

Community-wide effects of below-ground rhizobia on above-ground arthropods

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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 below-ground microbes may indirectly influence the above-ground arthropod community through changes in the quality and quantity of plants.

2. Rhizobia are root-nodulating bacteria that provide NH_4^+ to legume plants. We examined bottom-up effects of rhizobia on the community properties of the arthropods on host plants, using a root-nodulating soybean strain (R+) and 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 28 species of herbivores and three taxonomic groups of predators on R+ and R– plants. The herbivorous species were classified into sap feeders (12 species) and chewers (16 species).

4. The species richness of overall herbivores, sap feeders, and chewers on R+ plants was greater than that on R– plants. Rhizobia positively affected the abundance of chewers.

5. The community composition of herbivores was significantly different between R– and R+ plants, although species diversity and evenness did not differ.

6. Rhizobia-induced bottom-up effects were transmitted to the third trophic level. The abundance, taxonomic richness, and diversity of the predators on R+ plants were greater but evenness was lower than those on R– plants. The community composition of predators was not affected by rhizobia.

7. These results indicate that the below-ground microbes initiated bottom-up effects on above-ground herbivores and predators through trophic levels.

Key words. Above- and below-ground interactions, bottom-up effects, community structure, *Glycine max*, rhizobia.

Introduction

Ecological communities are structurally organised 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 higher trophic levels (Hunter *et al.*, 1992; Ohgushi, 1992; Price & Hunter, 2005; Ohgushi *et al.*, 2007). For example, Siemann (1998) and Haddad *et al.* (2000, 2009) reported how species diversity of plants influenced species richness and abundance of herbivores or predators. More recently, studies of community genetics have argued that not only interspecific diversity but also intraspecific genetic variation of plants influences community organisation of arthropods (Whitham *et al.*, 2003, 2006; Johnson & Agrawal, 2005). For instance, Crutsinger *et al.* (2008) demonstrated that species richness and relative abundance of

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herbivores and predators increased with increasing genotypic diversity of a goldenrod (*Solidago altissima*).

In addition to genetic determination of phenotypes, plant phenotypes are modified by herbivory (Ohgushi, 2005), and 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 organisation of arthropod communities through subsequent changes in performance, preference, and population dynamics of the herbivorous insects involved (Ohgushi, 2005; Kaplan & Denno, 2007).

Previous studies on the determination of community structure of arthropods have mainly focused on above-ground interactions (Hunter & Price, 1992; Hunter *et al.*, 1992). Recently, we have begun to pay more attention to how below-ground interactions influence above-ground ecological processes (Van der Putten *et al.*, 2001; Wardle, 2002; Hartley & Gange, 2009), because below-ground 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 below-ground 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 defence. There is a growing body of evidence that mycorrhizal fungi positively or negatively affect the performance of above-ground arthropods (Gange, 2007; Hartley & Gange, 2009). These studies suggest that the below-ground microbial effects can play a prominent role in organising the community structure of arthropods associated with plants (Gange *et al.*, 2002; Gange, 2007). Although several studies recently demonstrated bottom-up effects of below-ground microbes on above-ground interactions at the species level (Gange *et al.*, 2003; Gange, 2007; Kempel *et al.*, 2009, 2010), we know little about how below-ground microbes drive bottom-up effects 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 synthesise nitrogen compounds (NH_4^+) from N_2 in the atmosphere, and provide them to a host plant. In the root nodulation, there is a reciprocal signalling 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 above-ground arthropods. We carried out a common garden

experiment to examine the effects of rhizobia on the species richness or taxonomic richness, abundance, species diversity, and evenness, and community composition of above-ground arthropods on soybean, using a root-nodulating soybean strain (*Glycine max* L. cv. Fujimishiro) and a non-nodulating strain (cv. Touzan No. 90).

This study specifically addressed the following questions: (i) do these community properties of above-ground arthropods differ between root-nodulating strains and non-nodulating strain counterparts? and (ii) are the effects of rhizobia transmitted to a whole arthropod community through trophic levels?

Materials 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 that 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 is 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, which is another non-nodulating soybean (K. Takahashi, pers. comm.). 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; Sugauma *et al.*, 1991). Therefore, Touzan No. 90 is closely related to Fujimishiro except for root nodulation. In another potted plant experiment, Katayama *et al.* (2010) showed that the number of root nodules of the R+ plants was 82.4 ± 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 ± 25 $\mu\text{g}/\text{soil-g}$ (mean \pm SE, $n = 10$)], but may contain below-ground 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 unsterilised soil, without inoculating specific rhizobium species. We added ammonium sulphate (5 g 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 ($34^{\circ}58'17''\text{N}$, $135^{\circ}57'32''\text{E}$, 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 the experimental pots were placed. As additional fertilisation, we applied ammonium sulphate (10 g m^{-2}) to all potted R+ and R- plants three times on 8 August, 22 August, and 5 September.

From 21 June to 28 September we conducted 27 censuses at 3–4 day intervals on average. 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. We recorded the number of individuals of each arthropod species and number of species on each plant in each census. We measured plant size [height (cm)] and recorded the number of leaves as indicators of plant growth two times in June, four times in July, three times in August, and four times in September.

During the growing season, we observed 28 species of herbivores and three taxonomic groups (ant, ladybird beetle, and spider) of predators on R+ and R- plants (Table S1). The herbivorous species were classified into sap feeders (12 species) and chewers (16 species). For each arthropod species, the number of individuals on each plant was summed and the number of species was pooled for the data of all 27 censuses. Since we excluded dead plants during the experiment, the replications of R+ and R- plants were 28 and 48, respectively.

In addition to the richness and abundance of arthropods, we calculated the Simpson's diversity index (D : range: 0–1, Simpson, 1949), which is one of the commonly used indexes, as follows:

$$D = 1 - \sum_{i=1}^S P_i^2 \quad (1)$$

where S is the number of species, and P_i is the proportion of the number of individuals of i -th species relative to the total number of individuals on a given plant. This index varies from 0 to 1, representing minimum and maximum values of diversity, respectively.

We also calculated community evenness using Smith and Wilson's index (E_{var} : range: 0–1, Smith & Wilson, 1996) calculated as

$$E_{\text{var}} = 1 - 2/\pi \times \arctan \left[\sum_{i=1}^S (\log_e a_i - x)^2 / S \right] \quad (2)$$

$$x = \sum_{i=1}^S \log_e a_i / S$$

where S is the number of species, and a_i is the cumulated number of individuals of an i -th species on a given plant. This index varies from 0 to 1, representing minimum and maximum values of evenness, respectively.

To calculate the diversity and evenness of sap feeders, chewers, and predators, we excluded one, eight, and six plants, on which arthropods were absent, respectively.

Statistical procedures

A repeated measures ANOVA was used to compare plant size and leaf number between R+ and R- plants. The species richness and the cumulative number of individuals of overall herbivores, sap feeders, and chewers were compared between R+ and R- plants using a t -test. The taxonomic richness and the cumulative number of individuals of predators were compared between R+ and R- plants using a t -test. The Simpson's diversity index and the Smith and Wilson's evenness of overall herbivores, sap feeders, chewers, and predators were compared between R+ and R- plants using a t -test.

To examine whether treatment differences in herbivore richness were driven by a difference in arthropod abundance, we constructed rarefaction curves to correct for biases in species richness that arose 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).

In comparing the community structure of herbivorous and predacious arthropods between R+ and R- plants, we calculated a standardised 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 the $\log_e(n+1)$ -transformed average cumulative number of each arthropod species per plant. Then, the log-transformed data were divided by the values of the total log-transformed number of arthropods so that all species were weighed equally. Bray–Curtis dissimilarity matrices were calculated for dissimilarity in the species composition among plants. Analysis of similarity (ANOSIM) was used to test the difference in the dissimilarities of community structure of arthropods between R+ and R- plants. Differences in arthropod community structure between R+ and R- plants were graphically analysed by non-metric multidimensional scaling (NMDS). Similarity percentage analysis (SIMPER) was used to examine the percentage contribution of sap feeders and chewers to the dissimilarities of the herbivore communities between R+ and R- plants.

Rhizobia can affect not only biomass but also quality of host plants. Katayama *et al.* (2010) showed that rhizobia increased foliar nitrogen and decreased phenolics. The rhizobia-modified plant quality would be expected to influence species richness and abundance of arthropods, independent of plant biomass. To separate indirect effects of rhizobia exerted via plant quality and biomass (plant size), we carried out ANCOVA to compare the difference in species richness and abundance of herbivores between R- and R+ plants, using maximum plant size on 28 September as a covariate. We performed GLM to examine

how predator taxonomic richness and abundance were related to rhizobia, plant size, and herbivore abundance and richness.

Results

Plant growth

From late June to early August, R+ and R- plants grew gradually and reached a peak size 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 size of R+ and R- plants on 28 September was 34.9 ± 1.0 cm and 31.5 ± 0.8 cm (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 ± 1.5 and 34.6 ± 1.2 (mean \pm SE), respectively.

Community properties of herbivorous arthropods

The species richness of overall herbivores on R+ plants was 2.2-fold greater than that on R- plants (t -test, $t_{70} = 10.93$, $P < 0.001$; Fig. 1a). Sap feeders and chewers showed 1.7-fold and 2.9-fold increase in species richness, respectively (t -test, sap feeders: $t_{70} = 7.45$, $P < 0.001$; chewers: $t_{70} = 8.73$, $P < 0.001$; Fig. 1b,c).

The abundance of herbivores on R+ plants was 1.4-fold greater than that on R- plants, although no statistical effect was detected (t -test, $t_{70} = 1.47$, $P = 0.147$, Fig. 1d). Although the most abundant soybean aphid, *Aphis glycines*, did not

significantly differ between R+ and R- plants (t -test, $t_{70} = 0.86$, $P = 0.393$), other herbivores showed a 2.5-fold increase on R+ plants compared with R- plants (t -test, $t_{70} = 5.09$, $P < 0.001$). The abundance of sap feeders and chewers on R+ plants was 1.3-fold and 2.4-fold greater than that on R- plants, respectively, although there was not a significant difference for sap feeders (t -test, sap feeders: $t_{70} = 1.21$, $P = 0.232$; chewers: $t_{70} = 8.34$, $P < 0.001$; Fig. 1e,f). The number of sap feeders except for *A. glycines* was 2.1-fold greater on R+ plants than on R- plants, with a significant difference (t -test, $t_{70} = 3.18$, $P = 0.002$).

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 (Fig. 2). Thus, the increased species richness of herbivores on R+ plants was not due to different sampling efforts.

The Simpson's diversity index (D) of overall herbivores, sap feeders, and chewers on R+ plants was 1.2-fold, 1.2-fold, and 1.9-fold greater than that on R- plants, respectively, although there was not a significant difference for overall herbivores and sap feeders (t -test, overall herbivores: $t_{70} = 1.30$, $P = 0.198$; sap feeders: $t_{69} = 1.35$, $P = 0.249$; chewers: $t_{62} = 5.02$, $P < 0.001$; Table 1). The Smith and Wilson's evenness (E_{var}) of overall herbivores, sap feeders, and chewers on R+ plants was 1.3-, 1.2-, and 1.2- smaller than that on R- plants, respectively, although no significant differences were found for overall herbivores and sap feeders (t -test, overall herbivores: $t_{70} = -1.49$, $P = 0.141$; sap feeders: $t_{69} = -0.92$, $P = 0.361$; chewers: $t_{62} = -4.22$, $P < 0.001$; Table 1).

NMDS analysis showed that the herbivore community composition on R+ plants differed significantly from that on R- plants (ANOSIM, global $R = 0.485$, $P < 0.001$, Fig. 3).

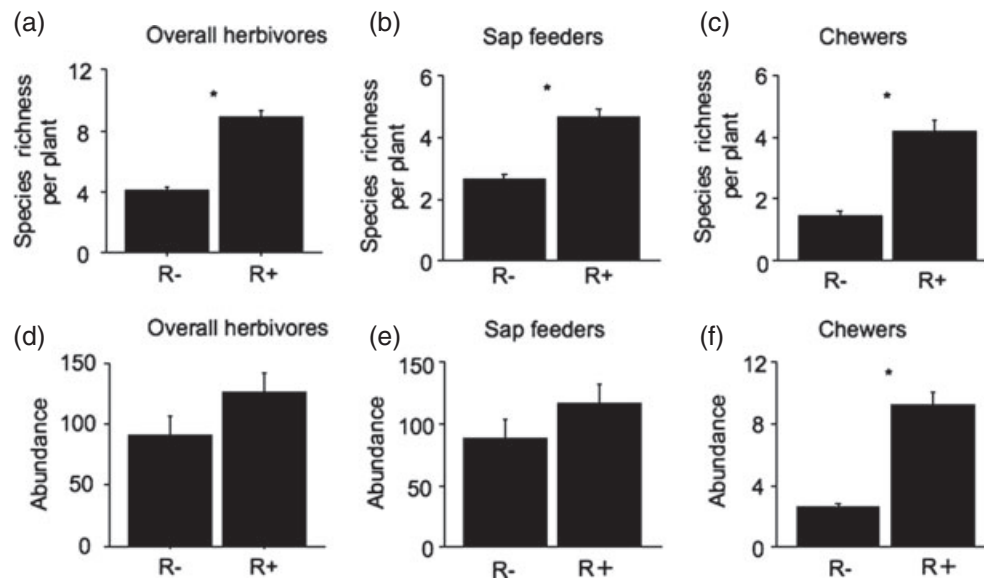


Fig. 1. Species richness of (a) overall herbivores, (b) sap feeders, and (c) chewers, and abundance of (d) overall herbivores, (e) sap feeders, and (f) chewers on an individual plant during the whole census. Bars show SE. Asterisks indicate significant difference between R+ and R- plants (t -test, $*P < 0.001$).

Table 1. Species diversity and evenness of herbivores and predators on R– and R+ plants.

	Diversity index (D)		Evenness index (E_{var})	
	R–	R+	R–	R+
Overall herbivores	0.35 ± 0.04	0.42 ± 0.05	0.46 ± 0.04	0.36 ± 0.05
Sap feeders	0.26 ± 0.03	0.32 ± 0.04	0.31 ± 0.04	0.26 ± 0.04
Chewers	0.30 ± 0.04	0.60 ± 0.04	0.93 ± 0.02	0.79 ± 0.02
Overall predators	0.32 ± 0.04	0.47 ± 0.04	0.93 ± 0.02	0.86 ± 0.02

Values show mean ± SE. Bold letters indicate significant difference between R– and R+ plants (t -test, $P < 0.05$).

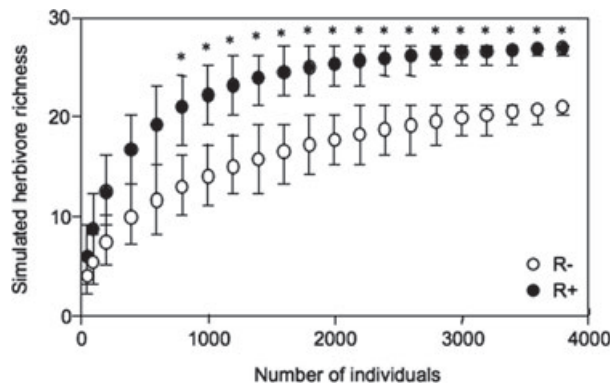


Fig. 2. Rarefaction curves between number of individuals and herbivore richness. We simulated herbivore richness from 10 000 re-sampling iterations using Ecosim 7.72 (Gotelli & Entsminger, 2004). Solid and open circles indicate the root nodulating (R+) and non-nodulating (R–) plants, respectively. Bars show 95% CI. Asterisks indicate significant difference in species richness between R+ and R– plants at the level of the same individual ($*P < 0.05$).

Sap feeders and chewers explained 43% and 57% of the overall dissimilarity between herbivores communities on R+ and R– plants, respectively.

ANCOVA showed that rhizobia increased the species richness of overall herbivores, sap feeders, and chewers, and the chews abundance (species richness of overall herbivores: $F_{1,68} = 121.50$, $P < 0.001$; sap feeders: $F_{1,68} = 57.432$, $P < 0.001$; chewers: $F_{1,68} = 75.617$, $P < 0.001$; abundance of chewers: $F_{1,68} = 66.859$, $P < 0.001$), although the abundance of overall herbivores and sap feeders was not affected by rhizobia (overall herbivores: $F_{1,68} = 2.142$, $P = 0.148$; sap feeders: $F_{1,68} = 0.078$, $P = 0.781$). When the most abundant aphid species (*A. glycines*) was excluded, the abundance of overall herbivores and sap feeders was significantly increased in the presence of rhizobia (overall herbivores: $F_{1,68} = 25.222$, $P < 0.001$; sap feeders: $F_{1,68} = 10.031$, $P = 0.002$). On the other hand, plant size did not affect the species richness or abundance of herbivorous arthropods (species richness of overall herbivores: $F_{1,68} = 2.05$, $P = 0.157$; sap feeders: $F_{1,68} = 2.60$, $P = 0.112$; chewers: $F_{1,68} = 0.36$, $P = 0.552$; abundance of overall herbivores: $F_{1,68} = 0.107$, $P = 0.745$; sap feeders: $F_{1,68} = 0.121$, $P = 0.729$; chewers: $F_{1,68} = 0.303$, $P = 0.584$). There were no interactions between rhizobia and plant size ($P > 0.05$).

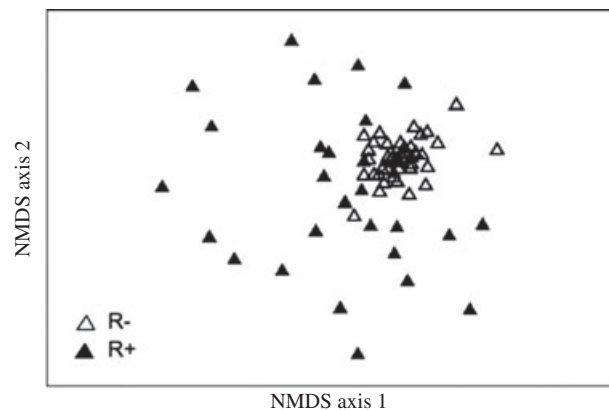


Fig. 3. Nonmetric multidimensional scaling (NMDS) ordination of the community composition of herbivorous arthropods. Solid and open triangles indicate the root nodulating (R+) and non-nodulating (R–) plants, respectively. Community composition on R+ plants significantly differed from that on R– plants (ANOSIM, global $R = 0.485$, $P < 0.001$). Each point is a two-dimensional (axis 1 and axis 2) representation of community composition on an individual plant. As this is a distance measure, NMDS axes are unitless.

Community properties of predacious arthropods

We found three taxonomic groups of predators: an ant (*Formica japonica*), a ladybird beetle (*Coccinella septempunctata*), and several unidentified spiders (Table S1).

The taxonomic richness and the abundance of predators on R+ plants were 1.4-fold and 1.8-fold greater than those on R– plants, respectively (t -test, taxonomic richness: $t_{70} = 2.97$, $P = 0.004$; abundance: $t_{70} = 3.56$, $P < 0.001$; Fig. 4).

The Simpson's diversity index (D) and the Smith and Wilson's evenness (E_{var}) of predators on R+ plants were 1.5-fold higher and 1.1-fold lower than those on R– plants, respectively (diversity index: t -test, $t_{64} = 2.42$, $P = 0.018$; evenness: $t_{69} = -2.55$, $P = 0.013$; Table 1). Predator community composition did not differ between R+ and R– plants (ANOSIM, global $R = -0.036$, $P = 0.849$).

GLM showed that there was a significant effect of herbivore species richness on predator taxonomic richness ($F_{1,71} = 4.213$, $P = 0.045$), indicating that taxonomic richness of predators increased with an increase in herbivore species richness (slope = 0.133 ± 0.07 (mean ± 95% CI)). Also, the taxonomic richness was affected by an interaction between herbivore abundance and plant size ($F_{1,71} = 5.446$,

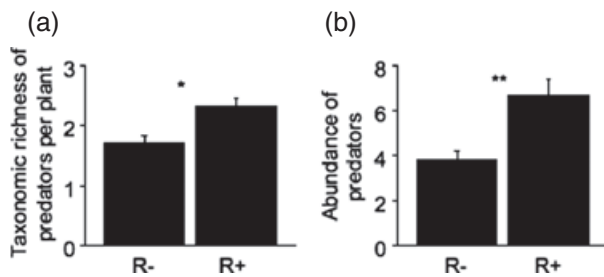


Fig. 4. (a) Taxonomic richness and (b) number of individuals of predators during the whole census. Bars show SE. Asterisks indicate significant difference between R+ and R- plants (t -test; * $P < 0.01$, ** $P < 0.001$).

$P = 0.023$). On the other hand, we did not detect any significant effects (all effects: $P > 0.05$) except for an interaction between rhizobia and plant size on predator abundance ($F_{1,71} = 5.230$, $P = 0.026$).

Discussion

This is the first study to demonstrate the effects of below-ground rhizobia on community properties of above-ground arthropods. R+ plants were larger and produced a greater number of leaves than R- plants. The species richness of herbivorous arthropods on R+ plants was significantly greater than that on R- plants, although abundance, species diversity, and evenness did not differ. This is because the most dominant sap-feeding species (*A. glycine*), accounting for 84% of all sap-feeding individuals, was not affected by the presence of rhizobia. The herbivore community composition was significantly different. In addition, rhizobia also increased the taxonomic richness, diversity, and abundance of predators, but decreased evenness, suggesting that effects of below-ground rhizobia are transmitted to not only herbivores but also predators.

Removing rhizobia is not practically possible in a common garden experiment, because soil bacteria easily colonise soybeans during the growing season in the field. Therefore, we used a non-nodulating strain to evaluate the effects of rhizobia on above-ground arthropods. Likewise, Kempel *et al.* (2009) examined the effects of rhizobia on the performance of above-ground 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 ± 8.6 : mean \pm SE), whereas R- plants had no nodules (Katayama *et al.*, 2010). Therefore, the observed differences in the community properties of above-ground arthropods are likely to have been caused by below-ground rhizobia, although the possibility that other plant traits of the non-nodulating strain may have affected the community properties of arthropods was not excluded.

Effects of rhizobia on above-ground herbivores

Recent studies have paid much attention to the effects of below-ground symbiotic microbes on above-ground

plant–arthropod interactions (Gange, 2007; Hartley & Gange, 2009). For example, arbuscular mycorrhizal fungi positively or negatively affected the abundance of leaf miners (Gange *et al.*, 2003) or two-spotted leaf mites (Nishida *et al.*, 2010). In a meta-analysis using 34 studies, Koricheva *et al.* (2009) showed that colonisation of mycorrhizal fungi decreased the abundance of mesophyll feeders, but increased that of sucking insects. There is increasing evidence that mycorrhizal fungi influence the survival or abundance of above-ground arthropods (Hartley & Gange, 2009). However, to date little is known about the effects of nitrogen-fixing bacteria on above-ground arthropods (but see Kempel *et al.*, 2009). Most of the previous studies mentioned above included only one or a few species of above-ground arthropods, and none have attempted to examine community consequences of the below-ground symbiotic microbes. Our study showed that rhizobia increased the abundance of above-ground leaf chewers, but not of sap feeders. This is because the most abundant sap feeder, *A. glycines*, was not affected by the presence of rhizobia, although other sap feeders increased. Thus, we can conclude that the rhizobia were likely to have increased the abundance of arthropod herbivores on the plants.

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 size and leaf number. In this study, rhizobia did not affect the abundance of arthropod herbivores via the increase in plant size. This indicates that the rhizobia probably modified other plant traits in addition to plant biomass, which may in turn have increased the abundance of the herbivorous arthropods.

In this context, let us consider plant quality. Nitrogen is an essential limiting element for survival and/or growth of many herbivorous arthropods (Mattson & Scriber, 1987; White, 1993). For example, the abundance of herbivorous arthropods was significantly greater on new willow leaves with high nitrogen content than on mature leaves with low nitrogen content (Nakamura *et al.*, 2006; Utsumi & Ohgushi, 2009). Leaf phenolics are defensive substances against arthropod herbivores (Feeny, 1970; Larson & Berry, 1984; Dudt & Shure, 1994). Herbivore abundance is negatively correlated with concentration of foliar phenolics (tannin) of oak species (Feeny, 1970; Forkner *et al.*, 2004). It is likely that rhizobia positively affected above-ground herbivores via changes in plant quality, because rhizobia-associated soybeans increased leaf nitrogen by 50% and decreased phenolics by 12% (Katayama *et al.*, 2010).

This study also found that the species richness of herbivores on R+ plants was significantly greater than that on R- plants. Increased plant biomass and improved nutrient conditions of plants can increase species richness of herbivores (Siemann, 1998; Fonseca *et al.*, 2005). Siemann (1998) conducted a fertilisation experiment in a grassland field, and demonstrated that improvement of resource availability increased the herbivore species richness. The increased herbivore richness on R+ plants in our experiment may be explained by the rhizobia-induced improvement of plant quality with

increased foliar nitrogen and decreased phenolics, as mentioned above.

Transmission of rhizobia effects to above-ground predators

Our data demonstrated that effects of rhizobia were also transmitted to predators, the third trophic level. The taxonomic richness, diversity, and abundance of predators increased, but community evenness decreased, in the presence of rhizobia, although the community composition did not change. This suggests that the bottom-up effects initiated by rhizobia can extend beyond trophic levels. There are several explanations for the bottom-up effects of plants on diversity and abundance of predators. First, plants support increased herbivore abundance, which may in turn increase the species richness of predators (Siemann, 1998; Knops *et al.*, 1999; Forkner & Hunter, 2000). This is because a variety of predator species can aggregate when prey becomes abundant (Ives *et al.*, 1993; Cardinale *et al.*, 2006). Second, plants support increased species richness of herbivores, which may provide a wider range of prey items for generalist predators (Hunter & Price, 1992). In addition, changes in plant size or architecture may directly affect the abundance or diversity of predators by providing shelter and foraging and/or oviposition sites (Langellotto & Denno, 2004; Denno *et al.*, 2005). In the present study, herbivore species richness partially explained the increase in taxonomic predator richness. However, whether and how rhizobia-induced changes in plant traits directly or indirectly influence the biodiversity of predators remains unclear. Thus, further analyses will be needed to reveal underlying mechanisms responsible for the effects of rhizobia on above-ground arthropod predators.

Microbe-driven bottom-up cascading effects in multi-trophic systems

Most terrestrial plants harbour microbial symbionts in some form, and symbiotic microbes such as mycorrhizae and endophytes can have strong impacts on plants and their consumer diversity (van der Heijden *et al.*, 2008). These symbiotic microbes strongly alter plant phenotypic expression and thus can influence multi-trophic interactions of arthropods on host plants (Omacini *et al.*, 2001; Gange *et al.*, 2003; Chaneton & Omacini, 2007; Hartley & Gange, 2009; Koricheva *et al.*, 2009). Recently, plant-mediated bottom-up effects induced by symbiotic microbes have begun to be recognised as an important agent structuring arthropod communities. In this context, several studies have illustrated the strong impacts on biodiversity and the abundance of higher trophic levels (Omacini *et al.*, 2001; Finkes *et al.*, 2006; Rudgers & Clay, 2008). For example, Rudgers and Clay (2008) showed the important role of a symbiotic grass endophyte on arthropod communities of *Lolium arundinaceum*. The presence of the endophyte reduced abundance and species diversity of arthropods. Finkes *et al.* (2006) also documented that the species richness of spiders on tall fescue grass without a fungal endophyte was greater than endophyte-infected grass. The endophyte may have decreased spider species richness by

reducing prey abundance. Total herbivore abundance declined 25–55% in the presence of the endophyte, which could indicate a reduction in prey. Jani *et al.* (2010) examined how endophyte alkaloids affect the abundance and species richness of arthropod communities on a sleepygrass, and found that endophyte-produced alkaloids were associated with increased herbivore and natural enemy abundance, and herbivore species richness. Hence, symbiotic microbes can play a prominent role in organizing the community of arthropods associated with host plants. Nevertheless, to date no studies have demonstrated such bottom-up cascading effects caused by below-ground symbiotic microbes.

Our work clearly illustrated that community-level bottom-up effects were generated by the below-ground rhizobia. This is the first evidence showing a strong impact of below-ground microbes on the biodiversity and community structure of above-ground arthropods. It should be noted that the two different strains of soybean used in this work may have affected arthropod communities through genotype-specific plant traits. Recent studies have emphasised the important role of plant genotypes in structuring arthropod communities (Whitham *et al.*, 2003, 2006; Johnson & Agrawal, 2005). As there is no available information on the genetic differences in traits of the two soybean strains that may affect preference and/or performance of associated arthropods, the effect of rhizobia and plant genotype interaction should be addressed in future studies.

We should focus on cascading effects induced by below-ground microbes in above-ground multi-trophic systems, to understand how common and widespread in nature the microbe-driven bottom-up effects, and what differences or similarities of the effects exist among different types of symbiotic microbes in a community context.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-2311.2010.01242.x

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

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