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Disentangling above- and belowground neighbor effects on the growth, chemistry, and arthropod community on a focal plant

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Abstract. Neighboring plants can influence arthropods on a focal plant, and this can result in associational resistance or associational susceptibility. These effects can be mediated by above- and belowground interactions between the neighbor and focal plant, but determining the relative contribution of the above- and belowground effects remains an open challenge. We performed a common garden experiment with a design that enabled us to disentangle the above- and belowground effects of five different plant species on the growth and chemistry of the focal plant ragwort (*Jacobaea vulgaris*), and the arthropod community associated with this plant. Aboveground effects of different neighboring plant species were more important for the growth and quality of *J. vulgaris* and for the arthropod abundance on this plant than belowground effects of neighbors. This remained true when only indirect neighbor effects (via affecting the biomass or quality of the focal plant) were considered. The aboveground neighbor effects on arthropod abundance on the focal plant were strongly negative. However, the magnitude of the effect depended on the identity of the neighboring species, and herbivore abundance on the focal plant was higher when surrounded by conspecific than when surrounded by heterospecific plants. We also observed interactions between above- and belowground neighbor effects, indicating that these effects may be nonadditive. We conclude that above- and belowground associational effects are not equally strong, and that neighbor effects on plant–arthropod interactions occur predominantly aboveground.

Key words: above- and belowground interactions; associational effects; *Jacobaea vulgaris*; multitrophic interactions; plant–insect interactions; plant–soil feedback.

INTRODUCTION

Understanding the mechanisms that shape resource–consumer interactions in diverse plant communities has been a long-lasting challenge in ecology (Tahvanainen and Root 1972, Andow 1991, Agrawal et al. 2006). The assembly of an arthropod community is not only determined by the influence of the host plant itself on demographic processes such as migration, growth, and reproduction, but also by the effects of other plants in the neighborhood (Agrawal et al. 2006, Barbosa et al. 2009, Underwood et al. 2014). Neighboring plants can cause a reduction (associational resistance; AR) or an increase (associational susceptibility; AS) in arthropod abundance or damage inflicted to another plant (the focal plant), and several mechanisms have been put forward to explain these effects (reviewed in, e.g., Agrawal et al. 2006, Barbosa et al. 2009, Hambäck et al. 2014, Underwood et al. 2014). Direct effects of the presence of neighboring plants on arthropod abundance on a focal plant can be mediated by chemical or visual

cues of the neighboring plants that can mask the focal plant or act as repellents or attractants (Tahvanainen and Root 1972, Khan et al. 1997), and by spillover of herbivores or carnivores from the neighboring plant on the focal plant (Andow 1991, White and Whitham 2000). Indirect effects occur when neighboring plants influence the biomass and chemistry of the focal plant and this, in turn, affects the insect–plant interactions on the focal plant. This can result from, e.g., plant–plant competition (Agrawal et al. 2006, Barbosa et al. 2009), shading (Ballare 2014), or from emission of volatile compounds by neighboring plants, which induce defense responses in the focal plant (Turlings and Ton 2006, Ninkovic et al. 2013).

However, the possible interactions between focal plants and neighbors do not stop at the soil–air interface. Belowground (BG) interactions between plants and biota have been shown to impact insect communities aboveground (AG). For example, exposure of plants to soil microorganisms, root-feeding herbivores, or decomposing macrofauna can result in changes in the nutritional quality of the foliage of these plants, ultimately causing changes in the performance and behavior of AG herbivores and carnivores (e.g.,

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Bezemer et al. 2005, Soler et al. 2007, Bardgett and Wardle 2010, Eisenhauer et al. 2010). Most of these AG–BG studies have examined how addition or exclusion of particular organisms or groups of organisms from the soil can affect AG plant–insect interactions. Plants themselves can also greatly influence the soil microbial community in the rhizosphere (Bardgett and Wardle 2010). The influence of a plant on the abiotic or biotic conditions in the soil and its subsequent effect on the performance of other plants that grow later in the soil is called plant–soil feedback (PSF) (Bever et al. 1997, Ehrenfeld et al. 2005, van der Putten et al. 2013). Several recent studies have shown that BG interactions with plant neighbors, via PSF effects on the soil microbial community, can affect AG plant–herbivore interactions on focal plants (Kostenko et al. 2012b, Bezemer et al. 2013, but see Schittko and Wurst 2014). As plant species differ in how they influence soil biota (Kardol et al. 2006, van de Voorde et al. 2011), the BG effects of neighboring plants on the AG plant–arthropod interactions on a focal plant may depend on the identity of the neighbor. Neighboring plants can also influence the nutritional quality or growth of a focal plant BG via competition for nutrients or water, via root–root communication mediated by root exudates or volatiles, or via common fungal networks (Wilson and Tilman 1993, Chen et al. 2012, Babikova et al. 2013, van der Putten et al. 2013). While there is evidence that neighboring plants can influence plant–arthropod interactions on a focal plant via both AG and BG interactions, an open challenge is to determine the relative contribution of the AG and BG effects.

AG and BG neighbor effects on plant–arthropod interactions are not necessarily equally strong. For instance, AG neighbor effects on arthropods can be both direct and indirect, via changes in focal plant growth and quality (Agrawal et al. 2006, Barbosa et al. 2009). BG neighbor effects are predominantly limited to plant-mediated (indirect) effects, unless, e.g., volatiles emitted by soil microbes, which have been shown to affect soil-dwelling insects (Davis et al. 2013), may affect AG arthropods as well, but this is currently unknown. The relative importance of AG and BG neighbor effects may also depend on the identity of the neighboring species. Furthermore, effects of AG and BG competition with neighboring plants on plant growth have been shown to interact (e.g., Cahill 1999, 2002, Kiaer et al. 2013), and this may result in interactions between AG and BG neighbor effects on AG plant–arthropod interactions as well.

We performed a common garden experiment with a design that enabled us to disentangle the AG and BG neighbor effects of five different plant species on the growth and chemistry of the focal plant ragwort (*Jacobaea vulgaris*), and the arthropod community associated with this plant. AG effects were tested based on the presence or absence of neighboring plants that were grown in isolated pots, i.e., without soil contact,

and BG effects by growing the focal plants in soil previously conditioned (“trained”) by the neighboring plant species or in control soil but without neighboring plants being present. We examined the combination of AG and BG neighbor effects by combining the two treatments, and by growing focal and neighboring plants in connected pots in which the soil and roots of the neighbor and focal plant were in contact. We tested six hypotheses. In the first hypothesis, BG neighbor effects, mediated by changes in soil microbial communities, will be more important for the growth and chemistry of focal plants than AG neighbor effects. In the second, AG neighbor effects, which can affect arthropods both directly and indirectly via plant growth and quality, will be more important for determining arthropod abundance on the focal plant than BG neighbor effects, which are limited to indirect effects. In the third, when considering only the indirect AG and BG effects on arthropod abundance (via plant growth and quality), BG neighbor effects will be stronger than AG neighbor effects. In the fourth, all selected neighboring species will decrease arthropod abundance on the focal plant, but the magnitude of AG and BG neighbor effects will depend on the identity of the neighboring species. In the fifth, *J. vulgaris* growth, quality, and arthropod abundance will be lower when roots and soil of the focal and neighboring plants are in contact (connected pots) than when the focal plant grows in soil previously conditioned by the neighbor (disconnected pots). In the sixth, the AG effect of neighboring plants on *J. vulgaris* growth, quality, and arthropod abundance will be influenced by whether the focal plant also has BG (root) contact with the neighboring plant.

MATERIAL AND METHODS

Plant species

Jacobaea vulgaris Gaertn. ssp. *vulgaris* (ragwort, synonym *Senecio jacobaea* L., Asteraceae) is a biennial monocarpic plant that produces pyrrolizidine alkaloids (PAs), which are toxic for generalist herbivores (Macel 2011). We selected four local grassland species as neighboring species of *J. vulgaris*: *Hypochaeris radicata* L., *Leucanthemum vulgare* Lamk., *Tanacetum vulgare* L., (all Asteraceae), and *Plantago lanceolata* L. (Plantaginaceae). These species were chosen because they represent phylogenetically related species that either share (*L. vulgare* and *T. vulgare*) or do not share herbivores with *J. vulgaris* (*H. radicata*), and a non-related species (*P. lanceolata*) that is unlikely to share herbivores with the focal plant. *Jacobaea vulgaris* was also included as a neighboring species, to study differences between intra- and interspecific neighbor effects. Seeds from all species were collected from a restoration grassland at Planken Wambuis (Ede, Netherlands), except for seeds from *P. lanceolata*, which were obtained from a specialized wild plant seed supplier (De Bolderik, Wervershoof, Netherlands). After surface sterilization (1 min in 0.5% sodium hypochlorite

solution and rinsed with water afterward) and germination on glass beads, individual seedlings were transplanted into seedling trays (3 cm diameter, 6 cm depth) filled with sterilized soil and placed in a greenhouse at 70% relative humidity and a 16 h light (21°C) and 8 h dark (16°C) photo regime.

Conditioned and control soil

The soil used in the experiment was a sandy loam with particle size distribution 3% < 2 μm , 17% 2–63 μm , and 80% >63 μm , with 4.5% organic matter (OM). We collected soil from around the roots (rhizosphere soil) from plants that were growing in experimental monocultures that were maintained for three years in a biodiversity field experiment within a nature restoration site on former arable land at Mossel, Ede (see Kostenko et al. [2012a] for a description of the experiment) to serve as conditioned soil. As there were no *J. vulgaris* monoculture plots, rhizosphere soil of this species was collected from large (>20 cm diameter) *J. vulgaris* rosette plants that had been planted into the monocultures. Soil collected from a species-rich grassland directly adjacent to the monocultures served as control soil. We selected this soil as a control because it was comparable in soil properties to the soil from the monoculture plots and because the grassland contained all plant species that were used in the experiment. We did not use sterilized soil as a control, as this does not represent the natural soil conditions in which plants grow in the field, and a sterile soil environment may facilitate the rapid increase of a limited number of fast-growing species of microorganisms (de Boer et al. 2003). Soil was collected (at a 0–15 cm depth), sieved (1-cm mesh size) to remove coarse fragments, and homogenized, and all macroarthropods were manually removed. For each of the six soil types (five monospecific soils and control soil), five samples of 200 g (fresh mass) pure field soil were analyzed for soil fungal and bacterial communities and soil abiotic characteristics.

Common garden experiment

A common garden experiment was established in the summer of 2012 at the Netherlands Institute of Ecology (NIOO-KNAW) in Wageningen, Netherlands. In this common garden, we grew *J. vulgaris* and neighboring plant species in experimental units to manipulate AG and BG interactions. Each experimental unit consisted of five 2-L square pots (11.3 \times 11.3 \times 21.5 cm) that were attached to each other so that they formed a cross (Appendix A: Fig. A1). Each experimental unit was randomly allocated to one of five treatments (Appendix A: Fig. A1). In the first treatment, aboveground neighbor effects (AG), the four outer pots were planted with one of the neighboring species growing in control soil (one plant per pot, all four pots planted with the same species), and the focal *J. vulgaris* was grown in the central pot in control soil. In the second treatment, belowground neighbor effects (BG), the focal *J. vulgaris*

was grown in soil that was conditioned by one of the neighboring species, and the four outer pots contained control soil without plants. In the third treatment, above- and belowground neighbor effects (AG and BG), *J. vulgaris* was grown in soil conditioned by one of the neighboring species, and the four outer pots were planted with the same neighboring species growing in control soil. In the fourth treatment, above- and belowground neighbor effects in connected pots (AG and BG connected), a rectangle (10 \times 5 cm) was removed from the four sides of the central pot and the inner side of the outer pots so that roots of the focal and neighboring plants were in contact during the course of the experiment, and all plants were grown in control soil. In the fifth treatment, control (C), *J. vulgaris* was grown in control soil and the four outer pots contained control soil without plants. For each neighboring species, the five treatments were replicated 10 times; the control treatment was replicated 15 times (three replicate controls were randomly allocated to each neighbor species treatment). In total, there were 215 units (4 AG/BG treatments \times 5 neighboring species \times 10 replicates + 15 control replicates).

Each of the five pots of a unit was filled with 1.5 kg soil (fresh mass). Pots were filled with a homogenized mixture of 60% live field soil and 40% sterilized bulk soil, which eliminated differences in nutrient limitation between treatments and at the same time allowed the introduction of soil biota. To obtain sterilized bulk soil, a part of the control soil was sterilized by gamma irradiation (>25 kGy; Isotron, Ede, Netherlands). A 1-cm layer of sterilized field soil was added to the surface of each pot to reduce possible germination of the seeds that may have been present in the field-collected soil. A fine fabric was added to the bottom of each pot to prevent roots and soil biota from entering or exiting the pots, while allowing passage of water through the small holes in the bottom of the pot.

In early June, three-week-old seedlings were transplanted from the seedling trays into the units. Plants were allowed to establish for two weeks in an outdoor tent and were subsequently placed into the common garden following a randomized design with an isolation distance of 65 cm bare soil between units. In order to maintain a realistic microclimate in the rhizosphere, experimental units were dug into the soil so that the soil level in the pots was the same as that outside the pots.

Chemical and molecular analyses in monospecific and control soil

To determine the abiotic soil characteristics of the five replicates of the monospecific and control soils, a subsample of 100 g of each soil sample was sieved (4-mm mesh size) and dried (5 d at 40°C) for chemical analysis of phosphorous (P), ammonium (NH₄⁺-N), nitrate (NO₃⁻-N), plant-available amounts of phosphorous (Olsen-P), percentage of organic matter (OM), percentage of carbon (C), percentage of nitrogen (N),

and pH. Terminal restriction fragment length polymorphism analysis (T-RFLP) was used to assess differences in the composition of soil fungal and bacterial communities between the soil types, for which five soil samples (0.25 g each) were analyzed from each monospecific soil and the control soil. One replicate of soil conditioned by *L. vulgare* was lost during the chemical analysis, and one replicate of soil conditioned by *J. vulgaris* was lost during the molecular analysis. See Appendix B for a detailed description of the chemical and molecular analyses of the soil samples.

Arthropod abundance

Starting two weeks after the units had been transported into the common garden (early July), the focal and neighboring plants of each unit were monitored biweekly for the presence of herbivorous and carnivorous arthropods. Each plant was visually inspected and the number and identity of the arthropods was recorded, without removing any arthropods from the plant. This was done until the end of August, four times in total. Data from these four samplings were pooled, and analyzed at the order or family level (see *Results*). Herbivore damage to plants was not recorded.

Plant biomass of focal and neighboring plants

At the end of the season (end of August), all plants (neighbor and focal) were clipped at soil level. For each focal plant, the roots were washed and the root and shoot biomass was oven-dried at 70°C and weighed. For the experimental units with connected (open) pots, we carefully separated the roots of the focal plant from the roots of the neighboring plants during washing. All *J. vulgaris* plants were in the rosette stage. For neighboring plants, leaves and reproductive parts (flowers and stems) were kept separate.

Chemical analysis of focal plants

At the end of July, when insect abundance peaked, we collected the fifth-youngest leaf of each focal *J. vulgaris* plant. Leaves were immediately frozen at -20°C, freeze-dried for 3 d, and finely ground for chemical analysis of C, N, P, and PAs. PA analysis was performed using liquid chromatography–tandem mass spectrometry (LC-MS/MS). A total of 41 PAs were detected in *J. vulgaris* leaves (Appendix C: Table C1). See Appendix B for a detailed description of the chemical analyses of focal plants.

Statistical analysis

To fulfil the requirements of normality and homogeneity of variances, plant biomass data, root : shoot ratio, and C:N ratio were log-transformed, proportion data were arcsine-square-root-transformed, and arthropod counts were square-root-transformed. Neighbor effects on plant biomass, plant chemistry, and arthropod abundance were tested using a three-way ANOVA with AG effects, BG effects, neighbor identity, and their

interactions as fixed factors. The fourth treatment, AG and BG effects in experimental units with connected (open) pots, was excluded in these analyses. Because of significant interactions between species identity and AG or BG effects, AG and BG effects were tested separately for each neighboring species by two-way ANOVA. For each species, we separately tested the effect of the type of BG contact with the neighboring plant (growing in connected pots or in conditioned soil in disconnected pots) on plant growth, plant chemistry, and arthropod abundance by comparing the third (AG and BG) and fourth treatments with a *t* test. We also tested separately for each species whether the AG effect of the neighboring plant on focal plant growth, plant chemistry, and arthropod abundance was influenced by direct root contact with the neighbor by comparing the first (AG) and fourth treatments with a *t* test. The bivariate correlations between neighbor shoot biomass (the average of the four plants in a unit, foliar and reproductive biomass combined) and focal plant biomass or arthropod abundance were tested with Pearson correlation tests, and data from all treatments were included in this analysis. Differences in plant biomass and arthropod abundance among the neighboring species were tested using one-way ANOVA with a post-hoc Tukey test for pairwise comparisons, and these results are shown in Appendix A: Fig. A2. Differences in soil abiotic characteristics among the six soil types (five monospecific soils and control soil) were tested with one-way ANOVA followed by pairwise comparisons with a Tukey test.

Multivariate analyses were used to compare fungal communities, bacterial communities, and abiotic characteristics of the different soils. Detrended correspondence analysis indicated that the longest gradient was <3, hence data were analyzed using principal component analysis (PCA) and redundancy analysis (RDA; Lepš and Šmilauer 2003). Significances in multivariate analyses were inferred by Monte Carlo permutation tests (999 permutations). For the analyses of fungal and bacterial communities, only terminal restriction fragments that appeared in at least three of the 30 soil samples were included in the analysis. For the analysis of soil chemistry, all measured soil abiotic characteristics (pH, P, Olsen-P, NH₄-N, NO₃-N OM [%], C [%], N [%], and C:N ratio) were included as species data. These data were log-transformed and standardized by species.

RDA was also used to test whether PA composition of focal plants and arthropod community composition on focal plants was affected by AG or BG effects, neighbor identity, or the interaction terms. The fourth treatment was kept out of this analysis. PA and arthropod data were log-transformed before the RDA analysis.

Structural equation modeling (SEM) was used to disentangle direct and indirect AG and BG neighbor effects on arthropod abundance on the focal plant (the fourth treatment was kept out of this analysis). SEM

was also used to explore which AG traits of the neighboring plants (foliar and reproductive biomass, herbivore and carnivore abundance, or other unmeasured traits modeled as neighbor identity) affected arthropod abundance on the focal plant (only data from the AG and AG and BG treatments were included). See Appendix B for a detailed description of the SEM analyses.

All univariate analyses were performed in IBM SPSS Statistics for Windows (19th edition, SPSS, Chicago, Illinois, USA). Multivariate analyses were performed in Canoco version 5.03 (Ter Braak and Šmilauer 2002). Structural equation modeling was performed using the sem package in R (version 3.0.1, R Development Core Team 2013).

RESULTS

AG and BG neighbor effects on J. vulgaris biomass

There was a negative overall AG effect on root ($F_{1,145} = 119.64$, $P < 0.001$; Fig. 1a) and shoot ($F_{1,145} = 56.90$, $P < 0.001$; Fig. 1b) biomass of the focal plant. The negative AG effect was stronger for shoot than for root biomass, resulting in a positive AG effect on root:shoot ratio ($F_{1,145} = 10.20$, $P = 0.002$). Even though fungal and bacterial communities and soil chemistry differed between the control soil and the monospecific soils, as well as among the five monospecific soils, (Fig. 2 and Appendix C: Table C2), there was no main BG effect on either root biomass, shoot biomass, or root:shoot ratio ($P > 0.05$). However, for root biomass, there was a significant AG \times BG interaction ($F_{1,145} = 4.49$, $P = 0.036$). The BG neighbor effect on root biomass of the focal plant was positive in the presence of the AG neighbor effect (focal plants had more root biomass in the AG and BG treatment than in the AG treatment), whereas the BG neighbor effect was negative in absence of the AG neighbor effect (focal plants had less root biomass in the BG than in the control treatment; Fig. 1a).

Neighbor identity did not affect root biomass, shoot biomass, or root:shoot ratio ($P > 0.05$), but there was a significant interaction between neighbor identity and the AG effect for root and shoot biomass (root: $F_{4,145} = 6.65$, $P < 0.001$; shoot: $F_{4,145} = 4.79$, $P = 0.001$). We therefore subsequently examined the effects for each neighboring species separately. The AG effect on *J. vulgaris* root and shoot biomass was significant for each neighboring species, but the magnitude of the effect differed among species (Fig. 1a, b). *Tanacetum vulgare*, which had the highest biomass of all neighboring species (Appendix A: Fig. A2), caused the largest negative AG effect on focal plant biomass. Focal plant root biomass, but not shoot biomass, was negatively related to the shoot biomass of the neighbor (root biomass: $r = -0.397$, $P < 0.001$, $n = 150$; shoot biomass: $r = -0.076$, $P = 0.354$, $n = 150$). Only *P. lanceolata* had a significant positive BG effect on root biomass of the focal plant ($F_{1,41} = 8.65$, $P = 0.005$; Fig. 1a), even though the biotic and

abiotic characteristics of soil conditioned by *P. lanceolata* were very similar to those of soil conditioned by *T. vulgare* and *J. vulgaris* (Fig. 2).

Overall, root biomass ($t_{98} = -3.68$; $P < 0.001$) and root:shoot ratio ($t_{98} = -4.53$; $P < 0.001$) were smaller when focal plants exposed to AG neighbors grew in connected pots (AG and BG connected) than when focal plants exposed to AG neighbors grew in soil conditioned by the neighbor in disconnected pots (AG and BG), although there were some species-specific differences (Fig. 1a). Shoot biomass did not differ between focal plants exposed to AG neighbors growing in connected pots and those growing in conditioned soil in disconnected pots (Fig. 1b). Shoot biomass generally was larger ($t_{98} = 2.97$; $P = 0.004$) and root:shoot ratio was smaller ($t_{98} = -5.29$; $P < 0.001$) when focal plants exposed to AG neighbors grew in connected pots (AG and BG connected) than when focal plants exposed to AG neighbors grew in control soil in disconnected pots (AG), although there were some species-specific differences (Fig. 1b). Overall, root biomass did not differ between focal plants exposed to AG neighbors growing in connected pots and those growing in control soil in disconnected pots ($P > 0.05$), except for the lower root biomass in connected pots when surrounded by *T. vulgare* (Fig. 1a).

AG and BG neighbor effects on J. vulgaris chemistry

There were no main AG or BG neighbor effects on total PA concentration ($P > 0.05$), but there were species-specific AG and BG effects (AG \times identity; $F_{1,145} = 2.65$, $P = 0.036$; AG \times BG \times identity; $F_{1,145} = 3.23$, $P = 0.014$; Appendix A: Fig. A3). There were no AG and BG effects on the proportion of tertiary amines or the concentration of any of the individual PAs in the leaves ($P > 0.05$). The composition of PAs was not affected by the main AG or BG effects or by neighbor identity (RDA: $P > 0.05$ for all factors), but there was a significant interaction between the AG and BG effect (pseudo- $F = 3.4$, $P = 0.004$; 2.1% explained variation), and between BG and neighbor identity (pseudo- $F = 1.5$, $P = 0.042$; 3.9% explained variation).

Overall, the presence of an AG neighbor increased foliar C:N ratio ($F_{1,145} = 9.69$, $P = 0.002$), but the AG effect on C:N ratio differed among species (AG \times identity: $F_{4,145} = 2.87$, $P = 0.025$; Appendix A: Fig. A3). There were no main BG or neighbor identity effects on C:N ratio ($P > 0.05$). Foliar percentage of P was not affected by any of the treatments ($P > 0.05$).

The type of BG contact with the neighboring plant, i.e., focal plants exposed to AG neighbors growing in connected pots (AG and BG connected) or in conditioned soil in disconnected pots (AG and BG), did not affect plant chemistry, except for the effect of *H. radicata* on total PA concentration (Appendix A: Fig. A3). Overall, total PA concentrations did not differ between focal plants exposed to AG neighbors growing in connected pots (AG and BG connected) and focal

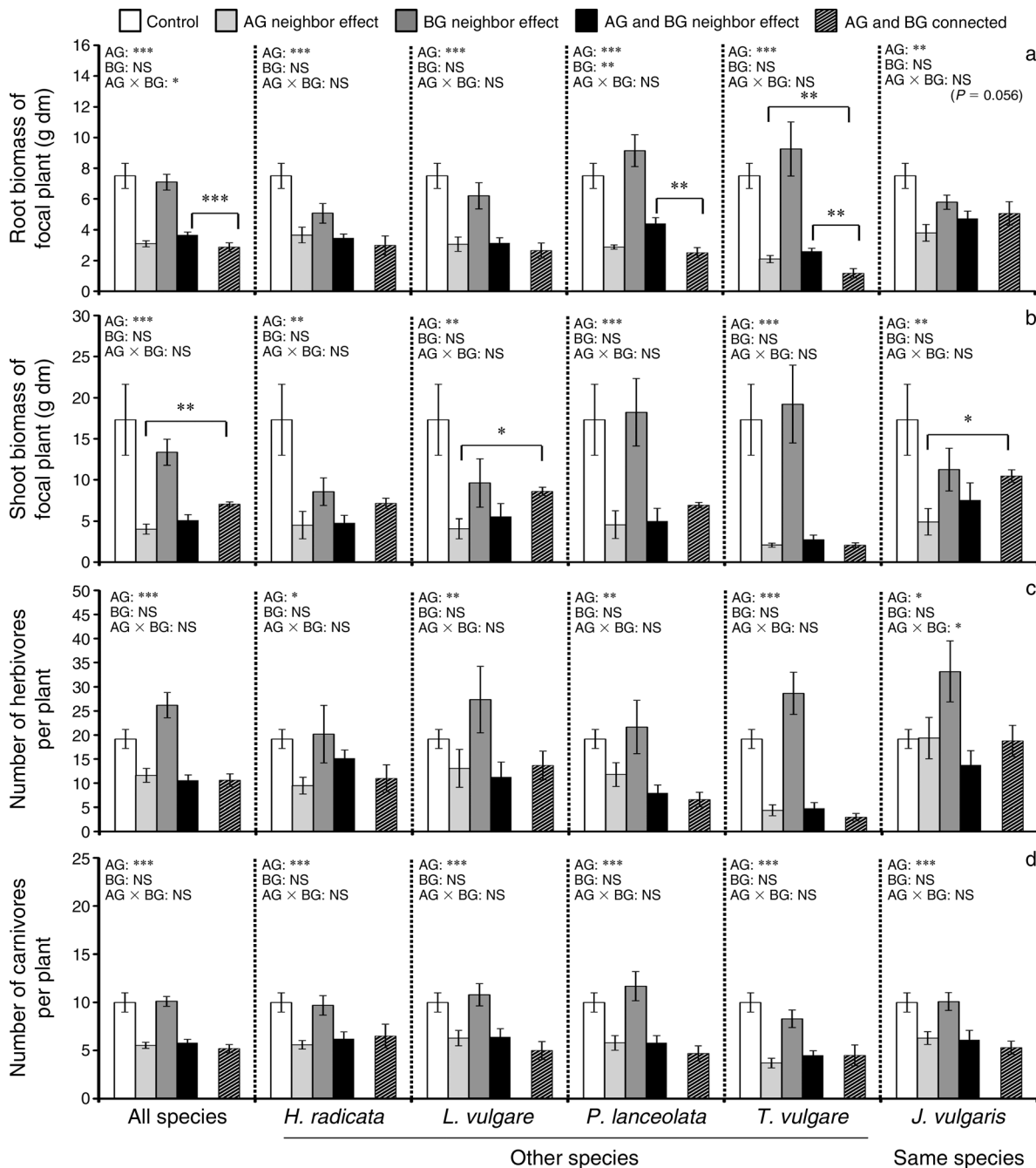


FIG. 1. Mean (\pm SE) (a) root biomass and (b) shoot biomass of focal *Jacobaea vulgaris* plants and total number of (c) herbivores and (d) carnivores on focal plants. Biomass is measured in dry mass (dm). Focal plants were growing either in control soil without a neighbor (control), surrounded by neighboring plants (aboveground [AG] neighbor effect), growing in soil conditioned by a neighboring plant (belowground [BG] neighbor effect), both surrounded by neighboring plants and growing in conditioned soil (AG and BG neighbor effect), or surrounded by neighboring plants and growing in control soil in connected (open) pots (AG and BG connected). Data are shown for all neighboring species combined, and separately for each neighboring species (*Hypochaeris radicata*, *Leucanthemum vulgare*, *Plantago lanceolata*, *Tanacetum vulgare*, and *J. vulgaris*). The main AG and BG neighbor effects and the interaction were tested with ANOVA; the AG and BG connected treatment was not included in this analysis. The differences between the AG and BG connected and AG treatment and between the AG and BG connected and AG and BG treatment were tested separately with a *t* test; the absence of asterisks denotes no significant difference.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. NS indicates a nonsignificant effect.

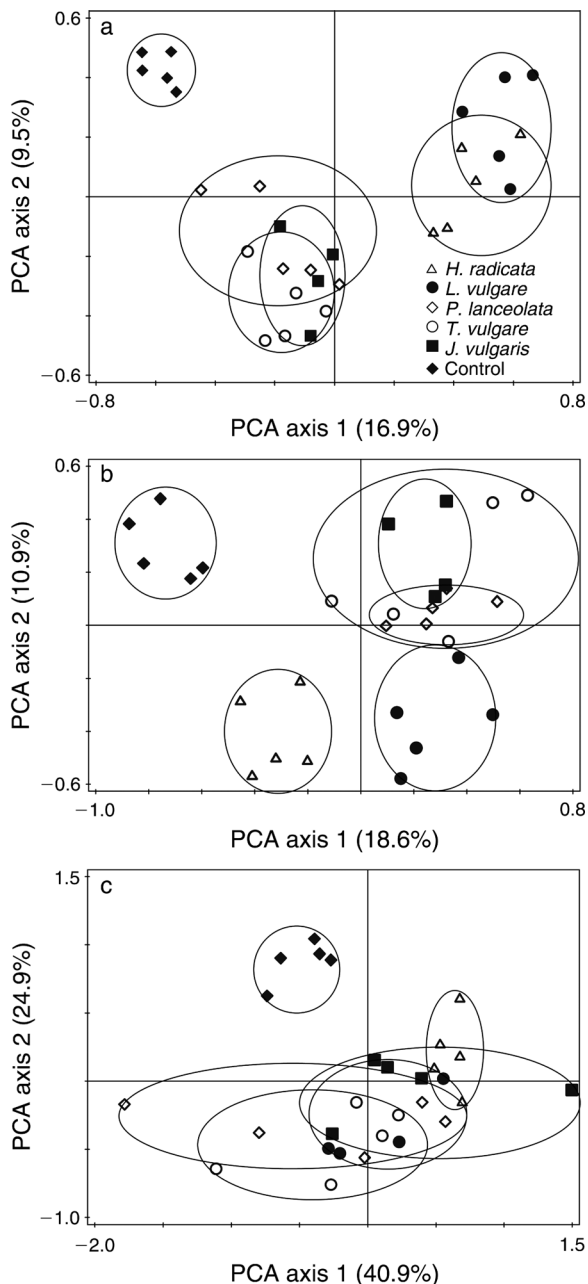


FIG. 2. Ordination diagram of principal component analysis (PCA) of (a) fungal community composition, as assessed by terminal restriction fragment length polymorphism analysis (T-RFLP), (b) bacterial T-RFLP community composition, and (c) chemistry of soil conditioned by the five neighboring plant species (*H. radicata*, *L. vulgare*, *T. vulgare*, *P. lanceolata*, and *J. vulgaris*) and of the control soil. Percentages of total explained variation by PCA axes are given in parentheses. The composition of fungal and bacterial communities and soil chemistry differed significantly between the different soil types. Redundancy analysis (RDA) for fungi; pseudo- $F = 2.9$, $P = 0.002$, 38.3% explained variation; for bacteria; pseudo- $F = 3.1$, $P = 0.002$, 40.5% explained variation; for soil chemistry; pseudo- $F = 4.0$, $P = 0.001$, 46.7% explained variation.

plants exposed to AG neighbors growing in control soil in disconnected pots (AG; $P > 0.05$), except for the lower PA concentrations in connected pots when surrounded by *T. vulgare* (Appendix A: Fig. A3). Foliar C:N ratios were lower ($t_{98} = -3.19$; $P = 0.002$) when focal plants exposed to AG neighbors grew in connected pots than when focal plants exposed to AG neighbors grew in control soil in disconnected pots, although there were some species-specific differences (Appendix A: Fig. A3).

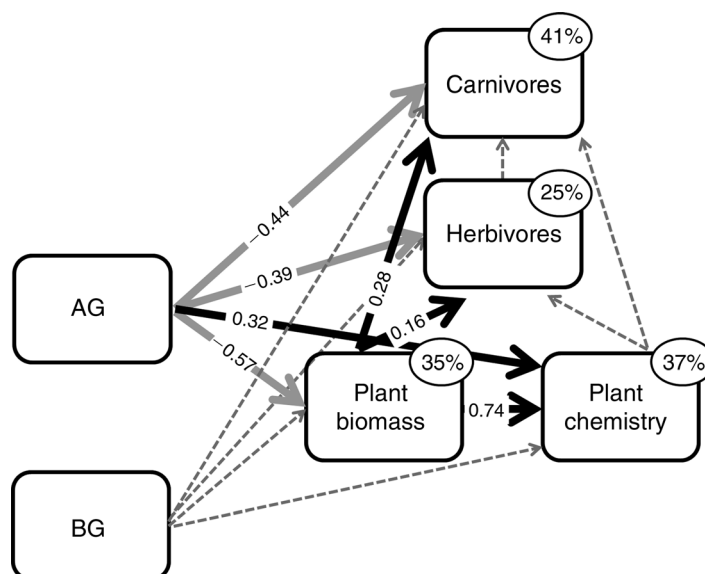
AG and BG neighbor effects on aboveground arthropods

The arthropod fauna on focal *J. vulgaris* plants consisted mostly of generalists, and was dominated by larvae of leaf-mining flies, aphids, and spiders (Appendix C: Table C3). For all neighboring species, AG presence negatively affected herbivore ($F_{1,145} = 37.69$, $P < 0.001$; Fig. 1c) and carnivore ($F_{1,145} = 74.89$, $P < 0.001$; Fig. 1d) abundances on the focal *J. vulgaris* plant. Overall, there was no BG effect on herbivore ($F_{1,145} = 1.11$, $P = 0.295$) or carnivore abundance ($F_{1,145} = 0.11$, $P = 0.740$). When *J. vulgaris* was used as a neighbor, there was a significant interaction between AG and BG for herbivore abundance ($F_{1,41} = 5.92$, $P = 0.019$; Fig. 1c): BG presence had a positive effect on herbivore abundance in absence of AG neighbors, and a negative effect in presence of AG neighbors. Neighbor identity had a significant effect on both herbivore ($F_{4,145} = 3.44$, $P = 0.010$; Fig. 1c) and carnivore abundance ($F_{4,145} = 3.00$, $P = 0.021$; Fig. 1d). Focal *J. vulgaris* plants harbored more herbivores when the neighboring species was *J. vulgaris* than when it was surrounded by *T. vulgare* or *P. lanceolata* (post-hoc Tukey test, $P < 0.05$; Fig. 1c). Focal *J. vulgaris* plants had the fewest carnivores when surrounded by *T. vulgare* (post-hoc Tukey test, $P < 0.05$; Fig. 1d). When all neighboring species were combined, herbivore and carnivore abundance on the focal plant were negatively related to the biomass of the neighbor (herbivores; $r = -0.398$, $P < 0.001$, $n = 150$; carnivores; $r = -0.232$, $P = 0.004$, $n = 150$).

The type of BG contact with the neighboring plant (growing in connected pots or in conditioned soil in disconnected pots) did not affect herbivore or carnivore abundance on the focal plant (Fig. 1c, d). Furthermore, herbivore and carnivore abundance did not differ between focal plants exposed to AG neighbors growing in connected pots (AG and BG connected) and focal plants exposed to AG neighbors growing in control soil in disconnected pots (AG; Fig. 1c, d).

There was a significant AG effect on the composition of the arthropod community on focal plants (RDA: pseudo- $F = 28.2$, $P = 0.002$; 15.2% explained variation). Most arthropod families were less abundant when an AG neighbor was present; aphids and the larvae of leaf-mining flies were most affected (Appendix A: Fig. A4). There was also a significant effect of neighbor identity (pseudo- $F = 2.8$, $P = 0.002$; 6.6% explained variation)

FIG. 3. Structural equation models of the relationships between aboveground neighboring plants (AG), belowground neighboring plants (BG), biomass and chemistry of the focal *J. vulgaris* plant, and herbivore and carnivore abundance on the focal plant. Plant chemistry is represented by the sample scores on the first axis of a principal component analysis on foliar plant chemistry. Solid arrows depict significant effects ($P < 0.05$), dashed arrows show nonsignificant effects. The path from plant biomass to herbivores was only marginally significant ($P = 0.054$). Standardized path coefficients are provided for significant paths (black indicates a positive relationship, gray indicates a negative relationship). Percentages indicate the variance explained by the model for each endogenous explanatory variable.



and a significant $AG \times$ identity effect (pseudo- $F = 2.0$, $P = 0.01$; 4.9% explained variation). There was no significant BG or $AG \times BG$ effect on the arthropod community ($P > 0.05$).

We used SEM to disentangle the direct and indirect neighbor effects on arthropod abundance on the focal plant. The SEM that included all neighboring species provided a good fit to the data ($\chi^2_6 = 3.43$; $P = 0.75$). AG neighbors negatively affected herbivore and carnivore abundance on the focal plant, both directly and indirectly via a negative effect on the biomass of the focal plant (but not via foliar chemistry; Fig. 3). For both herbivore and carnivore abundance, the direct AG effect was stronger than the indirect AG effect mediated via plant biomass (herbivores; direct effect = -0.39 , indirect effect = -0.09 ; carnivores; direct effect = -0.44 , indirect effect = -0.16). Carnivore abundance was more strongly affected by AG neighbors than herbivore abundance. There was no direct or indirect BG effect on arthropod abundance (Fig. 3). When the AG and BG connected treatment was included in the SEM analysis, the BG neighbor effect was still not significant (data not shown). The SEMs for each individual neighboring species showed similar negative direct and indirect AG effects on arthropod abundance, but there were some minor species-specific differences (Appendix A: Fig. A5).

We also used SEM to explore which AG traits of the neighboring plants most affected arthropod abundance on the focal plant. In this analysis, only data from the AG and AG and BG treatments were included. The SEM that included all neighboring species provided a good fit to the data ($\chi^2_{21} = 23.34$; $P = 0.33$). Arthropod abundance on the focal plant was affected directly by neighbor identity and neighbor biomass, but not indirectly via effects on *J. vulgaris* biomass or chemistry (Fig. 4). Carnivore abundance was negatively affected

by neighbor reproductive biomass, but was also affected by other traits of the AG neighbors (significant path from neighbor identity to carnivores on the focal plant). Herbivore abundance on the focal plant was negatively related to foliar biomass of the neighboring plant. Arthropod abundance on the focal plant was not related to arthropod abundance on the neighboring plant (Fig. 4).

DISCUSSION

Our study demonstrates that AG effects of different neighboring plant species were more important for the growth and quality of *J. vulgaris* and the arthropod abundance on this plant than BG effects of neighbors. So far, studies on neighbor effects on plant–arthropod interactions have not distinguished between AG and BG effects and only tested the combined effects (e.g., Hambäck et al. 2000, Agrawal 2004, Kostenko et al. 2012a). Our study provides evidence that AG and BG effects of neighboring plants are not necessarily equally strong, and may affect plant–arthropod interactions on a focal plant in a nonadditive way.

Our first hypothesis predicted that BG neighbor effects would be more important for the growth and chemistry of focal plants than AG neighbor effects, but our results showed the opposite effect. This is remarkable, because other work has shown that the growth and chemistry of *J. vulgaris* can be greatly influenced by soil conditioning (Bezemer et al. 2006, 2013, van de Voorde et al. 2011, Kos et al. 2013). Although it is generally accepted that plant performance is affected more by BG competition than by AG competition with neighbors, the intensity of AG competition increases with decreasing light supply, and the intensity of BG competition decreases with increasing nitrogen availability (Wilson and Tilman 1993, Kiaer et al. 2013). The focal plant in our study was surrounded by four neighboring plants, which may have decreased light availability substantial-

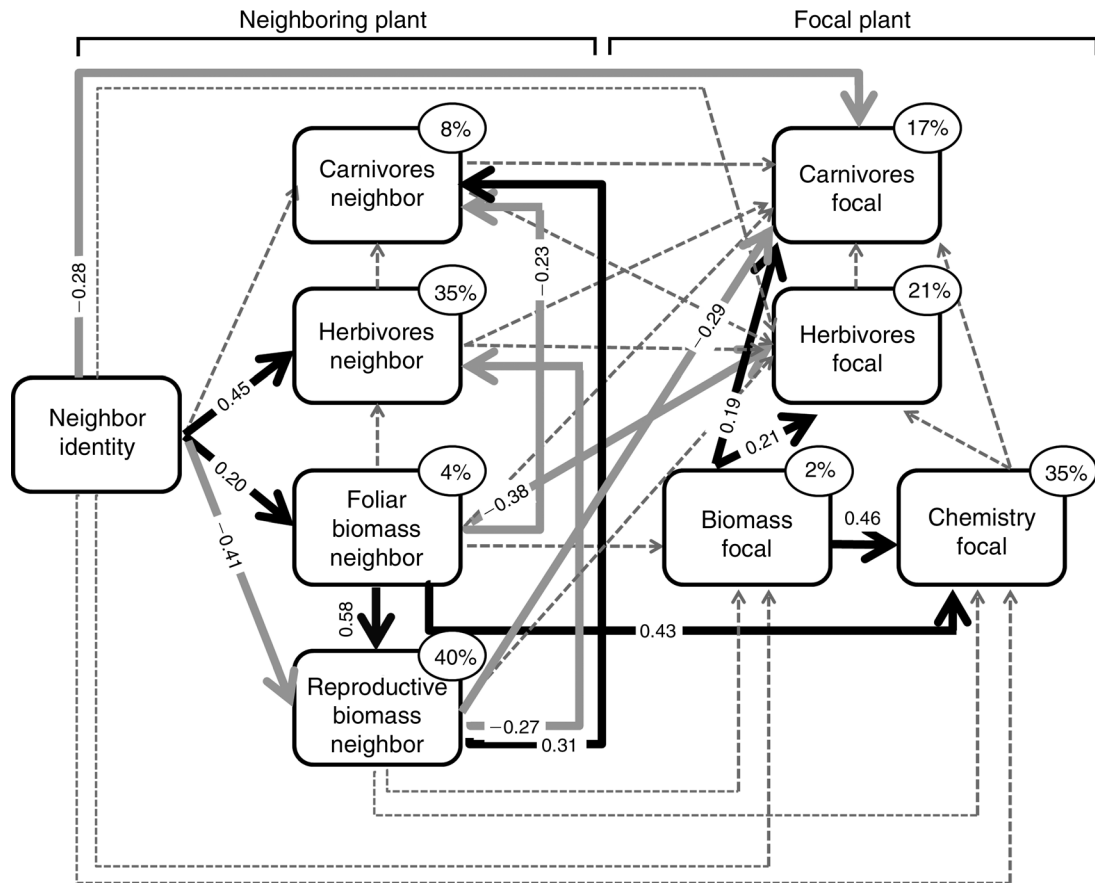


FIG. 4. Structural equation model of the relationships between neighbor identity, neighbor biomass (foliar and reproductive biomass), arthropod abundance on the neighboring plants, and biomass, chemistry, and arthropod abundance of the focal *J. vulgaris* plant. Chemistry of the focal plant is represented by the sample scores on the first axis of a principal component analysis on foliar plant chemistry. Solid arrows depict significant effects ($P < 0.05$), dashed arrows show nonsignificant effects. Standardized path coefficients are provided for significant paths (black indicates a positive relationship, gray indicates a negative relationship). Percentages indicate the variance explained by the model for each endogenous explanatory variable.

ly, increasing the potential for strong AG competition. Nitrogen availability was probably not limiting in our soils, as we added sterile soil to all pots, and the process of sterilization may have also increased available soil nutrients (Troelstra et al. 2001). The addition of sterile soil may have reduced the potential PSF effect on focal plant growth, because PSF effects on plant performance are mediated by biotic as well as abiotic conditions (Bever et al. 1997, Ehrenfeld et al. 2005, van der Putten et al. 2013). Interestingly, when all neighboring species were combined, we found an interaction between the AG and the BG neighbor effect for *J. vulgaris* root biomass, showing that AG and BG neighbor effects may cause nonadditive effects on the focal plant. Depending on the identity of the neighboring plant species, AG and BG neighbor effects on the growth of the focal plant may even be antagonistic; for instance, as we observed for AG and BG effects of *P. lanceolata* on *J. vulgaris* root biomass. It has been shown that competition with neighbors may alter a plant's sensitivity to PSF (Casper and Castelli 2007, Hol et al. 2013). In line with the

stronger AG effect on focal plant growth and quality, the AG neighbor effect on arthropod abundance on the focal plant was much stronger than the BG neighbor effect, in agreement with our second hypothesis. However, in contrast to our third hypothesis, SEM showed that the AG neighbor effect was stronger even when only the indirect neighbor effect (via focal plant biomass or quality) was taken into account. Only when the neighbor was *J. vulgaris* did we find a BG effect on herbivore abundance, but the direction of the BG effect depended on the presence of AG neighbors, resulting in an AG \times BG interaction. Thus, it is clear that, in our study system, AG neighbor effects were relatively more important for plant–arthropod interactions on *J. vulgaris* than BG neighbor effects. However, it may be possible that due to our experimental design, the AG treatment more rapidly affected plant biomass and chemistry, which then affected arthropod communities, than the BG treatment.

In line with our fourth hypothesis, we found a strong negative AG neighbor effect on focal plant growth and

nutritional quality, as well as on arthropod abundance and community composition on the focal plant, suggesting that AR occurred. In general, AR has been reported more frequently in ecological studies than AS, although AS is more common in interactions with insects (Barbosa et al. 2009). The herbivore community that we found on *J. vulgaris* consisted almost exclusively of generalists. Although it was previously suggested that AR is more likely for specialist herbivores, a recent meta-analysis showed that the dietary breadth of an herbivore does not affect the likelihood of AR or AS (Barbosa et al. 2009). Although AS has been suggested to be more likely between taxonomically related plant species (Barbosa et al. 2009), our results suggest that neighboring species from the same plant family as the focal plant may also confer AR. The SEM showed that the direct AG neighbor effect on arthropod abundance was stronger than the indirect AG effect mediated via the focal plant. The direct negative effect of AG neighbors on herbivore abundance was probably not caused by higher predation by carnivorous arthropods in the more diverse plant assemblages, as predicted by the enemies hypothesis (Root 1973, Andow 1991, Agrawal et al. 2006), because carnivore abundance was not higher, but lower, with AG neighbors. It is important to note that we cannot directly address AR or AS, as we did not quantify damage to plants or herbivore performance.

The magnitude of the negative AG effect conferred by neighboring plants depended on the identity of the neighbor. Focal *J. vulgaris* plants had the fewest herbivores and carnivores when surrounded by *T. vulgare*, which had the highest biomass of all neighboring species. Both herbivore and carnivore abundance on *J. vulgaris* were negatively related to biomass of the neighboring plants. SEM showed that the factor neighbor identity, which represents plant traits that were not measured in our study, such as volatile emission, plant architecture, or leaf or flower color (Barbosa et al. 2009), likely affected carnivore abundance. Neither herbivore nor carnivore abundance on the focal plant was related to the abundance of herbivores or carnivores on the neighboring plants, suggesting a lack of spillover of arthropods between plant neighbors (Andow 1991, White and Whitham 2000).

Herbivore abundance on the focal *J. vulgaris* plant was higher when surrounded by conspecific plants, and thus when plant patch size increased. The resource concentration hypothesis (RCH) predicts an increase of specialist herbivore abundance with increasing patch size, because immigration rates associated with large patches are higher, whereas emigration rates are lower (Root 1973). The density responses of insects, however, may be highly variable, depending on how herbivore foraging biology affects migration rates between patches (Bowman et al. 2002, Bukovinszky et al. 2005, Hambäck and Englund 2005) and how variation in plant traits,

such as nutritional quality, interacts with insect movement and reproduction (see, e.g., Bukovinszky et al. 2010, Hambäck et al. 2012). Ultimately, densities of herbivores on focal plants will depend on whether interactions with plant neighbors translate into frequency-dependent (associational) or density-dependent (concentration or dilution) effects on herbivore movement (Andersson et al. 2013, Hambäck et al. 2014). Our results indicate the possible role of associational and dilution effects of AG–BG interactions on insect population densities. The experimental approach described here may be extended to quantify the possible role of AG–BG associational effects between plants on herbivore foraging behavior and reproduction.

Our fifth hypothesis predicted that focal plant growth, quality, and arthropod abundance would be lower in connected (open) pots, where roots and soil of the focal and neighboring plants were in contact, than in disconnected pots filled with conditioned soil. As expected, root biomass and root:shoot ratio of the focal plant were lower when plants exposed to AG neighbors were growing in connected pots than when they were growing in conditioned soil in disconnected pots. However, shoot biomass, plant chemistry, and AG arthropod abundance were not affected by the type of BG contact. In agreement with our sixth hypothesis, shoot biomass of the focal plant was higher when plants exposed to AG neighbors were growing in connected pots (i.e., AG with root contact) than when they were growing in control soil in disconnected pots (i.e., AG without any BG neighbor effect), suggesting that root contact with the neighbor reduced the negative AG effect of that neighbor on plant biomass. It is known that BG and AG competition with neighbors can interactively affect plant growth and that these interactions can range from positive to negative, depending on, e.g., species identity and fertilization (e.g., Cahill 1999, 2002, Kiaer et al. 2013). AG arthropod abundance was not affected by direct root contact with the neighbor, even though foliar C:N ratios were smaller when focal plants exposed to AG neighbors grew in connected pots than when these plants grew without any BG effect in disconnected pots. These findings confirm that BG neighbor effects are less important for plant–arthropod interactions on *J. vulgaris* than AG neighbor effects. However, as discussed, our experimental design may have caused an underestimation of BG effects.

Even though there were very few significant BG neighbor effects on the biomass of the focal plant, the composition of the microbial communities in the soil differed significantly between the soil types. Soil fungal and bacterial communities of the control soil, collected from a species-rich grassland, separated most from the monospecific soils, which were collected from experimental monocultures. There were also clear differences in microbial community profiles among the monospecific soils, which supports the idea that PSF effects on soil microbial communities can be highly species specific

(Kardol et al. 2006, van de Voorde et al. 2011, van der Putten et al. 2013). However, the five monospecific soils could not be completely separated from each other based on their microbial community composition, but they clustered into two distinct groups. It is important to note there were no monocultures of *J. vulgaris* available, and we collected rhizosphere soil from individual *J. vulgaris* plants that had been planted into the monocultures of the other species. This may explain why soil conditioned by *J. vulgaris* was very similar in fungal and bacterial communities to soil conditioned by *P. lanceolata* and *T. vulgare*.

We conclude that neighboring plants can strongly influence the growth and quality of a focal plant, and the abundance and community composition of arthropods on the focal plant, but that these effects occur predominantly AG. Our study confirms that BG interactions are not a mirror image of those AG (van der Putten et al. 2001). The species-specific differences of neighbors on plant growth, plant quality, and arthropod abundance indicate that associational effects on plant–arthropod interactions are context specific, and we call for further studies that quantify AG and BG associational effects in other plant systems.

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SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–C are available online: <http://dx.doi.org/10.1890/14-0563.1.sm>