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1 **Review**

2

3 **The ecosystem and evolutionary contexts of allelopathy**

4

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25 **Abstract**

26
27 Plants can release chemicals into the environment that suppress the growth and establishment of
28 other plants in their vicinity, a process known as ‘allelopathy’. However, chemicals with
29 allelopathic functions have other ecological roles, such as plant defense, nutrient chelation, and
30 regulation of soil biota in ways that affect decomposition and soil fertility. These ecosystem-scale
31 roles of allelopathic chemicals can augment, attenuate or modify their community-scale
32 functions. In this review we explore allelopathy in the context of ecosystem properties, and
33 through its role in exotic invasions consider how evolution might affect the intensity and
34 importance of allelopathic interactions.

35
36 **Key words:** allelopathy, allelochemicals, community ecology, evolution, exudates, herbivory,
37 invasion, soil microbes

38 39 **Allelochemical interactions in the context of communities and ecosystems**

40
41 How populations are organized into higher units, or “communities”, is a central issue in ecology
42 [1]. The Russian ecologist T.A. Rabotnov [2] hypothesized that adaptation of plant species to the
43 chemistry of other species was crucial to this organization. Rabotnov focused on allelopathic
44 interactions, which involve biochemically based suppression of the establishment and growth of
45 one plant by another. But plant-released secondary chemicals also have powerful effects on
46 decomposition [3], herbivory [4], trophic interactions [5] and nitrogen cycling [6,7] (Figure 1).
47 Allelopathy has been studied a great deal over the last 50 years, but only a few studies have
48 attempted to understand allelochemical interactions among plants in the context of these broader

49 effects [8-14]. Consideration of allelopathy in this integrated community and ecosystem context
50 requires the recognition of the large number of different processes that can be affected by the
51 same chemical or its derivatives, and the potential for the direct allelochemical effects of plants
52 on each other to be augmented, attenuated, modified or offset [11]. These other interactors can
53 enhance or reduce allelochemical production, change the persistence or effectiveness of
54 allelochemicals in soil, and select for higher or lower allelochemical concentrations over
55 evolutionary time. Understanding allelopathy in the context of communities and ecosystems can
56 be further developed by comparing the potential allelopathic effects of invasive species between
57 their native and introduced ranges [15-18]. Such biogeographic comparisons suggest that
58 evolutionary relationships among plants, and between plants and soil biota, may affect the role of
59 allelopathy in community organization [16].

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

69
70

71 **Consumer, competitor and soil microbe effects on allelochemical production and activity**

72
73 The production, storage, and release of allelochemicals are key mechanisms of plant behavior
74 which affect almost all aspects of a plant's ecology [9]. These processes are affected by the
75 abiotic and biotic properties of the ecosystems in which plants grow [25], and chemicals
76 produced by plants in turn have strong effects on ecosystem properties. We propose that by
77 explicitly recognizing and integrating these ecosystem level effects, we will better understand
78 the various allelopathic, defensive, foraging, and signaling roles of chemicals in the organization
79 of natural communities (Figure 1).

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

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

93 There is considerable evidence for the direct inhibitory effects of specific allelochemicals
94 isolated from root exudates, leaf leachates and leaf volatiles of plants on other species. However,

95 in many cases, substantial variation has been found in the field concentrations and production of
96 the chemical, responses of target species, and the chemical's interactions with environmental
97 conditions, other phytochemicals, and other biota [10,12,32]. Such variation in allelochemical-
98 environment interactions makes allelopathy difficult to consistently demonstrate in the field [but
99 see 16,33,34], and has led to conflicting evidence for the ecological relevance of particular
100 chemicals (Box 1) [19,32,35-37]. However, variation in the allelopathic potential of chemicals
101 among environments allows for more realistic appraisals of the role of ecological context in
102 driving allelopathic interactions [12]. Such processes provide alternative hypotheses for the direct
103 effects of allelochemicals on other species, and a broader understanding of the conditional effects
104 of allelopathy. Here we discuss how interactions between chemicals and ecosystem factors affect
105 the production, release, accumulation and activity of allelochemicals (Figure 1).

106

107 *Above-ground ecosystem influences on allelopathy*

108

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

114 Many allelochemicals can be induced by low concentrations of soil nutrients (although the
115 ultimate cue is likely to be low concentrations in tissues). For example, iron deficiencies
116 stimulate highly complex exudation responses [14]. Under iron limitation the roots of *Centaurea*
117 *diffusa* prolong the release of 8-hydroxyquinoline that also mobilizes metals and makes them
118 available for plant uptake [14]. Thus, the metal content of soils from different ecosystems is

119 likely to strongly influence the production and soil availability of 8-hydroxyquinoline, and
120 complex interactions between this allelochemical and metals may also determine its biological
121 activity [14]. Light intensity increases the root exudation of 8-hydroxyquinoline [14], which
122 exhibits a diurnal rhythm and reaches a maximum after 6 hours of exposure to light. Evaluating
123 the role of an allelochemical in the context of its abiotic environment should aid our
124 understanding on its release and allelopathic activities.

125 Induced secondary metabolite-based defenses are common in plants [40], and if the same
126 secondary metabolites or their derivatives are also allelopathic, herbivory might substantially
127 modify allelopathic interactions [see 11]. Karban [8] found that volatiles produced by
128 experimental clipping of sagebrush also inhibited germination and establishment of neighboring
129 plant species, thus providing experimental evidence of an herbivore-enhanced allelopathic effect.
130 The effects of allelochemicals depend not only on environmental conditions but also the genetic
131 landscape. For example, effects of herbivore-induced volatiles on neighboring sagebrush plants
132 were greater when the plants were genetically identical than when genetically different [41].
133 Herbivory induces plant defenses that trigger the release of volatile organic compounds [38,42]
134 and accumulation of polyphenolics [43], and some of these chemicals may be allelopathic in
135 nature. Consistent with this, Bi *et al.* [44] found that exogenous application of methyl jasmonate,
136 a chemical that induces herbivore defenses in many plant species, led to the accumulation of
137 phenolics in rice and increased its allelopathic effects on other plants.

138

139

140 *Below-ground ecosystem influences on allelopathy*

141

142 Below-ground influences of ecosystem processes driven by soil biota, genetic effects on root
143 interactions, and complex interactions among different root exudates appear to shape allelopathic
144 interactions. The general importance of soil communities in influencing the qualitative and
145 quantitative availability of allelochemicals is well established [45,46]. Microbial transformation
146 of biologically active chemicals commonly degrades their function, and evaluation of the activity
147 of an allelochemical in microbe-free substratum may therefore not be ecologically relevant. For
148 example, allelopathic effects of m-tyrosine, a metabolite exuded by the roots of *Festuca rubra*
149 ssp. *commutata*, have been demonstrated through filter paper bioassays free from naturally
150 occurring microbes [47]. However, Kaur *et al.* [19] showed that allelopathic effects of m-
151 tyrosine were only evident in sterilized soil and diminished sharply in non-sterile soil with an
152 intact microbial community. Even this type of comparison must be interpreted with caution
153 because the scale of ecological interactions among roots, microbes and allelochemicals is
154 microscopic and ephemeral. For example, Bertin *et al.* [48] found that the predicted half-life of
155 m-tyrosine in soil in laboratory conditions was less than 1 day, indicative of rapid microbial
156 degradation. Sorgoleone, a major component of root exudates of *Sorghum bicolor*, is a potent
157 allelochemical [30] and microorganisms present in North American soils readily use it as a
158 carbon source [49]. It has been shown that the methoxy group of sorgoleone, which is responsible
159 for much of its activity, degrades rapidly in soil [49].

160 In addition to the direct effects of allelochemicals on plant growth, their indirect effects may
161 be mediated by microbial activity. Meier and Bowman [50] compared the effects of several
162 allelochemical fractions from a phenolic-rich alpine forb, *Acomastylis rossii*, on soil respiration
163 and the growth of the grass *Deschampsia caespitosa*. They found that some fractions had a direct

164 phytotoxic effect (i.e., which did not increase soil respiration but killed *D. caespitosa*) while
165 others appeared to work indirectly through the soil microbial community (i.e., which stimulated
166 soil respiration and reduced plant growth and plant N concentration). Their results provide a
167 compelling example of how phenolic compounds can inhibit root growth directly as well as
168 through interacting with soil biota. In another example, *Alliaria petiolata* can have negative
169 impacts on arbuscular mycorrhizal (AM) fungi and regeneration of seedlings native to North
170 America in soil from North America [51], but much weaker effects on AM fungi in soils from
171 Europe where it is native. Cantor *et al.* [52] showed that even very low field concentrations of
172 allyl isothiocyanate (*ca.* 0.001mM) produced in the presence of *A. petiolata* strongly inhibited the
173 spore germination of the AM fungus *Glomus clarum*. However, Barto *et al.* [53] did not find
174 effects of *A. petiolata* extracts on the AM fungal colonization of roots or soils, and suggested that
175 potential alleopathic effects of *A. petiolata* might be due to direct inhibition of plant seedlings
176 and fungus before the formation of symbiosis.

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

185 Belowground interactions among plants may also be genotype or ecotype dependent. For
186 example, when the roots of different *Ambrosia dumosa* plants make contact they often stop
187 growing, but there is a geographic and genotypic aspect to this response. For example, roots of

188 the plants from the same region show strong contact inhibition, but roots from plants from
189 different regions do not [56,57]. *Cakile edentula* plants allocate biomass differently to roots if
190 they are grown in the same pots shared by genetic relatives (kin) compared to pots shared by
191 strangers [58]. Lankau [59] reported that investment in high tissue concentrations of sinigrin
192 produced by *Brassica nigra* gave it an advantage in interspecific competition but a disadvantage
193 in intraspecific competition. Further, selection for *B. nigra* individuals that produced high levels
194 of sinigrin was stronger when grown with other species than with other individuals of its own
195 species.

196 Coexisting plant species can differ greatly in their growth response to allelochemicals
197 produced by a given plant species, and allelopathic effects can be highly species-specific
198 [16,22,60]. As such, there is a wide range of abilities (and perhaps mechanisms) among species
199 to protect themselves from chemical effects of their neighbors. Weir *et al.* [61] found that
200 *Gaillardia grandiflora* and *Lupinus sericeus* secrete oxalate in response to catechin exposure,
201 which could make these two species resistant to *C. stoebe* invasion. Exogenous application of
202 oxalate blocks the production of reactive oxygen species in the target plants, minimizing
203 oxidative damage caused by catechin. Such variation in the species-specific response of target
204 species may play a crucial role in the organization or assembly of plant communities in a similar
205 manner that it does for microbial communities [62], and provides an opportunity for allelopathy
206 to drive natural selection [63]. Variation in the ecological roles of secondary compounds is better
207 understood for consumer defense than for allelopathy, but for both types of interactions variation
208 is an important aspect of the effects of chemicals on communities and populations.

209 Issues of spatial scale and patchiness make studies of the roles of allelochemicals in soils
210 difficult to interpret. The effects of allelochemicals in soils are generally examined using “bulk
211 soils”, where allelochemicals are added to a volume of soil that is orders of magnitude greater

212 than the soil volume in which the interactions occur. 'Realistic' concentrations of
213 allelochemicals are estimated for the average of the large soil volume. However, the action of
214 root-exuded chemicals often takes place at root-root interfaces. The use of estimated soil
215 concentrations is just one way to explore allelopathy in a reasonably realistic manner, but they
216 have limitations for the determination of the allelopathic functions of chemicals. If an
217 allelochemical is experimentally applied to soil in such a way as to allow it to transform before
218 contact with roots [12,19,34,37], then the failure to find an effect cannot be taken as evidence that
219 effects do not occur when roots are in close proximity to each other. This issue is, however, less
220 relevant when allelochemicals enter the soil through release from foliage or decomposition of
221 plant litter.

222
223 **Biogeographic comparisons of allelopathy: evolutionary changes in allelochemical effects**

224
225 The effects of allelopathy are also dependent on the evolutionary history of the interaction.
226 Understanding the mechanisms by which many exotic invasive plants strongly suppress their
227 neighbors in invaded but not native ranges has attracted growing recent attention. Allelopathy
228 and other biochemically driven interactions may contribute to the success of some exotic invasive
229 plants, and when either specific allelochemicals or general allelopathic effects are stronger
230 against potentially evolutionarily naïve species in invaded ranges, we gain insight into how
231 evolutionary history affects biological organization [64]. Biogeographical comparisons of the
232 ecological and biochemical traits of species in introduced and native ranges have proven useful
233 for evaluating mechanisms of invasion [65]. Examining the production and/or accumulation of
234 allelochemicals in novel and native environments, and the sensitivity of native residents and soil
235 communities to novel chemicals, can help understand these mechanisms.

236 The Novel Weapons Hypothesis (NWH) provides a possible explanation for biogeographic
237 patterns of interactions in different ecosystems. The NWH was first proposed in the context of
238 allelopathy as a potential mechanism for the success of *Centaurea diffusa* as an invader in North
239 America [66], and subsequently as a component of invasion by *C. stoebe* [67]. Recent studies on
240 biogeographic comparisons of exotic species in native and introduced ranges have shown some
241 support for NWH [15,16,18,68,69]. A recent meta-analysis of hypotheses for invasions, focusing
242 on trees, found that published evidence for the NWH resulted in a stronger effect size in support
243 of the idea than the effects sizes of six other hypotheses [70]. Barto *et al.* [71] provided evidence
244 in support of NWH by showing that the allelochemical profile of invasive *A. petiolata* was not
245 shared by any native Brassicaceae in North America. Further, Callaway and Ridenour [67]
246 suggested that stronger allelopathic effects in invaded regions could lead to selection for greater
247 allelopathic production and thus increased competitive ability.

248 Biogeographic differences in the effects of particular compounds between native and invaded
249 ranges may occur in part through a lack of adaptation by species and soil communities in the
250 invaded ranges. However, these types of biogeographic differences may also emerge or intensify
251 because of particular conditions in the novel environment. As such, soil biota can be powerful
252 ecosystem mediators of biogeographic differences in allelopathic effects [46]. For example, soil
253 microbial taxa that metabolize specific chemicals are likely to have undergone evolution to do so,
254 or at least to utilize a related group of chemicals. If plants that occur in a given region do not
255 produce a particular allelochemical, then those soil microbes that are required to metabolize it
256 may not be present when it is introduced by an invader. Thus, novel chemicals produced by
257 invaders may have prolonged resident times in invaded ranges and therefore be more biologically
258 active. Such indirect processes may reinforce biogeographic differences in plant-soil feedbacks
259 involving invasive species [72].

260 Soil communities from non-native ranges have also been shown to eliminate allelopathic
261 effects of exotic plants. For example, the invader *A. petiolata* exerts allelopathic effects through
262 glucosinolate exudation on the native species *Platanus occidentalis* in sterilized soil but not in
263 non-sterile soil from the invaded range [73]. Future research would be required to determine
264 whether soil microbial communities from locations that differ in their invasion history of *A.*
265 *petiolata* also differ in their ability to degrade glucosinolate.

266

267 **Potential evolutionary relationships: temporal declines in allelochemicals from invasive**
268 **species**

269

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

281 Biogeographic variation in the production of volatile sesquiterpenes in particular could be due
282 to differences in herbivore densities between the native and introduced ranges [77]. Recently, it
283 has been shown [15] that lower amounts of volatile chemicals were released by plants from

284 exotic populations of the invasive plant *Ageratina adenophora* than by plants from native
285 populations grown in a common environment. However, it is not known whether such differences
286 in volatile emissions are evolutionary consequences of interactions with other species or due to
287 founder effects.

288 An allelochemical produced by a species can provide multiple ecological functions, making its
289 effects highly dependent on specific environmental conditions. Further, allelochemicals with
290 multiple functions should be selected for because this spares the plant the cost of producing
291 several different compounds [11]. Glucosinolates and their derivatives have been found to have
292 multiple functions as mediators of plant–plant, plant–microbe, and plant–insect interactions [59].
293 Lankau and Kliebenstein [78] found that competition and herbivory determined the accumulation
294 and fitness consequences of sinigrin for *B. nigra*. Further, it has been shown that the fitness costs
295 and benefits of sinigrin conformed to optimal defense theory only in the absence of competition,
296 apparently due to its multiple functions [11,78]. Further, Oduor *et al.* [79] found that invasive
297 populations of *B. nigra* had higher levels of sinigrin which defends the invader against generalist
298 herbivores. An increase in resistance against generalist herbivores and growth performance of *B.*
299 *nigra* in its introduced ranges compared to its native range further supports the hypothesis that
300 defenses have shifted [79]. Sinigrin from *B. nigra* is also reported to possess allelopathic
301 activities, which provide a competitive advantage to *B. nigra* over heterospecific neighbors [59].

302 Lankau *et al.* [23,24] examined the production, release and impact of glucosinolates from *A.*
303 *petiolata* along a gradient of invasion history i.e., from early invaded to recently-invaded
304 populations. They found a significant decline in the production of glucosinolates and an increase
305 in the community's resistance to *A. petiolata* invasion over time. Following an initial decline in
306 the number of operational taxonomic units (OTUs) of bacteria, fungi and AM fungi, an increase
307 was observed in older invaded sites [24]. The observed development of resistance to exotic

308 invasion in late invasion stages could lead to more species rich native communities. However, the
309 eventual outcome of the evolutionary changes is still unclear. Lankau *et al.* [80] found that the
310 higher production of sinigrin by introduced *B. nigra* suppressed mycorrhizal abundance, which
311 adversely affected the growth of heterospecific competitors but not non-mycorrhizal conspecifics.
312 Such rapid selection based on tradeoffs between competitive advantages against either
313 conspecifics or heterospecifics contributes to intransitive competitive networks which affect
314 genetic and species diversity in communities [80]. Studying evolutionary relationships between
315 native and non-native communities and ecosystems along gradients of invasion history has
316 significant potential for improving understanding of the role of allelopathy in community
317 organization.

318

319 **Conclusions**

320

321 It is important to identify how variation in the environment establishes conditionality in
322 allelopathic interactions. Sources of such variation include (1) the impact of soil chemistry on
323 production and effects of allelochemicals, (2) the impact of consumers, competitors, and soil
324 microbes on production and effects of allelochemicals, (3) evolutionary changes in
325 allelochemical effects, and (4) declines in allelochemical production and activity from invasive
326 species over time. A major gap in current allelopathy research involves the role of conditional
327 ecosystem factors that drive allelopathic processes and how these change over space and time
328 (Figure 1). Further, despite recent advances, we still have a limited understanding of the role of
329 evolution over time in the production, release and eventual loss of activity of biogeographically
330 novel chemicals.

331 The production, fate, and effectiveness of allelopathic compounds in soils is influenced by
332 environmental conditions and evolutionary history, generating a need for allelopathic interactions
333 to be studied across spatial and temporal scales (Figure 1). Over very small scales (microns to
334 millimeters; seconds to hours), processes in the rhizosphere, such as microbial-driven breakdown
335 of allelochemicals or metal chelation, dominate the influences of allelochemicals. Over small
336 scales (millimeters to meters; hours to months), organismal responses are important, for instance,
337 the increased production of chemicals following herbivore attack. At the medium scale (meters to
338 kilometers; months to years), variation in the plant and soil communities, and abiotic soil
339 conditions become increasingly important, if different species are more or less susceptible to the
340 allelochemicals. Finally, at the large scales (kilometers and beyond; years and beyond), the
341 evolutionary history of the allelopathic plant and the recipient soil and plant community assumes
342 increasing significance (Figure 1).

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

355
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357
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362

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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.

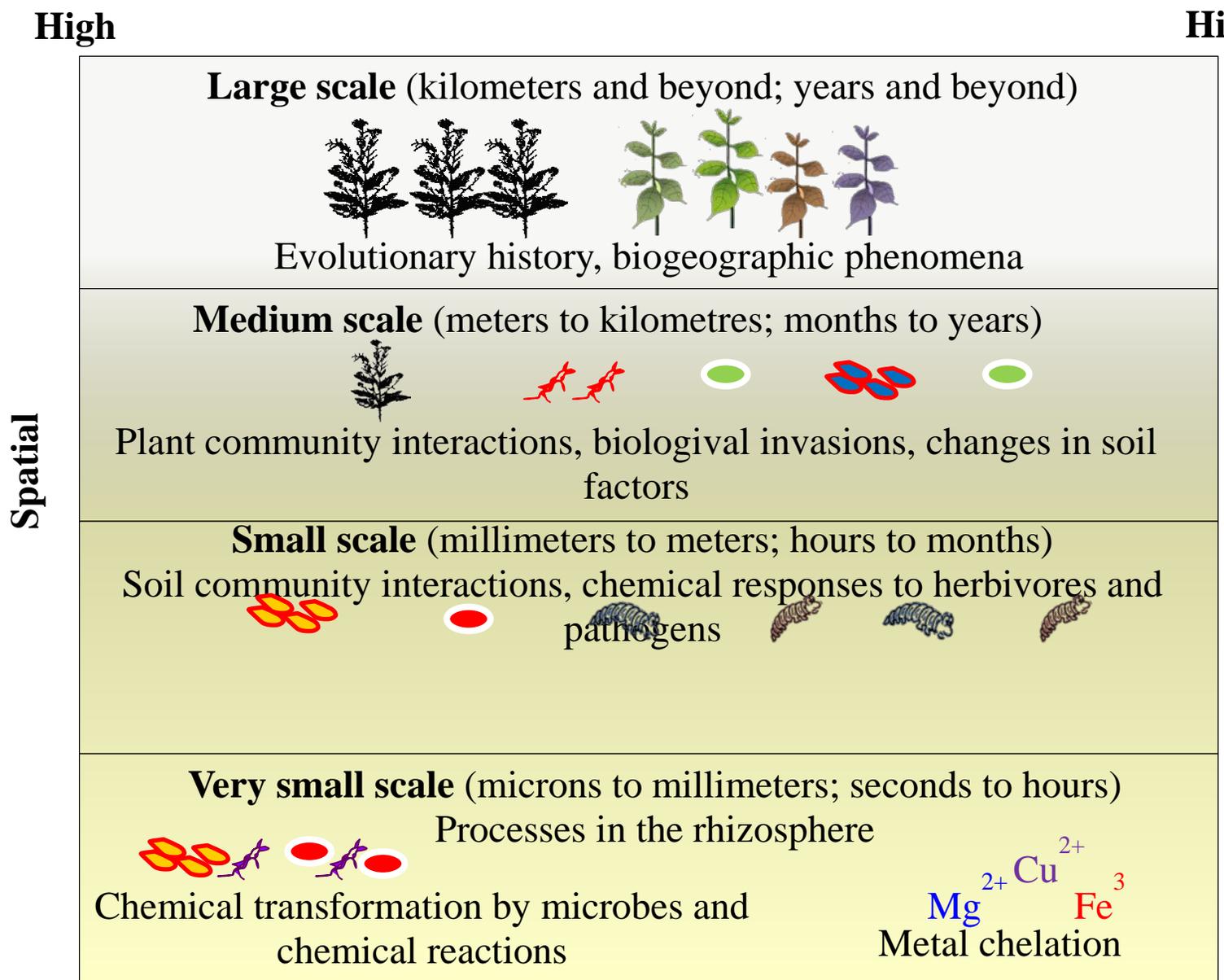
Allelochemicals: Secondary compounds of plant origin that interact with their environment and possess allelopathic activities.

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.

581 **Figure 1.** The impact of ecosystem factors, biogeographic variations and coevolutionary
 582 relationships on the production, release and activity of allelochemicals along spatial and temporal
 583 scales.

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586 **Low**
 587 **Figure 1**
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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.

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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

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Figure I (for Box 1)