

The "Nitrogen Fixation-Nitrogen Preemption" Mechanism of thomson kudzu in Suppressing the invasive weed Mikania micrantha: Nitrogen Cycle Remodeling and Light-Nitrogen Synergistic Suppression

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Abstract

Aims

This study investigated how the native legume thomson kudzu and non-legume sweet potato suppress the invasive weed *M. micrantha*, focusing on nitrogen fixation's role in competitive outcomes.

Methods

We conducted controlled experiments measuring biomass, nitrogen content (using ^{15}N natural abundance method), PNUE and microbial communities (via high-throughput sequencing). Competitive indices and random forest modeling identified key success factors. A 12-month field validation experiment was conducted to assess long-term suppression efficacy under natural conditions.

Results

Thomson kudzu showed superior suppression: (1) Thomson kudzu planted with *M. micrantha* significantly increased soil nitrogen levels, elevating total nitrogen, ammonium nitrogen, nitrate nitrogen, and microbial biomass nitrogen by 16.88%, 25.87%, 8.98%, and 37.63% respectively, whereas sweet potato planted with *M. micrantha* reduced these indicators by 3.4%, 14.4%, 12.3%, and 15.6%. (2) Co-planting with *M. micrantha* markedly enhanced nodulation in thomson kudzu, increasing nitrogenase activity by approximately 253%. (3) Thomson kudzu exhibited significantly higher phosphorus-nitrogen utilization efficiency (PNUE) than *M. micrantha*. (4) Thomson kudzu demonstrated stronger competitive ability (with a competitive index of 0.76, substantially higher than sweet potato's 0.36). (5) Field validation confirmed the sustained suppression of *M. micrantha* growth (coverage < 1%).

Conclusions

Thomson kudzu's "nitrogen amplification-preemption" strategy effectively controls invasives through nitrogen cycling optimization and multidimensional competition, offering an eco-friendly alternative to chemical control.

Introduction

Plant invasions have become one of the primary drivers of ecosystem degradation and biodiversity loss globally, with particularly severe impacts in tropical and subtropical regions. *Mikania micrantha* H.B.K., listed among the world's 100 most harmful invasive alien species by the International Union for Conservation of Nature (IUCN), poses a serious threat due to its rapid growth and reproductive capacity. By competitively excluding native species, it quickly occupies ecological niches, thereby presenting

major challenges to agricultural production and ecological restoration (Zan & Li, 2010; Day et al., 2012; Day et al., 2016).

Current control methods for *M. micrantha* mainly rely on manual removal and herbicide application. Although these approaches can effectively suppress its spread in the short term, they often result in negative environmental consequences such as pollution, soil degradation, and loss of biodiversity (Tsugawa, 1986; Sharma et al., 2019). Moreover, they fail to prevent the weed's persistent regeneration (Li et al., 2013), primarily due to the lack of effective management of the vacant ecological niches left behind. In contrast, replacement control, an ecological niche-based management strategy, offers a sustainable and ecologically safe alternative. By introducing competitive native plants to occupy available niches, this approach aims to achieve long-term suppression of invasive species (Li et al., 2015; Shen et al., 2015; Jia et al., 2022; Shen et al., 2023).

Regarding the replacement control of *M. micrantha*, previously reported replacement plants primarily include herbaceous plants, shrubs, and fast-growing trees. Examples include Poaceae grasses such as *Festuca arundinacea*, *Mosla chinensis*, *Lolium multiflorum*, and the Asteraceae species *Artemisia annua*, along with the *Ipomoea batatas* (L.) Lam. (hereafter sweet potato) (Xu et al., 2011; Shen et al., 2012; Song et al., 2020). Leguminous shrubs like *Stylosanthes guianensis* (Song et al., 2020) and tree species for community restructuring, such as *Macaranga tanarius* and *Heteropanax fragrans* (Li, 2012), have also been investigated. However, most of these plants exhibit relatively low ecological niche overlap with *M. micrantha*. Among them, sweet potato has demonstrated significant suppression of *M. micrantha* growth and reproduction through resource competition (Shen et al., 2015; Shen et al., 2023). Nevertheless, the herbaceous growth form of sweet potato is markedly different from the vine-like characteristics of *M. micrantha*. According to the principle of limiting similarity, species with similar ecological niches are expected to experience stronger competition due to their high overlap in resource requirements (MacArthur & Levins, 1967), making them more effective in resisting invasions (Price & Pärtel, 2013; Young et al., 2009). Therefore, selecting native species with similar ecological niches for replacement control could facilitate sustained ecological management of invasive plants (Santamarina et al., 2023).

Nitrogen limitation is a fundamental constraint in most natural ecosystems. The invasion of *M. micrantha* has been shown to intensify this limitation by accelerating nitrogen uptake and promoting denitrification, thereby exacerbating soil nitrogen depletion (Yu et al., 2021). In contrast, legume–rhizobium symbiotic systems contribute substantially to global nitrogen inputs, with estimated annual biological nitrogen fixation ranging from 40 to 50 million tons (Graham & Vance, 2003). These systems not only compensate for nitrogen loss in invaded ecosystems (Kebede, 2021), but also enable the rapid occupation of vacated ecological niches, thereby facilitating the recovery of native plant assemblages and enhancing community diversity. This process can promote positive successional trajectories in degraded habitats (Zedníková et al., 2023). Nevertheless, the mechanistic pathways through which legumes regulate soil nitrogen dynamics via symbiotic microorganisms to suppress invasive species remain insufficiently understood and warrant further investigation.

Therefore, this study selected the native leguminous vine *Pueraria lobata* var. *Thomsonii* Benth (hereafter thomson kudzu)—which occupies a similar ecological niche to the invasive *M. micrantha* and possesses economic value—as a replacement plant, with the non-leguminous sweet potato serving as a control. We systematically evaluated the endogenous mechanisms by which thomson kudzu suppresses *M. micrantha* regeneration through nitrogen fixation-mediated soil nitrogen replenishment.

Methodologically, we conducted **single-species planting** and co-culture experiments to measure plant biomass, net photosynthetic rate, specific leaf area, and tissue nitrogen content, calculating competitive balance indices and photosynthetic nitrogen use efficiency (PNUE). The ^{15}N natural abundance method was employed to quantify nitrogen fixation efficiency and estimate nitrogen fixation rates in thomson kudzu and sweet potato. High-throughput sequencing was applied to characterize the response of nitrogen-fixing microbial communities to thomson kudzu-*M. micrantha* competition. Finally, random forest modeling was used to quantify the combined explanatory power of soil nitrogen dynamics, nodule traits, and nitrogen-fixing microbes on competitive biomass outcomes. We proposed a "microbe-driven nitrogen fixation-nitrogen preemption-competitive dominance" cascade hypothesis, positing that thomson kudzu enhances nitrogen fixation by activating symbiotic microbes, thereby accumulating soil nitrogen. This enables thomson kudzu to preempt nitrogen resources through elevated tissue nitrogen content, ultimately establishing multidimensional suppression via optimized PNUE and shading effects. Our findings provide novel theoretical insights and technical frameworks for ecological management of *M. micrantha*, facilitating a paradigm shift from chemical control to sustainable models based on niche competition.

Materials and Methods

Experimental Design

The *M. micrantha* specimens used in this study were collected from the Botanical Garden of South China Normal University (Guangzhou, China; 23°8'N, 113°21'E). In late March 2021, healthy stem segments were selected and cut into 7–10 cm long cuttings, each containing two nodes. The cuttings were placed in culture flasks wrapped in aluminum foil and propagated asexually under controlled conditions in an RXZ intelligent climate chamber (Ningbo Jiangnan Instrument Factory). The growth conditions were set to a light intensity of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, a 12 h light/12 h dark photoperiod, a constant temperature of 25°C, and 65% relative humidity. Cuttings of thomson kudzu (a leguminous plant) and sweet potato (a non-leguminous plant, used as a control) were purchased from an online vendor on the Taobao platform (Store ID: 4165405168, China). These cuttings were propagated asexually under the same environmental conditions as *M. micrantha*.

In April 2021, after two weeks of cutting propagation, 100 vigorous and morphologically uniform seedlings were selected for the experiment and transplanted into pots (19-33.5 cm in diameter, 22 cm in height). Each pot was filled with 7.5 kg of a cultivation substrate composed of red soil (collected from the Botanical Garden of South China Normal University) mixed with horticultural soil at a 3:1 ratio (v/v).

Thomson kudzu and sweet potato were each co-planted with *M. micrantha* at a 1:1 ratio (referred to as PI + Mm and Ib + Mm, respectively). Additionally, each species was planted on their own as controls (PI, Ib, and Mm; two individuals per pot), resulting in five treatment groups. Each treatment was replicated 25 times, totaling 125 pots. After transplanting, all seedlings were randomly arranged and cultivated under greenhouse conditions at the Biology Garden of South China Normal University, with uniform irrigation management maintained throughout the experiment.

Plant Biomass, Nitrogen Content in Plant Tissues, and Competition Balance Index

The experimental plants were grown for a total duration of 240 days. During this period, aboveground biomass (stems + leaves) was harvested every 40 days, with five pots sampled at each time point. The final harvest, which included both above and belowground biomass, was conducted at day 240 (December 2022) using the remaining five pots. During the harvesting process, thomson kudzu plants were carefully excavated along with the intact rhizosphere soil. Root systems were then gently washed under a mild stream of water to remove soil, and root nodules were meticulously separated from the roots. The detached nodules were rinsed with sterile distilled water to eliminate surface contaminants. Intact nodules were counted and their number recorded; subsequently, surface moisture was removed using sterile filter paper, and fresh weight was measured (Xing et al., 2022). Following these procedures, samples were rapidly frozen in liquid nitrogen and stored at -80°C for long-term preservation, in preparation for subsequent functional sequencing and assays of nitrogenase activity in thomson kudzu root nodules. Then, all tissue samples (roots, stems, leaves) were dried to constant weight at 60°C and then weighed to obtain the biomass of each organ. Subsequently, a portion of the roots, stems, and leaves were ground and sieved (0.15 mm). The nitrogen content in the root, stem, and leaf tissues of the plants was determined using the Kjeldahl nitrogen analyzer-distillation method (Bao, 2000). Some of the sieved stem and leaf samples were set aside for subsequent determination of nitrogen fixation efficiency by the ^{15}N natural abundance method.

The Competitive Balance Index (CB) was employed to assess the relative competitive abilities among plant species (Fowler, 1982; Wilson, 1988; Wang & Chen, 2019). The index is calculated using the following equations: $\text{RYab} = \text{Yab}/\text{Ya}$, $\text{RYba} = \text{Yba}/\text{Yb}$, $\text{CBa} = \ln(\text{RYab}/\text{RYba})$, where Yab represents the average per-plant biomass of species a when co-planted with species b, and Ya denotes the average per-plant biomass of species a planted on their own. Similarly, Yba represents the average per-plant biomass of species b when co-planted with species a, and Yb denotes the average per-plant biomass of species b planted on their own.

The value of CBa indicates the relative competitive strength of species a compared to species b:

- $\text{CBa} > 0$ indicates that species a is more competitive than species b;
- $\text{CBa} = 0$ indicates that both species exhibit equal competitive ability;

- CBa < 0 indicates that species a is less competitive than species b.

Determination of Plant Nitrogen Fixation Efficiency and Fixed Nitrogen

Determination of nitrogen fixation efficiency and calculation of nitrogen fixation in thomson kudzu and sweet potato using the ^{15}N natural abundance method (Boddey et al., 2000). Ground and sieved (0.15 mm) stem and leaf samples (Section 2.2) were analyzed using a stable isotope ratio mass spectrometer (Thermo Fisher Scientific 253Plus) to measure the $^{15}\text{N}/^{14}\text{N}$ isotope ratios in the plant tissues. The $\delta^{15}\text{N}$ value (‰) was calculated as $\delta^{15}\text{N} (\text{‰}) = \left[\frac{\%^{15}\text{N}_s}{\%^{15}\text{N}_a} \right]$, where $\%^{15}\text{N}_s$ and $\%^{15}\text{N}_a$ represent the ^{15}N abundance in the sample and in the atmosphere (0.3663‰), respectively (Mariotti et al., 1981). The percentage of nitrogen derived from atmospheric fixation ($\%N_f$) in thomson kudzu and sweet potato was calculated as $\%N_f = \left[\frac{\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_f}{\delta^{15}\text{N}_{\text{ref}} - \beta} \right] \times 100$ (Shearer & Kohl, 1986), where $\delta^{15}\text{N}_{\text{ref}}$ is the $\delta^{15}\text{N}$ value of the reference plant (*M. micrantha*), $\delta^{15}\text{N}_f$ is the $\delta^{15}\text{N}$ value of the test plant (thomson kudzu or sweet potato), and β is the atmospheric ^{15}N abundance (assumed to be 0.0‰; Kato-Noguchi, 2023). Based on the measured stem and leaf biomass of thomson kudzu co-planted with *M. micrantha* and sweet potato co-planted with *M. micrantha* (Section 2.2) and their nitrogen content, we quantified the biologically fixed nitrogen (N_f) using the following calculation:

$N_f (\text{mg N}) = \%N_f \times \text{DM (g DW)} \times \text{N concentration (mg g}^{-1} \text{ DW)}$ (Yang et al., 2022) where:

- DM denotes the dry matter weight of stem or leaf tissue (g DW)
- N concentration indicates the nitrogen content in stem or leaf tissue (mg g⁻¹ DW)

Note

DM represents stem and leaf dry weight at 240 days, and N represents nitrogen content in stems and leaves at 240 days.

Determination of Plant Net Photosynthetic Rate, SLA, and PNUE

Net photosynthetic rate (Pn) was measured monthly starting one month after plant establishment, with four measurement intervals at 40, 80, 120, and 200 days. Measurements were conducted using a Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) under clear weather conditions between 9:00 and 11:00 AM. For each measurement, the third fully expanded healthy mature leaf from the top of the plant was selected, with eight biological replicates per treatment group. The light intensity in the leaf chamber was maintained at 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using the built-in LED light source. Data were recorded once the photosynthetic parameters stabilized.

Specific leaf area (SLA) was determined for 12 mature leaves each from thomson kudzu and *M. micrantha* in the co-cultivation system. Leaf area was measured via the scanning method (ImageJ software), and dry mass was obtained after oven-drying. SLA was calculated as leaf area/leaf dry mass ($\text{m}^2 \text{kg}^{-1}$). Concurrently, leaf nitrogen content (leaf N) was quantified using the Kjeldahl distillation method (Bao, 2000), expressed as g kg^{-1} dry weight (DW). Photosynthetic nitrogen use efficiency (PNUE) was calculated using the formula: $\text{PNUE} = \text{Pn} (\text{leaf N} / \text{SLA}) (\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1})$ as described by (Feng et al., 2009).

Determination of Soil Chemical Properties and Nitrogenase Activity

Rhizosphere soil samples were collected on days 120, 180, and 240 after potting. After removing impurities and plant roots, the soil samples were homogenized and sieved through a 2 mm mesh for preliminary processing. The sieved soil was divided into three portions: One portion was stored fresh at 4°C and used within one week to determine soil microbial biomass nitrogen via the chloroform fumigation method (Bao, 2000). A second portion was air-dried and used to analyze total nitrogen, ammonium nitrogen, and nitrate nitrogen. For total nitrogen determination, soil was passed through a 0.15 mm sieve and analyzed using the Kjeldahl method. For ammonium and nitrate nitrogen, soil was sieved through a 1 mm mesh, extracted with 2 mol L^{-1} KCl solution, and analyzed by the indophenol blue colorimetric method (for $\text{NH}_4^+\text{-N}$) and ultraviolet spectrophotometry (for $\text{NO}_3^-\text{-N}$), respectively (Bao, 2000). The remaining portion was stored at -80°C for subsequent functional gene sequencing.

Nitrogenase activity in soil and root nodules was determined using the acetylene reduction assay (Lyu et al., 2019). Specifically, 1.5 g of nodule or 20 g of fresh soil was weighed and placed in a 250 mL gas-tight vial. After sealing, 10% of the headspace air was evacuated and replaced with acetylene gas. The samples were incubated in the dark at 21°C (for nodules) or 24°C (for soil) for 24 hours. Ethylene production was then measured using a gas chromatograph (Perkin Elmer Clarus 680GC, equipped with an FID detector and a Porapak N column). Nitrogenase activity was expressed as the amount of ethylene produced per unit dry weight of the sample per unit time ($\text{nmol C}_2\text{H}_4 \text{h}^{-1} \text{g}^{-1}$ or $\mu\text{mol C}_2\text{H}_4 \text{h}^{-1} \text{g}^{-1}$).

Alpha Diversity, Community Composition, and Functional Gene Expression of Nitrogen-Fixing Microorganisms

This study employed *nifH* functional gene sequencing to analyze the characteristics of nitrogen-fixing microbial communities. The *nifH* gene (Chien & Zinder, 1996) was selected due to its high conservation (encoding the nitrogenase iron protein) and superior taxonomic resolution compared to the *nodA* gene. DNA extraction and high-throughput sequencing were outsourced to Sangon Biotech Co., Ltd. (Shanghai, China). Bacterial DNA from soil and root nodules was extracted using the OMEGA DNA extraction kit, and its quality was assessed via 0.8% agarose gel electrophoresis and Qubit quantification. PCR amplification was performed using the primers *nifH-F* (5'-AAAGGYGGWATCGGYAARTCCACCAC-3') and *nifH-R* (5'-TTGTTSGCSGCRATCATSGCCATCAT-3') (Chien & Zinder, 1996). Amplified products were

verified by 2% agarose gel electrophoresis, purified, and quantified using a Qubit® 4.0 fluorometer. Paired-end sequencing was conducted on the Illumina MiSeq platform, followed by bioinformatic processing and analysis. Sequence denoising and clustering were performed using the DADA2 method (Callahan et al., 2016) in QIIME2 (v2019.4), which included primer removal, quality filtering, denoising, merging, and chimera removal. Amplicon sequence variant (ASV) length distribution was analyzed to identify aberrant sequences. Taxonomic annotation of ASVs was executed using a pre-trained naïve Bayes classifier and QIIME2's *classify-sklearn* algorithm with the SILVA (bacteria) and UNITE (fungi) reference databases. The Shannon index was applied to assess community richness and diversity, and microbial community structure and composition were visualized.

The quantification of *nifH* gene copy numbers in root nodule and soil samples was conducted by Sangon Biotech Co., Ltd. (Shanghai, China) using fluorescence-based absolute quantitative PCR (qPCR). This technique involved the construction of a standard curve using known copy number standards. Reactions were performed on a thermal cycler (ETC 811, Beijing Easton Biotech Co., Ltd.), with the initial copy numbers of samples calculated based on the Ct values and standard curve. The PCR reaction mixture (10 µL total volume) consisted of: 5 µL 2×SYBR real-time PCR premixture, 0.2 µL of each 10 µM PCR-specific primer (27F and 1492R, Frank et al., 2008), 1 µL sample DNA, 3.6 µL nuclease-free water.

Field Validation

Based on the pot-controlled experiment, a field replacement plot was established in the Bio-garden of South China Normal University (23°8'N, 113°21'E), Tianhe District, Guangzhou City, Guangdong Province. The area experiences a subtropical monsoon climate with an average annual precipitation of 1,922.7 mm, featuring distinct dry (October-March) and wet (April-September) seasons. A monospecific *M. micrantha* community with over two years of invasion history was selected as the invaded plot, covering approximately 100 m² with an irregular, near-rectangular shape. Within the invaded plot, 15 quadrats (2 m × 2 m each, spaced 1 m apart; Fig. S1) were established. Among these, the aboveground parts of *M. micrantha* were completely removed from 10 quadrats, which were then planted with seedlings of thomson kudzu and sweet potato, respectively (Fig. S1). The remaining 5 quadrats were left untreated (Fig. S1). The soil from untreated *M. micrantha*-invaded quadrats was designated as "invaded soil" (control), while the soil from quadrats replanted with thomson kudzu or sweet potato after *M. micrantha* removal was termed "thomson kudzu-replaced soil" or "sweet potato-replaced soil," respectively.

At 3, 6, 9, and 12 months after plot establishment, the relative coverage of plant communities and *M. micrantha* regeneration were surveyed in each quadrat. Plant coverage was quantified using a 10×10 grid method in Photoshop after photographing, with relative coverage calculated as (coverage of a species/total coverage × 100%). Concurrently, soil samples were collected at each time point using an "X"-shaped five-point sampling method (5 replicates per treatment), homogenized, and analyzed for soil nitrogen and microbial biomass nitrogen content following the protocols of the pot experiment.

Data Statistics and Analysis

All statistical analyses were performed using SPSS 22.0 (IBM, New York, USA). One-way analysis of variance (ANOVA) was employed to compare plant biomass, soil nitrogen content, soil microbial biomass nitrogen, soil nitrogenase activity, nitrogen content in various tissues, soil *nifH* gene copy number, and soil microbial Shannon diversity index, followed by Duncan's multiple range test for post hoc comparisons ($P < 0.05$). For PNUE, nodule number, nodule biomass, nodule nitrogenase activity, nitrogen fixation rate, nitrogen fixation amount, nodule *nifH* gene copy number, and nodule microbial Shannon diversity index, differences were assessed using independent samples T-test ($P < 0.05$). To identify the key predictors influencing biomass during the replacement process, Random Forest analysis (Breiman et al., 2002) was conducted using the "randomForest," "A3," and "rfPermute" packages in R software (version 4.2.1). Additionally, linear correlation analysis was performed to evaluate the relationships between total nitrogen, thomson kudzu nodule number, thomson kudzu nodule biomass, soil nitrate nitrogen, soil nitrogenase activity (SNA), soil *nifH* gene copy number, and plant biomass. Data visualization was generated using GraphPad Prism version 10.

Results and Analysis

Plant Growth and Interspecific Competition

The results demonstrated that co-planted treatments significantly affected plant growth after 240 days of cultivation. Compared with *M. micrantha* planted on their own, thomson kudzu and *M. micrantha* planted together significantly reduced the aboveground biomass (Fig. 1A), belowground biomass (Fig. 1B), and total biomass (Fig. 1C) of *M. micrantha*. Notably, when thomson kudzu was grown under competition with *M. micrantha*, its biomass accumulation was significantly enhanced compared to thomson kudzu planted on their own (Fig. 1A, B, C). Specifically, the aboveground biomass increased by 24.9% (Fig. 1A), belowground biomass increased by 44.9% (Fig. 1B), and total biomass increased by 27.5% (Fig. 1C). In contrast, sweet potato showed an opposite trend when grown under competition with *M. micrantha* (Fig. 1A, B, C). The competition balance index revealed that both thomson kudzu and sweet potato exhibited greater competitive ability than *M. micrantha* (Fig. 1D). The competition balance index of thomson kudzu against *M. micrantha* (0.76) was 2.11 times higher than that of sweet potato (0.36), indicating that thomson kudzu possessed stronger competitive advantage over *M. micrantha* compared to sweet potato (Fig. 1D).

Nitrogen Fixation Capacity and Net Photosynthetic Rate

The research results show that the number (Fig. 2A, B) and fresh weight (Fig. 2C) of root nodules of thomson kudzu co-planted with *M. micrantha*, as well as the nitrogenase activity of root nodules, are significantly higher than thomson kudzu planted on their own. In addition, the nitrogenase activity in the rhizosphere soil of thomson kudzu after co-planted with *M. micrantha* is significantly higher than

thomson kudzu planted on their own, and both are significantly higher than that in the rhizosphere soil of *M. micrantha* planted on their own (Fig. 2E), suggesting that the enhancement of soil nitrogenase activity is mainly influenced by thomson kudzu.

The changes in plant tissue nitrogen content were as follows: The nitrogen contents in the roots, stems, and leaves of thomson kudzu co-planted with *M. micrantha* were the highest (Fig. 3A). In the two co-planted systems, the root nitrogen content of *M. micrantha* was lower when co-planted with thomson kudzu, while its stem nitrogen content was lower when grown under competition with sweet potato. There was no significant difference in leaf nitrogen content between the two co-planted patterns (Fig. 3A).

The results based on the ^{15}N natural abundance method (Fig. 3B) revealed the differences in nitrogen fixation efficiency between thomson kudzu and sweet potato. In the thomson kudzu-*M. micrantha* co-planted system, thomson kudzu exhibited significant nitrogen fixation advantages: the nitrogen fixation efficiencies of its leaves and stems reached approximately 128% and 203%, respectively, significantly higher than the 68% nitrogen fixation efficiency of sweet potato stems when sweet potato grown competitively with *M. micrantha* (the nitrogen fixation efficiency of sweet potato leaves was 0) (Fig. 3B). In terms of fixed nitrogen, the daily fixed nitrogen of thomson kudzu leaves and stems reached 49.68 mg and 36.04 mg, respectively, while that of sweet potato stems was only 1.86 mg (no nitrogen fixation in leaves) (Fig. 3C). These data fully demonstrate that under co-planted conditions with *M. micrantha*, thomson kudzu exhibits more efficient nitrogen fixation capacity than sweet potato.

Throughout the entire growth period, the net photosynthetic rate of *M. micrantha* co-planted with either thomson kudzu or sweet potato was lower than that *M. micrantha* planted on their own, with the most significant inhibition observed at 160 days (Fig. 3C). The net photosynthetic rates of both thomson kudzu planted on their own and thomson kudzu co-planted with *M. micrantha* increased continuously over time, and the latter showed a more significant increase. After 80 days, the net photosynthetic rate of thomson kudzu co-planted with *M. micrantha* was higher than that thomson kudzu planted on their own (Fig. 3D), indicating that *M. micrantha* has a promoting effect on the net photosynthetic rate of thomson kudzu. Additionally, the photosynthetic nitrogen use efficiency of thomson kudzu was significantly higher than that of *M. micrantha* (Fig. 3F). In contrast, sweet potato showed the opposite trend, with a decrease in net photosynthetic rate when co-planted with *M. micrantha* (Fig. 3E).

Soil Nitrogen Dynamics

The soil nitrogen dynamics revealed that compared to *M. micrantha* planted on their own, thomson kudzu planted on their own and thomson kudzu and *M. micrantha* planted together exhibited significant nitrogen accumulation effects. Specifically: Total nitrogen content increased by 11.69% and 16.88%, respectively (Fig. 4A); Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) rose by 41.82% and 25.87% (Fig. 4B); Nitrate nitrogen ($\text{NO}_3^-\text{-N}$) increased by 12.86% and 8.98% (Fig. 4C); Microbial biomass nitrogen showed enhancements of 18.53% and 37.63% (Fig. 4D). In contrast, the sweet potato-*M. micrantha* co-planted

system displayed nitrogen depletion characteristics: Total nitrogen content decreased by 3.4% (Fig. 4A); Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) declined by 14.4% (Fig. 4B); Nitrate nitrogen ($\text{NO}_3^-\text{-N}$) was reduced by 12.3% (Fig. 4C); Microbial biomass nitrogen decreased by 15.6% (Fig. 4D).

Alpha Diversity, Community Structure, and Functional Gene Expression of Nitrogen-Fixing Bacteria

Microbial diversity analysis revealed that in rhizobial communities, the Shannon diversity index (Fig. S4) and *nifH* gene abundance (Fig. 5A) of thomson kudzu when grown under competition with *M. micrantha* were significantly higher than those planted on their own. Soil microbial community analysis demonstrated that soil of thomson kudzu and *M. micrantha* planted together exhibited the highest *nifH* gene abundance, with a significant increase compared to treatments planted on their own (Fig. 5B). Analysis of nodule microbial communities showed that *Bradyrhizobium* not only dominated thomson kudzu planted on their own (99.67%) but also increased its relative abundance to 99.86% ($P = 0.034$) under competition with *M. micrantha* (Fig. 5C). Furthermore, the relative abundances of *Geobacter* and *Bradyrhizobium* in thomson kudzu and *M. micrantha* planted together soil significantly increased by 30.27% and 58.21%, respectively, compared to *M. micrantha* planted on their own (Fig. 5D).

Random Forest Analysis

This study employed the Random Forest algorithm to quantitatively identify key determinants of biomass during the replacement of *M. micrantha* by thomson kudzu (Fig. 6). Model analysis revealed that the best predictors of biomass variation were, in descending order of importance: total nitrogen content (11.81%), nodule number of thomson kudzu (11.66%), nodule biomass of thomson kudzu (11.63%), soil nitrate nitrogen ($\text{NO}_3^-\text{-N}$) (11.03%), soil nitrogenase activity (SNA, 10.91%), and soil *nifH* gene copy number (10.12%) (Fig. 6A). Pearson correlation analysis further confirmed that all these parameters exhibited significant positive correlations with total biomass ($P < 0.05$; Fig. 6B).

Field Validation of Replacement Control Efficacy

Field monitoring data revealed significant differences in the suppression efficacy of thomson kudzu and sweet potato against *M. micrantha* (Fig. 7). Results demonstrated that during the 12-month replacement period, thomson kudzu exhibited consistent and stable suppression (Fig. 7A): Its relative coverage remained at a high level of 87.20%-95.39%, while successfully restricting *M. micrantha* to an extremely low 0.10%-0.90% relative coverage range (Fig. 7B). In contrast, the suppression efficacy of sweet potato displayed pronounced time-dependence: During the initial planting phase (≤ 3 months), it maintained 79.10% coverage and suppressed *M. micrantha* to 4.90%. However, over time, its coverage sharply declined to 7.49%, while *M. micrantha* coverage rebounded to 85.79% (Fig. 7B).

The soil nitrogen analysis (Fig. S3) revealed distinct differences in the effects of thomson kudzu and sweet potato replacement plots on soil nitrogen compared to the *M. micrantha* control plots. Thomson kudzu replacement significantly increased soil nitrogen levels, with notable rises in total nitrogen (Fig. S3A), ammonium nitrogen (Fig. S3B), nitrate nitrogen (Fig. S3C), and microbial biomass nitrogen (Fig. S3D), except for nitrate nitrogen (Fig. S3C) in December and microbial biomass nitrogen (Fig. S3D) in March. In contrast, sweet potato replacement exhibited seasonal fluctuations: total nitrogen (Fig. S3A) in March, ammonium nitrogen (Fig. S3B) in September, and microbial biomass nitrogen (Fig. S3D) in June showed significant decreases, while microbial biomass nitrogen (Fig. S3D) in December increased significantly. The remaining indicators did not display significant changes (Fig. S3).

Discussion

Competitive Displacement Advantage of Thomson Kudzu over Sweet Potato Against *M. micrantha*

Morphological traits and biomass are widely regarded as robust indicators of competitive capacity (Jiang et al., 2008). These metrics can be quantified using the Competition Balance Index (CB) to evaluate their inhibitory effects on neighboring plants (Williams & McCarthy, 2001). Previous studies have demonstrated that sweet potato exhibits stronger competitiveness than several highly invasive species, including *Ageratina adenophora*, *Ageratum conyzoides*, *Bidens pilosa*, and *Brassica parviflora* (Shen et al., 2015, 2019a, 2019b). However, in this study, the Competition Index (CB) of thomson kudzu (0.76) was 2.11-fold higher than that of sweet potato (0.36) (Fig. 1D), unequivocally demonstrating thomson kudzu's significantly greater competitive advantage over sweet potato.

The findings of this study demonstrate that thomson kudzu exhibits stronger competitive superiority over sweet potato, significantly suppressing both aboveground and belowground biomass accumulation of *M. micrantha* (Fig. 1A, B) while enhancing its own biomass in co-planted systems (Fig. 1C). This aligns with classical competition theory, where dominant species optimize resource utilization to maintain or increase their biomass in competitive environments (Aerts, 1999). The results further corroborate that plant interspecific competition is jointly regulated by aboveground and belowground interactions (Bartelheimer et al., 2010). This finding parallels studies showing that *Festuca arundinacea* suppresses *Ambrosia trifida* through rapid growth and strong canopy coverage, thereby limiting resource acquisition (Wang et al., 2024). The dual competitive advantage of thomson kudzu likely stems from its climbing growth habit, which creates shading effects, whereas the creeping growth pattern of sweet potato restricts its ability to occupy vertical space effectively (Shen et al., 2016; Shen et al., 2023). Notably, sweet potato only inhibited the aboveground growth of *M. micrantha* (Fig. 1A) and experienced a decline in its own biomass when grown under competition (Fig. 1C), indicating a mutually inhibitory interaction. This resembles findings that *Spartina alterniflora* negatively impacts *Phragmites australis* through belowground interference (Medeiros et al., 2013). Field experiments also revealed that while sweet potato initially suppressed *M. micrantha* (< 3 months), long-term monitoring showed unsustainable control: Sweet potato coverage declined progressively, whereas *M. micrantha* exhibited significant niche rebound (Fig. 7). Previous studies highlight that co-planted leguminous plants with

grasses can sustain long-term control of invasive species like *Solanum rostratum* and promote ecological restoration (Zhang et al., 2019). Our field data further validate the persistent efficacy of thomson kudzu in replacing *M. micrantha*: Thomson kudzu rapidly established monoculture dominance with sustained high relative coverage (> 87%). *M. micrantha* populations were suppressed to extremely low levels (< 1%), with suppression efficacy strengthening over time (Fig. 7). These results indicate that thomson kudzu stably occupies ecological niches and persistently impedes the regeneration and expansion of *M. micrantha* (Yu et al., 2020).

Regulation of Competitive Outcomes by Soil Nitrogen Dynamics

Nitrogen, a critical mineral nutrient for plant growth, participates in tissue construction and physiological metabolism (Leghari et al., 2016). As a leguminous species, thomson kudzu can mitigate nitrogen depletion caused by invasive plants through biological nitrogen fixation (Kebede, 2021). This study revealed that compared to sweet potato, both the *M. micrantha*-thomson kudzu co-planted system and Thomson kudzu planted on their own significantly enhanced total nitrogen, ammonium nitrogen ($\text{NH}_4^{+}\text{-N}$), and nitrate nitrogen ($\text{NO}_3^{-}\text{-N}$) levels. Notably, microbial biomass nitrogen increased by 37.63% and 18.53% compared to the soil of *M. micrantha* planted on their own (Fig. 4D). Elevated soil nitrogen promotes plant growth by enhancing nitrogen allocation to photosynthetic organs, optimizing morphological plasticity and biomass allocation (Kuzyakov & Xu, 2013), thereby driving asymmetric competition between species (Yu et al., 2021). In contrast, the sweet potato-*M. micrantha* co-planted system exhibited a 15.6% reduction in microbial biomass nitrogen relative to *M. micrantha* planted on their own (Fig. 4D). This decline stems from sweet potato (a non-leguminous species) lacking efficient biological nitrogen fixation capacity, failing to replenish soil nitrogen. This aligns with findings that sweet potato and *Phaseolus vulgaris* planted together depletes soil nutrients, inducing nitrogen impoverishment (Shen et al., 2015).

The *Pueraria lobata*-*M. micrantha* co-planted system significantly enhances soil nitrogen availability, primarily due to thomson kudzu's unique symbiotic nitrogen fixation capability. Distinct from nitrogen-fixing plants like alder (*Alnus spp.*), thomson kudzu not only enriches soil nitrogen through fixation but also exhibits superior nitrogen competition capacity. Studies by Crocker & Major in Alaska's Glacier Bay demonstrated that while alder promotes nitrogen accumulation via fixation, its limited nitrogen competitiveness ultimately facilitates the invasion of *Picea sitchensis* (Crocker & Major, 1955; Hodge & Fitter, 2013). In contrast, although the invasive *M. micrantha* efficiently depletes soil nitrogen pools (potentially causing 12%-18% nitrogen loss, Yu et al., 2021), it is outcompeted by thomson kudzu. This is evidenced by thomson kudzu's dominance plant together: Higher net photosynthetic rate (Fig. 3D), Elevated leaf nitrogen content (Fig. 3A), Enhanced photosynthetic nitrogen use efficiency (PNUE) (Fig. 3F). Critically, thomson kudzu leverages enhanced photosynthesis to drive root expansion (Kato-Noguchi, 2023), efficiently converting nitrogen into biomass (Fig. 1–2; Fig. S2-S3). This dual strategy-simultaneous nitrogen fixation and optimized nitrogen utilization-enables thomson kudzu to achieve

both nitrogen enrichment and invasive plant suppression. Furthermore, *M. micrantha*'s rapid growth under high-light conditions is counteracted by its photosynthetic sensitivity to shading stress (Liao et al., 2003). Thomson kudzu creates a shading environment through rapid canopy expansion, effectively suppressing *M. micrantha*'s photosynthetic efficiency (Deng et al., 2004; Kato-Noguchi, 2023). This mechanism starkly contrasts with the *Alnus-Picea* system: while *Alnus* fixes nitrogen but lacks competitive vigor, thomson kudzu synergizes nitrogen fixation with competitive dominance to sustainably inhibit invasive species.

Microbial Community Modulation and Its Impact on Competitive Outcomes

The competitive outcomes of leguminous plants in interspecific interactions are profoundly influenced by microbial community dynamics, as demonstrated in the thomson kudzu and *M. micrantha* co-planted system. Utilizing $\delta^{15}\text{N}$ natural abundance analysis, thomson kudzu exhibited exceptional nitrogen fixation capabilities, with leaf and stem fixation rates reaching 128% and 203%, respectively—significantly surpassing the 68% observed in sweet potato stems (Fig. 3B). This disparity was further quantified by daily nitrogen fixation capacity: Thomson kudzu stems fixed 36.04 mg, a ~19.4-fold advantage over sweet potato stems (1.86 mg; Fig. 3B). While *M. micrantha* enhances nitrogen availability through nitrogen-cycling microbial enrichment (Yu et al., 2021), thomson kudzu establishes a multifaceted nitrogen acquisition system. Molecular analyses revealed upregulated expression of the nitrogenase-coding *nifH* gene in thomson kudzu root nodules when grown under competition (Fig. 5A, C), coinciding with microbial community restructuring: root nodules showed elevated *Bradyrhizobium* abundance (Fig. 5C) and Shannon diversity (Fig. S4), while soils were enriched with nitrogen-fixing genus including *Bradyrhizobium* and *Geobacter* (Fig. 5D). These microbes form a synergistic "nitrogen fixation-mineralization" network, where *Bradyrhizobium* directly supplies nitrogen via symbiotic fixation (Andrews et al., 2011), and *Geobacter* activates soil nitrogen reserves through iron reduction-coupled cycling and organic matter degradation (Ueki & Lovley, 2010). This coordinated system establishes a stable nitrogen supply chain, enabling thomson kudzu to dominate in competitive environments through both biochemical and ecological optimization.

Multidimensional evidence systematically elucidates the intrinsic mechanisms underlying enhanced nitrogen fixation in thomson kudzu when planted together with *M. micrantha*. Random Forest model analysis (Fig. 7A) quantitatively revealed that nitrogen fixation-related metrics (44.32%) and soil nitrogen parameters (22.84%) collectively explained 67.16% of biomass variation. Among these, nodule traits (23.29%), nitrogenase activity (10.91%), and soil *nifH* gene abundance (10.12%) emerged as the most predictive positive correlates, validating a microbe-mediated legume-soil feedback mechanism (Lan et al., 2023). This study observed that planting together with *M. micrantha* significantly enhanced thomson kudzu nodulation (Fig. 2A-C) and increased nitrogenase activity by ~253% (Fig. 2D). This phenomenon likely arises from a dual induction mechanism: *M. micrantha* competition induces soil nitrogen depletion. Thomson kudzu secretes flavonoid compounds (e.g., daidzein and genistein, upregulated under nitrogen deficiency) to activate rhizobial *Nod* gene expression (Issifu et al., 2024), thereby promoting nodule

formation (Han et al., 2024). These findings not only confirm the intensification of legume-rhizobia symbiosis under competitive pressure (Andrews et al., 2011) but also unveil a positive feedback loop driven by rhizobia-rhizosphere microbiome synergy (Iqbal et al., 2022): Nodule development enhances plant growth. Increased biomass improves rhizosphere conditions, optimizing microbial community structure. Refined microbiota further strengthens nitrogen fixation efficiency. This self-reinforcing system establishes a high-efficiency nitrogen fixation-growth interaction network, enabling thomson kudzu to dominate in competitive environments.

In summary, this study demonstrates that thomson kudzu's replacement strategy not only enhances its competitiveness through efficient nitrogen fixation but also effectively suppresses *M. micrantha* regeneration by improving soil nitrogen dynamics and microbial community structure. These findings provide critical insights into invasion management strategies centered on microbial regulation. Unlike traditional replacement control approaches that predominantly focus on aboveground competition (e.g., light interception), our work highlights the equally critical role of soil microbiome-mediated nitrogen fixation processes. This advances novel frameworks for developing nitrogen-fixing plant-based invasion control strategies, particularly in nitrogen-limited ecosystems such as red soil regions, where leguminous plants with efficient nitrogen-fixing microbial interaction networks exhibit unique advantages.

Declarations

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

WL designed the study. XY, QX and WZ conducted the experiments. YM analyzed the data. YM and WL wrote the first draft of the manuscript.

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Figures

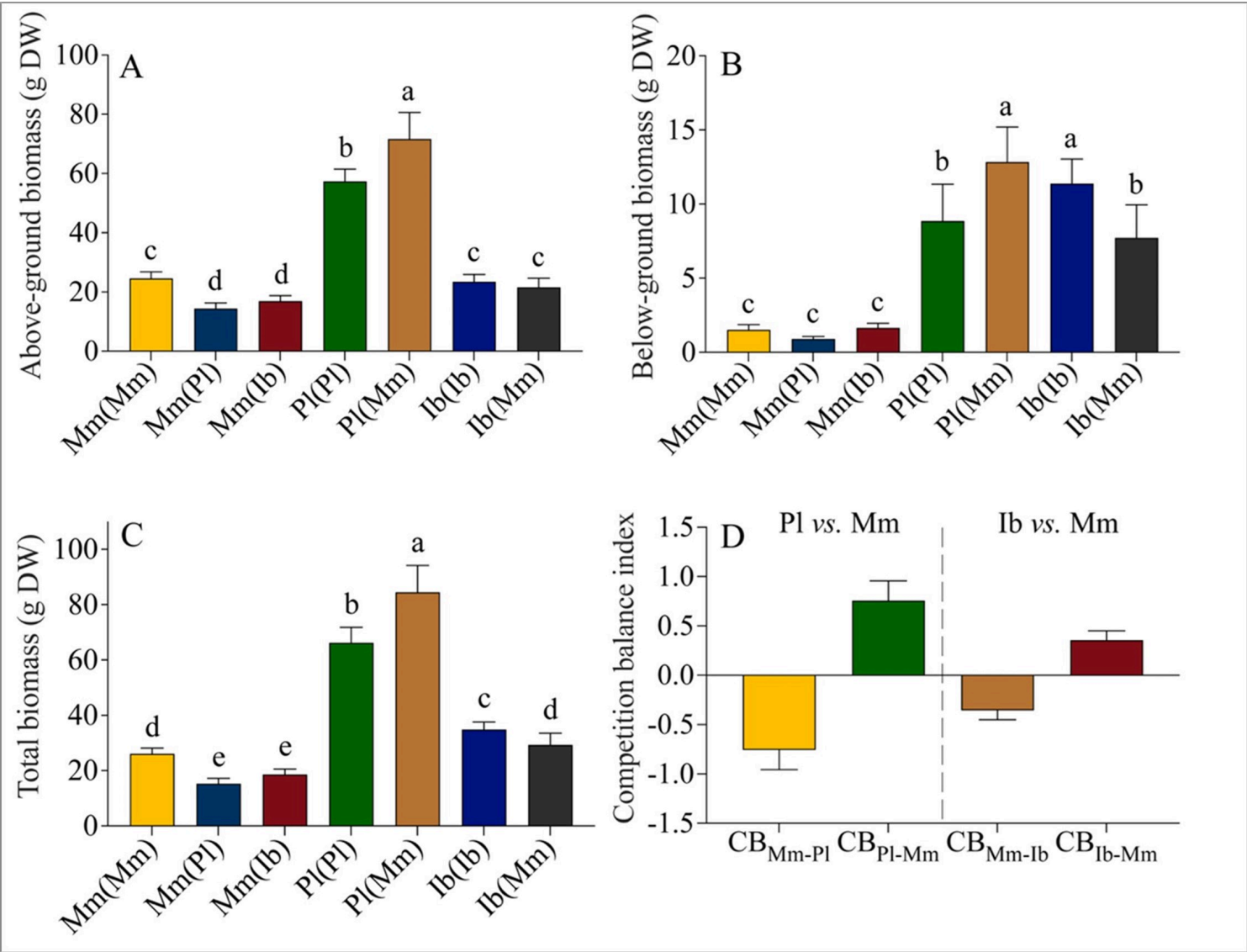


Figure 1

Changes in biomass of Pl, Ib and Mm under intra-/inter-specific competition and competition balance index between Pl or Ib and Mm grown for 240 days (AV±SD, n=5). Note: Mm(Mm) means *M. micrantha*

under intraspecific competition; Mm(PI) means *M. micrantha* growing with thomson kudzu under interspecific competition; Mm(lb) means *M. micrantha* growing with sweet potato under interspecific competition; PI(PI) means *Thomson kudzu* under intraspecific competition; PI(Mm) means thomson kudzu growing with *M. micrantha* under interspecific competition; lb(lb) means sweet potato under intraspecific competition; lb(Mm) means *Sweet potato* growing with *M. micrantha* under interspecific competition; CB_{Mm-PI} means the competitive ability of *M. micrantha* against *thomson kudzu*; CB_{PI-Mm} means the competitive ability of thomson kudzu against *M. micrantha*; CB_{Mm-lb} means the competitive ability of *M. micrantha* against sweet potato; CB_{lb-Mm} means the competitive ability of sweet potato against *M. micrantha*; Different letters above columns indicate significant differences between competition treatments according to Duncan's test ($P < 0.05$).

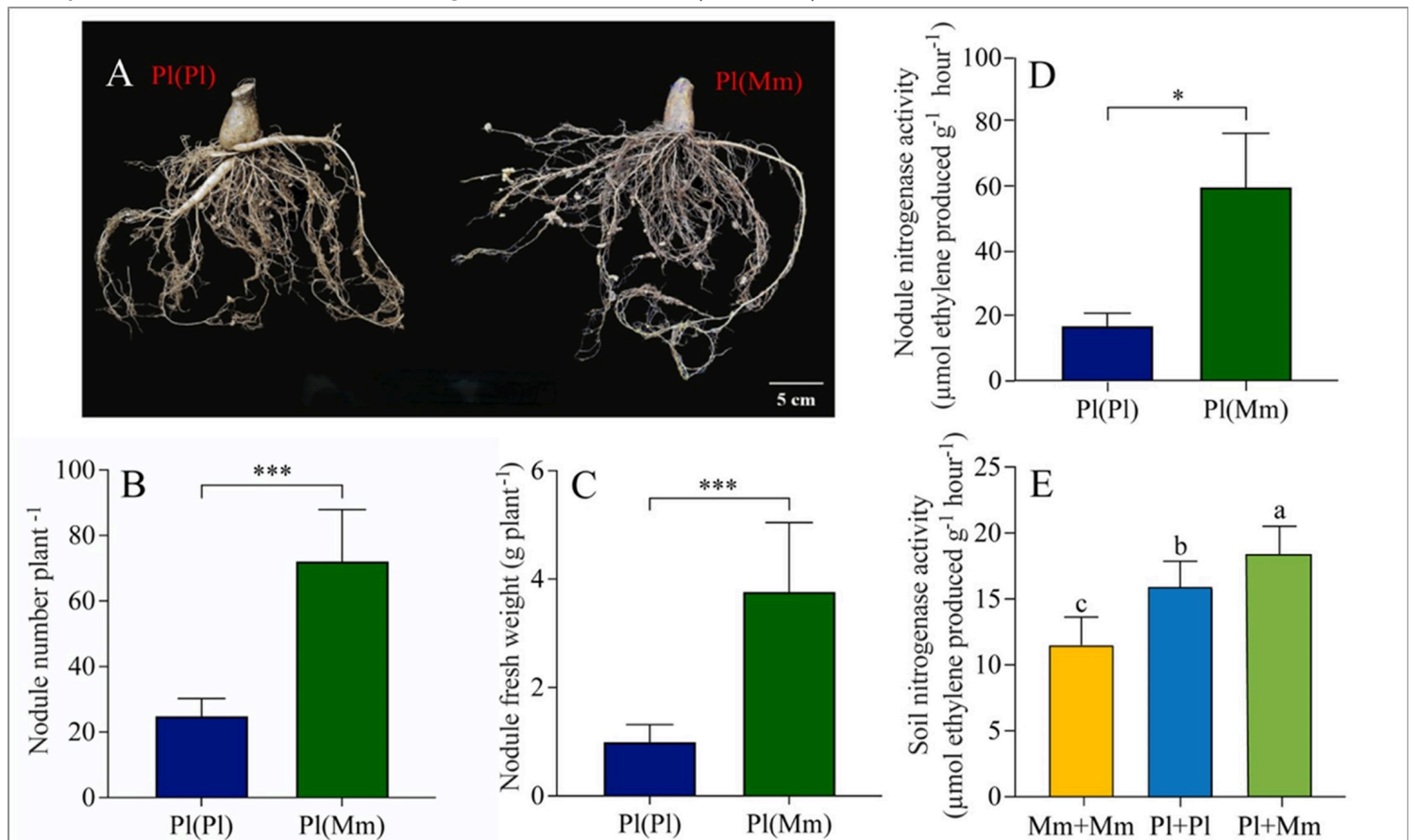


Figure 2

Competition-regulated nodule traits in PI and soil nitrogenase under intra-/inter-specific competition with Mm (AV±SD, n=5). Note: PI(PI) means thomson kudzu under intraspecific competition; PI(Mm) means thomson kudzu growing with *M. micrantha* under interspecific competition; Mm+Mm and PI+PI means the soil of *M. micrantha* and thomson kudzu under intraspecific competition, respectively. PI+Mm means the soil of thomson kudzu growing with *M. micrantha* under interspecific competition. Different letters above columns in the figure 4E indicate significant differences between competition treatments according to Duncan's test ($P < 0.05$). * $P < 0.05$, *** $P < 0.001$ (Independent Sample T-test).

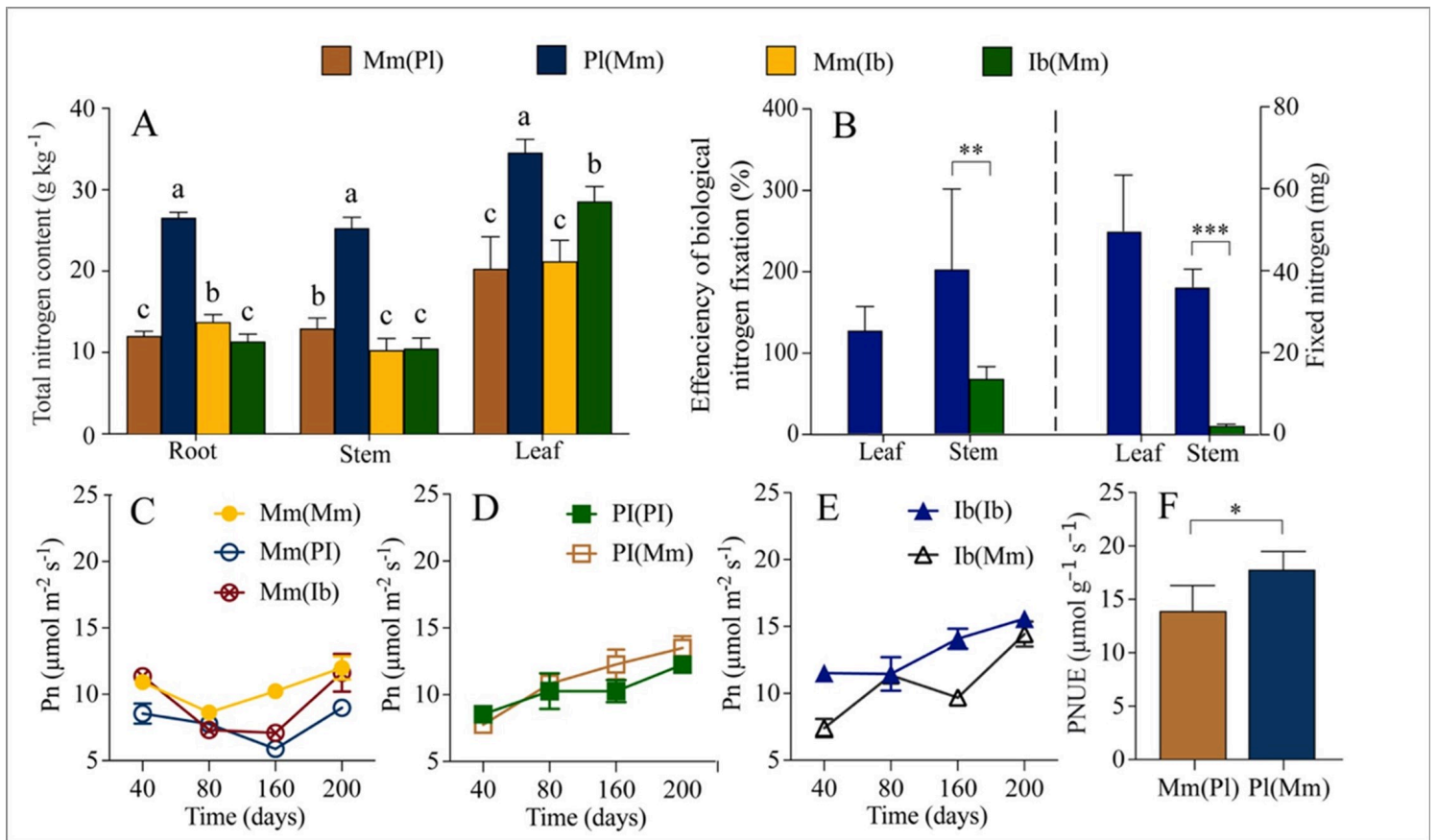


Figure 3

Competition-mediated nitrogen allocation dynamics and N-fixation divergence between thomson kudzu and sweet potato, as well as the changes in the net photosynthetic rate (Pn) and PNUE of *M. micrantha*, thomson kudzu and sweet potato under intraspecific and interspecific competition with the planting time (AV±SD, n = 5). Note: Mm(Mm) means *M. micrantha* under intraspecific competition; Mm(PI) means *M. micrantha* growing with thomson kudzu under interspecific competition; Mm(Ib) means *M. micrantha* growing with sweet potato under interspecific competition; PI(PI) means thomson kudzu under intraspecific competition; PI(Mm) means thomson kudzu growing with *M. micrantha* under interspecific competition; Ib(Ib) means sweet potato under intraspecific competition; Ib(Mm) means sweet potato growing with *M. micrantha* under interspecific competition. Different letters above columns in the figure 5A indicate significant differences between interspecific competition treatments according to Duncan's test ($P < 0.05$). * $P < 0.05$, *** $P < 0.001$ (Independent Sample T-test).

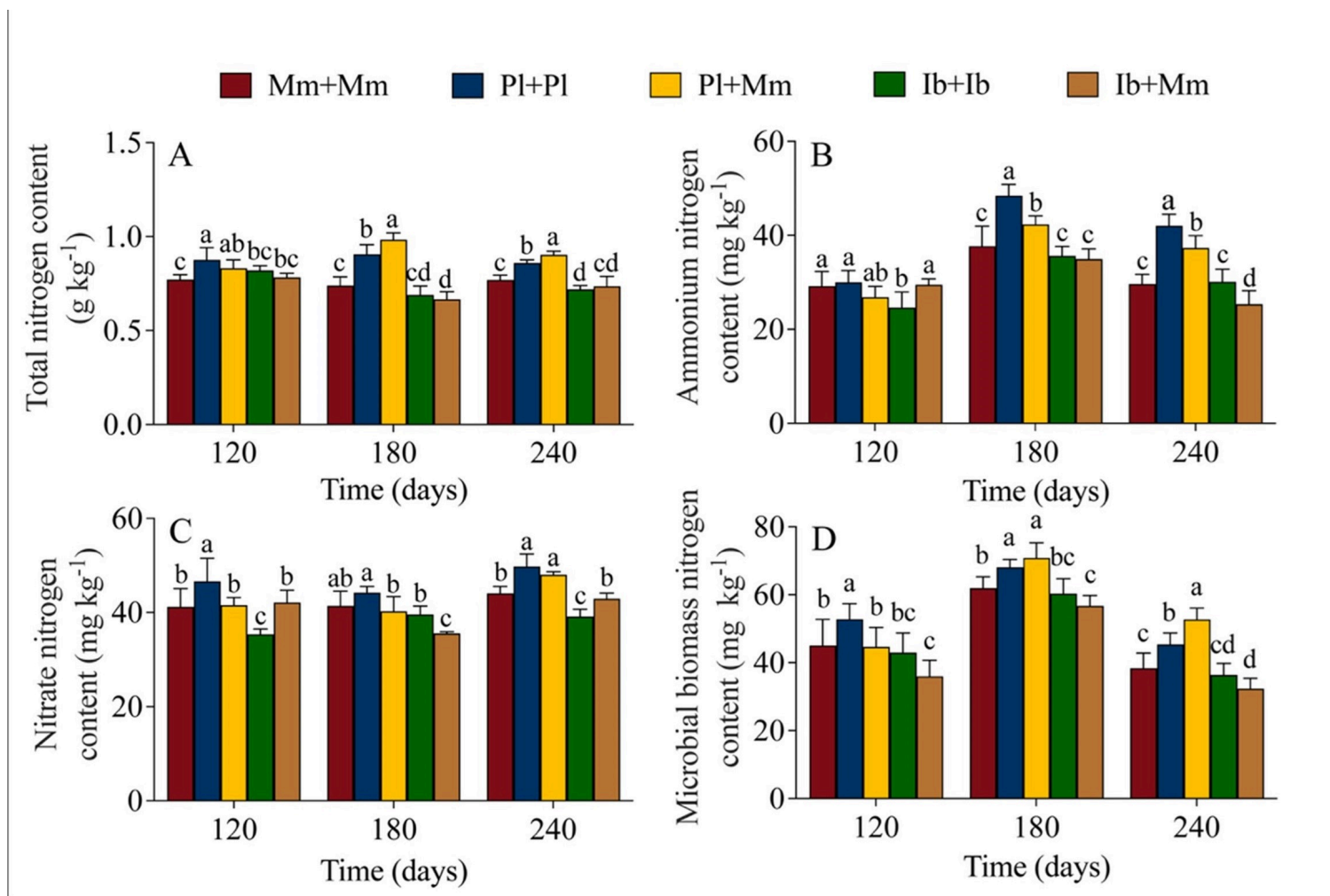


Figure 4

Changes in soil nitrogen and microbial biomass nitrogen of Pl, Ib and Mm under intra-/inter-specific competition with planting duration (AV±SD, n=5). Note: Mm+Mm, Pl+Pl, Ib+Ib means the soil of *M. micrantha*, thomson kudzu and sweet potato under intraspecific competition, respectively. Pl+Mm and Ib+Mm means the soil of thomson kudzu and sweet potato growing with *M. micrantha* under interspecific competition, respectively. Different letters above columns in the same time indicate significant differences between competition treatments according to Duncan's test ($P < 0.05$).

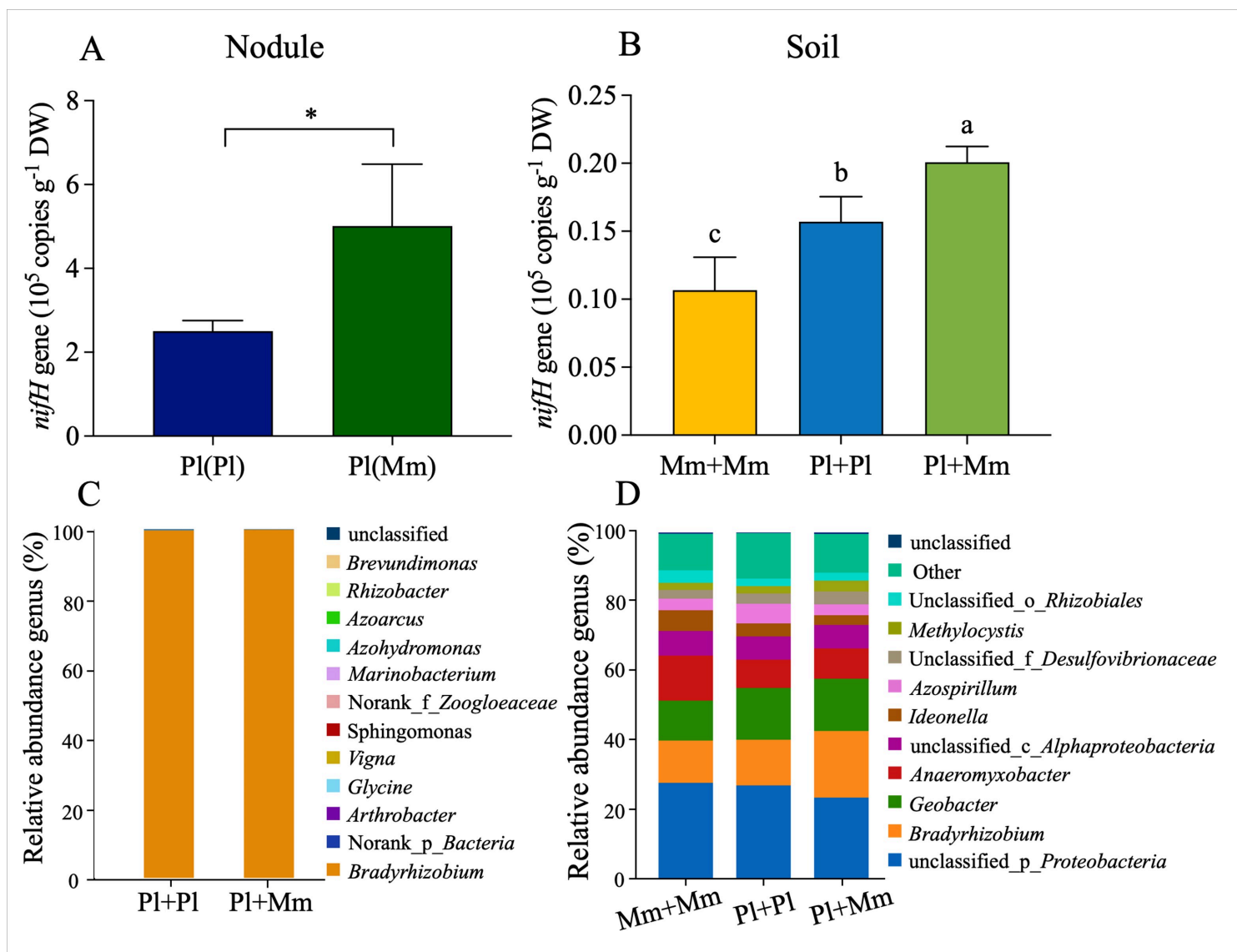


Figure 5

Variation of *nifH* gene copy numbers and relative microbial abundance within thomson kudzu root nodules and rhizosphere soil under intra-/inter-specific competition with *M. micrantha* ($AV \pm SD$, $n=3$). Note: PI(PI) means thomson kudzu under intraspecific competition; PI(Mm) means thomson kudzu growing with *M. micrantha* under interspecific competition; Mm+Mm and PI+PI means the soil of *M. micrantha* and thomson kudzu under intraspecific competition, respectively. PI+Mm means the soil of thomson kudzu growing with *M. micrantha* under interspecific competition. Different letters above columns in the figure 6B indicate significant differences between competition treatments according to Duncan's test ($P < 0.05$). * $P < 0.05$ (Independent Sample T-test).

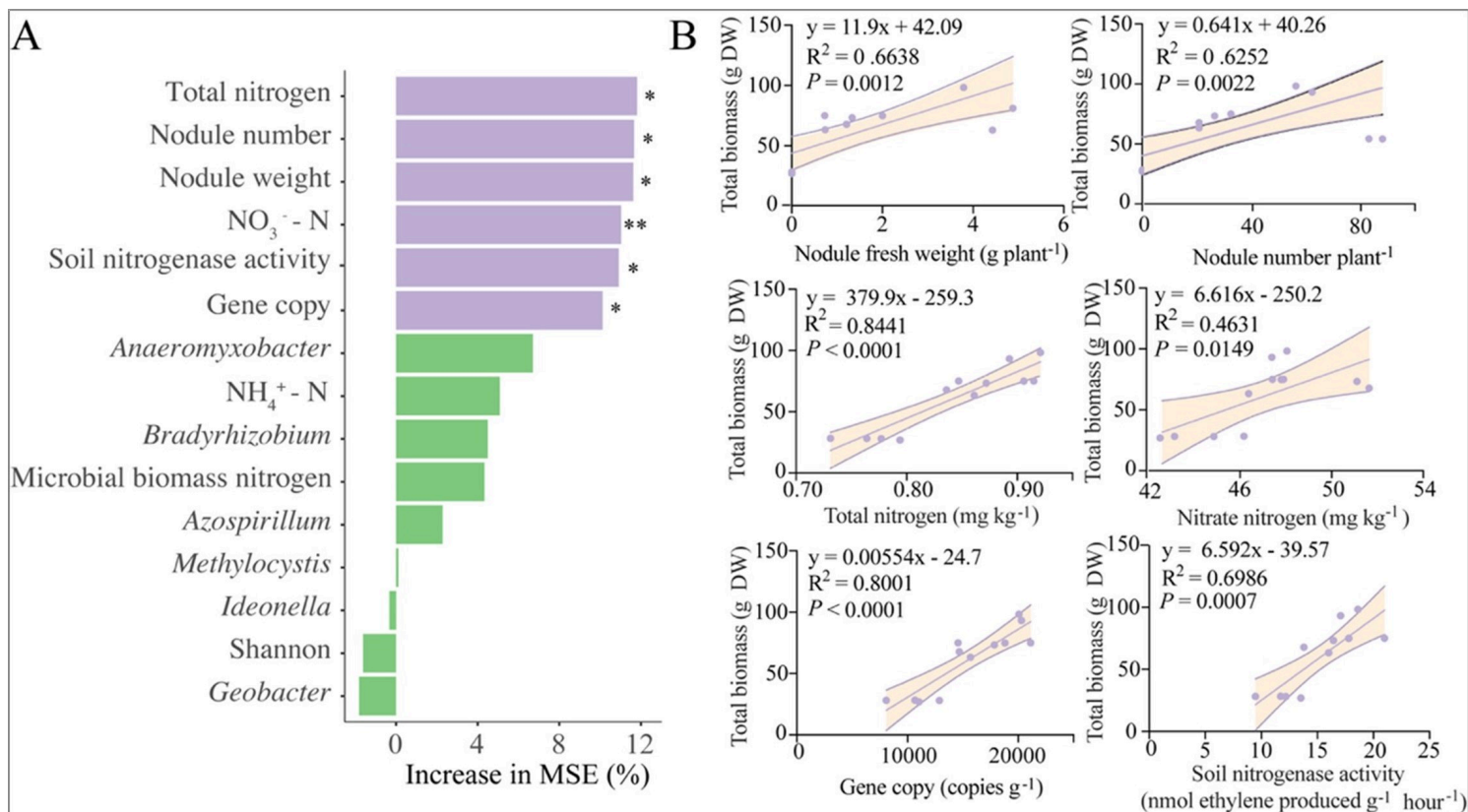


Figure 6

Screening of major variables for biomass and correlation analysis under intra-/inter-specific competition with *M. micrantha* (Mean $\pm$ SD, n = 3). Predictors include: Total nitrogen, nodule number, nodule weight, nitrate nitrogen (NO₃⁻-N), soil nitrogenase activity, Gene_copy (soil *nifH* gene copy number), *Anaeromyxobacter*, ammonium nitrogen (NH₄⁺-N), *Bradyrhizobium*, microbial biomass nitrogen, *Azospirillum*, *Methylocystis*, *Ideonella*, Shannon index (soil *nifH* gene sequencing diversity index), and *Geobacter*. * indicates $P < 0.05$, ** indicates $P < 0.01$ Permutation Test.

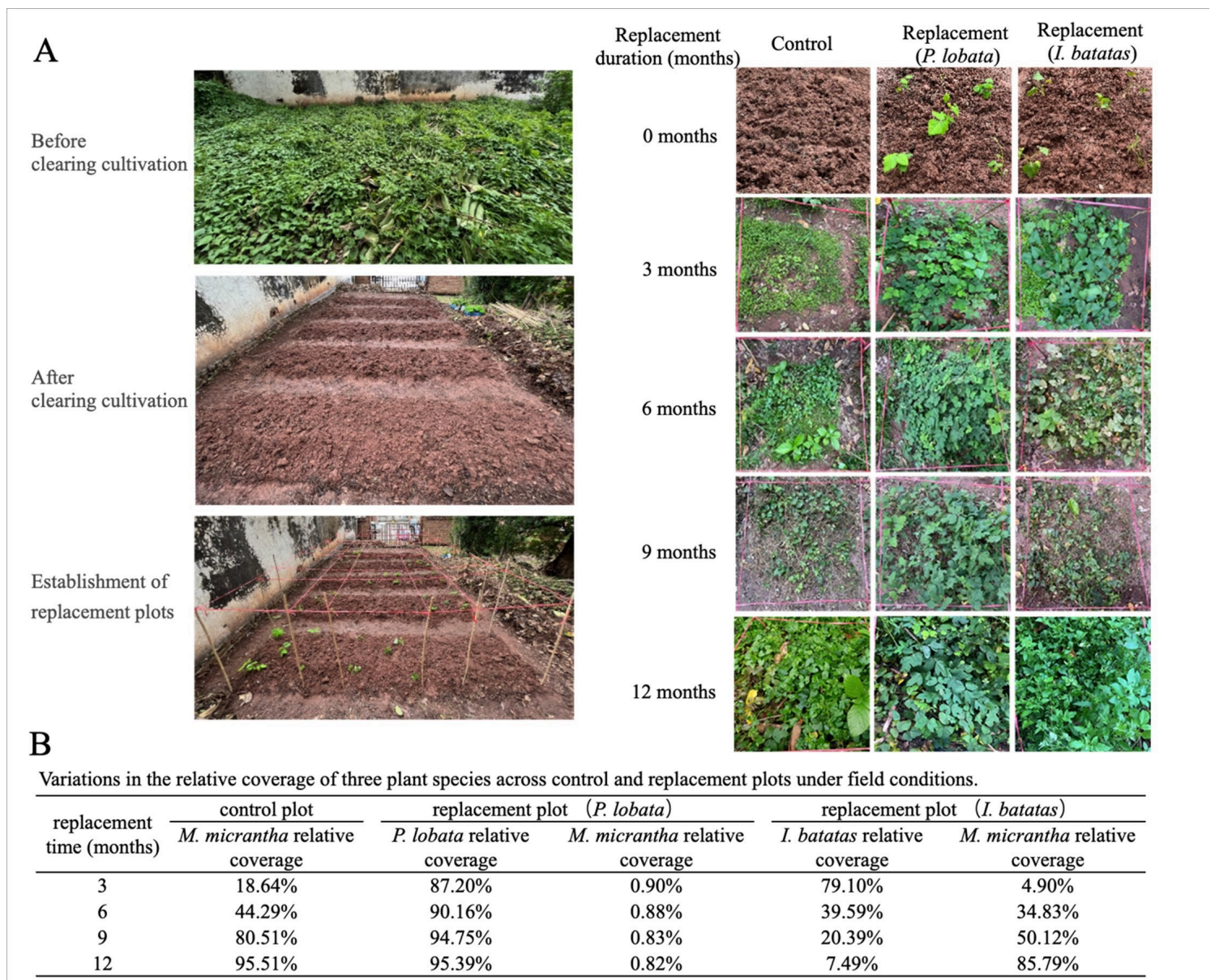


Figure 7

Field-observed coverage dynamics of three plant species in control vs. replacement plots (AV±SD, n= 5).

Supplementary Files

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- [SupplementalFigures.docx](#)