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

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J.H. conceived the idea, designed the study and performed the experiment; J.H. collected data, with N.K.S., S.S. and M.M.G. provided additional data; J.H., D.P. and J.J. analyzed the data; J.H. led the writing of the manuscript. All authors contributed critically to the draft.

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 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.

Key-words: plant-soil feedback, herbivorous insects, field conditions, selective herbivory, nutritional quality

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 versus 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 is 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 in order 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.

Material 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 x 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 in 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 influences 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 x 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, two-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 one 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. absence of aboveground insect herbivores we performed a 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 x 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 (> 1.3mm) 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 x 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 (Börschig 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 x 4 species x 2 soils x 2 herbivory treatments x 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 – 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 48h, 80°C; root 72h, 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 with 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 x 2 soils x 2 herbivory-exclusion treatments x 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. Additionally, 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): $\mathrm{PSF}_A = \log(\mathrm{home}_A / \mathrm{away}_A)$; where '$\mathrm{home}_A$' 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 x I interaction) was checked by separate ANOVAs for + herbivory and – herbivory. The relationship between intensity of herbivory and PSFs in the two herbivore-exclusion levels were analysed for each species using linear regressions, and differences in slopes were tested with ANOVAs (H x 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 x 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. 2 a-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 aboveground herbivore damage when grown in home vs. away soils (S x T: $F_{3,603} = 13.96$, $P < 0.001$; Online Resource 1: Table S6). *A. elatius*, *D. glomerata* and *H. lanatus* showed highest shoot biomass removal in away soils, where their shoots had the highest N concentration (Fig. 2 i, k, l), whereas for *A. odoratum* damage by aboveground herbivores was highest in home soils where its shoots had the highest N concentration (Fig. 2 j).

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 x 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 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 and along the gradient in intensity of herbivory (S x H x 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* exhibits 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 x 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 for all four species were significantly altered by herbivory intensity (S x 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 highest N concentrations. Third, home and away soils also affected biomass production (i.e. PSFs) of all four species, with highest biomass production in soil in which the species also exhibited highest shoot-N-concentration. Forth 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 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 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-Srbinović 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 and 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, a pattern also found in 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; Bernay 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 highest shoot damage (i.e. estimated shoot biomass removal) caused by aboveground herbivorous insects in soils in which they had highest shoot-N-concentrations. This result is consistent with studies showing that the quantity of herbivore damage is positively related with 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 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 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 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). That *A. odoratum* in comparison to the other species exhibited positive PSFs might be due to its high concentrations of coumarin they exudate via roots in comparison to other species (Tava 2001). Coumarin was recently found to have a negative effect on soil pathogens but a positive impact on beneficial rhizobacteria (Stringlin 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., Aarsen 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, Rodriguez and Brown 1998). Therefore, further studies should examine PSF-herbivory interactions by 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 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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Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

Aarssen LW (2015) Body size and fitness in plants: Revisiting the selection consequences of competition. Perspect Plant Ecol Evol Syst 17:236–242. https://doi.org/10.1016/j.ppees.2015.02.004

Adesemoye AO, Tobert HA, Kloepper JW (2010) Increased plant uptake of nitrogen from 15 N-depleted fertilizer using plant growth-promoting rhizobacteria. Appl Soil Ecol 46:54–58. doi: 10.1016/j.apsoil.2010.06.010

Baltensperger AA, Schank SC, Smith RL, Littell RC, Bouton JH, Dudeck AE (1978) Effect of inoculation with Azopirillum and Azotobacter on turf-type Bermuda genotypes. Crop Sci 18:1043–1045. doi: 10.2135/cropsci1978.0011183X010800180035x

Baxendale C, Orwin KH, Poly F, Pommier T, Bardgett RD (2014) Are plant–soil feedback responses explained by plant traits? New Phytol 204:408–423. doi: 10.1111/nph.12915

Bazzaz A (1996) Plants in changing environments: Linking physiological, population and community ecology. Cambridge University Press, Cambridge, UK

Behmer ST (2009) Insect herbivore nutrient regulation. Annu Rev Entomol 54:165–187. doi: 0066-4170/09/0107-0165

Bernays EA, Chapman RF (1994) Host-plant selection by phytophagous insects – Chapman & Hall, New York, 312pp

Berner D, Blanckenhorn WU, Körner C (2005) Grasshoppers cope with host quality by compensatory feeding and food selection: N limitation challenged. Oikos 111:525–533. doi: 10.1111/j.1600-0706.2005.14144.x

Berner LT, Law BE (2016) Plant traits, productivity, biomass and soil properties from forest sites in the Pacific Northwest, 1999-2014. *Scientific Data*, 3:160002. doi: 10.1038/sdata.2016.2

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. J Ecol 85:561–573. doi: 10.2307/2960528

Bever JD, Mangan SA, Alexander HM (2015) Maintenance of plant species diversity by pathogens. Annu. Rev. Ecol. Evol. Syst. 46:305–325. doi: 10.1146/annurev-ecolsys-112414-054306

Bezemer TM, van der Putten WH, Martens H, van de Voorde TFJ, Mulder PPJ, Kostenko O (2013) Above- and below-ground herbivory effects on below-ground plant-fungus interactions and plant-soil feedback responses. J Ecol 101:325–333. Doi: 10.1111/1365-2745.12045

Blüthgen N, Dormann CF, Prati D, Klaus VH, Kleinebecker T, Hölzel N, Alt F, Boch S, Gockel S, Hemp A, Müller J, Nieschulze J, Renner SC, Schöning I, Schumacher U, Socher

SA, Wells K, Birkhofer K, Buscot F, Oelmann Y, Rothenwöhrer C, Scherber C, Tscharntke T, Weiner CN, Fischer M, Kalko EKV, Linsenmair KE, Schulze E-D, Weisser WW (2012) A quantitative index of land-use intensity in grasslands: Integrating mowing, grazing and fertilization. Basic Appl Ecol 13:207–220. doi: 10.1016/j.baae.2012.04.001

Borgström P, Strengbom J, Viketoft M, Bommarco R (2016) Aboveground insect herbivory increases plant competitive asymmetry, while belowground herbivory mitigates the effect. PeerJ DOI 10.7717/peerj.1867

Brandt AJ, del Pino GA, Burns JH (2014) Experimental Protocol for Manipulating Plant-induced Soil Heterogeneity. JoVE (85), e51580, doi:10.3791/51580

Branson DH, Sword GA (2009) Grasshopper herbivory affects native plant diversity and abundance in a grassland dominated by the exotic grass *Agropyron cristatum*. Restor Ecol 17:89–96. doi: 10.1111/j.1526-100X.2007.00343.x

Brinkman EP, van der Putten WH, Bakker E-J, Verhoeven KJF (2010) Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. J Ecol 98:1063–1073. doi: 10.1111/j.1365-2745.2010.01695.x

Börschig C, Klein A-M, Kraus J (2014) Effects of grassland management, endophytic fungi and predators on aphid abundance in two distinct regions. J Plant Ecol 7:490-498. doi:10.1093/jpe/rtt047

Cahill JF Jr., Cale JA, Karst J, Bao T, Pec GJ, Erbilgin N (2017) No silver bullet: different soil handling techniques are useful for different research questions, exhibit differential type I and II error rates, and are sensitive to sampling intensity. New Phytol 216:11–14. doi: 10.1111/nph.14141

Cebrian J, Lartigue J (2004) Patterns of herbivory and decomposition in aquatic and terrestrial ecosystems. Ecol Monogr 74:237–259. doi: 10.1890/03-4019

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst. 31:343–366. doi:10.1146/annurev.ecolsys.31.1.343.

Chisté MN, Mody K, Gossner MM, Simons NK, Köhler G, Weisser WW, Blüthgen N (2016) Losers, winners, and opportunists: How grassland land-use intensity affects orthopteran communities. Ecosphere 7(11):e01545. 10.1002/ecs2.1545

Cornelissen JHC, Lavorel S, Granier E, Diaz S, Buchmann N, Gurvich DE, Reich PB, ter Steege H, Morgan HD, van der Heijden MGA, Pausas JG, Poorter H (2003) A handbook of protocols for standardised and easy measurements of plant functional traits worldwide. Aust J Bot 51:335–380. https://doi.org/10.1071/BT02124

Cortois R, Schröder-Georgi T, Weigelt A, van der Putten WH, de Deyn G (2016) Plant-soil feedbacks: role of plant functional group and plant traits. J Ecol 104:1608–1617. doi: 10.1111/1365-2745.12643

Crawley MJ (1989) Insect herbivores and plant population dynamics. Annu Rev Entomol 34:531–564. doi: 10.1146/annurev.en.34.010189.002531

DeLong JR, Fry EL, Veen GF, Kardol P (2019) Why are plant-soil feedbacks so unpredictable, and what to do about it? Func Ecol 33:118-128. doi:10.1111/1365-2435.13232

Djonova E, Petkova G, Stancheva I, Geneva M, Michovsky T (2016) Repsonse of pasture grasses to inoculation with mycorrhizal fungi and N-fixing bacteria. BJSS 1:64-77

Egerov E, Gossner MM, Meyer ST, Weisser WW, Brändle M (2017) Does plant phylogenetic diversity increase invertebrate herbivory in managed grasslands? Basic Appl Ecol 20:40-50. http://dx.doi.org/10.1016/j.baae.2017.03.004

Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulze J, Pfeiffer S, Prati D, Renner S, Schöning I, Schumacher U, Wells K, Buscot F, Kalko EKV, Linsenmair KE, Schulze E-D, Weisser WW (2010) Implementing large-scale and long-term functional biodiversity research: the Biodiversity Exploratories. Basic Appl Ecol 11:473–485. doi: 10.1016/j.baae.2010.07.009

Gossner MM, Weisser WW, Meyer ST (2014) Invertebrate herbivory decreases along a gradient of increasing land-use intensity in German grasslands. Basic Appl Ecol 15:347–352. http://dx.doi.org/10.1016/j.baae.2014.03.003

Gundale MJ, Wardle DA, Kardol P, Nilsson MC (2019) Comparison of plant-soil feedback experimental approaches for testing soil biotic interactions among ecosystems. New Phytol221:557–587. doi: 10.1111/nph.15367

Heinze J, Bergmann J, Rillig MC, Joshi J (2015a). Negative biotic soil-effects enhance biodiversity by restricting potentially dominant plant species in grasslands. Perspect Plant Ecol Evol Syst 17:227–235. doi: 10.1016/j.ppees.2015.03.002

Heinze J, Werner T, Weber E, Rillig MC, Joshi J (2015b) Soil biota effects on local abundances of three grass species along a land-use gradient. Oecologia, 179:249–259. doi: 10.1007/s00442-015-3336-0

Heinze J, Sitte M, Schindhelm A, Wright J, Joshi J (2016) Plant-soil feedbacks: a comparative study on the relative importance of soil-feedbacks in the greenhouse vs. field. Oecologia 181:559–569. doi: 10.1007/s00442-016-3591-8

Heinze J, Gensch S, Weber E, Joshi J (2017) Soil temperature modifies effects of soil biota on plant growth. J Plant Ecol 10:808–821.doi: 10.1093/jpe/rtw097

Heinze J, Joshi J (2018) Plant-soil feedback effects can be masked by aboveground herbivory under natural field conditions. Oecologia 186:235–246. doi: 10.1007/s00442-017-3997-y

Huberty AF, Denno RF (2006) Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent fife-history strategies. Oecologia 149:444–455. doi: 10.1007/s00442-006-0462-8

Hulme PE (1994) Herbivores and the performance of grassland plants: a comparison of arthropod, mollusc and rodent herbivory, J Ecol 84:43–51. doi: 10.2307/2261698

Johnson MTJ, Bertrand JA, Turcotte MM (2016) Precision and accuracy in quantifying herbivory. Ecol Entomol 41:112–121. doi: 10.1111/een.12280

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70. doi:10.1038/417067a

Klötzli F, Dietl W, Marti K, Schubiger-Bosshard C, Walther G-R (2010) Vegetation Europas – Das Offenland im vegetationskundlich-ökologischen Überblick. Ott Verlag, Bern, 1192 pp.

Kos M, Tuijl MAB, de Roo J, Mulder PPJ, Bezemer TM (2015) Species-specific plant-soil feedback effects on above-ground plant-insect interactions. J Ecol 103:904–914. doi: 10.1111/1365-2745.12402

Kostenko O, van de Voorde TFJ, Mulder PPJ, van der Putten WH, Bezemer TM (2012) Legacy effects of aboveground-belowground interactions. Ecol Lett 15: 813-821. doi: 10.1111/j.1461-0248.2012.01801.x

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant-soil feedbacks: a meta-analytic review. Ecol Lett 11:980–992. doi: 10.1111/j.1461-0248.2008.01209.x

Kulmatiski A, Kardol P (2008) Getting Plant-Soil Feedbacks out of the Greenhouse: Experimental and Conceptual Approaches. Pages 449–472 in U. Lüttge, W. Beyschlag and J. Murata, editors. Progress in Botany 69. Springer-Verlag, Heidelberg, Germany. doi: 10.1007/978-3-540-72954-9_18

Kulmatiski A (2016) Factorial and ‚self vs. other’ plant soil feedback experiments produce similar predictions of plant growth in communities. Plant Soil 408:485–492. doi: 10.1007/s11104-016-2946-6

MacDonald AAM, Kotanen PM (2010) The effect of disturbance and enemy exclusion on performance of an invasive species, common ragweed, in its native range. Oecologia 162:977–986. doi: 10.1007/s00442-009-1557-9

Manning P, Gossner MM, Bossdorf O, Allan E, Zhang YY, Prati D, Blüthgen N, Boch S, Böhm S, Börschig C, Hölzel N, Jung K, Klaus VH, Klein AM, Kleinebecker T, Krauss J, Lange M, Müller J, Pašalić E, Socher A, Tscharpka M, Türke M, Weiner C, Werner M, Gockel S, Hemp A, Renner SC, Wells C, Buscot F, Kalko EKV, Linsenmair KE, Weisser

WW, Fischer M (2015) Grassland management intensification weakens the associations among the diversities of multiple plant and animal taxa. *Ecology*, **96**, 1492–1501. doi:10.1890/14-1307.1.

Massey FP, Ennos E, Hartley SE (2007) Grasses and the resource availability hypothesis: the importance of silica-based defences. J Ecol 95:414–424. doi: 10.1111/j.1365-2745.2007.01223.x

Mattson WJ (1980) Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. 11:119–61. doi: 10.1146/annurev.es.11.110180.001003

Morriën E, Engelkes T, van der Putten WH (2011) Additive effects of aboveground polyphagus herbivores and soil feedback in native and range-expanding exotic plants. Ecology 92:1344–1352. doi: 10.1890/10-1937.1

Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular myccorhizas. Trends Ecol Evol 10:407–411. doi:10.1016/S0169-5347(00)89157-0

Orwin KH, Buckland SM, Johnson D, Turner BL, Smart S, Oakley S, Bardgett RD (2010) Linkages of plant traits to soil properties and the functioning of temperate grassland. J Ecol 98:1074–1083. doi: 10.1111/j.1365-2745.2010.01679.x

Petermann JS, Fergus AJF, Turnbull LA, Schmid B (2008) Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. Ecology 89:2399–2406. http://dx.doi.org/10.1890/07-2056.1

Pierce S, Negreiros D, Cerabolini BEC, Kattge J, Diaz S, Kleyer M, Shipley B, Wright SJ, Soudzilovskaia NA, Onipchenko VG, van Bodegom PM, Frenette-Dussault C, Weiher E, Pinho BX, Conrnelissen JHC, Grime JP, Thompson K, Hunt R, Wilson PJ, Buffa G, Nyakunga OC, Reich PB, Caccianiga M, Mangili F, Cerian RM, Luzzaro A, Brusa G, Siefert A, Barbosa NPU, Chapin III FSC, Cornwell WK, Fang J, Fernandes GW, Garnier E, Le Stradic S, Peñuelas J, Melo FPL, Slaviero A, Tabarelli M, Tampucci D (2017) A field-portable list of CRS strategies. Func Ecol 31:444 – 457. doi: 10.1111/1365-2435.12722

Pinheiro J, Bates D, DebRoy S, Sarkar D, R CoreTeam (2017) nlme: Linear and nonlinear mixed effects models. R.package version3.1-131. Retrieved from https://CRAN.R-project.org/package=nlme

R Developmental Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Reinhart KO, Rinella MJ (2016) A common soil handling technique can generate incorrect estimates of soil biota effects on plants. New Phytol 210:786–789. doi: 10.1111/nph.13822

Rodríguez MA, Brown VK (1998) Plant competition and slug herbivory: effects on the yield and biomass allocation pattern of Poa annua L. Acta Oecol 19:37–46. https://doi.org/10.1016/S1146-609X(98)80006-4

Russel FL, Rose KE, Louda SM (2010) Seed availability and insect herbivory limit recruitment and adult density of native tall thistle. Ecology 91:3081–3093. doi: 10.1890/09-1101.1

Schädler M, Jung G, Auge H, Brandl R (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. Oikos 103:121–132. doi: https://doi.org/10.1034/j.1600-0706.2003.12659.x

Simons NK, Gossner MM, Lewinsohn TM, Boch S, Lange M, Müller J, Pašalić E, Socher SA, Türke M, Fischer M, Weisser WW (2014a) Resource-mediated indirect effects of grassland management on arthropod diversity. PLoS ONE, 9(9): e107033

Simons NK, Gossner MM, Lewinsohn TM, Lange M, Türke M, Weisser WW (2014b) Effects of land-use intensity on arthropod species abundance distributions in grasslands. J Anim Ecol 84:143–154. doi: 10.1111/1365-2656.12278

Smith-Ramesh LM, Reynolds HL (2017) The next frontier of plant-soil feedback research: unraveling context dependence across biotic and abiotic gradients. J Veg Sci 28:484–494. doi: 10.1111/jvs.12519

Stajković-Srbinović O, Delić D, Kuzmanović D, Sikirić B, Rasalić N, Nikolić B, Knežević-Vukčević J (2016) Growth and nutrient uptake of orchardgrass (Dactylis glomerata L.) and meadow fescue (Festuca pratensis Huds.) as affected by rhizobacteria. Not Bot Horti Agrobo, 44:296–301. doi: 10.15835/nbha44110252

Stringlis IA, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk MC, Berendsen RL, Bakker PAHM, Feussner I, Pieterse CMJ (2018) MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. PNAS 22:E5213–E5222. doi: 10.1073/pnas.1722335115

Tava A (2001) Coumarin-containing grass: volatiles from sweet vernalgrass (*Anthoxanthum odoratum* L.). J Essent Oil Res 13: 357–370. doi: 10.1080/10412905.2001.9712236

Van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soilmicrobes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. doi: 10.1111/j.1461-0248.2007.01139.x

Van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA Suding KN, van de Voorde TFJ, Wardle DA (2013) Plant-soil feedbacks: the past, the present and future challenges. J Ecol 101:265–276. doi: 10.1111/1365-2745.12054

Van der Putten WH, Bradford MA, Brinkman EP, van der Voorde TFJ, Veen GF (2016) Where, when and how plant-soil feedback matters in a changing world. Func Ecol 30:1109–1121. doi: 10.1111/1365-2435.12657

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629–1633. doi: 10.1126/science.1094875

White JF, Chen Q, Torres MS, Mattera R, Irizarry I, Tadych M, Bergen M (2015) Collaboration between grass seedlings and rhizobacteria to scarvenge organic nitrogen in soils. AoB 7: plu093; doi:10.1093/aobpla/plu093

Xu G, Fan X, Miller A (2012) Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63:153–182. doi: 10.1146/annurev-arplant-042811-10553

Zhu F, Heinen R, van der Sluijs M, Raaijmakers C, Biere A, Bezemer TM (2018) Species-specific plant-soil feedbacks alter herbivore induced gene expression and defense chemistry in Plantago lanceolata. Oecologia 188:801–811. doi: 10.10007/s00442-018-4245-9

FIGURE LEGENDS

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 affect the outcome of PSF effects this set-up (i.e. PSF experiment x herbivory treatment) was installed at nine grasslands that formed a gradient in aboveground herbivory intensity. In total, the experiment contained 1296 plants (4 species x 2 soils x 9 replicates x 2 herbivory treatments x 9 grasslands). For further details see "Material and Methods". Color version of this figure is available online

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. lanatus* (right) grown in “home” (left bars) and “away” (right bars) soils. Data represent mean ± SE; with n = 6 for a – h and n = 81 for i – l. Asterisks between bars represent significance: (*) $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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

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 ± SE (n = 9)

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 competition 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, herbivory). Color version of this figure is available online

Fig.1

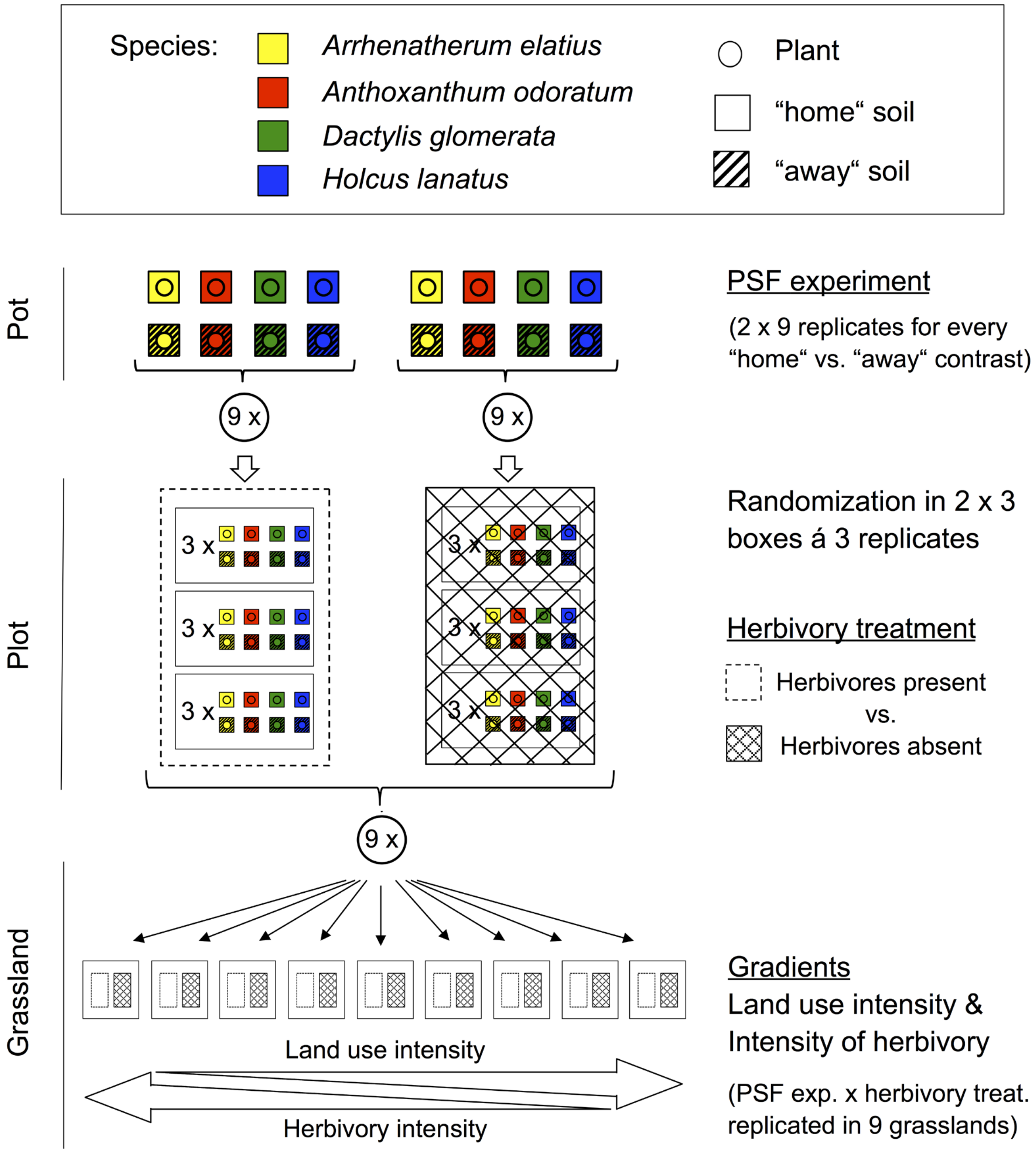

Species:
Arrhenatherum elatius
Anthoxanthum odoratum
Dactylis glomerata
Holcus lanatus
Plant
“home“ soil
“away“ soil
Pot
PSF experiment
(2 x 9 replicates for every “home“ vs. “away“ contrast)
9 x
9 x
Plot
3 x
3 x
3 x
3 x
3 x
3 x
Randomization in 2 x 3 boxes á 3 replicates
Herbivory treatment
Herbivores present
vs.
Herbivores absent
9 x
Grassland
Land use intensity
Herbivory intensity
Gradients
Land use intensity &
Intensity of herbivory
(PSF exp. x herbivory treat. replicated in 9 grasslands)

Fig. 2

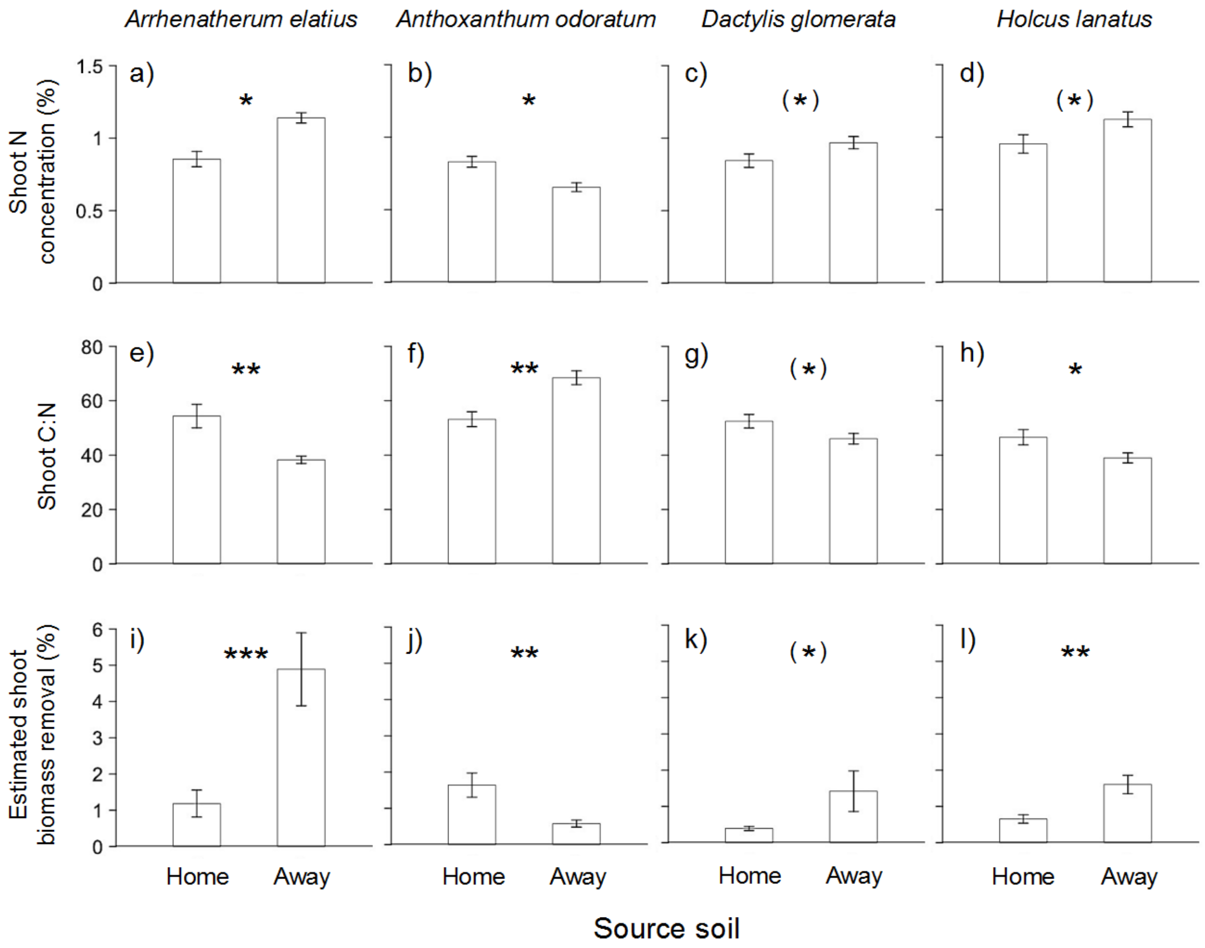

Arrhenatherum elatius
Anthoxanthum odoratum
Dactylis glomerata
Holcus lanatus
a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
k)
l)
*
*
(*)
(*)
**
**
(*)
*
***
**
(*)
**
Shoot N concentration (%)
1.5
1
0.5
0
Shoot C:N
80
60
40
20
0
Estimated shoot biomass removal (%)
6
5
4
3
2
1
0
Home
Away
Home
Away
Home
Away
Home
Away
Source soil

Fig. 3

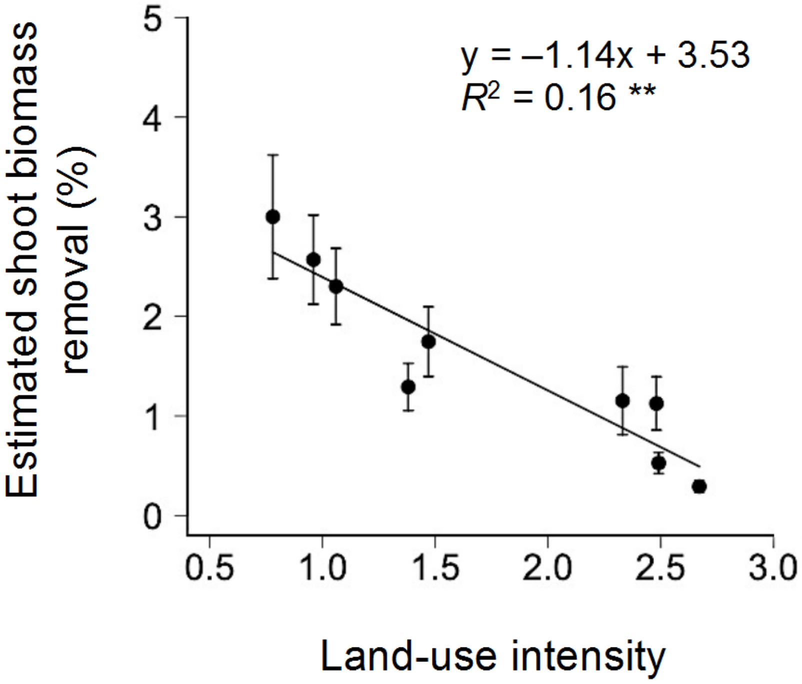

y = –1.14x + 3.53
R^2 = 0.16 **
Estimated shoot biomass removal (%)
0
1
2
3
4
5
0.5
1.0
1.5
2.0
2.5
3.0
Land-use intensity

Fig. 4

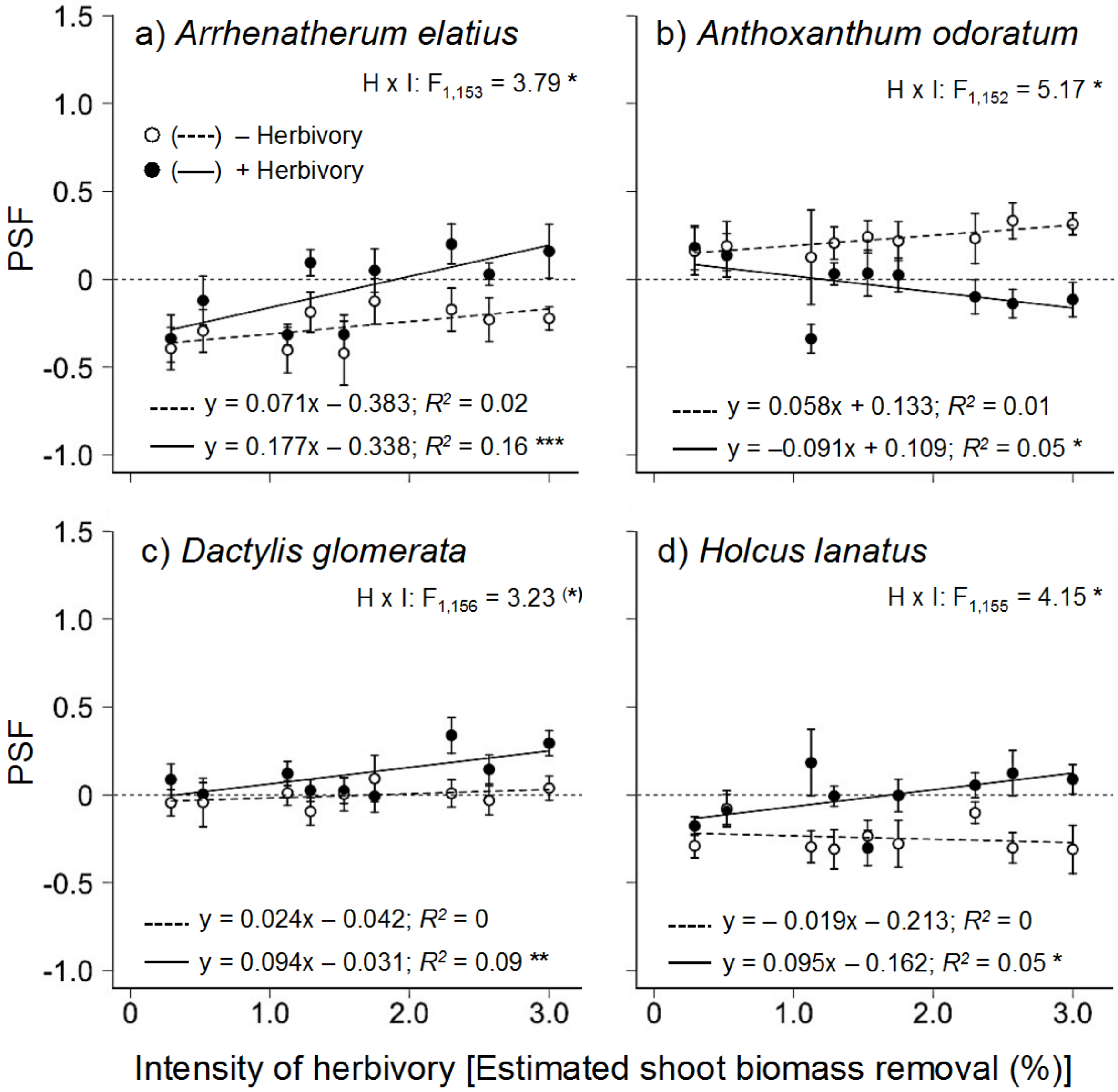
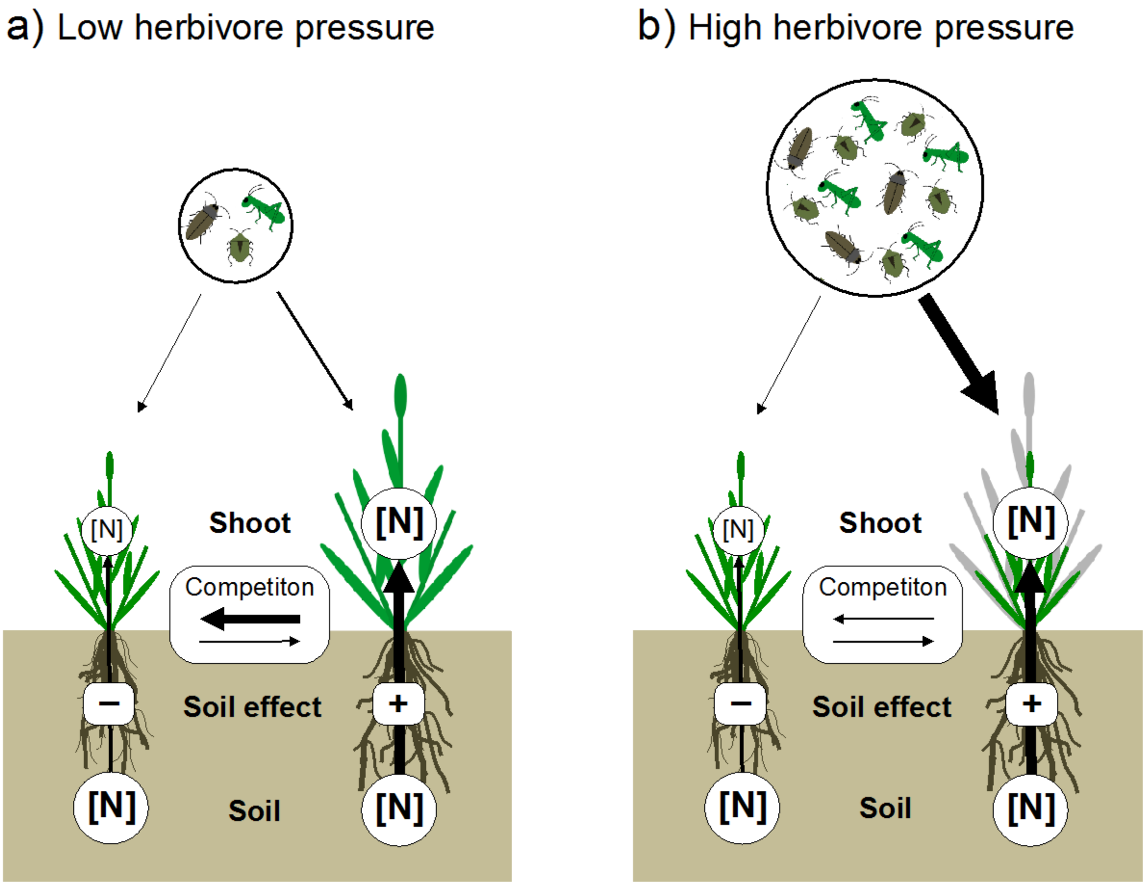

a) Arrhenatherum elatius
H x I: F1,153 = 3.79 *
○ (----) – Herbivory
● (—) + Herbivory
----- y = 0.071x – 0.383; R² = 0.02
— y = 0.177x – 0.338; R² = 0.16 ***
b) Anthoxanthum odoratum
H x I: F1,152 = 5.17 *
----- y = 0.058x + 0.133; R² = 0.01
— y = –0.091x + 0.109; R² = 0.05 *
c) Dactylis glomerata
H x I: F1,156 = 3.23 (*)
----- y = 0.024x – 0.042; R² = 0
— y = 0.094x – 0.031; R² = 0.09 **
d) Holcus lanatus
H x I: F1,155 = 4.15 *
----- y = – 0.019x – 0.213; R² = 0
— y = 0.095x – 0.162; R² = 0.05 *
PSF
1.5
1.0
0.5
0
-0.5
-1.0
0
1.0
2.0
3.0
Intensity of herbivory [Estimated shoot biomass removal (%)]

Fig. 5


a) Low herbivore pressure
b) High herbivore pressure
[N]
Shoot
[N]
Competiton
−
Soil effect
+
[N]
Soil
[N]
[N]
Shoot
[N]
Competiton
−
Soil effect
+
[N]
Soil
[N]