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Herbivory promotes more negative plant-soil feedbacks particularly for legumes and forbs

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Abstract (150-250 words):
Purpose: Plant-soil feedbacks and herbivory impact plant growth. We conducted a meta-analysis to test for an interaction between plant-soil feedbacks and herbivory, including effects on the magnitude and direction of feedbacks and the biomass or growth of herbivores.

Methods: Our literature search identified 244 studies to address our first question about herbivore impacts on plant-soil feedbacks as well as 179 studies to address our second question about plant-soil impacts on herbivores. We developed a database, calculated plant-soil feedback values for each study, then calculated Hedge’s D values for 1) the difference in plant-soil feedback values with and without herbivory for plants, and 2) the difference between home and away soils for insects.

Results: We found an overall significant weak negative effect of herbivory on plant-soil feedbacks, and effects differed between plant functional type. In legumes herbivory changed the outcome of plant-soil feedbacks from positive to negative, in forbs it further decreased negative feedbacks, and grasses were unaffected. We also found soil preconditioned in the lab produced consistently negative feedbacks whereas there was no significant change for soil preconditioned in the field. By contrast, there was no significant overall effect of plant-soil feedbacks on herbivores.

Conclusion: This first meta-analysis of the impact of herbivores on plant-soil feedbacks and vice versa identified an important potential role of plant functional type in determining the impact of conditioned soil on the magnitude and direction of feedbacks as well as some clear gaps that need to be addressed experimentally.

Keywords: meta-analysis; plant-functional type; soil conditioning; legumes; forbs; herbivore feeding guild
**Introduction**

Plants form the basis of most food chains, both above and belowground. Aboveground plant tissues are consumed principally by insect and mammalian herbivores. Belowground tissues are also consumed by insect and mammalian herbivores as well as provide carbon for a wide variety of microbes that cycle nutrients, promote plant growth, and act as pathogens. The composition of plant communities is correlated with the composition of aboveground herbivore (Ebeling et al. 2018; Kempel et al. 2015) and belowground microbial communities (Cordovez et al. 2019). Thus, plants act as a connection between above and belowground systems (Bardgett et al. 1998; Bennett 2010; De Deyn and Van der Putten 2005).

Plant-soil feedbacks are a measure of the impact of changes in soil abiotic and biotic elements driven by the presence of one plant species on the next generation of plants grown in the same soil (Bever et al. 1997). In studies examining plant-soil feedbacks, a comparison is made between plants grown in “home” (conspecific) and “away” (heterospecific) soils. Most plant-soil feedback experiments consist of two stages: a conditioning stage and an experimental or feedback stage, but studies vary in how these stages are conducted (Brinkman et al. 2010). The conditioning stage can be conducted in the greenhouse (by growing a focal plant species in field soil) or by collecting soil from the field, ideally beneath a focal plant. Multiple meta-analyses have shown differences in results between these two approaches (Beals et al. 2020; Forero et al. 2019). In the experimental or feedback stage, plant biomass is compared between home and away soils. Positive plant-soil feedbacks occur when growth in home soils is greater than growth in away soils, whereas negative plant-soil feedbacks occur when growth in away soils is greater than growth in home soils. Whether plant-soil feedbacks are positive or negative they can
interact with competition and environment to predict a given plant species’ abundance on the
landscape (Crawford et al. 2019; Lekberg et al. 2018).

Herbivory has long been known to exert top-down control on plant abundance, and recent
research efforts have identified potential influences of insects on the outcome of plant-soil
feedbacks. For example, herbivory has been shown to influence the feedbacks experienced by
some plant functional groups more than others (Heinen et al. 2020), while the invasive status of a
plant has been shown to strongly interact with both the soil biota involved in plant-soil feedbacks
and herbivores (Waller et al. 2020). In individual experiments, high levels of herbivory have
been shown to reduce the impact of plant-soil feedbacks (Heinze and Joshi 2018; Heinze et al.
2019; Heinze et al. 2020), however the degree of these impacts appear to be specific to the
conditioning species (Kos et al. 2015b; Zhu et al. 2018). To date there has been no attempt to
look across studies and plant species to draw general conclusions about how herbivory is likely
to impact the magnitude or direction of plant-soil feedbacks.

We also expect a bottom-up influence of plant-soil feedbacks on herbivore feeding and growth
likely through changes in plant quality and quantity (Heinen et al. 2020). While few studies have
measured plant quality and quantity explicitly, a growing number of studies are examining
impacts on the herbivores themselves (Heinen et al. 2020; Ingerslew and Kaplan 2018; Kos et al.
2015b; Zhu et al. 2018). Results are mixed: some studies have found no influence of plant-soil
feedbacks on herbivore growth (Zhu et al. 2018), while other studies have found positive
correlations between plant and insect performance and a strong influence of plant functional type
conditioning the soil (Heinen et al. 2020; Kos et al. 2015b), or variation in herbivore
performance based on the plant family conditioning the soil (Ingerslew and Kaplan 2018). Thus, while studies are limited, there is evidence of a bottom-up effect of plant-soil feedbacks on plant herbivores.

These studies have improved our understanding of the interaction between plant-soil feedbacks and herbivory, but they are ultimately limited in their scope to the study systems examined. Using a meta-analysis approach allows us to test for an interaction between plant-soil feedbacks and herbivory in a wider context as well as to examine the role of plant family, soil conditioning length, herbivore functional type and plant functional type in shaping that interaction. We applied the Population-Intervention-Comparator-Outcome (PICO) method to define questions regarding how herbivores influence plant-soil feedback and vice versa. In particular, we asked:

1) What is the plant-soil feedback value (outcome) of plants (population) exposed to herbivory (intervention) or no herbivory (comparator)?

2) What is the growth or biomass (outcome) of herbivores (population) feeding on plants exposed to home (intervention) or away (comparator) soils in plant-soil feedback studies?

Following previously published meta-analyses and other literature we examined qualifiers (experimental parameters) we expected to influence the outcome of our questions. In particular, we focused on plant family and plant functional groups (forb or grass, legume or non-legume) (Heinen et al. 2020; Ingerslew and Kaplan 2018; Kos et al. 2015b) and whether the soil used in the experiment was collected from the field or conditioned in the greenhouse (Beals et al. 2020).
Methods

To conduct our meta-analysis we began with a literature search to identify papers from which to collect data, developed a database, calculated plant-soil feedback values for each study, calculated Hedge’s D values for the difference in plant-soil feedback values with and without herbivory for plants, and calculated Hedge’s D values for the difference between home and away soils for insects.

Literature Search

In April of 2020 we conducted a search in Web of Science for “plant” AND “soil” AND “herbiv*” to identify papers published between 1994 (when the first paper on plant-soil feedbacks was published (Bever 1994) and 2020. The search returned 8347 journal articles, abstracts, books, and data files. In addition, we also examined references of articles cited by journal articles in our list to expand our search for suitable papers and contacted authors of several articles to enquire about data. Data used in our analysis was unpublished or only associated with journal articles. Papers used in the analysis were selected based on the following criteria: 1) plant species were grown in their own (home) and heterospecific (away) soil; 2) data was reported as biomass or growth means in each soil type (authors were contacted if data was in a different format); and 3) herbivory occurred in the feedback, not the conditioning, stage of the experiment. We prioritized data collection for plants and insects by the type of data collected. For plants, we prioritized total dry weight followed by aboveground dry weight followed by aboveground fresh weight. For insects, we prioritized population size followed by biomass. To address potential publication bias we included an unpublished dataset. We identified: 7 published (Heinen et al. 2020; Heinze and Joshi 2018; Heinze et al. 2019; Heinze et al. 2020; Kos et al. 2020;
2015a; Morrien et al. 2011; Schittko and Wurst 2014; Zhu et al. 2018) and 1 unpublished paper and 208 studies within those papers to address our first question about herbivore impacts on plant-soil feedbacks, and we identified 4 papers (Heinen et al. 2020; Ingerslew and Kaplan 2018; Kos et al. 2015b; Zhu et al. 2018) and 200 studies to address our second question about plant-soil impacts on herbivores.

Database and Data Collection

Prior to data collection we developed a database with defined variables in order to make it easier for multiple contributors to provide data, and to minimize database curation time following data collection. Throughout data collection we frequently revisited the database structure to ensure all factors were incorporated. The columns of the database were grouped by sections: Publication (information about the journal article or study from which the data was collected), Data (the variable measured, units, means, errors, and replicates of each study), Home Plants (qualifiers describing information about the home or focal plant species in the study), Away Plants (qualifiers describing information about the away or heterospecific plant species in the study), Soil (qualifiers describing the soil used in the study), and Herbivores (qualifiers describing information about the herbivores used in the study). For a detailed list of variables collected see the database link at the end of this paper.

From each study within each identified paper we collected means, estimates of error, and sample sizes for plant and insect variables in home and away soil. When raw data was not available we used WebPlotDigitizer (https://apps.automeris.io/wpd/) to collect means and estimates of error,
and, when necessary, we calculated the standard deviation from the standard error and sample size.

*Plant-soil feedback calculation*

We calculated plant-soil feedbacks as a ratio of the plant growth variable (total dry weight, aboveground dry weight, or aboveground fresh weight) in home soils divided by away soils. In this calculation positive plant-soil feedbacks are greater than one and negative plant-soil feedbacks are less than one. We estimated plant-soil feedbacks separately for herbivory and no herbivory treatments.

*Data analysis*

Data analysis was performed using R 4.0.3 (R Core Team 2020) and the package metafor (Viechtbauer 2010). To address our first question, the influence of herbivory on plant-soil feedbacks, we calculated Hedge's $D$ as an estimate of the difference in plant-soil feedbacks between herbivory and control treatments. We then used the function rma.mv() to test the overall influence of herbivory on plant-soil feedbacks as well as multiple qualifiers. Plant qualifiers included home and away plant family, functional type (legume, forb, woody, or grass), life history (annual or perennial), habitat (crop or not), metabolism (C3 or C4), and invasion status (native, non-native or invasive). Herbivore qualifiers included family, feeding strategy (chewer, galler, or phloem feeder), and plant tissue fed upon. Soil qualifiers included the type of soil source (managed or unmanaged), whether the soil was conditioned in the lab or field, and the country of origin of the soil.
To address our second question, the influence of plant-soil feedbacks on herbivores, we calculated the Hedge's D between insects feeding on plants grown on home soil and those grown on away soil. The influence of plant-soil feedbacks on herbivores and the same qualifiers listed above for the plant analysis was tested using the function rma.mv().

Results

Effects on plant-soil feedbacks

Generally, we found an overall highly significant weak negative effect ($Q = 818.561; df = 207; P < 0.0001$) of herbivory on plant-soil feedbacks. The strength of the effect is not determined by the significance, but by where the Hedge’s D value falls within the Cohen benchmarks (Cohen 1988). Here the Hedge’s D value of the feedback is $-0.2471 \pm 0.0448$ SE which falls very close to -0.2 and is thus considered a weak effect (Figure 1a). This effect suggests herbivory shifts plant-soil feedbacks to be more negative (Figure 1a, b). We examined three significant qualifiers of this pattern. The home plant family ($Q_M = 154.227, P < 0.0001$) effect was driven by Fabaceae. This effect was confirmed when we examined plant functional type ($Q_M = 72.171, P < 0.0001$; Figure 1c) as herbivory drove plant-soil feedbacks from positive to negative in legumes (Figure 1d). Among the Fabaceae, *Trifolium pratense*, *T. dubium* and *Lotus corniculatus* were strong drivers of the negative effect on plant-soil feedbacks. Herbivory also drove more negative feedbacks for forbs, but had little impact on the neutral feedbacks observed in grasses (Figure 1d).

We also found that the location where soil was conditioned significantly influenced plant soil feedbacks ($Q_M = 33.660, P < 0.0001$; Figure 1e). Soil conditioned in the greenhouse produced
consistently negative feedbacks regardless of herbivory whereas soil conditioned in the field experienced a slight insignificant shift from positive to negative with herbivory (Figure 1f).

Effects on herbivores

There was no significant overall effect of plant-soil feedbacks on herbivores, thus we did not test further for impacts of qualifiers.

Discussion

Effects on plant-soil feedbacks

We found that herbivory weakly shifts plant-soil feedbacks to become more negative, thus increasing the impact of soil legacy on plant biomass.

Studies included in this analysis have individually suggested that the plant species conditioning the soil may explain the impact of herbivory on the outcome of plant-soil feedbacks (Heinen et al. 2020; Heinze and Joshi 2018; Heinze et al. 2019; Heinze et al. 2020; Morrien et al. 2011). Our results agree, but extend beyond previous work to create predictions for plant functional groups. Specifically, we observed that whether the soil was conditioned by legumes, grasses or forbs predicted both the initial direction of feedbacks and the impact of herbivory on those feedbacks. In home soil legumes experienced positive feedbacks, forbs negative feedbacks and grasses neutral feedbacks. The grass results may be driven by the inclusion of solely cool season grasses, and may differ if warm season grasses were included. When herbivores were added legumes grown in their own (home) soil experienced a shift towards negative plant-soil
feedbacks so strong that it drove the overall feedback effect to become negative. Herbivory
drove feedbacks to become even more negative in forbs, and herbivory had no effect on the
neutral feedbacks of grasses. Based on these results we can use plant functional type to predict
how herbivory will shift the magnitude and direction of plant-soil feedbacks.

The shift towards negative or more negative plant-soil feedbacks for legumes and forbs suggests
two alternative hypotheses: 1) conditions in home soil that promoted growth in the absence of
herbivory no longer promote growth in the presence of herbivory, or 2) herbivores more strongly
(and possibly directly) impact plants grown in home soil. The first hypothesis suggests that
benefits provided by the soil microbial community are context-dependent and perhaps switch
from positive to negative when plant-derived carbon is lost to herbivory. The second hypothesis
suggests that plants cultivate home microbial communities that suppress defenses or promote
plant nutritional quality for herbivores (e.g., Heinze et al. 2020). For legumes this home-field
advantage may exhibit a trade-off: in the absence of herbivores the plants are promoted, but in
the presence of herbivores they are suppressed. The two studies that included legumes used
chewing herbivores (Heinze et al. 2020; Schittko and Wurst 2014), so we do not know for
example whether we would see the same trade-off with sucking herbivores (e.g., Koricheva et al.
2009).

Interestingly, the influence of herbivores on plant-soil feedbacks was entirely dependent on
home soils, as we found none of the away soil conditioning qualifiers had any influence on plant-
soil feedbacks. This suggests that plants cultivate a soil community that interacts with herbivory
to reduce the impact of both plant-soil interactions and herbivory. However, this conclusion
needs to be taken with caution as the strongest driver of the home soil effect was legumes, and no away soils were conditioned solely by legumes.

Like previous meta-analyses we found that the soil conditioning protocol was important, but we found that field conditioned soil drove opposite results compared to other published studies (Beals et al. 2020; Forero et al. 2019). In the absence of herbivory, field conditioned soil produced positive feedbacks, but when herbivory was present feedbacks shifted from positive to negative when conditioned soil was field soil. Herbivory had no impact on the negative plant-soil feedbacks observed in greenhouse-conditioned soil. Previous meta-analyses have shown the opposite: greenhouse conditioned soil produces positive feedbacks while field conditioned soil produces negative feedbacks, and stress shifts feedbacks to be more neutral (Beals et al. 2020) or has no effect (Forero et al. 2019). Both previous meta-analyses were broader and incorporated a larger number of studies, and thus our sample size may not have been large enough to observe the same patterns. However, the impact of herbivory in predominantly field-conditioned soils is intriguing.

None of the studies measured herbivory on plants under which field conditioned soil was collected. Thus, we also cannot rule out that herbivory occurred in the conditioning phase, potentially altered root exudation (Pang et al. 2021; van Dam and Bouwmeester 2016), and therefore drove unrecorded changes in the soil microbial composition. Such changes in soil microbial composition could then have influenced the changes we observed between soil conditioning protocols.
Effects on herbivores

We found no effects of plant-soil feedbacks on herbivore growth or biomass. The literature, including studies found in our database, reports wide effects of plant-soil feedbacks on herbivores. We expected to find differences based on herbivore feeding guild as a previous meta-analysis examining the role of arbuscular mycorrhizal fungi on herbivores observed differences based on feeding guild (Koricheva et al. 2009). In addition, some of the individual studies from our database show different effects based on feeding guild. For example, soil conditioning influenced the performance of chewing herbivores *Mamestra brassicae* (Heinen et al. 2020) and *Manduca sexta* (Ingerslew and Kaplan 2018), but did not influence the sucking herbivore *Aphis jacobaeae* under ambient nutrient conditions (Kos et al. 2015b). However, this pattern was not upheld in our data analysis, and may be a result of different patterns of defense metabolites promoted by different conditioning treatments (Zhu et al. 2018).

Gaps

This meta-analysis has highlighted a number of important gaps in the literature. Our results demonstrate the importance of plant functional type for conditioning home soils; however, we were unable to draw conclusions about the importance of plant functional type for conditioning away soils as several functional types were missing from the tested away soil conditioning treatments. In particular, we expected that, as legumes were a strong driver of the home conditioning effect, legumes would also impact the direction of feedbacks when they solely conditioned away soil. However, we were unable to test this as no away soil was conditioned solely by legumes.
Second, all of the studies from our database on interactions between plant-soil feedbacks and herbivores have been conducted in Germany, the Netherlands and the USA. However, only one study was conducted in the USA, and thus the studies represent a very narrow geographical range in the northern hemisphere with similar climates, soils, and plant communities. This is likely to produce a bias in the results.

Third, nearly all plant species used to condition soils had a C3 metabolism thereby limiting our ability to make comparisons between C3 and C4 plants. This is a particularly important point when we consider grasses. C3 (cool season) and C4 (warm season) grasses are known to vary in their response to many soil organisms, especially arbuscular mycorrhizal fungi (Bennett et al. 2013). We are cautious about concluding that all grasses will have neutral feedbacks that are unaffected by herbivory due to this gap in the literature.

Fourth, only a handful of plant and herbivore species were used in our analyzed studies. For example, the majority of studies (but see Ingerslew and Kaplan 2018) were conducted on plants from natural grassland systems thereby excluding agricultural and other natural systems. A smaller proportion of studies measured impacts of plant-soil feedbacks on herbivores themselves, thereby limiting our dataset. In our dataset all the herbivores were a mix of species or *M. brassicae* only which limited our ability to draw conclusions about the impacts of plant-soil feedbacks on herbivores or herbivore feeding guilds.

Finally, the studies included in our meta-analysis used a diversity of experimental approaches (primarily related to soil conditioning) (Beals et al. 2020; Reinhart et al. 2021; Smith-Ramesh...
and Reynolds 2017) which limits our conclusions. Due to these different approaches we were unable to disentangle the effects of two away conditioning effects: a mixture of plants and field collected soil. Specifically, all studies using a mixture of plants to condition away soil collected the soil under a mixture of plants in the field (Supplemental Figure). Thus, we can only draw limited conclusions about the influence of individual versus a mixture of species conditioning away soil.

Conclusions

This first meta-analysis of the impact of herbivores on plant-soil feedbacks and vice versa identified an important potential role of plant functional type in determining the impact of conditioned soil on the magnitude and direction of feedbacks. While there are some clear gaps that need to be addressed experimentally (we need to test legume and forb conditioning of away soils and expand the types of grasses tested), there is support for different responses of plant functional types grown in differently conditioned home soil.

By contrast, there was not enough data to make any strong conclusions about the impact of plant-soil feedbacks on herbivores. To address this gap, we encourage future studies incorporating herbivores from multiple functional guilds on soils conditioned by plants of different functional guilds.

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Data Deposition Information: Data has been deposited in Dryad ([https://doi.org/10.5061/dryad.qjq2bvqjb](https://doi.org/10.5061/dryad.qjq2bvqjb)) and will be made publicly available upon publication of this manuscript.

References


Figure 1. On the left are the Hedge’s D values for the impact of herbivory on a) overall plant-soil feedbacks, c) plant-soil feedbacks by plant functional type (legumes, C3 grasses, and forbs), and e) plant-soil feedbacks based on where the soil was conditioned (greenhouse or field). The red lines in a), c), and e) represent Cohen’s benchmarks (Cohen 1988) that indicate weak (-0.2), medium (-0.5), and large (-0.8) effects. On the right are the graphs of the least squared mean values and standard errors calculated for the values of plant-soil feedbacks in the database for b) overall plant-soil feedbacks, d) plant-soil feedbacks by plant functional type, and f) plant-soil feedbacks based on where the soil was conditioned.
Figure 1.

- **a.** PSFs
- **b.** Plant-Soil Feedbacks
- **c.** legumes
- **d.** grasses
- **e.** forbs
- **f.** Greenhouse

**Shift to More Negative PSFs**

**Shift to More Positive or Neutral PSFs**

**Hedge's D**

**No Herbivory**

**Herbivory**

- **a.** PSFs
- **b.** Plant-Soil Feedbacks
- **c.** legumes
- **d.** grasses
- **e.** forbs
- **f.** Greenhouse

**Shift to More Negative PSFs**

**Shift to More Positive or Neutral PSFs**

**Hedge's D**

**No Herbivory**

**Herbivory**
**Supplemental Figure 1.** Alluvial plot showing the different conditioning strategies used in the analyzed studies, and demonstrating the confounding influence of plant community conditioning with field soil conditioning.