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Simulated Acid Rain Accelerates Litter Decomposition and Enhances the Allelopathic Potential of the Invasive Plant *Wedelia trilobata* (Creeping Daisy)

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Invasive species and acid rain cause global environmental problems. Creeping daisy, an invasive exotic allelopathic weed, has caused great damage in southern China, where acid rain is prevalent. The impact of the acidity of simulated acid rain (SAR) on soil nutrients, the decomposition of creeping daisy litter, and on the allelopathic potential of the surrounding soils was investigated. Litter was treated with SAR at different acidity (pH 2.5, 4.0, 5.6) or with water (pH 7.0) as a control. After 70 d, the remaining amount of creeping daisy litter, nutrient contents, and allelopathic potentials in the surrounding soil were determined. The litter decomposition was commensurate to the increase in the acidity of the SAR. Total C and N contents, NO_3^--N and available P increased, levels of NH_4^+-N, the ratio of C/N and soil pH values decreased, water contents increased and then decreased, whereas available K did not significantly change in the soil surrounding the litters in response to the increase in the acidity of the SAR. Bioassays showed that SAR promoted the allelopathic activity in the soil surrounding the litter, as measured by seedling growth of turnip and radish. In conclusion, our results indicated that SAR influenced soil nutrient status, accelerated creeping daisy litter decomposition, and enhanced the allelopathic potential of its litter in the surrounding soil, suggesting that acid rain may enhance the invasiveness of creeping daisy plants.

Nomenclature: Creeping daisy, *Wedelia trilobata* (L.) Hitchc.; turnip, *Brassica campestris* L.; radish, *Raphanus sativus* L.

Key words: Biology invasion, bioassay, allelochemical-soil interactions, phytotoxin.

Acid rain was first reported in China in the late 1970s (Cao et al. 2009; Fan and Wang 2000). Acid rain in China emerged later than in North America and Europe (Cao et al. 2009) and has now become a serious environmental problem affecting 30% land of China, especially in the developing industrial coastal regions in southern China (Cao et al. 2009). The annual average pH value of precipitations in these regions was generally below 4.49, and levels as low as 3.52 have been observed (Cao et al. 2009; Fan and Wang 2000).

It is generally recognized that leaf litter decomposition and its accompanied release of nutrients are fundamental processes in humus formation, nutrient cycling, and ecosystem carbon flux (Hoorens et al. 2003; Wang et al. 2010). However, the relationship between acid rain and litter decomposition is not well understood. In some studies, acid rain accelerated litter decomposition (Lee and Weber 1983; Wolters 1991), whereas the opposite was observed in other studies (Dangles et al. 2004; Wang et al. 2010).

Biological invasion of exotic species has become a serious global problem, and it negatively impacts economy, ecosystems, and human health (Callaway and Ridenour 2004; Chen et al. 2009). Most invasive plants are characteristically adaptable, aggressive, and have a high reproductive capacity (Callaway and Ridenour 2004; Wang et al. 2011). Invasive plants reduce biodiversity of natural ecosystems by displacing native plant communities (Chen et al. 2009; Grant et al. 2003; Wang et al. 2011). Allelopathy has been regarded as an important mechanism for successful invasion of alien plants by suppressing the growth of neighbouring plants through the release of allelochemicals into the environment (Bainard et al. 2009; Dudai et al. 2009; Kim and Lee 2011). Litter from invasive plants can decompose and release nitrogen significantly faster than litter from the native species (Allison and Vitousek 2004; Ashton et al. 2005), resulting in improved nutrient availability. It has been proposed as a factor contributing to the invasiveness of exotic plants (Chen et al. 2009; El-Keblawy and Al-Rawai 2007).

Creeping daisy is a perennial herb species originating from South America (Weber et al. 2008; Xie et al. 2010). In southern China, creeping daisy is an invasive species that has caused significant damages to farmlands, forests, and orchards (Wu et al. 2008; Xie et al. 2010). Creeping daisy has a strong allelopathic potential on neighboring native plants (Weber et al. 2008; Wu et al. 2008; Xie et al. 2010). The aggressive growth characteristic and allelopathic potential of creeping daisy have increased the plant's invasive and widespread distribution during the recent years (Weber et al. 2008; Xie et al. 2010).

We hypothesized that acid rain may increase the decomposition of creeping daisy litter and thereby enhance the release of allelochemicals from the litter into the soil. This, in turn, may increase its suppression of growth of native plants and further promote the invasiveness of this exotic allelopathic plant. In the present study, the relationships between the acidity of SAR and the soil nutrient status, decomposition of creeping daisy litter, and the allelopathic potential of the surrounding soil were investigated.

Materials and Methods

Field Experiment. The effects of SAR on decomposition of leaf litter was evaluated at the experimental farm on the campus of South China Agricultural University in Guangzhou (N 23°16′, E 113°34′), China. Guangzhou has a subtropical marine climate with an annual average rainfall of 1,694 mm and an average temperature of 21.8 C (Cao et al. 2009). The soil used in all experiments was an acidic red soil (pH 4.85), a typical soil type in southern China, which contained at the beginning of the experiment 6.72 g kg^{-1} total C, 0.42 g kg^{-1}

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N, 2.33 mg kg^{-1} NH_4^+-N, 24.76 mg kg^{-1} NO_3^--N, 48.32 mg kg^{-1} P, 402.67 mg kg^{-1} K, and 88.2 g kg^{-1} H_2O. The ratio of C/N was 15.86. A 30-cm upper layer of the soil was mixed and subdivided into 32 sections (1 by 1 m). To avoid cross-contamination, waterproof plastic films were buried between sections at a depth of 50 cm. Fresh leaves of creeping daisy collected from a wild population on the university campus in June 2010 were air dried. Total C and N contents in dried leaves of creeping daisy were 345.30 g kg^{-1} and 31.67 g kg^{-1}, respectively. The ratio of C/N was 10.94. In July 2010, nylon-net bags (15 by 20 cm, 1-mm mesh) containing litter of creeping daisy (10 g dried leaves) were buried into the soil at approximately 5 cm in depth. Sixty-four bags were buried in 16 sections (four bags per section). Another 16 sections contained no litter bags.

A stock solution of SAR was prepared by mixing 1 M HNO_3 and 1 M H_2SO_4 at a ratio of 1:2.5 (v/v), which corresponded to the general anion composition of rainfall in Guangzhou city (Cao et al. 2009). The final solutions of SAR with pH 5.6, 4.0, and 2.5 were prepared by diluting the stock solution with distilled water. The SAR solutions and distilled water (pH 7.0) were sprayed twice a week on each section (16 mm per treatment) as performed in a previous study (Fan and Wang 2000).

After 70 d of SAR treatment, litterbags in every section were recovered. The litter was removed from each bag, gently brushed to remove soil, and dried at 60 C for further analysis. At the same time, samples from the upper 15-cm layer of the soil were collected from each section. Soil samples were transported to the laboratory in plastic bags under low temperature and then passed through a 2-mm sieve to remove litter and gravel. All samples were then homogenized and kept in sealed plastic bags at 4 C until further use. All experiments were repeated four times independently.

Analysis of Litter and Soil Samples. Litter samples were weighed and analyzed for their total C and N contents. Total C contents were determined by the potassium dichromate-volumetric method (Kalembasa and Jenkinson 1973) and total N contents by the Kjeldahl method (Kjeltec 2300 Autoanalyzer; Foss Tecator AB, Hoganas, Sweden) (Bremmer 1996). Soil pH values were measured using a glass electrode in a 1:2.5 (w/v) soil-water suspension for approximately 30 min (Dick et al. 2000). Soil water contents were measured gravimetrically after oven-drying at 105 C for 24 h (Li et al. 2004). Total C and N contents of soil samples were analyzed as described above. Available K was determined by atomic adsorption spectrophotometry (Hitachi Z-2300, Flame Atomic Absorption Spectrophotometer, High-Technologies C., Ltd., Shanghai, China), and available P was measured colorimetrically (UV-2450 UV-VIS Spectrophotometers, Shimadzu Instruments Manufacturing Co., Ltd., Kyoto, Japan) by the ammonium molybdate tartaric/ascorbic acid method following extraction with sodium bicarbonate (Olsen et al. 1954). NH_4^+-N and NO_3^--N were determined using a colorimetric technique following extraction with a potassium chloride solution (Keeney and Nelson 1982). All experiments were repeated four times independently.

Bioassay for Allelopathic Potential of Soil Samples. Turnip and radish, which are commonly used receiver species (Belz and Hurle 2004), were used in this study to test the allelopathic potential of different soil samples (Wang et al. 2011). Water agar (0.5%, w/v) was prepared and autoclaved at 115 C for 15 min. The autoclaved agar was cooled down to 45 C in a water bath. Soil (3 g) was sampled from each section and mixed with 5 ml agar in a 50-ml beaker (10 cm^2 area per beaker). After agar solidification, another 3.2 ml water agar was added to each beaker. Five germinated seeds were placed on the surface of the water agar. The beaker was then covered with plastic film. The seeds were incubated in a growth chamber (25 ± 1 C, dark condition) (Rashid et al. 2010), and shoot height and root length were recorded 3 d after incubation. All experiments were conducted with eight independent replicates.

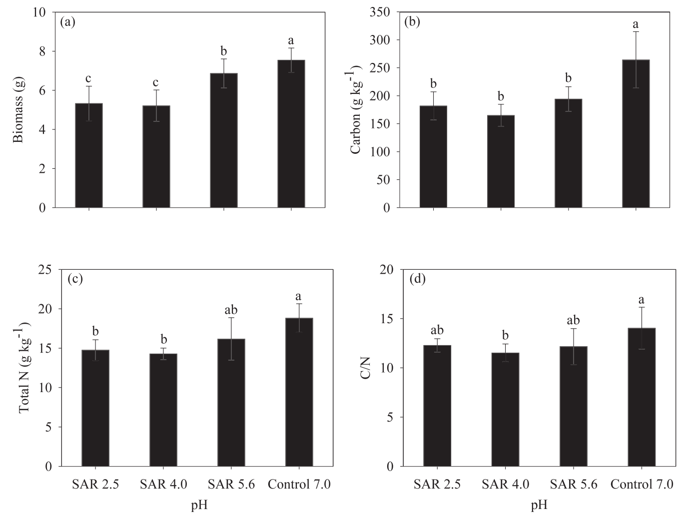

Figure 1. Effects of different simulated acid rain (SAR) treatments on biomass (a), carbon (b), and total N (c) contents, and C/N (d) of creeping daisy litter after 70 d of decomposition. Litterbags were placed into soil and SAR at indicated pH was sprayed twice a week. At the end of the experiment, litterbags were collected and C and N contents measured. Each bar represents means ± standard deviation. Different letters above bars indicate significant differences ($P < 0.05$) according to one-way ANOVA followed by the Duncan's multiple range tests.

Statistical Analysis. Effects of different SAR treatments on biomass, ratio of C/N, and C and N contents of creeping daisy litter were analyzed using one-way ANOVA followed by the Duncan's multiple range tests. Effects of different SAR treatments on chemical soil and the allelopathic potential of the soil on the receiver plants were analyzed by two-way ANOVA tests using the SPSS 13.0 software package (SPSS, Inc., Chicago, IL).

Results and Discussion

Litter Decomposition. After 70 d, the biomass of creeping daisy litter was 7.54, 6.86, 5.21, and 5.33 g after treatment with SAR at pH 7.0, 5.6, 4.0, and 2.5, respectively. The biomass of creeping daisy leaves decreased faster with increasing acidity of SAR (Figure 1a), indicating that SAR promoted the litter decomposition. Consequently, C contents of the litter decreased during litter decomposition. Compared to the water control (pH 7.0), the C contents of litter treated with SAR solutions at pH 5.6, 4.0, and 2.5 decreased by 26.6, 37.6, and 31.2%, respectively (Figure 1b). The total N contents of the litter showed a similar trend (Figure 1c). Accordingly, the C/N ratios in the litter depended on the SAR treatment. Compared to the water control (pH 7.0), the C/N ratio in the litter

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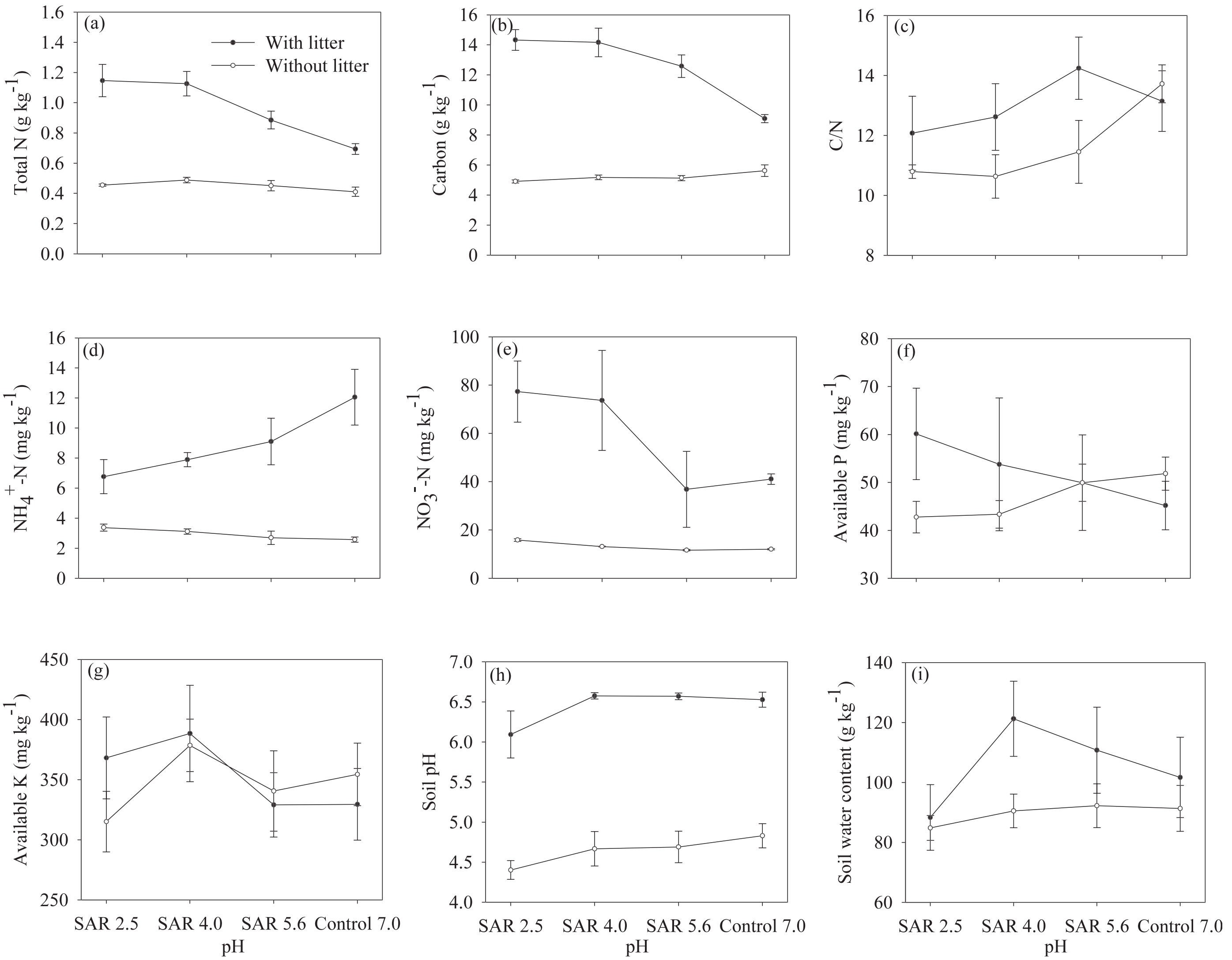

Figure 2. Effects of different simulated acid rain (SAR) treatments on the nutrient status of soils (with or without litter). Soil with litter was collected surrounding litterbags containing creeping daisy litter. Nutrient contents of collected soil [total N (a), carbon (b), C/N (c), NH_4^+-N (d), NO_3^--N (e), available P (f), available K (g), soil pH (h) and soil water content (i)] samples were determined at the end of the experiment (two SAR treatments per week; 70 d of litter decomposition). All data were analyzed by two-way ANOVA tests and represent means ± standard deviation.

significantly decreased by 17.9% for the SAR treatment at pH 4.0 (Figure 1d).

Acid rain can seriously alter ecosystem stability (Cao et al. 2009) by increasing nitrification (Brierley et al. 2001), reducing soil pH, affecting the composition of microbial communities (Kuperman and Edwards 1997; Pennanen et al. 1998), and altering species abundance of forest plants (Fan and Wang 2000). Furthermore, acid rain can change enzymatic indicator activities in the soil, which may reflect changes in the decomposition rate of litter (Dutta and Agrawal 2001; Reddy et al. 1991; Wang et al. 2010). Litter decomposition is strongly controlled by litter quality (Kaspari et al. 2008; Strickland et al. 2009) and is influenced by many factors, such as species composition of a plant community, temperature, and moisture (He et al. 2009; Kaspari et al. 2008). In this study, creeping daisy litter decomposed faster when exposed to SAR, especially at pH 4.0 and 2.5, relative to pH 7.0 (Figure 1a). Our results indicate that low pH levels in the SAR promoted the release of acid-soluble substances from the litter (Lee and Weber 1983; Wang et al. 2010; Wolters 1991), which may accelerate the litter decomposition.

Chemical Soil Analyses. After SAR treatments, total N (Figure 2a) and C (Figure 2b) contents in the soil surrounding the litter were significantly higher than those in the soil without litter. The C/N ratios of the soil (with litter) significantly decreased by the SAR treatments, especially at SAR at pH 4.0 and 2.5 (Figure 2c). The measured NH_4^+-N concentrations in the soil (with litter) steadily decreased with lower pH values of the SAR solutions (Figure 2d). In contrast, NO_3^--N concentrations showed the opposite trend (Figure 2e). Compared to the water control (pH 7.0), the NO_3^--N concentrations in the soil (with litter) increased by 79.4 and 88.3% for the SAR treatments with a pH of 4.0 and 2.5, respectively. Available P (Figure 2f) in the soil samples was not significantly affected by the SAR ($F = 0.27$, $P = 0.844$) or litter ($F = 3.95$, $P = 0.058$) treatments (Table 1). Available P in the soil samples, however, was significantly changed after litter treatment by SAR ($F = 4.03$, $P = 0.019$). Available K in the soil samples surrounding the litter was not significantly affected after litter treatment by SAR treatments ($F = 2.55$, $P = 0.079$) (Figure 2g).

The pH values in the soil (without litter) were not significantly affected by SAR except for SAR at pH 2.5, where

Table 1. Effects of different simulated acid rain (SAR) treatments on the nutrient status of soil (with or without litter) were analyzed using two-way ANOVA tests. Soil samples with litter were collected from soil surrounding litterbags containing creeping daisy litter.

	Effect								
	SAR treatment			Litter treatment			Interaction		
Soil chemistry	F	df	P	F	df	P	F	df	P
Carbon (g kg^{-1})	33.22	3	< 0.001	1533.00	1	< 0.001	52.39	3	< 0.001
Total N (g kg^{-1})	37.46	3	< 0.001	663.55	1	< 0.001	22.49	3	< 0.001
NH_4^+-N (mg kg^{-1})	7.89	3	0.001	301.91	1	< 0.001	14.29	3	< 0.001
NO_3^--N (mg kg^{-1})	9.92	3	< 0.001	147.54	1	< 0.001	7.33	3	0.001
Available P (mg kg^{-1})	0.27	3	0.844	3.95	1	0.058	4.03	3	0.019
Available K (mg kg^{-1})	4.35	3	0.014	0.38	1	0.544	2.55	3	0.079
C/N	8.58	3	< 0.001	17.36	1	< 0.001	4.77	3	0.010
Soil pH	2.57	3	0.078	937.43	1	< 0.001	10.01	3	< 0.001
Soil water content (g kg^{-1})	4.46	3	0.013	19.32	1	< 0.001	3.55	3	0.029

the pH was 8.9% lower than in the soil sample obtained from the water control (pH 7.0) treatment (Figure 2h). The pH values of the soil samples surrounding litter were consistently higher than those without litter. SAR treated litter had a significant effect on the soil pH values surrounding the litter ($F = 10.01$, $P < 0.001$) (Table 1), especially for SAR at pH 2.5, where the pH was 6.5% lower than in the soil sample obtained from the water control (pH 7.0) treatment (Figure 2h). The addition of litter to the soil resulted in increased soil water contents ($F = 3.55$, $P = 0.029$) (Table 1). The soil water contents (with litter) from the SAR treatments at pH 4.0 and 5.6 were 37.3 and 25.4% higher than for the SAR treatment at pH 2.5 (Figure 2i).

Litter deposition and corresponding decomposition rates can vary with plant species, and this may have strong effects on nutrient dynamics in the soil (Allison and Vitousek 2004; Kaspari et al. 2008). For example, the exotic grasses slender oat (*Avena barbata* Pott ex Link.) and soft chess (*Bromus hordeaceous* L.) decreased the concentration of NO_3^- in the soil but increased the concentration of NH_4^+ (Hawkes et al. 2005). NO_3^- and NH_4^+ levels beneath the invasive plant Japanese barberry (*Berberis thunbergii* DC.) were higher than those beneath native species (Kourtev et al. 2003). The invasive exotic mesquite [*Prosopis juliflora* (Sw.) D.C.] ameliorated some soil characters, such as acidification, increased N, P, and K contents, and elevated organic matter (El-Keblawy and Al-Rawai 2007). In our study, SAR (at pH 4.0 or 2.5) stimulated litter decomposition of creeping daisy, and this had a direct impact on nutrient contents in the surrounding soil. Total N contents and NO_3^--N levels in the soil increased, whereas NH_4^+-N levels were lower as compared to the control treatment with water (pH 7.0). Liao et al. (2008) showed that plant invasion enhances the concentrations of NH_4^+ and NO_3^- in the soils of grasslands, forests, and wetlands, and it thereby increases soil N availability (Sperry et al. 2006). Soils with higher concentrations of NH_4^+ and NO_3^- in invasive plant dominated areas might have positive feedback to further invasion (Ehrenfeld 2003). Hence, decomposition of creeping daisy litter in association with SAR increased the nitrogen pools available for plant uptake, and this might further increase the competitiveness of invasive plants (Chen et al. 2009).

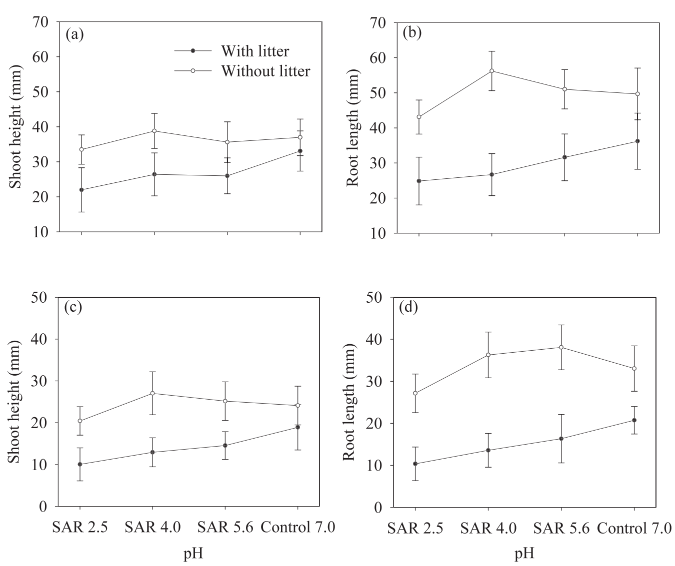

Figure 3. Effects of soil samples on plant growth. Soil samples (with or without litter) were obtained from different simulated acid rain (SAR) treatments. Soil with litter was collected surrounding litterbags containing creeping daisy litter. For the bioassay, the receiver plants radish (a, b) and turnip (c, d) were used. All data were analyzed by two-way ANOVA tests and represent means ± standard deviation.

Effects of SAR Treated Soil on Plant Growth. As shown in Figure 3, SAR treated soil (with or without litter) significantly affected seedling growth in the bioassay with turnip and radish (Table 2). The allelopathic potential of the native soil increased in the presence of creeping daisy litter as compared to the no-litter control and was further enhanced when the SAR pH values decreased. The shoot heights of radish were 33.1, 26.0, 26.4, and 22.0 mm when grown in soil samples (with litter), which were treated by the water control (pH 7.0) and SAR at pH 5.6, 4.0, and 2.5, respectively. Compared to the water control (pH 7.0), the shoot height of radish was significantly inhibited by 21.5, 20.2, and 33.5% when the soil samples were treated with SAR pH at 5.6, 4.0, and 2.5, respectively (Figure 3a). In contrast, shoot heights of radish were 37.0, 35.6, 38.8, and 33.3 mm when grown in soil samples (without litter), which were treated with water control (pH 7.0) and SAR at pH 5.6, 4.0, and 2.5, respectively. As compared to the water control (pH 7.0), the shoot height of radish was significantly inhibited by 10.0% when plants were grown in the soil (without litter) treated with SAR at pH 2.5 (Figure 3a). After the soil treatment with SAR at pH 4.0, the root lengths of radish were 56.2 and 26.7 mm, when plants were grown in soil samples without litter and with litter, respectively. Compared to the corresponding control treatment (pH 7.0), the root lengths of radish were stimulated by

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Table 2. Effects of soil (with or without litter) with different simulated acid rain (SAR) on plant growth were analyzed using two-way ANOVA tests. Soil samples with litter were collected from soil surrounding litterbags containing creeping daisy litter.

		Effect								
		SAR treatment			Litter treatment			Interaction		
Receiver plant	Bioassay parameter	F	df	*P*	F	df	*P*	F	df	*P*
Radish	Root length	31.33	3	< 0.001	785.29	1	< 0.001	22.05	3	< 0.001
	Shoot height	24.99	3	< 0.001	232.42	1	< 0.001	9.69	3	< 0.001
Turnip	Root length	53.52	3	< 0.001	1173.00	1	< 0.001	20.06	3	< 0.001
	Shoot height	31.57	3	< 0.001	436.46	1	< 0.001	14.47	3	< 0.001

13.1% (without litter) and inhibited by 26.2% (with litter), respectively (Figure 3b).

Compared to the water control (pH 7.0), the root lengths of turnip were significantly inhibited by 20.8, 34.3, and 49.8%, when plants were grown in the soil (with litter) treated with SAR at pH 5.6, 4.0, and 2.5, respectively (Figure 3d). As compared to the water control (pH 7.0), the root length of turnip was significantly stimulated by 10.0% and inhibited by 17.6% when root growth was tested for soil (without litter) treated with SAR at pH 4.0 and 2.5, respectively. A similar trend was observed for the shoot height of turnip (Figure 3c). The SAR and creeping daisy litter interaction (Table 2) showed increased inhibition effects on the seedling growth of both receiver plant species. The allelopathic potential of soil samples (with litter) on the receiver plants increased when the same soil treatment with decreased SAR pH values were compared.

Allelochemicals are considered as crucial determinants of competitiveness and fitness for invasive plants (Kim and Lee 2011; Singh et al. 2003; Skulman et al. 2004). Allelochemicals released from litter or root exudates might be absorbed or released from soil particles (Dilipkumar et al. 2012; Inderjit 2001; Kumar et al. 2009; Tharayil et al. 2008). Plant litter can release allelopathic compounds into soil that may suppress the weed growth (Inderjit 2005; Kumar et al. 2009; Singh et al. 2003). The root length and shoot height of turnip and radish increased when soil samples (without litter) were treated with SAR at pH 4.0. However, the root length and shoot height of these plants decreased when the soil (without litter) was treated with SAR at pH 2.5 (Figure 3). These findings are in agreement with a previous study with camphor tree (*Cinnamomum camphora* L.), glossy privet (*Ligustrum lucidum* Ait.), chinaberry (*Melia azedarach* L.), and Chinese flame tree (*Koelreuteria bipinnata* Franch), which suggested that stimulation of seedling growth by SAR at pH 4.0 may be caused by NO_3^- fertilization, whereas SAR at pH 2.0 decreased seedling growth (Fan and Wang 2000). Previous studies showed that creeping daisy had strong allelopathic effects on many plants (Weber et al. 2008; Wu et al. 2008; Xie et al. 2010). In our study, the bioassay with turnip and radish provides evidence for the presence of released allelochemicals. The result indicated that allelochemicals released from the neighboring litterbags exhibited a higher inhibition effect on the growth of the receiver plants. Allelochemicals are usually released in the soil in the form of mixtures of active compounds, whose persistence is normally longer than when applied to soils as individual compounds (Dilipkumar et al. 2012; Inderjit 2001; Tharayil et al. 2008). This is most often due to different rates of mineralization (Gimsing et al. 2009). Similarly, our data with soil containing creeping daisy litter suggest that SAR at low pH considerably increased the amount or activity of allelochemicals in the soil. It is worth noting that strong acid rain (pH 2.5) does not occur consistently in southern China. The mean pH values of rainwater in this region vary between pH 4.49 and 3.52 (Cao et al. 2009). Thus, the SAR treatment at pH 2.5 in our experiments did not directly reflect natural conditions. However, the unnaturally acidic soils generated by this treatment did illustrate the influence of very low soil pH on allelochemicals, which may be a useful indicator of what may happen under more extreme acid rain conditions.

These results show that SAR may accelerate litter decomposition of creeping daisy, alter chemical properties and nutrient status of the soil, and increase allelopathic potentials of litters in soils, suggesting that SAR may increase the invasiveness of creeping daisy. Future studies are required to determine effects of SAR on production and release of allelochemicals from creeping daisy.

Acknowledgments

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