


# The analysis of plant root responses to nutrient concentration, soil volume and neighbour presence: Different statistical approaches reflect different underlying basic questions

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## Abstract

1. To investigate the responses of plants to their below-ground neighbours independently of nutrient availability, experiments generally require a solitary treatment with one plant grown alone with one unit of nutrients, and a neighbour treatment with two plants grown together with two units of nutrients. This can either be done by doubling nutrient concentration (C) or by doubling soil volume (V) in the neighbour treatment as compared to the solitary treatment.
2. Statistically analysing the same dataset from an experiment that grew plants in solitary or neighbour treatment with a series of V given a fixed amount of nutrients per plant (e.g. 1 g), Chen et al. (2015a) found significant neighbour effects when they controlled for V, while McNickle (2020) found the effects to be insignificant when he controlled for C.
3. The discrepancy in the results of the two studies is caused by a difference in their analytical approaches. This includes (a) different choices of data transformation for the controlling factor, and (b) a mathematical deviation of model structures between V-based and C-based analyses, due to the different inversely proportional V-C relationships between solitary ( $C = \frac{1}{V}$ ) and neighbour ( $C = \frac{2}{V}$ ) treatments.
4. Choices for either V or C as a controlling factor in the analyses for 'neighbour effect' are based on two different perspectives, focussing either on neighbour-induced nutrient depletion (like McNickle, 2020) or on identity recognition (like Chen et al., 2015a).
5. We also raise concerns about the use of mesh-divided root interaction design and replacement series design in the studies of plant-plant root interactions. We propose to adjust the experimental designs and analytical methods based on the focal perspectives of neighbour effect.

**KEYWORDS**

game theory, neighbour detection, nutrient concentration, nutrient depletion, plant–plant interaction, pot-based experiment, root competition, soil volume

## 1 | INTRODUCTION

Competition is one of the fundamental forces determining the performance of plants, the structure and dynamics of plant communities, and the functioning of ecosystems (Aschehoug, Brooker, Atwater, Maron, & Callaway, 2016; Grace & Tilman, 1990; Rajaniemi, Allison, & Goldberg, 2003). Substantial evidence suggests that root competition is a ubiquitous phenomenon in almost all terrestrial plant communities (Chen et al., 2020; Kiær, Weisbach, & Weiner, 2013; Schenk, 2006). For decades, it was assumed that plants can only indirectly interact with neighbours below-ground via nutrient depletion (de Kroon, Mommer, & Nishiwaki, 2003). However, evidence accumulates that they can also interact with neighbours in more direct ways (Padilla et al., 2013). To empirically study the responses of plants to their below-ground neighbours independently of nutrient availability, experiments generally require a solitary treatment with one plant and one unit of nutrients, and a neighbour treatment with two plants and two units of nutrients. This can either be done by doubling the nutrient concentration or by doubling soil volume, both of which are known to have secondary effects as well. We set up an experiment that grew pea plants in solitary and in neighbour conditions with a fixed amount of nutrients per plant, applying a range of soil volumes for which we then statistically corrected (Chen et al., 2015a). We found that root and total biomass of plants increased with increasing soil volume and was lower in the presence than in the absence of a neighbour. This finding not only contrasts with a prediction of neighbour-induced root over-proliferation (often coined as a ‘tragedy of the commons’) from game theoretical root foraging models (e.g. Dybzinski, Farrior, Wolf, Reich, & Pacala, 2011; Gersani, Brown, O’Brien, Maina, & Abramsky, 2001), but also differs from a prediction of no response of root mass production to neighbours (often coined as an ‘ideal free distribution’) from the simple optimal root foraging theory (e.g. McNickle & Brown, 2014).

A recent paper by McNickle (2020) re-assessed a subset of our data (Chen et al., 2015b) and suggested that our results of neighbour effects were confounded by nutrient concentration. That is, if nutrient amount is fixed, changing volume will lead to an inversely proportional change in nutrient concentration (e.g. doubling volume leads to halving concentration). Based on this relationship embedded in the experimental design, he replaced the volume variable by nutrient concentration in the re-analyses, and found the effect of neighbour to disappear (i.e. to become insignificant) for all growth variables of plants. These contrasting findings led him to conclude that a researcher’s choice between correlated variables, that is, nutrient concentration and soil volume in this case, as a controlling factor in the analyses can influence the interpretation of neighbour effect in plant–plant interaction studies.

Here, we reply in order to develop more insight into the mechanisms that drive neighbour effects and the experimental designs that are required to demonstrate them. In doing so, we first focus on the direct analytical causes for the discrepancy in neighbour effects between McNickle (2020) and Chen et al. (2015a). We then highlight that the ‘neighbour effect’ in actual plant–plant below-ground interactions is composed of a variety of effects associated with different mechanisms, and that their different research focuses of the effects lead to the different choices of variables for control in the two studies. Thirdly, we provide further thoughts on the experimental designs required for testing for neighbour effects in plant–plant root interactions.

## 2 | ANALYTICAL CAUSES FOR THE DISCREPANCY IN THE DETECTED NEIGHBOUR EFFECT

### 2.1 | Impacts from different choices of data transformation for the independent variable

With a subset of data that only includes the records from one-pot-owner (neighbour absence) and two-pots-sharer (neighbour presence) treatments, that is, the a and b scenarios in table 1 of Chen et al. (2015a) as originally introduced by Gersani et al. (2001), McNickle (2020) performed analyses using the mixed linear model approach used in Chen et al. (2015a) but replacing the log-transformed independent variable ‘soil volume’ by untransformed variable ‘nutrient concentration’ in his new model. In contrast to Chen et al. (2015a), he found no significant neighbour effect on any of the dependent variables. Here, we repeated his analyses with the same dataset and model structure, but using ‘nutrient concentration’ after a log-transformation (ln-transformation in practice) in the purpose of being consistent with the transformation method for soil volume in Chen et al. (2015a). Interestingly, as shown in Table 1 here, the effects of neighbour on plant pod mass and root mass fraction became significant again, as found by Chen et al. (2015a). This new analysis still held unchanged when plant size (ln-transformed) was included in the analyses as an allometric covariate (see Table S1 in Supporting Information). Thus, it seems that the different choices of data transformation for the independent continuous variable can affect the detection of neighbour effect in the analyses.

The basic purpose of data transformation (particularly for dependent variables) is to fulfil the statistical requirements (e.g. normality and homoscedasticity) for the analyses. However, the way of data transformation also has consequences for the underlying assumptions about the biological (or ecological) relationship between

**TABLE 1** Summary of the effects of neighbour presence, nutrient concentration and their interaction on plant biomass and relative allocation in linear mixed models. All dependent variables and the nutrient concentration variable were ln-transformed in the analyses. Significant *p* values are indicated in bold. Note that the data used here are the same as used in the analyses shown in table 2 of McNickle (2020)

	Neighbour presence (N)			Nutrient concentration (C)			N × C		
	df	F	p	df	F	p	df	F	p
Vegetative mass	1, 100.05	0.07	0.791	1, 100.49	11.20	<b>0.001</b>	1, 100.43	1.73	0.192
Total mass	1, 100.05	1.42	0.235	1, 100.44	11.79	<b>&lt;0.001</b>	1, 100.39	0.44	0.510
Root mass	1, 100.11	0.36	0.551	1, 100.92	16.39	<b>&lt;0.001</b>	1, 100.81	1.04	0.311
Shoot mass	1, 100.03	0.72	0.400	1, 100.28	6.00	<b>0.016</b>	1, 100.24	2.06	0.155
Pod mass	1, 100.07	4.61	<b>0.034</b>	1, 100.59	2.99	0.087	1, 100.52	0.99	0.322
Root mass fraction	1, 100.08	4.44	<b>0.038</b>	1, 100.67	9.75	<b>0.002</b>	1, 100.59	0.99	0.323
Shoot mass fraction	1, 100.03	0.08	0.779	1, 100.29	0.70	0.406	1, 100.25	3.29	0.073
Pod mass fraction	1, 100.08	0.65	0.424	1, 100.67	5.20	<b>0.025</b>	1, 100.59	3.24	0.075

Note: *p* values are calculated from *F* statistics using a type III sum of squares, based on the Kenward-Rogers approximation for the degrees of freedom (*df*, presented as numerator *df*, denominator *df*).

dependent and independent variables. In this case, we ln-transformed both the dependent variable (plant trait *Y*) and the independent variable (nutrient concentration *C*). This implicitly assumed a power relationship between *Y* and *C*:

$$Y = b \cdot C^a, \text{ that is, } \ln(Y) = \ln(b) + a \cdot \ln(C), \quad (1)$$

in which both *a* and *b* are constants. In the analysis of McNickle (2020), only the dependent variable was ln-transformed, implicitly assuming an exponential relationship between *Y* and *C*:

$$Y = b' \cdot e^{a' \cdot C}, \text{ that is, } \ln(Y) = \ln(b') + a' \cdot C, \quad (2)$$

in which *a'* and *b'* are constants. These different assumptions can lead to a difference in the interpretation of the effect size of *C* on *Y*. This also implies that the variance partitioning of *Y* to the effect of neighbour will differ between the two analyses. In other words, the discrepancy in the detected neighbour effects between Chen et al. (2015a) and McNickle (2020) can be partially attributed to their different choices of data transformation in the analyses. A reasonable choice of data transformation should always be based on the real biological relationship between *Y* and *C*. Clearly, transformation also depends critically on whether differences in treatments are considered to be absolute or proportional. To reach consensus, the research community should give this aspect more attention.

## 2.2 | Impacts from difference choices for either soil volume or nutrient concentration as a controlling factor

Although the use of ln-transformed 'nutrient concentration' as variable to some extent can repeat the findings of Chen et al. (2015a), the detected neighbour effects on the majority of dependent variables remained insignificant (Table 1), which are still consistent with

McNickle (2020). To further explore the analytical causes that generate the discrepancy in neighbour effects between soil volume-based (*V*-based, hereafter) and nutrient concentration-based (*C*-based, hereafter) analyses, the mathematical formulae underlying the two analyses are compared in the following discussion. To facilitate the comparison, here we continue with the power relationship assumption that was used in Chen et al. (2015a).

We assume that in *C*-based analysis *Y* depends on both *C* and the presence of a neighbour (*N*); and for calculation convenience we further assume that the response of *Y* to *N* is independent of *C*:

$$\ln(Y) = \ln(b) + a \cdot \ln(C) + i \cdot N, \quad (3)$$

in which *a*, *b* and *i* are constants. Similarly, in *V*-based analysis, let *Y* and soil volume (*V*) also follow a power relationship, with a response of *Y* to *N* independent of *V*:

$$\ln(Y) = \ln(f) + d \cdot \ln(V) + j \cdot N, \quad (4)$$

in which *d*, *f* and *j* are constants. Furthermore, let the total amount of nutrients per plant be *m*. Then, we would expect an inversely proportional relationship between *V* and *C* as  $V \cdot C = m$  in the owner scenario (i.e. neighbour absence), while  $V \cdot C = 2m$  in the sharer scenario (i.e. neighbour presence).

Thus, for an owner plant with a soil volume size  $V_O$ , an observed trait  $Y_O$  in *V*-based analysis (i.e. Equation 4 with  $N = 0$  and  $V_O \cdot C_O = m$ ) would be:

$$\ln(Y_O) = \ln(f) + d \cdot \ln(V_O), \quad (5)$$

and in *C*-based analysis (i.e. Equation 3 with  $N = 0$  and  $V_O \cdot C_O = m$ ) would be:

$$\ln(Y_O) = \ln(b) + a \cdot \ln(C_O) = \ln(b) + a \cdot \ln\left(\frac{m}{V_O}\right) = \ln(b \cdot m^a) - a \cdot \ln(V_O). \quad (6)$$

From Equations 5 and 6 we know that  $f = b \cdot m^a$  and  $d = -a$ .

For a sharer plant growing in a soil volume  $V_S$  with the same size of  $V_O$  (i.e.  $V_S = V_O$ ), its observed trait  $Y_S$  in V-based analysis (i.e. Equation 4 with  $N = 1$ ,  $V_S = V_O$  and  $V_S \cdot C_S = 2m$ ) would be:

$$\ln(Y_S) = \ln(f) + d \cdot \ln(V_S) + j = \ln(f) + d \cdot \ln(V_O) + j, \quad (7)$$

while in a C-based analysis (i.e. Equation 3 with  $N = 1$ ,  $V_S = V_O$  and  $V_S \cdot C_S = 2m$ ) it would be:

$$\begin{aligned} \ln(Y_S) &= \ln(b) + a \cdot \ln(C_S) + i = \ln(b) + a \cdot \ln\left(\frac{2m}{V_S}\right) + i \\ &= \ln(b) + a \cdot \ln(m) + a \cdot \ln(2) - a \cdot \ln(V_S) + i \\ &= \ln(b \cdot m^a) - a \cdot \ln(V_S) + i + a \cdot \ln(2) \\ &= \ln(f) + d \cdot \ln(V_O) + i - d \cdot \ln(2). \end{aligned} \quad (8)$$

From Equations 7 and 8 we can find a mathematical cause for the discrepancy in the detected neighbour effect between C-based and V-based analyses. That is,

$$i = j + d \cdot \ln(2). \quad (9)$$

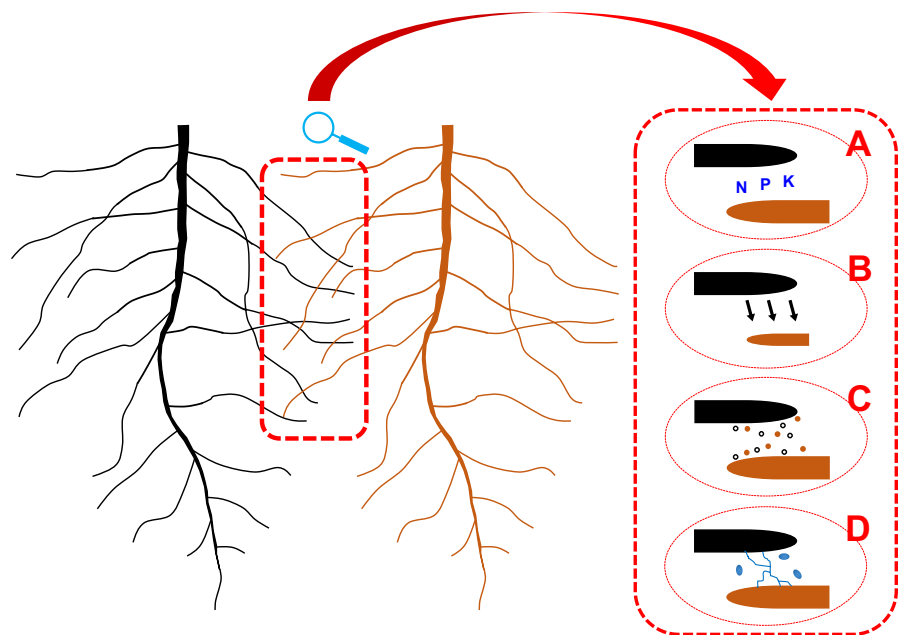
Equation 9 suggests that in a study of neighbour effects by comparing the performance of owner and sharer plants with  $m$  gram of total nutrient per plant and the same level of soil volume, the strength of the neighbour effect  $i$  in C-based analysis always deviates mathematically by  $d \cdot \ln(2)$  units from the strength  $j$  in V-based analysis. This formula reveals that the deviation originated from the different inversely proportional V-C relationships between owner ( $V_O \cdot C_O = m$ ) and sharer ( $V_S \cdot C_S = 2m$ ) scenarios, that is, a by-product of controlling for nutrient amount per plant constant in the methodology. The extent of the difference between  $i$  and  $j$  will depend on

the relative strength ( $d$ ) and direction of the volume effect as compared to those of the detected neighbour effect in V-based analysis. Especially when their directions are opposite, and their strengths are similar in V-based analysis, we would expect to have a high chance to find the effect of neighbour presence being weakened to an insignificant level in C-based analysis (see Table S2).

### 3 | THEORETICAL CAUSES FOR THE DIFFERENT CHOICES FOR CONTROLLING EITHER SOIL VOLUME OR NUTRIENT CONCENTRATION

In the preceding section, we have shown that the disagreement on the neighbour effect between McNickle (2020) and Chen et al. (2015a) are largely attributed to their different analytical approaches. We believe it is necessary to further the discussion started by McNickle (2020) about the origin of their different choices for either soil volume or nutrient concentration as a controlling factor in the analyses of neighbour effects. To that end, however, the so called 'neighbour effect' first needs to be clearly defined.

So far, numerous studies of plant-plant interactions at the root level have demonstrated at least four types of effects (Figure 1). The most well-known one is the nutrient depletion effect, which describes a phenomenon that nutrient uptake by the roots of neighbours will inevitably lead to a reduction of nutrient availability and thus growth for the focal plant (de Kroon et al., 2003). Interestingly, neighbours sometimes may increase nutrient availability of plants too. For example, compared to maize plants in pure stands, those intercropped with faba beans produce more roots and also direct more roots towards faba beans (Zhang et al., 2016). This is not a sign of enhanced competition but the consequence of increased mobilization of insoluble soil P by the root exudates from faba beans. Another



**FIGURE 1** Summary of neighbour effects in plant-plant root interactions. Studies so far have identified at least four types of component effects, including (A) nutrient depletion effect due to the nutrient uptake from neighbours' roots; (B) interference effect that can directly inhibit the growth of focal plants by means of, for example, allelopathy; (C) root exudates mediated identity recognition between interacting plants and (D) the involvements of soil microbes

well-studied influence from neighbours is the interference effect, of which allelopathy is probably the most prominent example. It depicts a situation that the (root) growth of plants can be directly inhibited by toxic allelochemicals secreted from the roots of both conspecific and heterospecific neighbours (Schenk, 2006). Recent studies of plant–plant interactions also revealed a so-called neighbour detection effect. It shows that plants are able to perceive the presence, and even discriminate the genetic relatedness of a neighbour based on the identity-related chemical signals secreted as root exudates from the neighbour (Chen, During, & Anten, 2012). Moreover, due to the fast development of molecular techniques in recently years, the diversity of soil microbes in the rhizosphere have been well revealed, and their functions on plant–plant root interactions are receiving increasing attention (Mommer, Kirkegaard, & van Ruijven, 2016). For instance, compared to solitary plants, those grown with intraspecific neighbours on the one hand may suffer more stress from higher accumulation of species-specific soil pathogens (Hendriks et al., 2015); on the other hand they may also receive photosynthates and soil resources transferred from neighbours via a common mycorrhizal network (Simard et al., 2015). Rather than mutually exclusive, these various effects elicited by the presence of a neighbour are more likely to function simultaneously. Of course, the relative importance of these effects may vary depending on the species and environmental conditions. Clearly, the neighbour effect is a composite variable, that is, a mixture of a variety of component effects.

With this clarification in mind, a revisit to the theoretical bases and hypotheses for neighbour effect in McNickle (2020) and Chen et al. (2015a) revealed that the two studies emphasized two different components of neighbour effects, though both used the same term ‘neighbour presence’ as a shortcut. As already explained in McNickle (2020), the neighbour effect therein is rooted in the classic root foraging theory, which predicts that plants should produce roots according to the nutrient status in soils, typically responding with more growth in nutrient rich patches while less in poor locations (Nabel et al., 2018); and the extent of total root production of a plant should be based on a balance between the marginal benefit of producing new roots for nutrient uptake and the marginal cost of that new production (McNickle, 2020). Then, when the assessment of root production balance of a plant is purely based on the decline of soil nutrient concentration caused by the consumption from both the plant and its neighbours, the presence of a neighbour would be analogous to a reduction of total nutrient availability for the plant. That means owner and sharer plants would have the same root production, given the same amount of nutrients per plant (see details in McNickle & Brown, 2012, 2014). However, when root production balance is not only determined by the decline of soil nutrient concentration but also a net return of resource investment in roots from that decline, game theoretical (GT) models predict that the presence of a neighbour would change the root production of the plant from a simple optimal strategy to an evolutionarily stable strategy. That is, given the same amount of nutrients, sharer plants will produce more roots than owner plants, which goes at the expense of, for example, seed production (see details in Gersani et al., 2001; McNickle & Brown, 2012).

Therefore, the ‘neighbour effect’ in McNickle (2020) is more related to the component nutrient depletion effect. Controlling for the initial level of soil nutrient concentration between owner and sharer scenarios is a prerequisite for the theoretical deductions.

Although the incentive for root over-proliferation in sharer plants in the aforementioned deductions can be purely attributed to an intra-plant assessment of cost and benefit balance in root investment, it has spurred a wave of interest in below-ground neighbour detection in plants. However, the central tenet of root-mediated neighbour detection is that plants possess an ability to directly perceive or detect the presence of a neighbour by sensing a (group of) special signal(s) which is (are) released by the neighbour and more importantly conveying the identity information (i.e. non-self) of the neighbour (Depuydt, 2014). It also implies the operation of a neighbour detection process is independent of nutrient depletion effects. However, the evolution of below-ground neighbour detection is believed to help plants to precisely compete for resources with neighbours rather than with themselves (Chen et al., 2012). Thus, for neighbour detection studies, the predictions from GT-based root foraging models can still provide a reasonable hypothesis to be tested. Regarding the fact that a larger soil volume for sharer than owner treatments in the experimental design of GT-based root competition studies (e.g. Gersani et al., 2001) has raised a methodological concern associated with an alternative volume effect hypothesis (see Hess & de Kroon, 2007; Semchenko, Hutchings, & John, 2007), the research focus on neighbour detection finally led Chen et al. (2015a) to control for soil volume at the expense of varying nutrient concentration in their experiment and statistical analyses.

## 4 | FURTHER THOUGHTS ON THE EXPERIMENTAL DESIGNS FOR TESTING THE NEIGHBOUR EFFECT

### 4.1 | Concerns about the proposed mesh-divided root interaction design and replacement series design

McNickle (2020) well reviewed five experimental designs (table 1 in McNickle, 2020) dedicated to controlling for various confounding factors in the studies of neighbour effects independently of nutrient availability. Among those, he recommended the use of a mesh-divided root interaction design (table 1E in McNickle, 2020) and a replacement series design to avoid all confounding effects ‘once and for all’. We value the advantages of these two recommended designs. However, we also see some potential drawbacks that may complicate the interpretation of neighbour effects from the two designs.

In the mesh-divided root interaction design, a basic setup is to construct both solitary and neighbour treatments with two plants in one pot, but with an impenetrable below-ground divider between plants in the solitary treatment and a mesh divider that allows liquids and mycorrhiza (Cui & Caldwell, 1996) but not roots to pass the divider between plants in the neighbour treatment (e.g. Semchenko et al., 2007; Zhu, Weiner, & Li, 2019). It represents

a laudable attempt that aims to ultimately control for nutrient amount per plant, nutrient concentration and also soil volume between neighbour and solitary treatments. However, it relies on the assumption that even without direct root contact, two interacting plants are still able to compete for nutrients via the diffusion of nutrients passing through the mesh driven by nutrient depletion, and/or to detect each other's root exudates diffusing from the other side of the mesh. These assumptions entail several drawbacks. First, the results can be undermined by a potting substrate which does not allow high nutrient mobility (McNickle, 2020). Second, due to the intrinsic differences in the mobility among nutrients (e.g. high in nitrate, medium in potassium and low in phosphate), the competition between mesh-divided plants will be limited to mobile nutrients, as compared to a competition scenario for all types of nutrients between root-intermingled plants. Such a difference can substantially diverge the nutrient foraging and root competition strategies of plants (Postma, Dathe, & Lynch, 2014). Third, the competition arena between mesh-divided plants is more likely to be limited to a narrow zone near the mesh, as compared to the whole pot space for root-intermingled plants, unless plants have developed sufficient mycorrhiza that penetrate the mesh (Cui & Caldwell, 1996). The whole pot arena provides a spatially homogeneous condition, while the narrow zone arena imposes a spatially heterogeneous condition that can influence the root deployments of plants (Gersani, Abramsky, & Falik, 1998; Zhang et al., 2020). Besides, if the self-inhibition for root growth which requires an accumulation of self-inhibitory signals secreted from a root in vicinity of an obstacle (Falik, Reides, Gersani, & Novoplansky, 2005) indeed commonly exists in plants, the process would be interrupted for roots in vicinity of a mesh which is an obstacle but still allow diffusion rather than accumulation of chemicals. Then, the use of a mesh divider may potentially stimulate some extra root production even with no competitor on the other side of the mesh. Therefore, we conclude that mesh divider experiments can create a number of additional confounding effects that hinder an unambiguous interpretation of the results.

Since the first introduction by de Wit (1960), the replacement series design has been widely used in plant-plant interspecific competition studies, and nowadays also becomes a key method in below-ground kin recognition studies (e.g. Yang, Li, Xu, & Kong, 2018). Indeed, we agree with McNickle (2020) that with a fixed plant density, this design also successfully bypasses both confounding nutrient concentration and soil volume effects. However, it unfortunately causes another point of consideration. That is, with a fixed plant density, an increased number of plants of species A will inevitably lead to a decreased number in species B. This new confounding effect will hinder our understanding of the responses of plants to the change of species composition. Moreover, the intrinsic difference between species (sometimes also cultivars and genotypes) may introduce a size bias that can undermine the assumption of a fixed plant density (Gibson, Connolly, Hartnett, & Weidenhamer, 1999), such as an extreme example of interaction between a tree and a herb species. The intrinsic

difference may also be embedded within a competition hierarchy, so that nutrient uptake of a species will vary rather than being fixed among species composition treatments. Moreover, interspecific differences can lead to over-yielding through niche complementarity (Oram et al., 2018); but changes in yield may be hard to interpret mechanistically, especially in short term experiments (Vermeulen, van Ruijven, Anten, & van der Werf, 2017). This will further complicate the situation. Thus, we are still on the way of seeking for better (and meanwhile convenient) practical solutions.

## 4.2 | Different components of the neighbour effect may require different designs

As clarified in Section 3, the presence of a neighbour may simultaneously generate a variety of component effects associated with different mechanisms and theories. This suggests that there is probably no universal design for the study of 'neighbour effect' in below-ground plant-plant interactions. Instead, we should search for different designs based on the key requirements for the functioning of different component effects. For example, controlling for nutrient concentration (McNickle & Brown, 2014), exposure to non-self-identity cues (Chen et al., 2012), exposure to allelochemicals (Schenk, 2006), and infection/colonization by soil microbes (Mommer et al., 2016) respectively are the prerequisites for testing the effects of nutrient depletion, neighbour detection, interference (allelopathy in particular) and microbial mediation from the presence of neighbours. Meanwhile, we should also make sure that a proper design for a specific component effect should also help to eliminate or at least minimize the impacts from other confounding (or unwanted) effects. Since the methodologies for studying the effects of allelopathy (Inderjit & Callaway, 2003) and mediations from soil microbes (Hoeksema, 2015; Ke & Wan, 2020) have been well established, here we only focus on the other two, which are also the main interests in the current study.

For the research of neighbour-induced nutrient depletion effects, we recommend using non-allelopathic plants from a single clone or genotype to eliminate the effects from allelopathy and neighbour detection (if the identity cues are genetically based, but see Chen et al., 2012). A well-controlled growth condition (e.g. growth chamber) with carefully sterilized potting substrates can help to avoid pathogen infections or mycorrhizal colonizations. Then, the major concern left is to avoid the confounding soil volume effect. For instance, one may consider to observe root interactions in bigger containers at an early stage before the occurrence of root restrictions (Padilla et al., 2013); or continue to adopt the classic 'split-root sharer versus owner' design (see Gersani et al., 2001) but with an additional check to make sure that the size of pots used does not lead to significant effects on plant growth from different soil volumes between solitary and neighbour treatments (see McNickle & Brown, 2014).

When focusing on the effect of below-ground neighbour detection, one could observe root interactions in bigger containers before apparent declines of nutrient concentration to avoid confounding nutrient depletion effect (Padilla et al., 2013). Another



promising avenue is to expose focal plants to the root exudates collected from neighbours (or themselves) rather than expose focal plants to neighbours per se (e.g. Biedrzycki, Jilany, Dudley, & Bais, 2010; Kong et al., 2018; Semchenko, Saar, & Lepik, 2014), since mounting evidence suggests chemicals in the exudates convey the identity information (Kong et al., 2018). To eliminate possible confounding impacts from soil microbes, these collected liquids can be further filter-sterilized (Semchenko et al., 2014). The collection of root exudates, in the forms of solutions (Biedrzycki et al., 2010) or leachates (Semchenko et al., 2014), from themselves and neighbours may differ from each other not only in the identity cues but also in nutrient composition. Thus, the nutrient contents, nitrogen and phosphorus in particular (Palmer et al., 2016), in the collections should be adjusted to a similar level before exposing focal plants to these collections.

## 5 | CONCLUSIONS

We cherish this valuable forum for helping us to clarify the mechanisms and theoretical bases of different components of neighbour effect. We conclude that in studies of neighbour effects, it is crucial to consider and specify which component effect(s) of neighbour presence one wants to explore as this determines the adequate choice of experimental design and analytical methodology. We show that the discrepancy in the detected neighbour effects between McNickle (2020) and Chen et al. (2015a) is rooted in their different analytical methodologies specifically: different choices of data transformation for the controlling factor, and a mathematical deviation between C-based and V-based analyses originated from the different inversely proportional relationships of soil volume and nutrient concentration between solitary and neighbour treatments. The different choices for either soil volume or nutrient concentration as a controlling factor between the two studies are based on two different perspectives, with a main focus either on neighbour-induced nutrient depletion in McNickle (2020) or on identity recognition in Chen et al. (2015a). We encourage further developments of evolutionarily game theoretical models of plant-plant root interactions, for example, by including root morphological and physiological parameters as well as integrating them with functional-structural plant models (e.g. Evers, Letort, Renton, & Kang, 2018), to enhance the predictive value of models as well as our understanding of neighbour-induced nutrient depletion effects.

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## AUTHORS' CONTRIBUTIONS

All authors contributed critically to the drafts and gave final approval for publication.

## DATA AVAILABILITY STATEMENT

Data are deposited in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.dr491> (Chen et al., 2015b).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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