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Carbon Isotope Constraints on Plant Water Use Efficiency in a Tropical Invaded Ecosystem

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Key Points:

- Leaf carbon isotopes and ^{13}C discrimination models were used to quantify plant water use efficiency (WUE) in an invaded ecosystem
- Invading C_3 plants gain invasive advantages with higher WUE than cooccurring native C_3 plants, while coexisting C_3 and C_4 natives had lower (by 42%) and higher (by 65%) WUE than those under no invasion, respectively
- Water losses for nutrient uptake and water use for photosynthetic growth co-regulate plant WUE under invasion

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract Exotic plant invasions have caused substantial changes in plant diversity and the functioning of terrestrial ecosystems. One of the key determinants of plant invasion success is its resource utilization strategy, such as water utilization strategies. However, how iWUE differs between exotic and native plants, and between natives under invasion and non-invasion, remains unclear, limiting our understanding of the role of water use strategies in plant invasion and coexistence. In this study, leaf δ^{13}C was measured to quantify the iWUE of 19 native C_3 and nine native C_4 species under no invasion, two exotic C_3 species (*Ageratina adenophora* and *Chromolaena odorata*), and 16 C_3 and 10 C_4 co-occurring natives in a tropical ecosystem of southwestern China. The significantly higher iWUE of invading plants compared with co-occurring C_3 species was associated with their invasion success and spread. Under invasion, the iWUE of coexisting native C_3 plants decreased by 42 ± 30% possibly due to enhanced water losses associated with nitrogen acquisition. Oppositely, native C_4 plants increased their iWUE by 65 ± 140%, along with enhanced photosynthetic N and NH_4^+ assimilations, which supported greater productivity. These results highlight the critical role of water use and its coupling with other resource use strategies in facilitating exotic plant invasion and promoting native plant coexistence. This work is of great significance for advancing the understanding of mechanisms shaping plant community composition and for informing the management of water and nutrient resources to control exotic plant invasion and sustain plant diversity in tropical ecosystems.

Plain Language Summary Exotic plant invasions have led to the extinction of native species and caused severe ecological problems and economic losses. Understanding how exotic plants invade and spread, and how native plants coexist or decline under invasion, is crucial for preventing and controlling exotic plant invasions. In this study, leaf carbon isotopes were analyzed to assess water use strategies of exotic plants, native plants under invasion, and natives under no invasion. A relatively higher iWUE in invasive plants was associated with their invasion success and survival. Under invasion, decreased iWUE for native C_3 plants resulted in their disappearance, while increased iWUE for native C_4 plants promoted their coexistence with exotic plants. Under uniform environmental conditions, variation in plant iWUE under invasion was linked to altered nitrogen use driven by changes in soil nitrogen status. These results highlight the critical role of coupled water and nitrogen use strategies in supporting the invasion of exotic plants and the coexistence of native plants.

1. Introduction

In the context of global change and human interference, exotic plant invasions represent one of the most significant threats to terrestrial plant biodiversity and ecosystem functioning (Dukes & Mooney, 1999; Lawler et al., 2006). The establishment and spread of exotic plants often lead to the extinction of native species, and such shifts in plant composition, driven by rapid declines in natives' abundance, are considered largely irreversible (Shuvar et al., 2021). In parallel, the processes and functioning of invaded ecosystems differ markedly from those of non-invaded ecosystems, leading to reduced ecosystem stability (Ehrenfeld, 2010; Hector et al., 2008; Valone & Balaban-Feld, 2017). Alterations in ecosystem processes (e.g., nutrient cycles) often facilitate invasion and create positive feedback that enhances the invasive capacity of exotic plants (Callaway et al., 2004; Kulmatiski et al., 2008). Accordingly, elucidating the mechanisms underlying exotic plant invasion and native extinction is critical for developing effective strategies to control invasions.

Resource utilization mechanisms are key aspects of exotic plant invasion (Funk & Vitousek, 2007), whereas the resource use strategies of natives and their adjustments under invasion are important determinants of native species coexistence or extinction (Oduor, 2021). Among the resources influencing plant competition, water is particularly crucial for plant growth and biomass production (Brendel, 2021). Invasive species often outcompete co-occurring natives through an enhanced capacity to use water (Richardson & Pyšek, 2006), and their water use strategies vary across invaded ecosystems (Ens et al., 2015; McAlpine et al., 2008; Stratton & Goldstein, 2001). As a key physiological parameter integrating plant water use and water losses (Guerrieri et al., 2016; McDowell, 2002), water use efficiency (WUE) is critical for understanding the mechanisms of invasion and coexistence among plant species. A global meta-analysis of plant water use showed that invading species generally exhibit higher stomatal conductance and consequently lower WUE than native species at the leaf observation scale (Cavaleri & Sack, 2010). Higher stomatal conductance is typically accompanied by greater CO_2 and nutrient acquisition, as well as faster growth rates, thereby contributing to invasion success (McAlpine et al., 2008). However, WUE measured on the plant and ecosystem scales does not necessarily favor greater water consumption in invading species (Cavaleri & Sack, 2010). Many invading species also exhibit higher WUE due to leaf surface traits, canopy complexity, and variations in plant age and size, which can enhance their survival and spread under water-limited conditions (Antunes et al., 2018; McAlpine et al., 2008). Moreover, invasive species with high water consumption can substantially alter ecosystem water balance by reducing water availability to native species. This can affect the WUE, carbon assimilation, and productivity of co-occurring native species (Brauman et al., 2007; Rascher et al., 2011). However, how the WUE of native plants differs between invaded and non-invaded conditions remains unclear (Rascher et al., 2011). Accordingly, it is essential to examine WUE differences between invading and native plants, and between natives under invaded and non-invaded ecosystems in natural conditions.

Water use efficiency can be assessed from instantaneous measurements at the leaf scale to more integrative estimates at the whole-plant and ecosystem-scales, which was divided based on measuring methods or scales of the research (Farquhar, 1989; Medrano et al., 2015). Ecosystem-scale WUE is calculated by GPP and ET of the whole ecosystem, while it was challenging to separate evaporation from transpiration (Guerrieri et al., 2016; Medrano et al., 2010, 2015). Leaf-scale WUE is calculated by instantaneous observations of net photosynthesis rate (A) and stomatal conductance (g_s), which makes it inadequate for explaining long-term physiological changes in plants (Medrano et al., 2010). Currently, plant $\delta^{13}C$ is widely recognized as a reliable proxy for measuring time-integrated intrinsic WUE (iWUE) (Guerrieri et al., 2016; Medrano et al., 2010). Notably, there are significant differences between the photosynthetic processes of C_3 and C_4 plants and their water utilization. The assimilation of CO_2 in C_3 plants can be simplified into two main processes, stomatal diffusion and Rubisco carboxylase fixation. Differently, C_4 plants possess a specialized CO_2 concentrating mechanism. Ambient CO_2 is initially fixed by efficient PEPCase in mesophyll cells to form C_4 acid, which is subsequently decarboxylated and refixed by Rubisco in bundle-sheath cells (Gong et al., 2017). As a result, C_4 plants exhibit higher photosynthesis capacity and greater water- and nitrogen-use efficiency than C_3 plants, making them well adapted to high temperature, high irradiance, and arid environments (Still et al., 2003). Nevertheless, calculations of iWUE for C_3 and C_4 plants rely on the differential discrimination of carbon isotopes by the enzymes that fix CO_2, as well as on the physical differences in isotope diffusivity (Farquhar, 1989; Farquhar & Richards, 1984). Considering both enzymatic and diffusive fractionations of ^{13}C, total discrimination against the heavier isotopes links plant $\delta^{13}C$ to the ratio of intercellular to ambient CO_2 concentrations (C_i/C_a, C_i refers to intercellular CO_2 concentrations, C_a refers to ambient CO_2 concentrations) (Farquhar, 1989). Meanwhile, iWUE is given by the ratio of A to g_s, which is closely related to C_i and C_a (Farquhar, 1989). Thus, $\delta^{13}C$ and iWUE are mathematically equivalent (Seibt et al., 2008; Ubierna & Farquhar, 2014). Alterations in ecosystem functioning under invasion can modify A and g_s, thereby influencing $\delta^{13}C$ and iWUE (Kloeppel & Abrams, 1995). Therefore, leaf $\delta^{13}C$-based iWUE provides a valuable approach for investigating the mechanisms of exotic plant invasion, coexistence, and their interactions with nitrogen use parameters under invasion.

As an essential aspect of invasibility, plant iWUE is regulated by multiple abiotic processes and biotic traits (Farquhar, 1989). In general, plant WUE is influenced by both physiological parameters (e.g., leaf N contents) and environmental factors (e.g., soil nutrient contents) (Cernusak et al., 2013; Elmore et al., 2017; Guerrieri et al., 2016). Changes in physiological traits and environmental conditions under invasion (Vitousek et al., 1987) can alter plant WUE. Thus, clarifying the coupling between plant water use and other resource use strategies under invasion is essential for understanding the mechanism of invasion and coexistence (McAlpine et al., 2008).

Pot and field experiments have demonstrated that increased photosynthetic capacities induced by elevated leaf N enhance plant WUE (Elmore et al., 2017; Guerrieri et al., 2016; Hamerlynck et al., 2004; Schmidt et al., 1993). Accordingly, invading plants with higher N acquisition capacity have higher WUE by increasing leaf N contents and photosynthetic rates (Feng, 2008). Meanwhile, leaf N is not the only trait regulating plant WUE. Atmospheric CO_2 concentration is also an important factor in predicting plant WUE, and WUE responses often vary with changes in intercellular CO_2 concentrations associated with ambient CO_2 (Gong et al., 2022). Environmental conditions such as temperature, humidity, and salinity influence plant iWUE by regulating stomatal closure, whereas irradiance and soil nutrient status influence plant iWUE by affecting photosynthesis rate (Farquhar, 1989). Cultivation experiments demonstrated that leaf C isotopes and iWUE of plants supplied with ammonium (NH_4^+) were significantly lower than those supplied with nitrate (NO_3^-), due to higher stomatal conductance (Guo et al., 2002). Modeling studies further revealed that NH_4^+ and NO_3^- assimilation resulted in different $\delta^{13}C$ values and WUE in plants (Raven et al., 1992). However, few studies have examined the coupling between nitrogen-use parameters and WUE in invaded ecosystems, despite significant differences in N metabolisms between invasive and native plants (Hu et al., 2018).

Ageratina adenophora (*A. adenophora*) and *Chromolaena odorata* (*C. odorata*) are perennial herbs native to Mexico that have rapidly spread across southeastern and eastern Asia (Cronk & Fuller, 1995). Their invasion has caused severe ecological problems in southwestern China since the 1940s (Wang & Wang, 2006). Leaf gas exchange measures suggested that *A. adenophora* exhibits higher WUE than co-occurring native species. Pot experiments indicate that higher N-use efficiency and photosynthetic WUE than native species may promote the adaptation and invasion of *A. adenophora* (Feng, 2008; Feng et al., 2011). Stable ^{15}N analyses further revealed that both invading species exhibit high N levels and a preference for NH_4^+, while invasion also elevates NH_4^+ utilization in native species (Hu et al., 2018, 2022). However, differences in iWUE between exotic and native plants, as well as within the same native species under invaded and non-invaded conditions, remain unclear. Moreover, the extent to which plant iWUE and its coupling with N utilization influence survival and growth under invasion remains poorly understood. To address these gaps, we quantified leaf $\delta^{13}C$ to assess iWUE in 23 native C_3 species, 10 native C_4 species, and two invading species, examining their variations along an invasion gradient in a tropical ecosystem of SW China, respectively. Specifically, we aimed to (a) compare iWUE between native and exotic C_3 plants, as well as between native C_3 and C_4 plants under invaded and non-invaded conditions, and (b) evaluate the relationships between plant N-use parameters and iWUE to elucidate water-nitrogen coupling mechanisms underlying plant invasion and coexistence.

2. Materials and Methods

2.1. Study Site

This study was conducted at Mt. Kongming (101.12°E, 22.14°N) in Xishuangbanna, Yunnan Province, southwestern China. The site is a limestone montane ecosystem characterized by a typical subtropical monsoonal climate, with a mean annual temperature of 21°C and mean annual precipitation of 1,600 mm. The study site was located on the mountain summit at an altitude of 1,100 ± 10 m, with a gentle slope of less than 10°. The area is pristine, dominated by yellow soil, and has no history of agricultural use or other anthropogenic disturbance.

Herbaceous and graminoid plants dominate the vegetation at Mt. Kongming, with a few deciduous and evergreen shrubs and scattered conifer forests (mainly *Pinus massoniana* Lamb.). Information on native plants is provided in Table S1 in Supporting Information S1, all natives are common species across tropical and subtropical China. Mt. Kongming is a representative site with distinct gradients of natural invasion pressure of *A. adenophora* or *C. odorata* under uniform climatic and environmental conditions. This setting provides an ideal place to investigate the water and nutrient utilization strategies of exotic and native plants under varying levels of invasion pressure (Hu et al., 2018, 2022).

2.2. Sample Collection

Three experimental blocks were established on three low hills at Mt. Kongming. Each block covered an area of approximately 100 m × 100 m at an elevation of about 1,100–1,130 m. The distance between the blocks is approximately 50–80 m. Within each block, distinct patches of *A. adenophora* invasion (about 20 m × 20 m), *C. odorata* invasion (about 20 m × 20 m), and no invasion (about 10 m × 10 m) were identified. Plots of no invasion and plots with different invasive pressures of *A. adenophora* or *C. odorata* were selected within corresponding patches. Dominant plants in patches of no invasion are species of Poaceae, Euphorbiaceae, Rubiaceae, Rosaceae, Malvaceae, and Cyperaceae (Table S1 in Supporting Information S1).

Aboveground biomass and species composition were investigated in each block in July 2011, based on a subjective classification of no invasion, light invasion, and severe invasion. Investigations were conducted for seven plots (1 m × 1 m for each) in no invasion patches of three blocks (3, 2, 2 plots for each), 14 plots (1 m × 1 m for each) in *A. adenophora* invasion patches of three blocks (5, 5, 4 plots for each), and 14 plots (1 m × 1 m for each) in *C. odorata* invasion patches of three blocks (5, 5, 4 plots for each). Harvested biomass was separated by plot and species, then dried at 105°C for 30 min followed by 75°C until a constant weight was achieved. Invasive pressure was determined at the plot level and calculated as the biomass percentage of invasive plants in each plot.

Based on the plant biomass and species data, we collected a new set of leaf and soil samples for chemical and isotopic analyses in the same no invasion, light invasion, and severe invasion patches. We sampled mature and green leaves of dominant species for a total of three plots (2 m × 2 m for each) in no invasion patches of three blocks (one plot for each), six plots (2 m × 2 m for each) in *A. adenophora* invasion patches of three blocks (two plots for each), and six plots (2 m × 2 m for each) in *C. odorata* invasion patches of three blocks (two plots for each). To obtain an adequate sample amount for analyses of each species, we aggregated leaf samples of multiple individuals of the same species and generated one sample per species in each plot. The 15 plots adjacent to plots sampled for chemical analyses have consistent variations of biomass and species number for all 35 plots. Accordingly, the invasive pressure of those 15 plots was used for interpreting chemical parameters.

2.3. Chemical Analyses

Sample pretreatments were conducted within 8 hr after the sampling in the laboratory at the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Fresh plant samples were washed strictly with deionized water and ultrapure water several times successively. After washing, samples were oven-dried at 60°C to constant weight and ground to a fine powder using a ball mill (MM200; Retsch, Haan, Germany). Leaf C and N concentrations were measured by using an elemental analyzer (Elementar Analysensysteme GmbH, Germany). Based on C contents, C isotopes of each leaf sample with 80 μg C were measured on a Thermo MAT 253 isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) coupled with an elemental analyzer (Flash EA 2000). Leaf C isotopes are expressed in per mil (‰) and calculated as follows.

$$\delta^{13}\mathrm{C} = (R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1) \times 1000$$

where R_{sample} and R_{standard} are $^{13}\mathrm{C}/^{12}\mathrm{C}$ in leaf samples and the standard (the IAEA standards Vienna Pee Dee Belemnite), respectively. Standard samples of IAEA-C_3 ($\delta^{13}\mathrm{C} = -24.97$‰, cellulose) were measured in the same way as leaf samples for the calibration of leaf $\delta^{13}\mathrm{C}$ values. The analytical precision of replicate $\delta^{13}\mathrm{C}$ analyses for individual samples averaged ±0.1‰.

Detailed analytical methods of N concentrations and isotopes in plant and soil samples referred to Hu et al. (2018, 2022). In this paper, data of plant and soil N concentrations and contributions of soil $\mathrm{NO_3^-}$ and $\mathrm{NH_4^+}$ to plant N were used to explore the potential influences of soil N availability and plant N assimilation on plant $\delta^{13}\mathrm{C}$ and iWUE variations.

2.4. Calculations of Plant iWUE Values

Following the method of Farquhar (1989), the photosynthetic C isotope discrimination (Δ) relative to plant C source (atmospheric CO_2) can be expressed as Equation 1.

$$\Delta = \left(\delta^{13}\mathrm{C}_{\mathrm{air}} - \delta^{13}\mathrm{C}_{\mathrm{plant}}\right)/\left(1 + \delta^{13}\mathrm{C}_{\mathrm{plant}}\right) \tag{1}$$

where $\delta^{13}\mathrm{C}_{\mathrm{air}}$ and $\delta^{13}\mathrm{C}_{\mathrm{plant}}$ denote $\delta^{13}\mathrm{C}$ values of atmospheric CO_2 and leaf C, respectively. The $\delta^{13}\mathrm{C}_{\mathrm{air}}$ value monitored at Mauna Loa Observatory, Hawaii (20°N, 156°W) in 2011 (−8.32‰; www.esrl.noaa.gov/gmd/index.html) was used in our calculation.

For C_3 plants, the Δ value is associated with the ratio of intercellular to ambient CO_2 concentrations (C_i/C_a) and can be expressed as Equation 2.

$$\Delta_{(C_3\ \text{plants})} = a + (b - a) \times C_i/C_a \tag{2}$$

where *a* refers to the C isotope fractionation during the CO_2 diffusion through leaf stomata (4.4‰) and *b* is the net C isotope effect of CO_2 carboxylation by Rubisco in C_3 plants (27‰) (Farquhar, 1989).

For C_4 plants, the Δ value is also associated with the C_i/C_a and can be expressed as Equation 3.

$$\Delta_{(C_4\ \text{plants})} = a + (b_4 + b_3 \times \phi - a) \times C_i/C_a \tag{3}$$

where b_4 is the combined C isotope fractionation of the two main processes of the PEP carboxylation and the dissolution and hydration of CO_2. These two processes have a temperature-dependent equilibrium fractionation of −5.7‰ at 25°C. b_3 is the isotope effect of CO_2 fixation by Rubisco in C_4 plants (27‰). ϕ is the proportion of C fixed by PEP carboxylation (Farquhar, 1989), which reflects the leakiness of the bundle cell. The ϕ value differs with both environmental conditions and among plant species (Ellsworth & Cousins, 2016; Fravolini et al., 2002; Henderson et al., 1992). So far, plant ϕ values have been measured by different methods, including the radiolabeling-inorganic C pool method, radiolabeling-leak rate method, quantum yield method, and carbon isotope discrimination method, showing values of 0.2–0.3 for plant species growing in normal ambient conditions (Kromdijk et al., 2014) (Table S2 in Supporting Information S1). However, the C isotope discrimination method is most commonly used, especially with the recent development of laser technologies that have greatly facilitated the measurement of leaf CO_2 isotope exchange (Kromdijk et al., 2014). In our calculations, we refer to the results of Henderson et al. (1992), which measured ϕ values of different C_4 subtypes (including PCK, NAD-ME, and NADP-ME subtypes) simultaneously by using the C isotope discrimination method.

Following the method of Farquhar and Richards (1984), the iWUE value was calculated as the ratio of net photosynthesis rate (*A*) to the leaf gas conductance of water vapor (g_{s-H_2O}) (Equation 4).

$$\text{iWUE} = A/g_{s-H_2O} \tag{4}$$

The *A* and g_{s-H_2O} were calculated by Equations 5 and 6, respectively.

$$A = g_{s-CO_2} \times (C_a - C_i) \tag{5}$$

$$g_{s-H_2O} = 1.6 \times g_{s-CO_2} \tag{6}$$

where g_{s-CO_2} is the leaf gas conductance for CO_2 (g_{s-CO_2}).

Then, we combined Equations 1–6 to get Equations 7 and 8 for calculating iWUE values of C_3 and C_4 plants, respectively.

$$\text{iWUE}_{(C_3\ \text{plants})} = C_a \times (b - \Delta_{(C_3\ \text{plants})})/1.6/(b - a) \tag{7}$$

$$\text{iWUE}_{(C_4\ \text{plants})} = C_a \times (b_4 + b_3 \times \phi - \Delta_{(C_4\ \text{plants})})/1.6/(b_4 + b_3 \times \phi - a) \tag{8}$$

where the C_a values monitored at Mauna Loa Observatory, Hawaii (19.5°N, 155.6°W) in 2011 (392.72 ppm; www.esrl.noaa.gov/gmd/index.html) were used.

2.5. Methods for Estimating Fractional Contributions of Soil NH_4^+ and NO_3^- to Plant N

Soil NH_4^+ and NO_3^- have been recognized as major N sources for plants in subtropical and tropical ecosystems where soil N mineralization and nitrification are generally higher than in high-latitude and high-altitude ecosystems (Houlton et al., 2007; Liu et al., 2018; Zhang et al., 2018). Accordingly, soil NH_4^+ and NO_3^- were assumed as the dominant N sources of plants in this study, and their relative contributions to leaf N (denoted as $f_{\text{leaf}-NH_4^+}$ and $f_{\text{leaf}-NO_3^-}$ hereafter, respectively) were estimated by the following isotope mass-balance equations.

$$\delta^{15}N_{\text{leaf}} + \Delta_U = \left(\delta^{15}N_{NH_4^+} - \Delta_{A-NH_4^+}\right) \times f_{\text{leaf}-NH_4^+} + \left(\delta^{15}N_{NO_3^-} - \Delta_{A-NO_3^-}\right) \times f_{\text{leaf}-NO_3^-}$$

where we assume that $f_{\text{leaf}-\text{NH}_4^+} + f_{\text{leaf}-\text{NO}_3^-} = 1$. $\delta^{15}\text{N}_{\text{leaf}}$, $\delta^{15}\text{N}_{\text{NH}_4^+}$, and $\delta^{15}\text{N}_{\text{NO}_3^-}$ are δ^{15}N values of leaves, soil NH_4^+, and soil NO_3^-, respectively. The data of $\delta^{15}\text{N}_{\text{NH}_4^+}$ and $\delta^{15}\text{N}_{\text{NO}_3^-}$ values are cited from Hu et al. (2018). Δ_{U} values are isotope effects caused by the process of plant N uptake, which was assumed as 0.0‰ for plants with no mycorrhizal association and 2.0 ± 2.5‰ for plants associated with arbuscular mycorrhizae (Craine et al., 2009; Evans, 2001; Evans et al., 1996).$\Delta_{\text{A}-\text{NO}_3^-}$ and $\Delta_{\text{A}-\text{NH}_4^+}$ values are N isotope effects caused by allocations of soil NO_3^--derived and NH_4^+-derived N between leaves and roots, respectively. Previously, $\delta^{15}\text{N}_{\text{leaf}}$ values would be higher by ca. 2.0 ± 0.7‰ than δ^{15}N values of source NO_3^- (Hu et al., 2018) due to the intra-plant allocation or redistribution of NO_3^--derived N (Bergersen et al., 1988; Evans et al., 1996; Kalcsits et al., 2015; Kohl & Shearer, 1980; Mariotti et al., 1982; Yoneyama & Kaneko, 1989). Accordingly, the mean $\Delta_{\text{A-NO3-}}$ values were considered as 2.0 ± 0.7‰ in our calculations. $\delta^{15}\text{N}_{\text{leaf}}$ values would be higher by ca. 0.2 ± 0.8‰ than δ^{15}N values of source NH_4^+ due to the intra-plant allocation or redistribution of NH_4^+-derived N (Evans et al., 1996; Kalcsits et al., 2015; Yoneyama et al., 1991). Accordingly, $\Delta_{\text{A}-\text{NH}_4^+}$ was considered as 0.2 ± 0.8‰ in our calculations. Errors of f values in equations were estimated by a Monte Carlo method.

2.6. Statistical Analyses

We defined the real δ^{13}C (or iWUE) differences as the variations in δ^{13}C (or iWUE) of native plants before and after invasion, which are calculated by using the mean δ^{13}C (or iWUE) of native plants under invasion ($n = 1 \sim 6$) minus those under no invasion ($n = 1 \sim 3$). We defined the relative differences as the magnitude of the variations in δ^{13}C (or iWUE) of native plants before and after invasion, which are calculated as the percentages of real δ^{13}C (or iWUE) differences in the corresponding mean δ^{13}C (or iWUE) values under no invasion. Pearson correlation analyses were conducted to examine relationships of leaf δ^{13}C and plant iWUE variations with the invasive pressure, plant, and soil N variables. Multiple comparisons were conducted to determine differences in leaf δ^{13}C and iWUE values between native and invading plants, native plants under invasion and no invasion, and different photosynthetic types and living forms of plants, respectively. Before the analysis of multiple comparisons, the homogeneity of variances was tested. If the variances are homogeneous, the least significant difference method (LSD) was used. If the variances are inhomogeneous, the Tamhane's Ta method was used. Statistical analyses were conducted using the software SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The statistically significant difference was set at $p < 0.05$ unless otherwise stated.

3. Results

3.1. Plant Biomass and Species Number

The aboveground biomass of invading plants increased from 0 to 1,082 g/m^2 (accounting for 0%–82%, Figure S1a), while that of native C_3 plants decreased distinctly in the invaded plots ($R^2 = 0.15$, $p < 0.05$, Figure S1b). Seven of 19 native C_3 species disappeared in invaded plots, whereas none of the nine native C_4 plants disappeared (Table S1 in Supporting Information S1), and their aboveground biomass did not change substantially in invaded plots ($R^2 = 0.09$, $p = 0.08$, Figure S1b). From no invasion to severe invasion plots, the average number of species ranged from 17 to 7 for native C_3 plants and 8 to 5 for native C_4 plants (Figure S1c).

3.2. Leaf δ^{13}C and Plant iWUE

Leaf δ^{13}C and plant iWUE of two invaders averaged −30.9 ± 0.6‰ and 40.6 ± 6.6 μmol CO_2 mol^{-1} H_2O, respectively (Figures 1b and 1c), with no difference between *A. adenophora* and *C. odorata*. Distinctly, co-occurring native C_3 plants showed lower ($p < 0.05$) leaf δ^{13}C (species-based mean values were −35.3 ± 1.4 ∼ −29.2 ± 0.0‰, averaging −31.6 ± 1.5‰) and plant iWUE (−10.5 ± 15.7 ∼ 59.6 ± 0.0 μmol CO_2 mol^{-1} H_2O, averaging 32.0 ± 17.6 μmol CO_2 mol^{-1} H_2O) (Figures 1a–1c), with no differences between herbs and shrubs (Table S3 in Supporting Information S1).

Native C_4 plants showed intrinsically higher leaf δ^{13}C and iWUE than all C_3 plants ($p < 0.05$), averaging −12.9 ± 0.7‰ and 334.3 ± 199.3 μmol CO_2 mol^{-1} H_2O, respectively (Figures 1b and 1c). The co-occurring C_3 species under invasion had lower leaf δ^{13}C (by −1.7 ± 0.8‰ on average) and iWUE (by −19.3 ± 9.6 μmol CO_2 mol^{-1} H_2O and −42 ± 30% proportionally) than the same species under no invasion (Figures 2a, 2c, and 2d).

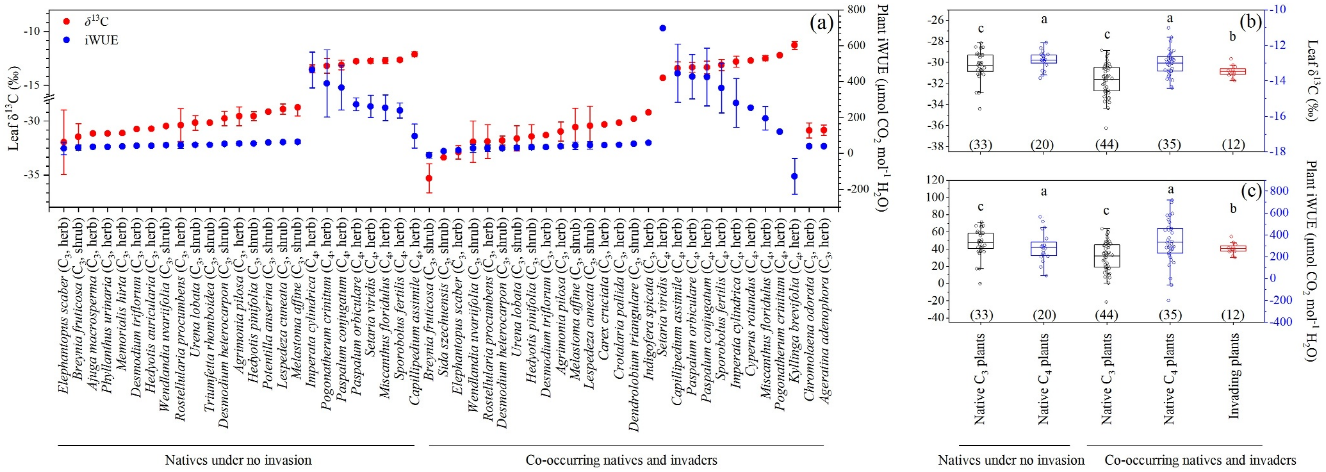

Figure 1. Leaf δ^{13}C and plant iWUE values of invading plants, native plants under no invasion, and co-occurring natives under invasion at Mt. Kongming of Xishuangbanna in tropical SW China. (a) Species-based mean leaf δ^{13}C and plant iWUE values under no invasion and invasion plots. Replicate plots ($n = 1 \sim 6$) depending on the presence of different plant species. Photosynthetic types and living forms are shown in parentheses after species names. (b) Leaf δ^{13}C and (c) plant iWUE of invading plants, native C_3 and C_4 plants under no invasion and invasion plots. The boxes encompass the 25th–75th percentiles, whiskers are SD values, and the line in each box marks the mean value for sample replicates (n). Different letters above the boxes indicate significant differences between different plants. The statistically significant difference was set at $p < 0.05$.

Moreover, the co-occurring C_4 plants under invasion showed higher iWUE (by 76.7 ± 244.7 μmol CO_2 mol^{-1} H_2O and 65 ± 140% proportionally) than the same species under no invasion, although their leaf δ^{13}C also decreased (by −0.3 ± 0.9‰ on average) (Figures 2a, 2c, and 2d).

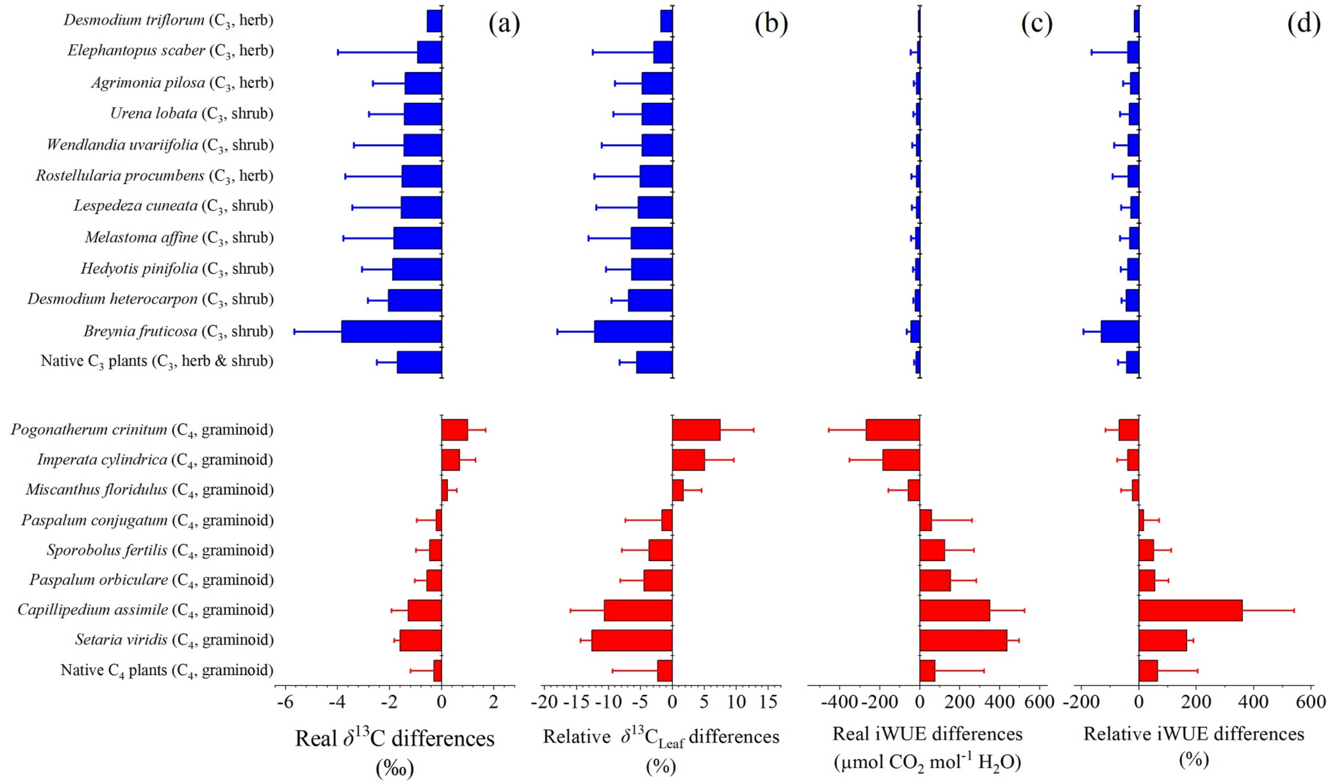

Figure 2. Differences in leaf δ^{13}C and plant iWUE of the same native species between invasion and no invasion at Mt. Kongming of Xishuangbanna in tropical SW China. (a) Real leaf δ^{13}C and (c) plant iWUE difference. (b) Relative leaf δ^{13}C and (d) plant iWUE difference. In panels (a and c), real differences were calculated by using the mean values under invasion ($n = 1 \sim 6$) minus those under no invasion ($n = 1 \sim 3$). In panels (b and d), relative differences were calculated as percentages of real differences (panels a and c) in the corresponding mean values of the same species under no invasion (Figure 1a). Whiskers are SD values.

Table 1
Influences of Major Factors on Plot-Based Plant iWUE Variations

Variable	Plant	Invasive pressure (%)	Soil NH_4^+ content (mg-N g^{-1}, dw)	N_{leaf} (%, dw)	$F_{leaf-NH_4^+}$ (%)
Plant iWUE (μmol CO_2 mol^{-1} H_2O)	Native C_3 (all plots, 14)	−0.710**	−0.572*	−0.607*	−0.555*
	Native C_3 (invaded plots, 11)	n.s.	n.s.	n.s.	n.s.
	Native C_4 (all plots, 13)	n.s.	0.653*	0.555*	0.616*
	Native C_4 (invaded plots, 10)	n.s.	0.646*	n.s.	n.s.
	Invaders (invaded plots, 12)	n.s.	n.s.	n.s.	n.s.

Note. N_{leaf}, leaf N content. $F_{leaf-NH_4^+}$, fractional contributions of soil NH_4^+ to leaf total N. Plot-based mean values were used for analyses. Positive and negative correlation coefficients are shown. n.s. not significant, *: $P < 0.05$, **: $P < 0.01$.

3.3. Variations of Plant iWUE With N Parameters

For native C_3 species, plant iWUE decreased with soil NH_4^+ ($p < 0.05$ in plot-based analyses, $p < 0.01$ in species-based analyses) and fractional contributions of soil NH_4^+ to leaf N ($p < 0.05$ in plot-based analyses, $p < 0.01$ in species-based analyses) (Tables 1 and 2). While for native C_4 species, it increased with soil NH_4^+ ($p < 0.05$ in plot-based analyses, $p < 0.05$ in species-based analyses) and fractional contributions of soil NH_4^+ to leaf N ($p < 0.05$ in plot-based analyses, $p < 0.01$ in species-based analyses) (Tables 1 and 2). Differently, the plant iWUE of the two invading species did not vary with the above factors (Tables 1 and 2).

4. Discussion

4.1. Higher iWUE in Invading C_3 Plants Than Co-Occurring Native C_3 Plants

It has been verified that leaf $\delta^{13}C$ increased with WUE for terrestrial C_3 plants (Farquhar & Richards, 1984). Physiologically, invading plants are often characterized by higher photosynthetic rates and greater stomatal conductance compared with co-occurring native plants (see the data syntheses by Cavaleri & Sack, 2010). Such traits are generally expected to reduce WUE, since enhanced stomatal opening increases transpirational water loss relative to carbon gain (Cavaleri & Sack, 2010). Unexpectedly, we observed that the leaf $\delta^{13}C$ of two invading species (−30.9 ± 0.6‰) was significantly higher than that of co-occurring C_3 native species (−31.6 ± 1.5‰) (Figure 1b). Correspondingly, the calculated iWUE of the invaders (averaging 40.6 ± 6.6 μmol CO_2 mol^{-1} H_2O) was markedly higher than that of co-occurring native C_3 species (averaging 32.0 ± 17.6 μmol CO_2 mol^{-1} H_2O) (Figure 1c).

Under uniform climatic conditions, exotic species can substantially increase community-level productivity and transpiration under invasion (e.g., a total of 42% contributed by invaders; Rascher et al., 2011). Consistently, in our study, the aboveground biomass of invading plants increased from 0 g/m^2 to 1,082 g/m^2 (accounting for 0%–82%), while the biomass of native C_3 plants declined markedly in invaded plots (Figure S1). This shift in community dominance likely altered the light environment, as the rapid growth of invaders reduced understory light availability. Such shading effects can depress photosynthetic rates in native C_3 plants, thereby lowering their leaf $\delta^{13}C$ and iWUE (Ehleringer et al., 1987; Funk & Vitousek, 2007).

Table 2
Influences of Major Factors on Species-Based Plant iWUE Variations

Variable	Plant	Invasive pressure (%)	Soil NH_4^+ content (mg-N g^{-1}, dw)	N_{leaf} (%, dw)	$F_{leaf-NH_4^+}$ (%)
Plant iWUE (μmol CO_2 mol^{-1} H_2O)	Native C_3 (all plots, $n = 77$)	−0.435**	−0.377**	n.s.	−0.312**
	Native C_3 (invaded plots, $n = 44$)	n.s.	−0.419**	n.s.	n.s.
	Native C_4 (all plots, $n = 55$)	n.s.	0.302*	n.s.	0.353**
	Native C_4 (invaded plots, $n = 35$)	n.s.	0.344*	n.s.	n.s.
	Invaders (invaded plots, $n = 12$)	n.s.	n.s.	n.s.	n.s.

Note. N_{leaf}, leaf N content. $F_{leaf-NH_4^+}$, fractional contributions of soil NH_4^+ to leaf total N. Species-based mean values were used for analyses. Positive and negative correlation coefficients are shown. n.s. not significant, *: $P < 0.05$, **: $P < 0.01$.

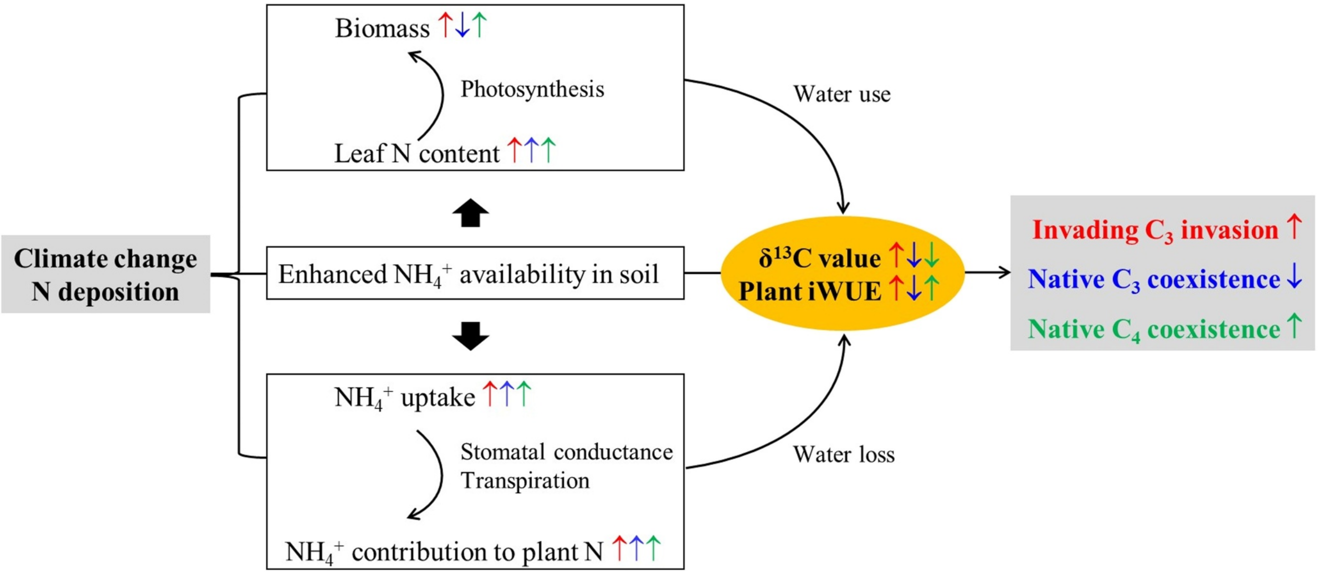

Figure 3. A schematic map summarizing water and N coupling mechanisms of different plants in the invaded ecosystem. The upward red arrows indicate relatively higher values (leaf N content, soil NH_4^+ contribution to leaf N, biomass, iWUE) of invading C_3 plants compared with co-occurring native C_3 plants. The upward and downward arrows of native C_3 (in blue) and C_4 (in green) plants indicate relatively higher and lower values of the same species under invasion than those under no invasion, respectively.

Previous studies have also reported higher WUE in invading plants compared to natives, particularly under conditions of low precipitation and soil water availability, where invaders benefited from greater tolerance to water and salinity stress (Busch & Smith, 1995). Even under no water limitation, higher iWUE in invading species were observed in a forest of eastern America because of the higher net photosynthesis in invading plants than native plants (Kloeppel & Abrams, 1995). Our study site was not water-limited, suggesting that the higher iWUE of invaders was instead linked to their enhanced photosynthetic capacity and productivity, supported by more efficient N and P acquisition (Feng, 2008; Hu et al., 2018). Accordingly, besides nutrient use advantages (Feng et al., 2009, 2011; Hu et al., 2022), our findings identify higher iWUE as an additional key strategy supporting the success of *A. adenophora* and *C. odorata* invasion.

4.2. Decreased Plant iWUE for the Same Native C_3 Species Under Invasion

Compared with the same species under no invasion, co-occurring C_3 natives under invasion exhibited lower $\delta^{13}C$ (by $-1.7 \pm 0.8‰$) and reduced iWUE (by -19.3 ± 9.6 μmol CO_2 mol^{-1} H_2O, a proportional decline of $42 \pm 30\%$) (Figure 2). Due to the proximity of the selected plots and similar environmental conditions, many common constraints on plant iWUE were minimized, such as temperature, humidity, and light. In this context, heterogeneous soil nutrient conditions and the inherent traits of the plants are likely to be the primary regulatory factors. Interestingly, generally higher soil N availability and leaf N contents in native plants under invasion were observed (Hu et al., 2018). In theory, greater N uptake should enhance WUE by stimulating photosynthetic capacity and reducing the relative cost of water losses (Rascher et al., 2011). However, we observed a negative correlation between leaf N and iWUE in native C_3 plants (Table 1). This indicates that the decline in iWUE of natives under invasion cannot be attributed to higher N absorption or allocation to photosynthesis but instead reflects other physiological or ecological constraints.

On one hand, invasion reduces the relative availability of both water and nutrients for native plants (Hu et al., 2018, 2022). To compete for limited soil N, co-occurring C_3 natives appear to increase transpiration, incurring greater water loss while gaining little additional carbon assimilation. This shift explains their reduced WUE under invasion (Matimati et al., 2014). Supportively, the above-ground productivity did not increase for coexisting C_3 natives, so their higher N contents demonstrated a reduced N-use efficiency for photosynthesis (Hu et al., 2018). Thus, native plants may lower their photosynthetic WUE as a trade-off to enhance N acquisition under invasion (Figure 3). This provides a new mechanism for how native plants reduce photosynthetic WUE to achieve soil N acquisition under invasion. A comparable pattern of reduced leaf $\delta^{13}C$ and iWUE in native C_3

plants under invasion was also observed in a pine forest of the semi-arid Mediterranean area with the MAP lower than 400 mm (Rascher et al., 2011).

On the other hand, N forms also influence water use efficiency. Further correlation analysis ultimately indicated that nitrogen utilization affects plant WUE. However, many issues remain, and the existing conclusions lack mechanistic explanations. Plants supplied with NH_4^+ exhibit higher stomatal conductance than those supplied with NO_3^-, leading to lower WUE in C_3 plants (Guo et al., 2002). In our study, invaded plots contained relatively higher soil NH_4^+ contents (Hu et al., 2018), which corresponded with reduced iWUE in native C_3 plants (Table 1). Such effects have been attributed to differences in isotopic fractionations during N assimilation (Raven et al., 1992). Consistently, we observed decreases in both leaf $\delta^{13}C$ and iWUE with increasing fractional contributions of soil NH_4^+ to leaf N (Table 1). The result was consistent with the modeling results that plant $\delta^{13}C$ and iWUE were lower for NH_4^+ assimilation than NO_3^- assimilation as the N source (Raven et al., 1992). These findings enriched the recognition of responses of water and nutrient physiology in native C_3 plants to changes in soil N status by exotic invasion (Figure 3). These physiological disadvantages in nutrient acquisition (Hu et al., 2018) and water use strategies (Figure 1c) suggests that, without management intervention, native C_3 species may continue to decline as observed (Figure S1b in Supporting Information S1) in the invaded ecosystem.

Our current interpretation connecting nutrient-use and water-use strategies via effects of nutrient content and uptake on carbon assimilation and stomatal conductance remains largely hypothetical in the absence of direct physiological measurements. While our inferences are grounded in established physiological frameworks, empirical validation is essential to substantiate these linkages. However, experimental observations of gas exchange data (photosynthesis, A, and stomatal conductance, gs) only represent water exchange processes in specific leaves at a given time (Liang et al., 2023). Gas exchange data are highly variable across temporal scales (Kläring & Körner, 2020; Liang et al., 2023). The instantaneous gas exchange does not correspond to the long-term iWUE, thereby limiting the reliability of gas exchange data as evidence for long-term water-use patterns. Also, differences in physiological traits and phenological behaviors among species contribute to the uncertainty associated with using simultaneous gas exchange measurements to infer water use strategies across species (Matthews et al., 2017).

Direct evidence for long-term plant water use will likely require future cultivation experiments. By thoroughly documenting plant biomass production and water consumption during the growth process under cultivation or by combining long-term monitoring with model predictions to obtain long-term averages of plant gas exchange, direct evidence of long-term changes in plant water use may be obtained. In addition, $\delta^{18}O$ evidence may provide more information on plant water use (Lin et al., 2022).

4.3. Increased Plant iWUE for the Same Native C_4 Species Under Invasion

Differing from C_3 plants, leaf $\delta^{13}C$ of C_4 plants decreased with plant iWUE, depending on the proportion of C fixed by the PEP carboxylation (ϕ) (Farquhar, 1989). The divergence arises from the decoupling of CO_2 absorption and assimilation in C_4 plants (Ellsworth & Cousins, 2016). In our study, co-occurring C_4 plants under invasion exhibited slightly lower leaf $\delta^{13}C$ (by $-0.3 \pm 0.9‰$ on average) but markedly higher iWUE (by 76.7 ± 244.7 μmol CO_2 mol^{-1} H_2O and $65 \pm 140\%$ proportionally) compared with the same species under no invasion (Figure 2). Potentially, increased plant iWUE under invasion may promote the survival of native C_4 plants (Figure 1). An increase in aboveground biomass (up to 40%) further demonstrated that native C_4 plants were more adaptable to the water restriction or deficiency than native C_3 plants (Figure S1 in Supporting Information S1). Similar to invasive plants, higher leaf N uptake enhanced photosynthetic rates and WUE of native C_4 plants (Table 1, Figure 3). Different from C_3 species, C_4 species often have high nitrogen and water use efficiency due to their efficient carboxylase and CO_2 concentrating mechanisms. This means that C_4 plants have lower demands for nitrogen and moisture, which promoted the successful co-occurrence of native C_4 and invading plants (Figure S1b in Supporting Information S1). It is speculated that native C_4 plants would continuously coexist with invading plants in this ecosystem. While future changes in climate conditions and soil nutrients may break the balance. Drier climate or drought events would promote the survival of native C_4 plants, whereas increase in soil NH_4^+ would be in favor of the expansion of invasive plants. Our results underscore a key mechanism underlying the coexistence of C_4 plants in invaded ecosystems and reveal how C_4 plants responded differently from C_3 plants to the changed soil N status under exotic invasion.

4.4. Uncertainty in Estimated iWUE Based on Leaf Carbon Isotopes

Uncertainties should be considered when predicting plant iWUE using leaf $\delta^{13}C$. Here we employed the classical discrimination model ($iWUE_{sim}$) (Farquhar, 1989). This model considered only the fractionation caused by carboxylation and stomatal diffusion, while neglecting several processes, such as mesophyll conductance, boundary-layer conductance, and respiration (Ma et al., 2023). Neglect of these processes, for example, mesophyll conductance, potentially results in systematic overestimation of iWUE up to ~65% compared to models that explicitly include g_m (such as $iWUE_{mes}$) (Ma et al., 2021). Subsequent research has optimized the model by incorporating additional parameters to improve the accuracy of iWUE calculations. While current $iWUE_{mes}$ and $iWUE_{com}$ models introduced numerous parameters that remain under debate and uncertainty (Ma et al., 2023). Although the classical discrimination model is not accurate enough, we still applied this model to calculate iWUE. In future work, we also hope to achieve more accurate iWUE estimation of plants by integrating models and parameters.

Plant iWUE reflects the effect of C_i on WUE independent of D (leaf to air vapor concentration difference) and has been widely used in both inter- and intra-specific comparison (Guerrieri et al., 2019; Keenan et al., 2013). Plant iWUE varies with rapid stomatal responses to environmental fluctuations, which implies that spatial or temporal environmental differences can compromise comparisons among genotypes (Liang et al., 2023). This confounding effect arises because C_i, a key determinant of iWUE, is influenced by multiple environmental and biochemical factors (Liang et al., 2023). In this study, samples were collected in three adjacent blocks separated by approximately 50–80 m. It was assumed that ecological conditions (temperature, humidity) were uniform across the study area, and that plant stomata responded consistently to environmental factors. Thus, changes in iWUE caused by environmental conditions for different plants should be consistent. Meanwhile, environmental factors, especially the absence of D, may affect the accuracy of the estimated iWUE. We suppose that a combination of environmental indicators and response of the stoma to ambient conditions for different plants should be considered in detail.

It is worth noting that in the ^{13}C isotope fractionation model, interspecific differences among plants usually cannot be ignored. In particular, interspecific differences are more pronounced in C_4 plants because of the various subtypes (Ellsworth & Cousins, 2016). Therefore, the uncertainty of parameters and the complexity of interspecific differences limit the utility of models for calculating iWUE. In this study, we investigated different species and considered them as parallel samples of native C_3, C_4, and exotic plants in the analysis. Therefore, uncertainty brought about by species differences in estimated iWUE could be seen as a part of SD values. In future research on iWUE uncertainty, interspecific differences in plants are also an important part that cannot be ignored.

5. Conclusion

Based on the natural ^{13}C interpretation of the iWUE of terrestrial plants, this study provides new insights into water-use strategies among invading and coexisting native plants in a tropical ecosystem. We found that two invading C_3 plant species (*A. adenophora* and *C. odorata*) had significantly higher iWUE than co-occurring C_3 species, which is recognized as a new physiological trait for their invasion success and spreading. With no increases in productivity, the iWUE of coexisting native C_3 plants was reduced passively under invasion because of enhanced water losses for acquiring N resources, particularly soil NH_4^+ whose assimilation was increased and itself consumed more water and had larger C isotope fractionations than those of soil NO_3^-. In contrast, native C_4 plants enhanced their WUE and photosynthetic N and NH_4^+ assimilations to support productivity increases. These findings underscore the critical role of water-use strategies in supporting the invasion of exotic plants and the coexistence of native C_4 plants. However, because of more water investments in nutrient competition and acquisition than in photosynthetic growth, most of the native C_3 plants disappeared, and the coexisting ones showed no productivity increase and lower iWUE. This work is of great significance for understanding the evolving mechanisms of plant species composition and effectively managing water and nutrient resources to control exotic plant invasion and maintain plant diversity in tropical ecosystems.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The data sets generated and analyzed during the current study are available at Yan (2025) https://figshare.com/s/ad66fc134605ca0fc3d4.

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