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Plant-soil feedbacks, their mechanisms and role in plant communities

Zpětná vazba mezi rostlinami a půdou, její mechanismy a role v rostlinných společenstvech

Doctoral thesis/Disertační práce

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I hereby declare that I made this thesis independently, using only the mentioned refereces. I did not submit this thesis nor its part for any other degree or diploma.

V Praze dne 13. 12. 2019 Eliška Kuťáková

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ABSTRACT

Plants can influence both the abiotic and biotic properties of the soil they grow in and in such way modified soil can affect performance of the plants growing simultaneously or subsequently in this soil, in a mechanism called plant-soil feedback. Plant-soil feedback can occur between two individuals of the same species (conspecifi c feedback) or between individuals of two different species (heterospecific feedback). So far, plant-soil feedbacks have been shown to play role in vegetation succession, plant invasions and coexistence of species in plant communities. However, mostly due to complexity of processes involved in plant-soil feedbacks, there are still blank pages in our understandings of these plant-soil interactions.

This thesis aimed to (i) investigate relationship between heterospecific feedbacks and plant phylogeny, species traits and co-occurrence patterns in plant communities; (ii) disentangle the biotic and abiotic components of soil feedback and evaluate their importance for plant species from a primary successional sequence; (iii) study the individual components of plant-soil feedbacks in a species rich grassland and evaluate their persistence in soil; and (iv) investigate if plant-soil feedbacks can be shaped by presence of soil mesofauna.

I found that (i) heterospecific plant-soil feedbacks can be linked to species relatedness over short phylogenetic distances, species difference in height, and to plant co-occurrence patterns in communities; (ii) early-successional plants accumulate more pathogens in their soils that further suppress establishment of their seedlings, and also alter (mostly abiotic) soil conditions in a way favoring later-successional species; (iii) plant-soil feedbacks in diverse communities are not easily predictable since the legacies of individual plant species may interact, with different persistence of individual components of feedback; (iv) the strength and direction of plant-soil feedbacks may be significantly altered by organisms present in the soil.

Overall, I showed that plant-soil feedbacks can be highly context-dependent. Specifically, their role can depend on the complexity of plant community since in diverse communities, plant-soil feedbacks of different species can influence each other. Similarly, soil legacies may be shaped by soil organisms, such as springtails. Despite such variability in plant- soil feedbacks, they seem to be an important factor influencing the dynamics of plant communities and plant species coexistence.

Key words: plant-soil feedback, plant coexistence, primary succession, soil abiotic conditions, soil bacteria and fungi

ABSTRAKT

Během svého života rostliny ovlivňují abiotické i biotické podmínky půdy, ve které kořenují. Tyto změněné podmínky mohou ovlivnit růst dalších rostlin v mechanismu zpětné vazby (tzv. plant-soil feedback). Zpětná vazba rostlina-půda se může odehrávat mezi jednotlivci stejného druhu (vnitrodruhová zpětná vazba) nebo mezi jedinci různých druhů (mezidruhová zpětná vazba). Předchozí studie ukazují, že zpětná vazba mezi rostlinami a půdou může hrát roli v sukcesi vegetace a ovlivnit rostlinné invaze nebo koexistenci druhů v rostlinných společenstvech.

Cílem této dizertační práce je (i) studovat vztah mezi mezidruhovými zpětnými vazbami rostlina-půda a příbuzností rostlinných druhů, rozdíly v jejich funkčních vlastnostech a četností jejich společných výskytů v rostlinných společenstvech; (ii) zhodnotit důležitost jednotlivých složek zpětné vazby v primární sukcesi; (iii) studovat jednotlivé složky zpětné vazby v druhově bohaté louce a zhodnotit jejich vytrvalost v půdě; a (iv) zjistit, zda zpětná vazba rostlina-půda může být ovlivněna přítomností půdní mesofauny.

Zjistila jsem, že (i) mezidruhová zpětná vazba je ovlivněna příbuzností interagujících rostlinných druhů (ale pouze na krátkých fylogenetických vzdálenostech), závisí na rozdílech ve funkčních vlastnostech a ovlivňuje četnost společných výskytů rostlin ve společenstvech; (ii) raně sukcesní druhy akumulují více patogenů v půdě, což snižuje další uchycování jejich semenáčků, ale tyto druhy zároveň ovlivňují (zejména abiotické) půdní podmínky tak, že usnadňují nástup druhů pozdější sukcese; (iii) zpětná vazba rostlina-půda je mnohem obtížněji predikovatelná v druhově bohatších společenstvech, protože „půdní dědictví“ jednotlivých druhů mohou vzájemně interagovat a jednotlivé jejich složky mají různou vytrvalost; (iv) působení zpětné vazby může být ovlivněno přítomností půdních organismů.

Tato práce ukazuje, že zpětná vazba rostlina-půda může být do velké míry závislá na dalším kontextu. Její role závisí na složitosti rostlinných společenstev, protože v druhově bohatších společenstvech se různé druhy rostlin mohou vzájemně ovlivňovat ve svém působení na půdní prostředí. Podobně mohou být zpětné vazby ovlivněny přítomností více trofických úrovní v půdním prostředí. Navzdory této variabilitě se zpětná vazba rostliny-půda zdá být důležitým faktorem ovlivňujícím dynamiku rostlinných společenstev a koexistenci rostlinných druhů.

Klíčová slova: plant-soil feedback, koexistence rostlin, primární sukcese, půdní abiotické podmínky, půdní bakterie a houby

CONTENTS

INTRODUCTION

During their life, plants influence abiotic and biotic properties of the soil they grow in. The plant impact on the soil is a result of numerous processes, such as nutrient uptake by the plants, decomposition of plant litter, accumulation of plant pathogens or mutualists or changes in soil microbiome activity and composition in response to specific root exudates (reviewed in Ehrenfeld et al., 2005). This modified soil can affect performance of the plants growing simultaneously or subsequently in the soil, in a mechanism called plant-soil feedback (Bever et al. 1997).

Such plant-soil interactions can be either negative or positive. Negative feedback occurs when a plant alters soil conditions in a way that results in decreased performance of other plants. Vice versa, positive feedback means that plant-altered soil conditions increase performance of subsequent plants. Plant-soil feedbacks take place between individuals (or populations) of different plant species, with species A having soil-mediated effects on performance of species B (i.e., heterospecific plant-soil feedback). Alternatively, soil conditions induced by growth of species A can impact further performance of species A (i.e., conspecific feedback).

First observations of plant-soil feedback mechanism come from agriculture and are the reason why crop rotation system was developed. The crop rotation uses certain sequence of crops in time and space to provide soil fertility for maintaining the crop yield and to prevent problems with pests and soil-borne diseases (Olesen et al. 1999). The idea that such plant-soil interactions could influence the natural plant communities as well, was firstly suggested by (Bever 1994) who studied plant-soil feedback among species from an old-field succession. Since 1990's research interest in this topic is rapidly increasing (Ehrenfeld et al. 2005, van der Putten et al. 2013, 2016, Mariotte et al. 2018).

To measure the magnitude and direction (positive vs. negative) of plant-soil feedbacks, Bever *et al.* (1997) devised a simple experimental design. Firstly, different plant species are grown separately to specifically alter the biotic and abiotic components of the soil. This step is usually called "the conditioning phase". Secondly, after removal of the conditioning plants, the conditioned soils are grown by subsequent plants in a so called "feedback phase". The magnitude and direction of feedback is then tested by comparing the response of plants grown in their own soils and the response in soils conditioned by different species (Bever et al. 1997). Based on this design, various experimental approaches have been developed, depending on the research question (reviewed in Brinkman et al. (2010)). For example, researchers can add microbial inoculum from the conditioned soil to a background soil to set up the feedback phase (Bever 1994, Klironomos 2002). Such design serves to separate the microbe-mediated soil feedback from the effects of plant-induced changes in soil abiotic conditions. There is also a variability in soil treatments used as controls: studies compare plant performance in soils of conspecific and heterospecific origin (Bever 1994, Bezemer et al. 2006), non-sterilized vs. sterilized conditioned soils (van der Putten et al. 1993) or conditioned and unconditioned soils (Kardol et al. 2007). Again, the choice of control treatment allows

focusing on a certain phenomenon: plant specificity of plant-soil feedbacks, role of microorganisms or the potential of plant to shift all soil properties. Indeed, each experimental approach has its strengths and weaknesses (Brinkman et al. 2010) and they need to be carefully chosen and considered in interpretation of the results since there is evidence that both the size and direction of the plant-soil feedback outcome is determined by the experimental method used (Kulmatiski et al. 2008).

PREDICTING PLANT-SOIL FEEDBACKS WITH THE USE OF PLANT TRAITS AND PHYLOGENY

Plant-soil feedbacks and plant traits

Since plant-soil feedbacks are plant species-specific (e.g., Bever 1994, Klironomos 2002, Anacker et al. 2014), there have been attempts to find some general patterns by relating them to plant functional traits (Kulmatiski et al. 2008, 2017, Meisner et al. 2014, Baxendale et al. 2014). For example, plant species with different life span could differ in their conspecific feedback: while annuals can afford more negative conspecific feedbacks because they can easily escape their own soil legacies via seed dispersal, perennials cannot. In support of this hypothesis, the conspecific feedback has been found to positively correlate with plant life span (Kulmatiski et al. 2008, 2017).

There are also other traits that can be expected to influence plant-soil feedbacks. Plants directly influence the composition and activity of microbial communities via litter or exudates entering the soil (Orwin et al. 2010, Laughlin 2011, de Vries et al. 2012). It is thus likely that plant traits that reflect the amount or quality of litter or root exudates, such as plant size, concentration of nitrogen in tissues or growth rate can be linked to plant-soil feedbacks. Semchenko et al. (2018) showed that plant species with higher nitrogen content in their shoots accumulate less mycorrhizal fungi and, moreover, attract more pathogens which results in prevailing negative soil feedbacks. The study of Baxendale et al. (2014) found relationship between plant-soil feedbacks and plant growth rate, with fast-growing species generating positive soil feedbacks towards other fast-growing species. Fast-growing plants are often associated with bacteria-dominated microbial communities, faster nutrient cycles and higher availability of nitrogen (Orwin et al. 2010, de Vries et al. 2012, Grigulis et al. 2013) and they are obviously capable of shifting soil properties in their own favor by e.g. enhancing the nitrogen availability (Baxendale et al. 2014, Kulmatiski et al. 2017). In contrast, Lemmermeyer et al. (2015) found a negative correlation between the plant growth rate and its response to conspecific soil biota, illustrating that fast-growing species tend to suffer more from specific pathogens. The same study, however, did not find the same pattern in species responses to the whole soil (Lemmermeyer et al. 2015), suggesting that the fast-growing species generate also positive soil legacies that out-weight the negative impact of specific pathogens.

There have not been many studies connecting plant-soil feedbacks with belowground plant traits, but Cortois et al. (2016a) showed that plants with fine roots suffer more from negative feedbacks. Recent study of Heinen et al. (2018), however, did not find a

relationship between the size of plant root system and either the strength of soil legacies left by plants, or the responsiveness of plants to the feedbacks.

Nevertheless, most of studies focusing on the role of plant traits in shaping soil feedbacks are focusing on conspecific feedbacks. To understand the role of soil feedbacks in plant communities, we, however, need to understand the heterospecific interactions as well. Thus, in the Study 1, I aimed to evaluate whether heterospecific plant-soil feedbacks can be linked to species differences in plant traits, using meta-analytical approach. Specifically, I investigated the differences in traits that can be related to the size or growth rate: plant life span, plant height and specific leaf area.

Plant-soil feedbacks and plant phylogeny

The eco-evolutionary dynamics of plant-soil feedbacks are subject of ongoing research and are well discussed in the review of terHorst and Zee (2016). Briefly, plant-soil feedbacks are at least partly driven by symbiotic pathogens or mutualists that accumulate in the soil (Bever 1994, Klironomos 2002, Kardol et al. 2007). Since these organisms are often dependent on plants as their food sources, there are likely to be strong evolutionary links between the two groups of organisms. For example, plant species-specific pathogens are likely transmittable between closely related plant species (Liu et al. 2012) and the probability of infection of a different plant species decreases with increasing phylogenetic distance of the donor plant (Gilbert and Webb 2007, Liu et al. 2012).

Similarly, previous studies found a phylogenetic signal in plant responses to mycorrhizal fungi (Reinhart et al. 2012, Anacker et al. 2014) or in responses to the whole previously conditioned soil (Brandt et al. 2009, Anacker et al. 2014). There have been also several studies examining the role of plant relatedness in heterospecific feedbacks but they are inconsistent in their results, showing either negative correlation between heterospecific feedback and the phylogenetic distance of the two plant species (Burns and Strauss 2011, Mehrabi et al. 2015, Münzbergová and Šurinová 2015), or no relationship at all (Dostál and Palečková 2010, Mehrabi and Tuck 2014, Fitzpatrick et al. 2017). This inconsistency could be a result of great variability between the individual experiments. Alternatively, the relationship between feedbacks and relatedness might depend on the scale of phylogenetic distance since over longer distances, convergence in plant traits between distant relatives might occur (Kelly et al. 2014). In the Study 1, I aimed to investigate the relationship between heterospecific plant-soil feedbacks and the phylogenetic distance of the two interacting species, using meta-analysis of already published data.

THE ROLE OF PLANT-SOIL FEEDBACKS IN PLANT COMMUNITIES

Plant-soil feedback has been shown to influence plant-plant interactions in both grassland (Bever 1994) and forest communities (McCarthy-Neumann and Kobe 2010, Bennett et al. 2017). However, the role of feedbacks is more obvious in systems with higher abundance of one or several plant species (i.e., in systems with a strong dominant and with lower species diversity). Good examples of such systems are early stages of plant communities undergoing successional development (Kardol et al. 2006, van de Voorde et al. 2011) or plant communities being colonized by invasive species (Agrawal et al. 2005, Perkins and

Nowak 2012, Yang et al. 2013). The reason is simple: with higher abundance of individuals of the same species, specific soil legacy is more likely to develop, and there are also fewer species whose soil legacies would interfere with legacies of the focal species (Wubs and Bezemer 2018).

Vegetation succession

Changes in vegetation composition during succession are strongly linked to changes of soil pH, levels of nutrients, as well as changes in communities of soil microorganisms and fauna (Merilä et al. 2002, Frouz et al. 2008). Such temporal synchronization illustrates the importance of plant-soil interactions during the community development. Plant species that appear at certain point during the course of succession can be merged into successional guilds. The species from one guild usually share some traits, such as good dispersal or competitive abilities. For example, early-successional species are usually short-lived, fast growing, good dispersers and do not invest into defence systems against pathogens. As such, they are more susceptible to soil-borne diseases when compared to later successional species (van der Putten et al. 1993), and the accumulation of pathogens can result in fast decline of their populations in time. Previous studies on plant-soil feedbacks showed that plant species from the early stages of succession suffer from negative conspecific plant soil feedbacks, while plant species from later stages can be linked with positive plant-soil feedbacks (van der Putten et al. 1993, Kardol et al. 2007, van de Voorde et al. 2011, Jing et al. 2015a). Besides the different susceptibility to pathogens of early- and later successional species, later successional species tend to form mycorrhizal symbiosis more often than early successionals (Cázares et al. 2005) that probably contributes to their positive conspecific feedbacks. Generally, late successional species are usually long-lived and as such they simply cannot exhibit too negative conspecific feedbacks that would significantly decrease their fitness with increasing age. In contrast, annual species can escape their local negative feedbacks each year via seed dispersal. Indeed, Kulmatiski et al. (2008) showed in their meta-analysis that annuals exhibit more negative conspecific feedbacks, compared to perennials (or biennials). Nevertheless, both the negative feedbacks of early successionals and the positive feedbacks of later successionals necessarily contribute to the species replacement, speeding up the process of succession.

Van de Voorde et al. (2011) suggested that species replacement during succession can be mediated by heterospecific feedback as well. Specifically, (i) early successionals generate positive heterospecific feedback towards later successional species, and (ii) later successional species generate negative heterospecific feedbacks towards early successional species. Example of the facilitative effects of early successionals towards later successional plants can be through increased soil fertility caused by N-fixing plant symbionts (Reynolds et al. 2003). Such positive feedback is, however, important mostly in the initial stages of succession. Later in the succession (as N-limitation decreases), the plant association with N-fixing symbionts becomes less beneficial strategy and such species become rarer (Vitousek and Field 1999).

Most of the published literature on importance of PSF during succession focuses on secondary succession (e.g., Kardol et al. 2007, van de Voorde et al. 2011, Jing et al. 2015a; but see van der Putten et al. 1993, Frouz et al. 2016). However, it could be expected that during primary succession, the role of plant-soil feedback could be different. Primary succession is necessarily connected with large changes in both abiotic and biotic soil conditions during the process of pedogenesis. It also posits a great opportunity to study the exact mechanisms of soil feedbacks since, at least at the initial stages, the soil is uninfluenced by preceding generations of plants, as is the case of secondary succession. In the Study 2, I focus on plant-soil feedbacks between 12 species from a primary successional sequence. Moreover, I dig deeper into the individual mechanisms of the feedback by distinguishing the changes in biotic and abiotic soil components.

Plant invasions

Plant-soil feedback is one of the mechanisms contributing to plant invasiveness (Agrawal et al. 2005, Perkins and Nowak 2012, Yang et al. 2013). Though I am not dealing with invasive plants in my research, I will briefly introduce this topic to complete the picture of our current knowledge of plant-soil feedbacks. The role of plant-soil feedbacks in ecology of invasive species is closely connected to the enemy release hypothesis (Elton 1958, Agrawal et al. 2005). According to this hypothesis, introduced species can become successful colonizers of the novel habitats because they lose their specific enemies during the invasion process (Elton 1958). Since soil pathogens are considered as important drivers of plant-soil feedbacks, plant species could thus experience less negative feedbacks in their invasive range than in their home range.

Generally, plant-soil feedback can contribute to invasion success in case that (i) invasive species are characterized with positive conspecific feedback (i.e., the enemy release hypothesis), (ii) generate negative heterospecific feedback towards native species (Batten et al. 2008), e.g. via allelopatics (Lankau et al. 2009), and (iii) exhibit positive responses to heterospecific feedbacks of the native species. In the latter case, invasive species can be less susceptible to soil pathogens of the native plants since they do not share specialized pathogens and the generalist pathogens usually have lower negative impact on the populations of their hosts (Colautti et al. 2004). Moreover, plant mutualists such as AM fungi, which contribute to positive soil feedback, are usually less host specific (Smith and Read 2008) and the invasive plants can thus benefit even from "heterospecific" AMF (Marler et al. 1999, Yang et al. 2013).

Plants probably change their feedbacks to more positive in the moment they are introduced into the novel community (Callaway et al. 2004). As the invasion proceeds, species increases in abundance and becomes dominant and plant-soil feedbacks could be an important factor determining the species invasion success. Indeed, there have been evidences that plant-soil feedbacks of invasive species are more positive than feedbacks of natives (Callaway et al. 2004, Agrawal et al. 2005, Van Grunsven et al. 2007, Kulmatiski et al. 2008, Yang et al. 2013, Meisner et al. 2014).

However, plant-soil feedbacks are not stable feature of plant species and can change during time of invasion. For example, *Alliaria petiolata*, a plant native to Europe that invades the forest understories in North America has been shown to select for decreased production of allelopatics over 50 years of invasion history, which resulted in its decreased abundances (Lankau et al. 2009). Dostál et al. (2013) showed that the conspecific feedback of an invasive plant *Heracleum mantegazzianum* can become more negative during time, probably as a result of accumulating soil pathogens.

Coexistence of plant species

Plant species coexistence and diversity of communities is stabilized and maintained if their conspecific interactions are more negative than the heterospecific ones (Chesson 2000, Bever et al. 2011). Positive conspecific feedbacks necessarily destabilize the system and lead to increase in population of certain species which is, for example, the case of plant invasions or vegetation succession. The idea that negative conspecific feedbacks contribute to species diversity is consistent with the Janzen-Connel hypothesis of herbivore/pathogen-driven diversity of tropical forests (Janzen 1970, Connell 1971). Indeed, the negative pathogen-mediated conspecific feedbacks have been shown to be an important factor causing tree seedling mortality in both tropical (Mangan et al. 2010) and temperate trees (Packer and Clay 2000).

Kulmatiski et al. (2008) showed in their meta-analysis that conspecific plant-soil feedbacks are predominantly negative. Moreover, the magnitude of the negative feedback seem to correlate with the species abundance in plant communities: the species with the strongest negative conspecific feedback are the rarest, while common plant species usually have less negative conspecific feedback (Klironomos 2002, Mangan et al. 2010, Kulmatiski et al. 2017). Mack and Bever (2014) used computer simulation to model the relationship between feedbacks and species abundance, and showed that the above mentioned positive relationship is valid only in case of local dispersal. In case of long-distance dispersal, the relationship was not different from zero: with increasing dispersal distance, the probability and thus the importance of conspecific interactions decreases. The fact that other factors, such as the dispersal distance, influence this relationship could be an explanation for missing relationship between plant-soil feedback and species abundance in other studies (Reinhart 2012).

Other models suggested that too negative plant-soil feedbacks can lead to larger oscillations in community composition (Bever 2003, Bonanomi et al. 2005). Such oscillations can stochastically result in species dominance or extinction. However, they can also result in cyclic replacements of the individual species (i.e., species A can replace species B that can replace species C that replaces species A), and thus allow their coexistence within community (Revilla et al. 2013). Interestingly, the oscillations in community composition caused by strong conspecific feedbacks disappeared if the species diversity was high (Bonanomi et al. 2005): the authors suggest that with high species diversity, the likelihood of finding a patch without strong negative feedback of conspecific plant is higher.

The composition of plant communities is shaped not only by the conspecific but also by the heterospecific soil feedbacks (Fitzpatrick et al. 2017). However, there are multiple other factors (such as plant competition, abiotic stress, herbivory etc.) occurring in the natural conditions and there is still a lot of work to be done to evaluate the relative importance of plant-soil feedbacks. The plant-soil feedback experiments are usually carried out under greenhouse conditions and as such they give us only part of the information (Heinze et al. 2016) and more research done directly in the field (or using field data) can contribute to our current knowledge. In the Study 1, I aimed to connect these two sides of one coin, the experimentally measured feedbacks with the real composition of plant communities. To do that, I used already published data on heterospecific feedbacks and related them to levels of species co-occurrence obtained from two European phytosociological databases (Czech and Dutch).

PLANT-SOIL FEEDBACKS IN THE ECOSYSTEM CONTEXT

The intensive research on plant-soil feedbacks in the last decades shows that this mechanism can influence plant coexistence in communities. There are also numerous additional factors such as abiotic conditions of the site, disturbances, plant-plant competition, seed dispersal, or herbivory that impact plant coexistence. Moreover these factors can interact with plant-soil feedbacks (Wardle et al. 1998). For example, Fry et al. (2018) showed that plant-soil feedbacks can be neutralized if plants experience drought. Hortal et al. (2017) analyzed soil communities of competing plants and found out that they resemble mostly to the communities of the stronger competitor. Other studies show that the negative effect of competition can be strengthen in case of negative soil feedback (Van der Putten and Peters 1997, Casper and Castelli 2007, Kardol et al. 2007, Pendergast et al. 2013), or that positive feedback can enhance competitive ability of invasive species (Marler et al. 1999). Herbaceous communities are usually maintained by disturbances that can out-weight the effects of soil legacies (Müller et al. 2016), or by long-term herbivory that has been shown to influence composition of soil biota and subsequently also the growth of plants (Veen et al. 2014, Chen et al. 2017). Bezemer et al. (2013) tested the effects of foliar insect herbivores and found shifts in soil legacies induced by the herbivory and, moreover, these legacies led to improvement of plant resistance to herbivores in the subsequent generation. Similarly, the belowground herbivores also altered plant-soil feedbacks (Bezemer et al. 2013). All these examples illustrate that there can be great variability in plant-soil feedbacks that is ecosystem context-dependent.

One of the possibilities how to study plant-soil feedbacks with inclusion of the other factors, is to study them directly in the field (Heinze et al. 2016). In the Study 3 of this thesis, I studied plant-soil feedbacks in soil conditioned by a plant community directly in the field, and compared them to feedbacks of plants grown in a growth chamber. Moreover, I aimed to evaluate the ability of plant-induced soil legacies to cover soil legacies of the previous plants. The knowledge of possible interactions of the individual specific soil feedbacks is necessary for our understanding of their role in plant communities.

Additionally, there is another complexity that has been largely overlooked in plant-soil feedback studies (mainly due to many methodological challenges): the complexity of soil food webs. The ecosystem of soil consists of mineral particles, soil aggregates, water, dead tissues of plants and animals (=soil organic matter, SOM), and a great variety of soil organisms: soil bacteria, fungi, Protozoa, nematodes, insects, earthworms and soil-dwelling vertebrates. However, most of plant-soil feedback studies study solely the effects of abiotic changes and changes in soil microbiota. In the Study 4, I asked how plant-soil feedbacks can be altered if a model community of soil arthropods is added to the experiment. In this study, I focused both on changes in soil microbial communities and on final plant responses to the altered conditions.

THE INDIVIDUAL COMPONENTS OF PLANT-SOIL FEEDBACKS

The biggest challenge in plant-soil feedback research is disentangling the individual plant-induced soil processes, understanding their interactions and evaluating their effects on plant performance. Plants generate feedbacks with numerous components of the soil: from soil aggregates, over nutrient cycles, to soil biota of various taxa (all reviewed in Ehrenfeld et al. 2005). However, the concept of plant-soil feedback has been developed by plant ecologists and the "soil" part of this concept has been treated as a black box for a long time.

The first step in disentangling the individual components is distinguishing between the biotic and abiotic soil feedback. This can be done, depending on the research question, by using microbial inoculum (Bever 1994), sterilization of conditioned soils (van der Putten et al. 1993), or by adding nutrients (Wubs and Bezemer 2018). Most studies found plant-soil feedbacks to be microbe-mediated (e.g., van der Putten et al. 1993, Kardol et al. 2007, van de Voorde et al. 2011, Jing et al. 2015b). Recent studies, however, showed that the importance of soil biota in feedbacks increases during vegetation succession (Castle et al. 2016, Frouz et al. 2016) which suggests that under certain conditions (such as the initial phases of succession), soil biota is not the driving mechanism of plant-soil feedbacks. Thus, in all my experimental work (i.e., in Study 2, 3 and 4), I am trying to disentangle the biotic and abiotic component of the soil and to assess its role in plant-soil feedbacks. Moreover, in the Study 4, I investigated how the individual components of plant-soil feedback might be altered by presence/absence of soil mesofauna.

Obviously, distinguishing the biotic and abiotic component of plant-soil feedback is just a beginning of our understanding. Future steps should lead researchers to examining them in more detail (such as I attempted in the Study 4), and to focus on other factors, such as plant root exudates that can influence availability of nutrients (Subbarao et al. 2015, Meier et al. 2017) or toxicity of plant DNA of conspecific origin (Mazzoleni et al. 2015).

OVERVIEW OF MY RESEARCH

Study 1: Heterospecific plant-soil feedback and its relationship to plant traits, species relatedness and co-occurrence in natural communities

The number of plant-soil feedback studies published during the last decades facilitated my goal to investigate possible general patterns in heterospecific feedbacks. In the study in Study 1, I asked whether the direction and magnitude of plant-soil feedbacks between two species might be explained by (i) phylogenetic relatedness of the two species, (ii) species difference in traits, or (iii) whether the heterospecific feedback correlates with species co-occurrence patterns in the field.

To answer these questions, we searched published literature and compiled a dataset of 618 PSF interactions. We gathered data on species relatedness, and on traits connected to plant size and growth rate: plant total height, specific leaf area and plant life span. Further, we used two European phytosociological databases to obtain data on species natural co-occurrence in plant communities.

The study revealed that while there was no relationship between the phylogenetic distance and heterospecific feedback on the whole dataset, among the close relatives, we found a negative correlation: plants grew better in soils conditioned by their close relatives than in conspecific soils. This suggests that the traits related to feedback could be subject of a fast evolution (i.e., strong shift in traits between the plant and its closest relative at the moment of speciation event). From the tested plant traits, we only found that plants benefit from soils conditioned by species with greater height. There was no relationship between heterospecific feedback and plant life span or specific leaf area. Finally, we found a positive relationship between heterospecific feedback and the frequency of species co-occurrence in plant communities: species that shared more positive feedback were more frequently found within one community. However, all predictors explained only a small fraction of the total variability in heterospecific feedbacks. We thus concluded that other factors such as environmental conditions possibly alter plant-soil feedback responses.

Study 2: Evaluating the role of biotic and chemical components of plant-soil feedback of primary successional plants

In my research, I focused on the role and mechanisms of plant-soil feedbacks in herbaceous communities. Since one of my main goals was to take a deeper look into the black box of plant-induced changes in soil, the first experiment was focused on the simplest possible system: the initial stage of primary succession. During my Master's studies, I dealt with spontaneous plant colonization and vegetation changes in an abandon limestone quarry in the Czech Karst (Czech Republic), and I thus had a good knowledge of vegetation dynamics during the time of succession on one specific site.

Based on this knowledge, we designed a pot experiment that focused on plant-soil feedbacks between 4 different successional guilds of plants: 3 early colonizers, 3 mid-

successional species, 3 species of developed dry grassland (as a potential late stage of succession) and 3 invasive species that occur at the locality and threaten conservational value of the local communities. We tested the hypothesis that during primary succession, plants are facilitated via heterospecific feedback by the preceding successional guilds. Additionally, we analyzed changes in soil chemical properties and in soil bacterial and fungal communities induced by plant conditioning with the aim to evaluate (i) the species- or guild- specificity of these changes and (ii) the individual impact of these changes on seedling establishment and total biomass of subsequent plants.

We found that the soils conditioned by early-successional species contained higher proportion of pathogenic fungi than soils conditioned by mid-successional plants. Bacterial communities were mainly plant species- specific (14.6 % of variation), though the successional guilds explained 7% of variation in their communities. Five plant species (out of 12) responded to the plant-induced changes in soil chemical properties. Seedling establishment of two plant species was influenced by changes in soil fungal communities. These results suggest that in primary succession, the negative biotic feedback might be more important in the initial stages of plant life, while growth of adult plants depends mostly on the abiotic soil feedback.

Study 3: Soil remembers but not too much: plant-soil feedback legacies over two generations of plants

Many grassland communities can be characterized by relatively high plant species diversity. However, it is not known how plant-soil feedbacks influence species co-existence in diverse communities. Thus, in the Study 3, I asked a question whether a specific legacy of certain plant species can be masked by legacies of other species. Moreover, we took advantage of a long-term field experiment in which a dominant grass, *Festuca rubra*, was yearly removed from permanent plots in a mountain grassland. We tested if the legacy of present *Festuca* can be detected on the level of the whole community, i.e., when other species that can influence its feedback are present.

To do that, we performed two experiments. In the first one, we took soil samples from the permanent plots with either the presence or absence of dominant *Festuca*. We put the soil into pots and grew four species from the grassland in the two soil types. In the second experiment, we conditioned soil by growing *Festuca* individuals. Then we removed the plants and planted the conditioned soil, as well as unconditioned control, with each of the four grassland species separately. Further, we removed the plants again and planted the soil by *Festuca* again. We tested whether the final performance of *Festuca* will be influenced by the first, the second or interaction of both conditionings. Additionally, we analyzed soil samples taken after both the first and second conditioning to see the plant-induced changes in soil abiotic properties and composition of microbial communities.

The presence of the dominant species, *Festuca rubra*, could not be detected in plant-soil feedback effect of the whole grassland community. This shows that in species rich grasslands, plant-soil feedbacks are not likely to be easily predictable. Our second experiment showed that though the most recent conditioning had the strongest impact on

both the soil properties and the final plant performance, the original conditioning had also significant effect on both these levels, illustrating the interactive nature of plant-soil feedbacks. Based on these results, we suggest further research should focus on the interactions of plant-soil feedbacks within individual communities, i.e. under conditions, where many species coexist and their plant-soil feedbacks may interact in complex ways.

Study 4: Soil microarthropods alter the outcome of plant-soil feedback experiments

In the study in Study 4 I focus on the complexity of soil food webs and their role in plant-soil feedbacks. Most of published plant-soil feedback studies use an experimental approach testing the plants growth responses to soil previously conditioned by another plant. These studies are usually carried out in pots and, moreover, often focus solely on effects of soil microbiota and exclude effects of larger soil fauna, such as nematodes, soil arthropods, and earthworms. However, such organisms are part of the soil environment as well and are also known to influence plant performance. In the Study 4, I aimed to explore effects of a model microarthropod community on plant-soil feedbacks, both on the level of specific changes in soil components and plant responses to the soil conditions.

To do that, we performed a plant-soil feedback experiment in microcosms with two plant species, *Phleum pratense* and *Poa pratensis*. We added a model microarthropod community consisting of three fungivorous springtail species (*Proisotoma minuta, Folsomia candida, Sinella curviseta*) and a predatory mite (*Hypoaspis aculeifer*) to half of the microcosms. We expected the springtails to feed on soil fungi and the predatory mite to feed on soil nematodes, as well as the springtails, enhancing thus nutrient cycling. We measured the final seedling establishment and plant biomass, nematode and microbial community composition, microbial biomass, and mycorrhizal colonization of roots.

The addition of model microarthropod community caused changes in the composition of btoh nematode and microbial communities. This effect was particularly strong in *Phleum* plants where microarthropods altered the composition of bacterial communities. Microarthropods also generally influenced plant performance, and their effects depended on previous soil conditioning and differed between the two plant species. Microarthropods did not affect soil microbial biomass and mycorrhizal colonization of roots. Due to their numerous effects on both the individual soil properties and the final plant responses to feedbacks, we conclude that the role of soil microarthropods should be considered in future plant-soil feedback experiments.

AUTHOR CONTRIBUTION STATEMENT

This thesis consists of two published papers, one accepted and one unsubmitted manuscript. All papers are co-authored. My contributions to particular papers are as follows:

1. **Eliška Kuťáková**, Tomáš Herben and Zuzana Münzbergová (2018) Heterospecific plant-soil feedback and its relationship to plant traits, species relatedness and co-occurrence in natural communities. *Oecologia 187 (3):* 679-688. https://doi.org/10.1007/s00442-018-4145-z

   All authors contributed to the design of this study. EK was responsible for gathering the data, and wrote the first draft. EK and TH performed the analyses. TH and ZM also contributed to obtaining the data, and they participated in writing the manuscript.

2. **Eliška Kuťáková**, Lenka Meszárošová, Zuzana Münzbergová and Petr Baldrian (accepted) Evaluating the role of biotic and chemical components of plant-soil feedback of primary successional plants. *Biology and fertility of soils.*

   ZM and EK designed the plant-soil feedback experiment. EK performed the feedback experiment. PB and LM designed the microbiological part of the study. LM analyzed soil microbial communities. EK and LM did the statistical analyzes and wrote the first draft of the manuscript. All authors commented on data analyzes and contributed to the final version of the manuscript.

3. **Eliška Kuťáková**, Lenka Mészárošová, Petr Baldrian, Zuzana Münzbergová and Tomáš Herben (manuscript) Soil remembers but not too much: plant-soil feedback legacies over two generations of plants.

   EK, TH and ZM designed the plant-soil feedback experiments. EK performed the experiments. PB and LM designed the microbiological part of the study. LM analyzed soil microbial communities. EK and LM did the statistical analyzes and wrote the first draft of the manuscript. All authors commented on data analyzes and contributed to the final version of the manuscript.

4. **Eliška Kuťáková**, Simone Cesarz, Zuzana Münzbergová and Nico Eisenhauer (2018) Soil microarthropods alter the outcome of plant-soil feedback experiments. *Scientific Reports 8:11898.* https://doi.org/10.1038/s41598-018-30340-w

   NE designed the study and supervised the project. ZM and SC commented on the experimental design. EK performed the experiments, processed all soil and plant samples, and performed data analyzes. SC supervised the determination of nematodes, extraction of soil PLFAs, and measurements of soil respiration, and helped with the data processing. ZM supervised the data analyzes. EK wrote the first draft of the manuscript. All the authors discussed the results and commented on the manuscript.

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STUDY 1: HETEROSPECIFIC PLANT-SOIL FEEDBACK AND ITS RELATIONSHIP TO PLANT TRAITS, SPECIES RELATEDNESS AND CO-OCCURRENCE IN NATURAL COMMUNITIES

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Abstract

Plant-soil feedback is one of the mechanisms affecting coexistence of species, ecological succession and species invasiveness. However, in contrast to conspecific plant-soil feedback, general patterns in heterospecific feedback are mostly unknown. We used a meta-analysis to search for correlations between heterospecific feedback and species relatedness, functional traits and field co-occurrence patterns. We searched published literature and compiled a dataset of 618 PSF interactions. We gathered data on species traits reflecting plant size and growth rate (height, specific leaf area, life span), co-occurrence in habitats and phylogenetic distance between species pairs. We found that species grew better in soil conditioned by (i) close relatives than in conspecific soil, whereas there was no relationship with phylogeny for distantly related species, (ii) species of greater plant height (but there was no relationship with species SLA or life span), and (iii) species more frequently co-occurring in the field. The results show that heterospecific plant-soil feedback can be explained by plant traits (height) and is reflected in co-occurrence patterns. Phylogeny was a significant predictor of feedbacks over short phylogenetic distance, suggesting fast evolution of traits related to feedback. The low variability explained by the models, however, indicates that other factors such as environmental conditions possibly alter plant-soil feedback responses.

Key words

Daphne phylogeny, plant coexistence, plant traits, Czech National Phytosociological Database, Dutch National Phytosociological Database

Introduction

The concept of plant-soil feedback is based on the assumption that plants are, during their life, influencing abiotic and biotic properties of the soil they grow in. The plant impact on the soil is a result of numerous processes, such as nutrient exploitation during plant growth, decomposition of plant litter, accumulation of plant pathogens or mutualists or changes in soil microbiome activity and composition in response to specific root exudates (reviewed in Ehrenfeld et al., 2005). This modified soil then affects performance of the plants growing simultaneously or subsequently in the soil (Bever et al. 1997). Experimentally, plant-soil feedback effects are measured as plant biomass production in a soil conditioned by conspecifics relative to plant biomass in a control soil (conditioned by a different species, mixture of species or unconditioned, soil).

In the last two decades, a number of studies showed that plant-soil feedback is likely to play an important role in the course of succession (e.g. van der Voorde et al. 2011; Kardol et al. 2006), spread of invasive species (e.g. Agrawal et al. 2005; Perkins and Nowak 2013; Yang et al. 2013), and is also considered one of the key mechanisms affecting composition and structure of natural communities (Revilla et al. 2013; Mack and Bever 2014; Bennett et al. 2017). All this indicates that plant-soil feedback is an important mechanism influencing species co-existence. Thus, there is a need to identify factors that would allow us to predict the direction of species plant-soil feedback response. For instance, meta-analyses of the data on plant-soil feedbacks showed that different plant life forms differ in their conspecific feedback (Kulmatiski et al. 2008; Meisner et al. 2014). It has also been repeatedly shown that early successional species exhibit more negative conspecific feedback, i.e. perform worse in the soil of conspecifics, than late successional species do (van der Putten et al. 1993; Kardol et al. 2006). Klironomos (2002) showed that conspecific plant-soil feedback can be related to plant abundance, with rarer plants having more negative feedback.

As an opposite of the conspecific feedback, heterospecific plant-soil feedback, i.e. interactions between two species mediated by soil environment, are much less known. Heterospecific feedback can be measured as plant performance in soil conditioned by a certain heterospecific species, relative to control soil (i.e. conspecific or unconditioned soil). It may seem that determinants of conspecific feedback may be identical to those of heterospecific feedback, as the two values can be identical but of an opposite sign. However, the challenges are completely different. In conspecific feedback, the primary challenge is to identify important characteristics of the focal species. For heterospecific feedback, the important question is the relationship between the focal and the heterospecific species. This relationship could be expressed as species phylogenetic distance, difference in functional traits, or their co-occurrence patterns in natural communities.

The role of phylogenetic distance in determining the intensity of heterospecific plant-soil feedbacks has been subject of recent research (Dostál and Palečková, 2010; Burns and Strauss, 2011; Mehrabi and Tuck, 2014; Mehrabi et al., 2015; Münzbergová and Šurinová, 2015; Fitzpatrick et al. 2017). Their results are, however, largely inconsistent. Some studies showed that plants perform better in soil of their close relatives than in soil of distantly related species (Burns and Strauss 2011, Mehrabi et al. 2015, Münzbergová and Šurinová 2015). Other studies do not confirm any effect of relatedness on feedbacks (Dostál and Palečková, 2010; Mehrabi and Tuck, 2014; Fitzpatrick et al. 2017). This suggests that the effect of phylogeny on plant-soil feedback could vary, possibly

depending on the scale of phylogenetic distance. Generally, the similarity in species traits is stably correlated with phylogenetic distance among close relatives, whereas the relationship over longer distances can be weakened by convergence in species traits among more distantly related species (Kelly et al., 2014). This relationship could be reflected in plant-soil feedbacks: they might be explained by species phylogeny only among close relatives.

Heterospecific feedback may depend on the species differentiation in plant traits. For example, soil microbial communities are dependent on the quality and amount of nutrients entering the soil, either in a form of plant litter or as root exudates (Orwin et al. 2010; Garnier et al. 2004; Laughlin 2011; de Vries et al. 2012). Large or fast-growing plants are usually associated with bacteria-dominated microbial communities with faster nutrient cycles and higher soil N availability (de Vries et al. 2012; Grigulis et al. 2013). Such soil environment has positive effect on growth of subsequent plants (Baxendale et al. 2014). Thus, plant traits reflecting the amount of plant litter and root exudates entering the soil, could be related to plant-soil feedbacks. An example of such traits is plant height, life span or specific leaf area, i.e. traits that respond to plant size or growth rate (Wright et al. 2004; Laughlin et al. 2010).

Heterospecific plant-soil feedback can be also related to plant community composition (Klironomos 2002; Mangan et al. 2010, Fitzpatrick et al. 2017). While it has been shown by theoretical studies that coexistence of two plant species can be stabilized by stronger negative conspecific than heterospecific interactions (Chesson 2000; Bever et al. 2010), its true action in the field may be much more complex, both due to interactions with other factors (climatic conditions, species competition or seed dispersal), and due to potentially confounding effects of co-evolutionary history. However, species that co-occur frequently could generally be expected to show more positive heterospecific feedback. Strong negative heterospecific feedbacks would destabilize the system and the system would need additional mechanisms for stabilization. Plant species thus co-occur either because they never had highly negative heterospecific feedback, or they might have been selected to reduce their negative heterospecific effect. This would imply that species that co-occur will show more positive heterospecific feedback than species with lower co-occurrence.

In this study, we aim to investigate the relationship between heterospecific plant-soil feedback and species relatedness, traits and patterns of co-occurrence, analyzing already published experimental data. Specifically, we hypothesized that heterospecific plant-soil feedback will be (i) influenced by species relatedness over short phylogenetic distances but not over longer phylogenetic distances; (ii) related to species differentiation in size and growth rate, i.e. species will benefit from soil conditioned by species which are larger/grow faster; and (iii) positively correlated with species co-occurrence, i.e., plants will perform better in soil conditioned by their frequent neighbours than in soil conditioned by less frequently co-occurring species. We examined these hypotheses using a meta-analysis of published data on heterospecific plant-soil feedback, and linked them to species-level parameters.

Materials and Methods

DATASET

To obtain data on plant-soil feedbacks, we searched Web of Science using search query "TI=(soil NEAR/3 feedback OR soil borne diseas* NEAR plant) OR TS=(plant-soil feedback OR microb* NEAR feedback)" (last search on 01 June 2017). In total, we found 808 studies. The studies were screened at three stages (title, abstract and full-text screening) by one of the authors (EK). During the title screening, the obviously irrelevant studies were removed (e.g., papers from other fields than plant ecology). Further, abstracts of 334 papers were examined to exclude studies not focusing on plant-soil feedback, or dealing with heterospecific interactions of woody plant species. Finally, full texts of 57 papers yielding from abstract screening were assessed for eligibility, according to the following criteria:

(i) We included only experimental studies dealing with plant-soil feedback using soil either conditioned in greenhouse conditions, or obtained from monospecific stands in the field.

(ii) Studies examining effects of a single specific group of soil organisms (e.g., studies dealing with plant-mycorrhiza feedbacks only) were excluded. We, however, included studies using either whole pre-conditioned soil, or whole extracted microbial inoculum of this pre-conditioned soil.

(iii) We included only papers containing data on plant performance when grown in soil previously conditioned by the same species ("conspecific" soil, further used as control treatment) and data on performance of the same species on soil previously conditioned by another species ("heterospecific" soil) of known identity. Thus, we excluded experiments using species mixture or randomly assigned "heterospecific" species with unknown identity to condition the soil.

(iv) Only studies with data available directly from the manuscripts (tables, figures, or published primary data), or obtained from authors, could be included into our dataset.

Using these criteria, we found 30 studies containing data from 618 plant-soil feedback experiments (for the list of included publications see Table S1 in Supporting information). The whole selection procedure is illustrated by PRISMA flow diagram (Fig. S1 in Supporting information). Though we searched only publications included in the Core Collection of Web of Science, this systematic search corresponds with requirements for a meta-analysis (Koricheva and Gurevitch 2014) and the obtained dataset forms a representative sample of plant-soil feedback studies.

PHYLOGENETIC DISTANCE

Phylogenetic distance between each pair of interacting species was calculated from the Daphne phylogeny (Durka and Michalski 2012) and it is expressed in millions of years (Mya) since a common ancestor of the two species. In 18 cases (out of 168 species in total), species from our dataset were not present in the Daphne phylogeny database (e.g. *Stipa krylovii, Vulpia microstachys*). Therefore, we used congeneric species (e.g. *Stipa pennata*, *Vulpia myuros*) for the phylogenetic distance calculation (in 11 cases), or closely related species of different genus (in 7 cases, for the species list see Table S2 in Supporting information). As the congeneric or the confamilial species we chose the closest relatives present in the Daphne phylogeny.

SPECIES TRAITS

Data on species life span (annual or perennial), plant height and specific leaf area (SLA) were obtained directly from the papers, from Kubát *et al.* (2002) or the LEDA Traitbase (Kleyer et al. 2008). Plant height was calculated as a mean of minimal and maximal height for each species. For the species list and information on further species data see Table S2 in Supporting information.

SPECIES OCCURRENCE IN EUROPEAN HABITATS

To estimate the extent of species co-occurrence in natural habitats, we used the Czech National Phytosociological Database (the Czech NPD; Chytrý and Rafajová 2003). We used a stratified and filtered subset used by Chytrý et al. (2005). It contains only vegetation records of comparable plot size, recorded after 1970 and corrected for oversampling of individual habitat types or localities. These filters enabled us to focus on recent patterns in species coexistence within a habitat.

It is important to note that the scale of plots in the databases might be larger than the scale of plant-soil feedback effects (commonly 4×4 m or 5×5m for herbaceous communities; Chytrý and Otýpková 2003). However, the plots for vegetation sampling are usually chosen in order to represent the respective habitat, i. e. they should be relatively homogenous and representative in terms of species composition. As such, the plots should be thus a good proxy of the level of species local co-occurrence in natural conditions. Additionally, an advantage of the databases is that they contain thousands of plots, which makes the estimation of species co-occurrence intensity more robust.

From our dataset of 168 species, we found 102 species present in the Czech NPD with number of vegetation records (containing at least one of these species) equal to 29 759. These 102 species formed 365 experimental pairs. For further analyses of co-occurrence patterns, we only included PSF experiments performed in Europe to prevent relating PSF effects measured in species invaded range (namely North America) to co-occurrence data obtained in species home range (i.e., Europe). However, we did include experiments focusing on non-European species invasive to Europe (6 species in our database). These invasive species can be (from the plant-soil feedback point of view) perceived as legitimate members of current plant communities and our first hypothesis can be thus applied to them as well. Of course, occurrence of invasive species in vegetation records can be underestimated in case of old records and a fast expanding species. We partially avoid this effect by using the stratified database.

For comparison, we collected data from the Dutch National Phytosociological Database (the Dutch NPD) for a separate analysis. This database contained 91 species from our dataset (forming 299 experimental pairs). The number of vegetation records containing at least one of species from our dataset was 14 277.

INDEX CALCULATIONS AND DATA ANALYSIS

EXPRESSION OF PLANT-SOIL FEEDBACK

We calculated index of heterospecific feedback (PSF_{het}) in each experiment as $\ln(h/c)$, where h is the biomass of a plant when grown in soil previously conditioned by a different species (heterospecific soil) and c is the biomass of a plant when grown in soil previously conditioned by an individual of the focal species (conspecific soil) as suggested by Brinkman et al. (2010). Positive values of this index mean that plant

performs better in the heterospecific soil (i.e., experiences positive heterospecific feedback) and vice versa. Note that other studies commonly use the reversed formula ($PSF_{con}=\ln(c/h)$) and thus operate with opposite values of PSF. However, we believe that when exploring heterospecific feedback our calculation is more intuitive for interpretation: positive PSF_{het} means that plant is facilitated in the soil of heterospecific origin. We will further refer to PSF_{con} as conspecific plant-soil feedback, to PSF_{het} as heterospecific plant-soil feedback. We will use the term plant-soil feedback without specification in cases where both conspecific and heterospecific feedback may be relevant.

INDEX OF SPECIES CO-OCCURRENCE

For each experimental pair of species, we calculated an index of co-occurrence as Jaccard similarity coefficient (Jaccard 1901): $N_{AB}/(N_A + N_B + N_{AB})$ where N_{AB} is the number of vegetation records with co-occurrence of both species from the experimental pair, and N_A and N_B are numbers of vegetation records with occurrence of each of the species separately. The values of this index range from 0 (the two species never co-occur) to 1 (the two species are always found together). We assume that this index reflects the frequency of co-occurrence of the two species, related to their total frequency in habitats and therefore reflects the frequency of their natural interaction.

SIMILARITY IN SPECIES TRAITS

For each experimental pair, we calculated the difference in plant height and SLA as $X_h - X_c$, where X_h represents trait value of the conditioning (heterospecific) species and X_c the trait value of the focal species (conspecific). This calculation expresses species similarity in that specific trait (index close to 0 indicates similar trait values), but also on the directionality of that difference (index>0 means conditioning species with greater trait value; index<0 focal species with greater trait value). Further, we distinguished the four following categories of life span combinations in the species pairs: two annuals, two perennials, annual grown after perennial and perennial grown after annual species.

DATA ANALYSIS

Prior to analyses, we square root- transformed the data on phylogenetic distance and species co-occurrence. In addition to correcting the non-normal distribution, the square root transformation of phylogenetic data is meaningful as it allows down-weighting of long-ago evolutionary events which would otherwise bias the ecological interpretation of the results (Letten & Cornwell 2015). Similarly, this transformation down-weights co-occurrence differences of frequent (or frequently co-occurring) species.

We fitted linear models for trait differences and co-occurrence data. Phylogenetic distance was examined using a polynomial model of the third order to account for nonlinearity in the relationship. For proper analyses of the relationship between plant-soil feedback and all the measured variables (co-occurrence index, phylogenetic distance and trait differences), we needed to out-filter possible publication bias (Koricheva and Gurevitch 2014). To do that, we used a linear mixed model testing the fixed effect of one of the variables on PSF, with a random effect of the original study. Datasets for each tested variable differed due to different numbers of data points (species) for which the appropriate information was available. Variance explained by the relationship was calculated using the formulas proposed by Johnsson (2014) that calculate the explained variation in mixed models correctly, as implemented in the package piecewiseSEM

(Lefcheck 2016); marginal R^2 was used as only variation in diversity explained by the fixed factors were of interest. Predicted values were constructed without including random effects to make them independent of random study-specific offsets. Confidence intervals of predicted values were calculated using parametric bootstrap (function bootMer from the package lmerTest; Kuznetsova et al., 2016). They were calculated for the fixed effect(s) only (not including the random effect of the study) and loess smoothing was used for their visualisation over the full range of the predictor variable.

The phylogenetic relatedness of the two interacting species might also impact the analyses of traits and co-occurrence patterns by introducing a correlated structure to the data: closely related species are more likely to have similar traits which leads to violating the assumption of independence of residuals (Chamberlain et al. 2012). Thus, we used an additional mixed model, corrected for phylogeny (phylogenetic distance of the two species was used as fixed effect, original study as random effect).

The mixed models were performed using lme4 package (Bates et al. 2015) in R 3.2.4 for Windows (R Development Core Team 2016). Significance of the effects was calculated in lmerTest package using F-test, based on Satterthwaite approximation for denominator degrees of freedom (Kuznetsova et al. 2016).

Results

The overall conspecific plant-soil feedback was not significantly different from zero (one sample t-test: t=0.23; df=446; p=0.814), with 95% confidence intervals ranging from -0.08 to 0.10. This illustrates that within our dataset, there was similar number of species that performed better in conspecific soil (positive PSF_{con}) and of those that performed better on heterospecific soil (negative PSF_{con}; Fig. 1).

Fig. 1

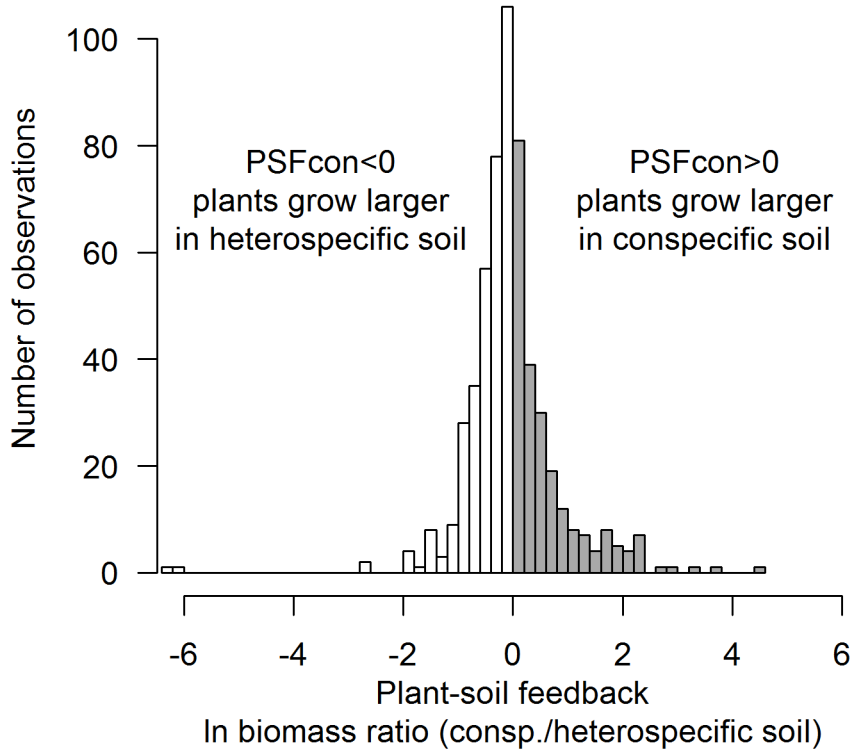

Fig. 1: Histogram of PSF values from the dataset. Conspecific PSF index (ln biomass ratio conspecific/heterospecific soil) is presented.

Heterospecific plant-soil feedback (PSF_{het}) was influenced by phylogenetic distance, but in a nonlinear fashion (R^2=0.051; Table 1, Fig. 2). Both quadratic and cubic terms of the polynomial model were significant. Within the close relatives (circa <50 Mya since the common ancestor), the value of PSF_{het} was decreasing with increasing phylogenetic distance and the plants were performing the best in soil conditioned by their closest relatives. For the more distant relatives (circa>50 Mya since the common ancestor), the PSF_{het} approximated zero (neutral PSF).

Table 1. Relationship between heterospecific plant-soil feedback and species co-occurrence, phylogenetic distance (cubic model) and difference in traits. Both results of basic model and model with phylogenetic correction are presented. D.f. stands for residual degrees of freedom estimated using Satterthwaithe approximation. R^2 was calculated according to Johnsson (2014).

	Basic model				Model with phylogenetic correction			
	d.f.	F	p	R^2 (%)	d.f.	F	p	R^2 (%)
Species co-occurrence	1;266	4.97	0.027	1.9	1;316	3.24	0.073	1.2
Phylogenetic distance	1;549	12.03	0.001	5.1	NA	NA	NA	NA
Difference in traits								
Height	1;433	12.71	<0.001	2.5	1;431	12.70	<0.001	4.1
SLA	1;316	0.83	0.362	0.2	1;316	0.81	0.368	0.2
Life span	3;520	1.35	0.257	0.8	3;532	1.56	0.197	1.0

Fig. 2

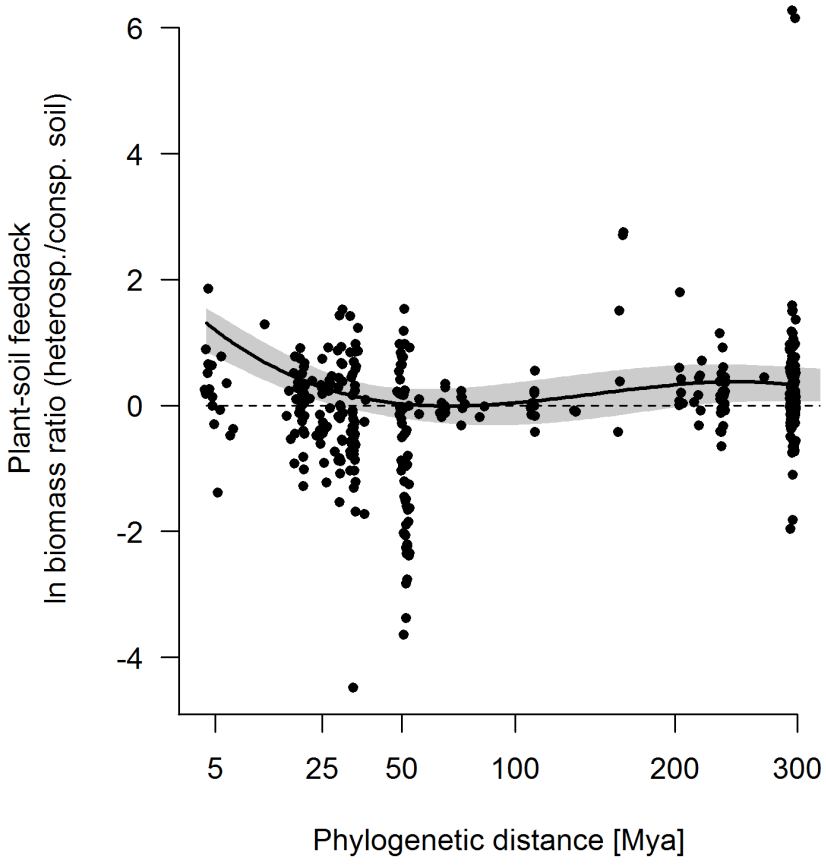

Fig. 2: Relationship between the phylogenetic distance (square root-transformed) and heterospecific plant-soil feedback (p=0.001; $F_{1;548.79}$=12.03). Positive values of plant-soil feedback indicate higher biomass in heterospecific than in own soil. The grey area represents 95% confidence interval. Random noise was added to the values of phylogenetic distance to prevent overplotting of the data points.

PSF_{het} was positively correlated with difference in plant height (R^2=0.025; Table 1; Fig. 3). In cases where the heterospecific species were larger than the focal species, the focal species tended to grow larger in soil conditioned by the heterospecific than conspecific. This relationship remained significant even in case the phylogenetic distance (Table 1) was used as a covariate. We found no relationship between the difference in species SLA or plant life span and PSF_{het} (Table 1).

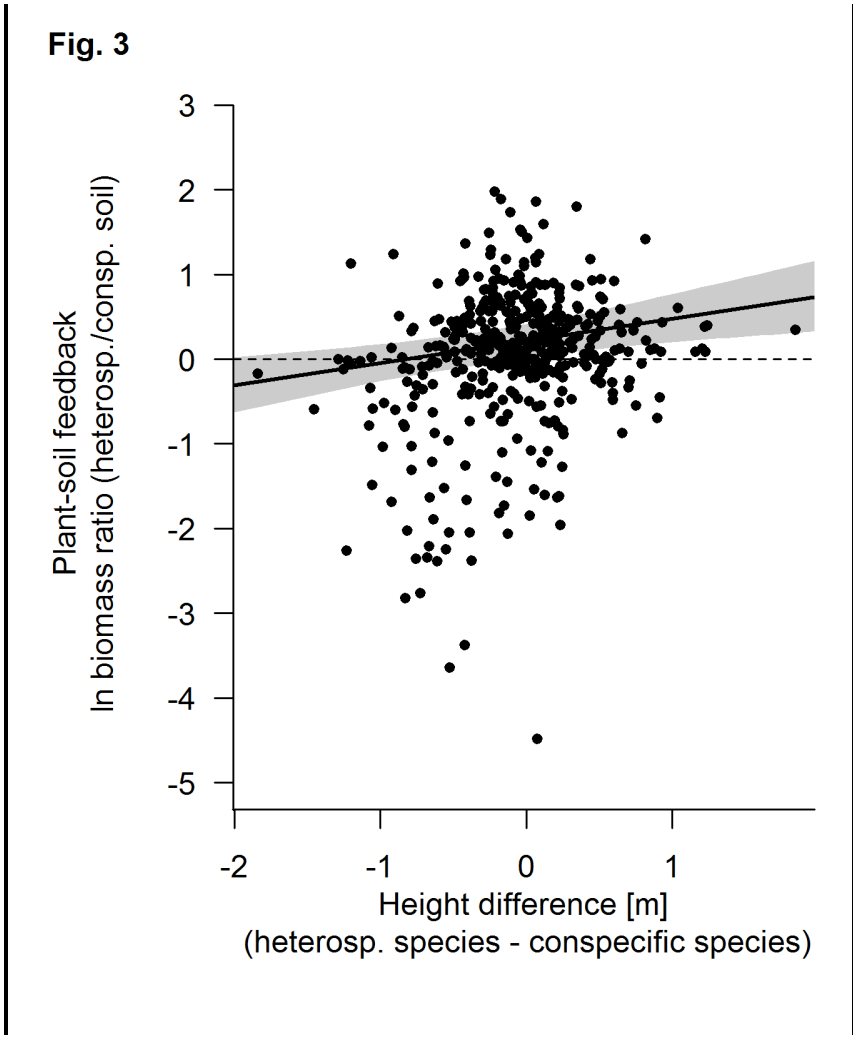

Fig. 3: Relationship between the difference in plant height and heterospecific plant-soil feedback ($p<0.001$; $F_{1;432.61}=12.71$). Positive values of plant-soil feedback indicate that species grew better in heterospecific than in own soil, negative values that species grew better in conspecific soil. The grey area represents 95% confidence interval. Random noise was added to the values of height to prevent overplotting of the data points.

PSF_{het} was positively correlated with the co-occurrence index (based on the data from the Czech National Phytosociological Database) and explained 1.9 % of the variance (Table 1). The positive correlation between co-occurrence and PSF_{het} means that with increasing co-occurrence of the two species, plants tended to benefit more (e.g., had greater biomass) when grown in soil conditioned by heterospecific species than when grown in conspecific soil (Fig. 4). This relationship was not influenced by the phylogenetic distance (Table 1). However, both tests performed on the co-occurrence data obtained from the Dutch National Phytosociological Database were not significant ($p \geq 0.380$;

$R^2 \leq 0.02$; Fig. S2). In addition, when we excluded experiments containing species invasive to the Czech Republic, the relationship between co-occurrence index for the Czech NPD and PSF_{het} was also not significant ($F_{1;223.7}=1.97$, $p=0.161$, $R^2=0.008$, Fig. S3).

Fig. 4

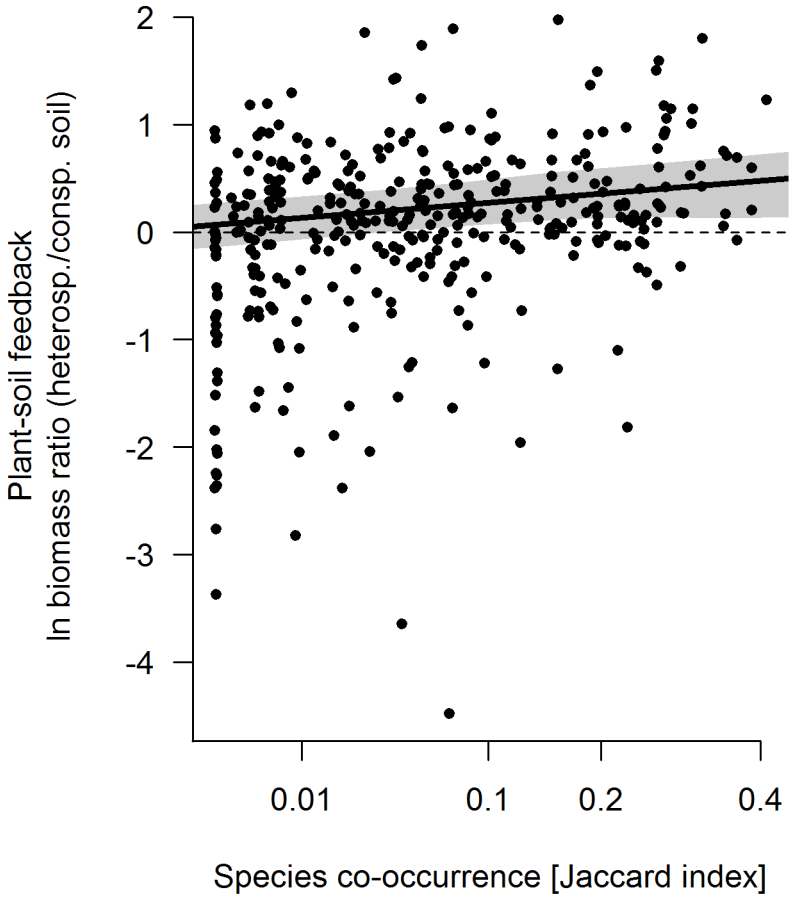

Fig. 4: Influence of heterospecific plant-soil feedback on species co-occurrence ($p=0.027$; $F_{1;266.03}=4.97$). Co-occurrence data were obtained from the Czech National Phytosociological Database. Values of Jaccard index range from 0 (the two species never co-occur) to 1 (they always co-occur). Positive values of heterospecific feedback indicate higher biomass in heterospecific than in conspecific soil. The grey area represents 95% confidence interval. Random noise was added to the values of species co-occurrence to prevent overplotting of the data points.

Discussion

Our study aimed to uncover the role of plant phylogeny, traits and co-occurrence patterns as predictors of variation in heterospecific plant-soil feedback. The analyses showed two significant but weak patterns, namely a positive relationship with species co-occurrence in natural habitats ($R^2=0.019$), and a negative relationship with species phylogenetic distance among closely related species, which became neutral over longer phylogenetic distances (polynomial model with $R^2=0.051$). Further, heterospecific feedback was

positively affected by difference in plant height (R^2=0.025), but showed no relationship to differences in species specific leaf area or life span.

The overall direction of conspecific feedback (PSF_{con}, the reversed formula commonly used in other studies) was not different from zero and we reported both positive and negative feedbacks. This is in agreement with finding of Meisner et al. (2014), who found neutral average plant-soil feedback between native and invasive species in their meta-analysis. However, our result contradicts findings of Kulmatiski et al. (2008) and Mehrabi & Tuck (2014) whose meta-analyses both reported moderately negative conspecific plant-soil feedback (mean value ± 95% CI equal to -0.56±0.12, and -0.33±0.17).

PHYLOGENETIC DISTANCE

We found a relationship between phylogenetic distance and heterospecific plant-soil feedback only for close relatives (approx. within families), suggesting that there is no phylogenetic pattern in heterospecific feedback over the entire phylogenetic tree. This also agrees with the results of Mehrabi & Tuck (2014), who, using a smaller data set, were not able to find significant relationship between plant-soil feedback and phylogenetic distance over a wide range of phylogenetic distances. In general, such finding is not unexpected as traits that evolve fast lose their phylogenetic signal over larger phylogenetic distances (Freckleton et al. 2002, Ackerly 2009, Kelly et al. 2014).

However, in contrast to our hypothesis, species relatedness within close relatives influenced heterospecific feedback negatively: plants tended to grow larger in the soil of close relatives (e.g. congeners) than of more distantly related taxa (e.g. confamiliar). A similar, although not significant, effect was reported by Mehrabi & Tuck (2014) for Poaceae (note that their y-axis is inversely scaled). In our data, the benefit of growing in soil conditioned by related species decreased as the phylogenetic similarity decreased, effectively becoming zero around phylogenetic distances of 50 Mya. While previous studies dealing with phylogenetic patterns in heterospecific feedback are not fully consistent in their results, there is some support for the same pattern in the literature (Mehrabi et al., 2015; Burns and Strauss, 2011, Münzbergová and Šurinová 2015). Such positive effects of soil conditioned by close relatives are contrary to the expectation that closely related species are likely to share pathogens and thus will generate negative heterospecific feedback. Possibly, the shared pathogens could have different impact on different closely-related species (Redman et al. 2001; Augspurger and Wilkinson 2007; Bradley et al. 2008), which would weaken the negative impact on plants. However, this mechanism itself could hardly result in more positive heterospecific feedback between close relatives than between distantly related plants and other feedback mechanisms are necessarily involved. For example, closely related species could enhance soil nutrient cycles in a similar way, profitable for the other species, or the positive feedback could be driven by accumulation of mutualistic microbiota. The different mechanisms may also interact with each other. However, further experimental work is needed to evaluate separate effects of these drivers.

Regardless the specific mechanisms causing the negative relationship between heterospecific plant-soil feedback and plant relatedness, the positive direction of heterospecific feedback between the closest relatives (conspecific soil being the control) is rather unexpected. If plant-soil feedbacks are driven by any trait conserved in plant phylogeny, then the value of PSF_{het} should be around zero: the closest relatives should condition the soil in a very similar way to the focal species itself. However, in our data,

the plants produced more biomass in heterospecific soil of a close relative than in conspecific soil. This suggests that there might be a shift in the respective traits related to plant-soil feedbacks at the time of plant speciation. For example, the two species could more efficiently distinguish their niches in terms of nutrient demands and specific pathogens, but still profit from very similar mutualistic biota and priming effects on the soil nutrients. At any rate, this means that we should pay more attention to potential changes in associated soil biota during speciation events, and the potential role which its divergence can play in formation of a new species (see e. g. terHorst and Zee 2016; Van Nuland et al. 2016 for broader discussion on evolution in plant-soil feedbacks).

PLANT TRAITS

The analysis of plant height showed that plants grew larger in soil conditioned by heterospecific species in case this species was larger than the focal species. Similar pattern was described in a study of Fitzpatrick et al. (2017), who showed that plants perform better in soil preconditioned by plants with greater belowground biomass; and of Baxendale et al. (2014), who found that plants perform better in soil of fast growing species (i.e., larger plants). This is in agreement with our hypothesis that plants will grow better in soil preconditioned by large plants that will enhance nutrient cycling and soil N availability more than small plants (de Vries et al. 2012; Grigulis et al. 2013). Such an explanation is supported by the fact that most studies in our dataset did not dilute the conditioned soil to account for changes in soil nutrient status (only 3 studies out of 30 either used fertiliser or worked with microbial inoculum).

We found no relationship between heterospecific plant-soil feedback and species specific leaf area (SLA). This result is in contrast to study of Fitzpatrick et al. (2017), who found that species with high SLA reduce performance of other plants via plant-soil feedback. SLA is commonly used as a proxy for plant growth rate and nutrient acquisition strategy (Wright et al. 2004) and as such could be linked to soil feedbacks. Of course, the missing relationship in our study could be caused by variability in SLA data obtained from databases. This could be supported by the fact that Fitzpatrick et al. (2017) found the relationship with SLA experimentally measured in their own study. At any rate, we did not find any relationship between species SLA and heterospecific feedback.

No difference in heterospecific plant-soil feedbacks among annuals and perennials could be caused by the fact that both annual and perennial plants are usually grown for the same period of time in these experiments. The resulting comparison between adult annual and juvenile perennial plants might lead to underestimation of perennial species effect.

SPECIES CO-OCCURRENCE

More positive (or less negative) soil feedback in co-occurring species implies that patterns of plant-soil feedback, although typically determined under artificial conditions, can be relevant also for the plant distribution in the field. Positive PSF_{het} between co-occurring species is an indication of potentially stabilizing heterospecific effects and thus supports our initial hypothesis that strong negative heterospecific feedback between co-occurring species are unlikely. As such stabilizing effects are a necessary condition for species coexistence (Chesson 2000, Adler et al. 2007), our results imply that heterospecific plant-soil feedback can play a role as one of the coexistence mechanisms in multispecies plant communities (see also Fitzpatrick et al. 2017). Interestingly, similar pattern as in our study was shown by Semchenko et al. (2013), who demonstrated a positive relationship between species small-scale co-occurrence in the field and their

performance under competition in a greenhouse.

However, using data currently available, we cannot determine direction of the causality, i.e. whether species co-occur because they have less negative heterospecific PSF, or whether co-evolutionary processes between plants and their symbiotic microorganisms lead to better performance of plants when grown in association with their shared symbionts (Klironomos 2003; Pánková et al. 2011). The pattern observed was relatively weak, and it was only significant in the central European (Czech) database but not in the western European (Dutch) database (the effect was not significant though went the same direction). The discrepancy in the strength of the patterns between the two databases is not clear. A possible explanation may be linked to the fact that the central European habitats are more stable, with longer history of species co-existence, while the habitats in western Europe underwent much stronger transformation and are likely newer and thus less stabilized. The weak pattern may be caused by confounding effects of the experimental setup, which cannot be controlled in a meta-analytical study. Namely, plant-soil feedback experiments typically involve one soil or one inoculum. As the soil microorganisms and soil abiotic conditions responsible for the PSF are often site- and environment specific, use of soil from one environment for species from another environment may have negative (or less positive) effects per se, hampering inference on the heterospecific effects. Comparisons of feedback effects in different soils illustrate that PSFs can be altered by origin of soil used for the experiment (Bauer et al., 2015; Bezemer et al., 2006; Harrison and Bardgett, 2010; Perkins and Nowak, 2013). Johnson et al. (2010) moreover showed that the site-specific environmental conditions can lead to local adaptations in plant-mycorrhiza symbiosis, which favour plants growing in soil and with mycorrhizal fungi from their home site. Therefore the interpretation of plant-soil feedback experiments should be more cautious: feedback measured in an experiment not using the site-specific soil can provide fewer clues to whether the process found in the experiment really takes place in the field.

Conclusions

In this study, we identified several determinants of heterospecific plant-soil feedback: plant relatedness (among close relatives), species co-occurrence and difference in plant height. These relationships are well interpretable and illustrate the possible role of heterospecific feedback in species coexistence and thus in structuring ecological communities. In this respect, heterospecific feedback is similar to the conspecific plant-soil feedback.

However, all the predictors explained only a small amount of variation. This could be caused by differences in methodologies among the individual studies, with different abiotic conditions affecting the final plant-soil feedback response. Under natural conditions, other factors, such as plant competition, herbivory or environmental stress, can interact with plant-soil feedbacks, resulting in greater variability of feedback responses. We suggest further research should focus on the importance of plant-soil feedbacks, relative to the other factors across wide range of habitats.

Interestingly, the heterospecific plant-soil feedback between closely related species in our dataset strongly differed from zero, possibly indicating sudden change in feedback mechanisms at the moment of formation of a new species. The mechanisms behind this pattern, however, remain to be explored.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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STUDY 2: EVALUATING THE ROLE OF BIOTIC AND CHEMICAL COMPONENTS OF PLANT-SOIL FEEDBACK OF PRIMARY SUCCESSIONAL PLANTS

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Abstract

During primary succession, vegetation and soil form important feedbacks that enhance plant species turnover. However, the mechanisms underlying such plant-soil feedbacks (PSFs) remain uncler. We studied PSFs among 12 species from different successional stages in a limestone quarry. We explored the changes in abiotic and biotic soil conditions induced by individual species, and the effects of these changes on further plant germination and biomass production. We performed a two-phase PSF experiment. Firstly, we conditioned the quarry soil by three early- and three mid-successional species. Secondly, we planted the conditioned soils, as well as unconditioned control, by the same early- and mid-successional species, and by three late-successional grassland and three invasive species. We recorded seedling establishment and total biomass of all plants. The conditioned soils were analysed for pH, nutrient content and composition of bacterial and fungal communities. Soils conditioned by early-successionals were characterized by higher proportion of pathogenic fungi than soils conditioned by mid-successionals. Bacterial communities were rather species- (14.6 % of variation) than guild-specific (7 %). From the individual properties, the most frequent predictor of plant performance were the changes in soil chemical properties and the biomass of conditioning plants (5 species out of 12). In case of two species, we found significant links between seedling establishment but not plant growth and changes in fungal communities (2 species), suggesting that biotic feedbacks might be more important in the initial stages of plant life.

Key words: plant-soil interactions, primary succession, limestone quarry, soil fungal community, soil bacterial community

Introduction

During their life, plants continuously change conditions of the soil they grow in, either by their metabolic activity (rhizodeposition, litter production) or through their effects on the activity of soil microorganisms. The changes in soil conditions include shifts in chemical properties of the soil and in the abundance and composition of microbial communities (Chapin et al. 1994; Frouz et al. 2008). Besides the plant-induced direct changes in soil nutrient content, plant-associated soil microorganisms, which act as the main decomposers of organic matter, regulate nutrient availability and cycling, and contribute to soil formation (van der Heijden et al. 2008). Furthermore, soil microbes can influence plants directly, acting as mutualists or pathogens. All these alterations of soil properties neccessarily influence the local performance of other plants in a mechanism called plant-soil feedback (Bever 1994; Bever et al. 1997).

Plant-soil feedback has been shown as an important mechanism influencing plant-plant interactions in various systems, including grassland (Kuťáková et al. 2018a) and forest communities (McCarthy-Neumann and Kobe 2010). It also influences the spread of invasive plant species (Yang et al. 2013; Dostálek et al. 2016) and course of succession (Kardol et al. 2006; van de Voorde et al. 2011). Plant-soil feedback is expected to be especially important during vegetation succession as successional communities are characterized by profound changes in soil pH, levels of nutrients and communities of soil microorganisms and fauna, that are all temporarily synchronized with changes in vegetation (Merilä et al. 2002; Frouz et al. 2008).

Previous studies on (mainly secondary) succession showed that plant species from the early stages of succession suffer from negative plant soil feedbacks, while plant species from later stages can be linked with positive plant-soil feedbacks (van der Putten et al. 1993; Kardol et al. 2007; van de Voorde et al. 2011; Jing et al. 2015). These effects contribute to the replacement of an early-successional guild by the later-successional guild, speeding up the process of succession. These plant-soil feedbacks are usually considered to be mediated by the soil microbiota: negative plant-soil feedbacks among early-successional species are driven by the accumulation of soil pathogens, while late-successional species benefit from mutualist-driven positive plant-soil feedbacks (van der Putten et al. 1993; Kardol et al. 2007; van de Voorde et al. 2011; Jing et al. 2015). However, the plant-soil feedbacks are likely driven also by altered abiotic conditions.

Recently, positive plant-soil feedback was recorded in a conditioned and subsequently sterilized soil, which suggests that the positive feedback was driven by changes in abiotic conditions in the absence of specific soil biota (Castle et al. 2016). These authors also show that the importance of soil biota in plant-soil feedback increases during the succession (Castle et al. 2016). This result is supported also by Frouz et al. (2016) who showed that early successional species respond less to the changes in soil biota than late successional species. Still, our knowledge of the mechanisms underlying plant-soil feedbacks and how they change during the succession is very scarce.

In this study, we aimed to experimentally investigate the abiotic and biotic mechanisms underlying plant-soil feedback interactions among plants from a primary sucession serie in a limestone quarry landfill in the Czech Republic. The study site was left to spontaneous development in 2009 and the vegetation development has been studied since the first year of succession. Based on the knowledge of vegetation development, we defined four guilds of species: initial colonizers (early successional species), mid-successionals, potential late-successional dry-grassland species, and a guild of invasive species present at the locality. In this study, we have followed the changes that early- and mid-successional plant species induce in the chemical composition of soil and in the composition of soil microbiota. Both of these factors may be potentially responsible for the plant-soil feedback affecting the performance of plants that arrive into the conditioned soil. Further, we planted the conditioned soil by each of the four functional guilds of plants and followed their performance. We hypothesized that: (i) early successional species would cause larger shifts in soil chemical properties than mid-successionals due to higher growth rate; (ii) soil conditioned by early-successional plants would contain higher share of pathogenic microorganisms and soils conditioned by mid-successionals higher share of mutualistic organisms; (iii) plants would generally perform better in soil conditioned by plants from the previous successional stage (i. e., mid-successional species would benefit from soil conditioned by early-successionals; grassland species from soil conditioned by mid-successionals) and invasive species would profit from conditioning by any species; (iv) plant growth would be driven primarily by soil chemical properties, while seedling establishment would be primarily influenced by composition of microbial communities.

Methods

Study locality and model plant species

The study locality was situated in the Czech Karst Protected Landscape Area in the Central Bohemia, Czech Republic, 49° 57' 46'' N, 14° 10' E; in a hilly karstic landscape characterized by relatively warm climate and mild winters (395 m above sea level, mean annual temperatures 8-9°C, mean annual precipitation 530 mm). The vegetation consists of thermophilic and xerophilic grasslands alongside deciduous forests and human-used areas.

Study site itself was a partly abandoned quarry. It was partly restored by filling the post-mining pit up with clay material from the bottom of the quarry in 2009 and then left to spontaneous development. The clay landfill (approximately 100 × 150 m large) is by its longer side directly adjacent to a species rich calcareous grassland, which serves as a source community for plant species colonizing the landfill. There are two additional potential sources of plant propagules: hornbeam forest on one side of the quarry and, on the other side, a mesic grassland down on the slope of the quarry hill. However, according to our long-term studies of this locality (Kuťáková unpubl. data), the calcareous grassland remains the most important seed source for two main reasons: (i) conditions on the landfill are too harsh for the forest species and (ii) migration of the species from the mesic grassland is difficult due to terrain steeply elevating towards the landfill.

With respect to the developing community at the study site, we chose plant species for the plant-soil feedback experiment. Using our data from 30 permanent plots (1 × 1 m^2) arranged in a grid on the landfill (Kuťáková unpubl. data), we defined four species guilds: early-successional, mid-successional, grassland, and invasive species. Early-successionals, defined as the initial colonizers with great abundance during the first years of succession (dominants on more than 15 plots), were *Tussilago farfara* L., *Melilotus albus* Medik., and *Daucus carota* L. Mid-successional species were species that colonized the landfill in the first three years of succession, but were present in low abundances on the plots (*Inula conyzae* (Griesselich) Meikle, *Securigera varia* (L.) Lassen, *Sanguisorba minor* Scop.). Grassland species were species from the neighbouring dry grassland, representing more developed successional community: *Centaurea jacea* L., *Erysimum crepidifolium* Rchb. and *Salvia verticillata* L., which started to appear at the landfill during the first seven years in numbers of several individuals. The guild of invasive

species consists of two species alien to the Czech Republic, *Conyza canadensis* (L.) Cronq. and *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & K. Presl (Pyšek et al. 2012), and a native but expansive grass species *Calamagrostis epigejos* (L.) Roth. These species were already present at the locality in the first three years of succession and their abundance has been increasing in time. Both *Arrhenatherum* and *Calamagrostis* are of a potential threat to the dry grassland community due to their high competitive ability (Fiala et al. 2004). Together with the invasive *Conyza*, these species lower the potential conservation value of the developing plant community in the studied quarry. All species are referred to by their genus names only further in the text.

Plant-soil feedback experiment

Soil for the experiment was obtained directly from the locality in autumn 2013. Approximately 10 centimeters of topsoil from 50 m^2 of an unvegetated part of the landfill were collected using a power shovel, in the total amount of 4 m^3. The topsoil was gathered from one-year old part of the quarry landfill where the vegetation was still absent, thus representing the initial stage of primary succession. Use of this soil is crucial for examining plant-soil feedbacks in such a system, especially due to its extreme abiotic characteristics: low nutrient content (N 0.05%; organic C 0.18%), slightly alkaline pH (pH/KCl=7.6) and low soil water-holding capacity (33.1 ml/100 cm^3) compared to values from the neighboring dry grassland (N content 1.5 g/kg; organic C content 7.6 g/kg; pH (KCl)=7.25; soil water-holding capacity 40.6 ml/100 cm^3) (Kuťáková unpubl. data). The chemical composition of the soil forming the landfill is homogeneous: in the previous study, Kuťáková (unpubl. data) found no differences in soil chemical properties along the transects of permanent plots. We are thus convinced that using samples from the younger part of the landfill is representative for the whole site.

The experiment consisted of two phases that were both performed in the experimental garden of Institute of Botany, Czech Academy of Sciences in Průhonice (49°59′38.972″N, 14°33′57.637″E). The climate in the garden is very similar to the climate of the study locality. It is 320 m above sea level, mean annual temperature 8.6°C and mean annual precipitation 610 mm. In the first (conditioning) phase, we used six plant species to condition the soil: three early-successional species from the study locality (*Tussilago, Melilotus, Daucus*) and three mid-successional species (*Inula, Securigera, Sanguisorba*). We obtained the seeds directly from the locality (*Melilotus*, *Tussilago*,

Inula) or from a local commercial seed provider, Planta naturalis, Markvartice, Czech Republic (*Daucus*, *Sanguisorba*, *Securigera*). The seeds were not stratified or surface-sterilized prior planting. In May 2014, we sowed seeds of each species into 4-liter pots (16×16 cm) in 120 replicates per plant species. The number of seeds sown per pot was based on the seed size. Specifically, we sowed 40 seeds of *Daucus*, 20 seeds of *Melilotus* or *Securigera*, and 10 seeds of *Sanguisorba*. For *Tussilago* and *Inula*, which have very small seeds that are difficult to count, we used 2-ml tube full of seeds (corresponding to approximately 150 seeds of each species). We left additional 120 pots unsown as controls. Because of a heavy slug grazing on *Tussilago* seedlings in the pots, we transplanted one-year old plants to pots from a monoculture stand at the locality in the beginning of August 2014. We grew the plants in monocultures (without reduction of seedlings) until the end of May 2015. The whole experiment, including the empty control pots, was regularly watered. In May 2015, we harvested the aboveground plant biomass, dried it at 60°C to a constant weight and weighed. We also removed majority of the roots from the soil. For each type of conditioned soil (7 types including the control), we created 10 replicates by mixing soil from 12 pots per replicate (see Fig. S1 in Supporting information for the scheme). Each soil replicate was subsampled for further soil analyses (approximately 30 ml of soil per sample were frozen for analyses of microbial communities and 100 ml were air-dried for analyses of soil chemical properties; see further for details).

In the second phase, we used 12 plant species: three early successional dominants and three mid-successional species from the first phase (*Tussilago, Melilotus, Daucus, Inula, Securigera, Sanguisorba*), three grassland species from a dry grassland adjacent to the study locality (*Centaurea, Erysimum, Salvia*) and three invasive species (*Arrhenatherum*, *Calamagrostis*, *Conyza*). Each of the soil replicates created after the harvest of the conditioning phase was used to fill 12 pots (2-liter, 12×12 cm). This resulted in 840 pots = 7 soil types (6 conditionings + an empty control) × 12 species in feedback × 10 replicates. Each pot was sown with one of the 12 species with each species sown into soil from each soil replicate. The number of seeds sown per pot depended on the seed size (50 seeds of *Erysimum* and *Calamagrostis*, 30 seeds of *Arrhenatherum*, *Centaurea* and *Salvia*, 1-ml tube of *Conyza* seeds, the other species in the same densities as in the conditioning phase). We sowed the seeds into the pots in the beginning of June 2015. After 4 weeks, we counted the emerging seedlings and reduced them to 5 per pot,

and subsequently, in week 8, we reduced the established seedlings to one per pot. If no seedlings germinated in a pot, we carefully transplanted a randomly chosen seedling from another pot within the same soil conditioning (this was a case of 93 pots out of 840 in total). We harvested both the aboveground and belowground biomass of all the plants after 3 months of growth at the end of September 2015. We dried the biomass at 60 °C to a constant weight and weighed it.

Analyses of soil samples

To assess the possible determinants of plant-soil feedbacks, we analysed soil samples taken from each replicate after the conditioning phase. We analysed soil chemical properties in all replicates (10 replicates × 6 conditioning species + 10 replicates from unplanted control pots) and composition of fungal and bacterial communities in 6 replicates (sampled from pots 1-6 in each conditioning treatment).

To analyse soil pH and content of nutrients, we dried approximately 100 ml of each sample at room temperature, and sieved it through 2-mm mesh to analyze active and exchangable pH and Ca, Mg, K, and P extractable content (Olsen 1954); or through 0.1-mm mesh for analyses of total N and total C content, C fixed in carbonates and organic C (Ehrenberger and Gorbach 1973). Analyses of pH and nutrient content were provided by the Analytical laboratory of the Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic.

Prior to the analyses of bacterial and fungal communities, the samples were sieved through a 4-mm mesh, freeze-dried and stored at -40°C. Fungal and bacterial communities were characterized by sequencing the internal transcribed spacer (ITS2) region and 16S ribosomal RNA gene, respectively. DNA from each sample was extracted in duplicates using the modified method of Miller (Sagova-Mareckova et al. 2008). Freeze-dried soil (0.18 g) was resuspended in 800 µl of extraction buffer (50 mM Na-phosphate buffer [pH 8], 50 mM NaCl, 500 mM Tris-HCl [pH 8], and 5% sodium dodecyl sulfate) and 300 µl of of phenol-chloroform–isoamyl alcohol (25:24:1), and homogenized three times in FastPrep®-24 bead beater (MP Biomedicals LLC, Santa Ana, CA, USA), each time for 20 s at maximum speed at 4°C, with 5-minute pause between homogenization runs. The homogenate was centrifuged at 10 000 × g for 3 min. The

supernatant was mixed with the same volume of phenol-chloroform–isoamyl alcohol (25:24:1) and centrifuged at 6000 × g for 5 min. The supernatant was mixed with an equal volume of chloroform-isoamyl alcohol (24:1) and centrifuged at 6 000 g for 5 min. The supernatant was then incubated with NaCl added to a final concentration of 1.5 M and CTAB added to 1% at 65°C for 30 min. The incubated solution was cooled, mixed with an equal volume of chloroform-isoamyl alcohol (24:1), and centrifuged at 4 500 × g for 20 min. DNA was then precipitated with isopropanol/sodium acetate. DNA extracts were purified using Geneclean Turbo Kit (Biogenic) following the manufacturer´s instructions, duplicates were pooled and stored in -20ºC before further use. For the microbial community analysis, PCR amplification of the fungal ITS2 region from DNA was performed using barcoded primers gITS7 and ITS4 (Ihrmark et al. 2012). It must be noted here that the choice of fungal genetic marker and classification database might bias the relative abundance of certain taxa, however, it should not affect the ability to detect the overall patterns it the community composition (Tedersoo et al. 2015; Xue et al. 2019). The V4 region of bacterial 16S rRNA was amplified using the barcoded primers 515F and 806R (Caporaso et al. 2012). PCR was performed in triplicates for each sample as recommended by Schöler et al. (2017) and Nilsson et al. (2019), and resulting amplicons were purified, pooled, and subjected to sequencing on the Illumina MiSeq. The presence of contaminant sequences was excluded using appropriate controls.

The amplicon sequencing data pre-processing was done using the pipeline Seed 2.0.3 (Větrovský et al. 2018) as described previously (Žifčáková et al. 2016). The processing included several steps including quality filtering (adapter trimming, quality and length filtering, removal of chimeric sequences and sequences not matching the target), clustering, and identification as recommended by Vestergaard et al. (2017). Consensus sequences were constructed for each cluster, and the closest hits at a genus or species level were identified using blastn against the Ribosomal Database Project (Cole et al. 2014) and GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Sequences identified as nonbacterial or nonfungal were discarded. To account for the variability of per-genome copy number of the 16S gene, the numbers of sequences were corrected for the 16S copy number in each taxon (Větrovský et al. 2013). Fungal ecology was determined using the FUNGuild assignment app (Nguyen et al. 2016). Sequence data have been deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under accession number PRJNA560475.

Calculation of plant-soil feedback (PSF) indices

For seedling establishment rate and total (aboveground+belowground) biomass harvested after the second phase, we calculated log-response ratios, following the formula $ln(x/c)$, where x is a value of a certain variable measured in a pot with previously conditioned soil, and c is a value of this variable measured in a control (unconditioned) pot. Note, that this index is different from the indices commonly used to calculate plant-soil feedback effects (Kulmatiski et al. 2008). However, we assume that this formula is more intuitively describing the PSFs during primary succession, where plants are often colonizing unconditioned (and usually unfavourable) soil. Thus, positive values of the index indicate that plants benefit from soil conditioning compared to unconditioned soil (positive PSF), values around zero indicate no feedback, and negative values indicate that plants grow better in unconditioned soil.

We calculated this index for total plant biomass but not for aboveground and belowground biomass separately, since all these variables were highly correlated (Pearson's correlation coefficient$\geq$0.33; $p<0.001$). In case of seedling establishment, we calculated the index as $ln((x+0.01)/(s+0.01))$ to avoid zero values in the denominator.

Data analyses

To reveal how conditioning influenced soil chemical properties, we tested the differences of each preconditioned soil from control soil in pH and nutrient content using t-test, and quantified the effect sizes by calculating Cohen's d (Cohen 1988). Prior to these analyses, we investigated possible correlations (Pearson's correlation) among the soil characteristics and excluded highly correlated variables from further analyses (specifically, we excluded active pH due to high correlation with the exchangeable pH; Table S1).

For statistical analyses of fungal and bacterial communities, we considered only OTUs occurring in at least three samples at a relative abundance of at least 0.1%. Differences in bacterial and fungal community composition caused by plant species or species guilds were tested using the permutational analysis of variance (PERMANOVA). To identify bacterial and fungal species that responded either positively or negatively to growth of individual plant species we carried out the indicator species analysis using the 'multipatt' function of the 'indicspecies' library (Cáceres and Legendre 2009) comparing

each of the conditioning plant species to control samples. We used the Indicator Value index which quantifies the specificity and fidelity of microbial species in relation to a priori defined groups of samples (in our case plant species and control). The significance of each OTU-group association was tested using a permutation test (n = 9999) and non-significant OTUs ($P > 0.05$) were discarded, as well as OTUs with specificity and fidelity < 0.8. Based on literature research, we determined potentially plant-interacting bacterial indicators belonging into one of following groups: N_2-fixers, plant pathogens, OTUs with antifungal activity, plant-polymer degraders and plant growth promoters. Additional information on analyses of microbial data can be found in Supplementary methods.

To investigate plant responses to preconditioned soils, we firstly analyzed whether the species from individual guilds (early-successionals, mid-successionals, grassland and expansive) respond differently to soils conditioned by early- versus mid-successionals. We used ANOVA for each guild, with PSF index of plant biomass or seedling establishment as a dependent variable, species identity in the feedback phase as a covariate, and conditioning species nested in the conditioning species guild as explanatory variables. Secondly, we tested whether the individual species responded differently to preconditioned soils (ANOVA for each species separately, with PSF index as a dependent variable, and conditioning treatment as an explanatory variable).

For each conditioning-responding species combination, we tested the strength of plant-soil feedback, i.e. the difference of PSF index from zero, using one-sample t-test. In case of plant-soil feedback for species guilds, we performed t-test on species means. The pairwise differences between treatments were investigated using Tukey HSD.

To evaluate the relative importance of the individual components of plant-soil feedback for plant performance, we partitioned the variance using the 'varpart' function of the 'vegan' library (Oksanen et al. 2018). The model consisted of four groups of explanatory variables: plant biomass in the conditioning phase (further conditioning biomass), and sample scores on the first two canonical axes of PCA analyses of data on soil chemical properties, and bacterial and fungal community compositions. As the dependent variable, we used PSF indices for seedling establishment and plant biomass. We performed variation partitioning for each plant species separately and for the individual species guilds, where we used PSF indices corrected for species identity. Further, we tested the effects of the four explanatory variables using linear regression.

All univariate analyses of plant seedling establishment, biomass and soil characteristics were performed using R 3.2.5 (R Core Team 2017). Statistical analyses of microbial communities were carried out using Past 3.11 (http://folk.uio.no/ohammer/past) and R 3.2.5 (R Core Team 2017).

Results

Plant effects on soil chemical properties

All plant species grown in the conditioning phase significantly lowered the pH of the soil. All species but *Inula* also significantly increased the extractable Mg and K content. All species but *Daucus* and *Securigera* increased the content of C fixed in carbonates. The highest increase of organic C content was observed in soils planted with *Melilotus* and *Sanguisorba*. The only species that increased total N content in soil was the leguminous species *Melilotus*. In contrast, total N content decreased in soil conditioned by the other legume, *Securigera*. Exchangable P content was altered only by *Inula* that caused its decrease. All early-successionals and *Inula* increased the content of calcium (Fig. 1). The differences between the chemical properties of control soil and plant-inoculated soil correlated significantly with the plant biomass (Fig. S2).

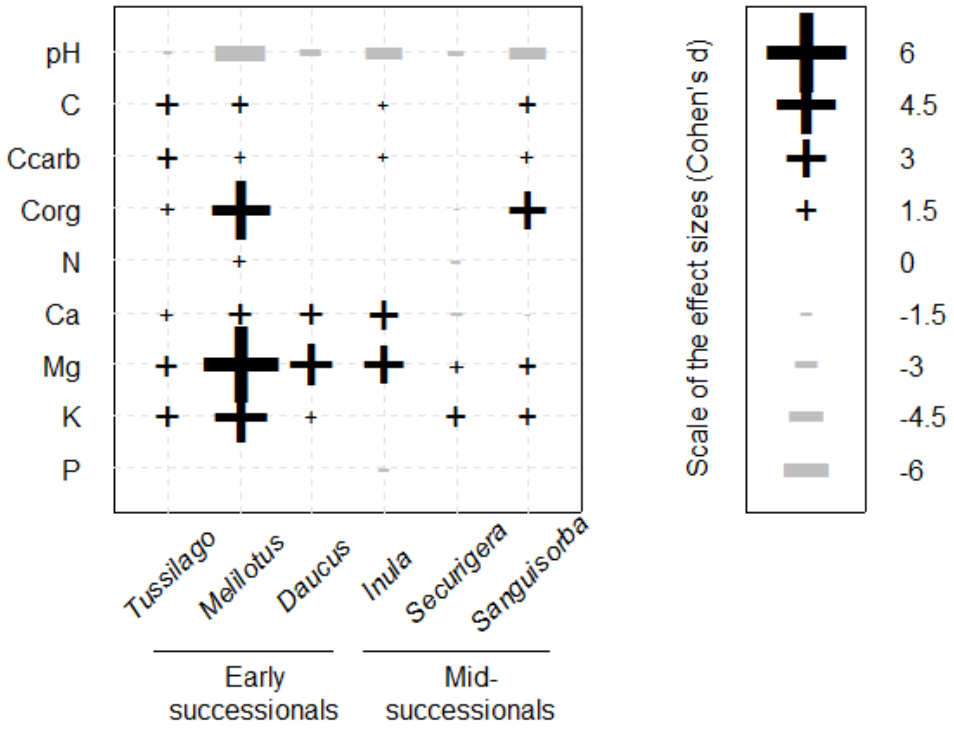

Fig. 1: Changes in soil properties induced by plant conditioning. Symbols represent increase or decrease of each variable in the conditioned relative to the control soil. Only differences with $p<0.1$ are shown. The symbol sizes correspond to the effect sizes (based on Cohen's d). C = total C content, Ccarb = C fixed in carbonates, Corg = organic C. Number of analyzed soil samples was 10 per each conditioning type.

Plant effects on fungal community composition

We obtained 230 957 fungal sequences which clustered into 4 537 OTUs including 2 248 singletons. Fungal communities in soils after plant cultivation differed significantly from control and among plant species (PERMANOVA $R^2 = 0.45$, $p<0.001$, Fig. S3) as well as between species guilds ($R^2 = 0.16$, $p<0.001$). The differences between the fungal community composition in control soil and plant-inoculated soils significantly covaried with soil chemical properties (Fig. S4). All plant species except *Daucus*, *Tussilago* and *Securigera* significantly altered the composition of the fungal community compared to control soil (Fig. S5); the differences between the composition of fungal communities in control soil and plant-inoculated soils correlated significantly with the plant biomass (Fig. S2). Plant species identity and soil chemical properties together explained 35.1% of variation in fungal community composition. Plant species identity alone explained 9.5% of the variation and was the only variable whose pure effect was significant ($p<0.001$). A substantial fraction of variation was explained by shared effects of plant species identity and soil chemical properties (26.5%), suggesting a strong correlation of the two variables and chemistry alone had no effect (Fig. S6). Across all treatments, Agaricales, Capnodiales, Boletales, Hypocreales, Helotiales, Mortierellales, Pleosporales, Russulales, and Mucorales were the most abundant fungal orders, constituting 3 – 11% (in ascending order) of all sequences (Fig. S7). The most abundant genera, each constituting more than 2% of sequences, were *Mucor*, *Pseudogymnoascus*, *Russula*, *Mortierella*, *Boletus*, *Cladosporium*, *Paraphoma*, *Boeremia*, *Phaeohelotium*, *Trichoderma* and *Tetracladium* (Fig. S7).

The pairwise indicator species analysis revealed, that fungal community was most altered by *Melilotus* and *Sanguisorba* that changed the abundance of 83 and 43 OTUs, respectively, as compared to control soil, and least by *Securigera* (14 OTUs). Given the high number of indicator OTUs, we focused on OTUs belonging to functional groups closely associated with plants, such as plant pathogens and mycorrhizal fungi. From these, early-successional species *Melilotus* and *Tussilago* promoted more phytopathogenic OTUs such as *Didymella*, *Boeremia*, *Paraphoma* or *Ascochyta* than any of the mid-successional species which, conversely, promoted more the arbuscular mycorrhizal *Claroideglomus* and *Funeliformis* species (Fig. 2, Fig. S7). Furthermore, the relative abundance of phytopathogenic genera was significantly higher in the early-successional guild as compared to the mid-successional guild ($p < 0.001$, Fig. 3).

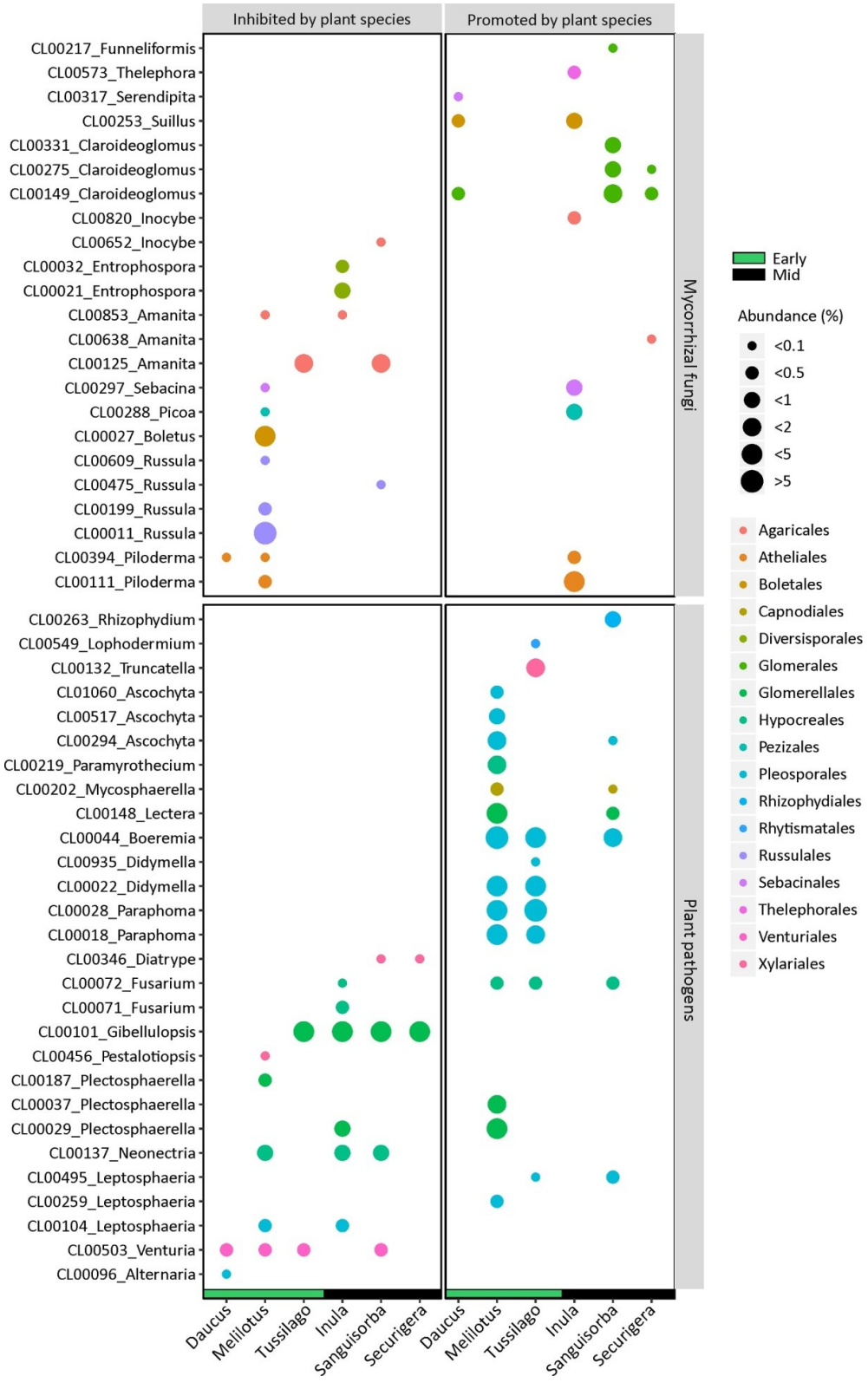

Fig. 2: Fungal indicator species. Selected indicator OTUs inhibited or promoted by different plant species as compared to control soil. Each point represents an OTU identified by indicator species analysis, colours mark fungal orders, size is proportional to the abundance of a given OTU in a given treatment (control / plant).

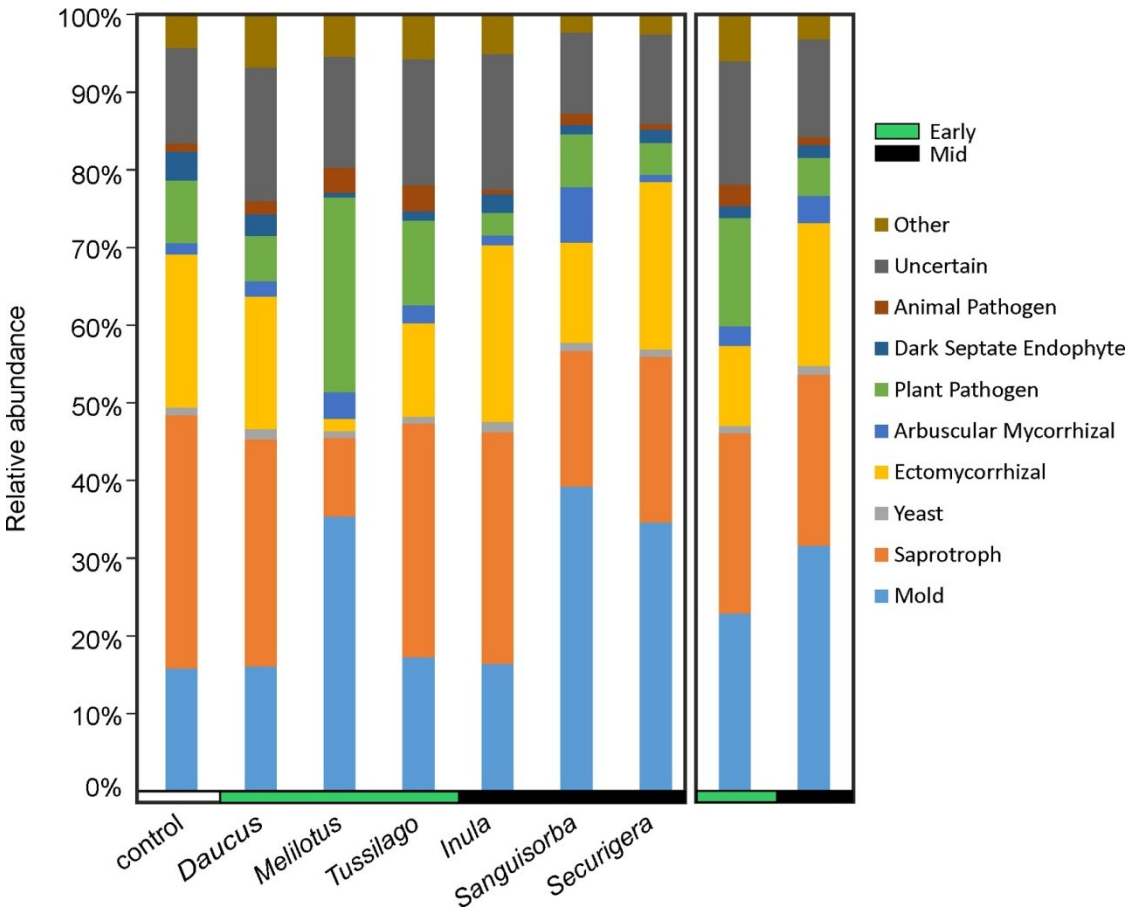

Fig. 3: Trophic guilds of soil fungal communities in soil conditioned by early (*Daucus*, *Melilotus*, *Tussilago*) and mid-successional (*Inula*, *Sanguisorba*, *Securigera*) species.

Plant effects on bacterial community composition

Sequencing yielded 368 145 bacterial sequences which clustered into 14 214 OTUs including 7 977 singletons. Soil bacterial communities differed between control and conditioned soil and among plant species (PERMANOVA, $R^2 = 0.51$, $p < 0.001$, Fig. S3), but only marginally between plant species guilds ($R^2 = 0.07$, $p = 0.055$). Unlike fungi, the differences between the bacterial community composition in control soil and plant-inoculated soils did not correlate with soil chemical properties (Fig. S4), or with the plant biomass (Fig. S2). However, plant species identity and soil chemical properties together explained more than 45% of variation in bacterial community composition. Plant species identity alone explained 14.6% ($p<0.001$) and further almost 28% were explained by combined effects of plant identity and chemistry. Soil chemical properties alone explained 3.2% of the variation, however, its effect was only marginally significant

($p<0.07$; Fig. S6). *Inula* and *Melilotus* had the strongest effect on bacterial community composition (Fig. S5), with *Inula* being the most distinct (Fig. S3). Bacterial communities were dominated by Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Acidobacteria, Verrucomicrobia, Planctomycetes and Chloroflexi, each representing more than 1.5% of all sequences in most treatments (Fig. S7).

According to a pairwise indicator species analysis for each plant species and control, *Inula* and *Melilotus* were the most influential plant species with respect to the number of bacterial OTUs that were inhibited or promoted by their cultivation (157 and 95 OTUs, respectively), *Securigera* was the least influential (8 OTUs). Indicator species profiles varied between individual plants, however, some of the more generally promoted OTUs belonged to diazotrophic genera such as *Rhizobium* and *Novosphingobium*, OTUs with antifungal activites such as *Duganella*, *Chondromyces* and *Chitinophaga*, and plant growth promoters such a *Flavobacterium* or *Arachidicoccus* (Fig. 4). The potential plant polymer degrading OTUs seemed to be inhibited by most plant species except for *Inula* which promoted them.

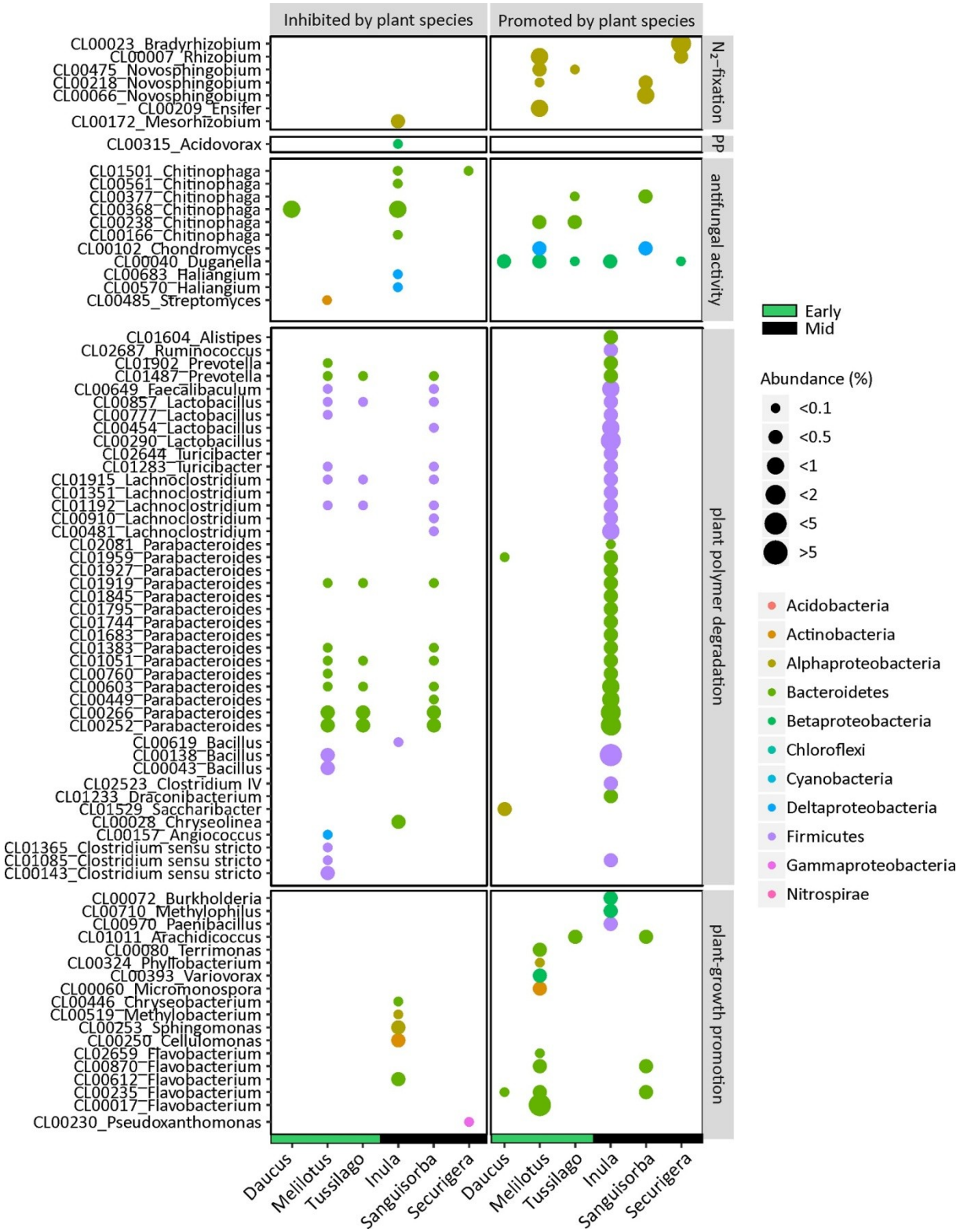

Fig. 4: Bacterial indicator species. Selected indicator OTUs inhibited or promoted by different plant species as compared to control soil. Each point represents an OTU identified by indicator species analysis, colours mark bacterial phyla / classes, size is proportional to the abundance of a given OTU in a given treatment (control / plant). PP – plant pathogen.

Plant responses to soil preconditioning

Plant-soil feedback indices for all species guilds were generally neutral to significantly positive (Fig. 5), illustrating better plant performance in conditioned than control soil. Both early-successionals and grassland plant species produced more biomass in the soil conditioned by the early-succesional plants than in the soil conditioned by the mid-successional species, but none of the two guilds singficantly responded to the soil conditioning in terms of seedling establishment (Table S2, Fig. 5). Neither seedling establishment nor biomass of mid-successionals and expansive species differed between the soils (Table S2).

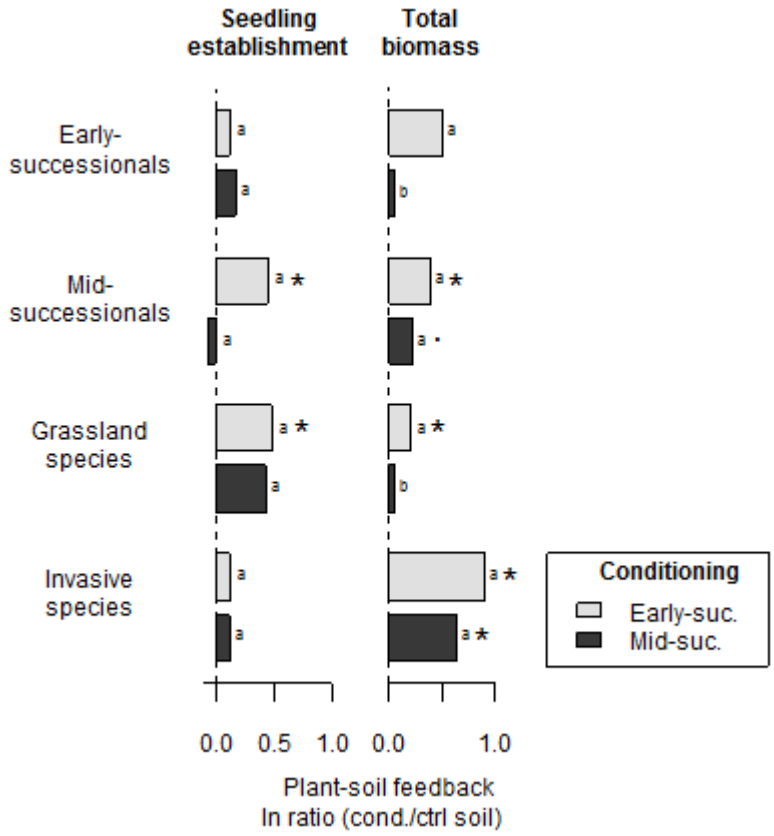

Fig. 5: PSF of individual successional guilds in soil conditioned by early-successionals (light bars) and mid- successionals (dark bars). PSF is calculated for the seedling establishment and the total biomass. Positive values indicate better plant performance in conditioned soil, negative values indicate better performance in control (unconditioned) soil. Asterisks (*) next to bars indicate significant differences from zero (t-test, $p<0.05$). Different letters indicate significant differences among the two soil treatments within each species guild (Tukey's HSD).

Five of the twelve tested species singificantly responded to the conditioning species. Specifically, we found significant effect of the conditioning species on both seedling establishment and total biomass of *Tussilago* (early-successional), *Inula* (mid-successional) and *Conyza* (expansive); and on the total biomass of *Centaurea* (grassland) and *Salvia* (grassland) (Table S2; Fig. 6).

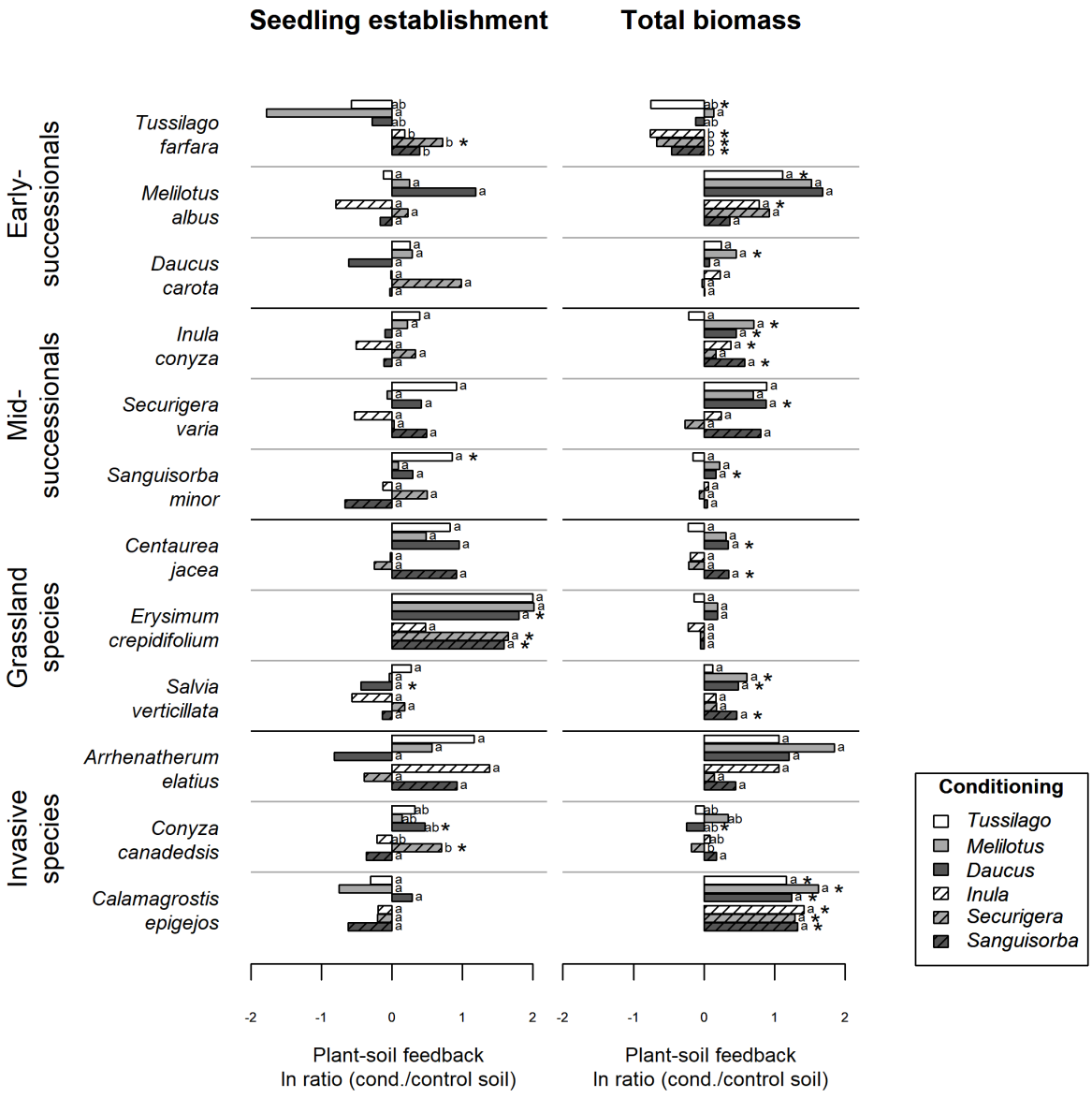

Fig. 6: Plant-soil feedback of all 12 species grown in the six conditioning treatments, calculated for both seedling establishment and total biomass. The bars represent mean ln ratios. Positive values indicate better plant performance in conditioned soil, negative values indicate better performance in control (unplanted) soil. Asterisks (*) next to bars represent significant differences from zero (t-test, $p<0.05$). Different letters indicate differences among individual treatments within one species (Tukey's HSD).

Among the species guilds, we found only few marginally significant effects of the individual components of PSF (conditioning plant biomass, changes in soil chemical properties, bacterial and fungal communities) on the performance of the plants in the feedback phase ($p<0.1$; Table S3). Specifically, the biomass of the conditioning plants influenced the biomass of the mid-successional ($p=0.095$) and the grassland species ($p=0.091$) in the feedback phase (tested as PSF indices). Grassland species also marginally significantly responded to the changes in soil chemical properties ($p=0.095$). Among the individual species, the biomass of the conditioning plants was the most frequent significant predictor of either seedling establishment or plant biomass (six cases out of 24; Table S3). Either seedling establishment or plant biomass were in six cases out of 24 influenced by changes in soil chemical properties (Table S3). Seedling establishment of two early-successional species, *Tussilago* and *Melilotus*, was significantly influenced by changes in the composition of fungal community, *Tussilago* also by the changes in bacterial communities (Table S3).

Variation partitioning for the species guilds showed that only small fractions (adjusted $R^2 < 0.01$) of variation can be linked to the pure effect of individual explanatory factors. Most variation in all the guilds was linked to the biomass of conditioning plants and changes in soil chemical properties, either through individual or shared effects with the other factors. In contrast to the low levels of explained variation for the species guilds, the variation explained by the four factors was 10 times higher in case of individual species (adjusted R^2 often > 0.1), showing that plant response patterns are rather individual than shared within the species guild.

Discussion

Plant effects on soil abiotic conditions

Plant conditioning induced changes in soil pH and nutrient content. Significant changes in soil chemical properties are usually associated with successional ecosystems (Chapin et al. 1994; Tscherko 2003; Zhou et al. 2018) and can be related to the accumulation of organic matter and increased abundance and activity of soil biota (Tscherko 2003; Waring et al. 2015). In this study, all plant species significantly decreased soil exchangeable pH, relative to the unplanted soil. The acidification of soils induced by plants is a well

described phenomenon (Hinsinger et al. 2003) and results from release of protons in case of prevailing NH_4^+ uptake by plants (Bolan et al. 1991), depletion of cations from the soil exchange complex (Kelly et al. 1998; Ehrenfeld et al. 2005), or from increased amounts of plant-derived carbonic or organic acids in the soil (Hinsinger et al. 2003). Moreover, it has been shown that plants can release exudates that supress the process of nitrification in the soil, keeping nitrogen in the form of NH_4^+ (reviewed in Subbarao et al. 2015). The ammonium cation is electrostatically held by clay surfaces and soil organic matter (unlike NO_3^-) where it remains available for plants and microbes and as such is a source of soil acidification. The plant-induced acidification may enhance the rate of weathering, leading to release of various nutrients from the soil (Hinsinger et al. 2001). In line with this, we observed increase of Mg, K, and partly Ca cation content in all conditioned soils, compared to control, likely as a result of such plant-induced or plant-associated microbe-induced mineral weathering (Hinsinger et al. 2001; Remiszewski et al. 2016).

The content of organic C increased most during soil conditioning by *Melilotus* and *Sanguisorba*. These two plant species produced the highest biomass among all the conditioning species which probably resulted in the highest production of rhizodeposits and root litter and thus the highest input of organic components into the soil. The content of total soil N increased in the soil conditioned by the legume *Melilotus*, known to support symbiotic N-fixing bacteria. Contrary to our expectations, *Securigera* (also a legume species with a potential of symbiosis with N-fixers) did not increase N content in the soil compared to the control. Augusto et al. (2013) has shown that N_2-fixation is positively corelated with plant biomass and indeed, *Securigera* in our experiment produced ca. 30× less biomass than *Melilotus*. This difference in biomass production might imply higher amount of root litter, i.e. higher amounts of N, in *Melilotus*-conditioned soil, compared to *Securigera*-conditioned soil.

Plant effects on soil microbial community composition

Both fungal and bacterial communities in the soils conditioned by the different plant species differed from the control soil and among each other. The plant species identity was stronger driver of both fungal and bacterial community composition than soil chemical properties alone. The extent to which soil chemical properties and fungal community composition were changed by plants as compared to the control soil was significantly correlated with plant biomass, as well as with each other. Changes in

bacterial community composition, on the other hand, were independent of plant biomass and did not covary with the changes in soil chemical properties. These results suggest that fungi are more affected by plant-induced changes to the soil properties than bacteria. This is in agreement with previous study of Harantová et al. (2017) and might be especially pronounced for functional groups tightly linked to plants such as plant mutualists and phytopathogens (Leff et al. 2018). Accordingly, the composition of the fungal communities significantly differed between plant successional guilds. Compared to the mid-successional species, soils conditioned by the early-successional species had significantly higher relative abundance of plant pathogens. This is in agreement with previous studies showing that early-successional species are more susceptible to soil-born pathogens than late-successional species due to their poor defense mechanisms and little or no mutualist interactions with mycorrhizal fungi (van der Putten et al. 1993; Kardol et al. 2006). Mid- and late-successional species, on the other hand, produce more defensive secondary metabolites (Rasmann et al. 2011) and invest more resources into mutualist relationships (Koziol and Bever 2015). Indeed, indicator species analysis showed that two of three mid-successional species in this study specifically upregulated the arbuscular mycorrhizal genera *Claroideglomus* and *Funeliformis*. In contrast to fungal communities, we were not able to detect any association between successional guild and bacterial community composition. However, although the relationships between plants and bacteria were less evident than the plant-fungal interactions, we were able to see that plant growth stimulated OTUs with potential antifungal activity such as *Duganella* (Haack et al. 2016), *Chondromyces* (Jansen et al. 1999), and *Chitinophaga* (Mohr et al. 2015), N_2-fixers such as *Rhizobium*, *Novosphingobium* and *Ensifer* (Wasai and Minamisawa 2018) and various plant-growth promoting bacteria, e.g. *Flavobacterium*, *Paenibacillus* or *Bulkhorderia* (Rodríguez and Fraga 1999; Glick 2012). These taxa might improve plant performance by defending them against fungal pathogens, supplying them with nutrients or producing plant growth promoting hormones.

Plant responses to feedbacks and its individual components

We found generally facilitative feedback effects of soils conditioned by early-successionals. This supports the hypothesis that plant-soil feedbacks are one of the drivers of succession since the early colonizers facilitate success of later arriving species. Previous studies dealing with secondary succession showed that late successional species can either benefit (Herzberger et al. 2015) or be supressed (Kardol et al. 2006) by

microbes from early successional sites. In our study, we assume that the positive feedback effect was mediated mostly by changes in abiotic conditions, since (i) changes in soil chemical properties as well as biomass of conditioning plants were the most frequent significant predictors of plant performance, and (ii) the soils conditioned by early successionals (that generally promoted plant growth) were characterized by higher pathogen abundance that would otherwise cause negative microbe-mediated feedback.

In contrast to the facilitative effects of early successionals, the soils conditioned by mid-successional species induced neutral feedback effects, possibly due to high differences in individual species responses. However, similarly to soils conditioned by the early successional species, soils conditioned by the mid-successional species caused strong facilitation of the expansive species, especially of *Arrhenatherum* and *Calamagrostis*. These grasses largely profit from increased fertility of the soils (Xia and Wan 2004) and the increased organic component in the conditioned soils, as well as higher availability of nutrients, could thus improve their growth (Frouz et al. 2016).

In contrast to other studies, which reported negative plant-soil feedbacks for early successionals (Kardol et al. 2006; Jing et al. 2015), in our study, these species exhibited neutral (non-significantly positive) feedbacks in soils conditioned by both early and mid-successional species. This could be explained by the fact that the soil at the locality is rather unfavourable (it lacks organic matter almost completely). Thus, the negative feedbacks, possibly driven by accumulation of pathogenic fungi, could be out-weighed by improved abiotic conditions. It is, however, neccessary to point out that two of the three early successional species in our study (*Melilotus* and *Tussilago*) were also significantly influenced by the composition of soil fungal communities. Despite these general patterns, there were differences in plant-soil feedback responses between the individual species. *Tussilago*, species typical for newly abandoned or disturbed habitats, was the only species that grew better in control soil. Other species, such as *Melilotus*, *Inula*, *Securigera*, and *Calamagrostis*, produced generally more biomass in previously conditioned soils. Some species exhibited neutral feedbacks in all soils (e.g., *Daucus*, *Sanguisorba*, *Erysimum*, and *Centaurea*). This illustrates that although positive or negative plant-soil feedbacks can be typical for a certain successional guild, the differences between individual species can still be large. Probably, each species is sensitive to a different component of plant-soil feedback and thus promotes specific shifts in soil conditions and responds more likely to certain soil features. However, the species from individual successional stages still share

some traits (such as adaptations to anemochory or competitive ability) that merge them into one guild during the vegetation development. Plant-soil feedback can thus generally contribute to species replacements, though it can be further altered by other factors, such as plant-plant competition (Lekberg et al. 2018), herbivory (Heinze and Joshi 2018), the level of abiotic stress (Florianová and Münzbergová 2018; Fry et al. 2018) or the complexity of soil food webs (Kuťáková et al. 2018b), none of which was manipulated in our study. Interestingly, feedback response of all the species from *Asteraceae* family in our study depended on the previous soil conditioning. The role of plant phylogeny in plant-soil feedbacks has been recently discussed (Mehrabi and Tuck 2014; Anacker et al. 2014; Münzbergová and Šurinová 2015; Kuťáková et al. 2018a) and our result indicates that some plant families could be more sensitive to changes in biotic or abiotic conditions of the soil than other.

Soil cultivated by *Melilotus* had consistently neutral to facilitative effect on growth of all the species. Surprisingly, soil conditioned by this species was characterized by the highest abundance of pathogenic fungi among all the conditioned soils. On the other hand, it was also linked with largest changes in abiotic conditions (decreased pH and increased content of cations, organic C and N), that could out-weight the negative effects of pathogens, as we suggested earlier. In contrast, soil conditioned by *Melilotus* had neutral to negative effects on seedling establishment. This could mean that the pathogens are more detrimental to seedlings than to older plants. Besides this, species of *Melilotus* genus are known to produce phytotoxic allelopathics, such as coumarin, that can significantly supress seed germination (Blackshaw et al. 2001; Esposito et al. 2008). The allelopathics may also lower the final plant biomass (Blackshaw et al. 2001) but their effect on plants in our experiment could be out-weighed by the enhanced nutrient content in the soil. Overall, these results, together with results from variation partitioning among the individual components of feedback, suggest that for plant germination and seedling establishment, the soil biotic conditions (and production of allelopathics) could be more important than the variations in pH and nutrient content. In contrast, final plant biomass seems to be more dependent on the changes in soil abiotic conditions.

Conclusions

The results indicate that plant-soil feedback is a complex phenomenon that includes the changes in soil nutrient content and microbial community composition. It may be an important driver of primary succession: the successional guilds were generally facilitated by guilds that precede them in the field. The most influential components of plant-soil feedback were the changes in soil chemical properties, and the biomass of conditioning plants. This illustrates the crucial importance of litter in plant-soil feedbacks during primary succession. The guild of expansive species especially benefitted from previous conditioning by any plant, probably due to the increased content of organic components in the soil. Soil fungi had impact on plant seedling establishment but not on plant final biomass, suggesting that biotic feedbacks might be more important in the initial stages of plant life.

Despite these general patterns among the species guilds, a lot of variation in both plant-induced changes in soil conditions and plant responses to the conditioned soils, was species-specific. This suggests that plants might be grouped into the individual successional stages by different traits, such as competitive ability, or that there might be another important component of plant-soil feedback that we did not measure (e.g. plant production of allelopatics).

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Supporting information

Supporting information may be found in this article:

Table S1: Correlation matrix between the individual soil chemical properties.

Table S2: Plant-soil feedback for seedling establishment and total biomass as influenced by soil conditioning treatment and species guild. Results from ANOVA.

Table S3. Effects of conditioning plant biomass, changes in soil chemical properties / composition of bacterial and fungal communities on plant performance in feedbacks.

Fig. S1: Scheme of the experimental design.

Fig. S2: The relationship between the soil chemical properties / composition of microbial communities and plant biomass.

Fig. S3: Principal component analysis of the composition of microbial communities in soils of different plant species. The vectors of environmental variables are shown when significant according to a permutational test.

Fig. S4: The soil chemical properties and the composition of microbial communities.

Fig. S5: Dissimilarity among and within treatments. The effect of different plants on soil microbial communities and soil chemical properties.

Fig. S6: Variation partitioning of the factors affecting the composition of microbial communities. The numbers indicate the share of variation explained by each factor out of the total variation.

Fig. S7: Taxonomic composition of bacterial and fungal communities in soils cultivated by different plant species. The plots show relative sequence abundances of each taxon.

Table S1: Pearson's correlation coefficients for soil chemical properties in conditioned soil. Cond. biomass = aboveground biomass of conditioning plants. Bold values indicate significant relationships ($p<0.05$), values in bold italic marginal significance ($p<0.1$).

	act. pH	ex. pH	N	C (total)	C (org.)	C (carb.)	Ca	Mg	K	P	Cond. biomass
act. pH	1	**0.838**	**-0.537**	-0.118	**-0.789**	0.030	-0.044	**-0.462**	**-0.672**	**-0.548**	**-0.797**
ex. pH		1	**-0.360**	-0.134	**-0.732**	0.002	***-0.226***	**-0.674**	**-0.649**	**-0.412**	**-0.804**
N			1	0.119	**0.560**	0.016	0.158	**0.263**	**0.399**	**0.388**	**0.454**
C (total)				1	**0.305**	**0.983**	-0.011	0.056	**0.227**	0.003	**0.232**
C (organic)					1	0.125	0.062	**0.451**	**0.721**	**0.517**	**0.745**
C (carbonates)						1	-0.024	-0.029	0.097	-0.096	0.098
Ca							1	**0.605**	**0.333**	-0.104	0.113
Mg								1	**0.591**	0.179	**0.573**
K									1	**0.402**	**0.601**
P										1	**0.461**
Cond. biomass											1

Table S2: Plant-soil feedback for seedling establishment and total biomass as influenced by soil conditioning treatment. Results from ANOVA (differences among log-response ratios) for species guilds and for each species separately. Bold values indicate significant relationships ($p<0.05$), values in bold italic marginal significance ($p<0.1$).

		Seedling establishment			Total biomass		
		d.f.	F	p	d.f.	F	p
Early-successionals		1;172	1.12	0.291	**1;135**	**6.89**	**0.010**
Mid-successionals		1;172	1.66	0.199	1;170	1.51	0.159
Grassland species		1;172	1.90	0.170	**1;172**	**4.40**	**0.037**
Invasive species		1;172	0.00	0.977	1;128	0.32	0.570
Early-successionals	*Tussilago farfara*	**5;54**	**4.05**	**0.003**	**5;51**	**4.97**	**0.001**
	Melilotus albus	5;54	0.58	0.716	5;26	0.41	0.838
	Daucus carota	5;54	1.04	0.407	5;48	0.76	0.582
Mid-successionals	*Inula conyza*	**5;54**	**2.39**	**0.050**	**5;54**	**5.54**	**0.001**
	Securigera varia	5;54	0.22	0.954	05;52	1.06	0.395
	Sanguisorba minor	5;54	1.95	0.102	5;54	1.78	0.133
	Centaurea jacea	5;54	0.45	0.813	***5;54***	***2.32***	***0.055***
Grassland sp.	*Erysimum crepidifolium*	5;54	0.42	0.834	5;54	0.82	0.543
	Salvia verticillata	5;54	0.86	0.515	**5;54**	**4.10**	**0.003**
	Arrhenatherum elatius	5;54	1.12	0.363	5;10	0.57	0.723
Invasive sp.	*Calamagrostis epigejos*	5;54	0.42	0.835	5;54	0.22	0.951
	Conyza canadensis	**5;54**	**2.70**	**0.030**	***5;54***	***2.34***	***0.054***

Table S3. Effects of conditioning plant biomass (biom), changes in soil chemical properties (abio12), bacterial (bact12) and fungal (fung12) community composition on PSF indices of seedling establishment and total biomass in feedback phase. Significant effects ($p<0.05$) are in bold and marginally significant effects ($p<0.1$) in italic bold.

	Seedling establishment					**Total biomass**				
	factor	df	F	p	R2	factor	df	F	p	R2
Early-successional	biom	1;88	1.55	0.217	0.017	biom	1;68	0.52	0.474	0.008
	abio12	2;87	0.28	0.757	0.006	abio12	2;67	0.77	0.467	0.022
	bact12	2;87	0.29	0.746	0.007	bact12	2;67	1.03	0.362	0.030
	fung12	2;87	0.10	0.908	0.002	fung12	2;67	0.09	0.915	0.003
Mid-successional	biom	1;88	0.05	0.826	0.001	***biom***	***1;87***	***2.85***	***0.095***	***0.032***
	abio12	2;87	0.13	0.877	0.003	abio12	2;86	1.60	0.207	0.036
	bact12	2;87	1.85	0.163	0.041	bact12	2;86	0.57	0.569	0.013
	fung12	2;87	1.37	0.261	0.030	fung12	2;86	0.16	0.849	0.004
Grassland	biom	1;88	0.88	0.352	0.010	***biom***	***1;88***	***2.92***	***0.091***	***0.032***
	abio12	2;87	2.22	0.115	0.048	***abio12***	***2;87***	***2.41***	***0.095***	***0.053***
	bact12	2;87	0.25	0.779	0.006	bact12	2;87	1.06	0.350	0.024
	fung12	2;87	0.60	0.552	0.014	fung12	2;87	1.83	0.167	0.040
Expansive	biom	1;88	1.77	0.187	0.020	biom	1;66	0.04	0.841	0.001
	abio12	2;87	2.11	0.127	0.046	abio12	2;65	1.67	0.196	0.049
	bact12	2;87	0.49	0.616	0.011	bact12	2;65	0.62	0.543	0.019
	fung12	2;87	1.73	0.183	0.038	fung12	2;65	1.04	0.361	0.031
Tussilago	**biom**	**1;28**	**4.99**	**0.034**	**0.151**	biom	1;26	1.15	0.294	0.042
	abio12	**2;27**	**5.11**	**0.013**	**0.275**	***abio12***	***2;25***	***2.58***	***0.096***	***0.171***
	bact12	***2;27***	***2.64***	***0.090***	***0.164***	bact12	2;25	0.01	0.993	0.001
	fung12	**2;27**	**6.98**	**0.004**	**0.341**	fung12	2;25	2.30	0.121	0.156
Melilotus	biom	1;28	0.00	0.991	0.000	biom	1;15	0.08	0.785	0.005
	abio12	2;27	0.32	0.729	0.023	abio12	2;14	0.37	0.698	0.050
	bact12	2;27	1.08	0.353	0.074	bact12	2;14	1.86	0.192	0.210
	fung12	**2;27**	**3.68**	**0.039**	**0.214**	fung12	2;14	0.66	0.532	0.086
Daucus	biom	1;28	0.24	0.625	0.009	biom	1;23	0.05	0.820	0.002
	abio12	2;27	0.17	0.846	0.012	abio12	2;22	0.29	0.749	0.026
	bact12	2;27	0.03	0.975	0.002	bact12	2;22	0.45	0.645	0.039
	fung12	2;27	0.04	0.956	0.003	fung12	2;22	0.78	0.469	0.066
Sanguis orba	**biom**	**1;28**	**5.86**	**0.022**	**0.173**	**biom**	**1;28**	**7.54**	**0.010**	**0.212**
	abio12	2;27	0.92	0.411	0.064	abio12	2;27	2.14	0.138	0.137

	bact12	2;27	0.75	0.483	0.053	bact12	2;27	0.73	0.489	0.052
	fung12	2;27	0.14	0.873	0.010	fung12	2;27	0.73	0.490	0.052
Securigera	biom	1;28	0.08	0.781	0.003	biom	1;27	0.14	0.714	0.005
	abio12	2;27	0.11	0.899	0.008	**abio12**	**2;26**	**0.30**	**0.023**	**0.741**
	bact12	2;27	0.99	0.386	0.068	bact12	2;26	0.31	0.738	0.023
	fung12	2;27	1.41	0.262	0.095	fung12	2;26	0.23	0.798	0.017
Inula	biom	1;28	0.12	0.728	0.004	**biom**	**1;28**	**9.63**	**0.004**	**0.256**
	abio12	2;27	0.89	0.424	0.062	**abio12**	**2;27**	**4.26**	**0.025**	**0.240**
	bact12	2;27	2.06	0.148	0.132	bact12	2;27	0.98	0.389	0.068
	fung12	2;27	1.21	0.313	0.083	fung12	2;27	1.62	0.217	0.107
Erysimum	biom	1;28	0.39	0.539	0.014	biom	1;28	0.03	0.872	0.001
	abio12	2;27	1.76	0.191	0.115	abio12	2;27	1.68	0.205	0.111
	bact12	2;27	0.18	0.836	0.013	bact12	2;27	0.24	0.787	0.018
	fung12	2;27	0.59	0.563	0.042	fung12	2;27	0.24	0.789	0.017
Centaurea	biom	1;28	0.68	0.417	0.024	biom	1;28	1.50	0.231	0.051
	abio12	2;27	0.36	0.698	0.026	abio12	2;27	0.43	0.652	0.031
	bact12	2;27	0.04	0.965	0.003	bact12	2;27	0.23	0.799	0.017
	fung12	2;27	0.00	0.998	0.000	fung12	2;27	0.47	0.629	0.034
Salvia	biom	1;28	0.04	0.849	0.001	***biom***	***1;28***	***3.61***	***0.068***	***0.114***
	abio12	2;27	1.17	0.327	0.079	***abio12***	***2;27***	***2.77***	***0.081***	***0.170***
	bact12	2;27	1.96	0.160	0.127	bact12	2;27	1.91	0.168	0.124
	fung12	2;27	1.62	0.216	0.107	fung12	2;27	2.45	0.105	0.154
Conyza	**biom**	**1;28**	**5.35**	**0.028**	**0.160**	biom	1;28	0.12	0.736	0.004
	abio12	***2;27***	***3.06***	***0.063***	***0.185***	abio12	2;27	0.83	0.448	0.058
	bact12	2;27	0.67	0.519	0.047	bact12	2;27	0.37	0.693	0.027
	fung12	2;27	1.24	0.307	0.084	fung12	2;27	0.23	0.793	0.017
Arrhenatherum	biom	1;28	0.00	0.971	0.000	biom	1;6	3.03	0.133	0.335
	abio12	2;27	0.55	0.581	0.038	abio12	2;5	3.61	0.107	0.591
	bact12	2;27	0.13	0.877	0.010	bact12	2;5	0.95	0.447	0.275
	fung12	2;27	0.81	0.457	0.056	fung12	2;5	0.76	0.515	0.233
Calamagrostis	biom	1;28	1.17	0.288	0.040	biom	1;28	1.30	0.263	0.045
	abio12	2;27	1.58	0.225	0.105	abio12	2;27	1.11	0.344	0.076
	bact12	2;27	1.25	0.302	0.085	bact12	2;27	0.37	0.692	0.027
	fung12	2;27	2.71	0.085	0.167	fung12	2;27	1.16	0.330	0.079

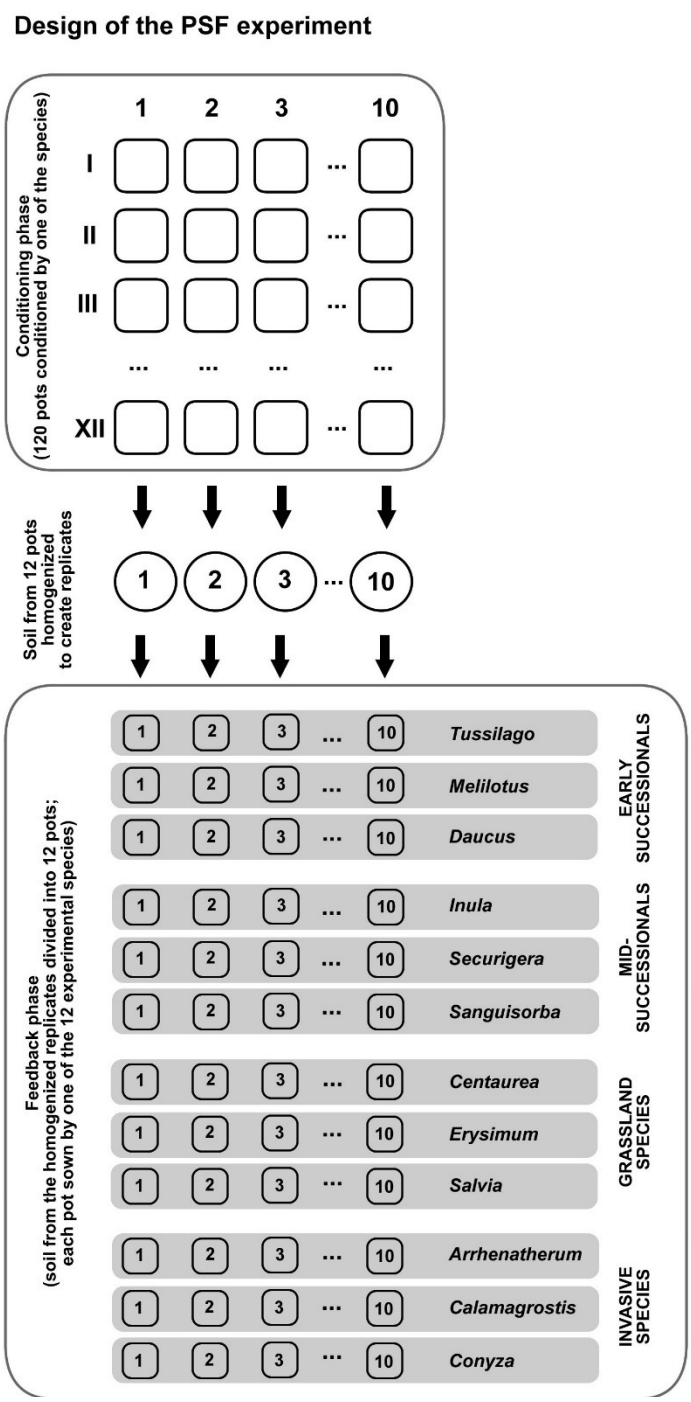

Fig. S1: Scheme of the experimental design. The upper box illustrates pots in the conditioning phase, conditioned by one of the six species. Arrows illustrate mixing of 12 pots to create 10 replicates. The lower box indicates dividing the soil from mixed replicates into 12 pots again. The grey bars show how the pots were sown by each of the 12 experimental species in the feedback phase. This scheme shows just one seventh of the whole experiment, since we used six different species plus an unconditioned control in the conditioning phase.

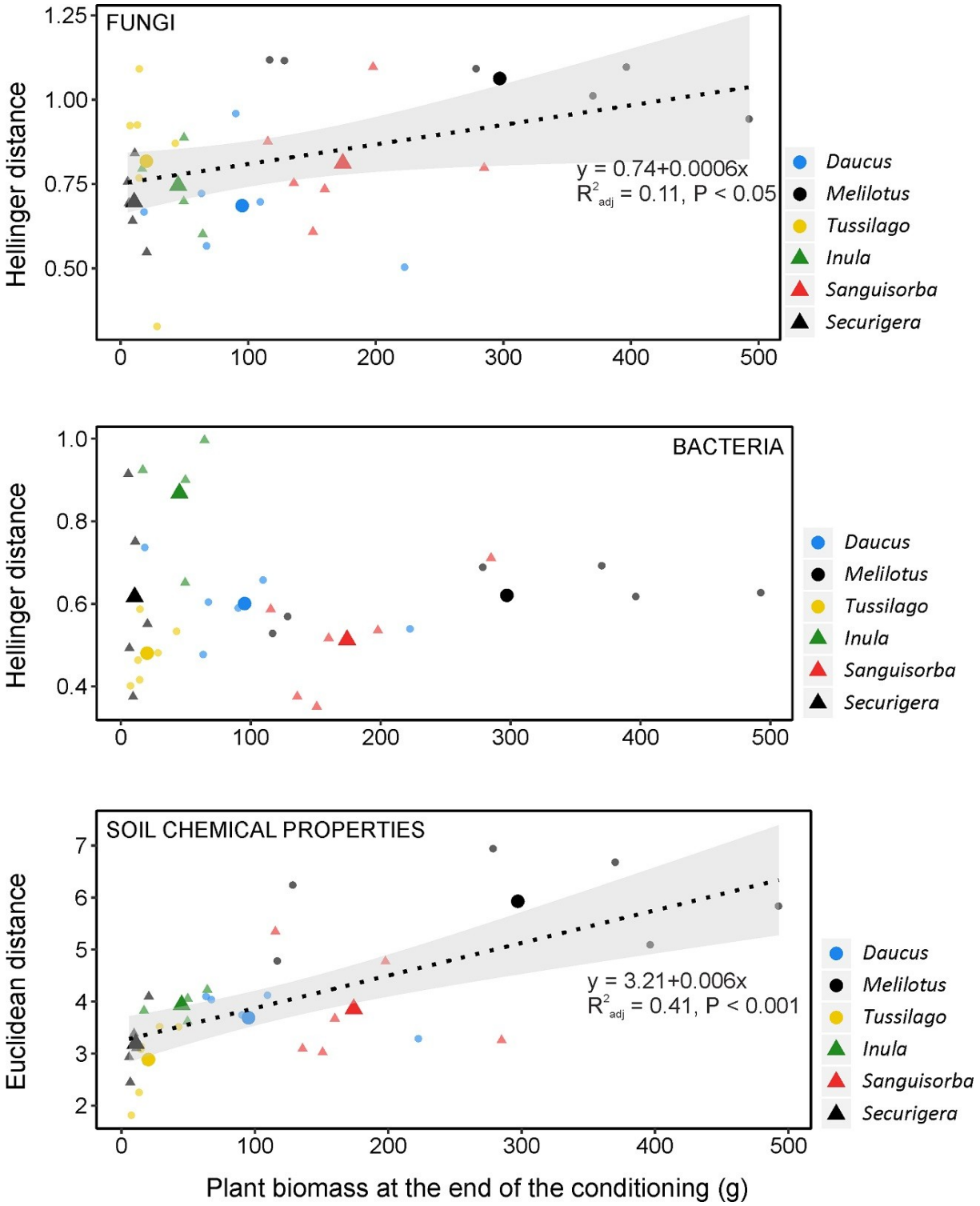

Fig. S2: The relationship between soil chemical properties / microbial communities and plant biomass. The composition of soil chemical properties and microbial communities are expressed as Euclidean or Hellinger distances, respectively, from control soil, large symbols represent centroids. Dashed lines represent fitted linear regression curves, grey bands represent the 95% confidence intervals of the fits, only the significant regression fits shown.

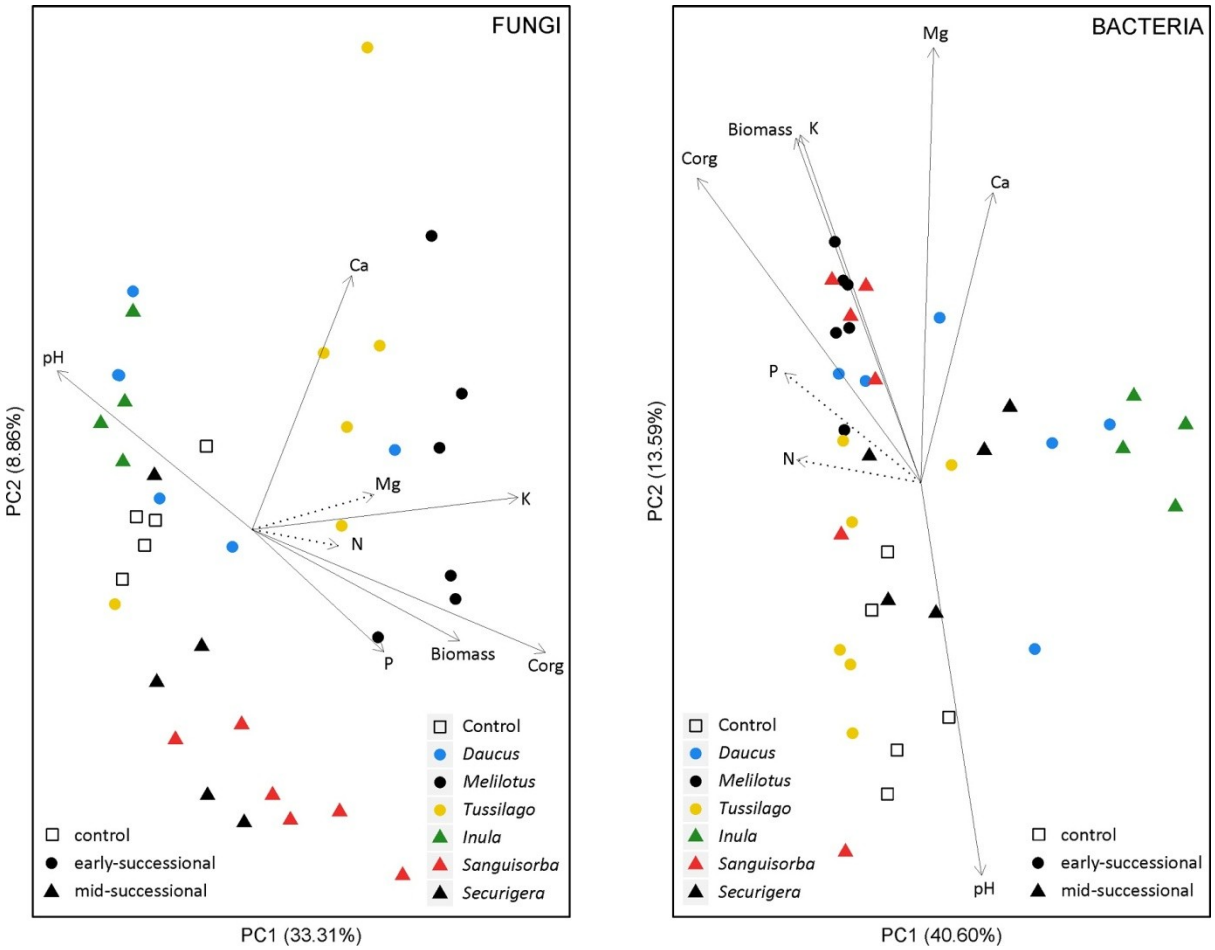

Fig. S3: Microbial communities in soils of different plant species. Principal component analysis calculated on Hellinger-transformed OTU abundances. Vectors represent environmental variables fitted using envfit function (R, ´vegan´) with variables that are significant according to the permutation test ($p < 0.05$) depicted with solid lines, non-significant ones with dotted lines; biomass = conditioning plant aboveground biomass.

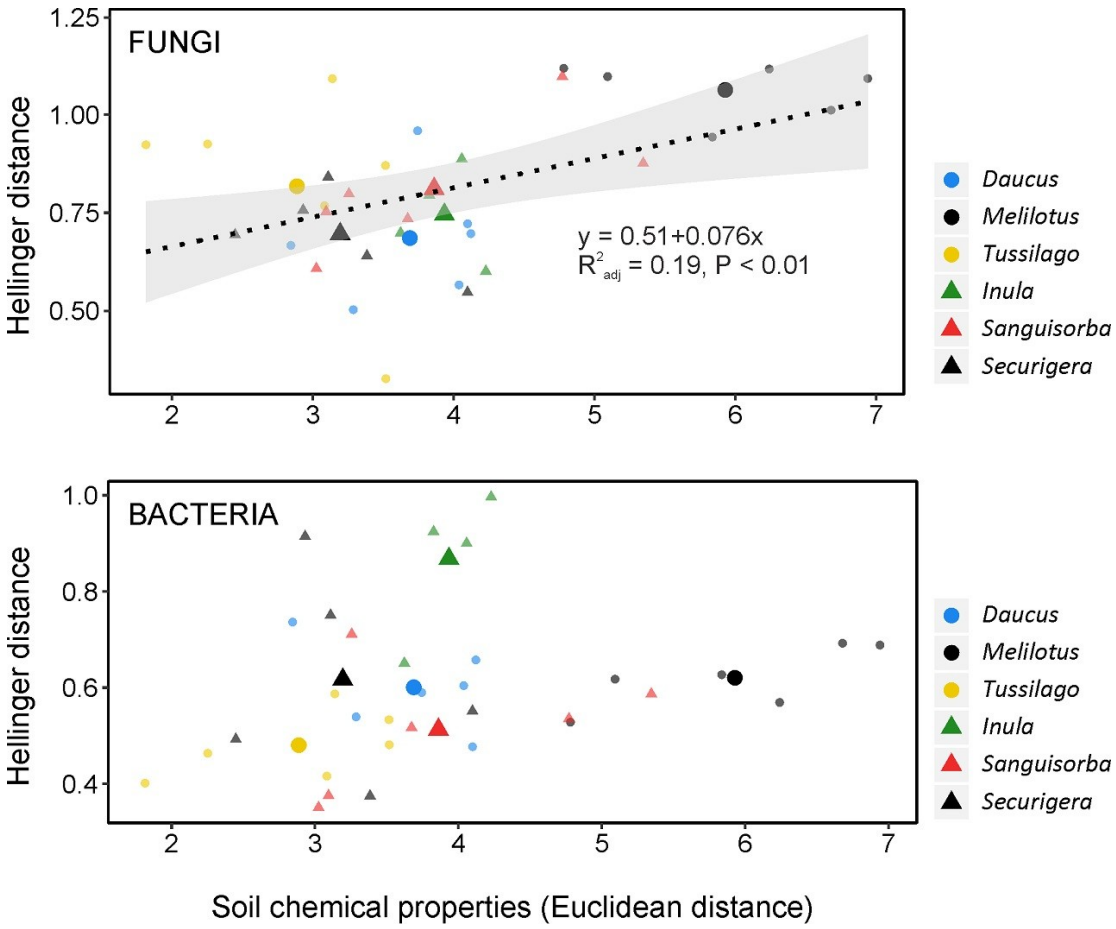

Fig. S4: The soil chemical properties and the composition of microbial communities. The composition of soil chemical properties and microbial communities are expressed as Euclidean or Hellinger distances, respectively, from control soil, large symbols represent centroids. Dashed line represents fitted linear regression curves, grey bands represent the 95% confidence intervals of the fits, only the significant regression fits shown.

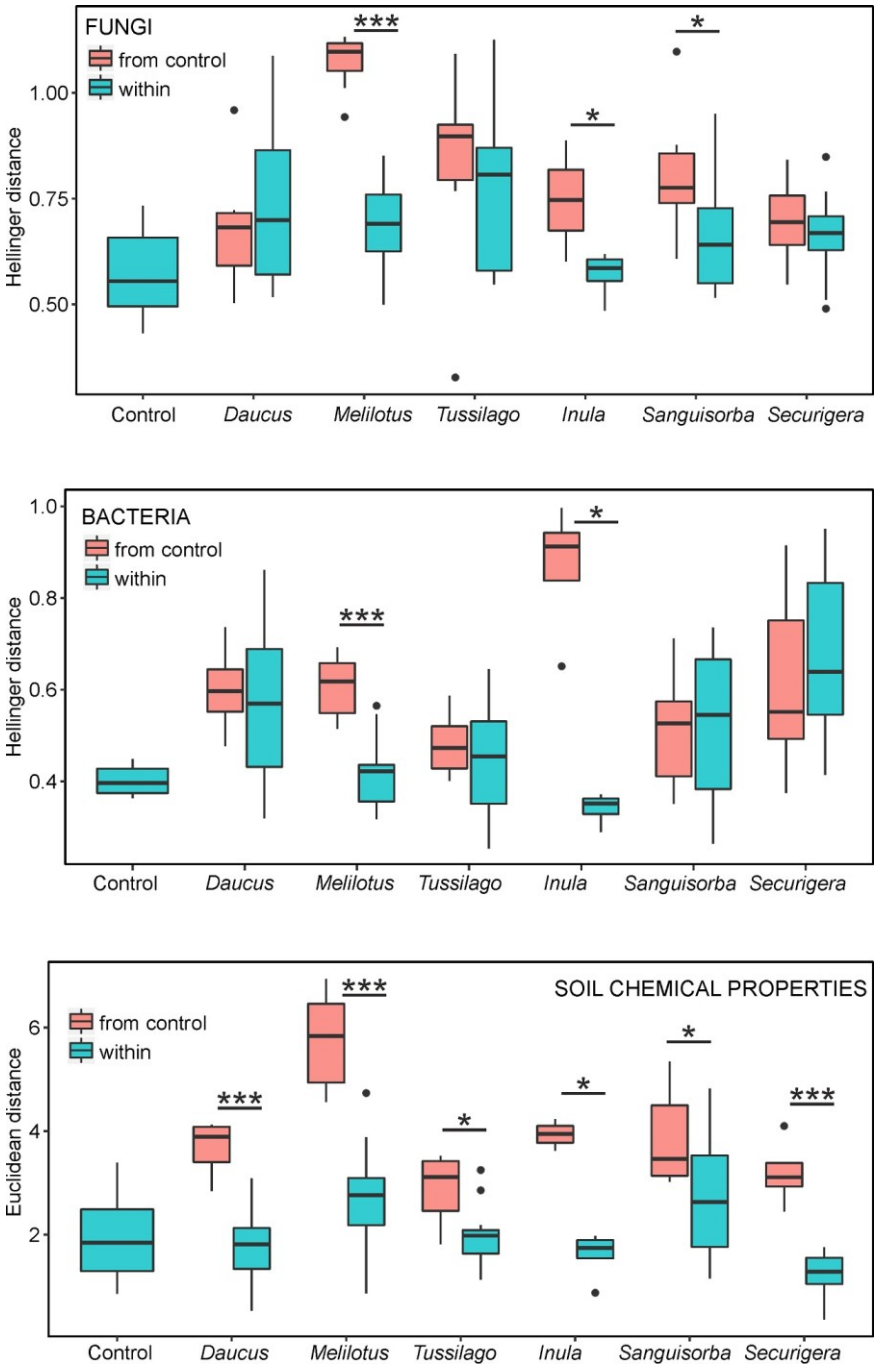

Fig. S5: The effect of different plants on the composition of soil microbial communities and soil chemical properties. Dissimilarity in the composition of soil microbial communities and soil chemical properties within each treatment and between treatments and control. The difference between dissimilarity within sample and between sample and control (from control) represents the strength of influence each plant species exerts on soil microbial community composition or soil chemical properties (i.e. the bigger the difference, the bigger the plant´s influence). The effects are considered significant if the difference from control is larger than among samples of the same treatment. Significant differences as determined by Welch's t-test are marked by asterisks, $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

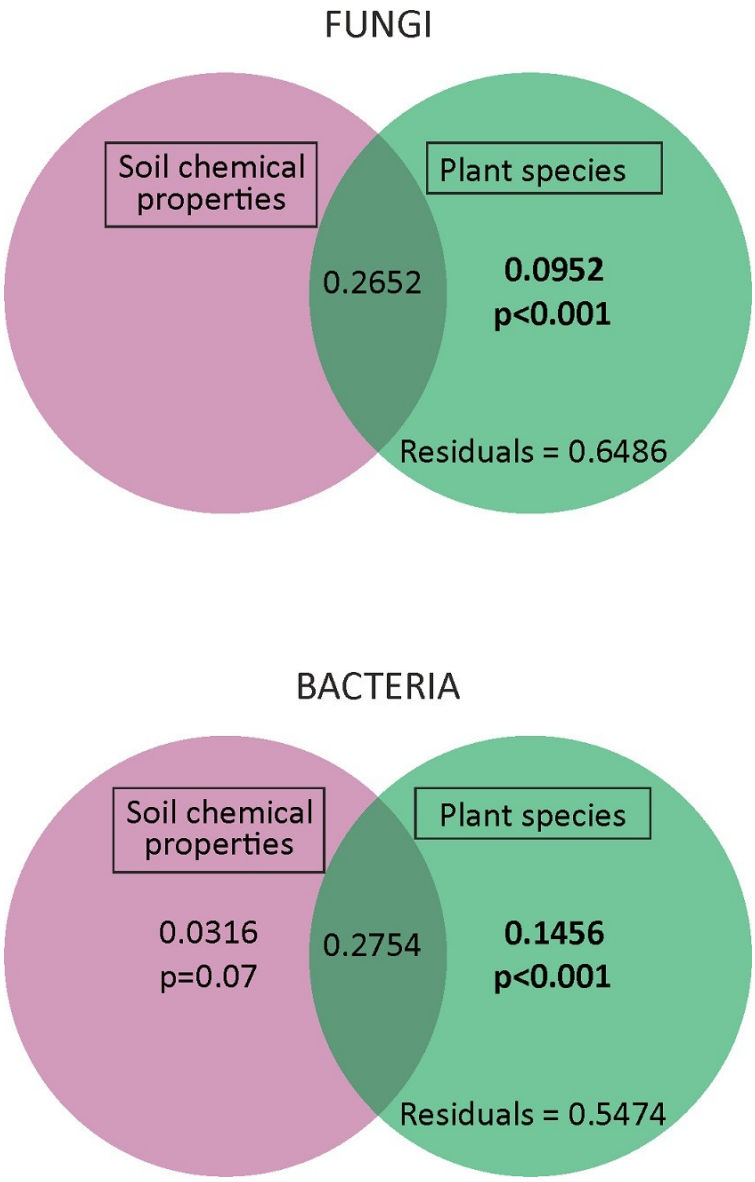

Fig. S6: Partitioning of variation in microbial communities. The diagrams show percentage contributions soil chemical properties, and plant species to variation in soil bacterial and fungal communities. Values<0 are not shown, "residuals" represent the percentage of variation unexplained by the two variables, significant values ($p<0.05$) are in bold.

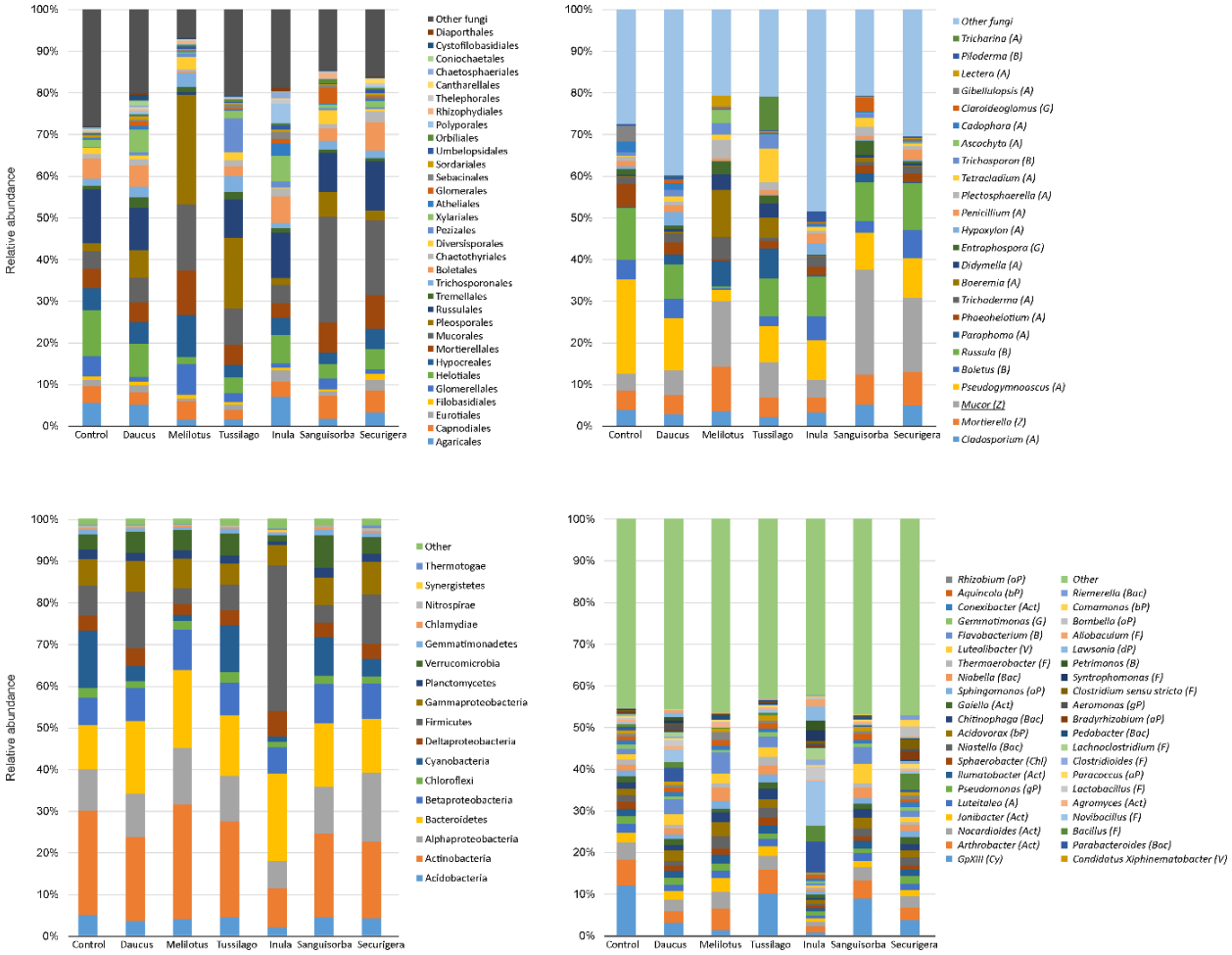

Fig. S7. Taxonomic composition of fungal and bacterial communities -in soils planted with different plant species. The values represent mean relative abundances (n=4-6) of fungal orders and genera (upper left and right, respectively) and bacterial phyla and genera (lower left and right, respectively). A – Ascomycota, B – Basidiomycota, G – Glomeromycota, Z – Zygomycota; Act – Actinobacteria, aP – Alphaproteobacteria, Bac – Bacteriodetes, bP – Betaproteobacteria, Chl – Chloroflexi, Cy – Cyanobacteria, dP – Deltaproteobacteria, F – Firmicutes, V – Verrucomicrobia.

Supplementary Methods:

Differences in the bacterial and fungal community composition among the samples were visualized using principal component analysis (PCA) based on Hellinger-transformed OUT abundances, and the environmental variables (soil chemical properties, plant biomass) were fitted as vectors using the envfit function (R, vegan library). The variation in composition of microbial communities was partitioned between soil chemical properties (variables to be included were preselected using redundancy analysis separately for bacteria and fungi) and plant identity using the varpart function in R (R, vegan library). To quantify the effect of individual treatments (i. e., soil conditionings) on microbial communities, we calculated the heterogeneity within each treatment and between treatment and control soil; the differences were tested for significance using t-test. The differences in relative abundance of fungal functional groups between successional guilds were tested using t-test.

STUDY 3: SOIL REMEMBERS BUT NOT TOO MUCH: PLANT-SOIL FEEDBACK LEGACIES OVER TWO GENERATIONS OF PLANTS

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Manuscript

Abstract

- Plant-soil feedbacks have been shown to play role in vegetation succession and in spread of invasive species. However, their role in species co-existence in more stable and diverse communities is more complex, since plant-induced effects on soil can interact.
- We investigated plant-soil feedbacks between a dominant grass, *Festuca rubra*, and three species from diverse mountain grassland, using 18-year field removal experiment and a classical pot experiment. We tested whether long-term presence/absence of *Festuca* can shape feedback of the grassland community. To understand these results, we assessed interactions of soil legacies induced by subsequently grown plants, both on the level of plant responses and the specific changes in soil properties.
- Under field conditions, the presence/absence of the dominant did not influence feedback of the whole community. In the pot experiment, *Festuca* generated negative feedbacks towards other species but they were influenced by effects of the other species from the community.
- We conclude that though plant-soil feedbacks in species-rich grasslands have potential to influence plant coexistence, they also interact in complex ways, making the predictions of their outcome complicated. Thus, we suggest further research to study soil feedbacks under more natural conditions than the classical experiments offer.

Key words

red fescue, mountain grassland, plant-soil feedback, soil bacteria, soil chemical properties, soil fungi

Introduction

Plant-soil feedbacks, the interactions among plants mediated by changes in soil abiotic and biotic conditions, have been known to contribute to species turnover in early successional communities (e.g., Kardol et al., 2006; van de Voorde et al., 2011) and communities affected by invasion (e.g., Dostálek et al., 2016; Yang et al., 2013). Both of these types of communities are characterized by relatively low species diversity and high species turnover during time. As a result, the importance of plant soil feedbacks in these communities could be larger than the role of feedbacks in more diverse and stable communities, where species are likely to interact in more complex ways. Indeed, several previous studies failed to link plant-soil feedbacks to species abundance in natural communities (e.g., Chiuffo et al., 2015; Reinhart, 2012), suggesting that though the feedback effects can be measured under experimental conditions, they might not play a major role in the field. In developed grassland communities, plant-soil feedbacks can be altered (or even masked) by other factors such as plant-plant competition (Lekberg *et al.*, 2018), herbivory (Heinze & Joshi, 2018), the level of abiotic stress (Florianová & Münzbergová, 2018; Fry *et al.*, 2018) or the complexity of soil food webs (Kuťáková *et al.*, 2018a). All these sources of variability can explain why soil feedbacks detected in pot experiments differ from the ones in the field (Heinze *et al.*, 2016; Forero *et al.*, 2019).

However, there are also studies suggesting that plant-soil feedbacks could play a role in the composition and dynamics of more diverse communities. For example, Klironomos (2002) showed that species with more negative conspecific plant-soil feedback are rarer in grassland communities than species with feedback which is less negative. Kuťáková *et al.* (2018b) demonstrated that heterospecific feedbacks are more positive among species more frequently co-occurring within the same community. There is also evidence that plants replace each other in a non-random order in a species-rich community (Herben *et al.*, 1997) and that some plants exhibit regular population cycles (Herben *et al.*, 2017). All these findings could point to a role of plant-soil feedbacks in grasslands, though the exact mechanisms and importance of these interactions are not known.

Predicting the outcomes of plant-soil feedback in species-rich grasslands where plant-soil feedbacks are likely to occur on a very fine scale and to interact with each other as well as with other factors are indeed very complicated. A possible starting point for investigations of plant-soil feedbacks in such diverse communities is to focus on feedbacks between a dominant species and its subordinates. Dominant species should not exhibit too negative conspecific interactions (such as plant-soil feedbacks but see Hemrová *et al.* (2016)) to maintain its dominant position, while it can exert negative effects on the subordinate species. Due to its high abundance in the community, soil legacies of the dominant species are likely to be detected not only in pot experiments, but also on the level of the whole grassland community in natural conditions, even though other species co-occur in the community. To what extend this expectation is justified, remains to be tested.

In this study, we aimed to investigate the importance of plant-soil feedback in multi-species grassland communities. Specifically, we asked whether (i) plant-soil feedback of a dominant plant species detected under classical experimental conditions is the same as the effect of this

dominant detected in a species rich herbaceous community, and (ii) the specific legacy generated by a plant species persists in the soil or if it is masked by the legacy of subsequently growing plant species. Additionally, we aimed to peak into the black box of soil feedbacks and asked whether (iii) the soil feedbacks change with the depth of soil, depending on the distribution of plant roots, and (iv) the individual components (changes in soil chemical properties and composition of microbial communities) change in their persistency in the soil.

To do that, we took advantage of a long-term research of a mountain grassland in the Czech Republic, where the non-random transitions between species have been observed on a scale of individuals (Herben *et al.*, 1997) together with the regular population cycles of legumes (Herben *et al.*, 2017). The grassland is dominated by a grass, *Festuca rubra* L., that has been shown to exert soil feedbacks towards other species (e.g., van der Putten *et al.* (1993); Petermann *et al.* (2008); Harrison & Bardgett (2010); Kos *et al.* (2015); Wubs & Bezemer (2018)). We performed two plant-soil feedback experiments. In the first one, we used soil originating from permanent plots in the grassland with either presence or 18 year annual removal of the dominant grass *Festuca rubra* (Herben *et al.*, 2003) and investigated the performance of four model species in these two soils. In the second experiment, we studied interactions of two subsequent plant conditionings of soil, changes in soil abiotic and biotic components during the two conditioning phases, and the final responses of *Festuca rubra* to the conditioned soil. We hypothesized that (i) plant-soil feedbacks measured in the field-conditioned soil will be weaker than the feedbacks measured in the pot experiment, due to diluted soil legacies of *Festuca* in the community, (ii) the soil legacies of *Festuca* will be masked by legacies of subsequently grown species, (iii) the legacy of *Festuca* in the field will be stronger in the upper soil layer due to presence of majority of plant roots, (iv) changes in the soil abiotic conditions will be more persistent than changes in the composition of microbial communities.

Methods

To answer the questions proposed, we used two plant-soil feedback experiments. In the first one, we used field soil conditioned by the whole grassland community with presence/absence of the dominant species, *Festuca rubra*. The second experiment was a pot experiment, where the soil was conditioned by individual plants in a growth chamber set to simulate the field conditions. In the Methods section, we describe the two experiments separately for better understandings. Further in Results and Discussion, we combine results of both experiments to answer the research questions.

Study locality and a removal experiment

The study site is semi-natural mountain grassland, located in the Krkonoše Mountains, Czech Republic (50°41′25.165″N, 15°47′41.525″E, and 895 m above sea level). The growing season starts after snow melt in mid-April and ends in November. The grassland is approximately 300-400 years old and is maintained by yearly summer mowing. The vegetation consists mainly of perennial plants (the only common annual is hemiparasitic *Euphrasia rostkoviana*) and is dominated by a grass species, *Festuca rubra*, that forms around 33% of the total aboveground biomass (Herben *et al.*, 2018). Species richness is 32-36 spp. per m^2 (Herben *et al.*, 2018).

In 1993, 14 permanent plots (50×50 cm) were established in the study grassland on the area of approx. 30×12 m). Between 1994 and 1996, the removal experiment was set up, with all individuals of the dominant grass, *Festuca*, removed from half of the plots. The removal was done carefully, using tweezers. Whole plants with roots were removed, with as little disturbance to other plants as possible (see Herben *et al.* (2003) for details). The plots were visited several times per year to assure removal of all individuals of *Festuca*. The removal continued every year until 2001, and then again from 2009 until 2014, i.e., twice five years with a break of eight years. There was a little recolonization of *Festuca* in the period during which the plots were not treated, but the density of *Festuca* in 2009 (i.e., in the year when the removal started again) was much lower in the removal plots than in untreated plots (Herben *et al.*, 2018).

Experiment 1: Effect of dominant removal on plant-soil feedbacks

In June 2014, we collected soil samples from the 14 permanent plots (7 control plots and 7 plots with *Festuca* removal). Thus, we had two types of soil with a different history: soil that was previously vegetated (i.e., conditioned) with natural grassland community and soil conditioned by a community without the dominant species. We sampled the soil by excavating soil monoliths of approx. 30×30 cm in area and 20 cm in depth from the center of each permanent plot. Further, we divided each monolith into two parts according to the soil depth: 0-6 cm and 6-12 cm (for details see Herben *et al.* (2018)). We assumed that since the majority of plant roots in this grassland is placed in the soil layer up to the depth of 4-8 cm (Herben *et al.*, 2018), the soil taken from the upper 6cm layer will have different effect on plant performance than the lower layer containing fewer roots of fewer species. We separately sieved the soil from each plot and layer through 1-cm mesh and put the soil into four 10×10×10 cm pots, resulting in a total 4 (species, see below) × 2 (conditionings) × 2 (depths) × 7 replicates, i.e. 112 pots.

To test the effect of previous soil history (conditioning) on plant performance, we used four model species: grasses *Festuca rubra* and *Anthoxanthum odoratum* L., and forbs *Leontodon hispidus* L. and *Ranunculus acris* L. (all the four species are further referred to by the genus name only). All of these species are common in the study grassland, with *Festuca* being dominant and the latter three species subordinates (Table 1). We collected seeds of these species directly at the study locality during June/beginning of July 2014. Prior to sowing the seeds into the pots, we performed a germination test on Petri-dishes in a growth chamber and adjusted the number of seeds according to the germination rates of each species. We sowed one of the four model species into each pot in the beginning of July: *Festuca* (15 seeds per pot), *Anthoxanthum* (15 seeds), *Leontodon* (10 seeds), or *Ranunculus* (20 seeds). We placed the pots into a growth

chamber with conditions simulating vegetation season in the study mountain grassland (22°C the day maximum/7 °C the night minimum temperature changing continuously among the two extremes, 12 hours of full daylight and 8 hours of darkness with 2 hours representing the transition between the dark and light period in either direction). After four weeks, we reduced the number of seedlings to a maximum of five individuals per pot, and after another four weeks, only the largest individual was left in each pot (in the case of *Festuca* and *Leontodon*, three seedlings per pot were left due to their very small size). After four months, we harvested the total plant biomass by clipping the shoots and carefully rinsing the roots. We dried the biomass to a constant weight at 60°C and weighed. The design of this experiment is illustrated in Fig. 1.

Table 1: Percentage aboveground biomass of the model species in the study grassland (mean from four permanent plots from 2011; source: Herben, unpublished) and their rooting patterns up to 12 cm (source: Herben *et al.*, 2018).

	biomass in grassland [%]	**vertical root distribution**
Anthoxanthum	2.8	most roots in top 4 cm
Festuca	27.1	homogenous, slightly more roots in layers below 4 cm
Leontodon	8.0	homogenous, slightly more roots in layers below 4 cm
Ranunculus	3.6	most roots in top 4 cm

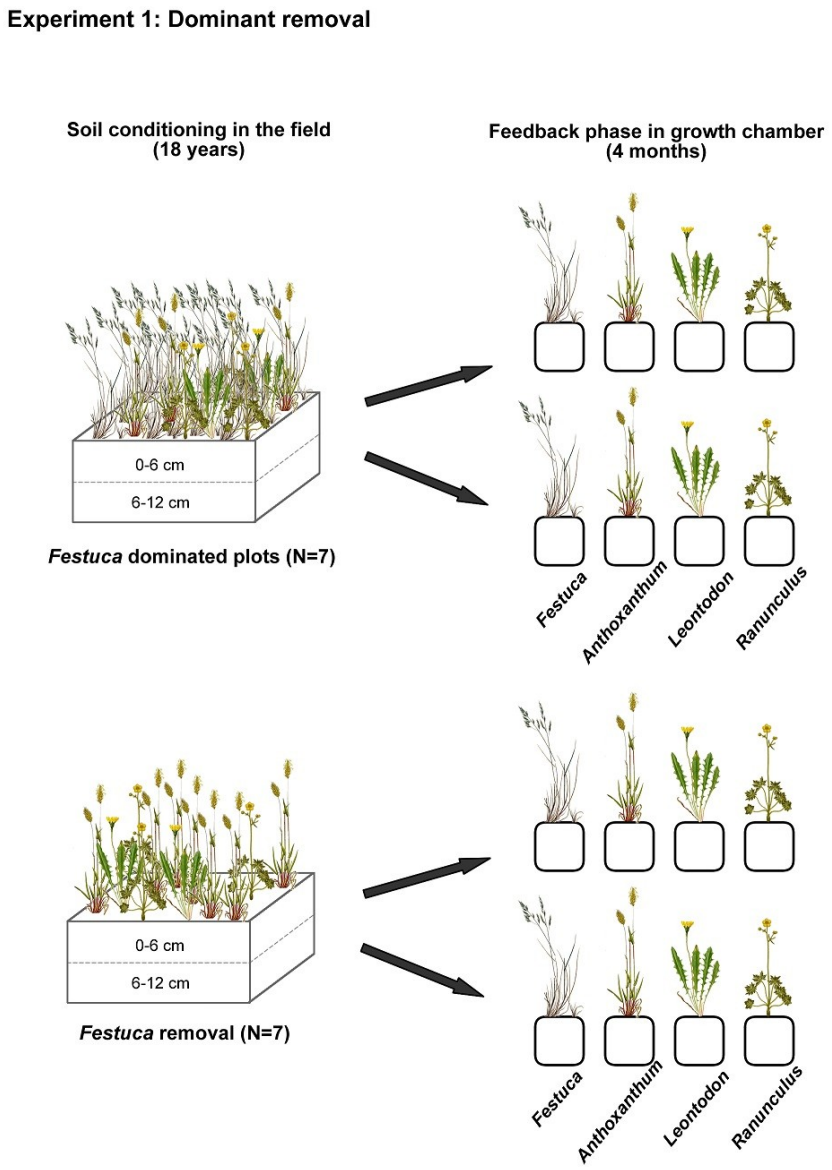

Fig. 1: Scheme of the experimental design (Experiment 1).

Experiment 2: Masking of plant-soil feedback effects

To study the interactions between subsequent conditionings, we conducted a three-phase plant-soil feedback experiment consisting of first conditioning phase, second conditioning phase and a feedback phase. The experiment ran in a growth chamber with conditions simulating vegetation season in the study mountain grassland (the same setting as in the Experiment 1). We performed the experiment between November 2015 and June 2016.

We obtained the soil for the experiment directly from the study grassland outside the permanent plots by sampling the topsoil (approx. from the depth 0-20 cm) in November 2015. Since *Festuca* was present in the patches from which the samples were taken, soil used for this

experiment was comparable to the soil conditioned by the whole community in Experiment 1. However, since we used further experimental conditionings of this soil, we call it "unconditioned" prior the experimental conditionings. We removed the majority of roots, sieved the soil through 1-cm mesh and put it into 80 pots (5×5×8 cm). To condition the soil by *Festuca* in the first conditioning phase, we sowed 15 seeds into 40 of the pots. We kept the remaining 40 pots unplanted as a control. Using an unplanted control allowed us to evaluate not only the specificity of plant-induced changes in the soil (both abiotic and biotic) but also the magnitude of these changes. In this respect, using an unplanted control is more appropriate than using the inoculation method (excluded abiotic changes), sterilized control (abiotic conditions changed by nutrients released due the sterilization), or control conditioned by a different species (both abiotic and biotic compounds altered in control soil in a species-specific way). However, our approach poses a risk of an additional shift in soil microbiota, promoting microbes thriving in unvegetated soil.

We placed all the pots into a growth chamber and watered them regularly with distilled water from the bottom. After four weeks, we thinned the established seedlings to one individual per pot. Three months after sowing the seeds, we harvested both aboveground and belowground biomass of the plants, dried it to a constant weight at 60°C and weighed it. We homogenized the conditioned soil, as well as the control soil, within each pot, but we did not mix soil from different pots together. We collected soil samples from 10 randomly chosen conditioned and 10 control pots for further analyses of soil pH and nutrient content and composition of microbial communities.

Immediately after harvesting the first conditioning phase, we set up the second conditioning phase. We sowed both the previously unconditioned control soil and the *Festuca*-conditioned soil with four model species (each species separately), *Festuca* (15 seeds per pot), *Anthoxanthum* (15 seeds), *Leontodon* (10 seeds) and *Ranunculus* (20 seeds). This resulted in 2 first phase conditionings × 4 model species × 10 replicates = 80 pots. All seeds used in this experiment were collected at the study locality during the vegetation season of 2015. After two weeks, we thinned the seedlings to a maximum of five individuals per pot, and after another two weeks, we left only the largest individual in each pot. Two months after sowing the seeds, we harvested plant above- and belowground biomass, dried it at 60°C and weighed it. Again, we homogenized the soil within each pot and sampled it for further analyses of soil chemical properties and composition of soil bacterial and fungal communities. This second conditioning phase allowed us to (i) assess plant-soil feedback effect of the first *Festuca* conditioning on the four plant species (i.e., the second conditioning taken as a feedback phase in a classical design) and (ii) study interactions of soil legacies of the first and second generation of plants.

To set up the feedback phase, we put two tablespoons of sterilized river sand (sterilized using steam sterilization) to the bottom of each pot to compensate for the volume of soil collected for analyses and topped it with the soil from the second conditioning phase. We decided not to mix the soil with the sterilized sand not to dilute the effect of the preconditioned soil on the establishing seedlings. With this design, seedlings interacted mostly with the preconditioned soil and only the longer roots of older plants reached the sand at the bottom of the pots, minimizing the bias. We sowed all the pots with 15 seeds of *Festuca*. Again, we reduced the number of

seedlings in two steps to one plant per pot. Two months after sowing, we harvested the above- and belowground plant biomass, dried it at 60°C and weighed it. The duration of the individual phases in this experiment were shorter than in Experiment 1 since we used smaller pots (to fit into the growth chambers) and the plant roots thus faster filled the whole soil volume. The design of this experiment is illustrated in Fig. 2.

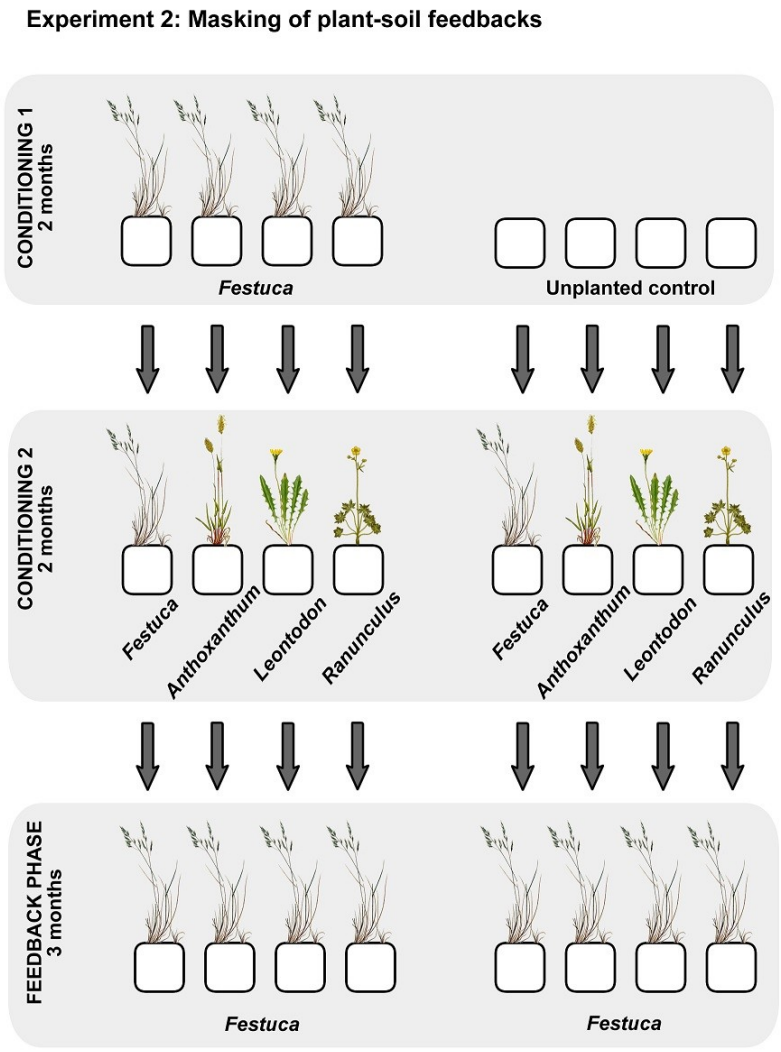

Fig. 2: Scheme of the experimental design (Experiment 2).

Soil analyses

We sampled the soil from the Experiment 2 (Masking of plant-soil feedbacks) after both the first and the second conditioning. Due to high costs and workload to perform these analyses, we only used 6 replicates per treatment (replicate 1-6, out of the total of 10 replicates). We analyzed the samples for soil chemical properties and composition of bacterial and fungal soil communities. The samples for analyses of soil pH and nutrient content were air-dried, and the samples for microbial communities were frozen at -20°C.

Soil chemistry

To analyze soil pH and the content of nutrients, we dried approximately 100 ml of each sample at room temperature and sieved it through a 2-mm mesh to analyze active pH and exchangeable Ca, Mg, K, and P content (Olsen, 1954; Zbíral, 1995); or through 0.1-mm mesh for the analyses of total N and total C content (Ehrenberger, 1973). The exchangeable content of Ca, Mg, K and P was measured following the Mehlich 3 procedure (Mehlich, 1984). The analyses of pH and nutrient contents were provided by the Analytical laboratory of the Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic.

DNA extraction, amplification of 16S and ITS2 regions and Illumina sequencing

Prior to processing, soil samples were freeze-dried. DNA from each sample was extracted in duplicate using the modified method of Miller (Sagova-Mareckova *et al.*, 2008), see Supporting information for a detailed description. For the microbial community analysis, PCR amplification of the fungal ITS2 region from DNA was performed using barcoded primers gITS7 and ITS4 (Ihrmark *et al.*, 2012). The V4 region of bacterial 16S rRNA was amplified using the barcoded primers 515F and 806R (Caporaso *et al.*, 2012). PCR was performed in duplicates for each sample as recommended by Schöler et al. (2017), and resulting amplicons were purified, pooled, and subjected to sequencing on the Illumina MiSeq. The presence of contaminant sequences was excluded using appropriate controls.

The amplicon sequencing data pre-processing was done using the pipeline Seed 2.0.3 (Větrovský *et al.*, 2018) as described previously (Žifčáková *et al.*, 2016). The processing included several steps including quality filtering (adapter trimming, quality and length filtering, removal of chimeric sequences and sequences not matching the target), clustering, and identification as recommended by Vestergaard, *et al.* (2017). The most abundant sequences were selected for each cluster, and the closest hits at a genus or species level were identified using blast against the Ribosomal Database Project (Cole *et al.*, 2014) and UNITE (Nilsson *et al.*, 2019). Sequences identified as nonbacterial or nonfungal were discarded. Raw sequencing data have been deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under accession number (the data will be deposited with the acceptance of this manuscript and accession number specified).

Data analyses

Experiment 1: Effect of dominant removal

We tested the performance of the four plant species in the two soil conditionings (whole community × removal) and the two soil layers using mixed-effect models (lme function in nlme R package). The plant total biomass (square-root transformed) was used as the dependent variable, identity of permanent plot as a random factor, and plant species in feedback, soil conditioning, soil layer and their interactions as fixed factors.

Experiment 2: Masking of plant-soil feedbacks

Festuca responses to the soil conditionings

To evaluate feedback of *Festuca*, we tested effect of the first conditioning on plant growth in the second conditioning phase (i.e., a classical plant-soil feedback experiment) using ANOVA with conditioning 1 as explanatory variable. To compare plant responses in feedback phase to the two subsequent soil conditionings, we performed ANOVA with total biomass as the dependent variable and conditioning 1, conditioning 2 and their interaction as explanatory variables. The total plant biomass was square-root transformed to meet the assumption of normality.

Changes in soil chemical properties

First, we tested whether the chemical properties of soil sampled after the first conditioning were influenced by *Festuca* growth by comparing the conditioned and control soil. We performed a redundancy analysis (RDA) with the conditioning treatment as explanatory variable and standardized values of soil pH and nutrient contents as response variables.

Second, we tested whether the chemical properties of soil sampled after the second conditioning were influenced by the first and the second conditioning treatment, and their interaction. Therefore, we performed RDA with the first or second conditioning as an explanatory variable, and the other variables as covariates. We also tested the interaction of the two conditionings, using the main effects as covariates.

Changes in soil microbial communities

To compensate for differences in sequencing depths of individual samples, we subsampled all samples to 10 000 sequences, leaving the samples with less than 10000 sequences as they were. As a result, we obtained 1 117 558 bacterial sequences, including 6 362 singletons, that clustered into 18 724 OTUs, and 1 033 105 fungal sequences clustering into 10 080 OTUs including 5 768 singletons. Statistical analyses were performed on datasets containing only OTUs occurring at the abundance of at least 0.1% in at least three samples (751 bacterial OTUs representing 76.33% of all sequences; 385 fungal OTUs representing 93.69% of all sequences).

Bacterial and fungal abundance data were subjected to Hellinger transformation prior to all statistical analyses. Soil microbial communities were visualized using principal component analysis (PCA) and the influence of the first and the second conditionings on soil fungal and

bacterial communities was tested using RDA using the same approach as described for soil chemistry.

Festuca responses to the individual components of plant-soil feedback

We also aimed to assess the relative importance of the individual components of the feedbacks on *Festuca* performance. Specifically, we focused on the effects of soil chemical properties, composition of fungal and bacterial communities and the biomass of plants in the second conditioning. To get univariate variables in the case of soil chemical properties and the composition of microbial communities, we used sample scores from the 1st and 2nd PCA axes (PCA: composition of standardized soil chemical properties / bacterial communities / fungal communities). We included the biomass of conditioning plants as another explanatory variable since it is a proxy for the amount of root litter left in the soil after the conditioning.

First, we tested the effects of all variables on the total biomass of *Festuca* in the feedback phase in separate tests. Second, if more variables showed significant effects on *Festuca* biomass, we tested the effect of each variable independently.

All analyzes were performed using R 3.6.1 (R Core Team 2019).

Results

Plant-soil feedback in the grassland vs. in the experiment

The performance of the four model species did not differ between soils conditioned by the whole community and community with *Festuca* removed (Exp. 1, Fig. 3a; p=0.704). In contrast, the conditioning by *Festuca* (first conditioning phase) in Experiment 2 had significant impact on the total biomass of the four model species (Fig. 3b; p<0.001; R^2=0.58).

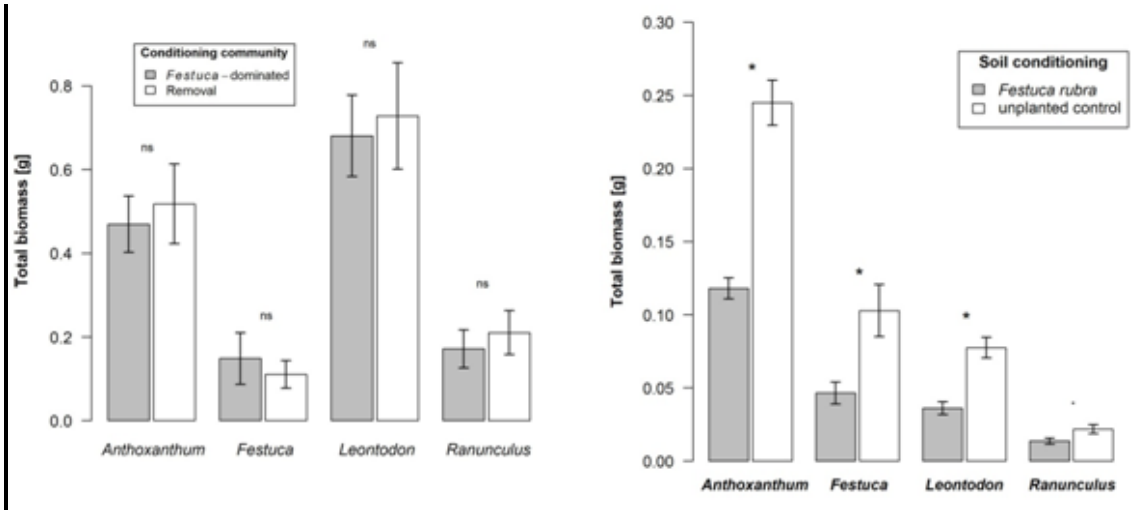

Fig. 3: Total plant biomass of the four model species (a) in soils conditioned by the *Festuca*-dominated community vs. removal (Exp. 1), (b) as response to *Festuca* conditioning in the pot experiment (Exp. 2). Means ± SE are presented. Asterisks (*), dot (.) and „ns“ above bars indicate significant (p<0.05), marginally significant (p<0.1), and non-significant pairwise comparisons, respectively.

In the field soil conditioning experiment (Exp. 1), the total plant biomass was dependent on the interaction of the plant species × soil layer ($p<0.001$; Table S1). While *Anthoxanthum* and *Ranunculus* produced more biomass in soil from the lower layer, *Festuca* and *Leontodon* produced more biomass in soil from the upper layer (Fig. 4). The total biomass was also influenced by the interaction of soil conditioning and soil layer ($p=0.019$; Table S1) - in the soil conditioned by the whole community, plants produced more biomass in the lower soil layer, but in the soil originating from the removal plots, they produced more biomass in the upper soil layer (Fig. S1).

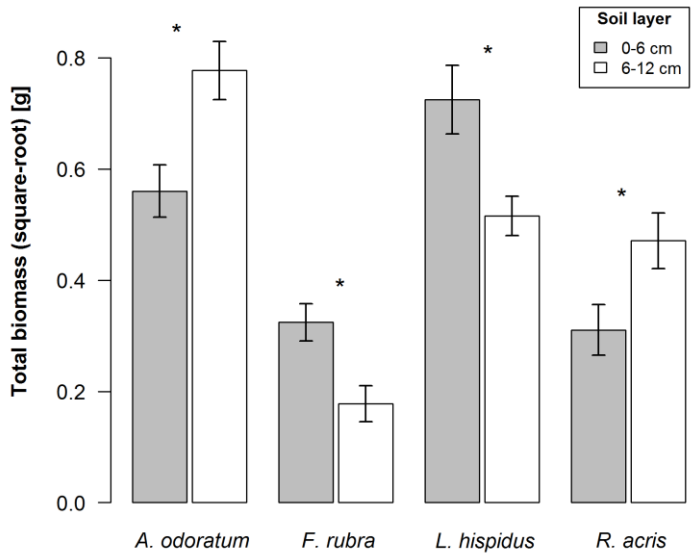

Fig. 4: Total biomass in the field-conditioned soils as influenced by plant species × soil layer ($p<0.001$). Means±SE are presented. Asterisk (*) above bars indicates significant pairwise comparison, "ns" indicates non-significant pairwise comparison.

Interactions of subsequent conditionings

The total biomass of *Festuca* responded to both conditionings and their interaction but the majority of variance was explained by the second conditioning (Fig. 5). Specifically, *Festuca* produced the least biomass in soil conditioned by *Anthoxanthum* in the second phase, regardless of the first conditioning, and the most biomass in 1st control - 2nd *Ranunculus* conditioned soil, while in all the other treatments, the biomass was more or less the same (Fig. S2). The difference of *Festuca* biomass in soil conditioned in both phases by *Festuca* and in soil conditioned firstly by *Festuca* and subsequently by any of the other model species did not differ (Fig. 6).

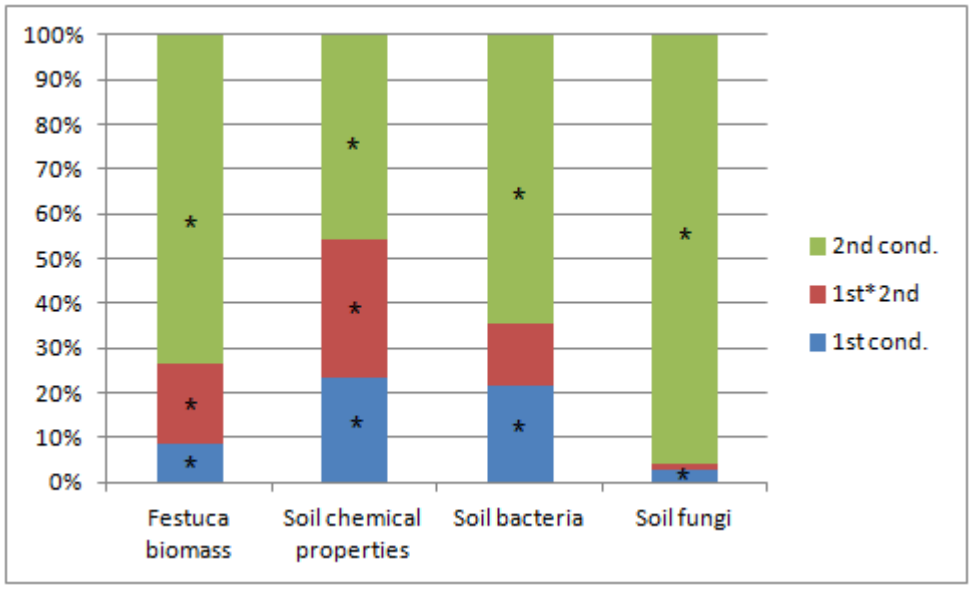

Fig. 5: Total biomass of *Festuca rubra* and the individual components of soil feedback as explained by the first, the second and the interaction of both conditionings. The proportions of variation from the total variation explained by the model are shown. Asterisks (*) in bars mark the significant effects.

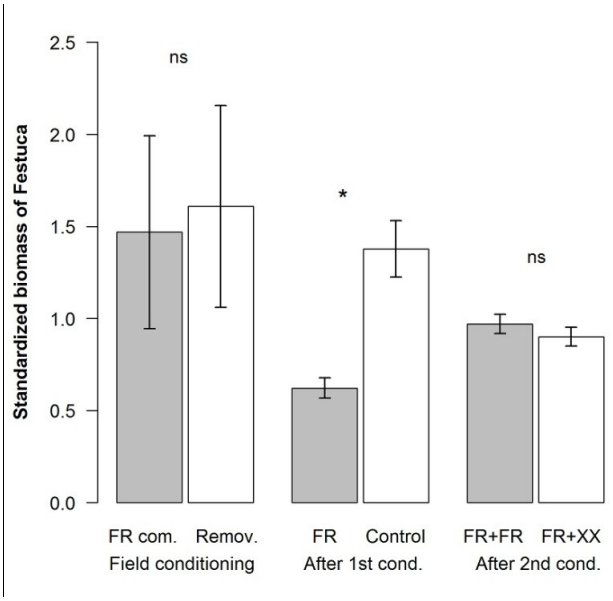

Fig. 6: Masking the original *Festuca* conditioning: responses of total biomass of *Festuca* (standardized by mean *Festuca* biomass from the respective experiment/phase) to the field conditioned soil (FR com. = whole community conditioning vs. Remov. = removal treatment without *Festuca*), and to the pot-conditioned soil after the first conditioning (FR = biomass in soil conditioned by *Festuca* vs. Control = biomass in control soil) and after the second conditioning (FR+FR = "pure *Festuca* conditioning", i.e., biomass in soil conditioned by *Festuca* in both conditioning phases vs. FR+XX = "masked *Festuca* conditioning", i.e., biomass in soil firstly conditioned by *Festuca* and subsequently by any other species). Means±SE are presented. Asterisk (*) above bars indicates significant pairwise comparison, "ns" indicates non-significant pairwise comparison.

After the first conditioning phase, we found significant differences between *Festuca*-conditioned and control soil in the composition of soil chemical properties ($p=0.046$; $R^2=0.14$) and the

bacterial communities (p=0.005; R^2=0.10), but not in the composition of fungal communities (p=0.25; R^2=0.02). Soil chemical properties analyzed after the second conditioning phase were still significantly influenced by the first conditioning. We also detected a significant effect of the second soil conditioning and of the interaction of the two conditionings (Fig. 5). The bacterial and fungal communities sampled after the second conditioning were influenced by both the first and the second conditioning but not by their interaction (Fig. 5). Soil microbial communities, especially the fungi, were more affected by the second conditioning than by the first one (Fig. 5). Generally, both bacterial and fungal communities from samples conditioned by forbs (*Leontodon* or *Ranunculus*) and grasses (*Festuca* or *Anthoxanthum*) formed two significantly distinct groups irrespective of the first conditioning (Fig. 7).

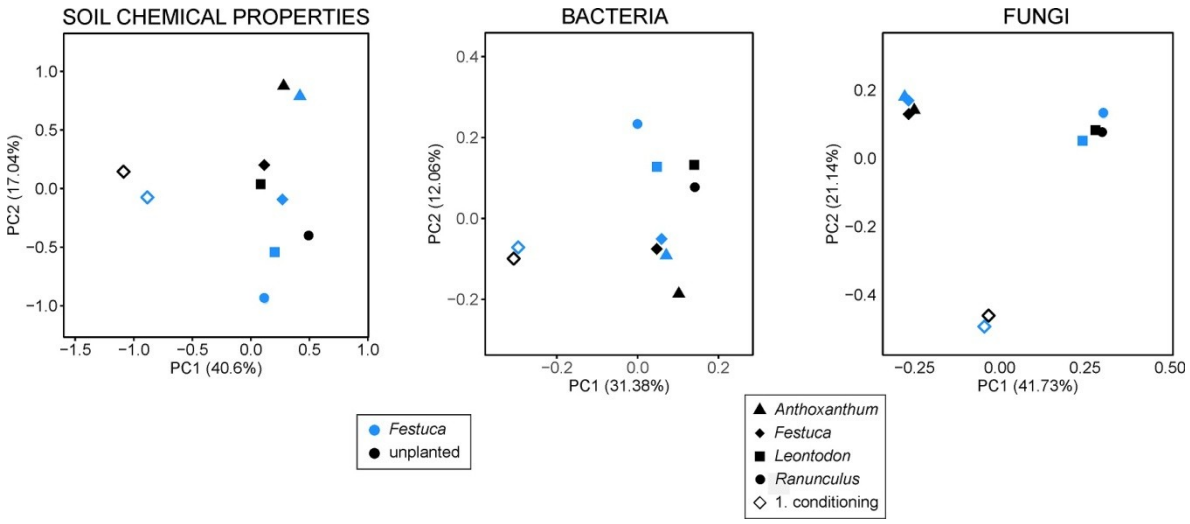

Fig. 7: PCA of (a) soil chemical properties, (b) bacterial and (c) fungal community composition in samples taken after the first (empty symbols) and the second (filled symbols) conditioning. The symbols represent centroids of 6 samples from the respective phase/treatment. Blue symbols represent soil conditioned by *Festuca* in the first conditioning; black symbols the unconditioned soil in the first conditioning. Various symbols represent the second conditioning species.

The total biomass of *Festuca* was influenced by the biomass of conditioning plants (R^2=0.36; Table S3) and by soil fungi (R^2=0.29). The total biomass of *Festuca* was also marginally significantly influenced by the soil chemical properties and the bacterial communities (Table S3).

Discussion

We found that plant-soil feedbacks measured in the classical pot experiments are not solely explaining the outcome of feedbacks within diverse communities. This might be caused by the interactions between soil legacies of the individual plant species. Indeed, we found effects of such interactions of two subsequently grown plant species on soil chemical properties, composition of soil bacteria and fungi, as well as on responses of plants to those soils. Additionally, our data suggest that vertical distribution of roots can shape plant-soil feedback relationships in the field. Further, we discuss the individual results in more detail.

Festuca legacies in the field-conditioned and in pot-conditioned soils

While we found negative effect of *Festuca* conditioning on all four species when the soil was conditioned in pots, we did not find any effect of presence/absence of *Festuca* in the grassland on the final feedback of the whole community. This result clearly indicates that plant-soil feedbacks in diverse grasslands are hardly predictable from such simple pot experiments (Heinze *et al.*, 2016).

There are several reasons why we did not detect the effect in the field-conditioned soil. Firstly, there could be another factor influencing the soil legacy in the field more strongly than the actual composition of the community, such as plant competition, the annual mowing (but see Ilmarinen & Mikola (2009)), activity of earthworms and other soil biota (Kuťáková *et al.*, 2018a), or microclimatic conditions of the site (Fry *et al.*, 2018). Secondly, legacies of other plant species present in the community could interact with - or even mask the effects of *Festuca* (we discuss this topic further). Thirdly, the plant-soil feedback of *Festuca* might change during time, with more negative effects prevailing in the beginning of their development (i.e., during the first months of plant life) and get more neutral over years to allow *Festuca* to maintain its dominant position. This can be supported by the fact that the negative effects of *Festuca* conditionings were not additive in the Exp. 2. Fourthly, the negative effect induced by *Festuca* conditioning in the pots could be just an artifact of the experiment where we used an empty control, and this could strengthen the difference between the conditioned and the control soil. However, both of these soils carried the original legacy of the whole community from the field and the unplanted control was not completely naïve. Also, the fact that the second conditioning had greater effect than the first one, speaks against this argument. Finally, feedbacks measured in pots ignore plant natural distribution of roots, and the concentrated roots within the pot can increase the strength of soil feedbacks.

Masking of plant-soil feedbacks

In our study, the final biomass of *Festuca* was influenced by interaction of both soil conditionings. The fact that soil legacies of subsequently growing plants can interact and both influence performance of next generations of plants is known mostly from agricultural research. Angus at el. (2015) showed in their meta-analysis that inducing a break-crop to continuous wheat crop sequences not only increased the wheat yield in the year following the break-crop (i.e., soil feedback of break-crop), but also had positive effect on wheat yield in the second year (i.e., the legacy of break-crop to the second generation of wheat), and under certain climatic conditions,

even affected the wheat yield in the third year. Similarly, they also showed that inducing two generations of break-crops can increase the wheat yield more than just one break-crop generation (Angus *et al.*, 2015).

Regarding the plant-soil feedback research on natural ecosystems, there are just two studies we are aware of, both confirming that the two subsequent conditionings can interact (Packer & Clay, 2004; Wubs & Bezemer, 2018). Packer & Clay (2004) showed that the negative intraspecific feedback of black cherry (*Prunus serotina*) can accumulate over multiple generations of growth of black cherry seedlings. In our study, this was rather not the case since *Festuca* did not have that negative soil feedback, compared to e.g. *Anthoxanthum*, and thus we did not find a strong additive negative feedback. The absence of additive negative legacy of *Festuca* is an interesting observation and could be linked to the fact that this species is perennial and as such has to avoid developing too negative soil feedback over time (but see Wubs & Bezemer (2018) for an additive negative effect of *Festuca rubra*). Generally, *Festuca* responded with its biomass mostly to the second conditioning, though there was also an important interaction effect of both conditionings, and a weaker effect of the first conditioning. This result is similar to results of Wubs and Bezemer (2018) who showed that plant response to feedback is mostly explained by interaction of the two conditionings, with stronger effect of the most recent one. In the case of *Ranunculus* being the second conditioning species, there was still a strong effect of the first conditioning on *Festuca* biomass. We assume that this can be explained by the fact that *Ranunculus* had much lower biomass than the other species in the second conditioning, and thus could not mask the legacy of the first conditioning.

The effects of plants on soil chemistry and microbial communities

In this study, the first conditioning by *Festuca* caused shifts in soil chemical properties and bacterial communities compared to the unplanted control but did not change the composition of soil fungi. Soil bacteria are small, unicellular organism and most of them associate with macroaggregates or live within microaggregates (Wilpiszeski *et al.*, 2019) and are thus influenced by the conditions in their immediate vicinity. The overall changes in soil chemical properties, albeit significant, were rather modest; however, they could appear much larger at a μm scale relevant for most soil bacteria (Vos *et al.*, 2013). Fungi, on the other hand, are generally larger than bacteria and often form extensive hyphal networks allowing them to interact with larger volumes of soil in search of nutrients or favorable chemical environment; these characteristics should make them more resistant to disturbances. Indeed, de Vries et al. (2018) observed that fungal communities are more stable under drought than bacterial communities and that although both bacteria and fungi responded to changes in vegetation, only bacterial community was directly affected by the biomass of a dominant plant species. The soil used for this experiment originated from the study grassland where *Festuca* is the dominant species and we hypothesize that over the years in the grassland, a stable fungal community developed which could not be disturbed by one generation of *Festuca* absence.

Generally, the properties of soil samples taken after the second conditioning phase were influenced not only by the second conditioning but also by the first one. This shows that plants can generate soil legacies that persist in the soil and, more importantly, are not completely

masked by the next generation of plants. The soil chemical properties after the second conditioning phase were influenced by the interaction of both conditionings, i.e. they reflected the legacies of both generations of plants. So far, there have been some evidences of changes of soil chemical properties induced by multiple subsequently grown plant species, coming from the crop rotation agricultural systems (Struik & Bonciarelli, 1997). For instance, it has been shown that different cropping sequences result in different levels of phosphorus, as well as in different efficiency of its use (Łukowiak *et al.*, 2016). Such research illustrates that the plant species-specific nutrient use can interact to form the final nutrient status of the soil and that these interactions are likely to occur in natural communities.

In the case of microbial communities, the carry-over effect of the first conditioning phase was present but much weaker than the effect of the second conditioning. Plants are able to efficiently shape both bacterial and fungal communities through rhizodeposits in a species-specific manner (Hartmann *et al.*, 2009) which could explain that their composition was mostly linked to the second conditioning species. In the case of soil fungi, the variability explained by the second conditioning species was much greater and the fungal communities were mostly shaped by the distinction of monocot/dicot conditioning. Monocots and dicots have been shown to differ in the levels of colonization by both the arbuscular mycorrhizal fungi and the dark septate endophytes (Weishampel & Bedford, 2006), and also to differ their responses to soil fungi (Dostálek *et al.*, 2013; Anacker *et al.*, 2014). Grasses and forbs have been also shown to generate different feedbacks that influence both further plant performance and insect herbivory on those plants (Kos *et al.*, 2015; Heinen *et al.*, 2018). Such distinction between monocots and dicots in their interactions with soil environment could explain the large effect of the second conditioning (monocots vs. dicots) compared to the virtually non-existent effect of the first conditioning, where we compared *Festuca*-conditioned soil with an unconditioned soil that had recent history of occurrence of grasses.

Festuca responses to the individual components of soil feedback

From the individual feedback components, the final biomass of *Festuca* was linked to the effect of the biomass of conditioning plants and the composition of fungal communities. These results are in agreement with previous studies concluding that PSFs are driven by soil microorganisms (Packer & Clay, 2004; Kardol *et al.*, 2007; Wubs & Bezemer, 2018). However, although it is primarily the microbes that interact with plants and influence the outcome of feedbacks, plants themselves have impact on soil chemical properties as we show in this study as well. The chemical properties in turn often determine the composition of microbial communities (Semchenko *et al.*, 2018).

Plant-soil feedbacks in different soil layers

We tested the hypothesis that the feedback effect of the whole community is particularly strong in the top soil layer (0-6 cm) due to the presence of the majority of plant roots (Herben *et al.*, 2018). Indeed, we found an effect of the interaction of soil conditioning and the soil layer on the final biomass across all four model species: plants produced most biomass in the top layer-soil from the removal plots and least biomass in the top layer-soil from the whole community-plots. Plants grown in the soil from lower layer produced intermediate biomass. This result may

indicate that the presence of dominant *Festuca* in the community generates soil legacy that suppresses growth of conspecifics and other plants or, alternatively, that the community without the dominant species generates positive feedback as a result of increased abundances of subordinate species. Nevertheless, although the effect of the interaction of soil conditioning and soil layer had a significant effect on plant biomass, it was not particularly strong, suggesting that other factors may be more important in determining the growth of the plants.

The effect may also be weak because the effects of layer were largely species specific. *Festuca* and *Leontodon* produced more biomass in soil from the upper layer, while *Anthoxanthum* and *Ranunculus* produced more biomass in the soil from the deeper layer, regardless the *Festuca* removal treatment. Herben et al. (2018) studied natural distribution of plant roots at the same permanent plots as we used in this study, and they showed that the rooting patterns of these species differ: *Anthoxanthum* and *Ranunculus* place majority of their roots in the topmost soil layer, while the roots of *Festuca* and *Leontodon* are homogenously distributed through the profile, with non-significantly higher proportion in the deeper layers. Such opposing patterns can indicate that these species are affected by their own negative plant-soil feedbacks since they produced more biomass is soil from a layer with potentially less roots of their conspecifics. Moreover, this points to another strategy plants could apply to avoid negative soil feedbacks - changing the rooting depth. It has been shown that plants can escape their own negative soil feedbacks by placing their roots to patches with different conditioning history (Hendriks *et al.*, 2015) and possibly, the same mechanism could work in the vertical distribution of roots. In support of this, Herben et al. (2007) showed in their field transplant experiment that *Festuca rubra*, as a species placing slightly more roots to the deeper soil layers, performed better in patches with more shallow-rooting plants (high root biomass in the depth of 3-6 cm), while it grew less in patches with higher abundance of other *Festuca* individuals.

Conclusions

In this study we showed that though *Festuca* generates negative feedbacks towards other co-occurring species and itself, these effects can be masked by other species' effects, both under experimental conditions and probably also within the natural grassland community. We thus conclude that in species-rich grasslands, plant-soil feedbacks are not likely to be easily predictable. Some legacies can persist in the soil over more generations of plants, but they can be influenced by legacies generated by subsequently (or simultaneously) growing plants, forming a "community-level" legacy. However, species-specific legacies can still affect plant performance on a scale of individual (neighboring or subsequent) plants. Interestingly, we found evidence that plant-soil feedbacks can be linked to rooting patterns of the individual species, suggesting that plants can avoid negative feedbacks by placing their roots to more favorable soil depth.

Based on our results, we suggest further research should focus on the interactions of plant-soil feedbacks within communities, i.e. under conditions, where many species coexist and their plant-soil feedbacks may interact in complex ways.

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Supporting information

Following supporting information may be found in this article:

Supplementary methods: Soil DNA extraction

Table S1: Effect of plant species, soil conditioning, soil layer and their interaction on plant total biomass (square root-transformed) (Exp1).

Table S2: Correlations between the individual soil properties after the second conditioning.

Table S3: Effects of the individual components of plant-soil feedback on the total biomass of *Festuca*.

Fig. S1: Total biomass in the field-conditioned soils as influenced by soil conditioning × soil layer

Fig. S2: Total biomass of *Festuca* as influenced by the interaction of the first conditioning (*Festuca* vs control) and the second conditioning (each of the four plant species).

Supplementary methods: Soil DNA extraction

Prior to processing, soil samples were freeze-dried. DNA from each sample was extracted in duplicate using the modified method of Miller (Sagova-Mareckova *et al.*, 2008). Freeze-dried soil (0.2 g) was resuspended in 800 µl of extraction buffer (50 mM Na-phosphate buffer [pH 8], 50 mM NaCl, 500 mM Tris-HCl [pH 8], and 5% sodium dodecyl sulfate) and 300 µl of of phenol-chloroform–isoamyl alcohol (25:24:1), and homogenized three times in FastPrep®-24 bead beater (MP Biomedicals LLC, Santa Ana, CA, USA), each time for 20 s at maximum speed at 4°C, with 5-minute pause between homogenization runs. The homogenate was centrifuged at 10 000 × g for 3 min. The supernatant was mixed with the same volume of phenol-chloroform–isoamyl alcohol (25:24:1) and centrifuged at 6000 × g for 5 min. The supernatant was mixed with an equal volume of chloroform-isoamyl alcohol (24:1) and centrifuged at 6 000 g for 5 min. The supernatant was then incubated with NaCl added to a final concentration of 1.5 M and CTAB added to 1% at 65°C for 30 min. The incubated solution was cooled, mixed with an equal volume of chloroform-isoamyl alcohol (24:1), and centrifuged at 4 500 × g for 20 min. DNA was then precipitated with isopropanol/sodium acetate.

Table S1: Effect of plant species, soil conditioning, soil layer and their interaction on plant total biomass (square root-transformed) (Exp1). Results from mixed effect model.

	Total biomass (sqrt)		
	Df	F	p
Species	**3;84**	**45.09**	**<.0001**
Conditioning	1;12	0.15	0.704
Layer	1;84	0.03	0.854
Species*cond.	3;84	0.19	0.901
Species*layer	**3;84**	**13.41**	**<.0001**
Cond.*layer	**1;84**	**5.75**	**0.019**
Sp.*cond.*layer	3;84	0.12	0.946

Table S2: Correlations (Pearson's correlation index) among the individual soil properties after the second conditioning. Cond. biom.=biomass of conditioning plants in the 2nd conditioning phase. Abio1, fung2 etc.=sample scores from PCA axes 1 or 2, respectively. Bold values indicate significant correlations, values in italic a marginal significance.

	Cond. biom.	abio1	abio2	bact1	bact2	fung1	fung2
Cond. biom.	1	**0.32**	**-0.55**	**0.44**	-0.15	**0.60**	-0.06
abio1		1	0.00	**0.31**	0.07	0.16	-0.20
abio2			1	**-0.34**	-0.04	**-0.55**	0.04
bact1				1	0.00	**0.37**	**-0.31**
bact2					1	*-0.27*	-0.10
fung1						1	0.00
fung2							1

Table S3: Effects of the individual components of plant-soil feedback on total biomass of *Festuca*. Bold values indicate significant effects, values in italic marginal significance.

	p	R2
Cond. biom.	**<0.001**	**0.361**
Abio1	0.303	0.002
Abio2	*0.097*	*0.038*
Fung1	**<0.001**	**0.291**
Fung2	0.542	-0.013
Bact1	0.895	-0.021
Bact2	*0.063*	*0.053*

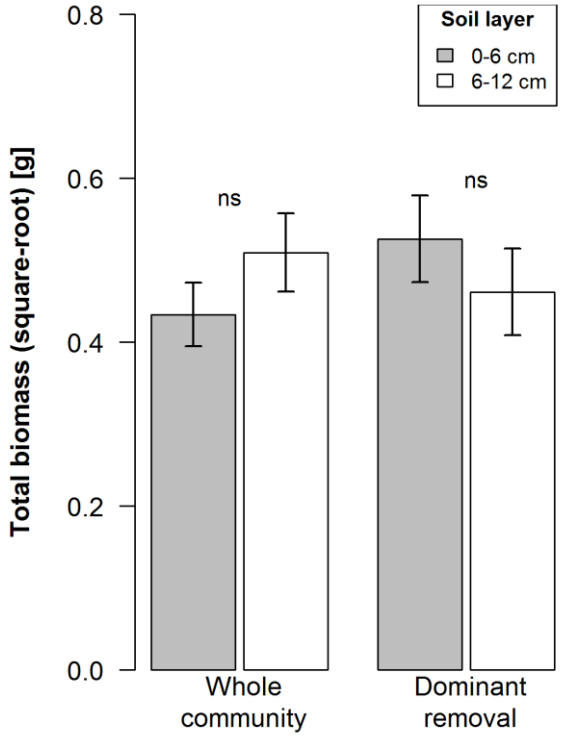

Fig. S1: Total biomass in the field-conditioned soils (across the four model species) as influenced by soil conditioning × soil layer (p=0.019). Means±SE are presented. "ns" above bars indicates non-significant pairwise comparison.

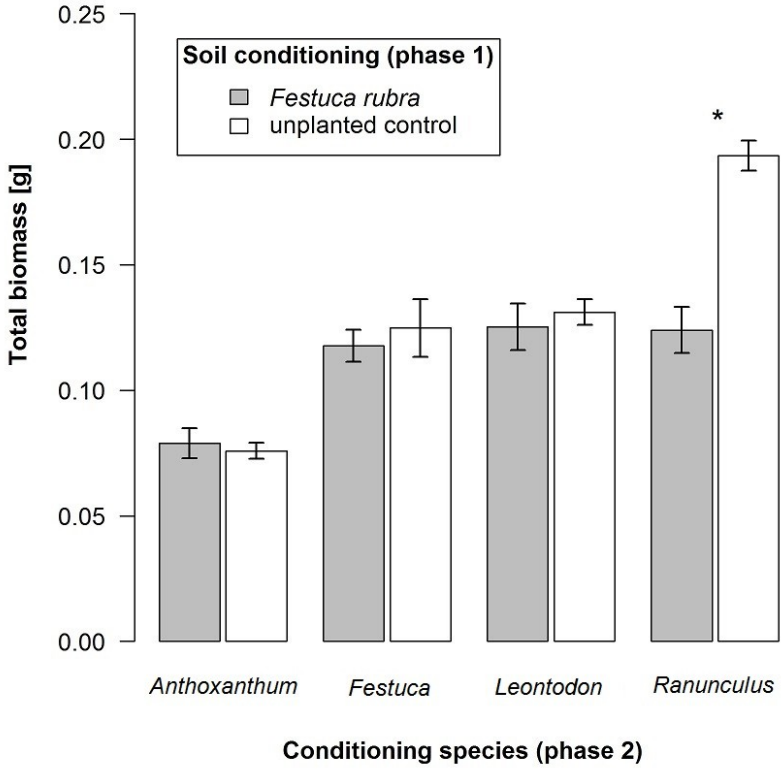

Fig. S2: Total biomass of *Festuca* as influenced by the interaction of the first conditioning (*Festuca* vs control) and the second conditioning (each of the four plant species). Means±SE are presented. Asterisks (*) represent significant ($p<0.05$) difference between the two neighboring bars (ANOVA).

STUDY 4: SOIL MICROARTHROPODS ALTER THE OUTCOME OF PLANT-SOIL FEEDBACK EXPERIMENTS

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Abstract

Plant-soil feedback (PSF) effects are studied as plant growth responses to soil previously conditioned by another plant. These studies usually exclude effects of soil fauna, such as nematodes, soil arthropods, and earthworms, although these organisms are known to influence plant performance. Here, we aimed to explore effects of a model microarthropod community on PSFs.

We performed a PSF experiment in microcosms with two plant species, *Phleum pratense* and *Poa pratensis*. We added a model microarthropod community consisting of three fungivorous springtail species (*Proisotoma minuta, Folsomia candida,* and *Sinella curviseta*) and a predatory mite (*Hypoaspis aculeifer*) to half of the microcosms. We measured seedling establishment and plant biomass, nematode and microbial community composition, microbial biomass, and mycorrhizal colonization of roots.

Microarthropods caused changes in the composition of nematode and microbial communities. Their effect was particularly strong in *Phleum* plants where they altered the composition of bacterial communities. Microarthropods also generally influenced plant performance, and their effects depended on previous soil conditioning and the identity of plant species. Microarthropods did not affect soil microbial biomass and mycorrhizal colonization of roots. We conclude that the role of soil microarthropods should be considered in future PSF experiments, especially as their effects are plant species-specific.

Introduction

The composition and dynamics of plant communities strongly depend on plant-soil interactions (Bever 2003). To better understand the role of plant-soil interactions, plant-soil feedback experiments have been widely applied (van der Putten et al. 2013). The concept of plant-soil feedback effects is based on the idea that plants change properties of the soil in which they grow, and such modified soil can, in turn, influence their growth (Bever et al. 1997). Specifically, plants can induce changes in soil biotic (e.g., composition of microbial communities) and abiotic (e.g., nutrient content or pH) properties (Ehrenfeld et al. 2005). Several studies have regarded plant-soil feedbacks as one of the key mechanisms affecting species coexistence in natural communities, the course of vegetation succession , and spread of invasive species (van der Putten et al. 2013). However, with such a wide applicability in various areas of plant and soil ecology, there is a need for precise interpretation of experimentally measured plant-soil feedback effects (Brinkman et al. 2010).

Plant-soil feedbacks are often studied using two-phase experiments. The first phase of such experiments is a conditioning phase, when a plant is grown in a pot to modify soil properties. The second phase is a feedback phase, when another plant (of the same or different species) is grown in the soil modified during the conditioning phase. The final biomass of a plant from the feedback phase is compared to the biomass of a plant grown in a control soil. Because soil microorganisms are perceived as the main drivers of plant-soil feedback effects (Bever 1994, Klironomos 2002b), many studies focus solely on the effects of the microbial community by transferring microbial inoculum between the two experimental phases. This excludes not only the abiotic compound of feedback effects from the experiment, but also major groups of soil fauna, such as microarthropods. Other plant-soil feedback studies use whole soil (Hawkes et al. 2013, Dostálek et al. 2016), which should lead to results which are more comparable to natural conditions. However, even this approach can exclude or at least reduce densities of soil fauna: unless these organisms are added to the experiment on purpose or their natural immigration is allowed during the experiment. Thus, to contain larger soil fauna like mesofauna, the soil should be obtained from a natural locality, carefully transported and treated. Treatments like drying or freezing can be lethal for soil mesofauna, but also soil sieving can lead to substantially decreased densities if they are larger than the used mesh size or sensitive to disturbances caused by processing the soil (de Rooij-van der Goes et al. 1997). As a result, most plant-soil feedback experiments may have unintentionally excluded or at least under-estimated the effect of soil mesofauna.

The suppression of soil mesofauna in plant-soil feedback experiments can be expected to significantly influence the respective results. Soil mesofauna is a significant component of the plant-soil system (Wardle et al. 2004, Bonkowski et al. 2009). Beside the direct effect of root-feeders on plants, some soil mesofauna can influence interactions between plants and microbial communities by feeding on specific microorganisms. For example, springtails are known to feed on fungal hyphae (Klironomos and Ursic 1998), and thus indirectly influence plant growth by altering mycorrhizal symbiosis (Gange 2000,

Sabatini and Innocenti 2001, Schreiner and Bethlenfalvay 2003, Maboreke et al. 2017). Springtails' grazing on mycorrhizal fungi can support growth of soil bacteria by lowering fungal biomass and thus making more resources accessible to bacterial communities (Maboreke et al. 2017). In this respect, springtails can indirectly impact plant-microbe competition for nutrients (Kuzyakov and Xu 2013). To sum up, soil microarthropods act on many stages of plant-soil interactions, having impact on both soil biota and nutrient cycling (Maire et al. 1999, Filser 2002). In previous studies, it has been shown that inclusion of larger soil organisms in experiments can change nutrient allocation patterns in plants (Ngosong et al. 2014, Maboreke et al. 2017), plant growth (Setala and Huhta 1991, Ngosong et al. 2014), and alter plant community composition (Bradford et al. 2002, De Deyn et al. 2003, Eisenhauer et al. 2011a). However, the role of soil fauna in plant-soil feedback effects is still unclear.

In this study, we investigated the effect of a model soil microarthropod community on plant-soil feedback effects. We set up a common plant-soil feedback experiment using two grassland plant species that are known to experience dissimilar plant-soil feedback effects (Cortois et al. 2016): *Phleum pratense* (in the following: *Phleum* for brevity) known for its positive intraspecific feedback effect, and *Poa pratensis* (in the following: *Poa* for brevity) that exerts a negative intraspecific feedback effect, both of them probably caused by interactions with plant species-specific soil microbial communities (Cortois et al. 2016). By following common methodology in plant-soil feedback studies as mentioned above, we established a two-phase experiment consisting of a soil conditioning phase and a feedback phase. Furthermore, we used a model microarthropod community comprising two trophic levels: three springtail species as primary consumers (fungivores), and a predatory mite as a predator of springtails, and other small invertebrates occurring in the soil. We hypothesized that (1) the addition of soil microarthropods will change the plant response to the pre-conditioned soil, and that (2) the effect of microarthropods will be context-dependent, i.e., it will be plant species-specific and depend on soil conditioning, as microarthropods will influence the composition of species-specific soil microbial communities responsible for the feedback effect.

Results

Effects on plants

The two plant species differed both in seedling establishment and final biomass. *Phleum* had significantly higher seedling establishment and also produced more above- and belowground biomass than *Poa* (Fig. 1-3, Table 1). Seedling establishment was influenced by the interaction of soil conditioning × sterilization: the sterilization treatment increased seedling establishment in *Phleum* soil, but decreased it in *Poa* soil (Fig. S1). Both species produced more above- and belowground biomass in sterilized soils (Fig. 2 and S2, Table 1). Aboveground biomass was influenced by the three-way interaction of

soil conditioning × sterilization × species: both plant species grew better in soil conditioned by the other species, and sterilization increased the differences between this intra- and interspecific soil feedback effect (Fig. S2, Table 1). Similarly, each species produced more belowground biomass in soil conditioned by the other species (Fig. S3) but there was no interaction with the sterilization treatment (Table 1). The root-to-shoot ratio was significantly higher in sterilized soils for both species, but the ratio for *Phleum* was generally higher across the other treatments (Fig. 3, Table 1). We found a significant effect of the interaction of soil conditioning × sterilization × species on the root-to-shoot ratio (Table 1): the ratio in both plant species was the highest when they were grown in non-sterilized soil conditioned by the other species (Fig. S4). The root-to-shoot ratio in sterilized soils was much lower, especially in the case of *Phleum* plants (Fig. S4).

The presence of arthropods influenced seedling establishment in interaction with sterilization and species (sterilization × arthropod addition × species interaction; Table 1). While seedling establishment of *Phleum* in sterilized soil and that of *Poa* in non-sterilized soil was not influenced by arthropods, their addition decreased seedling establishment of *Phleum* in non-sterilized soils and that of *Poa* in sterilized soils (Fig. 1). We also found a marginally significant interaction effect of soil conditioning × sterilization × arthropods on belowground biomass (Table 1). Arthropod addition generally decreased belowground biomass of plants grown in *Phleum* soil, although the decrease in sterilized *Phleum* soil was much weaker than in non-sterilized soil. In sterilized *Poa* soil, arthropod addition also decreased belowground biomass, but, in contrast, it increased belowground biomass in non-sterilized *Poa* soil (Fig. 2). The arthropod addition treatment also altered the root-to-shoot ratio in interaction with soil conditioning and plant species, or soil conditioning and sterilization (Fig. 3, Table 1). Specifically, arthropods lowered the root-to-shoot ratio of plants in non-sterilized *Phleum* soil, but they increased it in non-sterilized *Poa* soil. In sterilized soils, the changes were much weaker (Fig. 3a). In *Phleum* soils, arthropod addition lowered the root-to-shoot ratio of *Phleum* plants, but increased the ratio of *Poa* plants (Fig. 3b).

Table 1: The effect of treatments and their interactions on seedling establishment, plant aboveground and belowground biomass, root/shoot ratio, and soil microbial biomass. The results of ANOVA are shown. Bold values indicate significant relationships ($p<0.05$), values in italics and bold indicate marginal significance ($p<0.1$).

			Biomass							
	Germination		**Aboveground**		**Belowground**		**Root/shoot ratio**		**Microbial biomass**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Sterilization	2.20	0.143	**178.57**	**<0.001**	**15.77**	**<0.001**	**91.02**	**<0.001**	**15.06**	**<0.001**
Species	**165.72**	**<0.001**	**62.56**	**<0.001**	**45.09**	**<0.001**	**6.58**	**0.013**	**13.63**	**<0.001**
Conditioning	0.79	0.376	0.03	0.855	0.00	0.962	0.01	0.919	0.43	0.517
Arthropods	**4.32**	**0.042**	0.82	0.367	0.61	0.437	0.01	0.919	0.63	0.432
Steril. × species	2.05	0.157	**13.33**	**0.001**	0.79	0.377	**14.67**	**<0.001**	**30.40**	**<0.001**
Steril. × cond.	**5.02**	**0.029**	0.03	0.867	0.20	0.659	0.04	0.838	***3.95***	***0.051***
Species × cond.	1.11	0.297	**4.62**	**0.035**	***3.92***	***0.052***	***3.93***	***0.052***	0.59	0.445
Steril. × arthropods	0.12	0.727	0.49	0.486	0.20	0.654	0.02	0.892	0.03	0.857
Species × arthropods	0.39	0.537	0.05	0.828	0.08	0.777	1.20	0.278	2.48	0.120
Cond. × arthropods	0.21	0.648	0.01	0.907	0.84	0.363	1.20	0.278	1.01	0.318
Steril. × species × cond.	2.20	0.143	**4.17**	**0.045**	0.02	0.896	**7.30**	**0.009**	1.04	0.312
Steril. × species × arthropods	**11.02**	**0.001**	1.17	0.283	1.05	0.310	0.14	0.708	1.20	0.278
Steril. × cond. × arthropods	0.39	0.537	1.02	0.317	***2.98***	***0.089***	***2.81***	***0.099***	0.81	0.373
Species × cond. × arthropods	0.89	0.348	0.25	0.616	2.07	0.155	***3.80***	***0.056***	0.41	0.522
Steril. × species × cond. × arth.	0.70	0.406	1.02	0.315	0.04	0.841	1.69	0.198	0.48	0.490

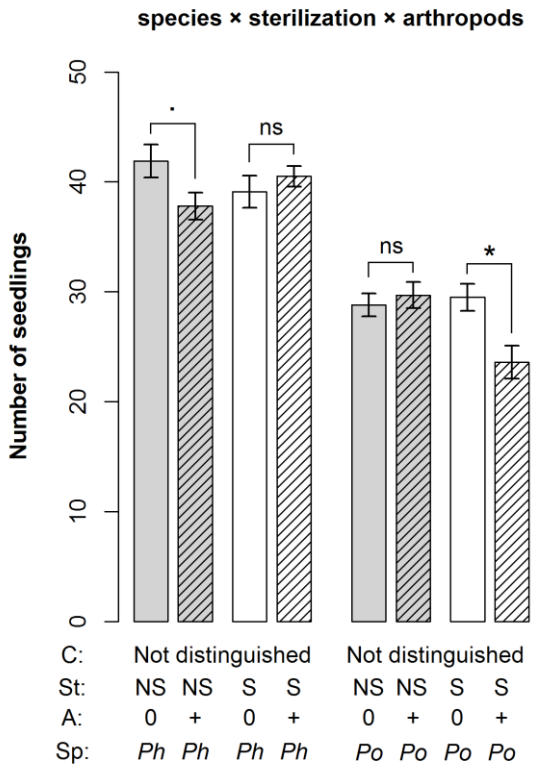

Figure 1. Number of established seedlings as influenced by the interaction of sterilization, arthropod addition and species grown in the feedback phase. Shown are means ± SE. Symbols above bars indicate significant differences between treatments with and without arthropods addition (*, $p<0.05$; . $p<0.1$; ns, $p>0.1$). Grey bars represent non-sterilized soil, white bars sterilized soil; hatched bars represent treatments with arthropod addition. C=conditioning species, *Ph=Phleum*, *Po=Poa*; St=sterilization, NS=non-sterilized, S=sterilized; A=arthropod treatment, 0=no arthropods added, +=arthropods added; Sp=plant species in feedback phase; Not distinguished=this treatment is not distinguished in the respective analysis.

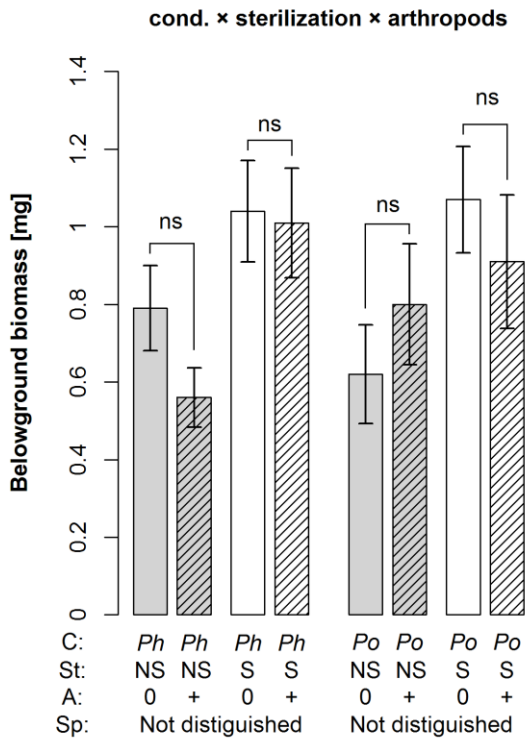

Figure 2. Belowground biomass as affected by the interaction of soil conditioning, sterilization and arthropod addition. Shown are means ± SE. Symbols above bars indicate significant differences between treatments with and without arthropods addition (*, $p<0.05$; . $p<0.1$; ns, $p>0.1$). If distinguished, grey bars represent non-sterilized soil, white bars sterilized soil, and hatched bars treatments with arthropod addition. C=conditioning species, *Ph=Phleum*, *Po=Poa*; St=sterilization, NS=non-sterilized, S=sterilized; A=arthropods, 0=no arthropods added, +=arthropods added; Sp=plant species in feedback phase; Not distinguished=this treatment is not distinguished in the respective analysis.

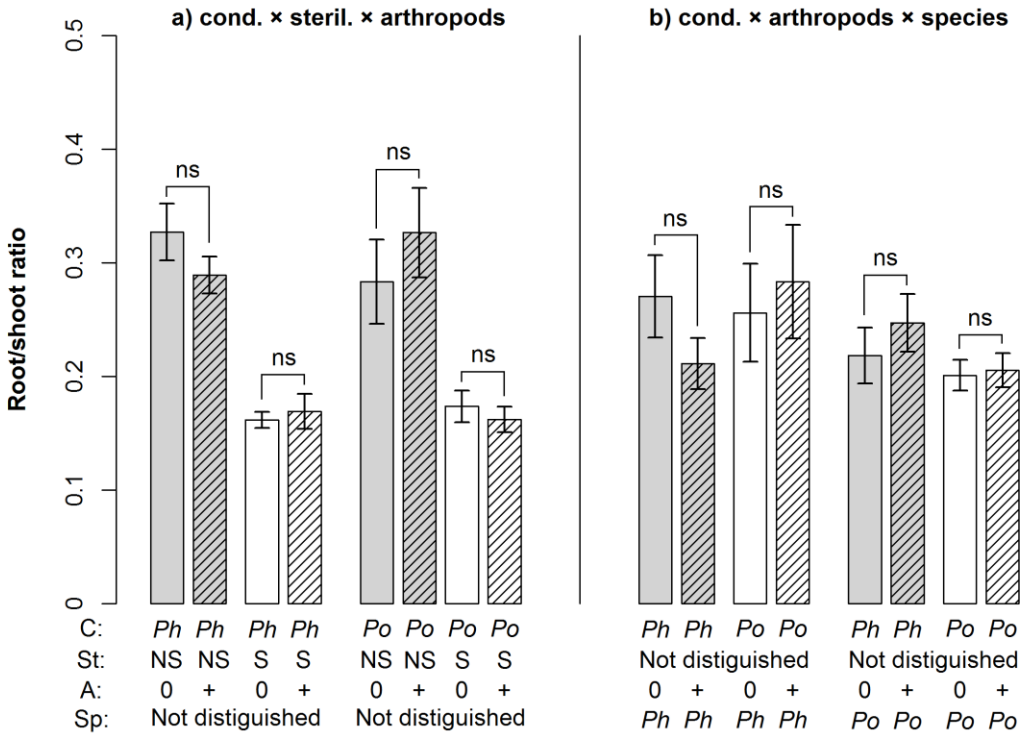

Figure 3. Root-to-shoot ratio as influenced by the interaction of a) conditioning, sterilization and arthropod addition; b) conditioning, arthropod addition and plant species grown in the feedback phase. Shown are means ± SE. Symbols above bars indicate significant differences between treatments with and without arthropods addition (*, $p<0.05$; . $p<0.1$; ns, $p>0.1$). Grey bars represent non-sterilized soil, white bars sterilized soil, and hatched bars treatments with arthropod addition. C=conditioning species, *Ph=Phleum*, *Po=Poa*; St=sterilization, NS=non-sterilized, S=sterilized; A=arthropods, 0=no arthropods added, +=arthropods added; Sp=plant species in feedback phase; Not distinguished=this treatment is not distinguished in the respective analysis.

Effects on soil microbial and nematode communities

All of the measured soil biological properties were affected by the sterilization treatment: sterilization caused an increase in soil microbial biomass (+8.3%; Table 1; Fig. S5), but largely decreased both nematode abundance (-94.1%; $\chi^2=27.65$; $p<0.001$) and mycorrhizal colonization of *Phleum* roots (-99.9%; decrease to 0.03 ± 0.02 % roots colonized; $\chi^2=36.10$; $p<0.001$;). Soil microbial biomass was significantly influenced by the interaction of soil conditioning × sterilization (Table 1): it increased by the sterilization treatment in *Phleum*-conditioned soil, whereas there was no effect of sterilization in *Poa* soil (Fig. S5a). Similarly, microbial biomass associated with *Phleum* plants was higher in sterilized soil, while for *Poa* plants, the pattern was the opposite (Fig. S5b). In non-sterilized soil, the nematode abundance was influenced by soil conditioning treatment (7.4 ± 2.8 nematodes per g soil dry weight in *Phleum* conditioned soil compared to 5.6 ± 1.7 in *Poa* soil; $F=4.92$; $p=0.03$), but not by the plant species, arthropod addition, or their interactions ($p\geq0.26$).

The functional composition of the nematode community was marginally significantly influenced by the four-way interaction of all experimental treatments, with the strongest direct effect of sterilization (Table 2, Fig. 4). In non-sterilized soil, the greatest differences were observed between the two soil conditioning treatments with omnivores, predators, and fungal feeding nematodes found preferentially in *Phleum*-conditioned soil (Fig. 4). There was no direct effect of the species grown in the feedback phase on nematode community composition. The composition of soil PLFAs also responded significantly to the four-way interaction of all experimental treatments (Table 2). The highest differences in PLFA composition were observable between the sterilized and non-sterilized soil and between the two plant species grown in the feedback phase (Table 2, Fig. 5). However, there was no direct effect of the soil conditioning treatment. Sterilization increased the arbuscular mycorrhizal marker 20:1ω9, the fungal marker 18:1ω9, and the gram-positive markers i15:0 and i17:0. The microbial communities in sterilized soils were clearly separated by the first axis from communities in non-sterilized soils. The soils containing *Phleum* plants were associated with gram-negative markers cy17:0 and cy19:0 increased, as well as i16:0 as an indicator of gram-positive bacteria. The arthropod addition treatment increased the amount of PLFA markers i14:0 (gram-positive bacteria) and 16:1ω7 (widespread bacteria). Interestingly, conditioning of the soil with *Phleum* or *Poa* did not strongly influence PLFAs; sterilization and plant species grown in the feedback phase were of higher importance.

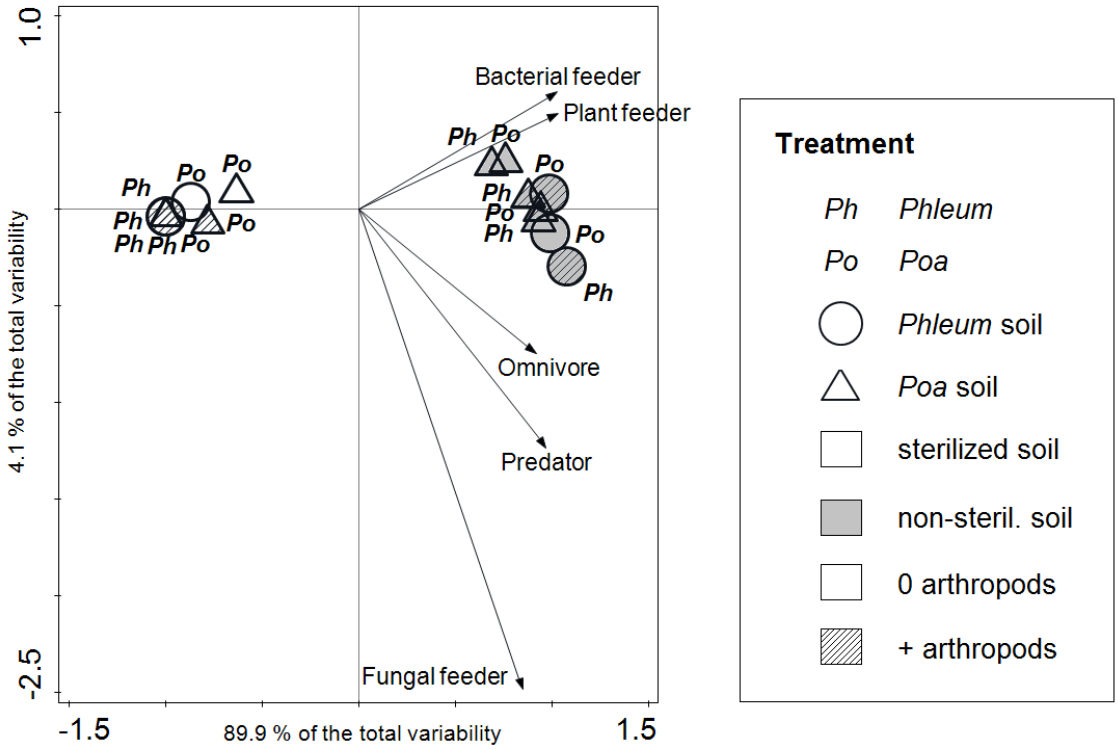

Figure 4. PCA of the composition of nematode trophic groups after the feedback phase. Symbols represent the individual experimental treatments (centroids). Arrows indicate the individual nematode trophic groups.

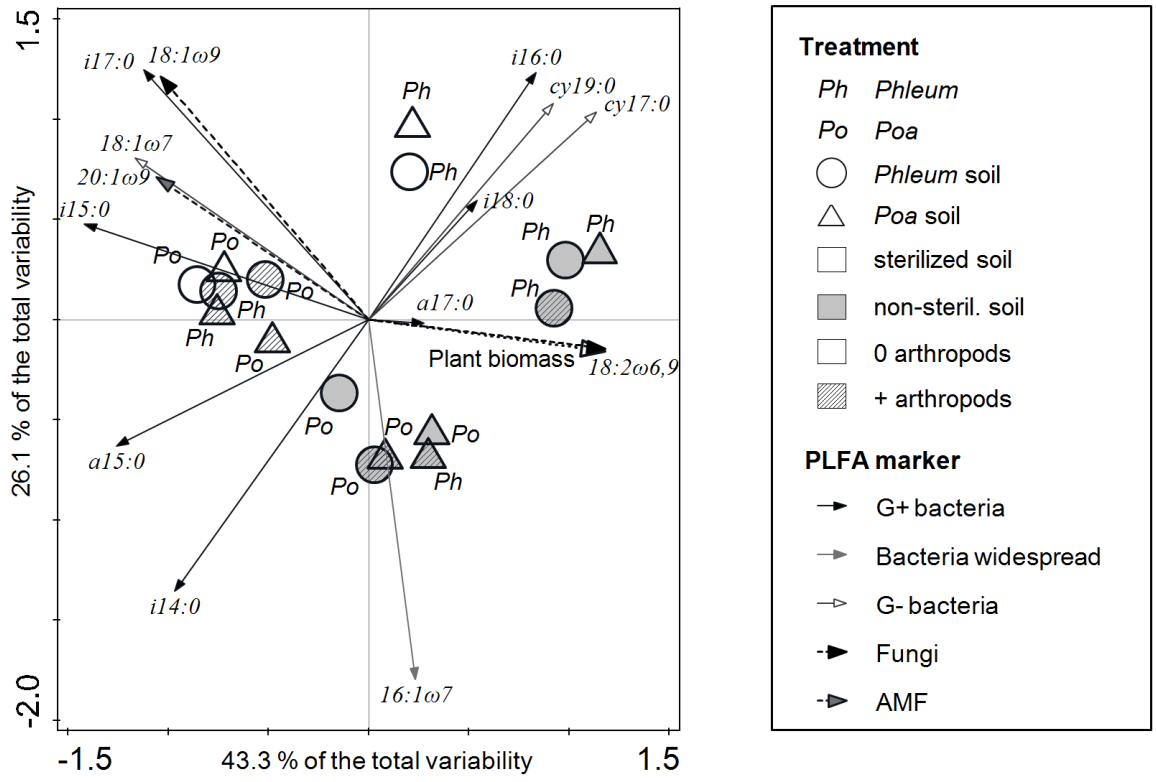

Figure 5. PCA of the composition of microbial communities after the feedback phase. Symbols represent individual experimental treatments (centroids of samples). Arrows indicate the individual PLFA markers.

Discussion

In this study, we show that the presence of soil microarthropods in a plant-soil feedback experiment can substantially alter the composition of soil microbial and nematode communities. More importantly and potentially as a consequence of these shifts in the microbial and nematode communities, the presence of microarthropods altered plant seedling establishment, belowground biomass, and biomass allocation patterns. The effect of microarthropods was plant species-specific and depended on previous soil conditioning.

The two plant species we used in our experiment exhibited contrasting plant-soil feedback effects (responded differently to soil conditioning treatments) as they did in the previous study of Cortois et al. (2016). However, although we detected significant plant-soil feedback effects on the biomass of plants at the end of the experiment, the seedling establishment rate was not significantly influenced. Similar differences between plant-soil feedback effects on seedling establishment and plant biomass have been described before (Yang et al. 2013, Münzbergová and Šurinová 2015), suggesting that in each of these life stages, plant performance may be influenced by different mechanisms. For instance, plant pathogens are often specialized to a certain life stage of a host plant (Agrios 2005), and several studies suggested that such specialization can be found also in mycorrhiza

(Zelmer et al. 1996, McKendrick et al. 2000). As a result, soil biota showing a certain effect at one life stage of a plant species might have a different impact at another life stage of the same species.

The two plant species were also associated with distinct microbial and nematode communities. The fact that plants facilitate specific communities of soil organisms (Westover et al. 1997, Marschner et al. 2001) is considered as one of the main drivers of the plant-soil feedback effects (Bever 1994, Klironomos 2002a). In our study, most of the soil samples from *Phleum* plants were associated with increased PLFA markers cy17:0, cy19:0 (gram-positive bacteria). Gram-negative bacteria are often faster growing than gram-positive bacteria, and their increased abundance in soils reflects higher nutrient availability and, generally, environments favouring r-strategists (Vries and Shade 2013). *Phleum* plants could thus facilitate gram-positive bacteria by higher production of root exudates compared to *Poa* plants that were generally smaller. Apparently, the bacterial communities responded to the plant species very quickly: in samples taken after the feedback phase, we could not detect any effect of original soil conditioning, but the effect of species grown in the feedback phase was very strong. By contract, nematode abundance and community composition of trophic groups at the end of the experiment were still affected by the plant species that conditioned the soil (conditioning phase). This difference in stability of these soil communities could be related to the different generation times of the respective organisms: while bacteria or fungi can produce multiple generations per day (Jousset et al. 2013), nematodes are slower, with generation turnover ranging from several days to months (Bongers and Bongers 1998). The composition of nematode communities could also be stabilized by other interactions with soil organisms, such as top-down regulation by the predatory mites. These differences in the stability of soil communities and, therefore different importance for plant-soil feedbacks, should be studied in future experiments.

Addition of the model microarthropod community in our experiment altered the composition of both nematode and microbial communities in the soil. The shifts in the soil communities were not systematic across all the treatments, but rather depended on interactions with the other experimental treatments. For example, microarthropods caused large shifts in PLFA composition in soils from *Phleum* plants, but not in soils from *Poa* plants. Microarthropods are generally known to influence the abundance and composition of microbial communities (Griffiths et al. 1999, Maboreke et al. 2017). The fact that their influence can vary, depending on plant species or soil conditioning, is an important finding for plant-soil feedback research. Interestingly, although the springtails we used in the present study are considered to be mostly fungivorous, they did not affect the mycorrhizal colonization of *Phleum* roots. However, previous studies showed both increases and decreases in arbuscular mycorrhiza colonization rates in the presence of springtails, depending on springtail densities (Bonkowski et al. 2000) or the availability of alternative resources (Schreiner and Bethlenfalvay 2003), suggesting that the impact of springtails are context-dependent. In contrast, the addition of microarthropods largely influenced composition of bacterial PLFAs, probably indirectly, via changes in resources

available to bacteria due to springtails' grazing on soil fungi and plant roots (Maboreke et al. 2017). The changes in microbial communities caused by microarthropods were reflected in their community composition rather than in the total biomass, since we did not detect differences in soil microbial biomass.

Microarthropod addition treatment not only affected soil microbial communities, but also had impact on plant performance in the feedback phase. Specifically, microarthropods altered seedling establishment, belowground plant biomass, and plant biomass allocation, with the effects depending on interactions with other treatments. Although it has been shown that springtails can feed on plant roots (Endlweber et al. 2009), our results suggest that herbivory was not the only determinant of plant performance in our experiment. Rather, it is very likely that microarthropods altered plant growth also indirectly by feeding on soil microbial or nematode communities, since we reported shifts in both microbial and nematode communities caused by microarthropod addition treatment. Springtails can, for example, influence plant germination by feeding on the fungal coat of the seeds (Mitschunas et al. 2006, 2008, Nietschke et al. 2011). Their negative influence on seedling establishment in the present study could thus be caused by springtails' feeding on mycorrhizal fungi (Klironomos and Ursic 1998). Feeding on the germinating seeds themselves is unlikely as this behaviour of springtails is considered very rare (Nietschke et al. 2011). Besides these effects, microarthropods can also alter the nutrient availability in the soil (Maire et al. 1999, Filser 2002). As plants are known to change their biomass allocation patterns according to the nutrient status of the soil (Glimskar and Ericsson 1999, Müller et al. 2000) and even according to the form of the nutrient source (Cambui et al. 2011), it is possible that the microarthropods impacted plant root-to-shoot ratio by altering soil nutrient availability.

We conclude that soil microarthropods can significantly alter plant-soil feedback effects. In our experiment, their presence altered the composition of soil microbial and nematode communities (i.e., the potential agents of plant-soil feedbacks) and plant performance. More importantly, their effect was highly specific as it differed between soil conditionings and was affected by prior soil sterilization. This illustrates that microarthropods do not impact plant-soil feedbacks systematically, but their effects can be context-dependent. Considering that soil microarthropods are significant members of soil biota, we propose that their role should be considered in future plant-soil feedback research.

Methods

Model species

We used two model plant species: *Poa pratensis* (*Poa* hereafter for brevity) and *Phleum pratense* (*Phleum* hereafter). The choice of these species was based on previous research in the framework of the Jena Experiment (Cortois et al. 2016), where they showed

opposing plant-soil feedback effects. This study found that *Poa* exhibited negative intraspecific plant-soil feedback effects (i.e., it grew worse in soil conditioned by individuals of *Poa* than in soil conditioned by individuals of other species). In contrast, *Phleum* showed a positive intraspecific plant-soil feedback effect (i.e., it grew better in soil conditioned by individuals of the same species than in soil of other species). Such opposing feedbacks could be driven by different mechanisms and thus could interact differently with added microarthropods. Seeds of both species were obtained from the same commercial provider (Rieger-Hoffmann GmbH, Blaufelden-Raboldshausen, Germany) as in Cortois et al. (2016).

As model soil microarthropods, we used one species of predatory mite (*Hypoaspis aculeifer*; Gamasida) and three springtail species (*Proisotoma minuta, Folsomia candida,* and *Sinella curviseta;* Collembola). The mite species is a generalist predator feeding on other soil microarthropods including springtails (Heckmann et al. 2007). The springtail species feed mostly on soil fungi (mycorrhiza as well as saprotrophic fungi), but they are omnivorous and can feed on a variety of other resources reaching from soil microfauna (such as Protozoa, Nematoda, or Rotatoria) and microflora (such as bacteria, and algae), to plant leaf litter or even living roots (Rusek 1998, Endlweber et al. 2009). In this study, we used a model community consisting of three springtail species to increase realism of the experiment: using a single species can lead to biased results due to specific feeding preferences of the model species (Hopkin 1997). Families of these three species (Isotomidae and Entomobryidae) can be found in the grassland where the experimental soil was obtained (Sabais et al. 2011). The springtail species were cultured on yeast in the laboratory. The mite species was obtained from a commercial provider (Schneckenprofi in Germany) and was also used in previous laboratory studies and shown to feed on the three springtail species (Thakur et al. 2015).

Plant-soil feedback experiment

Following common methodology in plant-soil feedback experiments (Kulmatiski et al. 2008), our experiment consisted of two phases: the conditioning phase and the feedback phase. To set up the conditioning phase, we used soil from a diverse semi-natural grassland adjacent to the Jena Experiment, Jena, Germany (Roscher et al. 2004). The soil has a pH value of 8.1, carbon concentration of 4.6%, nitrogen concentration of 0.3%, and a C-to-N ratio of 15.7; lime content of ~6%, a clay content of ~14%, a silt content of ~41%, and a sand content of ~45%. The two plant species were grown in monocultures in a climate chamber, each species in two 20 l mesocosms. The growth conditions were 12 hours of daylight, 25°C, and 70% humidity. The duration of the conditioning phase was 3 months. After that, plant shoots were harvested, and the soil was sieved with a mesh (size 5 mm) to remove plant roots. The soil of both mesocosms of each plant species was homogenized and sampled for further analyses. The approach of mixing soils after the conditioning phase has been used in other studies (Hawkes et al. 2013, Dostálek et al. 2016). It might be argued that such a treatment leads to artificially lowered variation in soil feedback effects among replicates. However, the main goal of this study was not to

measure effect sizes but to investigate the possible influence of soil microarthropods on plant-soil feedback effects, legitimizing the present approach(Cahill et al. 2016). Then, the soil was split in half, and one half of each soil type was sterilized by autoclaving to remove the specific soil microorganisms developed during the conditioning phase (twice, each 20 min at 120°C (Eisenhauer et al. 2009)).

The experiment for the feedback phase was set up in 300 ml microcosms (diameter: 7 cm, height: 10 cm) with a fine mesh at the bottom (50 µm) and a 10 cm plastic fence at the top of the round pots, both preventing soil fauna from escaping. Microcosms were filled with each of the pre-conditioned soil (further referred to as *Poa*-conditioned soil and *Phleum*-conditioned soil, respectively), both either sterilized or non-sterilized. Each of these soil types was crossed with further treatments: sowing of either *Phleum* or *Poa* seeds, and either an addition of soil arthropods or no addition of arthropods. With five replicates per treatment, the experiment resulted in 80 microcosms: 2 (soil conditioning) × 2 (soil sterilization) × 2 (species sown) × 2 (arthropod addition) × 5 (replicates).

Before sowing, all microcosms were repeatedly watered to leach nutrients released by the sterilization procedure (Eisenhauer et al. 2009). After two days of watering, 50 seeds of either *Phleum* or *Poa* were sown into each microcosm. Microcosms with microarthropod addition treatment received three individuals of the predatory mite and ten individuals of each springtail species (30 springtail individuals per microcosm in total, equivalent to 8000 individuals per square meter). The number of added springtails was expected to be sufficient to establish viable population in each microcosm: springtails are known to quickly reach population carrying capacity according to the available resources (Eisenhauer et al. 2011b, Sabais et al. 2012). We did not determine the densities of springtails before setting and after harvesting the experiment, but we expect the original numbers to be close to zero (due to large disturbances during the preparation of the conditioning phase) and the final numbers to be at the carrying capacity of each microcosm (Eisenhauer et al. 2011b).

The microcosms were kept in the climate chamber (for details see above) and were watered daily with 20 ml of distilled water. The establishing seedlings were counted every other day. After four weeks, the aboveground and belowground biomass of all seedlings was harvested, dried at 60°C, and weighed. Soil samples were taken from each microcosm after removal of plant roots: soil from the whole microcosm was homogenized and sampled for subsequent soil analyses. We also sampled plant roots for estimation of mycorrhizal colonization rates.

Analyses of soil communities

The soil samples taken after the feedback phase of the experiment were used to analyse the composition of soil microbial communities (5 g of fresh soil per sample, frozen at -20°C), nematode communities (25 g of fresh soil), and substrate-induced soil respiration (5 g of fresh soil) to determine active soil microbial biomass. We used root samples from the feedback phase to estimate the mycorrhizal root colonisation. In all cases, we used

five samples per treatment (i.e., from all replicates), except for only three samples per treatment for analysis of soil microbial communities (PLFAs).

Phospholipid fatty acids (PLFAs) were used as taxonomic markers for the quantification and classification of microorganisms (Ruess and Chamberlain 2010). Analysing PLFAs present in soil samples is an effective tool for studying the composition of soil microbial communities. Before PLFA extraction, soil samples were sieved with 2 mm mesh size to remove root and litter pieces. Lipid extraction followed the protocol by Frostegård et al. (2011). Therefore, 5 g fresh weight of soil was weighed and mixed with 18.5 ml Bligh & Dyer reagent (Bligh and Dyer 1959). Samples were shaken for 2 h to extract lipids from microorganisms. A centrifugation step with 1500 g for 10 min separated two phases. The upper organic phase was extracted and mixed with 6.2 ml chloroform and 6.2 ml citrate buffer. After centrifugation, 4 ml of the lower phase was transferred to a new tube. This organic phase was evaporated at 30°C under a nitrogen atmosphere. Silica-gel separation columns fractionated the lipid phase into phospholipid fatty acids by adding methanol which was again evaporated under a nitrogen atmosphere at 30°C. After evaporation, 30 ml of an internal standard (C 19:0), 1ml methanol/toluene, and 1ml methanol/KOH were added. The resulting fatty acid methyl esters (FAME) were heated for 15 min at 37°C. After adding 2ml hexanechloroform, 0.3 ml acetic acid, and 2 ml deionized water, the mixture was centrifuged and the upper phase was filled in a new tube and evaporated at 30°C under a nitrogen atmosphere. The evaporated extract was solved in 100µl hexane and filled into vials for analysis. FAMEs were identified by chromatographic retention time according to standard mixtures (Bacterial Acid Methyl Esters; methyl ester derivatives of a naturally occurring mix of bacterial fatty acids; Sigma Aldrich, St Louis, USA). Identification and quantification of PLFAs was performed by a gas chromatograph (SHIMADZU GC 17A) equipped with column DB 225MS (length: 60 m; diameter: 0.25 mm; film thickness: 0.25 mm) and hydrogen as carrier gas.The PLFAs detected in the samples were assigned to the major groups of soil organisms: gram-positive bacteria, gram-negative bacteria, saprotrophic fungi, and arbuscular mycorrhizal fungi (Ruess and Chamberlain 2010) (see Table S1 in Supporting Information).

Nematodes were extracted from 25 g of fresh soil samples (the average soil moisture was 13.3%) using a modified Baermann method (Ruess 1995). The extraction took 72 hours (Nijs 2013), which should allow even the slower nematode species to migrate from the soil sample and get trapped in the water. After that, nematodes were preserved in 4% formaldehyde. Nematodes were counted, identified to the family level (or genus, if needed for classification to trophic groups) following Bongers (1994), and classified to trophic groups (bacterial feeders, fungal feeders, plant feeders, omnivores, and predators) (Yeates et al. 1993). The mean number of nematodes extracted per sample was 139.2 (in the non-sterilized treatments). In each sample, we identified 100 individuals (or all nematodes present in the sample, if the total number did not exceed 100 individuals). We extrapolated the numbers of nematodes in each trophic group to the total nematode abundance in a sample and then expressed the numbers as individuals per gram soil dry weight.

We measured substrate-induced soil microbial respiration of 5 g of fresh soil using an O_2-microcompensation apparatus (Scheu 1992). After 24 h of adjusting basal respiration, we added 4 mg D-glucose g^{-1} soil dry weight as aqueous solution and determined the respiratory response to substrate addition. The mean of the lowest three readings within the first 10 h (between the initial peak caused by disturbing the soil and the peak caused by microbial growth) was assessed as the maximum initial respiratory response (MIRR; µl O_2 g^{-1} soil dry weight). Microbial biomass (µg C g^{-1} soil dry weight) was calculated as 38 × MIRR (Beck et al. 1997).

To determine the mycorrhizal root colonization, all fine roots from one microcosm were cut, pooled, and a new random sample was taken as a representative sample (Giovannetti and Mosse 1980) and stored in 50% ethanol until further processing. The samples were then cleared and stained with Trypan Blue. Therefore, root samples were carefully rinsed and cleared using 10% solution of KOH at 90°C for 60 min. Roots were washed again and acidified with 2% solution of lactic acid at 90°C for 20 min. Finally, the acidic solution was poured off, and the roots were put into 0.05% solution of Trypan Blue in lactoglycerol and heated at 90°C for 30 min. After staining, the remaining dye was washed out, and roots were stored in phials filled up with lactoglycerol. The mycorrhizal inoculation rates were determined using the gridline intersect method (Giovannetti and Mosse 1980) with 200 intersects per sample at 100× magnification. However, due to difficulties with distinguishing between arbuscular mycorrhizal fungi and other endophytic fungi in the roots of *Poa*, the mycorrhizal inoculation rates were determined only in *Phleum* roots.

Data analysis

We analyzed the data on individual plant measures using ANOVA with the main effects and all possible interactions of the four treatments, i.e., soil conditioning, sterilization, arthropod addition, and sown species. Prior the analyses, we checked the data for normality using QQplot. Further, we checked for correlations of the individual measures and as the total plant biomass was highly correlated with aboveground biomass, we excluded the total biomass from the analyses (see Table S2 in Supporting Information for the correlation matrix). Thus, we performed ANOVA for data on seedling establishment, aboveground and belowground plant biomass, root/shoot ratio, and microbial biomass. If significant differences were found, the means of individual treatments were compared by Tukey's HSD test. Both data on the total nematode counts and the mycorrhizal colonization of *Phleum* roots were not normally distributed: there were essentially no records of both measures in the sterilized soils, while the values in non-sterilized soils followed a normal distribution. Therefore, we used chi-square tests to analyze the effect of sterilization treatment on the presence of nematodes and the presence of mycorrhiza in *Phleum* roots. Values lower than 2 nematodes per sample (25 g of soil) and values lower than 2 mycorrhizal counts (out of 200 intersects) were treated as zeros. These zeros corresponded to the sterilized treatments. Further, we investigated the effects of soil conditioning, arthropod addition, sown species, and their interactions on nematode counts

in non-sterilized soils using ANOVA. We used ANOVA to test the effects of soil conditioning, arthropod addition, and their interaction on mycorrhizal colonization of *Phleum* roots in non-sterilized soils. All statistical analyses were performed using R 2.4.0 (R Development Core Team 2017).

To examine the influence of experimental treatments on the trophic composition of soil nematodes and PLFAs we used redundancy analysis (RDA) (Leps and Smilauer 2003). Here, arthropod addition was treated as an explanatory variable and the nematode abundance of each trophic group per gram dry soil (or PLFA measures) as dependent variables, respectively. We repeated this analysis to investigate the effect of sown species, soil conditioning, sterilization, and all possible interactions of these factors. In each analysis, the other main factors were used as covariates (in the case of higher-level interactions we used also all the lower-level interactions as covariates). For visualization of the data of both nematode trophic groups and soil PLFAs, we used principal component analysis (PCA) (Leps and Smilauer 2003). PCA is an indirect alternative of RDA searching for the main gradients in the data without explicitly considering the information on the different treatments. We used this method since it reveals the largest possible variance in the data and illustrates the composition of individual samples as well as their similarities. All the multivariate analyses were performed using Canoco 5 (TerBraak and Šmilauer 2012).

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Supporting information

Additional supporting information can be found for this article:

- Table S1: List of PLFAs and assigned groups of soil organisms.
- Table S2: Correlation matrix (Pearson's correlation coefficient) of all measured plant and soil characteristics.
- Figure S1. Seedling establishment as affected by the interaction of soil conditioning and sterilization treatment.
- Figure S2. Aboveground biomass of *Phleum pratense* and *Poa pratensis* as affected by the interaction of conditioning × sterilization × species.
- Figure S3. Belowground biomass as affected by the interaction of soil conditioning × species.
- Figure S4. Root/shoot ratio as affected by the interaction of soil conditioning × sterilization × species.
- Figure S5. Microbial biomass as affected by the interaction of conditioning × sterilization, and species × sterilization.

Table S1: List of PLFAs and assigned groups of organisms.

PLFA	marker
i14:0	G+ bacteria
i15:0	G+ bacteria
a15:0	G+ bacteria
i16:0	G+ bacteria
16:1ω7	bacteria widespread
i17:0	G+ bacteria
a17:0	G+ bacteria
cy17:0	G- bacteria
i18:0	G+ bacteria
18:1ω7	bacteria widespread
18:1ω9	fungal
18:2ω6,9	fungal
cy19:0	G- bacteria
20:1ω9	AM fungi

Table S2: Correlation matrix (Pearson's correlation coefficient) for all variables (or PCA scores in case of multivariate soil properties). Positive values mean a positive correlation of the two variables, negative values mean negative correlation. Bold values indicate significant correlations ($p<0.05$), values in italics and bold indicate marginal significance ($p<0.1$).

			Biomass							Nematode PCA scores		PLFAs PCA scores	
		Germination	Aboveground	Belowground	Total	Root/shoot ratio	Mycorrhiza	Soil respiration	Nematode counts	1st axis	2nd axis	1st axis	2nd axis
	Germination	1	**0.26**	**0.44**	**0.30**	**0.23**	0.04	**0.30**	0.01	0.03	-0.06	**0.40**	**0.49**
Biomass	Aboveground		1	**0.74**	**1.0**	**-0.47**	**-0.84**	**0.50**	**-0.64**	**-0.72**	-0.15	**0.75**	-0.13
Biomass	Belowground			1	**0.81**	***0.19***	***-0.31***	**0.24**	**-0.27**	**-0.31**	-0.13	**0.53**	***0.25***
Biomass	Total				1	**-0.39**	**-0.81**	**0.48**	**-0.61**	**-0.70**	-0.15	**0.75**	-0.08
	Root/shoot ratio					1	**0.72**	**-0.29**	**0.62**	**0.66**	0.10	**-0.34**	**0.52**
	Mycorrhiza						1	**-0.63**	**0.72**	**0.88**	0.15	**-0.68**	**0.45**
	Soil respiration							1	**-0.27**	**-0.31**	-0.02	***0.28***	0.10
	Nematode counts								1	**0.94**	-0.05	**-0.61**	**0.48**
Nem.	1st axis									1	-0.01	**-0.68**	**0.52**
Nem.	2nd axis										1	0.03	0.21
PLFAs	1st axis											1	0.01
PLFAs	2nd axis												1

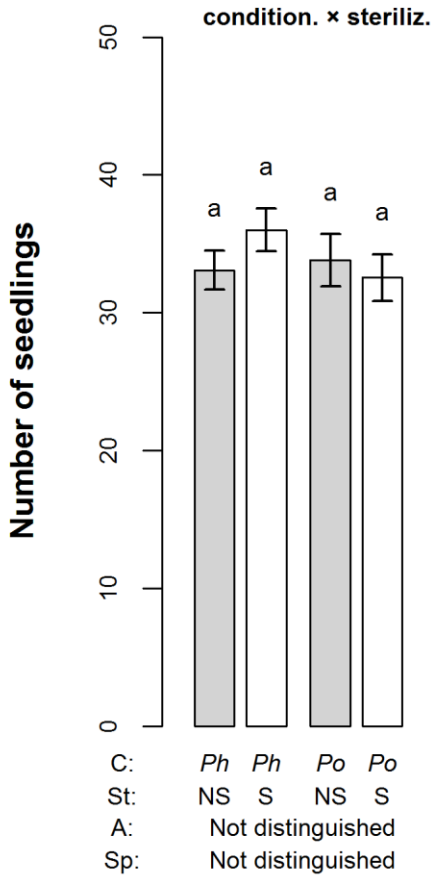

Figure S1: Seedling establishment as affected by the interaction of conditioning × sterilization. Shown are means ± SE. Bars with different letters differ significantly based on Tukey's HSD test ($p<0.05$). Grey bars represent non-sterilized soil, white bars sterilized soil. C=conditioning species, Ph=Phleum, Po=Poa; St=sterilization, NS=non-sterilized, S=sterilized; A=arthropod treatment; Sp=plant species in feedback phase; Not distinguished=this treatment is not distinguished in the analysis.

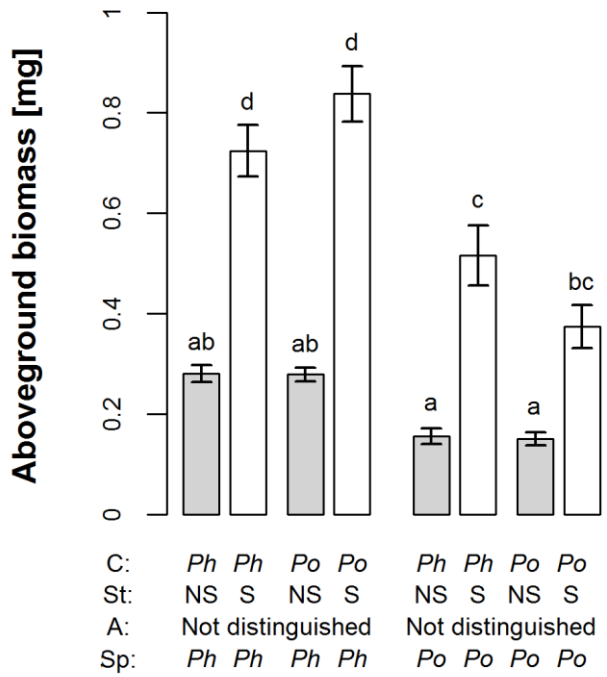

Figure S2. Aboveground biomass of Phleum pratense and Poa pratensis as affected by the interaction of conditioning × sterilization × species. Shown are means ± SE. Bars with different letters differ significantly based on Tukey's HSD test ($p<0.05$). Grey bars represent non-sterilized soil, white bars sterilized soil. C=conditioning species, Ph=Phleum, Po=Poa; St=sterilization, NS=non-sterilized, S=sterilized; A=arthropod treatment; Sp=plant species in feedback phase; Not distinguished=this treatment is not distinguished in the analysis.

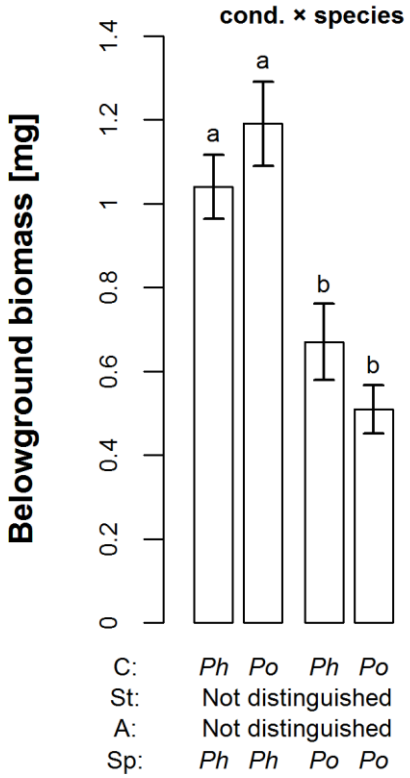

Figure S3. Belowground biomass as affected by the interaction of soil conditioning × species. Shown are means ± SE. Bars with different letters differ significantly based on Tukey's HSD test ($p<0.05$). C=conditioning species, Ph=Phleum, Po=Poa; St=sterilization, NS=non-sterilized, S=sterilized; A=arthropod treatment; Sp=plant species in feedback phase; Not distinguished=this treatment is not distinguished in the analysis.

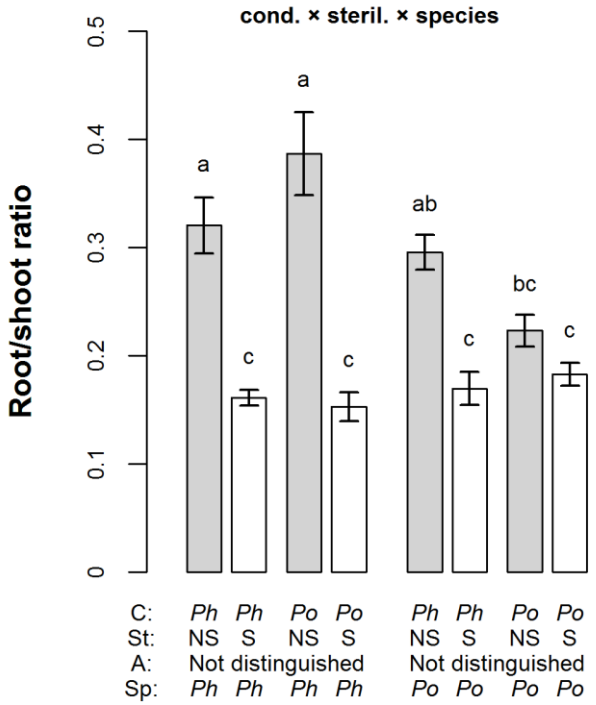

Figure S4. Root-to-shoot ratio as affected by the interaction of soil conditioning × sterilization × species. Shown are means ± SE. Bars with different letters differ significantly based on Tukey's HSD test ($p<0.05$). Grey bars represent non-sterilized soil, white bars sterilized soil. C=conditioning species, Ph=Phleum, Po=Poa; St=sterilization, NS=non-sterilized, S=sterilized; A=arthropod treatment; Sp=plant species in feedback phase; Not distinguished=this treatment is not distinguished in the analysis.

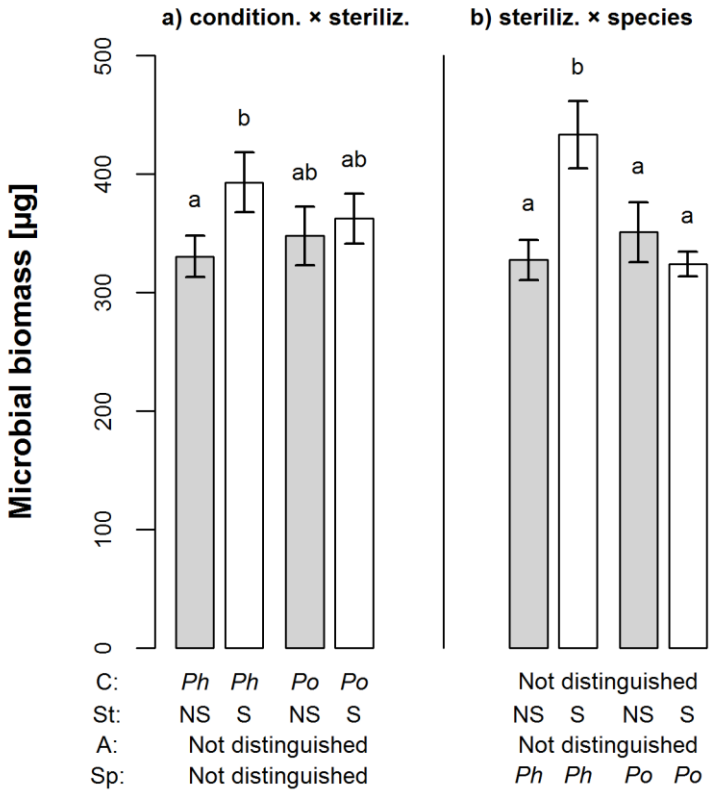

Figure S5. Microbial biomass as influenced by the interaction of a) conditioning and sterilization, and b) species and sterilization. Shown are means ± SE. Bars with different letters differ significantly based on Tukey's HSD test ($p<0.05$). Grey bars represent non-sterilized soil, white bars sterilized soil. C=conditioning species, Ph=Phleum, Po=Poa; St=sterilization, NS=non-sterilized, S=sterilized; A=arthropods; Sp=plant species in feedback phase; Not distinguished=this treatment is not distinguished in the respective analysis.

CONCLUSIONS

This thesis provides some insights into the role of plant-soil feedbacks in herbaceous communities, as well as their underlying processes. Using a meta-analysis, I found that plant species that share more positive heterospecific feedback are more frequently co-occurring in European communities, though only a small fraction of variance was explained. This suggests that though plant-soil feedbacks are likely influenced by many factors, such as abiotic conditions of the site, plant-plant competition, disturbances or herbivory, they still have some impact on structuring plant communities and influencing species coexistence.

The role of plant-soil feedbacks seems to be the most striking in simple systems, such as initial phases of vegetation succession. In such systems, only few plant species form the community and these species are often adapted to a certain phase of successional development. This is especially the case of primary succession where plants need to colonize previously unvegetated substrate that lacks developed soil profile, and the colonizing plants thus contribute also to the process of pedogenesis. I found that in primary succession, plant species can be facilitated via altered soil conditions by the species that precede them in the course of succession, mainly via improved abiotic conditions. In contrast, the decline of early-successional species in time can be also linked with their own negative conspecific feedbacks. In my study, these negative effects were caused by accumulation of pathogenic soil biota that was detrimental especially at the stage of establishing seedlings.

In more complex systems, the role of feedbacks is harder to disentangle. In herbaceous communities, plant-soil feedbacks are likely to occur on a small scale and to interact with soil feedbacks of the co-occurring plants. In my third study, I found that though the specific soil legacies can persis in the soil and influence even a second generation of plants, the subsequent plants modify the extent of this legacy. Interestingly, when I used soil conditioned by the whole grassland community with either presence or absence of a dominant grass, *Festuca rubra*, I could not detect the feedback effect of the dominant species. This illustrates that in species rich grasslands, plant-soil feedbacks are not likely to be easily predictable. Thus, further research should focus on the interactions of plant-soil feedbacks within individual communities, i.e. under conditions, where many species coexist and their plant-soil feedbacks may interact in complex ways.

Developing soil legacies are not only dependent on the plant species but also on the complexity of soil food webs. Due to methodological reasons, plant-soil feedback studies often exclude a large portion of soil organisms, especially meso- and macrofauna. In my fourth study, I showed that presence of model microarthropod community, consisting of three springtails and a predatory mite, can significantly shift plant-induced soil properties, as well as impact final plant responses to soil feedbacks. Future research of plant-soil feedbacks should thus consider the whole complexity of soil food webs.

I also showed that plant-soil feedbacks, though they might be altered by other factors, can influence plant fitness and play a role in plant species coexistence. Thus, soil feedback-related plant traits could be subjected to evolution. In my research, I found several pieces of evidence for that. Firstly, I found a relationship between plant species relatedness and heterospecific feedbacks. Interestingly, this relationship was only significant between closely related species: these species performed better in soils of heterospecific origin than in soil conditioned by conspecifics. This could indicate a sudden change in plant-soil feedback mechanisms at the moment of formation of a new species. Secondly, species from *Asteraceae* family were the most responsive species to soil feedbacks in the study on primary succession, and grasses and forbs developed distinct microbial communities in the study of the mountain grassland. Both these results point to phylogenetic patterns in plant-soil interactions. Finally, I found relationship between plant coexistence patterns and the strength of heterospecific soil feedbacks. This relationship can be caused either by plant-soil feedbacks being the structuring factor of plant communities, or by co-occurring plants being forced to develop more positive heterospecific interactions.

To conclude, my research contributed to the growing knowledge on plant-soil feedbacks and their role in plant communities but there are still a lot of questions unanswered. The complexity of soil environment brings many (methodological) challenges to researchers, especially if they focus on aboveground-belowground interactions. However, I believe that the research of plant-soil feedbacks forms a perspective bridge between plant and soil ecologist that can lead to our better understandings of these aboveground-belowground interactions.