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Growth performance, photosynthetic characteristics and water-use strategies of reintroduced *Malania oleifera* seedlings across contrasting habitats

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Habitat conditions are important factors influencing plant reintroduction outcomes, yet the physiological responses of endangered woody species across contrasting reintroduction habitats remain poorly understood. In this study, seedlings of *Malania oleifera* reintroduced into limestone (karst) and red-soil habitats were investigated, with naturally reintroduced wild seedlings serving as a reference. Photosynthetic light-response and CO₂-response curves, chlorophyll contents, leaf anatomical traits, morphological characteristics, and water-use efficiency (WUE) were measured to compare physiological and morphological variation among contrasting reintroduction habitats. Seedlings from the limestone reintroduction site exhibited higher maximum net photosynthetic rate (P_{max}), light saturation point (LSP), and isotope-derived water-use efficiency (WUE) than seedlings from the red-soil reintroduction site, whereas red-soil seedlings showed higher CO₂-saturated photosynthetic capacity (A_{max}). Limestone-grown seedlings also contained higher chlorophyll a and total chlorophyll contents than red-soil seedlings but lower levels than wild seedlings. Leaf anatomical traits also varied among habitats, with wild seedlings exhibiting greater palisade tissue thickness (PT) and higher palisade-to-spongy tissue ratios (PT/ST), whereas limestone- and red-soil-reintroduced seedlings showed similar PT/ST values. Seedlings from the limestone reintroduction site showed greater final plant height and basal stem diameter than those from the red-soil reintroduction site and wild reference habitat, whereas crown width did not differ significantly among habitats. Furthermore, isotope-derived WUE values were higher in seedlings from the limestone reintroduction site than in those from the red-soil site. This difference suggests variation in water-use strategies among habitats, although the underlying mechanisms require further investigation. Thus, seedlings from the three habitats exhibited distinct combinations of photosynthetic, morphological, anatomical, and water-use traits. The observed differences represent habitat-associated variation during the investigated period rather than evidence of universal habitat superiority. Because soil type was confounded with site

conditions and the study evaluated seedlings during a single sampling period, long-term monitoring across multiple sites is required to further assess the persistence and reintroduction outcomes of this endangered karst tree species.

KEYWORDS

contrasting habitats, karst ecosystem, *Malania oleifera*, photosynthetic performance, plant reintroduction, water-use efficiency

1 Introduction

Plant reintroduction is an effective approach for conserving endangered plant species and restoring degraded populations. However, the success of reintroduction largely depends on habitat suitability, which integrates multiple environmental factors, including soil conditions, microclimate, vegetation characteristics, and biotic interactions, that influence plant growth, photosynthesis, and resource utilization (Ren et al., 2014; Liu et al., 2025a). Environmental factors associated with different habitats, including soil properties, nutrient availability, pH, and water availability, may contribute to variation in plant carbon assimilation and biomass accumulation, although their relative contributions may vary among ecosystems (Chapin et al., 2002; Lambers et al., 2008b). Therefore, understanding plant physiological responses across contrasting habitats is important for improving habitat selection and conservation strategies for endangered species. *Malania oleifera* Chun & S. Lee is a rare and endangered evergreen tree endemic to the karst regions of southwestern China. It is valued for its high nervonic acid content and is listed as a nationally protected species. In recent years, habitat destruction and poor natural regeneration have caused a continuous decline in wild populations, making reintroduction an important conservation strategy (Xiong et al., 2023). Previous studies have mainly focused on seed germination, tissue culture, and seedling photosynthesis (Zhang et al., 2007; Huang et al., 2022). However, little information is available on how reintroduced seedlings of endangered woody species respond to contrasting habitats through coordinated changes in photosynthesis, leaf anatomical structure, growth, and water-use strategies.

Photosynthetic characteristics are widely used to evaluate plant adaptation to environmental conditions because they directly reflect carbon assimilation capacity. Leaf anatomical traits and chlorophyll content influence light capture and CO₂ diffusion, while stable carbon isotope composition ($\delta^{13}\text{C}$) is closely related to long-term water-use efficiency (Farquhar et al., 1989; Terashima et al., 2011). Combined analysis of these physiological and structural traits provides a comprehensive understanding of plant adaptation to different habitats. However, field-based reintroduction studies often involve multiple environmental factors that covary with soil characteristics, making it difficult to isolate the independent effects of soil properties on plant performance. Therefore, comparisons among reintroduction sites should consider the integrated influence of edaphic conditions, microclimate, vegetation context, and biotic interactions. In this study, wild seedlings and reintroduced seedlings growing in limestone and acidic red-soil habitats were compared. Seedling growth performance, photosynthetic characteristics, chlorophyll content, leaf anatomical traits, stable carbon isotope composition, and water-use strategies were systematically

evaluated. We aimed to characterize habitat-associated variation in functional traits of *M. oleifera* seedlings and identify potential physiological and structural traits associated with seedling establishment under different field conditions. We hypothesized that seedlings from different habitats would exhibit distinct functional characteristics reflecting their environmental conditions.

2 Materials and methods

2.1 Study site

The study was conducted in Leye County, Baise City, Guangxi Zhuang Autonomous Region, southwestern China, which represents one of the natural distribution areas of *Malania oleifera*. The region is characterized by a typical mid-subtropical monsoon climate, with a mean annual air temperature of 16.3 °C, an annual precipitation of 1051.7 mm, and an average annual sunshine duration of 1467.0 ah. The recorded extreme temperatures range from −3.0 °C to 41.1 °C. Rainfall is highly seasonal, with a wet season extending from May to October and a dry season from November to April. Three habitats were selected in this study, including two reintroduction sites with contrasting environmental conditions and one naturally reintroduced population as a reference. The limestone reintroduction site (SS; 107°28′46.72″E, 24°44′51.61″N), located in Jiaodawan Village, represents a typical karst landscape developed on limestone bedrock. This site is characterized by shallow soil layers and extensive exposed rock surfaces. The red-soil reintroduction site (TS; 106°35′18.17″E, 24°48′41.20″N), located in Shihen Village, developed from weathered sandstone and shale and contains relatively deeper soil profiles compared with the limestone site. A naturally reintroduced wild population (YS; 106°22′53.93″E, 24°50′43.82″N) located in Yachang Township was included as a reference. The wild population occurs on calcareous limestone substrates and represents a natural habitat of *M. oleifera* (Figure 1). Because each habitat was represented by a single field site, differences among habitats may reflect the combined effects of soil characteristics and other site-specific environmental factors, including microclimate, vegetation composition, and biotic interactions. Soil physicochemical properties and mineral nutrient contents were not measured in this study; therefore, the contribution of soil nutrient availability to habitat-associated differences could not be quantitatively evaluated. Accordingly, this study focuses on describing habitat-associated variation in seedling functional traits rather than isolating the independent effects of soil type. As a root hemiparasitic species, *M. oleifera* may depend on surrounding host plants during establishment. However, host

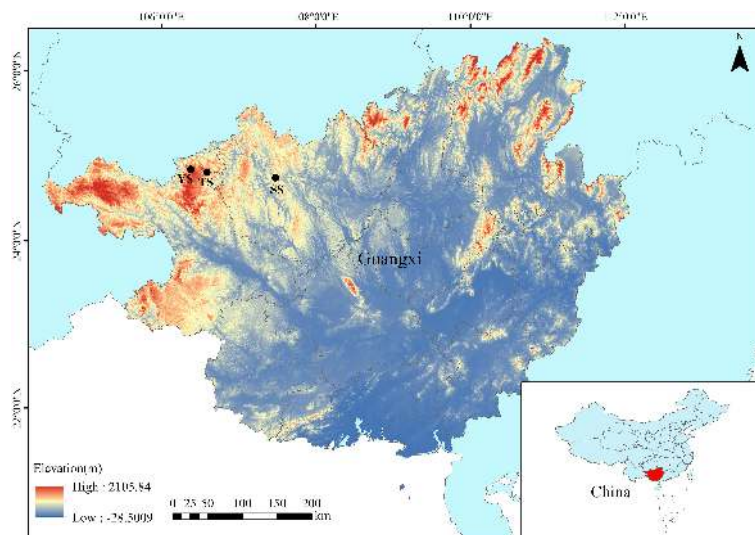


FIGURE 1
Overview of the test site.

plant composition, host availability, and haustorial connections were not quantified in the present study. Potential differences in host plant communities among habitats should therefore be considered as an additional factor influencing seedling performance.

2.2 Plant materials and experimental design

Experimental seedlings of *Malania oleifera* were collected from natural populations in Leye County, Guangxi, China. Healthy seedlings with similar initial growth status and without visible pest or disease damage were selected and cultivated under identical greenhouse conditions for one year to reduce variation in initial environmental history. In March 2024, one-year-old seedlings were transplanted to two reintroduction habitats, including the limestone reintroduction site (SS) and the red-soil reintroduction site (TS). Both reintroduction sites received routine field management, including annual manual weed control. Detailed fertilizer application information was unavailable, and therefore fertilization effects were not considered in this study. Naturally reintroduced three-year-old seedlings from the wild population in Yachang Township (YS) were selected as the reference group (Figures 2A–C). For each habitat, independent seedlings were considered as the biological experimental units. Physiological measurements, morphological measurements, anatomical analyses, and isotope analyses were conducted using individual seedlings as biological replicates, while repeated measurements obtained from the same seedling were treated as technical replicates.

2.3 Methods

2.3.1 Light response curve determination

Photosynthetic light-response curves were measured between 09:00 and 12:00 on clear, cloud-free days in mid-April 2026 using a LI-6800 Portable Photosynthesis System equipped with a 6800-01

fluorometer chamber (LI-COR Biosciences, Lincoln, NE, USA). Measurements were conducted on one fully expanded mature leaf per seedling. Before measurements, leaves were acclimated at a photosynthetic photon flux density (PPFD) of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 15 min to achieve a stable photosynthetic state. Preliminary measurements indicated that this light intensity was sufficient for photosynthetic induction in all treatments. The chamber conditions were maintained at a reference CO_2 concentration of $400 \mu\text{mol mol}^{-1}$, an air flow rate of $500 \mu\text{mol s}^{-1}$, a relative humidity of 60%, and a leaf temperature of 26°C . Light-response curves were generated by sequentially decreasing PPFD from 2000 to $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ (2000, 1800, 1600, 1400, 1200, 1000, 900, 800, 600, 400, 200, 100, 50, and $0 \mu\text{mol m}^{-2} \text{s}^{-1}$). Each light level was maintained for 120–180 s until gas exchange reached a steady state before data collection. Three independent seedlings from each habitat were measured as biological replicates, and repeated measurements obtained from the same leaf were considered technical replicates. The same set of seedlings was used for both light-response and CO_2 -response measurements. The relationship between net photosynthetic rate (P_n) and PPFD was fitted using the modified rectangular hyperbola model to estimate the maximum net photosynthetic rate (P_{max}), apparent quantum yield (AQY), dark respiration rate (R_d), light compensation point (LCP), and light saturation point (LSP) (Ye, 2010):

$$P_n = \text{AQY} \frac{1 - \beta \text{PPFD}}{1 + \gamma \text{PPFD}} \text{PPFD} - R_d$$

Where P_n represents net photosynthetic rate, AQY is apparent quantum yield, α , β , and γ are coefficients, PPFD is photon flux density, and R_d is dark respiration rate.

Following goodness-of-fit validation, the following equations were used to calculate light saturation point (LSP), maximum net photosynthetic rate (P_{max}), and light compensation point (LCP):

$$\text{LSP} = \frac{\sqrt{(\beta + \gamma)/\beta - 1}}{\gamma}$$

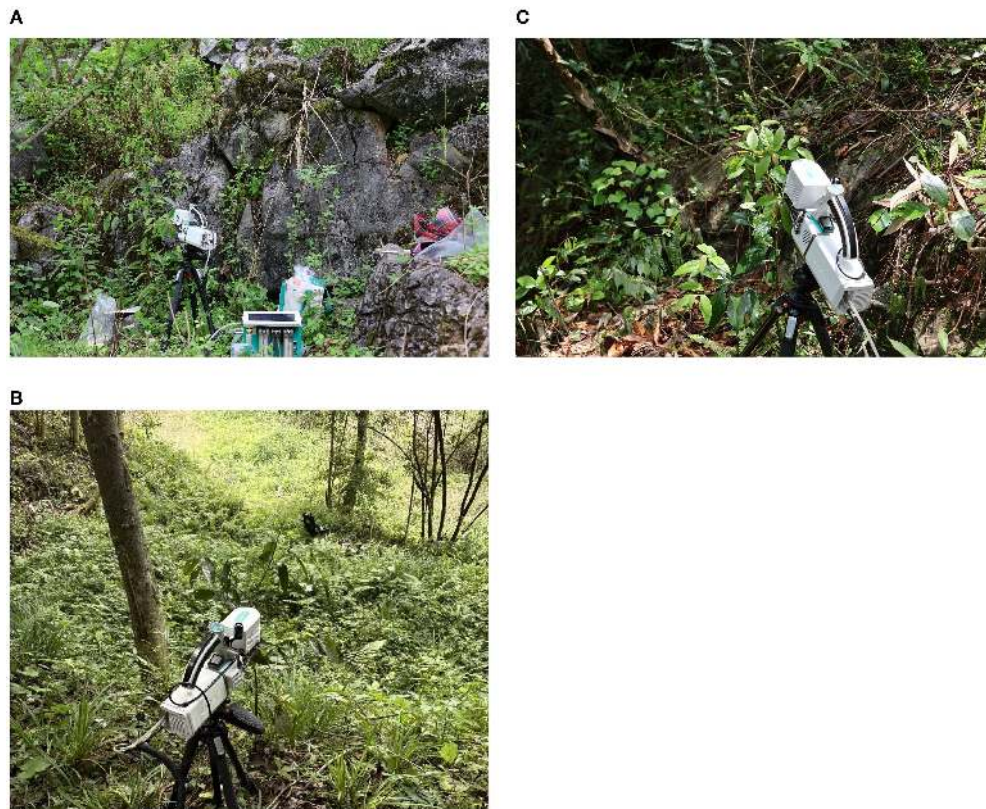


FIGURE 2
Photos of different soil types returning to the growth status of *Malania oleifera* seedlings. (A) SS; (B) TS; (C) YS.

$$P_{\max} = AQY \left(\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma} \right) - R_d$$

$$LCP = \frac{AQY - \gamma R_d - \sqrt{(\gamma R_d - AQY)^2 - 4\beta * AQY * R_d}}{2\alpha\beta}$$

2.3.2 CO₂ response curve determination

CO₂-response curves were measured between 09:00 and 12:00 on clear, cloud-free days in mid-April 2026 using a LI-6800 Portable Photosynthesis System equipped with a 6800-01 fluorometer chamber (LI-COR Biosciences, Lincoln, NE, USA). Before measurements, fully expanded, healthy leaves were acclimated at a photosynthetic photon flux density (PPFD) of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 15 min to achieve a stable photosynthetic state. During measurements, PPFD was maintained at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while the reference CO₂ concentration was sequentially adjusted to 400, 200, 100, 50, 0, 400, 600, 800, 1000, 1400, 1600, 1800, and 2000 $\mu\text{mol mol}^{-1}$. Each CO₂ level was maintained for 120–180 s until gas exchange stabilized before data collection. Three biological replicates were measured for each treatment. The relationship between net photosynthetic rate (P_n) and intercellular CO₂ concentration (C_i) was fitted using the modified rectangular hyperbola model to estimate the maximum net photosynthetic rate ($A_{\max}$), CO₂ saturation point (CSP), and CO₂ compensation point (CCP). $P_{\max}$ represents the maximum net photosynthetic rate derived from

light-response curves, whereas $A_{\max}$ represents the CO₂-saturated photosynthetic capacity derived from CO₂-response curves.

2.3.3 Growth measurements

Five independent seedlings from each habitat were selected for morphological measurements. Plant height was measured from the stem base to the terminal bud using a measuring tape. Basal stem diameter was measured at the soil line using a digital caliper. Crown width was determined by measuring the east–west and north–south crown diameters, and the mean value was used for subsequent analyses.

2.3.4 Leaf area measurements

Ten fully expanded, healthy mature leaves were randomly collected from the middle and upper canopy of each seedling. Leaf length, leaf width, and leaf area were measured using a LI-3000 Leaf Area Meter (LI-COR Biosciences, Lincoln, NE, USA). Ten leaves were measured per seedling, and the mean value of each seedling was used as the biological replicate.

2.3.5 Determination of photosynthetic pigments

Three to five leaves from each seedling were pooled as one sample. Approximately 0.20 g of fresh leaf tissue was extracted with 95% ethanol. Absorbance was measured at 665, 649, and 470 nm

using an Alpha 1502 UV-visible spectrophotometer (Shanghai Puyuan Instrument Co., Ltd., Shanghai, China). Chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl), carotenoid (Car) contents, and the Chl a/Chl b ratio were calculated according to the method of Li (2000). Pigment contents were expressed on a fresh-weight (FW) basis.

Chlorophyll a concentration: C_a , $\text{mg} \cdot \text{g}^{-1}$:

$$C_a = (13.95_{A665} - 6.88_{A649}) \times \frac{V}{1000W}$$

Chlorophyll b concentration: C_b , $\text{mg} \cdot \text{g}^{-1}$:

$$C_b = (24.96_{A649} - 7.32_{A665}) \times \frac{V}{1000W}$$

Carotenoid concentration: C_{x-c} , $\text{mg} \cdot \text{g}^{-1}$:

$$C_{x-c} = \left(\frac{1000_{A470} - 2.05C_a - 114.8C_b}{245} \right) \times \frac{V}{1000W}$$

2.3.6 Leaf anatomical structure

Leaf anatomical observations were conducted following the paraffin-section method described by Li (1987). Fully expanded leaves previously used for gas-exchange measurements were sampled from each seedling. Leaf segments (approximately 10×10 mm) adjacent to the midrib were immediately fixed in FAA solution (70% ethanol: formaldehyde: glacial acetic acid = 90:5:5, v/v/v), dehydrated through a graded ethanol series, cleared with xylene, embedded in paraffin, sectioned, stained with toluidine blue, and mounted with neutral balsam. Sections were observed and photographed under a light microscope, and anatomical traits were quantified using CaseViewer software (3DHISTECH Ltd., Budapest, Hungary). Measured variables included leaf thickness (LT), upper epidermis thickness (UET), lower epidermis thickness (LET), and the palisade-to-spongy tissue thickness ratio (PT/ST). Ten randomly selected microscopic fields were measured for each leaf, and the mean value of these fields was calculated for each leaf. Individual seedlings were considered the biological replicates for statistical analyses, whereas microscopic fields were treated as technical subsamples.

2.3.7 Isotope and water use efficiency measurements

In April 2026, three seedlings with similar growth status were randomly selected from each treatment (SS, TS, and YS) as biological replicates. Fully expanded and healthy sun-exposed leaves were collected from the outer canopy, immediately sealed in plastic bags, transported to the laboratory, rinsed with deionized water, oven-dried at 60 °C to constant weight, and finely ground for isotope analysis. Leaf carbon isotope composition ($\delta^{13}\text{C}$) was determined using a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) at the Institute of Botany, Chinese Academy of Sciences, with Vienna Pee Dee Belemnite (VPDB) as the international standard.

The stable carbon isotope composition of plant leaves was calculated according to the following equation:

$$\delta^{13}\text{C} = \frac{R_p - R_{\text{PDB}}}{R_{\text{PDB}}}$$

where $\delta^{13}\text{C}$ represents the carbon isotope composition of plant leaves; R_{sample} represents the $^{13}\text{C}/^{12}\text{C}$ ratio of the measured leaf sample, and R_{PDB} represents the $^{13}\text{C}/^{12}\text{C}$ ratio of the Pee Dee Belemnite (PDB) standard.

Carbon isotope discrimination ($\Delta^{13}\text{C}$) was calculated according to Tao et al. (2024):

$$\Delta^{13}\text{C} = \frac{(\delta^{13}\text{C}_a - \delta^{13}\text{C})}{(1 + \delta^{13}\text{C}/1000)}$$

where $\delta^{13}\text{C}$ and $\delta^{13}\text{C}_a$ represent the carbon isotope compositions of atmospheric CO_2 and plant leaves, respectively.

The atmospheric CO_2 concentration (C_a) and atmospheric carbon isotope composition ($\delta^{13}\text{C}_a$) were estimated using the following equations:

$$C_a = 277.28 + 1.350 \exp(0.01572t - 27.3528)$$

$$\delta^{13}\text{C}_a = -6.429 - 0.006 \exp(0.01572t - 37.758)$$

where t represents the sampling year (2026).

The chloroplastic CO_2 concentration (C_c) was calculated according to the equation proposed by Huang et al. (2019):

$$C_c = \left[C_a \times \frac{b - \Delta^{13}\text{C} - (f' \times \Gamma^*) / P_{C_a}}{b - a + (g_{sc}/g_m) \times (b - a_m)} - C_a \right]$$

where C_a represents the intercellular CO_2 concentration ($\mu\text{mol}/\text{mol}$); b (29‰) represents the fractionation factor associated with Rubisco carboxylation; $\Delta^{13}\text{C}$ represents carbon isotope discrimination of plant leaves; f' [$(12 \pm 4)\%$] represents the fractionation associated with photorespiration; Γ^* represents the CO_2 compensation point in the absence of mitochondrial respiration; P_{C_a} represents the fractionation associated with diffusion of CO_2 partial pressure (4.4‰) through stomata; g_{sc}/g_m (0.79 ± 0.07) represents the ratio of mesophyll conductance to stomatal conductance; and a_m (1.8‰) represents the fractionation associated with CO_2 diffusion across mesophyll cells.

The CO_2 compensation point in the absence of mitochondrial respiration (Γ^*) was calculated as follows (Mathias and Hudiburg, 2022):

$$\Gamma^* = \Gamma_{25}^* \times \left(\frac{P_{atm}}{P_0} \right) \times e^{\left[\frac{\Delta H_a \times (T - 298)}{R \times T \times 298} \right]}$$

where Γ_{25}^* (4.332 Pa) represents the CO_2 compensation point at 25 °C; P_{atm} represents atmospheric pressure calculated based on sampling altitude (108 m) and air temperature during sampling (8 °C); P_0 (101–325 Pa) represents atmospheric pressure at sea level; ΔH_a (37–830 J/mol) represents the activation energy; R [8.314 J/(mol·K)] represents the universal gas constant; and T represents the temperature during sampling (8 °C).

2.4 Data analysis

Preliminary data processing was performed using Microsoft Excel 2016. One-way ANOVA followed by Duncan's multiple range test was conducted in SPSS 27.0 to evaluate differences among

treatments. Light- and CO₂-response curves were fitted using the modified rectangular hyperbola model in Li-6400/6800 photosynthetic calculation software (v4.1.1). Pearson correlation analysis and data visualization were performed using Origin 2024 (OriginLab Corporation, Northampton, MA, USA).

3 Results

3.1 Photosynthetic characteristics of reintroduced *Malania oleifera* seedlings across contrasting habitats

3.1.1 Light-response curves across contrasting habitats

The light-response curves of reintroduced and wild *Malania oleifera* seedlings grown under different soil types are shown in Figure 3. The variation patterns of net photosynthetic rate (P_n) in seedlings from limestone soil (SS) and wild populations (YS) were generally similar. When photosynthetic photon flux density (PPFD) was below 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, P_n increased rapidly with increasing light intensity. With further increases in PPFD, P_n gradually increased at a slower rate and reached a plateau at approximately 1,000

$\mu\text{mol m}^{-2} \text{s}^{-1}$. After reaching the light saturation point, P_n of wild seedlings showed a slight decline with increasing PPFD. In contrast, seedlings reintroduced in soil from cultivated hills (TS) exhibited a lower photosynthetic response to increasing light intensity. P_n increased slightly when PPFD was below 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but rapidly reached saturation between 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with a substantially lower maximum value compared with SS and YS seedlings (Figure 3A). The responses of transpiration rate (E_{mm}) and stomatal conductance (g_{sw}) to increasing PPFD showed similar patterns among the three groups. Both parameters increased progressively with increasing light intensity, TS seedlings showed lower P_n values across the increasing PPFD gradient (Figures 3B, C). Across the entire light range, YS seedlings maintained higher Tr and g_{sw} values than SS and TS seedlings, whereas TS seedlings showed consistently lower values. The intercellular CO₂ concentration (C_i) decreased with increasing PPFD in seedlings from all treatments (Figure 3D). C_i declined rapidly under low light conditions (0–200 $\mu\text{mol m}^{-2} \text{s}^{-1}$), followed by a slower decrease when PPFD exceeded 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and eventually stabilized at higher light intensities.

3.1.2 Photosynthetic light-response parameters across contrasting habitats

Significant differences were observed in photosynthetic light-response parameters among soil treatments (Table 1). The P_{max}

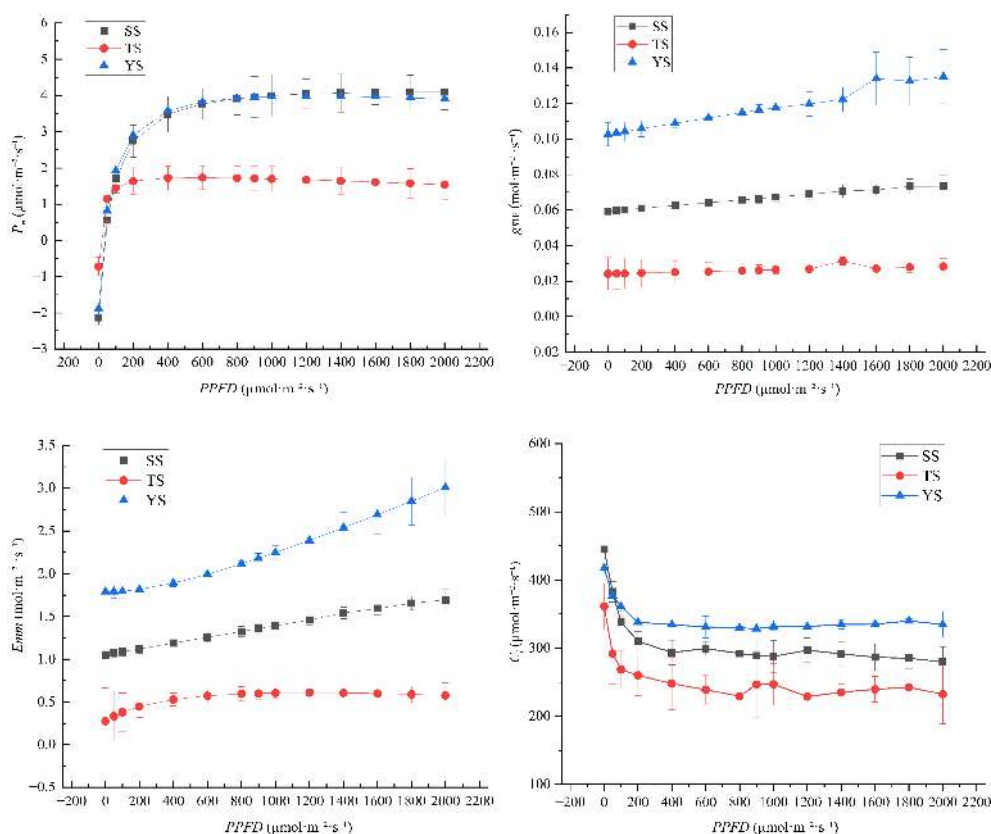


FIGURE 3

Light-response curves of reintroduced *Malania oleifera* seedlings across contrasting habitats. Data are presented as mean $\pm$ SD ($n = 3$). Error bars represent standard deviations. PPFD, photosynthetic photon flux density; P_n , net photosynthetic rate; C_i , intercellular CO₂ concentration; Tr , transpiration rate; g_{sw} , stomatal conductance. SS, seedlings reintroduced in limestone soil; TS, seedlings reintroduced in cultivated hill soil; YS, wild seedlings.

values of SS ($4.123 \pm 0.702 \mu\text{mol m}^{-2} \text{s}^{-1}$) and YS ($3.985 \pm 0.038 \mu\text{mol m}^{-2} \text{s}^{-1}$) seedlings were significantly higher than that of TS seedlings. The highest R_d was observed in SS seedlings, followed by YS and TS seedlings.

The LSP of SS seedlings was the highest ($2327.870 \pm 1210.654 \mu\text{mol m}^{-2} \text{s}^{-1}$), followed by YS ($1232.626 \pm 14.530 \mu\text{mol m}^{-2} \text{s}^{-1}$) and TS seedlings ($541.230 \pm 20.618 \mu\text{mol m}^{-2} \text{s}^{-1}$). The LCP of TS seedlings was significantly lower than those of SS and YS seedlings, while SS and YS showed no significant difference. AQY did not differ significantly between SS and YS seedlings but was significantly higher in both groups than in TS seedlings.

3.1.3 CO₂-response curves across contrasting habitats

The CO₂-response curves of reintroduced and wild *Malania oleifera* seedlings under different soil types are shown in Figure 4. The net photosynthetic rate (P_n) increased with increasing intercellular CO₂ concentration (C_i) in all treatments. At low to moderate C_i levels, P_n values showed apparent variation among habitats, with SS and YS seedlings generally exhibiting higher values than TS seedlings. However, with further increases in C_i , the increase rate of P_n differed among habitats. The fitted CO₂-response parameters indicated that TS seedlings had a significantly higher A_{max} than SS and YS seedlings (Table 2). The P_n of all seedlings gradually approached saturation between C_i of 1,200 and 1,600 $\mu\text{mol mol}^{-1}$. TS seedlings reached saturation at approximately 1,200 $\mu\text{mol mol}^{-1}$, while SS and YS seedlings reached saturation at approximately 1,600 $\mu\text{mol mol}^{-1}$. At C_i above 1,600 $\mu\text{mol mol}^{-1}$, P_n showed a slight decline in SS and YS seedlings, whereas TS seedlings maintained relatively higher P_n values.

3.1.4 Photosynthetic CO₂-response parameters across contrasting habitats

The photosynthetic CO₂-response parameters of reintroduced and wild *Malania oleifera* seedlings differed among soil treatments (Table 2). The CO₂-saturated photosynthetic capacity (A_{max}) of TS seedlings was significantly higher than that of SS and YS seedlings ($P < 0.05$), whereas no significant differences were observed between SS and YS seedlings. The initial carboxylation efficiency (α) showed no significant differences among treatments, with values of 0.016, 0.015, and 0.016 mol mol^{-1} for SS, TS, and YS seedlings, respectively. The CO₂ saturation point (CSP) was highest in TS seedlings ($2779.650 \pm 1349.022 \mu\text{mol mol}^{-1}$), followed by SS and YS seedlings; however, differences among treatments were not significant ($P > 0.05$). Similarly, the CO₂ compensation point (CCP) showed no

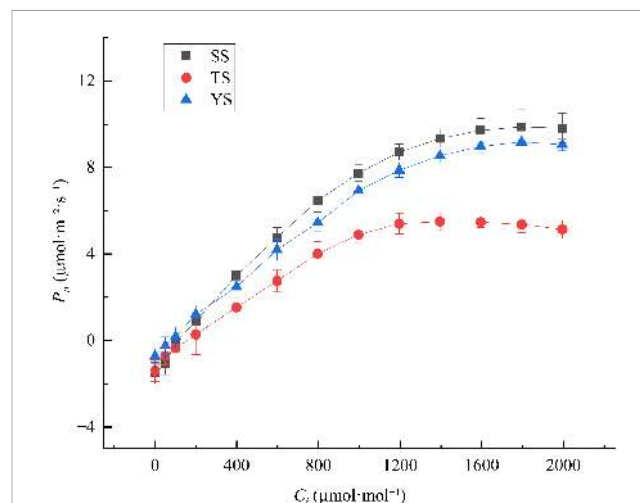


FIGURE 4
CO₂-response curves of reintroduced *Malania oleifera* seedlings across contrasting habitats. Data are presented as mean $\pm$ SD ($n = 3$). Error bars represent standard deviations.

significant differences among treatments, with values ranging from 122.640 to 147.814 $\mu\text{mol mol}^{-1}$.

3.2 Leaf photosynthetic pigment contents and ratios across contrasting habitats

The photosynthetic pigment contents of reintroduced and wild *Malania oleifera* seedlings differed significantly among soil treatments (Figure 5). The Chl a content showed the order YS > SS > TS, with significant differences among all treatments. Chl b content was highest in SS seedlings, which was significantly higher than those of YS and TS seedlings, while no significant difference was detected between YS and TS. Car content followed the order SS > YS > TS, with significant differences among treatments. Total chlorophyll content (Chl) was highest in YS seedlings, followed by SS and TS seedlings. The Chl a/b ratio showed the order YS > TS > SS, with significant differences among treatments.

3.3 Leaf anatomical characteristics across contrasting habitats

Leaf anatomical structures differed among reintroduced and wild *M. oleifera* seedlings grown under different soil types (Figure 6). Leaves consisted of a single layer of upper and lower epidermis, palisade tissue, and spongy tissue. YS seedlings exhibited relatively thicker palisade tissue with more compact and elongated cells, whereas reintroduced seedlings showed comparatively lower

TABLE 1 Photosynthetic light-response parameters of reintroduced *Malania oleifera* seedlings across contrasting habitats.

Soil types	$AQY/(\mu\text{mol}\cdot\text{mol}^{-1})$	$P_{\text{max}}/(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1})$	$LSP/(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1})$	$LCP/(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1})$	$R_d/(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1})$
SS	$0.0345 \pm 0.0110a$	$4.123 \pm 0.702a$	$2327.870 \pm 1210.654a$	$35.223 \pm 8.729a$	$2.133 \pm 0.209a$
TS	$0.0098 \pm 0.001b$	$1.737 \pm 0.321b$	$541.230 \pm 20.618b$	$13.421 \pm 3.407b$	$0.715 \pm 0.257b$
YS	$0.0236 \pm 0.001a$	$3.985 \pm 0.038a$	$1232.626 \pm 14.530ab$	$28.959 \pm 5.939a$	$1.880 \pm 0.167a$

Values are mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among treatments ($P < 0.05$). AQY , apparent quantum yield; P_{max} , maximum net photosynthetic rate; LSP , light saturation point; LCP , light compensation point; R_d , dark respiration rate.

TABLE 2 Photosynthetic CO₂-response parameters of reintroduced *Malania oleifera* seedlings across contrasting habitats.

Soil types	α ($\mu\text{mol mol}^{-1}$)	A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	CSP ($\mu\text{mol mol}^{-1}$)	CCP ($\mu\text{mol mol}^{-1}$)
SS	$0.016 \pm 0.016\text{a}$	$10.011 \pm 0.962\text{b}$	$1782.986 \pm 193.778\text{a}$	$122.640 \pm 12.851\text{a}$
TS	$0.015 \pm 0.003\text{a}$	$11.818 \pm 1.150\text{a}$	$2779.650 \pm 1349.022\text{a}$	$147.814 \pm 38.707\text{a}$
YS	$0.016 \pm 0.001\text{a}$	$9.200 \pm 0.295\text{b}$	$1589.060 \pm 26.858\text{a}$	$130.493 \pm 37.720\text{a}$

Values are presented as mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among treatments ($P < 0.05$). α , Initial carboxylation efficiency; A_{max} , Maximum net photosynthetic rate; CSP, CO₂-saturation point; CCP, CO₂-compensation point.

palisade tissue development. Quantitative analysis revealed significant differences in leaf anatomical traits among treatments (Table 3). The upper epidermal thickness (UET), palisade tissue thickness (PT), lower epidermal thickness (LET), and palisade-to-spongy tissue ratio (PT/ST) of YS seedlings were significantly higher than those of SS and TS seedlings. TS seedlings exhibited the highest spongy tissue thickness (ST), while SS and TS seedlings showed similar PT and PT/ST values, both of which were lower than those of YS seedlings. No significant differences were observed in total leaf thickness (LT) among treatments.

3.4 Growth traits and leaf phenotypic characteristics across contrasting habitats

Growth traits and leaf phenotypic characteristics differed significantly among soil treatments (Table 4). SS seedlings showed the largest final plant height (124.00 cm) and ground diameter at the sampling time, while YS seedlings showed an intermediate value (110.40 cm). Ground diameter was highest in SS seedlings (16.62 mm), significantly higher than those of TS and YS seedlings.

No significant differences were observed in crown width among treatments. Leaf traits showed different patterns among treatments. Leaf length, leaf width, and leaf area of YS and TS seedlings were significantly higher than those of SS seedlings. Leaf width followed the order YS > TS > SS, with significant differences among treatments.

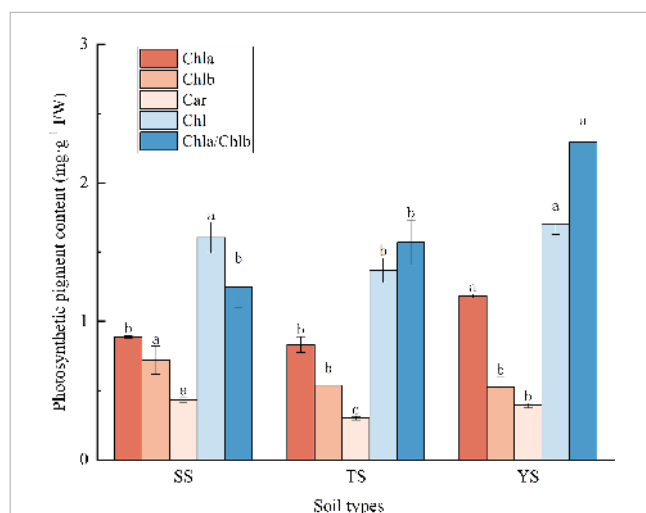


FIGURE 5 Photosynthetic pigment contents and ratios of reintroduced *Malania oleifera* seedlings across contrasting habitats. Chl a, chlorophyll a; Chl b, chlorophyll b; Car, carotenoids; Chl a+b, total chlorophyll content; Chl a/Chl b, chlorophyll a to chlorophyll b ratio.

3.5 Carbon isotope composition and water use efficiency across contrasting habitats

The carbon isotope composition ($\delta^{13}\text{C}$) and water use efficiency (WUE) differed significantly among soil treatments (Table 5). The $\delta^{13}\text{C}$ values followed the order SS > YS > TS. SS seedlings exhibited significantly higher $\delta^{13}\text{C}$ values than YS and TS seedlings, while YS seedlings showed significantly higher values than TS seedlings. WUE showed a similar pattern, with values following the order SS > YS > TS. WUE of SS seedlings was significantly higher than those of YS and TS seedlings, and YS seedlings exhibited significantly higher WUE than TS seedlings.

3.6 Correlations among photosynthetic parameters, pigment contents, leaf anatomical traits, leaf morphology, $\delta^{13}\text{C}$, and water use efficiency

Pearson correlation analysis revealed significant associations among photosynthetic parameters, pigment contents, leaf anatomical traits, leaf morphology, $\delta^{13}\text{C}$, and WUE (Figure 7). Photosynthetic pigment traits (Chl b, Car, and Chl a/b) were positively correlated with P_{max} and LSP, whereas AQY was negatively correlated with Chl a/b and Car. Leaf area (LA) showed negative correlations with P_{max} , LSP, and LCP, but a positive correlation with AQY. Both $\delta^{13}\text{C}$ and WUE were positively correlated with P_{max} and LSP, whereas $\delta^{13}\text{C}$ was negatively correlated with leaf area. Leaf anatomical traits were also associated with photosynthetic performance. PT, LT, and PT/ST showed positive correlations with photosynthetic parameters and chlorophyll content, whereas ST was negatively correlated with PT.

4 Discussion

4.1 Variation in photosynthetic characteristics of *Malania oleifera* seedlings across contrasting habitats

Light-response curves provide important information on plant photosynthetic responses to different light environments (Ye, 2010). Among the light-response parameters, AQY, LCP, and R_d mainly reflect the efficiency of light utilization under low irradiance, whereas P_{max} and LSP indicate the capacity for photosynthesis under high light conditions (Sun et al., 2021). In the present study, limestone habitats exhibited higher light-response-derived photosynthetic capacity P_{max} , whereas red-soil seedlings showed higher

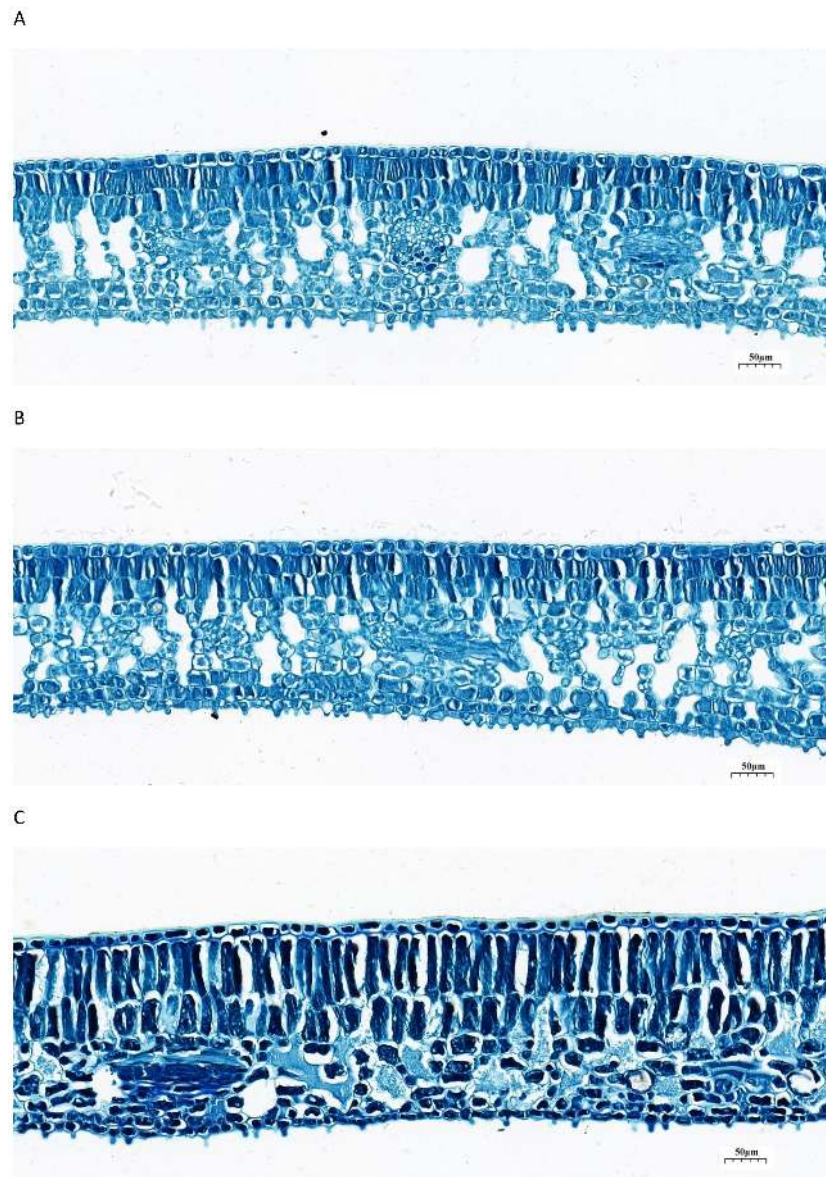


FIGURE 6
Leaf anatomical structures of reintroduced *Malania oleifera* seedlings across contrasting habitats. Scale bars = 50 μ m. (A) SS; (B) TS; (C) YS.

CO₂-saturated photosynthetic capacity $A_{\max}$. Moreover, the LSP of limestone seedlings was considerably higher than previously reported for *M. oleifera* seedlings (Zhang et al., 2007; Huang et al., 2022), indicating that limestone-site seedlings maintained higher light saturation thresholds under the measured conditions.

Although seedlings growing in acidic red soil exhibited the lowest LCP , they also had the lowest $P_{\max}$ and AQY . This result suggests that a low LCP does not necessarily represent high photosynthetic efficiency but may instead reflect an overall decline in photosynthetic activity. A similar phenomenon was reported in *Dipteronia dyeriana*, where lower LCP was not accompanied by greater photosynthetic capacity (Chen et al., 2026). Therefore, LCP should be interpreted together with other photosynthetic parameters rather than being used as an independent indicator of light adaptation. Significantly, $A_{\max}$ was significantly higher in acidic red-soil seedlings than in limestone seedlings, suggesting that the

potential capacity for carbon fixation remained relatively high, whereas suggesting that the lower photosynthetic performance of acidic red-soil seedlings may involve differences in light-response characteristics and related physiological processes. Flexas et al. (2012) proposed that under environmental stress, photosynthesis is often restricted by stomatal conductance and photochemical limitations before biochemical limitations become dominant, which is consistent with our observations. Differences in soil environmental conditions may partly contribute to these variations; however, the specific roles of soil nutrients could not be evaluated because soil nutrient data were unavailable. Previous studies have shown that available nitrogen and phosphorus are closely associated with photosynthetic performance (Zeng et al., 2024). In addition, Zhang et al. (2014) reported that increased chlorophyll content after reintroduction did not necessarily enhance net photosynthetic rate, suggesting that physiological adjustment during the early stage of

TABLE 3 Leaf anatomical characteristics of reintroduced *Malania oleifera* seedlings across contrasting habitats.

Soil types	UET (μm)	PT (μm)	ST (μm)	LET (μm)	LT (μm)	PT/ST
SS	14.940 $\pm$ 1.621b	53.850 $\pm$ 4.525b	132.380 $\pm$ 18.829a	11.730 $\pm$ 1.400a	212.900 $\pm$ 20.848a	0.413 $\pm$ 0.064b
TS	13.550 $\pm$ 1.767b	60.306 $\pm$ 14.306b	143.60 $\pm$ 25.942a	8.500 $\pm$ 1.323b	226.010 $\pm$ 37.279a	0.420 $\pm$ 0.075b
YS	15.20 $\pm$ 1.378a	100.530 $\pm$ 15.418a	107.360 $\pm$ 20.280b	12.710 $\pm$ 2.009a	235.800 $\pm$ 16.989a	0.980 $\pm$ 0.297a

Values are presented as mean $\pm$ SD ($n = 10$). Ten randomly selected fields were measured for each sample. Different lowercase letters within the same column indicate significant differences among treatments ($P < 0.05$). UET, upper epidermal thickness; PT, palisade tissue thickness; ST, spongy tissue thickness; LET, lower epidermal thickness; LT, leaf thickness; PT/ST, palisade-to-spongy tissue ratio.

TABLE 4 Growth traits and leaf phenotypic characteristics of reintroduced *Malania oleifera* seedlings across contrasting habitats.

Soil types	Plant height (cm)	Ground diameter (mm)	Crown width (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
SS	124.000 $\pm$ 17.131a	16.620 $\pm$ 3.670a	65.770 $\pm$ 18.934a	9.370 $\pm$ 0.761b	3.060 $\pm$ 0.164c	16.645 $\pm$ 1.846b
TS	89.000 $\pm$ 16.613b	13.010 $\pm$ 1.473b	62.212 $\pm$ 10.037a	14.510 $\pm$ 2.048a	4.450 $\pm$ 0.395b	39.893 $\pm$ 6.784a
YS	110.400 $\pm$ 16.056ab	12.340 $\pm$ 1.358b	46.947 $\pm$ 7.461a	14.230 $\pm$ 1.249a	4.910 $\pm$ 0.521a	41.516 $\pm$ 6.287a

Values are presented as mean $\pm$ SD. The growth index was based on single plant ($n = 5$). Leaf phenotypic indexes were measured in 10 leaves ($n = 10$). The difference of different lowercase letters in the same column was significant ($P < 0.05$).

reintroduction may be asynchronous. Differences in site environmental conditions, including soil-related factors, may partly contribute to the observed variation in photosynthetic traits, although the relative contributions of individual factors remain unclear (Lambers et al., 2008b); however, other site-specific environmental factors cannot be excluded, which may have contributed to the lower P_{max} and AQY observed in this study. Overall, our results indicate that seedlings at the limestone reintroduction site exhibited higher light-response-derived photosynthetic performance under the investigated conditions, whereas differences among sites likely reflected combined effects of soil properties and other environmental factors.

4.2 Variation in growth traits and leaf morphology of *Malania oleifera* seedlings across contrasting habitats

Plant morphological traits represent the integrated responses of plants to environmental conditions, including soil properties, light availability, and climatic factors (Tang et al., 2018). In the present study, seedlings from the limestone reintroduction site exhibited greater plant height and basal diameter than those from acidic red soil, indicating that limestone-site seedlings had larger final morphological size at the sampling time. However, these differences should be interpreted as variation in plant size rather than differences in growth rate, because initial measurements were not

available. In contrast, seedlings growing in acidic red soil showed larger leaf length, width, and area despite their lower P_{max} values, suggesting that seedlings from different sites exhibited distinct patterns of leaf trait variation.

Variation in leaf size among habitats may reflect different strategies of resource acquisition and utilization under contrasting environmental conditions. However, whether the larger leaves of acidic red-soil seedlings represent a compensatory response to lower photosynthetic capacity requires further investigation. The observed negative correlations between leaf area and several light-response parameters (P_{max} , LSP , and LCP) indicate associations among leaf morphological and photosynthetic traits, but do not necessarily demonstrate causal trade-offs or regulatory mechanisms. Similar studies have shown that soil microhabitats can influence biomass allocation patterns and plant functional traits (Zhang et al., 2021). Wang et al. (2024) reported that *Sinojackia huangmeiensis* grown under sufficient light developed thicker leaves and accumulated more dry matter, whereas shaded plants exhibited larger specific leaf area but lower photosynthetic capacity. Furthermore, Wang et al. (2020) suggested that variation in leaf nitrogen content, leaf area, and specific leaf area contributes to plant responses in heterogeneous environments. Together, these findings indicate that *M. oleifera* seedlings may exhibit different combinations of morphological and physiological traits under contrasting reintroduction sites.

The results are also comparable with the reintroduction study of *Ardisia gigantifolia*, where seedlings from historical habitats showed differences in growth and physiological performance, while excessive shading affected photosynthetic efficiency and survival (Liu et al., 2025a). Nevertheless, because each soil type in the present study corresponded to a specific field site, the observed differences may reflect the combined effects of soil characteristics, microclimate, vegetation conditions, and other site-specific factors. Therefore, the current findings provide evidence of habitat-associated variation in seedling morphological traits during the investigated period, but long-term monitoring is required to evaluate their implications for persistence and reintroduction outcomes in *M. oleifera*.

TABLE 5 Carbon isotope composition ($\delta^{13}\text{C}$) and water use efficiency (WUE) of reintroduced *Malania oleifera* seedlings across contrasting habitats.

Soil types	$\delta^{13}\text{C}$ (‰)	WUE ($\text{mol}\cdot\text{mol}^{-1}$)
SS	-29.592 $\pm$ 0.186a	39.408 $\pm$ 1.133a
TS	-35.196 $\pm$ 0.179c	4.706 $\pm$ 1.102c
YS	-34.378 $\pm$ 0.286b	9.725 $\pm$ 1.758b

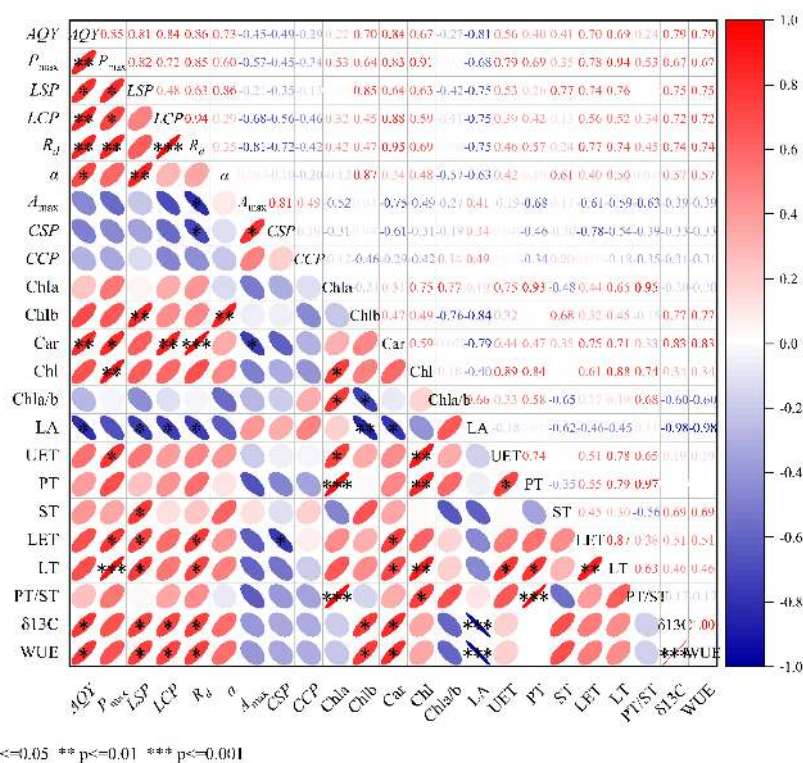


FIGURE 7

Correlations among photosynthetic traits, pigment contents, leaf traits, and water use efficiency. *indicates $P>0.05$; **indicates $P>0.01$; ***indicates $P>0.001$.

4.3 Variation in photosynthetic pigments and leaf anatomical traits across contrasting habitats

Photosynthetic pigments and leaf anatomical traits are important components associated with photosynthetic performance and plant responses to environmental variation. In this study, wild seedlings exhibited the highest chlorophyll a and total chlorophyll contents, followed by limestone-reintroduced seedlings, whereas seedlings growing in acidic red soil showed the lowest pigment contents. Similar patterns have been reported by Lai et al. (1999), who suggested that higher exchangeable manganese concentrations in acidic soils may affect chlorophyll synthesis. However, the specific factors underlying pigment variation in *M. oleifera* seedlings remain unclear. However, whether this mechanism contributed to the pigment variation observed in *M. oleifera* seedlings requires further investigation. Leaf anatomical characteristics also differed among reintroduction sites. Wild seedlings had greater palisade tissue thickness (PT) and higher PT/ST ratios than reintroduced seedlings, indicating differences in leaf anatomical characteristics among habitats. Limestone- and acidic red-soil-reintroduced seedlings showed similar PT and PT/ST values, whereas acidic red-soil seedlings exhibited higher spongy tissue thickness (ST) than wild seedlings. These findings are consistent with Dong et al. (2023), who reported associations between leaf anatomical structure and photosynthetic capacity. Correlation analysis showed that PT was positively correlated with chlorophyll a content, while the PT/ST ratio was associated with total chlorophyll content, indicating relationships between leaf

anatomical traits and pigment characteristics. Similar relationships have been reported in *Camellia* species (Chen et al., 2026) and the endangered orchid *Paphiopedilum armeniacum* (Wang et al., 2021). Overall, these results indicate that leaf anatomical and pigment traits varied among reintroduction sites, and such variation may contribute to differences in photosynthetic performance and such variation may be associated with differences in photosynthetic performance. However, further long-term observations are required to determine their implications for seedling persistence after reintroduction.

4.4 Variation in water-use efficiency of *Malania oleifera* seedlings across contrasting habitats

Water-use efficiency (WUE) is an important indicator reflecting plant water-use characteristics under different environmental conditions (Zhang et al., 2006). During photosynthesis, carbon isotope discrimination results in a close relationship between leaf $\delta^{13}\text{C}$ and long-term WUE (Chen et al., 2014). In the present study, the ranking of WUE was consistent with that of $\delta^{13}\text{C}$, with limestone-reintroduced seedlings showing the highest values and acidic red-soil seedlings showing the lowest values. The significant positive correlation between $\delta^{13}\text{C}$ and WUE further supports the use of $\delta^{13}\text{C}$ as an indicator of long-term water-use efficiency in *M. oleifera*. Limestone-reintroduced seedlings exhibited higher $\delta^{13}\text{C}$ and WUE values, suggesting higher isotope-derived water-use efficiency under the investigated conditions. Similar patterns have been reported for dominant tree species in karst ecosystems, where

higher WUE was associated with responses to water-limited environments (Dong et al., 2023). Although wild seedlings showed higher chlorophyll contents and more developed leaf anatomical traits than limestone seedlings, their lower WUE suggests that seedlings from different habitats may adopt different water-use characteristics under contrasting environmental conditions. Seedlings growing in acidic red soil exhibited the lowest WUE, which may be associated with differences in photosynthetic performance and site-specific environmental conditions. Thus, the variation in WUE, photosynthetic traits, and leaf characteristics indicates that *M. oleifera* seedlings exhibited distinct functional trait combinations among reintroduction sites. Because soil type was confounded with site conditions in this study, further long-term monitoring is required to determine whether these physiological differences influence survival, persistence, and population establishment after reintroduction.

4.5 Study limitations and implications for future research

Several limitations should be considered when interpreting the results of this study. First, the reintroduction sites investigated here represented specific locations, and soil type was inherently associated with site conditions. Therefore, the observed differences among habitats cannot be attributed solely to soil properties, as other environmental factors may also contribute to seedling variation. In addition, the limited number of available seedlings and sampling sites, caused by the conservation status of *M. oleifera*, may reduce the strength of statistical inference and introduce potential pseudoreplication. Second, wild and reintroduced seedlings differed not only in habitat conditions but also in environmental history and management background. Moreover, direct measurements of soil nutrients, soil moisture, and microclimatic conditions were not included, meaning that explanations related to resource availability and environmental adaptation should be interpreted cautiously. Although isotope-derived WUE provides an integrated indication of water-use characteristics, it also relies on assumptions related to carbon isotope discrimination. Finally, this study evaluated seedling functional traits during a single sampling period and did not include demographic indicators of reintroduction success, such as survival, mortality, reproduction, or recruitment. Therefore, the differences observed among habitats should be interpreted as short-term variation in seedling performance rather than direct evidence of long-term habitat suitability. Future multi-site and long-term monitoring studies are needed to clarify the factors influencing the persistence and restoration of *M. oleifera* populations.

5 Conclusion

This study revealed that seedlings of *Malania oleifera* exhibited significant variation in photosynthetic performance, morphological

traits, leaf characteristics, and water-use efficiency among different reintroduction sites. Seedlings from the limestone reintroduction site showed higher light-response-derived photosynthetic capacity (P_{max}), larger final plant size, and higher isotope-derived water-use efficiency than seedlings from the acidic red-soil site, whereas acidic red-soil seedlings exhibited higher CO_2 -saturated photosynthetic capacity (A_{max}). These contrasting patterns indicate that seedlings from different habitats developed distinct combinations of photosynthetic and morphological traits during the investigated period. Wild seedlings maintained higher chlorophyll contents, greater palisade tissue thickness, and higher PT/ST ratios, representing distinct leaf structural and pigment characteristics compared with reintroduced seedlings. Overall, the observed differences among habitats highlight the complexity of seedling responses during reintroduction and suggest that multiple functional traits should be considered when evaluating habitat-associated variation in *M. oleifera*. Because soil type was associated with specific field sites in this study and only short-term seedling traits were evaluated, further multi-site and long-term monitoring is required to determine how these physiological and morphological differences influence survival, persistence, and population establishment after reintroduction.

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

YW: Conceptualization, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. JY: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. YY: Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Writing – original draft. RZ: Data curation, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft. CZ: Data curation, Project administration, Resources, Supervision, Validation, Writing – review & editing. XW: Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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