O. cordata*, keeping appropriate water depth or transplanting *O. cordata* to spring should be an effective strategy. In the long run, in order to make the population recover, it was necessary to restore the degraded wetland, such as reducing water pollution and removing sludge to keep the water clean. In addition, by investigating whether the response of above- and below-ground traits to overgrazing vary between the species of annual vs. perennial life-forms, Zhang et al. demonstrates that overgrazing resulted in large removal of perennial species at all elevations, which forced perennial species to alter their above- and below-ground traits so that they could shift from resource conservation to resource acquisition to avoid overgrazing. In contrast, overgrazing promoted annual species at all elevation, thus annual species did not make any response to overgrazing. These findings bridge the gap in the literature for the influence of annual vs. perennial life-forms on species-specific response at both above- and below-ground levels during overgrazing. More important, these results can facilitate selecting species for sustainable ecosystem management and restoration from overgrazing.

The general and effective protocol for selecting suitable native plant species to perform effective reforestation and biotic invasion prevention has also been attained by using functional traits. For instance, by using functional trait, a native tree species (*Bombax ceiba*) has been found to be a good native tree species for performing reforestation to restore highly degraded tropical forests in Hainan Island, China (Luo et al.). A general trait-based protocol has also been developed for quick and effective selection of suitable native tree species to perform reforestation and biotic invasion prevention (Wang et al.; Zhang et al.). They also use their developed trait-based protocol to successfully restore

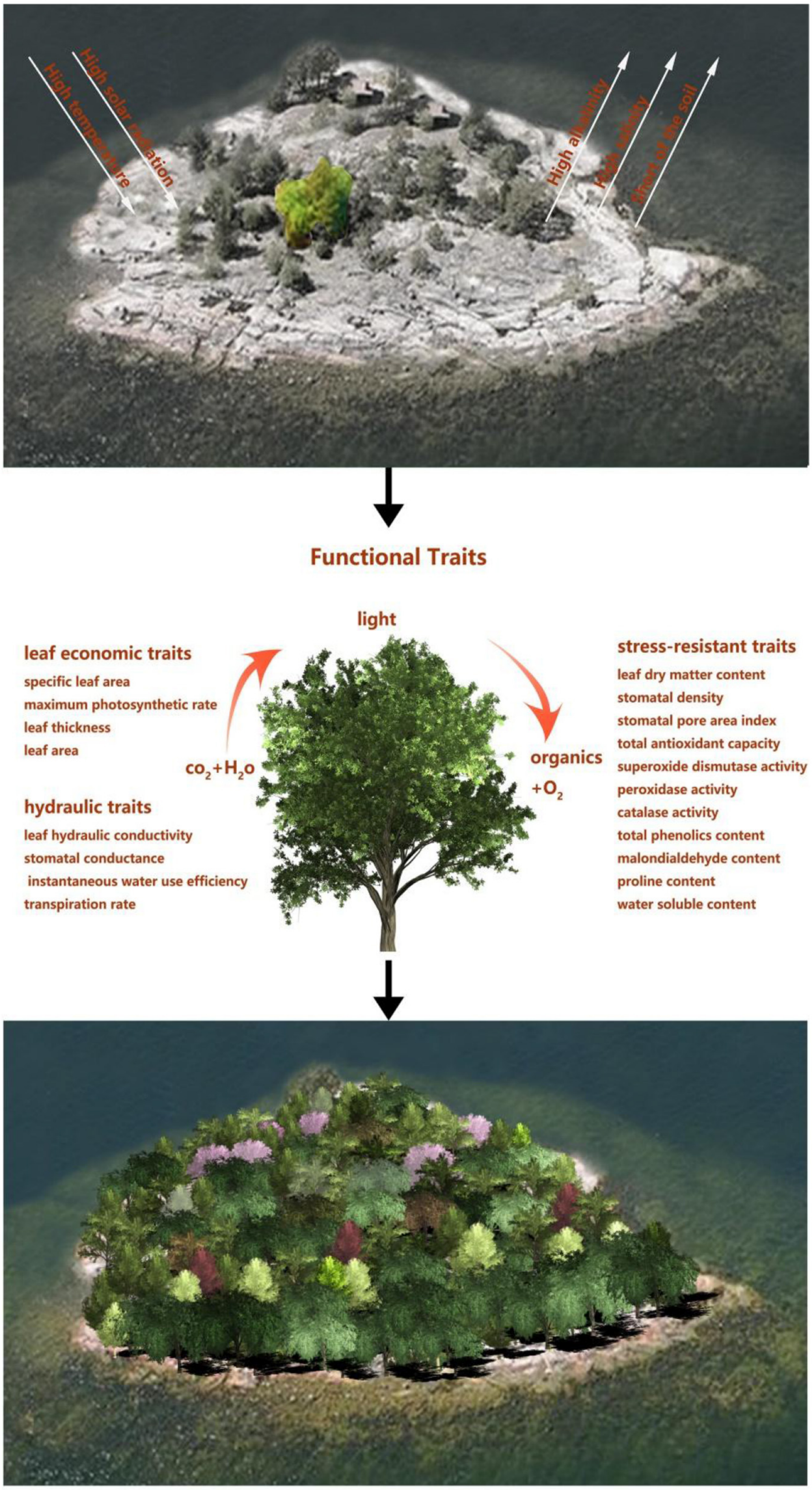

FIGURE 1 | A successful example for describing how to use the trait-based protocol developed by Wang et al. to quickly and effectively select many suitable native tree species to successfully restore an extremely degraded tropical coral island to tropical forest.

extremely degraded coral island (**Figure 1**) and the invasion of *Leucaena leucocephala*.

Functional traits can also be used to evaluate the effectiveness and success of reforestation. In light of functional traits, the reforestation success of a reforestation project which is used to restore 0.2 m^2 mining-induced extremely degraded tropical forest (merely consisting of bare rock) has been evaluated (Zhao et al.). Zhao et al. also pointed out the next step for this reforestation project should use trait-based protocol to select suitable late-successional native tree species to be mix-planted understory of the present tree species in this reforestation project. Through this, this extremely degraded tropical forest can be gradually restored to its originally undisturbed level. Similarly, functional traits can also facilitate the assessment of the influences of reforestation on soil water content and resistance to typhoon (Liu et al.; Li et al.). For example, Liu et al. has found reforestation based on mono-plantation of fast-growing non-native tree species is unhelpful in recovering soil water content. That is because mono-plantation of fast-growing non-native tree species will trigger much higher soil water absorption than the native tree species. Finally they recommend a three-step method for recovering soil water content of extremely degraded tropical forest ecosystems via reforestation. First, using the slope, the deep soil layers of the secondary tropical forest should be used as a reference to reconstruct slope and soil layers of extremely degraded tropical forest. Then the same soils should be refilled from the secondary tropical forest to plant fast-growing tree species to minimize impacts from landslides and other soil disturbance events. Third, dominant slow-growing tree species from the adjacent secondary area should be planted within the fast-growing species stands to increase soil water content. In addition to measuring height and DBH (diameter at breast height), Li et al. have found DBH of less than 5 cm and a height of less than 2 m are more prone to damage during severe weather events. In contrast, taller trees having a height greater than 20 m and a DBH of more than 20 cm faced minimal damage from typhoon Mangkhut. As a result, the restoration of natural forests that have the ability to withstand strong weather phenomena such as typhoons has been explored.

All in all, several new and key insights of trait-based principle and protocol which is used to guide how to use reforestation to recover degraded tropical forest have been achieved from our Research Topic. However, the generality and effectiveness of trait-based principle and protocol have to be tested in many other degraded tropical forests. We believe with many scientists participating in the trait-based reforestation principle and protocol research, trait-based effective and useful restoration protocol can be gradually attained.

AUTHOR CONTRIBUTIONS

HZ, GW, and WL organized and managed the Research Topic, did analysis of all published papers in the Research Topic. HZ wrote the paper. All authors contributed to the article and approved the submitted version.

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REFERENCES

Brown, R. W., and Amacher, M. C. (1999). "Selecting plant species for ecological restoration: a perspective for land managers," in *Revegetation With Native Species: Proceedings, 1997 Society for Ecological Restoration Annual Meeting,* eds L. K. Holzworth and R. W. Brown (Fort Lauderdale, FL; Ogden, UT), 1–16.

Crouzeilles, R., Curran, M., Ferreira, M. S., Lindenmayer, D. B., Grelle, C. E., and Benayas, J. M. R. (2016). A global meta-analysis on the ecological drivers of forest restoration success. *Nat. Commun.* 7, 1–8. doi: 10.1038/ncomms11666

Crouzeilles, R., Ferreira, M. S., Chazdon, R. L., Lindenmayer, D. B., Sansevero, J. B., Monteiro, L., et al. (2017). Ecological restoration success is higher for natural regeneration than for active restoration in tropical forests. *Sci. Adv.* 3, e1701345. doi: 10.1126/sciadv.1701345

Dobson, A. P., Bradshaw, A. D., and Baker, A. Á. (1997). Hopes for the future: restoration ecology and conservation biology. *Science* 277, 515–522. doi: 10.1126/science.277.5325.515

Fry, E. L., Power, S. A., and Manning, P. (2014). Trait-based classification and manipulation of plant functional groups for biodiversity–ecosystem function experiments. *J. Veget. Sci.* 25, 248–261. doi: 10.1111/jvs.12068

Hobbs, R. J., and Harris, J. A. (2001). Restoration ecology: repairing the earth's ecosystems in the new millennium. *Restorat. Ecol.* 9, 239–246. doi: 10.1046/j.1526-100x.2001.009002239.x

Jones, T. A. (2013). Ecologically appropriate plant materials for restoration applications. *Bioscience* 63, 211–219. doi: 10.1525/bio.2013.63.3.9

Karina, O. S., Martha, B. M., Fabiola, L. B., and Cristina, M. G. (2021). Accelerating tropical cloud forest recovery: performance of nine late-successional tree species. *Ecol. Eng.* 166, 106237. doi: 10.1016/j.ecoleng.2021.106237

Lamb, D., Erskine, P. D., and Parrotta, J. A. (2005). Restoration of degraded tropical forest landscapes. *Science* 310, 1628–1632. doi: 10.1126/science.1111773

Martínez-Garza, C., and Howe, H. F. (2003). Restoring tropical diversity: beating the time tax on species loss. *J. Appl. Ecol.* 40, 423–429. doi: 10.1046/j.1365-2664.2003.00819.x

Mesquita, R. D. C. G., Massoca, P. E. D. S., Jakovac, C. C., Bentos, T. V., and Williamson, G. B. (2015). Amazon rain forest succession: stochasticity or land-use legacy? *Bioscience* 65, 849–861. doi: 10.1093/biosci/biv108

Ostertag, R., Warman, L., Cordell, S., and Vitousek, P. M. (2015). Using plant functional traits to restore Hawaiian rainforest. *J. Appl. Ecol.* 52, 805–809. doi: 10.1111/1365-2664.12413

Padilla, F. M., Ortega, R., Sánchez, J., and Pugnaire, F. I. (2009). Rethinking species selection for restoration of arid shrublands. *Basic Appl. Ecol.* 10, 640–647. doi: 10.1016/j.baae.2009.03.003

Reid, J. L., Fagan, M. E., and Zahawi, R. A. (2018). Positive site selection bias in meta-analyses comparing natural regeneration to active forest restoration. *Sci. Adv.* 4, eaas9143. doi: 10.1126/sciadv.aas9143

Rosenthal, G. (2003). Selecting target species to evaluate the success of wet grassland restoration. *Agric. Ecosyst. Environ.* 98, 227–246. doi: 10.1016/s0167-8809(03)00083-5

Shimamoto, C. Y., Padial, A. A., da Rosa, C. M., and Marques, M. C. (2018). Restoration of ecosystem services in tropical forests: a global meta-analysis. *PLoS ONE* 13, e0208523. doi: 10.1371/journal.pone.0208523

Shoo, L. P., Freebody, K., Kanowski, J., and Catterall, C. P. (2016). Slow recovery of tropical old-field rainforest regrowth and the value and limitations of active restoration. *Conserv. Biol.* 30, 121–132. doi: 10.1111/cobi.12606

The Bonn Challenge (2011). *United Nations Environment Programme (UNEP)*.

Tsuboike, Y., Fujii, M., Yoshida, Y., and Kamata, N. (2021). An indirect impact of Sika Deer overpopulation on eutrophication of an aquatic ecosystem via understory vegetation: an individual-based approach using nitrate reductase activity. *Front. Ecol. Evol.* 9, 626905. doi: 10.3389/fevo.2021.626905

UN Climate Summit (2014). *New York Declaration on Forests*.

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Can Overgrazing Responses Be Disentangled by Above- and Below-Ground Traits?

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Understanding functional traits that capture species response to overgrazing can help optimize ecosystem functioning. However, most studies have ignored the effects of life-form on the response of above- and below-ground traits to overgrazing. Here, we determined whether the above- and below-ground traits respond similarly to overgrazing; and does the response of above- and below-ground traits to overgrazing vary between the species of annual vs. perennial life-forms. We compared common grassland species between fenced and overgrazed meadows at four elevations (from 3000 to 3600 m), spanning an area of ∼200 square kilometers on the Qinghai-Tibetan Plateau. We found overgrazing resulted in large removal of perennial species at all elevations, which forced perennial species to alter their above- and below-ground traits so that they could shift from resource conservation to resource acquisition to avoid overgrazing. In contrast, overgrazing promoted annual species at all elevation, thus annual species did not make any response to overgrazing. These findings bridge the gap in the literature for the influence of annual vs. perennial life-forms on species-specific response at both above-, and below-ground levels during overgrazing. More important, these results can facilitate selecting species for sustainable ecosystem managements and restoration from overgrazing.

Keywords: above-and below-ground traits, grazing avoidance strategy, land use, life form, Qinghai-Tibetan Plateau, resource conservation vs resource acquisition trade-off

INTRODUCTION

Optimizing ecosystem functions (e.g., productivity and nitrogen retention) has become a challenging task for agro-ecosystem management, as demands for food supply are ever-increasing (Orwin et al., 2018). Grazed land is one of the biggest agro-ecosystems that covers approximately 25% of the terrestrial surfaces on Earth (Asner et al., 2004). Overgrazing has been witnessed to have profound effects on ecosystem dynamics and species composition in grasslands ecosystems, as it leads to significant decrease in diversity, which in turn leads to increased greenhouse gas emissions and nitrogen loss, reduced productivity, water quality and lower soil carbon (C) storage (Ward et al., 2016; Zhou et al., 2017; Liu et al., 2018). Understanding of functional traits that capture responses of species to overgrazing can help in understanding vegetation composition dynamics. This in turn requires better understanding of above- and below-ground traits that respond to overgrazing as compared to landscapes that are not overgrazed (Orwin et al., 2010; Mouillot et al., 2011). Thus, use of the key functional traits that can capture species-specific response to overgrazing can help in selecting species to restoration from overgrazing (Orwin et al., 2018).

Above-ground traits, such as higher specific leaf area (SLA) and short plant height (H) that are associated with a resource acquisition vs. resource conservation trade-off, have widely been found as key traits that capture species-specific response to overgrazing (Briske, 1999; Westoby, 1999). In contrast, in the fenced condition, lower SLA and taller stature are regarded as a strategy for high resource conservation to adapt to resource limitation (Westoby, 1999). On the other hand, below-ground traits might also play a key role in mediating grazing-induced alterations to vegetation composition (Klumpp et al., 2009; McInenly et al., 2010). However, due to the notable lack of relevant data (Bergmann et al., 2017), it remains unclear whether below-ground traits also exhibit similar resource acquisition strategy (e.g., high root density, specific root length, specific root area, and low root tissue density) for avoiding overgrazing (McInenly et al., 2010; Kramer-Walter et al., 2016; Roumet et al., 2016; Feng et al., 2018).

Life-form plays a key role in influencing above-ground as well as below-ground traits. Usually annual species tend to have above- and below-ground traits that favor high resource acquisition, whereas perennial species tend to possess traits that promote a resource conservation strategy (Roumet et al., 2006, 2016; Zhang et al., 2018). Moreover, a meta-analysis has found that at the global scale, overgrazing promotes annual species over perennial species (Díaz et al., 2007). Thus, the response of above- and below-ground traits to overgrazing may vary between the species of annual vs. perennial life-forms. However, it remains unclear whether annual and perennial species differ in their above- and below-ground traits in response to overgrazing.

We hypothesize that after overgrazing, perennial species might largely get removed, whereas annual species could largely get promoted. Thus, annual and perennial species may alter their above- and below-ground traits for responding to overgrazing. To test this hypothesis, we determined how parameters of above- and below-ground traits of species dwelling in fenced and overgrazed meadows differed across annual and perennial life-forms. We investigated an area of ∼200 square kilometers, across an elevation of 3000–3600 m in Qinghai-Tibetan plateau. Overgrazing has led to nearly half million square km of degraded grasslands that account for the one-third of the total alpine grassland cover on Qinghai-Tibetan Plateau (Cui and Graf, 2009). Finding out the key above- and below-ground traits that can classify vegetation composition between overgrazed and fenced meadows can help in effective sustainable ecosystem managements and restoration from overgrazing in Qinghai-Tibetan Plateau.

MATERIALS AND METHODS

Study Sites

The maximum carrying capacity for cold season is approximately two yaks per hectare on Qinghai-Tibetan Plateau (Dong et al., 2015). Four overgrazed meadows in an area of ∼ 200 square kilometers were studied along an increasing elevation ranging from 3000 to 3600 m above mean sea level in the eastern part of the Qinghai-Tibetan Plateau. These meadows have undergone overgrazing at a comparable intensity as they have been overgrazed by approximately the same number of yaks (approximately four yaks per hectare) in winter season every year, for nearly 30 years. These four overgrazed meadows were distributed in Hezuo City, Gansu province (34°°5′N, 102°42′E; 3000 a.s.l.), Maqu County Gansu province (34°0′N, 102°44′E; 3400 and 34°5′N, 102°10′E 3600 a.s.l.), Ruergai County, and Sichuan province (34°1′N, 102°43′E; 3200 a.s.l.) of China (**Figure 1A**). The histories for the overgrazed meadows were obtained by interviewing local farmers. All overgrazed meadows are characterized by a cold and dry climate; with a mean annual temperature of 2.4–3.2°C. Mean annual precipitation of 530 to 560 mm is primarily distributed from July to August. The local vegetation in overgrazed sites is dominated by herbaceous species, such as *Ajania tenuifolia, Swertia tetraptera, and Taraxacum mongolicum* (Zhang et al., 2013). Soils are classified as alpine meadow soils (Zhang et al., 2018).

Since year 2000, the Chinese government has established fences to protect some areas of overgrazed meadows. Thus, in each elevation, there are also fenced meadows and the fenced meadow has been protected from grazing for nearly 17 years. The climate and soil type of each fenced meadow are the same as each overgrazed meadow. However, the local vegetation in fenced sites in 3000 and 3200 m elevations (sub-alpine meadow) are dominated by herbaceous species, such as *Elymus nutans*, *Kobresia pygmaea, Scirpus pumilus,* and *Thermopsis lanceolata* (Zhang et al., 2018). In contrast dominating species in fenced sites in 3400 m and 3600 m elevations (alpine meadow) are *Scirpus pumilus, Kobresia capillifolia, Festuca ovina, Poa poophagorum, Roegneria nutans, and Kobresia macrantha* (Chu et al., 2007).

Field Sampling

At each elevation, we adopted paired-sampling method to use the fenced meadow which has been constructed since 2000 for preventing grazing and its adjacent overgrazed meadow for comparison (**Figure 1B**). Sampling was conducted in August, during the peak of the growing season in 2017. At each of the four elevations, 30 plots, each of 0.5 m × 0.5 m, were arranged along four parallel transects in the fenced area and the adjacent overgrazed area of one meadow, with a 20 m spacing between the closest edges of the two areas (**Figure 1B**). The plots were spaced at 20 m intervals. For each of the 30 plots, species presence/absence was recorded. Total above-ground biomass of each species in each of the 30 plots was also measured to

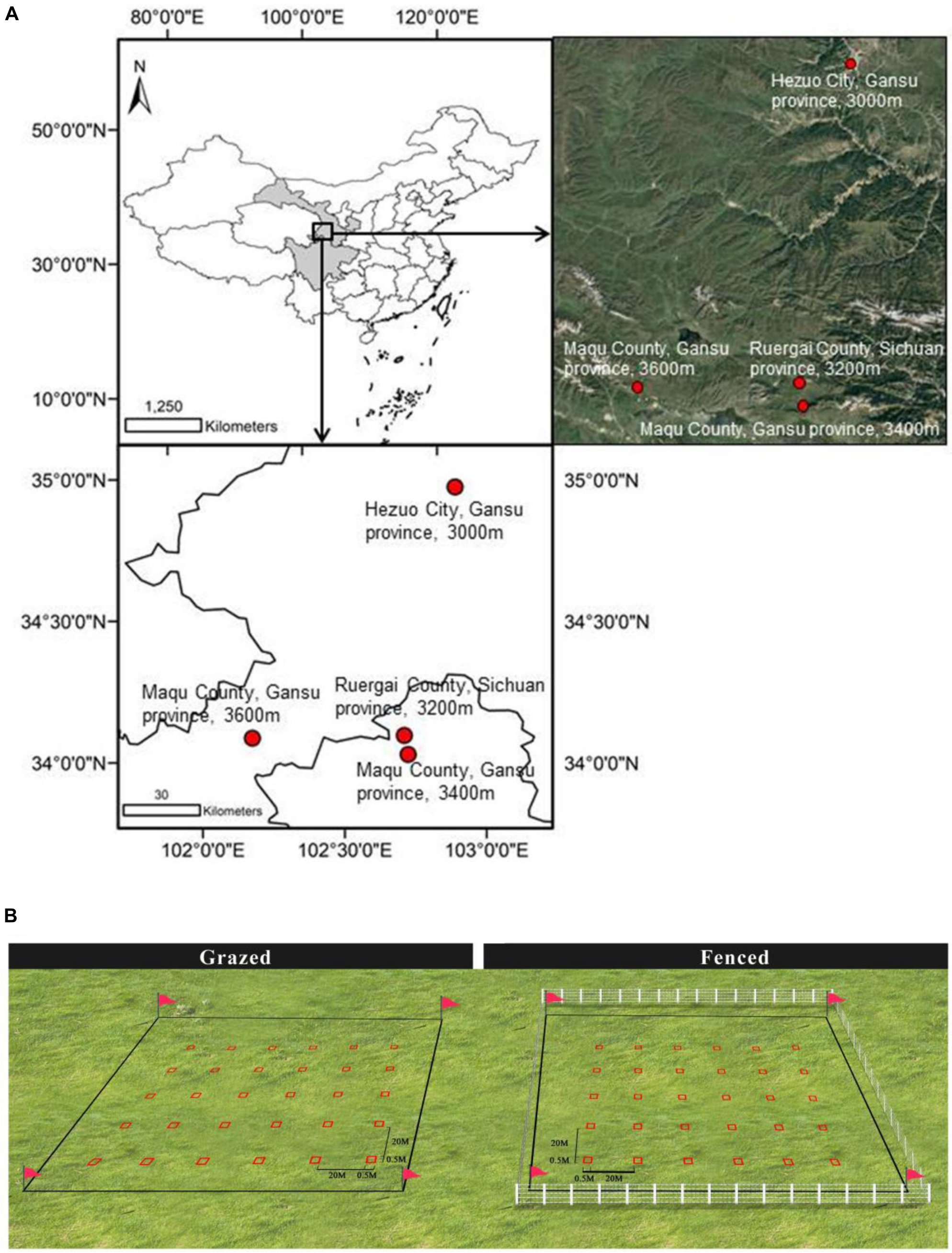

FIGURE 1 | The topographic map of the four study sites along an increasing elevation ranging from 3000 m to 3600 m above mean sea level in the eastern part of the Qinghai-Tibetan Plateau, China **(A)**, and the quadrat sampling design **(B)**. At each site, there was one overgrazed and one matched fenced meadow and 30 0.5 m × 0.5 m quadrats ware randomly arranged in both overgrazed and fenced meadows **(B)**.

represent abundances (numbers of individuals) for each species to determine whether overgrazing change the dominance of annual and perennial species.

Measurement of Above-Ground and Root Traits of Plants

All common species shared between fenced and grazed meadows were sampled to measure three above-ground traits: leaf photosynthesis rate (A), specific leaf area (SLA) and above-ground maximum height (H). Further, five root traits which include root average diameter (RD), root biomass (RB), specific root length (SRL), root tissue density (RTD), and specific root area (SRA) were also recorded. All these traits could be good predictors of resource acquisition vs. resource conservation trade-off (Díaz et al., 2001; Roumet et al., 2006). Traits were measured from three to five mature individuals per species, following the procedures of measurements described in Bergmann et al. (2017) and Zhang et al. (2018) as well as mentioned in the **Supplementary Material**.

Statistical Methods

First, we utilized non-parametric analysis (a Wilcoxon signed-rank test) to test whether there were differences in the above- and below-ground traits and abundances for all common species shared between fenced and overgrazed sites at all four elevations using. Next, a Spearman correlation test was used to examine the bivariate relationships among all above- and below-ground plant traits at all elevations. Finally, a principal component analysis (PCA) was employed to evaluate which of the eight above-ground and root traits play an important role in species distribution between fenced and overgrazed meadows at all elevations. Given the unique set of traits between annual and perennial plant species (Roumet et al., 2006; Zhang et al., 2018), the above analyses were also performed separately for annuals and perennials to understand whether the response of above- and below-ground traits to overgrazing vary between annual and perennial species.

RESULTS

In total, we sampled 53–77 species in the fenced meadows from 3000 to 3600 m elevations, whereas only 40–56 species, could be identified in the sampling of the overgrazed meadows (**Supplementary Figure 1**). However, species composition was similar between the fenced and overgrazed meadows as the two meadows shared 28–55 species (**Supplementary Figure 1**). When dividing all species into annual and perennial species separately, we found annual species was promoted in the overgrazed meadow. That was because although overgrazing nearly has no influences on numbers of species for annual species (**Supplementary Figure 1**), but abundances for shared annual species in overgrazed meadows were approximately 4.3 times as those in the fenced meadows at all elevations (**Supplementary Figure 2**). However, perennial species were got largely removal in the overgrazed meadow, as overgrazing had not only removed 18–22 perennial species (**Supplementary Figure 1**), abundances for shared perennial species in the overgrazed meadows but also were only 1/3 of those in the fenced meadows (**Supplementary Figure 2**). For further analysis of below- and above-ground traits, we focused on these common species for direct comparisons of the parameters across overgrazed and fenced meadows at all the elevations.

All shared species had significantly lower root biomass (RB), root average diameter (RD), root tissue density (RTD), and above-ground maximum height (H) in the overgrazed meadows than those in the fenced meadows at all four elevations. In contrast, overgrazed meadows had higher specific root length (SRL), specific root area (SRA), leaf photosynthesis rate (A) and specific leaf area (SLA) than those in the fenced meadow (**Figure 2**). Consistent results could still be observed in perennial species, when we performed Wilcoxon signed-rank test separately for annuals and perennials (**Figure 2**). However, for annual species, differences in all above-ground and below-ground traits between fenced and overgrazed meadows were not significant (**Figure 2**).

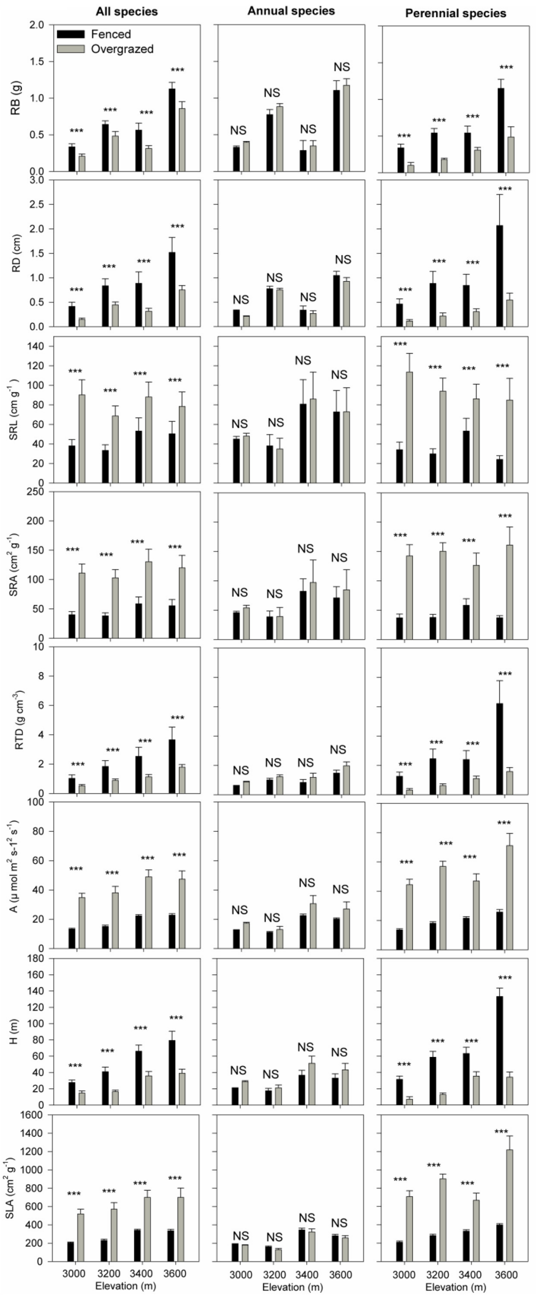

FIGURE 2 | The differences in above-ground traits [maximum height (H), leaf maximum photosynthesis rate (A) and specific leaf area (SLA)] and below-ground traits [root average diameter (RD), root biomass (RB), specific root length (SRL), specific root area (SRA) and root tissue density (RTD)] between fenced and overgrazed meadows for all species (left panel) and separately for annual (middle panel) and perennial (right panel) species from 3000 to 3600 m elevations. *** and NS (non-significant) indicates $P < 0.001$ and $P > 0.05$ based on Wilcoxon signed-rank tests.

We observed strong correlations amongst the five root traits, as well as between the three above-ground traits, for all common species in both, fenced and grazed meadows at all four elevations (**Figure 3**). For example, RD, H, RB, and RTD are all significantly positively correlated (**Figure 3**). Similarly, SRL, A, SRA, and SLA are also all significantly positively correlated (**Figure 3**). However, RD, H, RB, and RTD are significantly negatively associated with SRL, A, SRA, and SLA (**Figure 3**). However, when we tested these correlations separately for annuals and perennials, consistent significant traits correlations could be observed in perennial species in both fenced and overgrazed meadows (**Figure 3**). However, for annual species all trait correlations were non-significant in both fenced and overgrazed meadows (**Figure 3**).

Results of PCA revealed that "species of fenced and overgrazed meadows" were significantly separated by above- and below-ground traits measured at all elevations (**Figure 4**, and **Supplementary Table 2**). When performing PCA separately for annual and perennial species, we found that perennial species in fenced and overgrazed meadows were significantly separated by all the above- and below-ground traits measured at all elevations (**Figure 4** and **Supplementary Table 2**). In contrast, annual species in fenced and overgrazed meadows could not be significantly separated by any of measured above- and below-ground traits at all elevations (**Supplementary Figure 3** and **Supplementary Table 2**).

DISCUSSION

In this study, we found both above-ground and below-ground traits exhibited a similar resource acquisition vs. resource conservation trade-off for responding to overgrazing. Moreover, the response of above- and below-ground traits to overgrazing differed between annual and perennial species, with perennial species determining the response of above- and below-ground traits to overgrazing.

A Shift From Resource Conservation to Resource Acquisition at Above- and Below-Ground Level Determine Plant Overgrazing Response

A shift from resource conservation to resource acquisition at above-ground level have resulted higher SLA, A, but lower H after overgrazing as compared to those in the fenced condition (Díaz et al., 2001; Evju et al., 2009; Spasojevic et al., 2010; Laliberté and Tylianakis, 2012; Li et al., 2017). This shift from resource conservation to resource acquisition has also led to significant relationships among these three traits at both fenced and grazed meadows (Wright et al., 2004; Osnas et al., 2013). Here, we observed all fenced meadows, had higher H, but lower SLA and A, compared to overgrazed meadows. We also found significant correlations among these three traits in both fenced and overgrazed meadows at all four elevations. This indicated that a trade-off between resource acquisition vs. resource conservation at above-ground level likely determined plant overgrazing response in Qinghai Tibetan plateau.

A number of studies have found that the trade-off between resource acquisition vs. resource conservation can also be found in below-ground traits (Withington et al., 2006; Mommer and Weemstra, 2012). For example, resource acquisition may warrant high SRL, SRA, but low RTD, RB, and RD to allow fast-growth, whereas, resource conservation may result in high RTD, RB, and RD, but low SRL and SRA (Reich, 2014; Roumet et al., 2016). Likewise, this shift from resource acquisition to resource conservation at below-ground level can also lead to strong correlations among these below-ground traits (Withington et al., 2006; Prieto et al., 2015; de la Riva et al., 2018). Here we found all fenced meadows, had higher SRL, SRA, but lower RTD, RB, and RD, compared to overgrazed meadows. We also found significant correlations among these five below-ground traits. Thus, plants also alter their below-ground traits so that they can shift from resource conservation to resource acquisition to avoid overgrazing, in a manner like shifts in response of above-ground traits.

Principal component analysis revealed that fenced and overgrazed species were significantly separated, which in turn was significantly positively associated with SRL, SRA, A, and SLA, but negatively related RD, RB, RTD, and H. High SRL, SRA, A, and SLA represents high resource acquisition, whereas, high RD, RB, RTD, and H indicates high resource conservation (Wright et al., 2004; Reich, 2014; de la Riva et al., 2018). Thus, the shift from resource conservation to resource acquisition determines the classification of species between fenced and overgrazed meadows. These results together indicate that above- and below- ground traits studied here can capture the key plant response (a shift from resource conservation to resource acquisition) and thus help plants to avoid overgrazing. As a result, these traits can be used to select potential species to re-design overgrazed meadows for restoration in Qinghai-Tibetan Plateau.

Above-Ground Traits Can Be a Good Proxy for Below-Ground Traits

In the fenced meadows, resource conservation can also result in low photosynthetic rates and SLA. Moreover, high overall conservation of resources requires conservative root structures such as roots with high root tissue density, root diameter and biomass, and/or low specific root length and area (Eissenstat, 2002; Fort et al., 2013). In contrast, in the overgrazed meadows, resource acquisition relates to high photosynthetic rates and SLA, which requires roots with high specific root length and area, and low root tissue density, diameter and biomass to ensure sufficient nutrient supply to achieve fast plant growth (Reich, 2014; de la Riva et al., 2018). Therefore, significant above-ground -below-ground traits relationship may be observed in both fenced and grazed meadows. Here we observed consistently significant relationships between above- and below- ground traits in both fenced and overgrazed meadows at all elevations. We infer that above-ground traits might act as a proxy for below-ground traits in revealing plant-soil feedbacks and the effects of below-ground traits on ecosystem functioning in overgrazed meadows, as evident in Qinghai-Tibetan Plateau. Meanwhile, above-ground traits can also be a good proxy for below-ground traits to

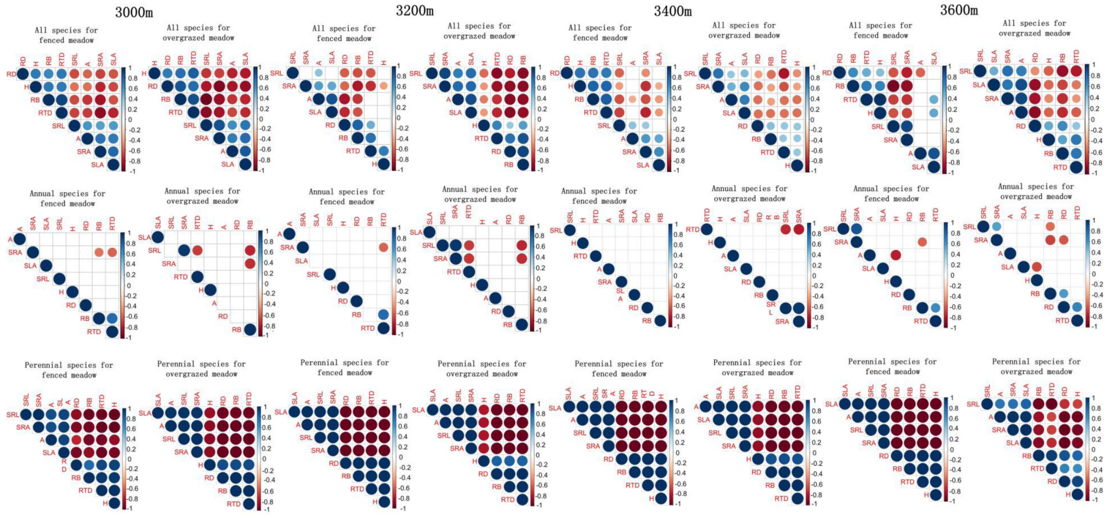

FIGURE 3 | The interrelationships among above-ground traits (H, A, and SLA) and below-ground traits (RD, RB, SRL, SRA, and RTD) in fenced and overgrazed meadows for all species (upper panel), and separately for annuals (middle panel) and perennial species (lower panel) from 3000 to 3600 m elevations.

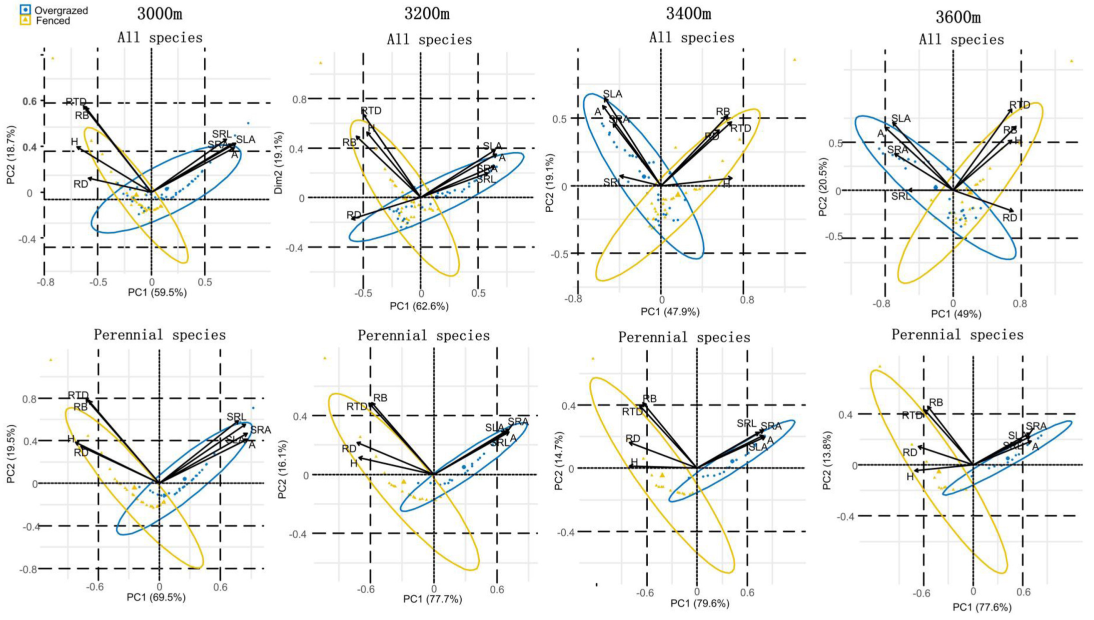

FIGURE 4 | Principal component analysis of below-ground (RD, RB, SRL, SRA, and RTD) and above-ground traits (H, A, and SLA) in fenced and overgrazed meadows for all species, and separately for perennial species from 3000 to 3600 m elevations.

select species to re-design communities to perform restoration of overgrazed meadows.

Responses of Above-Ground and Below-Ground Traits to Overgrazing Differ Between Annual and Perennial Species

It has been found that grazing promotes annual over perennial species in global grassland ecosystems (Díaz et al., 2007). Our study supports this trend where we find that abundances for shared annual species in the overgrazed meadows are 4 times as much as those in the fenced meadows. In contrast, abundances for shared perennial species in the overgrazed meadows are merely 1/3 of those in the fenced meadows. However, we observed the responses of above-ground and below-ground traits to overgrazing differ between annual and perennial species. That is because when performing trait variations, trait correlations and PCA analysis separately for annuals and perennials, consistently significant trait variations and trait correlations can still be observed in perennial species. Both aboveground and belowground traits can still significantly classify perennial species between fenced and overgrazed meadows. However, annual species did not have any trait variations and trait correlations in both fenced and overgrazed meadows. Moreover, both aboveground traits and belowground traits cannot differentiate annual species between fenced and overgrazed meadows. Thus, perennial species alter their above-ground and below-ground traits to develop a shift from resource conservation to resource acquisition to avoid overgrazing. While, annual species do not make any response to overgrazing.

CONCLUSION

Overgrazing is common from low elevation (3000 m) to high elevation (4000 m) (Cui and Graf, 2009). Here our results clearly observed that at all four elevations (3000, 3200, 3400, and 3600 m) annual and perennial species have consistent responses to overgrazing. Thus, we conclude that overgrazing can result in largely removal of perennial species, which force perennial specie to develop above- and below-ground traits that are highly associated with high resource acquisition to avoid overgrazing. In contrast, overgrazing promotes annual species, thus annual species do not necessarily make any response to overgrazing. These results filled the gap of the effects of life-forms (annual vs. perennial) on species-specific response at both above-, and below-ground levels during overgrazing. More important these findings have important implications for the sustainable ecosystem management and recovery of overgrazed ecosystems in Qinghai-Tibetan Plateau and beyond. First, since above-ground traits can be a good proxy for below-ground traits in revealing key overgrazing responses of plants, we can use above-ground traits and trait-based species selection model (Laughlin, 2014) to select many potential perennial species that have high resource acquisition ability from natural meadow ecosystems for increasing diversity of overgrazed meadows. Above-ground traits and trait-based species selection model can also be used to select potential perennial species for specific sustainable ecosystem management. It has been found that decreased species diversity and biomass resulting from overgrazing is the key driver of soil N loss and greenhouse gas emission (e.g., N_2O) in Qinghai-Tibetan Plateau (Liu et al., 2018). As a result, active seeding of these selected perennial species may have high potential to diminish soil N loss and N_2O emission. Moreover, using above-ground traits to select potential perennial species that have high resource acquisition and high biomass can provide feed for cultivating as many yaks or sheep as possible to meet ever-increasing food demand, while simultaneously increasing ecosystem productivity and soil C storage. Similarly, selecting potential perennial legume species that have high resource acquisition can help in increasing soil nitrogen retention. In addition, selecting desirable species with high resource acquisition to undesirable weeds may help control weeds in overgrazed meadows.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HZ, SP, and CW designed the research. HZ, QH, and KJ performed the research. HZ and SP analyzed the data and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.573948/full#supplementary-material

REFERENCES

Asner, G. P., Elmore, A. J., Olander, L. P., Martin, R. E., and Harris, A. T. (2004). Grazing systems, ecosystem responses, and global change. *Annu. Rev. Environ. Resour.* 29, 261–299. doi: 10.1146/annurev.energy.29.062403.102142

Bergmann, J., Ryo, M., Prati, D., Hempel, S., and Rillig, M. C. (2017). Root traits are more than analogues of leaf traits: the case for diaspore mass. *New Phytol.* 216, 1130–1139. doi: 10.1111/nph.14748

Briske, D. D. (1999). "Plant traits determining grazing resistance: why have they proved so elusive?," in *Proceedings of the VIth International Rangeland Congress*, eds D. Eldridge and D. Freudenberger (Charters Towers, QLD: Australian Rangeland Society), 901–905.

Chu, C. J., Wang, Y. S., Du, G. Z., Maestre, E. T., Luo, Y. J., and Wang, G. (2007). On the balance between niche and neutral processes as drivers of community structure along a successional gradient: insights from alpine and sub-alpine meadow communities. *Ann. Bot.* 100, 807–812.

Cui, X., and Graf, H. F. (2009). Recent land cover changes on the Tibetan Plateau: a review. *Clim. Change* 94, 47–61. doi: 10.1007/s10584-009-9556-8

de la Riva, E. G., Marañón, T., Pérez-Ramos, I. M., Navarro-Fernández, C. M., Olmo, M., and Villar, R. (2018). Root traits across environmental gradients in Mediterranean woody communities: are they aligned along the root economics spectrum? *Plant Soil* 424, 35–48. doi: 10.1007/s11104-017-3433-4

Díaz, S., Lavorel, S., Mcintyre, S., Falczuk, V., Casanoves, F., Milchunas, D. G., et al. (2007). Plant trait responses to grazing – a global synthesis. *Glob. Change Biol.* 13, 313–341. doi: 10.1111/j.1365-2486.2006.01288.x

Díaz, S., Noy-Meir, I., and Cabido, M. (2001). Can grazing response of herbaceous plants be predicted from simple vegetative traits? *J. Appl. Ecol.* 38, 497–508. doi: 10.1046/j.1365-2664.2001.00635.x

Dong, Q. M., Zhao, X. Q., Wu, G. L., and Chang, X. F. (2015). Optimization yak grazing stocking rate in an alpine grassland of Qinghai-Tibetan Plateau, China. *Environ. Earth Sci.* 73, 2497–2503. doi: 10.1007/s12665-014-3597-7

Eissenstat, D. (2002). Root structure and function in an ecological context. *New Phytol.* 148, 353–354. doi: 10.1046/j.1469-8137.2000.00781.x

Evju, M., Austrheim, G., Halvorsen, R., and Mysterud, A. (2009). Grazing responses in herbs in relation to herbivore selectivity and plant traits in an alpine ecosystem. *Oecologia* 161, 77–81. doi: 10.2307/40310116

Feng, C., Wang, Z., Zhu, Q., Fu, S., and Chen, H. Y. H. (2018). Rapid increases in fine root biomass and production following cessation of anthropogenic disturbances in degraded forests. *Land Degrad. Dev.* 29, 461–470. doi: 10.1002/ldr.2878

Fort, F., Jouany, C., and Cruz, P. (2013). Root and leaf functional trait relations in Poaceae species: implications of differing resource-acquisition strategies. *J. Plant Ecol.* 41, 107–127. doi: 10.1093/jpe/rts034

Klumpp, K., Fontaine, S., Attard, E., Le Roux, X., Gleixner, G., and Soussana, J. F. (2009). Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. *J. Ecol.* 97, 876–885. doi: 10.1111/j.1365-2745.2009.01549.x

Kramer-Walter, K. R., Bellingham, P. J., Millar, T. R., Smissen, R. D., Richardson, S. J., and Laughlin, D. C. (2016). Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *J. Ecol.* 104, 1299–1310. doi: 10.1111/1365-2745.12562

Laliberté, E., and Tylianakis, J. M. (2012). Cascading effects of long-term land-use changes on plant traits and ecosystem functioning. *Ecology* 93, 145–155. doi: 10.1890/11-0338.1

Laughlin, D. C. (2014). Applying trait-based models to achieve functional targets for theory-driven ecological restoration. *Ecol. Lett.* 17, 771–784.

Li, W., Xu, F., Zheng, S., Taube, F., and Bai, Y. (2017). Patterns and thresholds of grazing-induced changes in community structure and ecosystem functioning: species-level responses and the critical role of species traits. *J. Applied. Ecol.* 54, 963–975. doi: 10.1111/1365-2664.12806

Liu, S., Zamanian, K., Schleuss, P. M., Zarebanadkouki, M., and Kuzyakov, Y. (2018). Degradation of Tibetan grasslands: consequences for carbon and nutrient cycles. *Agric. Ecosyst. Environ.* 252, 93–104.

McInenly, L. E., Merrill, E. H., Cahill, J. F., and Juma, N. G. (2010). Festuca campestris alters root morphology and growth in response to simulated grazing and nitrogen form. *Funct. Ecol.* 24, 283–292. doi: 10.1111/j.1365-2435.2009.01642.x

Mommer, L., and Weemstra, M. (2012). The role of roots in the resource economics spectrum. *New Phytol.* 195, 725–727. doi: 10.1111/j.1469-8137.2012.04247.x

Mouillot, D., Villeger, S., Scherer-Lorenzen, M., and Mason, N. W. H. (2011). Functional structure of biological communities predicts ecosystem multifunctionality. *PLoS One* 6:e17476. doi: 10.1371/journal.pone.0017476

Orwin, K. H., Buckland, S. M., Johnson, D., Turner, B. L., Smart, S., Oakley, S., et al. (2010). Linkages of plant traits to soil properties and the functioning of temperate grassland. *J. Ecol.* 98, 1074–1083. doi: 10.1111/j.1365-2745.2010.01679.x

Orwin, K. H., Mason, N. W. H., Jordan, O. M., Lambie, S. M., Stevenson, B. A., and Mudge, P. L. (2018). Season and dominant species effects on plant trait-ecosystem function relationships in intensively grazed grassland. *J. Appl. Ecol.* 55, 236–245.

Osnas, J. L. D., Lichstein, J. W., Reich, P. B., and Pacala, S. W. (2013). Global leaf trait relationships: mass, area, and the leaf economics spectrum. *Science* 340, 741–744. doi: 10.1126/science.1231574

Prieto, I., Roumet, C., Cardinael, R., Dupraz, C., Jourdan, C., Kim, J. H., et al. (2015). Root functional parameters along a land-use gradient: evidence of a community-level economics spectrum. *J. Ecol.* 103, 361–373. doi: 10.1111/1365-2745.12351

Reich, P. B. (2014). The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211

Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S., et al. (2016). Root structure–function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytol.* 210, 815–826. doi: 10.1111/nph.13828

Roumet, C., Urcelay, C., and Díaz, S. (2006). Suites of root traits differ between annual and perennial species growing in the field. *New. Phytol.* 170, 357–368. doi: 10.1111/j.1469-8137.2006.01667.x

Spasojevic, M. J., Aicher, R. J., Koch, G. R., Marquardt, E. S., Mirotchnick, N., Troxler, T. G., et al. (2010). Fire and grazing in a mesic tallgrass prairie: impacts on plant species and functional traits. *Ecology* 91, 1651–1659. doi: 10.2307/25680406

Ward, S. E., Smart, S. M., Quirk, H., Tallowin, J. R. B., Mortimer, S. R., Shiel, R. S., et al. (2016). Legacy effects of grassland management on soil carbon to depth. *Glob. Change Biol.* 22, 2929–2938. doi: 10.1111/gcb.13246

Westoby, M. (1999). "The LHS strategy in relation to grazing and fifire," in *Proceedings of the VIth International Rangeland Congress*, eds D. Eldridge and D. Freudenberger (Charters Towers, QLD: Australian Rangeland Society), 893–896.

Withington, J. M., Reich, P. B., Oleksyn, J., and Eissenstat, D. M. (2006). Comparisons of structure and life span in roots and leaves among temperate trees. *Ecol. Monogr.* 76, 381–397.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403

Zhang, H., Gilbert, B., Wang, W., Liu, J., and Zhou, S. (2013). Grazer exclusion alters plant spatial organization at multiple scales, increasing diversity. *Ecol. Evol.* 3, 3604–3612. doi: 10.1002/ece3.743

Zhang, H., John, R., Zhu, S., Liu, H., Xu, Q., Qi, W., et al. (2018). Shifts in functional trait–species abundance relationships over secondary subalpine meadow succession in the Qinghai–Tibetan Plateau. *Oecologia* 188, 547–557. doi: 10.1007/s00442-018-4230-3

Zhang, H., and Long, W. (2019). Can the response of above- and below-ground traits to overgrazing vary between annual and perennial species? *bioRxiv [Preprint]* doi: 10.1101/771444

Zhou, G., Zhou, X. H., He, Y. H., Shao, J. J., Hu, Z. Z., Liu, R. Q., et al. (2017). Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. *Glob. Change Biol.* 23, 1167–1179. doi: 10.1111/gcb.13431.dref

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A Method for Performing Reforestation to Effectively Recover Soil Water Content in Extremely Degraded Tropical Rain Forests

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Deforestation continues to be extensive in the tropics, resulting in reduced soil water content. Reforestation is an effective way to recover soil water content, but the recovery depends on the type of reforestation efforts that are implemented. Monoculture of fast-growing species is a common reforestation strategy, because it is an effective means of preventing landslides resulting from the frequent typhoons and heavy rains in the tropics and easy to implement. To quantify whether monoculture plantings can help recover soil water content, we initiated a reforestation project within a 0.2 km^2 area of an extremely degraded tropical monsoon forest. We hypothesized that much higher transpiration rate of fast-growing tree species would deplete soil water more than the dominant slow-growing species in the adjacent secondary tropical rain forest during both wet and dry seasons, thereby resulting in much lower soil water content. To test this hypothesis, we compared transpiration rates and key functional traits that can distinguish transpiration rates between fast-growing and dominant slow-growing species in both wet and dry seasons. We also quantified whether soil water content around these species differed. We found that fast-growing species had transpiration rate and transpiration-related trait values that were 5–10 times greater than the dominant slow-growing species in both seasons. We also found that soil water content around dominant slow-growing species was 1.5–3 times greater than for fast-growing species in both seasons. Therefore, reforestation based on monoculture plantings of fast-growing species seems difficult to effectively recover the soil water content. We also provide a simple method for guiding the use of reforestation efforts to recover soil water content in extremely degraded tropical rain forests. We expect that this simple method can be an effective means to restore extremely degraded tropical rain forests in other parts of the world.

Keywords: deforestation, fast-growing trees, freshwater scarcity, hydraulic response to seasonal drought, limited leaf water supply, soil water content

INTRODUCTION

Forests provide sustainable and high quality freshwater across the globe (Vose, 2019). Forests store significant amounts of water in soils, and are thus a key regulator of the global freshwater supply (Evaristo and McDonnell, 2019). Compared to agricultural and grassland ecosystems, forests have extensive and deep root systems and the soil is characterized by a thick leaf litter layers, which allow them to retain high levels of soil water content (Neary et al., 2009). Together, these properties of forests result in high soil infiltration rates and low soil surface runoff (Neary et al., 2009). Of the precipitation infiltrating in the soil, a fraction is taken up by roots and transpired, while the rest is stored in deep soil layers to provide freshwater to human society via subsurface runoff (Jackson, 2006). As a result, soil water content stored in forests is vital for providing sustainable freshwater to human society (Chapin et al., 2011).

Past and current human disturbances (e.g., ore mining, and plantation of commercial trees) have resulted in high rates of deforestation and ecosystem degradation across the world, which have, in turn, resulted in a major threat to the global supply of freshwater (Sahin and Hall, 2004; Foley et al., 2011; Lambin and Meyfroidt, 2011; Vörösmarty et al., 2015). It is expected that deforestation in the tropics will continue to accelerate in the near future (Laurance et al., 2014), and a large amount of the water stored in forest soils may thus be lost as a result. Indeed, many studies have found that extensive deforestation in the tropics has already led to large-scale losses of freshwater in these systems (Castello and Macedo, 2016; Fugère et al., 2016; Luke et al., 2017; Gebrehiwot et al., 2019; Mapulanga and Naito, 2019). It is thus urgent to initiate and maintain reforestation projects aimed at recovering soil water content and increasing freshwater supply to human society (Chapin et al., 2011).

A serious challenge in managing soil water loss after degradation in tropics is that frequent typhoons and heavy rains can result in landslides that remove the soil layer (Guidicini and Iwasa, 1977; Chang et al., 2008; Yumul et al., 2012; Acosta et al., 2016; Villamayor et al., 2016). Such landslide events may make reforestation of large areas extremely difficult. To alleviate these problems, monocultures of fast-growing tree species with high survival rates perform better than slower-growing late-successional tropical tree species (Stokes et al., 2009; Walker et al., 2009; Lohbeck et al., 2013; Pang et al., 2018). As a result, monocultures of fast-growing tree species that are easy to cultivate are used extensively in reforestation projects across the tropics (Lu et al., 2017).

Although it is widely believed that reforestation will recover the soil water content (Bruijnzeel, 2004), only the reforestation in Flores, eastern Indonesia is proven to have successfully restored soil water content, as mentioned by Carson (1989) and Nooteboom (1987). Moreover, whether reforestation can recover the soil water content depends on time and spatial scales (Bruijnzeel, 2004). As a result, it is expected that reforestation based on monocultures of fast-growing species may be not able to recover soil water content in highly degraded tropical forest. Nevertheless, nearly no study has evaluated the influence of large-scale reforestation projects on soil water content recovery in humid tropical regions (Solange et al., 2017).

Plant functional traits including morphological and physiological traits across species can directly capturing critical dimensions of the vulnerability and response to seasonal drought across species and ecosystems (Maherali et al., 2004; Engelbrecht et al., 2007; Anderegg, 2014), comparing the differences in functional traits between fast-growing tree species used for reforestation and dominant slow-growing tree species in the secondary tropical rain forest can reveal whether and why reforestation with fast-growing tree species can recover soil water content in highly degraded tropical rain forest. It has been found that fast-growing tree species usually have higher transpiration rates than the dominant slower growing tree species in secondary tropical rain forests (Tardieu and Parent, 2017), which often leads to higher rates of soil water uptake for fast-growing species (Krishnaswamy et al., 2013). These higher transpiration rates are expected to result in lower soil water content in monoculture stands of fast-growing tree species (Evaristo and McDonnell, 2019). Thus, we hypothesized that during the wet season, fast-growing tree species used for reforestation will have higher transpiration rates than dominant slower-growing species in the secondary tropical rain forest. As a result, we expect soil water content around fast-growing species to be much lower than for dominant slow-growing species (**Figure 1**). In the dry season, we expect that dominant slow-growing species will exhibit suppressed transpiration rates which make soil water content around slow-growing species decline only slightly during the dry season. In contrast, for fast-growing tree species, high transpiration rates are required to meet their very high growth rates, which is impossible when precipitation is limited. As a result, fast-growing tree species must absorb large amounts of water from soils formed because of stored precipitation in the wet season in the soil layers. In such cases, we expect soil water content around fast-growing species to decline precipitously during the dry season.

To test our hypothesis, we initiated a large-scale reforestation project used to restore a 0.2 km^2 area of extremely degraded tropical rain forest in Sanya City, Hainan, China in 2016. Outside of this degraded area is secondary tropical rain forest. To prevent landslides, we planted monocultures of eight fast-growing tree species separately. This project thus provides a suitable system in which to test whether monocultures of fast-growing species can help recover soil water content compared with that in the secondary tropical rain forest. Specifically, we compared differences in transpiration rates between the eight fast-growing tree species used for reforestation and eight dominant slow-growing tree species sampled from the secondary tropical rain forest. Separate comparisons were made in both the wet and dry seasons. We also determined whether water content differed between soils surrounding fast-growing vs. slow-growing tree species in both seasons. Tree transpiration rates can be determined by using a suite of functional traits associated with plant transpiration, including photosynthesis rate, stomatal conductance, leaf hydraulic conductivity, and drought stress tolerance (Tuzet et al., 2003; Miyashita et al., 2005; Fisher et al., 2007; Maherali et al., 2008; Santos et al., 2018).

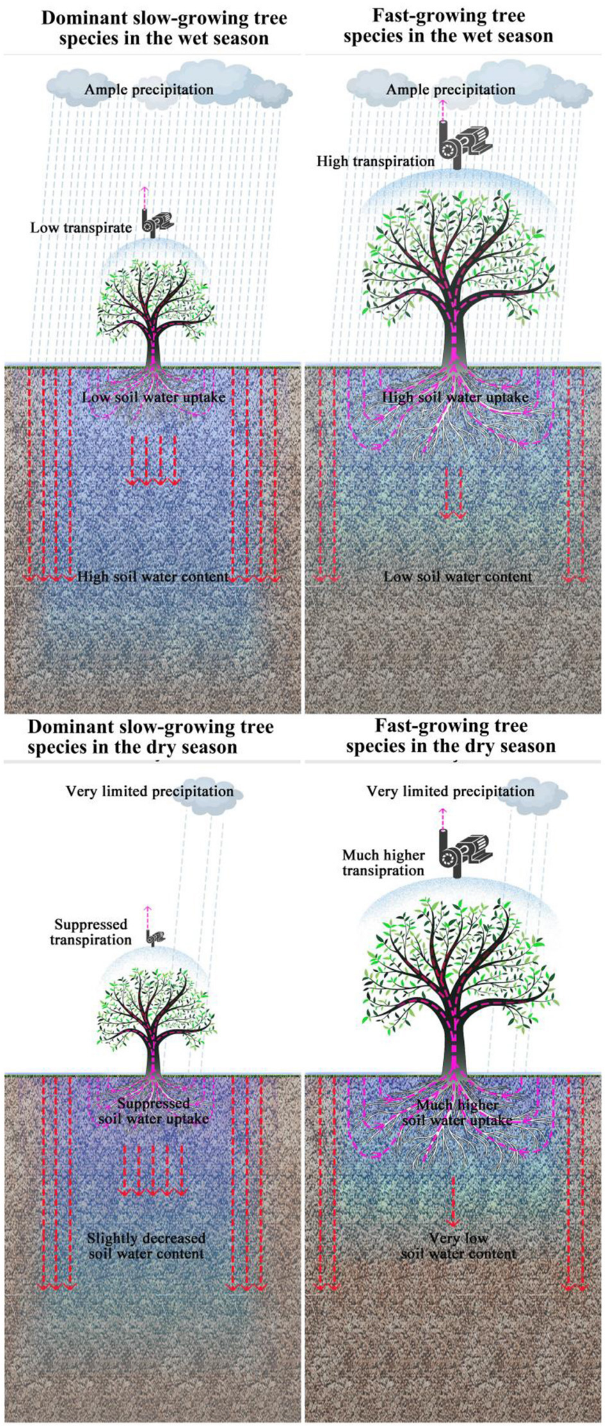

FIGURE 1 | Hypothesized differences in transpiration, soil water uptake, and soil water content between fast-growing tree species used for reforestation in the 0.2 km^2 extremely degraded tropical rain forest and dominant slow-growing species in the adjacent secondary tropical rain forest in wet and dry season respectively.

Therefore, we also compared these functional traits between fast-growing species used for reforestation and dominant slow-growing species in the secondary tropical rain forest in both wet and dry seasons to reveal which factors regulate transpiration for these two type of trees across wet and dry season.

MATERIALS AND METHODS

Study Site

Our study site is located on Baopoling mountain (BPL), which is a limestone mountain in Sanya City, Hainan, China (109°51′01′′E, 18°31′99′′N). The area has a tropical monsoon oceanic climate with a mean annual temperature of ∼28°C. The average annual precipitation on the island is 1,500 mm, ∼91% of which occurs from June to October (Luo et al., 2020). Vegetation of the BPL is described as species-rich tropical rain broad-leaf forest (Luo et al., 2020). Due to 20 years of limestone mining by the cement industry, a 0.2 km^2 area of the BPL has now become extremely degraded, consisting of bare rocky soils where minimal plant life is found. Areas of the BPL outside of the degraded site are composed of secondary tropical rain forest (see **Figure 2A**). Here we utilized a nearly natural reforestation method to perform the reforestation to restored the extremely degraded tropical rainforest and detail reforestation processes are described in detail as below. In May 2016, reforestation was firstly enacted by using the slope and the deep soil layer for the secondary tropical forest as a reference to reconstruct slope and soil layers (**Figures 2B,C**). Then separately refiling the same soils from the secondary tropical monsoon forest to monoculture seedlings [3 m height and 2 cm diameter at breast height (DBH)] of eight fast-growing species, which included *Terminalia neotaliala, Bombax malabarica, Cleistanthus sumatranus, Ficus microcarpa, Muntingia colabura, Acacia mangium, Leucaena glauca,* and *Bougainvillea spectabilis* (**Figures 2C–E**). Seedlings from these eight fast-growing species can be purchased commercially, and are known to be fast-growing and have high survival rates within the study region. Thus, these eight fast-growing species should have high potential to prevent landslide resulting from frequent typhoon and heavy rain. The eight fast-growing species was separately monocultured from the top to the bottom of BPL (**Figure 2D**) and planting density for each of the eight species was all kept at 80 stems per hectare. The restoration project was finished at end of year 2016. In 2019, thirty plots, each of 20 × 20 m^2 (an area of 400 m^2 for each plot) that were at least 100–300 m apart from one another, were randomly sampled across the adjacent secondary forest. Within each plot, all freestanding trees with diameter of $\geq$ 1 cm at breast height (DBH) were measured and identified to species. We finally found 80 tree species in the secondary forest and we select the 8 tree species (200–300 stems per hectare) (*Bridelia tomentosa, Radermachera frondosa, Lepisanthes rubiginosa, Rhaphiolepis indica, Pterospermum heterophyllum, Fissistigma oldhamii, Psychotria rubra,* and *Cudrania cochinchinensis*) as our candidate dominant slow-growing species.

Sampling

In the peak of wet season (August) in 2019, we sampled 20 fully expanded, healthy leaves from the same five independent

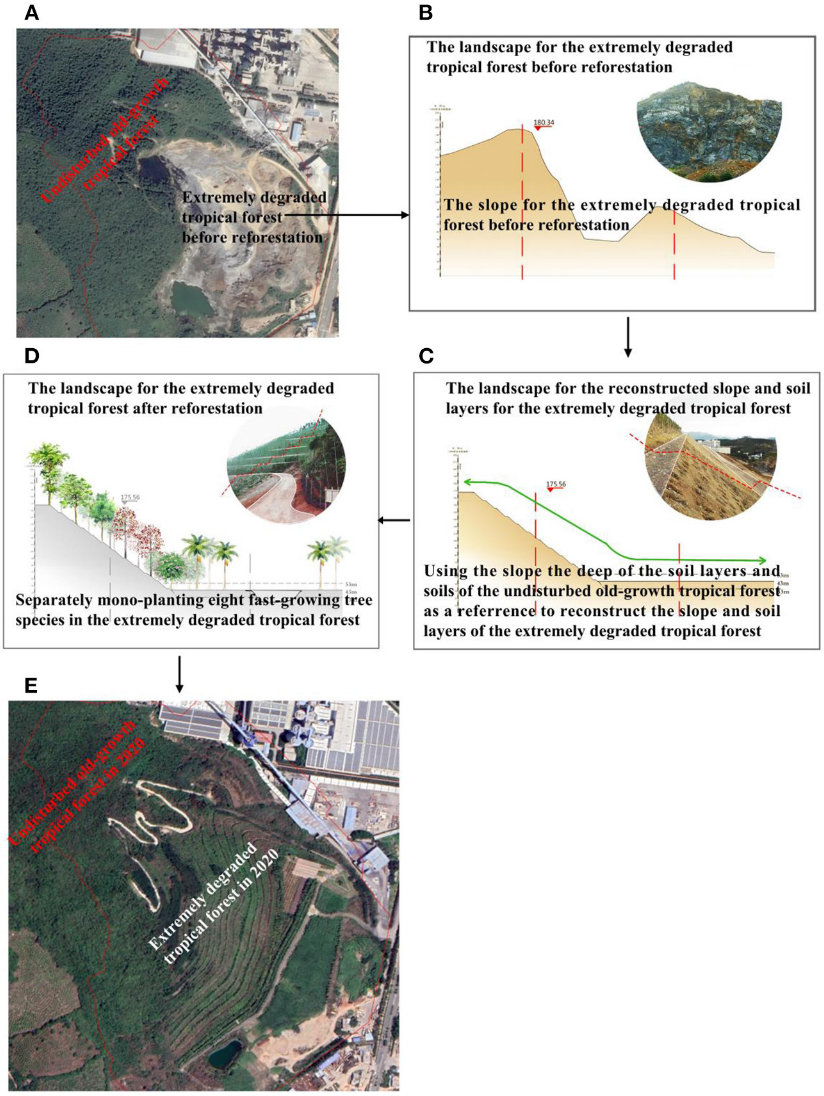

FIGURE 2 | Baopoling mountain (BPL), the surrounding landscape and detailed procedures regarding the reforestation project in the 0.2 km^2 extremely degraded tropical monsoon forest. **(A)** BPL and the surrounding landscape including the 0.2 km^2 area of extremely degraded tropical monsoon forest and the adjacent secondary tropical monsoon forests. **(B)** the original landscape and slope of the 0.2 km^2 extremely degraded tropical monsoon forest; **(C)** using the slope, the deep of soil layers as a reference to reconstructing slope and soil layers, and to refill soils from the secondary tropical forests to form planting area in the extremely degraded tropical monsoon forest; **(D)** separately mono-planting eight fast-growing tropical tree species; **(E)** the landscape of secondary tropical rain forest and the 0.2 km^2 extremely degraded tropical rain forest after reforestation efforts in 2020.

individuals for each of the eight fast-growing species and eight dominant slow-growing species found in the surrounding secondary region. Resampling was performed again in the dry season (February) in 2020. We selected trees that had a DBH that was comparable to the mean DBH of the species (**Table 1**). Leaf samples were used to measure five hydraulic traits, including transpiration rate (TR; μmol m^{-2} s^{-1}), maximum photosynthesis rate (A_{area}; μmol m^{-2} s^{-1}), stomatal conductance (SC; mmol m^{-2} s^{-1}), leaf hydraulic conductivity (LHC; mmol m^{-2} s^{-1} MPa^{-1}), and leaf turgor loss point (TLP; Mpa).

Measurement of Maximum Photosynthesis Rate, Stomatal Conductance, and Transpiration Rate

We used a Li-6400 portable photosynthesis system (Li-6400, Li-Cor, Lincoln, Nebraska, USA) to measure maximum photosynthesis rate, transpiration rate, and stomatal conductance for the 20 fully expanded and sun-exposed leaves collected from five mature individuals between 9:00 and 11:00 on sunny days. We set photosynthetic photon flux density at 1,500 μmol m^{-2} s^{-1} to ensure that light-saturated photosynthetic rates were measured for all forest species. We maintained ambient CO_2

TABLE 1 | The mean diameter at the breast height DBH (in cm) of the eight non-native species (*Terminalia neotaliala*, *Bombax malabarica*, *Cleistanthus sumatranus*, *Ficus microcarpa*, *Muntingia colabura*, *Acacia mangium*, *Leucaena glauca,* and *Bougainvillea spectabilis*) and the eight dominant native species (*Bridelia tomentosa*, *Radermachera frondosa*, *Lepisanthes rubiginosa*, *Rhaphiolepis indica*, *Pterospermum heterophyllum*, *Fissistigma oldhamii*, *Psychotria rubra,* and *Cudrania cochinchinensis*) and the DBH values of the sampled individuals (*n*) of each study species.

Species name	*n*	mean DBH	DBH of sampled individuals				
			Tree 1	Tree 2	Tree 3	Tree 4	Tree 5
Terminalia neotaliala	5	8.9	8.6	8.3	9.2	9.5	8.7
Bombax malabarica	5	8.2	8.1	7.5	8.8	9.2	7.8
Cleistanthus sumatranus	5	6.5	6.1	6.8	5.5	5.8	5.2
Ficus microcarpa	5	7.4	7.7	7.9	6.5	6.9	8.1
Muntingia colabura	5	8.2	8.7	8.3	7.8	7.5	9.1
Acacia mangium	5	9.6	10.3	10.7	9.8	9.1	8.9
Leucaena glauca	5	6.2	4.5	4.9	5.5	7.2	6.4
Bougainvillea spectabilis	5	7.8	7.5	7.1	8.2	9.3	8.5
Bridelia tomentosa	5	7.9	6.2	7.1	7.6	8.2	9.5
Radermachera frondosa	5	6.5	5.3	5.8	6.5	6.9	7.5
Lepisanthes rubiginosa	5	5.2	4.8	4.7	5.1	5.9	5.3
Rhaphiolepis indica	5	3.7	3.3	4.1	4.3	4.5	3.8
Pterospermum heterophyllum	5	4.2	3.2	3.1	5.1	4.9	3.5
Fissistigma oldhamii	5	4.7	3.8	4.2	4.6	4.3	5.6
Psychotria rubra	5	3.2	3	3.5	3.1	3.8	2.7
Cudrania cochinchinensis	5	4.3	3.6	4.5	3.5	4.8	3.8

and air temperature at 390 μmol mol^{-1} and 28°C, respectively. Before data were recorded, we exposed leaves to the above conditions for about 5 min to allow photosynthetic parameters to stabilize.

Measurement of Leaf Hydraulic Conductivity and Leaf Turgor Loss Point

We collected leaf-bearing branches from five individuals of each forest species where the basal ends of the branches were immersed in distilled water and re-cut to measure leaf hydraulic conductivity and leaf turgor loss point. We rehydrated all branch samples until leaf water potential was greater than -0.05 MPa. Then 20 fully expanded and healthy leaves were selected to measure leaf hydraulic conductivity using rehydration technique following Brodribb and Holbrook (2003). We also selected another 20 fully expanded and health leaves to determine leaf turgor loss point using the pressure-volume relationship analysis program developed by Schulte and Hinckley (1985).

Measurement of Soil Water Content in Both Wet and Dry Seasons

We first randomly selected three individuals for each of the eight fast-growing and eight dominant forest species in the secondary forest. Then we collected one soil sample at a depth of 0–100 cm in soils around each focal individual every day in the peak of wet (August) in 2019 and dry seasons (February) in 2020, respectively to measure gravimetric soil water content (g kg^{-1}) in both sites in both wet and dry seasons, respectively.

Statistical Analyses

We used a Wilcoxon signed-rank test to detect differences in functional traits values between the dominant slow-growing and fast-growing species. Separate comparisons were also made within the dry and wet seasons. We also utilized Wilcoxon signed-rank test to compare soil water content in soils surrounding the fast-growing and slow-growing species. As above, comparisons were made in both wet and dry seasons.

RESULTS

The restoration project was finished in 2016, and till now typhoons and heavy rains during the wet season have not resulted in destructive landslides (Figure 1E). DBH for these eight fast-growing forest species are 2.2 times higher than the dominant slow-growing forest species in the secondary tropical rain forest in 2020 (**Table 1**). For dominant slow-growing species, all five traits were lower during the dry season than in the wet season (**Figure 3**, $p < 0.001$ based on Wilcoxon signed-rank tests). In contrast, only transpiration rate and leaf hydraulic conductivity were different for fast-growing species (**Figure 3**, $p < 0.001$). During the dry season, functional traits values of fast-growing species were 1.5 to 10 times higher than those of dominant slow-growing species (**Figure 4**, $p < 0.001$). And during the wet season, most functional traits values for fast-growing species were 3 to 10 times higher than for dominant slow-growing species. However, we did not observe any difference in leaf turgor loss point between fast-growing and dominant slow-growing species (**Figure 4**, $p > 0.05$).

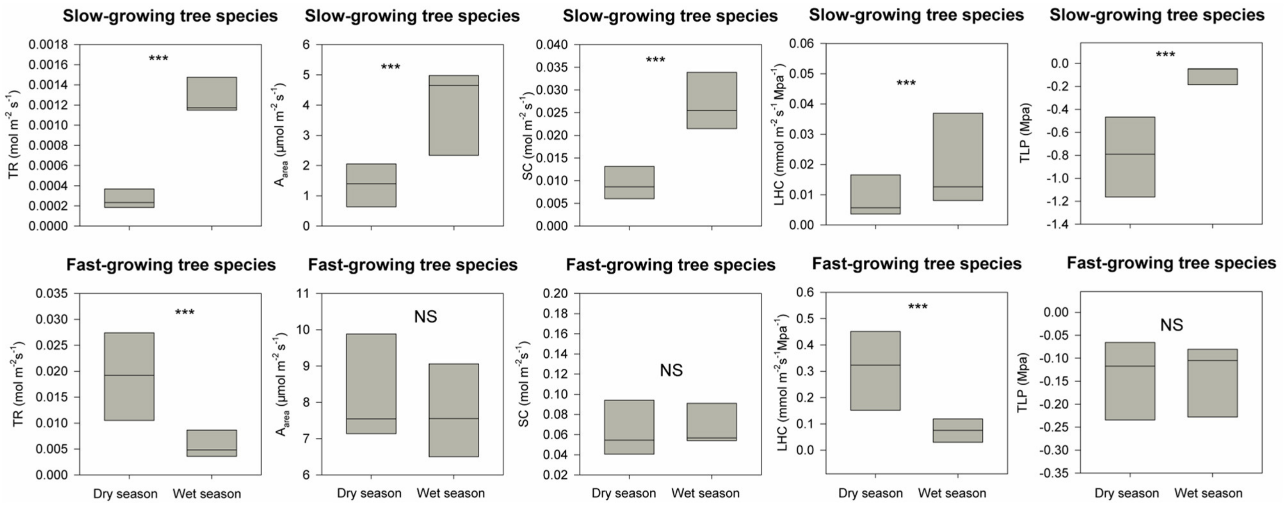

FIGURE 3 | Differences in five hydraulic traits (i.e., transpiration rate, TR; maximum photosynthesis rate, A_{area}; stomatal conductance, SC; leaf hydraulic conductivity, LHC; leaf turgor loss point, TLP) between the wet and dry season for both the fast-growing species used for reforestation and the dominant slow-growing species in the adjacent secondary tropical rain forest. *** indicates $P < 0.05$ and NS (short for non-significant) indicates $P > 0.05$ based on Wilcoxon signed-rank tests.

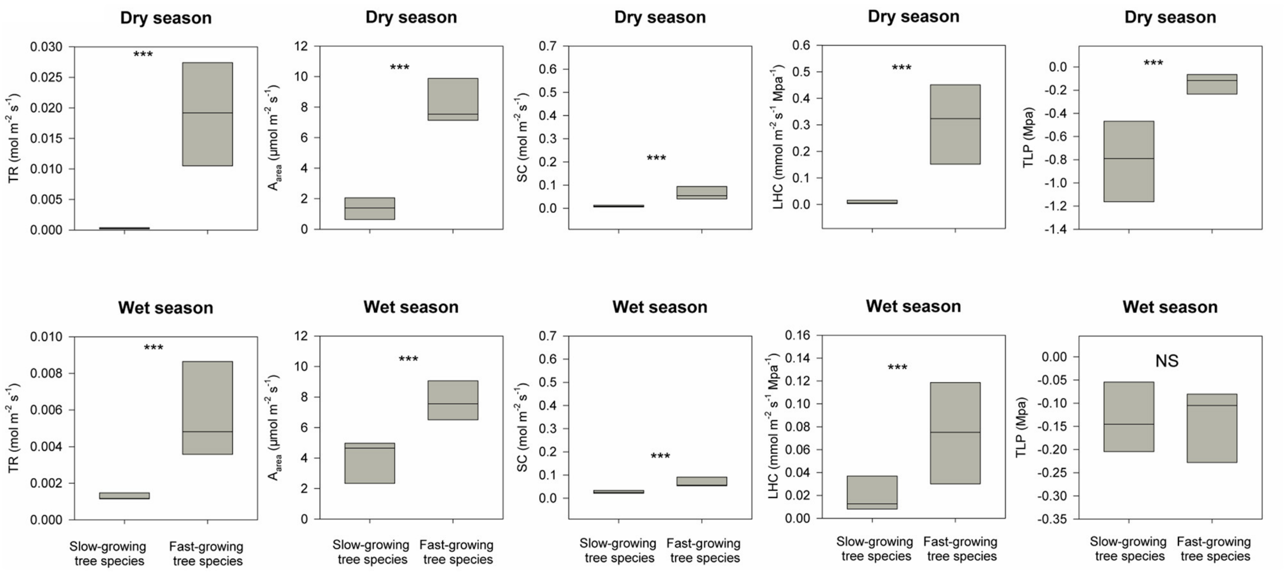

FIGURE 4 | Differences in distributions of five hydraulic traits (i.e., transpiration rate, TR; maximum photosynthesis rate, A_{area}; stomatal conductance, SC; leaf hydraulic conductivity, LHC; leaf turgor loss point, TLP) between the fast-growing species used for reforestation and dominant slow-growing species in the adjacent secondary tropical rain forest in dry and wet seasons respectively. *** indicates $P < 0.05$ and NS (short for non-significant) indicates $P > 0.05$ based on Wilcoxon signed-rank tests.

Soil water content around both the fast-growing and dominant slow-growing species decrease significantly from wet to dry seasons, with an 8% decrease in dominant slow-growing species, but a 190% decrease in fast-growing species (**Figures 5A,B**). That is, soil water content around the dominant slow-growing species remained almost constant across the seasons, whereas soil water content surrounding the fast-growing species decline dramatically from the wet to dry seasons. Soil water content in soils around the dominant slow-growing species in the secondary tropical rainforest ecosystem was as much as 1.5–3 times higher than in soils around the fast-growing species in the restoration area in the both wet and dry seasons (**Figures 5C,D**). Moreover, soil water content surrounding the dominant slow-growing species in the dry season was still 1.3 times higher than that for the fast-growing species in the wet season (**Figure 5E**).

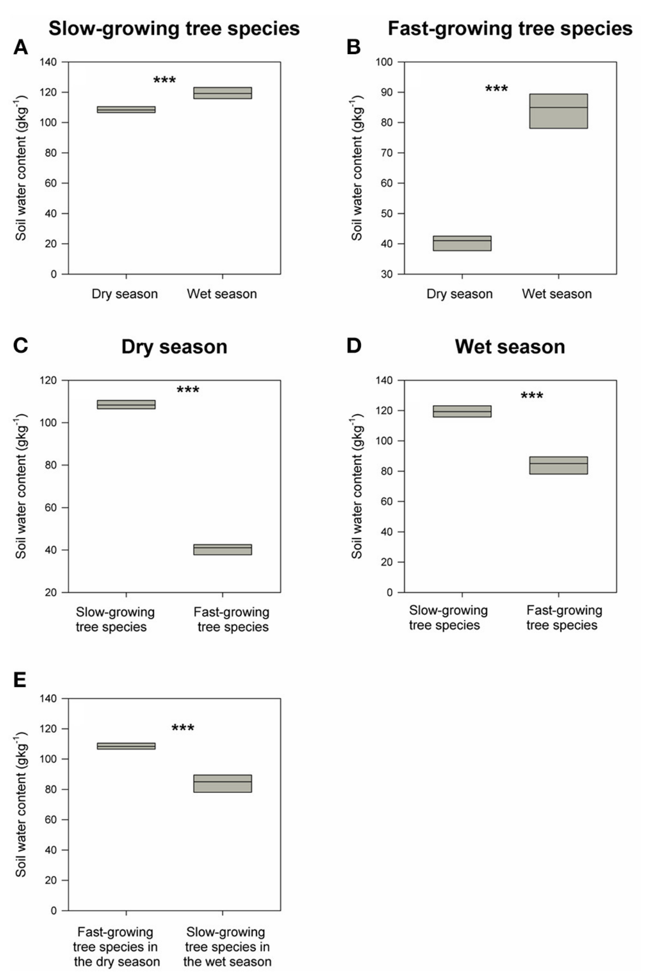

FIGURE 5 | (A,B) demonstrates the differences in soil water content between wet and dry season for slow-growing tree species and fast-growing tree species respectively. **(C,D)** shows the differences in soil water content between slow-growing tree species and fast-growing tree species in wet and dry season individually. **(E)** describes the differences in soil water content between fast-growing tree species in the dry season and slow-growing tree species in the wet season. ***indicates $P < 0.05$ and NS (short for non-significant) indicates $P > 0.05$ based on Wilcoxon signed-rank tests.

DISCUSSION

Our study provides convincing evidence that transpiration rates of the fast-growing species used for reforestation are much higher than that of the dominant slow-growing species in the secondary tropical rain forest, in both wet and dry seasons. This results in more uptake of water from the soil by the fast-growing species than by the slow-growing species, ultimately lower soil water content around the fast-growing species than that around the dominant slow-growing species, particularly during the dry season.

We found DBH for these eight fast-growing forest species are 2.2 times higher than the dominant slow-growing forest species in secondary tropical rain forest, indicating our selected fast-growing species can indeed grow much faster than the dominant forest species in the adjacent secondary tropical rainforest. We also found typhoons and heavy rains during the wet season had not resulted in destructive landslides on the reforestation project which was implemented by separately mono-planting these eight fast-growing forest species. These results demonstrated that mono-planting fast-growing species can indeed be effective for alleviating the potential landslide when performing reforestation in tropics due to frequent typhoon and heavy rain. (Stokes et al., 2009; Walker et al., 2009; Pang et al., 2018).

During the wet season, the fast-growing species had a much higher photosynthesis rate and stomatal conductivity than the dominant slow-growing species. Higher photosynthesis rates and stomatal conductance usually require higher leaf hydraulic conductivity to maintain high rates of transpiration (Tuzet et al., 2003; Miyashita et al., 2005; Maherali et al., 2008). Indeed, we found that the fast-growing species had much higher leaf hydraulic conductivity and transpiration rates than those of the dominant slow-growing species during the wet season. This suggests that the fast-growing species have much higher photosynthetic water demands and transpiration rates than the dominant slow-growing tree species. However, we observed no significant differences in leaf turgor loss point between them. Leaf turgor loss point reflects leaf water supply, with low leaf turgor loss point when plant leaf water supply is not enough (Bartlett et al., 2012). This indicated that leaf water supply was likely adequate for both types of trees. This is not surprising, as 90% precipitation occur in the wet season (Luo et al., 2020), which should meet the water demand of the trees. Nevertheless, higher plant transpiration rates will result in higher soil water uptake, which will lead to overall lower soil water content (Krishnaswamy et al., 2013; Peña-Arancibia et al., 2019). Indeed, we found that mean soil water content around the dominant slow-growing species was 1.5 times higher than in soils surrounding the fast-growing species. Thus, fast-growing species' much higher transpiration rates can indeed result in much lower soil water content, although precipitation is not limited.

During the dry season, it is likely that leaf water supply was insufficient for both fast-growing and dominant slow-growing species. However, we found that dominant slow-growing species had a much lower leaf turgor loss point during the dry season, while for fast-growing species it did not differ between seasons. This suggests that fast-growing species likely get adequate leaf water supply, while dominant slow-growing species do not. This was surprising, given that photosynthesis rate and stomatal conductivity was very high for fast-growing species during the dry season. This should make leaf water supply for fast-growing species not be fulfilled by the very limited precipitation in the dry season. Thus, fast-growing species must have a high leaf hydraulic conductivity, resulting in a high transpiration rate to meet their invariably high photosynthetic water demand. Indeed, we found that the fast-growing species had much higher leaf hydraulic conductivity and transpiration rates than in the wet season. As a result, the fast-growing species absorbed higher amounts of soil water than the dominant slow-growing species. Actually, we found that soil water content around fast-growing species during the dry season were only half of the amount observed during the wet season. Moreover, soil water content around the dominant slow-growing species during the dry season was also much higher than the fast-growing species. These results demonstrate that the high photosynthetic water demand of the fast-growing species cannot be fulfilled during the dry season without uptaking large amounts of water stored in soils. As a result, we expect that widespread plantings of fast-growing species will lead to long-term depletion of water in forest ecosystems.

A lower leaf turgor loss point also causes suppressed photosynthetic water demand, which results in suppressed leaf hydraulic conductivity and transpiration rates (Santos et al., 2018). Indeed, we found that dominant slow-growing species had much higher rates of leaf hydraulic conductivity and transpiration during the wet season than in the dry season. It is possible that dominant slow-growing species' suppressed transpiration rates can be sufficiently met by the limited water supply. Thus, dominant slow-growing species only requires to absorb a small amount of soil water content formed because of stored precipitation in the wet season in the soil layers. Indeed, we found that soil water content in soils around dominant slow-growing species decreased merely 8% in the dry season compared with the wet season. These results indicate that dominant slow-growing species' suppressed transpiration rates by lowering leaf turgor loss point can be largely met by the limited precipitation and thus they only absorb a small amount of soil water content formed in the wet season, thereby maintaining relatively high soil water content in the dry season.

Together, our results suggest that using monocultures of fast-growing tree species for large scale reforestation projects is difficult to recover the soil water content. Because of dominant species' lower transpiration rates, mixtures of fast-growing and dominant slow-growing species may be useful. Indeed, many studies from other sites have found that planting mixtures of the dominant slow-growing plant species and fast-growing species can enhance soil water content capacity (Sprenger et al., 2013; Amazonas et al., 2018; Jonsson et al., 2019). We expect that at our site, mixing dominant slow-growing species with the fast-growing monocultures would be useful for recovering soil water content. Still, initial plantings of fast-growing species remains important for preventing adverse effects from landslides and heavy rains (Stokes et al., 2009; Walker et al., 2009; Pang et al., 2018).

Based on all findings, we recommend a three-step method for recovering soil water content of extremely degraded tropical forest ecosystems via reforestation. First, using the slope, the deep of soil layers of the secondary tropical forest as a reference to reconstruct slope and soil layers. Then refiling the same soils from the secondary tropical monsoon forest to plant fast-growing tree species to minimize impacts from landslides and other soil disturbance events. Third, dominant slow-growing tree species from the adjacent secondary area should be planted within the fast-growing species stands to increase soil water content. We expect that this simple three-steps method can be an effective means of restoring extremely degraded tropical forests in other parts of the world.

CONCLUSION

Here we clearly demonstrate that our nearly nature reforestation method can help quickly and successfully perform reforestation to restore extremely degraded tropical rainforest. However, native forest species should be further mixed to effectively recover the soil water content. We also provide a simple method for guiding the use of reforestation efforts to recover soil water content in extremely degraded tropical rain forests.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

TL, HZ, and CW: designed research, analyzed data, and wrote the paper. KJ, ZT, and QH: performed research. All authors contributed to the article and approved the submitted version.

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REFERENCES

Acosta, L. A., Eugenio, E. A., Macandog, P. B. M., Macandog, D. B. M., Lin, E. K. H., Abucay, E. R., et al. (2016). Loss and damage from typhoon-induced floods and landslides in the Philippines: community Perceptions on climate impacts and adaptation options. *Int. J. Glob. Warm.* 9, 33–65. doi: 10.1504/ijgw.2016.074307

Amazonas, N. T., Forrester, D. I., Oliveira, R. S., and Brancalion, P. H. S. (2018). Combining Eucalyptus wood production with the recovery of native tree diversity in mixed plantings: implications for water use and availability. *For. Ecol. Manage.* 418, 34–40. doi: 10.1016/j.foreco.2017.12.006

Anderegg, W. R. (2014). Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. *New Phytol.* 205, 1008–1014. doi: 10.1111/nph.12907

Bartlett, M. K., Scoffoni, C., and Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecol. Lett.* 15, 393–405. doi: 10.1111/j.1461-0248.2012.01751.x

Brodribb, T. J., and Holbrook, N. M. (2003). Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol.* 132, 2166–2173. doi: 10.1104/pp.103.023879

Bruijnzeel, L. A. (2004). Hydrological functions of tropical forests: not seeing the soil for the trees? *Agric. Ecosyst. Environ.* 104, 185–228. doi: 10.1016/j.agee.2004.01.015

Carson, B. (1989). *Soil Conservation Strategies for Upland Areas of Indonesia*. Occasional Paper No. 9. Honolulu, HI: East–West Center, Environment and Policy Institute, 120.

Castello, L., and Macedo, M. N. (2016). Large-scale degradation of Amazonian freshwater ecosystems. *Glob. Change Biol.* 22, 990–1007. doi: 10.1111/gcb.13173

Chang, K. T., Chiang, S. H., and Lei, F. (2008). Analysing the relationship between typhoon-triggered landslides and critical rainfall conditions. *Earth Surf. Proc. Land* 33, 1261–1271. doi: 10.1002/esp.1611

Chapin, F. S. III., Matson, P. A., and Vitousek, P. (2011). *Principles of Terrestrial Ecosystem Ecology*. New York, NY: Springer. doi: 10.1007/b97397

Engelbrecht, B. M., Comita, L. S., Condit, R., Kursar, T. A., Tyree, M. T., Turner, B. L., et al. (2007). Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* 447, 80–82. doi: 10.1038/nature05747

Evaristo, J., and McDonnell, J. J. (2019). Global analysis of streamflow response to forest management. *Nature* 570, 455–461. doi: 10.1038/s41586-019-1306-0

Fisher, R. A., Williams, M., Da Costa, A. L., Malhi, Y., Da Costa, R. F., Almeida, S., et al. (2007). The response of an Eastern Amazonian rain forest to drought stress: results and modelling analyses from a throughfall exclusion experiment. *Glob. Change Biol.* 13, 2361–2378. doi: 10.1111/j.1365-2486.2007.01417.x

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., et al. (2011). Solutions for a cultivated planet. *Nature* 478, 337–342. doi: 10.1038/nature10452

Fugère, V., Nyboer, E. A., Bleecker, J. C., and Chapman, L. J. (2016). Impacts of forest loss on inland waters: identifying critical research zones based on deforestation rates, aquatic ecosystem services, and past research effort. *Biol. Conserv.* 201, 277–283. doi: 10.1016/j.biocon.2016.07.012

Gebrehiwot, S. G., Ellison, D., Bewket, W., Seleshi, Y., Inogwabini, B.-I., and Bishop, K. (2019). The Nile Basin waters and the West African rainforest: rethinking the boundaries. *WIREs Water* 6:e1317. doi: 10.1002/wat2.1317

Guidicini, G., and Iwasa, O. Y. (1977). Tentative correlation between rainfall and landslides in a humid tropical environment. *B. Eng. Geol. Environ.* 16, 13–20. doi: 10.1007/BF02591434

Jackson, C. R. (2006). "Wetland hydrology," in *Ecology of Freshwater and Estuarine Wetlands,* eds D. P. Batzer and R. Sharitz (Berkeley, CA: University of California Press), 43–81.

Jonsson, M., Bengtsson, J., Gamfeldt, L., Moen, J., and Snäll, T. (2019). Levels of forest ecosystem services depend on specific mixtures of commercial tree species. *Nat. Plants* 5, 141–147. doi: 10.1038/s41477-018-0346-z

Krishnaswamy, J., Bonell, M., Venkatesh, B., Purandara, B., Rakesh, K. N., Lele, S., et al. (2013). The groundwater recharge response and hydrologic services of tropical humid forest ecosystems to use and reforestation: support for the "infiltration-evapotranspiration trade-off hypothesis". *J. Hydrol.* 498, 191–209. doi: 10.1016/j.jhydrol.2013.06.034

Lambin, E. F., and Meyfroidt, P. (2011). Global land use change, economic globalization, and the looming land scarcity. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3465–3472. doi: 10.1073/pnas.1100480108

Laurance, W. F., Sayer, J., and Cassman, K. G. (2014). Agricultural expansion and its impacts on tropical nature. *Trends. Ecol. Evol.* 29, 107–116. doi: 10.1016/j.tree.2013.12.001

Lohbeck, M., Poorter, L., Lebrija-Trejos, E., Martinez-Ramos, M., Meave, J. A., Paz, H., et al. (2013). Successional changes in functional composition contrast for dry and wet tropical forest. *Ecology* 94, 1211–1216.doi: 10.1890/12-1850.1

Lu, Y., Ranjitkar, S., Harrison, R. D., Xu, J., Ou, X., Ma, X., et al. (2017). Selection of native tree species for subtropical forest restoration in Southwest China. *PLoS ONE* 12:e0170418. doi: 10.1371/journal.pone.0170418

Luke, S. H., Barclay, H., Bidin, K., Chey, V. K., Ewers, R. M., Foster, W. A., et al. (2017). The effects of catchment and riparian forest quality on stream environmental conditions across a tropical rainforest and oil palm landscape in Malaysian Borneo. *Ecohydrology* 10:e1827. doi: 10.1002/eco.1827

Luo, J. H., Cui, J., Pandey, S. P., Jiang, K., Tan, Z. Y., He, Q. F. et al. (2020). Seasonally distinctive growth and drought stress functional traits enable Leucaena Leucocephala to successfully invade a Chinese tropical forest. *Trop. Conserv. Sci.* 13, 1–7. doi: 10.1177 /1940082920949176

Maherali, H., Pockman, W. T., and Jackson, R. B. (2004). Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85, 2184–2199.doi: 10.1890/02-0538

Maherali, H., Sherrard, M. E., Clifford, M. H., and Latta, R. G. (2008). Leaf hydraulic conductivity and photosynthesis are genetically correlated in an annual grass. *New Phytol.* 180, 240–247. doi: 10.1111/j.1469-8137.2008.02548.x

Mapulanga, A. M., and Naito, H. (2019). Effect of deforestation on access to clean drinking water. *Proc. Natl. Acad. Sci. U.S.A.* 116:8249. doi: 10.1073/pnas.1814970116

Miyashita, K., Tanakamaru, S., Maitani, T., and Kimura, K. (2005). Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environ. Exp. Bot.* 53, 205–214. doi: 10.1016/j.envexpbot.2004.03.015

Neary, D. G., Ice, G. G., and Jackson, C. R. (2009). Linkages between forest soils and water quality and quantity. *For. Ecol. Manage.* 258, 2269–2281. doi: 10.1016/j.foreco.2009.05.027

Nooteboom, H. P. (1987). Further views on environmental impacts of (de)forestation in the humid tropics. *Wallaceana* 47, 10–11.

Pang, C. C., Ma, X. K.-K., Lo, J. P.-L., Hung, T. T.-H., and Hau, B. C.-H. (2018). Vegetation succession on landslides in Hong Kong: plant regeneration, survivorship and constraints to restoration. *Glob. Ecol. Conserv.* 15:e00428. doi: 10.1016/j.gecco.2018.e00428

Peña-Arancibia, J. L., Bruijnzeel, L. A., Mulligan, M., and van Dijk, A. I. J. M. (2019). Forests as 'sponges' and 'pumps': assessing the impact of deforestation on dry-season flows across the tropics. *J. Hydrol.* 574, 946–963. doi: 10.1016/j.jhydrol.2019.04.064

Sahin, V., and Hall, M. J. (2004). The effects of afforestation and deforestation on water yields. *J. Hydrol.* 178, 299–309. doi: 10.1016/0022-1694(95)02825-0

Santos, V. A. H. F.D., Ferreira, M. J., Rodrigues, J. V. F. C., Garcia, M. N., Ceron, J. V. B., Nelson, B. W., et al. (2018). Causes of reduced leaf-level photosynthesis during strong El Niño drought in a Central Amazon forest. *Glob. Change Biol.* 24, 4266–4279. doi: 10.1111/gcb.14293

Schulte, P., and Hinckley, T. (1985). A comparison of pressure-volume curve data analysis techniques. *J. Exp. Bot.* 36, 1590–1602. doi: 10.1093/jxb/36.10.1590

Solange, F., Ometto, B. M., Weiss, K. C. B., Palmer, M. A., and Silva, L. C. R. (2017). Impacts of forest restoration on water yield: a systematic review. *PLoS ONE* 12:e0183210. doi: 10.1371/journal.pone.0183210

Sprenger, M., Oelmann, Y., Weihermüller, L., Wolf, S., Wilcke, W., and Potvin, C. (2013). Tree species and diversity effects on soil water seepage in a tropical plantation. *For. Ecol. Manage.* 309, 76–86. doi: 10.1016/j.foreco.2013.03.022

Stokes, A., Atger, C., Bengough, A. G., Fourcaud, T., and Sidle, R. C. (2009). Desirable plant root traits for protecting natural and engineered slopes against landslides. *Plant Soil* 324, 1–30. doi: 10.1007/s11104-009-0159-y

Tardieu, F., and Parent, B. (2017). Predictable 'meta-mechanisms' emerge from feedbacks between transpiration and plant growth and cannot be simply deduced from short-term mechanisms. *Plant Cell Environ.* 40, 846–857. doi: 10.1111/pce.12822

Tuzet, A., Perrier, A., and Leuning, R. (2003). A coupled model of stomatal conductance, photosynthesis and transpiration. *Plant Cell Environ.* 26, 1097–1116. doi: 10.1046/j.1365-3040.2003.01035.x

Villamayor, B. M. R., Rollon, R. N., Samson, M. S., Albano, G. M. G., and Primavera, J. H. (2016). Impact of Haiyan on Philippine mangroves: implications to the fate of the widespread monospecific Rhizophora plantations against strong typhoons. *Ocean Coast. Manage.* 132, 1–14. doi: 10.1016/j.ocecoaman.2016.07.011

Vörösmarty, C. J., Hoekstra, A. Y., Bunn, S. E., Conway, D., and Gupta, J. (2015). Fresh water goes global. *Science* 349, 478. doi: 10.1126/science.aac6009

Vose, J. M. (2019). *Forest and Water in the 21st Century: A Global Perspective*. Washington, DC: Oxford University Press.

Walker, L. R., Velázquez, E., and Shiels, A. B. (2009). Applying lessons from ecological succession to the restoration of landslides. *Plant Soil* 324, 157–168. doi: 10.1007/s11104-008-9864-1

Yumul, J. G. P., Servando, N. T., Suerte, L. O., Magarzo, M. Y., Juguan, L. V. V., and Dimalanta, C. B. (2012). Tropical cyclone–southwest monsoon interaction and the 2008 floods and landslides in Panay island, central Philippines: meteorological and geological factors. *Nat. Hazards* 62, 827–840. doi: 10.1007/s11069-012-0109-5

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A Web-Based Software Platform for Restoration-Oriented Species Selection Based on Plant Functional Traits

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Plant functional traits are fundamental to the understanding of plant adaptations and distributions. Recently, scientists proposed a trait-based species selection theory to support the selection of suitable plant species to restore the degraded ecosystems, to prevent the invasive exotic species and to manage the sustainable ecosystems. Based on this theory, in a previous study, we developed a species screening model and successfully applied it to a project where plant species were selected for restoring a tropical coral island. However, during this process we learned that a software platform is necessary to automate the selection process because it can flexible to assist users. Here, we developed a generalized software platform called the “Restoration Plant Species Selection (RPSS) Platform.” This flexible software is designed to assist users in selecting plant species for particular purposes (e.g., restore the degraded ecosystems and others). It is written in R language and integrated with external R packages, including the packages that computing similarity indexes, providing graphic outputs, and offering web functions. The software has a web-based graphical user interface that allows users to execute required functions via checkboxes and buttons. The platform has cross-platform functionality, which means that it can run on all common operating systems (e.g., Windows, Linux, macOS, and others). We also illustrate a successful case study in which the software platform was used to select suitable plant species for restoration purpose. The objective of this paper is to introduce the newly developed software platform RPSS and to provide useful guidances on using it for various applications. At this step, we also realized that the software platform should be constantly updated (e.g., add new features) in the future. Based on the existing successful application and the possible updates, we believe that our RPSS software platform will have broader applications in the future.

Keywords: vegetation restoration, plant functional traits, maximum entropy model, restoration plant species selection platform, R language

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INTRODUCTION

Climate change and human activities (e.g., ore mining and agricultural use) have caused the land ecosystem degraded worldwide (Chapin et al., 2000). These degraded ecosystems are known to seriously affect human economic and social life (Laughlin and Laughlin, 2013; Laughlin, 2014). As such, ecological restoration has rapidly developed to solve the urgent and complex degraded problems (Brown and Amacher, 1999; Cardinale et al., 2012). Among all, identifying the suitable species that can successfully restore the degraded ecosystems is the primary challenge in restoration science (Fry et al., 2013). However, this step requires a comprehensive understanding of the ecological restoration theory, including information on species interactions, successional processes, and resource-use patterns. Because these processes differ greatly across different ecosystems, it remains an enormous challenge. As this type of information is typically lacking, the selection of candidate species for restoration purposes are typically chosen using traditional trial-and-error method. Nevertheless, the trial-and-error method based largely on expert knowledge of the ecosystem (Rosenthal, 2003), and such information requires the involvement of a restoration practitioner who has a wealth of practical experience which is based on years of training (Padilla et al., 2009; Ostertag et al., 2015).

Plant functional traits (including morphological, physiological and phenological characteristics that are linked to plant life history strategies) are fundamental to understanding plant adaptations and distributions. In theory, plant functional traits can be useful for selecting suitable species to restore degraded ecosystems by computing the similarity index between the target species (e.g., species have been proved to have high survival rates in the degraded ecosystems) and the potential species (e.g., all possible suitable species) (Zhang et al., 2018; Wang et al., 2020). Several studies have successfully used functional traits to select suitable plant species for restoring degraded ecosystems (Bochet and García-Fayos, 2015; Ostertag et al., 2015; Guimarães et al., 2018; Werden et al., 2018; Rayome et al., 2019; Wang et al., 2020). However, each of these studies used different screening methods or different sets of functional traits to select species. A generalized framework and a software platform which can assist this selection process is necessary to people which are new to this field.

Recently, Laughlin (2014) proposed a quantitative trait-based species selection process for selecting suitable species to restore degraded ecosystems. In a previous study (Wang et al., 2020), we developed a species screening model based on this quantitative trait-based theory (Shipley et al., 2006). Also, we successfully applied this model to select best fit plant species for restoring a tropical coral island which is part of Hainan Island, China (Wang et al., 2020). However, along this way we realized that a software platform which can help automate this modeling process is necessary as it will save time for beginners.

In this study, we introduced a newly developed, web-based species selection software platform and named it as "Restoration Plant Species Selection (RPSS) Platform." The platform aims to select suitable plant species for restoration purpose based on plant functional traits. It was developed using the high-level R programming language[1] which is popular for statistical computing and graphics. It is a web-based application that can run on a wide variety of operating systems, like Windows, Linux, and macOS and it can work based on various general browsers (e.g., IE, Chrome, Firefox, and others). The software platform makes use of several external R packages that perform various functions, including computing similarity rankings and drawing multiple graphic functions. For users, there are only two sets of information needed to run the software: (1) the trait values of the target species (species which proved to meet the specific goals) and (2) the trait values of the potential species (species to be chosen from). In addition, the RPSS platform also has a graphic user interface that helps users execute each of the functions.

TRAIT-BASED PLANT SPECIES SELECTION PROCESS

In this section, we will introduce the abiotic filtering theory which is the key selection theory for our software platform, and the trait-based plant species selection process in details.

Abiotic Filtering Theory

Abiotic filtering theory claims that for adapting well to the limiting abiotic environment (e.g., light and soil nutrient), all species have to develop similar traits to optimize fitness and performance (Laughlin, 2014). If we have found at least one or two species that can adapt well to the specific environment in the degraded ecosystem, many species that are appropriate for restoring the degraded ecosystem can easily be selected, as long as we compare the similarities in functional traits among them.

The Trait-Based Plant Species Selection Process

Step 1. Determine the Target Species

The target species are needed as a prerequisite in this process. Generally, the target species are species that have met certain standards of durability in ecosystems. For restoration purposes, the target species are which we already know have high survival rates in the degraded ecosystems. However, in some cases, the restoration is initiated from bare soil conditions which means that the target species are not easily found based on poor background knowledge. We therefore use the traditional trial-and-error method to determine them (Wang et al., 2020).

Step 2. Prepare the Pool of Potential Species

Potential species pool consists all the possible plant species which may be suitable for our specific purposes. For restoration purposes, the potential species pool should include both historical native species and non-native species that have the potential to restore the degraded ecosystems. The non-native species are which found in regions that have similar environments with the study site (Ostertag et al., 2015). However, in some cases, native species or non-native species are not easily found. For

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[1] https://www.r-project.org/

example, the study site may be located on a highly degraded island on which almost no native plants can be found. In such situations, only non-native species will be considered for the potential species pool.

Step 3. Select the Appropriate Functional Traits That Can Capture the Key Characteristics of the Particular Purpose

The selected functional traits should capture the key characteristics of the degraded ecosystems' environmental conditions. For example, leaf turgor loss point should be measured to reflect plant resistance to the drought stress, if drought stress is a key characteristic for the degraded ecosystem. Additionally, the appropriate traits should be well measurable for laboratories. If there is not any information on the characteristics already, the users may try to measure all possible functional traits (e.g., commonly measured morphological and physiological traits). We focused on different species that expressed similar trait values that were relevant to our objectives.

Step 4. Collect and Prepare Functional Trait Dataset

All trait information was obtained from field observations and laboratory measurements. Many previous studies have addressed how to collect and measure the functional traits. For the detailed information of how to prepare the functional trait dataset, please refer to some previous studies (Zhang et al., 2019; Wang et al., 2020) and **Supplementary Tables 2,3** in the **Supplementary Materials**.

Step 5. Use Functional Traits to Select the Best Fit Species

In our software platform, the maximum entropy (Maxent) model (Shipley et al., 2006) was used to screen plant species that are most functionally similar to the target species. The model outputs relative abundances to screen suitable species from the potential species pool. We defined relative abundance as the index of similarity between the target species and the potential species. The larger the value of similarity index, the more ecologically similar the two species are, and vice versa. Using this index, we assumed that the most similar species to the target species was also the most suitable species for restoring the degraded ecosystems.

Step 6. Monitor Seedling Survival of the Selected Plant Species

At the final step, the survival rates of the selected species should be monitored to check (1) if they have the high survival rates than the unselected species; and (2) if they have the comparable survival rates to the target species. If both requirements are met, that means our species screening process is succeed.

OVERVIEW OF THE PLANT SPECIES SELECTION PLATFORM

In this study, we developed a web-based software platform named "Restoration Plant Species Selection (RPSS) Platform" to aid in the trait-based plant species selection process. The RPSS platform is written in R language, and the main page of the platform is shown in **Figure 1**. The user only needs to prepare trait values for the target and potential species in text format (see data examples in **Supplementary Tables 2,3**) when using the software platform. The software outputs both text and figure results of similarity rankings for each potential plant species, ranked from highest to lowest. The returned similarity index can indicate the similarity rank of each candidate plant species to target species. To establish the whole functions of the platform, we make use of numerous external R packages to perform various functions. For example, the FactoMineR package[2] is used to perform the PCA analysis, the FD package[3] (Laliberté and Legendre, 2010; Laliberté and Shipley, 2010; Legendre, 2010) is used to compute the similarity rankings based on the Maxent model (Shipley et al., 2006), the ggplot2 package[4] is used for graphic outputs, and the shiny package[5] helps accomplish the web functions. The software is open source. Anyone who needs the software can contact the corresponding author to acquire the whole software platform.

AN ILLUSTRATION OF USING THE "RESTORATION PLANT SPECIES SELECTION (RPSS)" PLATFORM TO SOLVE A RESTORATION PROBLEM

Problem Description

In this section, we illustrate how to use our RPSS software platform successfully selects suitable plant species to restore a degraded ecosystem. Our study site is located in a tropical coral island which is part of Hainan Island, China. Because of multiple harsh environmental characteristics (e.g., high temperatures, strong light, drought, no soil, and other harsh conditions) (Zhang et al., 2019; Wang et al., 2020), it is difficult for plants to establish and grow in this area. For human habitation and economic development, restoration of the vegetation on this island is important and urgent. Plantations of some species are considered a quick way to vegetate the island and make the island suitable for the development of plant communities native to the region (Wolfe et al., 2015; Wang et al., 2020). The objective of this section is to use our newly developed software platform to select suitable plant species that could be used to restore the degraded ecosystem of this area. Specifically, this island can be comparable to primary succession, as it is only made of rock and sand and does not have any soil for plants to colonize. If finally, the species selected by our software platform can indeed be suitable for restoring this extremely degraded island, we believe that our software can be widely used for restoring multiple different types of degraded ecosystems.

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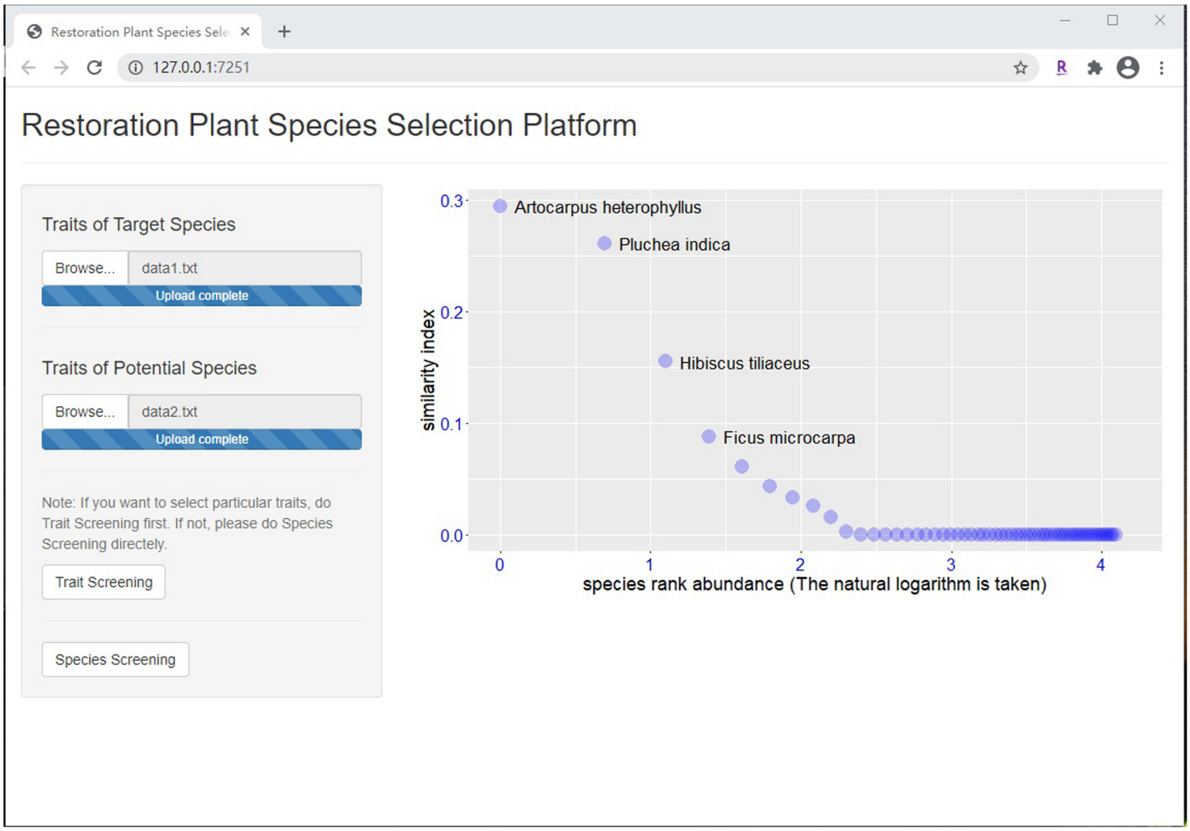
[2] https://cran.r-project.org/web/packages/FactoMineR/index.html

[3] http://cran.r-project.org/web/packages/FD

[4] https://cran.r-project.org/web/packages/ggplot2/index.html

[5] https://cran.r-project.org/web/packages/shiny



Restoration Plant Species Selection Platform

Note: If you want to select particular traits, do Trait Screening first. If not, please do Species Screening directly.

FIGURE 1 | The Graphic User Interface of the RPSS Platform.

Study Site

Our study site is located in a tropical coral island which lays to the southern of Hainan Island (lying between 108°37′—111°03′ E and 18°10′—20°10′ N), China. The study site has an area of approximately 1 km^2 and the mean altitude is about 5 meters (Wang et al., 2020).

The coral island our study site located has a tropical monsoon oceanic climate with a mean annual temperature of 28°C. The average annual precipitation on the island is about 2,800 mm, and most precipitation occurs between April and September. The adverse environments in this study site are characterized by high temperatures, intense light, drought and high salinity and alkalinity soil, in which it is difficult for plants to colonize and grow (Zhang et al., 2019). For human habitation and economic development, developing successful restoration management strategies for this area is exceedingly important.

Selecting Target and Potential Species

As described earlier, the trait-based species screening process requires target species as a baseline. However, our restoration is initiated from bare soil condition which no native plants can be found. Only the traditional trial-and-error method can be used to acquire the target species. So, we have identified 20 species on a nearby island with similar environmental conditions to the study site. These species were cultivated (watering and fertilizing) in Wenchang City, Hainan Island for 1–3 months and then transplanted to the study site. Three years later (from the year 2014 to 2017), target species were defined as those with survival rates >90% based on trial-and-error method. Ultimately, three species *Scaevola sericea*, *Ipomoea pes-caprae*, and *Cynodon dactylon*'Yangjiang' were selected as target species for trees, vines, and herbs (Li et al., 2016; Luo et al., 2018; Wang et al., 2020).

In order to prepare the potential species pool for restoring the degraded coral island, we reviewed literatures and studied surveys of plants in four tropical regions which the climatic and environmental conditions are similar to our study site in global. The four regions are the South China Sea Islands, the South Pacific Islands/Hawaii, the Indian Ocean Islands, and the Caribbean Sea Islands. At last, 66 species were selected to form the potential species pool. These species included a wide range of plant groups containing trees, shrubs, herbs, vines, legumes, semi-mangrove plants, and some medicinal and edible plants. The list of the 66 candidate species is shown in **Supplementary Table 1** (Wang et al., 2020). The seedlings of these potential species were cultivated mainly including watering and fertilizing in Wenchang City, Hainan Island.

Species' Functional Traits

We want to find out key traits that can assist plant species adapt well to the specific environments of the degraded ecosystem.

Thus, the traits will help to select suitable species to restore this place well. Based on our previous studies (Luo et al., 2018; Wang et al., 2020), we identified 28 traits that associated with the harsh environmental conditions on the tropical coral island. The selected functional traits are relate to drought resistance, resource allocation, antioxidant and photosynthetic capacity: specific leaf area (g/cm^2), leaf water conductance (mmol m-2 s-1 MPa-1), leaf dry matter content (%), stomatal conductance (mmol m^{-2} s^{-1}), stomatal density (numbers mm^{-2}), upper epidermic thickness (μm), palisade tissue (μm); spongy tissue (μm), lower epidermic thickness (μm), maximum photosynthetic rate (mol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), instantaneous water use efficiency (μmol mol^{-1}), transpiration rate (mol m^{-2} s^{-1}) and others. The 28 selected key traits are listed in **Table 1**. Because we don't have enough fresh herb leaf samples, some leaf related traits were not able to measure for herb species. As a result, we only measured 19 traits for herbs, but 28 traits for wood species. The omitted nine leaf structure-related traits are leaf/palisade/spongy tissue thickness, palisade tissue width, upper epidermis thickness, guard cell length, stomatal density, and stomatal area index. For three target species, only *Cynodon dactylon*'Yangjiang' had measured 19 traits while others all had 28 traits. Among all 66 potential species, 6 species had only 19 traits, others had 28 traits. For the selected target and potential species, traits on mature and healthy leaves of ten individuals for each species in a growing season were measured. The measurement methods and trait dataset can be available in the supporting files.

Results of the Plant Species Selection Process

We input the trait values of target and potential species into the RPSS platform, the input file format can be found in **Supplementary Tables 2,3**. The software returned the final screening results in both text and graphic formats. In this case study, we used the Windows operating system and Google Chrome browser to accomplish the whole analysis process. The example graphic results are shown in **Figures 2–4**. For the target species *Scaevola sericea*, four plant species (*Artocarpus heterophyllus*, *Pluchea indica*, *Hibiscus tiliaceus*, and *Ficus microcarpa*) were selected as the potential species. For target species *Ipomoea pes-caprae*, six plant species (*Ipomoea tuba*, *Pluchea indica*, *Pandanus tectorius*, *Hibiscus tiliaceus*, *Medicago sativa,* and *Sesuvium portulacastrum*) were screened to be more similar to the target species. For target species *Cynodon dactylon*'Yangjiang,' four plant species (*Spinifex littoreus*, *Lepturus repens*, *Miscanthus sinensis*, and *Cerbera manghas*) were outperformed than others. After removing duplicate species, we selected a total of 12 species for vegetation restoration in this study.

Finally, we monitored seedling survival for all species in order to test (1) whether the selected species had the comparable

TABLE 1 | Descriptions of the selected plant traits and abbreviations used in this study.

Leaf trait category	Trait	Abbreviation	Unit	Ecological relevance
Structural traits	Leaf/Palisade/Spongy tissue thickness	LT/PT/ST	μm	Radiation and drought tolerance
	Leaf palisade/spongy tissue thickness ratio	PST	/	Radiation and drought tolerance
	Palisade tissue width	PW	μm	Radiation and drought tolerance
	Upper epidermis thickness	UE	μm	Radiation and drought tolerance
	Leaf dry mater content	LDMC	mg g^{-1}	Nutrient acquisition and retention of resources
	Specific leaf area	SLA	cm^2 g^{-1}	Resource capture and environment adaptability
	Leaf area	LA	cm^2	Water retention and drought tolerance
	Guard cell length	SL	μm	Water retention and drought tolerance
	Stomatal density	SD	n mm^2	Water retention and drought tolerance
	Stomatal area index	SPI	%	Photosynthesis and evaporation intensity
Biochemical traits	Total antioxidant capacity	AOC	U g^{-1}	Antioxidant capacity
	Superoxide dismutase activity	SOD	U g^{-1}	Antioxidant capacity
	Peroxidase activity	POD	U g^{-1}	Antioxidant capacity
	Catalase activity	CAT	U g^{-1}	Antioxidant capacity
	Total phenolics content	TP	mg g^{-1}	Antioxidant capacity
	Water soluble protein	CPR	mg g^{-1}	Osmotic adjustment and nutrient retention
	Malondialdehyde content	MDA	nmol g^{-1}	Lipid peroxidation degree
	Proline content	PRO	μg g^{-1}	Osmotic adjustment
	Chlorophyll a/ Chlorophyll b/ Total chlorophyll content	CHLa/ CHLb/ CHLt	mg g^{-1}	Light capture and photosynthetic capacity
Hydraulic traits	Leaf hydraulic conductance	K_{leaf}	mmol s^{-1} m^{-2} MPa^{-1}	Water retention and acquisition capacity
Gas exchange traits	Maximum photosynthetic rate	$A_{\max}$	μmol m^{-2} s^{-1}	Photosynthetic capacity
	Stomatal conductivity	g_s	mol m^{-2} s^{-1}	Stomatal adjustment and water retention
	Instantaneous water use efficiency	WUEi	μmol mol^{-1}	Leaf carbon and water utilization capacity
	Transpiration rate	E	mmol m^{-2} s^{-1}	Leaf water utilization

Sources and physiological functions are also shown.

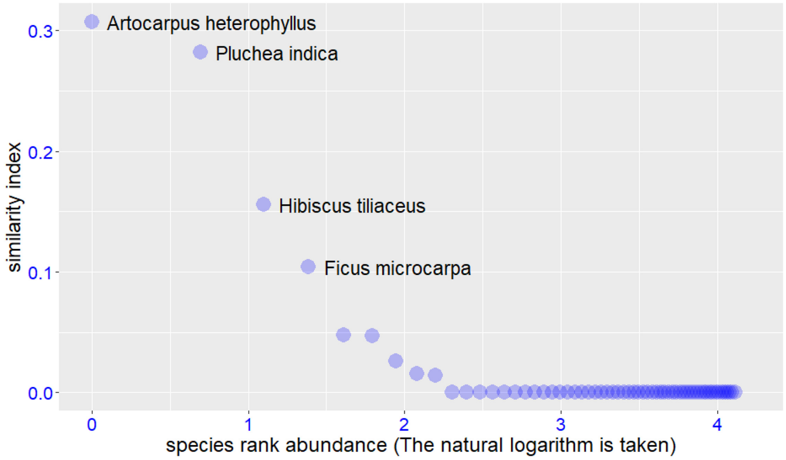


FIGURE 2 | Species selection results for the target species *Scaevola sericea*. The similarity index used in this study are relative abundances which output from the Maxent model.

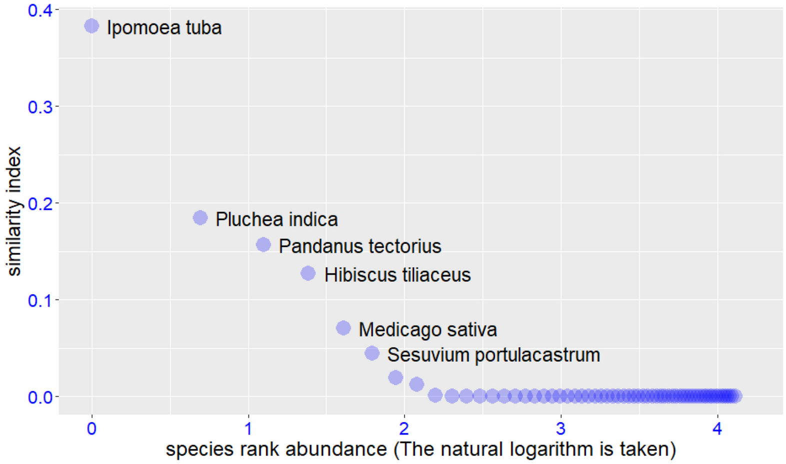


FIGURE 3 | Similar to figure 2, but the species selection results for the target species *Ipomoea pes-caprae*.

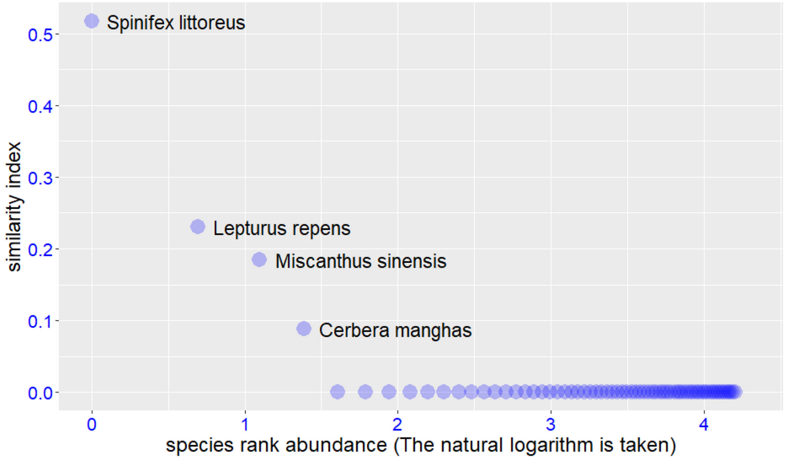


FIGURE 4 | Similar to figure 2, but the species selection results for the target species *Cynodon dactylon*'Yangjiang.'

survival rates with the target species; and (2) whether the selected species had higher survival rates than the unselected species. We cultivated 1,000 seedlings for each species in a nursery in Wenchang city, Hainan Island, China. In July 2017, we transplanted seedlings from our nursery to the tropical island. Each plant was planted on the same substrate type, and each row was divided into three repeat planting areas. The planting density was maintained at 80–100 plants per hectare. We recorded each plant's annual growth rates. After 2 years' follow-up, we recorded the final survival rate for each species using the following formula:

$$survival\ rate = \frac{currently\ remaining\ seedings}{original\ seedlings} \times 100\%$$

In final results, comparably high survival rates were found among the three target species and the twelve selected species (about 86 to 94%). However, the survival rates of the non-selected species were significantly lower than the target species in all cases (see **Supplementary Figure 1**). The results showed that the species selected by our software platform had significantly better survival rates than the non-selected species. This means that the software platform successfully performed the species selection for the purpose of restoration in our study site.

CONCLUSION

In this study, we introduce a newly developed web-based species screening software platform – the RPSS platform. It uses a trait-based framework to select suitable plant species for particular purposes (e.g., restore the degraded ecosystem and others). The software is implemented in the high-level R language and uses some external R packages (e.g., FD package, ggplot2 package, and shiny package) to realize the model calculations, the picture productions, and the web functions. The GUI equipped in the software allows an easy execution of purposed analyses. Because it is a web-based platform, it can run across different operating systems including Windows, Linux, and macOS. Compare with other similar work (e.g., Restoring Ecosystem Services Tool, REST, Rayome et al., 2019), our software is a web-based platform which don't need to install any programs on the local computer. In addition, our RPSS platform not only equipped with a web-based GUI to facilitate execution of various functions, it can also run as a script program in R language batch mode. Thus, users can clearly see which commands are executed, so as to better understand the science nature behind the plant species screening process.

We demonstrated a successful application of this software platform for selecting suitable species to restore a highly degraded coral island in Hainan Island, China. The example shown in the paper is designed to demonstrate the capabilities of the software platform. We have tested our software platform in different operating systems (e.g., Windows, Linux, and macOS) and various browsers (e.g., IE, Firefox, Google Chrome), all the test samples ran normally and the results were the same which proved that our software platform was stable across different systems. The RPSS platform is an evolving program with many directions for future development (e.g., add new features, output multiple types of figures). We believe that our software platform will have broad applications in the future, especially for selecting many appropriate plant species to restore degraded ecosystems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

CW conceived the ideas and designed the software platform, analyzed the data, and wrote the manuscript. NL collected the data. All authors contributed critically to the drafts and have final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.570454/full#supplementary-material

REFERENCES

Bochet, E., and García-Fayos, P. (2015). Identifying plant traits: A key aspect for species selection in restoration of eroded roadsides in semiarid environments. *Ecol. Eng.* 83, 444–451. doi: 10.1016/j.ecoleng.2015.06.019

Brown, R. W., and Amacher, M. C. (1999). "Selecting plant species for ecological restoration: a perspective for land managers," in *Revegetation with native species: Proceedings, 1997 Society for Ecological Restoration annual meeting; 1997 November 12- 15; Ft Lauderdale, FL. Ogden (UT): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-8*, eds L. K. Holzworth, R. Brown, and compilers (Washington, D.C: USDA), 1–16.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., et al. (2012). Biodiversity loss and its impact on humanity. *Nature* 486, 59–67. doi: 10.1038/nature11148

Chapin, F. S., Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M., Reynolds, H. L., et al. (2000). Consequences of changing biodiversity. *Nature* 405, 234–242.

Fry, E. L., Power, S. A., and Manning, P. (2013). Trait-based classification and manipulation of plant functional groups for biodiversity–ecosystem function experiments. *J. Veg. Sci.* 25, 248–261. doi: 10.1111/jvs.12068

Guimarães, Z. T. M., dos Santos, V. A. H. F., Nogueira, W. L. P., Martins, N. O. de A., and Ferreira, M. J. (2018). Leaf traits explaining the growth of tree species planted in a Central Amazonian disturbed area. *For. Ecol. Manage.* 430, 618–628. doi: 10.1016/j.foreco.2018.08.048

Laliberté, E., and Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91, 299–305.

Laliberté, E., and Shipley, B. (2010). *FD: Measuring Functional Diversity From Multiple Traits, and Other Tools for Functional Ecology*. Vienna: R Foundation for Statistical Computing.

Laughlin, D. C. (2014). Applying trait-based models to achieve functional targets for theory-driven ecological restoration. *Ecol. Lett.* 17, 771–784. doi: 10.1111/ele.12288

Laughlin, D. C., and Laughlin, D. E. (2013). Advances in modelling trait-based plant community assembly. *Trends Plant Sci.* 18, 584–593. doi: 10.1016/j.tplants.2013.04.012

Legendre, L. P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91, 299–305. doi: 10.1890/08-2244.1

Li, J., Liu, N., Ren, H., Shen, W. J., and Jian, S. G. (2016). Ecological adaptability of seven plant species to tropical coral island habitat. *Ecol. Environ. Sci.* 25, 790–794.

Luo, Q., Liu, H., Wu, G. L., He, P. C., Hua, L., Zhu, L. W., et al. (2018). Using functional traits to evaluate the adaptability of five plant species on tropical coral islands. *Acta Ecol. Sin.* 38, 1256–1263.

Ostertag, R., Warman, L., Cordell, S., and Vitousek, P. M. (2015). Using plant functional traits to restore Hawaiian rainforest. *J. Appl. Ecol.* 52, 805–809. doi: 10.1111/1365-2664.12413

Padilla, F. M., Ortega, R., Sánchez, J., and Pugnaire, F. I. (2009). Rethinking species selection for restoration of arid shrublands. *Basic Appl. Ecol.* 10, 640–647. doi: 10.1016/j.baae.2009.03.003

Rayome, D., Dimanno, N., Ostertag, R., Cordell, S., Fung, B., Vizzone, A., et al. (2019). *Restoring Ecosystem Services Tool (Rest): A Program For Selecting Species For Restoration Projects Using A Functional-Trait Approach.* Gen. Tech. Rep. PSW-GTR-262. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, 47.

Rosenthal, G. (2003). Selecting target species to evaluate the success of wet grassland restoration. *Agric. Ecosyst. Environ.* 98, 227–246. doi: 10.1016/s0167-8809(03)00083-5

Shipley, B., Vile, D., and Garnier, É (2006). From plant traits to plant communities: a statistical mechanistic approach to biodiversity. *Science* 314, 812–814. doi: 10.1126/science.1131344

Wang, C., Zhang, H., Liu, H., Jian, S., Yan, J., and Liu, N. (2020). Application of a trait-based species screening framework for vegetation restoration in a tropical coral island of China. *Funct. Ecol.* 6, 1193–1204. doi: 10.1111/1365-2435.13553

Werden, L. K., Alvarado, J. P., Zarges, S., Calderón, M. E., Schilling, E M., Gutiérrez, L. M., et al. (2018). Using soil amendments and plant functional traits to select native tropical dry forest species for the restoration of degraded vertisols. *J. Appl. Ecol.* 55, 1019–1028. doi: 10.1111/1365-2664.12998

Wolfe, B. T., Dent, D. H., Deago, J., and Wishnie, M. H. (2015). Forest regeneration under *Tectona grandis* and *Terminalia Amazonia* plantation stands managed for biodiversity conservation in western panama. *New Forests* 46, 157–165. doi: 10.1007/s11056-014-9448-2

Zhang, H., Chen, H., Lian, J. Y., Robert, J., Li, R. H., Liu, H., et al. (2018). Using functional trait diversity patterns to disentangle the scale-dependent ecological processes in a subtropical forest. *Funct. Ecol.* 32, 1379–1389. doi: 10.1111/1365-2435.13079

Zhang, W., Zhang, H., Jian, S., and Liu, N. (2019). Tree plantations influence the abundance of ammonia-oxidizing bacteria in the soils of a coral island. *Appl. Soil Ecol.* 138, 220–222. doi: 10.1016/j.apsoil.2019.02.014

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An Indirect Impact of Sika Deer Overpopulation on Eutrophication of an Aquatic Ecosystem via Understory Vegetation: An Individual-Based Approach Using Nitrate Reductase Activity

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Eutrophication of aquatic ecosystems is a serious global issue. Stream nitrate concentrations at the University of Tokyo Chichibu Forest have increased since 2000 after the opening of the new highway in 1998. Nitrogen oxide emissions from automobile exhausts were the most likely source of increased nitrate input in the forest ecosystem. Around the area, the sika deer *Cervus nippon* Temminck population has greatly increased since around 2000 and intensively browsed the understory vegetation. We hypothesized that the degradation of the understory vegetation caused by the deer overpopulation was one of the causes of increased nitrate output. Monthly observations were carried out from April to October 2013 to investigate the understory vegetation at the heights of 0–30 and 100–150 cm above the ground inside (without deer) and outside (with deer) of a deer exclusion fence. Plant taxa and % coverage of each taxon at each layer were recorded. The *in vivo* nitrate reductase activity (NRA) (≈ nitrate assimilation rate) was determined for each plant taxa each month. Compared to inside the fence, the understory vegetation outside was poor with smaller % coverage and less diverse community structure, and was occupied by unpalatable plant taxa that were uncommon or absent inside the fence. Contrary to our expectation, the phylogenetic diversity of the community assemblage outside the fence showed greater evenness (less clustering) than inside. The NRA peaked in early in the season or late in the season. In contrast to a previous report, no significant difference in the NRA was found between woody and herbaceous plants. Although the difference was no more than that of vegetation coverage, the estimated community-level NRA inside the fence was 5.6 times higher than that of the outside. The difference was greatest early in the season. These results support our hypothesis.

Keywords: *Cervus nippon*, NRA, nitrate, vegetation degradation, phylogenetic diversity, phylogenetic signal

INTRODUCTION

Eutrophication of aquatic ecosystems is a serious global issue (Chislock et al., 2013). Nitrogen overload that results in the alleviation of nitrogen limitations to biological functions and increase in nitrate mobility in soils is called nitrogen saturation, which has been reported in various temperate forests, especially in Europe and North America (Aber et al., 1998). Because the nitrogen budget of a forest ecosystem is determined by the balance between nitrogen input and output (Vitousek and Howarth, 1991), it is necessary to evaluate possible causes of input–output nitrogen saturation. Nitrogen deposition has increased approximately five times over the past 100 years (Galloway and Cowling, 2002). Approximately 80% of the deposited anthropogenic nitrogen originates from the industrial production of nitrogen-based fertilizers, whereas the other 20% relates to emissions from fossil fuel (Gruber and Galloway, 2008). However, almost all the nitrogen deposition in forest ecosystems is attributed to the latter. Nitrogen absorption by plants determines the output. Some studies have reported that understory vegetation in old-growth stands played a more important role in preventing nitrate leaching than canopy trees (Goodale et al., 2000; Furusawa et al., 2005). Therefore, the degradation of the understory vegetation by increased herbivore densities may influence leaching from soil and thereby affect the nitrate output. In Alaska, moose density has had a substantial effect on nutrient cycling, ostensibly through browsing and inputs from fecal and urinary nitrogen (Molvar et al., 1993).

Overpopulation by the Sika deer *Cervus nippon* Temminck has caused various problems in forest and agricultural ecosystems. The degradation of the understory vegetation by deer grazing and the mortality of adult trees by debarking are major direct effects of the deer (Takatsuki, 2009). In some places in Japan, forest ecosystems have changed to grassland ecosystems by regime shifts caused by deer overpopulations (Shibata and Hino, 2009).

In the University of Tokyo Chichibu Forest (UTCF), the influence of deer overpopulation on the forest ecosystem has become obvious since around 2000 (Kamata et al., 2020) with an increase in dead tree skeletons, the degradation of understory vegetation, and an increase in plants unpalatable to deer (Sakio et al., 2013). A population of an endangered species, *Cypripedium japonicum* Thunberg, grows in the understory of a *Zelkova serrata* (Thunberg) Makino plantation that was planted 1934. In 2006, UTCF constructed an exclosure fence (5 m × 20 m) to protect the endangered population from deer grazing. The great difference in the understory vegetation between the inside and outside of the fence is evidence of the substantial influence of deer grazing on the understory vegetation (**Supplementary Figure S1**).

On the other hand, the construction of a new highway started inside the territory of the UTCF from the middle of the 1980s. To monitor the impact of the road construction and gallets from the tunnels, UTCF began monitoring the water quality of the two branch rivers of the Arakawa River System in 1989 (The University of Tokyo Forests, 1998). One of these branches is actually the main stream of the Arakawa River. According to monitoring reports (The University of Tokyo Forests, 1998; Gomyo et al., 2011), nitrate (NO_3^--N) concentrations in the stream water have increased since 2000. The emission of NO_X from automobile exhausts was the most likely cause of the increase in nitrate input in the forest ecosystem. Although the influence of sika deer overpopulation became obvious around the same time (Kamata et al., 2020), no studies have been conducted to determine indirect impact of the overpopulation on nitrate leaching (output) via the degradation of understory vegetation.

Plants absorb inorganic nitrogen, such as ammonium (NH_4^+-N) and NO_3^-–N, in the soil. All vascular plants can utilize NH_4^+–N; however, not all plants can utilize NO_3^-–N. Organic nitrogen supplied to the soil will be mineralized to NH_4^+ and then nitrified to NO_3^- by bacteria. Soil particles are negatively charged in most parts of Japan because the soil is volcanic and acidic. Ammonium nitrogen is a positively charged cation that is usually attached to the negatively charged soil particles, whereas NO_3^--N is a negatively charged anion that is not attached to soil particles, so that NO_3^--N has higher mobility in the soil. For these reasons, the free NO_3^--N easily leaches from the soil (the forest ecosystem) to stream water (aquatic ecosystem). If a large amount of NO_3^- flows into streams, it will lead to eutrophication of the aquatic ecosystem. The utilization of NO_3^- varies greatly among plant taxa (Al Gharbi and Hipkin, 1984; Gebauer et al., 1998). It is known that the NO_3^- reductase activity (NRA), which is an index of the ability of NO_3^- utilization by plants, is generally higher in herbaceous plants than woody plants (Smirnoff et al., 1984). We hypothesized that the degradation of the understory vegetation, especially of herbaceous plants, by the deer overpopulation was one of the causes of the increased NO_3^- concentration in the stream water in UTCF.

In this study, we surveyed seasonal changes in the understory vegetation inside and outside the deer exclusion fence. The percentage of coverage and NRA of each plant taxon was determined. Taxonomic diversity and phylogenetic diversity were estimated from the vegetation data to evaluate the influence of deer on the understory plant community assemblage. A community-level NRA was estimated inside and outside of the fence by using the percent coverage and NRA values. The indirect influence of deer on the increase of stream water NO_3^- was discussed, as well as the direct influence of deer on the diversity of the understory vegetation.

MATERIALS AND METHODS

Research Site

The survey was conducted in an 80-year-old *Z. serrata* plantation (planted 1934) at the UTCF located about 1,030 m a.s.l. (35°56′35″N, 138°49′00″E). An endangered species, *C. japonicum*, grows in the understory, which is registered to the Red Data of Saitama Prefecture. Due to the rapid increase of Sika deer (Kamata et al., 2020), a 5 m × 20 m exclosure fence was installed in 2006 to protect *C. japonicum* from deer grazing. We expected that the effect of deer could be estimated by comparing the vegetation inside and outside of the fence.

Vegetation Survey and Samples for the *in vivo* Assay

The monthly vegetation surveys and plant material sampling for the *in vivo* NRA assays were conducted on April 11, May 7, June 4, July 8, August 8, September 9, and October 15, 2013.

Twelve 1 m × 1 m quadrats (six for inside the fence and six for outside) were set before seed germination or budburst. The distance between quadrats was approximately 4 m. The quadrats inside and outside the fence were approximately 1 and 5 m apart from the fence, respectively. The percentage of vegetation coverage and proportion of each plant taxa were recorded for the layer of 0–30 cm above the ground level (a.g.l.) and the layer 100–150 cm a.g.l.

Approximately 3 g (fresh weight) of leaf samples of each taxa were collected on each sampling date from plants outside of the 12 quadrats and placed in a cooler box with ice packs until the laboratory analysis. Therefore, the plant taxa used for the assay did not completely overlap with those found in the vegetation survey (**Supplementary Table S1**). The samples were collected between 13:00 and 15:00 to avoid the effect of diurnal changes in the NRA.

In vivo NRA Assay

The *in vivo* NRA assay was performed based on a modified version of the Jaworski method (Jaworski, 1971). We followed Koyama et al. (2019) as follows:

Reagents

Incubation buffer: 0.1 mol L^{-1} KNO_3, 0.1 mol L—^{1} KH_2PO_4, and 1.5% 1-propanol; the pH was adjusted to ca. 7.5 using a NaOH solution.

Diazotizing agent: 0.5 g of sulfanilamide was dissolved in 100 ml of 2.4N HCl.

Coupling agent: 0.3 g of N-1-naphthylethylenediamine dihydrochloride was dissolved in 100 ml of 0.12 N HCl.

NO_2-standard solution: 0.6900 g of $NaNO_2$ was dissolved in an appropriate amount of ion-exchanged water and then filled up to 100 ml. It was diluted with ion-exchanged water to 2, 10, 20, 30, 40, and 50 μM.

In vivo Assay

(i) Approximately 100 mg (fresh weight) of leaf laminae were cut into small fragments (approximately 2.5 mm × 2.5 mm segments of leaves) and transferred to test tubes.

(ii) The incubation buffer (5 ml) was added to the leaves, and the tube contents were vacuum-infiltrated. The samples were incubated for 1 h in darkness and then placed in hot water (>80°C) to terminate enzyme activity.

(iii) Absorbance (545 nm) was measured following diazotization and coupling as the end-point. The confounding effects of plant pigments were corrected by subtracting the absorbance of the controls, to which N-naphthyl ethylene diamine dihydrochloride was not added (Gebauer et al., 1998).

(iv) The six levels of the NO_2-standard solution were subjected to step (iii) to obtain a calibration curve.

NRA per Mass and per Leaf Area

A fraction of each leaf sample was oven-dried at 70°C for 1 week and then weighed. The concentration of NO_2^--N in the incubation buffer was calculated from the curve. The NRA per mass was obtained for each sample.

Using three fresh leaves for each taxon, which were not used for the NRA assay, the leaf area was determined using the free software ImageJ ver. 1.50i (Schneider et al., 2012) after digitizing each leaf. The leaves were oven-dried at 70°C for 1 week and then weighed. The leaf mass per area was calculated for each leaf. Finally, the NRA per area was also determined for each taxon.

Analyses

Phylogenetic Tree

The pairwise phylogenetic distance among plant taxa used in this study was calculated from trees stored by Zanne et al. (2014). Plant taxa that were not included in the database were replaced with other taxa in the same genus. *Sasa* was used as a substitute for *Sasamorpha borealis* because no taxa belonging to the genus *Sasamorpha* were registered.

Plant Community

Seasonal changes in the vegetation index metrics were compared between the inside and outside of the fence. The vegetation coverage was compared between the lower (0–30 cm a.g.l.) and higher (100–150 cm a.g.l.) layers of the understory separately. Species richness (R: the number of taxa) and the Shannon index (H') (Shannon, 1948) were calculated by combining the two layers. The Shannon index was calculated using the following equation:

$$\mathrm{H}' = -\sum_{i=1}^{R} p_i \ln p_i,$$

where p_i is the sum of the proportion of vegetation coverage of the ith taxon in the lower and higher layers (200% at maximum). On the other hand, the species richness was not the sum of the number of species found in each of the two layers, but the total number of species in the two layers. Namely, one species was counted as a single species even if the species was found in both layers. The Bray-Curtis dissimilarity index was also calculated to compare the similarity of the community assemblage between the outside and inside of the fence and among months.

The effects of the factors FENCE (inside/outside) and MONTH (April to October) on the plant community were tested using a permutational analysis of variance (PERMANOVA) following a test of homogeneity of dispersions by a permutational multivariate analysis of dispersion (PERMDISP). To visualize the effects of FENCE and MONTH in relation to each taxon, plant taxa were ordinated using non-metric multidimensional scaling (NMDS) with a Bray-Curtis dissimilarity index as the distance between the assemblages. The sum of the vegetation coverage of the two layers was used for these analyses.

Three phylogenetic diversity metrics, namely phylogenetic diversity (PD), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD), were estimated. The PD evaluates the total length of phylogenetic trees, MPD evaluates the average of the evolutionary distance between all pairs of species in the assemblage, and MNTD evaluates the average of the evolutionary distance between each species and its nearest relative (Barber et al., 2017). To remove the effect of species richness (the number of taxa in each assemblage) and evaluate the phylogenetic evenness or clustering of the species in the assemblages (Kellar et al., 2015), the standardized effect sizes (SES) of the three metrics were also estimated. The SES metrics were calculated by comparing the observed value and a randomly generated dataset (Kellar et al., 2015). There were 1,000 randomizations in each standardized effect size analysis.

A generalized linear mixed model (GLMM) was employed to test the effect of the factor FENCE on these metrics with the factor MONTH as a random effect. A gamma distribution was used as an error structure with a log link function except for species richness, where a Poisson distribution was used as an error structure with a log link function. A linear mixed model (LMM) was used for the SES values. Regarding vegetation coverage, a beta regression with a log link function was employed after transforming the percentage of coverage (0–100) to the proportion (0–1) by dividing by 100. In the beta regression, both the factors FENCE and MONTH were used as explanatory variables due to a limitation of the library.

NRA at an Individual Plant Level

To test the difference in the NRA between woody and herbaceous plants, a GLMM was employed, with the factor WH (woody/herbaceous) set as a fixed effect, and MONTH and SPECIES as random effects. Next, to test differences in the NRA between plant taxa that were found only inside the fence (I) and those outside (O), the fixed effect of the previous model was replaced with I/O.

To test the effects of season on the NRA, a GLMM with the same error structure was employed, with MONTH as a fixed effect and SPECIES as a random effect. A *post-hoc* Tukey HSD multiple comparison was employed for pairwise comparisons among months. The coefficients of each month and each plant taxon were used to estimate a community-level NRA in section "NRA at a Community Assemblage Level" (**Supplementary Table S2**).

To test for phylogenetic signals in the NRA, a K statistic (Blomberg et al., 2003) and p-value based on randomization were calculated to investigate the relationship between the NRA and phylogeny for each plant taxon. The K statistic was calculated from the phylogeny of taxa and NRA of each taxon, which is the variance of phylogenetically independent contrasts (PIC) (Felsenstein, 1985) divided by its expectation under a Brownian motion model. The coefficients of taxa as a random effect in the GLMM that were conducted to test the effects of MONTH on the NRA in the previous paragraph were used as the NRA values. A $K < 1$ indicates that closely related taxa have NRA levels that are less similar than expected under a Brownian motion model of evolution, while $K > 1$ indicates that closely related taxa have NRA levels that are more similar than expected. We then tested the p-value by comparing the variance of the PIC computed from the NRA coefficients on the topology of the phylogenetic tree to those computed by randomly assigning the NRA coefficients to the tips in the phylogenetic tree 999 times. If the variances in the PIC for the observed data were lower than those from the permutations, then significant conservatism was indicated (Blomberg et al., 2003). In order to help intuitively interpret the meaning of K, we examined a traitgram (Ackerly, 2009), in which the isolates were arranged along the NRA-coefficient axis and connected by the underlying phylogeny, thus reconstructing the evolution under a Brownian motion model. Traitgrams with more branches crossing indicate lower similarity between phylogenetic relatedness and traits.

NRA at a Community Assemblage Level

The index of net NRA (*NetNRA*) activity of each quadrat on each sampling date was calculated by summing the product of the NRA per leaf area (*NRAA*) and the coverage (*COV*) of each plant taxa on each sampling date as follows:

$$\text{NetNRA} = \sum_{i=1}^{R} NRAA_i \times COV_i,$$

where i is the ith plant taxon. However, we could not sample all taxa found in the quadrats because plant samples used for the NRA assays were taken from outside of the quadrats. Regarding taxa for which the *NRAA* value was not obtained by the *in vivo* assay, the value was estimated using coefficients of each month and each plant taxon obtained by the GLMM with MONTH as a fixed effect and SPECIES as a random effect in section "Plant Community" (**Supplementary Table S2**). Monthly *NRAA* estimates without a species effect were used for species for which the NRA was not analyzed at all.

Software and Library

All analyses were performed using the free software R (ver. 3.6.3) (R Development Core Team, 2019). A package "glmmADMB (Skaug et al., 2018)" was used for the GLMM, a package "betareg" was used for a beta regression (Cribari-Neto and Zeileis, 2010), a package "vegan (Oksanen et al., 2019)" was used for metrics of community diversity and dissimilarity, and packages "brranching (Chamberlain, 2020)," "ape (Paradis and Schliep, 2019)," and "picante (Kembel et al., 2010)" were used for phylogenetic analyses.

RESULTS

Fifty-four species were founds in this study (**Supplementary Table S1**). Among these, 48 species were recorded during the vegetation surveys and 51 were used for the *in vivo* NRA assay. All biennials and perennials were deciduous plants so that there were no evergreen plants in the site. The phylogenetic tree used in this study is shown in **Supplementary Figure S2**.

Seasonal Changes in Plant Community Assemblage Inside and Outside Fence

The mean coverage of each plant species inside and outside of the fence at 0–30 and 100–150 cm a.g.l. from May to October are shown in **Supplementary Table S3**. The number of taxa found

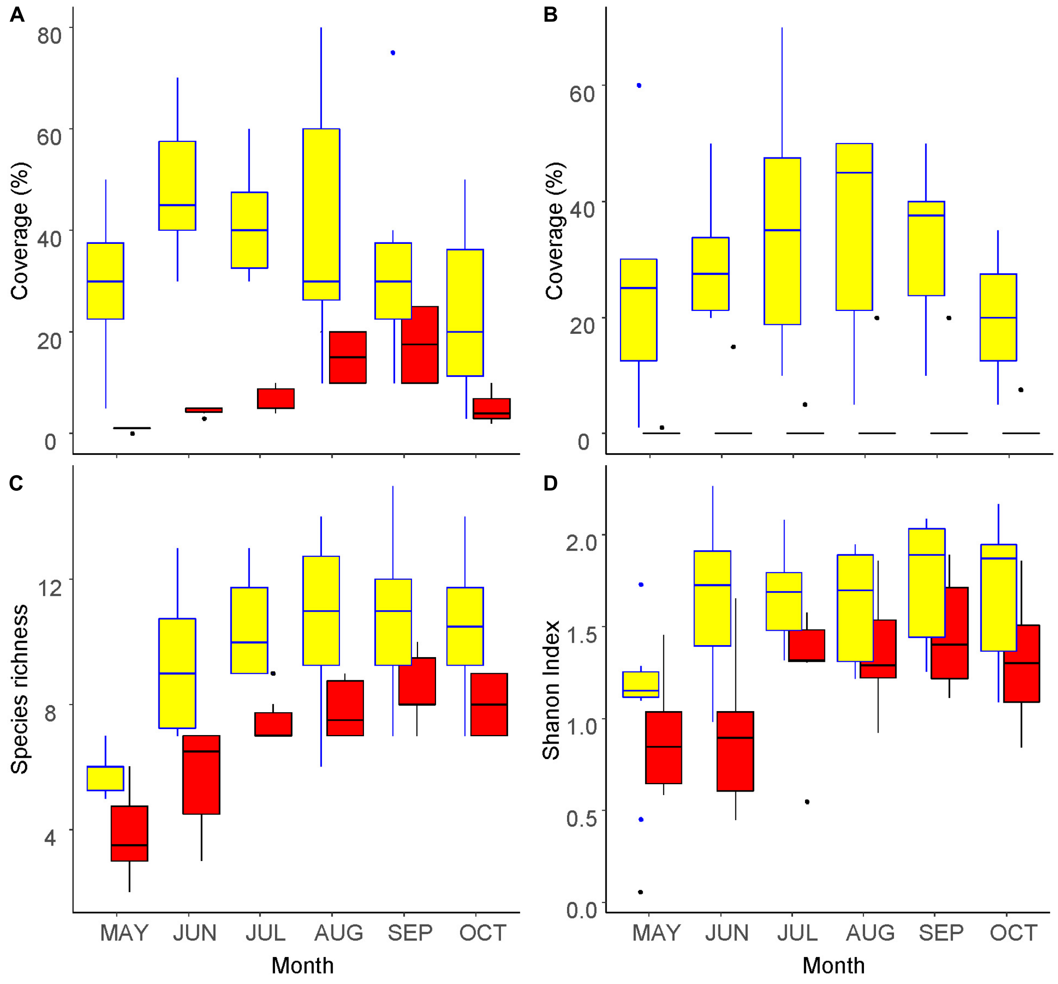

FIGURE 1 | Seasonal changes in vegetation inside (yellow) and outside (red) of the exclosure fence at the *Zelkova serrata* plantation in the University of Tokyo Chichibu Forest. **(A)** Percentage of vegetation coverage at 0–30 cm above ground level. **(B)** Percentage of vegetation coverage at 100–150 cm above ground level. **(C)** Number of species. **(D)** Shannon index.

inside and outside the fence were 38 and 31, respectively. Among these, 18 taxa appeared in both locations.

The vegetation coverage at 0–30 cm a.g.l. peaked in May and September inside and outside the fence, respectively (**Figure 1A**), whereas that at 100–150 cm a.g.l. peaked in August (**Figure 1B**). The vegetation coverage at 100–150 cm a.g.l. was almost 0% outside the fence and was without a clear peak. Species richness (the number of taxa) increased with season until August inside the fence and until September outside (**Figure 1C**). On the other hand, the Shannon index increased until May inside the fence and until Jun outside the fence, and did not change thereafter (**Figure 1D**). All four indicators were significantly greater inside the fence than outside, with significant differences (beta regression for vegetation coverage, GLMM for the number of species and Shannon index: $p < 0.05$). The seasonal peaks of all the metrics appeared earlier inside the fence than outside of it.

The pairwise Bray-Curtis dissimilarity indices indicated that dissimilarity was high between the inside and outside of the fence for the same month (**Table 1**). The pairwise dissimilarity among the months was lower inside the fence than outside. Dissimilarities between May and the other months were high both inside and outside of the fence. The plant community assemblage was significantly influenced by both FENCE ($p < 0.001$) and MONTH ($p < 0.05$) (PERMANOVA), although the dispersion of the assemblage did not differ among the groups by FENCE or MONTH (PERMDISP: $p > 0.05$). The NMDS results supported the Bray-Curtis dissimilarity indices (**Figure 2**). Namely, the 95% SE of the centroid did not overlap between the outside and inside of the fence. The 95% SE of the centroid among months other than May greatly overlapped each other, whereas overlaps between May and the other months were small.

Figure 3 shows seasonal changes in the three phylogenetic diversity metrics. Similar to the number of taxa shown in **Figure 1C**, the values of PD increased with the seasonal progression until August inside the fence and until September outside (**Figure 3A**), whereas those of MNTD changed in an opposite manner (**Figure 3E**). The change in MPD differed

TABLE 1 | Pairwise values of Bray-Curtis dissimilarity indices.

		Inside						Outside				
		MAY	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**
Inside	JUN	0.325										
	JUL	0.411	0.217									
	AUG	0.453	0.229	0.153								
	SEP	0.479	0.274	0.177	0.156							
	OCT	0.552	0.360	0.330	0.320	0.218						
Outside	MAY	0.980										
	JUN		0.903					0.816				
	JUL			0.879				0.832	0.305			
	AUG				0.763			0.919	0.464	0.482		
	SEP					0.770		0.927	0.518	0.523	0.174	
	OCT						0.862	0.807	0.384	0.284	0.515	0.546

The values between months were shown for each of inside and outside of the fence. The values between these of the same month were shown for the pairs between the inside and outside.

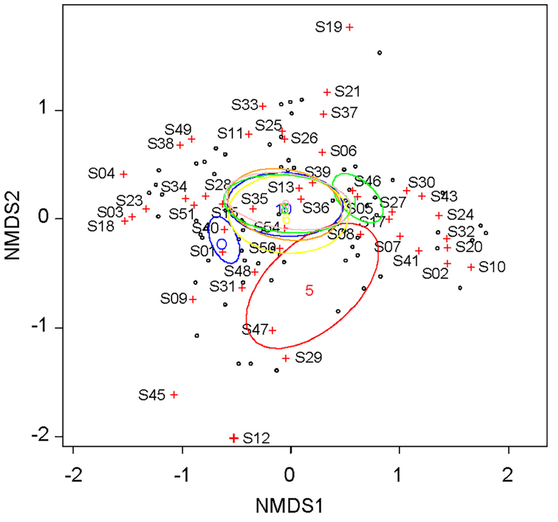

FIGURE 2 | Coordination of the plant community according to coverage by each plant taxon, which were observed monthly outside and inside the fence from May to October. Non-metric multidimensional scaling (NMDS) was used with a Bray-Curtis dissimilarity index as the distance between the assemblages. Ellipses for the 95% confidence interval of the centroid were shown for inside and outside of the fence and for each month from May to October. See **Supplementary Table S1** for taxa ID.

between the inside and outside of the fence, which showed a similar and an opposite trend to that of species richness, respectively (**Figure 3C**). Comparing the inside and outside of the fence, the PD values were greater inside, whereas those of MPD and MNTD were greater outside. The difference in each of the three metrics between the outside and inside of the fence was significant (GLM, $p < 0.001$). Regarding the SES values of the three metrics, the seasonal trends were less clear and the values were consistently smaller inside than outside of the fence (**Figures 3B,D,F**), indicating stronger phylogenetic clustering inside the fence, which was significantly different compared with outside the fence (LM, $p < 0.001$).

Nitrate Reductase Activity at the Species Level

The NRA per mass of leaves are shown in **Supplementary Table S4**.

The NRA tended to be higher early and late in the season but low in the middle (**Figure 4**). The NRA was significantly higher in April and May than in the other months and significantly lower in June and July than in the other months. However, at the species level, regardless of the timing (month) of the first appearance of each species, the values tended to decrease from the first sampling to the next sampling. The NRA at the first appearance was higher than both the second appearance ($n = 41$ taxa) and the next month ($n = 39$ taxa), and both differences were significant (GLMM, $p < 0.001$).

No significant differences in the NRA were found between woody and herbaceous plants (GLMM, $p > 0.05$), nor between taxa found only inside or outside the fence (GLMM, $p > 0.05$).

Figure 5 shows a traitgram of the NRA. The phylogenetic signal was smaller than the Brownian motion expectation because Blomberg's K was smaller than 1 ($K = 0.47$). The results of PIC also indicated no significant correlation between the NRA and plant phylogeny ($p > 0.05$).

NRA at the Community Assemblage Level

The community-level NRA peaked in May and September inside the fence (**Figure 6**). Only one peak was found outside the fence, such that the difference between the outside and the inside was greatest in May. The value inside the fence was 5.41 times higher than that outside, which was a significant difference (GLMM, $p < 0.001$).

DISCUSSION

There have been many reports on the influence of deer overpopulation on vegetation in Japan (e.g., Takatsuki, 2009). In

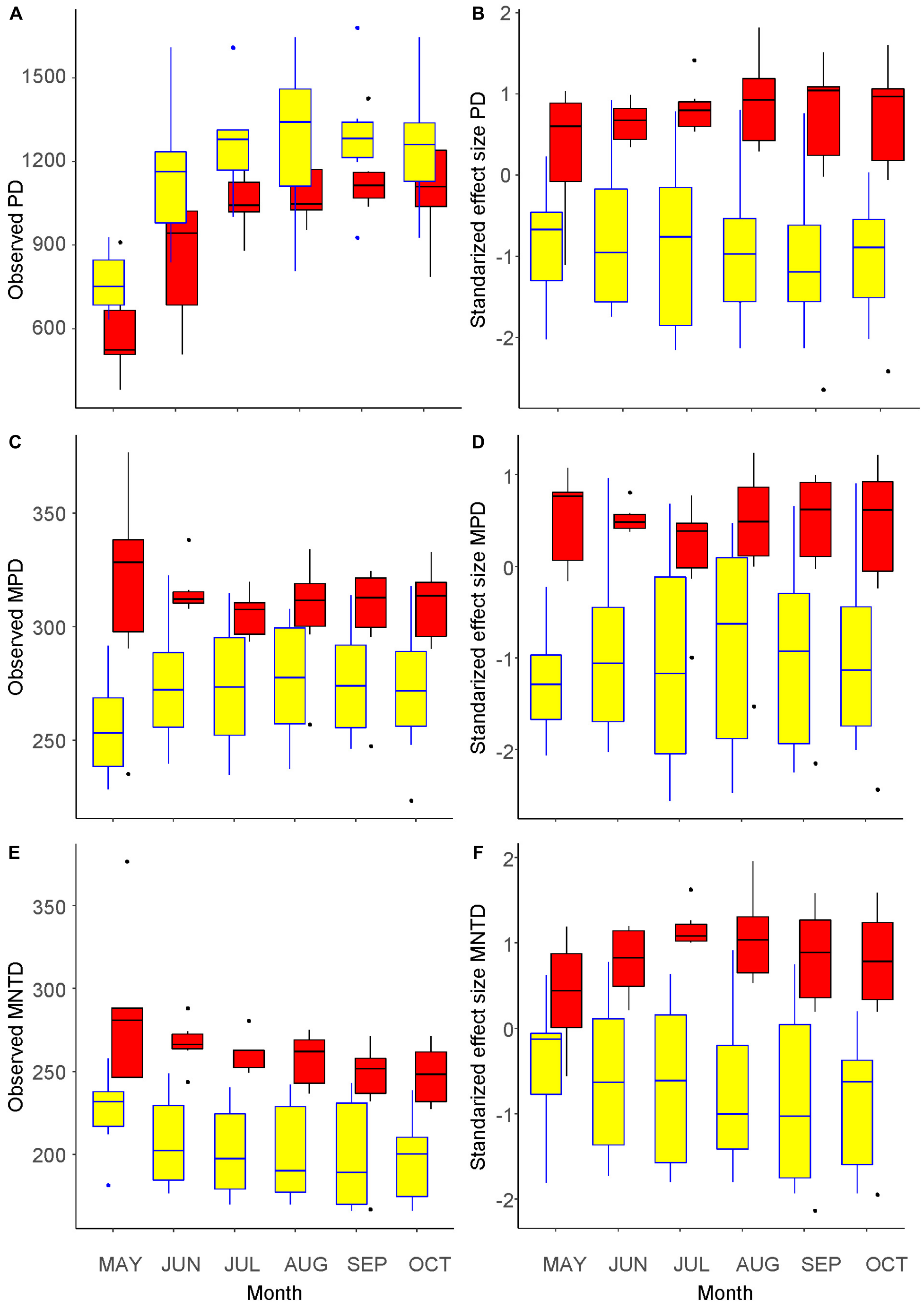

FIGURE 3 | Metrics of phylogenetic diversity of the understory vegetation inside (yellow) and outside (red) of an exclosure fence at a *Zelkova serrata* plantation in the University of Tokyo Chichibu Forest. **(A)** Phylogenetic diversity (PD). **(B)** Standardized effect sizes of phylogenetic diversity (SES.PD). **(C)** Mean pairwise distance (MPD). **(D)** Standardized effect sizes of mean pairwise distance (SES.MPD). **(E)** Mean nearest taxon distance (MNTD). **(F)** Standardized effect sizes of mean nearest taxon distance (SES.MNTD).

this study, the degradation of plant communities observed as reductions in plant coverage, species richness, and the Shannon index outside of the fence were likely caused by deer grazing. Selective grazing by deer was the likely cause of the decrease in the understory vegetation biomass and disappearance of some taxa from outside the fence. It is difficult for plants palatable to deer to grow outside the fence. Some taxa that were found only outside of the fence were probably not good at competition. Under strong disturbance (i.e., grazing pressure), inter-specific competition was probably weak and allowed the existence of

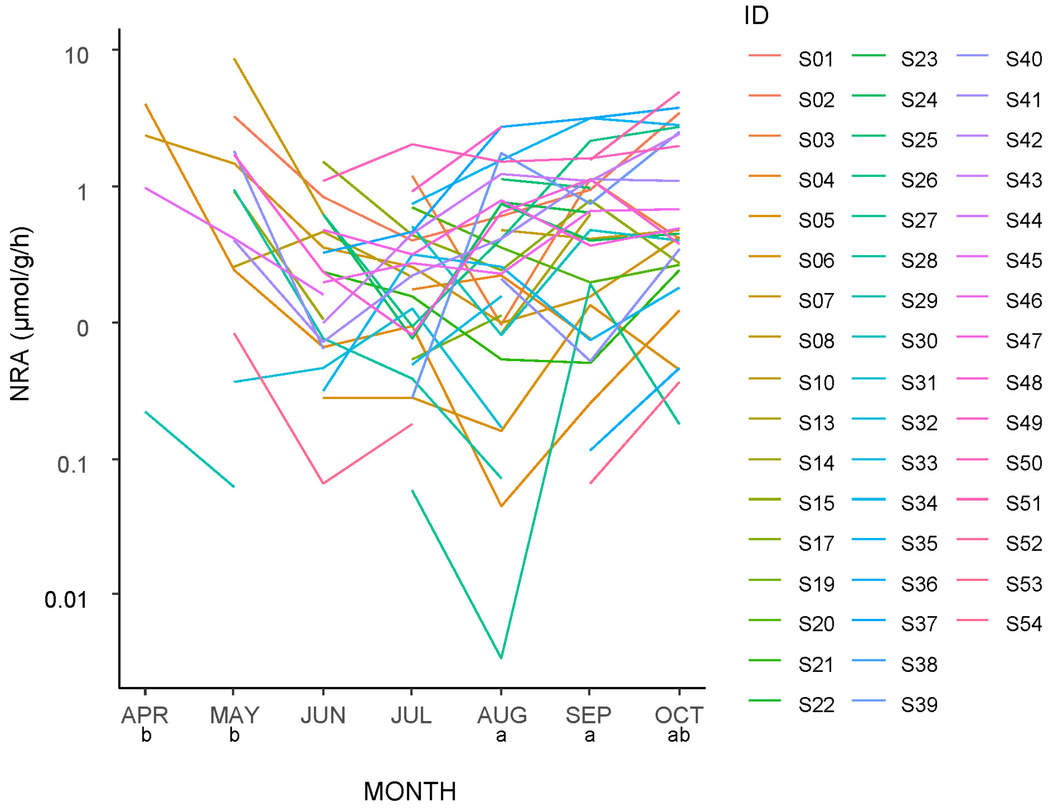

FIGURE 4 | Seasonal changes in the nitrate reductase activity (NRA) of leaves of each plant taxa in a logarithmic scale. The same letters below the month labels indicate no significant difference in the NRA among the months by a Tukey HSD multiple comparison following a generalized linear mixed model with SPECIES as a random effect (Gamma error structure and a log link function). See **Supplementary Table S1** for taxa ID.

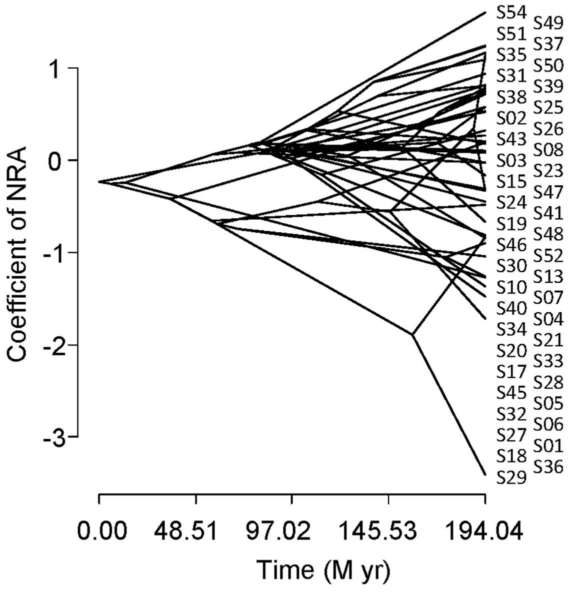

FIGURE 5 | Phylogenetic traitgram indicating the nitrate reductase activity of leaves collected inside and outside of the exclosure fence from the understory of a *Zelkova serrata* plantation at the University of Tokyo Chichibu Forest.

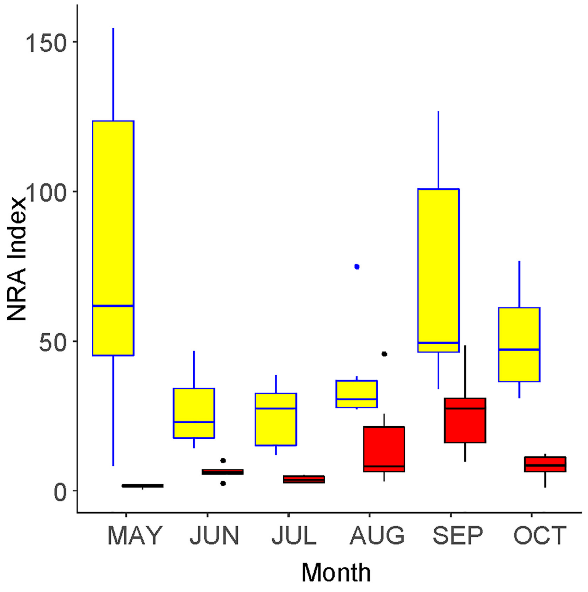

FIGURE 6 | Seasonal changes in the community-level nitrate reductase activity (NRA) inside (yellow) and outside (red) of an exclosure fence at a *Zelkova serrata* plantation in the University of Tokyo Chichibu Forest.

these species. We expected that deer grazing would cause phylogenetic clustering in the understory community assemblage if plant traits related to palatability to deer were phylogenetically conservative. Interestingly, contrary to our expectation, the community assemblages outside the fence were less clustered (higher evenness) than inside (**Figure 3**). These results suggest stronger phylogenetic conservativeness in plant traits related to interspecific competition than those related to palatability to deer.

The NRA tended to be high in spring and fall (**Figure 4**). A possible cause of the high NRA in spring was the high nitrogen requirement for the foliar nitrogen used for chlorophyll. This hypothesis is supported by the results that showed the NRA decreased from the first measurement to the next, irrespective of the timing (month) of the first appearance of the taxon, although the timing of the first appearance depended on the plant taxa. On the other hand, the high NRA in the fall was probably due to the high nitrogen demand for fruiting.

No significant phylogenetic signal was found in the NRA in this study (**Figure 5**, Blomberg's *K* statistic and PIC). It has been reported that plant taxa belonging to the family Ericaceae generally show low NRA (Routley, 1972; Al Gharbi and Hipkin, 1984; Smirnoff et al., 1984). On the other hand, great differences in the NRA were reported among two genera (*Salix* and *Populus*) in the family Salicaceae (Al Gharbi and Hipkin, 1984) and among congenic species, such as within the genera *Acer* (Koyama et al., 2019) and *Piper* (Fredeen et al., 1991). These facts are consistent with the results of our study.

It is generally believed that the NRA was greater in herbaceous plants than in woody plants (e.g., Smirnoff et al., 1984). In fact, herbaceous plants occupied the top eight of the highest NRA taxa in this study. However, no significant difference was found between herbaceous and woody plants. Of the 28 taxa with positive coefficient values of NRA (**Supplementary Table S2**), 14 taxa were woody plants. Koyama et al. (2019) reported that *Pterostyrax hispida* (S47) had an NRA as high as herbaceous plants. In this study, six woody plants showed higher NRAs than *P. hispida*. Another possible cause of the lack of difference between woody and herbaceous plants in our study was that we assayed shrub plants and saplings of canopy/subcanopy plants because we were studying the understory vegetation. The NRA of *Zelkova serrata* leaves differed greatly between young seedlings and canopy trees in May but were similar in June (**Supplementary Table S4**). It is likely that the NRA of canopy plants depends on plant age and/or size.

Al Gharbi and Hipkin (1984) reported that woody plants living in ruderal sites, such as *Sambucus nigra* (a species that is congenic with S02 in this study), *Acer pseudoplatanus* (a congenic species with S45), and *Populus alba* showed high NRAs that were similar to herbaceous plants. These high NRAs and fast growth rates were related to their high ability to invade after disturbance. In this study, the NRA was higher in plants that were only found outside the fence compared with those only found inside, although the difference was not significant. This seems reasonable because plants found only outside the fence were relatively early successional species under strong disturbance. The reduction of community-level NRA (**Figure 6**) was no more than that of the vegetation coverage (**Figure 1**), which was also influenced by the species-level NRA.

Gomyo et al. (2011) reported that the NO_3^- increase in stream water started around 2000. The increase in nitrogen deposition due to the opening of the new highway in 1998 was the most likely cause of nitrogen input in the area. However, unprocessed atmospheric nitrate stream flux represented a small percentage (<3%) of the atmospherically delivered wet nitrate flux to the studied region (Barnes et al., 2008). Most of the nitrogen deposition was accumulated in forest ecosystems; therefore, the nitrate output needs to be discussed. In contrast, damage to vegetation by deer became apparent in areas during similar times (Kamata et al., 2020). In another area in Japan, the nitrate concentration in stream water decreased when the catchment was fenced to exclude deer (Fukushima et al., 2013). Our study demonstrated that the degradation of the understory vegetation was a likely cause of the increase in nitrate output in forest soil and stream water in the UTCF (Gomyo et al., 2011). Our findings will help to understand nitrogen saturation in forest ecosystems with deer overpopulation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

A part of this work was a bachelor thesis of YT at Faculty of Agriculture, The University of Tokyo, FY2013. YY established the exclosure fence in 2006. YT, MF, and NK conceived the idea and analyzed the data for the bachelor thesis. YT, MF, YY, and NK conducted field surveys. YY identified plant taxa. YT and MF conducted *in vivo* assay. NK re-analyzed the data and wrote this manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.626905/full#supplementary-material

REFERENCES

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., et al. (1998). Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. *BioScience* 48, 921–934. doi: 10.2307/1313296

Ackerly, D. (2009). Conservatism and diversification of plant functional traits: evolutionary rates versus phylogenetic signal. *Proc. Natl. Acad. Sci.U.S.A.* 106(Suppl. 2), 19699–19706. doi: 10.1073/pnas.0901635106

Al Gharbi, A., and Hipkin, C. R. (1984). Studies on nitrate reductase in British angiosperms. *New Phytol.* 97, 629–639. doi: 10.1111/j.1469-8137.1984.tb03627.x

Barber, N. A., Jones, H. P., Duvall, M. R., Wysocki, W. P., Hansen, M. J., and Gibson, D. J. (2017). Phylogenetic diversity is maintained despite richness losses over time in restored tallgrass prairie plant communities. *J. Appl. Ecol.* 54, 137–144. doi: 10.1111/1365-2664.12639

Barnes, R. T., Raymond, P. A., and Casciotti, K. L. (2008). Dual isotope analyses indicate efficient processing of atmospheric nitrate by forested watersheds in the northeastern U.S. *Biogeochemistry* 90, 15–27. doi: 10.1007/s10533-008-9227-2

Blomberg, S. P., Garland, T., and Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57, 717–745. doi: 10.1554/0014-3820(2003)057[0717:tfpsic]2.0.co;2

Chamberlain, S. (2020). *Brranching: Fetch 'Phylogenies' from Many Sources.* R package version 0.6.0. Available online at: https://CRAN.R-project.org/package=brranching (accessed October 20, 2020).

Chislock, M. F., Doster, E., Zitomer, R. A., and Wilson, A. E. (2013). Eutrophication: causes, consequences, and controls in aquatic ecosystems. *Nat. Educ. Knowl.* 4:10.

Cribari-Neto, F., and Zeileis, A. (2010). Beta regression in R. *J. Stat. Softw.* 34, 1–24.

Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* 125, 1–15. doi: 10.1086/284325

Fredeen, A. L., Griffin, K., and Field, C. B. (1991). Effects of light quantity and quality and soil nitrogen status on nitrate reductase activity in rainforest species of the genus *Piper*. *Oecologia* 86, 441–446. doi: 10.1007/BF00317614

Fukushima, K., Sakaguchi, S., Inoue, M., Fujiki, D., Tokuchi, N., Nishioka, Y., et al. (2013). Deer overgrazing affects soil nitrogen dynamics and nitrate leaching in a cool-temperate forest ecosystem. *J. Jpn. Soc. Reveget. Technol.* 39, 360–367. doi: 10.7211/jjsrt.39.360

Furusawa, H., Hino, T., Kaneko, S., and Araki, M. (2005). Effects of dwarf bamboo (*Sasa nipponica*) and deer (*Cervus nippon* centralis) on the chemical properties of soil and microbial biomass in a forest at Ohdaigahara, central Japan. *Bull. FFPRI* 4, 157–165.

Galloway, J. N., and Cowling, E. B. (2002). Reactive nitrogen and the world: 200 years of change. *Ambio* 31, 64–71. doi: 10.1579/0044-7447-31.2.64

Gebauer, G., Hahn, G., Rodenkirchen, H., and Zuleger, M. (1998). Effects of acid irrigation and liming on nitrate reduction and nitrate content of *Picea abies* (L.) Karst. and *Oxalis acetosella* L. *Plant Soil* 199, 59–70. doi: 10.1023/a:1004263223917

Gomyo, M., Kuraji, K., Haruta, Y., Ohmura, K., Chishima, T., Saiki, M., et al. (2011). Impact of tunnel excavation muck dumped along the canyon on streamwater chemistry. *Bull. Univ. Tokyo For.* 125, 23–44.

Goodale, C. L., Aber, J. D., and McDowell, W. H. (2000). The long-term effects of disturbance on organic and inorganic nitrogen export in the White Mountains, New Hampshire. *Ecosystems* 3, 433–450. doi: 10.1007/s100210000039

Gruber, N., and Galloway, J. N. (2008). An earth-system perspective of the global nitrogen cycle. *Nature* 451, 293–296. doi: 10.1038/nature06592

Jaworski, E. G. (1971). Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.* 43, 1274–1279. doi: 10.1016/S0006-291X(71)80010-4

Kamata, N., Sanguansub, S., Beaver, R. A., Saito, T., and Hirao, T. (2020). Investigating the factors influencing trap capture of bark and ambrosia beetles using long-term trapping data in a cool temperate forest in central Japan. *J. For. Res.* 25, 163–173. doi: 10.1080/13416979.2020.1762288

Kellar, P. R., Ahrendsen, D. L., Aust, S. K., Jones, A. R., and Pires, J. C. (2015). Biodiversity comparison among phylogenetic diversity metrics and between three North American prairies. *Appl. Plant Sci.* 3:1400108. doi: 10.3732/apps.1400108

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., et al. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26, 1463–1464. doi: 10.1093/bioinformatics/btq166

Koyama, L. A., Terai, M., and Tokuchi, N. (2019). Nitrate reductase activities in plants from different ecological and taxonomic groups grown in Japan. *Ecol. Res.* 35, 708–712. doi: 10.1111/1440-1703.12083

Molvar, E. M., Bowyer, R. T., and Van Ballenberghe, V. (1993). Moose herbivory, browse quality, and nutrient cycling in an Alaskan treeline community. *Oecologia* 94, 472–479. doi: 10.1007/BF00566961

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019). *vegan: Community Ecology Package. R package version 2.5-5*. Available online at: https://CRAN.R-project.org/package=vegan (accessed October 20, 2020).

Paradis, E., and Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35, 526–528. doi: 10.1093/bioinformatics/bty633

R Development Core Team (2019). *R: a Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Routley, D. G. (1972). Nitrate reductase in leaves of Ericaceae. *Hort Sci.* 7, 84–87.

Sakio, H., Kubo, M., Kawanishi, M., and Higa, M. (2013). Effects of deer feeding on forest floor vegetation in the Chichibu Mountains, Japan. *J. Jpn. Soc. Reveget. Technol.* 39, 226–231. doi: 10.7211/jjsrt.39.226

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Method* 9, 671–675. doi: 10.1038/nmeth.2089

Shannon, C. E. (1948). A mathematical theory of communication. *Bell Syst. Tech. J.* 27, 379–423.

Shibata, E., and Hino, T. (eds.). (2009). *Ecology of Shika Deer and Forest Ecosystem of Mt. Ohdaigahara*. Hatano: Tokai University Press.

Skaug, H., Fournier, D., Nielsen, A., Magnusson, A., and Bolker, B. (2018). *Package 'glmmADMB' (ver. 0.8.3.4)*. Available online at: https://www.researchgate.net/profile/Arni_Magnusson/publication/265915218_glmmADMB_generalized_linear_mixed_models_using_AD_Model_Builder/ (accessed October 20, 2020).

Smirnoff, N., Todd, P., and Stewart, G. R. (1984). The occurrence of nitrate reduction in the leaves of woody plants. *Ann. Bot.* 54, 363–374. doi: 10.1093/oxfordjournals.aob.a086806

Takatsuki, S. (2009). Effects of sika deer on vegetation in Japan: a review. *Biol. Conserv.* 142, 1922–1929. doi: 10.1016/j.biocon.2009.02.011

The University of Tokyo Forests (1998). *Transaction of Natural Environment Assessment at the University of Tokyo Chichibu Forest: Environment of Chichibu Forest*. Tokyo: The University of Tokyo Forests, 388. (in Japanese).

Vitousek, P. M., and Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13, 87–115. doi: 10.1007/BF00002772

Zanne, A. E., Tank, D. C., Cornwell, W. K., Eastman, J. M., Smith, S. A., FitzJohn, R. G., et al. (2014). Three keys to the radiation of angiosperms into freezing environments. *Nature* 506, 89–92. doi: 10.1038/nature12872

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frontiers in Ecology and Evolution

ORIGINAL RESEARCH
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Light Intensity Influence on Growth and Photosynthetic Characteristics of *Horsfieldia hainanensis*

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Due to both anthropogenic and natural causes, the number of *Horsfieldia hainanensis* has been decreasing each year in the Tongza Branch nursery (109.534 525°E, 18.763 516°N) of the Hainan Academy of Forestry, China. Consequently, the protection of *H. hainanensis* is urgent, as is that of most rare tree species. To develop a more comprehensive understanding of the *H. hainanensis* growth environment, we took 3-year-old *H. hainanensis* saplings as the research object. We controlled the light intensity by setting different shade amounts to explore the growth and photosynthetic characteristics of *H. hainanensis* under different light intensities. We found that shade can promote growth and increase the contents of certain substances. Light transmittance of 44.41% can increase plant height (by 29.545%) and biomass (by 66.676%). Light transmittance of 16.19% can increase the pigment content; Chl increased by 40.864%, Chl a increased by 38.031%, and Chl b increased by 48.412%. Light transmittance of 7.30% can increase the soil plant analysis development (SPAD) value of each part of the leaf; the leaf base increased by 41.000%, the leaf margin increased by 32.574%, the blade tip increased by 49.003%, and the leaf average increased by 40.466%. The specific leaf area can reduce the specific leaf weight. We also found that compared to full light, reducing the light transmittance can increase the total chlorophyll (Chl), chlorophyll A (Chl a), and chlorophyll B (Chl b) contents, and the Chl-SPAD-leaf base, leaf edge, leaf tip, average content, and light-saturated net photosynthetic rate. This can in turn reduce the apparent quantum efficiency (AQY), light compensation point (LCP), and dark respiration rate (Rd). In addition, we found a strong correlation between seven of the photosynthetic pigment indicators (Chl, Chla, Chl b, Chl-SPAD-leaf base, leaf margin, leaf tip, and mean) and the three photosynthesis physiological parameters (AQY, LCP, and Rd). The light transmittance of 44.41% (one layer of shading net) treatment group was conducive to the growth of *H. hainanensis* and photosynthetic characteristic improvement. Therefore, our light transmittance selection of approximately 44.4% is significant for the natural return of *H. hainanensis*.

Keywords: ***Horsfieldia hainanensis*, illumination intensity, growth, photosynthetic physiology, photosynthetic pigment**

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INTRODUCTION

Horsfieldia hainanensis belongs to the Myristicaceae family and Horsfieldia Willd genus. The species is commonly known as Hainan horsfieldia, false jade fruit, Hainan huoerfei, water loquat, and mieus. In China, they are primarily distributed in southern Yunnan, western Guangxi, and southwestern Hainan. Because they are one of the most iconic humid tropical rainforest plants, they exhibit high reference value for studying the flora, geographical distribution, and ecological characteristics of tropical rainforests (Editorial Committee of Chinese Flora of Chinese Academy of Sciences, 1977; Xu et al., 2012). Due to their small distribution area and number, the excessive destruction of rainforests, the anthropogenic destruction and hacking of the plant, and their extremely poor natural regeneration capacity, *Horsfieldia hainanensis* are on the verge of extinction of China. In 1999, they were listed as nationally second-level protected wild plants (Jiang et al., 2016, 2018) in China. In recent years, many research studies have focused on *H. hainanensis* breeding (Liu et al., 2019), fertilizer management (Wang, 2017), community composition (Zhong et al., 2018), chemical composition (Liu et al., 2010), stress response (Huang et al., 2019), and genes (Jiang, 2018; Yang et al., 2019). However, research is lacking on the effect of shading on the physiology and photosynthesis of *H. hainanensis* that provides a theoretical basis for the selection of an appropriate light intensity for seedling breeding and natural return planting of *H. hainanensis*.

Shading is a common approach for exploring how weak light stress influences seedling photosynthesis. Scientific research and plant production in recent years has illustrated that the chloroplast structure, enzymatic activity, osmotic adjustment, and endogenous hormones are key elements for adapting to low-light conditions. Many experiments have demonstrated that plant vegetative growth under low-light treatments varies according to the variety and can cause morphological reconstruction (El-Gizawy et al., 1993; Ayuko et al., 2008; Kruse et al., 2008). Bepetel and Lakso (1998) found that under less than 40% low-light conditions, the accumulation of assimilate decreases due to photosynthetic inhibition, and the products distributed to the leaves increase, causing the proportion of distribution to the fruit to decrease, which leads to a 25% drop in fruit growth. Ishida (1989) found that eggplants exhibited slow reproductive growth, insufficient dry matter accumulation, and delayed flower bud differentiation under low-light conditions. Therefore, weak light is a key factor in plant photochemical efficiency and exerts various influences on leaf chlorophyll contents. These significant differences in leaf characteristics and photochemical efficiency can be viewed as adaptations to weak light.

Photosynthesis is the basic plant physiological and biochemical process and one of the main chemical reactions that provide plants with energy. It also determines plant growth and dry matter accumulation. The plant net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) change according to changes in environmental factors such as light, moisture, and temperature (Krause and Weis, 1991; Lore et al., 2020; Mansoure et al., 2020). The photosynthesis–light intensity response curve has long been used to evaluate the photosynthetic rates of plants and their related photosynthetic characteristics (Platt et al., 1980; Harrison and Platt, 1986), illustrating their potential photosynthetic capacity under a certain light intensity range (Falkowski and Raven, 1997) and growth and development status. It can be used as a critical indicator for breeding and identifying excellent varieties with high light efficiency and strong light resistance. However, studies on the response of plant photosynthesis to weak light conditions have predominately focused on crop plants (Li et al., 2020) and non-endangered species (Leite et al., 2019). Little is known about the responses of endangered species to weak light conditions, and the field should place greater emphasis on the scientific questions underlying the physiology of rare species such as *H. hainanensis*. Therefore, endangered plants should be studied under different light conditions, which can help us to protect natural resources.

In summary, to analyze the influence of different light intensities on *H. hainanensis*, this study controls the light intensity to compare the effects of various intensities on the growth indicators, photosynthesis, and photosynthetic pigments of its 3-year-old seedlings to reveal the response mechanism to different light intensities of *H. hainanensis*. The response mechanism to different light intensities can provide a theoretical basis for the selection of canopy closure in a nursery and for natural return planting of *H. hainanensis*.

MATERIALS AND METHODS

Plant Materials and Experimental Location

Wind blew the 3-year-old *H. hainanensis* phoebe seedlings, and the seeds were collected from the same tree. The experiment was conducted in the Tongza Branch nursery (109.534 525°E, 18.763 516°N) of the Hainan Academy of Forestry, China. The average altitude of the area is 300 m, and the annual average temperature is 22.4°C. The average temperature in January is 17.0°C, the average temperature in July is 26.0°C, and the maximum temperature is 35.9°C. The annual average rainfall is 1,690 mm, the maximum annual rainfall is 2,810.4 mm, the minimum annual rainfall is 1,055.5 mm, and the annual average relative humidity is 84%. Potting was conducted in November 2019. All test seedlings grew vigorously and consistently and then were moved to the specified location. The average plant height of the seedlings was 47.6 cm. Only seedlings with no diseases or insect pests were selected. Loess soil was used for bagging. The dimensions of each soil bag were 30 cm (w) × 30 cm (d) × 25 cm (h). Each bag weighed 10 ± 0.5 kg per seedling. The soil contents were as follows: organic matter, 52.89 g/kg; alkali-hydrolyzable nitrogen, 237.01 mg/kg; available phosphorous, 40.41 mg/kg; available potassium, 84.29 mg/kg; total nitrogen, 1.02 g/kg; total phosphorus, 0.05 g/kg; and total potassium, 18.14 g/kg. The soil pH was 7.18.

Experimental Design

Random sample plots were used in the study. Each plot was 1.8 m × 3.0 m, row, and plant spacing were both 60 cm, and the plant density was 27,780 plants/ha. Three treatment groups were set up using 1, 2, or 3 layers of shading net to control the light intensity, with full illumination (no shading net) as the control treatment group (CK). Each treatment was repeated three times for 5 plants each, with 60 plants in total. A digital illuminance meter (VICTOR 1010D, Shenzhen Yisheng Shengli Technology Co., Ltd., China) was used to measure the light intensity at 10:00, 12:00, and 14:00 (east eight districts) in fine weather conditions for 3 consecutive days, and then the average was determined. The specific experimental design and the light intensity used in each treatment are illustrated in **Table 1**.

Growth Index Determination

In July 2020, three plants were selected from each replicate for growth index determination. The plant height (H) and ground diameter (D) were measured using a tape measure and vernier caliper, respectively, and the H/D ratio was calculated. The biomass model D^2H was used to compare the biomass differences of the seedlings under different treatments (Hanson, 1975; Hase et al., 1985).

Determination of Leaf Morphology and Traits

On July 24, 2020, a hand-held leaf area meter (YMJ-B, Zhejiang Top Yunnong Technology Service Co., Ltd., China) was used to determine the leaf length (L), leaf width (W), leaf area (A), and aspect ratio (B). The leaf water content (C_{LWC}) was measured using the drying method (Cornelissen et al., 2003). One functional leaf was cut from each seedling, and three plants were used as replicates. First, we used an analytical balance to weigh the fresh weight (F_W) of the leaf, to three decimal places, and then placed the leaf in an oven at 100°C for 1 h. Then, we adjusted the temperature to 85°C until a constant weight was obtained. Finally, the dry weight (D_W) of the leaves was measured using an analytical balance, to four decimal places, and the following formulas were used to, respectively, calculate the water content (C_{LWC}), specific leaf area (S_{LAM}), and specific leaf weight (m_{LMA}): $C_{LWC} = 1 - D_W/F_W \times 100\%$, $S_{LAM} = A/D_W$, and $m_{LMA} = D_W/A$.

Photosynthetic Light Response Curve Determination

On a sunny day in September 2020, Photosynthetic light response curve determination was conducted from 8:30 to 11:30. Each selection of three functional leaves from the top of an *H. hainanensis* was fully tested, using the LI-6800 portable photosynthesis system (LI- COR, United States), using a light-emitting diode acted as the red and blue light source. We selected 90% red light and a 3 cm × 3 cm red and blue leaf chamber to measure the Pn, Tr, Gs, and Ci indicators and draw the corresponding light response curve. We set 12 intensity gradients of photosynthetic active radiation (PAR): 1,800, 1,500, 1,200, 1,000, 800, 600, 400, 200, 150, 100, 50, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The CO_2 concentration was set to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (provided by the CO_2 cylinder), the temperature was set to 27°C, the relative humidity was set to 65%, the gas flow rate was set to 500 $\mu mol \cdot s^{-1}$, and before measurement 1,800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ strong light was induced for at least 15 min until the data were stable. During measurement, the data were recorded after stabilizing for at least 2 min under each PAR. Then, using the leaf floating photosynthesis calculation method, the right-angle hyperbolic correction model (Ye and Zhao, 2010) was used to calculate the light-saturated net photosynthetic rate (Pnmax), AQY, LCP, light saturation point (LSP), Rd, and coefficient of determination (R^2).

$$P(I) = \alpha \frac{1-\beta I}{1+\gamma I} \text{I-R}_d \tag{1}$$

where α is the initial slope of the light response curve, β and γ are coefficients, I is PAR, and R_d is the dark respiration rate.

Determination of Photosynthetic Pigment Content

The photosynthetic pigment content and SPAD value were determined on July 22, 2020. The photosynthetic pigment content was determined using the 95% ethanol extraction method (Gu et al., 2017). Three plants were randomly selected from each treatment group, and one mature functional leaf was cut from each seedling. A total of 0.200 g of fresh leaves per sample were weighed using an analytical balance, cut, and immersed in 20.0 ml of 95% ethanol plastic tubes. The samples underwent dark treatment of 72 h until the leaves faded from their original green color, the absorbance (A) was measured at 470, 649, and 665 nm using an ultraviolet spectrophotometer (UV-2400, Shanghai Sunny Hengping Scientific Instruments Co., Ltd., China). The following formulas were used to calculate the

TABLE 1 | Different light test treatment table.

Group	Numbering	Shade net	Light intensity (100 lux)				Transmittance (%)
			10:00	12:00	14:00	Mean	
CK	11	No	852.67 ± 89.50	1, 121.00 ± 16.37	926.35 ± 35.63	996.67 ± 138.63	100.00
1	12	Layer	413.00 ± 36.66	439.00 ± 32.14	435.83 ± 45.81	429.28 ± 14.18	44.41
2	14	Two layers	137.00 ± 12.29	189.667 ± 5.15	142.73 ± 32.56	156.47 ± 28.90	16.19
3	13	Three layers	64.33 ± 2.52	78.33 ± 11.93	68.94 ± 3.79	70.53 ± 7.13	7.30

Chl, Chl a, Chl b, Chl a/b, and carotenoid (Caro) content.

$$\text{Chl mg/g} = \frac{13.95 \times A_{665} - 6.88 \times A_{649} + 24.96 \times A_{649} - 7.32 \times A_{665} \times V \times n}{S_W} \tag{2}$$

$$\text{Chl a mg/g} = \frac{13.95 \times A_{665} - 6.88 \times A_{649} \times V \times n}{S_W} \tag{3}$$

$$\text{Chl b mg/g} = \frac{24.96 \times A_{649} - 7.32 \times A_{665} \times V \times n}{S_W} \tag{4}$$

$$\text{Caro mg/g} = \frac{1000 \times A_{470} + 811.7385 \times A_{665} - 2851.304 \times A_{649} \times V \times n}{245 \times S_W} \tag{5}$$

where V is the extract volume (ml), *n* is the dilution factor, and S_W is the fresh weight of the leaf sample (g).

Three parts near the main leaf (leaf base), away from the petiole (leaf tip) and the middle part (leaf margin), were selected for analysis using the SPAD-502 chlorophyll content analyzer (SPAD-502 Plus, Konica Minoltia, Japan) to determine the Chl-SPAD value. Three plants were selected for each treatment group, and the measurement for each part was repeated times to calculate the average value of each part and then the average SPAD value of the entire leaf.

Data Analysis

The hyperbolic correction model of the Ye and Zhao (2010) software was first used to calculate the photosynthetic parameters (AQY, Pnmax, LSP, LCP, Rd, and R^2), and then to target the growth indicators (H, D, H/D, and D^2H), leaf morphology and traits (L, W, A, B, C_{LWC}, S_{LAM}, and m_{LMA}), photosynthetic parameters (AQY, Pnmax, LSP, LCP, Rd and R^2), and photosynthetic pigments (Chl, Chl A, Chl B, Chl A/B, Caro, and SPAD), We used IBM SPSS 26.0 for descriptive statistical analysis, followed by the Duncan test, with multiple comparisons at the 0.05 and 0.01 levels. IBM SPSS 26.0 was used to analyze the correlation between the photosynthetic physiological parameters and photosynthetic pigments. The results are expressed as mean ± *SD*. All statistical analysis and graphs were prepared using Excel 2016, IBM SPSS 26.0, and OriginPro 2021 software.

RESULTS

The various shade intensity treatment groups exhibited significant differences ($P < 0.05$) in their growth indicators (**Figure 1**). Maximum values of 76.00 cm, 14.47 mm, 53.75, and D^2H 154.37 kg were found in treatment group 1 for H, D, H/D, and D^2H, respectively. There are significant differences ($P < 0.01$) in H and D^2H between that of treatment group 1 and the CK group, while D and H/D are not significantly different between these two groups. The H in treatment group 2 is significantly lower ($P < 0.05$) than that in treatment group 1, while D^2H is significantly smaller in treatment group 2 than in treatment group 1. For treatment group 1, the H is 1.90 times that of treatment group 3, D is 1.54 times that of treatment group 3, H/D is 1.27 times that of treatment group 3, and D^2H is 4.06 times that of treatment group 3, with significance at the $P < 0.01$ level. This demonstrates that light shade is beneficial to the growth of the H, D, and H/D of *H. hainanensis* and in increasing its biomass.

It can be seen from **Figure 1** that the increase in D and H (29.55 cm and 5.32 mm, respectively), in treatment group 1 was the largest of the treatment groups. The increase in D was significantly ($P < 0.05$) greater than that in the other treatment groups, and it reached a greater significance level ($P < 0.01$) l when compared to that of the CK group and treatment group 3. The increase in D in treatment groups 2 and 3 was also significant. The increase in H in treatment group 1 was significantly ($P < 0.01$) larger than that in the other treatment groups, while there was no significant difference in H among the other treatment groups. These results indicate that light shade promotes H and D growth in *H. hainanensis* (**Figures 1E,F**).

The effects of different light intensities on the leaf morphology and characteristics of *H. hainanensis* are displayed in **Figure 2**. The values of S (31,296.48 mm^2), L (201.99 mm), W (100.62 mm), C_{LWC} (79.01%), and S_{LAM} (238.55 cm^2/g) in the CK group are the smallest, and they are significantly smaller ($P < 0.01$) than those in treatment group 2. S and L are both significantly smaller in the CK group ($P < 0.01$) than in treatment groups 1, 2, and 3 ($P < 0.05$). W is significantly smaller ($P < 0.05$) in the CK group than in treatment groups 1 and 2 (**Figure 2C**). There are no significant differences in B among the treatment groups. C_{LWC} displays significant differences among the treatment groups; only treatment groups 2 and 3 did not reach an extremely significant ($P < 0.01$) level (**Figure 2D**). The S_{LAM} of treatment group 2 is 1.40 times that of the CK group, 1.18 times that of treatment group 1, and 1.28 times that of treatment group 3, all of which exhibit significant differences ($P < 0.05$), including a particularly significant difference level ($P < 0.01$) between the S_{LAM} of the CK group and that of treatment group 3 (**Figure 2E**). m_{LMA} (30.06 g/m^2) in treatment group 2 is the smallest, and its difference with the CK group is extremely significant ($P < 0.01$) (**Figure 2F**). These results indicate that proper shading can increase the S, L, W, C_{LWC}, and S_{LAM} of *H. hainanensis*, but not those of m_{LMA}.

It can be seen from **Figure 3** that under different treatments, Tr, Pn, and Gs gradually increase with increasing light intensity, while Pn light suppression is not apparent. Under the same light intensity, Tr, Pn, and Gs display a decreasing trend with increased shading. Pn under full light is the lowest, Tr and Gs under heavy shading are the lowest, and Ci increases with an increase in light intensity. A decrease in shading results in the opposite patterns for Tr, Pn, and Gs. Ci and intercellular CO_2 concentration are greatest under heavy shade. These results suggest that light shading is beneficial to increasing the Tr, Pn, and Gs of *H. hainanensis* but not the Ci.

It can be seen from **Figure 4** that different light intensities exhibit different effects on the photosynthetic parameter characteristics of *H. hainanensis*. No significant difference between treatments was found for the LSP and R^2. For

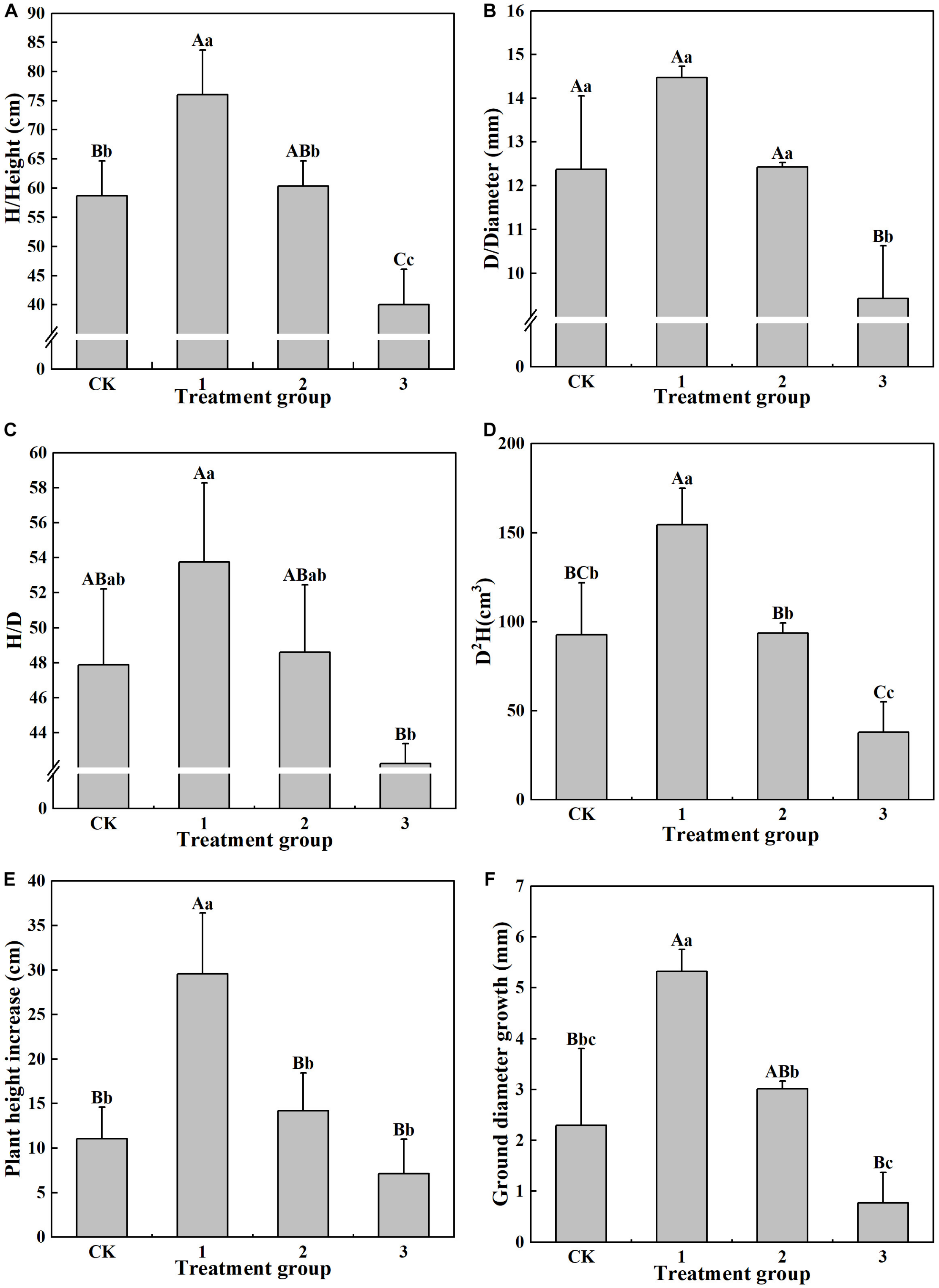

FIGURE 1 | The effect of different treatments on the growth index of *Horsfieldia hainanmensis* The uppercase letters in the figure indicate $P < 0.01$, and the lowercase letters indicate $P < 0.05$, which respective indicate extremely significant and significant differences **(A–D)**, respectively, represent the influence of different light transmittance on the plant height, ground diameter, height to diameter ratio, and biomass of *Horsfieldia hainanmensis* **(E,F)** represent the influence of different light transmittance on the growth of plant height and ground diameter of *Horsfieldia hainanmensis*.

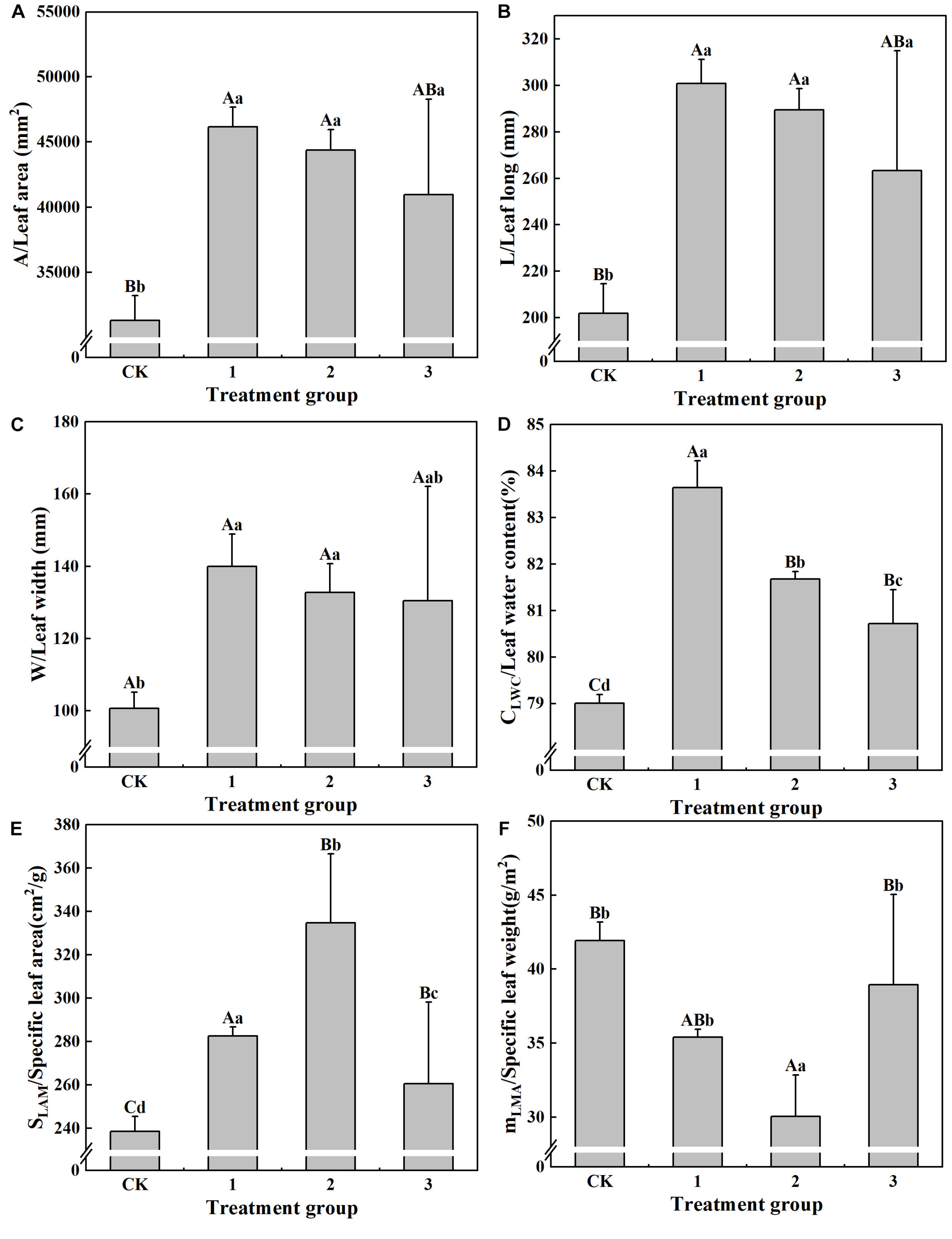

FIGURE 2 | The effect of different treatments on the leaf properties of *Horsfieldia hainanmensis* The uppercase letters in the figure indicate $P < 0.01$, and the lowercase letters indicate $P < 0.05$, which, respectively, indicate extremely significant and significant differences **(A–F)**, respectively, represent the different light transmittance to *Horsfieldia hainanmensis* the leaves A (Leaf area). L (Leaf long) W (Leaf width) C_{LWC} (Leaf water content) S_{LAM} (Specific leaf) and m_{LAM} (Specific leaf height).

AQY, the CK group displays the smallest value (0.047), which is significantly smaller than that of the other treatment groups. The Pnmax of treatment group 1 was the largest (7.854 μmol·m^{-2}·s^{-1}) and is significantly larger than that of the CK group and treatment group 3 (1.64 times and 1.62 times greater, respectively; **Figure 4B**). The LCP and Rd in the CK group are 5.42, 4.65, and 6.75 times greater and 2.77, 2.51, and 3.73 times greater than treatment groups 1–3, respectively,

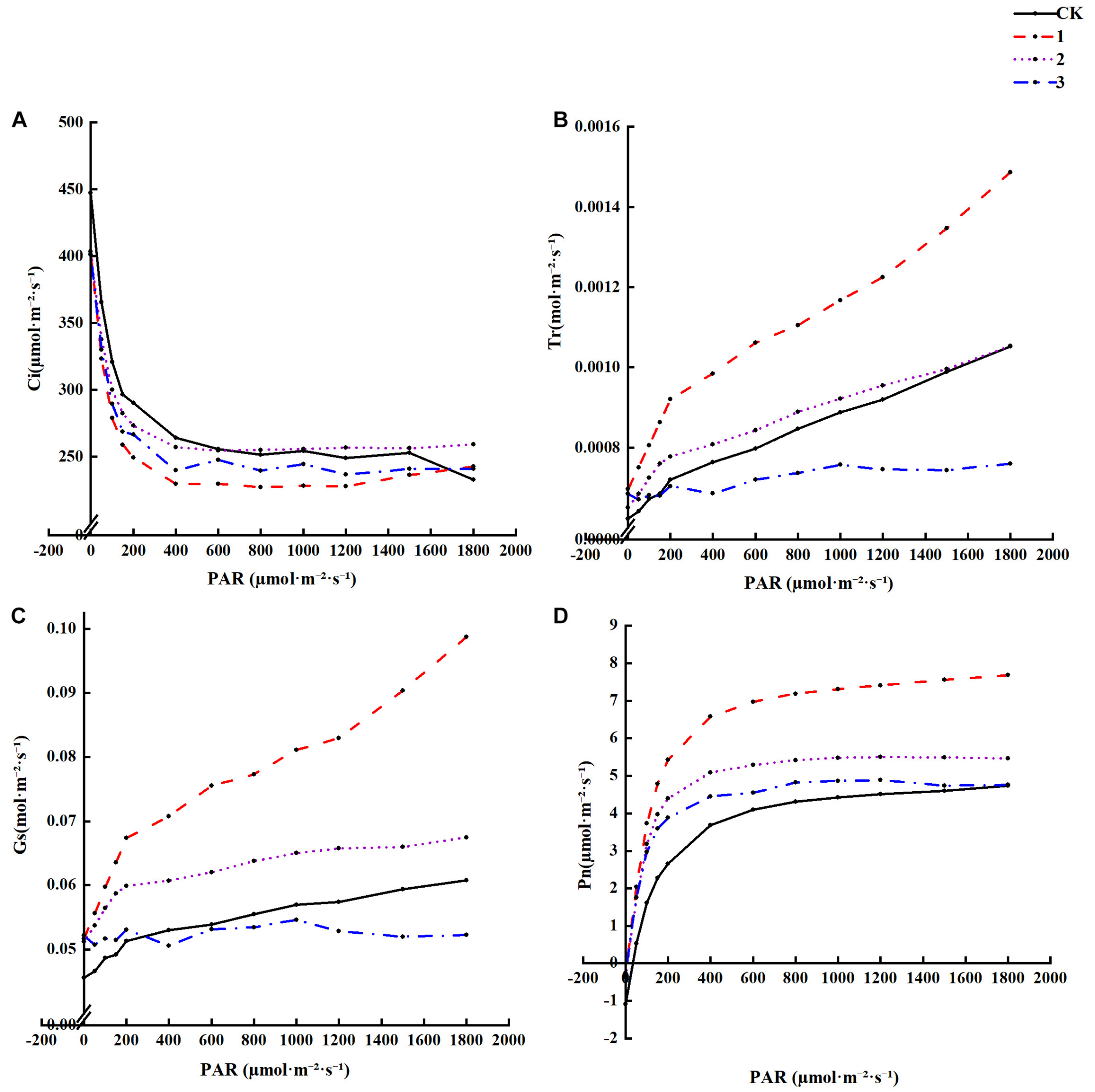

FIGURE 3 | The effect of different treatments on the Photosynthetic characteristics *Horsfieldia hainanmensis* **(A–D)**, respectively, represent the Ci (Intercellular CO_2 concentration), Tr (Transpiration rate), Gs (Stomatal Conductance), and Pn (Net Photosynthetic rate) of the influence of different light transmittance to *Horsfieldia hainanmensis* the light response curve.

and demonstrated extremely significant differences ($P < 0.01$). These results indicate that shading did not affect LSP and R^2. Shading helps to increase AQY and Pnmax but is not conducive to increasing LCP or Rd.

Differing light intensities exhibited little effect on the photosynthetic pigments of *H. hainanensis*. From **Figure 5A**, we can see that there are no significant differences among the photosynthetic pigments of treatment groups 1, 2, and 3. The Chl, Chla, and Chl b contents of the CK group (0.810, 0.589, and 0.221 mg/g, respectively), are significantly smaller ($P < 0.05$) than those of treatment group 3. The Chl a/b value of treatment group 2 was significantly lower ($p < 0.05$) than that of treatment group 3. There was no significant difference in the Caro level among treatment groups. These results indicate that shading can increase the Chl, Chl a, and Chl b contents in *H. hainanensis* leaves.

It can be seen from **Figure 5B** that the Chl-SPAD value of each leaf part in treatment group 3 is significantly greater than that of the CK group. The Chl-SPAD of the leaf tip (Chl-SPAD-leaf tip) of treatment group 2 exhibits a significant difference compared with that of treatment group 3. The mean value of the Chl-SPAD of the leaf base (Chl-SPAD-leaf base) of treatment group 2 was significantly greater than that of the CK group and significantly smaller than that of treatment group 3. The Chl-SPAD-leaf tip of treatment group 1 was significantly different compared to that of treatment group 3. These results indicate that shading can increase the Chl-SPAD value of each leaf part.

Based on **Table 2**, the photosynthetic physiological parameters of *H. hainanensis* display a sufficient correlation. AQY has a particularly significant negative correlation with LCP and Rd ($P < 0.01$) and a

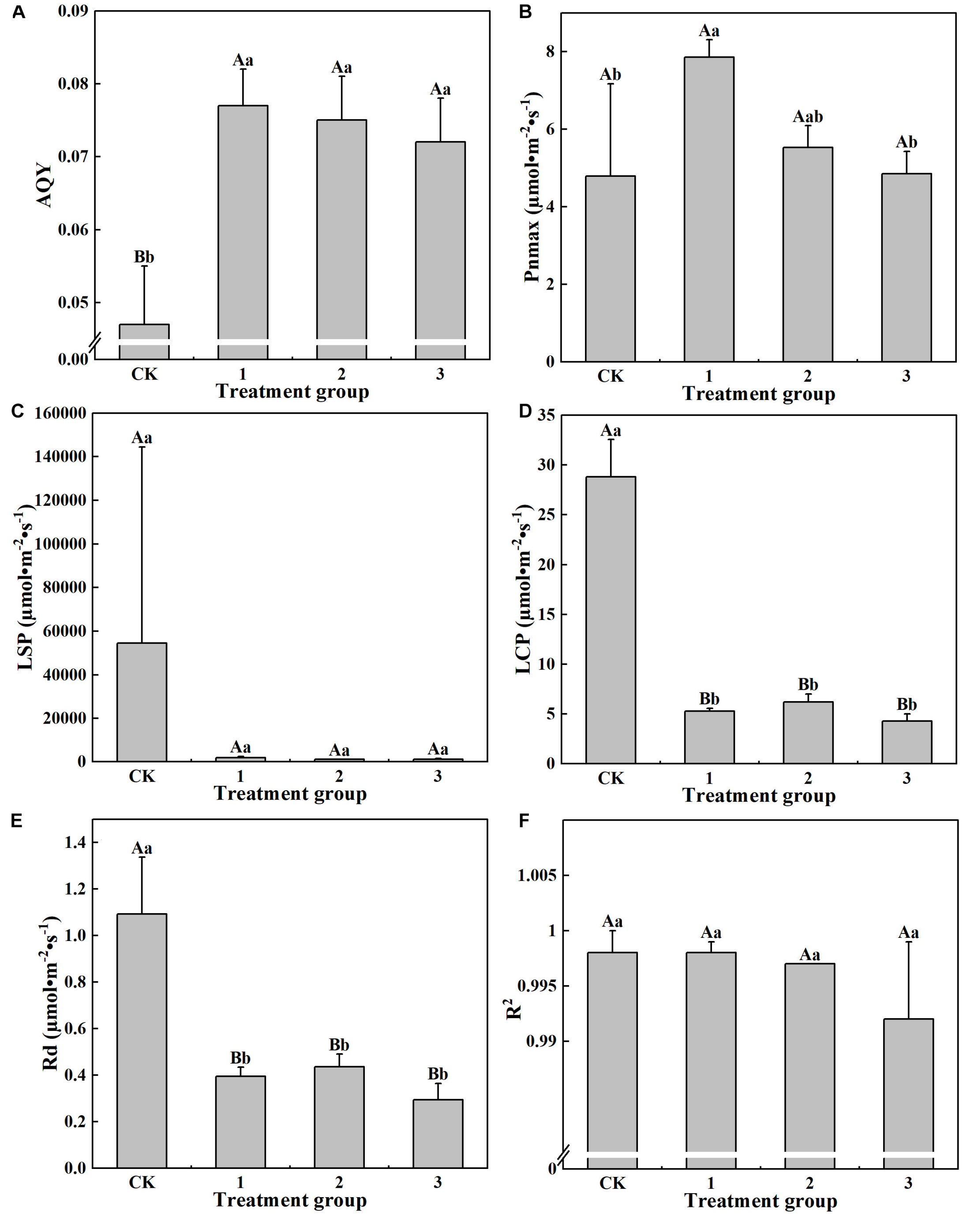

FIGURE 4 | The effect of different treatments on the Photosynthetic parameters of *Horsfieldia hainanmensis* The uppercase letters in the figure indicate $P < 0.01$, and the lowercase letters indicate $P < 0.05$, which, respectively, indicate extremely significant and significant differences **(A–F)**, respectively, represent. The apparent quantum efficiency (AQY) Light -saturated net Photosynthetic rate (Pnmax) light saturation point (LPS) compensation point (LCP), dark respiration rate (Rd) and coefficient of determination (R^2) of the influence of different light transmittance on *Horsfieldia hainanmensis*.

significant negative correlation with LSP ($P < 0.05$), while LCP exhibits a very significant positive correlation with Rd.

The photosynthetic pigments of *H. hainanensis* display a strong correlation (**Table 2**). Chl demonstrates an extremely significant positive correlation with Chl a and Chl b ($P < 0.01$), and Chl-SPAD-leaf base and Chl-SPAD-mean exhibit a significant positive correlation ($P < 0.05$). Chl a has a particularly significant positive correlation with Chl b and Chl-SPAD-leaf base ($P < 0.01$) and a significant positive correlation with the

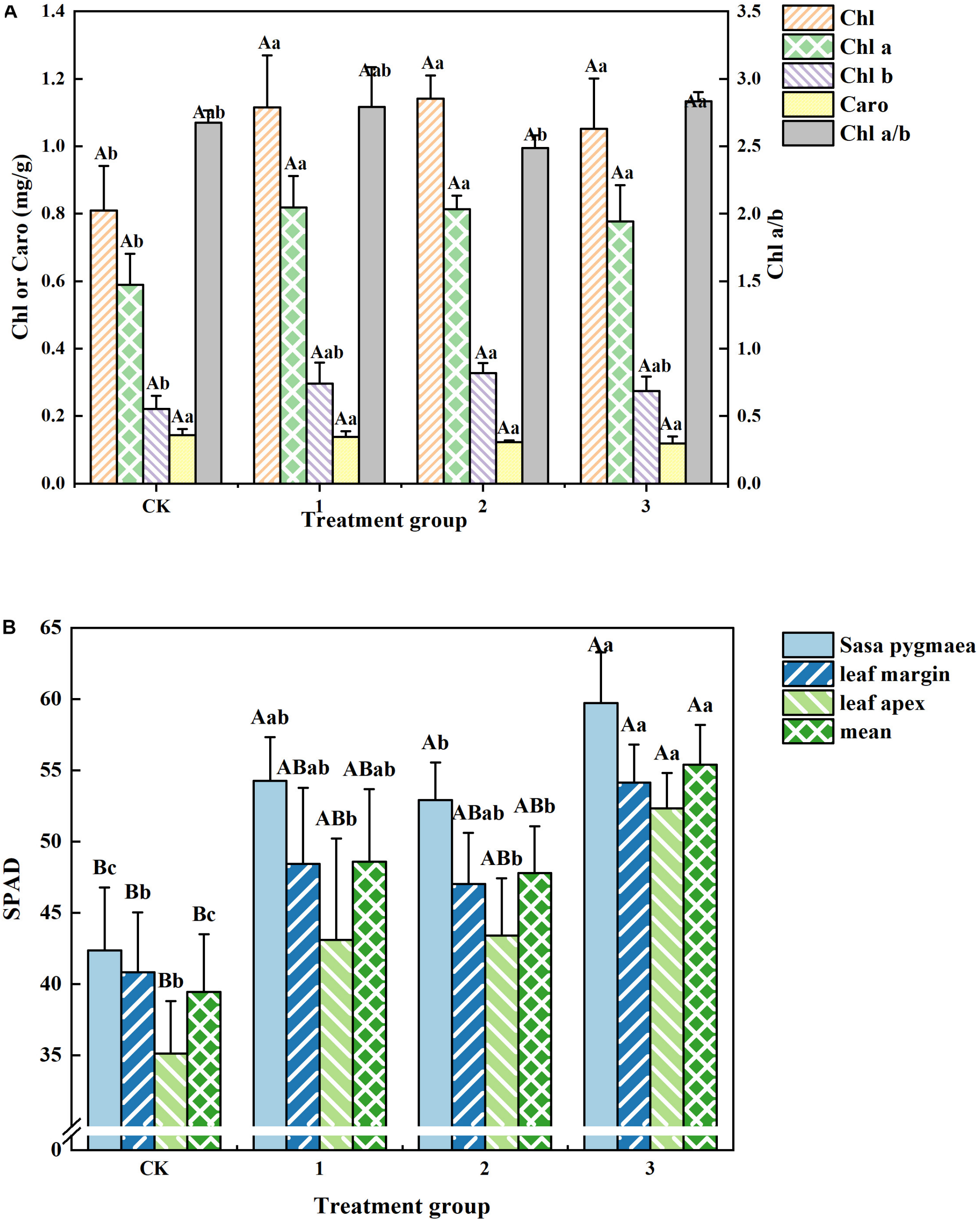

FIGURE 5 | The effect of different treatments on the Photosynthetic pigment content of *Horsfieldia hainanmensis*. The uppercase letters in the figure indicate $P < 0.01$, and the lowercase letters indicate $P < 0.05$, which, respectively, indicate extremely significant and significant differences **(A)** shows the influence of different light transmittance on *Horsfieldia hainanmensis* Chlorphyll, Chol a (Chlorphyll A) Chol b (Chlorphyll B), Chol a/b (Chlorphyll A/B), and Caro (carotenoid). **(B)** Represents the influences of different transmittance on the SPAD value of different position of the blades of *Horsfieldia hainanmensis*.

Chl-SPAD-leaf margin, Chl-SPAD-leaf tip, and Chl-SPAD-mean ($P < 0.05$). Caro only exhibits a significant negative correlation with the Chl-SPAD-leaf tip ($P < 0.05$). The Chl-SPAD-leaf base, Chl-SPAD-leaf margin, Chl-SPAD-leaf tip, and Chl-SPAD-mean all demonstrate significant positive correlations ($P < 0.01$).

There is also a correlation between the photosynthetic physiological parameters of *H. hainanensis* and the photosynthetic pigments (**Table 2**). AQY is significantly positively correlated with Chl, Chl a, and Chl-SPAD-mean ($P < 0.05$); it is also extremely significantly positively correlated with the Chl-SPAD-leaf base ($P < 0.01$). LCP and Rd are highly negatively correlated with Chl, Chla, Chl-SPAD-leaf base, Chl-SPAD-leaf margin, Chl-SPAD-tip, and Chl-SPAD-mean ($P < 0.01$), and

TABLE 2 | Pearson correlation analysis of photosynthetic physiological and biochemical parameters and chlorophyll and other parameters.

	AQY	Pnmax	LSP	LCP	Rd	R^2	Chl	Chl a	Chl b	Chl a/b	Caro	Chl-SPAD-leaf base	Chl-SPAD-leaf margin	Chl-SPAD-tip	Chl-SPAD-mean
AQY	1														
Pnmax	0.519	1													
LSP	−0.692*	−0.432	1												
LCP	−0.873**	−0.389	0.466	1											
Rd	−0.728**	−0.192	0.241	0.954**	1										
R^2	−0.053	0.262	0.021	0.352	0.458	1									
Chl	0.588*	0.109	−0.133	−0.734**	−0.761**	−0.076	1								
Chl a	0.629*	0.139	−0.148	−0.774**	−0.801**	−0.117	0.994**	1							
Chl b	0.473	0.039	−0.095	−0.612*	−0.639*	0.017	0.970**	0.937**	1						
Chl a/b	0.171	0.291	−0.112	−0.120	−0.093	−0.315	−0.394	−0.291	−0.603*	1					
Caro	−0.485	0.136	0.611*	0.391	0.278	0.181	−0.425	−0.427	−0.401	0.162	1				
Chl-SPAD-leaf base	0.716**	0.135	−0.232	−0.868**	−0.888**	−0.361	0.671*	0.731**	0.508	0.263	−0.465	1			
Chl-SPAD-leaf margin	0.542	−0.009	−0.109	−0.720**	−0.775**	−0.463	0.575	0.637*	0.414	0.285	−0.507	0.945**	1		
Chl-SPAD-tip	0.531	−0.104	−0.179	−0.703*	−0.751**	−0.449	0.552	0.604*	0.412	0.204	−0.591*	0.920**	0.976**	1	
Chl-SPAD-mean	0.610*	0.007	−0.18	−0.779**	−0.821**	−0.43	0.611*	0.670*	0.454	0.253	−0.532	0.973**	0.990**	0.983**	1

AQY, The apparent quantum efficiency; Pnmax, Light-saturated net photosynthetic rate; LSP, light saturation point; LCP, light compensation poin; Rd, dark respiration rate; R^2*, coefficient of determination; Chl, chlorophyll; Chl a, chlorophyll A; Chl b, chlorophyll B; Chl a/b, chlorophyll A/B; Caro, carotenoid.*

***Indicates that the correlation is significant at 0.01 level (two-tailed), and * indicates that the correlation is significant at 0.05 level (two-tailed).*

there is a significant negative correlation with Chlb ($P < 0.05$).

DISCUSSION

Light is one of the necessary conditions for plant growth and development. Plants use photosynthetic pigments to capture light energy to conduct photosynthesis. PAR size affects the formation, content, and distribution of photosynthetic pigments, which in turn effect photosynthesis and plant growth (Austin et al., 1996; Blot et al., 2009; Han et al., 2020). The ability of plants to utilize light energy is a direct manifestation of the level and ratio of their chlorophyll content. Weak light transmittance (shading) typically results in a decrease in the proportion of red light absorbed by Chl a and an increase in the proportion of blue light absorbed by Chl b (De Jesus Raposo et al., 2013; Watanabe and Ikeuchi, 2013; Mulders et al., 2014). This study found that the SPAD value of *H. hainanensis* is significantly correlated with its chlorophyll content (**Table 2**). In addition, we found that the Chl, Chl a, and Chl b contents of the CK group were significantly smaller than those of the treatment groups (**Figure 5A**), which may be due to the inhibition of photosynthetic pigment formation under full light conditions. Furthermore, the Chl-SPAD value of each leaf part in the CK group was significantly lower than that of the other treatment groups, while the SPAD values of the leaf parts demonstrated the following order: leaf base > leaf edge > leaf tip (**Figure 5B**). From this, we can conclude that *H. hainanensis* is not conducive to the formation of photosynthetic pigments under full light, and proper shading should be implemented during nursery and return to planting.

Light is one of the most important environmental factors in photosynthesis, which in turn affects the survival and growth of plants (Nawaz et al., 2020). Studying the effects of different levels of shade on plant physiological characteristics is conducive to revealing plant growth patterns, while the light response curve of plant photosynthesis particularly crucial for studying the photochemical reaction process (Epila et al., 2018; Du et al., 2020). The role of light in photosynthesis is primarily to provide the energy required for the formation of the assimilation force, activate certain enzymes involved in photosynthesis, promote the opening of the stomata, and regulate the development of photosynthetic machinery (Puji and Doland, 2017; Dörken and Lepetit, 2018). Pn measures the photosynthetic physiological stress ability of plants under adversity conditions (Farquhar and Sharkey, 1982). We found that the Pn of each treatment group gradually increased with an increase in PAR. Additionally, the Pn of each treatment group was greater than that of the CK group, and it continued to decrease as the shading level increased (**Figure 3D**). The main influencing factors of Pn were Ci and Gs (Zhang et al., 2017). Ci is indispensable for determining the primary cause of the photosynthetic rate change and whether it is a stomatal factor (Shubhangi et al., 2018). This study found that Ci gradually decreased as the PAR increased, and the Ci value of each treatment group was smaller than that of the CK group (**Figure 3A**).

The Ci size depends on four factors: the CO_2 concentration of the air around the leaves, Gs, mesophyll conductance (gm), and the photosynthetic activity of the mesophyll cells (Januskaitiene, 2011). Gs represents the degree of stomatal opening, which is the primary factor affecting plant photosynthesis and respiration, with a direct impact on transpiration (Kinose et al., 2020; Zhen and Bruce, 2020). This study found that the Gs of each treatment group gradually increased with an increase in PAR. When the PAR value was small, the Gs continued to decrease as the shading degree increased (**Figure 3C**). When the PAR value was greater than approximately 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the Gs of treatment group 3 appeared to be smaller than that of the CK group. Tr was the main driving force behind the absorption and transportation of water. It can accelerate the rate of inorganic salt transport to the above-ground part of the plant, reduce the plant body temperature, and induce leaf photosynthesis under strong light conditions without harming the plant (Lapidot et al., 2019; Jafarikouhini et al., 2020). We found that Tr gradually increased as PAR increased, and the Tr value of each treatment group gradually decreased as the shading degree increased. The Tr value of the CK group was smaller than that of each treatment group and greater than the maximum shading treatment group (**Figure 3B**). This experiment demonstrates that under certain shade conditions, the Tr, Pn, and Gs of *H. hainanensis* can be increased and the Ci can be reduced.

This study found that, compared with full light conditions, light shading can increase the Pnmax of *H. hainanensis*. Excessive shading exhibits no effect on the Pnmax of *H. hainanensis* (**Figure 4B**). This may be due to increases in the chlorophyll content per unit area (**Figure 5A**) and the Tr, Pn, and Gs (**Figure 3**). The AQY measures the conversion efficiency of light energy in photosynthesis under low light conditions. The larger the AQY, the stronger the ability of plants to use light energy, and the more pigment-protein complexes can absorb and transform light energy (Liu et al., 2013). This study found that the AQY values of *H. hainanensis* under the shading treatments were significantly greater than that of the CK group ($P < 0.01$; **Figure 4A**), indicating that *H. hainanensis* light utilization is enhanced under shading conditions. The LCP, LSP, and Rd reflect the ability of plants to adapt to a light environment. Plants with lower LCP, LSP, and Rd values can grow more efficiently in an environment with lower light transmittance (Sobrado and Turner, 1983; Dias-Filho, 2002; Iryna et al., 2004). In this study, the LCP, LSP, and Rd of the CK group were significantly higher than those of the treatment groups ($P < 0.01$; **Figures 4C–E**). We also found that the AQY value significantly increased under shading conditions ($P < 0.01$; **Figure 4A**). We speculate that this occurred to allow *H. hainanensis* to enhance its light capture ability under low light conditions. The lower levels of LCP, LSP, and Rd ensure that *H. hainanensis* can more effectively adapt to the decrease in light quantum density, reduce its loss of photosynthetic products due to respiration, and maintain a carbon metabolism balance and higher photosynthesis rate. The environmental conditions of plant growth and the plant physiological characteristics greatly impact the light response characteristic parameters (Sun et al., 2009). The reason for this can be discovered by studying the physiological activity of the *H. hainanensis* leaves.

When the shading level was increased, the plants adjusted through a series of growth and physiological responses, such as increasing plant height, ground diameter, leaf area, leaf length, leaf width, and specific leaf area to adapt to the environment (Mirzaei et al., 2007; Cho et al., 2008). This study found that the growth rates of H, H/D, and D in the 44.41% light transmittance treatment groups were significantly higher than those in the CK group (**Figure 1**). The weak light transmittance inhibited the CK group (**Figure 1**). This may be because when the plants are shaded, the assimilated carbon acts more on the vertical plant growth to obtain the maximum amount of light for the seedlings, thus reducing the carbon that acts on the growth of the plant base diameter (Gao et al., 2019). Additionally, low light adversely affects plant growth (Perrin and Mitchell, 2013). Leaves are one of the most sensitive and plastic plant organs (Brown et al., 2014). We found that with a decrease in the light transmittance (below 44.41%), the morphology and traits of the leaves did not significantly decrease, but they were significantly greater than those in the CK treatment group, especially for A, L, W, C_{LWC}, and S_{LAM} (**Figure 2**). Thus, under the condition of low light transmittance, *H. hainanensis* can increase the leaf area and expand the light absorption area to increase its photosynthetic efficiency to handle the stress caused by low light transmittance (Song et al., 2015; Konôpka et al., 2016).

In summary, the growth and development of *H. hainanensis* under both full and low light are restricted. Thus, we can infer why *H. hainanensis* development is rare under natural conditions. First, it may be that the surrounding trees are typically tall and dense, and the light under the forest is weak. Therefore, the *H. hainanensis* seedlings cannot photosynthesize during the growth and development process, and the energy stored by the plant is lower, causing the plant to often fail to grow and die. Second, the mother tree has fewer accompanying species. If the light intensity is too high, the photosynthetic rate can reach the maximum value, and a vast amount of energy can be accumulated. However, during the research process, it was found that full light can easily cause leaf burns while the leaves are small, which greatly reduces the energy available for plant growth and development, causing the seedlings to die. Therefore, during the *in situ* protection and natural return planting of *H. hainanensis*, we must properly shade the seedlings under full light conditions, and we must effectively cut down surrounding trees when planting and protecting the tall and lush forests to increase light intensity.

CONCLUSION

The results of this study impact two aspects of the *H. hainanensis* and provide guidance for its local protection and return to planting. First, full care and weak light restrict the growth of *H. hainanensis* seedlings at different levels, which cause the seedlings of *H. hainanensis* to wither and die. Second, the physiological response of *H. hainanensis* to light intensity is interrelated with photosynthesis parameters and photosynthetic pigments, which exhibit a very significant correlation ($P < 0.01$). Therefore, we must control the light at approximately 44.4% during *in situ* conservation and return planting of *H. hainanensis*. However, due to the limited number of plant samples, there is little research in this area. It is necessary to further increase the number of plants through conservation and natural regression to provide additional samples for research to reduce errors. In this study, enzymes, minerals, and other related indicators were not measured, and further research on these factors is required.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

XW, GC, and XY designed the research, analyzed the data, and wrote the manuscript. XW, GC, SD, HW, RF, and XY performed the research. All authors contributed to the article and approved the submitted version.

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REFERENCES

Austin, P. A., Ross, I. S., and Mills, J. D. (1996). Regulation of pigment content and enzyme activity in the cyanobacterium Nostoc sp. Mac grown in continuous light, a light-dark photoperiod, or darkness. *Biochim. Biophys. Acta Bioenerg.* 1277, 141–149. doi: 10.1016/S0005-2728(96)00093-X

Ayuko, U., Tadahiko, M., and Amane, M. (2008). Effects of temperature on photosynthesis and plant growth in the assimilation shoots of a rose. *Soil Sci. Plant Nutr.* 54, 253–258. doi: 10.1111/j.1747-0765.2007.00234.x

Bepetel, M., and Lakso, A. N. (1998). Differential effects of shade on early-season fruit and shoot growth rates in empire apple. *HortScience* 33, 823–825. doi: 10.21273/HORTSCI.33.5.823

Blot, N., Wu, X. J., Thomas, J. C., Zhang, J., Garczarek, L., Bohm, S., et al. (2009). Phycourobilin in trichromatic phycocyanin from oceanic cyanobacteria is formed post-translationally by a phycoerythrobilin lyase-isomerase. *J. Biol. Chem.* 284, 9290–9298. doi: 10.1074/jbc.M809784200

Brown, C. E., Mickelbart, M. V., and Jacobs, D. F. (2014). Leaf physiology and biomass allocation of backcross hybrid *American chestnut* (*Castanea dentata*) seedlings in response to light and water availability. *Tree Physiol.* 34, 1362–1375. doi: 10.1093/treephys/tpu094

Cho, M. S., Kwon, K. W., Kim, G. N., and Woo, S. Y. (2008). Chlorophyll contents and growth performances of the five deciduous hardwood species growing under different shade treatments. *Korean J. Agric. For. Meteorol.* 10, 149–157. doi: 10.5532/KJAFM.2008.10.4.149

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., et al. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51, 335–380. doi: 10.1071/BT02124

De Jesus Raposo, M. F., de Morais, R. M., and de Morais, A. M. (2013). Health applications of bioactive compounds from marine microalgae. *Life Sci.* 10, 479–486. doi: 10.1016/j.lfs.2013.08.002

Dias-Filho, M. B. (2002). Photosynthetic light response of the c4 grasses brachiaria brizantha and b. humidicola under shade. *Sci. Agric.* 59, 65–68. doi: 10.1590/S0103-90162002000100009

Dörken, V. M., and Lepetit, B. (2018). Morpho-anatomical and physiological differences between sun and shade leaves in Abies alba Mill . (Pinaceae, Coniferales): a combined approach. *Plant Cell Environ.* 41, 1683–1697. doi: 10.1111/pce.13213

Du, T. T., Meng, P., Huang, J. L., Peng, S. B., and Xiong, D. L. (2020). Fast photosynthesis measurements for phenotyping photosynthetic capacity of rice. *Plant Methods* 166:6. doi: 10.1186/s13007-020-0553-2

Editorial Committee of Chinese Flora of Chinese Academy of Sciences (1977). *Flora of China*. Beijing: Science Press.

El-Gizawy, A. M., Gomaa, H. M., El-Habbasha, K. M., and Mohamed, S. S. (1993). Effect of different shading levels on tomato plants 1. growth, flowering and chemical composition. *Acta Hortic.* 323, 341–348. doi: 10.17660/ActaHortic.1993.323.31

Epila, J., Hubeau, M., and Steppe, K. (2018). Drought effects on photosynthesis and implications of photoassimilate distribution in 11C-labeled leaves in the african tropical tree species maesopsis eminii engl. *Forests* 9:109. doi: 10.3390/f9030109

Falkowski, P. G., and Raven, J. A. (1997). *Aquatic Photosynthesis*. Oxford: Blackwell.

Farquhar, G. D., and Sharkey, T. D. (1982). Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol.* 33, 317–345. doi: 10.1146/annurev.pp.33.060182.001533

Gao, Z., Khalid, M., Jan, F., Saeed -Ur-Rahman, and Yu, X. (2019). Effects of light-regulation and intensity on the growth, physiological and biochemical properties of aralia elata (miq.) seedlings. *S. Afr. J. Bot.* 121, 456–462. doi: 10.1016/j.sajb.2018.12.008

Gu, Q., Chen, Z. P., Yu, X. L., Cui, W. T., Pan, J. C., Zhao, G., et al. (2017). Melatonin confers plant tolerance against cadmium stress via the decrease of cadmium accumulation and reestablishment of microRNA-mediated redox homeostasis. *Plant Sci.* 261, 28–37. doi: 10.1016/j.plantsci.2017.05.001

Han, T. W., Tseng, C. C., Cai, M., Chen, K., Cheng, S. Y., and Wang, J. (2020). Effects of cadmium on bioaccumulation, bioabsorption, and photosynthesis in sarcodia suiae. *Int. J. Environ. Res. Public Health* 17:1294. doi: 10.3390/ijerph17041294

Hanson, T. L. (1975). Needle biomass of lodgepole pine from tree dimensions. *Trans. Asae Gen. Ed. Am. Soc. Agric. Eng.* 18, 0491–0492. doi: 10.13031/2013.36616

Harrison, W. G., and Platt, T. (1986). Photosynthesis-irradiance relationships in polar and temperature phytoplankton populations. *Polar Biol.* 5, 153–164. doi: 10.1007/BF00441695

Hase, H., Foelster, H., and Lindheim, F. M. (1985). On the accuracy of estimating aboveground tree biomass in an evergreen forest near manaus, brazil. a simulation study. *Biotropica* 17, 191–195. doi: 10.2307/2388216

Huang, J., Wang, H. Y., Zhong, Y. D., Huang, J. G., Fu, X. F., and Teng, W. C. (2019). Growth and physiological response of an endangered tree, Horsfieldia hainanensis Merr., to simulated sulfuric and nitric acid rain in southern China. *Plant Physiol. Biochem.* 144, 118–126. doi: 10.1016/j.plaphy.2019.09.029

Iryna, I., Tartachnyk., and Michael, M, B. (2004). Effect of delayed fruit harvest on photosynthesis, transpiration and nutrient remobilization of apple leaves. *New Phytol.* 164, 441–450. doi:10.1111/j.1469-8137.2004.01197.x

Ishida, K. (1989). Influence of respiration rate and metabolic substances on nodal position of first flower bud of eggplant seedings. *J. Japanese Soc. Hortic. Sci.* 58, 657–660. doi: 10.2503/jjshs.58.657

Jafarikouhini, N., Pradhan, D., and Sinclair, T. R. (2020). Basis of limited-transpiration rate under elevated vapor pressure deficit and high temperature among sweet corn cultivars. *Environ. Exp. Bot.* 179:104205. doi: j.envexpbot.2020.104205

Januskaitiene, I. (2011). Effects of substrate acidity and UV-B radiation on photosynthesis of radishes. *Open Life Sci.* 6, 624–631. doi: 10.2478/s11535-011-0027-7

Jiang, Y. H. (2018). *Ecological Characteristics and Endangered Reason Analysis of Horsfieldia Hainanensis Merr. as an Extremely Small Population*, dissertation/master's thesis, CSUFT, China.

Jiang, Y. H., Liu, X. S., Xiang, W. H., and He, Y. H. (2018). Genetic diversity and structure analysis of the endangered plant species *Horsfieldia hainanensis* Merr. *Biotechnol. Biotechnol. Equ.* 32, 95–101. doi: 10.1080/13102818.2017.1391122

Jiang, Y. H., Xiang, W. H., Jiang, Y., He, Y. H., and Lin, J. Y. (2016). Floristic composition,structure and phytogeographic characteristics of horsfieldia hainanensis community in guangxi. *J. Beijing Forestry Univ.* 38, 74–82.

Kinose, Y., Fukamachi, Y., Watanabe, M., and Izuta, T. (2020). Ozone-induced change in the relationship between stomatal conductance and net photosynthetic rate is a factor determining cumulative stomatal ozone uptake by *Fagus crenata* seedlings. *Trees Struct. Funct.* 34, 445–454. doi: 10.1007/s00468-019-01927-1

Konôpka, B., Pajtík, J., Marušák, R., Bošel'a, M., and Lukac, M. (2016). Specific leaf area and leaf area index in developing stands of *Fagus sylvatica* L. and Picea abies Karst. *For. Ecol. Manag.* 364, 52–59. doi: 10.1016/j.foreco.2015.12.005

Krause, G. H., and Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: the basics. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42, 313–349.doi: 10.1146/annurev.pp.42.060191.001525

Kruse, J., Hopmans, P., and Adams, M. A. (2008). Temperature responses are a window to the physiology of dark respiration: differrences between CO2 release and O2 reduction shed light on energy conservation. *Plant Cell Environ.* 31, 901–914.doi: 10.1111/j.1365-3040.2008.01808.x

Lapidot, O., Ignat, T., Rud, R., Rog, I., Alchanatis, V., and Klein, T. (2019). Use of thermal imaging to detect evaporative cooling in coniferous and broadleaved tree species of the *Mediterranean maquis*. *Agric. For. Meteorol.* 271, 285–294. doi: 10.1016/j.agrformet.2019.02.014

Leite, F. T., Cavatte, P. C., Garbin, M. L., Hollunder, R. K., Santos, K. F., Capetine, T. B., et al. (2019). Surviving in the shadows: light responses of co-occurring Rubiaceae species within a tropical forest understory. *Flora* 261:151487. doi: 10.1016/j.flora.2019.151487

Li, Y. T., Xu, W. W., Ren, B. Z., Zhao, B., Zhang, J. W., Liu, P., et al. (2020). High temperature reduces photosynthesis in maize leaves by damaging chloroplast ultrastructure and photosystem II. *J. Agron. Crop Sci.* 206, 548–564. doi: 10.1111/jac.12401

Liu, S. B., Mei, W. L., Zeng, Y. B., Wang, H., Li, X. N., and Dai, H. F. (2010). Study on the fat-soluble constituents of li yao *Horsfieldia hainanensis* Merr. *Chin. J. Ethnomed. Ethnopharm.* 19, 147–149.

Liu, X. S., He, Y. H., Xiao, Y. F., Wang, Y., Jiang, Y. H., and Jiang, Y. (2019). Soil seed burial and competition with surrounding plants determine the emergence and development of seedling of an endangered species *Horsfieldia hainanensis* Merr. in China. *Sci. Rep.* 9, 703–715. doi: 10.1038/s41598-019-54644-7

Liu, X., Fan, Y., Long, J., Wei, R., Kjelgren, R., Gong, C., et al. (2013). Effects of soil water and nitrogen availability on photosynthesis and water use efficiency of *Robinia pseudoacacia* seedlings. *J. Environ. Sci.* 25, 585–595. doi: 10.1016/S1001-0742(12)60081-3

Lore, T. V., Leandro, V. L., Philippe, C., Elodie, A. C., Sara, V., Josep, P., et al. (2020). Coping with branch excision when measuring leaf net photosynthetic rates in a lowland tropical forest. *Biotropica* 52, 608–615. doi: 10.1111/btp.12774

Mansoure, H., Rezaei, N. A., Mohsen, K., Kazem, S. M., and Karim, S. (2020). Nitrate improves hackberry seedling growth under cadmium application. *Heliyon* 6:e03247. doi: 10.1016/j.heliyon.2020.e03247

Mirzaei, J., Tabari, M., and Daroodi, H. (2007). Early growth of quercus castaneifolia (c.a. meyer) seedlings as affected by weeding, shading and irrigation. *Pak. J. Biol. Sci.* 10, 2430–2435. doi: 10.3923/pjbs.2007.2430.2435

Mulders, K. J. M., Lamers, P. P., Martens, D. E., Wijffels, R. H., and Bassi, R. (2014). Phototrophic pigment production with microalgae:biological constraints and opportunities. *J. Phycol.* 50, 229–242. doi: 10.1111/jpy.12173

Nawaz, R., Abbasi, N. A., Hafiz, I. A., and Khalid, A. (2020). Impact of climate variables on growth and development of Kinnow fruit (*Citrus nobilis* Lour x Citrus deliciosa Tenora) grown at different ecological zones under climate change scenario. *Sci. Hortic.* 260:108868. doi: 10.1016/j.scienta.2019.108868

Perrin, P. M., and Mitchell, F. J. G. (2013). Effects of shade on growth, biomass allocation and leaf morphology in European yew (*Taxus baccata* L.). *Eur. J. For. Res.* 132, 211–218. doi: 10.1007/s10342-012-0668-8

Platt, T., Gallegos, C. L., and Harrison, W. G. (1980). Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J. Mar. Res.* 38, 687–701.

Puji, L. D., and Doland, N. J. (2017). Seedlings of subtropical rainforest species from similar successional guild show different photosynthetic and morphological responses to varying light levels. *Tree Physiol.* 37, 186–198.

Shubhangi, L., Marianne, H., Mats, R., Nitin, C., Sune, L., Danielle, W., et al. (2018). Physiological acclimation dampens initial effects of elevated temperature and atmospheric CO2 concentration in mature boreal Norway spruce. *Plant Cell Environ.* 41, 300–313. doi: 10.1111/pce.13079

Sobrado, M. A., and Turner, N. C. (1983). A comparison of the water relations characteristics of helianthus annuus and helianthus petiolaris when subjected to water deficits. *Oecologia* 58, 309–313. doi: 10.1007/BF00385228

Song, K. S., Jeon, K. S., Choi, K. S., Kim, C. H., and Kim, J. J. (2015). Characteristics of photosynthesis and leaf growth of peucedanum japonicum by leaf mold and shading level in forest farming. *Korean J. Med. Crop Ence* 23, 43–48. doi: 10.7783/KJMCS.2015.23.1.43

Sun, C. X., Qi, H., Hao, J. J., Miao, L., Wang, J., Wang, Y., et al. (2009). Single leaves photosynthetic characteristics of two insect-resistant transgenic cotton (*Gossypium hirsutum* L.) varieties in response to light. *Photosynthetica* 47, 399–408. doi: 10.1007/s11099-009-0061-0

Wang, L. S. (2017). *Effects of Different Fertilization Treatments on the Growth and Physiological Characteristics of Horsfieldia hainanensis Merr. Seedlings*. dissertation/master's thesis, Guangxi University, China.

Watanabe, M., and Ikeuchi, M. (2013). Phycobilisome: architecture of alight-harvesting supercomplex. *Photosynth. Res.* 116, 265–276. doi: 10.1007/s11120-013-9905-3

Xu, Y. L., Cai, N. H., Wu, Y., and Duan, A. A. (2012). Fatty acid composition of several plants of horsfieldia. *China Oils Fats* 37, 80–82.

Yang, Y., Liu, Q., Chen, Y. K., Wang, Y., and Chen, Q. (2019). The complete chloroplast genome of *Horsfieldia hainanensis* an endangered species with extremely small populations. *Mitochondrial DNA B* 4, 2654–2655. doi: 10.1080/23802359.2019.1644556

Ye, Z. P., and Zhao, Z. H. (2010). A modified rectangular hyperbola to describe the light-response curve of photosynthesis of *Bidens pilosa* L. grown under low and high light conditions. *Front. Agric. China* 4, 50–55. doi: 10.1007/s11703-009-0092-0

Zhang, X., Cao, Q., and Jia, G. (2017). A protocol for fertility restoration of F1 hybrid derived from Lilium ×formolongi 'Raizan 3' ×Oriental hybrid

'Sorbonne'. *Plant Cell Tissue Organ. Cult.* 129, 375–386. doi: 10.1007/s11240-017-1184-9

Zhen, S. Y., and Bruce, B. (2020). Steady-state stomatal responses of C3 and C4 species to blue light fraction: interactions with CO2 concentration. *Plant Cell Environ.* 43, 3020–3032. doi: 10.1111/pce.13888

Zhong, S. Y., Chen, G. D., Qiu, M. H., Liu, M. H., Lin, L., and Lin, Z. W. (2018). Investigation on the geographical distribution and habitat characteristics of *Horsfieldia hainanensis* in Hainan Island. *J. Fujian Forestry Sci. Technol.* 45, 86–106.

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Bombax ceiba is a Good Native Tree Species for Performing Reforestation to Restore Highly Degraded Tropical Forests in Hainan Island, China

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Reforestation is an effective way to alleviate deforestation and its negative impacts on ecosystem services. It is widely recognized that the most key step for reforestation is using suitable native species, but selecting suitable native tree species is much more complex and challenging than the selection of non-native tree species that have been widely used for reforestation. Here, we quantify whether the native tree species (*Bombax ceiba*) can be suitable for performing reforestation to restore a 0.2 km^2 highly degraded tropical monsoon forest in Baopoling Mountain (BPL), Sanya, China, due to 20 years of limestone mining for cement production. We found that stomatal closure helped *Bombax ceiba* develop higher drought stress tolerance than the most dominant native tree species (*Bridelia tomentosa*) in an undisturbed tropical rainforest in BPL, thereby better adapting well to drought stress in the dry season. These characteristics in turn facilitated it to have high survival rate (92% ± 4%) and fast growth rate, after three years of monoculture in BPL. Thus, *Bombax ceiba* is very suitable for performing reforestation to recover highly degraded tropical forests in Hainan Island, China.

Keywords: drought stress tolerance, fast-growing, functional trait, native species, non-native species, reforestation

INTRODUCTION

Historic human disturbance (e.g., ore mining and agricultural use) has resulted in very high rates of deforestation and degradation worldwide, which has become a major threat to global biodiversity and ecosystem services (e.g., freshwater supply and slowing down of global warming) (Sahin and Hall, 1996; Foley et al., 2011; Lambin and Meyfroidt, 2011; Vörösmarty et al., 2015). Reforestation is assumed to be able to partly alleviate deforestation-induced consequences (Griscom et al., 2017; Taubert et al., 2018). Thus, a number of reforestation projects have been performed worldwide to alleviate these native influences (Postel and Thompson, 2005; Calder and Aylward, 2006; Grime, 2006; Calder, 2007; Jian et al., 2015). However, to date, monoculture plantations of many non-native fast-growing and commercial tree species are the main strategy for reforestation in many areas across the world (Lu et al., 2017).

Although these monoculture plantations of non-native species can quickly facilitate reforestation, they can also increase risks including high cost for maintaining soil water and its nutrients and pest and pathogen outbreaks, besides having few benefits for ecosystem services (Lamb et al., 2005;

Wingfield et al., 2015). Thus, many studies emphasize the most key step for reforestation is using suitable native species but not non-native species (Miyawaki, 2004; Hall et al., 2011). However, selecting suitable native tree species is much more complex and challenging than the selection of non-native tree species, in particular (Meli et al., 2014; Stanturf et al., 2014). That is because the reforestation project usually has to be finished in a very short time and with limited economic resources. Moreover, in theory, mono-planting fast-growing tree species with high survival rates may be very effective for preventing high risks of landslide due to frequent typhoon and heavy rain in humid tropics (Stokes et al., 2009; Walker et al., 2009; Pang et al., 2018). Thus, non-native tree species which have high survival and growth rates and germination traits enabling easy propagation in the nurseries are the first choice for performing reforestation (Meli et al., 2014). Although pioneer native tree species in the early successional tropical rainforest are assumed fast-growing (Lohbeck et al., 2013; Zhu et al., 2013), their survival and growth rates and germination traits are very less investigated and thus cannot promise they are better than the non-native tree species for quickly finishing the reforestation project. Tree species in late successional or natural tropical rainforests may better adapt well to a specific environment of the highly degraded ecosystem than the non-native tree species, but they are treated as slow-growing, but with high resources competitive ability (Mason et al., 2012; Mason et al., 2013). As a result, they are also not very suitable for performing reforestation in humid tropics.

The currently developed trait-based method can open a good avenue for facilitating selection of suitable native tree species (McGill et al., 2006; Laughlin, 2014). It assumes that plant functional traits can directly reflect plants adapting well to different abiotic and biotic environments (Larjavaara and Muller-Landau, 2010; Laughlin and Laughlin, 2013; Cadotte, 2017). Thus, using the functional traits to find out native species that can not only have high survival and growth rates and germination traits enabling quick and easy propagation in the nurseries but also adapt well to the specific environment of the highly degraded ecosystem can be useful for reforestation. This goal may be easily achieved by comparing the functional traits which are highly correlated with survival and growth rates, germination, and the ability of adapting well to the environment of the highly degraded ecosystem between potential native tree species and dominant native tree species in the late successional or undisturbed old tropical rainforest.

Bombax ceiba is a native species, which is widely distributed in tropics, and it is also a high-demand plant species used for sculpture**,** so its seedling has been widely cultivated to get economic profits (Griffiths et al., 2003). In addition, it can adapt well to a very severe environment (e.g., drought stress), and thus, it may have high potential to be utilized for reforestation in the degraded tropical forest which has seasonal drought stress. Here, we mono-planted *Bombax ceiba* to restore a 0.2 km^2 highly degraded tropical rainforest in Baopoling Mountain (BPL) in Sanya, Hainan Province, China, to quantify whether *Bombax ceiba* can be a good native tree species for reforestation in Hainan Island. It has been found that BPL can have frequent typhoon and heavy rain and very limited precipitation in the dry season (Hong et al., 2020), which may result in a high risk of landslide and drought stress during reforestation. Based on this, we use the following standards to judge whether *Bombax ceiba* can be a good native tree species for reforestation in extremely degraded tropical rainforests. First, *Bombax ceiba* can have high growth and survival rates, thereby being suitable for quickly performing reforestation. Second, landslides can be prevented after mono-planting *Bombax ceiba* to perform reforestation. Third, *Bombax ceiba c*an adapt better to the seasonal drought in BPL than the most dominant native species in the undisturbed old tropical rainforest. Specifically, we survey survival and growth rates during reforestation. We also compare differences in functional traits that are highly associated with growth (i.e., photosynthesis rate) and drought stress tolerance (e.g., leaf turgor loss point) between *Bombax ceiba* and the most dominant native tree species in the adjacent undisturbed old tropical rainforest in BPL.

MATERIALS AND METHODS

Study Sites

Our study site is located in Baopoling Mountain (BPL) which is a limestone mountain in Sanya, Hainan Province, China (109°51′01″E, 18°31′99″N) (**Figure 1**). It has a tropical monsoon oceanic climate with the mean annual temperature of 28°C. The average annual precipitation in Sanya is 1500 mm, with approximately 91% of the precipitation occurring between June and October; these estimates are based on the climate data form 1959 to 2015 (Luo et al., 2018). The typical vegetation is the species-rich tropical rainforest (Luo et al., 2020), but *Bombax ceiba* is not the native tree species in this forest but the native tree species that is widely distributed in the whole Hainan Island, China. Due to 20 years of limestone mining for producing cement, one part (0.2 km^2 area) of BPL has become a highly degraded bare rocky mountain, where plants cannot be grown. Areas of BPL outside of this degraded area have not received any significant disturbance and remain as an undisturbed old tropical monsoon forest which has more than 120 tree species (**Figure 1**). In 2016, we have mono-planted the seedling of *Bombax ceiba* to perform a reforestation project in this 0.2 km^2 area. The planting density was kept at 80–100 stems per hectare; we recorded their survival rates from 2016 to 2019 as

$$\text{survival rate} = \frac{\text{remaining seedlings}}{\text{original seedlings}} \times 100\%.$$

Trait Collection and Measurement

Since there is a remarkable dry season in BPL, plant species must develop high drought stress tolerance, thereby being able to survive well in BPL. Thus, in the peak of dry season (February) in 2019, we measured functional traits that are

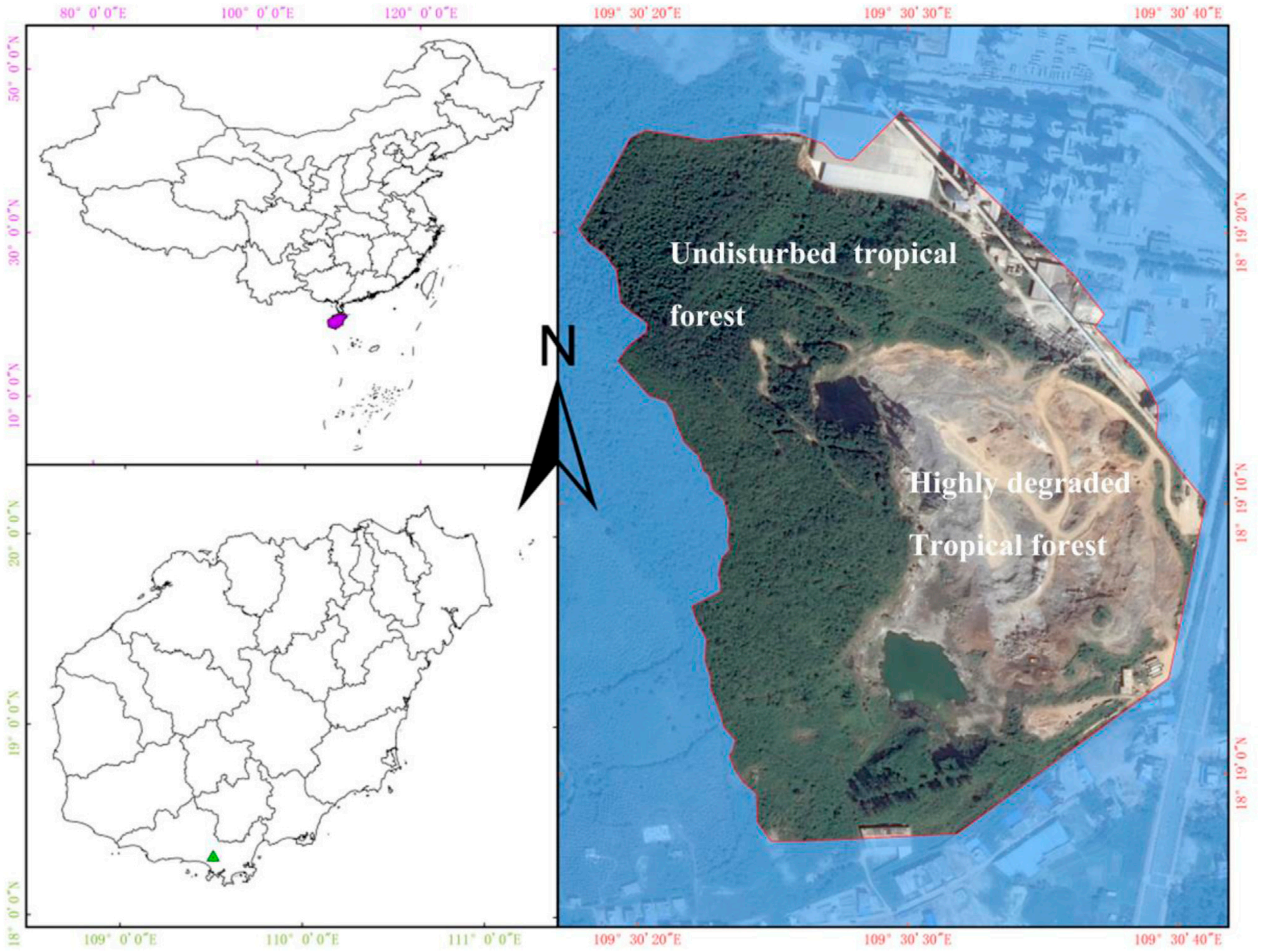

FIGURE 1 | Location and landscape of our study sites (Baopoling Mountain, Sanya, China) before reforestation.

Table 1 | Mean DBH (in cm) of *Bombax ceiba* and *Bridelia tomentosa* in Baopoling Mountain and DBH values (in cm) of the sampled individuals (n) for *Bombax ceiba* and *Bridelia tomentosa*, respectively.

Species name	*n*	Mean DBH	DBH of sampled individuals				
			Tree 1	Tree 2	Tree 3	Tree 4	Tree 5
Bombax ceiba	5	11.2	12.1	11.5	10.8	10.2	10.5
Bridelia tomentosa	5	7.9	6.2	7.1	7.6	8.2	9.5

highly related to fast growth and drought stress tolerance (i.e., upper epidermis thickness (μm), palisade tissue thickness (μm), spongy tissue thickness (μm), lower epidermis thickness (μm), stomatal density (numbers mm^{-2}), transpiration rate (mol m^{-2} s^{-1}), maximum photosynthesis rate (mol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), and leaf turgor loss point (MPa)) for *Bombax ceiba* and the most dominant (relative abundance >10%) plant species (*Bridelia tomentosa*) located in the nearby undisturbed forests. For measuring these traits, we first measured the diameter at breast height (DBH) of all individuals for both *Bombax ceiba* and *Bridelia tomentosa* to get the mean DBH for *Bombax ceiba* and *Bridelia tomentosa*, respectively (see **Table 1**). Then, 20 fully expanded, healthy, and sun-exposed leaves were collected from five mature individuals of *Bombax ceiba* and *Bridelia tomentosa* whose DBH values were comparable to the mean DBH values of *Bombax ceiba* and *Bridelia tomentosa* (**Table 1**).

Measurement of Leaf Anatomical Traits

Twenty mature and sun-exposed leaves were collected from five independent individuals of *Bombax ceiba* and *Bridelia tomentosa* in BPL. All leaf samples were progressively dehydrated in an ethanol series (50, 70, 85, 95, and 100%) and infiltrated with warm paraffin. Then, the thickness of the leaf, upper epidermis, lower epidermis, spongy tissue, and palisade was measured with a Leica DM2500 light microscope (Leica Microsystems GmbH, Wetzlar, Germany). The density of stomata was measured using a razor to slice the abaxial *epidermis*. All the sections were

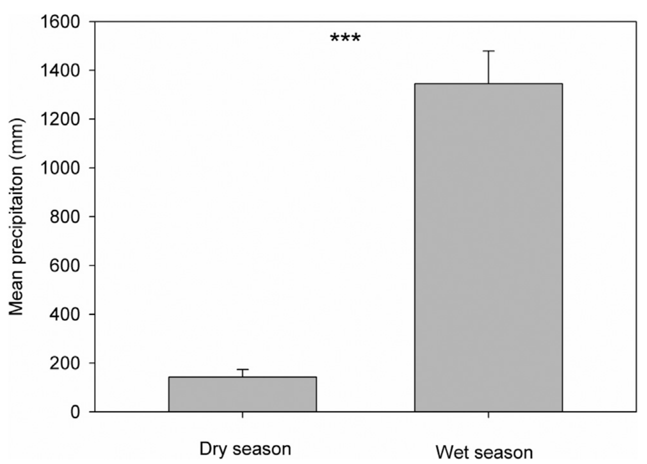

FIGURE 2 | Variations in mean precipitation in the dry season (February) and wet season (July) from 2016 to 2019. *** indicates $p < 0.001$, based on paired *t*-tests.

mounted on slides and observed under a Leica DM2500 microscope.

Measurement of Maximum Photosynthesis Rate, Transpiration Rate, and Stomatal Conductance

Measurements of the maximum photosynthesis rate, transpiration rate, and stomatal conductance were conducted between 9:00 and 11:00 on sunny days with an Li-6400 portable photosynthesis system (Li-Cor, Lincoln, Nebraska, United States). Based on preliminary trials, the photosynthetic photon flux density was set at 1500 μmol m^{-2} s^{-1} to ensure that light-saturated photosynthetic rates were measured for *Bombax ceiba* and *Bridelia tomentosa*. Ambient CO_2 and air temperature were maintained at 390 μmol mol^{-1} and 28°C, respectively (Zhang et al., 2018a). Before data were recorded, leaves were exposed to the above conditions for about 5 min to allow photosynthetic parameters to stabilize.

Measurement of Leaf Turgor Loss Point

Leaf-bearing branches from three to five individuals of each species were harvested and transported to the laboratory where the basal ends of the branches were immersed in distilled water and re-cut. The branch samples were rehydrated until the leaf water potential was greater than −0.05 MPa. Leaves were first weighed to obtain the initial fresh mass and then immediately placed in a pressure chamber to determine the initial water potential. The leaf mass and water potential were measured periodically during slow desiccation in the laboratory. Finally, leaves were oven-dried for 72 h at 70°C to determine their dry mass. The leaf turgor loss point was determined using a pressure–volume relationship analysis program developed by Schulte and Hinckley (1985).

Statistical Methods

First, we log-transformed all measured nine functional traits (upper and lower epidermis thickness, palisade and spongy tissue thickness, stomatal conductance, stomatal density, maximum photosynthesis rate, transpiration rate, and leaf turgor loss point) for *Bombax ceiba* and *Bridelia tomentosa* in BPL. Then, we used the paired *t*-test to quantify whether *Bombax ceiba* could have higher growth rates and drought stress tolerance than *Bridelia tomentosa*. Then, a principal component analysis (PCA) was employed to evaluate which of the 10 traits were best at discriminating between *Bombax ceiba* and *Bridelia tomentosa*.

RESULTS

Based on the precipitation records per month from 2016 to 2019, the mean precipitation in BPL in the dry season (February) was significantly much lower (only 1/10th) than that in the wet season (July) (**Figure 2**). However, after 2 years of plantation in BPL, the survival rate for all seedlings of *Bombax ceiba* was very high (92% ± 4%), and till now, we did not observe any destructive landslides (**Figure 1**). *Bombax ceiba* had a much higher growth rate than *Bridelia tomentosa*. That was because all seven traits (upper and lower epidermis thickness, palisade and spongy tissue thickness, stomatal conductance, maximum photosynthesis rate, and transpiration rate) that are highly associated with the growth of *Bombax ceiba* were all significantly much higher (3–5 times) than those of *Bridelia tomentosa* (**Figure 3**). In addition, *Bombax ceiba* had much higher drought stress tolerance than *Bridelia tomentosa*, as the leaf turgor loss point for *Bombax ceiba* was significantly much lower (merely 1/3rd) than that of *Bridelia tomentosa* (**Figure 3**). The results from the PCA indicated that only stomatal conductance was best at discriminating between *Bombax ceiba* and *Bridelia tomentosa* (**Figure 4** and **Table 2**).

DISCUSSION

Our results clearly revealed that when planting *Bombax ceiba* in Baopoling Mountain (BPL) for performing reforestation, we found that *Bombax ceiba* had a very high survival rate (92% ± 4%) and that mono-planting *Bombax ceiba* can indeed prevent landslides. Moreover, *Bombax ceiba* had a higher growth rate and drought stress tolerance than the most dominant native tree species (*Bridelia tomentosa*) in the nearby undisturbed tropical rainforest when facing the limited water supply in the dry season. Thus, *Bombax ceiba* can be suitable for performing reforestation to restore the highly degraded tropical forests in BPL.

We found that the photosynthetic rate for *Bombax ceiba* was much higher (3 times) than that of *Bridelia tomentosa*. The photosynthetic rate impacts the energy balance of a plant, where, typically, a high photosynthetic rate is linked to a fast-growing rate (Kirschbaum, 2011; Zhang et al., 2018b). This indicated that *Bombax ceiba* could have a higher growth rate

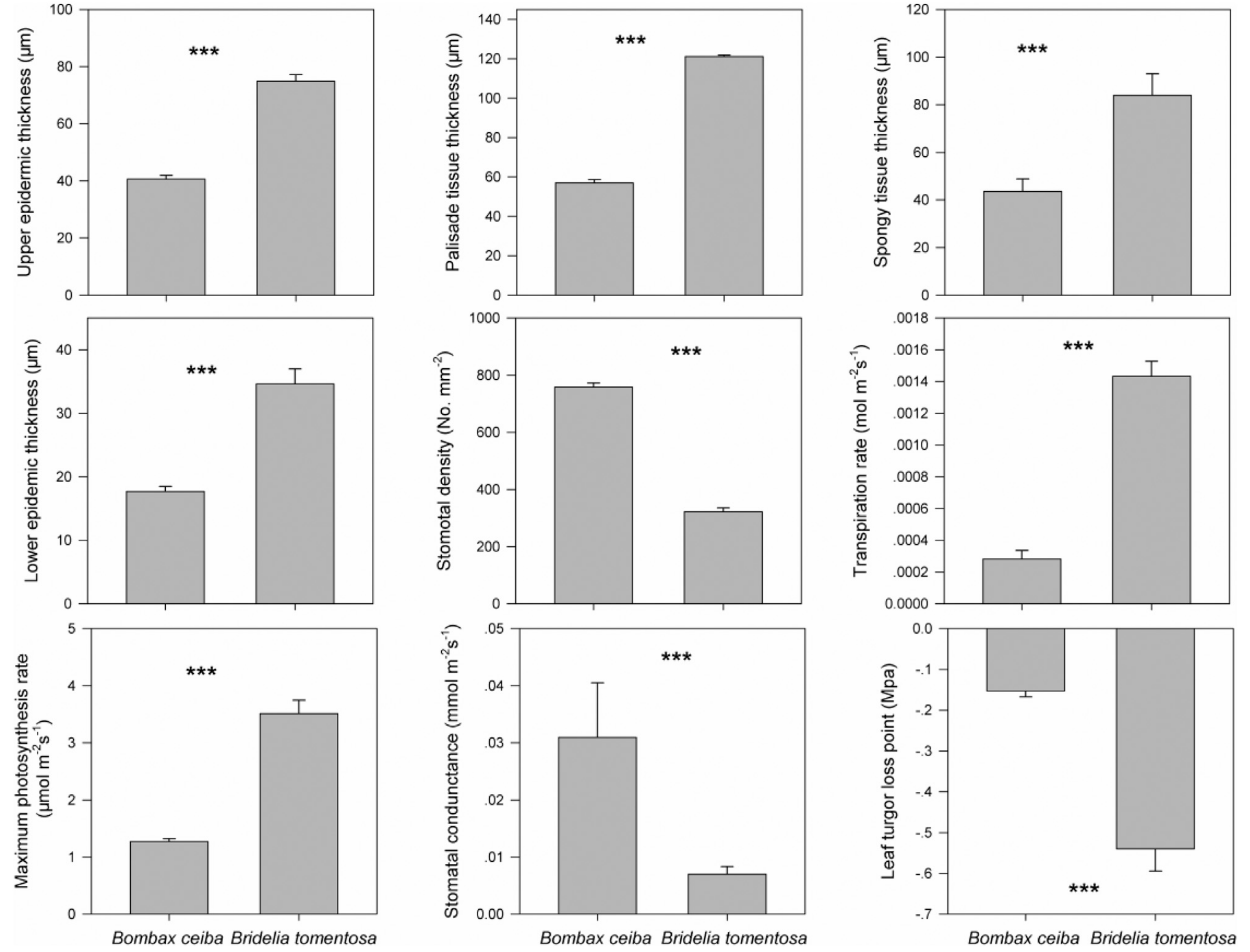

FIGURE 3 | Differences in the nine functional traits (upper epidermis thickness (*μm*), palisade tissue thickness (*μm*), spongy tissue thickness (*μm*), lower epidermis thickness (*μm*), stomatal density (numbers mm^{-2}), transpiration rate (mol m^{-2} s^{-1}), maximum photosynthesis rate (mol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), and leaf turgor loss point (MPa)) between *Bombax ceiba* and *Bridelia tomentosa*. *** indicates $p < 0.001$, based on paired t-tests.

than *Bridelia tomentosa*, even though it had to face drought stress in the dry season. Higher photosynthesis rates will also result in higher leaf anatomy traits (He et al., 2017; Hua et al., 2017), stomatal traits (McAusland et al., 2016; Lawson and Vialet-Chabrand, 2019), and leaf transpiration rates (Santos et al., 2018). That was why we observed these traits for *Bombax ceiba* are all much higher (from 3 to 5 times) than those of *Bridelia tomentosa*. These results indicated that *Bombax ceiba* had developed appropriate functional traits to have very high growth rates even when facing the limited water supply.

We found that the mean precipitation in the dry season was merely 1/10th of that in the wet season, indicating high drought stress in the dry season. We also found *Bombax ceiba* in BPL had a much lower leaf turgor loss point than the native dominant plant species (*Bridelia tomentosa*) in the dry season. A lower leaf turgor loss point is usually observed when leaf cannot get enough water supply (Bartlett et al., 2012). Moreover, plants with a lower leaf turgor loss point can maintain higher stomatal conductance, photosynthesis rates, and higher transpiration rates, when facing very limited water supply (Sack et al., 2003; Mitchell et al., 2008; Blackman et al., 2010). Thus, these results indicated that *Bombax ceiba* had developed higher drought stress tolerance than *Bridelia tomentosa* and thereby can maintain higher growth rates than *Bridelia tomentosa* even when facing the limited water supply. This also demonstrated that *Bridelia tomentosa* can even adapt better to the seasonal drought than the most dominant native species (*Bridelia tomentosa*) in the undisturbed tropical rainforest in the dry season.

Our PCA results revealed that *Bombax ceiba* and *Bridelia tomentosa* were significantly distinguished merely by stomatal conductance. *Bombax ceiba* had much lower stomatal conductance, compared to *Bridelia tomentosa*. Lower stomatal conductance is highly correlated with stomatal closure, which results in a lower leaf turgor point to tolerate drought stress (Brodribb and Holbrook, 2003; Farrell et al., 2017; Trueba et al.,

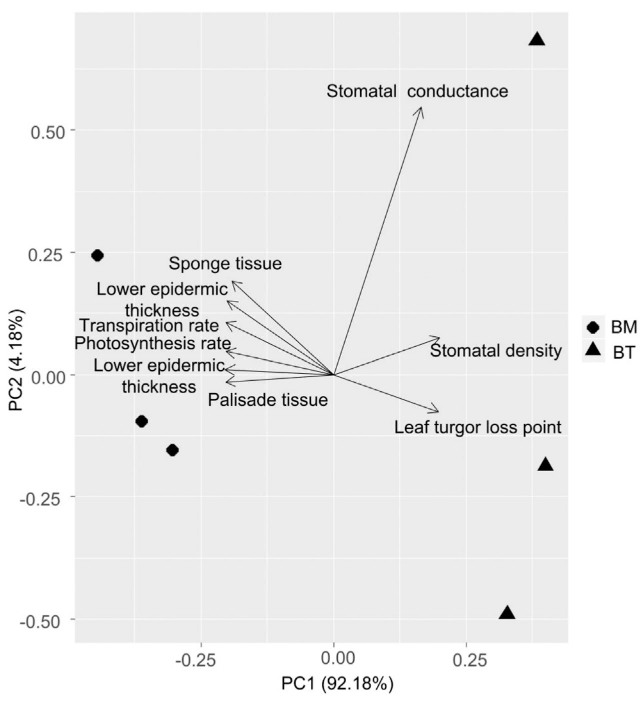

FIGURE 4 | Principal component analysis (PCA) of the nine functional traits between *Bombax ceiba* (BM) and *Bridelia tomentosa* (BT). Diamonds and triangles represent BM and BT respectively.

2019). As a result, a higher intensity of stomatal closure in the dry season should be the key to help *Bombax ceiba* to possess higher drought stress tolerance and growth rates than the most dominant native tree species (*Bridelia tomentosa*) in the undisturbed tropical rainforest (Bartlett et al., 2012).

CONCLUSION

Taken all results together, when planting *Bombax ceiba* to restore the highly degraded tropical forest in BPL, *Bombax ceiba* tends to trigger higher stomatal closure to have higher drought stress tolerance than the most dominant native tree species in the undisturbed tropical rainforest, thereby better adapting well to the drought stress in the dry season and having higher growth and survival rates. Moreover, consistent with previous observations (Stokes et al., 2009; Walker et al., 2009; Pang et al., 2018), mono-planting fast-growing tree species (in our study, *Bombax ceiba*) with high survival rate can indeed help prevent landslides due to frequent typhoon and heavy rain. As a result, *Bombax ceiba* can indeed be a good candidate native species for performing reforestation to restore the degraded tropical rainforest in the whole Hainan Island, China.

Table 2 | The first two axes of a principal component analysis (PCA) for *Bombax ceiba* and *Bridelia tomentosa*, based on nine plant traits. Bold indicates significant.

Functional traits	PC1	PC2
Upper epidermis thickness	−0.35	0.01
Palisade tissue thickness	−0.34	−0.02
Spongy tissue thickness	−0.33	0.28
Lower epidermis thickness	−0.34	0.24
Stomatal density	0.34	0.11
Transpiration rate	−0.34	0.17
Maximum photosynthesis rate	−0.34	0.07
Stomatal conductance	**0.28**	**0.90**
Leaf turgor loss point	0.34	−0.11

The traits include the upper epidermis thickness (μm), palisade tissue thickness (μm), spongy tissue thickness (μm), lower epidermis thickness (μm), stomatal density (numbers mm^{-2}), transpiration rate (μmol m^{-2} s^{-1}), maximum photosynthesis rate (μmol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), and leaf turgor loss point (MPa).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

JL, WH, JY, KJ, and HZ designed the research. JL, WH, KJ, ZT, QH, and JC performed the research. ZT, QH, HZ, and JC analyzed the data. All authors have contributed to the writing of this manuscript.

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REFERENCES

Bartlett, M. K., Scoffoni, C., and Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecol. Lett.* 15 (5), 393–405. doi:10.1111/j.1461-0248.2012.01751.x

Blackman, C. J., Brodribb, T. J., and Jordan, G. J. (2010). Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytol.* 188 (4), 1113–1123. doi:10.2307/40960863

Brodribb, T. J., and Holbrook, N. M. (2003). Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol.* 132 (4), 2166–2173. doi:10.1104/pp.103.023879

Cadotte, M. W. (2017). Functional traits explain ecosystem function through opposing mechanisms. *Ecol. Lett.* 20 (8), 989–996. doi:10.1111/ele.12796

Calder, I. R., and Aylward, B. (2006). Forest and floods. *Water Int.* 31 (1), 87–99. doi:10.1080/02508060608691918

Calder, I. R. (2007). Forests and water - ensuring forest benefits outweigh water costs. *For. Ecol. Manag.* 251 (1), 110–120. doi:10.1016/j.foreco.2007.06.015

Farrell, C., Szota, C., and Arndt, S. K. (2017). Does the turgor loss point characterize drought response in dryland plants? *Plant Cell Environ.* 40 (8), 1500–1511. doi:10.1111/pce.12948

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., et al. (2011). Solutions for a cultivated planet. *Nature* 478 (7369), 337–342. doi:10.1038/nature10452

Griffiths, A. D., Philips, A., and Godjuwa, C. (2003). Harvest of *Bombax ceiba* for the aboriginal arts industry, central arnhem land, Australia. *Biol. Conserv.* 113 (2), 295–305. doi:10.1016/S0006-3207(02)00419-6

Grime, J. P. (2006). Trait convergence and trait divergence in herbaceous plant communities: mechanisms and consequences. *J. Veg. Sci.* 17 (2), 255–260. doi:10.1111/j.1654-1103.2006.tb02444.x

Griscom, B. W., Adams, J., Ellis, P. W., Houghton, R. A., Lomax, G., Miteva, D. A., et al. (2017). Natural climate solutions. *P. Natl. Acad. Sci. USA.* 114 (44), 11645–11650. doi:10.1073/pnas.1710465114

Hall, J. S., Ashton, M. S., Garen, E. J., and Jose, S. (2011). The ecology and ecosystem services of native trees: implications for reforestation and land restoration in Mesoamerica. *For. Ecol. Manag.* 261 (10), 1553–1557. doi:10.1016/j.foreco.2010.12.011

He, N., Liu, C., Miao, T., Li, M., Hao, Y., Yu, G., et al. (2017). Variation in leaf anatomical traits from tropical to cold-temperate forests and linkage to ecosystem functions. *Funct. Ecol.* 32 (8), 1175–1181. doi:10.1111/1365-2435.12934

Hong, W. J., Yang, J. D., Luo, J. H., Jiang, K., Xu, J. Z., and Zhang, H. (2020). Reforestation based on mono-plantation of fast-growing tree species make it difficult to maintain high soil water content in tropics, a case study in Hainan island, China. *Water.* 12 (11), 3077. doi:10.3390/w12113077

Hua, L., Chen, Y., Zhang, H., Fu, P., and Fan, Z. (2017). Stronger cooling effects of transpiration and morphology of the plants from a hot dry habitat than from a hot wet habitat. *Funct. Ecol.* 31 (12), 2202–2211. doi:10.1111/1365-2435.12923

Jian, S., Zhao, C., Fang, S., and Yu, K. (2015). Effects of different vegetation restoration on soil water storage and water balance in the Chinese Loess Plateau. *Agr. For. Meteorol.* 206, 85–96. doi:10.1016/j.agrformet.2015.03.009

Kirschbaum, M. U. (2011). Does enhanced photosynthesis enhance growth? Lessons learned from CO2 enrichment studies. *Plant Physiol.* 155 (1), 117–124. doi:10.1104/pp.110.166819

Lamb, D., Erskine, P. D., and Parrotta, J. A. (2005). Restoration of degraded tropical forest landscapes. *Science* 310 (5754), 1628–1632. doi:10.1126/science.1111773

Lambin, E. F., and Meyfroidt, P. (2011). Global land use change, economic globalization, and the looming land scarcity. *P. Natl. Acad. Sci. USA.* 108 (9), 3465–3472. doi:10.1073/pnas.1100480108

Larjavaara, M., and Muller-Landau, H. C. (2010). Rethinking the value of high wood density. *Funct. Ecol.* 24 (4), 701–705. doi:10.1111/j.1365-2435.2010.01698.x

Laughlin, D. C. (2014). Applying trait-based models to achieve functional targets for theory-driven ecological restoration. *Ecol. Lett.* 17 (7), 771–784. doi:10.1111/ele.12288

Laughlin, D. C., and Laughlin, D. E. (2013). Advances in modeling trait-based plant community assembly. *Trends Plant Sci.* 18 (10), 584–593. doi:10.1016/j.tplants.2013.04.012

Lawson, T., and Vialet-Chabrand, S. (2019). Speedy stomata, photosynthesis and plant water use efficiency. *New Phytol.* 221 (1), 93–98. doi:10.1111/nph.15330

Lohbeck, M., Poorter, L., Lebrija-Trejos, E., Martínez-Ramos, M., Meave, J. A., Paz, H., et al. (2013). Successional changes in functional composition contrast for dry and wet tropical forest. *Ecology* 94 (6), 1211–1216. doi:10.1890/12-1850.1

Lu, Y., Ranjitkar, S., Harrison, R. D., Xu, J., Ou, X., Ma, X., et al. (2017). Selection of native tree species for subtropical forest restoration in southwest China. *PLoS ONE* 12 (1), e0170418. doi:10.1371/journal.pone.0170418

Luo, H. X., Dai, S. P., Li, M. F., and Xi, Z. H. (2018). The climate characteristics of Hainan Island from 1959-2015. *Jiangsu Agr. Sci.* 46 (15), 261–268. doi:10.15889/j.issn.1002-1302.2018.15.066

Luo, J. H., Cui, J., Pandey, S. P., Jiang, K., Tan, Z. Y., He, Q. F., et al. (2020). Seasonally distinctive growth and drought stress functional traits enable Leucaena Leucocephala to successfully invade a Chinese tropical forest. *Trop. Conserv. Sci.* 13, 1–7. doi:10.1177/1940082920949176

Mason, N. W., Bello, F., Mouillot, D., Pavoine, S., and Dray, S. (2013). A guide for using functional diversity indices to reveal changes in assembly processes along ecological gradients. *J. Veg. Sci.* 24, 794–806. doi:10.1111/jvs.12013

Mason, N. W., Richardson, S. J., Peltzer, D. A., de Bello, F., Wardle, D. A., and Allen, R. B. (2012). Changes in coexistence mechanisms along a long-term soil chronosequence revealed by functional trait diversity. *J. Ecol.* 100, 678–689. doi:10.1111/j.1365-2745.2012.01965.x

McAusland, L., Vialet-Chabrand, S., Davey, P., Baker, N. R., Brendel, O., and Lawson, T. (2016). Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. *New Phytol.* 211 (4), 1209–1220. doi:10.1111/nph.14000

McGill, B. J., Enquist, B. J., Weiher, E., and Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends Ecol. Evol.* 21 (4), 178–185. doi:10.1016/j.tree.2006.02.002

Meli, P., Martínez-Ramos, M., Rey-Benayas, J. M., Carabias, J., and Ewald, J. (2014). Combining ecological, social and technical criteria to select species for forest restoration. *Appl. Veg. Sci.* 17 (4), 744–753. doi:10.1111/avsc.12096

Mitchell, P. J., Veneklaas, E. J., Lambers, H., and Burgess, S. S. (2008). Leaf water relations during summer water deficit: differential responses in turgor maintenance and variation in leaf structure among different plant communities in south-western Australia. *Plant Cell Environ.* 31 (12), 1791–1802. doi:10.1111/j.1365-3040.2008.01882.x

Miyawaki, A. (2004). Restoration of living environment based on vegetation ecology: theory and practice. *Ecol. Res.* 19 (1), 83–90. doi:10.1111/j.1440-1703.2003.00606.x

Pang, C. C., Ma, X. K. -K., Lo, J. P. -L., Hung, T. T. -H., and Hau, B. C. -H. (2018). Vegetation succession on landslides in Hong Kong: plant regeneration, survivorship and constraints to restoration. *Glob. Ecol. Conserv.* 15 (2), e00428. doi:10.1016/j.gecco.2018.e00428

Postel, S. L., and Thompson, B. H. (2005). Watershed protection: capturing the benefits of nature's water supply services. *Nat. Resour. Forum.* 29 (2), 98–108. doi:10.1111/j.1477-8947.2005.00119.x

Sack, L., Cowan, P., Jaikumar, N., and Holbrook, N. (2003). The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant Cell Environ.* 26 (8), 1343–1356. doi:10.1046/j.0016-8025.2003.01058.x

Sahin, V., and Hall, M. J. (1996). The effects of afforestation and deforestation on water yields. *J. Hydrol.* 178 (1–4), 293–309. doi:10.1016/0022-1694(95)02825-0

Santos, V. A. H. F. D., Ferreira, M. J., Rodrigues, J. V. F. C., Garcia, M. N., Ceron, J. V. B., Nelson, B. W., et al. (2018). Causes of reduced leaf-level photosynthesis during strong El Niño drought in a Central Amazon forest. *Glob. Change Bio.* 24 (9), 4266–4279. doi:10.1111/gcb.14293

Schulte, P., and Hinckley, T. (1985). A comparison of pressure-volume curve data analysis techniques. *J. Exp. Bot.* 36 (10), 1590–1602. doi:10.1093/jxb/36.10.1590

Stanturf, J. A., Palik, B. J., and Dumroese, R. K. (2014). Contemporary forest restoration: a review emphasizing function. *For. Ecol. Manag.* 331, 292–323. doi:10.1016/j.foreco.2014.07.029

Stokes, A., Atger, C., Bengough, A. G., Fourcaud, T., and Sidle, R. C. (2009). Desirable plant root traits for protecting natural and engineered slopes against landslides. *Plant Soil.* 324 (1), 1–30. doi:10.1007/s11104-009-0159-y

Taubert, F., Fischer, R., Groeneveld, J., Lehmann, S., Müller, M. S., Rödig, E., et al. (2018). Global patterns of tropical forest fragmentation. *Nature* 554, 519–522. doi:10.1038/nature25508

Trueba, S., Pan, R., Scoffoni, C., John, G. P., Davis, S. D., and Sack, L. (2019). Thresholds for leaf damage due to dehydration: declines of hydraulic function, stomatal conductance and cellular integrity precede those for photochemistry. *New Phytol.* 223 (1), 134–149. doi:10.1111/nph.15779

Vörösmarty, C. J., Hoekstra, A. Y., Bunn, S. E., Conway, D., and Gupta, J. (2015). Fresh water goes global. *Science* 349 (6247), 478–479. doi:10.1126/science.aac6009

Walker, L. R., Velázquez, E., and Shiels, A. B. (2009). Applying lessons from ecological succession to the restoration of landslides. *Plant Soil.* 324 (1), 157–168. doi:10.1007/s11104-008-9864-1

Wingfield, M. J., Brockerhoff, E. G., Wingfield, B. D., and Slippers, B. (2015). Planted forest health: the need for a global strategy. *Sci.* 349 (6250), 832–836. doi:10.1126/science.aac6674

Zhang, H., Chen, H. Y. H., Lian, J., John, R., Ronghua, L., Liu, H., et al. (2018a). Using functional trait diversity patterns to disentangle the scale-dependent ecological processes in a subtropical forest. *Funct. Ecol.* 32, 1379–1389. doi:10.1111/1365-2435.13079

Zhang, H., John, R., Zhu, S., Liu, H., Xu, Q., Qi, W., et al. (2018b). Shifts in functional trait–species abundance relationships over secondary subalpine meadow succession in the Qinghai Tibetan Plateau. *Oecologia*. 188 (2), 547–557. doi:10.1007/s00442-018-4230-3

Zhu, S. D., Song, J. -J., Li, R. -H., and Ye, Q. (2013). Plant hydraulics and photosynthesis of 34 woody species from different successional stages of subtropical forests. *Plant Cell Environ.* 36, 879–891. doi:10.1111/pce.12024

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A Trait-Based Protocol for the Biological Control of Invasive Exotic Plant Species

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Selecting appropriate native species for the biological control of invasive exotic plants is a recurring challenge for conservationists, ecologists, and land managers. Recently developed trait-based approaches may be an effective means of overcoming this challenge. However, we lack a protocol and software platform that can be used to quickly and effectively select potential native plant species for performing biological control of the invasive exotic plant species. Here, our study introduces a protocol and a software program that can be used for trait-based selection of appropriate native plant species for performing biocontrol of invasive exotic plant species. In particular, we illustrate the effectiveness of this software program and protocol by identifying native species that can be used for the biological control of *Leucaena leucocephala* (Lam.) de Wit, a highly invasive plant species found in many parts of the world. *Bougainvillea spectabilis* was the only native species selected by our software program as a potential biocontrol agent for *L. leucocephala.* When separately planting 4 seedlings of *B. spectabilis* and two unselected species (*Bombax ceiba,* and *Ficus microcarpa*) as neighbors of each individual of *L. leucocephala* for 3 years, we found that *B. spectabilis*, which was functionally similar to the invasive *L. leucocephala*, significantly limited the invasion of the latter, while the unselected native plant species could not. That was because all the seedling of *B. spectabilis* survived, while half seedlings of unselected species (*B. ceiba* and *F. microcarpa*) died, during the experimental period when planted with *L. leucocephala* seedlings. Moreover, the growth of *L. leucocephala* was restricted when planted with *B. spectabilis,* in contrast *B. ceiba* and *F. microcarpa* did not influence the growth of *L. leucocephala.* Overall, our software program and protocol can quickly and efficiently select native plant species for use in the biological control of invasive exotic plant species. We expect that this work will provide a general protocol to perform biological control of many different types of invasive exotic plant species.

Keywords: biocontrol invasion, invasive exotic plant species, *Leucaena leucocephala*, native plant species, trait-based native plant species selection software platform, tropical seasonal forest

INTRODUCTION

Invasive exotic plant species greatly impact global native plant diversity and ecosystem functioning (Pyšek et al., 2012; Barney et al., 2013). Chemical and mechanical methods have long been used to quickly remove invasive plant species from natural ecosystems (Paynter and Flanagan, 2004; Flory and Clay, 2009). However, these methods are extremely expensive, requiring large amounts of time, money, and resources (Seastedt, 2015). Furthermore, these efforts can often fail, due to a high risk of re-invasion (Kettenring and Adams, 2011). As a result, many ecologists and invasion biologists have instead suggested using suitable native plant species as biological control agents. Such efforts may be a more effective and/or more cost-effective way of preventing the invasion of exotic plant species (Moran et al., 2005; Seastedt, 2015; van Wilgen et al., 2020).

It has been shown that functional traits can explain why and how some exotic plant species successfully invade ecosystems outside their native range (Küster et al., 2008; van Kleunen et al., 2010; Hulme et al., 2013; Ostertag et al., 2015). That is because, functional traits that help to better adapt (e.g., having higher growth rate) to the local environment, and are also functionally distinctive from the native dominant plant species, can successfully facilitate invasive exotic plant species to outperform the native dominant plant species, thereby to successfully invade the local plant ecosystem (Funk et al., 2008). Thus, trait-based methods can be useful for selecting appropriate native species for use as biological control agents (Funk et al., 2008; Laughlin, 2014; Ostertag et al., 2015). Recently, Laughlin (2014) proposed such a trait-based native species selection framework for ecological restoration and preventing further invasions. Similarly, Wang et al. (2020) were able to use these methods to select suitable plant species in the restoration of a highly degraded tropical coral island in China. Here, we seek to examine the effectiveness of trait-based native species selection framework in performing the biocontrol of invasive exotic plant species in a tropical seasonal forest.

van Kleunen et al. (2010) used a meta-analysis to examine trait differences between exotic and native plant species, and identified six types of functional traits that capture key characteristics of invasive exotic plant species. As such, these functional traits can be utilized for the selection of native plant species for use in biocontrol efforts. Here, we introduce a trait-based native plant species protocol (TBNPSP) that can identify native candidate species for biocontrol agents of invasive exotic plant species. Our TBNPSP is based on the theory of limiting similarity, whereby due to competitive exclusion, invasive plant species that are functionally similar to native plant species cannot invade a native plant community (MacArthur and Levins, 1967). Consequently, mixing invasive exotic plant species with functionally similar native plant species may be an effective method for performing biological control of exotic invasive species (D'Antonio and Chambers, 2006; Funk et al., 2008).

Here, we quantify the effectiveness of our model by testifying whether the native species selected by our program can be used for the biological control of *L. leucocephala*, a highly invasive plant species found in many parts of the world (Richardson and Rejmánek, 2011). Due to its high nitrogen-fixing ability, fast-growth rate, and tolerance to drought, *L. leucocephala* has been used widely in the restoration of degraded forest ecosystems worldwide (Goel and Behl, 2002; Wolfe and van Bloem, 2012; Ishihara et al., 2018; Liu et al., 2018; Peng et al., 2019). For example, 20 years of limestone mining associated with the cement industry degraded a 0.2 km^2 area of tropical forest located in Baopoling hill (BPL), Sanya city, China, to bare rock that could hardly support any plant life. In 2015, the local government initiated a large-scale reforestation project in this area. Monocultures of *L. leucocephala* were first planted at the top of BPL in an attempt to quickly restore vegetative cover. Within 2 years, however, *Leucaena leucocephala* has become highly invasive in the BPL, and no other plant species have been able to establish or survive. Moreover, the species has invaded the adjacent undisturbed old-growth tropical forests. The reforestation project on BPL thus provides a perfect system for testing the utility of TBNPSP for restoration effort. Specifically, we judge whether the native plant species selected by our TBNPSP can indeed prevent the invasion of *L. leucocephala* by the following two standards. First, we quantify whether functional traits of selected native species should be very similar to *L. leucocephala*, but dissimilar to the unselected species? Second, we test whether mixture of selected native species and *L. leucocephala* can indeed restrict the growth rate and survival rate of *L. leucocephala,* whereas mixture of unselected native species and *L. leucocephala* cannot achieve this function.

MATERIALS AND METHODS

Step by Step Introduction of the TBNPSP Protocol

Step 1. Choosing Candidate Native Plant Species

Invasive exotic plant species can finish successfully invasion, only when it can not only better adapt (e.g., having higher growth rate) to the local environment than the native dominant plant species, but also are functionally distinctive from the native dominant plant species. Thus, selecting plant species which are functionally similar to invasive exotic plant species among the native dominant plant species can help biocontrol of invasive exotic plant species.

Step 2. Selecting Suitable Functional Traits

Functional traits used for selecting native plant species for biocontrol of invasive exotic species should capture key characteristics of the invasive exotic species. If there is no information on the characteristics of the invasive exotic species, users can apply the six different functional traits identified by van Kleunen et al. (2010) as a start point (see **Table 1**).

Step 3. Using Functional Traits to Select Native Plant Species to Perform Biocontrol of Invasive Exotic Plant Species

Based on the trait-based community framework and limiting similarity theory, Funk et al. (2008) suggested building native communities with traits similar to potential invader to limit

TABLE 1 | Six functional trait that are known to reflect the common characteristics of invasive exotic plant species (van Kleunen et al., 2010).

Trait category	Traits
Physiological traits	Photosynthetic rate, transpiration, leaf construction costs, tissue nitrogen content, nitrogen use efficiency, and water use efficiency
Leaf-area allocation	Leaf area index, leaf area ratio, specific leaf area, leaf mass ratio, and specific leaf mass
Shoot allocation	Shoot-root ratio, root fraction, root-weight ratio and root-shoot ratio
Growth rate	Growth rate Increase in size or biomass over time
Size	Biomass of roots, shoots and complete plants, plant height, and total leaf area
Fitness	All characters measuring number of flowers or seeds per plant, per flower head, per inflorescence, per fruit, all characters associated with seed germination, and all traits associated with survival (stem survival, seedling establishment, mortality, and survival time)

exotic species invasion. To verify this hypothesis, we developed a software program to select native plant species for biocontrol of functionally similar invasive species. Briefly, for a series of candidate species, the software platform calculates the similarity index as a measure of the functional trait similarity between each of the candidate native plant species and the invasive exotic plant species, and ranks them from the highest to the lowest according to the similarity index. A higher similarity index indicates higher similarity between the native plant species and the invasive exotic plant species, therefore suggesting better performance of the native species in the biocontrol of the invasive species. This software program uses the maximum entropy model developed by Shipley et al. (2006) to obtain a set of native plant species that are most functionally similar to the invasive plant species. Our species selection function was integrated into a web-based software platform named the "Plant Species Selection Platform" (Wang et al., 2021). Input data for the program are trait values of the invasive species and the potential native species. The program outputs text and figure results showing similarity ranks between native and invasive species. The software was written in the R language[1], and the main page of the platform is shown in **Supplementary Figure 1**.

Step 4. Testing Whether Mixture of Selected Native Species and Invasive Exotic Plant Species Can Successfully Restrict the Growth and Survival Rate of Invasive Exotic Plant Species

Planting invasive exotic plant species with candidate native plant species selected for and selected against biocontrol by the software program, respectively, to determine whether native species selected for biocontrol are efficient in limiting the invasion of invasive exotic species.

Study Site Description

We tested whether our TBNPSP protocol can indeed select suitable species to perform biocontrol of an invasive exotic plant species (*L. leucocephala*) on Baopoling hill (BPL, 110°58′01″E, 19°38′48″N), which is a limestone hill in Sanya City, Hainan island, China that has an elevation of 300 m (Luo et al., 2020; **Figure 1**). The site has a tropical monsoon oceanic climate with a mean annual temperature of 28°C. The average annual precipitation in Sanya city is 1,500 mm, with approximately 91% of the precipitation occurring between June and October (Luo et al., 2018). The vegetation of the study area is classified as tropical monsoon broad-leaf forest. Limestone mining associated with the cement industry lasted from 1995 till 2015, resulting in a 0.2 km^2 extremely degraded area covered by bare rock (**Figure 1A**). Outside of this degraded area is the undisturbed old-growth tropical monsoon forest (**Figure 1A**). In year 2015 we invested 3 million USA dollars to performing reforestation to recover this 0.2 km^2 extremely degraded area. At the beginning, we used explosive and excavator to remove the top of the degraded area to reconstruct the slope and soil layers to perform reforestation (**Figures 1B,C**). For preventing any of the local people's misunderstanding that we are blasting the hill, *L. leucocephala* is first used to quickly perform the reforestation in 2015 (**Figure 1D**). Only within 2 years, *L. leucocephala* has become highly invasive and no other plant species can survive and grow around *L. leucocephala* (**Figure 1E**). Moreover, *L. leucocephala* has successfully invaded the adjacent undisturbed old-growth tropical forests (**Figure 1F**).

Step by Step Guidance on How to Use the TBNPSP Protocol to Prevent *L. leucocephala*

Step 1. Choosing Candidate Native Plant Species to Prevent *L. leucocephala*

For getting the local dominant native plant species, in 2017, thirty plots, each of 20 × 20 m^2 that were arranged along five parallel transects in the adjacent undisturbed old-growth forest, with 300 m intervals between adjacent plots. Within each plot, all freestanding plants were measured and identified to species. Here, we used the 11 dominant native plant species (relative abundance range from 6 to 10%) (*Bougainvillea spectabilis, Bombax ceiba,* and *Ficus microcarpa*, *Bridelia tomentosa, Radermachera frondosa, Lepisanthes rubiginosa, Rhaphiolepis indica, Pterospermum heterophyllum, Fissistigma oldhamii, Psychotria rubra,* and *Cudrania cochinchinensis*) as our biological control agents.

Step 2. Functional Trait Selection and Measurements

It has been found that high growth rates and drought stress tolerance help *L. leucocephala* outcompete native species in multiple places (Wolfe and van Bloem, 2012; Chiou et al., 2016; Barros et al., 2020). Previous work also suggests that

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[1] https://www.r-project.org/

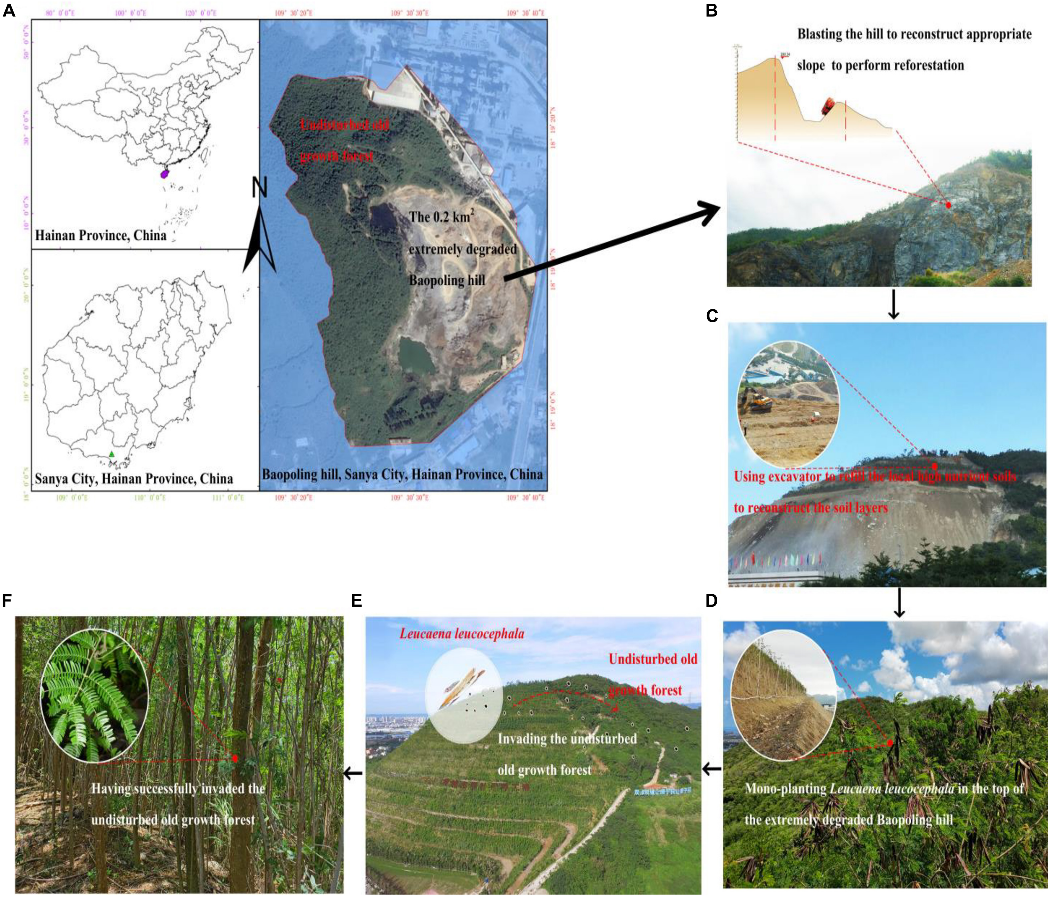

FIGURE 1 | Map of the study site (Baopoling hill) and the surrounding landscape, including the 0.2 km^2 area of extremely degraded land and the undisturbed old growth forest **(A)** and the detail processes for describing why and how *Leucaena leucocephala* not only becomes highly invasive in Baopoling hill, but also has successfully invaded the undisturbed old growth forest **(B–F)**.

traits that are highly associated with fast-growth (i.e., maximum photosynthesis rate, stomatal conductance and transpiration) and drought tolerance (i.e., leaf turgor loss point) are key characteristics facilitating the invasion of *L. leucocephala* in the BPL (Luo et al., 2020). As such, we expect that these traits can be used to select native plant species to perform biocontrol of this invasive species. To verify this, in the peak of both the wet (July) and the dry (February) seasons in 2018, we measured maximum photosynthesis rate, stomatal conductance, transpiration, and leaf turgor loss point for both *L. leucocephala* and the 11 candidate native plant species. We collected 20 fully expanded and healthy leaves from five individuals from each of the 12 species in February 2017 (the peak of the dry season) for trait measurement. For avoiding intraspecific variances in functional traits measurements, samples were collected from individuals that had a diameter at breast height (DBH) close to the species mean value (**Supplementary Table 1**), which was obtained from an extensive vegetation sample conducted at the site. Leaf samples were used to measure a suite of functional traits related to plant growth (i.e., maximum photosynthesis rate (Amass; μmol m^{-2} s^{-1}), stomatal conductance (SC; mmol m^{-2} s^{-1}), transpiration (TR; μmol m^{-2} s^{-1}), and drought stress tolerance (TLP, Mpa). These traits were listed and introduced in **Table 2**. Traits were measured following the methods described in Zhang et al. (2018), and the detailed procedures for measuring these functional traits can be found in the **Supplementary Materials**.

Step 3. Using Functional Traits to Select Native Plant Species to Prevent *L. leucocephala*

Inputting all measured traits for *L. leucocephala* and all candidate native plant species into our developed software program to automatically select native plant species for biocontrol of *L. leucocephala*.

Step 4. Testing Whether Mixture of Selected Native Species and *L. leucocephala* Can Successfully Restrict the Growth and Survival Rate of *L. leucocephala*

It was showed that mixed plantations of *Eucalyptus camaldulensis* and *Eucalyptus citriodora* can restrict the

TABLE 2 | Descriptions of the selected plant traits and abbreviations used in this study.

Trait	Abbreviation	Unit	Information compilation source	Ecological relevance	Ecophysiological mechanism
Leaf turgor loss point	*TLP*	MPa	Field sampling and laboratory measurements	Drought stress tolerance	Lower TLP could have higher drought stress tolerance
Maximum photosynthetic rate	$A_{\max}$	μmol m^{-2} s^{-1}	Field measurements	Photosynthetic capacity	Directly reflect growth rate
Stomatal conductivity	g_s	mol m^{-2} s^{-1}	Field measurements	Stomatal adjustment and water retention	Directly reflect gas exchange rates of water and oxygen
Transpiration rate	*E*	mmol m^{-2} s^{-1}	Field measurements	Leaf water utilization	Directly reflect leaf water loss

Sources and physiological functions are also shown.

growth of *L. leucocephala*, making them effective biocontrol agents of *L. leucocephala* (Liu et al., 2018). Here, we planted *L. leucocephala* with our candidate native plant species selected for and selected against biocontrol by our software program, respectively, to determine (1) whether native species selected for biocontrol are efficient in limiting the invasion of *L. leucocephala*; and (2) whether those native species selected against biocontrol perform inferiorly to those selected for. To accomplish these, we measured the average height and diameter at breast height (DBH) of all individuals of mono-planted *L. leucocephala* in BPL in 2017. We first tried to purchase seedlings of *L. leucocephala* and each of the 11 candidate native plant species with the same average height and DBH as mono-planted *L. leucocephala* in BPL from a local market. We planted monocultures of *L. leucocephala* (MLL) seedlings 3 m apart and treated it as a control in a nursery nearby. Mixture of *L. leucocephala* and native species were created in the same nursery as below. We first regularly planted 25 individuals of *L. leucocephala*, and then regularly planted 4 seedlings of a specific native species as neighbors of each individual of *L. leucocephala.* In 2020, we collected survival status (death or survival), and the height and DBH of each survival seedling. Detail statistic methods for analyzing whether selected native plant species can prevent *L. leucocephala.* were shown as below:

First, we used our native plant species selection software to select native plant species that were functionally similar/dissimilar to *L. leucocephala.* We then verified the results of the species selection via manual test. To do so, we performed a Wilcoxon signed-rank test to compare differences in the traits (i.e., photosynthesis rate, stomatal conductance, transpiration rate, and leaf turgor loss point) between *L. leucocephala* and each of the 11 native species to test: (1) whether native plant species selected by our program are functionally similar to *L. leucocephala;* and (2) whether native species that were not selected by our program are functionally dissimilar to *L. leucocephala.* Finally, we compared the differences in average height and DBH among individuals of *L. leucocephala* growing in the monoculture, and those individuals growing with selected and unselected native species to test whether selected native species can limit the growth of *L. leucocephala,* whereas unselected native species cannot.

RESULTS

Bougainvillea spectabilis was the only species selected by our software program as a potential biocontrol agent for *L. leucocephala*, having a similarity index value of 0.99 (**Figure 2**). Similarity index values for the remaining 10 native tree species ranged from 0.001 to 0.01, indicating that they were extremely and functionally dissimilar to *L. leucocephala* (**Figure 2**). The Wilcoxon signed-rank test further confirmed that *B. spectabilis* was highly similar to *L. leucocephala,* whereas the remaining 10 native plant species were all extremely dissimilar to *L. leucocephala.* There was no difference in the measured four traits (maximum photosynthesis rate, stomatal conductance, transpiration rate and leaf turgor loss point) between *B. spectabilis* and *L. leucocephala* ($p > 0.05$, **Figure 3**).

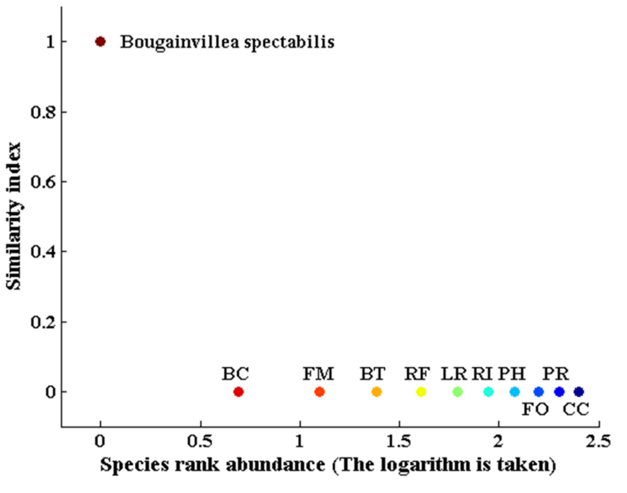

FIGURE 2 | Native plant species selection result based on our native plant species selection program. This result uses a similarity index to determine the functional trait similarity between each of the 11 candidate native plant species (i.e., BS, *Bougainvillea spectabilis*; BC, *Bombax ceiba*; FM, *Ficus microcarpa*; BT, *Bridelia tomentosa*; RF, *Radermachera frondosa*; LR, *Lepisanthes rubiginosa*; RI, *Rhaphiolepis indica*; PH, *Pterospermum heterophyllum*; FO, *Fissistigma oldhamii*; PR, *Psychotria rubra*; CC, *Cudrania cochinchinensis*) and the invasive exotic plant species (*Leucaena leucocephala*). A higher similarity index indicates higher similarity between the native plant species and the invasive exotic plant species.

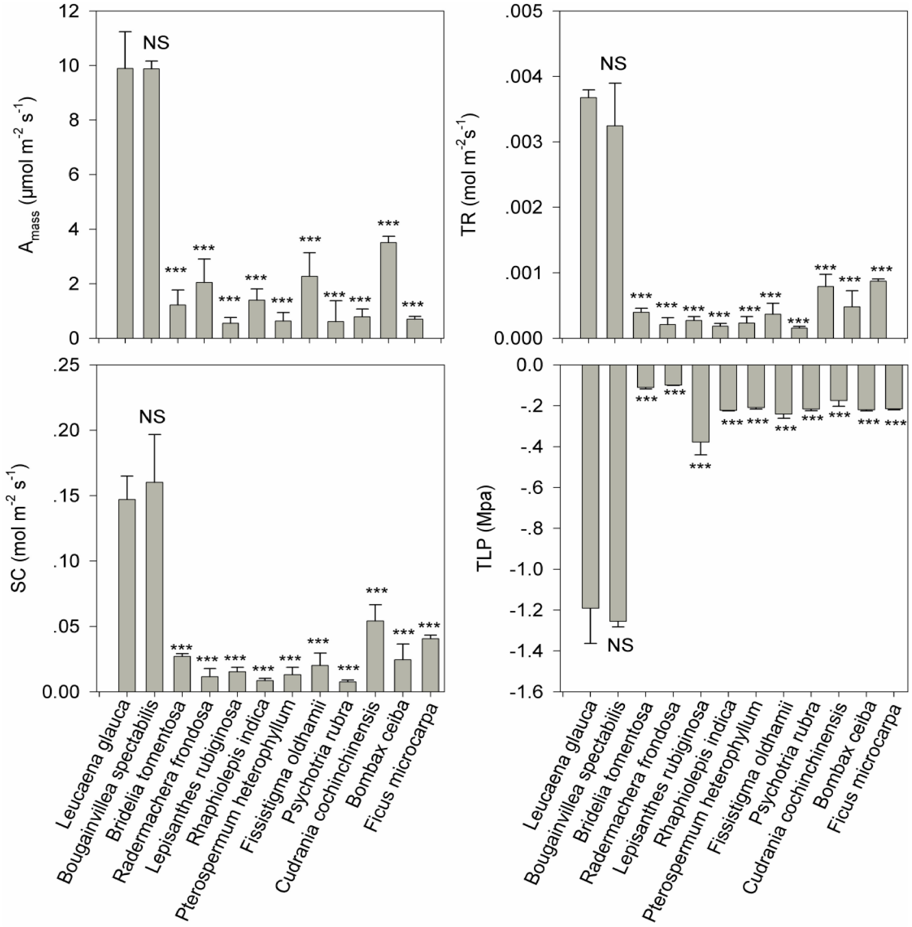

FIGURE 3 | Differences in the four functional traits (i.e., transpiration rate, TR; maximum Photosynthesis rate, Amass; stomatal conductance, SC; and leaf turgor loss point, TLP) between the invasive exotic plant species *L. leucocephala*, and each of the 11 native plant species (i.e., *B. spectabilis, B. ceiba* and *F. microcarpa, B. tomentosa, R. frondosa, L. rubiginosa, R. indica, P. heterophyllum, F. oldhamii, P. rubra,* and *C. cochinchinensi*). *** indicates $P < 0.05$ and NS (short for non-significant) indicates $P > 0.05$ based on Wilcoxon signed-rank test.

In contrast, *L. leucocephala* had much higher A_{mass}, SC and TR, and much lower TLP, than the remaining native species ($p < 0.05$, **Figure 3**).

Among the 10 unselected native candidate species, we were only able to purchase seedlings of *B. ceiba,* and *F. microcarpa* in the local market. Thus, we followed the mixing planting design in **Figure 4** to first monocultures of *L. leucocephala* (MLL) seedlings 3 m apart and treated it as a control in a nursery nearby. We then regularly planted 25 individuals of *L. leucocephala*, then regularly planted 4 seedlings of the selected native plant species (*B. spectabilis*) and unselected native plant species (*B. ceiba,* and *F. microcarpa*) as neighbors of each individual of *L. leucocephala.* After 3 years' plantation, we found that all 100 *B. spectabilis* seedlings survived with *L. leucocephala* seedlings. In contrast, only 40–45 seedlings of *B. ceiba* and *F. microcarpa* survived when planted with *L. leucocephala*, respectively. The average height and DBH of monocultured *L. leucocephala* were 1.4 times greater than individuals planted with *B. spectabilis* ($p < 0.05$, **Figures 5A,D**). In contrast, there was no significant difference in the average height or DBH of *L. leucocephala* when planted with *B. ceiba* (LL2) or *F. microcarpa* (LL3) ($p > 0.05$, **Figures 5A,D**). The average height and DBH of *L. leucocephala* were 33% ($p < 0.05$, **Table 1** and **Figures 5B,E**), and 17–23% ($p < 0.05$, **Figures 5B,E**) lower than the selected native species (*B. spectabilis*) and unselected species (*B. ceiba,* and *F. microcarpa*), respectively. In addition, the average height and DBH of *B. spectabilis* were 1.3 times greater than *L. leucocephala* in the monoculture ($p < 0.05$, **Figures 5C,F**). In summary, the survival and growth of *L. leucocephala* was restricted when planted with *B. spectabilis,* whereas *B. ceiba* and *F. microcarpa* did not significantly influence the survival and growth of *L. leucocephala.*

DISCUSSION

Here we introduce a trait-based native plant species selection protocol and a software program that can be used for selection of appropriate native plant species for performing biocontrol of invasive exotic plant species.

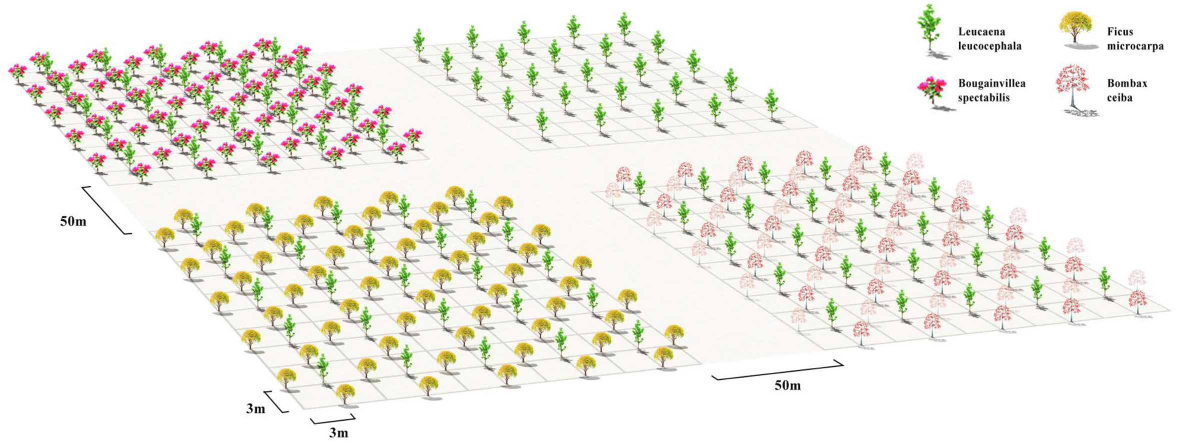

FIGURE 4 | The design of *L. leucocephala* monocultures (MLL), mixed plantation of *B. spectabilis* and *L. leucocephala,* mixed plantation of *B. ceiba* and *L. leucocephala,* and mixed plantation of *F. microcarpa* and *L. leucocephala.* MLL is made of 25 monocultured seedlings of *L. leucocephala* at 3 m spacing. BS vs. LL1 is mixing 100 *B. spectabilis* seedings with 25 *L. leucocephala* seedlings at 3 × 3 m spacing, with 4 *B. spectabilis* seedings surrounding one *L. leucocephala* seedling. Mixed plantation of *B. ceiba* and *L. leucocephala,* and mixed plantation of *F. microcarpa* and *L. leucocephala* uses the same mixing design as mixed plantation of *B. spectabilis* and *L. leucocephala*.

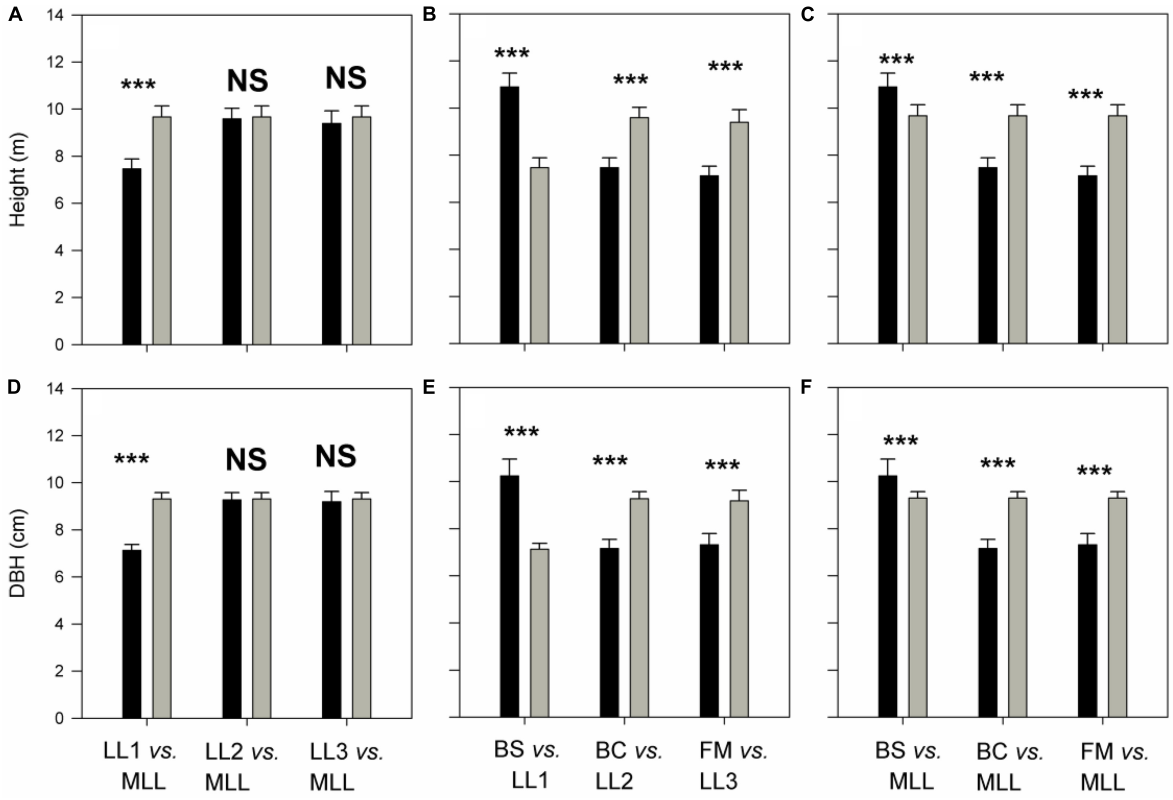

FIGURE 5 | Differences in average height and diameter at breast height (DBH) among monocultured *L. leucocephala* (MLL), *L. leucocephala* that is mixed with selected native species (*B. spectabilis*) (LL1), *L. leucocephala* mixed with unselected native species (*B. ceiba and F. microcarpa*) (LL2 and LL3, respectively) and selected and unselected native species (*B. spectabilis, B. ceiba, and F. microcarpa*) on Baopoling hill after 3 years of planting. **(A,D)** Demonstrate the differences in average height and DBH between individuals of *L. leucocephala* in LL1 and MLL (LL1 vs. MLL), between LL2 and MLL (LL2 vs. MLL) and between LL3 and MLL (LL3 vs. MLL). **(B,E)** represent the differences in average height and DBH between *L. leucocephala* in LL1 and *B. spectabilis* (BS vs. LL1), between *L. leucocephala* in LL2 and *B. ceiba* (BC vs. LL2) and between *L. leucocephala in* LL3 and *F. microcarpa* (FM vs. LL3). **(C,F)** show the differences in average height and DBH between *L. leucocephala* in MLL and BS, between MLL and BC and between MLL and FM. Detail seedlings planting design are provided in **Figure 4**. *** indicates $P < 0.05$ and NS (short for non-significant) indicates $P > 0.05$ based on Wilcoxon signed-rank test.

Using *L. leucocephala* as an example, we verified the power of our protocol and the software program in selecting potential biocontrol agents based on specific functional trait targets. By using the selected four functional traits (maximum photosynthesis rates, stomatal conductance, transpiration rate, and leaf turgor loss points), *B. spectabilis* was the only species selected by our software program as a potential biocontrol agent for *L. leucocephala.* By comparing the differences in these four traits between *L. leucocephala* and each of the 11 native plant species, we verified the results of our software program that *B. spectabilis* was the only species that was functionally similar to *L. leucocephala.* Interestingly, the remaining 10 native plant species were extremely dissimilar to *L. leucocephala.* As expected, *B. spectabilis*, which is functionally similar to the invasive *L. leucocephala*, significantly limited the invasion of the latter, while the unselected native plant species could not. All the seedling of *B. spectabilis* survived, while half seedlings of unselected species (*B. ceiba* and *F. microcarpa*) died, during the experimental period when planted with *L. leucocephala* seedlings. Moreover, the growth of *L. leucocephala* was restricted when planted with *B. spectabilis,* in contrast *B. ceiba* and *F. microcarpa* did not influence the growth of *L. leucocephala.* Ultimately, our results demonstrated the power of our software program in selecting appropriate native plant species for the biological control of invasive exotic plant species.

Although the widely used traditional trial-and-error method could also be applied for selecting appropriate native species for the biocontrol of the invasive species, it is time and effort consuming because it may need to conduct a large experiment for several years. Moreover, such methods are likely limited to a small number of species because the number of treatments and replicates will exponentially increase with the number of candidate species (Giardina et al., 2007; Funk et al., 2008; Hall et al., 2011; Williams, 2011; Kanowski, 2014). Here we showed that, using our protocol, selecting appropriate native species as biological control agents can be fulfilled easily and efficiently. Most importantly, a large number of candidate native species can be considered simultaneously, given the availability of specific functional traits targeted for the biocontrol of the invasive exotic plant species.

We noted that merely using growth rates as indictor for the biocontrol of *L. leucocephala* may be not fully sufficient. Ecological factors that structure plant communities, determine growth and survival of individual plants, and affect plant evolution (e.g., root and shoot competition, light competition, soil heterogeneity), biomass, the number of fruits or seeds, roots development merits future survey. In addition, it is very necessary to quantify whether *B. ceiba* and *F. microcarpa* seedling mortality is due to competition with *L. leucocephala* in future.

In summary, we developed a protocol that includes the following steps for selecting suitable native plant species for biocontrol. First, identify functional traits that can capture key characteristics of the invasive exotic species. If there is no information on the characteristics of the invasive exotic species, users can apply the six different functional traits identified by van Kleunen et al. (2010) as a start point (see **Table 2**). Second, a potential native plant species pool should be selected using communities found in an adjacent area to the study site or similar habitats. Third, careful measurements of the selected functional traits should be performed for both exotic and native species. Fourth, input trait data to the native plant species selection software platform to select functionally similar native plant species. Finally, mix the selected native plant species with populations of the invasive exotic plant species, with the hope that they will act as biocontrol agents to reduce the growth and spread of the invasive species. Taken together, we demonstrated that our native plant species selection protocol and software program can quickly and efficiently select native plant species for use in the biological control of invasive exotic plant species. We expect that this work provides a general protocol to help conservationists and land managers perform biological control of various types of invasive exotic plant species. However, we have to admit our current results can only prove the native plant species selection software program and protocol is feasible and effective for biocontrol of tree species. Usually, many invasive exotic plant species in forest ecosystem are vine and herbaceous species, but not tree species. Thus, future researches are still necessary to testify the effectiveness of our native plant species selection software program and protocol in preventing the invasion of vine and herbaceous species.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HZ, KJ, YZ, JC, TDL, and CW designed the research. HZ, KJ, YZ, YTX, HJG, JC, TDL, and CW performed the research. HZ, KJ, YZ, JC, TDL, and CW analyzed the data. HZ, KJ, YZ, JC, TDL, and CW wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.586948/full#supplementary-material

REFERENCES

Barney, J. N., Tekiela, D. R., Dollete, E. S., and Tomasek, B. J. (2013). What is the "real" impact of invasive plant species? *Front. Ecol. Environ.* 11:322–329. doi: 10.1890/120120

Barros, V., Melo, A., Santos, M., Nogueira, L., Frosi, G., and Santos, M. G. (2020). Different resource-use strategies of invasive and native woody species from a seasonally dry tropical forest under drought stress and recovery. *Plant Physiol. Biochem.* 147, 181–190. doi: 10.1016/j.plaphy.2019.12.018

Chiou, C.-R., Chen, Y.-J., Wang, H. H., and Grant, W. E. (2016). Predicted range expansion of the invasive plant Leucaena leucocephala in the Hengchun peninsula. Taiwan. *Biol. Invasions* 18, 381–394. doi: 10.1007/s10530-015-1010-4

D'Antonio, C. M., and Chambers, J. C. (2006). "Using ecological theory to manage or restore ecosystems affected by invasive plant species," in *Foundations of Restoration Ecology*, eds D. A. Falk, M. A. Palmer, and J. B. Zedler (Covelo, CA: Island Press), 260–279.

Flory, S., and Clay, K. (2009). Invasive plant removal method determines native plant community responses. *J. Appl. Ecol.* 46, 434–442. doi: 10.1111/j.1365-2664.2009.01610.x

Funk, J. L., Cleland, E. E., Suding, K. N., and Zavaleta, E. S. (2008). Restoration through reassembly: plant traits and invasion resistance. *Trends Ecol. Evol.* 23, 695–703. doi: 10.1016/j.tree.2008.07.013

Giardina, C. P., Litton, C. M., Thaxton, J. M., Cordell, S., Hadway, L. J., and Sandquist, D. R. (2007). Science Driven restoration: a candle in a demon haunted World—Response to cabin. *Restor. Ecol.* 15, 171–176. doi: 10.1111/j.1526-100X.2007.00227.x

Goel, V. L., and Behl, H. M. (2002). Selection of Leucaena species for afforestation and amelioration of sodic soils. *Land Degrad. Dev.* 13, 387–393. doi: 10.1002/ldr.518

Hall, J. S., Ashton, M. S., Garen, E. J., and Jose, S. (2011). The ecology and ecosystem services of native trees: implications for reforestation and land restoration in Mesoamerica. *For. Ecol. Manag.* 261, 1553–1557. doi: 10.1016/j.foreco.2010.12.011

Hulme, P. E., Pyšek, P., Jarošík, V., Pergl, J., Schaffner, U., and Vila, M. (2013). Bias and error in understanding plant invasion impacts. *Trends Ecol. Evol.* 28, 212–218. doi: 10.1016/j.tree.2012.10.010

Ishihara, K. L., Honda, M. D., Bageel, A., and Borthakur, D. (2018). "*Leucaena leucocephala*: a leguminous tree suitable for eroded habitats of Hawaiian islands," in *Ravine Lands: Greening for Livelihood and Environmental Security*, eds J. C. Dagar and A. K. Singh (Berlin: Springer), 413–431. doi: 10.1007/978-981-10-8043-2

Kanowski, J. (2014). *Restoration Ecology: The New Frontier*, 2nd Edn, Vol. 15, eds J. Van Andel and J. Aronson, Oxford: Blackwell Publishing, 1–7.

Kettenring, K. M., and Adams, C. R. (2011). Lessons learned from invasive plant control experiments: a systematic review and meta-analysis. *J. Appl. Ecol.* 48, 970–979. doi: 10.1111/j.1365-2664.2011.01979.x

Küster, E. C., Kühn, I., Bruelheide, H., and Klotz, S. (2008). Trait interactions help explain plant invasion success in the German flora. *J. Ecol.* 96, 860–868. doi: 10.1111/j.1365-2745.2008.01406.x

Laughlin, D. C. (2014). Applying trait-based models to achieve functional targets for theory-driven ecological restoration. *Ecol. Lett.* 17, 771–784. doi: 10.1111/ele.12288

Liu, F., Gao, C., Chen, M., and Li, K. (2018). Above- and below-ground biomass relationships of *Leucaena leucocephala* (Lam.) de Wit in different plant stands. *PLoS One* 13:e0207059. doi: 10.1371/journal.pone.0207059

Luo, H. X., Dai, S. P., Li, M. F., and Xi, Z. H. (2018). The climate characteristics of 302 Hainan Island from 1959-2015. *Jiangsu Agric. Sci.* 46, 261–268. doi: 10.15889/j.issn.1002-1302.2018.15.066

Luo, J. H., Cui, J., Jiang, K., Tan, Z. Y., He, Q. F., Zhang, H., et al. (2020). Invariably fast-growing from wet to dry season and high drought stress tolerance in the dry season make *Leucaena leucocephala* successfully invade tropical forest. *Trop Conserv. Sci.* 13, 1–7.

MacArthur, R., and Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat.* 101, 377–385. doi: 10.1086/282505

Moran, V. C., Hoffmann, J. H., and Zimmermann, H. G. (2005). Biological control of invasive alien plants in South Africa: necessity, circumspection, and success. *Front. Ecol. Environ.* 3:71–77. doi: 10.2307/3868513

Ostertag, R., Warman, L., Cordell, S., and Vitousek, P. M. (2015). Using plant functional traits to restore Hawaiian rainforest. *J. Appl. Ecol.* 52, 805–809. doi: 10.1111/1365-2664.12413

Paynter, Q., and Flanagan, G. J. (2004). Integrating herbicide and mechanical control treatments with fire and biological control to manage an invasive wetland shrub, mimosa pigra. *J. Appl. Ecol.* 41, 615–629. doi: 10.1111/j.0021-8901.2004.00931.x

Peng, S. H., Wang, H. H., and Kuo, Y. L. (2019). Methods for preventing the invasion of *Leucaena leucocephala* in Coastal Forests of the Hengchun Peninsula. Taiwan. *Taiwan J. For. Sci.* 34, 99–112.

Pyšek, P., Jarošík, V., Hulme, P. E., Pergl, J., Hejda, M., Schaffner, U., et al. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Glob. Change Biol.* 18, 1725–1737. doi: 10.1111/j.1365-2486.2011.02636.x

Richardson, D. M., and Rejmánek, M. (2011). Trees and shrubs as invasive alien species-a global review. *Divers. Distrib.* 17, 788–809. doi: 10.1111/j.1472-4642.2011.00782.x

Seastedt, T. R. (2015). Biological control of invasive plant species: a reassessment for the Anthropocene. *New Phytol.* 205, 490–502. doi: 10.1111/nph.13065

Shipley, B., Vile, D., and Garnier, É (2006). From plant traits to plant communities: a statistical mechanistic approach to biodiversity. *Science* 314, 812–814. doi: 10.1126/science.1131344

van Kleunen, M., Weber, E., and Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecol. Lett.* 13, 235–245. doi: 10.1111/j.1461-0248.2009.01418.x

van Wilgen, B. W., Raghu, S., Sheppard, A. W., and Schaffner, U. (2020). Quantifying the social and economic benefits of the biological control of invasive alien plants in natural ecosystems. *Curr. Opin. Insect Sci.* 38, 1–5.

Wang, C., Jian, S., Ren, H., Yan, J., and Liu, N. (2021). A web-based software platform for restoration-oriented species selection based on plant functional traits. *Front. Ecol. Evol.* 9:570454. doi: 10.3389/fevo.2021.570454

Wang, C., Zhang, H., Liu, H., Jian, S., Yan, J., and Liu, N. (2020). Application of a trait-based species screening framework for vegetation restoration in a tropical coral island of China. *Funct. Ecol.* 34, 1193–1204. doi: 10.1111/1365-2435.13553

Williams, B. K. (2011). Adaptive management of natural resources—framework and issues. *J. Environ. Manag.* 92, 1346–1353. doi: 10.1016/j.jenvman.2010.10.041

Wolfe, B. T., and van Bloem, S. J. (2012). Subtropical dry forest regeneration in grass-invaded areas of Puerto Rico: understanding why *Leucaena leucocephala* dominates and native species fail. *For. Ecol. Manag.* 267, 253–261. doi: 10.1016/j.foreco.2011.12.015

Zhang, H., Chen, H. Y., Lian, J., John, R., Ronghua, L., Liu, H., et al. (2018). Using functional trait diversity patterns to disentangle the scale-dependent ecological processes in a subtropical forest. *Funct. Ecol.* 32, 1379–1389. doi: 10.1111/1365-2435.13079

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Effects of Typhoon Mangkhut on a Monsoon Evergreen Broad-Leaved Forest Community in Dinghushan Nature Reserve, Lower Subtropical China

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Sensitivity to climate change is one of the key features of the Dinghushan National Nature Reserve that is located in the lower subtropical China. Having faced typhoon Mangkhut in 2018, there emerged a need to study the effects that the typhoon had on the evergreen broad-leaved forest community in Dinghushan. The forest composition data for 2015, 2018, and 2020 was used in this study. The aim was to establish a scientific basis for the restoration of natural forests that have the ability to withstand strong weather phenomena such as typhoons and the hypothesis stated that typhoon Mangkhut had a long-term detrimental effect on the forest community in Dinghushan forest. The results showed that trees that have a DBH of less than 5 cm and a height of less than 2 m were more prone to damage during a severe weather events. In 2015, there was a total of 5,682 trees per hectare while in 2018 there was a total of 5,022 trees per hectare showing a decline in the number of trees per hectare of 660 due to the typhoon. Number of trees in each DBH class, height class, total above ground dry weight and average tree height was also lower in 2018 as compared to 2015 and 2020. The species abundance was also adversely affected with a loss of 672 species per hectare in 2018 compared to 2015. In 2020, about 83% of all trees had a DBH of less than 5 cm and an average height of 3.8 m making up a large proportion of trees that could be damaged if another storm occurred of similar or greater magnitude as the one that hit the region in 2018. Of interest was the forests remarkable ability to bounce back as there is an increase in biomass when 2018 is compared to 2020. The results support the hypothesis of the study. It is therefore of utmost importance that strategies be put in place to protect this ecosystem and others of a similar nature, by employing the use of natural forests that have great resilience against typhoons.

Keywords: Dinghushan Nature Reserve, typhoon Mangkhut, community composition and structure, biomass, monsoon evergreen broad-leaved forest

INTRODUCTION

Climate change is threatening tropical forest communities in a variety of ways, such as increasing the frequency of severe drought, large fires, storms, and flooding (Davidson et al., 2012). There are various scientific arguments on the relationship between typhoons and global climate change, but relevant studies support the fact that typhoons are closely related to global climate change. It has been demonstrated through climatic simulations that under the influence of climate change, the severity of typhoons will increase in frequency and magnitude especially in lower latitudes, such as the latitude where lower subtropical China is found, more than in higher latitudes, a prediction that puts evergreen broad-lived forests such as Dinghushan in direct harm's way in future typhoon events that will inevitably occur (Takemi et al., 2016). As a natural disturbance factor, a typhoon has a short influence time but great destructive power. It not only has strong winds, but also brings a sharp increase in precipitation (Lin et al., 2011); it directly causes mechanical damage to the forest community, and affects the species composition and structure, water, litter, and soil nutrient cycling processes and ecosystem stability in the forest community (Kauffman and Cole, 2010; Wang et al., 2018). Tropical cyclones also have the capacity to cause canopy gaps of various scales that have an impact on understory light permeability and nutrient availability thus impacting forest regeneration and species composition, moreover, forests that have a high density such as Dinghushan experience greater damage due to a high ratio of tree height to diameter breast height and relatively weak roots (Liu et al., 2012). The deposition of large amounts of litterfall into the forest floor as a result of a typhoon has been recorded to increase nutrient availability such as P or K in the soil beyond normal amounts as the fresh vegetation fall contains more nutrients thus leading to a situation where the microorganisms and vegetation are unable to breakdown, absorb, and retain the nutrients in the ecosystem leading to nutrient loss through runoff and stream water (Liu et al., 2012; Peng et al., 2012; Lin K. C. et al., 2017). For a given stem diameter, trees that were shorter with dense and stiffer wood were found to be more likely to be uprooted while those with strong wood were more likely to be snapped during a storm (Putz et al., 1983). In the past two decades, many scientists have carried out extensive research on the impact of typhoons on forest communities (Lin et al., 2011; Lin K. C. et al., 2017). The impact of storm events on vegetation has also been systematically studied all over the world. In the Caribbean for example, hurricanes have been found to impact ecosystems and alter the structure of plant and animal communities (Walker et al., 1991). The impact of a typhoon on forests in Australia has also been studied and the effects documented (Bellingham, 2008). China is one of the main countries affected by typhoons, with frequent typhoons along the southeast coast, especially in Hainan. For the tropical forest in Hainan, a typhoon affects the redistribution of rainstorms, causing changes in hydrological function, mechanical damage of the forest community, increased litter and increased soil loss (Xu et al., 2008; Liu et al., 2012). In China, however, the impact of a typhoon on the subtropical monsoon evergreen broad-leaved forest community has not been reported, and the research on typhoons in China is less developed compared to other natural disasters such as fire and drought.

The super typhoon "Mangkhut" that occurred on September 16, 2018 had a profound impact on the forest ecosystem of Dinghushan Nature Reserve, especially as it is a monsoon evergreen broad-leaved forest. The occurrence of the typhoon led to a large number of fallen trees, broken branches, and fallen leaves. Long term monitoring of vegetation favored the studying of the influence of a typhoon on the monsoon evergreen broad-leaved forest systematically and comprehensively in lower subtropical China. In this study, the vegetation composition and structure of a monsoon evergreen broad-leaved forest before and after the typhoon were compared and analyzed, to find out the community structure and species diversity change law, analyze the impact factors of wind fallen trees and the impact of a typhoon on tropical forest biomass and carbon return processes. The study aimed to provide a scientific basis for vegetation restoration of natural forests that are composed of strong wind resistant tree species in typhoon prone areas and degraded natural forests by monitoring the long-term dynamic changes of community characteristics of monsoon evergreen broad-leaved forests. The hypothesis tested in this study was, Typhoon Mangkhut had a long term detrimental impact on forest community structure in Dinghushan forest. This study will be helpful in improving the ability to deal with natural disasters in the future and to study the impact of typhoons on vegetation in connection to the possible extreme climate events under the situation of global climate change.

MATERIALS AND METHODS

Study Site Description

Dinghushan National Nature Reserve (23°09′21′′–23°11′30′′N, 112°30′39′′–112°33′41′′E) is located in Dinghu District, Zhaoqing City, Guangdong Province, covering a total area of 1,155 ha. The main terrain is made up of hills and low mountains, with an elevation of 100–700 m. The area has a south subtropical monsoon climate, with an annual rainfall of 1,714 mm and an average annual humidity of 76%. The wet season is from April to September, and the dry season is from October to March. The wet season rainfall accounts for about 80% of the annual precipitation. The average annual temperature is 22.5°C, the average temperature of the coldest month (January) and the hottest month (July) is 13.8 and 28.8°C. The geological basis is mainly Devonian sandstone, sand shale, shale and quartz sandstone, and the zonal soil is southern subtropical lateritic red soil (Zou et al., 2018). The plant community in Dinghushan is evergreen all the year round, and the canopy density is about 95%. According to the succession of the forest ecosystem, the plant community in Dinghushan can be divided into masson pine coniferous forest in the early stage of succession, coniferous, and broad-leaved mixed forest in the middle stage of succession and monsoon evergreen broad-leaved forest in the late stage of succession. Monsoon ever-green broad-leaved forest, close to the Buddhist temple, is distributed in the core area of the reserve and has not been

FIGURE 1 | The landscape of the site, the upper plate showed before typhoon Mangkhut (September 2016), and the lower plate showed after (September 2018).

disturbed for more than 400 years. A series of studies was done to demonstrate that the forest has not been disturbed for that long (Zhou et al., 2013). The main species in the tree layer of monsoon evergreen broad-leaved forest are *Castanopsis chinensis, Schima superba, Cryptocarya concinna, Cryptocarya chinensis, Gironniera subaequalis, Macarashell cnga sampsonii, Aidia canthioides*, etc. (Zou et al., 2018). To investigate the long-term impacts of climate change and human activities on ecological resilience, biodiversity, and ecosystem services, a long-term monitoring project on the dynamics of monsoon evergreen broadleaved forest began in 1978. And a 1-ha permanent monitoring site in the monsoon evergreen broad-leaved forest was established. The well-preserved monsoon evergreen broad-leaved forest suffered from the typhoon Mangkhut heavily in 2018, the landscape of the site before and after the typhoon are shown in **Figure 1**. Based on the old monitoring data, brief description of the permanent site is shown in **Table 1** (Zhang, 2011; Zhou et al., 2013, 2014).

TABLE 1 | Brief description of the permanent site.

No.	Site name	Monsoon evergreen broad-leaved forest site
1	Succession stage	Top-stage
2	Location	23°10′ 24.41′′N, 112°32′ 50.85′′E
3	Altitude/m	230–350
4	Aspect	NE
5	Slope/(°)	25–35
6	Area/m^2	1
7	Stand age/a	>400
8	Crown density/%	>95
9	Leaf area index	6.2
10	Stand density/plant·ha	4,538
11	Biomass/t·ha	290.0
12	Soil type	Red soil

Methods

Community Surveys

We surveyed the tree community since 1978, the data collection of this study was surveyed in 2015, 2018, and 2020. All individuals with a diameter at breast height (DBH at 1.3 m above the ground) $\geq$ 1 cm and a height $\geq$ 1.5 m in the permanent sample plot were labeled, during the measurement period, all individuals were accurately traced. The difference between 2015 and 2018, resulting from the typhoon, was an area of focus during the data collection procedures and we also emphasized the data collected in 2018 and 2020 in our analysis for post-typhoon community structure survey. To facilitate comparisons, we classified all individuals into four classes based on diameter at breast height (DBH): $1 \leq \text{DBH} \leq 5$ cm, $5 < \text{DBH} \leq 10$ cm, $10 < \text{DBH} \leq 20$ cm, and $\text{DBH} > 20$ cm). According to the protocols for standard biological observation and measurement in terrestrial ecosystems (Committee of Chinese Ecological Research Network, 2019), the unified methods were applied to community field survey and biomass calculation (Sam, 1973). The detailed field survey date were November, 2015, 2018, and October, 2020.

Statistical Analysis

The statistical analysis was done using Sigma Plot for Windows version 14.0 Build 14.0.0.124. Data derived from 100 10 × 10 m plots was divided into DBH classes and clustered into 2018 and 2020 equivalent variants for variable analysis. Descriptive statistics as well as graphical analysis were used for the quantitative analysis of the data. Linear and non-linear correlation was performed using standard and polynomial quadratic curve functions. The slopes of the regression that had an R square value of >0.7 and a p-value of <0.05 were accepted as statistically significant.

RESULTS

Tree Damage Analysis in 2018 as a Result of Typhoon Mangkhut

Trees that had a DBH of less than 5 cm and a height of less than 2 m were most likely to be destroyed in a storm as shown in **Figures 2A,B**; this may perhaps be because of how feeble these small trees were lacking the structure and vigor to anchor to the soil when a large storm hits. **Figures 2A,B** clearly illustrates that any tree that had a DBH of less than 10 cm and a height of less than 10 m suffered damage or even complete destruction in the presence of a large storm. It is, however, counter-intuitively to note that taller trees that had a height of greater than 20 m and a DBH of more than 20 cm faced minimal damage in a storm despite having a large surface area of their above ground foliage exposed to the elements a situation that may have had something to do with their greater capacity to anchor deep into the soil using their larger roots and therefore withstand a storm with minimal damage.

Out of the 1,290 recorded damaged trees in Dinghushan forest, only five trees that had a height of 20 m or above were damaged and only seven that had a DBH of greater than 40 cm were damaged with *Canarium album* (Loureiro) Raeuschel and *Castanopsis chinensis* (Spreng.) (**Table 5**) hence being the only two large trees that had damage inflicted on them by the storm a fact that probably had to do with their structure or how firmly they anchored to the soil and how deep their roots grew. As a whole, there were 1,290 damaged trees (25.71%) and 488 fallen trees (9.73%) making up a total of 5,017 trees per hectare affected by the storm in 2018.

DBH classes of less than 5 cm had the highest number of fallen trees per hectare (**Figure 3A**). The average DBH of fallen trees was 9.38 cm while the tree with the largest DBH that fell was 35.4 cm with *Blastus cochinchinensis* Lour. having a height classes of between 2 and 5 m having the highest number of fallen trees per hectare (**Table 5**). The average height of a fallen tree was 8.89 m whereas the tallest tree that fell was 20 m belonging to *Pygeum topengii* Merr species. DBH and the height of a tree therefore had role in determining whether a tree would fall during a typhoon in Dinghushan forest. *Macaranga sampsonii* Hance had the highest number of fallen trees at a count of 127 per hectare with an average DBH of 3.5 cm and an average height of 4.65 m followed by *Blastus cochinchinensis* Lour. with 99 fallen trees per hectare having an average height of 4.86 m and a DBH of 3.89 cm (**Table 5**). In general, shorter trees of less than 5 m in height with a small DBH of less than 5 cm had a greater probability of falling during a typhoon or strong storm in Dinghushan forest.

Statistical and Analytical Comparisons of Dinghushan Forest Composition in 2015, 2018, and 2020

In 2015 there was a total of 5,682 trees per hectare (**Table 2**) while in 2018 there was a total of 5,022 trees per hectare (**Table 3**) showing a decline in the number of trees per hectare of 660. In 2020, about 83% of all trees had a DBH of less than 5 cm and an average height of 3.8 m (**Table 4**) making up a large proportion of trees that could be destroyed or damaged if another storm occurred of similar or greater magnitude as the one that hit the region in 2018. **Table 3** illustrates clearly a decline in number of trees and above ground biomass as compared to **Table 4** partly as a result of the storm that hit the forest in

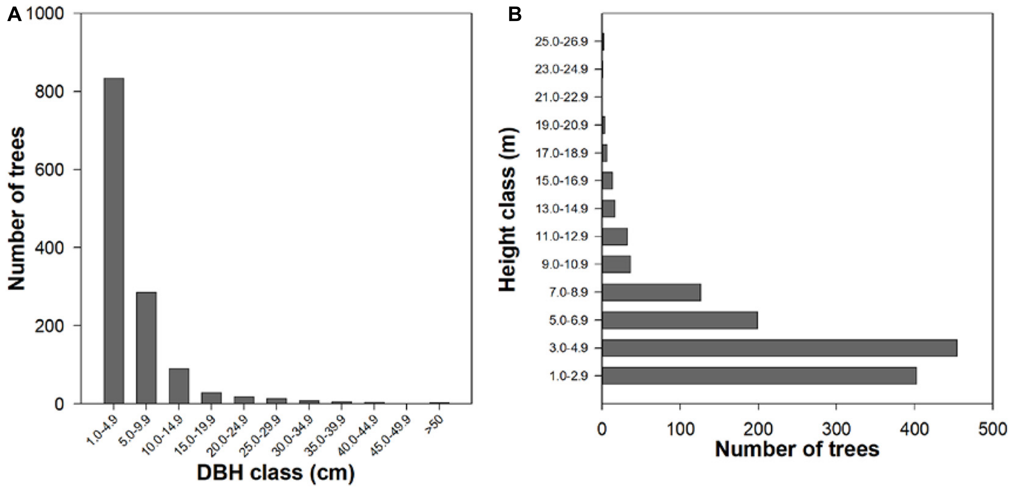

FIGURE 2 | Number of trees damaged **(A)** in DBH class, **(B)** in height class.

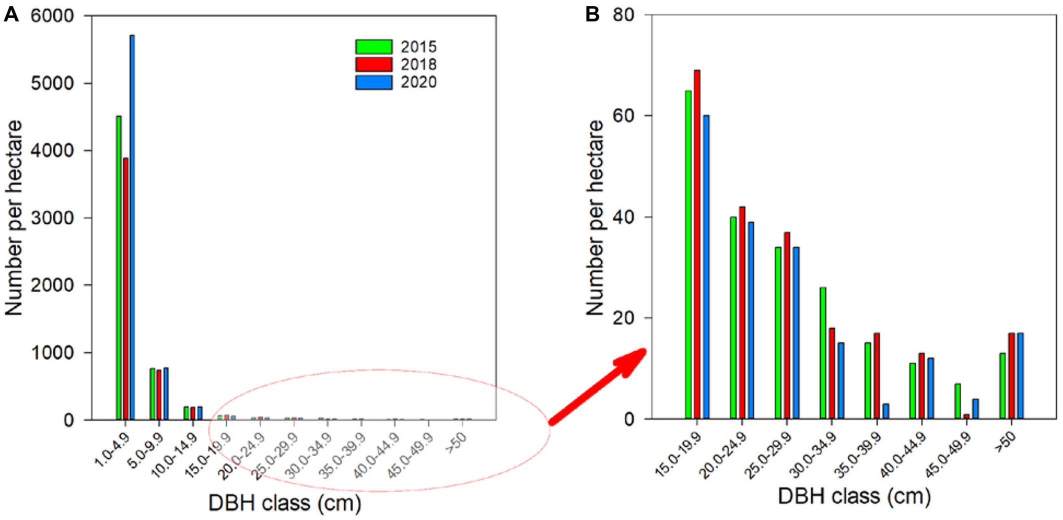

FIGURE 3 | Number of trees in each DBH class per hectare in 2015, 2018, and 2020 **(A)** all DBH classes, **(B)** 15 to >50 cm DBH class.

TABLE 2 | Data on the number, tree height, above ground weight, and below ground weight of Dinghushan forest station in the year 2015 divided into DBH classes.

DBH class	Number	%	Tree height range	Average tree height (m)	Total above ground biomass (kg)	Total below ground biomass (kg)
1.0–4.9	4,510	79.37	1.30–11.0	3.9	6,558.794	1,774.52
5.0–9.9	768	13.52	2.0–16.0	7.7	11,301.46	2,848.504
10.0–14.9	193	3.4	5.3–23.0	11.5	11,615.35	2,631.341
15.0–19.9	65	1.14	4.5–23.0	14.7	12,964	2,993.64
20.0–24.9	40	0.7	9.5–27	17.6	18,769.83	4,384.15
25.0–29.9	34	0.6	10–29	18.9	33,119.46	7,665.16
30.0–34.9	26	0.5	5–35	20.9	28,598.81	7,034.23
35.0–39.9	15	0.26	17–30	23	13,695.77	3,017.49
40.0–44.9	11	0.19	16–33	22	11,563.32	2,971.93
45.0–49.9	7	0.12	15.5–32	24.9	10,384.93	2,684
>50	13	0.23	21–37	27.1	44,125.59	26,646.79
Total	5,682				20,2697.3	64,651.76

TABLE 3 | Data on the number, tree height, above ground weight, and below ground weight of Dinghushan forest station in the year 2018 divided into DBH classes.

DBH class	Number	%	Tree height range (m)	Average tree height (m)	Total above ground biomass (kg)	Total below ground biomass (kg)
1.0–4.9	3,886	77.38	1.3–10.3	3.7604	6,002.6373	1,589.9009
5.0–9.9	735	14.64	1.3–13.5	6.9588	9,762.2462	2,427.657297
10.0–14.9	187	3.72	2–20	9.6155	8,844.7617	2,060.7894
15.0–19.9	69	1.37	2.5–20	12.6072	10,554.3517	2,359.3096
20.0–24.9	42	0.84	3.2–27	14.1	7,887.4436	3,203.3995
25.0–29.9	37	0.74	2.5–27.8	17.2351	14,372.76331	5,042.3359
30.0–34.9	18	0.36	5.5–27	15.6	22,638.5977	7,063.215
35.0–39.9	17	0.33	12–23.6	33.5	27,226.5883	3,247.4078
40.0–44.9	13	0.26	9–27	19.05	14,198.3727	2,047.2766
45.0–49.9	1	0.02	25–28	26.9	8,905.0222	1,185.7276
>50	17	0.33	13–31	23.8824	79,964.9465	19,878.4857
Total	5,022				210,357.7312	50,105.5053

TABLE 4 | Data on the number, tree height, above ground weight, and below ground weight of Dinghushan forest station in the year 2020 divided into DBH classes.

DBH class	Number	%	Tree height range	Average tree height (m)	Total above ground biomass (kg)	Total below ground biomass (kg)
1.0–4.9	5,713	83	1.3–12	3.8	8,258.8179	2,160.6477
5.0–9.9	773	11.2	1.8–14	7.6	11,282.4295	2,803.9167
10.0–14.9	197	2.9	1.6–20	10.3	10,123.7724	2,323.2888
15.0–19.9	60	0.9	3–19.5	13.5	10,184.8573	2,224.7707
20.0–24.9	39	0.6	2.5–23	14.2	12,990.8622	2,908.6599
25.0–29.9	34	0.5	11–28	18	23,332.3817	5,385.8542
30.0–34.9	15	0.2	7.2–23	14.9	15,783.4297	3,821.2085
35.0–39.9	3	0.04	8.5–33.5	21.4	16,344.3611	3,295.0315
40.0–44.9	12	0.2	10–27.5	20.9	33,557.2576	9,106.0976
45.0–49.9	4	0.06	25.5–28	27.3	4,826.9879	1,597.946
>50	17	0.2	15–28	24.7	88,650.6655	23,986.3109
Total	6,867				235,335.8228	59,613.7325

2018. Trees with a DBH of less than 5 cm made up the majority of the plants within the area in Dinghushan forest (**Figure 2**); within this DBH of less than 5, there were a total of 4,510 trees per hectare making up 79.37% of all trees in 2015, 3,886 trees per hectare making up 77.38% of all the trees in 2018 and 5,713 trees per hectare making up 83% in 2020 (**Tables 2–4**) thus illustrating the potential that Dinghushan forest has both to thrive when the conditions allow and at the same time to be heavily damaged in a storm.

Figure 3 shows a lower population of trees in 2018 compared to 2015 and 2020 in the 1–5 cm DBH classes illustrating their vulnerability to a severe storm or typhoon. Trees with a larger DBH of greater than 25 cm are shown to have fared on well despite the typhoon perhaps their larger physiological structures helped them withstand the typhoon better than their smaller counterparts. In **Figure 4**, the number of trees per hectare are displayed according to their height. The year 2018 repeatedly had fewer individual trees per hectare as compared to both 2015 and 2020 illustrating the effect the typhoon had on the Dinghushan ecosystem with even the taller trees with a height greater than 27 m being adversely affected and reducing in number.

Biomass per hectare was consistently lower in the year 2018 as shown on **Figure 5** with only one DBH class (35–39.9 cm) being higher than the rest of the years being compared in the study. The ability of Dinghushan forest to bounce back after facing a destructive weather event such as a typhoon is illustrated in **Figure 5** as well. Even though the total above ground biomass is lower in 2018 just after the storm hit the forest, the forest quickly recovers and the increases is reflected in **Tables 2–4** and **Figure 5**.

Tree height was also affected by the typhoon in 2018 because the height of trees per DBH class in 2015 and in 2020 was higher except only in one DBH class (35–39.9 cm) which was higher than expected (**Figure 6**). This result shows that in general, typhoons and storms impact tree height resulting in on average shorter trees in every DBH class. Linking to the physical damage, after the typhoon, the community structure changed significantly, with 1,290 damaged trees (25.71%) and 488 fallen trees (9.73%).

Species Composition Analysis in Dinghushan Forest in 2015, 2018, and 2020

We found dominant species including *Cryptocarya concinna* Hance, *Lasianthus chinensis* (Champ.) Benth., *Meliosma rigida* Siebold et Zucc., *Pygeum topengii* Merr., *Schima superba*, Gardner et Champ., *Sterculia lanceolata* Cav., and *Syzygium*

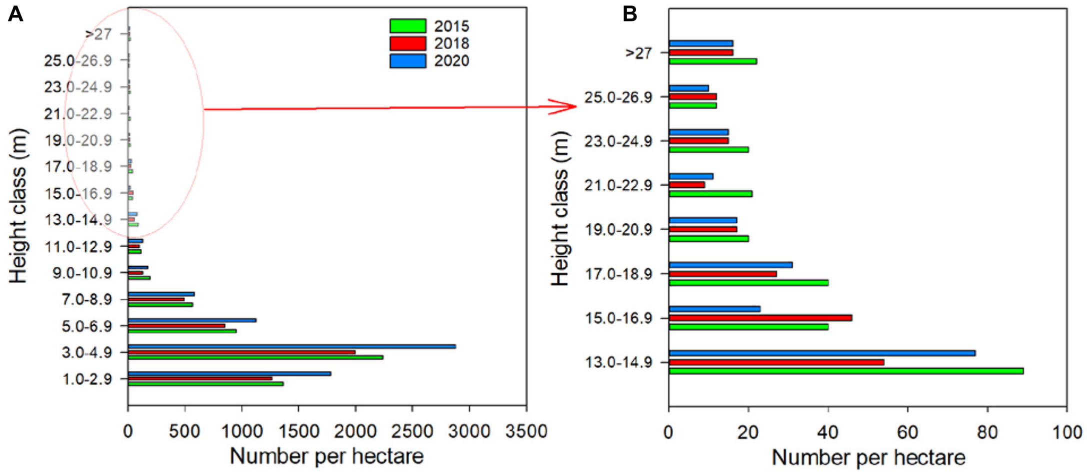

FIGURE 4 | Number of trees per hectare in each height class in 2015, 2018, and 2020 **(A)** all height classes, **(B)** 13 to <27 m height class.

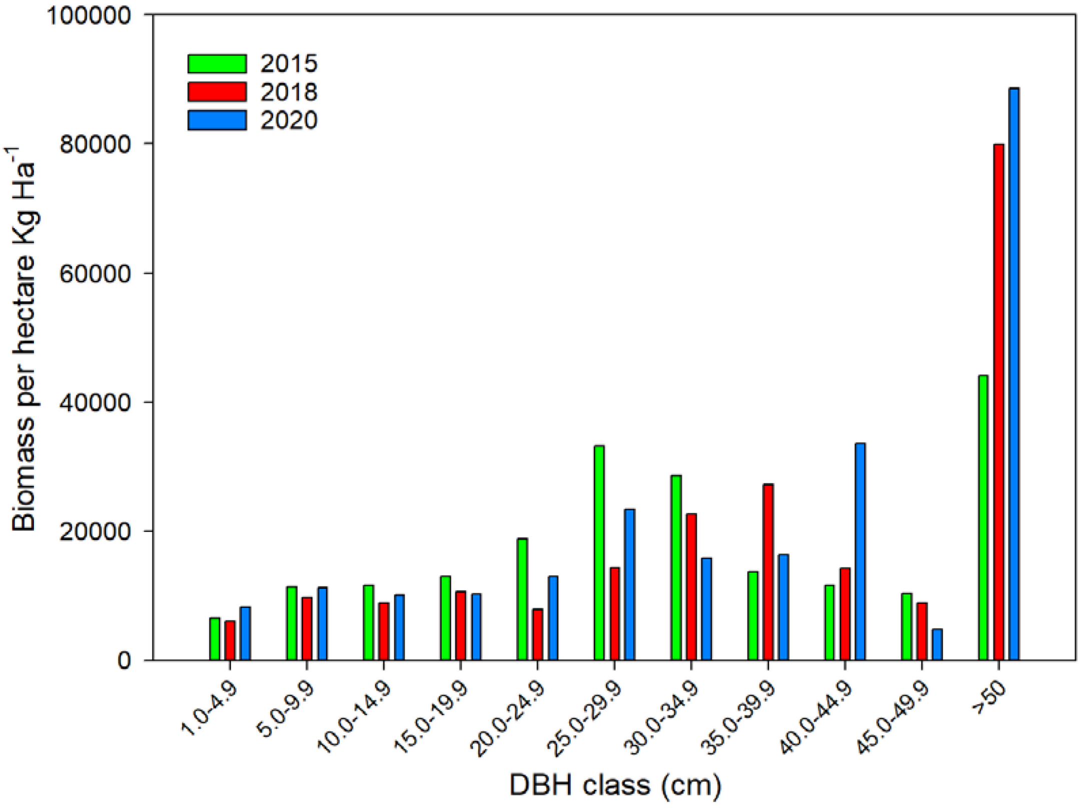

FIGURE 5 | Biomass per hectare divide into DBH classes for 2015, 2018, and 2020.

levinei (Merr.) Merr. et L. M. Perry were resilient tree species against a strong storm, and therefore they did not easily fall down in spite of the typhoon as illustrated in **Figure 7B**. On other hand, *Blastus cochinchinensis* Lour., *Cryptocarya chinensis* (Hance) Hemsl., *Gironniera subaequalis* Planch., *Macaranga sampsonii* Hance, *Mallotus paniculatus* (Lam.) Müll. Arg., *Pterospermum lanceifolium* Roxb. were non-wind resistant tree species (**Figure 7A**). The tree fall of the non-wind resistant tree species contributed 9.37% of the total biomass returned to soil.

The total average biomass, number of species and species abundance was lower in 2018 as compared to both 2015 and 2020 as shown in **Figures 7A,B**. The biomass was 192,584.5474 kg ha^{-1} in 2015, 188,487.1375 kg ha^{-1} in 2018, and 236,131.0484 kg

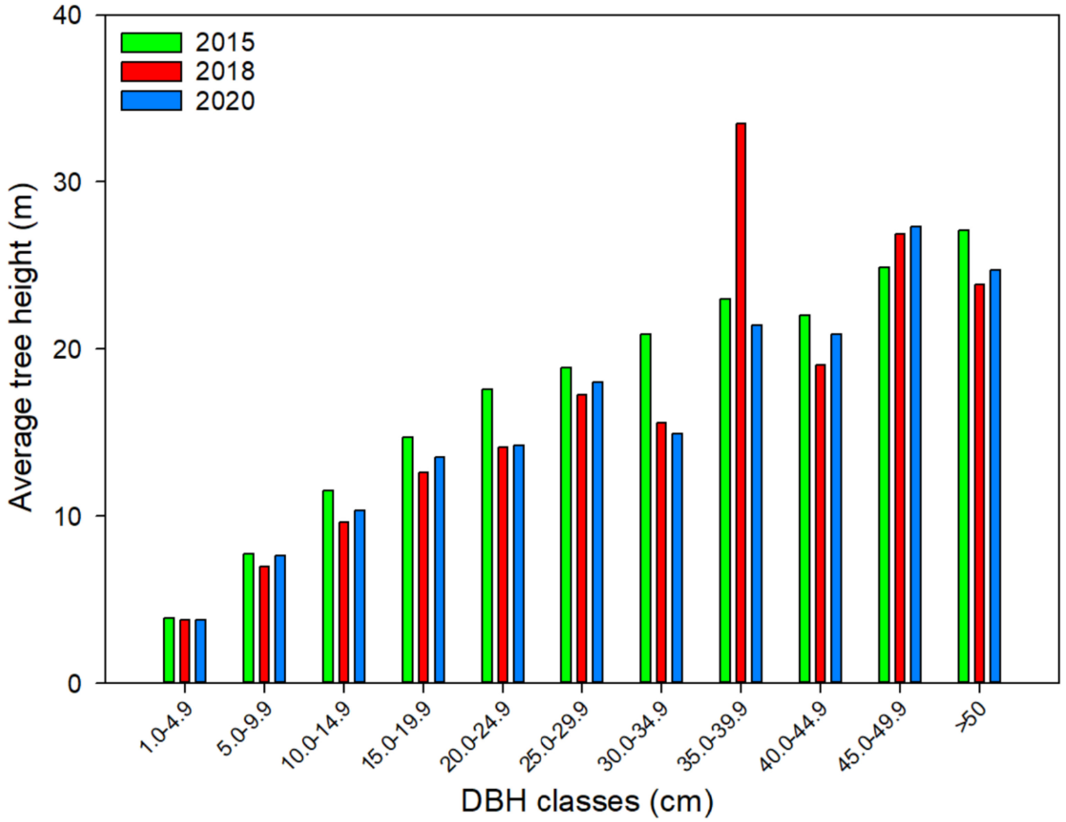

FIGURE 6 | Average tree height in each DBH class in 2015, 2018, and 2020.

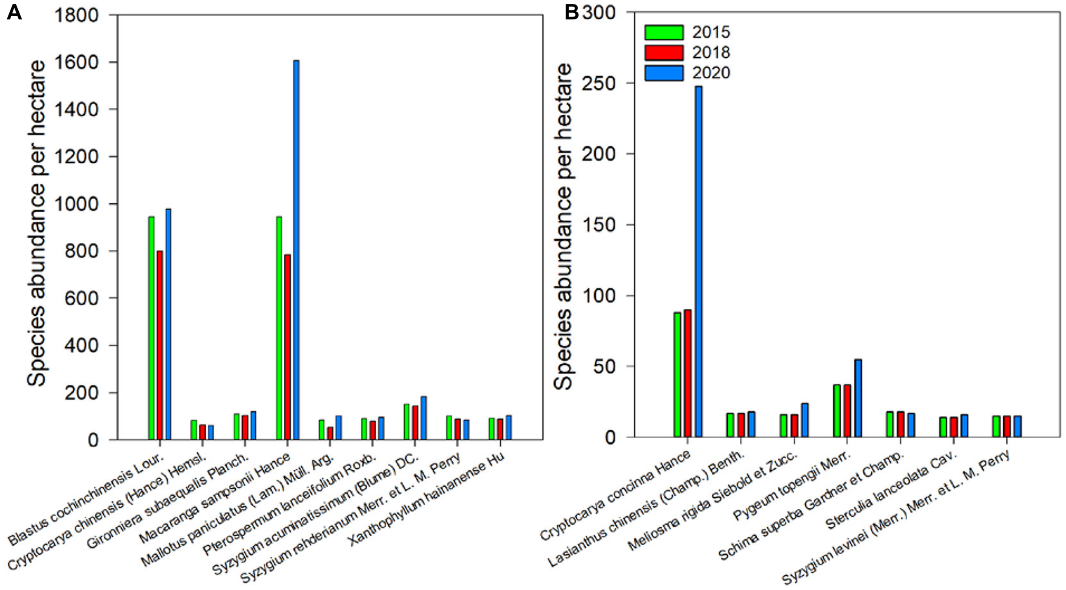

FIGURE 7 | Species abundance in **(A)** species most affected by the typhoon **(B)** Species least affected by the typhoon.

TABLE 5 | Number of trees damaged during the 2018 typhoon.

Species	Number	Average DBH (cm)	Average height (m)
Macaranga sampsonii Hance	298	3.7607	4.4675
Aidia canthioides (Champ. ex Benth.) Masam.	181	2.6033	3.695
Aporosa yunnanensis (Pax and K.Hoffm.) F.P.Metcalf	175	6.6468	5.1069
Blastus cochinchinensis Lour.	169	1.7721	2.7721
Mischocarpus pentapetalus (Roxb.) Radlk.	45	2.4466	3.377
Syzygium acuminatissimum (Blume) DC.	42	11.8142	7.3095
Gironniera subaequalis Planch.	36	17.6944	9.03611
Ormosia glaberrima Y. C. Wu	35	3.1714	3.91714
Syzygium rehderianum Merr. et L. M. Perry	33	7.7606	6.0727
Pterospermum lanceifolium Roxb.	27	8.7814	6.7525
Mallotus paniculatus (Lam.) Müll. Arg.	25	12.4	7.136
Ficus esquiroliana H. Lév.	20	6	4.35
Canarium album (Loureiro) Raeuschel	19	10.6157	6.1789
Cryptocarya concinna Hance	18	2.0722	3.5277
Sarcosperma laurinum (Benth.) Hook. f.	16	8.7187	6937
Pygeum topengii Merr.	13	9.3923	6.5153
Xanthophyllum hainanense Hu	13	4.6461	4.1307
Cryptocarya chinensis (Hance) Hemsl.	12	18.875	8.1
Ardisia quinquegona Blume	8	1.8375	2.9
Schefflera heptaphylla (Linn.) Frodin	8	11.1125	5.1875
Microdesmis caseariifolia Planch. ex Hook. f.	7	5.474	3.1571
Bridelia balansae Tutcher	6	5.0666	3.4833
Castanopsis chinensis (Spreng.) Hance	5	43.38	9.9
Macaranga andamanica Kurz	5	5.52	3.44
Machilus chinensis (Champ. ex Benth.) Hemsl.	5	12.3	5.52
Neolitsea cambodiana Lecomte	5	2.02	4.02
Acronychia pedunculata (L.) Miq.	4	15.05	11.75
Memecylon ligustrifolium Champ. ex Benth.	4	7.025	5.3
Sterculia lanceolata Cav.	4	4.175	3
Archidendron lucidum (Benth.) I.C. Nielsen	3	4.48333	4.6333
Ardisia waitakii C. M. Hu	3	11.6	12.1666
Craibiodendron scleranthum var. kwangtungense (S. Y. Hu) Judd	3	9.86666	5.233333
Lasianthus chinensis (Champ.) Benth.	3	1.5	2.0333
Lindera chunii Merr.	3	7.3	6.7333
Schima superba Gardner et Champ.	3	17.833	8.733
Canthium horridum Blume	2	10.25	9.5
Carallia brachiata (Lour.) Merr.	2	4.3	3.9
Casearia velutina Blume	2	2.7	4.05
Chrysophyllum lanceolatum var. stellatocarpon P. Royen	2	6.5	3.8
Croton lachnocarpus Benth.	2	2.5	2.65
Engelhardia roxburghiana Wall.	2	21.5	10.25
Ilex cochinchinensis (Lour.) Loes.	2	3.05	4
Psychotria asiatica L.	2	3.45	3.25
Syzygium levinei (Merr.) Merr. et L. M. Perry	2	4.1	5.1
Artocarpus styracifolius Pierre	1	3.1	6.5
Caryota maxima Blume ex Mart.	1	15.5	8.6
Casearia glomerata Roxb.	1	1.1	1.6
Elaeocarpus dubius A. DC.	1	3.9	5.9
Elaeocarpus sylvestris (Lour.) Poir.	1	19.5	15
Euonymus laxiflorus Champion et Bentham	1	1.6	2.7
Ficus nervosa B. Heyne ex Roth	1	5.2	2.6
Ficus variolosa Lindl. ex Benth.	1	1.5	1.8
Glochidion eriocarpum Champion ex Bentham	1	1.3	1.9
Ilex chapaensis Merr.	1	16.2	10.5
Litsea verticillata Hance	1	2.1	3
Meliosma rigida Siebold et Zucc.	1	9.5	9
Michelia odora (Chun) Noot. et B. L. Chen	1	110	16
Nauclea officinalis (Pierre ex Pit.) Merr. et Chun	1	2	1.6
Pavetta hongkongensis Bremek.	1	1.6	1.6
Pterospermum heterophyllum Hance	1	3.8	3.8

(Conitnued)

TABLE 5 | Conitnued

ha^{-1} in 2020 numbers that give a glimpse of the destruction and loss experienced in the forest during the typhoon. The species abundance was also adversely affected with a loss of 672 species per hectare compared to 2015 and a gain of 1,851 species per hectare compared to the year 2020 (**Figure 7B**). The findings illustrated when observed in their totality, support the hypothesis that typhoon Mangkhut did have a long-term detrimental impact on the forest community in Dinghushan forest.

DISCUSSION AND CONCLUSION

Effects of Typhoon on Community Composition and Structure and Physical Environment in the Forests

In this study, we found that effects of typhoon Mangkhut on the Dinghushan monsoon evergreen broad-leaved forest community were multi-faceted. Firstly, small trees with a DBH of less than 5 cm and a height of less than 2 m experienced the greatest damage due to the typhoon (**Figures 2A,B**). A large number of fallen trees, broken branches and fallen leaves were produced in the forest after the typhoon, which led to the decrease of canopy density, thus enhancing light transmittance and produced a large number of gaps (**Table 5**), a result that agrees with the findings of Lin et al. (2011) and Tang and Zhou (2005). In agreement with Xu et al. (2008), Dinghushan forest also exhibited a strong gap edge effect, as inferred from the results, meaning the community structure response to the typhoon showed the importance of gaps for regeneration

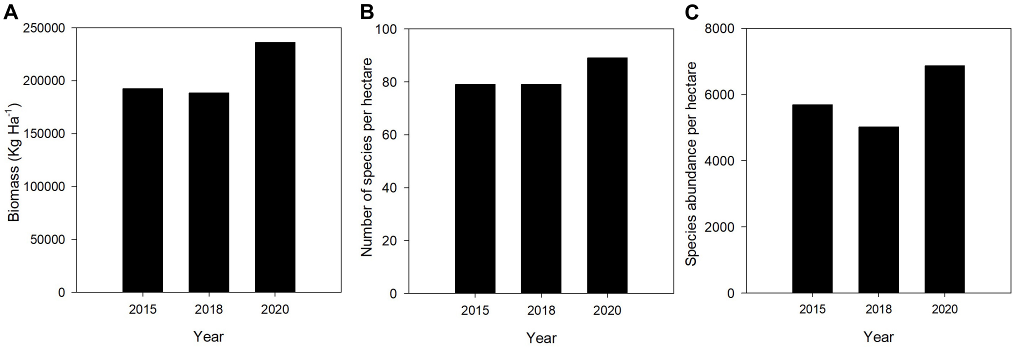

FIGURE 8 | A comparison between **(A)** Biomass, **(B)** Number of species per hectare, and **(C)** Species abundance per hectare in 2015, 2018, and 2020.

of trees and maintenance of species diversity as shown in **Figures 8A–C**.

Comparisons of Community Structure Between 2015, 2018, and 2020

The response of community composition and structure to a typhoon, differed from what has been reported in southern China perhaps due to climate change thus, when big trees fell down, it was observed that young trees grew up very fast in the two coming years (**Figures 3**, **4**). The number of individuals per unit area also increased significantly, but the number of species did not increase significantly as the data for the year 2020 suggests (**Figures 8B,C**).

The microclimate environment in the forest also changed to some extent. In this case, considering community composition and structure change, as reported by many researchers, the forest density is getting higher with many seedlings flourishing because the forest gaps provide growth opportunities for regenerating individuals (Walker et al., 1991; Peng et al., 2012; Chi et al., 2015) as illustrated in **Figures 3**, **4**. A study conducted in Panama by Putz et al. (1983) recorded that trees that have a smaller DBH and a shorter height sprouted faster after damage as a way of compensating for their susceptibility to snapping or mechanical damage and this observation agrees with the findings at Dinghushan that show that after the typhoon, smaller trees bounced back faster and contributed significantly to the ecosystem recovery (**Figures 3**, **4**). Canopy openness as a result of disturbance has been recorded to have a larger impact on a forest ecosystem more than debris deposition with a tremendous change in ferns, woody plant and understory herbivory (Shiels et al., 2014) and this was also observed in Dinghushan particularly after the typhoon more so in the year 2020. On the other hand, after the typhoon, the species composition and spatial distribution pattern of the population changed, leading to the change of species diversity (**Figure 8C**), due to the vertical structure of the forest changing, the arbor layer was changed significantly. Other studies have recorded that a tree's lifespan is significantly affected by floods or mudslides that happen during and after a typhoon (Tzeng et al., 2018) and this factor needs further investigation in Dinghushan forest. In our results, we highlighted the effect typhoon Mangkhut had on tree height, however, Lin C. et al. (2017) warns that caution should be observed when interpreting maximum tree height as a consequence of environmental harshness (**Figure 6**) and instead suggests this metric be applied mostly in regions that have frequent disturbances on tree height. Tree species that were recorded to be resistant to typhoon induced disturbance suggest that they possess certain adaptations that other trees lack, however, with climate change and an intensification of tropical cyclones, these adaptations may not be as effective in the years to come and therefore such tree species need further studies to safeguard their sustainability and the carrying forward of these advantageous traits. Owing to observation time limit, we didn't explore how different layers respond to a typhoon effect, but based on the long-term forest monitoring, the research on community succession direction and long-term adaptive response of different plant species will be explored further in the future.

The results suggest that at least 9.37% biomass returned to the forest floor after Typhoon Mangkhut (**Figure 8A**), which greatly influenced the carbon cycle of the forest ecosystem. Old-growth forests have traditionally been considered negligible as carbon sinks because carbon uptake has been thought to be balanced by respiration. The monsoon evergreen broad-leaved forest community in Dinghushan Nature Reserve is an old-growth forest in lower subtropical China. Previous studies in Dinghushan have shown that old-growth forests can accumulate carbon in soils (Zhou, 2006). While under the influence of the typhoon, a lot of biomass returned to soil, this rapid return leads to the acceleration of carbon cycle and the redistribution of carbon storage in different spaces of forest environment. The recovery degree of its carbon sequestration capacity and the recovery time needed are still unclear in the case of Dinghushan forest, and further long-term fixed monitoring is needed. The impact of typhoon Mangkhut on the forest community of Dinghushan is clear, testable, and overall detrimental thus, supporting the hypothesis of the study. Having got a clear understanding of the impact of a typhoon on an evergreen broad-leaved forest from this study, if we only account of forest carbon sequestration, *Cryptocarya concinna* Hance, *Lasianthus chinensis* (Champ.) Benth., *Meliosma rigida* Siebold et Zucc., Pygeum topengii *Merr., Schima superba* Gardner et Champ., *Sterculia lanceolata* Cav., *and Syzygium levinei* (Merr.) Merr. et L. M. Perry might be the right afforestation species in the face of typhoon pressure.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

YL, BM, QZ, JL, SW, ZL, and TW: data process. YL, BM, and JL: writing. SZ and CW: data analysis. SL and GC: data collection. SL: vegetation investigation. XT and DZ: experimental design. ZM: site maintenance. All authors contributed to the article and approved the submitted version.

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REFERENCES

Bellingham, P. J. (2008). Cyclone effects on Australian rain forests: an overview. *Austr. Ecol.* 33, 580–584. doi: 10.1111/j.1442-9993.2008.01914.x

Chi, C. H., Mcewan, R. W., Chang, C. T., Zheng, C., and Lin, T. C. (2015). Typhoon disturbance mediates elevational patterns of forest structure, but not species diversity, in humid monsoon Asia. *Ecosystems* 18, 1410–1423. doi: 10.1007/s10021-015-9908-3

Committee of Chinese Ecological Research Network (2019). *Protocols for Standard Biological Observation and Measurement in Terrestrial Ecosystems*. Beijing: China Environmental Science Press.

Davidson, E. A., Araujo, A., and Artaxo, P. (2012). The Amazon basin in transition. *Nature* 481, 321–328.

Kauffman, J. B., and Cole, T. G. (2010). Micronesian mangrove forest structure and tree responses to a severe typhoon. *Wetlands* 30, 1077–1084. doi: 10.1007/s13157-010-0114-y

Lin, C., Wang, L., Zheng, C., McEwan, R., Chang, C., Chiang, J., et al. (2017). Tropical cyclones disrupt the relationship between tree height and species diversity. *Ecosphere* 8:e01938. doi: 10.1002/ecs2.1938

Lin, K. C., Hamburg, S. P., Wang, L., Duh, C. T., Huang, C. M., Chang, C. T., et al. (2017). Impacts of increasing typhoons on the structure and function of a subtropical forest: reflections of a changing climate. *Sci. Rep.* 7:4911. doi: 10.1038/s41598-017-05288-y

Lin, T. C., Hamburg, S. P., Lin, K. C., Wang, L. J., Chang, C. T., Hsia, Y. J., et al. (2011). Typhoon disturbance and forest dynamics. *Ecosystems* 14, 127–143. doi: 10.1007/s10021-010-9399-1

Liu, B., Pan, L., and Xue, L. (2012). A review of the effect of typhoon on forests. *Acta Ecol. Sin.* 32, 1596–1605. doi: 10.5846/stxb201012231832

Peng, S. L., Wang, Y. H., You, W. H., Liu, P. S., and Wen, Z. Z. (2012). Effects of typhoon storm on hydrological process and nutrient transportation of coastal forest. *Hubei Agricult. Sci.* 51, 4381–4383.

Putz, F. E., Coley, P. D., Lu, K., Montalvo, A., and Aiello, A. (1983). Uprooting and snapping of trees: structural determinants and ecological consequences. *Canad. J. For. Res.* 13, 1011–1020. doi: 10.1139/x83-133

Sam, D. (1973). The integration of fieldwork and survey methods. *Am. J. Sociol.* 78, 1335–1359. doi: 10.1086/225467

Shiels, A. B., Grizelle, G., and Michael, R. W. (2014). Responses to canopy loss and debris deposition in a tropical forest ecosystem: synthesis from an experimental manipulation simulating effects of hurricane disturbance. *For. Ecol. Manag.* 260, 2214–2223.

Takemi, T., Ito, R., and Arakawa, O. (2016). Effects of global warming on the impacts of Typhoon Mireille (1991) in the Kyushu and Tohoku regions. *Hydrol. Res. Lett.* 10, 81–87, (Omitted). doi: 10.3178/hrl.10.81

Tang, X. L., and Zhou, G. Y. (2005). Coarse wood debris biomass and its potential contribution to the carbon cycle in successional subtropical forests of southern China. *Acta Phytoecol. Sinica* 29, 559–568. doi: 10.17521/cjpe.2005.0075

Tzeng, H., Wang, W., Tseng, Y., Chiu, A., Kuo, C., and Tsai, T. (2018). Tree mortality in response to typhoon-induced floods and mudslides is determined by tree species, size, and position in a riparian Formosan gum forest in subtropical Taiwan. *PLoS One* 13:e0190832. doi: 10.1371/journal.pone.0190832

Walker, L. R., Brokaw, N. V. L., Lodge, D. J., and Waide, R. B. (1991). Ecosystem, plant, and animal responses to hurricanes in the Caribbean. *Biotropica* 23, 313–521.

Wang, Q., Yu, D., Li, Z., and Wang, L. (2018). The effect of typhoons on the diversity and distribution pattern of aquatic plants on hainan island, South China. *Biotropica* 40, 692–699. doi: 10.1111/j.1744-7429.2008.00430.x

Xu, H., Li, Y. D., Luo, T. S., Lin, M. X., Chen, D. X., Mo, J. H., et al. (2008). Influence of typhoon Damrey on the tropical montane rain forest community in Jianfengling, Hainan island, China. *J. Plant Ecol.* 32, 1323–1334.

Zhang, Q. (2011). *China Ecosystem Positioning Observation and Research data set Forest Ecosystem Volume Guangdong Dinghushan Station: 1998-2008*. Beijing: China Agricultural Publishing House.

Zhou, G. Y. (2006). Old-growth forests can accumulate carbon in soils. *Science* 314, 1417–1417. doi: 10.1126/science.1130168

Zhou, G., Houlton, B. Z., Wang, W., Huang, W., Xiao, Y., Zhang, Q., et al. (2014). Substantial reorganization of China's tropical and subtropical forests: based on the permanent plots. *Glob. Change Biol.* 20, 240–250. doi: 10.1111/gcb.12385

Zhou, G., Peng, C., Li, Y., Liu, S., Zhang, Q., Tang, X., et al. (2013). A climate change-induced threat to the ecological resilience of a subtropical monsoon evergreen broad-leaved forest in southern China. *Glob. Change Biol.* 19, 1197–1210. doi: 10.1111/gcb.12128

Zou, S., Zhou, G. Y., Zhang, Q. M., Shan, X. U., Xiong, X., Xia, Y. J., et al. (2018). Dynamics of community structure of monsoon evergreen broad-leaved forest in Dinghushan from 1992 to 2015. *J. Plant Ecol.* 42, 442–452.

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Tropical Rainforest Successional Processes can Facilitate Successfully Recovery of Extremely Degraded Tropical Forest Ecosystems Following Intensive Mining Operations

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Reforestation is an effective way to alleviate deforestation and its negative impacts on ecosystem services. In tropical rainforest ecosystem, however, frequent typhoons and heavy rainfall can result in landslides and uprooting of many seedlings, making reforestation efforts very difficult, especially within extremely degraded sites where soil conditions cannot support any plant life. Here, we described a reforestation protocol which is based on tropical rainforest successional processes to not only prevent landslides and tree uprooting due to frequent typhoon and heavy rain, but also accelerate tropical forest succession. This protocol first used the slope and soil layer of the undisturbed old-growth tropical rainforest as a reference to reconstruct slope and soil layers. Then multiple tropical tree species with high growth and survival rate were separately monocultured in the reconstructed soil layers. In the year of 2015 and 2016, we tested the effectiveness of this protocol to recover a 0.2 km^2 extremely degraded tropical rainforest which consists of bare rock and thus does not support any plant life, in Sanya city, China. Our results showed that, both typhoons and heavy rains did not result in landslide or any tree damages in the area this reforestation protocol was used. Moreover, our separately monocultured eight fast-growing tree species have much higher fast-growing related functional traits than those for tree species in the adjacent undisturbed tropical seasonal forest, which in turn resulted in large soil water and nutrient loss within 3 years. This seemed to simulate a quick transition from primary succession (consist of bare rock and cannot support any plant life) to mid-stage of secondary tropical rainforest succession (many fast-growing pioneer tree species induced high soil water and nutrient loss). Thus, mixing the late-successional tropical tree species with each of the separately monocultured eight fast-growing tree species can accelerate recovery to the undisturbed tropical rainforest as soon as possible. Overall, based on tropical rainforest successional processes, our research provides an effective protocol for quickly and effectively restoring an extremely degraded tropical rainforest ecosystem. We expect that this work will be important for the future recovery of other extremely degraded tropical rainforest ecosystems.

Keywords: deforestation, fast-growing trees, functional traits, primary succession, reforestation, secondary succession

INTRODUCTION

Tropical rain forests are highly important ecosystems due to their high biodiversity and ability to store large amount of carbon thereby to play a key role in regulating global climate change (Houghton et al., 2000; Mitchard 2018). Human disturbances in tropical rain forests, including mining, and agricultural use have resulted in heavy deforestation and degradation of these systems and it has been predicted that tropics will have the most deforestation in the near future (Laurance et al., 2014). This degradation, in turn, has led to a global loss in tropical biodiversity, increased carbon emissions, and excessive water loss (Aleman et al., 2018; Symes et al., 2018; Taubert et al., 2018). As a result, it is urgent to develop some effective way to reduce deforestation and its native influences on tropical rain forests.

Reforestation has been assumed to be an effective way to alleviate deforestation and its negative impacts on ecosystem services (Griscom et al., 2017; Taubert et al., 2018). However, frequent typhoons and heavy rains in tropical rainforest can result in landslides and uprooting of many trees (Chang et al., 2008; Yumul et al., 2012; Acosta et al., 2016; Villamayor et al., 2016), making reforestation very difficult. In extremely degraded tropical rainforest (i.e., long ore mining induced extremely degraded tropical rainforest), only a bare rock environment can be found and any plant life can be supported. This is another challenge for performing reforestation that must be overcome. Extremely degraded tropical rainforest (e.g., the ore mining induced degraded tropical rainforest) can be comparable to the initial stage of primary forest succession which originates from the bare rock environment. Thus, an effective approach to reforestation in such conditions is to follow the natural forest successional trajectory (Funk et al., 2008; Johnson and Miyanishi, 2008). However, primary succession usually requires more than 200 years to change the bare rock into the appropriate soil environments to sustain the colonization and survival of tree species (Chapin et al., 1994). Thus, it is highly needed to develop a quick and effective reforestation protocol to not only prevent the landslide resulting from the frequent typhoon and heavy rain, but also facilitate quick forest restoration.

The final target for reforestation in the extremely degraded tropical rainforest is restoring to the undisturbed old-growth tropical rainforest. Usually, frequent typhoons and heavy rains are less disruptive in undisturbed old-growth tropical rain forests. Thus, undisturbed old-growth rainforest in the similar but adjacent locations to the ore mining sites can be a reference to reconstruct slope and soil layers to perform reforestation. It has been found that planting monocultures of fast-growing tree species with high survival rate can help prevent landslide (Stokes et al., 2009; Walker et al., 2009; Pang et al., 2018), thereby to facilitate successfully performing reforestation in tropics. Additionally, based on the tropical rainforest successional processes, fast-growing species usually dominate early succession which in turn may lead to gradually reduced soil water and nutrients loss (Lohbeck et al., 2014). Planting monocultures of fast-growing species can quickly cause high soil nutrient and water loss (Lamb et al., 2005; Wingfield et al., 2015), and thus it can quickly accelerate tropical rainforest succession (Lugo, 1997). Here we develop a simple reforestation protocol, which first uses the slope and soil layer of the undisturbed old-growth tropical rainforest as a reference to reconstruct slope and soil layers. Then separately monoculture multiple tropical tree species with high growth and survival rate in the reconstructed soil layers to not only prevent landslides and tree uprooting due to frequent typhoon and heavy rain, but also accelerate tropical rainforest restoration.

We hypothesized that our protocol could prevent landslides and tree uprooting due to frequent typhoon and heavy rain, while at the same time accelerated tropical rainforest succession. Thus, it can successfully perform reforestation to quickly and effectively recover extremely degraded tropical rain forests. To test this hypothesis, we used our reforestation protocol in a 0.2 km^2 extremely degraded tropical rainforest in the Baopoling hill (BPL) in Sanya city, Hainan island. The site has been extremely degraded due to 20 years of limestone mining associated with the cement industry and now consists of bare rock that does not support any plant life. In this study we aim to quantify whether using our reforestation protocol to recover this 0.2 km^2 extremely degraded tropical rainforest can prevent landslides resulting from frequent typhoon and heavy rain while also simulating a quick transition from primary succession to mid-stage of the secondary succession and thereby accelerate tropical rainforest succession.

MATERIALS AND METHODS

Study Sites

BPL (300 m elevation), is a limestone mountain in Sanya City, Hainan island, China (110°58′01′′E, 19°38′48′′N) (**Figure 1**). The climate of the region is a tropical monsoon oceanic climate with a mean annual temperature of 28°C. The average annual precipitation in Sanya city is 1,500 mm, with approximately 91% of the precipitation occurring between June to October (Luo et al., 2018). The vegetation is described as broad-leaf tropical rainforest (Guo et al., 2013). Limestone mining associated with the cement industry lasted from 1995 till 2015, resulting in a 0.2 km^2 extremely degraded area covered by bare rock (**Figure 1**). Outside of this degraded area is undisturbed old-growth tropical rainforest (**Figure 1**).

Description of Our Reforestation Method on BPL

We followed our protocol to use the slope and soil layer of the undisturbed area as a reference to reconstruct slope and soil layers to plant tree seedlings. Thus, the first step of our reforestation project is to analyze the slope and soil depth of the adjacent undisturbed areas of BPL. The slope and soil depth of the adjacent undisturbed areas were measure by theodolite and soil compactness measuring instrument (Spectrum SC900, The United States). Second, the original slope of the highly degraded 0.2 km^2 area was cut so as to match the slope from the adjacent undisturbed area (**Figure 2A**). Third, excavators

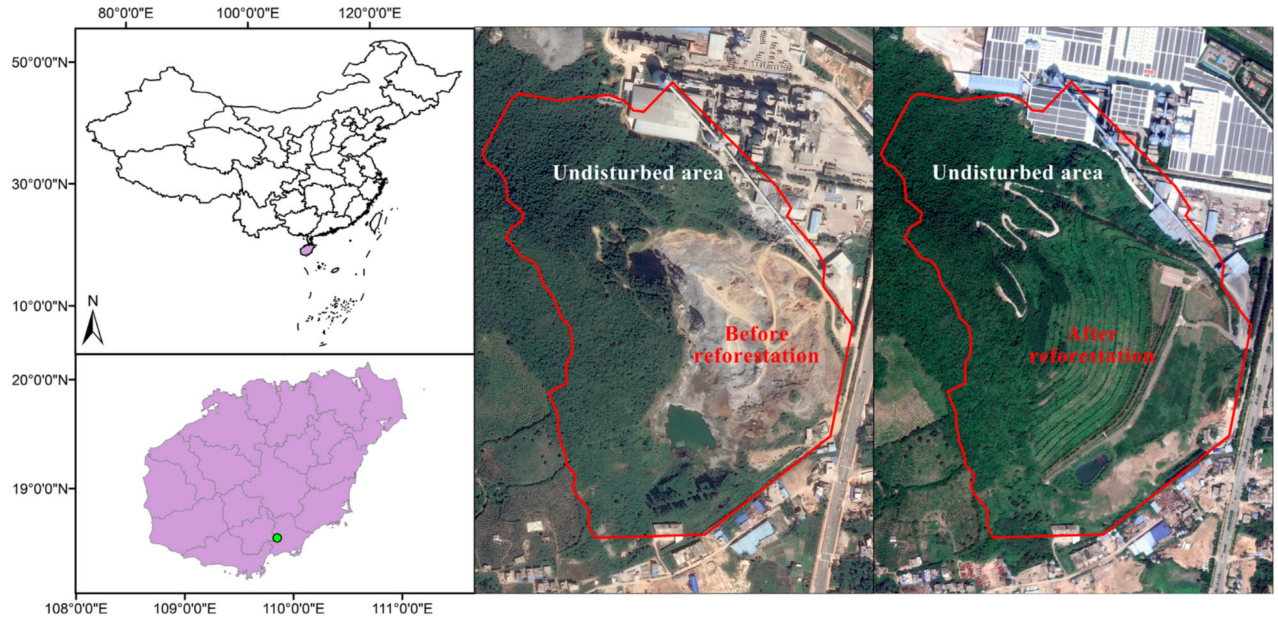

FIGURE 1 | Map of the study site (Baopoling hill, Sanya city, Hainan island, China) and the surrounding landscape of the 0.2 km^2 highly degraded tropical bare hill, both before **(A)** and after **(B)** reforestation efforts.

were used to dig a lot of holes that approximated the soil depth of the adjacent undisturbed area to refill the local high nutrient soils to form eight planted areas form the top to the foot of the 0.2 km^2 area (**Figure 2B**). Finally, eight fast-growing tropical tree species (*Terminalia neotaliala*, *Bombax malabarica*, *Cleistanthus sumatranus*, *Ficus microcarpa*, *Muntingia colabura*, *Acacia mangium*, *Syzygium hainanense* and *Bougainvillea spectabilis*) are separately mono-planted in the reconstructed soil layers to quickly perform the reforestation (**Figure 2C**). These eight fast-growing tree species are proven good afforestation tropical trees in Hainan island and they can have high growth rate and surviving rate after reforestation (Lin et al., 2006; Liu et al., 2017). Moreover, their seedlings are very easy to get in the local market. Specifically, the initial height and a diameter at breast height (DBH) for seedlings of the eight separately monocultured fast-growing tropical tree species ranged from 2 to 3 m and 3–5 cm respectively. Planting density was kept at 80–100 stems per hectare. The entire project was finished in 2016 (**Figure 2D**) and we recorded their survival rates from 2016 to 2019 as:

$$\text{survival rate} = \frac{\text{Remaining seedings}}{\text{Original seedings}} \times 100\%$$

Plant Leaf Sampling and Functional Traits Measurement

In 2018, 30 plots, each of 20 × 20 m^2 (an area of 400 m^2 for each plot) that were at least 100–300 m apart from one another, were randomly sampled across the adjacent undisturbed old-growth forest. Within each plot, all freestanding trees with diameter of ≥1 cm at breast height (DBH) were measured and identified to species. We finally found 80 tree species in the undisturbed old-growth forest and we select the eight tree species (200–300 stems per hectare) *(Bridelia tomentosa*, *Radermachera frondosa*, *Lepisanthes rubiginosa*, *Rhaphiolepis indica*, *Pterospermum heterophyllum*, *Fissistigma oldhamii*, *Psychotria rubra* and *Cudrania cochinchinensis) as our candidate dominant slow-growing species.* In the maximum growth season (August) of 2019, we measured plant height and DBH for these eight separately monocultured fast-growing tropical tree species and the eight dominant old-growth tropical rainforest tree species found in the undisturbed site. We also sampled 20 fully expanded and healthy leaves from five individuals for each of the eight separately monocultured fast-growing tropical tree species and dominant old-growth tropical tree species in the undisturbed site, and only individuals with DBH close to the species mean value were selected (**Supplementary Table S1**). Leaf samples were used to measure ten functional traits related to plant growth, including leaf anatomy traits [palisade tissue (μm), spongy tissue (μm), leaf thickness (μm), leaf tightness (%), and stomatal density (numbers mm^{-2})], specific leaf area (g cm^{-2}), leaf hydraulic conductivity (k_{leaf}; mmol m^{-2} s^{-1} MPa^{-1}), maximum photosynthesis rate (A_{mass}; μmol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), and transpiration rate (μmol m^{-2} s^{-1}). Traits were measured following the methods described in Hua et al. (2017) and Zhang et al. (2018a).

Soil Sampling and Properties Measurements

In the peak of growing season (August) of 2015, before planting the seedlings of the eight separately monocultured fast-growing

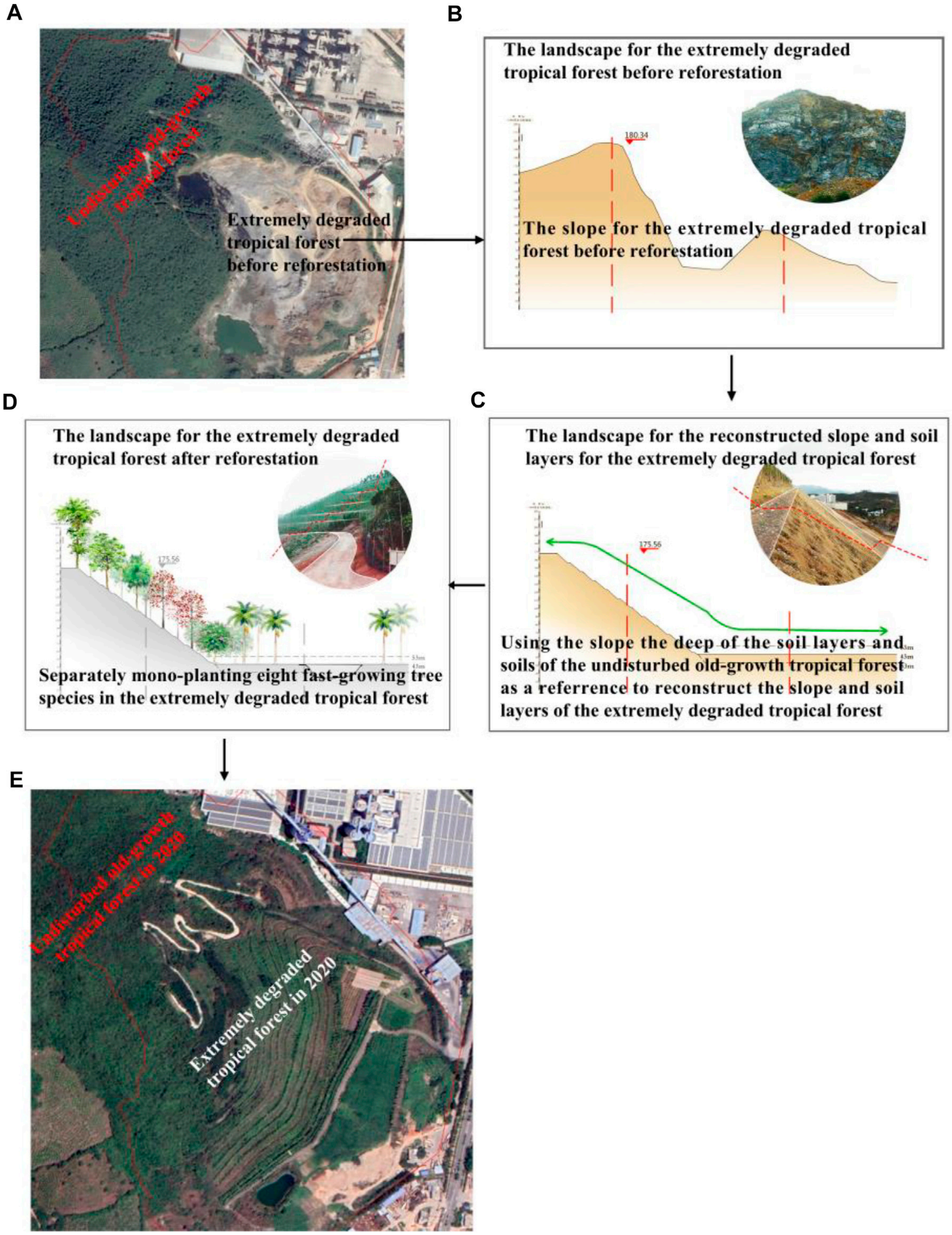

FIGURE 2 | The surrounding landscape and detailed procedures regarding the reforestation project in the 0.2 km^2 extremely degraded tropical rain forest in Baopoling mountain (BPL). **(A)** BPL and the surrounding landscape including the 0.2 km^2 area of extremely degraded tropical rain forest and the adjacent undisturbed old-growth tropical rain forests. **(B)** the original landscape and slope of the 0.2 km^2 extremely degraded tropical rain forest; **(C)** using the slope, the deep of soil layers as a reference to reconstructing slope and soil layers, and to refill soils from the undisturbed old-growth tropical forests to form planting area in the extremely degraded tropical rain forest; **(D)** separately mono-planting eight fast-growing tropical tree species; **(E)** the landscape of undisturbed old-growth tropical rain forest and the 0.2 km^2 extremely degraded tropical rain forest after reforestation efforts in 2020.

TABLE 1 | Survival rates for the eight fast-growing tree species (*Terminalia neotaliala* (Combretaceae), *Bombax malabarica* (Bombacaceae), *Cleistanthus sumatranus* (Euphorbiaceae), *Ficus microcarpa* (Moraceae), *Muntingia colabura* (Elaeocarpaceae), *Acacia mangium* (Leguminosae), *Syzygium hainanense* (Myrtaceae) and *Bougainvillea spectabilis* (Nyctaginaceae)) after 3°years of reforestation.

Species	Survival rate (%)
Terminalia neotaliala	92
Bombax malabarica	94
Cleistanthus sumatranus	92
Ficus microcarpa	95
Muntingia colabura	93
Acacia mangium	100
Syzygium hainanense	100
Bougainvillea spectabilis	100

tropical tree species, we followed the methods described in Burt (2009) to determine soil available nitrogen (ammonium and nitrate nitrogen; mg kg^{-1}), soil available phosphorus (mg kg^{-1}), and soil water content (g kg^{-1}) in randomly collected 24 soil samples at a depth of 0–20 cm of the high nutrient local soils that are used for reforestation and soils in the adjacent undisturbed old-growth tropical rain forest. After reforestation, in the peak of growing season (August) of 2019, three soil samples were collected randomly at a depth of 0–20 cm around each of the eight dominant old-growth tropical tree species and monocultured fast-growing tropical tree species to measure soil available nitrogen, soil available phosphorus, and soil water content in the undisturbed old-growth forests. Detailed procedures for measuring both functional traits and soil properties can be found in the Supplementary Material.

Statistical Analysis

Since we did not know the detail distribution of all of our data, we first utilized non-parametric analysis (Wilcoxon signed-rank test) to quantify whether there were any important differences in plant height, DBH, and ten growth traits between the eight separately monocultured fast-growing tropical tree species and eight old-growth tropical tree species. Then, we used principal component analysis (PCA) to evaluate which of the ten growth traits best discriminated between the eight separately monocultured fast-growing tropical tree species and eight old-growth tropical tree species. Finally, we also compared differences in soil water content, available nitrogen, and phosphorus content among the initial local high nutrient soil before reforestation sampled in July, 2015 (reforestation soil in 2015), soils in undisturbed old growth forest sampled in July, 2015 (undisturbed soil in 2015), soils in reforestation area sampled in July, 2019 (reforestation soil in 2019) and soils in undisturbed old growth forest sampled in July, 2019 (undisturbed soil in 2019).

RESULTS

So far both typhoons and heavy rains have not resulted in landslide or tree damages in this reforestation project (**Figure 1**). Moreover, all of the eight separately mono-planting fast-growing have survived and have had positive growth rates after reforestation. The survival rates among all separately mono-planting fast-growing tree seedlings were greater than 92% after 3 years' reforestation (**Figure 1**; **Table 1**). Furthermore, the height and DBH for the eight separately mono-planting fast-growing tree seedlings increased 2–6 times in the 3 years' plantation (from 2 to 3 m and 3–5–13 m and 10 cm). In addition, the height and DBH for the eight monocultured fast-growing tree species were also 1.6–2 times larger than the eight dominant old-growth tree species ($p < 0.001$, based on Wilcoxon signed-rank test, **Supplementary Figure S1**).

We also found all ten growth trait metrics for the monocultured tree species were greater than the dominant old-growth tree species ($p < 0.001$, based on Wilcoxon signed-rank test, **Figure 3**). However, results from the PCA show that five of the traits were best at discriminating between the mono-planting tree species and the old-growth tree species. These include stomatal conductance, stomatal density, leaf water conductance, maximum photosynthesis rate, and transpiration rate (**Figure 4**; **Table 2**). Thus, stomatal conductance, stomatal density, leaf water conductance, maximum photosynthesis rate, and transpiration rate are the key characteristics to facilitate the much higher growth rate in the monocultured tree species compared to the dominant old growth tree species.

In addition, we found that our reforestation method could quickly result in soil water and nutrient loss. That was because soil water content, soil available nitrogen (ammonium and nitrate nitrogen), and available phosphorus content in the local high nutrient soils before reforestation were 1.5–2 times higher than those for soils in undisturbed old growth forest sampled in the same time (August, 2015) (**Figure 5** and $p < 0.05$ based on Wilcoxon signed-rank test in **Supplementary Table S2**). However, in 2019, after 3 years of growth, the reforestation area had merely 1/4 of the available nitrogen (ammonium and nitrate) and available phosphorus content those for the initial local nutrient soils before reforestation (**Figure 5** and $p < 0.05$ based on Wilcoxon signed-rank test in **Supplementary Table S2**). In contrast, there were no significant variation in soil water content, soil available nitrogen (ammonium and nitrate nitrogen), and available phosphorus content in the undisturbed area from 2015 to 2019 (**Figure 5** and $p > 0.05$ based on Wilcoxon signed-rank test in **Supplementary Table S2**). Moreover, soil water content, soil available nitrogen (ammonium and nitrate nitrogen), and available phosphorus content in the reforestation area were only 1/2–1/3 of those in the undisturbed area (**Figure 5** and $p < 0.05$ based on Wilcoxon signed-rank test in **Supplementary Table S2**).

DISCUSSION

Here we introduce a reforestation protocol which is based on tropical rainforest successional processes to quickly and effectively restore extremely degraded tropical rainforest. Our approach resulted in a successfully restored 0.2 km^2 extremely degraded tropical rainforest which consists of bare rock and cannot support any plant life. Importantly this large scale

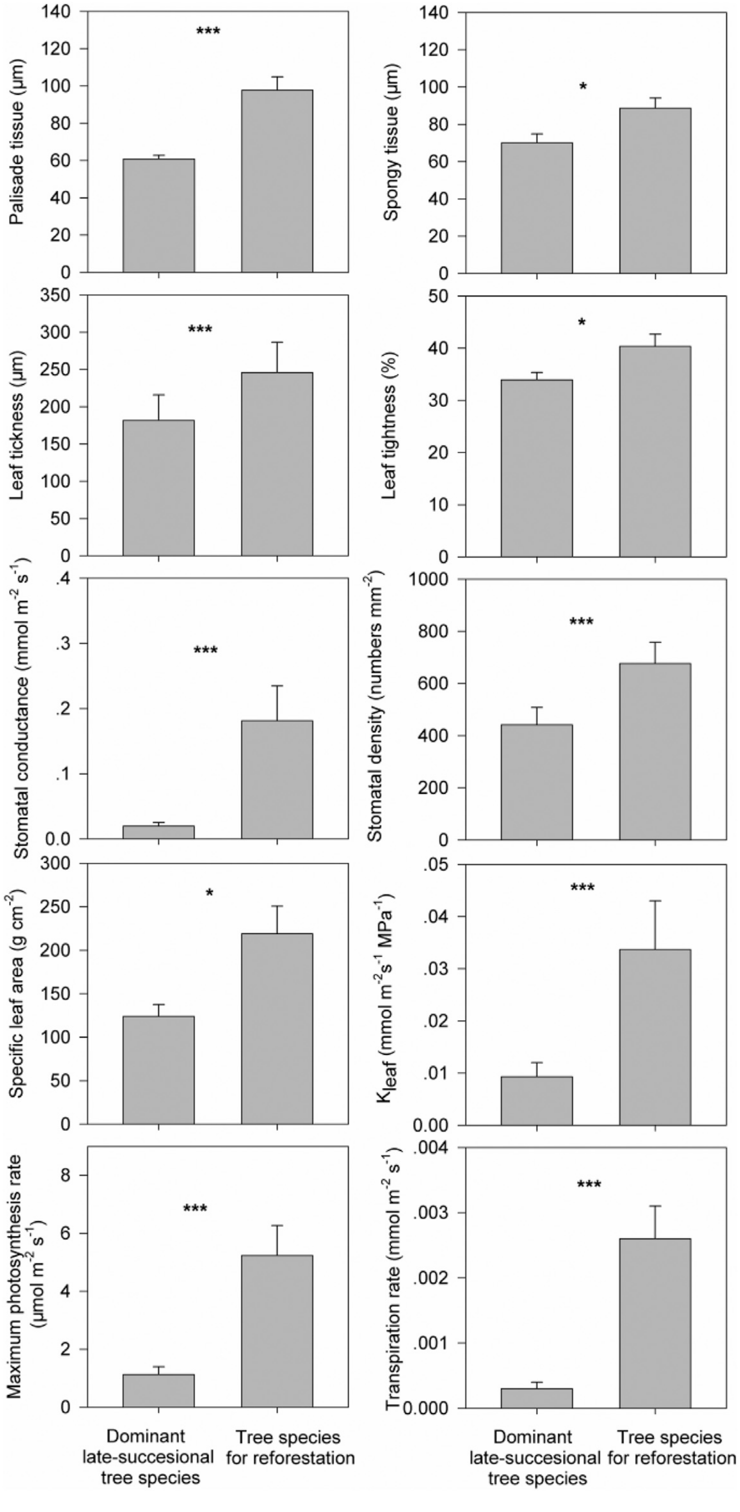

FIGURE 3 | Differences in ten functional traits associated with a fast-growth strategy (i.e., palisade tissue (PT; μm), spongy tissue (ST; μm), leaf thickness (LT; μm), leaf tightness (LTI; %), stomatal conductance (SD; mmol m^{-2} s^{-1}), stomatal density (numbers mm^{-2}), specific leaf area (SLA; g cm^{-2}), leaf hydraulic conductivity (k_{leaf}; mmol $m^{-2}s^{-1}$ MPa^{-1}), maximum photosynthesis rate (A_{mass}; μmol m^{-2} s^{-1}) and transpiration rate (μmol m^{-2} s^{-1})) between the eight separately monocultured fast-growing tropical tree species used for reforestation (tree species for reforestation) and the eight dominant tropical tree species in the undisturbed old-growth tropical rainforest (dominant late-successional tree species). *** indicates $p < 0.001$ based on Wilcoxon signed-rank tests.

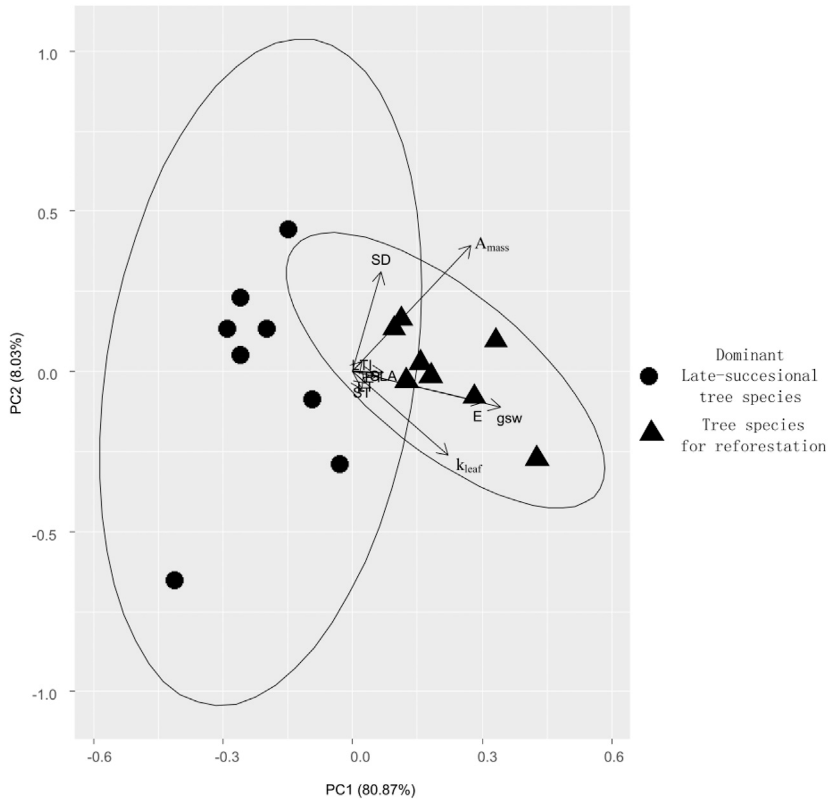

FIGURE 4 | Principal component analysis (PCA) of the ten functional traits associated with fast growth between the eight separately monocultured fast-growing tropical tree species (tree species for reforestation) and the eight dominant tropical tree species in the undisturbed old-growth tropical rain forest (dominant late-successional tree species).

TABLE 2 | The first two axes of a principal component analysis (PCA) for the eight fast-growing tree species and eight dominant old-growth tree species, based on 10 plant traits. These include palisade tissue (μm), spongy tissue (μm), leaf thickness (μm), leaf tightness (%), stomatal conductance (mmol m^{-2} s^{-1}), stomatal density (numbers mm^{-2}), specific leaf area (g cm^{-2}), leaf hydraulic conductivity (k_{leaf}; mmol m^{-2} s^{-1} MPa^{-1}), maximum photosynthesis rate (A_{mass}; μmol m^{-2} s^{-1}) and transpiration rate (μmol m^{-2} s^{-1}).

Functional traits	PC1	PC2
Palisade tissue	0.08	–0.01
Sponge tissue	0.04	–0.10
Leaf thickness	0.05	–0.06
Leaf tightness	0.03	0.05
Transpiration rate	0.51	–0.16
Maximum photosynthesis rate	0.47	0.67
Stomatal conductance	0.58	–0.19
Specific leaf area	0.12	0.00
Stomatal density	0.11	0.53
Leaf water conductance	0.58	–0.45

reforestation project helps to solve the challenge of improving forest conditions and the negative outcomes (landslides and tree uprooting) due to frequent typhoon and heavy rain, but also quickly simulates a transition from primary tropical rainforest succession (consisting of merely bare rock and no plant can grow and survive) to mid-stage of secondary tropical rainforest succession (dominant fast-growing tree species have resulted in soil water and nutrient loss). Finding the appropriate tree species in late-stage of secondary tropical rainforest succession can have high potential to quickly simulate late secondary forest succession thereby accelerating the forest trajectory towards undisturbed old-growth tropical rainforest. Ultimately, our reforestation protocol can be treated as a general protocol to perform reforestation to recover extremely degraded tropical rainforest.

The high risk of landslides and uprooting of many trees due to frequent typhoons and heavy rains in tropical rain forests (Chang et al., 2008; Yumul et al., 2012; Acosta et al., 2016; Villamayor et al., 2016) and bare rock environment are two big challenges for performing reforestation in extremely degraded tropical rainforest. Here both typhoons and heavy rains did not result in landslide and any tree damages, when using our reforestation protocol. Moreover, all separately mono-planting multiple fast-growing tropical tree species had a very high survival and growth rate rate (the height and DBH for the eight separately monocultured tree species were 1.6–2 times larger than the dominant tree species in the undisturbed old-growth tropical rain forests after 3 years' plantation). This confirmed that mono-planting multiple tree species with high survival and growth rate could prevent typhoons and heavy rains induced landslide in tropical rainforest (Stokes et al., 2009; Walker et al., 2009; Pang

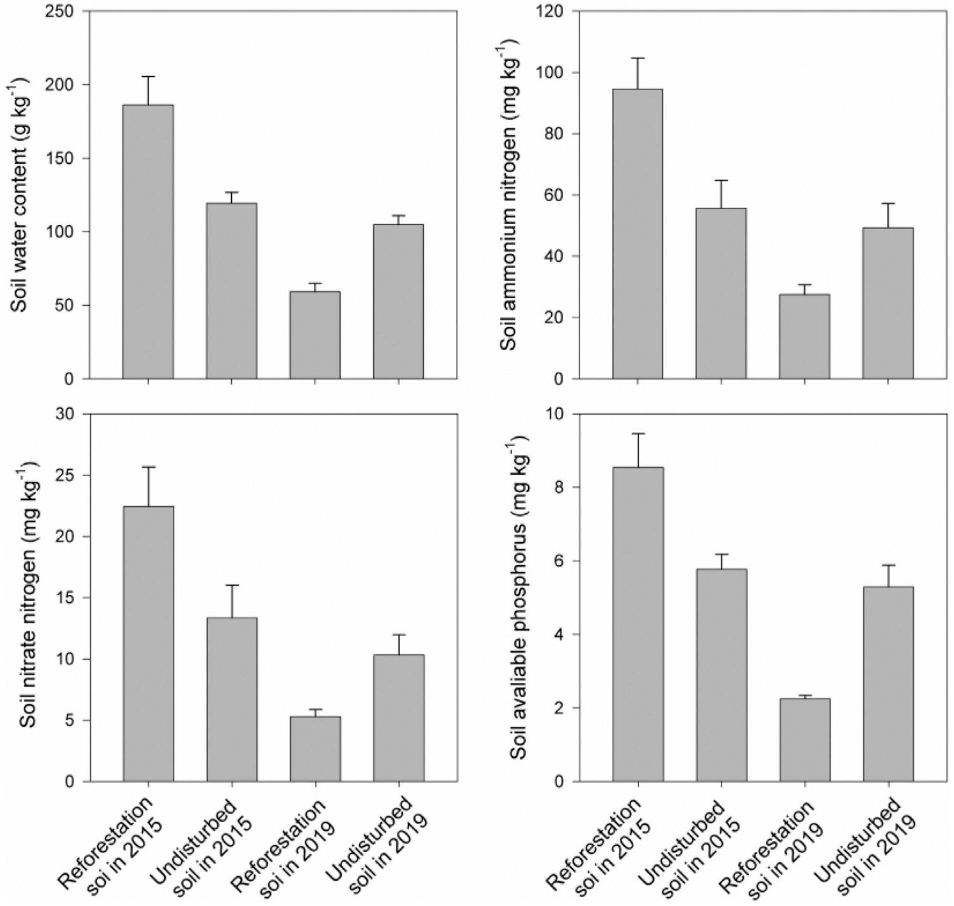

FIGURE 5 | Variation in soil water content, soil available nitrogen (ammonium and nitrate N), and soil available phosphorus among the initial local high nutrient soil before reforestation sampled in 2015 (reforestation soil in 2015), soils in undisturbed old-growth tropical rainforest sampled in 2015 (undisturbed soil in 2015), soils in reforestation area sampled in 2019 (reforestation soil in 2019) and soils in undisturbed old-growth tropical rainforest sampled in 2019 (undisturbed soil in 2019).

et al., 2018), but also indicated our reforestation protocol is successful in extremely degraded tropical rainforest.

Photosynthetic rate influences the energy balance of a plant, with higher photosynthetic rates being associated with a fast growth rate (Kirschbaum, 2011; Zhang et al., 2018b). Here, we found that the photosynthetic rates for the eight species were as much as five times larger than the eight native species. This can explain why the height and DBH of the initial seedlings of the species increase 3–5 times merely in 3 years' plantation (from the year of 2016 to 2019). Higher photosynthetic rates also result in higher leaf anatomy traits (Oguchi et al., 2003; He et al., 2017; Hua et al., 2017), stomatal traits (McAusland et al., 2016; Lawson and Vialet-Chabrand, 2019), transpiration rate and leaf hydraulic conductivity (Maherali et al., 2008; Bucci et al., 2019). Indeed, we found that these traits for the eight fast-growing species were three to five times greater than for native species. These results also indicate that these fast-growing tropical tree species likely developed appropriate leaf traits for maximizing photosynthetic rates and overall growth.

It is well known that soil available nitrogen, phosphorus, and soil water content are key constraints on plant maximum photosynthesis rates (Guan et al., 2015; Riley et al., 2018). This suggests that the eight fast-growing species may absorb a large amount of soil water and nutrients needed for maintaining a higher photosynthetic rate. This, in turn, may decrease soil water and nutrient storage. Indeed, we found that soil water, available nitrogen, and phosphorus largely decrease after 3 years' reforestation. Thus separately monocultures of multiple fast-growing tree species can indeed result in soil water and nutrient loss (Lamb et al., 2005; Wingfield et al., 2015). Generally in the mid-stage (usually more than 40 years) of secondary tropical rainforest succession, the increased species richness and abundances for the fast-growing pioneers tree species will lead to soil water and nutrient loss, and the late-successional slow-growing but highly resource competitive tree species will gradually replace the early successional fast-growing pioneers tree species (Grime, 2006; Mason et al., 2012). As a result, our reforestation protocol can effectively lead to a transition from primary tropical rainforest succession (consist of bare rock and cannot support any plant life) to the mid-stage of secondary tropical rainforest succession (dominant fast-growing pioneer tropical trees lead to soil water and nutrient loss).

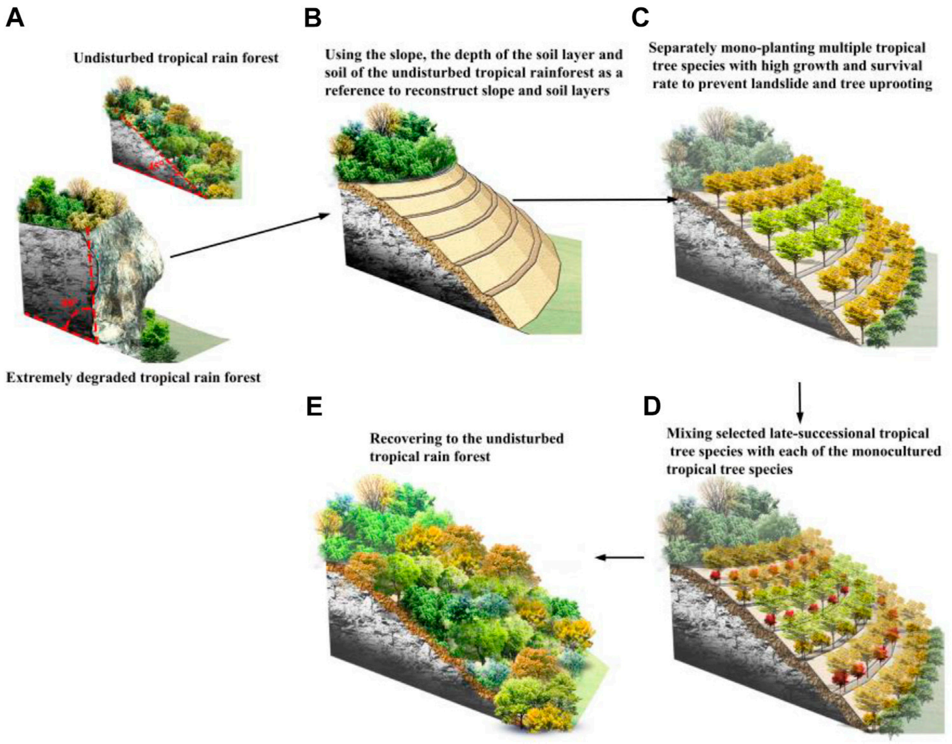

FIGURE 6 | A protocol for successfully performing reforestation to recover extremely degraded tropical rainforest. **(A)** Extremely degraded tropical rain forest and the adjacent undisturbed old-growth tropical rain forests. **(B)** using the slope, the deep of soil layers as a reference to reconstructing slope and soil layers, and to refill soils from the undisturbed old-growth tropical forests to form planting area in the extremely degraded tropical rain forest; **(C)** separately mono-planting multiple tropical tree species with high growth rate and survival rate; **(D)** mixing selected late-successional tropical tree species in the undisturbed tropical rainforest with each of the monocultured tropical tree species; **(E)** Recovering the extremely degraded tropical rainforest to the undisturbed old-growth tropical rainforest.

Our PCA results revealed that the eight separately mono-planting tree species and the eight dominant old-growth tree species were significantly distinguished by stomatal conductance, stomatal density, leaf water conductance, maximum photosynthesis rate and transpiration rate. This suggests that these five are the key traits related to higher growth rate in the monocultured fast growing species compared to the old-growth trees. Thus, these five traits can be utilized by a recently developed native species screening framework (Wang et al., 2020) in future to select many late-successional tropical tree species that are maximally dissimilar to these eight separately monocultured tropical tree species from all undisturbed old-growth tropical rainforest in whole Sanya City. Based on the limiting similarity theory, species that are functionally dissimilar can stable coexist (MacArthur and Levins, 1967). However, given the low soil water and nutrient and the much higher water and soil nutrient demand due to the eight separately monocultured tropical tree species' very high growth rate, these eight separately monocultured tropical tree species cannot compete with the late-successional tropical tree species. Thus, mixing these late-successional tropical tree species with each of the eight separately monocultured tropical tree species can quickly simulate late successional forests and thereby accelerate recovery to the undisturbed tropical rainforest as soon as possible.

CONCLUSION

In summary, based on tropical rainforest successional processes, we developed a reforestation protocol that includes the following steps for successfully performing reforestation to recover extremely degraded tropical rainforest. First, use the slope, the depth of the soil layers and soil of the adjacent undisturbed tropical rainforest as a reference to reconstruct slope and soil layers (**Figures 6A,B**). Second separately mono-planting multiple tropical tree species with high growth rate and surviving rate to prevent landslide and tree uprooting (**Figure 6C**). Third, evaluate whether the high fast-growth rate for these fast-growing species have resulted in large soil water and nutrient loss. If this these fast-growing species have indeed resulted in large soil water and nutrient loss, identify key traits that can be used Wang et al. (2020) to select many functionally dissimilar tropical tree species from the undisturbed old-growth tropical rainforest. Finally, we recommend mixing selected late-successional tropical tree species with each of the monocultured tropical tree species to quickly simulate the late succession thereby to recover to the undisturbed old-growth tropical rainforest as soon as possible (**Figures 6D,E**). We expect that this work will be important for the future recovery of other extremely degraded tropical rainforest ecosystems.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

YaZ, YiZ, LW, RS, HZ, TL, JC, CW, QH, and KJ designed research; YaZ, YiZ, LW, RS, HZ, BC, YX, TL, JC, CW, QH, and KJ performed research; YaZ, YiZ, LW, RS, HZ, TL, JC, CW, QH, and KJ analyzed data; YaZ, HZ, TL, JC, and CW wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2021.701210/full#supplementary-material

REFERENCES

Acosta, L. A., Eugenio, E. A., Macandog, P. B. M., Macandog, D. B. M., Lin, E. K. H., Abucay, E. R., et al. (2016). Loss and Damage from Typhoon-Induced Floods and Landslides in the Philippines: Community Perceptions on Climate Impacts and Adaptation Options. *Int. J. Glob. Warming* 9 (1), 33–65. doi:10.1504/ijgw.2016.074307

Aleman, J. C., Jarzyna, M. A., and Staver, A. C. (2018). Forest Extent and Deforestation in Tropical Africa Since 1900. *Nat. Ecol. Evol.* 2, 26–33. doi:10.1038/s41559-017-0406-1

Bucci, S. J., Carbonell Silletta, L. M., Garré, A., Cavallaro, A., Efron, S. T., Arias, N. S., et al. (2019). Functional Relationships Between Hydraulic Traits and the Timing of Diurnal Depression of Photosynthesis. *Plant Cell Environ.* 42 (5), 1603–1614. doi:10.1111/pce.13512

Burt, R. (2009). *Soil Survey Field and Laboratory Methods Manual*. Washington, DC: National Soil Survey Center, Natural Resources Conservation Service, US Department of Agriculture.

Chang, K. T., Chiang, S. H., and Lei, F. (2008). Analysing the Relationship Between Typhoon-Triggered Landslides and Critical Rainfall Conditions. *Earth Surf. Process. Landforms* 33 (8), 1261–1271. doi:10.1002/esp.1611

Chapin, F. S., Walker, L. R., Fastie, C. L., and Sharman, L. C. (1994). Mechanisms of Primary Succession Following Deglaciation at Glacier Bay, Alaska. *Ecol. Monogr.* 64, 149–175. doi:10.2307/2937039

Funk, J. L., Cleland, E. E., Suding, K. N., and Zavaleta, E. S. (2008). Restoration Through Reassembly: Plant Traits and Invasion Resistance. *Trends Ecol. Evol.* 23 (12), 695–703. doi:10.1016/j.tree.2008.07.013

Grime, J. P. (2006). Trait Convergence and Trait Divergence in Herbaceous Plant Communities: Mechanisms and Consequences. *J. Vegetation Sci.* 17 (2), 255–260. doi:10.1111/j.1654-1103.2006.tb02444.x

Griscom, B. W., Adams, J., Ellis, P. W., Houghton, R. A., Lomax, G., Miteva, D. A., et al. (2017). Natural Climate Solutions. *Proc. Natl. Acad. Sci. USA* 114 (44), 11645–11650. doi:10.1073/pnas.1710465114

Guan, K., Pan, M., Li, H., Wolf, A., Wu, J., Medvigy, D., et al. (2015). Photosynthetic Seasonality of Global Tropical Forests Constrained by Hydroclimate. *Nat. Geosci.* 8 (4), 284–289. doi:10.1038/ngeo2382

Guo, K. J., Xiong, J. W., Wu, J. Z., Liu, Z. F., Li, Z. G., and Zhang, B. (2013). Scenery Resources Survey and Feature Analysis of forest in Sanya City. *Cent. South For. Inventory Plann.* 32 (3), 11–17. doi:10.16166/j.cnki.cn43-1095.2013.03.001

He, N., Liu, C., Tian, M., Li, M., Yang, H., Yu, G., et al. (2017). Variation in Leaf Anatomical Traits from Tropical to Cold-temperate Forests and Linkage to Ecosystem Functions. *Funct. Ecol.* 32 (8), 10–19. doi:10.1111/1365-2435.12934

Houghton, R. A., Skole, D. L., Nobre, C. A., Hackler, J. L., Lawrence, K. T., and Chomentowski, W. H. (2000). Annual Fluxes of Carbon from Deforestation and Regrowth in the Brazilian Amazon. *Nature* 403 (6767), 301–304. doi:10.1038/35002062

Hua, L., Chen, Y., Zhang, H., Fu, P., and Fan, Z. (2017). Stronger Cooling Effects of Transpiration and Leaf Physical Traits of Plants from a Hot Dry Habitat Than from a Hot Wet Habitat. *Funct. Ecol.* 31 (12), 2202–2211. doi:10.1111/1365-2435.12923

Johnson, E. A., and Miyanishi, K. (2008). Testing the Assumptions of Chronosequences in Succession. *Ecol. Lett.* 11 (5), 419–431. doi:10.1111/j.1461-0248.2008.01173.x

Kirschbaum, M. U. F. (2011). Does Enhanced Photosynthesis Enhance Growth? Lessons Learned from CO2 Enrichment Studies. *Plant Physiol.* 155 (1), 117–124. doi:10.1104/pp.110.166819

Lamb, D., Erskine, P. D., and Parrotta, J. A. (2005). Restoration of Degraded Tropical Forest Landscapes. *Science* 310 (5754), 1628–1632. doi:10.1126/science.1111773

Laurance, W. F., Sayer, J., and Cassman, K. G. (2014). Agricultural Expansion and its Impacts on Tropical Nature. *Trends Ecol. Evol.* 29 (2), 107–116. doi:10.1016/j.tree.2013.12.001

Lawson, T., and Vialet-Chabrand, S. (2019). Speedy Stomata, Photosynthesis and Plant Water Use Efficiency. *New Phytol.* 221 (1), 93–98. doi:10.1111/nph.15330

Lin, J. Y., Zeng, R., Liu, H. W., Chen, H. Y., and Li, S. X. (2006). The Important Role of Local Tree Species in the Construction of Tropical Characteristic Garden. *Trop. For.* 4, 1–11. doi:10.3969/j.issn.1672-0938.2006.04.007

Liu, X. Z., Xue, Y., Wang, X. Y., Lin, Z. P., and Su, X. F. (2017). Analysis of Different Reforestation experiment in the Northeast Tropical Coast of Hainan Province. *Ecol. Sci.* 36, 130–134. doi:10.14108/j.cnki.1008-8873.2017.03.018

Lohbeck, M., Poorter, L., Martínez-Ramos, M., Rodriguez-Velázquez, J., van Breugel, M., and Bongers, F. (2014). Changing Drivers of Species Dominance During Tropical forest Succession. *Funct. Ecol.* 28 (4), 1052–1058. doi:10.1111/1365-2435.12240

Lugo, A. E. (1997). The Apparent Paradox of Reestablishing Species Richness on Degraded Lands with Tree Monocultures. *For. Ecol. Manage.* 99 (1), 9–19. doi:10.1016/S0378-1127(97)00191-6

Luo, H. X., Dai, S. P., Li, M. F., and Xi, Z. H. (2018). The Climate Characteristics of 302 Hainan Island from 1959-2015. *Jiangsu Agric. Sci.* 46 (15), 261–268. doi:10.15889/j.issn.1002-1302.2018.15.066

MacArthur, R., and Levins, R. (1967). The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The Am. Naturalist* 101 (921), 377–385. doi:10.1086/282505

Maherali, H., Sherrard, M. E., Clifford, M. H., and Latta, R. G. (2008). Leaf Hydraulic Conductivity and Photosynthesis Are Genetically Correlated in an Annual Grass. *New Phytol.* 180 (1), 240–247. doi:10.1111/j.1469-8137.2008.02548.x

Mason, N. W. H., Richardson, S. J., Peltzer, D. A., de Bello, F., Wardle, D. A., and Allen, R. B. (2012). Changes in Coexistence Mechanisms along a Long-Term Soil Chronosequence Revealed by Functional Trait Diversity. *J. Ecol.* 100 (3), 678–689. doi:10.1111/j.1365-2745.2012.01965.x

McAusland, L., Vialet-Chabrand, S., Davey, P., Baker, N. R., Brendel, O., and Lawson, T. (2016). Effects of Kinetics of Light-induced Stomatal Responses on Photosynthesis and Water-use Efficiency. *New Phytol.* 211 (4), 1209–1220. doi:10.1111/nph.14000

Mitchard, E. T. A. (2018). The Tropical Forest Carbon Cycle and Climate Change. *Nature* 559 (7715), 527–534. doi:10.1038/s41586-018-0300-2

Oguchi, R., Hikosaka, K., and Hirose, T. (2003). Does the Photosynthetic Light-Acclimation Need Change in Leaf Anatomy? *Cell Environ.* 26 (4), 505–512. doi:10.1046/j.1365-3040.2003.00981.x

Pang, C.-C., Ma, X. K.-K., Lo, J. P.-L., Hung, T. T.-H., and Hau, B. C.-H. (2018). Vegetation Succession on Landslides in Hong Kong: Plant Regeneration, Survivorship and Constraints to Restoration. *Glob. Ecol. Conservation* 15 (2), e00428. doi:10.1016/j.gecco.2018.e00428

Riley, W. J., Zhu, Q., and Tang, J. Y. (2018). Weaker Land-Climate Feedbacks from Nutrient Uptake During Photosynthesis-Inactive Periods. *Nat. Clim. Change* 8, 1002–1006. doi:10.1038/s41558-018-0325-4

Stokes, A., Atger, C., Bengough, A. G., Fourcaud, T., and Sidle, R. C. (2009). Desirable Plant Root Traits for Protecting Natural and Engineered Slopes Against Landslides. *Plant Soil* 324 (1), 1–30. doi:10.1007/s11104-009-0159-y

Symes, W. S., Edwards, D. P., Miettinen, J., Rheindt, F. E., and Carrasco, L. R. (2018). Combined Impacts of Deforestation and Wildlife Trade on Tropical Biodiversity Are Severely Underestimated. *Nat. Commun.* 9 (1), 4052. doi:10.1038/s41467-018-06579-2

Taubert, F., Fischer, R., Groeneveld, J., Lehmann, S., Müller, M. S., Rödig, E., et al. (2018). Global Patterns of Tropical forest Fragmentation. *Nature* 554 (7693), 519–522. doi:10.1038/nature25508

Villamayor, B. M. R., Rollon, R. N., Samson, M. S., Albano, G. M. G., and Primavera, J. H. (2016). Impact of Haiyan on Philippine Mangroves: Implications to the Fate of the Widespread Monospecific Rhizophora Plantations Against Strong Typhoons. *Ocean Coastal Manage.* 132 (2), 1–14. doi:10.1016/j.ocecoaman.2016.07.011

Walker, L. R., Velázquez, E., and Shiels, A. B. (2009). Applying Lessons from Ecological Succession to the Restoration of Landslides. *Plant Soil* 324 (1), 157–168. doi:10.1007/s11104-008-9864-1

Wang, C., Zhang, H., Liu, H., Jian, S., Yan, J., and Liu, N. (2020). Application of a Trait-based Species Screening Framework for Vegetation Restoration in a Tropical Coral Island of China. *Funct. Ecol.* 34 (6), 1193–1204. doi:10.1111/1365-2435.13553

Wingfield, M. J., Brockerhoff, E. G., Wingfield, B. D., and Slippers, B. (2015). Planted Forest Health: The Need for a Global Strategy. *Science* 349 (6250), 832–836. doi:10.1126/science.aac6674

Yumul, G. P., Servando, N. T., Suerte, L. O., Magarzo, M. Y., Juguan, L. V. V., and Dimalanta, C. B. (2012). Tropical Cyclone-Southwest Monsoon Interaction and the 2008 Floods and Landslides in Panay Island, central Philippines: Meteorological and Geological Factors. *Nat. Hazards* 62 (3), 827–840. doi:10.1007/s11069-012-0109-5

Zhang, H., Chen, H. Y. H., Lian, J., John, R., Ronghua, L., Liu, H., et al. (2018a). Using Functional Trait Diversity Patterns to Disentangle the Scale-dependent Ecological Processes in a Subtropical forest. *Funct. Ecol.* 32 (5), 1379–1389. doi:10.1111/1365-2435.13079

Zhang, H., John, R., Zhu, S., Liu, H., Xu, Q., Qi, W., et al. (2018b). Shifts in Functional Trait-Species Abundance Relationships over Secondary Subalpine Meadow Succession in the Qinghai-Tibetan Plateau. *Oecologia* 188 (2), 547–557. doi:10.1007/s00442-018-4230-3

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Nitrogen Addition and Arbuscular Mycorrhizal Fungi Beta Diversity: Patterns and Mechanisms

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Global nitrogen eutrophication, which is disrupting the intimate plant–arbuscular mycorrhizal fungi (AMF) symbiosis, can alter the diversity and physiological functions of soil AMF greatly. However, shifts of beta diversity and the intrinsic patterns of AMF community dissimilarities in response to nitrogen addition remain unclear. Based on a 7-year nitrogen addition experiment in a Qinghai–Tibet Plateau alpine meadow, we detected the changes in soil AMF alpha diversity (richness and genus abundance) and the community composition beta diversity by partitioning the two components of Simpson and nestedness dissimilarities along (turnover) and within (variation) nitrogen addition treatments, and fitted with environmental factor dissimilarities. We found that nitrogen addition decreased AMF richness by decreasing the most dominant AMF genus of *Glomus* but increasing the abundance of the rare genera. The turnover of the AMF community overall beta diversity along the nitrogen addition gradients was induced by the increased nestedness dissimilarity, while the variation within treatments was explained by both increased Simpson and nestedness dissimilarities, which was significantly correlated with soil pH. Our study found both Simpson and nestedness dissimilarities worked on the AMF community dissimilarity after nitrogen addition and the significant variation within the same treatment, which would be important in the future for predicting global AMF or microbial diversity changes in response to nitrogen eutrophication.

Keywords: beta diversity, community composition, nestedness, turnover, nitrogen addition

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INTRODUCTION

Arbuscular mycorrhizal fungi, which belong to the phylum Glomeromycota, are widely distributed in soil and plant roots (Smith and Read, 2008; Davison et al., 2015). The fungi, forming symbiosis with plants to swap nutrients by supplying soil nitrogen and phosphorus and capturing the photosynthetic products in return, can protect plants from abrupt environmental changes or biotic attacks such as pathogens, which is an essential part of ecosystem processes (Johnson, 2010; Tedersoo et al., 2020). Global changes in nitrogen eutrophication caused by anthropogenic activities have largely impacted the physiological functions and diversity of AMF (Treseder, 2004; Jiang et al., 2018), yet the underlying mechanisms of these processes are still not fully understood.

Previous studies have shown that nitrogen addition changed the AMF alpha diversity. Most studies illustrated a decreased AMF colonization rate after nitrogen addition, owing to the decreased dependence of plants (Treseder, 2004; Liu et al., 2012), which could also result in a decrease in AMF alpha diversity in soil. However, a meta-analysis showed a negative but nonsignificant relationship between the AMF richness and nitrogen addition (Han et al., 2020), which may be due to variation among different ecosystems, treatment forms, rates, soil properties (Egerton-Warburton et al., 2007; Camenzind et al., 2014; Zheng et al., 2014), and/or the abundance shift of specific AMF taxons with specific functions. In plant roots, the more redundant AMF families may decrease in abundance more quickly, while the less abundant taxa increase in abundance after nitrogen addition, even though the overall richness remains unchanged (Lu et al., 2020). Hence, identifying the changes in alpha diversity and taxon abundance of AMF species in soil under long-term nitrogen addition is meaningful but not useful for discovering the functional properties and their role in the ecosystem process.

Beta diversity, which links the biodiversity between local (alpha diversity) and regional (gamma diversity) diversity, reflects the degree of species similarity (or dissimilarity) among species assemblages at different places and times (Whittaker, 1960; Whittaker, 1972; Vellend, 2010). Despite the low endemism of global AMF species (Davison et al., 2015), fungi still showed compositional dissimilarities due to spatiotemporal heterogeneity (Hiiesalu et al., 2014; Bahram et al., 2015). For example, AMF community composition differs among different nitrogen addition treatments by interrupting the intimate mutualistic relationship (Jiang et al., 2018; Lu et al., 2020). Actually, two metrics of beta diversity have been proposed for measuring composition dissimilarity (Anderson et al., 2011). The first one measures directional turnover (thereafter “turnover”) or changes in community composition along an environment or treatment gradient, from one sampling unit to another (Vellend, 2001). The other is defined as the non-directional variation (thereafter “variation”) within a specific region or gradient range, which was directly related to the multivariate dispersion of community composition (Legendre et al., 2005; Anderson et al., 2006). Different measures emphasize particular properties (Anderson et al., 2011), but whether nitrogen addition affects turnover and/or variation of AMF communities remains unknown.

The overall beta diversity can additionally be partitioned into two intrinsic components, that is, the Simpson and nestedness dissimilarities (Baselga, 2010). The Simpson dissimilarity, previously referred to as “spatial turnover” (Baselga, 2010), defines the replacement of some species by others as a consequence of spatial and historical constraints or environmental differentiation, with species losses balancing the gains (Lennon et al., 2001; Koleff et al., 2003). The nestedness dissimilarity emphasizes that the biotas of sites with fewer species are subsets of the richer sites, forming an inverted stepped structure (Baselga, 2010; Baselga, 2012). Both Simpson and nestedness dissimilarities can exist in turnover and variation. Exploring the intrinsic causes of AMF community beta diversity can provide a better understanding of whether community dissimilarity is due to difference in species diversity and/or function replacement or the loss and gain of AMF species, which requires opposing biodiversity conservation strategies (Thébault and Fontaine, 2010; Baselga, 2012). Likewise, many previous experiments also failed to disentangle the contribution of Simpson and nestedness beta diversity patterns to the overall AMF community dissimilarity under nitrogen addition.

Environmental changes (abiotic or biotic) after nitrogen addition played important roles in shaping the beta diversity of microbiota (Xu et al., 2016; Fierer, 2017). First, nitrogen addition can reduce plant species richness and change community composition (Tilman, 1987; Stevens et al., 200), and it led to a reduction in AMF root colonization via both the direct AMF physiological effects and shift in plant community composition (Liu et al., 2012; Lu et al., 2020). Second, the alternation of soil chemical properties after nitrogen addition is also closely related to the AMF diversity (Egerton-Warburton et al., 2007; Zheng et al., 2014). For instance, soil pH was considered an important factor shaping AMF community composition, with a higher pH value being positive to AMF diversity (Xu et al., 2016; Chen et al., 2017). The content of different nitrogen forms and other microbes in soil can also affect soil AMF abundance and composition (Egerton-Warburton et al., 2007; Zheng et al., 2014; Chen et al., 2017). However, it remains unknown whether and how the environmental factors affect overall and partitioned AMF community beta diversity, which may help detect and understand any complicated diversity changes after nitrogen addition.

Alpine meadows are known as a “hot spot” for above- and belowground biodiversity. However, the sophisticated alpha and beta diversity changes of the AMF community under nitrogen addition and the intrinsic patterns have not been fully explored. Here, based on a 7-year nitrogen addition experiment in a Qinghai–Tibet Plateau alpine meadow, we investigate the soil AMF alpha diversity (richness and taxon abundance change) and beta diversity by partitioning the two components of Simpson and nestedness dissimilarities along (i.e., turnover) and within (i.e., variation) nitrogen addition treatment and their relationships with environmental factors. We aim to test the following hypotheses: 1) nitrogen addition decreases AMF richness by decreasing the dominant AMF taxon but increasing the abundance of the rare taxa; 2) the partitioned Simpson and nestedness beta dissimilarities may provide different explanation powers on the soil AMF community overall dissimilarity along and within different nitrogen addition treatments; and 3) soil AMF beta diversity is related to dissimilarities in environmental factors.

MATERIALS AND METHODS

Study Site

The study was conducted at the Gannan Grassland Ecosystem Field Science Observation and Research Station of the Ministry of Education (101° 52’ E, 33° 40’ N; 3500 m. a.s.l.) in Maqu County, Gansu Province, the eastern part of the Qinghai–Tibet Plateau, China. The site is a typical alpine meadow vegetation ecosystem, and the nitrogen-limited soils are classified as “chestnut” or “subalpine meadow” soils, with an average thickness of 80 cm (Liu et al., 2017). The annual mean precipitation is 620 mm, which mainly falls during the short growing season from May to

September. The annual mean temperature is 1.2°C, ranging from–10.7°C in January to 11.7°C in July. The alpine meadow is dominated by some perennial herbaceous species such as Asteraceae, Poaceae, and Ranunculaceae, and herbivore animals include yaks, marmots, and zokors (Lu et al., 2020).

Experimental Design

The long-term nitrogen addition experiment had been established on a south-facing meadow since June 2011, with grazing (mainly yaks) permitted only in winter. We regularly arranged twenty-four 5 × 5-m plots in a 50 × 50-m area with roughly the same plant community composition, and the adjacent edges of the plots were separated by a 1-m buffer zone. Four concentrations of nitrogen addition of ammonium nitrate (NH_4NO_3) with six replicates were randomly assigned to the 24 plots: 0 (ambient), 5, 10, or 15 gm^{-2} yr^{-1} (Liu et al., 2016; Jiang et al., 2018). Nitrogen addition has been applied annually in mid-June since 2011.

Data Collection

In August 2017, we randomly arranged one 0.5 × 0.5-m subplot in each of the 24 plots, parallel to but at least 0.5 m away from the plot edges. We harvested all the aboveground stems in each subplot and recorded the species richness and abundance (i.e., number of individuals) of each species. The 7-year nitrogen addition treatment had significantly changed the plant species richness and community composition and soil chemical properties (Liu et al., 2020; Lu et al., 2020). Additionally, we drilled four soil cores in each plot which were 5 cm in diameter and 10 cm in depth, mixing into one soil sample. The soil samples were transported to the laboratory for measuring soil chemical properties and molecular AMF diversity identification.

Soil chemical properties include soil pH (measured using a pH meter forming a slurry with a dry soil-to-water ratio of 1:5), soil moisture (%; measured as the percent change of weight after 72 h of desiccation at 70°C), soil ammonium nitrogen (NH_4^+–N mg/kg; measured using the indophenol blue colorimetric method), soil nitrate nitrogen (NO_3^-–N mg/kg; measured using the salicylate colorimetric method), soil microbial biomass carbon and nitrogen (MBC and MBN mg/kg; measured using the chloroform fumigation–direct extraction method), and soil total carbon and total nitrogen (TC and TN, g/kg, measured using an element analyzer) (Elementar Vario EL III, Hanau, Germany).

Molecular and Bioinformatic Analysis

For each composite soil sample, around 50 g root-free soil was resampled for DNA extraction. The AMF community diversity identification was followed by a polymerase chain reaction (PCR) sequencing approach described by Lu et al. (2020). In brief, the soil DNA from each sample was extracted using a Qiagen Plant DNeasy Kit (Qiagen, Hilden, Germany) and evaluated with 1% (w/v) agarose gel to confirm efficiency. The fungal 18 S rRNA genes of distinct regions were amplified by PCR using the specific primer pair AMV4.5NF and AMDGR (Sato et al., 2005; Lumini et al., 2010), with a 12-bp barcode. After detection, pooling, and purification of PCR products, we generated sequencing libraries using an NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, United States). At last, the library was sequenced on an IlluminaHiseq2500 platform, and 250-bp paired-end reads were generated.

High-quality clean reads were obtained using the Trimmomatic (V0.33) quality control process that sequences with no valid primer sequencing, that is, an average quality score of <25 or length <200 bp was discarded. We merged paired-end clean reads with FLASH (V1.2.11) and assigned sequences to each sample with mothur software (V1.35.1) based on the unique barcode and primer. Sequences with a similarity threshold above 97% were clustered to the same operational taxonomic units (OTUs) by removing potential chimeric and singleton reads. The representative sequences for all 1313 detected OTUs were compared using the SILVA database for further annotation (Pruesse et al., 2007). To compare our data with those of other published studies, AMF OTUs were blasted and classified into the virtual taxa (i.e., VT) using the online database MaarjAM (http://maarjam.botany.ut.ee), and an *e*-value less than 1*e*–50 was considered significant (Öpik et al., 2010). The representative sequence of each AMF VT has been deposited in the NCBI Sequence Read Archive (SRA) database (accession code SRP318342).

Statistical Analysis

We rarefied each sample to a minimum of 7000 sequences in total for downstream analysis to allow for equalization of different sequencing depths and to enable comparison between samples with the rarecurve function in the vegan package (Oksanen et al., 2013). For each plot, the AMF VT richness, Shannon diversity, Simpson diversity, and the relative abundance of each AMF genus were calculated, and we used linear models to test the effects of nitrogen addition on them.

Based on the community matrix of AMF VT, the beta diversity between samples was calculated with the abundance-weighted (i.e., relative sequence reads) Bray–Curtis, and Sørensen dissimilarity matrices. The Sørensen dissimilarity represents the sum of dissimilarities due to spatial turnover (i.e., Simpson dissimilarity) and nestedness (Baselga, 2010; Baselga, 2012). We partitioned the Sørensen dissimilarity of the AMF community into Simpson and nestedness diversity using the beta. pair function (index.family = “sorensen”) in the betapart package (Baselga and Orme, 2012). To confirm our results, we also employed another beta partition method that separates the overall Jaccard dissimilarity (corresponds to Sørensen dissimilarity) into species replacement (corresponds to Simpson dissimilarity) and richness difference (corresponds to nestedness dissimilarity) (Podani and Schmera, 2011; Carvalho et al., 2012) with function beta.multi in the BAT package (Cardoso et al., 2015) and built linear models to detect the correlations of the three sets of data.

To identify the turnover of the AMF community beta diversity along four nitrogen addition treatments, we calculated the treatment distance as the difference between the intensities of different nitrogen addition treatments. We built linear models with the nitrogen addition treatment distance as the independent

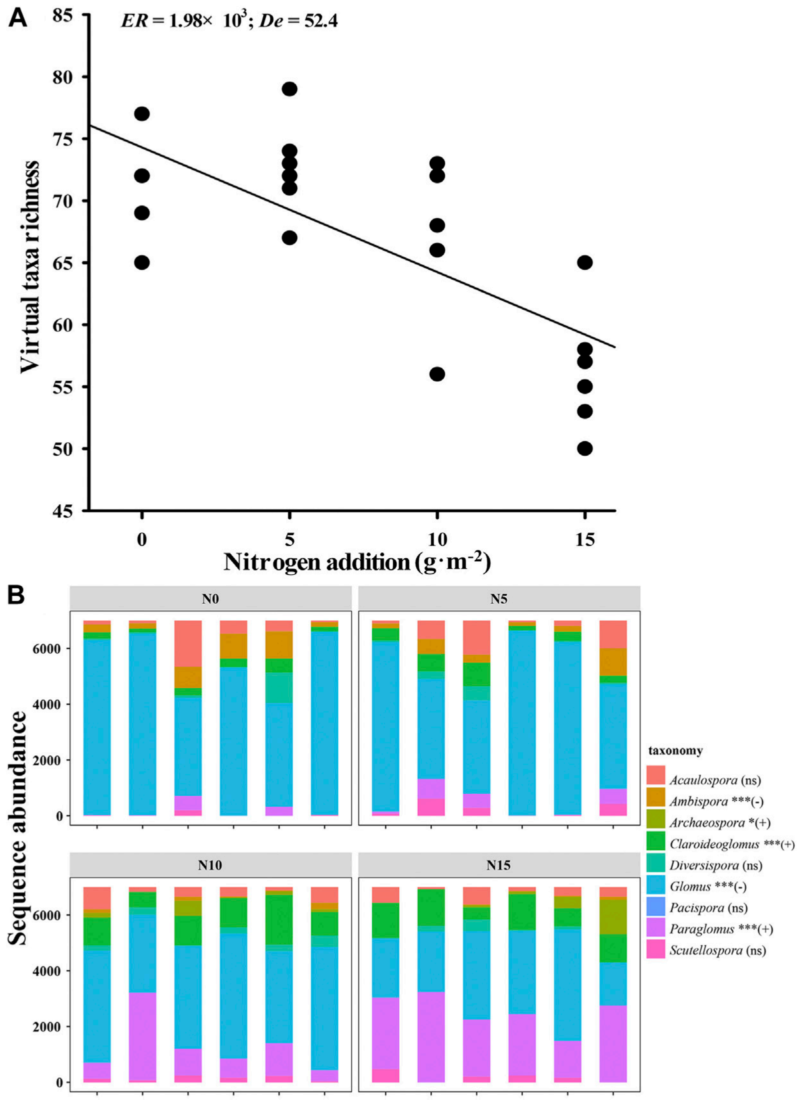

FIGURE 1 | (A) Virtual taxa richness decreased with nitrogen addition (information-to-theory evidence ratio (*ER*) = 1.98 × 10^3; percent deviance explained as (*De*) = 52.4; with some overlapping points). *ER* > 1.5 means a significant relationship. **(B)** AMF abundance change of the rarified sequence assigned to the represented genera with four nitrogen addition treatments. The "+" or "–" sign in parentheses means significant increase or decrease in sequence abundance with nitrogen addition, while "ns" means insignificance.

variable, and the Bray–Curtis dissimilarity (β-bray; abundance-weighted), Sørensen dissimilarity (β-sor; presence/absence), and partitioned Simpson (β-sim), and nestedness (β-nes) dissimilarities as the response variables, separately. Similarly, variation of AMF community beta diversity among replicates at each nitrogen addition level was also tested with linear models, with the β-bray, β-sor, β-sim, and β-nes dissimilarities among the same treatment samples as response variables, and the exact nitrogen addition level (i.e., within treatment) as the independent variable. The significance of the linear models was evaluated using the information-to-theory evidence ratio (*ER*), calculating the relative support between the linear slope model and the intercept-only (null) model, which was better than the arbitrary *p*-value, and *ER* > 1.5 means a significant relationship (Burnham et al., 2011). The goodness of fit of the linear models was calculated with the percent deviance explained (*De*) in the response variable. We also used one-way ANOVA to decompose the Simpson and nestedness components of the overall Sørensen dissimilarities after nitrogen addition with the turnover and variation patterns, respectively (Lepš et al., 2011; Lu et al., 2020). The percentage explained in the Sørensen dissimilarity partitioned by Simpson and nestedness dissimilarities and their covariation was calculated.

We used PERMANOVA (permutation multivariate analysis) with 9999 permutations for β-bray, β-sor, β-sim, and β-nes dissimilarity matrices to test for the beta diversity differences of AMF communities under different nitrogen addition treatments, using the adonis function in the vegan package

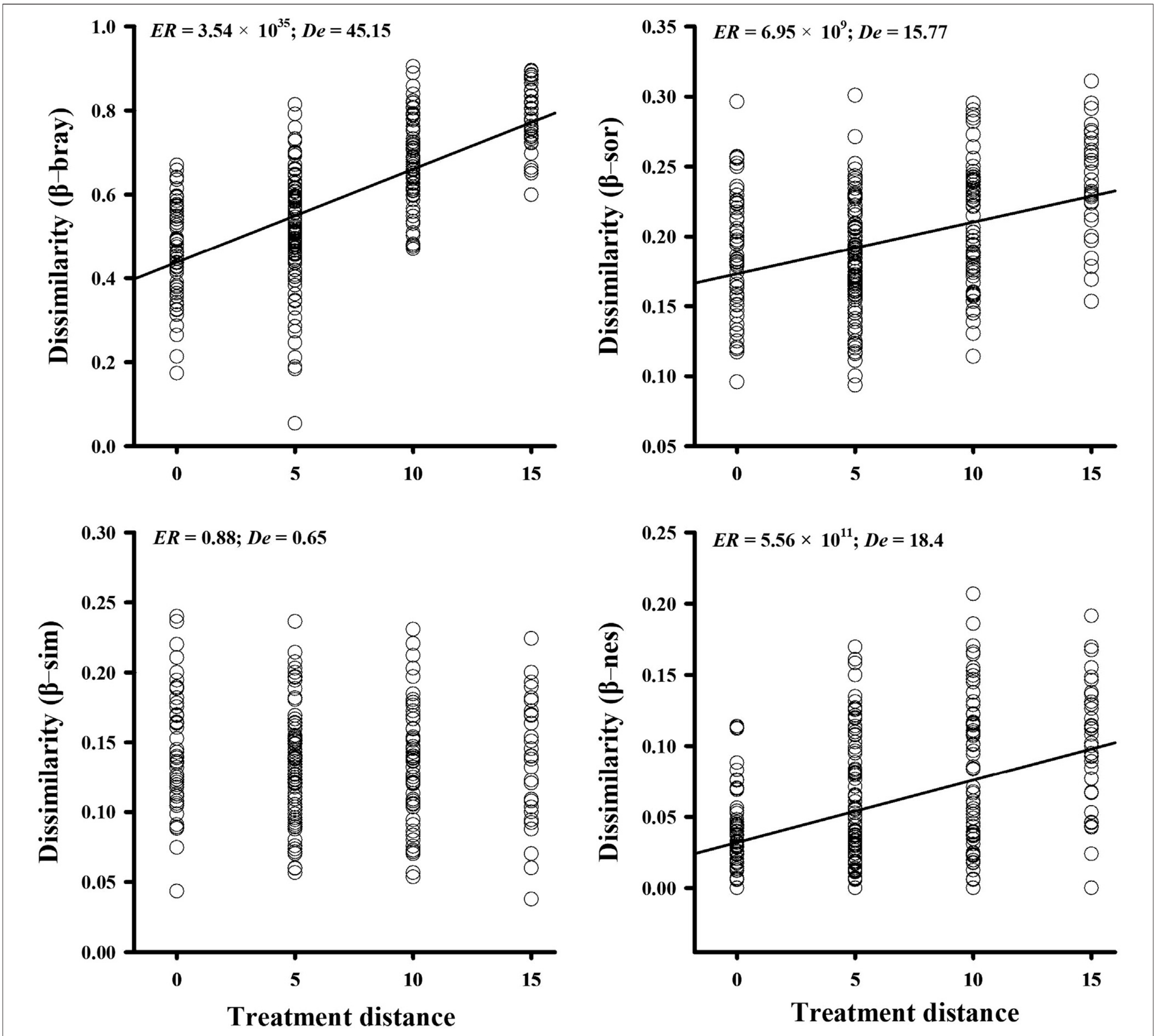

FIGURE 2 | Turnover of the soil AMF community beta diversity of Bray–Curtis (β-bray; abundance-weighted), Sørensen (β-sor; presence/absence-based), partitioned Simpson (β-sim), and nestedness (β-nes) along the nitrogen addition gradient. *ER* > 1.5 means a significant relationship.

(Oksanen et al., 2013). To graphically illustrate the AMF community β-bray, β-sor, β-sim, and β-nes dissimilarities between and within nitrogen addition treatments, dissimilarity matrices were formed by PCoA (principal coordinate analysis) with the pcoa function in the ape package (Paradis et al., 2004).

We calculated Spearman's rank correlation between the environmental factor dissimilarities of plant community composition, soil chemical properties (pH, moisture, NH_4^+–N, NO_3^-–N, MBC, MBN, TC, and TN), and β-bray, β-sor, β-sim, and β-nes dissimilarities (Oksanen et al., 2013). The plant community dissimilarities were calculated in line with the AMF community dissimilarities, with β-bray, β-sor, β-sim, and β-nes dissimilarities. The soil chemical property dissimilarities were calculated by subtracting between samples, and the correlations of the soil properties were tested using the cor. test function by Pearson's rank correlation examination. All the calculations were done in R version 3.5.1 (R Development Core Team, 2014).

RESULTS

Out of the 24 soil samples, a total of 885, 077 sequencing reads of the phylum Glomeromycota and 114 AMF virtual taxa (VTs) belonging to nine genera were observed after rarefaction. Nitrogen addition significantly decreased the soil AMF VT

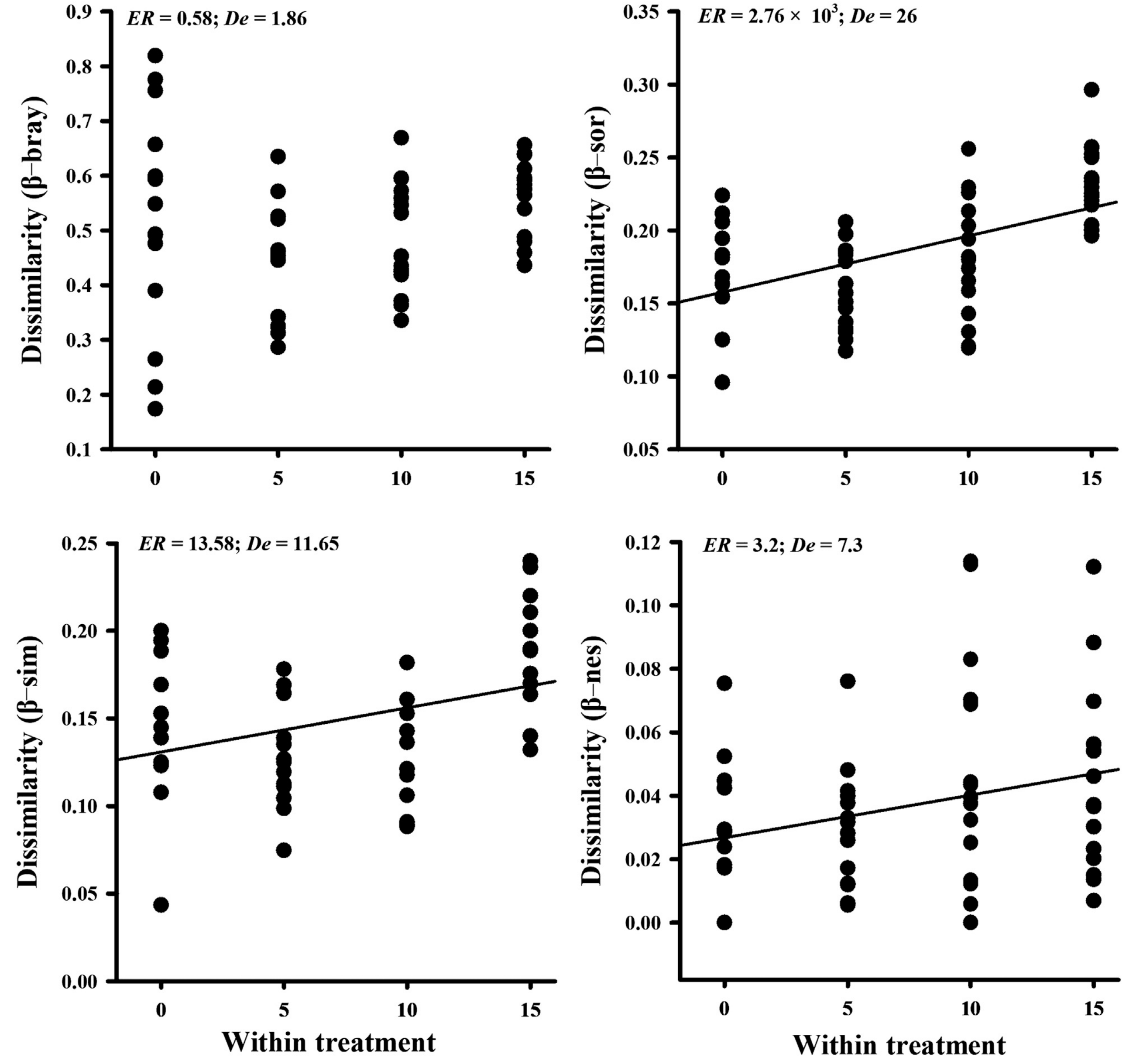

FIGURE 3 | Variation of the soil AMF community beta diversity of Bray–Curtis (*β*-bray; abundance-weighted), Sørensen (β-sor; presence/absence-based), partitioned Simpson (β-sim), and nestedness (β-nes) within four nitrogen addition treatments. *ER* > 1.5 means a significant relationship.

richness ($ER = 1.98 \times 10^3$; $De = 52.4$; **Figure 1A**) but not the Shannon ($ER = 0.30$; $De = 0.92$) and Simpson diversities ($ER = 0.46$; $De = 4.41$; **Supplementary Figure S2**). The abundance of different AMF genera also had a significant variation along and within nitrogen addition gradients, with the AMF genera of *Ambispora* ($p < 0.001$) and *Glomus* ($p < 0.001$) decreasing significantly but the genera of *Archaeospora* ($p = 0.03$), *Claroideoglomus* ($p < 0.001$), and *Paraglomus* ($p < 0.001$) increasing significantly, while the genera of *Acaulospora* ($p = 0.493$), *Diversispora* ($p = 0.637$), *Pacispora* ($p = 0.665$), and *Scutellospora* ($p = 0.283$) did not change with nitrogen addition (**Figure 1B**).

We found that the results obtained using the beta partition method proposed by Baselga (2010) and those obtained using the method proposed by Podani and Schmera (2011) and Carvalho et al. (2012) showed significant correlations, with β-sor highly correlated with Jaccard dissimilarity ($p < 0.001$), β-sim correlated with replacement ($p < 0.001$), and β-nes correlated with richness difference ($p < 0.001$) (**Supplementary Figure S3**). Hence, we only report the results of β-sor, β-sim, and β-nes below.

Based on linear models, the turnover of the AMF community β-bray (abundance-weighted) ($ER = 3.54 \times 10^{35}$; $De = 45.15$) and the β-sor (presence/absence data) ($ER = 6.95 \times 10^9$; $De = 15.77$) dissimilarities both significantly increased with nitrogen addition,

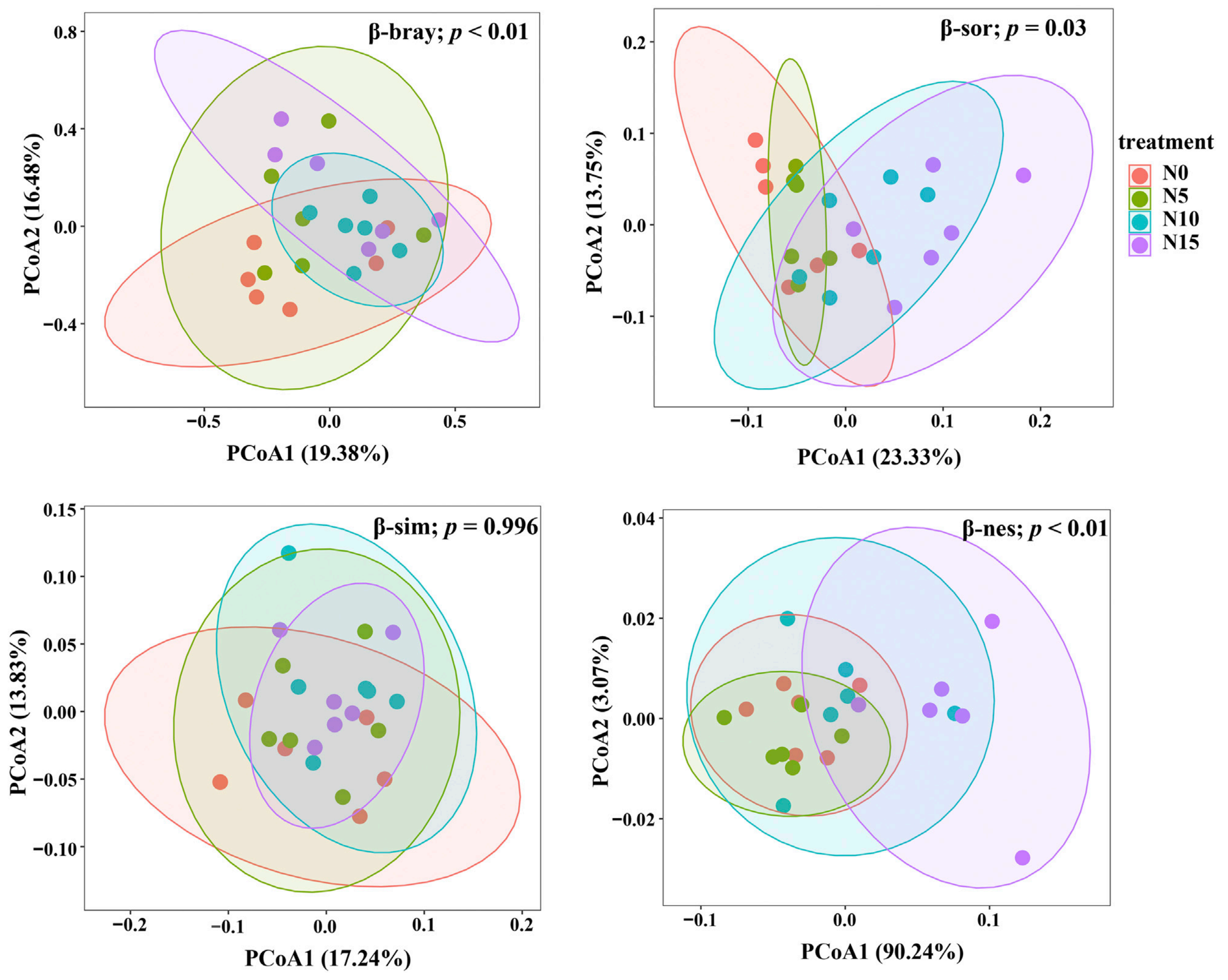

FIGURE 4 | Principal coordinate analysis (PCoA) by PERMANOVA showing the association of soil AMF community dissimilarities along and within nitrogen addition treatments. Significance of the Bray–Curtis (β-bray; abundance-weighted), Sørensen (*β*-sor; presence/absence-based), partitioned Simpson (β-sim), and nestedness (β-nes) dissimilarities with nitrogen addition are shown with *p* values ($p \leq 0.05$ means a significant effect).

which can be attributed to the significant increase in β-nes ($ER = 5.56 \times 10^{11}$; $De = 18.4$) but not in β-sim ($ER = 0.88$; $De = 0.64$) dissimilarities (**Figure 2**). The results of one-way ANOVA also showed that β-nes ($p < 0.001$; $De = 22.1$) significantly increased the overall β-sor dissimilarity but not β-sim ($p = 0.183$; $De = 0.53$) along the nitrogen treatment distance (**Supplementary Table S2**).

β-sor within the nitrogen addition treatments showed a significant increase with nitrogen addition ($ER = 2.76 \times 10^3$; $De = 26$), which can be explained by the increase in both *β*-sim ($ER = 13.58$; $De = 11.65$) and *β*-nes ($ER = 3.2$; $De = 7.3$) dissimilarities. The results of one-way ANOVA also showed that both *β*-sim ($p = 0.008$; $De = 11.4$) and *β*-nes ($p = 0.037$; $De = 3.16$) significantly increased the overall *β*-sor dissimilarities (**Supplementary Table S3**). However, there was no significant variation in abundance-weighted *β*-bray metrics within the treatments ($ER = 0.58$; $De = 1.86$) (**Figure 3**).

The PCoA results of PERMANOVA depicted an overview of the AMF community dissimilarity between and within treatments. Nitrogen addition significantly affected the β-bray ($F = 5.18$; $p < 0.003$) and β-sor ($F = 1.91$; $p = 0.003$) dissimilarities, which was explained more by the partitioned effect of *β*-nes ($F = 15.1$; $p < 0.001$) but not β-sim ($F = –0.2$; $p = 0.996$) (**Figure 4**).

The Mantel test showed that AMF community beta diversity was correlated with environmental dissimilarities. The dissimilarity of the plant community composition was positively correlated with the β-bray dissimilarities ($p = 0.012$) but had a weak correlation with β-sor ($p = 0.095$) and partitioned β-sim ($p = 0.094$) but no significant correlation with β-nes ($p = 0.236$) dissimilarities. Soil pH was the only factor that was significantly correlated with all four beta dissimilarities ($p < 0.001$ for β-bray and β-sor, respectively), having a negative relationship with partitioned β-sim ($p = 0.014$) and a positive relationship with β-nes ($p < 0.001$). Soil moisture was

TABLE 1 | Mantel tests for the soil arbuscular mycorrhizal fungi community Bray–Curtis (β-bray; abundance-weighted), Sørensen (β-sor; presence/absence-based), partitioned Simpson (β-sim), and nestedness (β-nes) dissimilarities with environmental factor dissimilarities. The Spearman correlation r-value and significant effects are shown (. $p < 0.1$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ns, insignificant).

	β-bray	β-sor	β-sim	β-nes
Plant species	0.188*	0.175	0.109	0.068 ns
pH	0.474***	0.435***	–0.187*	0.527***
Moisture	0.196**	0.120 ns	–0.131	0.185*
NH_4^+-N	–0.325 ***	–0.171 *	0.111	–0.249 **
NO_3^--N	–0.595 ***	–0.282 ***	0.066 ns	–0.313 **
MBC	0.192*	0.301**	–0.033 ns	0.316**
MBN	0.178*	0.326**	0.015 ns	0.291*
TC	0.218**	0.124	–0.169**	0.244***
TN	0.139*	0.076 ns	–0.162*	0.194 *

NH_4^+–N, ammonium nitrogen; NO_3^-–N, nitrate nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; TC, soil total carbon; and TN, soil total nitrogen content.

significantly correlated with *β*-bray (p = 0.007) and *β*-nes (p = 0.024) but not β-sor (p = 0.136) and β-sim (p = 0.066). Dissimilarities of NH_4^+–N, NO_3^-–N, MBC, and MBN all correlated significantly with β-bray, β-sor, and partitioned β-nes dissimilarities (all $p < 0.05$), but NH_4^+–N and NO_3^-–N correlated negatively, while MBC and MBN correlated positively with AMF community dissimilarities. Soil TN (p = 0.043) and TC (p = 0.003) correlated positively with β-bray but not with β-sor, as the partitioned β-sim (TN, p = 0.017; TC, p = 0.006) and β-nes (TN, p = 0.017; TC, p = 0.001) components changed in opposite directions (**Table 1**).

DISCUSSION

We found that AMF richness decreased and the relative abundance of different AMF genera was altered differently after nitrogen addition. By disentangling multiple aspects of soil AMF diversity along a nitrogen addition gradient, we found that nitrogen addition significantly increased the AMF community dissimilarities. The turnover of the AMF community along the treatment gradient was explained by an increase in nestedness but not Simpson dissimilarity, while the variation of the AMF community within treatments was explained by the increase in both Simpson and nestedness dissimilarities. Under nitrogen addition, overall and partitioned AMF community dissimilarities also significantly correlated with dissimilarities of the plant community and soil chemical properties.

Decreased AMF richness suggests that plants reduce their dependence on AMF and tend to absorb nutrients through their own roots as soil nutrient availability is enriched (Johnson, 2010), which supports previous findings (Egerton-Warburton and Allen, 2007; Camenzind et al., 2014; Chen et al., 2017). After short-term nitrogen fertilization, an insignificant or even positive relationship between AMF richness may still persist (Zheng et al., 2014; Xiang et al., 2016) because plant species may still have a friendly interaction with AMF (Jiang et al., 2018). However, after long-term nitrogen fertilization, the loss of some plant species caused by nitrogen addition may lead to the corresponding loss of associated specific AMF species because of host identification (Hiiesalu et al., 2014; Martínez-García et al., 2015).

The functional equilibrium model emphasized that aboveground plants still need to rely on some functionally specific AMF groups to obtain other scarce elements (e.g., phosphorus) when only some elements (e.g., nitrogen) of the soil are enriched (Johnson, 2010). This may result in the abundance and functional turnover of the AMF taxa (Lilleskov et al., 2019). The genus of *Glomus* was considered the most dominant and redundant in natural conditions (Davison et al., 2015; Gosling et al., 2016); thus, taxa belonging to this genus may be prone to extinction because of competitive exclusion of functionally and/or phylogenetically similar groups after nitrogen addition (Liu et al., 2015). Previous global-scale meta-analysis also proposed that Glomeraceae (including the *Glomus* genus) would be lost under increasing soil nitrogen (Han et al., 2020). Other genera of *Ambispora*, *Claroideoglomus*, and *Paraglomus*, on the contrary, are less abundant in natural conditions but can increase significantly after nitrogen addition probably because of their specific functional traits in helping plants grow by absorbing more scarce elements (Dodd et al., 2000; Chagnon et al., 2013). Genus-specific response of AMF in abundance after nitrogen addition may also be affected by soil properties, like the soil nitrogen/phosphorus ratio (Egerton-Warburton and Allen, 2007; Han et al., 2020).

We found that the dissimilarities of the AMF community with presence/absence and abundance-weighted data significantly increased with nitrogen addition, which is consistent with previous studies (Chen et al., 2017; Jiang et al., 2018; Lu et al., 2020). This may be because long-term nitrogen addition leads to changes of some AMF species in abundance due to environmental filtering or competitive exclusion (Liu et al., 2015; Chen et al., 2017). Nevertheless, the partitioned nestedness dissimilarity increased significantly, indicating that the AMF species remaining in the higher nitrogen addition treatment is, to some extent, a subset of those remaining in relatively lower N addition treatments. However, the Simpson dissimilarity of AMF along the nitrogen addition gradient did not change significantly, which may be because the variation within the same treatments was large and diluted the effect between different treatments. Moreover, as more AMF species got lost at higher nitrogen addition levels, the increase of taxa cannot compensate for the loss of species, so there was no significant change in spatial turnover (β-sim) (Baselga, 2010).

We found that AMF community composition was more dissimilar within high nitrogen addition treatments, which could be explained by the increased Simpson and nestedness dissimilarities. The fungal diversity can vary greatly even over a small geographical distance because of changes in the microenvironment (e.g., soil environments or animal movements) (Bahram et al., 2015; Fierer, 2017) or stochastic processes like random drifts (Zhou and Ning, 2017). Increased dissimilarity means more unshared species within sampling units, which may be conducive to the conservation of AMF diversity

(Anderson et al., 2011). However, we found that the abundance-weighted variation of the AMF community structure did not change significantly within nitrogen treatments, indicating that the dominant groups were still dominant and vice versa, even though the Simpson and nestedness of species richness both changed after nitrogen addition.

Attributing different patterns of beta diversity to the respective biological processes is fundamental to understanding the mechanisms of biodiversity maintenance. For conservation purposes, it is also essential to evaluate the relative importance of the Simpson and nestedness dissimilarities, which requires opposing conservation strategies (Wright and Reeves, 1992; Baselga, 2010). Although different methods have been proposed for partitioning the overall beta diversity (Podani and Schmera, 2011; Carvalho et al., 2012; Baselga and Leprieur, 2015), they generally provide positively corrected results (Wu et al., 2017), and they also resulted in consistent results along the nitrogen addition gradient in this study. Hence, even though global AMF diversity shows low endemism, the partition methods assessing beta diversity may be a vital method in the extrapolation of global AMF richness (Davison et al., 2015).

Different environmental factors affect the AMF diversity, like an environmental filter, and possess different powers in predicting AMF community dissimilarity (Stegen et al., 2012; Chen et al., 2017). We found that the plant community dissimilarity positively correlated with the composition dissimilarity of the AMF community (presence/absence) but not with that measured with abundance or partitioned components. There may be a coherence of plant and AMF species that meant that more abundant plants also contained more dominant AMF groups (Hiiesalu et al., 2014). Soil pH, moisture, and total carbon and nitrogen dissimilarities were all positively correlated with the overall AMF dissimilarities, but Simpson dissimilarity negatively correlated, while nestedness dissimilarity positively correlated with AMF. The microbial biomass carbon and nitrogen were found to have a collaboratively opposite relationship with ammonium nitrogen and nitrate nitrogen (by negatively correlating with overall AMF community composition, structure, and nestedness). We confirmed a strong relationship between soil AMF and pH (Pan et al., 2020), but it may also be influenced by other surroundings and explained more by the partitioning of the Simpson and nestedness components.

In summary, our experiments demonstrate a decreased soil AMF richness and abundance of different genera in a 7-year nitrogen addition experiment in an alpine meadow. We found that decomposing the overall AMF community beta diversity into Simpson and nestedness dissimilarities along (turnover) and within (variation) nitrogen addition treatments uncovered more intrinsic mechanisms of AMF diversity variation. The turnover of the AMF community overall beta diversity among treatments was explained by an increased nestedness dissimilarity, while the variation of the community overall dissimilarity within treatments was due to both increased Simpson and nestedness dissimilarities. The overall and partitioned AMF community beta diversity also correlated with environmental factors, respectively. Our results provide a deep detection of AMF diversity change after nitrogen addition, which may be a vital key for global AMF diversity conservation.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The data presented in the study are deposited in the (NCBI SRA BioProject) repository, accession number (PRJNA726639).

AUTHOR CONTRIBUTIONS

SZ, YL, and XL designed this experiment. YL and XL collected the data. YL, XL, and SZ analyzed the data and wrote the manuscript. All authors approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2021.701653/full#supplementary-material

REFERENCES

Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., et al. (2011). Navigating the Multiple Meanings of β Diversity: a Roadmap for the Practicing Ecologist. *Ecol. Lett.* 14, 19–28. doi:10.1111/j.1461-0248.2010.01552.x

Anderson, M. J., Ellingsen, K. E., and McArdle, B. H. (2006). Multivariate Dispersion as a Measure of Beta Diversity. *Ecol. Lett.* 9, 683–693. doi:10.1111/j.1461-0248.2006.00926.x

Bahram, M., Peay, K. G., and Tedersoo, L. (2015). Local-scale Biogeography and Spatiotemporal Variability in Communities of Mycorrhizal Fungi. *New Phytol.* 205, 1454–1463. doi:10.1111/nph.13206

Baselga, A., and Leprieur, F. (2015). Comparing Methods to Separate Components of Beta Diversity. *Methods Ecol. Evol.* 6, 1069–1079. doi:10.1111/2041-210x.12388

Baselga, A., and Orme, C. D. L. (2012). Betapart : an R Package for the Study of Beta Diversity. *Methods Ecol. Evol.* 3, 808–812. doi:10.1111/j.2041-210x.2012.00224.x

Baselga, A. (2010). Partitioning the Turnover and Nestedness Components of Beta Diversity. *Glob. Ecol. Biogeogr.* 19, 134–143. doi:10.1111/j.1466-8238.2009.00490.x

Baselga, A. (2012). The Relationship between Species Replacement, Dissimilarity Derived from Nestedness, and Nestedness. *Glob. Ecol. Biogeogr.* 21, 1223–1232. doi:10.1111/j.1466-8238.2011.00756.x

Burnham, K. P., Anderson, D. R., and Huyvaert, K. P. (2011). AIC Model Selection and Multimodel Inference in Behavioral Ecology: Some Background, Observations, and Comparisons. *Behav. Ecol. Sociobiol.* 65, 23–35. doi:10.1007/s00265-010-1029-6

Camenzind, T., Hempel, S., Homeier, J., Horn, S., Velescu, A., Wilcke, W., et al. (2014). Nitrogen and Phosphorus Additions Impact Arbuscular Mycorrhizal Abundance and Molecular Diversity in a Tropical Montane forest. *Glob. Change Biol.* 20, 5203646–5203659. doi:10.1111/gcb.12618

Cardoso, P., Rigal, F., and Carvalho, J. C. (2015). BAT - Biodiversity Assessment Tools, an R Package for the Measurement and Estimation of Alpha and Beta Taxon, Phylogenetic and Functional Diversity. *Methods Ecol. Evol.* 6, 232–236. doi:10.1111/2041-210x.12310

Carvalho, J. C., Cardoso, P., and Gomes, P. (2012). Determining the Relative Roles of Species Replacement and Species Richness Differences in Generating Beta-Diversity Patterns. *Glob. Ecol. Biogeogr.* 21, 760–771. doi:10.1111/j.1466-8238.2011.00694.x

Chagnon, P.-L., Bradley, R. L., Maherali, H., and Klironomos, J. N. (2013). A Trait-Based Framework to Understand Life History of Mycorrhizal Fungi. *Trends Plant Sci.* 18, 484–491. doi:10.1016/j.tplants.2013.05.001

Chen, Y.-L., Xu, Z.-W., Xu, T.-L., Veresoglou, S. D., Yang, G.-W., and Chen, B.-D. (2017). Nitrogen Deposition and Precipitation Induced Phylogenetic Clustering of Arbuscular Mycorrhizal Fungal Communities. *Soil Biol. Biochem.* 115, 233–242. doi:10.1016/j.soilbio.2017.08.024

Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Bâ, A., et al. (2015). Global Assessment of Arbuscular Mycorrhizal Fungus Diversity Reveals Very Low Endemism. *Science* 349, 970–973. doi:10.1126/science.aab1161

Dodd, J. C., Boddington, C. L., Rodriguez, A., Gonzalez-Chavez, C., and Mansur, I. (2000). Mycelium of Arbuscular Mycorrhizal Fungi (AMF) from Different Genera: Form, Function and Detection. *Plant Soil* 226, 131–151. doi:10.1023/a:1026574828169

Egerton-Warburton, L. M., Johnson, N. C., and Allen, E. B. (2007). Mycorrhizal Community Dynamics Following Nitrogen Fertilization: a Cross-Site Test in Five Grasslands. *Ecol. Monogr.* 77, 527–544. doi:10.1890/06-1772.1

Fierer, N. (2017). Embracing the Unknown: Disentangling the Complexities of the Soil Microbiome. *Nat. Rev. Microbiol.* 15, 579–590. doi:10.1038/nrmicro.2017.87

Gosling, P., Jones, J., and Bending, G. D. (2016). Evidence for Functional Redundancy in Arbuscular Mycorrhizal Fungi and Implications for Agroecosystem Management. *Mycorrhiza* 26, 77–83. doi:10.1007/s00572-015-0651-6

Han, Y., Feng, J., Han, M., and Zhu, B. (2020). Responses of Arbuscular Mycorrhizal Fungi to Nitrogen Addition: A Meta-analysis. *Glob. Change Biol.* 26, 7229–7241. doi:10.1111/gcb.15369

Hiiesalu, I., Pärtel, M., Davison, J., Gerhold, P., Metsis, M., Moora, M., et al. (2014). Species Richness of Arbuscular Mycorrhizal Fungi: Associations with Grassland Plant Richness and Biomass. *New Phytol.* 203, 233–244. doi:10.1111/nph.12765

Jiang, S., Liu, Y., Luo, J., Qin, M., Johnson, N. C., Öpik, M., et al. (2018). Dynamics of Arbuscular Mycorrhizal Fungal Community Structure and Functioning along a Nitrogen Enrichment Gradient in an alpine Meadow Ecosystem. *New Phytol.* 220, 1222–1235. doi:10.1111/nph.15112

Johnson, N. C. (2010). Resource Stoichiometry Elucidates the Structure and Function of Arbuscular Mycorrhizas across Scales. *New Phytol.* 185, 631–647. doi:10.1111/j.1469-8137.2009.03110.x

Koleff, P., Gaston, K. J., and Lennon, J. J. (2003). Measuring Beta Diversity for Presence-Absence Data. *J. Anim. Ecol.* 72, 367–382. doi:10.1046/j.1365-2656.2003.00710.x

Legendre, P., Borcard, D., and Peres-Neto, P. R. (2005). Analyzing Beta Diversity: Partitioning the Spatial Variation of Community Composition Data. *Ecol. Monogr.* 75, 435–450. doi:10.1890/05-0549

Lennon, J. J., Koleff, P., Greenwood, J. J. D., and Gaston, K. J. (2001). The Geographical Structure of British Bird Distributions: Diversity, Spatial Turnover and Scale. *J. Anim. Ecol.* 70, 966–979. doi:10.1046/j.0021-8790.2001.00563.x

Lepš, J., de Bello, F., Šmilauer, P., and Doležal, J. (2011). Community Trait Response to Environment: Disentangling Species Turnover vs Intraspecific Trait Variability Effects. *Ecography* 34, 856–863. doi:10.1111/j.1600-0587.2010.06904.x

Lilleskov, E. A., Kuyper, T. W., Bidartondo, M. I., and Hobbie, E. A. (2019). Atmospheric Nitrogen Deposition Impacts on the Structure and Function of forest Mycorrhizal Communities: A Review. *Environ. Pollut.* 246, 148–162. doi:10.1016/j.envpol.2018.11.074

Liu, X., Lyu, S., Zhou, S., and Bradshaw, C. J. A. (2016). Warming and Fertilization Alter the Dilution Effect of Host Diversity on Disease Severity. *Ecology* 97, 1680–1689. doi:10.1890/15-1784.1

Liu, X., Lyu, S. M., Sun, D. X., Bradshaw, C. J. A., and Zhou, S. R. (2017). Species Decline under Nitrogen Fertilization Increases Community-Level Competence of Fungal Diseases. *Proc. R. Soc. B* 284, 2016–2621. doi:10.1098/rspb.2016.2621

Liu, X., Zhang, L., Huang, M. J., and Zhou, S. R. (2020). Plant Diversity Promotes Soil Fungal Pathogen Richness under Fertilization in an alpine Meadow. *J. Plant Ecol.* 14 (2), 323–336. doi:10.1093/jpe/rtaa099

Liu, Y., Johnson, N. C., Mao, L., Shi, G., Jiang, S., Ma, X., et al. (2015). Phylogenetic Structure of Arbuscular Mycorrhizal Community Shifts in Response to Increasing Soil Fertility. *Soil Biol. Biochem.* 89, 196–205. doi:10.1016/j.soilbio.2015.07.007

Liu, Y., Shi, G., Mao, L., Cheng, G., Jiang, S., Ma, X., et al. (2012). Direct and Indirect Influences of 8 Yr of Nitrogen and Phosphorus Fertilization on Glomeromycota in an alpine Meadow Ecosystem. *New Phytol.* 194, 523–535. doi:10.1111/j.1469-8137.2012.04050.x

Lu, Y., Liu, X., Chen, F., and Zhou, S. (2020). Shifts in Plant Community Composition Weaken the Negative Effect of Nitrogen Addition on Community-Level Arbuscular Mycorrhizal Fungi Colonization. *Proc. R. Soc. B.* 287, 20200483. doi:10.1098/rspb.2020.0483

Lumini, E., Orgiazzi, A., Borriello, R., Bonfante, P., and Bianciotto, V. (2010). Disclosing Arbuscular Mycorrhizal Fungal Biodiversity in Soil through a Land-Use Gradient Using a Pyrosequencing Approach. *Environ. Microbiol.* 12, 2165–2179. doi:10.1111/j.1462-2920.2009.02099.x

Martínez-García, L. B., Richardson, S. J., Tylianakis, J. M., Peltzer, D. A., and Dickie, I. A. (2015). Host Identity Is a Dominant Driver of Mycorrhizal Fungal Community Composition during Ecosystem Development. *New Phytol.* 205, 1565–1576. doi:10.1111/nph.13226

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., et al. (2013). Vegan: Community Ecology Package. R Package Version 2.0-7. Available at: http://CRAN.R-project.org/package=vegan Accessed October 10, 2020.

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J. M., et al. (2010). The Online Database *MaarjAM* Reveals Global and Ecosystemic Distribution Patterns in Arbuscular Mycorrhizal Fungi (Glomeromycota). *New Phytol.* 188, 223–241. doi:10.1111/j.1469-8137.2010.03334.x

Pan, S., Wang, Y., Qiu, Y., Chen, D., Zhang, L., Ye, C., et al. (2020). Nitrogen-induced Acidification, Not N-nutrient, Dominates Suppressive N Effects on Arbuscular Mycorrhizal Fungi. *Glob. Change Biol.* 26, 6568–6580. doi:10.1111/gcb.15311

Paradis, E., Claude, J., and Strimmer, K. (2004). APE: Analyses of Phylogenetics and Evolution in R Language. *Bioinformatics* 20, 289–290. doi:10.1093/bioinformatics/btg412

Podani, J., and Schmera, D. (2011). A New Conceptual and Methodological Framework for Exploring and Explaining Pattern in Presence - Absence Data. *Oikos* 120, 1625–1638. doi:10.1111/j.1600-0706.2011.19451.x

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., et al. (2007). SILVA: a Comprehensive Online Resource for Quality Checked and Aligned Ribosomal RNA Sequence Data Compatible with ARB. *Nucleic Acids Res.* 35, 7188–7196. doi:10.1093/nar/gkm864

R Development Core Team (2014). *A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Sato, K., Suyama, Y., Saito, M., and Sugawara, K. (2005). A New Primer for Discrimination of Arbuscular Mycorrhizal Fungi with Polymerase Chain Reaction-Denature Gradient Gel Electrophoresis. *Grassland Sci.* 51, 179–181. doi:10.1111/j.1744-697x.2005.00023.x

Smith, S. E., and Read, D. J. (2008). *Mycorrhizal Symbiosis*. Cambridge, UK: Academic Press.

Stegen, J. C., Lin, X., Konopka, A. E., and Fredrickson, J. K. (2012). Stochastic and Deterministic Assembly Processes in Subsurface Microbial Communities. *Isme J.* 6, 1653–1664. doi:10.1038/ismej.2012.22

Stevens, C. J., Dise, N. B., Mountford, J. O., and Gowing, D. J. (2004). Impact of Nitrogen Deposition on the Species Richness of Grasslands. *Science* 303, 1876–1879. doi:10.1126/science.1094678

Tedersoo, L., Bahram, M., and Zobel, M. (2020). How Mycorrhizal Associations Drive Plant Population and Community Biology. *Science* 367, 367. doi:10.1126/science.aba1223

Thébault, E., and Fontaine, C. (2010). Stability of Ecological Communities and the Architecture of Mutualistic and Trophic Networks. *Science* 329, 853–856. doi:10.1126/science.1188321

Tilman, D. (1987). Secondary Succession and the Pattern of Plant Dominance along Experimental Nitrogen Gradients. *Ecol. Monogr.* 57, 189–214. doi:10.2307/2937080

Treseder, K. K. (2004). A Meta-analysis of Mycorrhizal Responses to Nitrogen, Phosphorus, and Atmospheric CO 2 in Field Studies. *New Phytol.* 164, 347–355. doi:10.1111/j.1469-8137.2004.01159.x

Vellend, M. (2001). Do commonly Used Indices of β-diversity Measure Species Turnover? *J. Vegetation Sci.* 12, 545–552. doi:10.2307/3237006

Vellend, M. (2010). Conceptual Synthesis in Community Ecology. *Q. Rev. Biol.* 85, 183–206. doi:10.1086/652373

Whittaker, R. H. (1960). Vegetation of the Siskiyou Mountains, Oregon and California. *Ecol. Monogr.* 30, 279–338. doi:10.2307/1943563

Whittaker, R. H. (1972). Evolution and Measurement of Species Diversity. *Taxon* 21, 213–251. doi:10.2307/1218190

Wright, D. H., and Reeves, J. H. (1992). On the Meaning and Measurement of Nestedness of Species Assemblages. *Oecologia* 92, 416–428.

Wu, L., Si, X., Didham, R. K., Ge, D., and Ding, P. (2017). Dispersal Modality Determines the Relative Partitioning of Beta Diversity in Spider Assemblages on Subtropical Land-Bridge Islands. *J. Biogeogr.* 44, 2121–2131. doi:10.1111/jbi.13007

Xiang, X., Gibbons, S. M., He, J. S., Wang, C., He, D., Li, Q., et al. (2016). Rapid Response of Arbuscular Mycorrhizal Fungal Communities to Short-Term Fertilization in an alpine Grassland on the Qinghai-Tibet Plateau. *PeerJ* 4, e2226. doi:10.7717/peerj.2226

Xu, T., Veresoglou, S. D., Chen, Y., Rillig, M. C., Xiang, D., Ondřej, D., et al. (2016). Plant Community, Geographic Distance and Abiotic Factors Play Different Roles in Predicting AMF Biogeography at the Regional Scale in Northern China. *Environ. Microbiol. Rep.* 8, 1048–1057. doi:10.1111/1758-2229.12485

Zheng, Y., Kim, Y.-C., Tian, X.-F., Chen, L., Yang, W., Gao, C., et al. (2014). Differential Responses of Arbuscular Mycorrhizal Fungi to Nitrogen Addition in a Near Pristine Tibetan alpine Meadow. *FEMS Microbiol. Ecol.* 89, 594–605. doi:10.1111/1574-6941.12361

Zhou, J., and Ning, D. (2017). Stochastic Community Assembly: Does it Matter in Microbial Ecology? *Microbiol. Mol. Biol. Rev.* 81, e00002–e00017. doi:10.1128/mmbr.00002-17

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Annual Population Dynamics and Their Influencing Factors for an Endangered Submerged Macrophyte (*Ottelia cordata*)

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Due to wetland loss, *Ottelia cordata* (*O. cordata,* Wallich) Dandy has been categorized as an endangered species on the List of Key Protected Wild Plants in China. Quantifying the relative importance of demographic (i.e., growth, survival, and reproduction) and habitat preference traits on the population dynamics (abundance) of *O. cordata* could guide how to develop the best recovery strategies of *O. cordata*, yet currently, there are no studies that investigate this. By monitoring monthly changes in *O. cordata* abundance and demographic traits (plant height, leaf area, flower sex ratio, and seed number) that were highly correlated with growth rate, photosynthetic rate, and water depth, we identified several relationships. Linear mixed-effect models and variance partition quantified the specific effects of four demographic traits and water depth on *O. cordata* abundance in three habitat types (paddyfield, stream, and spring). The linear mixed-effect models indicate that among the four demographic traits, height could be significantly positively correlated to abundance in all three habitat types. In contrast, other three traits (leaf area, sex ratio, and seed numbers) were non-significantly associated with abundance across each habitat. Height was determined by water depth, so water depth rather than photosynthetic rate and reproduction rate may promote the development and recovery of *O. cordata* populations. Variance partition results showed that water depth mediated the positive influence of growth rate on the abundance of *O. cordata* in the living habitats (paddyfield and spring). In contrast, water depth but not growth rate determined the abundance of *O. cordata* in the living habitat (stream). However, water depth had a significantly negative impact on the abundance of *O. cordata* in stream habitats, likely because all of the streams were shallow. Altogether, in the short term for avoiding the potential harm or even extinction of *O. cordata*, keeping appropriate water depth or transplanting *O. cordata* to spring should be an effective strategy because the water is not only deep enough but also clear in spring habitats. Additionally, water turbidity was shown to affect the density of *O. cordata* growth, wherein *O. cordata* was sparsely distributed when turbidity was high. Therefore, in the long run, to make the population gradually recovery, it will be necessary to restore the degraded wetland. This could be accomplished by reducing water pollution and removing sludge to reduce turbidity and increase hydrological connectivity.

Keywords: abundance, demographic traits, linear mixed-effect model, monthly variation, conservation strategy

INTRODUCTION

Ottelia cordata (*O. cordata,* Wallich) Dandy, distributed in China, Cambodia, Myanmar, and Thailand (Wang et al., 2010), is a submerged dioecious macrophyte that is insect-pollinated and shows leaf dimorphism in submerged and floating leaves. Found in clear and slowly flowing water, full lifecycle of *O. cordata* is inseparable from water, though flowering takes place on the water surface. Historically, *O. cordata* was widely distributed in the whole Hainan province, located in the southernmost part of China, at the northern edge of the tropics. Hainan province has a tropical monsoon climate with abundant rainfall from May to November followed by a dry season. The vegetation is mainly tropical monsoon rain forest. Due to wetland destruction (e.g., cutting forest for planting, production and domestic sewage discharge), *O. cordata* can only be found in part of northern Hainan Province (Haikou city, Ding'an County, and Chengmai County), China (**Figure 1a**), close to 90% of the distribution points are located in Haikou, so it has a very narrow distribution. In recent years, multiple factors, such as water eutrophication and wetland destruction, have resulted in the loss of many *O. cordata* populations and a significant reduction in its range (Wang et al., 2019). According to the observation of the research group of authors, the distribution points of *O. cordata* were decreased from 42 points to 38 points, and the number *O. cordata* was decreased by about 11% between 2017 and 2020. Given these trends, *O. cordata* has been categorized as an endangered species in the List of Key Protected Wild Plants in China (Zhang et al., 2019). To protect and recover *O. cordata*, several wetland reserves, such as the Yangshan Wetland Reserve and Changwangxi Wetland Reserve, have been established in Hainan Province (Wang et al., 2019). However, without knowing local population dynamics and their potential influencing factors, it is very difficult to develop accurate and effective protection strategies to save this endangered species.

Although *O. cordata* has been listed as endangered plant species in China, there are no specific evaluation criteria judging its endangered degree. It has been shown that without knowing the plant species population dynamics and demographic traits, any evaluation of endangered degree is essentially guesswork (Tear et al., 1993; Schemske et al., 1994; Neel et al., 2012). Moreover, population dynamics (e.g., abundance) of the population should be determined using demographic traits that are highly associated with growth and reproduction (Davies et al., 2000; Rytwinski and Fahrig, 2012; Soudzilovskaia et al., 2013; Curtis et al., 2015). Therefore, *O. cordata* population dynamics and demographic traits were measured to determine which demographic characteristics influence population dynamics, thereby shedding light on the potential influencing the endangered degree. Using these population dynamics and demographic traits for *O. cordata* to understand the determinants of *O. cordata* abundance dynamics, effective recovery strategies can be developed (Wang et al., 2010).

Since *O. cordata* is an annual submerged macrophyte, its population dynamics should be easy to measure as it only requires monthly monitoring across its population distribution within a 1-year period. To be the best of our knowledge, the whole genome of *O. cordata* and its response to CO_2 addition have been reported (Wang et al., 2019; Zhang et al., 2019; Huang et al., 2020), while currently there are no reports on its population dynamics and demographic traits. As a result, it is very difficult to develop effective and suitable recovery strategies to better protect *O. cordata*. Therefore, in this study, we monitored the monthly variation in population dynamic (i.e., abundance) and several key demographic traits (i.e., height, leaf area, the ratio of numbers of male and female flowers, and seed numbers) in all possible distributions of *O. cordata* that were highly associated with growth, photosynthesis rate reproduction of submerged macrophyte over a 1-year period. For example, several studies indicate that height and leaf are highly associated with growth and photosynthesis rate for submerged macrophyte (Fu et al., 2014; Huang et al., 2020; Zhang et al., 2020). Additionally, the ratio of numbers of male and female flowers and seed numbers are highly associated with the reproductive ability of submerged macrophytes (Xu et al., 2015; Liu et al., 2017; Zhang et al., 2020). It has also been widely noted that water depth is a key factor affecting the growth, reproduction, and re-establishment of submerged macrophyte populations (Nõges and Nõges, 1999; Dong et al., 2014). Deeper water may induce lower light intensity, however, increased water pressure and unstable temperature and sediment at such depths may not favor increased growth and reproduction for submerged macrophytes (Philbrick and Les, 1996; Kosuge et al., 2013). Similarly in very shallow water depth, light intensity and temperature at shallow depths may limit the growth and reproduction of submerged macrophytes (Søndergaard et al., 2010; Zhu et al., 2012; Li et al., 2017). Taken altogether, it may be that water depth is a key regulating factor. Therefore, we aimed to use the measured monthly variation in abundance, demographic traits, and water depth to quantify, (1) monthly variations of abundance, demographic traits, and water depth for *O. cordata*; (2) whether the key demographic characteristic or water depth was the determining

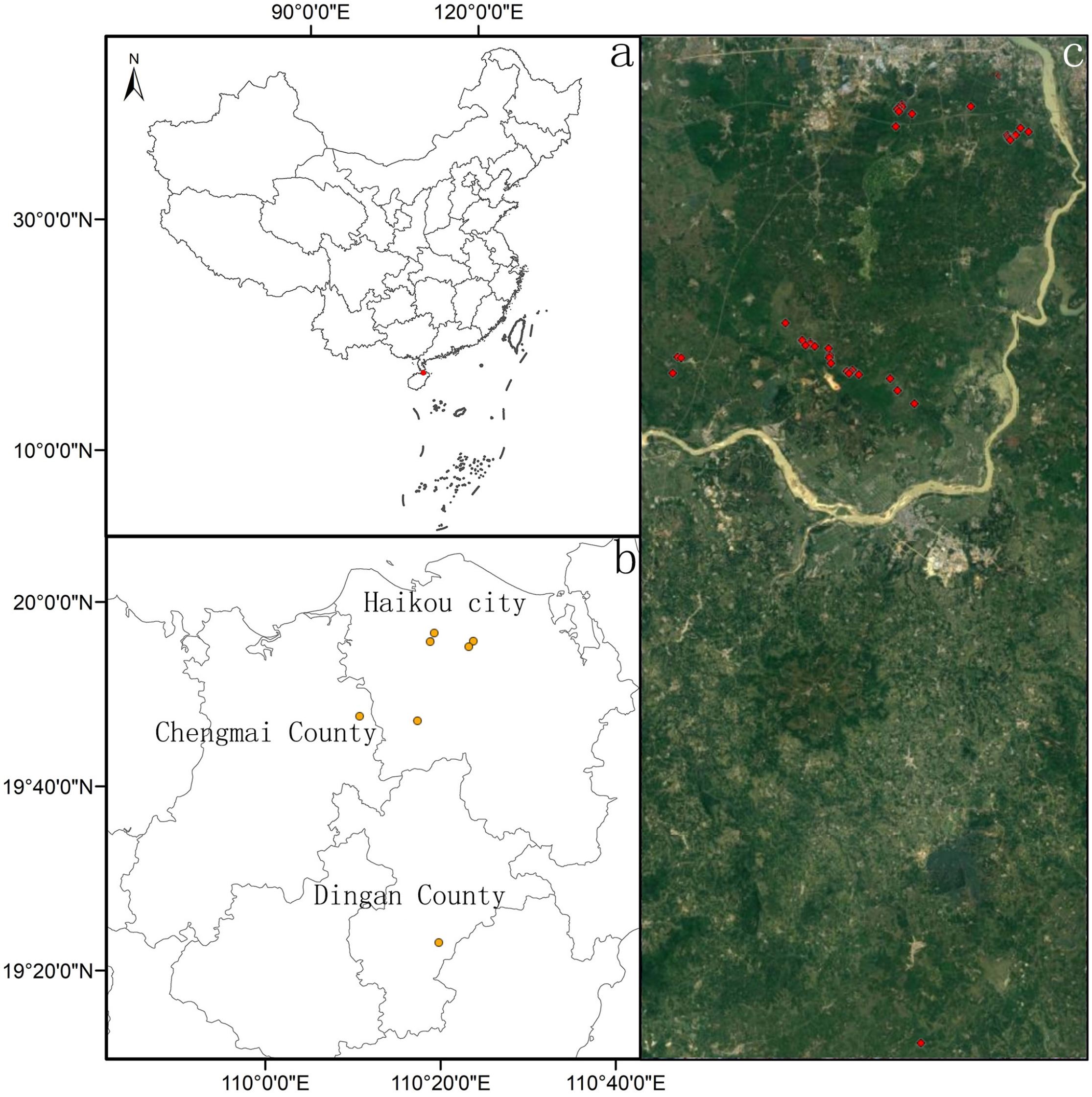

FIGURE 1 | Topographic map of all spatial distributions of *Ottelia cordata* **(c)** in our several study sites (five in Haikou city, one in Chengmai County, and one in Ding'an County) **(b)** in Hainan province, China **(a)**. Red symbols indicate all distribution points, and yellow symbols represent sample plots.

factor influencing abundance for *O. cordata* by investigating the interrelationships among these variables. We hypothesize that water depth and some demographic characteristics (height, sex ratio, or seed numbers) could affect the abundance of *O. cordata*, and their ability to predict *O. cordata* abundance may be different.

MATERIALS AND METHODS

Study Site

We determined all the distribution points for *O. cordata* by means of unmanned aerial vehicle and field investigation. Dji Mavic Air 2 UAV (SZ DJI Technology Co., Ltd. Shenzhen, China) was used for the investigation, with a flight altitude of 10–20 m. In the area around the distribution point of *O. cordata* or in the area where *O. cordata* may be distributed, the image accuracy could reach the centimeter level, then confirmed the suspected point by field investigation. Finally, we found a total of 38 distribution points (**Figure 1c**), one of which was located in Longmen Town, Ding'an County (19.386458N; 110.329212E), and three of which were located in Yongfa Town, Chengmai County (19.7872–19.7963N; 110.1748–110.1770E), and the remaining 34 sites were located in Yangshan Wetland Multi-functional Reserve of Haikou City (19.7556N–19.9682N, 110.2333E–110.4070E; **Figure 1a**).

The distribution areas are divided into the rainy season and dry season. The rainy season was distributed from June to November, and the dry season was distributed from December to April. The average annual precipitation in this region was about 1,966–2,067 mm, 70% of which occurs in the rainy season. The annual average evaporation was 1,763–1,843 mm.

Across this distribution, the water quality was good with clear water observed at about three-quarters of the total sites, and only a small part of the water with muddy substrate had higher turbidity. There were mainly three habitat types: stream, paddyfield, and spring. The stream type was accounted for about 70% of sites, the paddyfield type was accounted for about 20% of sites, and there were only two springs (but the number of *O. cordata* in spring accounts for about 20–25% of the total). The water flow speed was gentle with the stream speed at less than 0.5 m/s and the springs and paddy fields with a flow rate below 0.1 m/s. The substrates of the water body were sandy, muddy, or a mixture of the two; sand-mud mixture accounted for about half of the distribution sites. The springs sediment was mainly sandy or having a little mud in some areas. The substrate of paddyfield was mainly muddy, and a few distribution spots were mud-sand mixture. However, there were three types of sediment in streams, and the proportion difference was not obvious. The turbidity of springs and paddyfields was lower, the turbidity of streams with different substrates types varied greatly, among which muddy basement had the highest turbidity, and the sand basement had the lowest turbidity. The chemical oxygen demand (COD) of all water bodies was less than 13.0 mg/L, the springs water was the lowest, and the COD was less than 1.5 mg/L. The total phosphorus (TP) of all water bodies was less than 0.08 mg/L. Other indices were also up to the water standard of class II (or I), except total nitrogen (TN) was between 7.0 and 12.0.

Field Sampling

Seven sites were selected as sample plots based on the area, the number of plants, distribution distance, habitat type, and water bottom material and at least two sample sites for each habitat (three for streams because there are lots), then seven sites were selected as sample plots, which covered different types and population characteristics of the distribution of *O. cordata*. They were, respectively, Yufucun paddyfield, Changwangxi paddyfield, stream of Nayang Village, stream of Chengmai Yongfa Town, stream of Yangshan forest, Xihu Niang Temple Spring, and Ding'an Cold Spring (**Table 1** and **Figure 1b**). *O. cordata* was an annual plant species, so we studied its population dynamics for a year. Based on our preliminary observation, *O. cordata* blooms every 2–3 months, therefore, sampling was conducted in 6 months (January, March, May, July, September, and November) in 2020 to quantify variations in *O. cordata* population dynamics (monthly variation in abundance), its functional traits, and water depth. In each month, in each of 41 sites, five 1 m × 1 m plots (1 m^2 area) were randomly arranged to measure abundance, four traits (height, leaf area, the ratio of numbers of male flower, and numbers of female flower) and water depth every day to calculate average abundance, traits, and water depth for the corresponding month.

Trait Measurement

Individual leaf area (cm^2) was calculated from the leaf scans using Image-Pro Plus (IPP) 6.0 (Media Cybernetics, Inc., Silver Spring, MD, United States). We measured the height from the root to the stem, numbers of male and female flowers, and seeds in each individual in each of the five 1 m × 1 m plots. We then calculated the ratio of numbers of male and female flowers (*O. cordata* is dioecious, but according to our observation of each flower open only 1 day, it was the sex ratio of flowers rather than the sex ratio of plants that affected the population dynamics, so the sex ratio of flowers was used in this study).

Statistical Analysis

We first quantify monthly variations in abundance, four functional traits, and water depth. Then we tested whether the four traits and water depth were significantly correlated with abundance for *O. cordata*. Since our study design was hierarchical, with three different living habitat types (paddyfield, stream, and spring), we used linear mixed-effects models using maximum likelihood with the R package "lme4" (Bates et al., 2014). For each trait and the water depth, we used the model: [abundance ∼ trait/water depth + (1| living habitat type)] with trait/water depth and living habitat type as the predictor of abundance and random effect, respectively. Finally, we used a variance partitioning analysis to quantify the relative contribution of traits and water depth to abundance for using the function "varpart" in the "*vegan*" R package (Oksanen et al., 2016). Specifically, abundance with traits and water depth as explanatory variables could be divided into four complementary components: (a) "purely traits," variance explained by traits alone; (b) "shared traits and water depth," variance explained by both traits and water depth; (c) "purely water depth," variance explained by water depth alone; and (d) "unexplained residual variation" (Legendre et al., 2009; Zhang et al., 2018a). For each of the three living habitat types, variance partitioning was done separately.

RESULTS

Abundance for *O. cordata* showed a clear monthly variation, with the highest abundance occurring in November or January in paddyfield and spring habitats, but from September to November for the stream habitat. The lowest abundance came up from March to May (**Figure 2**). Similarly, the four demographic traits (height, leaf area, the ratio of numbers of male and female flowers, and seed numbers) for *O. cordata* also demonstrated clear monthly alteration. However, only height described similar monthly variations to those for abundance. Water depth also showed consistent monthly alterations with those for abundance, but monthly variations for water depths in the stream were much lower than those for paddyfield and spring (**Figure 2**).

The linear mixed-effect model results indicated that the slopes of the relationships between species abundance and height and abundance and water depth had significantly ($P < 0.05$) across the three living habitat types (**Table 2** and **Figure 3**). For instance, height was significantly correlated with abundance in all three living habitat types (**Table 2** and **Figure 3**). In contrast, water depth was significantly and positively associated with abundance in paddyfield and spring habitats, but was significantly and negatively related to abundance in stream habitats (**Table 2** and **Figure 3**). However, the slopes of the relationships between abundance and functional traits did not change significantly

TABLE 1 | Characteristics of the *Ottelia cordata* sample area (2020.01–2020.12).

The sample name	Latitude and longitude	Sample size (m^2)	Number of plant	Habitat	Water quality	Bottom material	Remarks
Yufucun paddyfield	110.38479E 19.928589N	800	9–520	Paddyfield	Clear, turbidity ≤ 3 NTU	Mud	*O. cordata* was cleaned when planting rice
Changwangxi paddyfield	110.28572E 19.78905N	1,200	820–1,300	Paddyfield	Clear, turbidity ≤ 3 NTU	Sand-mud	
Stream of Nayang Village	110.39771E 19.93171N	600	270–350	Stream	Some turbid, turbidity ≤ 10 NTU	Mud	
Stream of Chengmai Yongfa Town	110.17700E 19.79633N	60	10–30	Stream	Some turbid, turbidity ≤ 15 NTU	Mud	The plant has been falling, in danger of disappearing
Stream of Yangshan forest	110.31528E 19.94387N	120	520–1,100	Stream	Very clear, turbidity ≤ 1 NTU	Sand	
Xihu Niang Temple Spring	110.31331E 19.93485N	850	290–500	Spring	Clear, turbidity ≤ 2 NTU	Sand-mud	
Ding'an Cold Spring	110.32921E 19.38646N	1,000	900–1,500	Spring	Very clear, turbidity ≤ 0.5 NTU	Sand	

Water quality description: Very clean, everything at the bottom of the water is visible; Clean, most things at the bottom of the water can be seen; Some turbidity, most things at the bottom of the water cannot be seen.

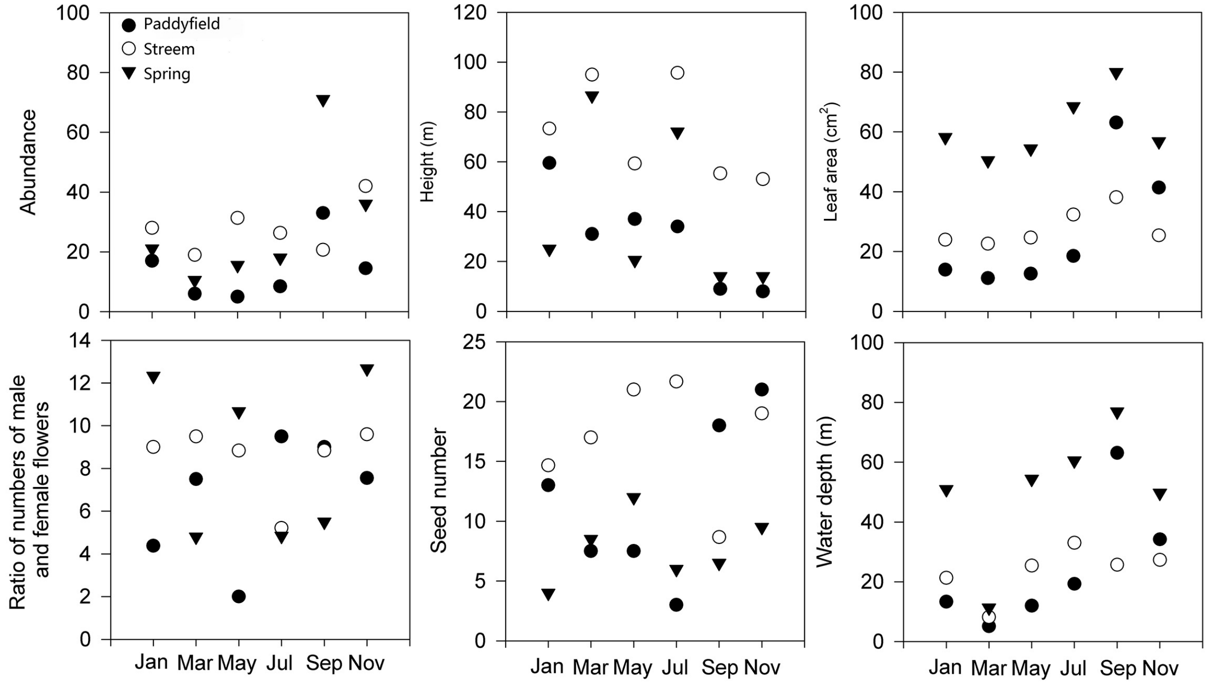

FIGURE 2 | Monthly variations in abundance, water depth, and four demographic traits (height, leaf area, ratio of numbers of male and female flowers, and seed number) for *Ottelia cordata* in a full year.

across ($P > 0.05$; **Table 2** and **Figure 3**). Additionally, variance partitioning results demonstrated that water depth explained large variations (53–64%) in abundance in all three living habitat types, whereas height merely explained relatively few proportions (21–40%) of variations in abundance in all three living habitat types (**Figure 4**).

There was no correlation between floral sex ratio and seed quantity, water depth, or plant height. Among the seven sites,

TABLE 2 | The relationships between species abundance and both demographic traits (photosynthetic rate, leaf proline content, and seed germination rate) and water depth, while accounting for the random effect of living habitat types (paddyfield, stream, and spring) tested using the linear mixed-effect model [formula: abundance $\sim$ trait/water depth + (1| living habitat type)].

Source		
Height	Degree of freedom	1
	Effect size	19.34
	F	**9.45**
	P	**<0.05**
Water depth	Degree of freedom	1
	Effect size	20.52
	F	**21.15**
	P	**<0.05**
Leaf area	Degree of freedom	1
	Effect size	17.93
	F	1.24
	P	>0.05
Ratio of numbers of male and female flowers	Degree of freedom	1
	Effect size	19.79
	F	0.46
	P	>0.05
Seed numbers	Degree of freedom	1
	Effect size	20.42
	F	0.84
	P	>0.05

The degree of freedom, effect size, F-statistics, and corresponding P-values are shown and boldface type indicates significant differences at $P < 0.05$.

the floral sex ratio of Ding'an Cold Spring was the highest, while that of Xihu Niang Temple Spring was the lowest. Therefore, the floral sex ratio was not related to habitat type but might be related to spatial distribution distance. The highest number of seeds was in the stream of Chengmai Yongfa Town and stream of Nayang Village, while the lowest number of seeds was in the stream of Yangshan forest, indicating that the muddy substrate was favorable for seed production.

DISCUSSION

Hereby using monthly variations in abundance, some key demographic traits (height, leaf area, the ratio of numbers of male and female flowers, and seed numbers), and water depth, our results show the population dynamics of *O. cordata* and its influencing factors. Results show that although water depth and demographic trait (height) could affect the abundance, water depth and height had different predictive abilities in the abundance of *O. cordata*.

Demographic traits may directly reflect *O. cordata* performance as determined by growth, reproduction, and photosynthesis rate. For example, height has been shown to be highly associated with growth rate (Falster and Westoby, 2003; Sun and Frelich, 2011; Han et al., 2018). Similarly, previous studies indicate that the ratio of the male and female flowers and seed numbers are good indicators of plant reproductive ability of submerged macrophyte (Xu et al., 2015; Liu et al., 2017). In addition, leaf area could influence plant photosynthesis rate, with a large leaf area resulting in a high photosynthesis rate (Fu et al., 2014; Huang et al., 2020; Zhang et al., 2020). It had been found that growth, reproduction, and photosynthesis rate could determine plant abundance (Westoby et al., 1999; Wright et al., 2004; Zhang et al., 2013, 2018b). Thus, the relationship between abundance and these functional traits could provide insight into which characteristics may determine abundance for *O. cordata*. Since only height showed consistent and monthly variations with those for abundance, it is possible that only plant height is highly related to abundance. Indeed, we found that height was significantly and positively associated with abundance in all three living habitat types, whereas the other three demographic traits (the ratio of the numbers of male flowers and numbers of female flowers, leaf area, and seed numbers) were not significantly related with abundance. This suggests that growth rate, but not photosynthesis rate or reproduction, could determine abundance for *O. cordata*. Dynamics for abundance and demographic traits have been widely reported for many submerged macrophytes (Santamaría et al., 2003; Boedeltje et al., 2008; Fu et al., 2014; Bai et al., 2015). However, our results for the first time revealed that demographic traits could be used to predict abundance for submerged macrophytes. In addition, the number of plants per unit area was correlated with turbidity, and the number of plants per unit area was less when turbidity was higher. The water with high turbidity was usually a muddy substrate (there were more seeds on the muddy substrate) but fever plants per unit area, indicating that water quality may affect the growth and development of *O. cordata*.

It has been widely reported that water depth may determine both the abundance and biomass of submerged macrophyte (Xu et al., 2015; Li et al., 2017; Zhang et al., 2020). Here, we observed that water depth and abundance for *O. cordata* had varying consistently from month to month. Thus, water depth should be highly associated with abundance. Indeed, we observed that water depth was significantly and positively related to abundance in paddyfield and spring habitats, but negatively associated with abundance in the stream habitat owing to streams having the shallowest depths (all less than 45 cm). These results indicate that water depth in paddyfields and springs could facilitate the re-establishment of *O. cordata*. In contrast, water depth in the streams would limit the population development of *O. cordata*. The key reason was that the distribution of *O. cordata* had a significantly dry and wet season, which in turn would lead to a significant alteration in water depth. It had been found that significant variation in water depth could facilitate growth and reproduction, which in turn would affect abundance and biomass for *O. cordata*. Thus, water depth could facilitate the population development and re-establishment of *O. cordata*. Here, we found that the paddyfield and spring water depth showed significant variations, with the highest and lowest water depth occurring in wet (September–November) and dry (March) seasons, respectively. Thus, it is not surprising to see the significantly positive water depth-abundance relationships in paddyfield and spring habitats. However, water depth in the stream habitat was relatively invariable across wet and dry

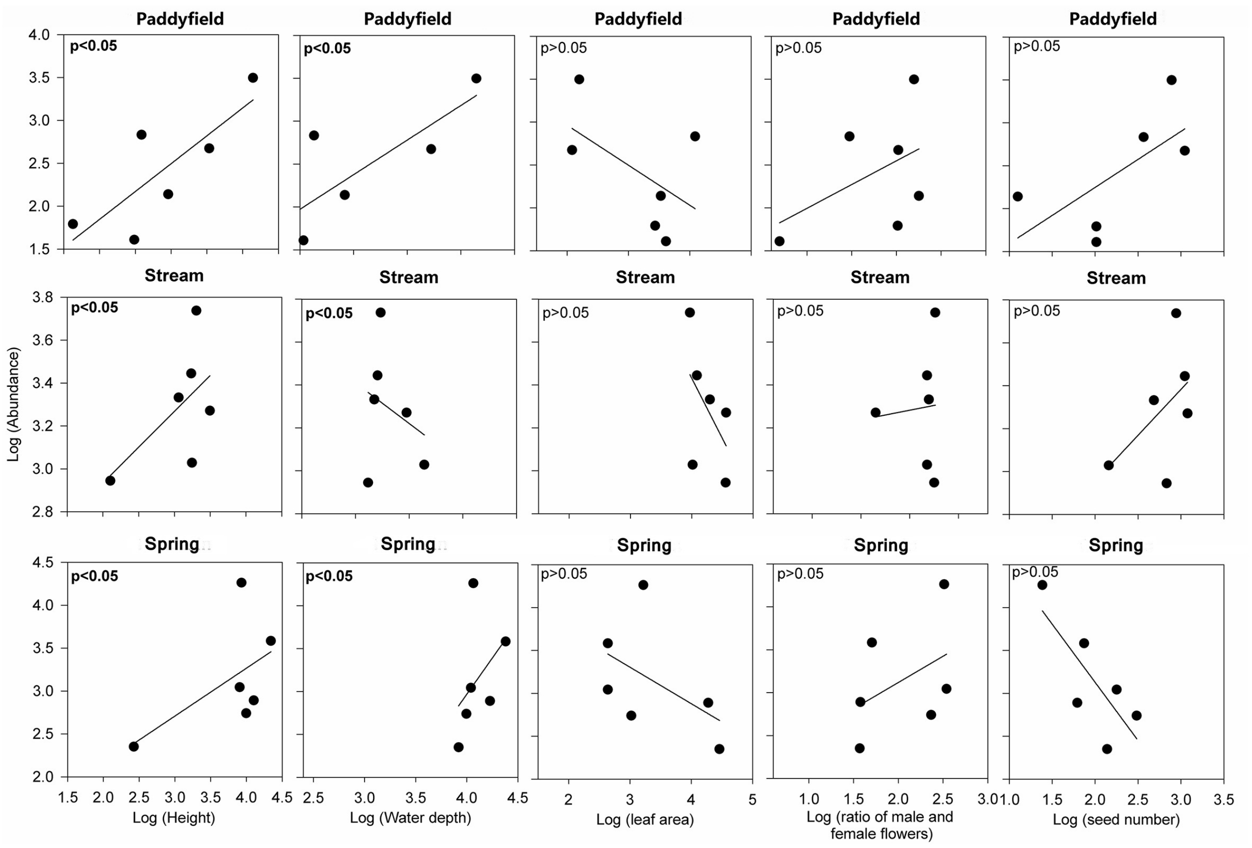

FIGURE 3 | The relationships between abundance for *Ottelia cordata* and four demographic traits (height, leaf area, ratio of numbers of male and female flowers, and seed number) along with water depth.

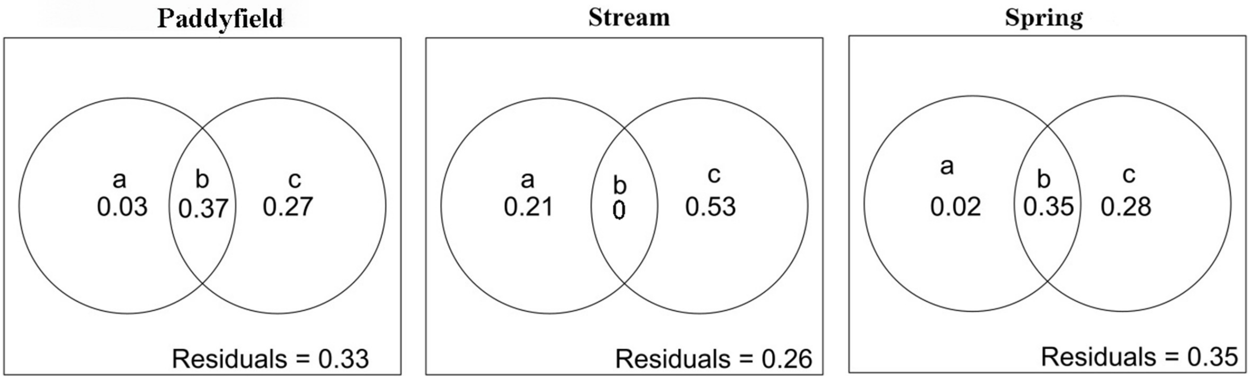

FIGURE 4 | The percentage of variation in abundance for *Ottelia cordata* is explained by four predictor types: "purely height," variance explained only by height **(a)**, "shared height and water depth," variance explained by both height and water depth **(b)** "purely height," variance merely explained by water depth **(c)**, and "unexplained variables" (Residuals), in the three living habitat types (paddyfield, stream, and spring) individually.

seasons. This is likely due to the previous unreasonable land use (i.e., deforestation, building apartment, and dam construction) causing serious wetland destruction, which in turn restricted the water depth (Wang et al., 2019). Here, the water depth would restrict population development and re-establishment of *O. cordata* thereby resulting in negative water depth-abundance relationships. However, deeper water would weaken the light and increase the sediment, which is also not conducive to the growth and reproduction of *O. cordata* and restricted the development of the population, similar to previous findings of aquatic plants (Kosuge et al., 2013). In addition, the height of *O. cordata* must be equal to or greater than the depth of water to produce floating leaves, which are the main organs for photosynthesis (Huang et al., 2020), and the stem must be out of the water to reproduce. In this study, when the water depth was close to 100 cm, the number of seeds would be significantly reduced and the abundance of *O. cordata* decreased. Of course, only 1 of the 38 distribution points could reach 100 cm in the wet season, so shallow water depth was still the most important factor limiting the restoration and reconstruction of *O. cordata*.

Usually, submerged macrophytes would alter their height in response to the variations of water depth (Carollo et al., 2005; Li et al., 2017, 2020; He et al., 2019; Su et al., 2019). Thus, water depth should have a higher predictive ability of abundance than plant height. Indeed, our variance partition results indicated that water depth could explain a large proportion, while the height can only explain very little. As a result, water depth but not height could determine the abundance of *O. cordata*. Our results also showed that water depth and height were highly correlated in living habitats (paddyfield and spring), indicating that water depth mediated the influence of growth rate on the abundance of *O. cordata* in these habitats. However, water depth and height were not significantly correlated in the stream habitat suggesting that the limited water depth did not obviously affect plant growth. Thus, water depth tends to directly limit *O. cordata* population development. Therefore, it is incredibly important to restore the degraded wetland to retrieve the restricted water depth, otherwise *O. cordata* may have a high risk of going extinct.

CONCLUSION

Our results show the dynamics in abundance and demographic traits of *O. cordata*. We have clearly demonstrated that water depth and growth rate indicated by height could affect the abundance of *O. cordata*, but water depth plays a determining role. The floral sex ratio and fruit dynamic change were not directly related to water depth; instead seem to have effects on the sex ratio and whether it is the influence requires further study. In addition, the number of seeds in mud substrates was significantly higher than that in sand substrates, but the number of plants per unit area in mud substrates was significantly lower than that in sand substrates. Because water turbidity in mud base was often higher, turbidity was also one of the factors causing the *O. cordata* to be endangered. Therefore, the restoration of wetland water quality should be strengthened. In conclusion, the abundance of *O. cordata* was limited by the relatively constant water depth due to wetland structure. Therefore, maintaining appropriate water depth levels (30–90 cm) or transplanting *O. cordata* to spring habitats may be a feasible and effective strategy to avoid potential harm to its abundance because the water is not only deep enough but also clear in spring habitats. However, in the long-term, it is critical that the whole degraded wetland in Yangshan Wetland Reserve to the wetland population gradually recover. For example, wetland water sources are managed to reduce water pollution, silt is removed to keep water quality clean (turbidity $\leq$ 10 NTU), and dredging systems strengthen connections between distribution points.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

YS and XS designed the research and wrote the manuscript. YS and JL performed the research. YS and MR analyzed the data. All authors contributed to the article and approved the submitted version.

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REFERENCES

Bai, X., Chen, K. N., Zhao, H. G., and Chen, X. M. (2015). Impact of water depth and sediment type on root morphology of the submerged plant Vallisneria natans. *J. Freshwater Ecol.* 30, 75–84. doi: 10.1080/02705060.2014.970672

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2014). *lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7*. Available online at: http://CRAN.R-project.org/package=lme4 (accessed date 2021-06-22).

Boedeltje, G., Qzinga, W. A., and Prinzing, A. (2008). The trade-off between vegetative and generative reproduction among angiosperms influences regional hydrochorous propagule pressure. *Glob. Ecol. Biogeogr.* 17, 50–58. doi: 10.1111/j.1466-8238.2007.00365.x

Carollo, F. G., Ferro, V., and Termini, D. (2005). Flow Resistance Law in Channels with Flexible Submerged Vegetation. *J. Hydraul. Eng.* 131, 554–564. doi: 10.1061/(ASCE)0733-94292005131:7(554)

Curtis, R. J., Brereton, T. M., Dennis, R. L. H., Carbone, C., and Isaac, N. J. B. (2015). Butterfly abundance is determined by food availability and is mediated by species traits. *J. Appl. Ecol.* 52, 1676–1684. doi: 10.1111/1365-2664.12523

Davies, K. F., Margules, C. R., and Lawrence, J. F. (2000). Which traits of species predict population declines in experimental forest fragments? *Ecology* 81, 1450–1461. doi: 10.1890/0012-96582000081[1450:WTOSPP]2.0.CO;2

Dong, B. L., Qin, B. Q., Gao, G., and Cai, X. L. (2014). Submerged macrophyte communities and the controlling factors in large, shallow Lake Taihu (China): Sediment distribution and water depth. *J. Great Lakes Res.* 40, 646–655. doi: 10.1016/j.jglr.2014.04.007

Falster, D. S., and Westoby, M. (2003). Plant height and evolutionary games. *Trends Ecol. Evol.* 18, 337–343. doi: 10.1016/S0169-5347(03)00061-2

Fu, H., Zhong, J., Yuan, G., Ni, L., Xie, P., and Cao, T. (2014). Functional traits composition predict macrophytes community productivity along a water depth gradient in a freshwater lake. *Ecol. Evol.* 4, 1516–1523. doi: 10.1002/ece3.1022

Han, L., Yang, G., Yang, H., Xu, B., Li, Z., and Yang, X. (2018). Clustering Field-Based Maize Phenotyping of Plant-Height Growth and Canopy Spectral Dynamics Using a UAV Remote-Sensing Approach. *Front. Plant Sci.* 9:1638. doi: 10.3389/fpls.2018.01638

He, L., Zhu, T., Wu, Y., Li, W., Zhang, H., Zhang, X., et al. (2019). Littoral Slope, Water Depth and Alternative Response Strategies to Light Attenuation Shape the Distribution of Submerged Macrophytes in a Mesotrophic Lake. *Front. Plant Sci.* 10:169. doi: 10.3389/fpls.2019.00169

Huang, W., Han, S., Xing, Z., and Li, W. (2020). Responses of Leaf Anatomy and CO_2 Concentrating Mechanisms of the Aquatic Plant *Ottelia* cordata to Variable CO^2. *Front. Plant Sci.* 11:1261. doi: 10.3389/fpls.2020.01261

Kosuge, K., Iida, S., Katou, K., and Mimura, T. (2013). Circumnutation on the water surface: Female flowers of Vallisneria. *Sci. Rep.* 3:1133. doi: 10.1038/srep01133

Legendre, P., Mi, X., Ren, H., Ma, K., Yu, M., Sun, I. F., et al. (2009). Partitioning beta diversity in a subtropical broad-leaved forest of China. *Ecology* 90, 663–674. doi: 10.1890/07-1880.1

Li, H., Li, Q., Luo, X., Fu, J., and Zhang, J. (2020). Responses of the submerged macrophyte Vallisneria natans to a water depth gradient. *Sci. Total Environ.* 701:134944. doi: 10.1016/j.scitotenv.2019.134944

Li, L., Bonser, S. P., Lan, Z., Xu, L., Chen, J., and Song, Z. (2017). Water depth affects reproductive allocation and reproductive allometry in the submerged macrophyte *Vallisneria natans*. *Sci. Rep.* 7:16842. doi: 10.1038/s41598-017-16719-1

Liu, J., Lin, F., Shi, S., Ayi, Q., Liu, S., Zeng, B., et al. (2017). Effects of water level regulation on the seed germination and production of annual plant *Xanthium sibiricum* in the water-level-fluctuating-zone of Three Gorges Reservoir. *Sci. Rep.* 7:5056. doi: 10.1038/s41598-017-04599-4

Neel, M. C., Leidner, A., Haines, A., Goble, D. D., and Scott, J. M. (2012). By the numbers: How is recovery defined by the US Endangered Species Act? *BioScience* 62, 646–657. doi: 10.1525/bio.2012.62.7.7

Nõges, T., and Nõges, P. (1999). The effect of extreme water level decrease on hydrochemistry and phytoplankton in a shallow eutrophic lake. *Hydrobiologia* 408, 277–283. doi: 10.1007/978-94-017-2986-4_30

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., and O'Hara, R. B. (2016). *Vegan: community ecology package. R package version. 2.4-0.*Available online at: https://cran.r-project.org/web/packages/vegan/index.html (accessed date 2020-November-28).

Philbrick, C. T., and Les, D. H. (1996). Evolution of aquatic angiosperm reproductive systems. *Bioscience* 46, 813–826. doi: 10.2307/1312967

Rytwinski, T., and Fahrig, L. (2012). Do species life history traits explain population responses to roads? A meta-analysis. *Biol. Conserv.* 147, 87–98. doi: 10.1016/j.biocon.2011.11.023

Santamaría, L., Figuerola, J., Pilon, J. J., Mjelde, M., Green, A. J., de Boer, T., et al. (2003). Gornall. Plant performance across latitude: The role of plasticity and local adaptation in an aquatic plant. *Ecology* 84, 2454–2461. doi: 10.1890/02-0431

Schemske, D. W., Husband, B. C., Ruckelshaus, M. H., Goodwillie, C., Parker, I. M., and Bishop, J. G. (1994). Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75, 584–606. doi: 10.2307/1941718

Søndergaard, M., Johansson, L. S., Lauridsen, T. L., Jørgensen, T. B., Liboriussen, L., and Jeppesen, E. (2010). Submerged macrophytes as indicators of the ecological quality of lakes. *Freshwater Biol.* 55, 893–908. doi: 10.1111/j.1365-2427.2009.02331.x

Soudzilovskaia, N. A., Elumeeva, T. G., Onipchenko, V. G., Shidakov, I. I., Salpagarova, F. S., Khubiev, A. B., et al. (2013). Functional traits predict relationship between plant abundance dynamic and long-term climate warming. *P. Natl. Acad. Sci. USA.* 110, 18180–18184. doi: 10.1073/pnas.1310700110

Su, H., Chen, J., Wu, Y., Chen, J., Guo, X., Yan, Z., et al. (2019). Morphological traits of submerged macrophytes reveal specific positive feedbacks to water clarity in freshwater ecosystems. *Sci. Total Environ.* 684, 578–586. doi: 10.1016/j.scitotenv.2019.05.267

Sun, S., and Frelich, L. E. (2011). Flowering phenology and height growth pattern are associated with maximum plant height, relative growth rate and stem tissue mass density in herbaceous grassland species. *J. Ecol.* 99, 991–1000. doi: 10.1111/j.1365-2745.2011.01830.x

Tear, T. H., Scott, J. M., Hayward, P. H., and Griffith, B. (1993). Status and prospects for success of the Endangered Species Act: A look at recovery plans. *Science* 262, 976–977. doi: 10.1126/science.262.5136.976

Wang, H.-X., Guo, J.-L., Li, Z. M., and Yu, Y.-H. (2019). Characterization of the complete chloroplast genome of an endangered aquatic macrophyte, *Ottelia cordata* (Hydrocharitaceae). *Mitochondrial DNA B* 4, 1839–1840. doi: 10.1080/23802359.2019.1612719

Wang, Q. F., Guo, Y. H., Haynes, R. R., and Hellquist, C. B. (2010). "Hydrocharitaceae," in *Flora of China*, eds Z. Y. Wu, R. H. Peter, and D. Y. Hong (Beijing: Science Press), 91–102.

Westoby, M., Eldridge, D., and Freudenberger, D. (1999). "The LHS strategy scheme in relation to grazing and fire," in *Proceed-ings of the International Rangeland Congress*, Vol. 2, (Townsville), 893–896.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403

Xu, W., Hu, W., Deng, J., Zhu, J., and Li, Q. (2015). How do water depth and harvest intensity affect the growth and reproduction of Elodea nuttallii (Planch.) St. John? *J. Plant Ecol.* 9, 212–223. doi: 10.1093/jpe/rtv048

Zhang, H., Chen, H. Y. H., Lian, J., John, R., Ronghua, L., Liu, H., et al. (2018a). Using functional trait diversity patterns to disentangle the scale-dependent ecological processes in a subtropical forest. *Funct. Ecol.* 32, 1379–1389. doi: 10.1111/1365-2435.13079

Zhang, H., Gilbert, B., Zhang, X., and Zhou, S. R. (2013). Community assembly along a successional gradient in sub-alpine meadows of the Qinghai-Tibetan Plateau, China. *Oikos* 122, 952–960. doi: 10.1111/j.1600-0706.2012.20828.x

Zhang, H., John, R., Zhu, S., Liu, H., Xu, Q., Qi, W., et al. (2018b). Shifts in functional trait-species abundance relationships over secondary subalpine meadow succession in the Qinghai-Tibetan Plateau. *Oecologia* 188, 547–557. doi: 10.1007/s00442-018-4230-3

Zhang, Q., Shen, Z., Li, F., and Li, G. (2019). Complete chloroplast genome sequence of an endangered Ottelia cordata and its phylogenetic analysis. *Mitochondrial DNA B* 5, 2209–2210. doi: 10.1080/23802359.2020.1768921

Zhang, X., Guo, K., Lu, C., Awais, R. M., Jia, Y., and Zhong, L. (2020). Effects of origin and water depth on morphology and reproductive modes of the submerged plant *Vallisneria natans*. *Glob. Ecol. Conserv.* 24:e01330. doi: 10.1016/j.gecco.2020.e01330

Zhu, G. R., Li, W., Zhang, M., Ni, L. Y., and Wang, S. R. (2012). Adaptation of submerged macrophytes to both water depth and flood intensity as revealed by their mechanical resistance. *Hydrobiologia* 696, 77–93. doi: 10.1007/s10750-012-1185-y

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