

**Title:** Community-wide effects of belowground rhizobia on aboveground herbivore and predator arthropods

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**Running title:** Rhizobia effects on arthropod community

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**Abstract.** 1. Plants take nutrients for their growth and reproduction from not only soil but also symbiotic microbes in the rhizosphere, and therefore, belowground microbes may indirectly influence the aboveground arthropod community through changes in the quality and quantity of plants.

2. Rhizobia are root-nodulating bacteria that provide  $\text{NH}_4^+$  to legume plants. We examined the effects of rhizobia on the structure of the arthropod community on host plants, using 28 pots of a root-nodulating soybean strain (*Glycine max* L.: R+) and 48 pots of a non-nodulating strain (R-) in a common garden.

3. R+ plants grew larger and produced a greater number of leaves than R- plants. We observed 33 and 29 arthropod species on R+ and R- plants, respectively. They were classified into sap feeders (12 species), chewers (16 species), predators (3 species), and unknown (5 species).

4. The species richness and abundance of herbivorous arthropods on R+ plants were greater than those on R- plants. Rhizobia positively affected the abundance of both sap feeder and chewer herbivores. The community composition of arthropod herbivores was significantly different between R- and R+ plants.

5. Likewise, the abundance and species richness of the predators on R+ plants were greater than those on R- plants.

6. Greater species richness of herbivores increased the species richness and abundance of predators. Greater abundance of herbivores increased the species richness of predators, although it did not influence predator abundance.

7. These results indicate that aboveground arthropod communities were largely driven

by the belowground microbes.

**Key words.** Above- and belowground interactions, bottom-up effects, community structure, *Glycine max*, rhizobia, species richness

## Introduction

Ecological communities are structurally organized by complex networks of direct and indirect interactions (Ohgushi, 2005; Bascompte & Jordano, 2007; Ings *et al.*, 2009). It has been argued that top predators principally regulate the population or community dynamics of insect herbivores (Lawton & Strong, 1981; Strong *et al.*, 1984; Schmitz *et al.*, 2000; Finke & Denno, 2004). This top-down view in community ecology has been strongly influenced by “the green world hypothesis” (Hairston *et al.*, 1960), arguing that herbivores are not resource-limited. On the other hand, recent studies have revealed the prominent role of bottom-up effects of plants on the population and community dynamics of herbivores (Hunter *et al.*, 1992; Ohgushi, 1992; Price & Hunter, 2005; Crutsinger *et al.*, 2006; Ohgushi *et al.*, 2007; Walker *et al.*, 2008). More recently, there is increasing appreciation that bottom-up effects initiated by herbivore-induced plant responses have a community-wide impact on arthropods in terrestrial systems (e.g. Van Zandt & Agrawal, 2004; Ohgushi, 2005, 2008; Utsumi *et al.*, 2009). Herbivore-induced changes in a wide range of plant traits, such as C/N ratio, secondary metabolic substances, volatile compounds, leaf toughness, and secondary regrowth, have a great impact on the organization of arthropod communities through subsequent changes in performance, preference, and population dynamics of the herbivorous insects involved (Ohgushi, 2005).

The bottom-up effects can extend to higher trophic levels, depending on the plant traits in terrestrial systems (Hunter & Price, 1992; Siemann, 1998; Kagata &

Ohgushi, 2006; Chaneton & Omacini, 2007; Bukovinszky *et al.*, 2008; Utsumi & Ohgushi, 2009). For example, Nakamura *et al.* (2006) examined the regrowth of willows following artificial cutting and subsequent effects on the abundance and species richness of associated arthropods. The artificial damage induced the plants to produce a greater number of new leaves with higher water and nitrogen contents, which, in turn, increased the abundance and species richness of not only herbivorous but also predaceous arthropods. Thus, the bottom-up effect initiated by trait change in plants can alter the community structure of higher trophic levels.

Previous studies on the determination of community structure of arthropods have mainly focused on aboveground interactions (Hunter & Price, 1992; Hunter *et al.*, 1992). Recently, we have begun to pay more attention to how belowground interactions influence aboveground ecological processes (Van der Putten *et al.*, 2001; Wardle, 2002; Hartley & Gange, 2009). This is because belowground microbes also change a wide variety of plant traits (Gange & West, 1994; Gange, 2007). Plants take nutrients for their growth and reproduction from not only soil but also symbiotic microbes in the rhizosphere (Smith & Read, 1997). Symbiotic belowground microbes (mycorrhizal fungi and/or nitrogen-fixing bacteria) provide nitrogen and phosphorus to host plants, and plants in return provide photosynthetic carbon to microbial symbionts. Plants use these nutrients for their growth, reproduction, and defense. There is a growing body of evidence that mycorrhizal fungi positively or negatively affect the performance of aboveground arthropods (Hartley & Gange, 2009). These studies suggest that the belowground microbial effects can extend upward to higher trophic

levels associated with a plant (Gange *et al.*, 2003; Bennett *et al.*, 2006; Hartley & Gange, 2009). Although several studies recently demonstrated bottom-up effects of belowground microbes on aboveground interactions at the species level (Gange *et al.*, 2003; Gange, 2007; Kempel *et al.*, 2009), we know little about how belowground microbes drive bottom-up effects to higher trophic levels in a community context.

Rhizobia are root-nodulating bacteria that have obligate symbiosis with legume plants, and live in root nodules that appear as small growths on legume roots. Rhizobia synthesize nitrogen compounds ( $\text{NH}_4^+$ ) from  $\text{N}_2$  in the atmosphere, and provide them to a host plant. In the root nodulation, there is a reciprocal signaling system between the rhizobia and legume hosts (Miklashevichs *et al.*, 2001). The nodulating process is regulated by multiple nod genes of the legumes and rhizobia (van Rhijn & Vanderleyden, 1995). Therefore, legume mutants lacking nod genes cannot produce root nodules. Recently, Kempel *et al.* (2009) documented the positive effects of rhizobia on the body weight of lepidopteran caterpillars and colony size of aphids in a greenhouse experiment using root nodulating and non-nodulating clovers (*Trifolium repens* L.). However, no studies to date have investigated the effects of rhizobia on biodiversity components of aboveground herbivorous and predacious arthropods. We carried out a common garden experiment to examine the effects of rhizobia on the species richness, abundance, and community composition of aboveground arthropods on soybean, using a root-nodulating soybean strain (*Glycine max* L. cv. Fujimishiro) and a non-nodulating strain (cv. Touzan No. 90).

In this study, we specifically addressed the following questions. (1) Do

abundance, species richness, and community structure of aboveground arthropods differ between root-nodulating strains and non-nodulating strains counterparts? (2) Do rhizobia affect sap feeders and chewers in a different way? and (3) Do effects of rhizobia expand upward to predators in a community-context?

## **Material and methods**

### *Materials*

Soybean is an annual legume plant native to East Asia. In central Japan, seeds are sown in late June to early July, and begin to bear flowers in August. In September, soybean produces pods which gradually mature over the autumn. Several bacteria species, including *Bradyrhizobium japonicum*, *B. elkani*, and *Rhizobium fredii*, form root nodules on soybean roots. In this study, we used two soybean strains to compare the effects of rhizobia. One was a root nodulating strain (cv. Fujimishiro: R+) and the other is a non-nodulating strain (cv. Touzan No 90: R-). Touzan No. 90 was made by backcrossing to Fujimishiro after crossing between Fujimishiro and T201 (another non-nodulating soybean (K. Takahashi, personal communication)). T201 has a mutation in the *rj1* locus, which is responsible for root hair-curling when taking rhizobia into the root (Williams & Lynch, 1954; Mathews *et al.*, 1987; Suganuma *et al.*, 1991). Therefore, Touzan No. 90 is closely related to Fujimishiro except for root nodulation. In another potted plant experiment, Katayama *et al.* (in press) showed that

the number of root nodules of the R+ plants were  $82.4 \pm 8.6$  (mean  $\pm$  SE,  $n = 15$ ), but there were no nodules on the roots of the R- plants ( $n = 24$ ). Also, foliar nitrogen and phenolics of the R+ plants were 50% higher and 12% lower than those of the R- plants in the presence of rhizobia.

### *Experimental design*

We carried out a common garden experiment to examine the effects of rhizobia (R) on arthropod community structure. In May 2006, 100 and 200 seeds of R+ and R- soybeans, respectively, were sown into polyethylene pots with a diameter of 7 cm and depth of 6.5 cm and the pots were placed outside. These seeds were provided by the Laboratory of Plant Breeding of the Faculty of Agriculture, Kyoto University. Two weeks after the emergence of seedlings, we removed non-germinated seeds or badly dwarfed seedlings, and transplanted each healthy seedling into an unglazed pot (24 cm in diameter and 20 cm in depth) filled with a 1:1 mixture of black soil and sand. The black soil and sand are low in nutrients (available nitrogen (ammonium-N + nitrate-N) concentration:  $55 \pm 25$   $\mu\text{g}/\text{soil-g}$  (mean  $\pm$  SE,  $n = 10$ )), but may contain belowground microbes including rhizobia. Since we focused on overall effects of rhizobia rather than species-specific effects of rhizobia, we cultivated R+ and R- soybeans in unsterilized soil, without inoculating specific rhizobium species. We added ammonium sulphate ( $5 \text{ g}/\text{m}^2$ ) to all pots to adjust to a normal soil nitrogen level for cultivation of agricultural soybeans.



We selected 29 pots of R+ plants and 64 pots of R- plants that grew normally and randomly placed them in six rows in a common garden of the Center for Ecological Research of Kyoto University (34°58'17"N, 135°57'32"E, Otsu, Japan). The rows were spaced at 80-cm intervals, and the pots within a row were spaced at 80-cm intervals. We had cleared all plants growing in the garden before we placed the pods. As additional fertilization, we applied ammonium sulphate (10 g/m<sup>2</sup>) to all potted R+ and R- plants three times on August 8, August 22, and September 5.

From June 21 to September 28 we conducted 27 censuses at 3-4 day intervals on average to determine both arthropod abundance and species richness per plant. In the first census, we took one individual of each morphologically distinct species, and brought them to a laboratory for identification. Then, we counted the number of arthropods on each plant. In later censuses, we counted the number of each identified arthropod species without capturing. When we found an unidentified species, one individual was collected for identification. At 6-13-day intervals, we measured plant height and number of leaves as indicators of plant growth. We recorded the number of individuals of each arthropod species and number of species (species richness) on each plant in each census.

During the season, we observed 33 and 29 arthropod species on R+ and R- plants, respectively (Appendix: Table S1). These species were classified into sap feeders (12 species), chewers (16 species), and predators (3 species). Five arthropod species could not be classified into any guild, and they were excluded from the analysis. For each arthropod species, the number of individuals on each plant was summed and

the number of species (species richness) was pooled for all 27 census data.

Since we excluded dead plants during the experiment, the replications of R+ and R- plants were 28 and 48, respectively.

### *Statistical procedures*

A repeated measures ANOVA was used to compare plant height and leaf number between R+ and R- plants. The species richness and the cumulative number of individuals of each guild were compared using a t-test between R+ and R- plants.

We calculated the log response ratio of the abundance of each herbivore species to compare the strength of rhizobia effects on sap feeders and chewers. Log response ratio is widely used to compare effect sizes in manipulation experiments (Hedge *et al.*, 1999). When the value is  $< 0$ , the effect is negative relative to the control, and when the value is  $> 0$ , the effect is positive. In our study, the log response ratio of the abundance of  $i$ -th herbivore species was obtained as follows:

$$\text{log response ratio} = \log_e [(n_{R+i}+1)/(n_{R-i}+1)]$$

$n_{R+i}$  indicates the cumulated number of individuals of the  $i$ -th arthropod species on R+ plants, and  $n_{R-i}$  indicates the cumulated number of the individuals of the  $i$ -th arthropod species on R- plants. The average log response ratio was compared using a t-test between sap feeders and chewers.

In comparing the community structure of herbivorous arthropods between R+ and R- plants, we calculated a standardized value to prevent common species from swamping less abundant species (Whitham *et al.*, 1994). The relative abundance value for each species was expressed by  $\log_e(n+1)$ -transformed numbers. We calculated  $\log_e(n+1)$ -transformed average cumulative number of each arthropod species per plant. Then, the log-transformed data were divided by the values of total log-transformed number of arthropods so that all species were weighed equally. Bray–Curtis dissimilarity matrices were calculated for the dissimilarity in the species composition among plants. ANOSIM was used to test the difference in the dissimilarities between R+ and R- plants. SIMPER was used to examine the percentage contribution of sap feeders and chewers to the dissimilarities of the herbivore community between R+ and R- plants.

To examine whether treatment differences in herbivore richness were driven by difference in arthropod abundance, we constructed rarefaction curves to correct for biases in species richness that arise from differences in the number of individuals (Gotelli & Colwell, 2001). We used the cumulative abundance of each species within each treatment (Ecosim 7.72, 10,000 iterations; Gotelli & Entsminger, 2004).

Linear regression analysis was conducted to reveal the relationships between species richness of predators and species richness or abundance of herbivores, and between abundance of predators and species richness or abundance of herbivores.

## **Results**

### *Plant growth*

From late June to early August, R+ and R- plants grew gradually and reached a peak height thereafter (repeated measures two way ANOVA, time:  $F_{12,61} = 220.35$ ,  $P < 0.001$ ). R+ plants were significantly larger than R- plants ( $F_{1,72} = 4.63$ ,  $P < 0.001$ ). The average height of R+ and R- plants on 28 September was  $34.9 \pm 1.0$  and  $31.5 \pm 0.8$  (mean  $\pm$  SE), respectively. Similarly, the leaf number of both kinds of plants increased throughout the season (repeated measures two way ANOVA, time:  $F_{12,61} = 246.92$ ,  $P < 0.001$ ). The leaf number of R+ plants was significantly greater than that of R- plants ( $F_{1,72} = 11.73$ ,  $P = 0.001$ ). The average leaf number of R+ and R- plants on 28 September was  $41.0 \pm 1.5$  and  $34.6 \pm 1.2$  (mean  $\pm$  SE), respectively.

### *Richness and abundance of herbivorous arthropods*

The species richness of sap feeders and chewers on R+ plants were significantly greater than those on R- plants (t-test, sap feeders:  $t_{70} = 7.45$ ,  $P < 0.001$ ; chewers:  $t_{70} = 8.73$ ,  $P < 0.001$ ; Fig. 1A, B). Although the number of sap feeder individuals did not significantly differ between R+ and R- plants, the number of chewers on R+ plants was significantly greater than that on R- plants (t-test, sap feeders:  $t_{70} = 1.21$ ,  $P = 0.232$ ; chewers:  $t_{70} = 8.34$ ,  $P < 0.001$ ; Fig. 1C, D). Among sap feeders, *Aphis glycines* (Hemiptera) was the most dominant species, accounting for 84 % of all

sap feeders (Appendix Table S1). The number of *A. glycines* did not differ between R+ and R- plants (t-test,  $t_{70} = 0.86$ ,  $P = 0.393$ ), but the number of other sap feeders was significantly greater on R+ plants than on R- plants (t-test,  $t_{70} = 3.18$ ,  $P = 0.002$ ).

The log response ratios of the abundance of both sap feeders and chewers were significantly greater than 0 ( $P < 0.05$ ), but no difference was found between them (t-test,  $t_{26} = 0.68$ ,  $P = 0.500$ ; Fig. 2). This indicates that the intensity of rhizobial effect did not differ between sap feeders and leaf chewers.

The herbivore community composition on R+ plants differed significantly from that on R- plants (ANOSIM, global  $R = 0.485$ ,  $P < 0.001$ ). The percentage contributions of sap feeders and chewers to the dissimilarities of the herbivore community between R+ and R- plants were 43% and 57%, respectively.

The rarefaction curves indicated that rarefied estimates for R+ plants were significantly greater than those for R- plants in the range of more than 800 individuals. Thus, the increased species richness of herbivores on R+ plants was not due to different sampling efforts.

#### *Richness and abundance of predators*

We found three predator groups: an ant (*Formica japonica*), a ladybird beetle (*Coceinaella septempunctata*), and spiders (Appendix: Table S1). The species richness and abundance of predators on R+ plants were greater than those on R- plants (t-test, species richness:  $t_{70} = 2.97$ ,  $P = 0.004$ ; number of individuals:  $t_{70} = 3.56$ ,  $P < 0.001$ ; Fig.

4).

Species richness of predators increased with increases in species richness and abundance of herbivores (species richness of herbivores: slope =  $0.133 \pm 0.07$  (mean  $\pm$  95% CI),  $t_{70} = 3.88$ ,  $P < 0.001$ , abundance of herbivores: slope =  $0.0026 \pm 0.0021$ ,  $t_{70} = 2.38$ ,  $P = 0.020$ ; Fig. 5A, B). Although the abundance of predators increased with an increase in species richness of herbivores (slope =  $0.469 \pm 0.268$ ,  $t_{70} = 3.49$ ,  $P < 0.001$ ; Fig. 5C), it was not affected by the abundance of herbivores (slope =  $0.0064 \pm 0.0084$ ,  $t_{70} = 1.51$ ,  $P = 0.135$ ; Fig. 5D).

## Discussion

This is the first study to illustrate the effects of belowground rhizobia on biodiversity components of aboveground arthropods. R+ plants were larger and produced a greater number of leaves than R- plants. The species richness and abundance of herbivorous arthropods on R+ plants were significantly greater than those on R- plants. The strength of the positive effects of rhizobia on abundance did not differ between sap feeders and chewers. Furthermore, the herbivore community composition was significantly different between R- and R+ plants. Rhizobia also increased the species richness and abundance of predators. In general, species richness and/or abundance of predators were positively correlated with those of herbivores. Thus, our results suggest that belowground rhizobia largely govern not only herbivore but also predator aboveground arthropods.

Removing rhizobia is not practically possible in a common garden experiment, because soil bacteria easily colonize soybeans in the field. Therefore, we used a non-nodulating strain to evaluate the effects of rhizobia on aboveground arthropods. Likewise, Kempel *et al.* (2009) examined the effects of rhizobia on the performance of aboveground lepidopteran caterpillars and aphids, using a non-nodulating mutant of white clover. In another common garden experiment, R+ plants had many root nodules ( $82.4 \pm 8.6$ : mean  $\pm$  SE), while R- plants had no nodules (Katayama *et al.*, in press). Therefore, the observed differences in the community properties of aboveground arthropods are more likely to be caused by belowground rhizobia, although the possibility that other plant traits of the non-nodulating strain may have affected the abundance or species richness of arthropods was not excluded.

#### *Effects of rhizobia on richness and abundance of herbivores*

Recent studies have paid much attention to the effects of belowground symbiotic microbes on aboveground plant-arthropod interactions (Gange, 2007; Hartley & Gange, 2009). For example, the body weight of aboveground herbivorous arthropods can be positively or negatively affected by arbuscular mycorrhizal fungi (Goverde *et al.*, 2000; Vicari *et al.*, 2002). In a meta-analysis using 34 studies, Koricheva *et al.* (2009) showed that mycorrhizal fungi colonization decreased the abundance or body weight of mesophyll feeders, but increased those of sucking insects. There is increasing evidence that mycorrhizal fungi influence the survival or abundance

of aboveground arthropods (Hartley & Gange, 2009). However, to date we know little about the effects of nitrogen fixing-bacteria on aboveground arthropods (but see Kempel *et al.*, 2009). Our study showed that rhizobia increased the abundance of aboveground leaf chewers, but not of sap feeders. This was because the most abundant sap feeders, *A. glycines*, was not affected by rhizobia, although other sap feeders increased. Our study showed that the strength of the positive effect of rhizobia did not differ between sap feeders and leaf chewers.

Several studies have experimentally documented that increased plant biomass can increase the abundance of a wide variety of arthropod herbivores (Siemann, 1998; Forkner & Hunter, 2000; Fonseca *et al.*, 2005). Rhizobia increased plant biomass in terms of height and leaf number. Also, rhizobia increased leaf nitrogen of soybeans by 50 % and decreased phenolics by 12 % (Katayama *et al.*, in press). Nitrogen is an essential limiting element for survival and/or growth of many herbivorous arthropods (Mattson & Scriber, 1987; White, 1993). For example, the leaf beetle *Plagioderma versicolora* Laicharting (Coleoptera: Chrysomelidae) had a significantly greater mass and fecundity when fed on new willow leaves with high nitrogen content than when fed on mature leaves with low nitrogen content (Utsumi & Ohgushi, 2008). Leaf phenolics are defensive substances against arthropod herbivores (Feeny, 1970; Larson & Berry, 1984; Dudt & Shure, 1994). Actually, egg production of a spider mite (*Tetranychus urticae* Koch) increased when it was fed on R+ soybean leaves with greater nitrogen and low phenolics (Katayama *et al.*, in press). If higher performance of herbivorous arthropods will increase their abundance (Hunter *et al.*, 1996), the increased resource



availability derived from rhizobia is likely to increase the abundance of herbivorous arthropods associated with the plant.

This study also found that the species richness of herbivores on R+ plants was significantly greater than that on R- plants. Heterogeneity of nitrogen and secondary metabolic compounds of plants may explain the increased diversity of herbivores (Hunter & Price, 1992; Utsumi *et al.*, 2009). Utsumi *et al.* (2009) examined the effect of variation of leaf quality of willow trees using artificial cuttings and natural boring by a moth larva. The enhanced heterogeneity of leaf nitrogen of trees that were subjected to moderately artificial cutting and attack by the stem borer increased the overall abundance and diversity of insect herbivores. However, it is unknown whether rhizobia increase the heterogeneity of plant quality because our study did not examine the variation in quality among plants.

#### *Effects of rhizobia on richness and abundance of arthropod predators*

Recent studies have revealed that plastic responses of plants following herbivory affect the third trophic level via changes in the second trophic level (Omacini *et al.*, 2001; Nakamura *et al.*, 2006; Kagata & Ohgushi, 2006; Utsumi *et al.*, 2009; Utsumi & Ohgushi, 2009). For example, Utsumi *et al.* (2009) showed that herbivore-induced willow regrowth largely determined the entire arthropod community structure due to changes in plant quality. Our study revealed the bottom-up effects initiated by rhizobia, leading to increased species richness and abundance of predators.

There are several explanations for the bottom-up effects of plants resulting in an increase in abundance and/or species richness of predators. First, plants show increased herbivore abundance, which may in turn increase the abundance and/or species richness of predators (Siemann, 1998; Knops *et al.*, 1999; Forkner & Hunter, 2000). This is because a greater number of prey species of predators can aggregate when prey becomes abundant (Ives *et al.*, 1993; Cardinale *et al.*, 2006). Second, plants show increased species richness of herbivores, which may provide a wider range of prey items for generalist predators (Hunter & Price, 1992). Our results generally support the notion that the species richness and abundance of predators are greatly dependent on those of herbivores. In fact, species richness of herbivores was associated with increased species richness and abundance of predators. Also, greater abundance of herbivores was associated with increased species richness of predators, although it did not influence predator abundance.

The abundance and species richness of predaceous arthropods are also influenced by plant architecture, because a complex architecture provides shelter, and foraging and/or oviposition sites for arthropods (Langellotto & Denno, 2004; Denno *et al.*, 2005). Note that this is a direct effect of plant architecture on predators, not an indirect effect through a change in herbivores. Rhizobia may enhance the structural plant complexity by increasing plant biomass, which may result in an increase in the abundance and richness of predators by providing favorable habitats.

Many terrestrial plants harbor belowground symbiotic microbes. These microbes can largely modify biomass, nutrient conditions, and defensive compounds

(Gange & West, 1994), which can modify aboveground multi-trophic interactions (Gange, 2007; Hartley & Gange, 2009). To demonstrate the belowground microbial effects on aboveground multi-trophic interactions, previous studies have mainly focused on species-level interactions with a single species at each trophic level (Gange *et al.*, 2003; Bennett *et al.*, 2006). In this study, we illustrated that community-level bottom-up effects were initiated by belowground rhizobia. To our knowledge, this is the first evidence demonstrating a strong impact of belowground microbes on biodiversity of aboveground herbivorous and predacious arthropods. To understand the structure of arthropod communities on plants, we should pay more attention to belowground microbes as strong drivers generating bottom-up effects in a multi-trophic context.

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## Figure legends

**Fig. 1.** Species richness of (A) sap feeders and (B) chewers, and number of individuals of (C) sap feeders and (D) chewers on an individual plant during the whole census. Bars show SE. Asterisks indicate significant difference between R+ and R- plants (*t*-test, \* $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ ).

**Fig. 2.** Log response ratio of abundance of sap feeders and chewers. Bars show 95% CI. There was no significant difference between R+ and R- plants (*t*-test,  $P > 0.05$ ).

**Fig. 3.** Rarefaction curves between number of individuals and herbivore richness. We simulated herbivore richness from 10,000 re-sampling iterations using Ecosim 7.72 (Gotelli & Entsminger, 2004). Solid and open circles indicate the root nodulating (R+) and non-nodulating (R-) plants, respectively. Bars show 95% CI. Asterisks indicate significant difference in species richness between R+ and R- plants at the level of the same individual ( $P < 0.05$ ).

**Fig. 4.** (A) Species richness and (B) number of individuals of predators during the whole census. Bars show SE. Asterisks indicate significant difference between R+ and R- plants (*t*-test, \* $P < 0.05$ , \*\*  $P < 0.01$ , and \*\*\*  $P < 0.001$ ).

**Fig. 5.** Relationships between (A) species richness of predators and herbivores, (B) predator species richness and herbivore abundance, (C) predator abundance and herbivores species richness, and (D) abundance of predators and herbivores. Solid lines indicate linear regressions (A: slope =  $0.133 \pm 0.07$  (mean  $\pm$  95% CI),  $P < 0.001$ ; B: slope =  $0.0026 \pm 0.0021$ ,  $t_{70} = 2.38$ ,  $P = 0.020$ ; C: slope =  $0.469 \pm 0.268$ ,  $P < 0.001$ ).

Fig. 1

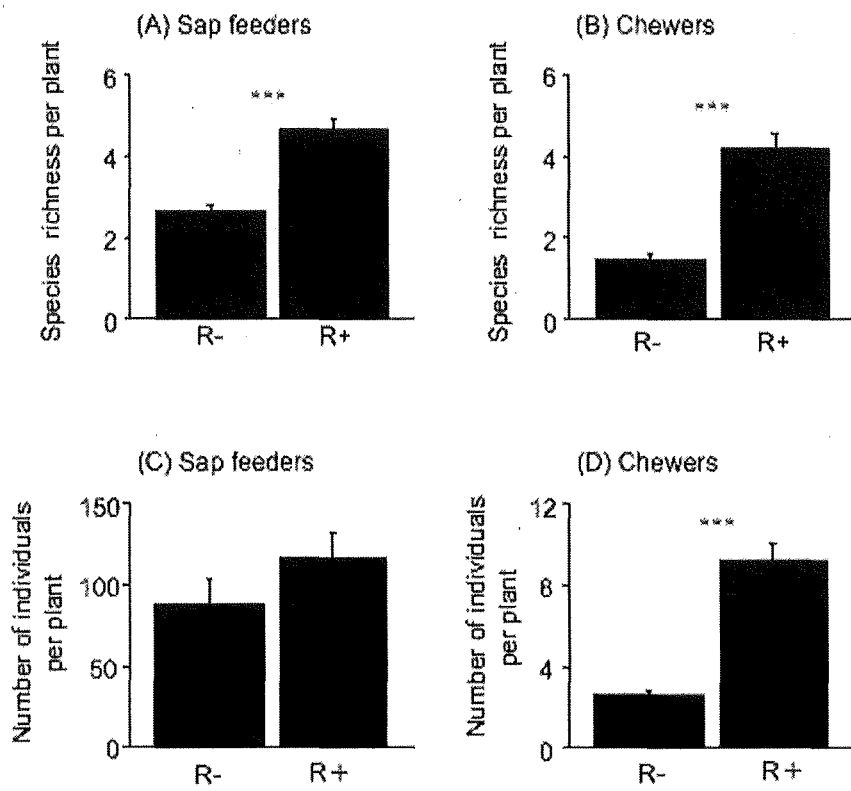


Fig. 2

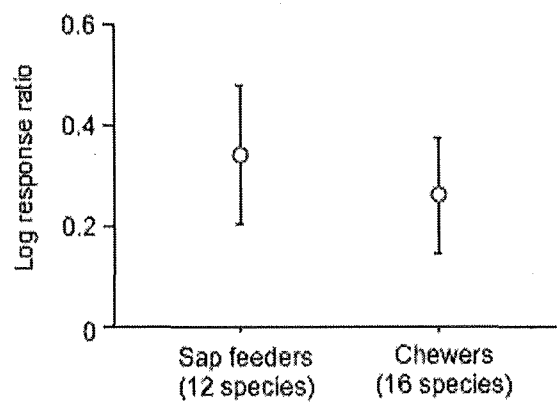




Fig. 3

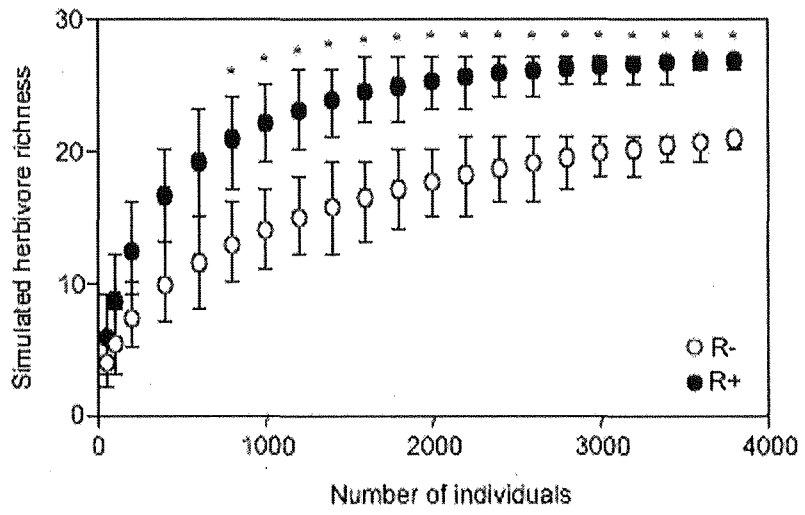


Fig. 4

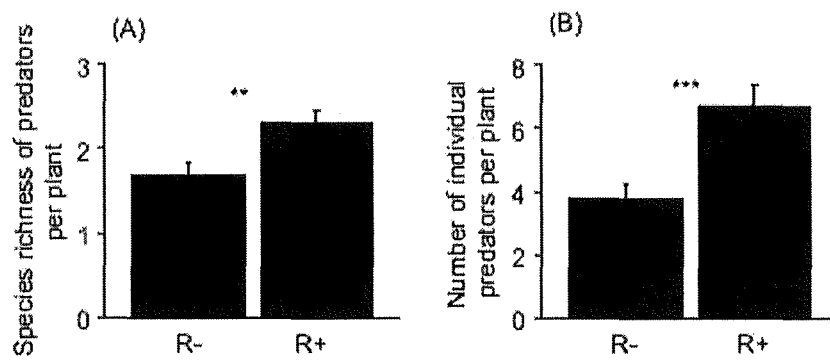


Fig. 5

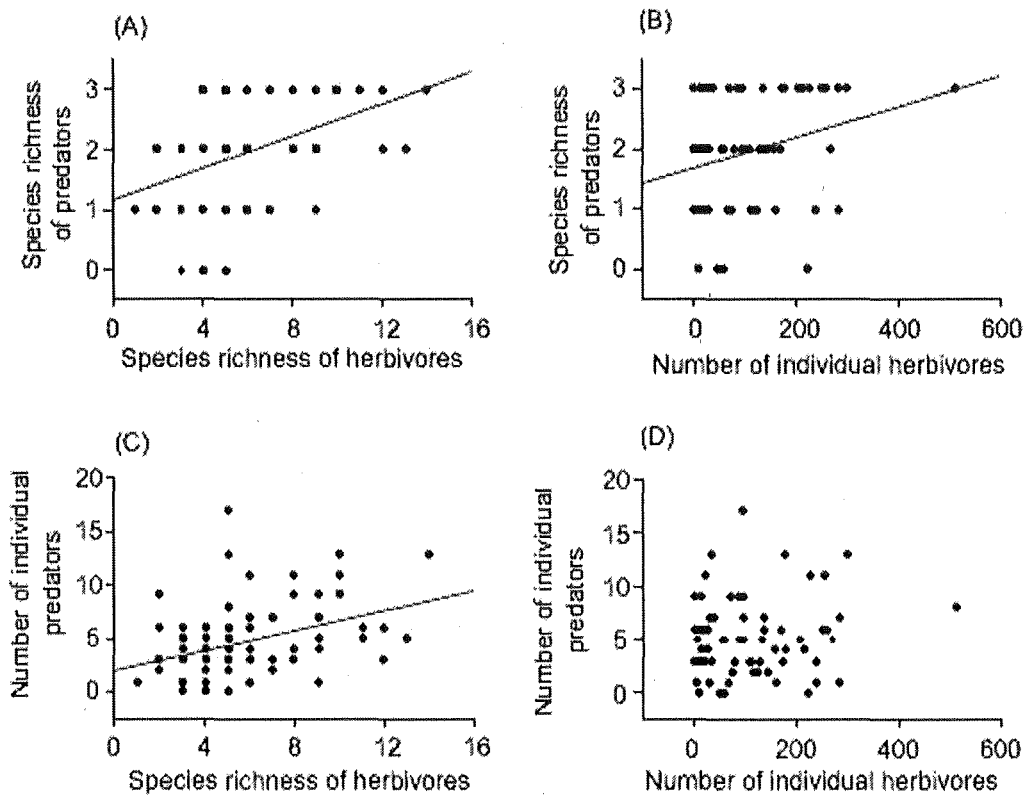


Table S1. Arthropod species found on soybeans in each treatment.

Order	Family	Species	Guild	Total observed number during the survey	Plant type	
					R+	R-
Acari	Tetranychidae	<i>Tetranychus ludeni</i>	Chew	49	Y	Y
Araneae		spp.	Predator	162	Y	Y
Coleoptera	Chrysomelidae	<i>Aulacophora indica</i>	Chew	2	Y	Y
Coleoptera	Chrysomelidae	<i>Medythia nicrobilineata</i>	Chew	59	Y	Y
Coleoptera	Chrysomelidae	<i>Monolepta dichroa</i>	Chew	1		Y
Coleoptera	Coccinellidae	<i>Coccinella septempunctata</i>	Predator	86	Y	Y
Coleoptera	Elateridae	sp. 1	Unknown	11	Y	Y
Coleoptera	Phalacridae	sp. 1	Unknown	2		Y
Diptera	Culicidae	sp. 1	Unknown	1		Y
Diptera		sp. 1	Unknown	45	Y	Y
Hemiptera	Alydidae	<i>Riptortus clavatus</i>	Sap	93	Y	Y
Hemiptera	Aphididae	<i>Aphis glycines</i>	Sap	6326	Y	Y
Hemiptera	Berytidae	<i>Yemma exilis</i>	Sap	5	Y	
Hemiptera	Cicadellidae	<i>Bothrogonia ferruginea</i>	Sap	9	Y	
Hemiptera	Coreidae	<i>Homoeocerus dilatatus</i>	Sap	24	Y	Y
Hemiptera	Coreidae	<i>Homoeocerus unipunctatus</i>	Sap	17	Y	Y
Hemiptera	Malcidae	<i>Chauliops fallax</i>	Sap	14	Y	Y
Hemiptera	Pentatomidae	<i>Halymorpha halys</i>	Sap	5	Y	Y
Hemiptera	Pentatomidae	<i>Piezodorus lybneri</i>	Sap	53	Y	Y
Hemiptera	Pentatomidae	<i>Plautia crossota</i>	Sap	3	Y	
Hemiptera	Pentatomidae	sp. 1	Sap	6	Y	Y
Hemiptera	Plataspidae	<i>Megacopta punctatissima</i>	Sap	572	Y	Y
Hymenoptera	Apistidae	sp. 1	Unknown	4	Y	Y
Hymenoptera	Formicidae	<i>Formica japonica</i>	Predator	104	Y	Y
Lepidoptera	Geometridae	<i>Ascolis selenaria</i>	Chew	12	Y	Y
Lepidoptera	Lymantriidae	<i>Cifana locuples</i>	Chew	5	Y	Y
Lepidoptera	Lymantriidae	<i>Oryzia thyellina</i>	Chew	10	Y	Y
Lepidoptera	Noctuidae	<i>Mamestra brassicae</i>	Chew	3	Y	
Lepidoptera	Pieridae	<i>Cotias erate</i>	Chew	10	Y	
Lepidoptera	Tortricidae	<i>Matsucorhynchus falcatus</i>	Chew	10	Y	Y
Lepidoptera		sp. 1	Chew	34	Y	Y
Orthoptera	Acrididae	<i>Acrida cinerea</i>	Chew	21	Y	Y
Orthoptera	Acrididae	<i>Eurenophila longipennis</i>	Chew	13	Y	Y
Orthoptera	Catantopidae	<i>Oxya japonica</i>	Chew	1	Y	
Orthoptera	Pyrgomorphidae	<i>Atractomorpha lata</i>	Chew	131	Y	Y
Thysanoptera	Thripidae	<i>Mysetothenes glycines</i>	Chew	7	Y	Y

Arthropod species were classified into three guilds: chewers (Chew), sap feeders (Sap) and predators, according to feeding manner. Five arthropod species could not be classified into any guild, and are described as unknown. Y indicates that individuals