DOES PISUM SATIVUM EXHIBIT A RESPONSE TO NEIGHBOUR AND POT VOLUME MANIPULATIONS? A BAYESIAN HIERARCHICAL META-ANALYSIS

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ABSTRACT: Plant-plant competition is ubiquitous in nature. However, studying below ground competition has been plagued with methodological problems, and arguments about the appropriate controls. One experimental design that controls total nutrients and soil nutrient concentrations among plants grown alone or with neighbours achieves this by intentionally confounding pot volume (V) with neighbour addition. Here, we examined 18 unique experiments from 7 different studies for a total of 254 replicate alone-$V/2$ and with neighbour-V pairs of *Pisum sativum* (L.) plants in a Bayesian hierarchical meta-analysis. The posterior distributions show that it is unlikely that *P. sativum* root growth is influenced by either pot volume or neighbours. Pods are slightly more likely to increase in the neighbour-V treatment compared to the alone-$V/2$ treatment, though it is impossible to know if this is due to neighbour interactions or potting volume effects. Some researchers have dismissed experiments that seek to control soil nutrients when plants are grown alone or with neighbours, however, we believe this is still an active area of debate, and there is value in further study that seeks to understand how plants assess and respond to cues in their environment. More data and not more rhetoric is needed to resolve this problem. We suggest that further work should not use *P. sativum* anymore, and suggest some promising model plant candidates based on results in the literature.

KEYWORDS: Pisum sativum, tragedy of the commons, Ideal free distribution, plant-plant competition, replication crisis, below ground competition, meta-analysis.

INTRODUCTION:

Plant-plant competition is ubiquitous in nature, and influences individual plants, populations, communities and ecosystems (Kraft *et al.* 2015; Tilman 1982; Wilson 1988). Competition below ground has always been more difficult to study than competition above ground for reasons that are somewhat obvious (Casper and Jackson 1997; Casper *et al.* 2003; Schenk 2006). These reasons include the difficulty in getting to roots through opaque soil, the fact that most roots of most species are visually identical (Mommer *et al.* 2011; Taggart *et al.* 2011), and persistent questions about the appropriate set of controls for the study of below ground competition (Chen *et al.* 2015; Chen *et al.* 2020; Chen *et al.* 2021; Hess and de Kroon 2007; Laird and Aarssen 2005; McNickle 2020; Semchenko *et al.* 2010).

There are at least five different designs that have been used for the study of below ground plant-plant competition in potted glasshouse studies and all of them have confounding factors (McNickle 2020). The first, oldest, and by far the most common is to grow plants alone or with neighbours in the same pots with the same soil (e.g. Tansley 1917). If resource competition is the only mechanism by which plants interact, then this is probably the ideal experimental design. The plants grown together show the effect of the neighbouring plant capturing soil resources compared to the plant grown alone with exclusive access to the soil volume. However, interpreting these results becomes complicated if plants have responses to the neighbouring plant themselves that are independent of the plant's response to the soil resource environment. For example, using this design Cahill *et al.* (2010) showed that plant roots alone travelled significantly further than plant roots grown with neighbours. However, plants alone were much larger than plants grown with neighbours and so it might just be the case that larger plants have wider root systems and that this had nothing to do with neighbours. They anticipated this concern and included shoot mass as a covariate in the analysis to attempt to control for this vast difference in plant size between the alone and neighbours treatment. However, this assumes

there is a linear relationship between shoot mass and the spatial width of a root system which may or may not be a valid assumption. The analysis approach used by Cahill *et al.* (2010) highlights the concern with this design: plants grown alone have access to twice as many nutrients per plant as the two plants grown together. Thus, if there are responses to neighbours that are independent of responses to nutrients it can be difficult to separate them from the well-known effects of resource availability on plant size.

A second experimental design was introduced by Chen *et al.* (2015). They sought to control the nutrients available on a per plant basis by growing plants in nutrient free media (sand), and adding fertilizer at a rate of 1g per plant. Thus, a pot with one plant received 1g of fertilizer, and a pot with two plants received 2g of fertilizer. This would at first appear to correct the problem in the design above because now each plant has access to the same amount of nutrients per-plant, and – as Chen et al. (2015) argued – this design would let the neighbour effects emerge independent from the nutrient effects without the need for complex statistical controls for the differences in plant size. Except this design halves the concentration of nutrients in soil for plants alone compared to plants with neighbours. The uptake kinetics of plant roots are strongly dependent on soil nutrient concentrations (Bassirirad 2000; Epstein and Hagen 1951), and nutrient concentration in soil is expected to also influence plant growth and root behaviour (McNickle and Brown 2012; McNickle and Brown 2014b). Indeed, when analysing the results without accounting for soil nutrient concentration Chen *et al.* (2015) found that plants with neighbours reduced root growth relative to plants alone, but in a subsequent reanalysis of the same data accounting for soil nutrient concentration Chen *et al.* (2020) concluded that plants with neighbours produced more roots relative to plants alone (but see McNickle 2020). These contrasting results from the same dataset highlight the concern of controlling soil nutrient concentration.

A third experimental design seeks to control both total nutrients per plant, and soil nutrient concentration, but it does this by growing two neighbouring plants in a pot of volume V, and a plant alone in a volume of $V/2$ (e.g. Gersani *et al.* 2001). The concern with this design should be obvious, it confounds neighbour addition with pot volume, and pot volume has well-known influences of the growth of potted plants such that plants in bigger pots are often larger than plants in smaller pots (Hess and de Kroon 2007; Poorter *et al.* 2012). Two other designs have been tried such as including a mesh barrier between competing plants and an impermeable barrier between alone plants (Chen *et al.* 2021; Zhu *et al.* 2019), or combining the first and third designs so that there is an alone plant grown in pots of volume V and $V/2$ to compare the neighbour plants (McNickle and Brown 2014a).

Despite strong preferences for one design over another in different research groups, the utility of these alternative experimental designs, as well as their interpretation and how important confounding effects are in result is still an active area of debate in the literature (Chen *et al.* 2020; McNickle 2020). There are many meta-analyses summarising the first design that confounds total nutrients per plant with neighbour addition and most plants are larger when grown with neighbours and half the nutrients compared to alone with double nutrients (Goldberg *et al.* 1999; Wilson 1988). Only a few studies have attempted the design that confounds neighbours with soil nutrient concentration or used the mesh-barrier approach and so more work is needed to be able to quantitatively synthesize these results. However, there are many studies that examined the design that confounds pot volume with neighbour addition, and for some reason many of these have even used the same plant species common pea (*Pisum sativum* L.). Given that this is an active area of debate; we see value in attempting to synthesize the results in this debated experimental design in the hopes of continued progress towards understanding the many alternative experimental designs and their confounding effects. Here, we used a hierarchical Bayesian meta-analysis approach to synthesize these results and we focused on

studies of common pea. All possible results have been reported in these neighbour-volume manipulations for pea including reduced root production in response to neighbours and increased volume (Chen *et al.* 2015 but see; McNickle 2020), increased root production in response to neighbours and increased volume (O'Brien *et al.* 2005 but see Hess and de Kroon 2007), and three found no effect (Jacob *et al.* 2017; McNickle *et al.* 2020; Meier *et al.* 2013). We expanded these five published results by performing five additional experiments (Supplemental information). We asked what is the likely posterior effect size for the neighbour-volume treatment in pea. Contrary to the dismissal of the neighbour-volume experimental design by some authors (Chen *et al.* 2015; Chen *et al.* 2020; Hess and de Kroon 2007), we believe that there is value in work that seeks to more completely understand how neighbours, nutrients and pot volume interact to shape plant growth.

METHODS

Studies included in the meta-analysis

We sought experiments that grew pea plants grown with neighbours in pots of volume V, and compared it to plants grown alone in pots of volume $V/2$. We identified five different studies in the literature that used this neighbour-volume experimental manipulation in pea (Chen *et al.* 2015; Jacob *et al.* 2017; McNickle *et al.* 2020; Meier *et al.* 2013; O'Brien *et al.* 2005). From these five studies we extracted the mean root production with and without neighbours, and the mean pod production with and without neighbours, and their standard deviations. We also recorded the cultivar used, and the pot volume used to define V in the neighbour-volume manipulation. In addition to the neighbour-volume manipulation, some studies imposed additional factorial treatments. We treated these as additional treatments as separate experiments performed within study. These means are recorded separately resulting in multiple data points for the following studies: (i) O'Brien *et al.* (2005) crossed the neighbour addition treatment with low and high nutrient addition; (ii) Chen *et al.* (2015) included three levels of

129 soil nutrient concentration (McNickle 2020) and; (iii) McNickle *et al.* (2020) grew plants with and without
130 mycorrhizae. Two studies only collected root data and did not have pod data (Jacob *et al.* 2017; Meier
131 *et al.* 2013) and one of these also did not report an estimate of variation (Jacob et al 2017).
132
133 In addition, we performed five new supplementary experiments using the same basic
134 neighbour-volume treatment as the rest of the studies (Supplementary information). One of our
135 experiments implemented the basic neighbour-volume treatment where V was 1L while four of our new
136 experiments crossed the neighbour-volume treatment with the presence or absence of above ground
137 shoot competition, used a V of 6.3L and used different potting media in each case (Table S1-3, Figures
138 S1-S4). Large pots were intentionally chosen in this second set of experiments to expand the number of
139 values for V we could examine directly in the meta-analysis.
140
141 *Meta-analysis test statistic*
142 To compare plants in the two neighbour volume treatments, we used Hedges g (Hedges 1981)
143 as our test statistic calculated according to:

$$g = \frac{\bar{X}_{n,V} - \bar{X}_{a,V/2}}{SD_{pooled}} c(n), \qquad Eqn\ 1$$

144 where $\bar{X}_{n,V}$ is the mean response variable in the presence of a neighbour and in a pot of volume V
145 (hereafter, neighbour-full); $\bar{X}_{a,V/2}$ is the mean response variable when grown alone in a pot of volume
146 $V/2$ (hereafter, alone-half); n was the sample size of the study and $c(n)$ is a correction factor for small
147 sample size. The correction factor derived by Hedges (1981) for a balanced experimental design is given
148 by:

$$c(n) = \frac{\Gamma(n-1)}{\Gamma\left(n - \frac{1}{2}\right)\sqrt{n-1}}, \qquad Eqn\ 2$$

149 where $\Gamma(x)$ is a Gamma function of the form:

$$\Gamma(x) = (x-1)!. \qquad Eqn\ 3$$

The correction factor asymptotes at $c(n) = 1$ as n approaches infinity, and was designed to remove known bias in the test statistic that arises from low sample sizes. By constructing g with $\bar{X}_{n,V} - \bar{X}_{a,V/2}$ as the numerator, it will be negative in the case of reduced root growth in the alone-half treatment, positive in the case of increased root growth in the neighbour-full treatment, and zero in the case of no effect of either neighbours or doubling/halving pot volume. Since all studies used a balanced design, the pooled standard deviation was calculated as:

$$SD_{pooled} = \sqrt{\frac{{SD_{n,V}}^2 + {SD_{a,V/2}}^2}{2}}, \qquad Eqn\ 4$$

where $SD_{n,V}$, and $SD_{a,V/2}$ are the standard deviation associated with the means of the same subscript. We calculated g for individual root biomass, and also for lifetime seed yield and analysed these two tissues independently.

Hierarchical Bayesian linear mixed effects model

A hierarchical Bayesian linear mixed effects approach was implemented in brms (Bürkner 2017; Bürkner 2018) in the R statistical environment (v 4.1.1 R-Core-Team 2021). To control for heterogeneity among studies, individual experiment was nested within study as a random effect. This allowed us to account for differences among both individual experiments (i.e. total nutrients (O'Brien *et al.* 2005), nutrient concentration (Chen *et al.* 2015; McNickle 2020), with and without mycorrhizae (McNickle *et al.* 2020), with and without shoot competition (Fig S1)), and individual research groups (E.g. soil media, fertilizer type, watering schedules, timing, and so on). Since studies also did not always use the same cultivar, and since cultivars represent a separate form of biologically interesting heterogeneity among studies, we also included pea cultivar as another random effect. Finally, we included the pot volume used by each study as a fixed effect continuous variable to explicitly identify the effect of the value of V

171 in the neighbour-volume response. We present both the posterior mean for each study independent of
172 value of V, and the linear relationship among studies accounting for the value of V as a covariate. The
173 posteriors were generated using four Markov chain Monte Carlo (MCMC) chains, 2500 burn-ins, 5,000
174 iterations per chain, resulting in 10,000 estimates for each posterior distribution. No thinning was used
175 as thinning has been shown to have no detectable effects on MCMC simulation (Link and Eaton 2012).
176 We assumed any value of g or standard deviation was equally likely as priors.
177

178 **RESULTS**

179 *Root responses*

180 The meta-analysis included 18 unique experiments from 7 different studies for a total of 254
181 replicate alone-half and with neighbour-full pairs of plants. Additionally, 6 different cultivars of pea were
182 used across the literature. The value of V used by studies ranged from 50mL (Meier *et al.* 2013) to 6.2 L
183 (this study; Supplemental Information).
184

185 The influence of pot volume on effect size for roots was 0 (95%CI: -0.12, 0.11) with an intercept
186 of -0.03 (95% CI: -0.61, 0.58; Fig 1a). Thus, the posterior average effect size across studies was -0.03
187 (Table 1; Fig 2a). Unlike a frequentist approach that assigns a p-value to either accept or reject one
188 hypothesis, the Bayesian framework allowed us use the posterior distributions to assign probabilities
189 that a given outcome might be observed in a future study with peas. Here, the logical hypothesis to
190 examine was the probability that the effect size is greater than zero (increased roots in the neighbour-
191 full treatment) or less than zero (decreased roots in the neighbour-full treatment). Using the posterior
192 distribution, there was a probability of 0.44 that the effect size for peas would be greater than zero, and
193 thus a probability of 0.56 that the effect size was less than zero.
194

With any meta-analysis there is obviously heterogeneity among studies, and here quantifying that heterogeneity was major motivation for our analysis. The hierarchical Bayesian mixed effects analysis is based on the assumption that each level of the random effect (experiment within study, study and cultivar) has its own effect size which emerged from sampling the world, and it models each of these as a standard deviation around the random intercept. Furthermore, since each random effect was modelled with its own prior distribution we can estimate this heterogeneity directly as standard deviations that also have 95% CIs (Table 1). Cultivar produced an estimated standard deviation of 0.28 (95% CI: 0.01, 0.97) around the intercept, differences among studies produced an estimated standard deviation of 0.28 (95% CI: 0.01, 1.00) and differences among experiments but within a study had a standard deviation of 0.35 (95% CI: 0.2, 0.6). These should be interpreted as standard deviations around the random intercept of the model.

Pod responses

The estimated slope of its relationship with pot volume was weakly positive at 0.04 (95% CI: -0.10, 0.19) and the intercept was 0.05 (95% CI: -0.96, 1.07; Fig 1b). Thus, pot volume increased the expected neighbour-volume effect size by 0.04 standard deviations L^{-1} of pot volume. For example, a 1L pot would have an estimated effect size of 0.09, a 2L pot would have an estimated effect size of 0.13 and so on. The average posterior effect size for most studies was also slightly positive, indicating more pod mass in the neighbour-full treatment compared to the alone-half treatment (Fig 2b). As above, we can use the posterior distributions to assign probabilities to a given outcome in pea. Here, the average posterior distribution shows that there is a probability of 0.68 that the effect size is greater than zero, and thus a probability of 0.32 that the effect size is less than zero.

As above, we estimated heterogeneity in the meta-analysis directly as standard deviations around the random intercept in the model with 95% Cis (Table 1). With pods, cultivar had an estimated standard deviation of 0.51 (95% CI: 0.02, 1.85), study had a standard deviation of 0.49 (95% CI: 0.02, 1.85), and differences among experiments but within a study had a standard deviation of 0.37 (95% CI: 0.19, 0.70).

DISCUSSION

Despite being outright dismissed by some, many researchers have used a neighbour-volume manipulation to study root growth responses in pea such that two neighbour plants are grown in a pot of volume V and one plant is grown alone in a pot of volume $V/2$ (Chen *et al.* 2015; Chen *et al.* 2020; Gersani *et al.* 1998; McNickle 2020; McNickle *et al.* 2020; O'Brien *et al.* 2005). With this design, researchers intentionally confound neighbour addition with pot volume so as to control both soil nutrients available per plant and soil nutrient concentrations (McNickle 2020). This experimental design is usually performed in contrast to the more common and older approach of confounding nutrients available per plant with neighbour addition (e.g. Cahill *et al.* 2010; McNickle *et al.* 2016; Tansley 1917; Wilson 1988). There remains an open debate about whether to confound nutrients with neighbours or pot volume with neighbours (Chen *et al.* 2020; McNickle 2020). For some reason pea has become a sort of model species for these neighbour-volume experiments but different studies have found almost all possible results with some finding reduced root production in response to neighbours and increased volume (Chen *et al.* 2015; Gersani *et al.* 1998), others finding increased root production in response to neighbours and increased volume (Chen *et al.* 2020; O'Brien *et al.* 2005), and others finding no response (Jacob *et al.* 2017; McNickle 2020; McNickle *et al.* 2020; Meier *et al.* 2013).

241 Since the literature is mostly populated by significant p-values, here we examined them in a
242 Bayesian hierarchical meta-analysis that estimated the average posterior effect size of pea neighbour-
243 volume effects as well as heterogeneity among studies and we assumed any result was equally likely for
244 our priors. We also performed five additional neighbour-volume experiments with pea to expand the
245 meta-analysis dataset (Supplemental information). An absolute effect size of $0 < |g| < 0.2$ is
246 considered small, and it corresponds to just 50-58% of the treatment group being larger or smaller than
247 the control and would not be visually obvious. By this convention, for both roots and pods the average
248 effect size across studies was small (-0.03 and 0.13 respectively; Fig 2). Furthermore, the 95% credible
249 intervals around estimates was so large that – as we already knew from the literature – effectively any
250 result might be found for root responses in any given experiment (Fig 2, Table 1). These Bayesian
251 posterior distributions suggest that if researchers take a frequentist approach to hypothesis testing with
252 a relatively small sample size, then whether one finds a neighbour-volume root response in pea is
253 essentially the same as flipping a coin (Fig 2a), and only slightly better than flipping a coin that a positive
254 pod response would occur (Fig 2b). Therefore, we conclude that in general pea has neither strong
255 neighbour root responses, nor strong responses to halving pot volume when the treatments compared
256 are two neighbour plants grown in a volume of V, and one plant grown in a pot of volume $V/2$.
257
258 However, in addition to the basic neighbour-full and alone-half comparison made within study,
259 we could compare the pot volumes used among studies. The volume of the pot that peas were grown in
260 varied from $V = 50$ mL (Meier *et al.* 2013) to $V = 6.2$ L (this study; Supplementary information). For
261 roots, the estimated posterior slope of this pot volume effect was 0.00 (95% CI: -0.12, 0.11; Table 1; Fig
262 1A). This means that one can expect the same effect size for roots whether or not plants are grown in
263 50mL pots or 6.2L pots. This is not the same as saying that plants did not have a growth response to pot
264 volume (Fig S5), only that the effect size calculated by Eqns 1-4 are zero on average no matter the pot

size. The influence of pot volume used in a study on the effect size for pods on the other hand was weakly positive at 0.04 (95% CI: -0.10, 0.19; Table 1; Fig 1b). This means that the effect size was increased by pot volume from 0.05 in a 50mL pot to 0.30 in a 6.2L pot for pods. Thus, we conclude that the neighbour-volume manipulation does begin to have a larger effect on pod production in a positive direction. Since these experiments confound neighbour addition and pot volume it is impossible to determine which factor caused the increasing difference in pod production in neighbour-full pots relative to alone-half pots. For example, one could argue that pots of volume V have more nutrients and space and that this lead to the increase in pod production relative to pots of volume $V/2$ and that neighbours had nothing to do with this (e.g. Hess and de Kroon 2007). One could also argue from the nutrient school of thought that perhaps some kind of facilitation occurred between the two plants and that pot volume had nothing to do with the results (Callaway and Walker 1997; Thorpe *et al.* 2011). One could also argue that both neighbour and volume effects simultaneously occurred, since there is no reason to think those two ideas are mutually exclusive.

However, these conclusions about the mean effect size (Fig 1, Fig 2) should also be considered in the context of the heterogeneity among experiments, studies and cultivars (Table 1). The Bayesian approach assumes that each individual experiment within study, combined with cultivar differences have their own effect size that was sampled from the global population of possible effect sizes. Therefore, these random effects produce standard deviations with their own posterior distributions that should be interpreted as standard deviations around the intercept (Fig 1) or mean effect (Fig 2). Interpreting these standard deviations is easiest when considering the posterior distribution of possible effect sizes for each study (Fig 2). The tails of the posterior distribution for each study include 1 and -1 for both roots and pods. Thus, though the mean is centred on 0 and 0.14 respectively, there is a wide degree of uncertainty in these average estimates. For roots and pods, the uncertainty for cultivar was

large which could indicate a genetic basis for the responses (Table 1). The differences among experiment performed within study indicate that other manipulations such as total nutrients (O'Brien *et al.* 2005), nutrient concentration (Chen *et al.* 2015; McNickle 2020), manipulation of mycorrhizae (McNickle *et al.* 2020), manipulation of shoot competition (Supplemental information) also influence the results, and none of this is surprising. We would direct interested readers to each study to interpret the influence of these other treatments.

Importantly, we only studied the neighbour-volume response of pea roots at the coarsest possible scale: total root biomass. Pea might have other finer scale plastic root growth in space in relation to either neighbours or barriers such as pot walls (Cabal *et al.* 2020; Falik *et al.* 2005; O'Brien *et al.* 2007). These fine-scale behaviours of individual root tips as they navigate the rhizosphere are obviously not captured by studies of total root system size. For example, Falik *et al.* (2005) found that individual root tips of pea were able to adjust growth near barriers as small as 0.8mm diameter nylon string. In another study using 20 herbaceous prairie species that tracked finer scale root growth and architecture Belter and Cahill (2015) found that many different root behaviours were possible, and that a focus on total root biomass obviously obscures finer scale root growth behaviours. That we found pea does not seem to have a neighbour-volume response at the coarsest possible scale (Fig 1a, Fig 2a), does not mean that pea lacks finer scale root response. O'Brien *et al.* (2007) presented a model for finer-scale root responses to neighbours that might occur in regions of root system overlap relative to regions of soil where one plant is alone which could aide hypothesis development in future studies. Cabal *et al.* (2020) recently presented a very similar model which also makes spatially explicit predictions about intermingled root systems. If researchers want to continue to study pea responses to neighbours, we suggest that future experiments should attempt to take volume out of the picture and study finer scale root responses at the scale of the rhizosphere (Downie *et al.* 2015).

313

314 Much of the literature that has used the confounded neighbour-volume manipulation
315 experiment that we examined here is based in an argument about how plants sense and respond to
316 their environment (McNickle 2020). Part of the argument is about the appropriate controls in a potted
317 competition experiment, it turns out that it is impossible to control more than two of three key variables
318 due to the limitations of geometry and chemistry: (i) total nutrients available per plant; (ii) soil nutrient
319 concentration, and; (iii) pot volume. For example, most commonly plants are grown either alone or with
320 a neighbour in pots of volume V (Tansley 1917). This design confounds total nutrients and neighbours
321 and so it is difficult to identify responses to neighbours that are independent of responses to nutrients.
322 The experiments examined in this meta-analysis control total nutrients per plant, but are forced to
323 confound pot volume with neighbour addition. Though we conclude from this meta-analysis that pot
324 volume conclusively has no effect on pea roots (Fig 1, 2, S5), volume might still have important effects
325 for other species (Poorter *et al.* 2012). Another possible design controls pot volume and total nutrients
326 per plant but confounds soil nutrient concentration (Chen *et al.* 2015; Chen *et al.* 2020; McNickle
327 2020). The problem posed by these experimental limitations is that pot volume influences plant growth
328 positively (Poorter *et al.* 2012), total nutrients influence plant growth positively (Chapin *et al.* 1986), soil
329 nutrient concentration influences uptake kinetics positively, and therefore also positively increases plant
330 growth (Bassirirad 2000; McNickle and Brown 2014b), and many theories also predict that neighbours
331 will positively increase plant growth as a strategy for maximizing competitive ability (Givnish 1982;
332 Zhang *et al.* 1999). Thus, whether one sees a positive effect of a neighbour-nutrient, neighbour-
333 concentration, or neighbour-volume manipulation it is impossible to test any of the hypotheses that the
334 effects were due to total nutrients, nutrient concentration, pot volume or neighbours since they all
335 make the same predictions in the same direction. Furthermore, the hypothesized effects of total
336 nutrients, nutrient concentration, pot volume and neighbours are not mutually exclusive, and there is no

337 reason why they might not all simultaneously influence root growth. Thus, simply observing root growth
338 in these different experiments is not a test of the many alternative hypotheses. One possible path
339 forward would be to stop performing experiments that cannot actually falsify alternative hypotheses,
340 and seek the mechanism or mechanisms involved in these root responses to the environment. It would
341 be sensible to hypothesize that the mechanisms involved in assessing and then responding to
342 neighbours, nutrients or pot volume are different. Characterising the mechanisms would then allow
343 each hypothesized effect to be falsified based on how plants sense their environment. Nested
344 association mapping populations could be a useful tool for identifying the mechanisms that generate
345 different growth responses in plants. Using variable genotypes, or perhaps eventually using knockout
346 mutants could begin to provide a clearer path forward in understanding how plant roots respond to
347 their environment and truly disentangle neighbour effects from volume and nutrient effects.
348
349 Pea is an attractive model species for experimental studies since it is relatively small, and
350 completes its lifecycle in 50-70 days. Obviously we cannot speak for other researchers, but this is the
351 one important reason we chose to develop it as a model species to explore root responses to
352 neighbours. The other reason was that it seemed from the published literature that this was a species
353 that might have relatively complex root plasticity because of the breadth of reported responses (Chen *et*
354 *al.* 2015; Falik *et al.* 2005; Gersani *et al.* 1998; O'Brien *et al.* 2005). However, after performing five
355 experiments ourselves that could not reproduce any of these previous findings (Supplementary
356 information), and the hierarchical meta-analysis synthesizing 18 unique experiments from 7 different
357 studies for a total of 254 replicate alone-half and with neighbour-full pairs of plants we are convinced
358 that pea has no coarse scale responses to neighbours. This does not mean that no plant species on earth
359 has neighbour responses below ground. This raises the question; if one is still interested in the cause of

these neighbour-volume manipulations what plants might be better model species for studying root responses to neighbours?

One obvious model would be the workhorse *Arabidopsis thaliana* since there is an enormous amount of pre-existing literature, and significant genetic resources that would aide in further understanding of the neighbour-volume response (Kaul *et al.* 2000). Indeed, there is a body of work on plant-plant competition in *Arabidopsis thaliana*, particularly above ground competition (Cahill *et al.* 2005; Pantazopoulou *et al.* 2017). Another appealing option is Soybean (*Glycine max* (L.)) which also has significant genetic resources (Grant *et al.* 2009), and it is an economically important food crop. Even a small effect size in root or pod growth could have large economic implications at the scale of global agriculture. However, as with pea there are variable results in the literature with one study finding the TOC response (Gersani *et al.* 2001) and another finding the IFD response (Chen *et al.* 2021). These two studies used different cultivars, and so it remains to be seen if these are genetic differences, or simply type I errors as we found with pea (Fig 2). *Medicago trunata* is another common model annual plant species with significant genetic resources (Frugoli and Harris 2001) that could be considered to examine neighbour responses. Yang *et al.* (2013) found that alfalfa (*M. sativa* (L.)), a perennial congener to *M. trunata*, exhibited a root response to neighbours and so *M. trunata* might share some of these genes. However, legumes might not be the best model species for root competition studies since they form mutualisms with nitrogen fixing bacteria which might limit the need for root foraging. Wheat (*Triticum aestivum* (L.)) might be another attractive model species with variable responses across contracts (Weiner *et al.* 2017), significant genetic resources (REF), and economic importance (REF). Many other clear root responses to neighbours have been reported in herbaceous perennial plants which could be explored as new models for the neighbour response (e.g. Belter and Cahill 2015; McNickle *et al.* 2016; Padilla *et al.* 2013; Semchenko *et al.* 2010; Semchenko *et al.* 2014). However, most models include

paired predictions of root growth and lifetime fitness (e.g. Gersani *et al.* 2001; McNickle and Brown 2012; McNickle and Brown 2014a; O'Brien and Brown 2008; O'Brien *et al.* 2007), and so perennials might not be the best model species to test these model predictions. However, as with all scientific problems these questions will be addressed by continued experimentation over time.

Conclusion

Common pea has been studied in the same basic neighbour-volume manipulation across 18 unique experiments from 7 different studies for a total of 254 replicate alone-half and with neighbour-full pairs of plants. Positive, negative and neutral results have all been published and the interpretation of these results has been debated in the literature for more than a decade and so we see value in understanding average pea responses to its environment (Chen *et al.* 2015; Chen *et al.* 2020; Hess and de Kroon 2007; Laird and Aarssen 2005; McNickle 2020). Here, we used a hierarchical Bayesian meta-analysis to generate posterior distributions from the published literature. We find that whatever the effect of the neighbour-volume manipulation in other plant species, pea likely has no responses to a neighbour-volume manipulation at any pot volume ranging from 50mL to 6300 mL. We suggest that to resolve these debates about how plant roots respond to their environment, researchers should (i) develop different a plant model for these questions, and (ii) work towards identifying the molecular basis for root responses to their environment.

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AUTHOR CONTRIBUTIONS

408 GGM and MM designed the experiments. MM performed experiments 1,2 and 4 and statistical analyses.
409 AK performed experiment 3, and statistical analyses. MM drafted the initial manuscript, and all authors
410 contributed to revisions.
411

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533 **TABLE 1:** Bayesian estimators, their error and associated 95% credible intervals (CI) from the hierarchical
534 Bayesian mixed effects model for roots and pods in the meta-analysis. Cultivar, study and individual
535 experiment nested within study were included as random effects. 'Standard deviation' is abbreviated as
536 'StDev'.

Tissue	Factor	Statistic	Estimate	Error	95% CI
Roots	Mean effect size	Intercept	-0.03	0.28	(-0.61, 0.58)
	Pot volume	Slope	0.00	0.06	(-0.12, 0.11)
	Cultivar	StDev	0.28	0.26	(0.01, 0.97)
	Study	StDev	0.28	0.26	(0.01, 1.00)
	Study/Experiment	StDev	0.35	0.11	(0.2, 0.6)
Pods	Mean effect size	Intercept	0.05	0.49	(-0.96, 1.07)
	Pot volume	Slope	0.04	0.07	(-0.10, 0.19)
	Cultivar	StDev	0.51	0.49	(0.02, 1.85)
	Study	StDev	0.49	0.52	(0.01, 1.00)
	Study/Experiment	StDev	0.37	0.13	(0.19, 0.70)

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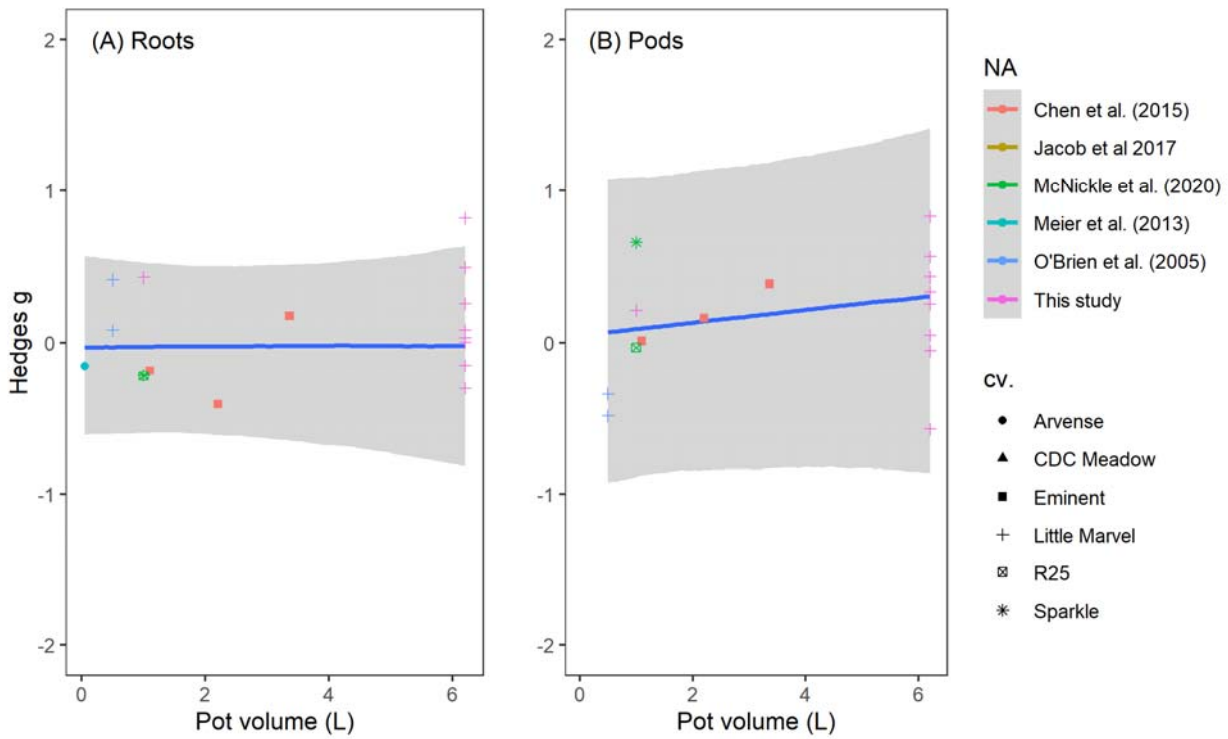

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540 **FIGURE 1**: Relationship between effect size and pot volume in each study. Points show the observed
541 mean effects of pot volume on log response ratio for (A) roots and (B) pods across all studies in the
542 meta-analysis and their standard deviations. Solid lines represent the Bayesian regression line, and gray
543 shading represents the 95% credible interval around the regression line. Cultivar, study and individual
544 experiment within study were treated as multi-level random effects, and the heterogeneity in results
545 introduced by these differences are shown in Table 1.

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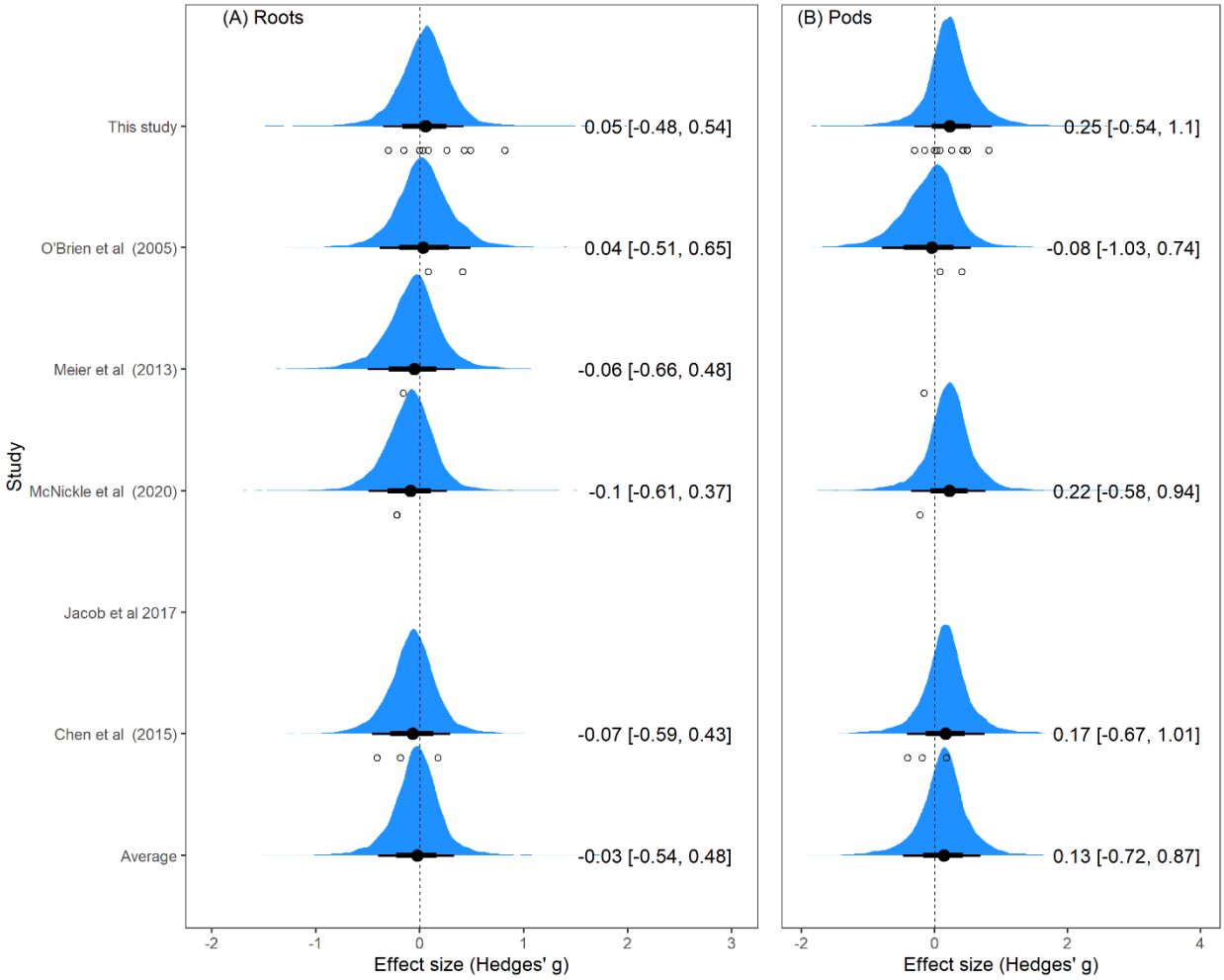

FIGURE 2: Forest plots showing the posterior distribution of effect sizes (blue) for each study and the average of all studies for (A) roots and (B) pods for each study individually (top seven rows), and averaged among all studies (bottom row). Black points and lines represent posterior means with 89% (thick) and 67% (thin) credible intervals. The posterior mean and its 95% credible interval are written to the right of each distribution. White points are the observed prior effect sizes from each study. Note: since Jacob et al. 2017 did not report any estimate of error, and Meier et al. (2013) did not report error for pod mass, we cannot estimate a posterior distribution for those studies.