Plant-litter-soil feedbacks driven primarily by litter type and plant compartment, but not modified by litter exposed to insect herbivory

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Abstract

Purpose Insect herbivory affects plant growth, nutrient and secondary metabolite concentrations and litter quality. Changes to litter quality due to insect herbivory can alter decomposition, with knock on effects for plant growth mediated through the plant-litter-soil feedback pathway.

Methods Using a multi-phase glasshouse experiment, we tested how changes to fast- and slow-growing grass shoot and root litter quality caused by insect herbivores affect the performance of response plants in the soil in which the litter decomposed.

Results We found that insect herbivory resulted in marginal changes to litter quality and did not affect growth when plants were grown with fast- versus slow-growing litter. However, there was a strong interactive effect between litter type (i.e., root versus shoot) and response plant compartment (i.e., root versus shoot growth): shoot litter resulted in a marginally negative effect on both roots and shoots, while the addition of root litter had a strong negative effect on root growth and a positive effect on shoot growth. Further, shoot litter exposed to insect herbivory interacted with response plant identity to affect root growth.

Conclusions Our results suggest that whether litter originates from plant tissues exposed to insect herbivory or not and its interaction with fast- versus slow-growing grasses is of little importance. In contrast, litter origin (root versus shoot) effects on allocation to root versus shoot growth and species-specific responses to litter exposed to insect herbivory appear to play a critical role in the plant-litter-soil feedback pathway. Taken collectively, plant-litter-soil feedbacks likely affect broader ecosystem processes.

Introduction

Insect herbivory is omnipresent in terrestrial ecosystems and can create substantial alterations to plant community composition and function (Vidal and Murphy 2018). It is well established that feeding by insects can induce increases in plant defence compounds in both living shoots (Gatehouse 2002; Kaplan et al. 2008; Karban 2011) and roots (Kaplan et al. 2008; van der Putten 2003). Furthermore, insect herbivory can either increase (Ohgushi 2005) or decrease (Johnson et al. 2009; Nykänen and Koricheva 2004) the nutrient content of living plant tissues, depending on plant species, tissue type, metabolites and nutrients considered. Changes to plant tissue chemistry that result in increased concentrations of defensive compounds and lower or higher nutrient content can persist after senescence (Chapman et al. 2003; Lattanzio et al. 2006). Consequently, changes in the quality of the leaf and root litter that reaches the soil could alter the decomposition process. In contrast to high quality litter, labile litter (e.g., readily decomposable), poor quality litter is typically more recalcitrant, meaning it decomposes more slowly because it is less suitable to decomposer organisms (Chomel et al. 2016). For example, Schweitzer et al. (2005) found that litter galled by the leaf galling aphid (*Pemphigus betae*) contained higher polyphenol and lower N concentrations, which led to decreased leaf litter decomposition rates. Inhibited

decomposition can result in fewer nutrients released into the soil, thereby hindering the growth of future plants growing in that soil (Hättenschwiler and Vitousek 2000). Further, herbivore-induced shifts in secondary compounds in plants (Thelen et al. 2005) could result in the build-up of allelopathic compounds in the soil, leading to effects in plants that grow in the soil in which the litter decomposes (John and Sarada 2012). Insect-induced changes to the litter decomposition pathway could result in alterations to the plant-litter-soil feedback (PLSF) pathway (Veen et al. 2019b). Despite recent advances in the understanding of the role of litter decomposition in soil nutrient cycling processes (Woodman et al. 2021), and how this may affect plant fitness, few studies have considered how insect herbivory might modify the PLSF pathway (e.g., (Burghardt et al. 2018).

Shoots and roots serve very different functions to the plant, the former providing energy through photosynthesis, and the latter serving to anchor the plant into the soil, gather nutrients and water, and serve as a major storage compartment. After senescence, both shoots and roots become an important source of nutrients in the soil. Shoots and roots – even of the same plant individual - are exposed to a set of very different biotic and abiotic conditions, in terms of herbivore communities and microclimatic conditions. Consequently, chemical composition varies markedly between the above- and belowground plant compartments (Faucon et al. 2017). Therefore, the decomposition of both shoot and root litter tends to be driven by tissue chemistry (Aerts 1997; Silver and Miya 2001), which can be highly plastic, depending on the environment. Shoot litter tends to have higher nitrogen concentrations than root litter, which makes it more labile (i.e., readily decomposable) (Aerts 1997; Cornwell et al. 2008; Reich 2014). Although the shoot and root litter decomposition rates within a species occasionally differ (Hobbie et al. 2010), there is considerable evidence that root and shoot litter decomposition characteristics show similar patterns within most plant species (Freschet et al. 2013).

Substantial research supports the idea of a “plant economic spectrum”, which proposes that plants typically fall on a continuum from “fast” to “slow” growth strategies (Díaz et al. 2016; Reich 2014; Wright et al. 2004). Specifically, fast-growing plants usually have higher productivity, promote faster nutrient cycling, and are less well defended, leading to greater susceptibility to pathogen accumulation (as opposed to mutualists), while slow-growing plants are generally the opposite. Plants at opposite ends of this spectrum tend to produce litter with contrasting decomposition rates; litter of fast growers decomposes quicker, whereas litter of slow growers decomposes slower (Cornwell et al. 2008; Freschet et al. 2012; Santiago 2007). As litter produced by fast-growing plants decomposes, it more easily releases nutrients to the soil and plants growing in the vicinity, leading to more positive plant-litter feedbacks (Freschet et al. 2013). Alterations to the PLSF pathway caused by plant community compositional shifts (i.e., different proportions of fast- versus slow-growing species) have the potential to substantially alter ecosystem function and services through litter input and/or shifts in decomposer communities (Dias et al. 2017; Jongen et al. 2021). Therefore, understanding how litter produced by fast- and slow-growing plant species feeds back to influence the growth of other plants is of critical importance.

We examined how herbivore-induced changes to plant shoot and root litter quality of grasses with contrasting growth strategies (fast versus slow), impact on response plant performance in the soil in

which the litter decomposes, via the PLSF pathway. Using a glasshouse experiment with six common, naturally co-occurring grass species with contrasting growth strategies, we tested the following hypotheses: 1) Litter from grasses that were exposed to above- or belowground herbivores will negatively influence response plant biomass, mediated through the PLSF pathway. This is because insect herbivores typically reduce litter nutrient content (Nykänen and Koricheva 2004) and increase secondary metabolites in both shoots and roots (Kaplan et al. 2008), which inhibits decomposition and thereby reduces nutrient access to plants growing in the decomposed litter; 2) Overall, root litter will generate more negative PLSFs than shoot litter because root litter is recalcitrant and shoot litter is labile (Freschet et al. 2013). These effects will interact differently with above- and belowground herbivory, because of the contrasting responses to herbivory in each plant compartment (Kaplan et al. 2008); 3) Litter from fast-growing plant species will generate more positive PLSFs than that of slow-growing plants, because their litter is more labile and less defended (Reich 2014), making it a better source of nutrients for plants growing in the decomposing litter (Cornwell et al. 2008; Freschet et al. 2012; Santiago 2007); and 4) We expect species-specific effects due to the wide interspecific variation in litter nutrient content and secondary metabolites (Faucon et al. 2017).

Materials And Methods

Litter conditioning phase

Six grass species were selected to represent fast- (*Arrhenatherum elatius* L. (P. Beav.), *Holcus lanatus* L., *Lolium perenne* L.) and slow- (*Agrostis capillaris* L., *Deschampsia flexuosa* L. (Trin.), *Festuca ovina* L.) growing species (Elberse and Berendse 1993; Heinen et al. 2020; Scheurwater et al. 2002). Despite grasses having inherently lower concentrations of secondary metabolites (Geisen et al. 2022) and generally lower molecular richness (Defossez et al. 2021) as compared to forbs and the fact that these compounds are often affected by herbivores (Gatehouse 2002; Kaplan et al. 2008; Karban 2011; van der Putten 2003), we opted to investigate only grass species because grasses are the dominant functional group in grassland systems. Seeds were obtained from Cruydt-Hoeck (Nijeberkoop, the Netherlands) or Pratensis AB (Lönashult, Sweden). The grasses were sown on 15 November 2017 directly into 5-litre plastic pots in soil that consisted of 90% gamma irradiated soil (Synergy Health, Ede, The Netherlands) characterised as holtpodzol sandy loam (84% sand, 11% silt, 2% clay, ~ 3% organic matter, pH 5.9, 1150 mg kg^{-1} N, 61 mg P_2O_5 100 g^{-1}, 2.4 mmol K kg^{-1}) collected from a grassland near Lange Dreef, Driebergen, The Netherlands (52° 02’ N, 5° 16’ E) and 10% live field soil that was collected from a restored grassland site abandoned from agricultural use in 1996 (“De Mossel”, Ede, The Netherlands, 52° 04´ N, 5° 45´ E) characterised as holtpodzol sandy loam (94% sand, 4% silt, 2% clay, ~ 5% organic matter, 5.2 pH, 1060 mg kg^{-1} N, 75 mg P_2O_5 100 g^{-1} P, 1.9 mmol K kg^{-1}). After the seeds sprouted, each pot of grass seedlings was thinned to obtain a similar visual density. During the entire growing period, the grasses were grown in a glasshouse with climate control (light regime 16:8 h day:night, day temperature 21 °C, night temperature 16 °C) and watered and weeded as needed. *D. flexuosa* was re-sown due to poor germination rates. Treatments consisted of aboveground herbivory, belowground herbivory, both above-

and belowground herbivory and a no herbivory control (n = 5 for each treatment per plant species, yielding a total of 120 pots). All plants were grown in hanging plastic mesh sleeves (BugDorm, Taiwan) for the duration of the herbivory treatment.

Belowground herbivory: The highly polyphagous root-feeding larvae of click beetles (Traugott et al. 2008) (c. 75% *Agriotes lineatus* and c. 25% *A. obscurus*), were chosen as the belowground herbivore (hereafter: wireworms). The wireworms were collected near Lelystad (52° 54' 50.35" N, 5° 53' 68.28" E) in marine sandy loam (c. 7% clay) a few weeks before the start of the experiment and stored at 4 °C until they were used in the experiment. On 20 December 2017, four holes approximately 2–3 cm deep were made in each corner of each pot receiving the belowground herbivory treatment and one wireworm was placed into each hole and covered with soil. Holes were also made in the same manner as described above in the remaining pots (i.e., those that did not receive the belowground herbivory treatment) in order to control for artefact effects. Addition of wireworms to *D. flexuosa* was delayed by 2 weeks due to re-seeding.

Aboveground herbivory: Caterpillars of the highly polyphagous cabbage moth (*Mamestra brassicae*), which is ubiquitous within the grasslands from which the plants chosen here originate (Wu et al. 2015), were placed on the plants receiving the aboveground herbivory treatments. The eggs from *M. brassicae* were obtained from the Department of Entomology at Wageningen University, The Netherlands. The colony has been maintained for years on *Brassica oleracea* var. *gemmifera* cv. cyrus and the larvae were originally collected from a cabbage field near Wageningen. Previous work has shown that *M. brassicae* performs well and sometimes even prefers grasses over forbs (Heinen et al. 2020). Upon hatching, the larvae were reared in separate groups of 200–300 larvae and provided with artificial diet (140 g agar dissolved in 5 L of boiling water with addition of 800 g maize flour, 250 g beer yeast, 250 g wheat germs, 10 g sorbic acid, 8 g nipagin (methyl-4-hydroxybenzoate), 40 g ascorbic acid and 0.5 g streptomycin), which was regularly refreshed. Caterpillars were placed on the plants in three successive rounds to ensure the plants were sufficiently damaged and thereby the quality of the litter they produced was affected. In the first round of herbivory (20th December 2017), two early L3 larvae were selected and placed on the grass monocultures using a fine-hair brush. In the second round of herbivory (26 December 2017), five late L1 larvae were added to the monocultures, followed by a third round of herbivory (3rd January 2018) in which an additional five L2 larvae were added to the monocultures. Larvae at different stages were added to increase the chances of successful establishment. Addition of caterpillars to *D. flexuosa* was delayed by 2 weeks due to re-seeding.

About one month after the herbivory treatments were initiated (19th January 2018), aboveground damage to the plants was assessed visually as an estimate of total surface area consumed by the caterpillars, and expressed as a percentage of the total surface area. (Note: Both *M. brassicae* and *Agriotes* spp. larvae were still present on the plants and under the ground, respectively.) Assessment of *D. flexuosa* was delayed by 2 weeks. Regrowth of biomass after herbivory was substantial in *A. capillaris* and *F. ovina*, whereas in *H. lanatus*, *A. elatius* and *L. perenne*, regrowth was comparatively less. As a result, in *A. capillaris* and *F. ovina*, the estimates of herbivory were comparatively low. Aboveground herbivore visual estimation of damage on the plants exposed to aboveground and above-belowground

herbivory ranged between 5–25%. Respectively, aboveground and above-belowground herbivory damage values were as follows: *A. capillaris*: 5 ± 0% and 5 ± 0%; *A. elatius*: 16 ± 2% and 15 ± 3%; *D. flexuosa*: 9 ± 2% and 13 ± 3%; *F. ovina*: 9 ± 2% and 9% ± 2%; *H. lanatus*: 21 ± 2.2% and 17 ± 7%; and for *L. perenne* 8 ± 1% and 10 ± 2%. No aboveground herbivore damage was observed on plants from the control and belowground herbivory treatments. It was not possible to assess belowground herbivore damage, but given that *Agriotes* spp. are polyphagous and voracious feeders (Hermeziu 2021; Traugott et al. 2008), it is very likely they caused severe damage to the roots of the plants in the belowground and above-belowground herbivory treatments.

After damage was assessed, we stopped watering the plants so that they senesced and their litter could be collected. Again, for *D. flexuosa* this was delayed by 2 weeks. It is well known that drought causes changes to the chemical composition of plant root and shoot litter (He and Dijkstra 2014; Varela et al. 2016). However, obtaining the litter for this experiment via drought was the only feasible way to ensure the production of enough dead litter in a timely manner. After *c.* 3 weeks (5 February 2018) the plants were fully senesced and root and shoot litter were harvested. A subsample of litter from each plant was taken, freeze dried and set aside for elemental analyses (see below). Shoots were carefully detached from roots and placed in a paper bag. Roots were then washed clean and left to air-dry overnight, then placed in a paper bag. Both roots and shoots were oven-dried at 40 °C for a minimum of 72 h, and weighed to determine total biomass. Six randomly selected subsamples of 0.5 g of both shoot and root litter were collected from each of the 120 pots. These samples served as litter sources in the decomposition phase of the experiment.

Litter chemistry analyses

In order to make mechanistic links between litter properties and changes to plant growth during the litter feedback phase (see below), analyses on litter chemistry were performed. After harvest, a subsample of the shoot and root tissue from each pot was also analysed for total carbon (C) and nitrogen (N) content. Each subsample was ground with a ball mill (Schwingmühle Qiagen Tissue Lyser II, Hilden, Germany), placed into a tin capsule and then analysed using a Flash EA1112 elemental analyser (Thermo Fisher Scientific, Inc., Waltham, MA, USA). An additional subsample of ground shoot and root tissue was analysed for total polyphenolic concentrations using the Folin-Denis method (Folin and Denis 1915; Hagerman and Butler 1989). Briefly, 25 mg of freeze-dried root and shoot litter was extracted in 5 ml of a 50:50 2.4 M HCl:MeOH solution heated to 90 °C for 2 h. Extracts were then centrifuged for 10 m at 5000 rpm and the top 2 ml pipetted into an Eppendorf tube and stored at 20 °C until analysis. Upon analysis, extracts were warmed to room temperature. Then, 200 µL was placed into a 2 mL Eppendorf tube along with 200 µL of Folin-Denis reagent and 1 mL of 1.6 M sodium carbonate. Tubes were vortexed for 10 s and then allowed to incubate on the lab bench for 30 m before being centrifuged for 5 min at 14000 rpm. The top layer of liquid was pipetted into a 96-well plate and absorption was read at 750 nm using on plate reader with Gen5 software (version 1.11.5, BioTek Instruments, Inc., Winooski, Vermont, USA).

Further, a subset of root litter samples (four herbivory treatments × six grass species × three replicates = 72) were analysed for (micro)nutrient concentrations. Root material was oven dried at 70°C for at least 48

h. Next, 20 mg of dried root material was transferred to glass digestion vials (MG5, Anton Paar GmbH). A mixture of 250 µL 69% HNO_3 and 125 µL of 30% H_2O_2 was added to each vial. The vials were closed with special PEEK screw caps (MG5, Anton Paar GmbH) and disposable PFTE lip-type seals (Anton Paar GmbH) capable of tolerating high temperatures and pressures. Sample digestion was carried out in a microwave oven (Multiwave ECO, Anton Paar GmbH) mounted with a 64-position rotor (64MG5, Anton Paar GmbH). A 10 min ramping period was used to a maximum temperature of 140°C. The samples were kept at this temperature for 80 min after which the digested samples were left to cool for 10 min. The samples were then transferred to a − 20°C freezer for 30 min followed by the quick release of the pressure of all samples. This cooling step prevents the loss of volatile elements such as S. Finally, samples were diluted with Milli-Q water to a final concentration of 3.33% HNO_3 and filtered using a Whatman Puradisc Aqua 30 filter with CA membrane. Samples were then analysed for Aluminum, (Al), Copper (Cu), Iron (Fe), Potassium (K), Manganese (Mn), Sodium (Na), Nickel (Ni), Phosphorus (P), Sulfur (S) and Zinc (Zn) by an inductively coupled plasma-optical emission spectrometer (ICP-OES, Thermo Scientific iCAP 6500 Duo Instrument with axial and radial view and CID detector microwave digestion system).

Litter feedback phase

On 5–8 March 2018, 1 L pots were filled with 1 kg of soil (a mixture of 90% gamma-irradiated soil and 10% live soil; live soil was collected from the field on 5 March 2018; see above). Using a randomized block design, pots were placed in the glasshouse under the same conditions as mentioned above and watered freely to allow the soil to settle and microbial activity to re-establish. On 12 March 2018, the collected six 0.5 g litter subsamples from all 120 pots from the herbivory treatment phase were placed into individual pots and allowed to decompose in preparation for the litter feedback phase. This resulted in a design as follows: four insect litter legacy treatments (aboveground herbivores, belowground herbivores, both above- and belowground herbivores, or no herbivores) · six 'litter' grass species (*A. capillaris*, *A. elatius*, *D. flexuosa*, *F. ovina*, *H. lanatus*, *L. perenne*) · two litter types (shoot, root) · six 'response' grass species (*A. capillaris*, *A. elatius*, *D. flexuosa*, *F. ovina*, *H. lanatus*, *L. perenne*) · five replicate blocks = 1,440 pots. In addition, each block included two control pots containing no litter · six response grass species (*A. capillaris*, *A. elatius*, *D. flexuosa*, *F. ovina*, *H. lanatus*, *L. perenne*) · five replicate blocks = 60 no litter control pots for a total of 1,500 pots (Fig. 1). Due to limited litter production of some species (i.e., *D. flexuosa* and *F. ovina*), some replicates were lost, leaving a total of 1,404 pots. Litter was placed onto the surface of each pot and gently pressed into the surface of the soil and then approximately 2 cm of fine quartz sand was placed on top of the litter in order to ensure that the litter was full covered and in contact with the soil substrate below. This helped to retain moisture to ensure decomposition took place and prohibited oviposition into the pots by fungus gnats (superfamily Sciaroidea). Pots were watered as needed over the next three weeks to ensure adequate moisture for decomposition.

On 15 March 2018, seeds from the six test species were sown onto sterilised glass beads, watered thoroughly, and placed into the glasshouse (same conditions as mentioned above) to allow for germination. Once species grew large enough for transplantation (approximately 2–3 cm in height), they

were moved to the cool room and kept at 4 °C to arrest further growth. On 5 April 2018, a single seedling of the six grass species mentioned above was planted into each pot. All pots were checked daily and watered as necessary and dead seedlings were replaced up until ten days after the initial planting. After the last replacement of dead seedlings, a total of 1.1% of the plants died by the end of the experiment. Beginning on 16 April 2018, each pot was watered every other day with 50 mL of tap water until the harvest of the experiment.

Between 22 and 28 May 2018, the experiment was destructively harvested. Shoots were clipped at the meristem and placed into a paper bag on the first day of the harvest. Pots were subsequently stored in the dark at room temperature until the roots could be washed. Roots were carefully washed clean of soil over a 4 mm sieve and placed on a paper towel overnight to air dry before they were placed into a paper bag. Remaining root litter from the litter treatments was separated from live roots (i.e., the colour and texture were different), while virtually all the shoot litter had decomposed during the course of the experiment and therefore posed no issue. All roots and shoots were placed into an oven and dried for a minimum of 72 h at 40 °C before dry weights were measured. (NB: for the species *D. flexuosa* when it was grown with root litter, there were numerous instances where the plants and their root systems were so small that it was not possible to disentangle the roots from the litter. In this case, root measurements could not be taken, but shoot measurements were still recorded. Further, due to contamination in the *D. flexuosa* seed batch, 12 individuals were actually a *Poa* spp. instead. These plants were dropped from subsequent analyses.)

Statistical analyses

All plant data from the glasshouse experiment were analysed using mixed effect models. Data collected after the litter conditioning phase on root and shoot litter characteristics carbon, nutrients and total polyphenols and root litter nutrients (i.e., Al, Cu, Fe, K, Mn, Na, Ni, P, S and Zn) were analysed in two models. To test for effects of herbivory on litter quality and plant growth speed on litter properties, the first model included herbivory (control (litter exposed to no insect herbivory), aboveground herbivory (*Mamestra brassicae*), belowground herbivory (*Agriotes* spp.), above- and belowground (*Mamestra brassicae* + *Agriotes* spp.)) and growth speed of the species from which the litter was obtained (fast versus slow) as fixed factors. The second model tested for the effects of herbivory and litter species identity on litter properties (i.e., the different grass species from which litter was derived), with both considered fixed factors. In both models, block (i.e., the randomized block design into which all the pots were placed in the glasshouse) was included as a random factor and all interactions were specified.

In order to standardize the response species data collected during the litter feedback phase, the root and shoot biomass of each response plant of each species was subtracted from the average respective root and shoot biomass of the no litter controls for the same species (i.e., two no litter control per species present in each of the five blocks for a total of ten no litter control units across all five blocks). For example, the root biomass of a *L. perenne* grown with litter exposed to belowground herbivory was subtracted from the average root biomass of the ten *L. perenne* grown without added litter designated for comparison to plants grown with root litter. To investigate our four hypotheses, we created three different

models. All models allowed us to test our first hypothesis on the effects of above-belowground herbivory on plant growth response. In the first model, herbivory (as described above), litter type (root versus shoot litter) and response compartment (root or shoot of response the plants) were included as fixed factors, with all interactions specified. The response variable was the standardized value (see above) of the roots and shoots of the response plant; both root and shoot responses were included in the same analysis. Therefore, to account for autocorrelation, the sample identity (i.e., individual from which a particular pairing of root and shoot measurements originated) was included as a random factor. Block, litter species identity and response species identity (i.e., the different grass species that were used as response species) were also included as random factors. This model allowed us to investigate our second hypothesis regarding interactive effects between root versus shoot litter exposed to herbivory on the different compartments (i.e., roots versus shoots) of the response plant species. In the second model, herbivory, litter growth speed and response species growth speed (fast versus slow) were included as fixed factors, with all interactions specified. Random factors were as specified in the first model. The response variables were the standardized responses of the roots and shoots of the response plants. Plant roots and shoots that were grown with root versus shoot litter were analysed separately, resulting in four analyses: standardized responses of roots and shoots grown with root litter and standardized responses of roots and shoots grown with shoot litter. This model allowed us to interrogate our third hypothesis regarding the effect of plant growth speed on the PLSF pathway. In the third model, herbivory, litter species identity and response species identity were included as fixed factors and block was included as a random factor. Response variables were the same as in the second model. This allowed us to investigate our fourth hypothesis regarding species-specific effects of litter and herbivory on the response plants. In the three models described immediately above, herbivory was originally analysed as a binary response (i.e., aboveground herbivory yes/no, belowground herbivory yes/no), but these models generated results that were not drastically different from the models that considered herbivory as a single variable with four categories. Therefore, for the sake of simplicity and ease of interpretation, the above models were used.

All of the models described above included an *a priori* selection of random factors based on the experimental design. However, all possible combinations of random factors listed for each model were compared using the AICcmodavg package in R (Mazerolle and Mazerolle 2017) and the best selection of random factors for each response variable was selected. Please see footnotes in ANOVA Tables. All data were transformed as necessary to meet the model assumptions; see ANOVA tables for details. Restricted maximum likelihood (REML) estimation was used to produce an unbiased estimate of variation and covariation (Patterson and Thompson 1971) and Kenward-Roger degrees of freedom approximation was used to reduce bias introduced by a relatively small sample size (Kenward and Roger 1997). Analyses were performed using R software (R Core Team 2015) with the packages lme4/lmerTest (Bates et al. 2015; Kuznetsova et al. 2017).

Results

Litter conditioning phase

Exposure to above- and belowground insect herbivory did not result in changes to root or shoot litter carbon, nitrogen or total polyphenol concentrations (Tables S1-S4). However, compared to roots, shoot carbon, nitrogen and total polyphenol concentrations were *c.* 3.5%, 24% and 3.5% higher, respectively (Table S1). Root C was 7.3% higher in slow- than in fast-growing plants (Table S2). Analyses on root and shoot carbon, nitrogen and total polyphenol concentrations revealed many species-specific differences; detailed descriptions go beyond the scope of the main text, but fully detailed descriptions can be found in Tables S3-S4 and Text S5.

Analyses on root (micro)nutrient concentrations revealed numerous differences generated by herbivory, litter species identity and their interactions, but litter growth speed never affected any of the nutrient concentrations (Tables S6-S9). According to the models testing for herbivory and litter growth speed, as well as herbivory and litter species identity effects, root litter sodium concentrations were 14% lower in litter explosed to belowground herbivory only versus litter exposed to both above- and belowground herbivory, but neither differed from control litter or litter exposed to aboveground herbivory (Table S6-S7). According to the model testing for herbivory and litter species identity effects, nickel root litter concentrations were affected by herbivory, but post-hocs revealed no true significant differences (Table S7). However, there was a significant interaction between litter species identity and herbivory on root litter iron and nickel concentrations, which resulted in numerous species-specific effects (Table S7). For the sake of brevity, these effects will not be described in detail, but means and standard errors, along with post-hoc results can be found in Table S9. Numerous additional species-specific differences between root and shoot litter nutrient concentrations were detected, which are described in detail in Text S5.

Herbivory and root versus shoot litter interactive effects on the PLSF pathway

Although there were no significant interactions between herbivory and litter type (root versus shoot litter) on response plant growth, there was a strong effect of litter type that interacted with response plant growth compartment (root versus shoot) (Table 1, Fig. 2). Root litter had a 22% more negative effect on response plant growth than shoot litter (-0.030 ± 0.015 versus − 0.024 ± 0.009). Further, response plant root growth was overall negatively affected by litter addition (regardless of litter type), while shoot growth was overall positively affected by litter (-0.195 ± 0.011 versus 0.139 ± 0.012). However, there was a significant interaction between litter type and response plant compartment (root versus shoot). Response plant root and shoot growth were not differently affected by shoot litter (slightly negative: -0.013 ± 0.013 versus − 0.035 ± 0.014), but the addition of root litter negatively affected response plant root growth (-0.408 ± 0.014) and positively affected response plant shoot growth (0.342 ± 0.016) (Fig. 2).

Table 1
Results of mixed effects models (*F*- and (*P*-) values) testing for the effects of litter exposed to herbivory (control (no herbivory), aboveground, belowground, above-belowground), litter type (root, shoot) and response compartment (root or shoot of response plants) on the standardized growth responses to the no litter controls; significant *P*-values at $p < 0.05$ bolded.

	Root or shoot standardized response‡	
	df	F-value (P)
Herbivory (H)	3, 1294	2.4 (0.067)
Litter type (LT)	1, 1294	11.9 (**< 0.001**)
Response compartment (C)	1, 1301	730.4 (**< 0.001**)
H × LT	3, 1294	0.0 (0.999)
H × C	3, 1302	0.4 (0.736)
LT × C	1, 1302	837.5 (**< 0.001**)
H × LT × C	3, 1302	0.3 (0.814)
‡Litter species identity was dropped as a random factor in line with AIC selection criteria.		

Herbivory and plant economic spectrum interactive effects on the PLSF pathway

The model to assess the effect of herbivory and plant growth speed (litter growth speed and response plant growth speed) on the PLSF pathway revealed a significant three-way interaction between herbivory, litter species growth speed and response plant species growth speed on shoot biomass growth when plants were grown with root litter (Table 2, Fig. 3). However, post-hoc tests revealed no true significant differences. No other significant main or interactive effects of herbivory, litter species growth speed and/or response species growth speed were detected.

Table 2
Results of mixed effects models (*F*- and (*P*-) values) testing for the effects of shoot and root litter that had been exposed to herbivory (control (no herbivory), aboveground, belowground, above-belowground), litter species growth speed (fast versus slow) and response plant species growth speed (fast versus slow) on standardized response species root and shoot biomass relative to the no litter controls; significant *P*-values at $p < 0.05$ bolded.

	Root litter				**Shoot litter**			
	Shoot biomass		Root biomass ‡		Shoot biomass ‡		Root biomass	
	df*	F-value (P)	df	F-value (P)	df	F-value (P)	df	F-value (P)
Herbivory (H)	3, 580	1.8 (0.155)	3, 574	0.5 (0.702)	3, 678	0.4 (0.782)	3, 680	1.3 (0.267)
Litter species growth speed (L)	1, 4	1.7 (0.267)	1, 574	0.7 (0.403)	1, 4	3.2 (0.150)	1, 680	1.2 (0.271)
Response species growth speed (S)	1, 4	0.8 (0.418)	1, 4	1.1 (0.356)	1, 4	1.0 (0.385)	1, 4	2.4 (0.193)
H × L	3, 579	0.3 (0.844)	3, 574	0.2 (0.926)	3, 677	0.6 (0.619)	3, 680	0.6 (0.651)
H × S	3, 581	2.1 (0.103)	3, 574	0.7 (0.538)	3, 677	1.1 (0.334)	3, 680	0.6 (0.632)
L × S	1, 580	0.3 (0.609)	1, 574	0.5 (0.482)	1, 677	3.6 (0.057)	2, 680	2.0 (0.157)
H × L × S	3, 581	3.9 (**0.009**)	3, 574	2.4 (0.069)	3, 677	1.3 (0.284)	3, 680	1.5 (0.225)

*Degrees of freedom sometimes differ due to inability to disentagle tiny root systems from the remaining litter, lost samples and/or death of plants during the experiment.

‡Litter species identity was dropped as a random factor in line with AIC selection criteria.

Species-specific effects on the PLSF pathway

The model to assess the species-specific effects of litter and response species identity revealed many response species identity effects (but no litter species identity effects) and an interactive effect between herbivory and response species identity (Table 3, Fig. 4). Shoot biomass response to root litter was positive across all species, but the response of *A. capillaris* was 131% more positive than that of *F. ovina* (0.839 ± 0.046 versus 0.364 ± 0.025) and the response of *F. ovina* was 77% more positive than that of *A. elatius* (0.206 ± 0.037), but no differences were detected between *F. ovina* and *H. lanatus* and *L. perenne*, nor *A. elatius* and the latter two species (Fig. 4A). Root biomass response to root litter was negative across all species, but the response of *A. elatius* was *c.* 3% more negative than that of *H. lanatus* (-0.279 ± 0.034 versus − 0.288 ± 0.029) and the response of *H. lanatus* was 67% less negative than that of *A.*

capillaris (-0.873 ± 0.025), but no differences were detected between *H. lanatus* and *F. ovina* and *L. perenne*, nor *A. capillaris* and the latter two species (Fig. 4B). Shoot biomass response to shoot litter was positive for *H. lanatus* (0.109 ± 0.026) when compared to *L. perenne* (-0.063 ± 0.028) and *F. ovina* (-0.325 ± 0.022), but there was no difference in response between *H. lanatus* versus *A. capillaris, A. elatius* and *D. flexuosa*, nor the latter three species and *L. perenne* (Fig. 4C). Root biomass response to shoot litter was *c.* 15 times more positive for *H. lanatus* compared to *A. capillaris*, *A. elatius*, *D. flexuosa* and *L. perenne* (0.244 ± 0.032 versus 0.016 ± 0.013 averaged across the latter four species). Compared to the other species, *F. ovina* had a strong negative response (-0.396 ± 0.019). There was a significant interactive effect between herbivory and response species identity on root biomass when plants were grown with shoot litter. For the sake of brevity, these effects will not be described here, but post-hoc letters can be found in Fig. 4D. In summary, species-specific effects were the most dominant aspect of the PLSF pathway.

Table 3
Results of mixed effects models (*F*- and (*P*-) values) testing for the effects of root and shoot litter that had been exposed to herbivory (control (no herbivory), aboveground, belowground, above-belowground) and litter and response species identity on response species shoot and root biomass relative to the no litter controls; significant *P*-values at $p < 0.05$ bolded.

	Root litter				**Shoot litter**			
	Shoot biomass		Root biomass		Shoot biomass		Root biomass	
	df*	F-value (P)	df	F-value (P)	df	F-value (P)	df	F-value (P)
Herbivory (H)	3, 466	1.3 (0.282)	3, 458	0.3 (0.865)	3, 557	0.3 (0.805)	3, 556	1.7 (0.321)
Litter species identity (LI)	5, 466	2.1 (0.062)	5, 458	0.3 (0.902)	5, 557	1.2 (0.311)	5, 556	1.4 (0.226)
Response species identity (I)	5, 466	53.0 (**< 0.001**)	5, 458	54.3 (**< 0.001**)	5, 557	24.4 (**< 0.001**)	5, 556	61.2 (**< 0.001**)
H × LI	15, 466	0.5 (0.952)	15, 458	0.8 (0.692)	15, 557	0.8 (0.731)	15, 556	0.8 (0.697)
H × I	15, 466	1.2 (0.253)	15, 458	0.6 (0.854)	15, 557	0.9 (0.577)	15, 556	1.7 (**0.050**)
LI × I	25, 466	0.7 (0.856)	25, 458	0.6 (0.950)	25, 557	0.7 (0.866)	25, 556	0.7 (0.893)
H × LI × I	68, 466	0.9 (0.629)	67, 458	0.8 (0.894)	75, 557	0.8 (0.893)	75, 556	0.8 (0.864)

*Degrees of freedom sometimes differ due to inability to disentagle tiny root systems from the remaining litter, lost samples and/or death of plants during the experiment.

D. flexuosa was included in the root litter models, but was excluded from the post-hocs tests on response species identity due to too many missing data points. See Materials and Methods for details on why numerous D. flexuosa root biomass values were lost.

Discussion

Here, we examined the effects of above- and belowground insect herbivory on the root and shoot litter quality of three fast- and three slow-growing grass species and the subsequent effects on response plant growth as mitigated via the plant-litter-soil feedback (PLSF) pathway. We found that insect herbivory resulted in few subtle changes to litter quality (i.e., nutrient concentrations), and that herbivory did not translate to alterations in the growth of the response plants via the PLSF pathway. Further, and in contrast to our hypothesis, there were no significant alterations to growth when plants were grown with fast- versus slow-growing plant litter. Interestingly, however, there was a strong interactive effect between litter type (i.e., root versus shoot litter) and response plant compartment (i.e., root versus shoot growth). When the effects of root versus shoot litter addition were considered separately, shoot litter addition

resulted in a negligible negative effect on both roots and shoots, but the addition of root litter generated a strong negative and positive effect on response plant root and shoot growth, respectively. We also found an interactive effect on root growth of shoot litter exposed to insect herbivory and response plant identity. Below we discuss possible mechanisms for these effects and relate our findings to potential implications for the PLSF pathway.

Herbivory effects on the PLSF pathway

Our first hypothesis that herbivory would negatively impact on the PLSF pathway was not supported because litter exposed to above- and/or belowground herbivory did not result in changes to plant growth. Despite evidence that insect herbivory can change both leaf nutrient (Nykänen and Koricheva 2004) and polyphenol (Kaplan et al. 2008) concentrations, we found no differences in C, N and polyphenol concentrations across herbivory treatments. This may have been the result of the plants resorbing nutrients before senescence (Vergutz et al. 2012). Further, secondary metabolites such as polyphenols (that are generally present in lower concentrations in grasses versus forbs (Geisen et al. 2022)) that might be responsible for litter allelopathic effects may have broken down rapidly (Bokhari 1978; García Palacios et al. 2016), resultantly erasing chemical differences usually found in live tissue. However, there was a nearly significant overall effect of insect herbivory across above- and belowground compartments (Table 1) because litter exposed to above-belowground herbivory had a stronger negative effect on plant growth than litter exposed to only above- or belowground, or no herbivory. Despite a generally strong impact of herbivores on live tissue chemistry, it seems that very few of these effects remain present after plant senescence, evidenced by the minimal effects of herbivory on litter quality and secondary chemistry. An alternative explanation for these herbivore impacts, although not measured in the current study, could be found in the presence of insect frass, which is increasingly being recognized as a driver of soil nutrient dynamics (Poveda 2021). Although frass pellets generally drop to the soil, a potential microbial (i.e., litter phylloplane) or chemical (i.e., litter quality) contamination of litter cannot be ruled out. Furthermore, frass itself could also be an important direct driver of plant performance (Kagata and Ohgushi 2012). Therefore, the direct and indirect involvement of frass in the interactions between herbivores and PSFs warrants future study.

Herbivory and root versus shoot litter interactive effects on the PLSF pathway

Our second hypothesis that root litter will generate more negative PLSFs than shoot litter, but will vary with herbivory, was partially supported because root and shoot litter resulted in contrasting effects on response plant growth. However, contrary to our hypothesis, there were no interactions between root or shoot litter and herbivory. Herbivory resulted in few changes to litter chemistry, making it unsurprising that interactions with root versus shoot litter did not manifest nor lead to subsequent changes in response plant growth. Yet, there was a strong interaction between litter type (root versus shoot) and response plant compartment: Shoot litter had marginally negative effects on both root and shoot growth, while root litter had a strong negative and positive effect on root versus shoot growth, respectively. This (partially)

supports other work that has shown inhibitory effects of root litter on plant growth (Zhang et al. 2016), and reinforces the idea that it is important to investigate the different roles of root and shoot litter in driving plant-soil feedbacks (Veen et al. 2019b). Root and shoot litter are generally considered recalcitrant and labile, respectively (Freschet et al. 2013), meaning root litter probably released fewer nutrients into the soil, thereby reducing plant (root) growth. Further, root litter often did not fully decompose during the course of the experiment, which may have created a physical barrier that contributed to negative impacts on plant growth; something that has been suggested before (Bardgett et al. 2014; Veen et al. 2019b), but a mechanism for which there is scant evidence. Root litter may have also contributed to negative effects on root growth via allelopathic (Bokhari 1978) and/or microbial effects (Hossain et al. 2010); parameters not measured here. Inhibition in root growth caused by root litter may have led to greater allocation to shoots in order to compensate. That is to say, we speculate that shoots grew larger so that they could produce sugars that were then shunted to the roots. However, the duration of this experiment was likely too short to realise the long-term compensatory effects of this allocation, which is likely relevant for these perennial grass species in the long-term.

Herbivory and plant economic spectrum interactive effects on the PLSF pathway

Our third hypothesis that litter from fast-growing plant species would generate more positive PLSF effects was not supported because litter from fast- versus slow-growing species did not generate contrasting growth responses. Despite slow-growing root litter having 7% higher total carbon concentrations, which can be an indicator of more recalcitrant litter and thereby inhibited nutrient release (Cornwell et al. 2008), we found minimal effects of litter growth speed on the PLSF pathway. There was a three-way interaction between herbivory, litter growth speed and response plant species speed on shoot growth when grown with root litter, but post hoc tests detected no true differences, and hence this effect is unlikely to be ecologically relevant. There were likely no direct or interactive effects of fast- versus slow-growing litter and response species, which may have been because most grasses are positioned closely on the fast-growing side of the plant economic spectrum. In essence, although the grasses used in our study significantly differed from one another in terms of growth rates, grasses in general are relatively fast-growing. As a result, these grasses may have very similar ranges of litter quality that are rather labile and easily decomposable, and lower in secondary metabolites (Defossez et al. 2021), which typically inhibit decomposition and nutrient release (Chomel et al. 2016; Osono and Takeda 2004). Stronger effects of plant growth speed on the PLSF pathway could be expected if species further apart on the plant economic spectrum were selected, with starker differences in litter nutrient content and secondary metabolites (Díaz et al. 2016).

Species-specific effects on the PLSF pathway

Our fourth hypothesis was partially supported because there were some significant main and interactive effects of herbivory, litter type and response species identity on plant growth. This finding supports other work showing species-specific litter feedback effects (Bueno de Mesquita et al. 2019). Here, for example, *L. perenne* showed reduced root growth in response to shoot litter that had been exposed to above-

belowground herbivory versus only belowground herbivory. This effect could have been caused by changes to the litter that we did not measure here, such as the litter microbiome (Veen et al. 2019a), that only manifested when plants were exposed to both above- and belowground herbivory. However, no other intraspecific effects of herbivory-exposed litter on response plant growth were detected, suggesting species-specific herbivory effects may be of little ecological significance. On the other hand, species-specific responses to different litter types played a stronger role. One noteworthy example: *H. lanatus* had an overall positive root growth response to shoot litter, while *F. ovina* showed a negative response. This contrasts the overall effect of litter type (root versus shoot). Shoot litter generally elicited a negligible negative response in root growth (essentially neutral), which indicates that species-specific effects can be masked when only composite effects are explored. Considering the response of individual species to the PLSF pathway is critical, as litter effects could influence dominant and subordinate species responses, with implications for plant community composition (Hassan et al. 2021).

Conclusions

Although it is well known that insect herbivory induces strong chemical changes in live plant tissues, we show here that effects on litter chemistry are minimal. This may have been due to resorption during senescence or a delay in manifestation of changes that would have occurred later in the life cycle of the plants (e.g., during reproduction). Consequently, effects of insect herbivory on the PLSF pathway were minimal and species-specific. Further, no interactions with fast- versus slow-growing plants (i.e., plant economic spectrum) and insect herbivory on response plant growth were seen, perhaps due to the relatively fast-growing nature of grasses when the entire economic spectrum is considered. An interesting follow up should consider herbivory effects on the litter of plant species that sit further apart on the economic spectrum, with starker differences in initial litter chemistry. However, shoot litter demonstrated a very marginally negative impact on the growth of both shoots and roots of the response plants, while root litter showed a strong positive and negative effect on shoots and roots, respectively. This finding demonstrates that root litter might shift resource allocation to aboveground tissues in plants, possibly due to a need to compensate for negative effects of root physical, chemical, microbial and/or allelopathic properties on response plant root growth. Overall, despite limited insect herbivore-induced changes to litter quality and subsequent effects on response plant growth, litter type (shoots versus roots) played an important role in determining resource allocation. These results pull focus on the potential implications of the PLSF pathway on determining plant performance, which may create knock on effects for the wider plant community and its functions.

Abbreviations

PLSF
plant-litter-soil feedback

Declarations

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Competing interests

The authors declare no competing interests of any nature.

Authors' contributions

J.R.D.L., R.H. and T.M.B. conceived the experimental design; J.R.D.L., R.H., S.E.H., R.J. and K.S. collected the data; J.R.D.L. and R.H. analysed the data; J.R.D.L. and R.H. led the writing of the manuscript. All authors contributed to revising the manuscript.

Data availability

Upon acceptance, data will be made available via the Dryad repository.

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Figures

Figure 1

Conceptual diagram of the design of the experiment, which involved exposing fast- (Ae = *Arrhenatherum elatius*, Hl = *Holcus lanatus*, Lp = *Lolium perenne*) and slow- (Ac = *Agrostis capillaris*, Df = *Deschampsia flexuosa*, Fo = *Festuca ovina*) growing grass species to different herbivory treatments (control = no herbivore, aboveground = *Mamestra brassicae*, belowground = *Agriotes* spp., above- and belowground *Mamestra brassicae* + *Agriotes* spp.), collecting their litter and then growing the same fast- and slow-

growing species with the collected litter in a full-factorial experiment. No-litter controls were included to calculate the standardized response of the species to the litter treatments (panel A). Experimental pots before planting, after the litter treatments had been administered. The block design can be clearly seen (panel B). The response plant species several weeks after the feedback phase of the experiment began. Sand was added to the upper layer of the pots to prevent fungus gnat colonisation (panel C)

Figure 2

The effect of root and shoot litter on the standardized responses of roots and shoots of grass species (*Agrostis capillaris*, *Arrhenatherum elatius*, *Deschampsia flexuosa*, *Festuca ovina*, *Holcus lanatus*, *Lolium perenne*) grown with litter that had received different herbivory treatments (control = no herbivore, aboveground = *Mamestra brassicae*, belowground = *Agriotes* spp., above- and belowground *Mamestra brassicae* + *Agriotes* spp.). Across both panels, groups of bars topped with different uppercase letters differ at $p < 0.05$ (Tukey's HSD). Panels show 1st quartile above and below the medians (i.e., line inside each bar), the minimum and maximum values, excluding outliers (i.e., whiskers) and the outliers (i.e., black dots). ANOVA results are presented in Table 1

Figure 3

Standardized responses of root and shoot biomass of fast- (*Arrhenatherum elatius*, *Holcus lanatus*, *Lolium perenne*) and slow- (*Agrostis capillaris*, *Deschampsia flexuosa*, *Festuca ovina*) growing grass species that were grown with root (panels A, B) and shoot (panels C, D) litter that had received different herbivory treatments (control = no herbivore, aboveground = *Mamestra brassicae*, belowground = *Agriotes* spp., above- and belowground *Mamestra brassicae* + *Agriotes* spp.). Panels show 1st quartile above and below the medians (i.e., line inside each bar), the minimum and maximum values, excluding outliers (i.e., whiskers) and the outliers (i.e., black dots). ANOVA results are presented in Table 2

Figure 4

Standardized responses of root and shoot biomass of grass species (*Agrostis capillaris*, *Arrhenatherum elatius*, *Deschampsia flexuosa*, *Festuca ovina*, *Holcus lanatus*, *Lolium perenne*) that were grown with root (panels A, B) and shoot (panels C, D) litter that had received different herbivory treatments (control = no herbivore, aboveground = *Mamestra brassicae*, belowground = *Agriotes* spp., above- and belowground *Mamestra brassicae* + *Agriotes* spp.). Within each panel, response species followed by different uppercase letters differ at $p < 0.05$ (Tukey's HSD). Within panel D, bars topped with different lowercase letters differ at $p < 0.05$ (Tukey's HSD). Panels show 1st quartile above and below the medians (i.e., line

inside each bar), the minimum and maximum values, excluding outliers (i.e., whiskers) and the outliers (i.e., black dots). ANOVA results are presented in Table 3

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