An ideal free distribution explains the root production of plants that do not engage in a tragedy of the commons game.

Gordon G. McNickle[1,2,*] and Joel S. Brown[2]

[1] University of Illinois at Chicago, Department of Biological Sciences, 845 W. Taylor St. (MC066), Chicago, IL, 60607, USA.

[2] Current address: Wilfrid Laurier University, Department of Biology, 75 University Avenue West, Waterloo, ON N2L 3C5, Canada.

*Correspondence author. E-mail: gmcnickle@wlu.ca

Running headline: Plant root growth and the ideal free distribution

Summary

1. Below-ground competition is intense, and may dramatically reduce plant performance. However, there is still no consensus on the best strategies plants should use to maximize competitive ability in soil. Some suggest plants should grow roots according to nutrient availability, while others suggest plants should grow roots taking into account both nutrients and neighbours. Unambiguously testing between these two alternative hypotheses has been challenging.
2. This manuscript had three objectives. First we presented a model of root growth under competition that is based on an ideal free distribution (IFD). Second, we develop the concept of the best response curve as a tool for clearer experimental tests of plant responses to neighbours. Third, we test these ideas by growing fast cycling *Brassica rapa* either alone or with neighbours to examine this species' root growth strategy both alone and under competition.
3. We hypothesize that those plants with no direct response to neighbours should grow roots according to an IFD. This means that if plants produce x roots in a soil volume of quality R, they should produce x/n roots in a soil volume of quality R/n. The experimental data were consistent with this prediction. Growing plants with neighbours was statistically identical to growing them with half as many nutrients. Thus, the only effect of neighbours was to reduce nutrient availability.
4. This model provides an alternative to previous game theoretic models, and suggests an experimental protocol based on the concept of the best response curve. We hope that testing the game theoretic model with a clear alternative model will guide experiment and debate.

5. *Synthesis:* The data in the literature are mixed, with species sometimes responding to nutrients only, and sometimes responding interactively to both nutrients and neighbours. At present we lack a general understanding of the causes or consequences of this diversity of strategies. We suggest that a greater understanding of trade-offs among traits that are important for other biotic interactions (above-ground competition, enemy defence, mutualisms) will lead to a greater understanding of why some species over-proliferate roots when in competition but other species do not.

Key-words: competition, evolutionary game theory, evolutionary stable strategies, ideal free distribution, over-proliferation, plant–plant interactions, self/non-self recognition, tragedy of the commons.

Introduction: Below-ground competition among plants can be both important and intense (Casper and Jackson, 1997, Schenk, 2006, Lamb and Cahill, 2008). In many terrestrial systems, plants are strongly limited by soil nitrogen availability (Vitousek and Howarth, 1991, Robinson, 1994) and competition below-ground can reduce plant growth and performance by several orders of magnitude (Wilson, 1988, Lamb and Cahill, 2008). As a result competitive interactions among plants in soil can have important consequences for the ecology (Casper and Jackson, 1997, Cahill and McNickle, 2011) and evolution (Gersani *et al.*, 2001, Thorpe *et al.*, 2011) of plants. Thus, it is logical to ask: what rooting strategies lead to the greatest competitive ability and highest returns on fitness?

The majority of plant ecologists have long worked under the assumption that plants have no direct response to below-ground competitors that is independent of their growth response to nutrients (Casper and Jackson, 1997, Gersani *et al*., 1998, Schenk, 2006, Hess and de Kroon, 2007, de Kroon *et al.*, 2012). That is, if competition from neighbours results in reduced access to nutrients, and since less access to nutrients depresses plant growth, then plants have been expected to simply be smaller in every way in the presence of competition from neighbours. It is rarely named as such, but this hypothesis is mathematically equivalent to the expectation that root growth follows an ideal free distribution (IFD) (Fretwell and Lucas, 1969a, Gersani *et al.*, 1998, McNickle and Brown, 2012). The IFD is so named because it is assumed that all individuals know the distribution and availability of nutrients, and that they are free to grow roots wherever and however they like (i.e. there is no interference competition). Under an IFD model of plant root growth under competition, plants would grow roots in proportion to nutrient availability at each soil location. Under this hypothesis, competition from below-ground neighbours (intra- or interspecific) may decrease nutrient availability and as a result neighbours might indirectly reduce plant root growth, but plants do not have any direct response to neighbours that is independent of their response to nutrients (Hess and de Kroon, 2007, de Kroon *et al.*, 2009). An important prediction of an IFD model for root production is that competition from neighbours could never increase root production in the presence of neighbour relative to when grown alone, they can only decrease root production by depressing the nutrients available in soil. There is evidence that some plants follow an IFD for root growth in at least some contexts. For example, Gersani *et al.* (1998) grew *Pisum sativum* plants in a split root design, where the roots of a focal plant had access to one pot that was free from competition, and a second pot that had 1, 2, 3, 4 or 5 intra-specific competitors. In this experiment, consistent with an IFD, *P. sativum* increasingly grew roots into the competition free pot as the number of competitors in the second pot increased. Indeed, similar findings have been reported for a diversity of plant species (Gersani *et al.*, 1998, Schenk *et al.*, 1999, Hess and de Kroon, 2007, Semchenko *et al.*, 2007, Cahill and McNickle, 2011). However, although the majority of the data seems to support this type of response, not all plants appear to follow an IFD when growing roots under competition.

Increasingly, studies have revealed evidence of interactive effects between nutrients and neighbours where some plants increase their root production in the presence of competition from neighbours (Dudley and File, 2007, Murphy and Dudley, 2007, Cahill *et al.*, 2010, Mommer *et al.*, 2010, Semchenko *et al.*, 2010, Padilla *et al.*, 2013). Such increases in root production are independent of the effect of neighbours on nutrient levels, which is impossible under IFD for root growth which assumes that the resource environment is the only variable that influences root production. Instead, over-production of roots in the presence of neighbours is better predicted by game theoretic models of over-proliferation root growth that allows plants to respond to the way that the competitive environment changes the return on investment into roots in terms of nutrient uptake (Gersani *et al.*, 2001, O'Brien *et al.*, 2007, O'Brien and Brown, 2008, McNickle and Brown, 2012). Specifically, for those plants that respond to neighbours independently of nutrients, the presence of neighbours increases the value of each unit of root growth by simultaneously enhancing nutrient uptake and competitive ability. For example, Cahill *et al.* (2010) grew plants either alone or with intraspecific neighbours in homogeneous or heterogeneous soil. Plants dramatically reduced their exploration of soil leading to segregation of neighbours in the presence of neighbours in poor quality patch-free soil, but dramatically increased root growth leading to overlap of root systems in the presence of neighbours in soils containing a high quality nutrient patch. Similarly, Padilla *et al.* (2013) and Mommer *et al*. (2010) grew plants either in mixtures of species or in monocultures. Both studies found that some species dramatically increased their root production in mixtures relative to monocultures, while some species dramatically reduced root production. Again, increases in root production are impossible under an IFD model because neighbours depress the nutrient availability which should lead to decreased and not increased root production under an IFD.

The experiments described in the preceding paragraph generated unambiguous evidence for direct responses by plants to neighbours, but many more studies that have sought to test for such direct plant-plant responses have been plagued by methodological problems (e.g. Gersani *et al.*, 2001, O'Brien *et al.*, 2005). It has been remarkably difficult to unambiguously test whether plants have direct responses to neighbours that are independent of their response to nutrients (Laird and Aarssen, 2005, Hess and de Kroon, 2007, Semchenko *et al.*, 2007, O'Brien and Brown, 2008). We suggest that part of this problem is that the majority of predictive mathematical models produced to date are of the game theoretic form (Gersani *et al.*, 2001, Craine *et al.*, 2005, Craine, 2006, O'Brien *et al.*, 2007, O'Brien and Brown, 2008, Dybzinski *et al.*, 2011, McNickle and Brown, 2012, Farrior *et al.*, 2013), while proponents of the IFD model of plant growth have largely produced only verbal (e.g. Hess and de Kroon, 2007, de Kroon *et al.*, 2012) or graphical models (Gersani *et al.*, 1998). This has meant that there is a lack of mathematical models available to directly compare to game theoretic models, and to develop quantitative predictions for experimental tests of plant responses to neighbours and nutrients. Ideally, we would have models derived from similar equations and assumptions that could be examined as a game, or as an IFD.

In this paper, we have three objectives. First, we propose a non-game theoretic model for plants that do not respond to neighbours, but instead grow roots according to an IFD. This model is derived from our previous game theoretic models (O'Brien *et al.*, 2007, McNickle and Brown, 2012), but is not game theoretic. Second, we propose an experimental protocol that we think is capable of unambiguously testing for the presence of independent responses of plants to nutrients and neighbours. This involves growing pairs of plants either alone or with neighbours, and two tests, one for the potential confounding effects of nutrient availability and pot volume and another to test for neighbour effects. Third, to test the IFD model of root production under competition we performed an experiment using fast cycling *Brassica rapa* (L., var Wisconsin Fast Plants®, Carolina Biological Supply Company, Burlington, NC, USA) that asked; How does *B. rapa* grow roots when grown alone or when grown with neighbours and how do these root allocation strategies influence reproductive output? Based on our experience with this plant (GGM Personal Observation), we did not expect this plant to be capable of engaging in a tragedy of the commons game. We think that this combination of modeling and experimental design can move the study of plant responses to neighbours in soil forward by permitting stronger experimental tests and quantitative predictions.

Materials and methods: *An IFD model of root growth under competition:*

There are many models of optimal root production, dating back to the 1960s (e.g. Gleeson and Fry, 1997, McNickle and Cahill, 2009). However, these generally have not included competition as a factor that can influence optimal root growth. This has limited the ability to use previous models as alternatives to recent game theoretic models of root production. Here we develop a model of root growth for plants that respond only to nutrients, but still face competition. These plants can still experience competitive effects due to depletion of the resource environment by neighbours, but in this model plants do not take into account the competitive strategies of their neighbours when allocating to root biomass. Instead, the plants in this model employ a resource matching strategy and do not play a game (Hereafter we refer to such plants as game-off). However, neighbours still depress the nutrient environment and these "game-off" plants simply perceive the environment as becoming worse, and produce fewer roots in the presence of a neighbour compared to alone. This model is derived from, and directly comparable to our previously published game theoretic models (Gersani *et al.*, 2001, O'Brien *et al.*, 2007, O'Brien and Brown, 2008, McNickle and Brown, 2012), but the model presented here is not game theoretic and instead allows plants to grow roots according to an IFD. The advantage of this approach is that it lets us directly compare the best root strategy for game-on and game-off plants under competition without the confounding problem of using two drastically different models. Our hope is that this can further develop future understanding of plant strategies for below-ground competition.

Let R be the initial resource abundance in the soil, let u_f be the root production of the focal plant, u_n be the root production of the neighbour plant and let r be the sum total amount of roots of all competing plants ($r = u_f + u_n$). Further, let, c be the per-unit cost of root production for all plants. We assume that there is no shoot competition and that plants are not light limited. In the simplest possible terms, the net harvest (π_f) of an annual plant at senescence is given by benefits minus costs. Competition adds the complication that available benefits might be influenced by the activities of competitors. Thus, net harvest under competition can be represented from the combination of three functions: (i) a function describing the focal plant's share of the nutrient harvest based on their root production (u_f), relative to the root production of all competitors (r), given by $f(u_f, r)$; (ii) a function describing the nutrients harvested, by roots given by $H(r)$ and, (iii) a function describing the costs of root production to the focal plant, given by $c(u_f)$ (Gersani *et al.*, 2001). Thus, a basic benefits minus costs equation for the focal plant under competition is given by,

$$\pi_f = f(u_f,\mathbf{u})\ H(r)\ -c(u_f) \qquad \text{(Eqn 1a)}$$

Here we select simple analytically tractable functional forms for the three functions in equation (1a). Specifically, we assume that nutrients are divided up proportionally to relative root production (i.e. $f(u_f,\mathbf{u})= u_f/r$), that nutrients are depleted over time according to a negative exponential trajectory leading to diminishing returns to root production (i.e. $H(r)= R\text{-}Re^{-r}$, O'Brien *et al.*, 2007, McNickle and Brown, 2012), and that costs scale linearly with root production (i.e. $c(u_f)=c_f u_f$). Other equations could be used, and as long as all three equations are monotonically increasing with u_f, and as long as $H(_r)$ has diminishing returns to root production, the qualitative predictions of equation 1b will not change (Gersani *et al.*, 2001, McNickle and Brown, 2012). Under the assumptions described above, we can expand equation 1a;

$$\pi_f = (u_f/r)R(1\text{-}e^{-r}) - c\, u_f \qquad \text{(Eqn 1b)}$$

We write this for the focal plant only, but an identical equation exists for the neighbour plant with the subscripts reversed. This formulation recognizes that competing plants will only acquire a fraction of the available nutrients because neighbours also deplete the environment with their root production. Plants that play a plastic tragedy of the commons game (hereafter we refer to these as game-on plants) will choose a root production strategy, u_f*, that satisfies

$$\partial\pi_f/\partial u_f = (1/r - u_f/r^2)[R(1\text{-}e^{-r})] + (u_f/r)[R(e^{-r})] - c_f = 0. \qquad \text{(1c)}$$

We present the game theoretic version of this model here purely as a point of comparison with the non-game theoretic version (derived below). However, we do not analyze it in detail; it is described and analyzed in detail in McNickle and Brown (2012). For plants that do not play a plastic tragedy of the commons game we need an alternative optimization criterion which is not game theoretic (Anten and During, 2011). We hypothesise that these "game-off" plants should be blind to the strategy of neighbours (or at least ignore it) and as a result these plants should not base their root production strategy on their expected share of the nutrients. Such plants should assess the initial environment, but ignore the net return on their investment in roots, and as a result ignore the effects of competition that cause depletion in the environment. As a result, we propose that such game off plants should ignore the term u_f/r in equation 1a, and focus only on the resource environment, any depletion trajectories (caused by self or non-self) and their own costs associated with root production. Thus, we define an expected payoff for these game-off plants, P_f, based on eqn 1a that omits this term,

$$P_f = R(1\text{-}e^{-r}) - c_f u_f \qquad \text{(Eqn 2a)}$$

This is not the plant's actual payoff, but it is the payoff that a plant that ignores the net return on their investment into root production will expect since it does not revise its strategy based on the returns they acquire from root production. Note that these "game-off" plants will still experience reduced performance due to depletion that is caused by neighbours, however they are incapable of distinguishing between resource depletion caused by their own roots or the roots of neighbours. Thus, the optimal root production strategy, u_f^*, of a plant that cannot detect neighbours should satisfy,

$$\partial P_f / \partial u_f = Re^{-r} - c_f = 0 \qquad \text{(Eqn 2b)}$$

Solving equation (2b) for u_f* gives the optimal root production strategy of plant *f*, and a version of equation (2b) must be simultaneously solved for plant *n*. However, though these game-off plants expect the payoff given in equation (2a), they still must compete with neighbours and their actual payoff is still given by equation (1a) and not by equation (2a).

Using the model: Best response curves

McNickle and Brown (2012) introduced the concept of a best response curve that plots one plant's root production strategy, against the root production strategy of its neighbour and we use this as one diagnostic criterion to test whether plants are playing a game. From our model (Equation 1, 2) this would plot the points u_f* vs $\boldsymbol{u_n}$ for the focal plant's best response to any possible strategy of the neighbour, and $\boldsymbol{u_f}$ vs u_n* where $\mathbf{u_n}$ and $\mathbf{u_f}$ are continuous vectors of all possible root production strategies either plant might choose. This set of best responses can be thought of as a competitive "play book" for each plant: if the neighbour produces *y* roots, the best response of the focal plant is to produce *x* roots and *vice versa*. Comparing the game theoretic, and non-game theoretic version of this nutrient competition model, the best response curves from each model are quite different and this can be a useful diagnostic tool for experimentally comparing game on or game off plants. It requires growing plants in pairs (paired pots for the alone treatment, and the two competitors are pairs in the competition treatment), and it requires manipulation of the available nutrients.

Experiment: What is the proper control?

To test the model we grew *B. rapa* plants either alone or with neighbours. Plants were grown in three treatments based on the goal of parameterizing a best response curve (Figure 2), and based on criticisms of previous experiments that did not test for volume effects (Laird and Aarssen, 2005, Schenk, 2006, Hess and de Kroon, 2007, Semchenko *et al.*, 2007). Early experiments testing for responses to neighbours, and attempted to estimate root growth based upon resource availability. To achieve this, these experiments attempted to control resource supply per-plant (R) but they did so by manipulating pot volume (V). For example, plants with neighbours were grown in pots of volume V containing R resources, and plants were grown alone in pots of volume $V/2$ containing $R/2$ resources (e.g. Gersani *et al.*, 2001, O'Brien *et al.*, 2005). This design was based on the hypothesis that plants which do not respond to neighbours should respond only to nutrient availability (e.g. equation 1b vs 2b) and thus it was critical to control nutrient availability per-plant to test whether plants respond only to nutrient availability or to both nutrient availability and neighbours (Gersani *et al.*, 2001, McNickle and Brown, 2012). However, though this design controls resource availability at the per-plant level, it confounds neighbour addition with pot volume manipulation.

In pot experiments, volume can affect plant growth if plants become pot-bound during the course of the experiment. Thus, many authors have been critical of this method for controlling nutrient availability and argue that it is more important to control pot volume than to control nutrient availability (Schenk, 2006, Hess and de Kroon, 2007). However, controlling only pot volume is also potentially problematic because it produces a design where plants grown alone have access to pots of volume *V* and *R* nutrient resources, while plants grown with neighbours have access to pots of volume *V* but only *R*/2 resources. In this more common design nutrient availability is confounded with neighbour addition even though pot volume is controlled. Indeed, plants grown alone are almost always significantly larger than plants grown with neighbours because they have been given access to twice as many nutrients making it difficult to compare plants grown alone to plants grown with neighbours. The reality is that either the volume or the nutrient experimental control in isolation is problematic. The traditional method of holding volume constant confounds neighbour addition with nutrient availability, while the method of Gersani *et al*. (2001) confounds neighbour addition with pot volume.

In reality, we suggest that the best approach is to employ both controls and here, we used both a volume and a nutrient availability control. Plants were grown with neighbours in pots of volume *V* (*V*=1L, 10x10x10cm), and plants grown alone were either grown in pots of volume *V* or *V*/2 (Figure 1). This let us parameterize a best response curve using three treatments: (i) plants were grown alone in pots of volume V (*R* soil nutrients per plant). This estimates the growth of plants alone (white circle, Figure 2); (ii) two plants were grown with neighbours in pots of volume *V* (i.e. *R*/2 resources per plant). This estimates the root growth with neighbours (black circle, Figure 2) and; (iii) plants grown alone in pots of volume V (*R*/2 soil nutrients per plant). This estimates the how the plants should grow if they have no direct responses to neighbours, and only respond to nutrient levels (‘X’, Figure 2).

There are two tests embedded in this experimental design. To test for confounding effects of pot volume, plants grown alone in pots of volume V/2 should produce half as many roots, and reproductive output as plants grown alone in pots of volume V. If this hypothesis is rejected, it suggests that volume is producing a confounding effect which is independent from resource availability (Schenk, 2006, Hess and de Kroon, 2007), and further tests must stop. However, if the hypothesis is accepted then we may proceed with the second test, which compares plants grown with neighbours in pots of volume V, to plants grown alone in pots of volume V/2. If root production and reproductive output in the presence of neighbours is statistically indistinguishable from plants grown in pots of volume V/2 then we accept the IFD model and reject the tragedy of the commons game. If root production is significantly higher than plants grown alone, and reproductive output is significantly lower, then we must reject the IFD model and accept the tragedy of the commons model of over-proliferation of roots. This design permits a test for volume affects that was absent from previous studies, while also permitting a simple control for nutrient availability.

Growing conditions

Soil was a 1:3 mixture of potting soil (Miracle-Gro® Moisture Control® Potting Mix, The Scotts Company LLC, Marysville, OH, USA) to washed sand (Quickrete, Atlanta, GA, USA) which were each pre-sieved through a 2mm screen to facilitate root extraction at harvest. Soils were amended with 19-6-12 (N-P-K) slow release fertilizer pellets on the surface of the soil (Osmocote, The Scotts Company LLC, Marysville, OH, USA). Fertilizer was supplied based on an equal supply per- plant (and per volume of soil), such that full sized pots (alone full pot, and with neighbours treatments) each were given 1.2 grams of fertilizer while half sized pots were given 0.6 grams. Plants were grown in a controlled growth chamber with continuous fluorescent lighting. Plants were spaced at least 15 cm apart on the bench, and opaque screens were erected between competing plant so they did not interact above-ground in any treatments. This forced all competitive interactions to occur below-ground, in order to satisfy the assumption of the model that there was no shoot competition. Additionally, plants in each treatment were grown in pairs (with neighbours or alone) where each plant was *a priori* designated as either focal or neighbour which produced pairs of plants that experienced the same conditions on the bench. Thus, each replicate (n=25) of each treatment contained two plants (focal and neighbour, 50 plants per treatment), which were paired to be plotted on the best response curve. Plants were watered daily using an automatic drip irrigation system that supplied ~126 mL of water per day to pots of volume *V*, and ~63mL of water per day to pots of volume *V*/2. Because we were trying to generate best response curves of each plants (see below), each alone treatment included 25 pairs of plants, and the 'with neighbours' treatment included 50 pairs of plants (total of 100 focal plants, and 100 neighbour plants).

On day zero, multiple seeds were sown per planting location, and these were thinned to one plant per location within one day of germination. Plants were grown for 40 days under continuous lighting conditions which is recommended for fast cycling *B. rapa* (Carolina Biological Supply Company, Burlington, NC, USA). Location on the bench of each pair of plants was re-randomized every five days, but paired plants always remained together. A randomized block design is a preferable approach for statistically controlling for bench effects compared to re-randomization, however this was not feasible in this experiment. These data represent the first generation of a multi-generation selection experiment, we could not allow block effects to contribute selection pressure to the experiment and it was absolutely necessary to re-randomize to average out selective pressures of any bench effects on plant growth. Given the relatively small error associated with our estimated means, our relatively large sample size (n=25), and that the observed patterns were consistent with our hypotheses, we do not think that this experimental approach had a large effect on the outcome of the experiment, however the limitations of a randomized design should be recognized. *B. rapa* is self-incompatible, and fruit takes approximately 20 days to mature. All flowers produced up to day 20 were hand pollinated using cotton swabs. After 20 days pollination was discontinued and fruit were permitted to mature for 20 more days. Mates were haphazardly chosen each day, so each plant had a new mating partner on each day of pollination. After 40 days of growth plants had begun senescence and fruits, leaves and stems were harvested. Roots were washed on a 2mm sieve, and the root systems of neighbouring plants were carefully separated by floating them in water and gently shaking them to separate. We had no difficulty carefully separating the roots of each plant grown in competition by marking the stem of each plant, and gently shaking the root system in water until they separated. Subsequently, biomass was dried at 60°C to constant mass, and weighed. Fruits were dried at room temperature, counted and weighed. Fruits were then opened and seeds were counted and weighed.

Seed yield and root production were analyzed with separate one-way ANOVAs with competition treatments (Alone, Alone-half, with-neighbours) as factors. A Tukey's test was used for post-hoc comparisons among treatments. All statistical models were run in the R statistical environment (R-Development-Core-Team, 2009).

Results: *Model results, and best response curves*

The model shows how a plant that produces roots according to an ideal free distribution should grow (Fretwell and Lucas, 1969b, Gersani *et al.*, 1998). This means that if a soil volume of quality R contains x roots, then a soil volume of quality R/n will contain x/n roots. Similarly, if a plant grown alone in a soil volume of quality R produces x roots, then a plant grown with N

neighbours in a soil volume of quality *R* should produce *x*/*N* roots. The best response curve of intraspecific competition among game-off annual plants is shown in Fig 2b. It is a straight line connecting the root production strategy of each plant when grown alone. Applied to our experimental treatments, the model predicts that each plant should produce half as many roots when grown with neighbours in pots of volume *V/2* as when grown alone in pots of volume *V* when there are no independent effects of volume. Additionally, plants grown with neighbours in pots of volume *V* should each produce a statistically indistinguishable amount of roots as plants grown alone in pots of volume *V*/2.

As a point of comparison, game-on plants theoretically choose a root production strategy based on both the resource environment, and the strategy used by neighbours (equation 1b). This causes game-on plants to over-proliferate roots in an attempt to pre-empt the resource supply of neighbours, and several versions of these game theoretic models have been described and analyzed in detail elsewhere (Gersani *et al.*, 2001, Craine *et al.*, 2005, O'Brien *et al.*, 2007, O'Brien and Brown, 2008, Dybzinski *et al.*, 2011) including an analysis and discussion of best response curves (McNickle and Brown, 2012). An example best response curve of intraspecific competition among game-on annual plants is shown in Fig 1c (McNickle and Brown, 2012). Here, curved lines bow-out from the origin because of the strategy of over-proliferation causes plants to produce more roots in the presence of neighbours compared to when they grow alone (McNickle and Brown, 2012). In the context of our experiment, a game theoretic model would predict that, plants grown with one neighbour in pots of volume *V* should produce significantly more than half the roots of a plant grown alone in a pot of volume *V*, and significantly more roots than plants grown alone in pots of volume *V*/2. However, the control of plants grown alone in pots of volume *V*, is absolutely critical to estimate the intercepts of the best response curve to test between the game-on and game-off model.

Experimental results

In this study, *B. rapa* root growth was game-off and followed an IFD (Figure 3). A one way ANOVA revealed that plants grown with neighbours had a statistically indistinguishable seed yield and root biomass as plants grown in half volume pots, and half of the seed yield ($F_{2,147}$=29.69, P<0.0001) and root biomass ($F_{2,147}$=49.01, P<0.0001) as plants grown in full volume pots (Figure 3a, b). Volume did not produce a confounding effect in this study, as root production and seed yield in pots of volume V/2 was statistically indistinguishable from half of root production or seed yield in pots of volume V (Figure 3). Patterns of leaf, stem and fruit production followed the same pattern as Figure 3a and Figure 3b, and are not shown. When plotted as best responses, the observed root production strategies of *B. rapa* fall on the ideal free distribution line which reject the game theoretic model and support the simpler game-off model for this species (Figure 3c,d).

Discussion Tests for direct responses of plants to neighbours that are independent from their responses to nutrients have been limited by a number of problems. Part of the problem has been that many models of optimal root production which are non-game theoretic, do not explicitly include depletion by competing neighbours and so may not be appropriate for generating predictions about game-off competition. Here we derived a game-off model from previous game-theoretic models (O'Brien *et al.*, 2007, McNickle and Brown, 2012) to permit the generation of game-off predictions for plants who do not play a tragedy of the commons game, but still face competition. The model predicts root growth should follow an IFD, and shows how the concept of best response curves can be used to potentially develop a more rigorous experimental test of plant responses to neighbours (Figure 2). Specifically, experimental tests of plant responses to neighbours have sometimes confounded pot volume and neighbour addition (Schenk, 2006, Hess and de Kroon, 2007, Semchenko *et al.*, 2007), and sometimes confounded resource availability and neighbour addition (E.g. Cahill *et al.*, 2010). Adjusting soil volume is the simplest way to manipulate nutrient availability, but volume can have effects of its own sometimes and this must be experimentally tested. We presented an experimental protocol based on the concept of a best response curve that requires: (i) a test to ensure there are no confounding effects of pot volume. Only if there are no detectable confounding effects of manipulating pot volume may we then (ii) test whether plants grown with neighbours over-proliferated roots relative to plants grown alone. This requires choosing pot volumes which are sufficient to minimize the negative effects of plants becoming pot-bound. We tested this approach using *B. rapa*, which we did not expect to play a tragedy of the commons game. Indeed, the test found no significant influence of pot volume independent from the effect of nutrients (Figure 2a,b), and that *B. rapa* grows roots according to the ideal free distribution (Figure 2c,d). This result is quite different from the volume based analysis of Hess and de Kroon (2007), which would have predicted the plants with neighbours in pots of volume V, produced the same amount of roots as the plants grown alone in volume V. We suggest that testing this volume hypothesis independent of the neighbours hypothesis (Figure 1) will be critical to advancing our understanding of the effects of pot volume, nutrients and neighbours.

The idea that root growth of plants might follow an IFD as an alternative to over-proliferation has been discussed before, though it is rarely named as such. For example, several authors have discussed the basic idea of growing roots according nutrient availability and not according to neighbours and developed verbal models (E.g. de Kroon *et al.*, 2009, Mommer *et al.*, 2010, de Kroon *et al.*, 2012, Padilla *et al.*, 2013). These verbal models do not reference or name the IFD specifically, but the predictions are the same. Gersani *et al.* (1998) presented a graphical model explicitly based on Fretwell's (1972) fitness density model that used nutrient uptake per unit root to estimate root allocation of plants via density dependent. The quantitative model presented here takes this one step further, and should permit further investigation of the IFD strategy versus the over-proliferation strategy for below-ground competition.

The data in the literature show that a mixture of these two strategies exists. Indeed, there are examples that support the game-off IFD model (Figure 3, Litav and Harper, 1967, Gersani *et al.*, 1998, Cahill *et al.*, 2010) and examples that support a game-on over-proliferation model (Cahill *et al.*, 2010, Mommer *et al.*, 2010, Semchenko *et al.*, 2010, Padilla *et al.*, 2013). How are we to integrate these apparently contradictory lines of evidence? We suggest that there are two lessons here: First, the world need not be 100% game-on or 100% game-off. Given the language of hypothesis testing, this seems to have been the expectation of some authors (e.g. Semchenko *et al.*, 2007), but we suggest that the current evidence supports the idea that the world appears to contain a mixture of both types of species (e.g. Mommer *et al.*, 2010, Semchenko *et al.*, 2010, Padilla *et al.*, 2013). Second, any particular species need not be 100% game-on or 100% game-off in all contexts, again there seems to be evidence that species sometimes behave one way and sometimes behave another way. For example, Cahill *et al.* (2010) showed that *Abutilon theophrasti* exhibited increased overlap of root systems of neighbours in a nutrient rich patchy environment, but avoidance of neighbour roots in a nutrient poor patch-free environment. *P. sativum* has also been shown to sometimes exhibit game-off behaviour (Gersani *et al.*, 1998) and sometimes exhibit game-on behaviour (Falik *et al.*, 2003). Currently we lack sufficient understanding to determine what contexts cause species like *P. sativum* or *A. theophrasti* to switch strategies, or to describe possible evolutionary pressures or life history trade-offs might produce game-on vs game-off species.

We suggest that one path forward might be to develop models and experimental investigations that examine trade-offs among ecologically important processes. For example, most models of root growth – including the one presented here - employ the simplifying assumption that competition below-ground is the only important process for plant fitness and this assumption is only met in the most controlled of glasshouse experiments (E.g. Figure 1). Similarly, the majority of experiments isolate one ecological process like competition or mutualism or enemy attack and investigate them in isolation. This is a good first step. Yet, the evolutionary history of plants requires trade-offs between traits associated with root and shoot competition, investment into mutualisms, defence against enemy attack and a myriad of biophysical and environmental pressures (De Deyn and Van der Putten, 2005, McNickle and Dybzinski, 2013). For example, when should a plant invest in above-ground competitive ability instead of below-ground competitive ability (but see Dybzinski *et al.*, 2011)? When should a plant invest in enemy defence instead of below-ground competitive ability? When should a plant invest in mutualisms instead of enemy defence? At present we lack a general understanding of how plants make trade-offs between investments in each biotic interaction, and how investment in one biotic interaction shifts investment into other biotic interactions. However, one hypothesis is that those plants which do not engage in a tragedy of the commons game for root competition, might be adapted to focus more strongly on other interactions which are important in plant ecology. This could include above-ground competitive ability, defence from enemy attack or investment into mutualistic associations (Oksanen, 1990, Falster and Westoby, 2003, Archetti *et al.*, 2011, McNickle and Dybzinski, 2013). We suggest that understanding why some plant species are adapted to be capable engaging in a below-ground tragedy of the commons game while other plant species do not engage in such games, will be an important question to move this debate on what strategies maximize plant competitive ability below-ground forward.

Finally, the signal that game-on plants use as a cue for the strategy of responding to neighbours remains unknown. Thus, a key objective for future work should be to investigate the signal used by game-on plants to respond to neighbours. There are several hypotheses in the literature, the two most common are: (i) plants recognize and respond to the root exudates of neighbours (Falik *et al.*, 2003, Semchenko *et al.*, 2007, Novoplansky, 2009) and; (ii) plants simply respond to their own physiological nutrient returns which might be influenced by neighbours (O'Brien and Brown, 2008, McNickle and Brown, 2012). There is limited evidence for either of these right now. The first hypothesis, that plants recognize the exudates of neighbours has been the most commonly investigated mechanism to date (Falik *et al.*, 2003, Falik *et al.*, 2005, Semchenko *et al.*, 2007, Novoplansky, 2009). However, exudates are notoriously difficult to manipulate independently from neighbours without also changing resource availability, which calls some of the results of these studies into question (Lau *et al.*, 2008). The ability to sense exudates is also a very complex signal neighbour recognition. It implies that plants possess a large array of receptors for a diversity of chemical exudates from a diversity of neighbours requiring shared evolutionary histories and a relatively complex set of cellular machinery. The second hypothesis, that plants simply recognize their own internal nutrient returns (O'Brien and Brown, 2008, McNickle and Brown, 2012) has received very little attention. Indeed, for plants to respond to neighbours, they need only be capable of assessing the net return from investing in additional roots (i.e. their own internal nutrient status), they need not "know" anything about what is changing the net returns, and they need not directly sense the roots of neighbours. Plants should simply produce roots until marginal benefits balance marginal costs, the point is that the balancing of benefits with costs is different under an IDF (equation 2b) compared to under a game theoretic model (equation 1c). The behavior predicted by either model does not actually require the direct sensing of neighbours or exudates, only the ability to assess benefits and costs of root production. This is a significantly simpler mechanism which has received almost no attention, despite the fact that the necessary mechanisms of sensing internal nutrient status and coordinating root growth based on nutrient demands are already quite well understood and described (Zhang and Forde, 1998, Zhang *et al.*, 1999, Zhang and Forde, 2000, Hardtke, 2006, Osmont *et al.*, 2007, de Kroon *et al.*, 2009, Forde and Walch-Liu, 2009). The fact that there is evidence for neighbour recognition from intraspecific competition (Cahill *et al.*, 2010), and even genetically identical clones (Gruntman and Novoplansky, 2004) suggests that the exudate recognition hypothesis is unlikely. How could genetically identical clones, with chemically identical exudates tell the difference between self and non-self if exudates were the cue? We suggest that tests of this physiologically based form of neighbour response mechanism could make use of mutant lines that are deficient in their ability to sense nitrogen in the environment, and/or deficient in hormones like auxin that allow coordination of root and shoot growth based on nutrient status (e.g. Cahill *et al.*, 2005, Forsum *et al.*, 2008, Forde and Walch-Liu, 2009). Understanding the mechanism that permits responses to neighbours would go a long way towards pre-screening for game-on and game-off behaviour and to developing a much richer understanding of these two alternative plant strategies.

Conclusions

We had three objectives in this paper. First we presented a game-off version of past game theoretic models that was based on an ideal free distribution. This allows for the generation of hypotheses concerning game-off plants that are directly comparable to predictions from game-on models. From the model, we developed the concept of the best response curve as one tool for examining plant responses to nutrients and neighbours. Second, based on the best response curve, we suggested an experimental design that parameterizes the best response curve, and explicitly tests for the potential confounding effects of volume and nutrients, as well as for direct responses to neighbours which are independent from responses to nutrient availability. Third, we tested the IFD model using *B. rapa*, and presented empirically derived best response curves. *B. rapa* root production followed an IFD. The literature contains evidence that some species are game-on while others are game-off. Future work should seek to understand the factors that produce either game-on and game-off plants, and investigate simpler mechanisms of neighbour recognition that are based on a physiological mechanism of internally estimating returns on investing in more root growth. We think that the IDF model paired with the game theoretic models, permits a more holistic set of tools for interpreting the diversity of experimental results associated with plant responses to neighbours.

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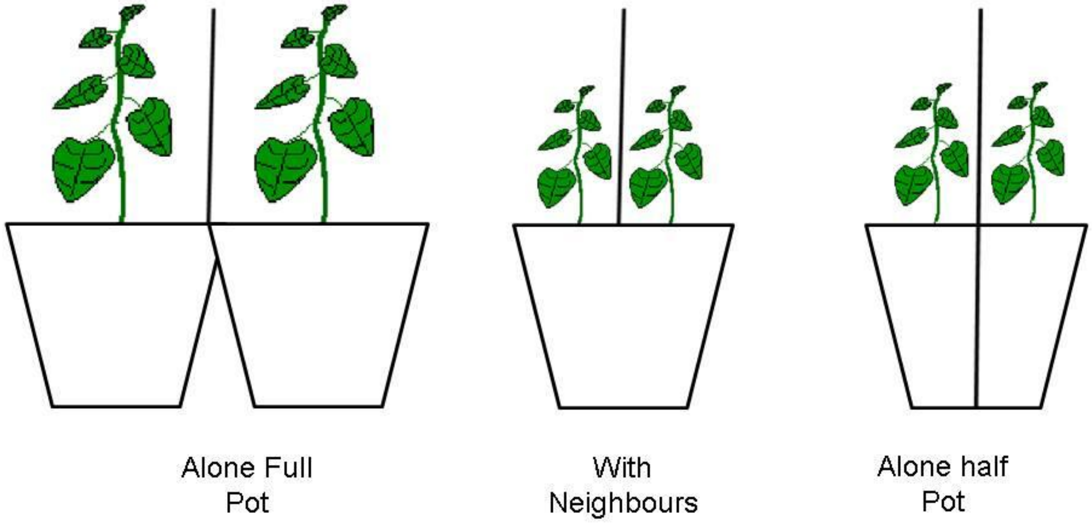

Fig 1: Schematic of experimental design. Pairs of plants were either grown in three treatments; (*i*) alone in full sized pots of volume *V*=1L (with *R*, soil resources per plant), (*ii*) with neighbours in pots of volume *V* (with *R*/2, soil resources per plant), or (iii) alone in pots of volume *V*/2 (with *R*/2, soil resources per plant). Opaque screens were erected between paired plants so that they did not compete or interact above-ground.

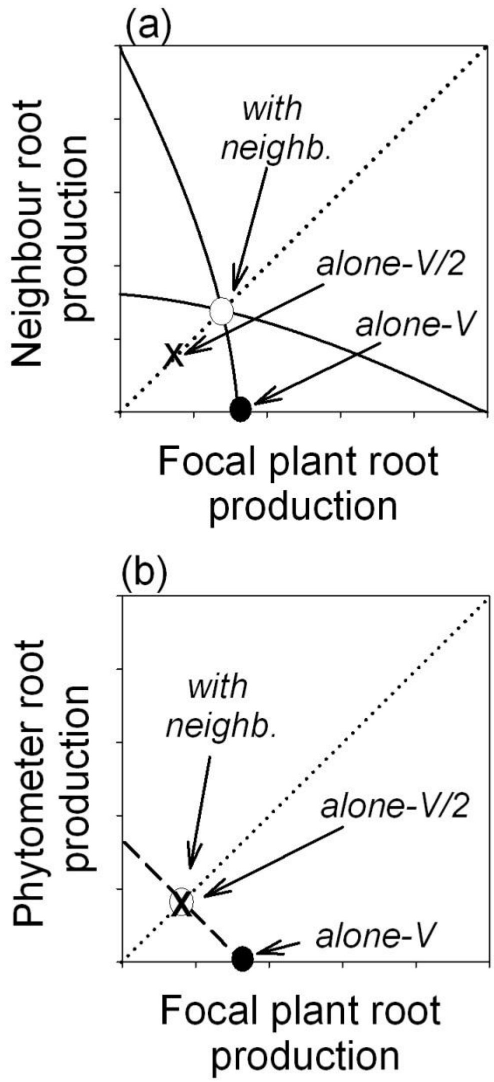

Fig 2: Examples of best response curves for game on plants (a) or game off plants (b). In either case, there are three points of interest that correspond to three experimental treatments (Fig 1). The closed circle represents expected root biomass when grown alone in pots of volume V. The x represents expected root biomass when grown alone in pots of volume V/2 assuming no confounding effects of volume. The open circle represents the expected root growth when grown with neighbours. Game-on plants over-proliferate roots in response to neighbours causing the best response curve to bow up and out from the origin (panel a). For plants that do not engage in a tragedy of the commons game the model predicts a linear best response curve that obeys an ideal free distribution of roots relative to soil nutrient concentrations (b). Game off plants will never produce more roots when neighbours are present compared to alone.

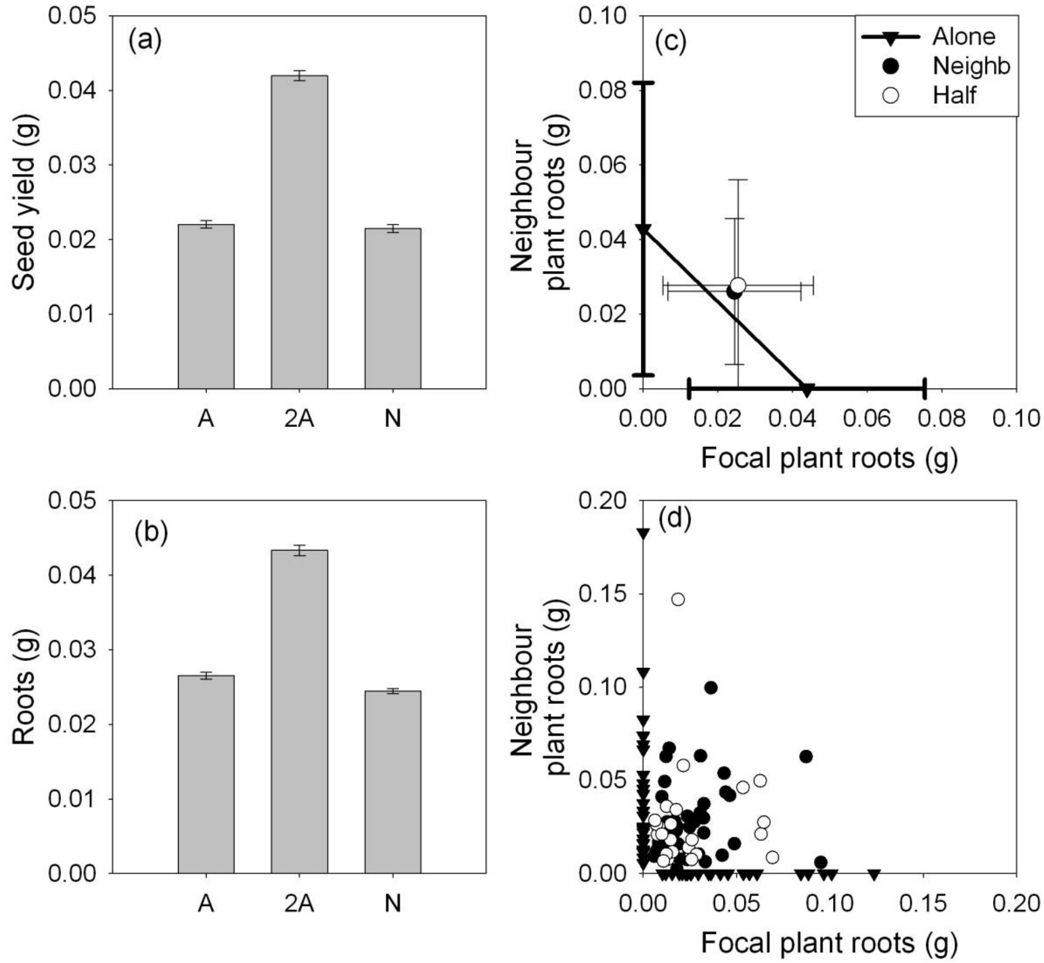

Fig 3: Mean seed yield (a) and root mass (b) of focal plants grown either alone in pots of volume V/2 (A), alone in pots of volume V/2 (A/2) or with neighbours (N). These data are also plotted as best response curves (c-d). Mean responses of plants grown with neighbours or alone in half pots are compared to the expected best response curve (c). Raw data are shown in panel (d). Error bars are ± 1 SD.

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