



The relative importance of plant-soil feedbacks for plant-species performance increases with decreasing intensity of herbivory

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Abstract

Under natural conditions, aboveground herbivory and plant-soil feedbacks (PSFs) are omnipresent interactions strongly affecting individual plant performance. While recent research revealed that aboveground insect herbivory generally impacts the outcome of PSFs, no study tested to what extent the intensity of herbivory affects the outcome. This, however, is essential to estimate the contribution of PSFs to plant performance under natural conditions in the field. Here, we tested PSF effects both with and without exposure to aboveground herbivory for four common grass species in nine grasslands that formed a gradient of aboveground invertebrate herbivory. Without aboveground herbivores, PSFs for each of the four grass species were similar in each of the nine grasslands—both in direction and in magnitude. In the presence of herbivores, however, the PSFs differed from those measured under herbivory exclusion, and depended on the intensity of herbivory. At low levels of herbivory, PSFs were similar in the presence and absence of herbivores, but differed at high herbivory levels. While PSFs without herbivores remained similar along the gradient of herbivory intensity, increasing herbivory intensity mostly resulted in neutral PSFs in the presence of herbivores. This suggests that the relative importance of PSFs for plant-species performance in grassland communities decreases with increasing intensity of herbivory. Hence, PSFs might be more important for plant performance in ecosystems with low herbivore pressure than in ecosystems with large impacts of insect herbivores.

Keywords Plant-soil feedback · Herbivorous insects · Field conditions · Selective herbivory · Nutritional quality

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Introduction

Under natural field conditions, the performance (i.e., biomass production) of a plant is influenced by many abiotic and biotic environmental factors that act simultaneously above- and belowground (e.g., Bazzaz 1996; Wardle et al. 2004). Biotic environmental factors such as belowground microbiota and mesofauna as well as aboveground insect herbivory have profound effects on plant performance (Heinze and Joshi 2018).

Via litter production, exudation and uptake processes' plants induce changes in abiotic and biotic soil properties that, in turn, influence subsequent seedling establishment and plant growth. These plant-soil feedbacks (PSFs; Bever et al. 1997) are typically examined by evaluating the growth of a plant species in response to its own, 'home' (i.e., conspecific) soil compared to growth with other, 'away' (i.e., heterospecific) soil (e.g., Kulmatiski et al. 2008; van der Putten et al. 2013). Besides abiotic soil effects, soil biota are important drivers of PSFs (e.g., DeLong et al. 2019). Since microbial soil biota can function as pathogens or parasites (e.g., pathogenic fungi, bacteria or nematodes) or as mutualists [e.g., arbuscular mycorrhizal fungi (AMF), plant-growth promoting rhizobacteria (PGPR)] (see, e.g., van der Heijden et al. 2008; van der Putten et al. 2013; Bever et al. 2015), PSFs can be negative, neutral, or positive. Positive PSFs, for example, increase plant-biomass production and thus enhance competitiveness of plant species, whereas negative PSFs weaken their competitive ability. Therefore, PSFs are suggested to influence plant competition and community composition (e.g., Klironomos 2002; Kulmatiski et al. 2008; van der Putten et al. 2013) and have been the subject of intense research (see, e.g., Brinkman et al. 2010; Smith-Ramesh and Reynolds 2017). Besides influencing plant biomass, studies on PSFs revealed that soil biota also can influence the nutritional quality of plants (Kos et al. 2015) as well as the composition of secondary metabolites that are involved in herbivory defense (Kostenko et al. 2012; Bezemer et al. 2013). Hence, it is likely that plants growing in home vs. away soils, mediated by soil biota, differ in nutritional quality and palatability, which in turn influences aboveground herbivory.

Aboveground insect herbivory can affect plant performance directly (e.g., Hulme 1996), but can also influence the composition of plant communities by altering competitive asymmetry between plant species via selective herbivory (Borgström et al. 2016). Therefore, insect herbivory is considered a prominent factor influencing plant-species performance and community diversity (Crawley 1989; Branson and Sword 2009). Due to their metabolic requirements, herbivorous insects are known to prefer

plants with low carbon (C) to nitrogen (N) ratios [i.e., high N content] and high phosphorus content (Schädler et al. 2003; Berner et al. 2005; Huberty and Denno 2006; Behmer 2009). Therefore, changes in plant nutritional quality due to soil conditioning in home and away soils (Kos et al. 2015) may alter aboveground herbivore preferences (e.g., Mattson 1980; Massey et al. 2007) and finally the amount of biomass reduction.

As calculations of PSFs are mostly based on biomass ratios ('home' vs. 'away'; see Brinkman et al. 2010), it is likely that any disproportional reduction of plant biomass in home relative to away soils by herbivores, due to soil-mediated differences in plant nutritional quality, will influence the results (i.e., outcome) of PSFs. A previous study found that herbivory influences the outcome of PSFs (Heinze and Joshi 2018), but it is currently unknown how the outcome and thus importance of PSFs for plant performance are affected by the strength of this biomass reduction, i.e., by the intensity of herbivory. Thus, (1) if home or away soils increase plant nutritional quality, the resulting increase in aboveground herbivory could mask PSF effects on plant growth and (2) the strength of this masking effect will depend on the intensity of herbivory.

Both PSFs and herbivory affect the performance of plants and can act as mechanisms enabling coexistence in plant communities (e.g., i.e., Janzen–Connell effects; see Petermann et al. 2008; selective herbivory; see Borgström et al. 2016). Understanding the relative impact of PSF–herbivory interactions on plant performance is key to understanding the contexts in which these interactions contribute to coexistence. However, to the best of our knowledge, whether and how the intensity of herbivory influences the outcome of PSFs in the field has never been tested.

Most previous studies on PSF–herbivory interactions were performed under controlled greenhouse conditions (e.g., Morriën et al. 2011; Kostenko et al. 2012; Bezemer et al. 2013; but see Heinze and Joshi 2018). There is, however, high agreement that PSFs should be tested together with herbivory under field conditions to gain a comprehensive understanding on the importance of PSFs for plant performance (see van der Putten et al. 2016), especially because PSFs differ between greenhouse and field conditions (Heinze et al. 2016). Therefore, for the first time, we tested PSF (i.e., home vs. away) effects with a standardized comparative PSF pot experiment in nine grasslands that differed in intensity of aboveground herbivory. We focussed on effects of soil biota (i.e., biotic PSFs) to avoid confounding effects with abiotic soil properties that can also influence nutrient content of plants (e.g., Mattson 1980). In each of the nine grasslands, we manipulated the presence/absence of aboveground herbivorous insects with an herbivore-exclusion treatment. The intensity of herbivory (i.e., the density/abundance of insects) corresponded to the natural condition

(i.e., was not experimentally manipulated) to avoid restricting the herbivory effects to one or only a few types of herbivores. To assess the impact of home and away soils on the nutritional quality of plants, we analysed C and N concentrations in roots and shoots of the experimental plants. We hypothesized that: (1) home and away soils differentially influence plant nutritional quality; (2) as herbivorous insects chose plants selectively consume plants according to their nutritional quality, these home and away soil effects will consequently affect aboveground herbivory by insects; and (3) the outcome of PSFs is influenced by the intensity of herbivory, due to herbivore-induced changes in home vs. away biomass ratios.

Materials and methods

Study region

The comparative PSF experiment was performed in the Biodiversity Exploratories Project (Fischer et al. 2010) in nine grasslands within the Hainich–Dün region (Thuringia, Central Germany). The studied grasslands are located on calcareous mineral soils with high clay content (Fischer et al. 2010).

Plant-soil feedback experiment

We selected four common grass species that are widespread within Central Europe (Klötzli et al. 2010): *Arrhenatherum elatius* (L.) J. Presl. et C. Presl., *Anthoxanthum odoratum* L., *Dactylis glomerata* L., and *Holcus lanatus* L. All four species are perennial tussock grasses that are frequently found in grasslands within the Biodiversity Exploratories (Heinze et al. 2015a, b). Seeds of all four grass species were collected in 2016 in a meadow at a field site of the University of Potsdam (N52°24'29.76", E13°1'13.74", Brandenburg, Germany). In May 2017, seeds of all four species were surface-sterilized for 3 min in 7% sodium hypochlorite solution and subsequently rinsed with sterile water to prevent microbial contaminations. Afterwards, seedlings were germinated on autoclaved sand (5 times within 24 h; 20 min, 121 °C) in sterile plastic chambers (32 cm × 50 cm × 14 cm; Meyer; Germany) in a greenhouse at the University of Potsdam.

We used the “self vs. other” approach (Kulmatiski 2016) to investigate PSF effects for the four grass species. Although this approach does not provide insight into soil-mediated interactions between species pairs, it focuses on conspecific PSF effects and minimizes the sample size (Kulmatiski 2016). We used species-specific field conditioned rhizosphere soils of all species for our PSF experiment in accordance with the “natural-experiment” approach (Kulmatiski and Kardol 2008). All four species are perennials

that form persistent tussocks and, therefore, generated PSFs over longer time periods. Immediately before the start of the experiment, species-specific rhizosphere soils were sampled in the same meadow (size approximately 1 ha) that served as origin for the seeds. For each species, we selected 20 patches (30 cm × 30 cm), spaced at least 2 m apart from each other, in which the vegetation was solely covered (i.e., 100%) by the respective species (see Heinze et al. 2016 for description on vegetation structure). Within each patch, we collected 1 L of species-specific soil (top 20 cm) from the rhizosphere and directly adjacent to the rhizosphere following Brandt et al. (2014). As we were interested in general PSF effects rather than within-site variation in PSFs, we mixed the 20 replicate soil samples per species to one bulk soil for each species and split into two halves with one half serving as ‘home’ soil (i.e., conspecific soil), whereas the other half was used to create ‘away’ soils (i.e., soils of the remaining heterospecific species) for the other species. Although this mixing procedure decreases variance in plant responses among individual soil samples (Reinhart and Rinella 2016), this procedure was appropriate for our specific research question, as we were interested in general (rather than within-site variation of) PSF effects and how they are influenced by the intensity of herbivory. Furthermore, this mixing procedure is reported to produce similar PSFs compared to independent soil samples (see, e.g., Kulmatiski 2016; Cahill et al. 2017; Gundale et al. 2019). In total, there were eight soils: four home soils (one for every species) and four away soils that each consisted of equal proportions of soils from the three heterospecific species. To reduce potential differences in soil nutrient availability among the eight soils, the soils were inoculated (10%) into an autoclaved soil:sand mixture. The soil:sand mixture consisted of a 1:1 mixture of sieved (mesh size: 5 mm) field soil collected from the same meadow at the field site of the University of Potsdam and purchased sand (grain size: 2 mm; Brun & Böhm; Potsdam, Germany).

Pots (Deepots D25L: volume 0.41 L; height 25 cm; diameter 5 cm; Stuewe & Sons; USA) were prepared with an autoclaved fleece strip (3 cm × 25 cm) covering 10 cm of the pots’ inside and hanging out 15 cm to enable watering from below. The pots were subsequently filled with the inoculated soils. To prevent cross contamination between the pots, each pot was placed in a separate plastic cup (volume 0.3 L; height 15.2 cm; diameter 5.9 cm) and received an additional layer (1 cm) of sterilized sand on top.

In early June 2017, 2-week old similar-sized seedlings of all four species were planted in the prepared pots, one seedling per pot. Each species was grown in pots inoculated with ‘home’ soil or with ‘away’ soil. Immediately after planting, the pots were moved from the greenhouse to a protected outdoor site near the field study site of the University of Potsdam. There, seedlings were allowed to acclimatize for 1 week. Seedlings that died during this week were replaced.

Herbivore-exclusion treatment

To compare the outcome of PSFs for the four grass species in the presence vs. the absence of aboveground insect herbivores, we performed an herbivory-exclusion treatment in accordance with Heinze and Joshi (2018). This herbivore-exclusion treatment was established in nine grasslands in the Hainich–Dün region (see below). In each grassland, we established two plots (120 cm × 160 cm) that were spaced 80 cm apart. The plots were equipped with cages (length 160 cm × width 120 cm × height 100 cm) that were either completely covered with fly mesh (mesh size: 1.3 mm; Meyer; Germany) or only shaded (i.e., no fly mesh at the lower 50 cm). The fully covered cages excluded herbivorous insects (see MacDonald and Kotanen 2010), whereas the shaded cages allowed aboveground herbivorous insects to reach the experimental plants while providing the same levels of shade and precipitation as the cage treatment (see Heinze and Joshi, 2018). In both plots, we removed the sward to slightly (ca. 5 cm) sink the prepared pots (in boxes; see below) into the soil and for the fully covered plots to exclude non-developed aboveground herbivorous insects, whose eggs might be attached to plants or buried in the soil. In the fully covered plots, the fly screen was buried into the soil. One side was prepared as a door to water the plants. The fully covered plots that excluded aboveground herbivorous insects (> 1.3 mm) are referred to as ‘– herbivory’, whereas the shaded plots are referred to as ‘+ herbivory’ treatment throughout the manuscript. The plots within each grassland were fenced off (3 m × 3 m) to prevent herbivory by vertebrates as well as disturbances by land-use activities (e.g., mowing).

Intensity of aboveground insect herbivory

To test our hypothesis that the intensity of aboveground insect herbivory gradually affects the outcome of PSF effects under natural conditions, we selected nine grasslands along a gradient of land-use intensity. For this gradient, it has been shown that land-use intensification influences the abundance and diversity of multiple taxa (Manning et al. 2015), especially herbivorous insects (Simons et al. 2014a, b; Chisté et al. 2016). These land-use effects were found to ultimately affect the severity of aboveground insect herbivory (Bör-schig et al. 2014; Egerov et al. 2017), which decreases with increasing land-use intensity (Gossner et al. 2014).

We used information about past land-use practices (2006–2015), abundance of herbivorous insects (2011–2013) and herbivory on plants measured in the grasslands in 2013 to select nine grasslands along the land-use gradient that are supposed to form a gradient of aboveground insect herbivory (see Online Resource 1: Table S1). The nine grasslands differed in average amount of fertilizer application

as well as mowing and grazing intensity, factors that were previously shown to affect abundance and diversity of insect herbivores as well as invertebrate herbivory (Gossner et al. 2014; Simons et al. 2014a, b; Chisté et al. 2016; see Online Resource 1: Table S1).

Between and within years, land-use practices and their frequency that influences abundance of herbivorous insects, and thus intensity of herbivory, can be highly dynamic and dependent on climate conditions (Blüthgen et al. 2012). Therefore, we also used information regarding planned land-use management in 2017 (personal communications from farmers) for the selection of grasslands. We also recorded land-use practices (e.g., mowing events) before and during the experiment (Online Resource 1: Table S2).

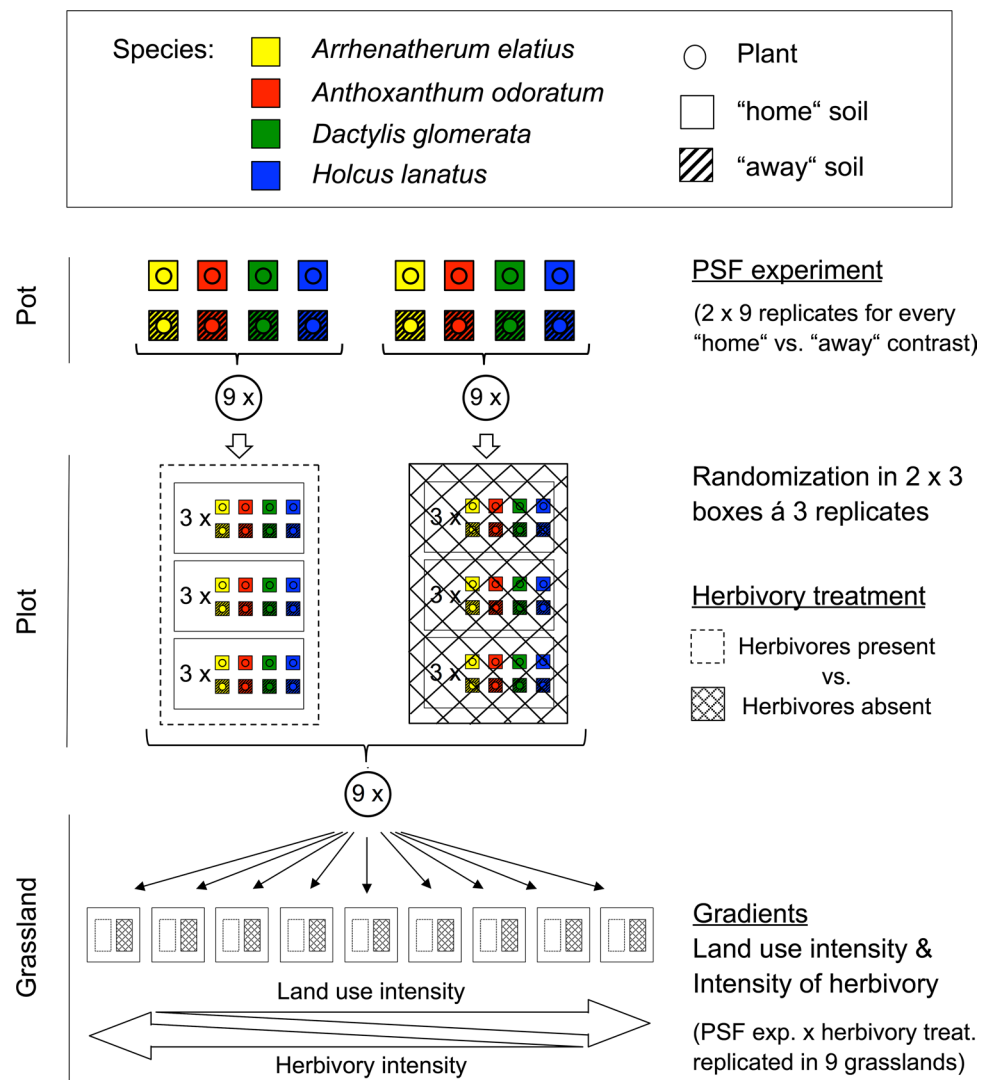
To test whether land-use intensity affects the intensity of aboveground herbivory in our experiment, we calculated an index of land-use intensity (LUI) according to Blüthgen et al. (2012). This index integrates three components of land use: mean amount of fertilizer (kg N ha⁻¹ year⁻¹), mean frequency of mowing (number cuttings year⁻¹), and mean intensity of grazing (live-stock units days of grazing ha⁻¹ year⁻¹) per grassland that are standardized by the mean of each component per region. The index is square-root transformed, to achieve more evenly distributed values. High values indicate intense land use and vice versa (see also Online Resource 1: Table S1).

PSF experiment and herbivore-exclusion treatment along the gradient of herbivory

In mid-June 2017, the planted pots (PSF experiment) were transported to the Hainich–Dün region and positioned in the prepared – and + herbivory plots (herbivore-exclusion treatment) at the nine grasslands (Fig. 1). In each of the nine grasslands, each treatment [herbivory exclusion and soil treatment (home vs. away)] was replicated nine times for every species, resulting in 1296 pots (9 grasslands × 4 species × 2 soils × 2 herbivory treatments × 9 replicates). In the experiment, each of the nine grasslands was equipped with exactly the same experimental setup. The planted pots were placed in individual plastic cups (see above) to enable watering from below and were arranged in a randomized block design [i.e., one block contained a single replicate per species and soil treatment (home vs. away)].

As we were interested in the effects of aboveground invertebrates (excluding slugs) and as we wanted to exclude direct competition between experimental and neighbouring plants in our experiment, pots and plastic cups were placed in boxes (78 cm × 50 cm × 30 cm). To protect the pots from slug herbivory, these boxes were filled with water (height: 5 cm). In addition to this water barrier, the edges of these boxes are effective barriers against slugs (personal observation J. Heinze). In every grassland, each + herbivory and

Fig. 1 Conceptual figure of the experimental design. To test plant-soil feedback (PSF) effects, four grass species were grown in pots in their ‘home’ and ‘away’ soils. To investigate the effect of herbivory on PSFs, nine replicates of each ‘home’ vs. ‘away’ contrast were exposed to a herbivory treatment in which aboveground insects could either reach the plants (+ herbivory plot) or not (– herbivory plot). Within each of the + and – herbivory plots, the nine replicates were arranged in a randomized complete block design and distributed over three boxes (i.e., one box contained 3 replicates/blocks). The boxes were necessary to prevent herbivory by slugs and competition with surrounding plants and to enable the watering from below. To test whether the intensity of herbivory affects the outcome of PSF effects, this setup (i.e., PSF experiment × herbivory treatment) was installed at nine grasslands that formed a gradient in aboveground herbivory intensity. In total, the experiment contained 1296 plants (4 species × 2 soils × 9 replicates × 2 herbivory treatments × 9 grasslands). For further details, see “Materials and methods”. Color version of this figure is available online



– herbivory plot contained three boxes, which again contained three blocks of pots each (see Fig. 1). At the beginning of the experiment, all plants were watered and all plastic cups underneath every pot were filled with 200 ml water. Every third week, the water level in the plastic cups was checked and water was added if necessary.

Measurements

We were interested in damage caused by herbivorous insects on the four grass species during the experimental time. We, therefore, measured herbivory on experimental plants, as these plants were not exposed to destructive land-use practices (like mowing) or slug herbivory. In early September 2017, after 11 weeks of variable invertebrate herbivory intensity exposure, we recorded herbivory on experimental plants. To check whether aboveground herbivory differed between the nine grasslands and the different home vs. away soils, we assessed the damage by aboveground chewing

insect herbivores without any further discrimination of feeding guilds. We visually estimated biomass removal (in percent; severity) at ten randomly chosen leaves per individual plant (see, e.g., Johnson et al. 2016). Furthermore, in accordance with Russel et al. (2010) for each single experimental plant, we also determined the proportion of damaged leaves by counting the number of damaged as well as total leaves (incidence). We used severity and incidence to assess the shoot biomass removal by aboveground insect herbivores for whole experimental plants according to Smith et al. (2005).

After herbivory measurements were complete, the pots were brought back to the University of Potsdam, where the shoots were harvested and the roots were washed. Shoot and root biomass was dried (shoot 48 h, 80 °C; root 72 h, 70 °C) and weighed.

To check whether inoculated soils differed in nutrient concentration, we analysed abiotic soil conditions of the eight different inoculated soils (four home soils and four away soils) prior to the experiment according to Heinze et al.

(2017). To test whether the different home and away soils affected the nutritional quality in plant shoots and roots, we analysed C and N (see Berner and Law 2016 for C and Cornelissen et al. 2003 for N). As the same soils were used in all of the nine grasslands, we analysed C and N in plant shoots and roots for subsamples of three grasslands. One replicate per species, soil, and herbivory treatment was sampled within these three chosen grasslands (see Online Resource 1: Table S1), resulting in 48 samples (4 species \times 2 soils \times 2 herbivory-exclusion treatments \times 3 grasslands). Complete shoots and roots were dried at 80 °C (48 h), separately ground (Retsch MM200; Germany) and subsequently analysed for C and N concentrations using an elemental analyser (HEKAtech GmbH; Wegberg; Germany; Euro EA 3000).

Statistical analysis

All analyses were performed in R version 3.1.2 (R Development Core Team 2014). To account for the split-plot design and the nesting of factors, we analysed the data on shoot-, root-, and total biomass, herbivory, PSFs, and C:N ratios of plants with linear mixed effects models using the “nlme” package (Pinheiro et al. 2017). Data on soil nutrients were analysed with linear models, as we tested initial conditions of soils prior to the experiment. Residuals were checked for homogeneity of variance and tested for normality.

We used ANOVAs and Tukey HSD tests to check whether the eight inoculated soils [i.e., the sterilized soil:sand mixture (90%) that was inoculated (10%) with the different home and away soils of all four species] differed in abiotic characteristics.

To test the first hypothesis that home and away soils differentially affect plant nutritional quality, we performed ANOVAs for N and C concentration as well as C:N ratios in shoots and roots. The ANOVAs included species (*A. elatius*, *A. odoratum*, *D. glomerata*, *H. lanatus*), soil treatment (home and away), and herbivory-exclusion treatment (+herbivory and –herbivory) as well as their interactions as predictor variables. We used “grassland” (three; see “Measurements”) as random factor. Afterwards, differences in N, C, and C:N between home and away soils were tested with two sample *t* tests for every species.

To test the second hypothesis, that home and away soils affect aboveground herbivory, and to verify whether intensity of aboveground herbivory differed between the nine grasslands along the land-use intensity gradient, we analysed the herbivory (i.e., estimated shoot biomass removal) of experimental plants that were exposed to herbivory (experimental plants in the—herbivory plots showed no damage by herbivores).

The ANOVA tested effects and interactions between the predictor variables ‘species (S)’, ‘soil treatment (T)’, ‘herbivory-exclusion treatment (H)’, and ‘land-use intensity

(LUI)’ as fixed factors on herbivory, as response variable. We used ‘boxes’ (three) nested in ‘grassland’ (nine) as random factors. In addition, we integrated shoot biomass as co-variable into the model, to test whether herbivory was related to shoot biomass. We used linear regressions to check whether herbivory was related to land-use intensity, for (1) all experimental plants and (2) separately for all species.

We used average percentage of estimated shoot biomass removal per grassland as a continuous variable in the following analyses to test for the effects of herbivory intensity on PSFs and biomass production (see below). Average percentage of estimated shoot biomass removal is, therefore, referred to as ‘intensity of herbivory’ throughout the manuscript.

PSFs were calculated using log biomass ratio of ‘home vs. away’ contrasts that has the advantage of directly comparing positive and negative feedback effects (see Brinkman et al. 2010): $PSF_A = \log(\text{home}_A / \text{away}_A)$, where ‘home_A’ is the biomass of species A with its own soil biota and ‘away_A’ is the biomass of species A with soil biota of the three remaining heterospecific species. PSFs were calculated pairwise per block (i.e., replicate) for shoot, root, and total biomass for the + and – herbivory treatments in every grassland.

To test the third hypothesis that the intensity of aboveground herbivory influences the outcome of PSFs, we performed ANOVAs using linear mixed effects models. The model included the predictors ‘species (S)’, ‘herbivory-exclusion treatment (H)’, and ‘intensity of herbivory (I)’ (average percentage of estimated shoot biomass removal per grassland) as fixed factors, as well as their interactions and tested their effects on PSFs. We used ‘boxes’ (three per herbivory plot), ‘herbivory plot’ (two per grassland), and ‘grassland’ (nine) as random factors that were nested as follows: boxes nested in herbivory plots and herbivory plot nested in grassland. Whether PSFs for the four species differed within the herbivory treatments along the gradient of herbivory intensity (S \times I interaction) was checked by separate ANOVAs for + herbivory and – herbivory. The relationship between intensity of herbivory and PSFs in the two herbivory-exclusion levels was analysed for each species using linear regressions, and differences in slopes were tested with ANOVAs (H \times I interaction).

The main focus of this study was to investigate effects of herbivory intensity on the outcome of PSFs. However, as PSFs are based on biomass ratios, it is likely that data on biomass (shoot) in home vs. away soils in response to herbivory intensity contain valuable information. These results are presented in the supporting information, along with the respective ANOVAs (see Online Resource 1: Table S3; Fig. S1).

Results

Home and away soil effects on plant nutritional quality and herbivory

At the beginning of the experiment, the eight inoculated soils neither differed in plant-available nor total nutrient concentrations (Table S4). However, plant shoot-N concentration but not C concentration was affected by the different home and away soils for all four species, resulting in different C:N ratios ($S \times T$: shoot N: $F_{3,30} = 10.06$, $P < 0.001$; shoot C:N: $F_{3,30} = 15.15$, $P < 0.001$; Online Resource 1: Table S5a). *A. elatius*, *D. glomerata*, and *H. lanatus* showed higher shoot-N concentration in away soils, whereas for *A. odoratum*, N concentration was highest in shoots when grown on home soils (Fig. 2a–h). N, C, and C:N ratios in roots were not affected by the different soils (Online Resource 1: Table S5b).

All four grass species showed differences in above-ground herbivore damage when grown in home vs. away

soils ($S \times T$: $F_{3,603} = 13.96$, $P < 0.001$; Online Resource 1: Table S6). *A. elatius*, *D. glomerata*, and *H. lanatus* showed the highest shoot biomass removal in away soils, where their shoots had the highest N concentration (Fig. 2i, k, l), whereas for *A. odoratum*, damage by aboveground herbivores was highest in home soils, where its shoots had the highest N concentration (Fig. 2j).

Aboveground herbivory on experimental plants along the gradient of land-use intensity

The estimated shoot biomass removal was highest in less intensively managed grasslands and decreased with increasing land-use intensity ($F_{1,7} = 12.71$; $P = 0.009$; Tables S6; Fig. 3). This pattern of herbivore damage in response to land-use intensity was similar for all four species ($S \times LUI$: $F_{3,603} = 1.74$; $P > 0.05$; Online Resource 1: Table S6; Fig. S2). When grown without herbivores, shoot biomass was similar in all grasslands along the land-use gradient, but

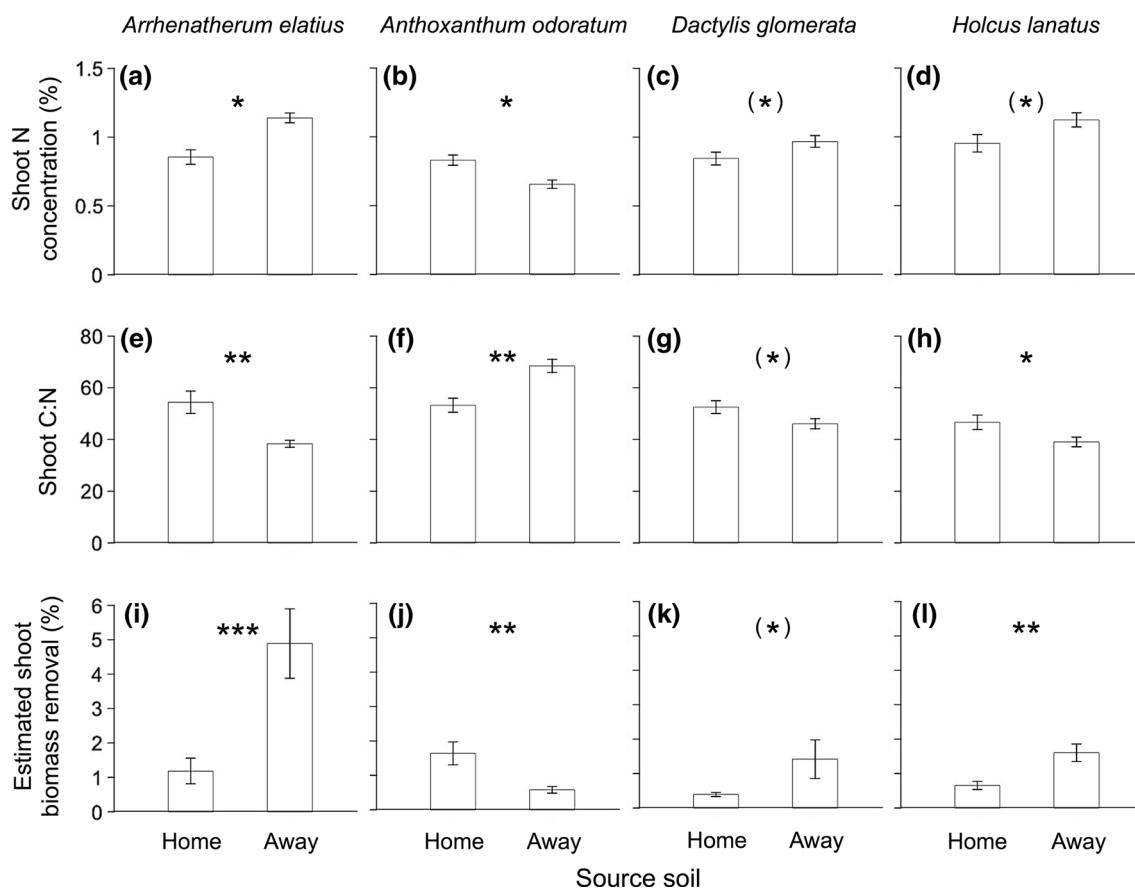


Fig. 2 a–d Shoot-nitrogen (N) concentration, e–h shoot carbon (C)-to-nitrogen ratio (C:N) as well as i–l estimated shoot biomass removal by aboveground insect herbivores of *A. elatius* (left), *A. odoratum* (middle left), *D. glomerata* (middle right), and *H. lana-*

tus (right) grown in “home” (left bars) and “away” (right bars) soils. Data represent mean \pm SE; with $n=6$ for a–h and $n=81$ for i–l. Asterisks between bars represent significance: (*) $P < 0.1$; ** $P < 0.05$; *** $P < 0.001$

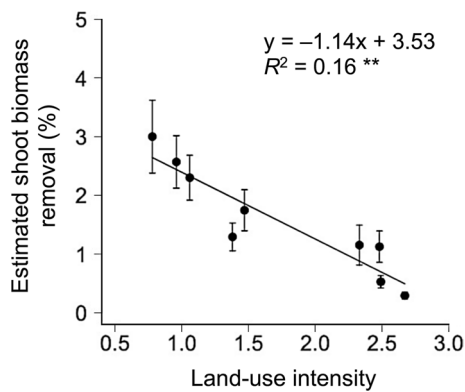


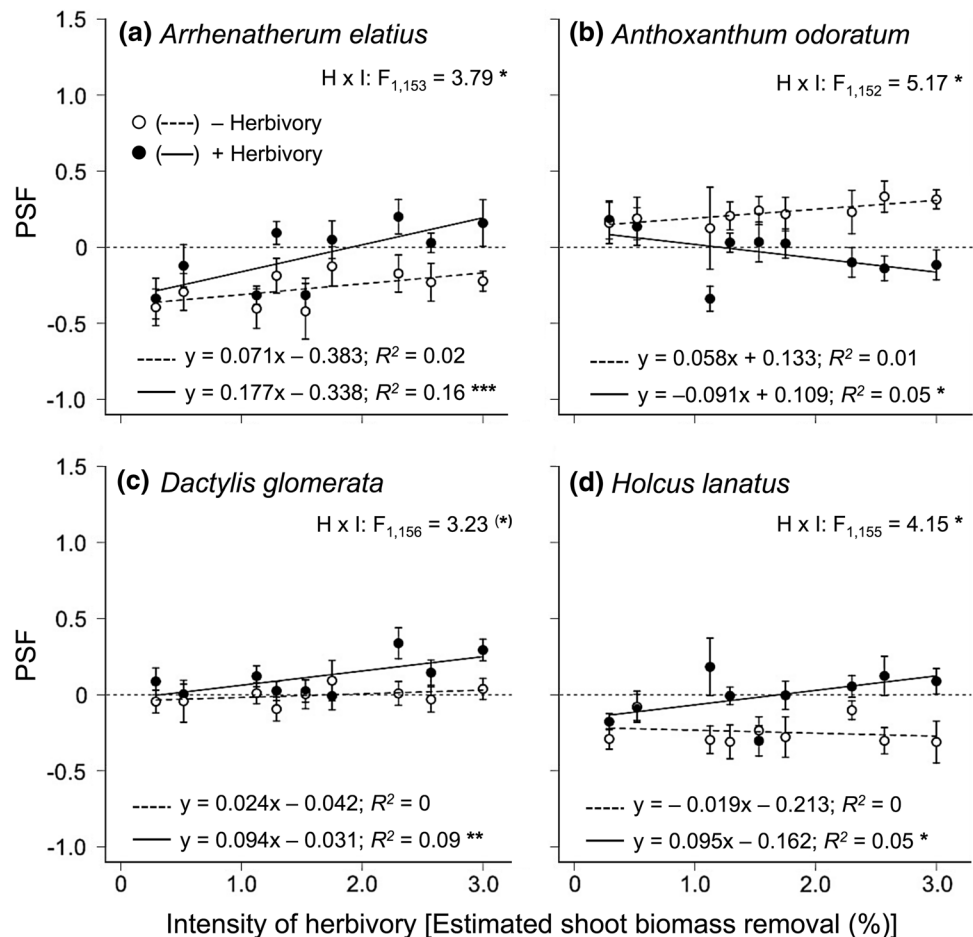
Fig. 3 Relationship between land-use intensity and estimated shoot biomass removal of all experimental plants exposed to herbivory. Data represent mean \pm SE ($n = 72$)

decreased with decreasing land-use intensity in the presence of herbivores (see Online Resource 1: Fig. S3).

Impact of intensity of aboveground herbivory on PSFs

For all four grass species, the presence of aboveground herbivory influenced the outcome of PSFs for total plants (shoots and roots), but these effects differed among the four species along the gradient in intensity of herbivory ($S \times H \times I$: PSF total: $F_{3,566} = 4.53$, $P = 0.004$; see Online Resource 1: Table S7). Without aboveground herbivores, the four species exhibited different individual PSFs (Fig. 4a–d). *A. elatius* and *H. lanatus* exhibit negative PSFs in home soils (i.e., showed higher biomass production in away soils), *A. odoratum* in contrast responded positively to home soils (i.e., showed positive PSFs), and *D. glomerata* showed neutral PSFs (Fig. 4a–d). Importantly, for all species, these PSFs remained similar in magnitude and direction along the gradient of aboveground herbivory intensity ($S \times I$: $F_{3,278} = 0.9$, $P > 0.5$; Online Resource 1: Table S7a; Fig. 4a–d). In contrast, when plants were exposed to aboveground herbivory, the direction and magnitude of PSFs

Fig. 4 Relationship between intensity of herbivory (i.e., average shoot biomass removal by aboveground herbivores per grasslands) and plant-soil feedback [PSF; log total biomass ratio (“home”/“away”)] in the presence (full circles) and absence (open circles) of aboveground herbivorous insects; for **a** *Arrhenatherum elatius*, **b** *Anthoxanthum odoratum*, **c** *Dactylis glomerata*, and **d** *Holcus lanatus*. Statistics shown are interactions of herbivory exclusion (H) and intensity of herbivory (I) derived from ANOVAs and for lines derived from linear regressions. Asterisks represent significance: (*) $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Data represent mean \pm SE ($n = 9$)



for all four species were significantly altered by herbivory intensity ($S \times I: F_{3,288} = 8.57, P < 0.001$; Online Resource 1: Table S7; Fig. 4a–d). The mostly negative and neutral PSFs of *A. elatius*, *H. lanatus*, and *D. glomerata* became more positive with increasing intensity of herbivory, whereas for *A. odoratum*, positive PSFs decreased. Increasing intensity of herbivory increased the difference between PSFs measured with and without herbivores, whereas in the presence of herbivores, increasing intensity resulted in mostly neutral PSF effects (Fig. 4a–d).

Discussion

The results of our study confirm all three hypotheses and reveal four important findings. First, shoot-N concentration of the four grass species was influenced by whether the plants were growing in home or away soils. Second, herbivory by aboveground invertebrate herbivores differed between home and away soils, with all species exhibiting most damage in soils in which their shoots contained the highest N concentrations. Third, home and away soils also affected biomass production (i.e., PSFs) of all four species, with the highest biomass production in soil in which the species also exhibited the highest shoot-N concentration. Fourth and most important, in the presence of herbivores, these PSFs changed in magnitude and in direction with increasing intensity of aboveground herbivory, while without herbivores, these PSFs remained similar along the gradient of herbivory. These results suggest that the relative importance of PSFs for individual plant-biomass production and thus for the performance in plant communities increases with decreasing intensity of herbivory.

Effect of home and away soils on plant quality

In our PSF experiment, all eight home and away soils did not differ in total or plant-available nutrients at initial conditions, an advantage of the inoculation method (Brinkman et al. 2010). Hence, the observed differences in plant nutritional quality (i.e., N and C concentrations) and biomass production of the grass species in the different soils (i.e., home and away) appear to be caused by soil biota.

In this study, we examined whether the N and C concentrations in plants, chemical plant traits that were broadly overlooked in the past and rarely tested in the context of PSF (see Baxendale et al. 2014; Cortois et al. 2016) were affected when grown in the different soils. We observed that the grass species exhibited the highest shoot-N concentration in soils, where also their biomass production benefitted from soil biota (positive away soil effects for *A. elatius* and *H. lanatus* and positive home soil effects for *A. odoratum*). This result is in accordance with findings of Stajković-Srbinić et al. (2016), who

showed that inoculation with plant PGPRs enhances both plant biomass and N content in shoots of grass species (see also Baltensperger et al. 1978; White et al. 2015). In our experiment, N concentration was enhanced in shoots in soils, where the species benefited from soil biota but not in roots, and a pattern also found in the previous inoculation studies with grasses (e.g., Baltensperger et al. 1978; Djonova et al. 2016). Overall, shoots show high turnover rates during growth and thus are sinks for N (Mattson 1980; Xu et al. 2012). This might explain why increased N concentration was confined to shoots.

Plant quality and aboveground insect herbivory

In general, due to their high protein content and poor N use efficiency, herbivorous insects need to ingest relatively large amounts of N (Mattson 1980; Bernays and Chapman 1994). Insect herbivores, therefore, generally prefer to feed on plants with high N content (Berner et al. 2005; Behmer 2009). In our experiment, all four grass species showed the highest shoot damage (i.e., estimated shoot biomass removal) caused by aboveground herbivorous insects in soils in which they had the highest shoot-N concentrations. This result is consistent with studies, showing that the quantity of herbivore damage is positively related to plant N content (Cebrian and Lartigue 2004; Berner et al. 2005). A reverse pattern was observed for C:N ratios. In line with Schädler et al. (2003), we found all species to have lowest levels of shoot damage in soils, where plants had the highest C:N ratios. This suggests that beside shoot-N concentration, the palatability is influenced by other physical and/or chemical plant properties (Massey et al. 2007). Soil conditioning can influence other primary and secondary compounds such as amino acids, glycosides, and pyrrolizidine alkaloids (e.g., Kostenko et al. 2012; Kos et al. 2015; Zhu et al. 2018) and, therefore, might affect the palatability of a plant. Furthermore, there are also indications that biotic or abiotic soil characteristics can affect the leaf toughness of plants (Orwin et al. 2010). However, to what extent physical anti-herbivore plant properties are influenced by soil conditioning remains unknown. Although we did not determine specific N-containing secondary metabolites, amino acids or silica content in our study, we, nevertheless, provide empirical evidence that soil-mediated differences in total N concentration in shoots can strongly affect herbivory by aboveground arthropods. Such specific home and away soil effects on aboveground plant damage and their intensity subsequently affected the outcome of PSFs in our experiment (see below).

Intensity of herbivory and its effects on the outcome and importance of PSFs

Increasing intensity of herbivory increased the difference between PSFs measured with and without aboveground

insect herbivores. These results confirm the previous studies on PSF and herbivory that aboveground herbivores can have negative direct effects on plant growth in the feedback phase (Bezemer et al. 2013). Hence, herbivory has the potential to affect the outcome of PSFs (Heinze and Joshi 2018), most likely due to soil-mediated differences in plant quality. However, more importantly with our present study, we were able to show, for the first time, that the intensity of herbivory gradually affected the outcome of PSFs. The change in direction and magnitude of PSFs in response to increasing herbivory intensity mostly resulted in neutral PSFs for the grass species, suggesting that aboveground herbivores reduce the soil-mediated benefits for biomass production depending on herbivore intensity. This is supported by analyses of shoot biomass along the gradient of herbivory intensity: herbivores solely reduced shoot biomass on one specific soil type, namely, soil in which the species showed the highest shoot-N concentration (in away soil for *A. elatius*, *D. glomerata* and *H. lanatus* and in home soil for *A. odoratum* see Online Resource 1: Fig. S1).

Grasses are known to have a large and often finely branched root systems with a large surface area and, therefore, may be more susceptible to root pathogens (Newsham et al. 1995). The *A. odoratum* in comparison with the other species exhibited positive PSFs might be due to its high concentrations of coumarin they exudate via roots in comparison with other species (Tava 2001). Coumarin was recently found to have a negative effect on soil pathogens but a positive impact on beneficial rhizobacteria (Stringlis et al. 2018) that are important for nutrient uptake and thus plant N concentrations (e.g., Adesemoye et al. 2010). This might also explain the neutral and negative PSFs of the other species, as the away soils they grew in most likely contained coumarin exudates from *A. odoratum*. However, we did not determine soil microbial communities in our experiment. Therefore, future studies should use sequence techniques to better understand the role of soil biota in PSF–herbivore interactions.

Nevertheless, the findings of our study provide new insights and allow assessments of the importance of PSFs for plant performance in relation to the intensity of herbivory, which has only been considered within a theoretical framework so far (see Smith-Ramesh and Reynolds 2017). Based on results of this study, we propose that (1) PSFs might be more important for plant performance in ecosystems, where the influence of aboveground herbivores is low and (2) as the magnitude and direction of PSFs are altered by herbivory, mostly resulting in neutral PSFs, the importance of PSFs will be changed or overridden by aboveground herbivores in ecosystems, where herbivorous insects have a large impact on plant communities (see Fig. 5).

In our experiment, species were best supplied with N in soils from which they received the highest biomass gain,

indicating that biotic PSFs influence plant performance and quality (Fig. 5a). As larger plants with more biomass are considered to be better competitors in plant–plant interactions (e.g., Aarssen 2015; Heinze et al. 2015a), aboveground herbivores, via specific selection of well-supplied plants (i.e., high N concentration), might prevent the development of dominance structures within plant communities (Fig. 5b). Potential soil-mediated competitive advantages might, therefore, be attenuated by selective herbivory, thus promoting coexistence in plant communities (see Fig. 5).

We suggest that negative density-dependent soil effects (i.e., Janzen–Connell effects) such as negative PSFs for more competitive plant species (*A. elatius*, *D. glomerata*, and *H. lanatus*; see Pierce et al. 2017) can act as a stabilizing mechanism (see Chesson 2000) enabling species coexistence in ecosystems with low abundances of herbivorous insects. However, in ecosystems with high abundance of herbivorous insects, plant species coexistence might be elevated due to additional equalizing mechanisms, such as selective herbivory that neutralizes soil-mediated competitive advantages, thus influencing the competitive asymmetry between competing plants (Borgström et al. 2016).

In our study, we focused on effects of intensity of aboveground insect herbivory on the outcome of PSFs. Soils in our experiment were conditioned with one specific herbivore community (i.e., intensity of herbivory). As the intensity of herbivory is suggested to influence PSFs (Smith-Ramesh and Reynolds 2017) further studies should perform soil conditioning under different intensities of herbivory and investigate these conditioning effects in a feedback phase. Furthermore, we solely excluded insect herbivores > 1.3 mm in our experiment. However, slugs or smaller insect herbivores such as aphids can also have large effects on plant performance (Crawley 1989; Rodríguez and Brown 1998). Therefore, further studies should examine PSF–herbivory interactions using stepwise exclusion of herbivores and test these interactions across different habitat types as well as with other functional groups to elucidate the relative contribution of herbivores on biomass production and thus their impact on the outcome PSFs.

Conclusions

This study is the first to provide empirical evidence that the outcome of PSFs depends on the intensity of aboveground insect herbivory even in our short-term experiment. Soil-mediated differences in plant quality affected herbivory. The intensity of herbivory in turn influenced the shoot biomass in home and away soils for all species and, therefore, the overall outcome of PSFs. We propose that PSF effects might be more important for plant performance in ecosystems with low insect herbivore pressure compared

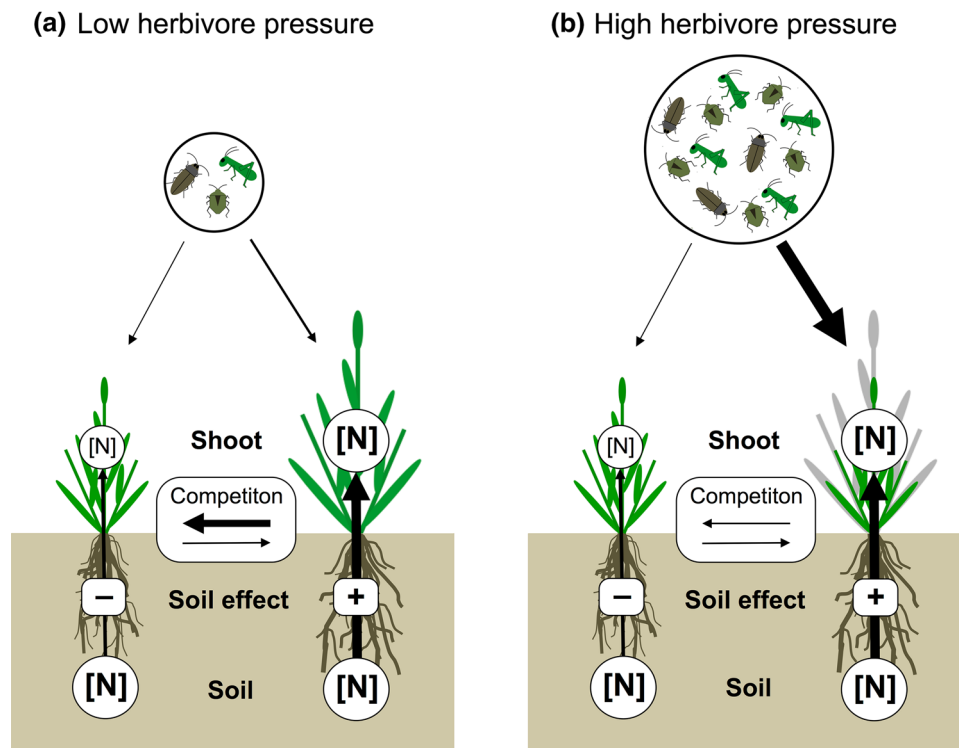


Fig. 5 Diagram showing how PSF may differently affect plant performance and plant–plant competition in ecosystems with **a** low vs. **b** high herbivore pressure. In general, soils can have negative or positive effects on nutrient uptake [e.g., nitrogen (N)] resulting in smaller plants with lower nutrient quality in shoots (left plant) or larger and better-supplied plants (right plant). These soil-mediated differences in plant quality and performance might affect competition between competing plants. In ecosystems with low herbivore pressure (**a**), this soil-mediated advantage in plant growth might be maintained due to marginal damage by insect herbivores resulting in enhanced compe-

titution effects for the larger plant. However, in ecosystems with large herbivore pressure (**b**), effects of insect herbivores might be larger for better-supplied plants. This selective herbivory might dampen the soil-mediated gain of plant growth (grey shadowed) and, therefore, attenuate competition between plants. Overall, effects from soils influence plant performance and competition, but depending on the intensity and selectivity of herbivory, these effects might be influenced by herbivory. The width of arrows and the size of letters indicated the strength or impact of the processes (nutrient uptake, competition, and herbivory). Color version of this figure is available online

to ecosystems with high insect herbivory pressure, where soil-mediated advantages for plants might be attenuated via selective herbivory. In addition to the stabilizing effect of negative PSFs, soil-mediated selective herbivory might act as an equalizing mechanism between competing species and might thus promote coexistence in plant communities (Fig. 5). Since under natural conditions both PSFs and herbivory interact and affect plant-biomass production over longer time periods, PSF–herbivory interactions might be stronger and may change over time. Future studies should, therefore, test potential changes in these interactions in long-term experiments and assess their impact for competitive outcomes. However, from the present results, we suggest that in general, the relative importance of PSFs for plant-species performance in grassland communities increases with decreasing intensity of herbivory.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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