

Manipulation of soil mycorrhizal fungi influences floral traits

Batoule F. Hyjazie  and Risa D. Sargent 

Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada

Author for correspondence:

Risa D. Sargent

Email: risa.sargent@ubc.ca

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Summary

- Most plants form root hyphal relationships with mycorrhizal fungi, especially arbuscular mycorrhizal fungi (AMF). These associations are known to positively impact plant biomass and competitive ability. However, less is known about how mycorrhizas impact other ecological interactions, such as those mediated by pollinators.

- We performed a meta-regression of studies that manipulated AMF and measured traits related to pollination, including floral display size, rewards, visitation, and reproduction, extracting 63 studies with 423 effects.

- On average, the presence of mycorrhizas was associated with positive effects on floral traits. Specifically, we found impacts of AMF on floral display size, pollinator visitation and reproduction, and a positive but nonsignificant impact on rewards. Studies manipulating mycorrhizas with fungicide tended to report contrasting results, possibly because fungicide destroys both beneficial and pathogenic microbes.

- Our study highlights the potential for relationships with mycorrhizal fungi to play an important, yet underrecognized role in plant–pollinator interactions. With heightened awareness of the need for a more sustainable agricultural industry, mycorrhizal fungi may offer the opportunity to reduce reliance on inorganic fertilizers. At the same time, fungicides are now ubiquitous in agricultural systems. Our study demonstrates indirect ways in which plant–belowground fungal partnerships could manifest in plant–pollinator interactions.

Introduction

A large body of evidence supports the hypothesis that microbial mutualisms directly contribute to plant fitness (Koide, 2000). However, the hypothesis that microbial partnerships could indirectly affect plant fitness through interactions with other key ecosystem players, such as pollinators, is relatively new (Koricheva *et al.*, 2009; Bennett & Meek, 2020). Studies where authors experimentally manipulate the mycorrhizal community have reported positive effects on floral traits important to pollinator visitation (Wolfe *et al.*, 2005), as well as changes to the pollinator community itself (Cahill *et al.*, 2008), but the extent of, and conditions under which, mycorrhizal interactions influence pollinator-mediated interactions is currently unknown. With upwards of 80% of plants, including most crops, capable of forming relationships with mycorrhizal fungi (especially arbuscular mycorrhizal fungi, or AMF), understanding the contributions of AMF to plant fitness through other ecological interactions is a critical knowledge gap.

In one of the first studies of the impacts of mycorrhizal inoculation on pollinator visitation, Wolfe *et al.* (2005) grew *Chamerion angustifolium* in soils either inoculated with AM fungi or not, and measured differences in floral display size and pollinator visitation. They found a positive impact of AMF inoculation on inflorescence size and pollinator visitation, attributing the results

to AMF-induced alterations to resource allocation within the plant. Then, in one of the few field studies on the effects of AMF on pollinator visitation, Cahill *et al.* (2008) suppressed AMF by adding the agricultural fungicide benomyl to a prairie meadow in a replicated block design over 3 yr, finding that the species composition of the visiting pollinator community varied among treatments, with plants in the AMF blocks receiving more visits from large-bodied bumble bees and fewer from small bees than the fungicide treated (AMF–) blocks. Since these two seminal papers were published, the possibility that AMF could alter floral traits and pollination has received increased attention; a recent qualitative review of the existing literature in this field revealed a range of potential pollinator-mediated responses to AMF (Bennett & Meek, 2020). In spite of its potential importance to ecology, agriculture, restoration and our basic understanding of plant biology, there has been no formal meta-analysis of the impacts of AMF on plant traits related to pollination.

Relationships with AMF could impact plant–pollinator interactions through a number of different pathways (Fig. 1). In particular, floral display size (i.e. the size and/or number of flowers produced by a plant) is a strong predictor of pollinator visitation, seed production and pollinator-mediated fitness (Bell, 1985) and can be significantly impacted by drought or nutrient stress, as shown by Burkle & Runyon (2016). Symbioses with AMF can support the maintenance of a larger floral display during periods

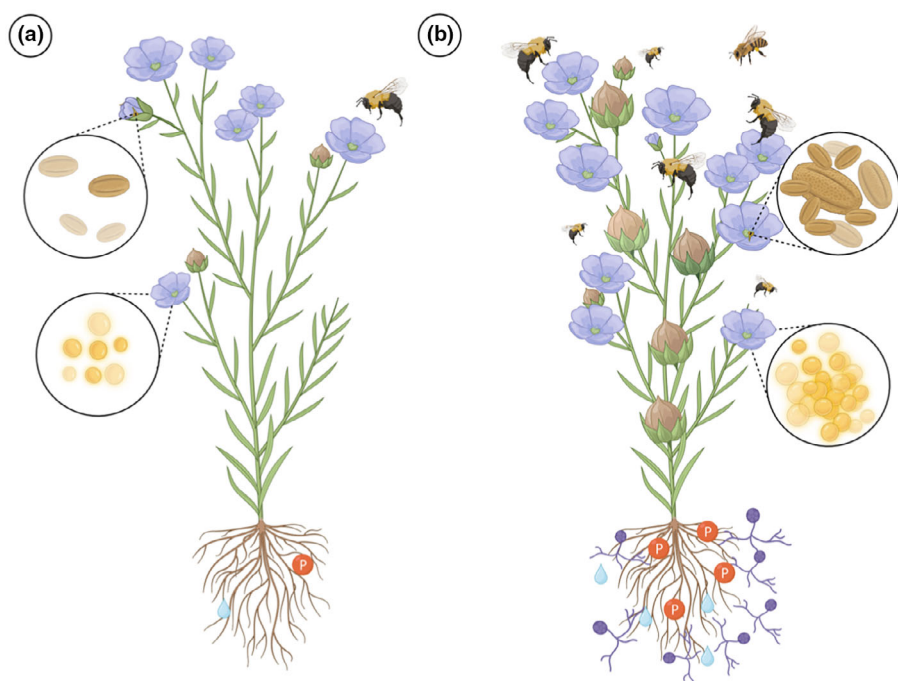


Fig. 1 Illustration of hypothesized pathways relating arbuscular mycorrhizal fungi (AMF) on floral trait response. Panel (a) depicts the absence of arbuscular mycorrhizal fungi (AMF⁻); panel (b) depicts presence (AMF⁺). In (a), AMF could mediate floral trait responses by aiding in nutrient acquisition and/or alleviating drought stress, compared with (b). The additional access to critical nutrients allows greater allocation of resources to floral display and pollinator rewards (i.e. pollen, depicted as oblong pollen grains and nectar, depicted as yellow droplets). The increase in resource allocation to pollinator attraction leads to higher rates of pollinator visitation and yield (floral fruit and seed counts and sizes, depicted as brown capsules on plant) in panel (a) compared with panel (b).

of drought or nutrient stress (Koziol *et al.*, 2012), which could provide a pollinator-mediated fitness advantage to plants that are able to form such relationships. In addition, AMF may influence plant secondary chemistry by altering the production and composition of floral volatile organic compounds (VOCs), providing another pathway for AMF to influence pollinator attraction and preferences (Becklin *et al.*, 2011; Davis *et al.*, 2019; Bennett & Meek, 2020). Finally, nectar rewards, including sugar concentration and the rate of replenishment, are vulnerable to drought and nutrient stress and may benefit from AMF symbiosis (Wolfe *et al.*, 2005; Bennett & Meek, 2020).

Perhaps the most plausible connection between AMF and floral traits is the propensity for AMF to enhance plant nutrient acquisition, especially phosphate (P) and nitrogen (N) (Lu & Koide, 1994). In the wild, plants receive *c.* 50% of their nitrogen and upwards of 90% of phosphorus from associations with AMF (Bennett & Groten, 2022). Nitrogen and phosphorus are critical to flower production and display (White & Hammond, 2008); the availability of these soil nutrients has previously been shown to positively impact the size of floral traits including floral display, nectar volume, and pollen count (Burkle & Irwin, 2009; Vaudo *et al.*, 2022). In a study of the impacts of inoculation with the AMF *Glomus etuniacatum* on the herbaceous annual *Abutilon theophrasiti*, Lu & Koide (1994) found that AMF inoculation in nutrient-poor soils was associated with a dramatic increase in the number of flowers produced, a greater proportion of flowers setting fruit, longer total flowering duration, earlier flowering, and more seeds per fruit. Furthermore, a recent study reported that increasing soil N and P concentrations lead to floral trait changes that resulted in increased visitation by bumblebees to cultivated cucumber plants (Vaudo *et al.*, 2022).

A common approach to quantifying the impacts of AMF on floral traits is to manipulate AMF through soil inoculation.

However, AMF inoculation is difficult to apply and even more difficult to control in field conditions. Ecological studies in field settings frequently use fungicide to eliminate AMF, as opposed to inoculation (Koricheva *et al.*, 2009). Although the methodological constraints of field inoculation are understandable, the ecological effects of fungicide are likely broader than those of inoculation, because fungicide wipes out a wide range of soil microbial mutualists as well as pathogens (Newsham *et al.*, 1995; Schmidt *et al.*, 2000; Qu *et al.*, 2022). In a study of plant–soil feedback effects using 24 grassland species grown in conspecific or interspecific soils, Petermann *et al.* (2008) found that the application of fungicide increased plant biomass in nearly every species, an effect they attributed to fungicides eliminating a broad swath of soil fungal pathogens, thereby increasing plant productivity. Similar results were reported by Maron *et al.* (2011) in their study of how soil fungal pathogens mediate plant diversity–productivity relationships. These methodological differences have the potential to impact experimental outcomes. Thus, in addition to assessing the effects of AMF on floral traits, we explore the role of experimental design on study outcomes.

The goal of our study is to establish the extent to which the experimental manipulation of soil mycorrhizal fungi impacts plant traits related to pollination. Using meta-regression, we summarize the average impact of AMF manipulation on four categories of traits: floral display size, pollinator visitation, nectar rewards, and seed/fruit production. Next, we ask, to what degree does the floral trait response depend on factors such as experimental design (fungicide vs inoculation design; nutrient supplementation, lab/glasshouse vs field study) and whether the plant was a wild, crop, or ornamental species. We hypothesize that, because they enhance a plant's ability to acquire nutrients (especially N and P) critical to floral display, the presence of mycorrhizal fungi will be associated with larger display size, higher

pollinator visitation rates and higher reproduction. We also predict that the effects of AMF will be less pronounced in the presence of high nutrient supplementation and that studies that manipulate AMF using fungicide will have more variable outcomes compared to those that use an inoculation approach.

Materials and Methods

Literature search

To generate a dataset of studies that measured floral trait responses to the manipulation of the soil mycorrhizal fungal community, we conducted a systematic search of the Web of Science database in July 2023, using the following search terms: [mycorrhiza* AND pollinat*] OR [mycorrhiza* AND (flower* OR floral)]. The search generated a list of 809 journal articles. The titles and abstracts of all articles were screened and retained only if they described results of a study that manipulated soil mycorrhizal fungi on floral traits (e.g. flower size, flower number, or reward chemistry (i.e. nectar sugar concentration)), pollination (e.g. pollinator visitation rate), or yield (either fruit or seed weight, number, or size). Afterward, a full-text screening of the remaining papers was conducted based on the following inclusion criteria:

- (1) The study described a manipulation of the mycorrhizal community using either inoculation or fungicide application (hereafter: fungal exclusion).
- (2) The observations described in the study referred to at least one of the following traits: floral display size (i.e. flower dry or fresh weight, diameter, number, mass, petal size, weight, inflorescence size, weight, etc.), pollinator visitation (i.e. rate of visits or probes by different pollinator communities, total number of visits, and length of visits), yield (e.g. fruit or seed mass, weight, or number), or reward (e.g. nectar volume or sugar content and pollen number or weight).
- (3) Studies reported effect size(s) (means) for plants in the AMF+ category (i.e. inoculated or no fungal exclusion), and for plants in the AMF− category (i.e. not inoculated, or fungal exclusion) along with the sample size of each treatment group, and a relevant measure of variance in effect size (e.g. standard error, standard deviation, or variance) either in the main text, a table, or displayed in a figure (values extracted through IMAGEJ; Schneider *et al.*, 2012).

After screening, 63 journal articles met the above criteria, reporting a total of 423 pairwise comparisons of treatments with AMF (i.e. either inoculated or no fungal exclusion) to treatments without AMF (i.e. either not inoculated or fungal exclusion).

Data extraction

Group means, sample sizes, and associated measures of variance from the 63 studies were extracted and entered into a database (Supporting Information Table S1). Most studies reported effect sizes, sample sizes, and variance within the text or tables. Some studies did not report this information within the text but illustrated the relevant information in one or more figures. In this

case, data were obtained using IMAGEJ software (Schneider *et al.*, 2012), which allows the user to measure means and errors from published figures.

In addition to examining the relevant floral traits (display, yield, reward, visitation), we extracted other relevant information pertaining to the design of each individual study. This included the focal plant species and its taxonomic family, life history (perennial, annual, semiannual, varying (i.e. multiple possible life histories according to environmental conditions)), the AMF species (if reported), and its taxonomic family (if AMF species was reported), the domestication status (wild, ornamental, crop), whether the design included supplemental nutrients (yes or no), and, if so, the treatment (i.e. high or low), and type of supplementation (i.e. chemical composition), and the experimental setting (glasshouse/growth chamber or field).

Meta-analysis

Standardized mean effects and meta-regressions were calculated/conducted using functions contained in the packages METAFOR and META (Viechtbauer, 2010; Schwarzer *et al.*, 2015). Following Koricheva *et al.* (2013), Hedge's *g* was calculated as the difference between the AMF+ group (i.e. inoculated or no fungicide applied) and the AMF− group (i.e. not inoculated or fungicide applied) divided by the pooled standard deviation which calculates the number of standard deviations separating the two groups (Hedges, 1981). Thus, a positive effect size indicates that the trait response was greater in the presence of AMF (AMF+); a negative effect indicates that the trait response was greater in the absence of AMF (AMF−). Compared to other measures of SMD (standardized mean differences), Hedge's *g* is considered less biased because it is not as influenced by unequal sample variances and includes a correction for small sample sizes (Koricheva *et al.*, 2013).

Using Hedge's *g*, we ran a series of subset meta-regression models testing for differences in plant response to AMF. We then ran separate moderator analyses for the following fixed effects: experimental setting (glasshouse/laboratory or field), AMF manipulation (fungicide or inoculation), plant species category (crop, ornamental or wild), nutrient supplementation (high or low), plant life history (perennial, annual, semiannual, varying) and plant family (with a subset of the database consisting of plant families with > 8 effect sizes to manage issues with poor replication) as moderator variables. We also ran a single, large meta-regression analysis that included specific moderators (domestication type, setting, nutrient supplementation, and AMF manipulation type) as fixed effects in the model and the nested random effects of study ID, arm ID, and effect ID (described below). We accounted for the potential of hierarchical effects of plant taxonomic family membership by including family as a random effect in the large single meta-regression model but not in the separate moderator effect models. We assessed the significance of moderator effects by comparing effect sizes, confidence intervals, and *P*-values for each model. Because the results of the moderator and the single meta-regression were nearly indistinguishable, we focus on the moderator analyses in our results and discussion below.

In addition to these analyses, we also ran analyses on subsets of the data, in order to test for differences among the reported metrics of floral display size (i.e. flower number vs flower size) and reproduction/yield (i.e. seed number vs seed weight vs fruit weight vs fruit number) (Tables S2, S3). We were unable to run parallel analyses for the categories of pollination visitation or reward due to insufficient replication.

In order to control for the potential nonindependence of effects extracted from within the same study, we added Study ID as a random factor to our meta-regression analyses. In addition, because our dataset included a number of studies which reported multiple effect sizes sharing a common control group (i.e. 'multi-armed' design), we included the control group as an additional random effect in our model structure. In this type of design, extracted effects are correlated with one another, which must be accounted for in the statistical models (Harrer *et al.*, 2021). Our approach accounts for nonindependence of multi-arm design by including 'effect nested in control group nested in study' (i.e. study/control/effect) as random effects in the model (Siviter *et al.*, 2021; Hasik *et al.*, 2023). For the nutrient supplementation moderator analysis, pairs within a study (high vs low comparison within an individual study) were compared by including an additional nested random effect (i.e. study/control/nutrient pair/effect). Statistical models were fit using restricted maximum likelihood (REML) (Viechtbauer, 2010). All analyses were conducted in R v.4.2.3 (R Core Team, 2023).

Publication bias

Standard methods for detecting publication bias (also known as small-study effects), including funnel plots, Egger's test, and 'trim and fill' are considered unsuitable for datasets with high heterogeneity and nonindependence of effect sizes, both of which are common in ecological datasets (Nakagawa *et al.*, 2022). In particular, asymmetry in a funnel plot distribution can occur even in the absence of publication bias due to correlations between effect sizes and standard error. Asymmetry is common where there is a clustering feature such as multiple effects from each study. In a recent paper Nakagawa *et al.* (2022) suggest new methods to test for publication bias in datasets that exhibit nonindependence and high heterogeneity, such as ours. Extending Egger's publication bias regression test, the Nakagawa *et al.* method uses multilevel meta-regression (which accounts for the hierarchical structure of the data) to detect publication bias and generates a corrected effect size which accounts for publication bias using sample size as a moderator. We applied this approach and generated funnel plots with conditional residuals (as opposed to the observed effect size in traditional funnel plot figures) that take into account the clustering factor (in our case, study ID), as described in Nakagawa *et al.* (2022). Specifically, two funnel plots were generated: a 'standard' funnel plot of standard error vs effect size (SMD) and a funnel plot of standard error vs conditional residuals which accounts for the hierarchical multilevel structure of the data and meta-regression models, as described by Nakagawa *et al.* (2022).

Results

Study trends

Our literature search resulted in effects from a total of 68 different plant species from 26 unique families, all of which were angiosperms. Of the 423 effect sizes from the 63 studies, 163 effect sizes originated from field settings and 260 from glasshouse or growth chamber studies (N.B., as some studies provided effects in multiple categories, subcategory study totals do not always sum to the study total). Our dataset includes five studies that applied a fungicide (AMF exclusion) to manipulate the fungal community (as opposed to inoculating with AMF), contributing a total of 30 effects. The remaining studies ($n = 393$ (number of effects), $k = 58$ (number of studies)) applied an inoculation approach. Five studies explored the effects of AMF with high and with low nutrient supplementation on floral traits (contributing 48 effects). All nutrient supplementation studies incorporated addition of phosphorus in combination with other nutrients such as calcium, carbon, nitrogen, sulphur, and potassium.

We extracted effects from studies that inoculated with 34 different AMF species. The vast majority of studies inoculated with one or more species of AMF (most commonly species in the genera *Acaulospora*, *Funneliformis*, or *Glomus*, see [Supporting Information](#)). In one study of highbush blueberry (genus *Vaccinium*), plants were inoculated with ericoid mycorrhizas – a specialist group of mycorrhizas associated with plants in the ericoid subfamily of the Ericaceae (Brody *et al.*, 2019). Some studies cultivated their own AMF inoculum by transplanting existing AMF from either a native plant (e.g. *C. angustifolium*), from a well-known host plant (typically from species in the genus *Allium*), or from soil collected from study locations known to be AMF rich (Wolfe *et al.*, 2005; Barber *et al.*, 2013). Studies focused on a broad range of plant species including several horticultural/agricultural cultivars (e.g. *Calendula officinalis*, *Crocus sativus*, *Cucumis pepo*, *C. sativus*, *Vaccinium* spp., *Glycine max*, etc.) but also a number of wild plant species (e.g. *Campanula rotundifolia*, *Cerastium arvense*, *Lobelia siphilitica*, *Lythrum salicaria*, *Solidago missouriensis*, etc.). The most commonly represented plant family was Asteraceae ($n = 90$) followed by Cucurbitaceae ($n = 82$). Other common families in the dataset were Fabaceae ($n = 47$), Geraniaceae ($n = 28$), and Solanaceae ($n = 38$) (Table S1). Two studies did not indicate sufficient plant taxonomic information: one reported the average effect size for eight species of ornamentals but did not identify the effects by species (Bennett & Cahill, 2018), and another examined multiple plant species but without identifying their effects individually (Puschel *et al.*, 2014).

The most commonly reported response to AMF manipulation in our dataset was floral display size, with a total of 251 effects extracted from 52 different studies. The second most common category of effects described traits related to yield or reproduction such as seed and fruit number and/or seed and fruit size with 83 effects from 22 studies. We extracted 56 effects that described pollinator visitation from eight studies. Reward was the smallest category, with just 29 effects from nine studies (Table S1).

Studies of the effects of AMF manipulation on floral phenology and floral chemistry (e.g. volatile organic compounds) were uncommon, and, due to low power, we could not include these as factors in our meta-analysis. Similarly, only a handful of studies described manipulations of water or drought stress, and we were, therefore, unable to further examine the implications of these experimental design choices on the effects of AMF manipulation. Finally, AMF taxonomic affiliation was reported with varying degrees of precision. Of the 423 effects, 233 involved a manipulation of AMF from a single family, usually species in the genus *Glomus* (Glomeraceae). Another 165 reported manipulating soil with AMF mixtures composed of species from a number of different families; the remaining 25 effects were manipulations performed using AMF from families other than Glomeraceae. The poor representation of single species manipulations from families other than Glomeraceae limited our ability to include AMF taxonomic affiliation in the analyses.

Effects of AMF on floral traits

On average, there was a positive effect of AMF on the traits we examined ($g = 0.640$, $SE = 0.100$, $P < 0.001$; Table 1; Fig. 2). Specifically, for floral display size, there was a large effect of AMF ($g = 0.786$, $SE = 0.132$, $P < 0.001$; Table 1; Fig. 2). A subset analysis of floral display categories (i.e. flower size or number) revealed a significant difference between the two, with flower size exhibiting a greater response to AMF than flower number (Table S2). We also found moderate to large positive effects of AMF on pollinator visitation ($g = 0.590$, $SE = 0.227$, $P = 0.012$; Table 1; Fig. 2) and reproductive yield ($g = 0.450$, $SE = 0.130$, $P < 0.001$; Table 1; Fig. 2). A subanalysis of reproductive yield revealed a significant difference between fruit number, fruit weight, seed number, and seed weight ($F_{81,4} = 3.61$, $P < 0.009$; Table S3). There was a positive, although not statistically significant, effect of AMF on measurements of floral rewards ($g = 0.551$, $SE = 0.307$, $P = 0.084$; Table 1; Fig. 2).

Effect of moderators

Studies that applied AMF inoculation as opposed to fungal exclusion exhibited a different overall direction and size of effect, with

fungicide treatments tending to have the opposite effect of inoculation ($g = 1.140$, $SE = 0.334$, $P < 0.001$; Table 2; Fig. 3). This is in line with our prediction that unintended impacts of fungicides (especially pathogen control) could complicate a study's ability to isolate the impacts of AMF.

A few studies tested the impacts of nutrient supplementation (low vs high), on the effect of AMF treatment ($n = 88$, $k = 5$). In a subset analysis of studies examining differences in nutrient supplementation, we found no significant difference on the response to AMF treatment ($g = 0.428$, $SE = 0.245$, $P = 0.084$; Table 2; Fig. 4).

The setting (lab vs field) in which the experiment took place had no detectable effect on average effect size ($g = 0.205$, $SE = 0.177$, $P = 0.247$; Table 2; Fig. S1); this was true regardless of whether the analysis included only studies that applied an inoculation approach (Koricheva *et al.*, 2009) or all studies. Similarly, a plant species' categorization as crop/agricultural, ornamental, or strictly wild had no impact on its response to AMF treatments ($g = 0.244$, $SE = 0.198$, $P = 0.220$; Table 2; Fig. S2).

We conducted a moderator analysis to consider the effect of plant family on the effect of AMF manipulation using a subset of our database consisting of plant families with > 8 effect sizes (i.e. those with sufficient replication). We found no significant effect of plant family on the response to AMF (Moderator Test: $F_{346,11} = 0.907$, $P = 0.5333$; Table S4). In addition, we tested the effect of plant life history on AMF response (i.e. perennial, annual, or semiannual) and found no significant effect (Moderator Test: $F_{399,4} = 0.5703$, $P = 0.6843$).

Publication bias

Following the methods described in Nakagawa *et al.* (2022), we determined that our dataset exhibits evidence of publication bias. We base this on the fact that the slope of a meta-regression (as described by Nakagawa *et al.* to account for study effects) was significantly > 0 ($\beta = 1.2067$, 95% CI = (0.221, 2.19), $P = 0.0166$), which indicates that effect sizes with larger uncertainty (larger inverse sample size) tend to be larger (suggesting publication bias). Given this, we recalculated the overall effect adjusted for publication bias (again following Nakagawa *et al.*), finding that the adjusted effect is still significantly > 0 (SMD = 0.416, 95% CI = (0.141, 0.692), $P = 0.00320$).

Table 1 Results of meta-regression analyses for all studies (All) and subgroup analyses for floral traits in response to AMF (display, visitation, yield, and reward).

Trait	g	n	k	SE	df	95% CI	P -value	Q
Display	0.786	251	52	0.1320	235	0.527, 1.05	< 0.001	1240 ^a
Visitation	0.590	56	8	0.2270	55	0.135, 1.05	0.012	187 ^a
Yield	0.450	87	22	0.1300	82	0.19, 0.709	< 0.001	258 ^a
Reward	0.551	29	9	0.3070	28	-0.0785, 1.18	0.084	86 ^a
All	0.640	423	63	0.0985	403	0.447, 0.834	< 0.001	1870 ^a

Heterogeneity (Q) for each meta-regression and subset analysis is also depicted.

^aTests for heterogeneity (Q) indicate significant heterogeneity.

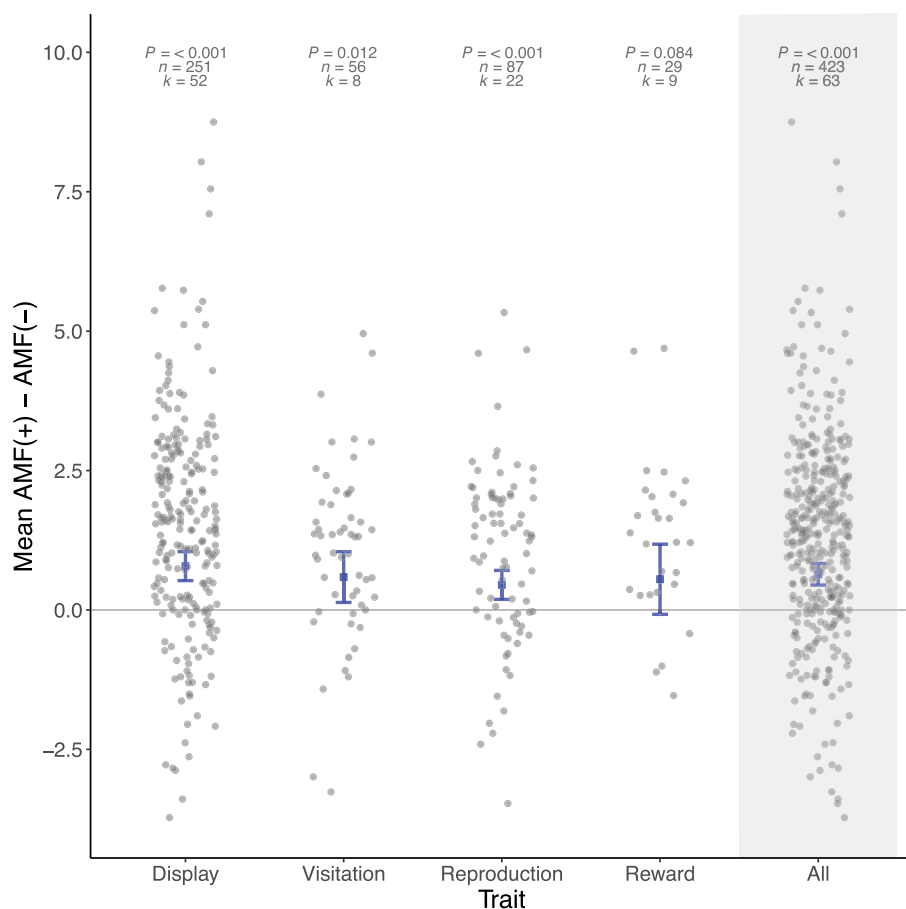


Fig. 2 Impacts of arbuscular mycorrhizal fungi presence (AMF+) vs absence (AMF-) on each floral trait. Points represent standardized mean differences (SMDs) from meta-regression results with 95% confidence intervals (in blue) with scatterplots of individual SMDs presented for each effect size and for each trait (in addition to all traits combined). In each trait category, the number of effects is indicated by n and number of studies by k .

Table 2 Results of moderator analyses for different experimental characteristics (nutrient supplementation (with vs without), AMF manipulation type (fungal inoculation vs exclusion), setting (greenhouse vs field), and plant category (crop, ornamental, or wild) in response to AMF.

Experimental design	g	n	k	SE	df	95% CI	P -value	Q
Nutrient supplementation (with vs without)	0.428	94	47	0.245	89	-0.0591, 0.915	0.084	232 ^a
AMF manipulation type (fungal inoculation vs exclusion)	1.140	423	63	0.334	402	0.483, 1.8	< 0.001	1807 ^a
Setting (glasshouse vs field)	0.205	423	63	0.177	402	-0.142, 0.552	0.247	1688 ^a
Plant category (crop, ornamental, or wild)	0.244	423	63	0.198	401	-0.146, 0.633	0.220	1841 ^a

Heterogeneity (Q) for each meta-regression and subset analysis is also depicted. Meta-regression structure is the same but a moderator (fixed effect) is added. Number of effect sizes (n) and number of studies (k).

^aTests for heterogeneity (Q) indicate significant heterogeneity.

In addition to these tests, again following Nakagawa *et al.* (2022), we generated two funnel plots: a standard funnel of standard error vs effect size (SMD) in which effect sizes are distributed across the funnel asymmetrically (Fig. S3a) and a second funnel plot of standard error vs conditional residuals (SMD), which accounts for the multilevel hierarchical structure of our data and models (Fig. S3b). The latter funnel plot (Fig. S3b) is less asymmetrical, indicating that the multilevel nature of our data is at least partly responsible for the asymmetrical distribution of the standard funnel plot (Fig. S3a).

Discussion

Effect of AMF on pollination

We found strong support for the hypothesis that soil arbuscular mycorrhizal fungi can impact plant–pollinator interactions through the enhancement of floral display size. Taken together, our findings suggest that the presence of AMF in soils can increase pollinator-mediated fitness through known pathways, that is larger floral displays driving increased pollinator attraction and seed set (Bell, 1985). While the impacts of ‘non-pollinators’

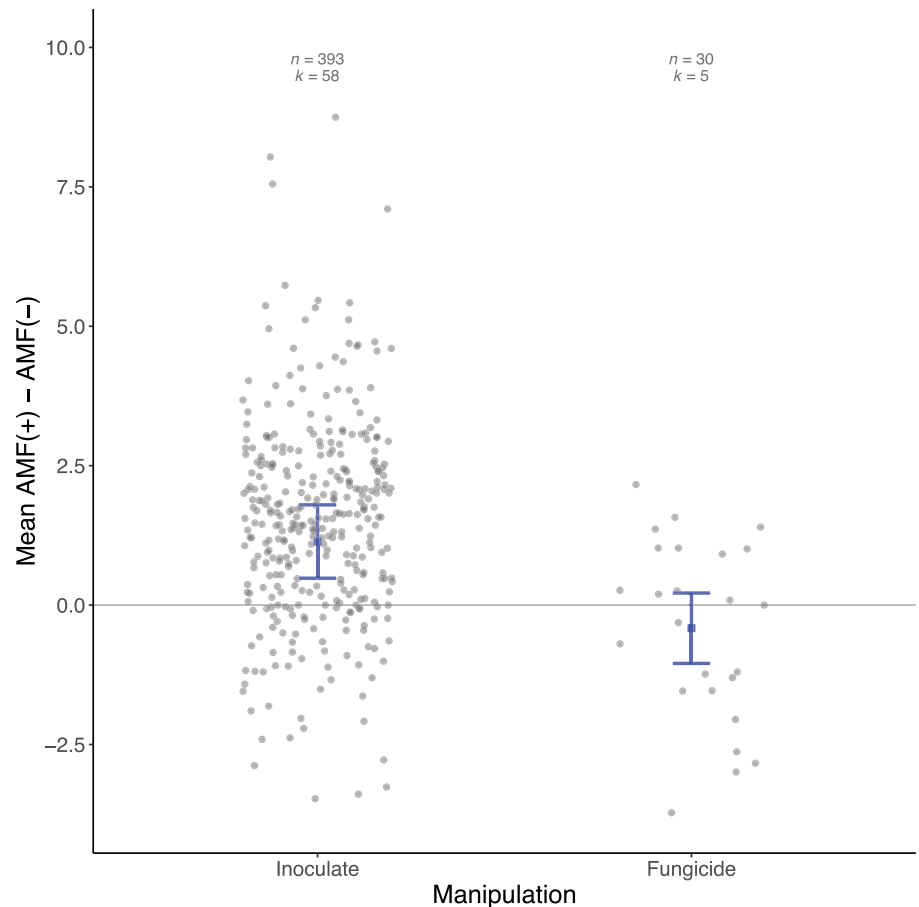


Fig. 3 Impacts of arbuscular mycorrhizal fungi presence (AMF+) vs absence (AMF–) for each AMF manipulation type (fungal inoculation or fungal exclusion) on all floral traits (Test of Moderators: $F_{1,402} = 11.62$, $P = 0.0007$). Points represent standardized mean differences (SMDs) from meta-regression results with 95% confidence intervals (in blue) with scatterplots of individual SMDs of each study and for each AMF manipulation type. In each category, the number of effects is indicated by n and number of studies by k .

on floral traits, especially herbivores, is a well-established field of study (Strauss & Whittall, 2007; Johnson *et al.*, 2015), the potential for a role of AMF is a relatively recent idea, and, consequently, is much less well understood. Our results also lend support to the idea that AMF could impact plant community dynamics through pollinator-mediated processes (Cahill *et al.*, 2008). Overall, our meta-analysis contributes to the growing body of evidence that interactions with belowground microbes can alter interactions between plants and their pollinators, opening up an array of exciting new research avenues in ecology, evolution and applied biology.

In a recent meta-analysis, Marro *et al.* (2022) reported an average effect of AMF on plant biomass of 1.34 (CI 1.13–1.56). Comparing our two studies, the effect of AMF on vegetative biomass is *c.* three times that of traits involved in pollination ($g = 0.640$ (CI 0.447–0.834)) and *c.* 1.5 times the effect on floral display size alone ($g = 0.786$ (CI 0.527–1.05)). We do note, however, that Marro *et al.* (2022) included studies from both high- and low-stress environments, which may have exaggerated their overall effect size. At the same time, our dataset exhibits some evidence of publication bias, which could have inflated our own effect size estimates (Figs S2, S3). Nonetheless, together, these two meta-analyses suggest that, while the average effect of AMF on traits involved in pollination is large by meta-analysis

standards (Cohen, 1977), they are not as strongly impacted as vegetative traits, based on currently available studies.

The difference in the effect of AMF on vegetative and floral traits is, at first glance, surprising. If AMF influences plant traits through enhanced nutrient acquisition, studies of nutrient supplementation indicate that floral traits should be at least as AMF-responsive as vegetative traits (Vaudo *et al.*, 2022). On the other hand, nutrient supplementation is probably a poor model for how AMF functionally impact plant traits, because plants divert carbon to the AMF partnership as part of the interaction (Hoeksema *et al.*, 2010), possibly at the expense of floral display size. More studies comparing the effects of AMF on vegetative and floral traits under different levels of nutrient limitation are needed to disentangle how relationships with AMF influence plant resource allocation and floral trait size.

While our overall effect sizes tended to be large, there was high among-study heterogeneity (Tables 1, 2; Fig. 2). High inter-study heterogeneity is a well-known feature of meta-analyses in ecology and evolution and has been attributed to the high system specificity of outcomes in biological systems (Senior *et al.*, 2016; Nakagawa *et al.*, 2022). In the following sections, we describe our attempts to better understand this inter-study heterogeneity by exploring the most likely system- and study-specific factors (for which we had data to test).

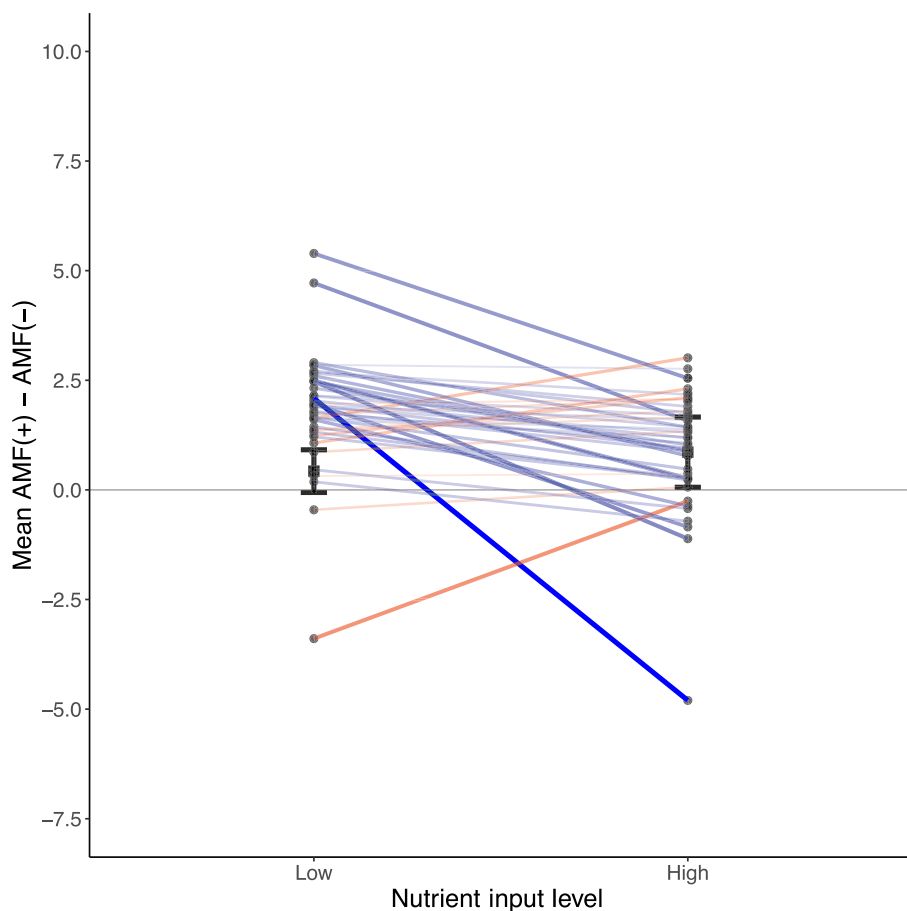


Fig. 4 Impacts of arbuscular mycorrhizal fungi presence (AMF+) vs absence (AMF-) for each nutrient supplementation category (with high vs low nutrient supplementation) on all floral traits (Test of Moderators: $F_{1,89} = 3.05$, $P = 0.084$). Points represent standardized mean differences (SMDs) from meta-regression results with 95% (in black) for each nutrient supplementation category. Each low-high comparison pair is linked by a blue-toned line (for decreasing effect when comparing low-to-high nutrient supplementation) and a red-toned line (for increasing effect when comparing low-to-high nutrient supplementation). Lines with greater thickness and opacity have a larger increasing (red-toned) or decreasing (blue-toned) effect of AMF.

Nutrient limitation

A leading explanation for why plants engage in AMF symbiosis is to enhance phosphorus (P) relations (Sawers *et al.*, 2017). Soil nitrogen (N) availability has also been shown to be an important predictor of AMF responsiveness; many studies report a greater response to AMF in nutrient-limited conditions (Hoeksema, 2018). Floral display and reward production relies heavily on nitrogen and phosphorus (Burkle & Irwin, 2009; Vaudo *et al.*, 2022), and, accordingly, some studies in our dataset included a manipulation of these factors to test whether they alter the relationship between AMF inoculation and floral traits.

Although only a handful of the studies in our dataset ($n=5$) explored trait responsiveness to AMF in the context of soil nutrients (most often P limitation), these studies contributed 88 effects. We were able to extract 44 pairwise comparisons (low vs high nutrients) in total. However, after we controlled for nonindependence among effects from the same study, we found a trend (Fig. 4), but no statistical support for our prediction of stronger effects under nutrient limitation, possibly due to insufficient power.

In a meta-analysis of the context dependency of plant responses to mycorrhizal inoculation, Hoeksema *et al.* (2010) found little evidence to support a simple cause-and-effect relationship between P supplementation and plant biomass

response to mycorrhizal inoculation. In fact, they found that P limitation was unimportant in any context, a finding that seemingly contradicts the most common assumptions about the benefits of AMF to plants. The authors hypothesized that nitrogen limitation in studies designed to measure the impacts of P supplementation lowers the photosynthetic rate, reducing the carbon available to foster the mycorrhizal symbiosis, and limiting the potential for a strong AMF response (Johnson, 2010). More well-controlled studies of AMF inoculation that also supplement nutrients are required to better understand this association.

Experimental design

High heterogeneity in meta-analyses in ecology are most likely driven by among-study methodological differences and the relative importance of system and setting to biological outcomes (Senior *et al.*, 2016). Many studies in our dataset were performed in a glasshouse setting. An earlier meta-analysis found that field studies on AMF response tended to report smaller effects compared to glasshouse studies (Lekberg & Koide, 2005). To explore this possibility, Pringle & Bever (2008) compared the response of four plant species to inoculation by AMF in lab and field conditions, finding that glasshouse studies were a reasonable approximation of field studies. In a different meta-analysis, Hoeksema

et al. (2010) found that setting was not a predictor of a plant biomass response to AMF inoculation. Our results are generally in agreement, suggesting that glasshouse vs field setting is not contributing significantly to the variation among-study outcomes.

Response to AMF manipulation depended on whether the experiment applied fungicide or inoculation. Overwhelmingly, inoculation had a positive impact on floral traits and pollination (Fig. 3). On the other hand, eliminating AMF through fungicide often showed the opposite, with traits responding more positively to fungicide treatment (i.e. AMF–) than plants in the control group. There was, however, a considerable difference in the number of studies in fungicide ($k=5$) vs inoculation ($k=58$) categories. This disparity in the number of contributing studies makes it difficult to assess our original hypothesis that outcomes would be more variable in fungal exclusion studies, as the higher variability in outcome could be an artifact of the smaller number of studies. Nonetheless, the difference in the mean effect is notable (Fig. 3). A similar result was reported by Koricheva *et al.* (2009) in a meta-analysis of the effects of AMF soil manipulation (through either inoculation or fungicides) on herbivore performance. Fungicides, in addition to reducing or eliminating AMF, also reduce or eliminate a whole range of soil organisms including pathogens. In another meta-analysis, Lekberg *et al.* (2017) reported that fungicide treatments stood out as having an average null effect on plant performance (while other treatments had a consistently negative effect), due to the highly variable ways that fungicides can impact plant performance. Our results echo existing concerns that the effect of fungicide on the plant-AMF response is complex and should be interpreted cautiously.

Effects of domestication

Theory suggests that evolutionary changes during domestication could reduce the AMF symbiosis – essentially, that modern agricultural environments (e.g. high nutrient, low pest pressure, high irrigation) alter the cost : benefit ratio of engaging in AMF symbiosis, leading to a functional loss of traits (Porter & Sachs, 2020). Indeed, the response to AMF appears to be reduced in domesticated crop species relative to their progenitors (Rillig & Mummey, 2006; Lehmann *et al.*, 2012). In a study of 27 crops, Martin-Robles *et al.* (2018) showed that, on average, wild relatives produced higher AMF responsiveness than a domesticated relative, although the effect was small. The difference in mycorrhizal responsiveness in wild vs domesticated noncrop plants is currently unknown.

A relatively large proportion (66%) of species in our dataset are ornamentals, which reflects growing interest from the commercial sector in using AMF to improve floral display. Plants bred for horticultural use may experience a shift in the cost : benefit of the AMF symbiosis similar to what has been hypothesized for crops (Porter & Sachs, 2020). However, we found no evidence to support this hypothesis in the studies we extracted – ‘ornamental’, ‘wild’ or ‘crop’ species did not differ in their overall response to AMF.

Publication bias

Publication bias results when effects represented in the meta-analysis are a biased sample of the true distribution of effects. Bias can arise because: (1) authors hold back certain results, affecting what studies are submitted for publication; (2) editors or reviewers favour certain results during the publication process, affecting which results are published; and/or (3) bias is introduced through the search procedure, impacting which studies are discovered (Jennions *et al.*, 2013). Using methods developed by Nakagawa *et al.* (2022) we found that, even accounting for the hierarchical nature of the data, there likely exists some publication bias in our dataset. This suggests that one or more of the described three sources of bias is present. As pointed out by Jennions *et al.* (2013), the finding of publication bias, while important, does not negate the relevance of a particular meta-analysis, but rather highlights the need for future research to examine and reduce the existing limitations on the available distribution of effects.

Conclusions

Overall, we found strong support for the hypothesis that the presence of AMF can positively impact floral display size. We also found support for effects of AMF on pollinator visitation and yield. The studies we extracted from our search cover a broad range of study objectives, methods (especially treatments), and plant/AMF taxonomic groups. This breadth of systems and approaches likely explains the high among-study heterogeneity we encountered.

Several key questions about the effects of AMF on pollinator-mediated interactions remain unanswered: (1) How widespread are they? (2) To what degree does the effect of AMF depend on the specific plant and AMF species involved? (3) How important are environmental drivers such as soil fertility and drought? And, more broadly, (4) what role do interactions with AMF play in plant demography and community assembly? To address the first and second questions, more studies examining the effects of AMF on floral traits across a variety of species, systems, and settings are needed. For example, a large number of the studies we extracted applied AMF from the family Glomeraceae (common in commercially available products). This concentration of studies examining the effects of species from a single order limits our ability to estimate whether, for example, other taxonomic groups of AMF are more or less potent drivers of floral traits. Conversely, the combination of AMF and plant species may be very important to the outcome (Stahlhut *et al.*, 2023). Thus, studies that apply a commercial AMF product could be a poor approximation for outcomes in natural systems. For point (3), more studies that specifically examine the impacts of AMF on floral traits under different environmental conditions are needed. Finally, for (4), while some evidence exists to suggest that interactions with AMF can be potent drivers of pollinator-mediated community dynamics (e.g. Cahill

et al., 2008), there is not currently sufficient information available to determine whether these effects are widespread. All of these questions require more study to adequately address them.

Our meta-analysis adds to a growing body of work connecting above- and belowground ecological interactions. While the effects of AMF on plant biomass are well-recognized, our results imply that a plant's interactions with its soil microbiome extend well beyond typical growth and biomass metrics and involve a broader set of critical aboveground interactions. The implications are potentially far-reaching – for example, if pollinator preference for floral traits such as specific floral pigments, VOCs, and/or floral display size are mediated by the plant's relationship(s) with mycorrhizal fungi, this suggests links between floral traits, pollinator-mediated fitness and the plant-AMF symbiosis. At the same time, we lack studies that connect AMF-associated changes in floral display size to changes in pollinator-mediated fitness across a variety of systems, approaches, and settings.

Expanding our understanding of the impacts of the AMF-plant symbiosis is critical in an era where belowground ecosystems are increasingly under assault from a rapid acceleration in the application of pesticides, including prophylactic fungicides (Banerjee & van der Heijden, 2023). Arable lands tend to harbour lower soil microbial diversity compared to nearby woodlands (Helgason *et al.*, 1998), which could hinder AMF-mediated nutrient acquisition from agricultural soils (Edlinger *et al.*, 2022). Whether this effect is due to the pesticides themselves, which have broad impacts on a surprising number of ecological interactions (Sargent *et al.*, 2023), or the erosion of the plant–soil symbiosis during domestication (Porter & Sachs, 2020), improving our understanding the role of the AMF symbiosis in plant ecological, community and evolutionary dynamics an increasingly urgent goal.

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Competing interests

None declared.

Author contributions

BFH updated a preliminary literature search and extracted the full dataset, performed the analyses, generated the figures, and contributed to the writing. RDS conceived the idea for the study, performed the original search for suitable studies, collected preliminary effect size and associated variance data, advised on the analyses, and contributed to the writing.

ORCID

Batoule F. Hyjazie  <https://orcid.org/0009-0002-8903-8373>
Risa D. Sargent  <https://orcid.org/0000-0001-8736-5712>

Data availability

Data have been uploaded to the Dryad database and is archived as Sargent, Risa; Hyjazie, Batoule (2024). AMF and floral traits (Dataset). Dryad. doi: [10.5061/dryad.280gb5mwp](https://doi.org/10.5061/dryad.280gb5mwp).

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Supporting Information

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

Fig. S1 Impacts of arbuscular mycorrhizal fungi presence (AMF+) vs absence (AMF–) for each experimental setting (glasshouse/laboratory vs field) on all floral traits (Test of Moderators: $F_{1,375} = 0.013$, $P = 0.912$).

Fig. S2 Impacts of arbuscular mycorrhizal fungi presence (AMF+) vs absence (AMF–) for each domestication type (crop, ornamental, wild) on all floral traits (Test of Moderators: $F_{2,401} = 0.770$, $P = 0.464$).

Fig. S3 Funnel plots of standard error vs standardized mean differences (SMD), and standard error vs conditional residuals (SMD).

Table S1 423 means (μ), standard deviations (SD), sample sizes (n) (all raw data) extracted from 63 studies for AMF supplementation or presence (AMF) and AMF exclusion or absence (no AMF).

Table S2 Results of meta-regression moderator analyses for display traits (display size vs display number) in response to AMF.

Table S3 Results of meta-regression moderator analyses for reproduction/yield traits (seed number vs seed weight vs fruit number vs fruit weight) in response to AMF.

Table S4 Results of meta-regression moderator analyses for plant family in response to AMF.

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