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Short-term parasite-infection alters already the biomass, activity and functional diversity of soil microbial communities

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Native parasitic plants may be used to infect and control invasive plants. We established microcosms with invasive *Mikania micrantha* and native *Coix lacryma-jobi* growing in mixture on native soils, with *M. micrantha* being infected by parasitic *Cuscuta campestris* at four intensity levels for seven weeks to estimate the top-down effects of plant parasitism on the biomass and functional diversity of soil microbial communities. Parasitism significantly decreased root biomass and altered soil microbial communities. Soil microbial biomass decreased, but soil respiration increased at the two higher infection levels, indicating a strong stimulation of soil microbial metabolic activity (+180%). Moreover, a Biolog assay showed that the infection resulted in a significant change in the functional diversity indices of soil microbial communities. Pearson correlation analysis indicated that microbial biomass declined significantly with decreasing root biomass, particularly of the invasive *M. micrantha*. Also, the functional diversity indices of soil microbial communities were positively correlated with soil microbial biomass. Therefore, the negative effects on the biomass, activity and functional diversity of soil microbial community by the seven week long plant parasitism was very likely caused by decreased root biomass and root exudation of the invasive *M. micrantha*.

Top-down effects of species at higher trophic levels on species at lower trophic levels in the food chain can induce cascade effects¹. For instance, aboveground consumers (e.g. animals) can affect the belowground consumers such as soil microbes^{2–5}. However, less attention has been paid to the effects of holoparasite-host interaction on belowground decomposers⁶. Holoparasites are not but very similar to the primary consumers because they are completely dependent upon their hosts for photosynthates, water, and mineral nutrients. Although the interaction between holoparasites and host alters carbon and nutrient cycling in the plant and soil system^{7,8}, very little is known about the ecological consequences such as its effects on microbial communities and their function.

Parasitic plants with over 4,500 known species are among the most ubiquitous generalist parasites in both natural and managed ecosystems worldwide. About 20% of parasitic plants are holoparasites⁹. Parasitic plants acquire part (hemiparasites) or all (holoparasites) of their demand of water, carbon, and nutrients from the hosts, and thus influence the hosts' performance¹⁰ and further the belowground properties. For example, Bardgett et al. found that the root hemiparasite *Rhinanthus minor* indirectly regulated the belowground chemical and microbial properties in a grassland ecosystem infected after three years¹¹. Two main mechanisms have been forwarded to explain the 'top-down' effects of parasitic plants on belowground microbial communities: (1) an enhanced supply of substrates in the rhizosphere could stimulate soil microbial activity. For instance, Bardgett et al. suggested that an increased host's root growth and root exudation was the primary reason for the enhanced activity of belowground decomposers in a mixed grassland community infected by hemiparasitic *R. minor*¹¹. Also, Jescke et al. found that the concentration of certain amino acids decreased in the roots of *Ricinus communis* infected by the holoparasite *Cuscuta reflexa*¹². (2) Beside belowground C inputs, hemiparasitic plants may also affect aboveground litter inputs potentially altering soil C cycling and soil microbial communities. An alternative mechanism could be a positive impact on soil nutrient cycling may occur¹³, where hemiparasitic plants such as *Bartsia alpine*

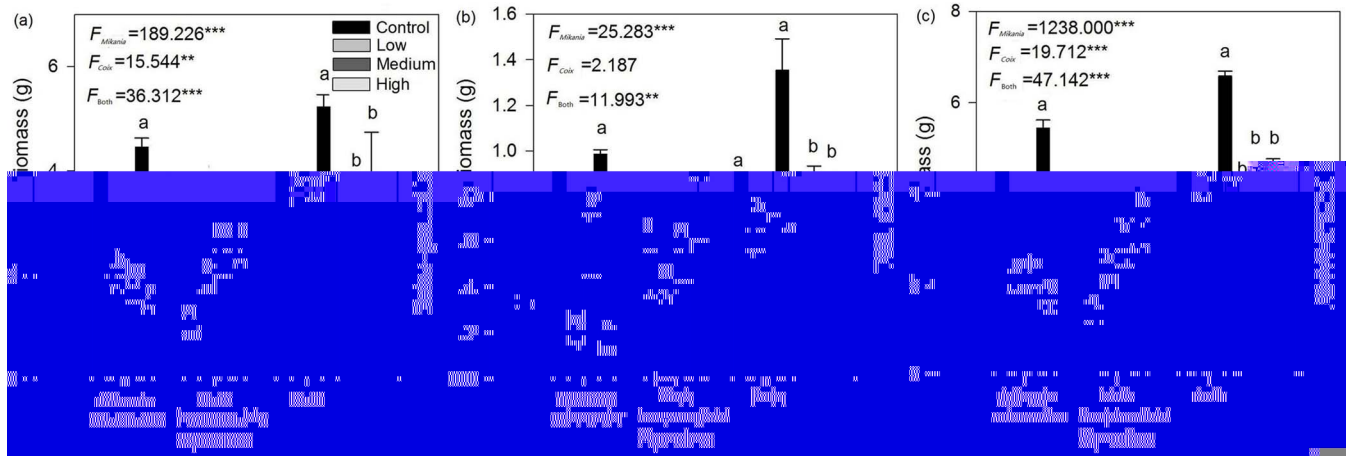


Figure 1 | Aboveground biomass (a), belowground biomass (b) and the total plant biomass (c) of an invasive *Mikania* growing with a native plant *Coix* in four treatments (Control, non-infected *Mikania*, and low-, medium-, and high-level infected *Mikania* by *Cuscuta*). Mean value \pm 1 SD are given. Different letters within each category indicate significant difference between treatments ($p < 0.05$). $F_{Mikania}$, F_{Coix} , F_{Both} indicates F values of treatments (non-infected *Mikania*, and low-, medium-, and high-level infected *Mikania* by *Cuscuta*) on biomass of the invasive *Mikania*, the co-existing native grass *Coix* and on biomass per pot (*Mikania* + *Coix*), tested using one-way ANOVA. p , 0.05; **, p , 0.01; ***, p , 0.001.

and *Amyema miquelii* are found to accumulate nutrients in their leaves and to produce high-quality litter that decomposes rapidly.

So far, the mechanisms underlying the ‘top-down’ effects of parasitic plants on belowground microbial communities are still poorly understood. Litter, root detritus, and root-derived exudates are the main sources of soil organic matter and the carbon sources of soil microbes¹⁴. In natural ecosystems, some hemiparasitic plants such as *Bartsia alpine* and *Amyema miquelii* accumulate nutrients in their leaves and produce high-quality litter that decomposes rapidly, and thus positively influence soil nutrient cycling¹². However, Bardgett et al. suggested that a mixed grassland community infected by hemiparasitic *R. minor* stimulated the activity of belowground decomposers, which was regarded as a result of enhanced supply of substrate rather than increased litter quality because both the host’s root growth and root exudation increased¹¹. Jescke et al. also found that the concentration of certain amino acids decreased in the roots of *Ricinus communis* infected by the holoparasite *Cuscuta reflexa*¹². Thus, we proposed that such changes in the root exudates could affect the belowground properties, although so far, there is no experimental evidence for this view.

The introduction of exotic plant species to a native ecosystem may lead to changes in the interactions between native plant species, herbivores, pathogens, parasites, and other biotic compartments at various tropic levels, which shape the structure and functioning of the invaded system¹⁵. Biological control of invasive plants uses parasitic plants to infect and control the invasive plants^{16–18}. This may affect the ecological interactions between invasive species and other biotic and abiotic factors at different trophic levels in invaded communities, as well as alter soil microbial communities, nutrient cycling and greenhouse gas emission. These changes, in turn, may provide alternative ways to control invasive plants.

Mikania micrantha (Asteraceae) (hereafter *Mikania*) is native to Central and South America and was introduced into China after 1910¹⁹. *Mikania* is now widely distributed in Guangdong Province in South China and threatens native ecosystems¹⁹. A native holoparasitic plant, *Cuscuta campestris* (Convolvulaceae, hereafter *Cuscuta*), has been suggested to infect the invader *Mikania* as a biological control measure in southern China⁸. Several studies found that the parasitism of *Cuscuta* effectively suppressed photosynthesis and growth of the invasive plant *Mikania*, leading to a reduced cover and an increasing diversity of native species^{8,18,19}. Yu et al. observed that the levels of soil nutrients in a field *Mikania*

community infected by *Cuscuta* have already changed after 1–4 years of infection⁸. In a field experiment with *Mikania* in southern China, Li et al. found that infection of *Cuscuta* changed soil chemical properties, enzyme activity and microbial biomass three years after infection⁷. The effects found in those experiments^{7,8} may be a combined result of changes in litter quality and quantity, root detritus, and root-derived exudates. In our study here, we conducted a short-term (7 weeks) microcosm experiment with *Mikania* infected by *Cuscuta* to exclude the effects of parasite litter inputs on the biomass, activity and functional diversity of soil microbial communities. We hypothesized that 1) the short-term infection of *Cuscuta* decreases the host’s biomass and as a result also C inputs to the rhizosphere, which in turn reduces soil microbial biomass, soil respiration, the activity of soil enzymes, and the functional diversity of soil microbial communities, 2) the magnitude of such effects increases with increasing levels of infection intensity. In addition, we aimed to better understand the mechanisms of biological control using parasitic plants against invasive plants.

Results

Infection effects on biomass Parasite infection decreased the aboveground, belowground and total biomass of the host plant, but increased the aboveground and total biomass of the co-occurring grass (Fig. 1). The total pot plant biomass was significantly decreased by the infection, but did not differ among the infection intensities (Fig. 1).

Infection effects on soil microbial communities Shannon’s ($F_{3,16} = 101.092$, $p < 0.001$), Simpson’s diversity ($F_{3,16} = 46.078$, $p < 0.001$) and evenness ($F_{3,16} = 340.286$, $p < 0.001$) indices of soil microbial communities decreased significantly with infection intensity (Table 1). The utilization of miscellaneous C sources by soil microbial communities did not differ among treatments (Fig. 2), whereas the low-level infection significantly decreased the utilization of carbohydrates and amines/amides, and high-level infection significantly decreased the utilization of polymers, carbohydrates, amines/amides, and carboxylic acids by soil microbial communities (Fig. 2). Medium-level infection had no significant effects on the utilization of various carbon sources, but the utilization of polymers, carbohydrates, amines/amides and carboxylic acids were significantly higher in the medium-level infection than in the low- and high-level infection (Fig. 2).

Table 1 | Functional diversity indices of soil microbial communities in soils under four treatments (non-infected *Mikania*, and low-, medium-, and high-level infected *Mikania* by *Cuscuta*). Different letters within a column indicate significant difference between treatments ($p < 0.05$)

Treatments	Shannon-Wiener diversity index	Evenness diversity index	Simpson's diversity index
Control	3.187 6 0.010a	0.934 6 0.003a	21.723 6 0.200a
Low-level infection	3.057 6 0.173b	0.905 6 0.009b	18.160 6 0.266b
Medium-level infection	3.115 6 0.012b	0.910 6 0.003b	19.526 6 0.204b
High-level infection	2.966 6 0.022c	0.878 6 0.007c	15.368 6 0.312c

Infection effects on soil microbial biomass, soil enzyme activity and soil respiration Parasite infection increased concentrations of soil C_{org} ($F_{3,16} = 4.245, p = 0.045$) (Fig. 3a) but decreased soil microbial biomass C ($F_{3,16} = 30.882, p < 0.001$) (Fig. 3b). Correspondingly, the ratio of C_{mic} to C_{org} was decreased ($F_{3,16} = 24.081, p < 0.001$) by parasite infection but not affected by the infection intensity (Fig. 3c). The soil b-D-glucosidase activity tended to decrease with infection intensity, but the decrease was only significant when *Mikania* was highly parasitized by *Cuscuta* (Fig. 4a). Soil respiration rates significantly decreased by the low-level infection but increased at the medium- and the high-level infection as compared to controls ($F_{3,16} = 12.161, p < 0.01$; Fig. 4b). The microbial metabolic activity did not change with low infection. However, at the two higher levels of infection, the microbial metabolic activity increased by 180% or so (Fig. 4c).

PCA analysis of soil properties and the relationships with the biomass of plants The PCA ordination of soil properties had eigenvalues on the first two axes of 2.607 and 2.100, and 78.4% of the variance was explained by the two axes. Soils under the medium- and high-level infection were clearly separated from soils under the low-level infection and controls according to the PC1 axis, whereas soils under medium-level infection and controls were distinguished from the soils subjected to the low- and high-level infection according to the PC2 axis (Fig. 5). The values of C_{mic} , C_{mic}/C_{org} ratio, and b-D-glucosidase activity were strongly positively correlated with the first axis. The carbon utilization ability of soil microbial communities had a strongly positive correlation with the

second axis, while soil C_{org} had a strongly negative correlation with the second axis.

Pearson correlation analysis showed that Shannon-Wiener diversity was significantly positively correlated with soil C_{mic} ; The evenness index was significantly positively correlated with soil C_{mic} , C_{mic}/C_{org} ratio, and AWCD; Simpson's diversity was significantly positively correlated with soil C_{mic} , C_{mic}/C_{org} ratio, b-D-glucosidase activity, and AWCD (Table 2). Both C_{mic} and C_{mic}/C_{org} ratio were significantly positively correlated with aboveground, belowground, and total biomass of host *Mikania* and both *Mikania* and neighboring *Coix*, but significantly negatively correlated with aboveground and total biomass of neighboring *Coix* (Table 2).

Discussion

In our study, short-term (7 weeks) parasitism on invasive plants significantly altered the biomass, functional diversity and activity of soil microbial communities, indicating a quick top-down effect of aboveground consumer on the belowground decomposers. In line with our results, previous long-term field studies found that *Mikania* infected by *Cuscuta* markedly affected soil physico-chemical properties, enzyme activity, soil microbial biomass⁷, and soil nutrients⁸ in *Mikania*-invaded communities. In a natural grassland ecosystem, Bardgett et al. observed significant changes in belowground properties by an infection with hemiparasitic *R. minor*². Similarly to plant parasite infection²⁰, foliar herbivores were also found to have strong top-down effects on soil decomposers in a NERC Soil Biodiversity field site located in Scotland²¹ and in a well-drained arctic tundra heath system²².

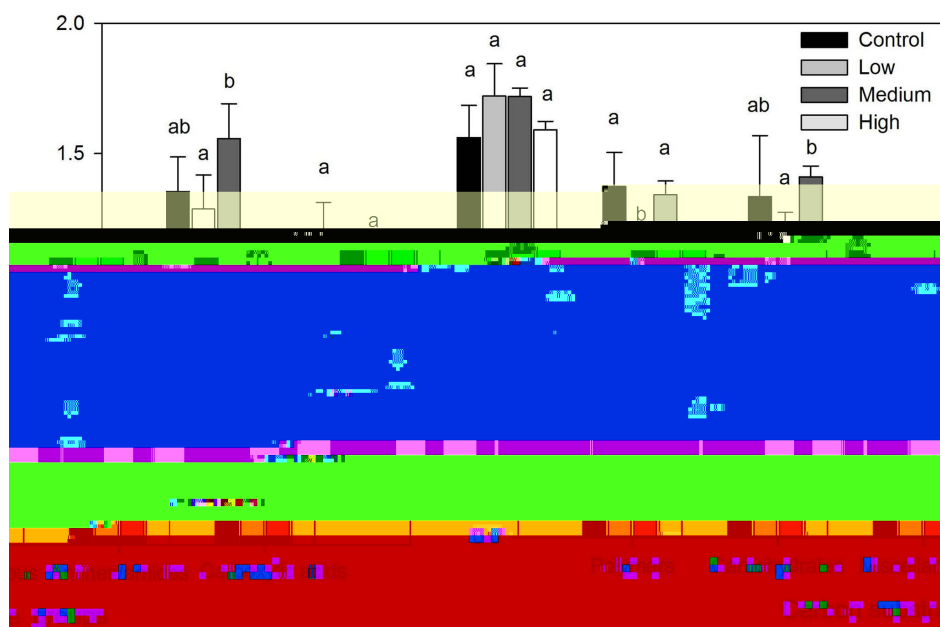


Figure 2 | Carbon utilization ability of soil microbial communities in relation to treatments (Control = non-infected *Mikania*, and low-, medium-, and high-level infected *Mikania* by *Cuscuta*). Data was shown with mean and standard deviation. Different letters within each category indicate significant difference between treatments ($p < 0.05$).

Figure 3 | Responses of soil C_{org} (a), C_{mic} (b), and C_{mic}/C_{org} (c) to treatments (Control, non-infected *Mikania*, and low-, medium-, and high-level infected *Mikania* by *Cuscuta*). Data was shown with mean and standard deviation. Different letters indicate significant difference between treatments ($p < 0.05$).

Previous studies have paid less attention to the effects of parasitism on soil microbial biomass but focused on the relationships between root-parasitic nematodes and soil microbial biomass. For example, no effects²³ of *Heterodera trifolii* infection but negative effects²⁴ of *Rotylenchulus reniformis* infection on soil microbial biomass C were reported. Denton et al. found that low intensity infection of *H. trifolii* increased but high level infection decreased the microbial biomass in *Trifolium repens* community²⁵. Our present study found that short-term infection of holoparasites significantly decreased soil microbial biomass C. With increasing level of infection, the microbial biomass C decreased, which may indicate that holoparasite-infection rapidly reduced the C supply to soil microorganisms. The likely reason for this decline was a smaller C input from roots since there were no litter inputs from aboveground foliage in our microcosm with young plants during a short experimental period, and hence, C inputs were only derived from the belowground system. Root biomass and root-derived exudates are the primary carbon sources for soil organic matter and soil microbial populations^{26–28}. Parasitic plants, especially holoparasitic plants absorb nutrients and water from the host²⁸. In addition, the parasite consumes photosynthetic products from the host's phloem, thereby reducing the amount of carbon supplied to the root²⁹. In this study, parasitism significantly decreased the belowground biomass of *Mikania* but had no effects on the biomass of *Coix* (Fig. 1).

Pearson correlation analysis showed that the decrease in soil microbial biomass was significantly correlated with a declining belowground biomass of *Mikania*, indicating that an altered root biomass of parasitized *Mikania* was primarily responsible for the observed changes in soil microbial biomass. The inhibition effect of parasitism of *Cuscuta* on *Mikania* released the neighboring grass *Coix* from competition and increased the aboveground, belowground, and the total biomass of *Coix* (Fig. 1). Pearson correlation analysis showed that the soil microbial biomass was highly positively correlated with the above- and belowground biomass and the total biomass of *Mikania* (Table 2), indicating that the invasive plants had strong effects on the structure and function of soil microbial communities. Increases in the aboveground biomass of *Coix* might have increased the supply of the rhizosphere with assimilates, but this increase in carbon supply was apparently not strong enough to compensate for the negative effects of decreased *Mikania* biomass on soil microbial biomass. In this study, pots were fertilized to avoid the differences in soil nutrient availability and the nutrient limitation on the growth of host and parasitic plants³⁰. Although the fertilization could boost the timing of infection and magnify the effect of parasitic plants on host, the close correlation of plant biomass with microbial biomass indicates that the nutrient addition has not modified the general responses of on microbial communities and their functions to parasite infection.

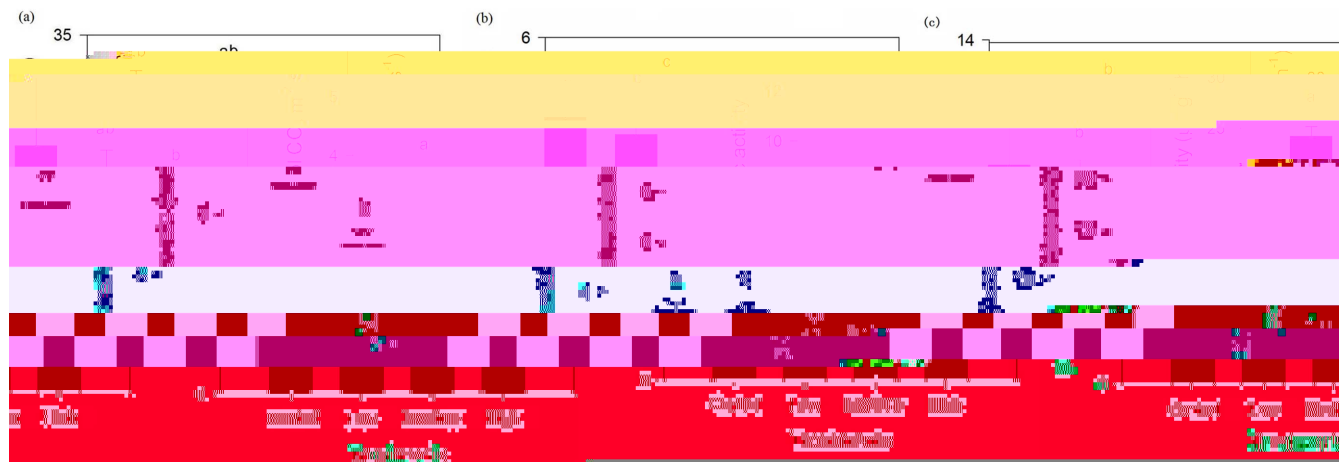


Figure 4 | Soil b-D-glucosidase activity (a), Soil respiration rate (b) and microbial metabolic activity (c) in relation to treatments (Control, non-infected *Mikania*, and low-, medium-, and high-level infected *Mikania* by *Cuscuta*). Data was shown with mean and standard deviation. Different letters within each category indicate significant difference between treatments ($p < 0.05$).

In contrast to microbial biomass, soil respiration increased at the two higher levels of infection and hence, the soil microbial metabolic activity, the respiratory activity per unit microbial biomass strongly increased. However, calculating metabolic activity using total soil respiration measured in the field is critical as soil respiration has an autotrophic component, which ideally should be subtracted. In the studied system, the autotrophic contribution to soil respiration is not known, but as root biomass even decreased by plant parasitism, it seems likely that an increase in root respiration was not responsible for the observed increase in microbial metabolic activity. The stimulated C metabolization without a corresponding increase in microbial biomass indicates a higher C flow through the belowground and higher respiratory C losses and hence, a smaller potential of the soil to sequester C under high levels of infection³¹. This finding also suggests that the infection of *Mikania* by a holoparasitic plant altered the availability but probably also the quality of substrate for microbial communities. One reason could be a decrease in belowground biomass by the plant parasitism but there might have also been a shift in root exudation. Root exudates are low-molecular-weight compounds that are passively and actively released by living roots^{32,33}. Carbon-rich substrates, such as sugars (50–70% of total exudates), carboxylic acids (20–30% of total exudates), and amino acids (10–20% of total exudates) make up the majority of exudates compounds³⁴ and can provide abundant resources for soil microbial communities^{26,32}. These root exudates are of primary importance for microorganisms, as they are readily assimilable without the need to be synthesized by exo-enzymes³⁵. In our study, parasitism significantly changed the functional diversity indices of soil microbial communities and the utilization of various carbon sources by soil microbial communities, again suggesting that the available carbon substrates in root exudates changed with parasitism. Using ¹⁴C-labelled compounds, van Hees et al. demonstrated that 60 to 90% of organic acids but only 10–30% of amino acids are respired in the short-term, and hence metabolic activities are higher when organic acids are the dominant root exudates³³. Exudation of low-molecular weight organic acids generally increases with environmental stress³⁴. Consequently, our observation of an increased microbial metabolic activity at high levels of infections could be interpreted as a result of a stress induced by parasitism.

Exotic plant species can rapidly alter the structure and function of

Method

Study site We conducted our microcosm experiment in the Dengshuilong village in the southeastern part of Dongguan City (E 113°19'11.4" N 22°49'23.0" S, Guangdong Province, China). The climate is marine subtropical with a mean annual precipitation of 1820 mm, mean annual temperature of 23.1°C and mean annual sunshine time of 1874 hours. *Mikania* started to invade this area in the early 1990s and has spread extensively in shrublands and abandoned fields.

Experimental design Our microcosm experiment consisted of seedling of the invasive *Mikania* and native annual grass *Coix* both planted together in pots (25 cm in diameter and 20 cm in height). *Coix* was chosen because it is one of the most common co-occurring species in communities invaded by *Mikania*. Three weeks after seedling planting, *Mikania* was infested at three different levels of intensity (low, medium and high) with *Cuscuta*, a native holoparasitic plant, which is widely distributed in Fujian, Guangdong Province and Xinjiang Uygur Autonomous Region, China⁴³.

Prior the microcosm experiment, seedlings of the invasive *Mikania* were propagated by cuttings (10 cm long), which were collected from a *Mikania* population in the field near Dengshuilong village, using sharp pruning shears sterilized with 70% ethanol. Only the upper stem segments of healthy and disease-free plants were used for cutting collection. Half of the leaves were removed from each collected cutting to reduce water losses. The cuttings were vertically inserted 3–4 cm deep in prepared nursery beds on July 16, 2006.

Native *Coix* seeds purchased from Heze Chinese Medicine Institute of Shandong were immersed in 20% CuSO₄ for 10 min to avoid disease infection. Thereafter, the seeds were left in water for 24 hours, placed in 70% ethanol for 1 min, in water for 5 min, and in 10% H₂O₂ for 5 min. Finally, the seeds were rinsed with sterilized water three times. On June 2006, seeds with similar size were sown on prepared nursery beds in the field.

In each pot, one *Mikania* and one *Coix* seedling were planted at a distance of 10 cm on July, 2006. At planting, seedlings of both species were 15 cm in height. The potting soil was a mixture of sand and local soil which was taken from an abandoned field without the invasive species near Dengshuilong village. After removing the vegetation and litter from the soil surface, the red clay soil was sampled to a depth of 15 cm. Then, we removed plant materials (e.g. roots) and stones, homogenized the soil, mixed it with sand (soil-to-sand ratio, 35:1, v/v) and filled 2.5 kg of the soil mixture into the pots. The potting soil had a pH (in distilled water without CO₂) of 5.3 and initial contents of total organic carbon, nitrogen and phosphorus of 16 g kg⁻¹, 0.56 g kg⁻¹, and 0.16 g kg⁻¹, respectively.

All pots were fertilized with half-strength Hoagland's nutrient solution weekly⁴⁴ and irrigated with tap water twice per day. Three weeks after the seedlings had been transplanted, native parasitic *Cuscuta* collected from a field population near Dengshuilong village was wound around the stems of *Mikania* for infection. In a pilot study, Li et al. (unpublished data) found that the haustoria number, the number of branches and the proportion of the cover of the parasite to the host plant were positively correlated with the number of the parasite's stem but not the length of the parasite's stem. Therefore, we used one, two and three 15-cm-long *Cuscuta* stems wound around *Mikania* stems to represent low-, medium-, and high-level infection, respectively. *Mikania* grown without infection was used as controls. Each treatment was replicated five times. Pots were randomly arranged in the field and moved every week.

Measurements After seven weeks of infection, soil respiration was monitored *in situ* using the LCi Portable Soil Respiration system (ADC BioScientific Ltd., Hoddesdon, Herts, England). The elliptical soil collars (14 cm in maximum diameter, 8 cm in minimum diameter and 10 cm in height) were inserted 10 cm into the soil. For each measurement of soil respiration, the soil chamber was placed on the collar and the increase in CO₂ was recorded for five minutes. We repeated the measurement cycles 10 times for 30 s-intervals until the CO₂ flux was constant.

Seven weeks after infection, *Cuscuta* was removed from the *Mikania* host. All plants were harvested, separated into roots and shoots, dried for 48 h at 80°C, and weighed to determine biomass. Soil samples were stored at 4°C and transported to the laboratory immediately. The soil samples were sieved through a sterilized 2-mm sieve to remove vegetation, small animals, plant roots and stones. A sub-sample of each soil sample was air-dried and ground for soil chemistry analysis, and a second sub-sample

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Li et al.⁷, Zornoza et al. found that b-D-glucosidase (EC 3.2.1.21) activity determined in air-dried soils was almost same with those obtained from soils under field-moist conditions⁵⁰. The specific substrate p-nitrophenyl b-D-glucoside was used for this determination.

The carbon utilization ability of soil microbial communities was assessed by Average Well-Color Development (AWCD) at 96 h using Biolog 96-well Ecoplates (Biolog, Inc., Hayward, California, USA). Each plate contains 31 different carbon sources each with three replicates, including eight carbohydrates, eight carboxylic acids, four polymers, six amino acids, two amines, and three miscellaneous substrates⁵¹. Fresh soil (10 g) was prepared and diluted according to the modified method described by Li et al.⁷. Each well of a Biolog EcoPlate was filled with 150 μ l of the final dilution⁵². Three replicate substrate sets were used to get a mean value for each soil sample. Plates were incubated at 25°C for 96 h, and color development was measured as absorbance (A) using a microplate reader (Multiscan MK3, Thermo Lab. Systems) at 590 nm⁵³. The individual absorbance value of the 31 single substrates was calculated by subtracting the value of the blank control (raw difference; RD). Negative RD values were set to zero. To minimize the effects of inoculum densities on the absorbance, data were normalized by dividing the RD values by their respective average well color development (AWCD) values. AWCD values were used to calculate Shannon's, Simpson's and evenness diversity indices, using Biological Tools version 0.20 software.

Data analysis One-way ANOVA was used to analyze the effects of infection on biomass growth and soil properties, followed by Fisher protected least significant difference (LSD) test at the 0.05 confidence level to examine the difference in means between treatments. A Pearson correlation analysis was used to test the correlation between plant biomass and the carbon-related soil properties. All statistical analyses were performed in SPSS 16.0 for Windows. Soil-carbon-related properties in all treatments were assessed using principal component analysis (PCA) to study the relationships between the properties and their grouping⁵⁴. The statistical software package PC-ORD was used for PCA analysis⁵⁵. All figures were created in Sigma Plot 11.0.

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Author contribution

L.J. and J.Z. designed and completed the experiment. L.J., F.H. and L.M. wrote the manuscript. All authors reviewed the manuscript.

Additional information

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