




REVIEW AND SYNTHESIS

Effect of allelopathy on plant performance: a meta-analysis

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Abstract

Allelopathy (i.e. chemical interactions between plants) is known to affect individual performance, community structure and plant invasions. Yet, a quantitative synthesis is lacking. Here, we performed a meta-analysis of 384 studies that measured allelopathic effects of one species (allelopathy plant) on another species or itself (test plant). Overall, allelopathy reduced plant performance by 25%, but the variation in allelopathy was high. The type of method affected the allelopathic effect: compared to leachates, allelopathy was more negative when residues of allelopathy plants were applied, and less negative when soil conditioned by allelopathy plants was applied. The negative effects of allelopathy diminished with study duration, and increased with concentrations of leachates or residues. Although allelopathy was not significantly related to lifespan, life form or domestication of the interacting plants, it became more negative with increasing phylogenetic distance. Moreover, native plants suffered more from leachates of naturalised alien plants than from leachates of other native plants. Our synthesis reveals that allelopathy could contribute to success of alien plants. The negative relationship between phylogenetic distance and allelopathy indicates that allelopathy might contribute to coexistence of closely related species (i.e. convergence) or dominance of single species.

Keywords

Crop, interference competition, invasive species, life history, meta-analysis, phylogeny, soil microbes, succession.

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INTRODUCTION

Allelopathy refers to either the inhibitory or stimulatory effect of one plant on another through the production of chemicals and their release into the environment (Molisch 1937; Rice 1984). The earliest written record of allelopathy can be traced back to c. 300 BC, when the Greek philosopher Theophrastus noticed that chick pea plants 'exhaust' the soil and destroy weeds (Hort 1916). To understand the roles of allelopathy in agriculture, forestry and old-field succession, numerous studies have been conducted since 1970 (Rice 1984; Cheng & Cheng 2015). In the late-1990s, research on allelopathy gained even more attention as some studies revealed allelopathy as a driving mechanism for the success of some invasive plants (Wardle *et al.* 1998; Callaway & Aschehoug 2000; Hierro & Callaway 2003). Despite the long-standing interest and the mounting number of studies on allelopathy, we still lack a quantitative synthesis to answer whether allelopathy generally inhibits or stimulates plants.

So far, inhibitory effects of allelopathy have been found in some studies (Mahall & Callaway 1992), but no or stimulatory effects have been found in others (Bauer *et al.* 2012; Zhang *et al.* 2015). Therefore, besides testing the overall effect of allelopathy, identifying the sources of heterogeneity can

also improve our understanding (Lankau 2009; Tharayil *et al.* 2009; Meiners *et al.* 2012). The first candidate source is the method to study allelopathy. For example, to test allelopathy, some studies soaked seeds in leaf leachates or root exudate from other plants, and some grew seedlings on substrate mixed with residues from other plants (see Table 1 for a summary of the major methods that have been used to test allelopathy). The differences in study method partly reflect the four pathways through which allelochemicals are released into the environment, that is, leaching from plants by rain, decomposition of plant residues (e.g. litter), exudation from roots and volatilisation (Fig. 1; Rice 1984 p309). However, studies also differ with regard to the different life stages of plants (e.g. germination *vs.* growth) and the duration of the experiment. Disentangling the importance of the different factors related to the study design can hardly be achieved in a single case study, but is feasible in a quantitative synthesis, such as meta-analysis (Gurevitch *et al.* 2018).

Variance in allelopathy can also arise from biological traits of plants. Several studies of plant succession revealed that the effect of allelopathy may depend on life history. For example, Jackson and Willemsen (1976) reported that ragweed (*Ambrosia artemisiifolia*) and wild radish (*Raphanus raphanistrum*), which are short-lived, early successional species, cannot

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Table 1 A description of the seven types of methods used to test for allelopathy. We also indicate which of the four pathways of allelopathy (see Fig. 1) the method tests

Method	Description	Pathway*
Leachate	Leachates were made by soaking plant biomass (usually leaves) of the allelopathy plant in water, or in a mixture of water and an organic solvent, for a certain time (ranging from a few hours to days). Then, the leachate was applied to the test plants.	P1
Residue	Biomass (litter or fresh biomass) of the allelopathy plant was directly incorporated into the substrate of the test plants.	P2
Exudate	The allelopathy plant was grown on artificial medium or in an exudate collection system. Then the medium was used to grow test plants, or exudate was applied to the test plants.	P3
Volatile	The test plant was grown in the presence of allelopathy plants, but without any physical contact.	P4
Soil	Soil was collected from where the allelopathy plant grew or the allelopathy plant was used to train the soil. Then the soil was used as substrate for the test plant.	P1, P2, P3
Activated carbon	Active carbon was used to absorb allelochemicals, so that the effect of allelopathy would be neutralised.	P1, P2, P3
Solvent extraction	Leachates of the allelopathy plant were extracted by organic solvents (if different organic solvents were used, the least polar solvent was used first, followed by more polar ones). Then, the solvent fraction was applied to the test plant.	P1

*The abbreviations correspond to the pathways in Figure 1. P1: leachate; P2: residue; P3: exudate; P4: volatile.

re-establish on soils on which the long-lived, late-successional frost aster (*Aster pilosus*) grew previously. Besides life history, domestication may also matter as crop species appear to be more sensitive to allelopathy than weeds (Sera 2012). From an evolutionary perspective, individuals of short-lived species are associated with other species for a shorter period than long-lived species are (Myster & Pickett 1992). Likewise, crop species are less exposed to interference with other species (e.g. weeds) as they are frequently protected by humans. Consequently, the abilities to inhibit other species and to tolerate inhibition from others are less likely to be selected for in short-lived species or crops (Meiners *et al.* 2012). However, whether this is true remains untested at a broad scale.

The strength and direction of allelopathic interactions may also depend on two other aspects of evolutionary history: phylogenetic distance and species origin. On the one hand, the composition of secondary metabolites (i.e. allelochemicals) is generally phylogenetically conserved in plants (Wink 2003; Grutters *et al.* 2017). Therefore, distantly related species might strongly inhibit each other due to little overlap in, and thus novelty of, their allelochemical profiles. On the other hand, evolutionary history is also shaped by the conditions that a species has experienced. In their native range, alien plants interacted with other species (e.g. competitors and enemies)

than they do in their non-native range (Liu & Stiling 2006; Blumenthal *et al.* 2009; Saul & Jeschke 2015). Therefore, the aliens might have evolved different arsenals of allelochemicals to which the natives, in the non-native range of the aliens, are not adapted. This is known as the Novel Weapons hypothesis (Callaway & Ridenour 2004). For example, Callaway and Aschehoug (2000) found that the Eurasian *Centaurea diffusa*, a noxious invasive weed in North America, had more negative effects on species native to North America than on species native to Eurasia. The other way around, the natives may also produce arsenals of allelochemicals to which introduced aliens are not adapted, and this might provide biotic resistance (Rabotnov 1981; Cummings *et al.* 2012; Ning *et al.* 2016). Still, the importance of phylogenetic distance and novelty for the strength of allelopathic interactions and the consequences for invasion success of alien plants remain unknown.

Although there are still many open questions in allelopathy research, the past decade has witnessed a slowdown of research on allelopathy (Fig. S1). There are possibly two major reasons for this. First, retractions and corrections of several high-profile publications – whose findings could not be repeated by subsequent studies (Blair *et al.* 2005; Perry *et al.* 2007) or were identified as being fabricated (Borrell 2015; Palus 2016) – have haunted the field. Second, some of the techniques to study allelopathic effects have been questioned (e.g. Lau *et al.* 2008, Blair *et al.* 2009). Therefore, to assess the overall evidence for allelopathy and to quantify its effects, we conducted a meta-analysis. Importantly, we also identified sources of variance in allelopathy by testing whether allelopathy is related to study design (including the techniques used), biological traits of species and evolutionary history. Finally, to provide new avenues for future studies, we identified some of the knowledge gaps in present allelopathy research.

METHODS

Literature search

We searched *ISI Web of Science* for between-plant-allelopathy studies that were published between January 1900 and December 2017. We used the search string ‘allelopath* AND plant’, which resulted in 3699 papers. This list of papers was further expanded by including all papers that were published in the *Allelopathy Journal* (ISSN 0971-4693) between 1994 (the year that the journal was first published) and 2017, which resulted in another c. 900 papers. We then screened the titles, abstracts and main texts. Studies were included if the following criteria were met:

- (1) The study tested a pairwise interaction, that is, the effect of one species on another or on conspecifics. Therefore, studies that tested effects of allelopathy on a community, that tested effect of mixed species and that tested effects of single allelochemicals on plants were excluded.
- (2) The study tested allelopathy using one of the seven major methods listed in Table 1.
- (3) The study measured traits related to germination, growth or fitness, and thus studies that measured other traits,

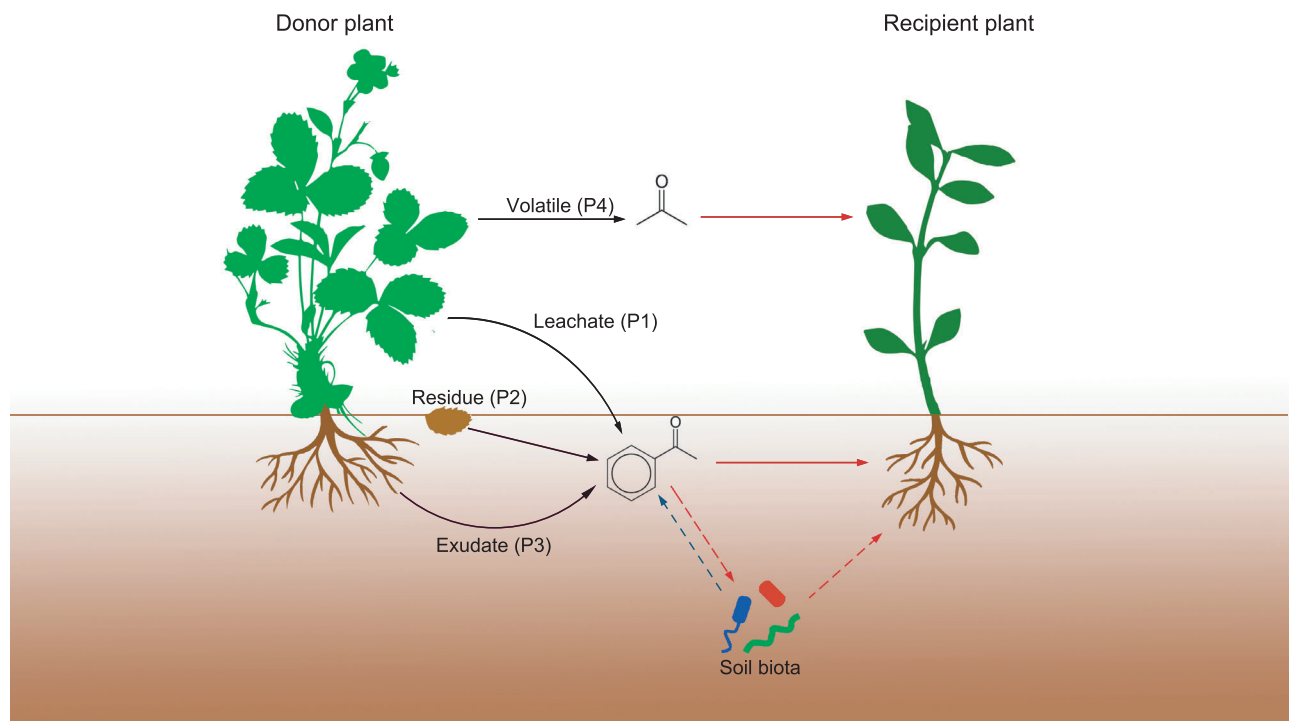


Figure 1 The different release pathways and effects of allelochemicals. The allelopathy plant (left) can release allelochemicals through four pathways (black arrows): leaching by rain (P1), decomposition of plant residues (P2), exudation from roots (P3) and volatilisation (P4). The allelochemicals can affect the test plant directly (red arrows) or indirectly through their effect on soil biota (dashed red arrows). Soil biota can also affect allelochemicals, such as through conversion or degradation of allelochemicals. The root illustration is designed by Brgfx/Freepik.

such as mycorrhizal colonisation and the concentration of chlorophyll, were excluded. Germination measurements include germination rate (i.e. proportion of seeds that germinated), germination speed and time to germination. Growth measurements include total biomass and biomass of different plant parts (shoots, roots, leaves and stem); lengths of roots and shoots; numbers of leaves and branches; leaf size; and stem diameter. Fitness measurements include biomass of seeds, fruit and flowers, and numbers of seeds, fruit, flowers and ramets.

- (4) The study used seed plants (i.e. the few studies that used algae or ferns were excluded).
- (5) The study reported the duration of the experiment.
- (6) Mean values and variances (standard deviations, standard errors or 95% confidence intervals) of control and allelopathy treatments could be obtained from the text, tables, figures or supplements.

Data collection

In total, 378 published papers met our criteria. We also added data of six experiments from our laboratories, two of which were published after 2017 (Zhang *et al.* 2018; Oduor *et al.* 2020). So, we included 384 studies in total. If a study sampled

data at multiple time points, we only extracted the final data points to avoid temporal autocorrelation. If a study tested multiple species pairs, applied multiple treatments (e.g. different concentrations of leachates), we considered them as independent observations. For each observation, we extracted the mean values, variances and sample sizes of the test species, under control and allelopathy treatments. Data from figures were extracted using ImageJ (Abràmoff *et al.* 2004). We recorded study duration, the plant part used for making the allelopathy treatment (shoot, root and whole plant), the concentration of leachates or residues if it was reported in the paper. Besides, we recorded the experimental environment, that is, controlled (Petri dishes, growth chamber or greenhouse), semi-natural (outdoor experiment that was not under natural conditions) or natural environment. Our final data set contained 16,810 data points covering 461 allelopathy species and 470 test species.

In addition to the data extracted from the papers, we acquired three biological traits – lifespan (short- or long-lived), life form (woody or non-woody) and domestication (crop or wild) – of the species by matching their names with several databases. Before matching our database with others, we harmonised taxonomic names of all species according to The Plant List (<http://www.theplantlist.org/>), using the *Taxonstand* R package (Cayuela *et al.* 2017).

We extracted the information on lifespan and life form from the following databases: TRY (Kattge *et al.* 2020; last accessed on 9 November 2015), the World Checklist of Selected Plant Families (WCSP; <http://wcsp.science.kew.org/>; last accessed on 22 August 2018), Plants for a Future (PFAF, <https://pfaf.org/>; last accessed on 22 August 2018), LEDA (Kleyer *et al.* 2008; last accessed on 22 August 2018) and PLANTS (<http://plants.usda.gov/>; last accessed on 22 August 2018). We classified annual and biennial species as short-lived, and perennials as long-lived. A few species were not included in the databases (29% in case of lifespan and 16% in case of life form). For them, we extracted the information on lifespan and life form from the description of the species in the allelopathy papers or we did internet searches to find this information. We classified a species as a crop species in the narrow sense if it is recorded as a human food in the World Economic Plants (WEP) database (National Plant Germplasm System GRIN-GLOBAL; <https://npgsweb.ars-grin.gov/grin/global/taxon/taxonomysearcheco.aspx>, last accessed on 7 January 2016), which is based on a book by Wiersema and León (2013). However, because crops are not restricted to plants grown for human food, we also used a broader classification in which a species was classified as a crop if it is recorded to have any economic use according to the WEP database. The results based on the broader definition of crop (Table S1) were very similar to those based on the narrower definition, indicating that the results on crop *vs.* non-crop species are robust.

We also acquired data on the origin of the species (i.e. native or alien at the location of study), and in the case of alien species, we distinguished between non-naturalised and naturalised species. The latter are species that have established self-perpetuating populations in the wild at the location of study (*sensu* Richardson *et al.* 2000). For this, we used three databases – the Global Naturalized Alien Flora (GloNAF), the Germplasm Resources Information Network (GRIN) and the Plants of the World Online portal (POWO). The GloNAF database contains lists with naturalisation status of 13,939 alien vascular plant taxa for 1,029 regions (countries or sub-national administrative units; van Kleunen *et al.* 2019). GRIN is an online database published by the United States Department of Agriculture (USDA; <https://www.ars-grin.gov/>; last accessed on 2 March 2020). POWO is an online database published by the Royal Botanic Gardens, Kew (<http://powo.science.kew.org/>; last accessed on 2 March 2020). All of them provide descriptions of the distribution of the taxa.

We classified a species at the location of study as a naturalised alien if GloNAF or GRIN reported that it is naturalised there; as native if GRIN, POWO or the study reported that it is native there; as non-naturalised alien if the species is recorded there neither as native nor as naturalised by any of the data sources. Five studies tested for allelopathic effects of an alien species collected in its non-native region on species that had been collected in the native region of the alien species (Callaway & Aschehoug 2000; Prati & Bossdorf 2004; Abhilasha *et al.* 2008; Gruntman *et al.* 2016; Oduor *et al.* 2020). As, in these cases, it is not clear whether the allelopathy and test species should be considered native or alien, we excluded such species pairs from analysis.

We constructed a phylogenetic tree for all species in our data set (Supplement S3) by pruning an existing phylogenetic supertree using the R function *S.PhyloMaker* (Qian & Jin 2015). The supertree, which was initially constructed by Zanne *et al.* (2014) and further corrected and expanded by Qian and Jin (2015), included 30,771 seed plants, and has been time-calibrated for all branches using seven gene regions (i.e. 18S rDNA, 26S rDNA, ITS, *matK*, *rbcL*, *atpB* and *trnL-F*) available in GenBank and fossil data. Species that were absent in the supertree (about 21.6%) were added as polytomies to the root of their genus or family. We calculated the pairwise phylogenetic distance between allelopathy and test species using the *cophenetic* function in the *ape* package (Paradis & Schliep 2018).

Effect size calculation

As effect size, we calculated the log response ratio ($\ln RR$; Borenstein *et al.* 2009) as:

$$\ln RR = \ln(\bar{X}_{\text{allelopathy}}) - \ln(\bar{X}_{\text{control}}).$$

where $\bar{X}_{\text{allelopathy}}$ and \bar{X}_{control} are the mean values of performance (germination, growth or fitness) of a test species in the allelopathy and control respectively. When a high value of a measured trait indicates low performance of plants (e.g. for a given species, a high value for germination time indicates low success of germination), we changed the sign of the effect sizes of this trait. Consequently, a negative value of $\ln RR$ indicates that allelopathy reduced plant performance, and a positive value indicates that allelopathy increased plant performance.

The sampling error variance of the $\ln RR$ was calculated as:

$$v = \frac{S_{\text{allelopathy}}^2}{n_{\text{allelopathy}} \bar{X}_{\text{allelopathy}}^2} + \frac{S_{\text{control}}^2}{n_{\text{control}} \bar{X}_{\text{control}}^2}$$

where S and n are the standard deviation and sample size respectively (Borenstein *et al.* 2009).

Statistical analyses

All analyses were carried out with Bayesian multivariate models implemented in the *brms* package (Burkner 2017) in R 3.6.1 (R Core Team 2019), which allowed us to account for phylogenetic non-independence of the species and to include both the sampling error and the residual error. We considered an estimated parameter as significant when its 95% credible interval of the posterior distribution did not overlap zero, and as marginally significant when the 90% credible interval did not overlap zero.

Meta-analysis

To test the overall effect of allelopathy, we ran an intercept-only meta-analysis model. We included effect size ($\ln RR$) as response variable; and study identity (i.e. paper), species identity and phylogenetic effect (added as phylogenetic variance—covariance matrices) of allelopathy and test species as random effects. We specified standard errors of the observations (squared root of the variance) to weight the effect size by its precision. Note that both the species identity and phylogenetic

effect of species are species-specific effects, but that the former represents the non-phylogenetic part (Nakagawa & Santos 2012).

We used the default priors set by the *brms* package, but changing the priors had negligible effect on our results. We ran four independent chains with 3000 iterations each. Half of the iterations were used as warm-up, so a total of 6000 samples were retained to estimate the posterior distribution of each parameter. We quantified heterogeneity of the model by estimating I^2 *sensu* Nakagawa and Santos (2012) (see Supplement S4 for details). The phylogenetic signal was quantified by H^2 , which is a metric of phylogenetic signal named phylogenetic heritability (Lynch 1991). Similar to the widely used metric of phylogenetic signal Pagel's λ (Pagel 1999), $H^2 = 0$ indicates that there is no effect of phylogenetic relatedness of species on their difference in effect sizes, while $H^2 = 1$ indicates that the differences in effect sizes are exactly proportional to the phylogenetic relatedness of the species. To report the effect of allelopathy on plant performance at the raw-data scale, we back-transformed the log response ratio ($\ln RR$) with the formula: $e^{\ln RR} - 1$. For example, $\ln RR = -0.5$ indicates that allelopathy reduced plant performance by 39.3% ($e^{-0.5} - 1$).

Meta-regressions

To test the importance of different sources of variance in allelopathy, we ran three meta-regression models (i.e. mixed-effects meta-analyses). The first model included data from all seven methods described in Table 1 combined. The second and third models analysed two subsets, that is, the leachate and the residue method respectively. These two models allowed us to analyse certain aspects of the experimental design in more detail. For example, the concentration of the allelopathic agents was not manipulated in all seven methods (e.g. activated-carbon method), and thus cannot be tested in the first model. We also ran meta-regression models for each of the other five methods, but because the number of studies was relatively small, we report their results in the supplements (Supplement S5). In all models, we included effect size as response variable; and used the same random effects, priors, warm-up and sampling as in the intercept-only meta-analysis described in the 'Meta-analysis' subsection above.

In the first model, we included the following explanatory variables as fixed effects: (1) type of method (leachate, residue, activated carbon, solvent, soil, volatile and exudate; see Table 1); (2) type of performance measure (germination and growth; as only 10 studies measured fitness, we combined fitness with growth); (3) experimental environment (controlled, semi-natural and natural); (4) study duration (days); (5) lifespan (short-lived [annual and biennial] and long-lived [perennial]) of allelopathy and test species; (6) life form (herbaceous and woody) of allelopathy and test species; (7) domestication (crop and wild) of allelopathy and test species; (8) phylogenetic distance between allelopathy and test species; and (9) origin (native, naturalised alien and non-naturalised alien) of allelopathy and test species, and interaction between origin of allelopathy and test species.

Variables 1–4 are those related to the experimental design. Variables 5–7 are those related to biological traits. Variables 8

and 9 are those related to evolutionary history. Note, however, that the variables 5–7 (lifespan, life form and domestication) are also related to evolution. For example, crops were selected for high yield by humans. However, the variables 8 and 9 (phylogenetic distance and origin) focus on the similarity in evolutionary history between allelopathy and test species, for example, their relative positions on the phylogenetic tree.

In the second and third models, which focused on studies that used the leachate and residue methods, respectively, we included the same fixed effects as in the first model, except for type of method. Consequently, the models included the following fixed effects: (1) type of measurement; (2) experimental environment (not for leachate, because there was no semi-natural or natural experiment); (3) study duration; (4) lifespan of allelopathy and test species; (5) life form of allelopathy and test species; (6) domestication of allelopathy and test species; (7) phylogenetic distance between allelopathy and test species; and (8) origin of allelopathy and test species, and their interaction.

We included another three fixed effects related to the study design, which could not be included in the first meta-regression model: (9) 'colour' of biomass (fresh biomass [green] and litter [brown]); (10) plant part (belowground, aboveground part and whole plant); (11) concentration of leachates or residues.

Because the concentration of secondary metabolites, study duration and phylogenetic distance have been found or hypothesised to be nonlinear (Rice 1984; Reich *et al.* 2018; Malecore *et al.* 2019), we considered nonlinear effect for all of them. To do this, we first ran models that applied data transformation (natural-log and square-root transformation), included quadric terms or specified nonlinear curves (Monod equation and Weibull curve). Then, we performed model selection with the leave-one-out cross-validation method using the *loo* package (Vehtari *et al.* 2016). In the end, we natural-log transformed study duration and concentration of leachates or residues and added quadric terms for those variables, while we used the original linear scale for phylogenetic distance. To improve comparability of the coefficient estimates, we bounded phylogenetic distance, which ranges from 0 (i.e. same species) to 781 million years, between 0 and 1.

To improve interpretation of the intercept (Schielzeth 2010) and to aid comparisons between different meta-regression models, we mean-centred all categorical explanatory variables with two levels after coding them as dichotomous (0 and 1) variables. For categorical variables with more than two levels, we set the most common levels as the reference levels. For type of method this was 'leachate', for experimental environment this was 'controlled', for plant part this was 'aboveground part' and for origin of allelopathy and test species this was 'native'. For all continuous variables, we applied median-centering instead of mean-centering because their distributions were skewed.

For the concentration of leachates or residues, studies used three types of units (weight/weight, g/g; weight/volume, g/cm³; and weight/area, g/cm²), which differ in their scales and might have different per-unit effects. To account for this, we included type of unit and set 'weight/weight', the most common level, as the reference level in the model for residue method. In the model for leachate method, all but two studies

used the unit of weight/volume, and we therefore removed the two exceptions from the analysis. Finally, of the studies that used the residue method, only a few used non-naturalised alien species. For example, only two studies tested the effect of non-naturalised aliens on natives. Therefore, we did not discriminate between non-naturalised and naturalised aliens in the model for the residue method.

Detection of publication bias

Because statistically significant results are more likely to be published than non-significant results (Rosenthal 1979), publication bias can affect the results of meta-analyses. To test for publication bias, we first used the funnel plot method by plotting meta-analytic residuals against the inverse of their precision (i.e. inverse of the sampling error). When the funnel plot is asymmetric, one can conclude that publication bias is present. The meta-analytic residual is the sum of a within-study effect (analogy to residual in linear models) and a sampling error effect (Nakagawa & Santos 2012), and was extracted from the meta-analysis (the intercept-only model). Second, we performed a modified Egger's regression (Egger *et al.* 1997; Nakagawa & Santos 2012) by using the meta-analytic residuals as the response variable, and the precision as the explanatory variable. When the intercept does not overlap zero, one can conclude that publication bias is present. Finally, we analysed the temporal trends in effect sizes. A so-called time-lag bias arises when the earliest published studies have larger effects than those of later studies (Trikalinos & Ioannidis 2005). To do so, we ran a meta-regression model that included effect size as response variable, and publication year as the only fixed effect.

RESULTS

Our meta-analysis showed that allelopathy was, overall, inhibitory, as it reduced plant performance by on average 25.3% (mean $\ln RR = -0.288$, 95% CI = $[-0.492, -0.052]$). As expected, the heterogeneity in effect size was high. Within-study heterogeneity (i.e. at the observation level) accounted for 53.6% of the variance, between-study heterogeneity accounted for 26.2% of the variance, between-test-species heterogeneity (i.e. the non-phylogenetic part) for 13.0% of the variance and between-allelopathy-species heterogeneity for 7.1% of the variance (Table S2). The phylogenetic signal was low, both among allelopathy species ($H^2 < 0.01\%$) and among test species ($H^2 = 0.02\%$; Table S2).

Effects of study design on allelopathy

The type of method significantly affected the effect of allelopathy (Fig. 2a; Table 2). Compared to the leachate method, the effect of allelopathy was more negative (-15.1%) when the study used residues ($\ln RR_{\text{residue-leachate}} = -0.163$ [$-0.220, -0.107$]), and less negative ($+13.0\%$) when the study used soil on which the allelopathy species was grown ($\ln RR_{\text{soil-leachate}} = 0.122$ [$0.024, 0.218$]). The effect of allelopathy in studies that used activated carbon, exudate, volatile or solvent extraction did not deviate significantly from those that used leachates.

Allelopathy had overall weaker negative effects ($+15.1\%$) on germination than on plant growth ($\ln RR_{\text{germination-growth}} = 0.140$ [$0.119, 0.161$]; Fig. 2a; Table 2). This was particularly true when allelopathy was tested with leachates (Table S4). However, when tested with residues, allelopathy had stronger negative effects (-25.7%) on germination than on plant growth ($\ln RR_{\text{germination-growth}} = -0.297$ [$-0.387, -0.208$]; Table S5). Allelopathy had less negative effects ($+26.7\%$) in experiments done under semi-natural conditions than under controlled conditions ($\ln RR_{\text{seminatural-controlled}} = 0.237$ [$0.058, 0.411$]; Fig. 2a; Table 2), whereas allelopathic effects did not significantly differ between experiments done under natural and controlled conditions. We also found that leachates from belowground parts of plants had weaker negative effects ($+13.6\%$) than leachates from aboveground parts ($\ln RR_{\text{below-above}} = 0.127$ [$0.095, 0.160$]; Table S4).

The effects of study duration and concentration of leachates or residues on allelopathy were weakly non-monotonic, as indicated by the significant quadric terms (Fig. 2b and c; Table 2). Allelopathic effect was negative for experiments of short duration, but was not significantly different from zero when the experiments lasted over 89 days. The effect of low concentrations of leachates or residues was not significantly different from zero, but became significantly negative when the concentration of leachates (or residues) exceeded 0.055 g/ml .

Effects of biological traits on allelopathy

Overall, allelopathy did not differ between short- and long-lived species, between herbaceous and woody species, and between crop and wild species (Fig. 3; Table 2). This holds for both the allelopathy and test species (Fig. 3; Table 2).

Effects of evolutionary history on allelopathy

The effect of allelopathy became slightly more negative as the phylogenetic distance between allelopathy and test species increased (Fig. 4a; Table 2). More specifically, the effect of allelopathy between the most closely related species (or individuals of the same species) reduced plant performance of each other by 28.2% ($\ln RR = -0.331$, [$-0.521, -0.119$]), whereas that between the most distantly related species reduced plant performance by 41.0% ($\ln RR = -0.528$, [$-0.731, -0.319$]). Across all seven methods (Table 1), allelopathy was not affected by the origin of the allelopathy and test species, or by the interaction between the origins of the two species (Table 2). However, for the subset of studies that tested the effect of leachates, we found that natives experienced more negative (-13.4%) effects from naturalised alien species than from other natives ($\ln RR_{\text{naturalizedonnative-nativeonnative}} = -0.143$ [$-0.286, -0.004$]; Fig. 4b; Table S4). In addition, allelopathic effects between two naturalised aliens tended to be less negative ($+13.2\%$) than allelopathic effects between two natives ($\ln RR_{\text{naturalizedonnaturalized-nativeonnative}} = 0.124$ [$-0.011, 0.260$]; Fig. 4b; Table S4), although this was only marginally significant.

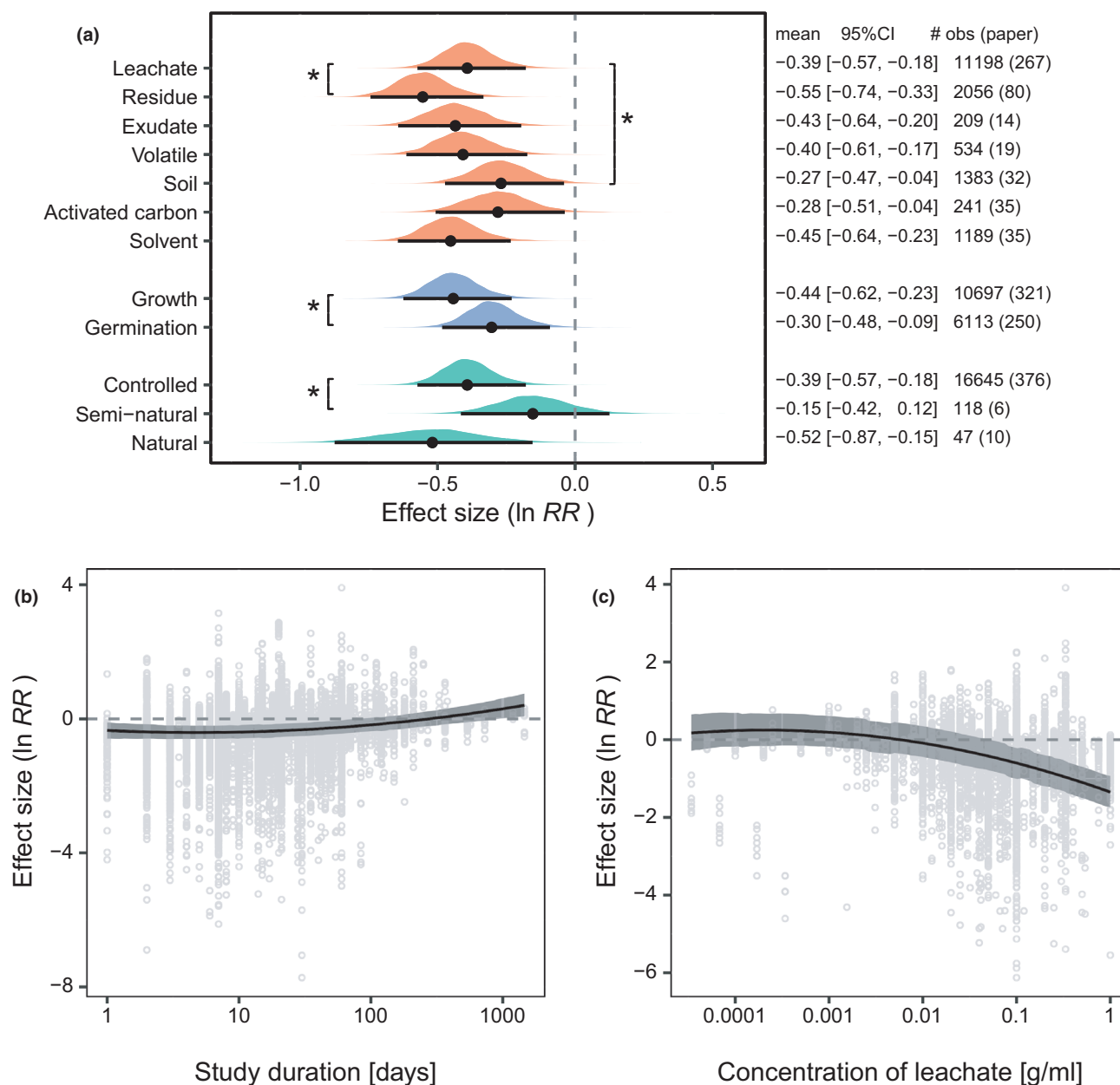


Figure 2 Effects of different aspects of study design on allelopathy. (a) Effects of the type of method (orange), the type of performance measure (blue) and the experimental environment (green) on allelopathy. (b) Effect of study duration on allelopathy. (c) Effect of the concentration of leachates on allelopathy. In (a) for each category (parameter), the posterior distribution is plotted with the mean and 95% credible interval. The text on the right displays the mean, 95% credible interval (CI), and the number of observations (obs) and papers. An asterisk indicates a significant difference between the level of interest and the reference level. In (b) and (c), curves of the estimated effects are shown with their 95% credible intervals. Negative values of the effect size (ln RR) indicate that allelopathy inhibits plant performance.

Publication bias

The funnel plot of the meta-analysis residuals is asymmetric (Fig. 5a), suggesting some publication bias in the literature. In particular low-precision studies are more likely to report strong negative than strong positive effects, suggesting that the latter are less likely to be published. The presence of a publication bias is confirmed by Egger's regression, as the intercept differed significantly from zero (Intercept = -1.485 [$-1.704, -1.266$]). However, we did not find a significant

relationship between effect size and year of publication (Fig. 5b).

DISCUSSION

Overall, allelopathy reduced plant performance by 25%. By comparison, competition – which might also include allelopathic effect – reduces plant performance by 43% (Lekberg *et al.* 2018), and herbivores consume 5% of leaf tissues produced annually by plants (Turcotte *et al.* 2014). Therefore, allelopathy can be a

Table 2 Output of the model testing effects of study design, biological traits and evolutionary history on effect sizes (ln *RR*) of allelopathy. Shown are the model estimates and standard errors as well as the lower (L) and upper (U) values of the 95% and 90% credible intervals (CI)

		Estimate	SE	L95% CI	U95% CI	L90% CI	U90% CI
Study design	Intercept (Leachate)	-0.388*	0.099	-0.573	-0.179	-0.541	-0.220
	Residue	-0.163*	0.029	-0.220	-0.107	-0.210	-0.115
	Exudate	-0.043	0.053	-0.145	0.061	-0.130	0.044
	Volatile	-0.015	0.050	-0.114	0.082	-0.098	0.067
	Soil	0.122*	0.049	0.024	0.218	0.040	0.203
	Activated carbon	0.109	0.071	-0.029	0.247	-0.007	0.227
	Organic solvent	-0.061†	0.032	-0.124	0.001	-0.113	-0.009
	Measurement	0.140*	0.011	0.119	0.161	0.123	0.158
	Experimental environment – semi-natural	0.237*	0.090	0.058	0.411	0.089	0.381
	Experimental environment – natural	-0.132	0.152	-0.432	0.168	-0.379	0.120
Biological traits	Study duration	0.041*	0.016	0.009	0.072	0.014	0.068
	Study duration ²	0.025*	0.008	0.009	0.040	0.012	0.038
	Lifespan (allelo) ^a	0.014	0.044	-0.072	0.099	-0.058	0.086
	Life form (allelo)	0.046	0.039	-0.030	0.125	-0.019	0.112
	Domestication (allelo)	0.031	0.041	-0.047	0.112	-0.036	0.097
	Lifespan (test)	0.039	0.041	-0.041	0.120	-0.029	0.107
	Life form (test) ^b	0.049	0.056	-0.060	0.158	-0.042	0.140
	Domestication (test)	0.053	0.044	-0.033	0.141	-0.018	0.126
	Phylogenetic distance	-0.195*	0.060	-0.310	-0.079	-0.291	-0.096
	Origin – naturalised (allelo) [O-N (allelo)]	-0.037	0.038	-0.110	0.038	-0.098	0.026
Evolutionary history	Origin – non-naturalised (allelo) [O-NN (allelo)]	-0.017	0.058	-0.128	0.095	-0.111	0.079
	Origin – naturalised (test) [O-N (test)]	0.051	0.039	-0.026	0.127	-0.012	0.117
	Origin – non-naturalised (test) [O-NN (test)]	0.013	0.037	-0.058	0.084	-0.046	0.073
	O-N (allelo)* O-N (test)	0.019	0.049	-0.080	0.114	-0.064	0.099
	O-N (allelo)* O-NN (test)	0.054	0.046	-0.036	0.143	-0.022	0.129
	O-NN (allelo)* O-N (test)	-0.040	0.068	-0.174	0.091	-0.150	0.071
	O-NN (allelo)* O-NN (test)	-0.009	0.066	-0.142	0.115	-0.118	0.097

Parameters whose 95% credible intervals do not overlap with zero are indicted with asterisks (*), and whose 90% credible intervals do not overlap with zero are indicated with daggers (†).

^aBiological traits or origin of allelopathy species.

^bBiological traits or origin of test species.

significant force affecting the germination and growth of plants, and consequently the population dynamics of species and community assembly. However, the variation in magnitude and direction of allelopathy is high. Allelopathy was more negative when tested with plant residues than with leachates, and was less negative when tested with soil. Furthermore, the negative effect of allelopathy, generally, became weaker with study duration, and increasingly stronger with the concentrations of leachates and residues. Allelopathy was not significantly related to lifespan, life form and domestication of the two plant species. However, allelopathy became slightly but significantly more negative when phylogenetic distance between allelopathy and test species increased. In addition, native plants suffered more from leachates of naturalised alien plants than from leachates of other native plants, and effects of naturalised alien plants on each other were weak.

Effects of study design on allelopathy

Allelochemicals can be released into the environment by leaching from plants by rain, decomposition of plant residues, exudation from roots and volatilisation (Fig. 1). Our meta-analysis revealed that the effects of exudates and volatiles did not differ from those of leachates, but that plant residues had the most negative effect on plant performance. As the leachates are usually made by soaking plant material in water for *c.* 1 day, smaller

amounts of chemicals might be released than from residues. The leachates treatment is nevertheless not unrealistic, because in nature leaching may only happen during periods of rain. Moreover, while leachates contain only water-soluble chemicals, residues could additionally release non-water soluble secondary metabolites, which increases the likelihood that some of those are toxic to test plants. In support of this idea, studies that extracted different fractions of plant secondary metabolites showed that fat-soluble chemicals exerted stronger negative effects than water-soluble ones (Table S10).

Studies that used soils on which allelopathy plants were grown had weaker negative effects than those that used leachates. The reasons are various. First, the two types of methods differed in the substrates. While about 90% of the soil-method studies used natural soil, most leachate-method studies used artificial substrates. The abiotic characteristics of natural soil, such as higher bulk density, may hinder diffusion of allelochemicals (Parepa & Bossdorf 2016). Second, the effect of natural soil might also arise from the presence or higher abundance of soil microbes (Mishra *et al.* 2013). A recent study found that a bacterial species (*Arthrobacter* sp. ZS) isolated from soil increased the degradation of allelochemicals produced by *Ageratina adenophora* (Li *et al.* 2017), suggesting that soil microbes can neutralise allelopathic effect. Third, few if any soil-method studies have ruled out the effect of the allelopathy plant on soil microbes through primary

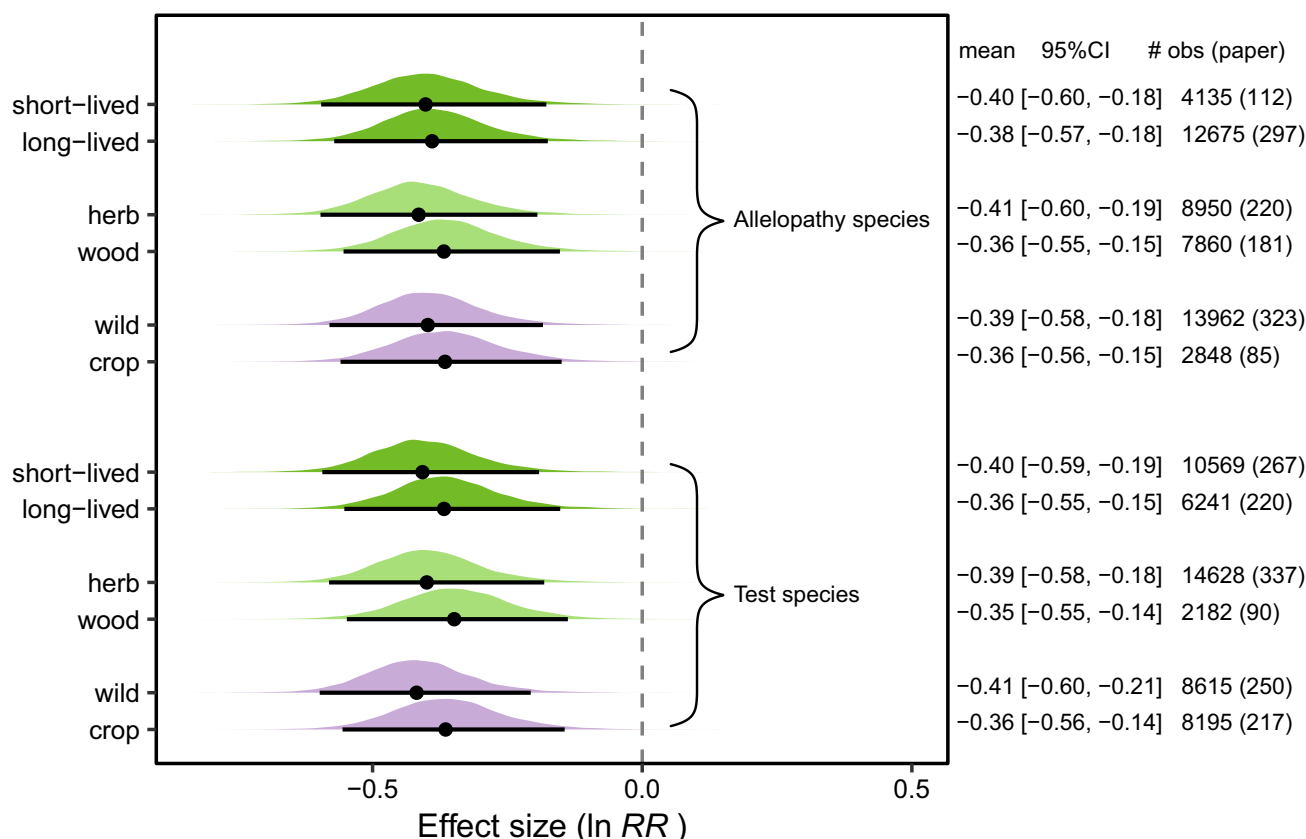


Figure 3 Effects of biological traits (lifespan, dark green; life form, light green; domestication, violet) on allelopathy. Biological traits of allelopathy species are shown in the upper half, and those of test species in the lower half. For each category (parameter), the posterior distribution is plotted with the mean and 95% credible interval. The text on the right displays the mean, 95% credible interval (CI), and the number of observations (obs) and papers. Negative values of the effect size ($\ln RR$) indicate that allelopathy inhibits plant performance. No significant difference was found.

metabolites (e.g. plants can foster arbuscular mycorrhizal fungi), which are not involved in allelopathy but could neutralise allelopathic effect. So, future research needs to account for effects of soil that are not due to allelopathy.

The use of activated carbon in allelopathy studies is appealing, because it is easy to apply, and because it would allow to mimic natural conditions more closely (e.g. can be tested with resource competition). We found that allelopathic effects did not differ between studies that used activated carbon and those that used leachates. This suggests that the allelopathic effect is significant in the presence of resource competition. An important caveat, however, is that activated carbon can result in artefacts, such as increased nutrient availability (Lau *et al.* 2008). To account for artefacts, two questions need to be addressed: (1) whether and to which extent are the plants affected by activated carbon when grown in the absence of competitors? (2) whether and to which extent are the effect of resource competition affected by activated carbon? The latter is hard to test, but could be examined by fertilising plants that are competing (assuming that activated carbon indeed increases nutrient availability). However, such studies are still challenging because allelopathy and resource competition could interact. Consequently, as suggested by Lau *et al.* (2008), using other methods in addition to the activated-carbon method will provide more compelling evidence.

Across the different types of methods, seed germination was less inhibited by allelopathy than plant growth was. This finding is not surprising given that seed germination is mainly determined by seed viability and abiotic environmental conditions, including temperature, water and oxygen concentrations (Bonnewell *et al.* 1983), which are unlikely to be affected by the allelopathy treatments. In contrast to the overall pattern, studies that tested allelopathy with plant residues showed that growth was less negatively affected than seed germination. As we also found that plants were less inhibited by litter than by fresh biomass, one potential explanation could be that plant residues (especially litter) increase nutrient availability, which partly offsets the inhibition of plant growth by allelochemicals. Still, the effect of allelopathy on fitness remains unclear as fitness was rarely measured.

The negative effect of allelopathy diminished with increasing duration of the experiment, and was absent in most studies that lasted over 89 days. Possibly, plastic responses of the test plants allow them to compensate for the early negative effects. This could suggest that allelopathy has no long-term impacts. However, if the plants also physically interact, like in nature, the allelopathy species could turn the short-term germination and growth inhibition of its competitors – which gives the allelopathic species more access to resources such as light – into a long-term competitive advantage (e.g. a priority effect; Fukami

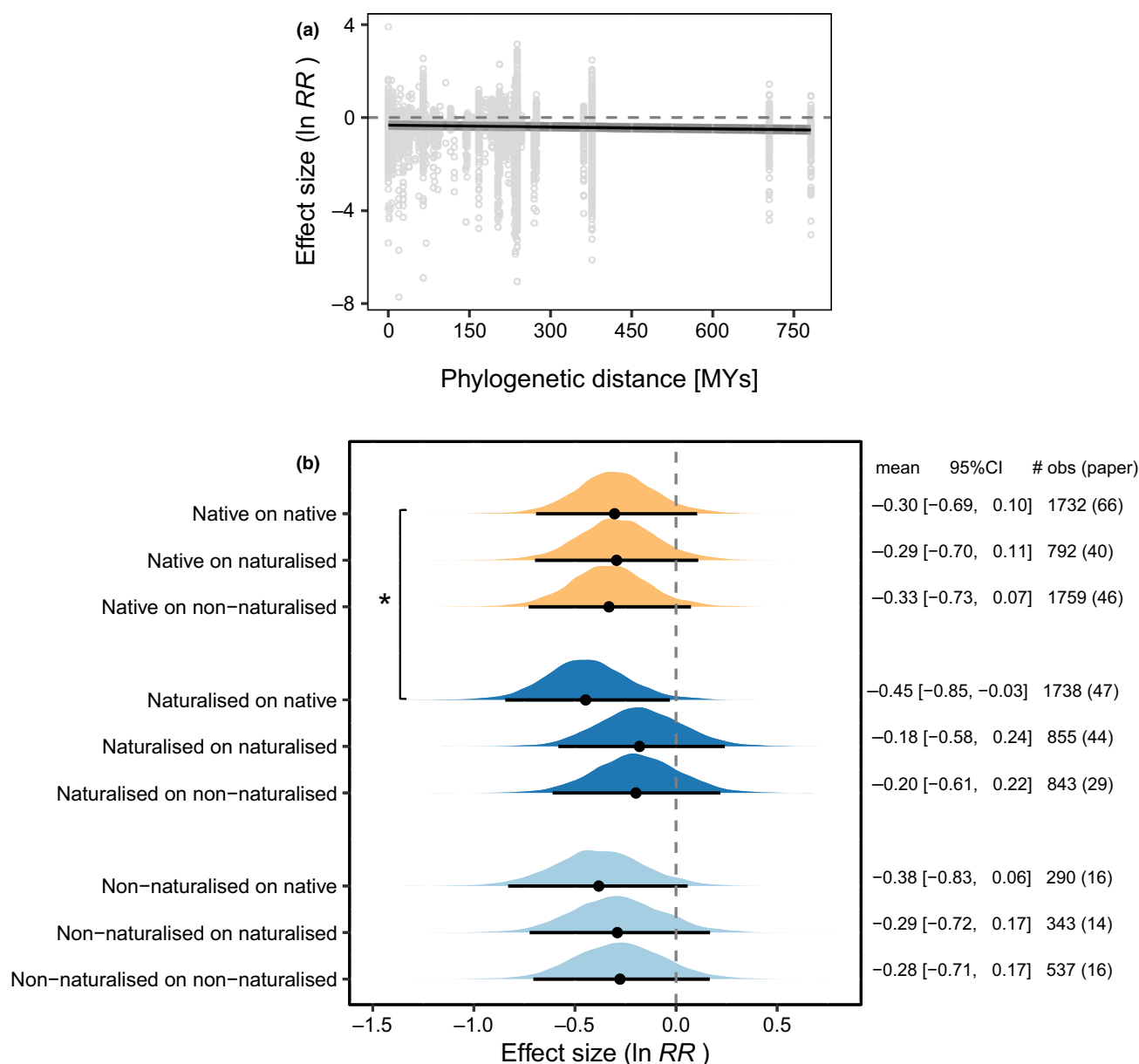


Figure 4 Effects of evolutionary history on allelopathy. (a) Effect of phylogenetic distance between allelopathy and test species on allelopathy. The curve of the estimated effect is shown with its 95% credible interval. (b) Effects of origin of allelopathy and test species on allelopathy when tested with the leachate method. Effects of native, non-naturalised alien and naturalised alien allelopathy species are shown in dark blue, light blue and orange. For each category (parameter), the posterior distribution is plotted with the mean and 95% credible interval. The text on the right displays the mean, 95% credible interval (CI), and the number of observations (obs) and papers. Negative values of the effect size (ln RR) indicate that allelopathy inhibits plant performance. An asterisk indicates significant difference between reference level (native on native) and the other.

et al. 2005). As long-term experiments are still rare and allelopathy is rarely tested in combination with other forms of competition (e.g. resource competition, but see the activated-carbon method), this speculation requires further research.

As expected, the inhibitory effect of allelopathy became stronger with increasing concentrations of leachates and residues. An important question then is whether the concentration chosen is realistic. The few studies that give some clues show that the annual amount of plant litter varies largely among study sites, ranging from 43 to 884 g/m² (Facelli & Carson 1991; San Emeterio *et al.* 2004; Chave *et al.* 2010; Overholt

et al. 2012; Aguilera *et al.* 2015). Based on this range of concentrations, our model predicts that allelopathy through plant residues will reduce plant performance by 2.9% to 31.1%, if we assume that the litter ends up in the upper 10 cm of soil that has a bulk density of 1.5 g/cm³ (Martín *et al.* 2017). By comparison, our analysis of the residue method showed that allelopathy reduced plant performance by 50% across concentrations (intercept in Table S5). Consequently, the inhibitory effect of allelopathy might be overestimated by some studies that have used unrealistically high concentrations. A more realistic understanding of allelopathic effect requires (1) identifying putative

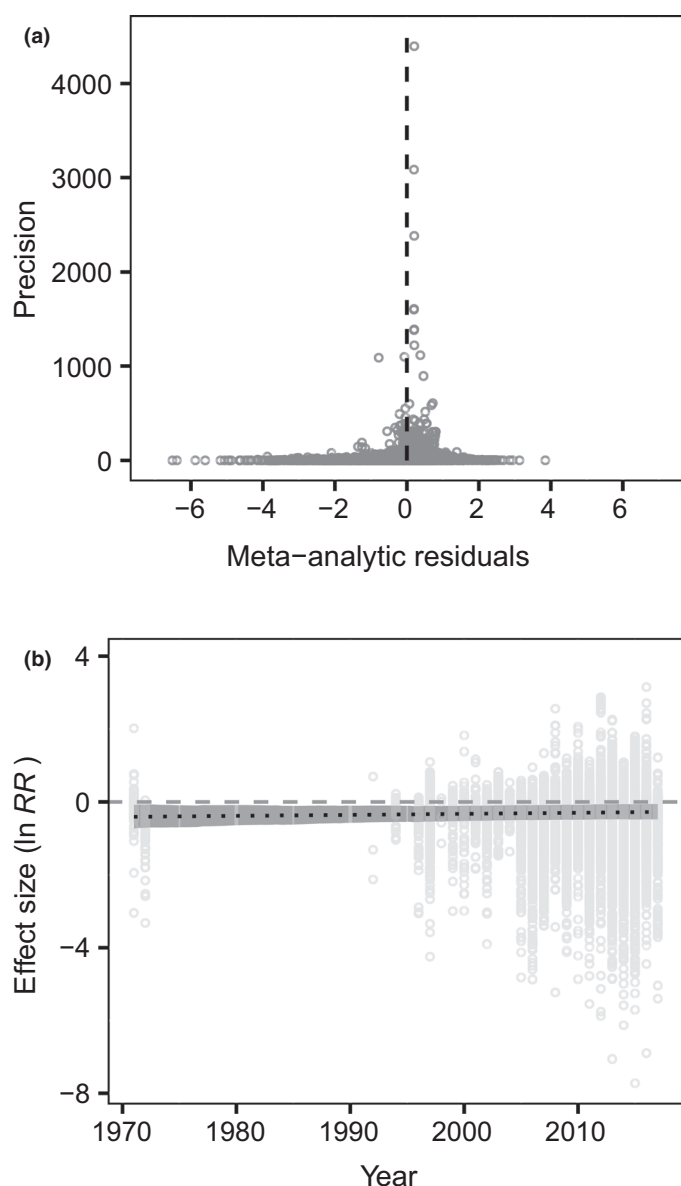


Figure 5 Tests of publication bias for the allelopathy studies used in our meta-analysis. (a) Funnel plot of the study precision (inverse of standard error) vs. the residuals of the meta-analysis. The asymmetry at low levels of precision indicates the presence of a publication bias. The dashed line was added as visual reference for symmetry. A few data points with extremely high precision are not plotted to aid visualisation and a complete plot is provided in the supplement (Fig. S3). (b) The effect size vs. the year of study revealed no significant relationship.

allelochemicals, (2) measuring their corresponding concentrations in natural soil and (3) testing effects of allelochemicals at realistic concentrations.

Effects of biological traits on allelopathy

Allelopathy was not related to lifespan, life form or domestication of the species. This was unexpected, because many textbook examples of allelopathy are from long-lived trees, such as *Juglans nigra* (black walnut; Jose & Gillespie 1998) and *Ailanthus altissima* (tree-of-heaven; Heisey 1990). Moreover,

farmers have long been annoyed by weed interference on crop yield. The reported strong negative effects of some long-lived woody species in nature might come from the accumulation of large amounts of allelochemicals over time, whereas short-lived weedy species leave fewer allelochemicals due to their short generation time and high spatial turnover. When differences in strength of allelopathy between species with different life histories are only due to differences in accumulation of allelochemicals, and not due to differences in toxicity, these differences will not be apparent in short-term experiments with standardised amounts of plant material. On the other hand, the sometimes reported vulnerability of crops might be a result of the crops being grown in monoculture, resulting in a low diversity of allelochemicals, and consequently a lower resistance against invasion by weeds.

Effects of evolutionary history on allelopathy

Charles Darwin suggested that closely related species are more ecologically similar, and thus will compete more strongly with each other than when they compete with distantly related species (known as Darwin's naturalisation hypothesis; Darwin 1859). With regard to allelopathy, we found the opposite pattern, that is, that closely related species (or individuals of the same species) had weaker effects on each other than distantly related species. Our finding does not challenge Darwin's naturalisation hypothesis, because that hypothesis is mainly based on resource competition, whereas allelopathy is a form of interference competition. However, the possibly opposing effects of phylogenetic distance on interference and resource competition could help explain why studies testing Darwin's naturalisation hypothesis have not found consistent results (Thuiller *et al.* 2010).

Still, ecological differences between species, or more specifically the difference in secondary metabolites, cannot be fully captured by phylogenetic distance (Pichersky & Lewinsohn 2011; Huang *et al.* 2016; Cadotte *et al.* 2017). Secondary metabolites are under selection by competitors and enemies (Uesugi & Kessler 2013; Zheng *et al.* 2015), and thus plants that evolved in different regions (e.g. native and alien plants) may not be adapted to each other's allelochemicals. This might give the alien plants an advantage, as posed by the novel weapons hypothesis (Callaway & Ridenour 2004), or increase biotic resistance of native communities, as posed by the homeland security hypothesis (Cummings *et al.* 2012; Ning *et al.* 2016). Although, across all types of methods, we did not find that the origins of the interacting species mattered, in the studies that used leachates, natives were more strongly inhibited by naturalised aliens than by other natives. On the other hand, the effect of natives on others did not depend on the native-alien status of the other species. So, these findings support the novel-weapons hypothesis but not the homeland-security hypothesis.

Surprisingly, although naturalised aliens usually do not share a co-evolutionary history with other naturalised aliens (unless they have the same native range), their leachates had weaker effects on each other than was the case for the effects of natives on other natives (Fig. 4b). The exact reason for this is not clear, but it could imply that naturalised species are less likely to suppress other naturalised species than that they

suppress natives. This could contribute to invasional meltdown (Simberloff & Von Holle 1999).

Future directions

Our meta-analysis of 16,810 effects sizes from 384 studies revealed some clear patterns, but also that there is still lots of unexplained variation in the effects of allelopathy. The explanatory variables regarding study design, and biological traits and evolutionary history of the species accounted for only 3.5% of the variance, and 26.2%, 13.0% and 7.1% of the variance was explained by identities of study, test and allelopathy species respectively. This means that about 50% of the variance remains unexplained. Although this partly reveals the prevalence of heterogeneity in ecology and evolution in general, it also indicates that some other potential explanatory factors were not included. Below we suggest several directions that might help to better understand allelopathic effects.

Positive effects of allelopathy

Molisch (1937) defined allelopathy as the positive or negative effects resulting from biochemical interactions. However, Rice (1974), in the first edition of his influential book 'Allelopathy', restricted the definition to negative effects. Although in the second edition, Rice re-embraced Molisch's definition, the narrow-sense definition of allelopathy may explain the publication bias in favour of studies that found negative effects. As a result, some of our findings might be biased. For example, if studies that used low concentrations of allelochemicals found positive effects, they might not have published this result or the publication might not use the term 'allelopathy'. Actually, in the field of agriculture and horticulture, there is already interest in plant extracts (so-called biostimulants or bioeffectors) that could stimulate crop production (Wise *et al.* 2020). Due to the bias in publication of positive effects, non-monotonic effect of concentration, if present, might be hard to detect using meta-analysis. Therefore, to move towards a more accurate understanding of allelopathy, we should follow Molisch's original definition of allelopathy, which covers both positive and negative effects.

Soil microbes

Soil microbes have long been acknowledged to mediate allelopathy (Fig. 1; Molisch 1937; Rice 1984; Cheng & Cheng 2015). For example, Stinson *et al.* (2006) showed that allelochemicals of the non-mycorrhizal European invader *Alliaria petiolata* suppressed growth of native North American plants by disrupting their mutualistic mycorrhizal associations. Microbes might, however, also be a missing link in studies that test allelopathy between alien and native species. Specifically, as most experiments are *ex situ*, without adding native soil microbes to mimic the natural condition, the environment will be novel to both alien and native species. Consequently, the origin (alien vs. native) will be less relevant. Yet, empirical tests of how soil microbes affect allelopathy between plants remain rare. Four out of five direct tests found that the presence of soil microbes neutralised allelopathic effects (Wu *et al.*

2008; Lankau 2009; Zhu *et al.* 2011; Corbett & Morrison 2012; Del Fabbro & Prati 2015). However, caution must be taken, because three of the tests removed soil microbes with autoclaving, which as a side effect can release nutrients (Alphei & Scheu 1993). Inoculation with soil microbes might be more appropriate to manipulate the presence or amount of microbes. In addition, with sequencing techniques becoming more and more affordable, we now have the tools to study the soil microbiome and its role in allelopathy in more detail.

Test species

Much of the work on allelopathy has focused primarily on the allelopathy species, as indicated by the fact that fast-growing crops (e.g. lettuce, wheat and rice) have been frequently used as test (indicator) species. We found, however, that the species identity of the test plant explained nearly twice as much variance in the allelopathic effect as the species identity of the allelopathy plant did (13.0% vs. 7.1%). Therefore, although testing crops is valuable to agriculture, using certain fast-growing crops as indicators, just for convenience, could bias the estimation of allelopathic effect. A stronger focus on the test species may provide new insights. Indeed, how test species respond to allelopathy is important in many circumstances, such as during succession and establishment of alien plants. If a species enters a certain community as a single individual, its establishment will initially, due to its low abundance, hardly depend on how strongly it can affect other species. What matters then is how the individual responds to the communities (e.g. allelopathic effects of the resident plants on the invader). This is a key component of invasion growth rate (i.e. growth rate when rare; Grainger *et al.* 2019).

The ecological and evolutionary significance of allelopathy

Although we found that allelopathy was on average negative in experiments done under controlled conditions (i.e. in Petri dishes, growth chambers or greenhouses), it was non-significant in experiments under semi-natural conditions (i.e. in outdoor gardens or agriculture systems), where there were more organisms, interactions and environmental stochasticity. This conclusion is still premature, because we found a negative effect of allelopathy in experiments done under natural conditions, though with a wide 95% confidence interval; and because experiments under semi-natural or natural conditions are rare (e.g. 16 studies in our meta-analysis). Therefore, more experiments under natural conditions are needed to understand the ecological significance of allelopathy. In addition, secondary metabolites have functions other than allelopathy, such as anti-herbivory defence (Lankau & Kliebenstein 2009). To understand the evolutionary significance of allelopathy, we require tests of whether the allelopathic effect is selected by competition *per se* or is a pleiotropic effect of secondary metabolites that are selected by herbivory.

Uses of multiple literature search engines

For our meta-analysis, we searched the ISI Web of Science for allelopathy studies. However, other search engines may

identify additional studies. Indeed, a search *a posteriori* using the same search string as we used in Web of Science revealed that Scopus could potentially provide more studies (Supplement S6). Therefore, although our meta-analysis includes already a large number of studies (384) across the globe, broader searches (e.g. by combining ISI Web of Science, Scopus and China National Knowledge Infrastructure) could allow more general conclusions (Yu *et al.* 2016), and should be considered for future meta-analyses.

CONCLUSIONS

Studies on allelopathy have rapidly accumulated since the 1970s. Despite the detection of a potential publication bias, our global synthesis demonstrates that, on average, allelopathy reduced plant performance. Among the four pathways through which allelochemicals are released into the environment, plant residues exerted the most negative effect. Still, the importance of allelopathy in nature requires further investigation, as allelopathic effects were weaker in studies where soil microbes were likely to be abundant, and when study duration was longer. Finally, we found that allelopathic effects were weaker between closely related species (or individuals of the same species) than between distantly related species. This suggests that allelopathy would favour the coexistence of closely related species (or dominance of single species), which is opposite to the predicted effect of resource competition. Therefore, investigating allelopathy and resource competition jointly may explain the mixed outcomes of species coexistence.

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AUTHORSHIP

MvK and EW conceived the idea. ZZ, YL and MvK designed the study. ZZ, YL and LY collected the data. ZZ led the analyses and writing, with inputs from all others.

DATA AND CODE ACCESSIBILITY

The data and code of the study are archived in Figshare (<https://doi.org/10.6084/m9.figshare.13014404>).

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ele.13627>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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