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Plant secondary compounds in soil and their role in belowground species interactions

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Abstract

Knowledge about the effect of plant secondary compounds (PSCs) on belowground interactions in the more diffuse community of species living outside the rhizosphere is sparse compared to what we know about how PSCs affect aboveground interactions. We illustrate here that PSCs from foliar tissue, root exudates, and leaf litter effectively influence such belowground plant-plant, plant-microorganism, and plant-soil invertebrate interactions. Climatic factors can induce PSC production and select for different plant chemical types. Climate change can therefore alter both quantitative and qualitative PSC production, and how these compounds move in the soil. This can change the

soil chemical environments, with cascading effects on both the ecology and evolution of belowground species interactions, and ultimately soil functioning.

Plant chemicals mediate species interactions

Secondary compounds in plants are major contributors to the chemical diversity of nature. They are formed from **primary metabolites** (see Glossary) in specific pathways and omnipresent in plants. The distribution of **plant secondary compounds** (PSCs) is heterogeneous across the plant kingdom, and these compounds exhibit extensive variation both among and within species [1]. Though many PSCs also play pivotal roles in the plant's primary metabolic processes (such as growth or development), the large majority are known to serve as infochemicals mediating interactions between plants and their biotic and abiotic environments. PSCs can profoundly modify the abiotic environment as they are released into the atmosphere, soil, and water of an ecosystem, which in turn determines the community structure of the biotic constituents. For example, PSCs that accumulate in stems and foliage of the vegetation can promote bushfires, that profoundly alter the physicochemical properties of soil and groundwater [2]. Many PSCs are also involved in ecologically crucial interactions for plants, as they can form chemical barriers against pathogens and herbivores, attract pollinators and predators of herbivores, and help disperse fruit and seeds [3]. To fine-tune the release of PSCs, plants "eavesdrop" on their neighbours and increase their own defences when they sense volatiles released from neighbouring plants damaged by leaf herbivores [4].

PSC-mediated interactions between plants and their associated organisms are best-documented for aboveground compounds associated with airborne transport and signalling [3, 5, 6]. PSCs, however, are also omnipresent in the soil, where they mediate interactions with soil organisms and neighbouring plants. Compared to aboveground interactions, these soil PSCs represent a "hidden" link between organisms. Recent reviews of PSCs and belowground interactions [7-9] have focused on PSCs in root exudates and on species interactions, mainly in the **rhizosphere**. However, less attention has been given to the impact of PSCs on the broader and more diffuse community of associated species further away from roots, which also includes the effects of PSC from foliar tissue and litter decomposition. Climate change may alter both the quantitative and qualitative production of PSCs, which could cascade to affect belowground PSC-mediated community interactions. Given the vital role PSCs play aboveground, we expect PSCs to be equally important to belowground plant-organism interactions.

Here, we focus on four major groups of PSCs: terpenes, flavonoids, glucosinolates, and alkaloids (Table 1) that enter the soil matrix. We discuss their origin and fate in the soil and their ecological and evolutionary impacts on belowground interactions. Release of PSCs by plants alters soil chemistry, and neighbouring species can either tolerate, adapt to or escape the chemical environment this imposes on them. However, the production, release, and transport of PSCs by plants are greatly affected by local conditions, such as microclimate, microbes, herbivore and pathogen pressure. Therefore, changes in climate will affect the quantitative and qualitative production and release of PSCs both directly through changes in temperature and humidity but also indirectly through potential concomitant changes in herbivore and pathogen pressure. As the effects of PSCs on neighbour species are often both compound-specific and dose-specific, there is the potential for cascading effects of PSC-driven alteration of soil chemistry on belowground interactions. So far, however, very little is known regarding such cascading effects in plant-soil community interactions. To encourage more studies on this topic, we outline knowledge gaps and describe best practices for sampling and detecting PSCs in soil, to designate directions for future studies advancing this field of research.

Origin of PSCs in soils

How do PSCs enter the soil? Those formed in roots can be excreted directly into the soil from living and deteriorating tissues [10]. PSCs formed in aboveground organs are deposited into the soil from belowground organs following internal transport [11] or during wet and dry deposition from the atmosphere after volatilization and further chemical reactions [12-14]. PSCs can also be washed from vegetation surfaces with water intercepted during precipitation [15], or they may outgas and leach from fresh and decomposing litter on the soil surface and in the upper horizons [16, 17]. Many plant species accumulate large quantities of PSCs in secretory organs that are either external, such as trichomes, or internal, such as oil blisters or resin ducts, and a strong discontinuous release is expected during the decline and decomposition of these organs.

Concentrations of PSCs can be high and persistent in the soil. For instance, mono- and sesquiterpenes released from pine needles, litter and roots are detectable in forest soil throughout the year [18] and can attain high concentrations especially in topsoil but also lower mineral soil, being highest in late summer, and in the winter during snow cover [19]. Glucosinolates, produced by all cruciferous plants, are hydrolysed in the soil to highly bioactive compounds, notably isothiocyanate, nitriles, and thiocyanate. The degradation and hydrolyzation of glucosinolates depend on the presence of the extracellular enzyme myrosinase [20]. Glucosinolates are water-soluble and can more easily be washed out from soil, but isothiocyanates are hydrophobic and adsorb strongly to organic matter [20, 21], and may thus persist in the soil for periods of time. The isothiocyanate sinigrin, for example, is persistent in the soil of forests invaded by *Alliaria petiolata* (garlic mustard), where even low concentrations suppress germination and growth of arbuscular mycorrhiza. This suppression disrupt the mycorrhizal association with native plant, negatively affecting the native plant's resource uptake [22]. The persistence and slow degradation of many PSCs in soil emphasize the ecological and evolutionary potential pressure PSCs can impose on belowground species.

PSC production under climate change

The production of PSCs improves the ability of plants to cope with different abiotic [23] and biotic stressors [5, 6]. Various abiotic factors, such as CO_2 and temperature, affect PSC release by up- or downregulating their production, although the specific PSC compounds that are affected may differ among factors. For example, in northern hemisphere trees, high CO_2 levels generally increase the foliar concentration of phenolic compounds such as flavonoids and tannins, and decrease the production of terpenoid compounds, whereas elevated temperatures lead to the opposite trend [24-26]. Drought can both increase and decrease PSC production dependent on the severity of drought and on the compound. Terpenoids and phenolics in temperate and Mediterranean trees and herbs increased under moderate drought stress but decreased under severe drought [25, 27, 28]. Although severe drought overall decreased total terpenoid emission, some specific mono- and sesquiterpenes either increased or remained unaffected, suggesting an adaptive role of specific compounds in mitigating abiotic stress [27, 28].

In addition to environmental effects on the production of specific compounds or compound classes, genetically determined variation in the PSC chemical phenotype (hereafter referred to as chemotype) is present in many species. For example, a strong genetic basis for variation in the identity of leaf chemical compounds has been demonstrated for terpenes produced in oak and pine trees [29] [30, 31], aromatic Lamiaceae [32], Asteraceae [33], for glucosinolates produced in the Brassicaceae [34], and for flavonoids in Berberidaceae [35], illustrating that genetic variation for these compounds are present in a wide range of plant families. Thus the (a)biotic environment can shape which compounds are released to the environments not only through differential induction of PSCs, but also by natural selection, favouring genotypes producing specific chemotypes (Figure 1)[36]. Differences in climatic conditions and herbivore pressure favour specific chemotypes both within and among species [29, 37, 38]. A recent example of evolutionary changes in PSC production in response to climate change is that of wild thyme (*Thymus vulgaris*), a small shrub widespread in the Mediterranean Basin. The dominant monoterpene in thyme is genetically determined to be a phenolic or a non-phenolic type. These two chemotypes are ecotypes with adaptation to warm dry summers and early winter frost, respectively [39]. The lack of very cold winters in the last 25 years is associated with a significant increase in phenolic chemotypes in regions that were previously dominated by non-phenolic types [38]. In addition to differences in their ability to tolerate frost and drought, the toxicities of phenolic and non-phenolic chemotypes differ towards associated plants, microorganisms and herbivores, suggesting that climate-driven changes in the genetic composition of this widespread species may have cascading effects on associated species interactions.

A correlation between variation in PSC production and environmental variation holds for many plant species, but we need more long-term experimental studies to understand whether current and future changes in climate and herbivore pressure will result in major induced or genetic changes in PSC production. If so, cascading effects of such changes are expected for belowground species interactions and decomposition of organic matter and ultimately nutrient cycling in soil [25, 40]. Moreover, climate change may also affect the concentration of PSCs in soil by altering soil-water content, temperature, and pH. As a result, the levels and circulation of liquid and gas among soil pores (see below) will change and hence the movement and diffusion of PSCs.

Diffusion of PSC in soil

In contrast to atmospheric PSCs, volatile PSCs in the soil can form stable concentration gradients due to the lack of air turbulence. The diffusion of PSCs in soil depends on their physicochemical properties (Table 1) and the texture of the soil matrix. Fine-textured and clayey soils facilitate the flow of volatile PSCs that are diluted in smaller air volumes due to the smaller pores of these soil types. This generates gradients that are steeper and propagate faster than those in soils with large air spaces. In well-ventilated soils, though, PSCs will rapidly fill large air volumes, albeit at lower concentrations.

PSC transfer in soil is also affected by adsorption to the mineral and organic surfaces of soil particles, whose fractions depend on soil type and layer, with A horizons (topsoil) generally containing more organic material than the underlying B horizons (subsoil) [41, 42]. PSCs will adhere to and dissolve on lipo- and hydrophilic surfaces during diffusion, thus forming intermediate pools in equilibrium with the gas-phase concentrations [43, 44]. Soil organisms, therefore, receive PSCs more slowly and with an attenuated amplitude compared to the actual variations in the emission strength of the PSC source.

Abiotic factors such as temperature, soil moisture, and pH interfere with the persistence and diffusion of PSCs in soil. The adsorption of volatile PSCs by the mineral and organic fractions of soil depends on pH, with higher adsorption in alkaline than acidic soils [45]. The acidity of soil-pore water can affect the uptake of volatiles on aqueous surfaces, e.g. by protonating highly reactive sesquiterpenes that are otherwise poorly soluble in water [46]. Periods of rain and drought change the movement patterns of PSCs through soil, with opposite behaviours expected for volatile and non-volatile water-soluble PSCs (Table 1). During rainy weather, volatile PSCs are first concentrated in the soil pores during water penetration and then move with air bubbles to the surface [47], whereas non-volatile water-soluble PSCs are diluted and transported away from their sources [48]. High levels of soil moisture also affect the availability of oxygen for roots and soil microorganisms, eventually generating anaerobic conditions, which influence both plant PSC-producing and microbial PSC-decomposing metabolisms [45, 49]. In contrast, drought may decrease the concentration of volatile PSCs in the soil due to the increase in the volume of air in the soil and better ventilation at the soil surface. Sesquiterpenes, for example, emitted by maize diffuse faster and farther at low moisture levels [50].

Climate change can deeply impact the movement and lifetime of PSCs in soils, either by affecting abiotic drivers directly or by affecting the macro- and micro-biotic communities involved in the liberation, processing and transport of PSCs [51] (see also section “Plant-soil microorganism interactions” below). For example, increasing soil temperature will enhance PSC diffusion and exchange rates and alter state and partitioning equilibria between surfaces. Extreme weather events such as thunderstorms and drought events are expected to increase in both frequency and intensity, which will amplify the asymmetric transport and concentration build-up of polar/non-polar PSCs in soils. Determining the presence, and the fate of PSCs in soil is an under-researched area but is highly relevant to evaluate their ecological and evolutionary impact on the belowground interactions discussed below. Understanding the main environmental factors driving these processes is necessary to assess how climate change will alter belowground interactions mediated by PSCs (Figure 1). Different methods may be needed for different types of PSCs, depending on their solubility and volatility (see Box 1, Table 1).

Ecological and evolutionary impacts of PSCs on belowground species interactions

Similar to the functions of PSCs aboveground, key organismal interactions in soil, i.e. plant-plant, plant-soil microorganism, and plant-soil invertebrate interactions, are also mediated by PSCs. The identity, concentration, and composition of PSCs belowground play pivotal roles in species interactions and community structuring, and ultimately affect entire ecosystem processes.

Plant-plant interactions

PSCs mediate both competitive and facilitative interactions among plants. **Allelopathic** effects of PSCs released by a focal plant to the local soil environment can inhibit germination and growth of competitors giving the focal plant a competitive advantage for space and nutrients [52]. The "Novel Weapons" hypothesis [53] is based on the idea that invasive plants release **allelochemicals** which the native community has not previously encountered and that give the invader a competitive advantage. Allelopathic effects of PSC have helped some invasive plant species to create virtual monocultures in the habitats they invade. For example, production of **phytotoxic** phenolic acids, and di-, and sesquiterpene lactones likely aided the invasive success of Canadian goldenrod *Solidago canadiensis* [54, 55] and yellow star thistle *Centaurea solstitialis* [56]. Allelopathic effects are often less dramatic within the native ranges of plants because coexisting species have evolved a tolerance to local PSCs, or the ability to detoxify them [57].

PSCs released to the soil by one plant species may alter competitive hierarchies among neighbouring plant species both directly when PSC inhibit growth of one species more than another, and indirectly by PSC modifying the soil nutrient availability that favour some species over others. For example, pine soil altered plant competition between spotted knapweed (*Centaurea stoebe)* and different grass species in favour of grasses. Soil containing pine litter had a higher content of phosphorus (P), and the higher availability of P made the grasses more competitive against *Centaurea* [58]. Phenolic compounds and terpenes influence soil nitrogen (N) cycling as these compounds inhibit nitrification and the net N mineralization [59]. This could differentially favour plant species that differ in their preference for nitrate vs ammonium [60].

PSC mediated plant-plant interactions also facilitate neighbour plants via so-called associational resistance [61] where neighbour plants benefit from reduced attack by herbivores and pathogens due to the deterrent effects of the PSCs released by a focal plant. However, the PSCs in soil may also increase susceptibility to herbivores and pathogens if these plant enemies are attracted rather than repelled by the PSC [52, 61]. How these selective forces are balanced in the multispecies networks present in a natural field needs further study.

PSCs can vary among species, genotypes within species, and damaged and healthy tissue, so they contain information about the identity and state of the emitting plant [62]. Plants may therefore use PSCs as a cue to recognize their neighbours and prepare for the competitive encounters by adjusting their competitive growth towards or away from the neighbouring plant [63]. Root exudates and the chemicals they contain have been shown to mediate recognition of neighbour plants [64-66]. The competitive responses to such PSC-mediated neighbour recognition may depend on the history of co-existence between interacting plants [67], and for some plant species also on the genetic relatedness among interacting conspecifics [64, 68]. However, we lack knowledge on the identity of which molecules in root exudates that mediate plant recognition and how plants perceive them. This knowledge is relevant in order to understand if environmental changes in the soil may interfere with plants ability to detect their neighbour.

Spatial consistency of PSC production allows PSCs to act as selective agents on other species and favour the genotypes that respond most beneficially to the effects of frequently encountered PSCs. For example, the grass *Bromus erectus* is a frequent neighbour of *T. vulgaris*, which releases either phenolic or non-phenolic monoterpenes to the local soil environment. *Bromus* plants originating from sites where their thyme neighbours produce a non-phenolic monoterpene germinate and grow better in soil rich in non-phenolic thyme monoterpenes compared to similar soil rich in phenolic thyme monoterpenes [69]. Hence, plants can adapt to the chemical environment imposed by frequent neighbours, and this adaptation is highly compound-specific. Changes in the consistency of frequently encountered PSCs could disrupt such adaptive responses.

From pairwise plant-plant interaction to communities

The PSCs released to the soil from different plant genotypes and species create a mosaic of soil chemical environments. Because different chemical environments favour different species due to the pairwise competitive and facilitative interactions described above, variation in chemical types both within and among populations can contribute to local and regional species richness. For example, the chemical diversity of Scots Pine (*Pinus sylvestris*) needle terpenes was positively correlated with plant species richness in the vegetation under the trees, and trees with different needle chemistry associated with a different plant species composition [70, 71]. Chemical diversity of *T. vulgaris* also correlated with plant species richness and composition of the vegetation around thyme plants. Plant species richness was higher, and species composition more similar around thyme plants, than in thyme-free area only few meters away. However, species richness and species composition differed in area with the different thyme chemical types [72]. These examples suggest that the chemical types of dominant plants have an "extended phenotype" [73, 74] where the chemicals released from a focal plant can shape the surrounding vegetation. However, we need more experimental manipulation studies investigating the consistency of the effects of variation in PSC released by foundation plants on richness and composition of associated plant communities. These studies will allow us to better understand cause and effect of variation in PSC and to predict the potential cascading effects of qualitative and quantitative changes in PSCs.

Plant-soil microorganism interactions

A large proportion of the nutrients present in the soil are not immediately available for plants but require enzymes to degrade compounds into usable parts, and soil microorganisms are major suppliers of these enzymes [80]. In addition, soil symbionts such as mycorrhizal fungi and rhizobacteria are important regulators of plant productivity especially in nutrient poor ecosystems, where they are responsible for 5 to 75% of nitrogen and up to 80% of phosphorous acquired by plants annually [81] The importance and complexity of the interactions between plants and soil microbes have been acknowledged for a long time, but only more recently it has been appreciated that many of these interactions are mediated by PSCs [82]. Given the importance of such interactions, it is likely that plants have evolved strategies to recognize friends from foes and to harness useful partners across distances. For example, flavonoids produced by legumes have a fundamental role in attracting rhizobia and promoting the production of Nod-factor molecules, which are critical for nodulation [83]. In this process, both plants and microbes produce chemical signals that allow the plant to recognize the rhizobia as a friend and prevent the activation of immune responses upon initiation of the symbiosis [84]. When rhizobia are not found in the proximity of the rhizosphere and distances between the roots of individual plants and their symbionts are too large to be covered by root exudates, legumes can resort to the help of an intermediary to carry the symbionts to them [83, 85]. The production of volatile PSCs by the legume *Medicago truncatula* attracts individuals of the free-living nematode *Caenorhabditis elegans*, which harbours the rhizobium *Sinorhizobium meliloti* either on its cuticle or in its intestine. Isoflavonoids produced by the plant prime the rhizobium to initiate the symbiosis when the nematode is near the roots [75].

Not only can plants affect the composition of specific soil microbial communities via PSC, but they can also alter it when exposed to stress such as herbivore attacks [82]. In many plant species, the presence of pathogens or root feedings fungi corresponds with a shift in the soil microbial composition close to the rhizosphere. This is a consequence of both the production of specific antimicrobial compounds by the plant rhizosphere and of recruitment of favourable bacteria [86] The blend of PSCs produced by the graminoid *Carex arenaria* shifts toward a higher concentration of terpenes and terpenoids [59] to attract specific bacteria with antifungal properties when the roots are infected with the pathogenic fungus *Fusarium culmorum* [87]. Bacteria successfully rallied by the plant can gain from this interaction via the additional nutrients obtained by feeding on the plant pathogens [87]. Recent studies compared wild types of *Arabidopsis thaliana* with mutants that are unable to synthesize PSCs involved in induced systemic resistance against soil pathogens. Metagenome analysis of root microbiome associated with wild types *vs.* mutants showed substantially different community composition, indicating the important role of PSC in modulating the composition of root microbiome [76].

PSC-induced shifts in the composition of soil microbiota can also be activated by attacks to plant leaves. For example, infestations of white flies (*Bemisia tabaci*) in pepper plants lead to a change in belowground microflora enhancing anti-pathogen responses [88].

The interactions between plants and soil microorganisms are not unidirectional, as soil microbes can also emit chemical compounds that can be detected and used by plants. For example, secondary compound emissions from soil microflora may help the woodland strawberry, *Fragaria vesca*, to detect nutrient-rich patches prior to rooting. Stolons can preferentially develop toward substrates emitting unbranched fatty acids and avoid substrates with higher concentrations of terpenes and aldehydes [89].

Many PSCs are used by bacteria and fungi as a source of nutrients [90, 91], and soil microorganisms can alter and repurpose the PSCs released by plants. In the legume *Lotus japonicus,* the inoculation with its symbiont *Mesorhizobium loti* leads to a change in the profile of the plant phenolic compounds [60]. The soil bacterial community associated with vetiver, *Chrysopogon zizanioides*, uses the sesquiterpenes produced by the grass as a source of carbon, and after metabolizing them releases several compounds from vetiver oil [63]. Colonization of roots by arbuscular mycorrhizal fungi can increase the production of glucosinolates, phenolic acids, and flavonoids in several plant species [64, 65].

On a large scale, the ability of soil microbial communities to process and degrade plant secondary metabolites can have important consequences on plant communities and ecosystem stability. For example, soil microorganisms can either favour or hinder the proliferation on invasive plant species, depending on their ability to degrade the allelopathic compounds produced by invasive plants [92].

A high species-specificity of plant-microbe interactions suggest that single plants can shape their soil bacterial communities, and microorganisms can respond to subtle genetic variation in plants. In cottonwood trees *Populus angustifolia*, 70% of the variation in soil microbial community composition was explained by plant genotype identity [77].

Although there is still much that we need to understand about the complex interactions between PSC and roots microbiome, progress is being made in elucidating the factors that drive the composition of root inhabiting bacterial communities [78, 79]. Future steps will need to go beyond pairwise interactions between plants and a specific guild of soil microorganism, targeting instead how whole communities of soil microorganisms are affected by PSC and in turn affect plant communities. Climate change may mediate shifts in plant-soil microorganism interactions via shifts in the chemical profile of plants and soil microorganisms. Such studies are highly valuable as the positive effect of certain plant-soil microorganism interactions can be harnessed to either enhance agricultural practices or as biocontrol agents. Understanding how to engineer beneficial soil communities for plant community conservation, sustainable agriculture and preservation of native communities require extensive experimentation and in depth understanding of potential unintended consequences of such manipulations.

Plant-soil invertebrate interactions

Many of the interactions between plants and soil invertebrates are mediated by PSCs, often with significant effects on soil processes that determine soil function and plant productivity. Two major ways can be distinguished through which PSCs mediate the interactions between plants and soil invertebrates. First, soil organisms can be exposed to substantial input of PSCs from aboveground plant tissues, through PSCs leaching from foliar tissue and PSC-rich litter deposition. These PSCs are mostly known as feeding repellents, and many of them with toxic effects to aboveground insect herbivores [80], and the available evidence indicates that they also have toxic effects on non-target organisms such as soil invertebrates [81, 82]. Toxic effects can occur if PSCs diffuse across the cuticle or if animals ingest PSC-rich litter, with the main route of exposure determined by cuticle morphology and diet of the soil invertebrate and by the chemical properties of the PSC (see Table 1 for mode of toxicity). Nonpolar (lipophilic) volatile PSCs likely enter animal bodies primarily by simple diffusion across the cuticle and easily partition into cellular membranes [81]. High concentrations of nonpolar PSCs can occur regularly in soil, especially in older litter and near roots, as is the case for terpenoids (1-5 mg g^{-1} fresh weight) [2]. The likelihood of exposure is increased because terpenoids will remain in the litter layer for several months until fully decomposed [83]. Exposure to polar PSCs is mainly through ingestion of litter or roots, for example in case of isothiocyanates. Isothiocyanates have a high toxicity towards non-target soil invertebrates, including beneficial macro-detritivores that promote nutrient cycling and soil fertility [84]. Currently, the relative importance of the two exposure routes, either via contact with volatile or water dissolved PSCs in pore water and diffusion across the cuticula or via ingestion of PSC-rich litters to soil fauna and how this affects soil fauna functional community composition is poorly known.

An unexplored aspect of the lipophilic PSCs is that their spread can be facilitated by animal transport. Soil animals can accumulate high internal concentrations of these PSCs in the vicinity of roots and litter, and subsequently, deliver them to other soil microenvironments with lower chemical activity when they move away from the PSC source. Aquatic Protozoa have demonstrated such animal-facilitated transport [85], but more research is needed to explore this phenomenon mediated by soil animals. In order to explore the toxicity of PSCs to soil invertebrates under field conditions, more precise analytical determinations of concentrations of PSCs and their degradation products in the environment are needed. This necessitates new experimental systems of exposure, e.g. by using the framework of chemical activity for volatile compounds [81].

The second way in which PSCs can mediate plant invertebrate interactions in the soil is by targeted release of PSCs from the roots. These PSCs can attract or deter soil invertebrates and serve as chemical signals released by the plant to control trophic interactions with plant-parasitic nematodes, root herbivores, and their predators. The use of PSCs in tri-trophic interactions between plants and belowground organisms may be similar to the extensively studied chemically mediated interactions between plants, foliar herbivores, and their natural aboveground predators. Attack by root herbivores has been shown to induce a change in the quality and quantity of PSCs released by plant roots, which can then function as a signal to attract soil-dwelling predators and reduce the pest population [9, 86, 87]. Entomopathogenic nematodes often function as indirect defences for plants [88, 89]. For example, larvae of the longhorn beetle *Tetraopsis tetraophthalmus* feed on the roots of the common milkweed *Asclepias syriaca* which releases volatiles into the soil that attract entomopathogenic nematodes, preventing the loss of plant biomass to herbivory [90]. Maize roots increase sesquiterpene exudation when attacked by insects, thus attracting entomopathogenic nematodes that feed on root herbivores [91]. However, notwithstanding this potential protective role of PSCs via attraction of beneficial nematodes, these PSCs can also attract the root herbivores themselves. The net effect of the attraction of both advantageous as well as detrimental nematodes on plant fitness has to be evaluated. Most examples of tri-trophic interactions belowground involve entomopathogenic nematodes, but one of the few papers that reports on other soil fauna in tri-trophic interactions is a case in which tulip bulbs infested by the rust mite *Aceria tulipae*, emits volatile signals to the predatory mite *Neoseiulus cucumeris* [92]. Given the importance of chemical cues in belowground signalling, the effect of PSCs on tri-trophic interactions is expected to be omnipresent, but currently, we do not know enough about the spatial dimensions of volatile and water-dissolved PSCs effects on plant-root herbivore or parasite-herbivore interactions [93]. Abiotic gradients in soils are rather short and steep compared to aboveground systems, but currently, we are ignorant of the spatial dimensions of volatile and water-dissolved PSCs effects on plant-root herbivore or parasite-predator interactions.

PSCs and belowground co-evolutionary interactions

The examples above demonstrate the different vital roles that PSCs play in belowground plant-plant, plant-soil microorganism, and plant-soil invertebrate interactions. Some of these interactions are examples of the evolutionary adaptation of species to frequently encountered PSCs. Except for interactions specific to the rhizosphere, no studies have yet clearly shown whether adaptations of organisms to specific PSCs reciprocally alter PSC composition and production in plants, i.e. if PSC-mediated belowground interactions result in pairwise or diffuse co-evolution. Understanding if belowground interactions are a source of selection on the chemical variation of PSC-producing plants is not straightforward because of the unspecific nature of the interactions. Belowground interactions often consist of multiple interactions, therefore, estimating the consequences for fitness in PSC-producing plants is difficult. Also, for PSCs entering the soil via leaf leachates, the main selective force determining which PSCs are produced and in what concentration may largely depend on aboveground biotic interactions and local climate. In addition, the multifunctionality of PSCs extends beyond biotic interactions, which is a further complication in the evaluation of fitness consequences in PSC-producing plants. For instance, maize produces benzoxazinoid compounds not only as a defence against generalist herbivores but also as chelating agents to acquire iron as a micronutrient. The larvae of western corn rootworm *Diabrotica virgifera*, are not injured by benzoxazinoids, but this specialist herbivore instead takes advantage of the presence of complexes between iron and these PSCs not only to detect the plant but also forage on these complexes [94].

Climate-induced shifts in the release of PSCs into the soil (Figure 1) may alter the outcome of within- and between-species interactions in ways that we still cannot predict. Long-term studies and the development of ecometabolomics [95] for elucidating the responses of PSC changes in plant communities due to warming and altered precipitation from climate change are particularly warranted for a better understanding of, and ability to predict, these shifts in PSCs production, diffusion in soil, and effects on belowground interactions.

Concluding remarks and perspectives

Soil is a theatre of facilitation, symbiosis and warfare deployed by plants and the various organisms living in it, and PSCs plays a major role mediating many of these interactions. Plants and soil organisms have adapted to withstand, detoxify or use the cocktail of PSCs originally meant to harm some of them. Understanding PSC mediated relationships at community scale and identifying the compounds involved in these interactions is therefore important for better insight into the functioning of these systems and their evolution, especially in changing environments. High-throughput technologies are increasingly becoming available and may help to identify and determine the concentrations, transport and modes of action of PSCs in soils. We encourage the creation of open, community-wide, curated, labelled, broad-spectrum PSC data sets across plant species and soils, as this would greatly increase the transfer of knowledge between scientist studying plants, microbes, and invertebrates in this biological belowground theatre. This could be a platform similar to, e.g. the TRY plant trait data base (http://www.try-db.org) or PSC data sets could be added and incorporated to such established well-functioning platforms. The warranted study of the quantitative and qualitative effects of climate change on PSCs and their interactions with the environment and organisms would also benefit from these PSC data sets. The alterations of the production of PSCs due to climate change are highly variable, depending on dose, timing, PSC, and species. These changes in the production of PSCs can lead to unforeseen consequences for soil structure and function and can disturb biological feedbacks on soil chemistry and biology, perhaps even on atmospheric chemistry and climate [96], with a direction and intensity that warrants in-depth investigation.

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BOX 1. Sampling of PSCs for identification and quantification.

The heterogeneous chemical and physical nature of soil creates spatial differences that complicate representative sampling. In addition, PSCs possess a high diversity of functional groups, volatilities, and polarities. Their belowground presence can thus be in air, in pore water, or adsorbed to solid matter. Representative sampling may therefore require a combination of sampling techniques.

Sampling techniques for PSCs in soil include extraction (solvent or solid phase), head-space analysis, and purge and trap techniques [7].

Solvent extraction relies on the principle of "like dissolves like". Polar compounds are thus extracted in polar solvents (e.g. water, methanol) and nonpolar compounds in nonpolar solvents (e.g. n-hexane, dichloromethane). Several hundreds of grams of soil, however, may be needed for the representative sampling of PSCs with low concentrations in soil [109]. Dilution beyond detection limits may occur, so subsequent sample concentration by solid-phase extraction (SPE) or evaporation (with the risk of loss of volatiles) may be necessary. Emission of PSCs directly from roots have been investigated using primarily solid-phase micro extraction (SPME) or adsorption tubes (with e.g. Tenax) coupled with GC-MS analysis in a number of studies as reviewed in [7].

Solid-phase micro extraction (SPME), head-space analysis, and purge and trap methods avoid the dilution effect of solvents but are more selective in the compounds sampled. SPME relies on the equilibrium between a fibre coated with a sorbing material and most often the gas phase (potentially also water or solid phase). This technique allows in-situ sampling for evaluating small-scale spatial differences. The sorbing material determines the selectivity of the compounds sampled [110], whereas soil type, water content, and pH influence the equilibrium. Stir-bar sorptive extraction (SBSE), where a magnetic rod is coated with sorbing material, may provide up to 1000-fold higher sensitivity but requires thermal desorption for subsequent instrument introduction. SBSE is useful for extraction from water, as demonstrated for many organic pollutants [111]. Identifying the mode of transport of PSCs (i.e. in gas or water phase) could be accomplished by sampling the soil gas phase using SPME and subsequently washing the soil and extracting using SBSE.

Collecting only head spaces using an air-tight syringe is a potential way to sample only volatile PSCs (i.e. VOCs). The concentration of VOCs from soil, however, may be <10 $\mu g\ m^{-3}$, which is below the detection limit for head-space analysis. This limitation may be overcome with purge and trap methods recommended for analysis when concentrations are <200 $\mu g\ kg^{-1}$ [112], which have recently been applied for sampling isoprene, monoterpenes, and sesquiterpenes in forest soil [19].

Glossary box:

Allelochemical is a compound produced by an organism that can have a detrimental physiological effect on individuals of another species when released into the environment.

Allelopathic effect is the interference effect of chemical compounds released by a plant on the growth and performance of other plants. An allelopathic effect usually implies a direct negative (harmful) effect, but some definitions also include positive effects.

Narcotic effect refers to the unspecific effects of lipophilic organic chemicals on membrane fluidity. These chemicals tend to partition into the lipid compartments of the organism, causing (nonpolar) narcosis or baseline toxicity.

Phytotoxic refers to chemical compounds toxic to plants, including any adverse effect on plants such as delayed seed germination, inhibition of growth, death, or destruction of plant tissue.

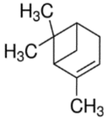
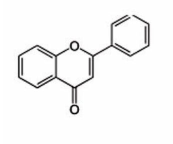
Plant secondary compounds (PSCs) are organic compounds typically formed from primary metabolites in specific pathways. Some PSCs are involved in plant's primary metabolic processes, but the large majority of these are known to serve as infochemicals mediating interactions and are important for plants to survive in the environment, e.g. by their repellent, attractant, or toxic effects on other organisms.

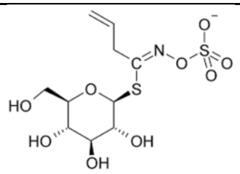
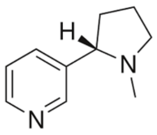
Primary metabolites are compounds from metabolic pathways present in all plants and are essential for growth, development, and reproduction. These metabolites comprise amino acids, nucleic acids, peptides, various carbohydrates, and lipids.

Rhizosphere is the microecological zone of soil in direct proximity to plant roots and includes the organisms in that soil that are directly affected by root exudates.

Legend to Figure 1. Direct and indirect effects of climatic conditions (red box) and aboveground herbivore pressure (green box) on plant secondary compound production (yellow boxes) and input into the soil, and its belowground interactions (brown box). In addition, climate affects how PSCs are transported and degrade in soil. Dashed arrows and boxes indicate processes and effects that are less well understood and are elucidated in the main text.

Table 1. Overview of the important classes of PSCs involved in mediating belowground interactions, and best practices for sampling and analysing them. The chemical properties of PSCs affect their position and transport in the soil matrix, with polar PSCs dissolved in water, volatile PSCs diffusing through air spaces in the soil, and less polar non-volatile PSCs adsorbed to the organic fraction of soil.

Compound class	**Example of structure**	**Polarity**	**Water solubility**	**Volatility**	**Ecological interactions/ impacts and effects on associated soil organisms**	**The analytical instrument for detection in soil (see also Box 1)**
Terpenes Synthesized from isoprene units (C_5H_8). Includes monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$).	 α-Pinene	Low	Low	High	Defensive and signalling compounds, many with a characteristic odour (e.g. α-pinene from pine, limonene from citrus). Antioxidant, antimicrobial, and phytotoxic properties [3]. Toxic to invertebrates due to baseline toxicity (**“narcotic” effect**) via interaction with cellular membranes [97]. Involved in formation of aerosol particles, thus affecting climate [98].	Monoterpenes and highly volatile sesquiterpenes: S with PDMS adsorbent, purge-and-trap with Tenax adsorbent and subsequent GC-MS [19], [99], On-line measurement with Proton-transfer-reaction MS [100]. Nonpolar sesquiterpenes likely adsorbed to soil particles require extraction with organic solvents a evaporation followed by GC-MS [41].
Flavonoids General structural C-15 skeleton consisting of two phenyl rings		Low to intermediate	Low to intermediate Presence of sugar moieties increases	Low	Both defensive and signalling; promote symbiosis (used by legumes to attract rhizobia), various antimicrobial activities [101, 102]. Can interact with Ca^{2+}	Most likely present belowground in water and ac Extraction using polar solvents (water, methanol) metals are formed [104]. Extracts can be analysed with reversed-phase LC-

and a heterocyclic ring (C6-C3-C6).	Basic flavonoid structure		solubility in water		channels in cell membranes causing cytotoxic influx of Ca^{2+} [103].	Aglycones may be sampled using SPE [101] and a to GC-MS after derivatization. Greater sensitivity r obtained with SBSE and subsequent GC-MS.
Glucosinolates Derived from glucose and an amino acid. Most are found in the order Brassicales (30).	 Sinigrin	High	High	Low	Due to polarity and water solubility, are readily taken up by soil organisms, where they can be converted to highly reactive compounds such as isothiocyanate and thiocyanates. Toxicity pathways include inhibition of cytochrome P_{450} detoxification complex, induction of programmed cell death (apoptosis), and genotoxic effects [106].	Mostly present in the water phase due to high po Extraction using polar solvents (water, methanol) with LC-MS [17] or GC-MS after derivatizat concentration in the sample.
Alkaloids Highly diverse, usually with a basic nitrogen atom either in	 Nicotine	Low to intermediate	Low to intermediate	Low	Mostly defensive compounds. Phytotoxic, toxic (e.g. strychnine), and narcotic effects (e.g. morphine, codeine). Can interfere with gene	Low concentration in soil, so clean-up and pr necessary. Polarity can be adjusted with pH changes with liquid-liquid extraction, SPE, or SPME [108 both LC-MS and GC-MS.

cyclic rings or open chains.					transcription and protein synthesis [107].	

Outstanding Questions

To what extent does the plant secondary compound (PSC) plume originally released into a soil compartment become chemically degraded during diffusion and mixed with secondarily formed and other ambient PSCs? Does this dilution and mixing with other PSCs impair the signal quality for the receiving soil organisms or have they adapted to and even benefited from signal noise, e.g. to improve their foraging efficiency?

How will climate change affect PSC production in both above- and belowground organs and how will these effects in turn affect the belowground interactions of associated communities of microbes, plants, and soil invertebrates?

What is the relative importance of specific PSCs for both plant-soil invertebrate and plant-plant interactions and can we understand their prevalence from knowledge on their mode of action? If the effects of PSC on associated species is very compounds specific, quantitative and qualitative changes in the input of PSC to the soil can have cascading effects on richness and composition of belowground soil communities that may ultimately alter soil functioning.

What is the importance of a common co-evolutionary history between PSCs in soil and the organisms exposed to them for shaping the outcome of their interactions?

How important are belowground interactions in determining the composition and concentration of PSCs released by the producer? In particular, what is the impact of selection (if any) that belowground interactions can exert on PSC composition and production in leaves relative to aboveground interactions and climate?

HIGHLIGHTS:

- Plants produce a high diversity of secondary compounds that are released to their surroundings.

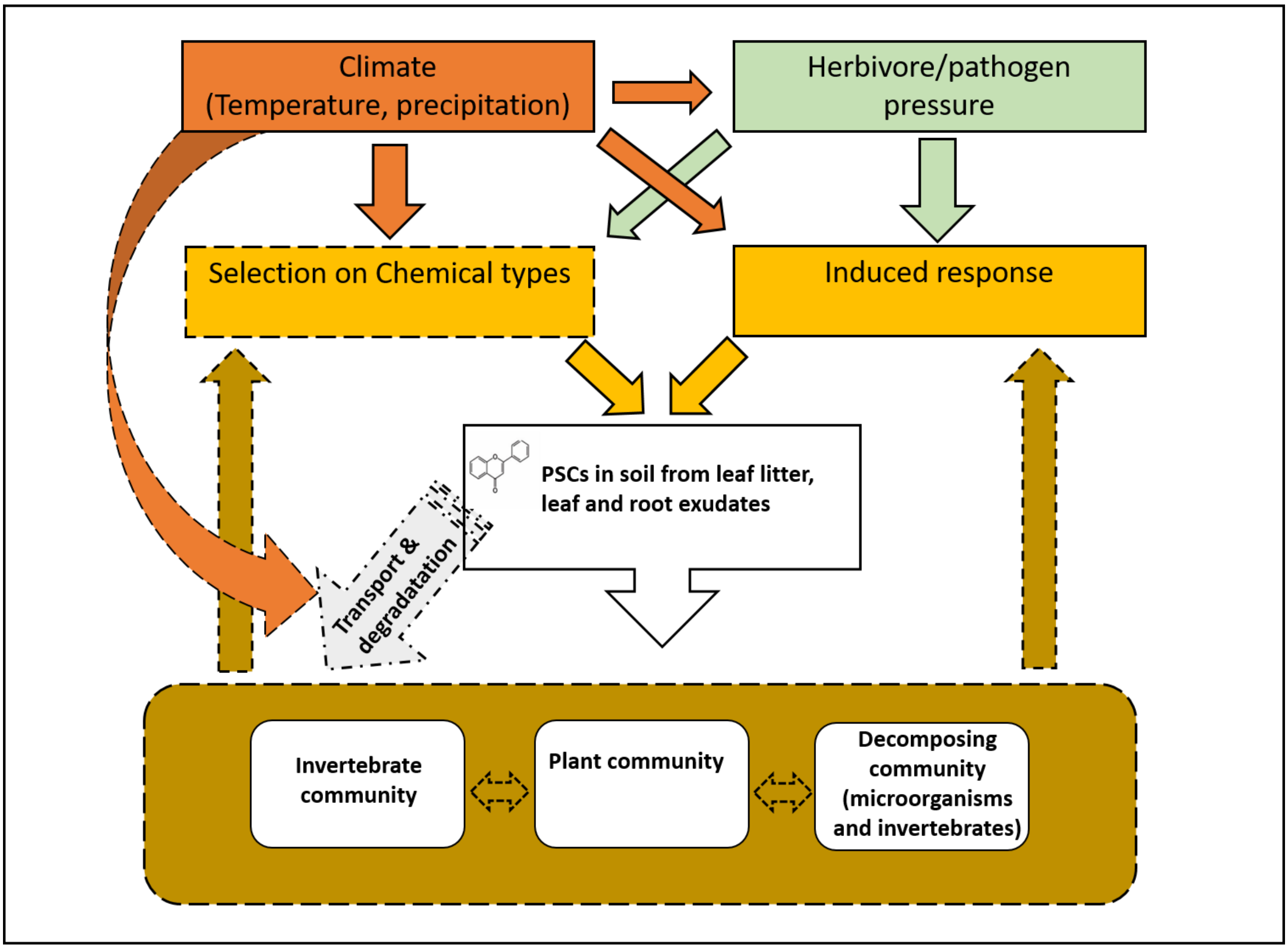
- The role of plant secondary compounds (PSCs) on aboveground interactions has been well studied, but less is known about the role of PSCs in mediating and determining the outcome of belowground interactions – especially interactions beyond the rhizosphere.

- PSCs enter the soil directly by excretion from belowground plant organs or indirectly by washing from the canopy and leaching from litter.

- Leaching of PSCs creates a heterogeneous soil chemical environment and mediate plant-plant interactions, plant-microbiome associations, and plant-soil invertebrate relationships. This affects and shape the diffuse community of species living in soil also further away from the rhizosphere.

- Climate change can substantially alter the quantitative and qualitative production of PSCs released to the soil, which may change belowground species interactions and affect species composition, richness, and ultimately soil functioning.

Figure


Climate
(Temperature, precipitation)
Herbivore/pathogen pressure
Selection on Chemical types
Induced response
PSCs in soil from leaf litter, leaf and root exudates
Transport & degradatation
Invertebrate community
Plant community
Decomposing community (microorganisms and invertebrates)