

Influence of *Lantana camara* on soil II. Effects of *Lantana camara* leaf litter on plants and soil properties

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ABSTRACT

We determined the impact of *Lantana camara* leaf litter on seedling growth of *Raphanus sativus*, *Lactuca sativa*, *Bidens pilosa*, *Bidens bipinnata* and *Urena lobata* and assessed changes in soil enzymes and soil chemical properties after 40-day litter decomposition. The shoot height and total biomass of each test plant, 40 days after transplanting were measured. Functional diversity of soil microbial community was determined by BIOLOG-ECO plates. The activity of soil microbial community was analyzed using the Average Well Color Development (AWCD). Decomposed *L. camara* leaf litter significantly inhibited the growth of all the test plants, and significantly increased the level of soil urease, invertase, protease, catalase and cellulase, and the contents of soil organic matter, total N, P, K, available N, P and K. The BIOLOG results indicated that Shannon index, McIntosh index, Simpson index and Richness index of microbial communities in soil samples were also higher in the treatments with increasing levels of *L. camara* leaf litter.

Key words: AWCD, Biological invasions, BIOLOG-ECO, *Lantana camara*, leaf, litter, litter decomposition, soil enzyme activities, soil nutrients

INTRODUCTION

L. camara L. (Verbenaceae) is notorious weed and a popular ornamental garden plant worldwide (3,9,13). Due to its high growth rate and high productivity, it is one of the 100 worst invasive species in the world as per IUCN (21). Earlier studies indicate that its successful invasion may be due to production of numerous seeds per plant (9,26) and its ability to grow under a wide range of environment conditions (30). *L. camara* also produces and releases several types of secondary metabolites (flavonoids, terpenes, terpenoids and phenolic acids), some of which are well known allelochemicals that inhibit the growth of other plants and organisms (26,29). During the leaf litter decomposition, nutrients are released (7,17,34). Soil enzymes activities in decomposing litter are directly related to the loss of litter mass (18) and the microbial communities responding quickly to the incorporation of exotic invasive alien plants litter by altering enzymes profile (6,8,18). *L. camara* produces large quality of leaf litter and the litter decomposition could enhance

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the allelopathic potential of soil and change the soil chemical properties to facilitate its further invasion. To test this hypothesis, experiments were done to determine (i) the effects of 40-day decomposed *L. camara* leaf litter on the growth of *Raphanus sativus*, *Lactuca sativa*, *Bidens pilosa*, *Bidens bipinnata* and *Urena lobata*, (ii) changes in soil enzymes and soil chemical properties and (iii) functional diversity of soil microbial community.

METHODS AND MATERIALS

I. Litter decomposition

Fresh leaves (about 500 g) of *L. camara* were collected from wild population in May 2012 from our Experimental Farm, South China Agricultural University, Guangzhou (N 23°16', E 113°34'), China. The soil used in the experiments was acidic red soil (pH 4.85), a typical soil type in southern China. It was collected from the same Experimental Farm and air-dried. Fresh leaves of *L. camara* were mixed with 10 kg soil to prepare different ratios of litter-soil : control (0g / kg) and treatments (10 g/kg, 20 g/kg, 30 g/kg and 40 g/kg) in pots, respectively. The soil with no fresh leaves of *L. camara* was control (0 g litter/kg soil). The pots (10 kg soils per pot) were placed in green house (25 ± 2 °C, 75 ± 5% relative humidity, and dark conditions) and watered daily. After 40 days, 100 g soil samples were collected from each pot. The samples were air-dried and kept at -18°C until further use. All treatments were replicated in triplicate.

II. Pot culture

One kg of 40-day-old soil litter mixture of different treatments was put into pots (10 cm dia, 20 cm deep) and young seedlings (10 days old) of *R. sativus*, *L. sativa*, *B. pilosa*, *B. bipinnata* and *U. lobata* were transplanted (one seedling per pot) on Aug. 21 2012. The pots were then transferred into green house (14/10 h day/night cycle, 25 ± 2°C, 75 ± 5% relative humidity) and watered daily. After 40 days, the shoot height of each plant was measured on Oct. 2 2012. Total biomass (leaves, stems and roots) was determined after dried at 85°C for a week. The magnitude of inhibition or stimulation was expressed as the response index (RI) and calculated as under (5,34):

$$RI (\%) = (T/C-1) \times 100$$

Where, T: Treatment, C: Control.

III. Soil chemical composition

Soil samples were passed through a 2-mm sieve to remove gravel and litter. All samples were then homogenized and 50 g soil was used for analyzing chemical composition. The contents of total C, N and P before transplanting the seedlings in soil were determined by the potassium dichromate-volumetric method, Kjeldahl method and sodium hydroxide fusion molybdenum stibium resist colorimetric method, respectively. The contents of available N, P and K were measured using alkaline hydrolysis diffusion method, colorimetrically by the ammonium molybdate tartaric/ascorbic acid and ammonium acetate extraction-flame spectrometry, respectively (2). All experiments were repeated thrice independently.

IV. Enzyme assays

Soil enzyme activities were measured in the various treatments before the seedling transplanting. Soil cellulase and invertase were measured colorimetrically using 3,5-dinitrosalicylic acid (32,33). Soil urease, protease and catalase were analyzed by phenol-sodium hypochlorite colorimetric method, ninhydrin colorimetric method and potassium permanganate titration, respectively (12,32). Soil protein was determined using the method as described by Liao *et al* (20). Three replicates were used for each treatment.

V. Microbial biomass

Functional diversity of soil microbial biomass in various treatments before seedling transplant was analyzed using BIOLOG-ECO method. Overall colour development in BIOLOG plates was expressed as Average Well Color Development (AWCD) (23). Plates were read on a BIOLOG™ Microplate Reader every 24 h. The Shannon-Wiener index, Simpson index, Pielou's evenness index and McIntosh index and Richness index of the different treatments were analyzed respectively (24) and there were three independent replicates for each treatment.

Statistical analysis: All data were presented as means \pm SE. Significant differences ($P < 0.05$) were analyzed using one-way ANOVA followed by Duncan's multiple range test with SPSS 13.0 Software Package (SPSS, Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Seeding growth of test species

The bioassay results indicated that 40-day decomposed *L. camara* leaf litter was inhibitory to seedling growth of all target species (Figure 1). Among all the treatments, maximum seedlings growth was found in the control. The growth decreased with increasing levels of decomposed leaf litter in soil and the maximum inhibition was in treatment 40 g litter/kg soil. The total biomass of *B. pilosa*, *B. bipinnata*, *U. lobata*, *R. sativus* and *L. sativa* decreased by 38.58%, 71.90%, 77.03%, 72.16%, and 71.48% in the 40 g litter/kg soil treatment, respectively, than control, The responses of shoot height of the test plants to the leaf litter doses were also similar and the degree of inhibition was related to the litter doses. These results suggested that decomposed *L. camara* leaf litter impairs the growth of the test plants through its allelopathic properties.

Kumar *et al* (19) reported that the seedling growth of the target plants grown in soil amended with the plant litter with allelopathic potential was less than those grown in the unamended soil. *Pueraria montana*, an invasive legume, significantly inhibited the germination and radicle growth of *B. pilosa* and *L. perenne* at various concentrations (10 - 50 g/L) (27). As seen here, the decomposed *L. camara* leaf litter displays the allelopathic effect and decreased the shoot height and biomass of all target plants and the degree of inhibition varied with the treatments and the test species. These results are in agreement with those reported by others that allelochemicals present in the plants have harmful effects on other species (22,27,28,31).

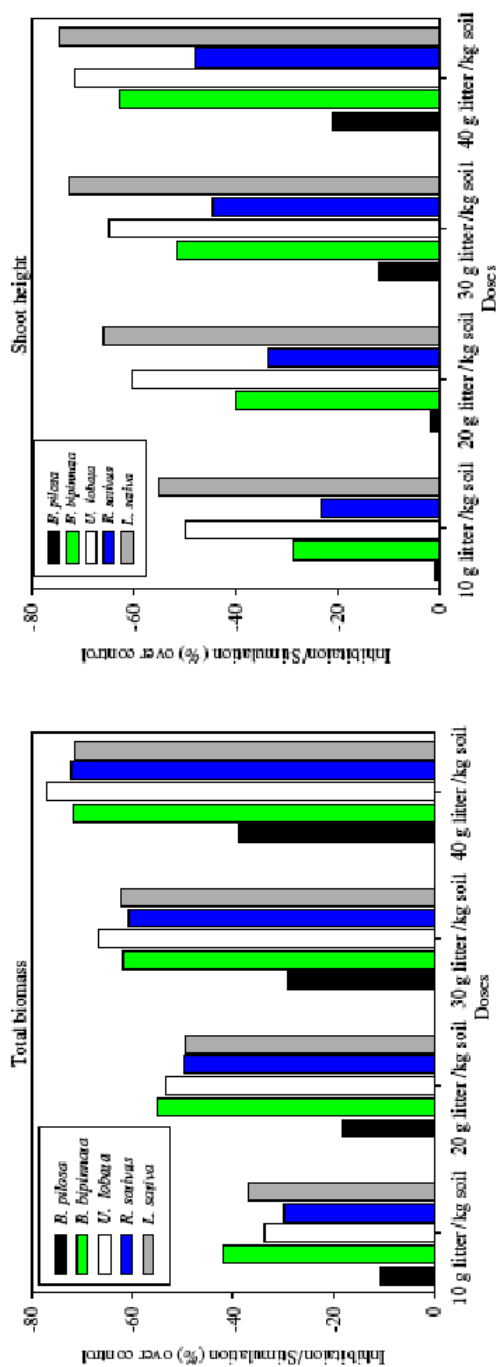


Figure 1. Inhibitory effects of *L. camara* litter on the total biomass and shoot height of *B. pilosa*, *B. bipinnata*, *U. lobata*, *R. saribus* and *L. sariva*

Table 1. Effects of *L. camara* invasion on soil protein content and soil enzyme activities in the different treatments

Treatments	Soil enzyme activity				
	Urease ($\mu\text{g NH}_3\text{-N g}^{-1} 24\text{h}^{-1}$)	Protease ($\mu\text{g NH}_3\text{-N g}^{-1} 24\text{h}^{-1}$)	Invertase ($\text{mg glucose g}^{-1} 24\text{h}^{-1}$)	Cellulase ($\text{mg glucose g}^{-1} 72\text{h}^{-1}$)	Catalase ($\text{ml } 0.02 \text{ M KMnO}_4 \text{ g}^{-1} 20 \text{ min}^{-1}$)
0 g/kg	143.67 e	1.18 d	26.16 d	22.25 e	7.85 d
10 g/kg	198.01 d	1.19 d	33.13 c	31.83 d	8.28 d
20 g/kg	360.27 c	1.35 c	39.95 b	37.61 c	10.44 c
30 g/kg	442.27 b	1.56 b	45.69 a	43.96 b	12.16 b
40 g/kg	547.11 a	1.72 a	46.03 a	51.11 a	15.96 a

All data represents means. Different letters indicated significant differences ($P < 0.05$) as per Duncan's multiple range test.

Soil enzyme activities

The activity level of various soil enzymes in 40-day decomposed leaf litter varied and *L. camara* litter significantly affected the level of soil enzymes (Table 1). The 40 g litter /kg soil had significantly higher level of soil urease, protease, invertase, cellulase, catalase and soil protein (280.81%, 45.76%, 75.96%, 129.71%, 103.31% and 151.30%, respectively) than the control. Previous studies have shown that the nature of litter strongly determines the type and level of soil enzymes in the decomposed litter (14,18). *Falcataria moluccana* invasion increases the litterfall biomass (1.3-8.6 folds) (15) and acid phosphatase activity to $> 90 \mu\text{mol g}^{-1} \text{soil h}^{-1}$ compared to $30\text{-}60 \mu\text{mol g}^{-1} \text{soil h}^{-1}$ in native-dominated stands (1). Our results showed that *L. camara* litter also increased the soil enzyme activities and the increase was related to the litter dose.

Soil chemical properties

The decomposed *L. camara* leaf litter significantly influenced the soil chemical properties (Table 2). As the dose of leaf litter in the soil increased, the contents of soil organic matter, total N, P, K, available N, P and K in the soil also sharply increased. *Typha × glauca* Godr. (hybrid cattail), an aggressive plant, through its litter production changes its ecosystem to benefit its own invasion (11). Similarly, the exotic plant *Mikania micrantha* H.B.K. also significantly affects the soil nutrients level and thereby promotes its invasion and establishment in new habitats (5). Similarly, *L. camara* litter also improves the soil fertility and nutrients cycling and thus promotes its own invasion and growth (25).

Table 2. Effects of *L. camara* invasion on soil nutrients in the different treatments

Treatments	Soil chemical Properties ($\text{g} \cdot \text{kg}^{-1}$)				Soil chemical Properties ($\text{mg} \cdot \text{kg}^{-1}$)		
	Soil OM	Total N	Total P	Total K	Available N	Available P	Available K
0 g/kg	21.04 e	0.98 d	0.40 d	15.45 e	82.31 e	6.43 e	161.36 e
10 g/kg	22.19 d	1.01 d	0.43 c	16.36 d	89.84 d	7.14 d	191.87 d
20 g/kg	24.09 c	1.11 c	0.46 c	18.16 c	98.09 c	7.67 c	207.02 c
30 g/kg	26.14 b	1.23 b	0.69 b	20.13 b	110.74 b	8.39 b	265.53 b
40 g/kg	28.91 a	1.47 a	0.86 a	23.07 a	129.84 a	8.96 a	396.22 a

All data represents means. Different letters indicated significant differences ($P < 0.05$) as per Duncan's multiple range test. Values are based on air-dried soil samples.

Functional diversity of soil microbial community

The activity of soil microbial community in soils with varying levels of decomposed *L. camara* leaf litter was determined using the Average Well Color Development (AWCD) (Figure 2). Microbial utilization rate of all carbon sources in 40 g litter/kg soil was higher than 10, 20, 30 g litter/kg and the control soil (without litter). Substrates in BIOLOG-ECO plates were divided into 6 groups: carbohydrates, polymers, carboxylic acids, phenols, amino acids and amines. The results indicated that AWCD changed with the incubation time of different treatments in following order: 40 g litter /kg soil $>$ 30 g litter /kg soil $>$ 20 g litter /kg soil $>$ 10 g litter /kg soil $>$ control soil (0 g litter /kg soil) (Figure 3). The microbial Shannon-Wiener index, McIntosh index, Richness index and Simpson index of 40 g litter /kg soil were also significantly higher than those

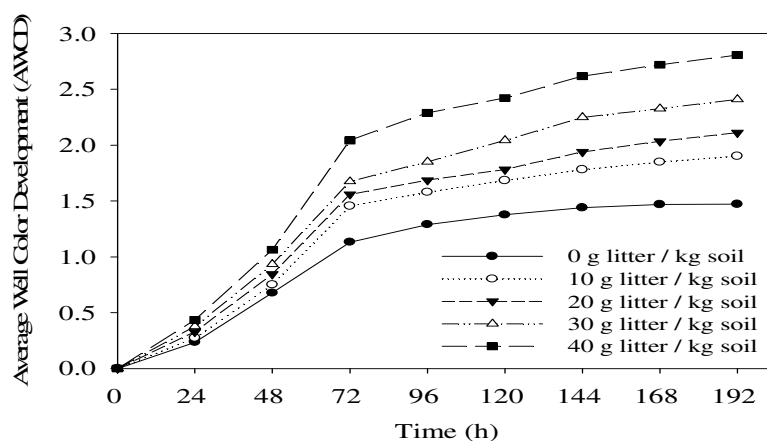


Figure 2. Average Well Color Development (AWCD) on BIOLOG-ECO plates in the different treatments.

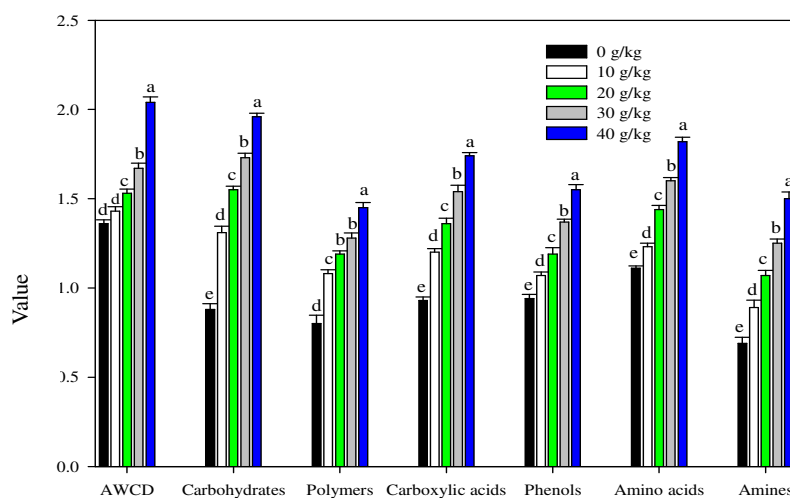


Figure 3. Average Well Color Development and different carbon sources use efficiency of soil microbial community in the different treatments (at 72 h). All data represent means + SE. Different letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

of 30 g litter /kg soil, 20 g litter /kg soil, 10 g litter /kg soil and control (0 g litter /kg soil) (Figure 4). The Richness indices were significantly increased by 14.70%, 21.26%, 22.57% and 25.81% in 10 g litter /kg soil, 20 g litter /kg soil, 30 g litter /kg soil and 40 g litter /kg soil samples, respectively, than control. However the Pielou's evenness index did not significantly differ among 40 g litter /kg soil, 30 g litter /kg soil, 20 g litter /kg soil, 10 g litter /kg soil and control (0 g litter /kg soil). The responses of soil microbes and enzyme activities showed that the biochemical effects of *L. camara* litter were pervasive, especially at the highest dose (40 g litter /kg soil).

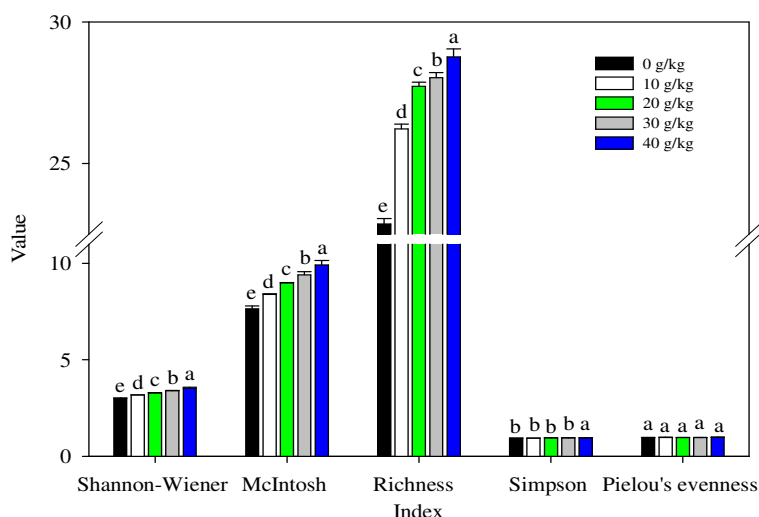


Figure 4. Diversity indices of soil microbial community in the different treatments. All data represent means + SE. Different letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

Previous studies have shown that allelochemicals play a crucial role in mediating the interspecific interactions (7,10). Allelochemicals exuded by plant roots, leaves, litters or by volatilization affect the germination or growth of neighbouring plants (19). Allelochemicals are considered as key determinants of competitiveness and fitness for invasive plants (16,34). Allelopathic mechanisms have been recognized as one of the drivers in the successional replacement of plant species (4,7). Pot experiments have shown that the seedling growth of *Trifolium repens* was significantly inhibited (65.7%), when the invasive *Ageratina adenophora* leaf litter was mixed in soil at 50 g/kg (32). *A. adenophora* leaf litter decomposition releases allelochemicals into soils which inhibited the seed germination and seedling growth of accompanying plants and created a favourable environment for its own invasion (32). Our results also showed that *L. camara* leaf litter significantly inhibited the seedling growth of four target plants and changed soil enzyme activities, nutrients contents as well as functional diversity of soil microbial community. The allelochemicals released during the decomposition of *L. camera* litter inhibited the plant growth and more importantly, but enhanced the level of soil enzymes and soil nutrients which provide a mechanism for its own invasive abilities.

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