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SHORT COMMUNICATION

Aphids decelerate litter nitrogen mineralisation through changes in litter quality

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Abstract. 1. Herbivorous insects may have significant impacts on litter decomposition through modification of plant litter quality and quantity. The effects of herbivorous insects on decomposition processes are of growing interest.

2. Here, experiments were conducted to examine how sap-feeding aphids modify plant litter and whether the aphid-induced modification influences litter decomposition processes, using a plant–herbivore system consisting of soybean [*Glycine max* (L.)] and soybean aphids (*Aphis glycines* Matsumura).

3. First, litter traits produced by aphid-free and aphid-infected plants were compared, and it was found that aphids did not affect litter mass and carbon concentration, but significantly decreased the nitrogen concentration. Such aphid-mediated modification of litter quality may cause deceleration of litter decomposition as the higher C/N ratio inhibits litter decomposition.

4. A decomposition experiment was then carried out to compare the decomposition of litter between the aphid-free and aphid-infected plants. No impacts of aphid herbivory were found on litter carbon mineralisation but negative impacts were found on nitrogen mineralisation. Litter nitrogen mineralisation of aphid-infected plants decreased by 40% and 28% compared with that of aphid-free plants 1 and 3 months after commencement of the experiment, respectively.

5. The experimental results clearly showed that aphids decelerated litter nitrogen mineralisation by modifying litter quality.

Key words. *Aphis glycines*, C/N ratio, decomposition, soybean, trait-mediated interaction.

Introduction

The abundance and species richness of insects are remarkably higher than those of other organisms (Schowalter, 2000), and insects are consequently one of the dominant components of biodiversity in terrestrial ecosystems. In spite of their abundance and their biodiversity, the importance of insects in governing ecosystem functions tends to be underestimated. Herbivorous insects not only consume plant resources, but also provide nutrients to soil via their excrement, and can influence the litter decomposition process by modifying plant traits (Mattson & Addy, 1975; Weisser & Siemann, 2004).

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Insect excrement falls to the soil, where plants absorb it as nutrients after mineralisation (Weisser & Siemann, 2004). This source of nutrients could be a major factor in determining the dynamics of nutrient uptake in plants (Frost & Hunter, 2008). In addition, herbivorous insects can also influence the litter decomposition process through the modification of litter traits (Findlay *et al.*, 1996; Frost & Hunter, 2008). In biological degradation, soil microbial decomposers (free-living soil bacteria and fungi) mineralise plant litter (Wardle, 2002). The tissue of these microbes has a lower C/N ratio than plant litter (Manzoni & Porporato, 2007), indicating that the soil microbes require more nitrogen when they consume the litter. Therefore, plant litter with a lower C/N ratio is more easily mineralised by microbial decomposers (Manzoni *et al.*, 2008). Plants increase or decrease carbon and/or nitrogen in their tissues in response to herbivory (Karban & Baldwin, 1997;

Ohgushi, 2005). Plant traits modified by herbivorous insects often carry over to leaf litter, which in turn influences the decomposition process via litter quality (Hunter, 2001).

Several previous studies have reported the modification of litter quality after leaf herbivory by chewing and gall-making insects, as a result of which litter decomposition was accelerated or decelerated (e.g. Findlay *et al.*, 1996; Chapman *et al.*, 2006). However, to date, the effect of sap-feeding aphids on the litter decomposition process by modifying litter quality is poorly understood (but see Choudhury, 1988; Schweitzer *et al.*, 2005), in spite of the fact that aphids affect soil nutrient dynamics by inputting honeydew into the soil (Choudhury, 1985; Stadler *et al.*, 2004; Wardle, 2002). The aim of this study was to evaluate how aphids modify plant litter traits, and determine whether the aphid-induced litter modification influences the decomposition process. We studied a system consisting of soybean, *Glycine max* (L.) (Fabaceae), and soybean aphids, *Aphis glycines* Matsumura (Hemiptera: Aphididae).

In this study, we conducted two experiments. The first experiment compared the traits of litter (litter mass, carbon, and nitrogen) produced by the aphid-free and aphid-infected plants. The second experiment examined whether aphid-mediated modification of litter traits influences the decomposition process, focusing in particular on litter nitrogen mineralisation.

Materials and methods

Experimental design

On 22nd of September 2008, 200 soybean seeds were planted individually into polyethylene pots (7 cm in diameter, 7 cm in depth) with non-sterilised soil. After 2 weeks, individual seedlings were transplanted into large polyethylene pots (20 cm in diameter, 20 cm in depth, containing 5 l of soil). Seedlings were grown in temperature-controlled greenhouses (25 °C and natural light conditions) until the beginning of the experiment.

One month after germination, 10 potted plants of a similar size were selected. Each pot was covered with a nylon net (mesh-size: 1 mm), to prevent colonisation by other insects. One thousand aphids were inoculated on each of the five plants (aphid-infected plants), and the remaining five plants were left as a control lacking aphids (aphid-free plants). These two treatments were placed randomly in the climate chamber. These plants were adequately watered every day. The number of aphids on each plant was checked every week to maintain the fixed number, and any additional aphids were removed.

One month after aphid inoculation, all aphids were removed from the plants. The plants were maintained until death (5–6 months from cultivation) to collect naturally senesced litter. The collected litter of each plant was stored for drying (24 °C, 26% RH). When all litter had been collected, it was freeze-dried and weighed. Freeze-dried litter (300 mg per sample) was powdered using an electric blender (Wonder Blender, Osaka Chemical, Osaka, Japan). Carbon and nitrogen concentrations in 20 mg of litter were determined using an elemental analyser (CHN Corder MT-3, Yanaco, Kyoto, Japan). The freeze-drying might have an impact on microbial community composition or metabolic activity in the following decomposition experiment, but using the freeze-dry method was unavoidable because we had to store the litter samples until the decomposition experiment, and there is currently no perfect technique available that conserves both chemical properties and microbial community composition and activity in leaf samples.

Litterbag experiment

On 9th of June 2009, 300 mg of each litter sample was placed in an 8 cm × 8 cm polyethylene fabric fine-meshed litter bag. Two samples of each replicate of the aphid-inoculated and aphid-free treatments were prepared, for a total of 20 litter bags (2 treatments × 5 replications × 2 collections). Twenty plastic pots (12 cm in diameter and 10 cm in depth) were filled with 150 g of non-sterilised soil, and each litter bag was placed on the soil surface in a plastic pot. The pots were weighted to check the initial weight. The pots were watered once a week to keep the initial pot weight and soil humidity constant (60%). Then, the pots were placed in an incubator (25 °C, in a dark condition). After 1 and 3 months of incubation, one litter bag from each replicate was collected. The litter in the bag was freeze-dried, and dry mass was measured. The litter was then ground to a powder, and carbon and nitrogen concentrations in 20 mg of dry litter were determined using the elemental analyser.

The relative amount of litter mass remaining in the litter bags (RLM, %) was calculated using the following formula:

$$\mathrm{RLM}\ (\%) = \mathrm{LM}_t/\mathrm{LM}_0 \times 100$$

LM_0 and LM_t represent the initial litter mass (300 mg) and the litter mass of the t-th collection, respectively. In addition, the loss of carbon mass (LCM, mg) and of nitrogen mass (LNM mg), and the relative amounts of carbon mass (RCM, %) and of nitrogen mass (RNM, %) were calculated using the following formula:

$$\mathrm{LCM\ (mg)\ or\ LNM\ (mg)} = [\mathrm{conc}]_0 \times \mathrm{LM}_0 - [\mathrm{conc}]_t \times \mathrm{LM}_t$$

$$\mathrm{RCM\ (\%)\ or\ RNM\ (\%)} = [\mathrm{conc}]_t \times \mathrm{LM}_t/[\mathrm{conc}]_0 \times \mathrm{LM}_0 \times 100$$

$[\mathrm{conc}]_0$ represents the initial concentration of litter carbon or nitrogen, and $[\mathrm{conc}]_t$ represents the concentration of the t-th collection.

We arcsine-transformed the percentage data (RLM, RCM and RNM) to satisfy the assumptions of repeated measures ANOVA. As one litter bag in the aphid-free treatment was accidentally lost during the experiment, it was removed from statistical analyses.

Results and discussion

Although aphids did not affect litter quantity (dry mass of dried leaves of a plant) (T-test, $t_8 = 1.32$, $P = 0.225$) and carbon concentration ($t_8 = 0.33$, $P = 0.747$), they decreased the nitrogen

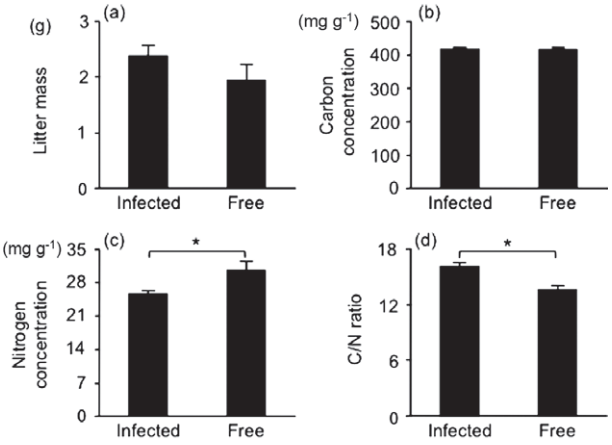

Fig. 1. Quantity and quality of litter produced by aphid-infected and aphid-free plants. (a) Dry mass, (b) carbon concentration, (c) nitrogen concentration, and (d) C/N ratio. Asterisks represent significant differences between treatments (*$P < 0.05$). Error show ± 1 SE.

concentration ($t_8 = 2.56$, $P = 0.034$; Fig. 1a–c). As a result, the litter C/N ratio was greater under aphid-infected plants than under aphid-free plants ($t_8 = 2.90$, $P = 0.020$; Fig. 1d). The reduction in litter nitrogen may be as a result of the effects of aphids decreasing the nitrogen uptake of plants. Aphids add droplets of sugar-rich honeydew into soil. The honeydew droplets increase the abundance of belowground microbes, which results in a decrease in soil nitrogen available for plants (Wardle, 2002; Stadler *et al.*, 2004). In addition, aphids consume the photosynthetic carbon which is needed for plants to maintain their symbiotic association with below-ground microbes (Macedo *et al.*, 2003). The plants studied here, soybeans, have a symbiotic association with nitrogen-providing rhizobia. Aphid herbivory may decrease nitrogen uptake from the rhizobia by weakening the plant–rhizobia association.

Litter mineralisation may be decelerated by such aphid-mediated modification as a higher C/N ratio in litter generally inhibits decomposition (Bragazza *et al.*, 2007; Manzoni *et al.*, 2008). The decomposition experiment illustrated that aphid-induced litter modification did not affect the temporal changes in relative litter mass ($F_{1,7} = 3.30$, $P = 0.111$; Fig. 2a). This is because the mineralisation of litter carbon, which comprised the majority of litter mass, was not affected by the aphid-induced modification (LCM: $F_{1,7} = 0.06$, $P = 0.814$; RCM: $F_{1,7} = 0.14$, $P = 0.718$; Fig. 2b). In contrast, the aphid-induced modification affected nitrogen mineralisation in the litter (LNM: $F_{1,7} = 93.90$, $P = 0.001$, RNM: $F_{1,7} = 36.56$, $P < 0.001$; Fig. 2c). Nitrogen decreased by 50%, compared with the initial nitrogen content, in the litter of aphid-free plants over the course of 1 month. In contrast, 70% of nitrogen was retained in the litter produced by aphid-infected plants after 1 month, and even after 3 months, 64% of nitrogen remained. These results suggest that aphids decelerated litter nitrogen mineralisation by decreasing litter quality.

Many researchers have reported that aphids cause plant damage, which results in low growth rates and even death, and a decrease in ecosystem productivity or vegetable yields

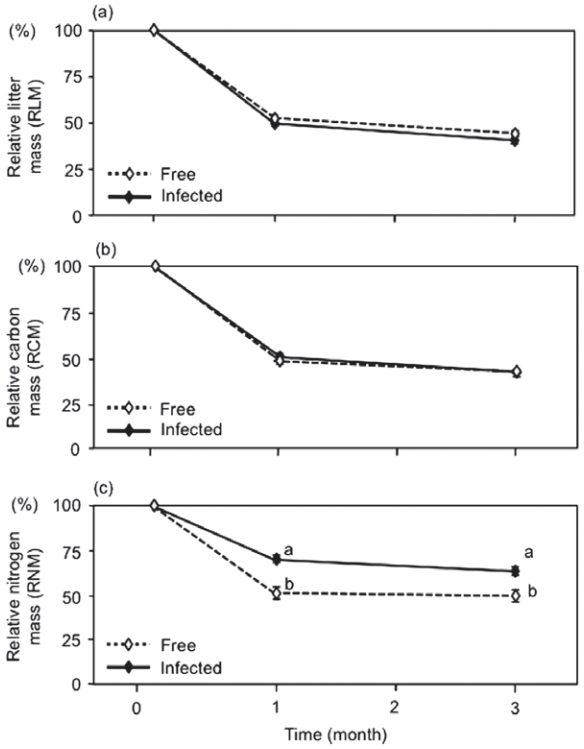

Fig. 2. Decomposition process during the experiment. (a) Relative litter mass (RLM, %), and (b) relative carbon mass (RCM, %), and (c) relative nitrogen mass (RNM, %) of the litter. Different letters in (c) represent significant differences among treatments (Tukey and Kramer test, $P < 0.05$). Open diamonds with dotted lines and solid diamonds with solid lines indicate litter from aphid-free and aphid-infected plants, respectively. Error bars show ± 1 SE.

(reviewed by Dixon, 1998). However, we know little about the effects of aphids on the process of litter decomposition. Our results suggest that aphids could significantly influence litter decomposition via the modification of litter quality. This study is of value for future studies examining aphid effects on nutrient flux in ecosystems.

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