The ecosystem and evolutionary contexts of allelopathy

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Abstract

Plants can release chemicals into the environment that suppress the growth and establishment of other plants in their vicinity, a process known as ‘allelopathy’. However, chemicals with allelopathic functions have other ecological roles, such as plant defense, nutrient chelation, and regulation of soil biota in ways that affect decomposition and soil fertility. These ecosystem-scale roles of allelopathic chemicals can augment, attenuate or modify their community-scale functions. In this review we explore allelopathy in the context of ecosystem properties, and through its role in exotic invasions consider how evolution might affect the intensity and importance of allelopathic interactions.

Key words: allelopathy, allelochemicals, community ecology, evolution, exudates, herbivory, invasion, soil microbes

Allelochemical interactions in the context of communities and ecosystems

How populations are organized into higher units, or “communities”, is a central issue in ecology [1]. The Russian ecologist T.A. Rabotnov [2] hypothesized that adaptation of plant species to the chemistry of other species was crucial to this organization. Rabotnov focused on allelopathic interactions, which involve biochemically based suppression of the establishment and growth of one plant by another. But plant-released secondary chemicals also have powerful effects on decomposition [3], herbivory [4], trophic interactions [5] and nitrogen cycling [6,7] (Figure 1). Allelopathy has been studied a great deal over the last 50 years, but only a few studies have attempted to understand allelochemical interactions among plants in the context of these broader
effects [8-14]. Consideration of allelopathy in this integrated community and ecosystem context requires the recognition of the large number of different processes that can be affected by the same chemical or its derivatives, and the potential for the direct allelochemical effects of plants on each other to be augmented, attenuated, modified or offset [11]. These other interactors can enhance or reduce allelochemical production, change the persistence or effectiveness of allelochemicals in soil, and select for higher or lower allelochemical concentrations over evolutionary time. Understanding allelopathy in the context of communities and ecosystems can be further developed by comparing the potential allelopathic effects of invasive species between their native and introduced ranges [15-18]. Such biogeographic comparisons suggest that evolutionary relationships among plants, and between plants and soil biota, may affect the role of allelopathy in community organization [16].

Mere production of chemicals by a plant is not sufficient to ensure their allelopathic potential. Abiotic and biotic environmental conditions determine the allelopathic potential of chemicals in soil [10]. Recent studies have advanced our understanding of allelopathy by examining it in environmental [12,19-21], biogeographic [15,16,22] and evolutionary [23,24] contexts. Our goal is to discuss how (i) biotic and abiotic environmental conditions and (ii) evolutionary history affect the production, fate, and effectiveness of allelopathic compounds in soils (Figure 1). Specifically, we consider how habitat or site-specific characteristics, non-native ecosystems, and environmental variables all influence the release, accumulation, and function of chemicals, and thus affect the organization of natural systems.
Consumer, competitor and soil microbe effects on allelochemical production and activity

The production, storage, and release of allelochemicals are key mechanisms of plant behavior which affect almost all aspects of a plant’s ecology [9]. These processes are affected by the abiotic and biotic properties of the ecosystems in which plants grow [25], and chemicals produced by plants in turn have strong effects on ecosystem properties. We propose that by explicitly recognizing and integrating these ecosystem level effects, we will better understand the various allelopathic, defensive, foraging, and signaling roles of chemicals in the organization of natural communities (Figure 1).

Under natural conditions, allelopathic effects can result from interactive effects among multiple compounds [26-29]. One of the best understood allelopathic systems involves the root exudates of Sorghum bicolor which can contain up to 85% sorgoleone [30,31], However, is now recognized that these exudates often contain both sorgoleone and its analogue (the lipid resorcinol) in a 1:1 ratio [31], yielding the opportunity for studying potential interactive effects among these two compounds.

Many chemicals released from the roots of plant species function to make nutrients available, often through chelation, and can be quite substrate specific. Some chelators also appear to be allelopathic. Chelating chemicals can degrade either slowly or rapidly, and this can increase or decrease their biological activity [9,12,14]. However, many chelators are non-specific and hence will bind with any of the metal ions with affinity decreasing along a lyptropic series. Most natural soils are abundant in metal ions and hence it is difficult to find an uncomplexed chelator under natural conditions. This aspect therefore needs more attention.

There is considerable evidence for the direct inhibitory effects of specific allelochemicals isolated from root exudates, leaf leachates and leaf volatiles of plants on other species. However,
in many cases, substantial variation has been found in the field concentrations and production of
the chemical, responses of target species, and the chemical’s interactions with environmental
conditions, other phytochemicals, and other biota [10,12,32]. Such variation in allelochemical-
environment interactions makes allelopathy difficult to consistently demonstrate in the field [but
see 16,33,34], and has led to conflicting evidence for the ecological relevance of particular
chemicals (Box 1) [19,32,35-37]. However, variation in the allelopathic potential of chemicals
among environments allows for more realistic appraisals of the role of ecological context in
driving allelopathic interactions [12]. Such processes provide alternative hypotheses for the direct
effects of allelochemicals on other species, and a broader understanding of the conditional effects
of allelopathy. Here we discuss how interactions between chemicals and ecosystem factors affect
the production, release, accumulation and activity of allelochemicals (Figure 1).

Above-ground ecosystem influences on allelopathy

Biotic components of the ecosystem such as herbivores, competitors, pathogens and belowground
decomposers can alter concentrations of chemicals already in plant tissues or released from
plants, or stimulate the production of chemicals that are otherwise not present or occur at very
low levels [38,39]. Here we discuss above-ground biotic influences of ecosystems on allelopathic
effects of herbivory-induced volatile chemicals in various environments.

Many allelochemicals can be induced by low concentrations of soil nutrients (although the
ultimate cue is likely to be low concentrations in tissues). For example, iron deficiencies
stimulate highly complex exudation responses [14]. Under iron limitation the roots of Centaurea
diffusa prolong the release of 8-hydroxyquinoline that also mobilizes metals and makes them
available for plant uptake [14]. Thus, the metal content of soils from different ecosystems is
likely to strongly influence the production and soil availability of 8-hydroxyquinoline, and complex interactions between this allelochemical and metals may also determine its biological activity [14]. Light intensity increases the root exudation of 8-hydroxyquinoline [14], which exhibits a diurnal rhythm and reaches a maximum after 6 hours of exposure to light. Evaluating the role of an allelochemical in the context of its abiotic environment should aid our understanding on its release and allelopathic activities.

Induced secondary metabolite-based defenses are common in plants [40], and if the same secondary metabolites or their derivatives are also allelopathic, herbivory might substantially modify allelopathic interactions [see 11]. Karban [8] found that volatiles produced by experimental clipping of sagebrush also inhibited germination and establishment of neighboring plant species, thus providing experimental evidence of an herbivore-enhanced allelopathic effect. The effects of allelochemicals depend not only on environmental conditions but also the genetic landscape. For example, effects of herbivore-induced volatiles on neighboring sagebrush plants were greater when the plants were genetically identical than when genetically different [41]. Herbivory induces plant defenses that trigger the release of volatile organic compounds [38,42] and accumulation of polyphenolics [43], and some of these chemicals may be allelopathic in nature. Consistent with this, Bi et al. [44] found that exogenous application of methyl jasmonate, a chemical that induces herbivore defenses in many plant species, led to the accumulation of phenolics in rice and increased its allelopathic effects on other plants.
Below-ground influences of ecosystem processes driven by soil biota, genetic effects on root interactions, and complex interactions among different root exudates appear to shape allelopathic interactions. The general importance of soil communities in influencing the qualitative and quantitative availability of allelochemicals is well established [45,46]. Microbial transformation of biologically active chemicals commonly degrades their function, and evaluation of the activity of an allelochemical in microbe-free substratum may therefore not be ecologically relevant. For example, allelopathic effects of m-tyrosine, a metabolite exuded by the roots of *Festuca rubra* ssp. *commutata*, have been demonstrated through filter paper bioassays free from naturally occurring microbes [47]. However, Kaur *et al.* [19] showed that allelopathic effects of m-tyrosine were only evident in sterilized soil and diminished sharply in non-sterile soil with an intact microbial community. Even this type of comparison must be interpreted with caution because the scale of ecological interactions among roots, microbes and allelochemicals is microscopic and ephemeral. For example, Bertin *et al.* [48] found that the predicted half-life of m-tyrosine in soil in laboratory conditions was less than 1 day, indicative of rapid microbial degradation. Sorgoleone, a major component of root exudates of *Sorghum bicolor*, is a potent allelochemical [30] and microorganisms present in North American soils readily use it as a carbon source [49]. It has been shown that the methoxy group of sorgoleone, which is responsible for much of its activity, degrades rapidly in soil [49].

In addition to the direct effects of allelochemicals on plant growth, their indirect effects may be mediated by microbial activity. Meier and Bowman [50] compared the effects of several allelochemical fractions from a phenolic-rich alpine forb, *Acomastylis rossii*, on soil respiration and the growth of the grass *Deschampsia caespitosa*. They found that some fractions had a direct
phytotoxic effect (i.e., which did not increase soil respiration but killed *D. caespitosa*) while others appeared to work indirectly through the soil microbial community (i.e., which stimulated soil respiration and reduced plant growth and plant N concentration). Their results provide a compelling example of how phenolic compounds can inhibit root growth directly as well as through interacting with soil biota. In another example, *Alliaria petiolata* can have negative impacts on arbuscular mycorrhizal (AM) fungi and regeneration of seedlings native to North America in soil from North America [51], but much weaker effects on AM fungi in soils from Europe where it is native. Cantor et al. [52] showed that even very low field concentrations of allyl isothiocynate (ca. 0.001mM) produced in the presence of *A. petiolata* strongly inhibited the spore germination of the AM fungus *Glomus clarum*. However, Barto et al. [53] did not find effects of *A. petiolata* extracts on the AM fungal colonization of roots or soils, and suggested that potential alleopathic effects of *A. petiolata* might be due to direct inhibition of plant seedlings and fungus before the formation of symbiosis.

The impacts of seasonal variation on the production and accumulation of allelochemicals [54] and soil microbial communities [55] also contribute to the context-specificity of allelopathic effects. For example, *Alliaria petiolata* accumulates glucotropaeolin three times more rapidly in autumn than in spring, while accumulation of alliarinoside is highest in spring [54]. Fungal communities and ectomycorrhizal colonization rates showed linear and curvilinear responses to alliarinoside and glucosinolate concentrations, respectively [24]. Increasing concentrations of alliarinoside were found to alter AM fungal communities, leading to a decline in AMF colonization of *Quercus rubra* roots [24].

Belowground interactions among plants may also be genotype or ecotype dependent. For example, when the roots of different *Ambrosia dumosa* plants make contact they often stop growing, but there is a geographic and genotypic aspect to this response. For example, roots of
the plants from the same region show strong contact inhibition, but roots from plants from
different regions do not \cite{56,57}. \textit{Cakile edentula} plants allocate biomass differently to roots if
they are grown in the same pots shared by genetic relatives (kin) compared to pots shared by
strangers \cite{58}. Lankau \cite{59} reported that investment in high tissue concentrations of sinigrin
produced by \textit{Brassica nigra} gave it an advantage in interspecific competition but a disadvantage
in intraspecific competition. Further, selection for \textit{B. nigra} individuals that produced high levels
of sinigrin was stronger when grown with other species than with other individuals of its own
species.

Coexisting plant species can differ greatly in their growth response to allelochemicals
produced by a given plant species, and allelopathic effects can be highly species-specific
\cite{16,22,60}. As such, there is a wide range of abilities (and perhaps mechanisms) among species
to protect themselves from chemical effects of their neighbors. Weir \textit{et al.} \cite{61} found that
\textit{Gaillardia grandiflora} and \textit{Lupinus sericeus} secrete oxalate in response to catechin exposure,
which could make these two species resistant to \textit{C. stoebe} invasion. Exogenous application of
oxalate blocks the production of reactive oxygen species in the target plants, minimizing
oxidative damage caused by catechin. Such variation in the species-specific response of target
species may play a crucial role in the organization or assembly of plant communities in a similar
manner that it does for microbial communities \cite{62}, and provides an opportunity for allelopathy
to drive natural selection \cite{63}. Variation in the ecological roles of secondary compounds is better
understood for consumer defense than for allelopathy, but for both types of interactions variation
is an important aspect of the effects of chemicals on communities and populations.

Issues of spatial scale and patchiness make studies of the roles of allelochemicals in soils
difficult to interpret. The effects of allelochemicals in soils are generally examined using “bulk
soils”, where allelochemicals are added to a volume of soil that is orders of magnitude greater
than the soil volume in which the interactions occur. ‘Realistic’ concentrations of allelochemicals are estimated for the average of the large soil volume. However, the action of root-exuded chemicals often takes place at root-root interfaces. The use of estimated soil concentrations is just one way to explore allelopathy in a reasonably realistic manner, but they have limitations for the determination of the allelopathic functions of chemicals. If an allelochemical is experimentally applied to soil in such a way as to allow it to transform before contact with roots [12,19,34,37], then the failure to find an effect cannot be taken as evidence that effects do not occur when roots are in close proximity to each other. This issue is, however, less relevant when allelochemicals enter the soil through release from foliage or decomposition of plant litter.

**Biogeographic comparisons of allelopathy: evolutionary changes in allelochemical effects**

The effects of allelopathy are also dependent on the evolutionary history of the interaction. Understanding the mechanisms by which many exotic invasive plants strongly suppress their neighbors in invaded but not native ranges has attracted growing recent attention. Allelopathy and other biochemically driven interactions may contribute to the success of some exotic invasive plants, and when either specific allelochemicals or general allelopathic effects are stronger against potentially evolutionarily naïve species in invaded ranges, we gain insight into how evolutionary history affects biological organization [64]. Biogeographical comparisons of the ecological and biochemical traits of species in introduced and native ranges have proven useful for evaluating mechanisms of invasion [65]. Examining the production and/or accumulation of allelochemicals in novel and native environments, and the sensitivity of native residents and soil communities to novel chemicals, can help understand these mechanisms.
The Novel Weapons Hypothesis (NWH) provides a possible explanation for biogeographic patterns of interactions in different ecosystems. The NWH was first proposed in the context of allelopathy as a potential mechanism for the success of *Centaurea diffusa* as an invader in North America [66], and subsequently as a component of invasion by *C. stoebe* [67]. Recent studies on biogeographic comparisons of exotic species in native and introduced ranges have shown some support for NWH [15,16,18,68,69]. A recent meta-analysis of hypotheses for invasions, focusing on trees, found that published evidence for the NWH resulted in a stronger effect size in support of the idea than the effects sizes of six other hypotheses [70]. Barto *et al.* [71] provided evidence in support of NWH by showing that the allelochemical profile of invasive *A. petiolata* was not shared by any native Brassicaceae in North America. Further, Callaway and Ridenour [67] suggested that stronger allelopathic effects in invaded regions could lead to selection for greater allelopathic production and thus increased competitive ability.

Biogeographic differences in the effects of particular compounds between native and invaded ranges may occur in part through a lack of adaptation by species and soil communities in the invaded ranges. However, these types of biogeographic differences may also emerge or intensify because of particular conditions in the novel environment. As such, soil biota can be powerful ecosystem mediators of biogeographic differences in allelopathic effects [46]. For example, soil microbial taxa that metabolize specific chemicals are likely to have undergone evolution to do so, or at least to utilize a related group of chemicals. If plants that occur in a given region do not produce a particular allelochemical, then those soil microbes that are required to metabolize it may not be present when it is introduced by an invader. Thus, novel chemicals produced by invaders may have prolonged resident times in invaded ranges and therefore be more biologically active. Such indirect processes may reinforce biogeographic differences in plant-soil feedbacks involving invasive species [72].
Soil communities from non-native ranges have also been shown to eliminate allelopathic effects of exotic plants. For example, the invader *A. petiolata* exerts allelopathic effects through glucosinolate exudation on the native species *Platanus occidentalis* in sterilized soil but not in non-sterile soil from the invaded range [73]. Future research would be required to determine whether soil microbial communities from locations that differ in their invasion history of *A. petiolata* also differ in their ability to degrade glucosinolate.

Potential evolutionary relationships: temporal declines in allelochemicals from invasive species

Plant species that are introduced into a novel environment would likely evolve in response to new conditions over time, and other species that are native to that environment may in turn evolve in response to the introduced species [16]. Such evolutionary responses have been reported for populations of *Trifolium repens* that have co-adapted to (and with) local competitors [74], and for populations of native soapberry bugs (*Leptocoris tegalicus*) that have adapted to various introduced host plants [75]. Some native residents in the naturalized range of *C. stoebe* have exhibited tolerance to it relative to individuals of other native species that have not previously encountered the invader [76]. Individuals grown from seeds of parents that have survived exposure to allelochemicals from *C. stoebe* have become more resistant to its invasion. This is consistent with the NWH, and suggests that allelopathy may play a role in evolution between neighbors in the non-native ranges.

Biogeographic variation in the production of volatile sesquiterpenes in particular could be due to differences in herbivore densities between the native and introduced ranges [77]. Recently, it has been shown [15] that lower amounts of volatile chemicals were released by plants from
exotic populations of the invasive plant *Ageratina adenophora* than by plants from native populations grown in a common environment. However, it is not known whether such differences in volatile emissions are evolutionary consequences of interactions with other species or due to founder effects.

An allelochemical produced by a species can provide multiple ecological functions, making its effects highly dependent on specific environmental conditions. Further, allelochemicals with multiple functions should be selected for because this spares the plant the cost of producing several different compounds [11]. Glucosinolates and their derivatives have been found to have multiple functions as mediators of plant–plant, plant–microbe, and plant–insect interactions [59].

Lankau and Kliebenstein [78] found that competition and herbivory determined the accumulation and fitness consequences of sinigrin for *B. nigra*. Further, it has been shown that the fitness costs and benefits of sinigrin conformed to optimal defense theory only in the absence of competition, apparently due to its multiple functions [11,78]. Further, Oduor *et al.* [79] found that invasive populations of *B. nigra* had higher levels of sinigrin which defends the invader against generalist herbivores. An increase in resistance against generalist herbivores and growth performance of *B. nigra* in its introduced ranges compared to its native range further supports the hypothesis that defenses have shifted [79]. Sinigrin from *B. nigra* is also reported to possess allelopathic activities, which provide a competitive advantage to *B. nigra* over heterospecific neighbors [59].

Lankau *et al.* [23,24] examined the production, release and impact of glucosinolates from *A. petiolata* along a gradient of invasion history i.e., from early invaded to recently-invaded populations. They found a significant decline in the production of glucosinolates and an increase in the community’s resistance to *A. petiolata* invasion over time. Following an initial decline in the number of operational taxonomic units (OTUs) of bacteria, fungi and AM fungi, an increase was observed in older invaded sites [24]. The observed development of resistance to exotic
invasion in late invasion stages could lead to more species rich native communities. However, the eventual outcome of the evolutionary changes is still unclear. Lankau et al. [80] found that the higher production of sinigrin by introduced *B. nigra* suppressed mycorrhizal abundance, which adversely affected the growth of heterospecific competitors but not non-mycorrhizal conspecifics. Such rapid selection based on tradeoffs between competitive advantages against either conspecifics or heterospecifics contributes to intransitive competitive networks which affect genetic and species diversity in communities [80]. Studying evolutionary relationships between native and non-native communities and ecosystems along gradients of invasion history has significant potential for improving understanding of the role of allelopathy in community organization.

**Conclusions**

It is important to identify how variation in the environment establishes conditionality in allelopathic interactions. Sources of such variation include (1) the impact of soil chemistry on production and effects of allelochemicals, (2) the impact of consumers, competitors, and soil microbes on production and effects of allelochemicals, (3) evolutionary changes in allelochemical effects, and (4) declines in allelochemical production and activity from invasive species over time. A major gap in current allelopathy research involves the role of conditional ecosystem factors that drive allelopathic processes and how these change over space and time (Figure 1). Further, despite recent advances, we still have a limited understanding of the role of evolution over time in the production, release and eventual loss of activity of biogeographically novel chemicals.
The production, fate, and effectiveness of allelopathic compounds in soils is influenced by environmental conditions and evolutionary history, generating a need for allelopathic interactions to be studied across spatial and temporal scales (Figure 1). Over very small scales (microns to millimeters; seconds to hours), processes in the rhizosphere, such as microbial-driven breakdown of allelochemicals or metal chelation, dominate the influences of allelochemicals. Over small scales (millimeters to meters; hours to months), organismal responses are important, for instance, the increased production of chemicals following herbivore attack. At the medium scale (meters to kilometers; months to years), variation in the plant and soil communities, and abiotic soil conditions become increasingly important, if different species are more or less susceptible to the allelochemicals. Finally, at the large scales (kilometers and beyond; years and beyond), the evolutionary history of the allelopathic plant and the recipient soil and plant community assumes increasing significance (Figure 1).

Continuing to quantify various aspects of how ecosystem factors influence allelopathy is key to better understanding of how plants interact with each other. Other important steps would include greater focus on conducting experiments under natural conditions, comparing single chemical effects to whole-exudate effects, profiling metabolites, and conducting bioassays in search of unidentified compounds that mediate these interactions. More generally, there is a greater need for understanding of how biotic and abiotic environmental conditions and evolutionary history affect the production, fate, and effectiveness of allelopathic compounds in soils. Recent work linking chemical ecology to biogeography and evolutionary biology has provided new perspectives on biochemical processes in ecosystems. Expanded use of biogeographical and evolutionary approaches will improve our understanding of the release of allelochemicals over a range of abiotic and biotic conditions and how those conditions determine the outcomes of allelochemical interactions.
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**Glossary**

**Allelopathy:** Suppression of the growth and/or establishment of neighboring plants by chemicals released from a plant or plant parts.

**Allelochemicals:** Secondary compounds of plant origin that interact with their environment and possess allelopathic activities.

**Homeostatis:** The tendency of a biological system (organism, population, community or ecosystem) to resist changes and to remain in the state of equilibrium or change its properties in such a way as to minimize the impact of outside factors [81].

**Novel weapons hypothesis (NWH):** The idea that some invasive plant species produce secondary metabolites that are novel in their non-native ranges and that this novelty provides advantages to the invasive species as it interacts with native plants, microbes or generalist herbivores.
Figure 1. The impact of ecosystem factors, biogeographic variations and coevolutionary relationships on the production, release and activity of allelochemicals along spatial and temporal scales.
Box 1. Catechin as a novel weapon. (-)-Catechin, reported to be exuded from the roots of a Eurasian invader in North America, *Centaurea maculosa* (*C. stoebe*), was the first isolated chemical discussed as a possible ‘novel weapon’ [67]. Initial work on this compound used (-)-catechin but subsequent experimental studies used (±)-catechin because root exudates of *C. stoebe* contain a racemic mixture of (+)- and (-)-catechin. Early reports of consistently high rates of exudation have not been reproducible using protocols similar to those in the original experiment [see retraction, 82]. Catechin has been reported at very low concentrations in soil in the rhizospheres of *C. stoebe* [35] but high concentrations may occur periodically [83,84]. The phytotoxic effects of the enantiomeric form (±)-catechin, and the (+) form have been demonstrated in vitro, in sand culture, in controlled experiments with field soils, and in the field [12,16,22,34 and citations within], but others have not found either the + or the – form to be phytotoxic [36,37].

Tharayil and Triebwasser [85] quantified catechin release at picomolar levels by roots of *C. stoebe* in hydroponic medium and showed a diurnal rhythm in its exudation in response to light. There is also evidence that this invader’s impact is also due to interactions with the soil ecosystem including through effects on nitrogen (N) and phosphorous (P) cycling and on soil fungi [72,86-89]. Recently, Thorpe and Callaway [90] examined biogeographical differences in the responses of soil communities to *C. stoebe* and catechin by studying the effects of catechin on soil ammonification and nitrification in both native (Romania) and non-native (Montana) ranges. Catechin and *C. stoebe* were linked to similar reductions of resin-extractable nitrates and gross nitrification in Montana soils but not in Romanian soils where *C. stoebe* is native. As discussed below, we do not know if the consistency and rate of catechin exudation and its concentration at root-root and root-bacteria interfaces is adequate to drive substantial effects in natural systems, but biogeographical differences in ecosystem effects controlled by soil bacteria suggests that novel chemicals might affect soil nutrients by influencing soil communities as well as other plants, and that these effects have an evolutionary context.

INSERT Figure I HERE

Figure I. Abiotic and biotic ecosystem components influence the release, accumulation and activity of catechin. Unresolved issues regarding whether catechin has an important role as a novel chemical and under which environmental conditions could be addressed by studying the natural release of catechin in different ecosystems, or across gradients of invasion history.
Factors that may influence production, release or activity of catechin

- Soil biota [32]
- Soil chemistry [12]
- Impact on nitrification [90]
- Cell wall elicitors from soil fungi [91]
- Biogeographic differences in neighbour sensitivity [63, 64]
- Abiotic factors (e.g., light) [85]

Key factors that we don’t know
- Phytotoxicity through natural release
- Evolutionary changes in impact over time in non-native ranges
- Potential for other chemicals in exudates or foliar leachates to alter or exceed in importance
- Differences in impact of soil biota from native and non-native ranges
- Seasonal differences in release or impact

Figure I (for Box 1)