Soil fungi of three native tree species inhibit biomass production and shift biomass allocation of invasive *Mikania micrantha* Kunth

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Received May 7, 2012; accepted July 9, 2012; published online October 29, 2012

Soil microbes contribute to native plant species successful resistance against invasive plant. Three native tree species, *Heteropanax fragrans* (HF), *Cinnamomum burmanii* (CB), and *Macaranga tanarius* (MT) were effective in controlling the notorious invasive vine *Mikania micrantha* (MM). Biomass production and allocation patterns (shoot/root biomass ratio (shoot/root)) are important indicators of MM climbing coverage and competitive light-capturing capacity. An investigation was conducted to test the role of soil microbes associated with the three native tree species to inhibit MM biomass production and shift MM shoot/root. Rhizosphere soils originating from preculture HF, CB, MT, and MM plots were collected separately for use as inocula. The inocula were mixed with sterilized river sand at a 1:9 (w/w) ratio to grow MM. The fungicide carbendazim (methyl benzimidazol-2-ylcarbamate) was applied to half the treatments to kill pathogenic soil fungi. Two nutrient levels were established based on the natural soil nutrient concentration from a field stand invaded by MM. MM were grown from seeds in a glasshouse, harvested 15 weeks after sowing, and separated into shoot and root portions. Results showed that under interaction of soil origin and nutrient levels, MM biomass production was unchanged, but biomass allocation patterns were significantly different. MM biomass production grown in the three native tree soils under two nutrient levels was similar or higher than MM biomass production in MM conspecific soil, indicating the absence of species-specific pathogens that inhibited MM biomass production in native tree soils. However, in both conspecific and tree soils, MM biomass production was significantly reduced in the presence of pathogenic soil fungi, i.e. MM experienced significant fungal inhibition, demonstrating the pathogenic soil fungi promoted native tree resistance to MM. MM exhibited decreased shoot biomass allocation when cultivated in native tree soil relative to MM conspecific soil under field stand nutrient level conditions. Reduced resource allocation to shoot biomass could result in diminished capacity to climb, cover, and subsequent smother to native trees, and reduced surface area exposed to available light. Following fungicide application, significant biomass allocation differences disappeared, suggesting the native tree soil fungi were responsible for decreasing MM shoot biomass. The overall results indicated tree soil fungi serve an integral role in controlling invasive MM through fungal inhibition on MM biomass production, and shifts in MM biomass allocation patterns.

biological control, biotic resistance, *Cinnamomum burmanii*, *Heteropanax fragrans*, invasion ecology, *Macaranga tanarius*, *Mikania micrantha*, nutrient levels, pathogenic fungi

Citation: Gao L, Zan Q J, Li M G, et al. Soil fungi of three native tree species inhibit biomass production and shift biomass allocation of invasive *Mikania micrantha* Kunth. Chin Sci Bull, 2013, 58: 758–765, doi: 10.1007/s11434-012-5394-6

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Mikania micrantha Kunth (Asteraceae) (hereafter MM), a herbaceous to semi-woody vine, is among the 100 most notorious invasive species in the world [1,2]. Native to the tropical Americas [1], the species range is extensive, and includes China [3]. Since its initial 1884 cultivation in Hong Kong [4], MM has significantly expanded its range in southern China [5]. MM grows a dense canopy over other plants, and eventually smothers all plants underneath [6], resulting in tremendous economic and environmental losses [7]. In the Neilingding Island National Nature Reserve of Shenzhen, China (22°23'49"–22°25'35"N, 113°46'18"–113°49'49"E), MM covers approximately 60% of a 468-ha forest, which is degenerating into shrubland and near pure MM stands [8].

Reports indicate three tree species native to China are effective in controlling MM [9]. Among 18 regional native tree species planted in Neilingding Island to test MM suppression [10], we found no MM-induced damage to *Heteropanax fragrans* (Roxb.) Seem. (Araliaceae), *Cinnamomum burmanii* (C. G. et Th. Nees) Bl. (Lauraceae), and *Macaranga tanarius* (L.) Muell. Arg. (Euphorbiaceae) (hereafter HF, CB, and MT, respectively).

Soil microbes have frequently been identified as integral in native species successful resistance against invasion; native species *Erigeron acer* and *Geum urbanum* reduced biomass production in the invasive *Stenactis annua* via the interaction with soil microbial flora [11]. Among soil microbial organisms, fungi have been shown to play a key role in resisting invasive plants. For example, due to soil fungal effects the native species *Festuca idahoensis* and *Koeleria cristata* reduced biomass production of the highly invasive introduced Eurasian alien *Centaurea maculosa* [12].

The majority of biotic resistance studies have focused on plant size and fecundity as a proxy for native species capacity to reduce alien species fitness and competitiveness [13,14], however, shifts in biomass allocation patterns may also reduce the competitive advantage of alien species. Shifts in biomass allocation patterns may result in restricted shade tolerance by reducing specific leaf area [15], decreased allocation to structures reducing plant height [16,17], and/or inhibited photosynthetic capacity by reducing leaf mass per unit area [18]. Former studies demonstrated that soil microbial community elicited shifts in biomass allocation patterns [19,20].

Biomass production and biomass allocation patterns, expressed as the ratio of shoot and root biomass (hereafter shoot/root), are important indicators of climbing coverage ability, and the competitive light-capturing capacity of MM. Previous studies demonstrated increased biomass and shoot proportion stimulated greater MM coverage over other plants, increased exposed MM surface area, and elevated primary production, which enhanced MM growth [21] and heightened its invasive potential [6]. Substantive evidence from field investigations indicated that increased biomass

production and shoot/root ratio are primary traits contributing to the competitive capacity of MM to capture available light and cover other plant species [22].

Therefore, we investigated the role of soil microbes associated with the three native tree species (HF, CB, and MT) in China: (i) do the soil microbes contribute to MM biomass production inhibition, and shift MM biomass allocation patterns (shoot/root) relative to MM soil microbes in two soil nutrient concentrations based on soil nutrient concentrations from a MM invaded field stand [23]; and (ii) the roles of the three native tree soil fungi that contribute to MM biomass production, and biomass allocation patterns in two soil nutrient concentrations.

1 Methods

1.1 Tree species

Three evergreen native tree species (HF, CB, and MT), naturally distributed in open or dense secondary forests in south China [24] were used in this study. All three species have demonstrated a significant capacity to control MM [9].

1.2 Seed collection

MM seeds were collected for growth experiments (described in section 1.7). The seeds were obtained in winter 2009 when MM seeds were fully mature from the Longyandong site (23°13'85.1"N, 113°21'90.6"E), in a suburb of Guangzhou, China, located in a typical southern subtropical monsoon climate zone. Seeds were subsequently stored under dry conditions at room temperature until use.

1.3 Soil origin and preculture

Soil originating exclusively from precultured HF, CB, and MT plots was obtained by establishing three monoculture plots, each containing one of the three native species, in 2005 at Longyandong. Each plot was approximately 30 m². Twenty HF (height=2 m) were planted 1.5–2 m apart; 25 CB (height=2.5 m) were planted 1.5–2 m apart; 36 MT (height=1.5 m) were planted 1.5–2 m apart.

Adjacent to the three native tree plots, a 4th plot with an approximately 16 m² area (4 m×4 m) was cleared of all plants. MM was planted in this plot exclusively to obtain preculture MM soil. Individual MM cuttings were planted 5–10 cm apart in June 2009, and grown from June 2009 to April 2010. Manual weeding was performed every month in order to maintain the monoculture.

In May 2010, rhizosphere soils were obtained from each of the monoculture plots (three native plants and MM). The soils were collected using a 5 cm×10 cm (diameter×length) soil probe. Between each plot the soil probe was flame sterilized to avoid cross contamination. In the MM monoculture

plot, 16 points were randomly selected, and 10 soil cores were randomly collected within 0.5 m of each point after MM aboveground portions had been cleared. In each of the three native tree monoculture plots, 16 trees were randomly selected, and 10 soil cores were randomly collected within 0.5 m of each chosen tree. Rhizosphere soils from each plot were pooled, and homogenized into a separate single bulk soil of ~40 L in volume per plot. The rhizosphere soil microbial profile was essentially preserved by slow air-drying the soils to mimic the drying process during a natural drought, as recommended by Callaway et al. [25]. The soil was sieved through a 2 mm mesh to remove roots and coarse fragments. From each bulk soil, five samples were taken and set aside for further nutrient analyses (Table S1).

Rhizosphere soil collected from each species plot (MM, HF, CB, and MT) served as inocula. The rhizosphere soil was mixed with sterilized river sand (autoclaved for 3 h at 121.5°C) at a 1:9 (120 g: 1080 g, w/w) ratio. Pots used for growth experiments were each filled with 1200 g of the resulting soil mixture. Each pot was 14 cm in diameter and 13 cm in height, and included holes at the bottom for drainage.

1.4 Fungicide treatments

The fungicide, carbendazim (methyl benzimidazol-2-ylcarbamate, Guoguang Products, Sichuan, China) was applied to kill pathogenic fungi in rhizosphere soil. This is a recommended experimental method for manipulating soil pathogenic fungi [26]. Carbendazim is a systemic broad-spectrum fungicide, which kills a wide range of pathogenic fungi, but causes only minor damage to arbuscular mycorrhizal (AMF), and other beneficial fungi such as *Trichoderma* sp. [27,28]. Reports indicate plants readily take up carbendazim, and its use at the recommended application rate does not affect plant growth, nor does subsequent uptake by plants reduce the toxic effects of the fungicide on any soil fungal organisms tested in this study [27]. Carbendazim was applied in a water suspension of 50 mg carbendazim/kg soil (manufacturer's recommended application strength).

Fungicide was applied once every two weeks, beginning one week before sowing until harvest. Control pots not receiving the fungicide were provided an equivalent volume of additional water concurrent with fungicide application. Pots were watered every other day by an automatic sprinkler system to maintain soil surface moisture.

1.5 Nutrient levels

Two nutrient levels were established based on natural soil nutrient concentrations from a field stand invaded by MM in the Neilingding Island National Nature Reserve of Shenzhen. The nitrogen concentration in both nitrate-N (NO_3^- -N) and ammonium-N (NH_4^+ -N) in the field stand soil was 26.59 mg/kg

soil [23]. Field stand soil nutrient concentrations were simulated by establishing nitrogen concentration in both NO_3^- -N and NH_4^+ -N at 24.28 mg/kg soil applied in 130 mL of 1.0 strength Hoagland nutrient solution per pot, hereafter referred to as high nutrient level. In addition, we established a quarter of the soil nutrient concentration of the field stand; the nitrogen concentration in both NO_3^- -N and NH_4^+ -N was 6.08 mg/kg soil applied in 130 mL of 0.25 strength Hoagland nutrient solution per pot, hereafter referred to as low nutrient level. River sand is highly porous, consequently nutrient loss can be rapid. Therefore, the nutrient solution was applied once a week to maintain the nutrient concentration. Nutrient solution was added prior to seed sowing, and maintained until harvest.

1.6 Experimental site and condition

Experiments were conducted in a glasshouse with 70% relative humidity and a 16 h/8 h light/dark cycle at 30/25°C, respectively, located at the Sun Yat-Sen University campus in Guangzhou, China (23°5'44.3"N, 113°17'53.3"E). Fluorescent lamps were used for additional lighting, providing $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ when sunlight was insufficient.

1.7 Growth experiment

Thirty MM seeds were placed in each pot filled with sterile river sand inoculated with soil precultured by MM, or by each native tree species (HF, CB, or MT) (as described in section 1.3). Two nested treatments were established as follows: with or without fungicide (as described in section 1.4), and high or low nitrogen nutrient levels (as described in section 1.5). MM was grown with the following experimental variables: four soil (inocula) origins (MM, HF, CB, or MT) × two fungicide treatments (with or without) × two nutrient levels (high or low) × six replicates, resulting in a total of 96 pots. Pots were placed on benches randomly arranged in the glasshouse. The effects of soil origin were evaluated using MM preculture soil as a control, which was compared to native tree preculture soils, similar to the experimental design of Klironomos [29]. We similarly followed Maron et al. where inocula with fungicide served as the control for inocula without fungicide [30].

Three weeks following seed sowing, seedlings in each pot were thinned to two seedlings of uniform size across all pots. Shoot and root portions were harvested 15 weeks after sowing, dried at 70°C for 72 h, and weighed to the nearest 0.001 g. The root (below ground portions) was separated from the shoot (above ground portions) by cutting the plant at the soil surface.

1.8 Data analysis

MM biomass allocation patterns were calculated as the shoot to root biomass ratio, as shown in the following equation:

$$\text{Shoot/root} = \frac{\text{Shoot biomass of MM}}{\text{Root biomass of MM}} \times 100\%.$$

The impacts of soil fungi (fungal effects) were measured as a proportional reduction in MM biomass when soil fungi were present (no fungicide in inocula), as shown in the following equation:

Fungal effects =

$$\frac{\text{Biomass in inocula} - \text{Biomass in inocula with fungicide}}{\text{Biomass in inocula with fungicide}} \times 100\%.$$

A three-way analysis of variance (ANOVA) was used to assess significant differences in MM biomass production and shoot/root ($\alpha=0.05$). Soil origin, fungicide treatment, and nutrient level were independent factors while biomass production and shoot/root were dependent variables. A *post hoc* Least-Significant Difference (LSD) test was used to identify significant differences among specific treatments. Data analyses were performed with SPSS for Windows, release 15.0 (SPSS Inc., 2006).

2 Results

2.1 Biomass production

Three-way ANOVA detected significant main effects on MM biomass production for soil origin ($F_{3,80}=9.393$, $P<0.01$), fungicide treatment ($F_{1,80}=150.096$, $P<0.01$), and nutrient level ($F_{1,80}=53.785$, $P<0.01$) (Table 1). However, significant interactions were not indicated between soil origin and nutrient level ($F_{3,80}=0.871$, $P=0.46$), nutrient level and fungicide treatment ($F_{1,80}=0.005$, $P=0.941$), or soil origin, fungicide treatment, and nutrient level ($F_{3,80}=0.637$, $P=0.593$). Significant interactions were only found between soil origin and fungicide treatment ($F_{3,80}=3.212$, $P<0.05$).

Under low nutrient level conditions, biomass production

Table 1 Three-way ANOVA results for *Mikania micrantha* (MM) biomass and shoot/root biomass ratio (shoot/root)^{a)}

	MM biomass			MM shoot/root		
	df	F	P	df	F	P
Soil origin	3	9.393	<0.01*	3	2.765	0.047*
Fungicide treatment	1	150.096	<0.01*	1	43.859	<0.01*
Nutrient level	1	53.785	<0.01*	1	159.551	<0.01*
Soil × fungicide	3	3.212	0.027*	3	3.411	0.021*
Soil × nutrient	3	0.871	0.460	3	2.807	0.045*
Fungicide × nutrient	1	0.005	0.941	1	0.303	0.583
Soil × fungicide × nutrient	3	0.637	0.593	3	3.460	0.020*
Error	80			80		

a) Soil origin, fungicide treatment and nutrient level are considered fixed, independent factors. Asterisk (*) indicates significant effect ($P<0.05$).

of MM grown in MM (conspecific) soil did not significantly differ from MM biomass grown in HF and MT soils; however, biomass production of MM grown in MM soil was significantly ($P<0.05$) reduced compared to MM biomass grown in CB soil (Figure 1(a)). Under the high nutrient level, biomass production of MM grown in MM soil was not significantly different from biomass in CB and MT soils, but was significantly ($P<0.05$) reduced compared to MM biomass production in HF soil (Figure 1(b)). Under both low and high nutrient levels, fungicide application resulted in a significant ($P<0.05$) increase in MM biomass compared to non-fungicide treatments in all soils (i.e., MM, HF, CB, and MT) (Figure 1(a) and (b)).

Figure 1 *Mikania micrantha* (MM) biomass in sterile sand with soil inocula from MM, and each of the three native tree species (HF, CB, and MT) with or without the presence of fungicide (carbendazim), and low or high nutrient levels. Data are means $\pm$ SE, $n=6$ per treatment. Values with the same letter indicate no significant difference; values with a different letter indicate significant differences ($\alpha=0.05$, LSD). An asterisk (*) indicates a significant ($\alpha=0.05$) difference in MM biomass between soils without and with fungicide (*t*-test).

2.2 Shoot/root

Significant main effects for MM shoot/root was associated with soil origin ($F_{3,80}=2.765$, $P<0.05$), fungicide treatment ($F_{1,80}=43.859$, $P<0.01$), and nutrient level ($F_{1,80}=159.551$, $P<0.01$) (Table 1). Significant interactions were also detected between soil origin and fungicide treatment ($F_{3,80}=3.411$, $P<0.05$), soil origin and nutrient level ($F_{3,80}=2.807$, $P<0.05$), and soil origin, fungicide treatment, and nutrient level ($F_{3,80}=3.46$, $P<0.05$). The interaction of fungicide treatment and nutrient level was not significant ($F_{1,80}=0.303$, $P=0.583$).

Under low nutrient level, MM shoot/root in MM soil was not significantly different from MM shoot/root in HF, CB, or MT soils. When fungicide was applied, MM shoot/root increased in all soils compared to the non-fungicide treatment (Figure 2(a)). Under high nutrient level, there was a significant MM shoot/root increase in MM soil compared to shoot/root in HF, CB, and MT soils. However, in the presence of fungicide shoot/root significant differences were not detected between MM and native tree soils (Figure 2(b)).

2.3 Fungal effects

Under low and high nutrient level conditions when soil fungi were present (no fungicide), a negative trend in MM biomass production was observed in all soils (MM, HF, CB, and MT). The negative fungal effects (reduced MM biomass) in HF soils were significantly ($P<0.05$) greater under the low compared to high nutrient level. However, the negative impacts on MM biomass production due to the presence of soil fungi in the other soils (MM, CB, and MT) were not significantly different between the low and high nutrient treatments (Figure 3).

3 Discussion

3.1 Soil origin and nutrient level interaction significantly affected MM (*Mikania micrantha*) biomass allocation patterns

Results demonstrated soil origin and nutrient level interaction did not significantly affect MM biomass production, however, the interaction effect on MM biomass allocation pattern was significant (Table 1). These observations suggest that even though MM biomass production was relatively unchanged under different soil origins and nutrient level conditions, biomass allocation patterns were significantly variable, and altered under certain conditions. These changes could affect the ability of MM to capture available light via affected tissue production and the capacity for the plant to grow vertically, and cover other plants.

3.2 The absence of species-specific pathogenic inhibition on MM biomass production

Biomass production of MM grown in native tree soils was

Figure 2 *Mikania micrantha* (MM) biomass allocation (shoot/root) in sterile sand with MM soil inocula, and each of the three native tree species (HF, CB, and MT) with or without the presence of fungicide (carbendazim), and low or high nutrient levels. Data are means $\pm$ SE, $n=6$ per treatment. Values with the same letter indicate no significant difference; values with a different letter indicate a significant difference ($\alpha=0.05$, LSD). An asterisk (*) indicates a significant ($\alpha=0.05$) difference in MM shoot/root between soils without and with fungicide (t -test).

similar or higher than MM biomass production in MM conspecific soil at low or high nutrient levels (Figure 1). These data suggested the absence of a species-specific pathogen that specifically inhibited MM biomass production in the three native tree soils. Similarly, biomass production of the alien species *Impatiens parviflora* following cultivation in conspecific soil was consistent with biomass production in native species soil [11]. However, the invasive species *Stenactis annua* was found inhibited by soil microbes characteristic of its two sympatric native species, *Erigeron acer* and *Geum urbanum* [11]. Data from the current and previous studies, therefore, indicate differences in how certain

invasive species respond to the presence of soil microbes. While some alien taxa encounter species-specificity resistance from soil microbes of native species during range expansion, others are confronted with no such resistance.

3.3 No relative allelopathic inhibition on MM performance

MM biomass production following cultivation in native tree soils was similar or higher than MM biomass production in MM conspecific soil at either nutrient level (Figure 1). Significant differences in biomass allocation (shoot/root) were not detected between MM grown in MM conspecific soil, or native tree soils, when the nutrient level was high and fungicide was added (Figure 2(b)). These results indicated that even if allelochemicals were present in tree soils, the chemicals did not inhibit MM performance (including biomass production and biomass allocation patterns) relative to allelochemicals in MM soil. Thus, allelopathic inhibition on MM performance in the current study, if any, was minor and negligible. In fact, considering that the ratio of rhizosphere soil to river sand in the experimental media was 1:9 by weight, any allelochemicals would likely be diluted to the point of non-functionality, i.e., irrelevant to the current study.

3.4 Soil fungal resistance to MM

In the presence of pathogenic soil fungi, a significant decrease in MM biomass production was revealed, i.e. MM experienced significant fungal inhibition, which occurred in both conspecific and native tree rhizosphere soils (Figure 1). These results suggested that the presence of pathogenic soil fungi promoted resistance to MM. Resistance to alien plant invasion is a well-studied, recognized phenomenon, although not all processes that contribute to resistance are clearly resolved [31]. Resistance to alien plant invasion might result from the strong effects of a relatively small number of native species [32], such as in HF, CB, and MT [9]. Our results support a hypothesis of soil biotic resistance [33,34], and are congruent with a study by Hou et al. [35], which showed MM was inhibited when exposed to soils from three different forests located in lower subtropical China.

Many studies have reported invasive species success is habitat-dependent, and nutrient-rich habitats often experience more invasion than nutrient-poor environments [36,37]. Previous studies have shown high soil nutrient concentrations promoted increased biomass production in alien species, as well as facilitated their invasion and expansion [38,39]. MM also appeared to prefer high-nutrient soils [5]. Our results showed that the negative effects on biomass production due to soil fungi were less pronounced under high nutrient compared to low nutrient level (Figure 3). As nutrient availability in the tree soil increased, fungal inhibition on MM biomass production diminished, suggesting nutrient

Figure 3 Fungal effects on *Mikania micrantha* (MM) biomass production, assessed using plant biomass following growth in sterile sand with soil inocula from MM, and each of the three native tree species (HF, CB, and MT) with or without the presence of fungicide (carbendazim), and low or high nutrient levels. Fungal effects were determined as the proportional reduction of biomass in inocula with fungicide application. Data are means $\pm$ 1SE, $n=6$ per treatment. An asterisk (*) indicates a significant ($\alpha=0.05$) difference in the fungal effect of each plant soil on MM between low and high nutrient level after Bonferonni-adjustment of t -test results.

application interfered with fungal-induced inhibition, providing a plausible explanation for MM preference of nutrient-rich habitats [5].

3.5 Native tree soil fungi shift MM biomass allocation patterns under field stand nutrient levels

Under nutrient levels reflective of field stand nutrient concentrations, we observed substantial shifts in MM biomass allocation given the effects of native tree soil fungi. MM exhibited decreased shoot biomass allocation when cultivated in native tree soil relative to MM soil (Figure 2(b)). This response is consistent with results reported in other studies that explored plant responses to soil microbes [15,40]. The significant influence of native tree soil fungi on MM occurred through reduced resource allocation directed to plant shoot biomass, resulting in diminished capacity to climb, cover, and smother native trees, and reduced surface area exposed to available light. Adequate light exposure is vital in nutrient-rich habitats along secondary forests and forest margins, where MM is distributed [5]. Diminished resource allocation to shoot biomass could inhibit species success, and further compromise the competitive growth advantage. As a heliophyte, MM prefers high-light environments [21], and does not reproduce under shaded conditions [41]. Closset-Kopp et al. [42] reported similar results for the invasive species *Prunus serotina*, which is unsuccessful at allocating sufficient resource to height growth on

waterlogged and calcareous soils.

However, following fungicide application significant biomass allocation differences were no longer evident (Figure 2(b)), suggesting the native tree soil fungi were responsible for decreasing MM shoot biomass.

Significant differences in MM biomass allocation patterns were not observed between MM conspecific and native tree soils under low nutrient level (Figure 2(a)), indicating MM biomass allocation patterns between conspecific and native tree soils were similar when nutrients were in short supply. Previous studies demonstrated the proportion of shoot biomass allocation decreased significantly when nutrient availability declined [43,44]. If nutrient availability is a limiting plant growth factor that significantly affects MM biomass allocation, any differences among experimental treatments associated with tree soil fungi could be masked due to limitations imposed by restrictive nutrient levels.

4 Conclusion

MM biomass production was relatively unchanged under the interaction between soil origin and nutrient levels, however, MM biomass allocation patterns were significantly changed. A species-specific pathogen that inhibited MM biomass production in the soils of native HF, CB, and MT was not identified. However, resistance evidence against MM in soil fungi of the native tree species HF, CB, and MT was detected. Fungal inhibition on MM biomass production, and shifts in MM biomass allocation patterns may consequently influence the climbing and coverage ability of MM, and plant surface area for exposure to available light. Fungi in soils of the native trees HF, CB, and MT serve an integral role in shifting the competitive balance between native tree species and the invasive plant MM through fungal inhibition on MM biomass production, and MM biomass allocation patterns, which were unfavorable to MM.

We are grateful to Mr. Deng Junlong, Ms. Yang Yinyin and Ms. Yu Ting for practical assistance in data collection, and Dr. Joanna Schultz for the improve clarity of our manuscript. Two anonymous reviewers greatly improve the content of the manuscript. This work was supported by the National Natural Science Foundation of China (30570330 and 30370243), Shenzhen Greening Committee: Non-Environmental Disruptive Control of *Mikania micrantha* (2010–2013), Special Fund for Agroscientific Research in the Public Interest (201103027), and the Zhang Hongda Scientific Research Fund.

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Supporting Information

Table S1 Rhizosphere soil characteristics from the four plant species (mean $\pm$ SE, $n=5$)

The supporting information is available online at csb.scichina.com and www.springerlink.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.