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Subtle variation in shade avoidance responses may have profound consequences for plant competitiveness

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Running title: Variation in shade avoidance responses influence competitiveness

Abstract

Background and Aims: Although phenotypic plasticity has been shown to be beneficial for plant competitiveness for light, there is limited knowledge on how variation in these plastic responses plays a role in determining competitiveness.

Methods: A combination of detailed plant experiments and functional-structural plant (FSP) modelling was used that captures the complex dynamic feedback between the changing plant phenotype and the within-canopy light environment in time and 3D space. Leaf angle increase (hyponasty) and changes in petiole elongation rates in response to changes in the ratio between red and far-red light, two important shade avoidance responses in *Arabidopsis thaliana* growing in dense population stands, were chosen as a case study for plant plasticity. Measuring and implementing these responses into an FSP model, allowed to simulate plant phenotype as an emergent property of the underlying growth and response mechanisms.

Key results: Both the experimental and model results showed that substantial differences in competitiveness may arise between genotypes with only marginally different hyponasty or petiole elongation responses, due to the amplification of plant growth differences by small changes in plant phenotype. In addition, it illustrated that strong competitive responses do not necessarily have to result in a tragedy of the commons; success in competition going at the expense of community performance.

Conclusions: Together these findings indicate that selection pressure could likely have played a role in fine-tuning the sensitive shade avoidance responses found in plants. The model approach presented here, provides a novel tool to further analyse how natural selection could have acted on the evolution of plastic responses.

Key-words: Arabidopsis, competition, functional-structural plant model, phenotypic plasticity, shade avoidance, tragedy of the commons

Introduction

Plants compete for resources with their neighbours, which influences species composition and vegetation dynamics in both natural (Kiaer *et al.* 2013; Kunstler *et al.* 2016) and managed plant communities (Olsen *et al.* 2005; Yu *et al.* 2015). Plants experience both above and belowground competition, and the relative importance of the degree of competition for plant performance depends on the availability of resources, e.g. nutrients or light (Kiaer *et al.* 2013). The degree of competition for resources and therefore plant functioning is influenced by differences in plant phenotype, created by the component traits and their values (Kunstler *et al.* 2016). These values can be genotype specific but may also be modulated by environmental factors through phenotypic plasticity. Phenotypic plasticity is the ability of a genotype to express multiple phenotypes in various environments (Bradshaw 1965; Sultan 2000).

Here we emphasize that expression of different phenotypes in different environments is mediated by dynamic organ-level responses to environmental signals. From an evolutionary perspective one can argue that plants have evolved to optimize plastic responses to maximize resource acquisition in different environments (Sultan 2000). Plastic responses to changes in vegetation density and the associated light conditions constitute a well-known form of phenotypic plasticity in plants, called the shade avoidance syndrome (SAS; Casal 2012; Ballaré & Pierik 2017). Increase in stem or petiole extension rate, reduction in branch production, increase in leaf inclination (hyponasty) and advanced flowering time are typical SAS responses that plants exhibit when encountering increased competition for light, though the combination of responses differ between species.

Relations between species, component traits and their values, and their relationship with competitiveness have been studied intensively to understand ecosystem processes

(Dybzinski *et al.* 2011; Farrior *et al.* 2013; Bardgett *et al.* 2014; Kunstler *et al.* 2016). For instance, game-theoretical studies suggest that because plants compete for resources, plants can evolve traits associated with a relatively large investment in resource harvesting (e.g. leaves, stems and roots) instead of reproduction. This means that under competition, natural selection can result in plant traits that will not optimize performance of the plant population, also referred to as a tragedy of the commons (ToC, Falster & Westoby 2003; McNickle & Dybzinski 2013). The existence of such a ToC may have profound consequences for vegetation performance (Anten and Vermeulen 2016). However, studies that evaluate the role of resource-harvesting traits for competition often do not take phenotypic plasticity into account (but see e.g. Dybzinski *et al.* 2013). Analysing how plastic responses affect competition is challenging because plastic responses affect trait values that influence the dynamic interaction between plant phenotype and environmental conditions and signals. Environmental signals elicit plastic responses that induce small trait changes which in turn change the light climate and thus modify the environmental signals. Furthermore small changes early in plant development eventually can be amplified into substantial consequences for competitiveness. Although phenotypic plasticity is identified to be beneficial for plant performance, illustrated by adequate stem or petiole length matching to different environments (Schmitt *et al.* 1995; Dudley and Schmitt 1996; Pierik *et al.* 2003; Weijschede *et al.* 2008), it is unknown to what extent subtle variation in the plastic response itself has consequences for plant performance in competitive settings. Large consequences of such subtle variation would likely result in strong selection for a fine-tuned detection and signal-transduction system.

Our main objective was to determine to what extent differences in plastic responses between neighbouring plants affect the outcome of competition for light, considering the dynamic feedback between plant phenotype and environment. We use SAS responses in

Arabidopsis thaliana (Arabidopsis) as a case study for phenotypic plasticity. Arabidopsis rosettes show two major SAS responses: increased leaf angle (hyponasty) and petiole elongation (Pierik & de Wit, 2014). When Arabidopsis plants are grown in dense stands, leaf angles will first increase due to physical touching among growing leaves (de Wit *et al.* 2012). This resulting vertical stand structure will change the ratio of red to far-red (R:FR) light scattered by the elevated leaves. This decrease of R:FR light is the most important signal for the subsequent induction of further leaf hyponasty and petiole elongation (Pierik & de Wit, 2014). To quantify the effect of differences in these SAS responses on plant competitiveness, we used a combination of detailed plant experiments and functional-structural plant (FSP) modelling (Bongers *et al.* 2014). FSP models can capture the dynamic feedback between the changing plant phenotype and the surrounding light environment by simulating plant phenotypic development and biomass growth over time in three dimensions at the organ level (Vos *et al.* 2010; Evers 2016). We implemented phenotypic plasticity as the ability to express organ-level plastic responses: changes in the rate of petiole elongation and changes in the rate of hyponasty. These plastic responses were modelled using response curves that relate organ change to R:FR (Gautier *et al.* 2000; Evers and Vos 2013). In parallel with model analysis, variation in these plastic responses was explored in experiments using Arabidopsis mutants. Ultimately, by simulating the R:FR distribution as a function of the dynamic 3D plant phenotypes that are created by the interaction of resource acquisition and growth at the organ level, plastic responses at the organ level were quantitatively linked to whole-plant performance during competition.

Material and Methods

Plant experiments

Three independent experiments were conducted to obtain organ-level growth data, petiole elongation response curves, and plant phenotype and performance of various genotypes of *Arabidopsis thaliana*, for model design and validation (outlined in Fig. 1). To obtain organ-level growth data, wild-type Col-0 plants were used. To explore the variation in SAS responses we tested various Arabidopsis mutants for their SAS responses (Fig. 2 and Fig. S1, **Supplementary Information**). For model validation the genotypes *hfr1-5* and *rot3-1* were used because of their clear distinct levels of petiole elongation (Fig. 2). Arabidopsis seeds were sown on potting soil (mix Z2254, Primasta B.V., the Netherlands), stratified for 4 days at 4°C in the dark after which they germinated and grew in a growth chamber with 9-hour photoperiod of 200 µmol m^{-2} s^{-1} PAR and R:FR ratio of 2.3, 20 °C and 70% relative humidity. Ten days after germination, seedlings were transplanted to individual 19 ml pots (Ø 2.5 cm) and plants grew in the same growth chamber with bottom up watering for soil water saturation.

Experiments for model design

To obtain organ-level growth data, Arabidopsis wild-type Col-0 was grown solitarily (referred to as 'low density' in the results) or in high density stands of 7 x 7 plants with inter plant distance (IPD) of 2.5 cm, until bolting. During stand development, R:FR measurements were taken in the high-density stands at seven locations with a LI-COR1800 spectroradiometer (LiCor, Lincoln, USA) using a glass fiber with cosine corrector (SKL 904, spectroSense2, Skye, United Kingdom). R:FR was calculated from the irradiance within the wavelengths of 654-664 nm for R and 724-734 nm for FR light. Per location in the stand, readings in four horizontal directions were taken and the average calculated. Between day 21 and day 46, plants were harvested every 2-4 days, and in each harvest two high-density stands and 10 individually grown plants were selected. In each stand the outer two rows of plants were excluded from the harvest to diminish border-effects. Before every harvest, leaf angle of rank

number 8 and 10 were measured with a protractor. For every harvested plant, laminas and petioles were scanned (at 600 dpi). For all leaves with a rank higher than 6 and with a distinct petiole, all laminas and petioles were pooled separately and dried for 48 hours to obtain lamina and petiole dry weight. The remaining aboveground plant material was pooled and dried to get total aboveground biomass. Root material was not harvested. Leaf scans were analysed with ImageJ (https://imagej.net) to collect petiole length and width and lamina area, length, width and shape. Petiole length and lamina area were used to determine parameter values for the organ-growth function (**Supplementary Information - Material and Methods**). Data of all harvested plants per developmental stage and density were used to calculate trait value averages. All parameter values used in the model and extracted from this experiment are given in Table S1, **Supplementary Information**.

To obtain petiole elongation-response curves for three Arabidopsis genotypes, 10-day-old seedlings were transplanted in 70 ml pots (Ø 5 cm) and grown for 28 days at which time they were subjected to one of eight R:FR ratios (2.3, 1.6, 1.2, 1.0, 0.7, 0.5, 0.2 and 0.1) for 24 hours, n = 12 per R:FR. These eight different R:FR ratios were created by supplementing normal light (R:FR 2.3) with FR LEDs (730 nm; Philips Green Power, The Netherlands). Two petioles per plant (start length 4 - 6 mm) were measured at the start and end of the experiment with a digital calliper. The relative elongation per petiole was calculated and the mean of the two petioles per plant was used for further analysis. Relative elongation of all genotypes was described with:

$$P = b * R{:}FR^{-a} \quad (1)$$

where P is the relative petiole elongation (mm mm^{-1} $24h^{-1}$), *a* a slope coefficient and b the elongation rate at R:FR 1. Parameters were fitted for each genotype separately.

157 *Experiments for model validation*

158 Three different Arabidopsis genotypes (Col-0, *hfr1-5*, *rot3-1*) were grown solitarily (low
159 density) or in high-density stands of 8 x 8 plants (IPD of 2.5 cm) composed of plants of the
160 same genotype (monoculture) or plants of two genotypes grown in a checkerboard pattern
161 (mixtures; Keuskamp *et al.* 2010). After 46 days of growth, five solitary plants per genotype
162 and five replicated plots per genotype specific monocultures and mixtures were harvested. For
163 all solitary plants and three plants per genotype per plot, laminas and petioles were scanned,
164 dried and measured similar to the first experiment. The mean values of the middle 16 or 8
165 plants per genotype per plot were calculated and used as independent values for further
166 analysis. Paired student's T-test was used to test significant difference between genotypes
167 within the mixture, and unpaired student's T-test was used to test significant difference
168 between monocultures.

169 ***Model description***

170 A functional-structural plant (FSP) model (Vos *et al.* 2010; Evers 2016) of Arabidopsis
171 rosette growth and development was constructed using the simulation platform GroIMP v1.5
172 (https://**sourceforge**.net/projects/**groimp**). The rosettes were represented as a collection of
173 leaves that were composed of petioles and laminas. An additional root compartment
174 functioned only as a sink for carbon assimilates. The leaves were provided with values for
175 reflectance, transmittance and absorbance of PAR, R and FR light, which were used by the
176 radiation model to simulate the light environment and calculate the absorption of PAR and
177 perception of R:FR. The appearance rate and shape of the leaves were based on empirical data
178 and the leaves grew in time in three dimensions based on light interception, photosynthesis
179 and carbon-allocation mechanisms (Explained in more detail in **Supplementary Information**
180 **- Material and Methods** and in Evers and Bastiaans (2016)). During each simulated time
181 step (representing 24 hours) individual leaves absorbed PAR that was converted to an amount

of carbon through photosynthesis, and perceived R:FR that determined the shade avoidance responses (see below). Therefore, simulated plant growth depended on the level of competition for light that individual plants experienced with neighbouring plants: plant phenotype, size and biomass were thus an emergent property of the simulated model scenarios. Parameter values for organ structure, physiological processes and environment signals were obtained from the experiments described above and from literature (Table S1). The complete model is available on request from the corresponding author.

Shade avoidance responses

Two SAS responses were included: hyponasty (by touching and by R:FR) and petiole elongation (by R:FR). Hyponasty by leaf touching is induced upon mechanical interaction at the tips of two growing leaves before the R:FR in a canopy decreases significantly (de Wit *et al.* 2012). This touch-induced hyponasty was simulated to occur when the distance between lamina tips of neighbouring leaves was smaller than 2 mm. Hyponasty induced by R:FR perception was simulated to happen when the perception of R:FR by the lamina was below a threshold value of 0.5. In every model time-step (24 hours), when touch or low R:FR threshold criteria were met, leaf angle increased by a fixed amount, for which either a default value of 16 degrees (based on measurements on Col-0) was used or a scenario-dependent value (see below *Model scenarios*). The leaf angle over time was therefore a function of the number of time steps in which touch or low R:FR perception occurred, with a maximum leaf angle of 80 degrees (see **Supplementary Information – Video** for hyponastic response of Arabidopsis plants in high density). Leaves with rank number up to six did not become hyponastic.

The second SAS response incorporated in the model was relative petiole elongation. RFR ratios perceived at lamina level were used as input for the response curves (Kozuka *et al.*

2010). The petiole response curve based on Arabidopsis type Col-0 was used as default setting (Fig. 2B), for other settings see *Model scenarios*. The fitted function for the relative petiole elongation obtained from the petiole elongation experiment was normalized for growth at control R:FR light (R:FR 2.3). This way the relative petiole elongation rate could be simulated in addition to petiole growth by carbon allocation. Petiole elongation and related extra investment of substrates was modelled in two steps. First the petiole elongated by multiplying the petiole length with the relative petiole elongation value (representing cell expansion without extra biomass demand; Sasidharan *et al.* 2010; Huber *et al.* 2014). Second, the longer elongated petiole increased its carbon demand to correct for the needed biomass corresponding to the length (representing increased biomass allocation to the petiole; Poorter *et al.* 2012; de Wit *et al.* 2015). Petioles could only show the elongation response during the actual growth phase. Petiole length over time was therefore a result of daily calculated carbon growth based on PAR absorption and petiole elongation based on R:FR perception.

Model scenarios

In all scenarios, plants were simulated solitarily (representing low density) or in high-density monocultures or mixtures (consisting of 8 x 8 plants and IPD of 2.5 cm) for 46 days (**Supplementary Information - Video**), and different plant types were created by adjusting relevant SAS response values. In Scenario 1, three plant types were simulated solitarily and in monocultures to test the extent to which the model could simulate Arabidopsis phenotype and growth: The first plant type had default SAS response values as measured for Arabidopsis wild-type Col-0 (referred to as ‘Col-0’) in the experiment, two additional plant types had either no hyponastic responses (‘noHypo’) or no petiole-elongation response (‘noPE’). The R:FR ratio in the vegetation stand was captured by placing virtual sensors at soil level that measured R:FR from four directions, to mimic the measurements of R:FR in the experimental Arabidopsis stands. Dynamic changes of leaf angle, petiole length, lamina area and total

aboveground biomass of these plant types were compared with data from experimentally grown Col-0 Arabidopsis grown in low or high density stands. In Scenario 2 we simulated two plant types with different values for their petiole elongation curves as measured for the *hfr1-5* and *rot3-1* Arabidopsis genotypes (0.073 for '*hfr1-5*' and 0.028 for '*rot3-1*' plant type) in low and high density stands to validate if variation in the petiole elongation responses curve could result in distinct petiole length differences at low and high density. Of these simulated plant types the petiole lengths per rank after 46 days of growth were compared with measured petiole lengths after 46 days of the two corresponding Arabidopsis genotypes.

To quantify the impact of variation in plastic response curves on plant performance in competitive settings, and to determine if stronger response curves would result in high plant competitiveness but sub-optimal population performance (tragedy of the commons), four additional scenarios were simulated (Scenario 3-6). In these scenarios, mixtures of two plant types, placed in a checkerboard design, and the associated monocultures, were simulated for 46 days. Organ growth, light absorption and total aboveground biomass during the development of the stands were recorded as model output. In Scenario 3, two plant types were only different in their petiole elongation response curve; 'Col-0' having a slope of 0.054 and '*hfr1-5*' of 0.073 (respectively matching the measured Col-0 and *hfr1-5* Arabidopsis genotypes). Simulated total aboveground biomass was compared with total aboveground biomass measured from the validation experiment with these same genotypes. In Scenario 4, two plant types had different hyponastic responses but similar petiole elongation response curves; plants increased their angle with 10 ('10deg') or 15 ('15deg') degrees per hyponastic event. These hyponasty values were chosen based on observed variation in hyponastic values of different Arabidopsis genotypes (data not shown). To analyse if competitiveness depends on the difference in plastic responses between two competing plant types, we simulated mixtures with distinct differences between the plastic response values of the two plant types.

In all mixtures a ‘wild-type’ plant type competed with a ‘competitor’ plant type that had a different value for the petiole elongation response (Scenario 5) or the hyponastic response (Scenario 6). The ‘wild-type’ plant type had a petiole elongation response value of 0.054 and a hyponastic response value of 20 degrees. The absolute difference in aboveground biomass of the ‘competitor’ compared to the ‘wild-type’ was a measure for the degree of competitiveness. In addition, over the same range of petiole elongation and hyponastic response values, monoculture stands were simulated. All model simulations were replicated 10 times to capture the variation in plant growth created by the stochastic nature of the light model and the random plant rotation angle. The mean values of the middle 16 (monocultures) or 8 (mixtures) plants per genotype per plot were calculated and used as independent values for further analysis.

Results

Variation in the petiole elongation response curve

Arabidopsis genotypes showed a gradually increasing relative petiole elongation with decreasing R:FR (Fig. 2A and S1). Col-0 and *hfr1-5* showed only a marginally different elongation response, where *rot3-1* clearly had a lower relative petiole elongation rate at the same R:FR conditions compared to the other two. However, all the fitted curves had distinct slope values for their response curves: 0.054 for Col-0, 0.073 for *hfr1-5* and 0.028 for *rot3-1*. The normalization procedure resulted in three response curves with distinct slopes that all increased with decreasing R:FR ratio (Fig. 2B).

Test model design (Scenario 1)

During the development of a dense Arabidopsis stand, leaf area index (LAI) increased and R:FR ratio decreased in time (Fig. S2). This decrease in R:FR is primarily created by

increased leaf angles through the touching of leaves (de Wit *et al.* 2012). Consequently, the R:FR decrease induced hyponastic and petiole elongation responses that further change plant phenotype. The dynamic change of leaf angle and petiole length of experimentally grown plants in low and high density stands were best simulated by the plant type that included both SAS responses (referred to as 'Col-0') (Fig. 3). When the hyponastic responses were set to zero ('noHypo'), plants did not become hyponastic in high density compared to the 'Col-0' type. The simulated 'Col-0' plants increased the leaf angles slightly later during stand development than the experimentally measured leaf angles. Plants that had no petiole elongation response ('noPE') could not grow longer petioles in high density compared to low density, illustrating that the petiole elongation response curve included in 'Col-0' plant type is needed to simulate long petiole lengths in high density population stands. Overall, when including the SAS response values based on wild-type Col-0 ('Col-0'), the model predictions were in good agreement with the experimental aboveground biomass accumulated during stand development in low and high density stands (Fig. 3C).

Validation of the petiole elongation response curve (Scenario 2)

Validation of the petiole elongation response curve (Scenario 2) revealed that the magnitude of the experimentally observed petiole length difference between *hfr1-5* plants grown in low or high density stands was predicted by the model that used the '*hfr1-5*' response curve, although petiole lengths of leaves with high ranks were underestimated (Fig. 4A). In addition, the model predicted no petiole length difference when using the '*rot3-1*' response curve, which is in agreement with the experimentally observed petiole lengths of *rot3-1* plants grown in low or high density stands (Fig. 4B). In absolute terms the model overestimated petiole lengths due to the higher constitutive growth of the simulated Arabidopsis plants compared to the natural *rot3-1* plants.

Impact of variation in plastic response values on plant performance (Scenarios 3 and 4)

'Col-0' and '*hfr1-5*' plant types had different simulated aboveground biomass after they were grown 46 days together in a mixture but not when simulated separately in monocultures (Scenario 3; Fig. 5A). This difference of plant performance in monocultures compared to mixtures was also observed in the experimental data with Col-0 and *hfr1-5* Arabidopsis genotypes (Fig. 5B). In this scenario, the '*hfr1-5*' type had slightly longer petioles than 'Col-0' both in the monocultures and mixture, but the laminas of '*hfr1-5*' absorbed more PAR than 'Col-0' only in the mixture (Fig. 6A,B). The higher PAR absorption at the individual lamina level resulted in higher simulated whole-plant PAR absorption for '*hfr1-5*' compared to 'Col-0' in the mixture, whereas in the monocultures there was no difference between the two plant types for lamina or whole-plant PAR absorption (Fig. 6C). Thus, in direct mixed competition the plant type with the slightly stronger petiole elongation response (as reflected in a higher slope in the petiole elongation-R:FR curve) had higher performance because it created slightly longer petioles that could put laminas in a better lit part of the canopy.

In the monocultures and the mixture of Scenario 4, in which the strength of the hyponastic response was tested, both plant types showed increased leaf angles at the same developmental stage during stand development, but the '15deg' plant type increased its leaf angle faster (Fig. 7A). In the mixture, this faster increase resulted in higher lamina PAR absorption that also resulted in higher whole-plant PAR absorption, compared to the weaker '10deg' plant type (Fig. 7B,C). In the monocultures, the slightly higher leaf angle of the stronger '15deg' type did not result in higher lamina or whole plant PAR absorption compared to the '10deg' type. These model simulations could not be validated due to the lack of appropriate Arabidopsis mutants that have distinct hyponastic responses but overall similar growth forms.

Competitiveness depends on the difference in plastic responses (Scenario 5 and 6)

To determine how subtle variation in plastic responses can affect plant competitiveness, we simulated multiple mixtures in which a 'wild-type' competed with a 'competitor' with a different value for the petiole elongation response (Fig. 8A, Scenario 5) or with a different value for the hyponastic response (Fig. 8B, Scenario 6). The plant type with the stronger petiole elongation response always had a higher aboveground biomass, but when the difference in response was very large, the difference in aboveground biomass increased only marginally (Fig. 8A). The plant type with the stronger hyponastic response had only a higher aboveground biomass with absolute hyponastic values up to 30 degrees (Fig. 8B). Increasing the difference in plastic responses when the absolute hyponastic response was larger than 40 had no effect or a negative effect on competitiveness. When plant types with increased SAS response values grew in monocultures, the aboveground biomass of the plants decreased slightly (Fig. S3), indicating that performance at population level is sub-optimal when plants increase their plastic response strength.

Discussion

In this study we showed that small differences in petiole elongation or hyponastic responses to changes in R:FR conditions can strongly affect plant phenotype and competitiveness. Model simulations illustrated that subtle variation in SAS response curves could influence competitiveness for light because a small change in a structural trait (petiole length or leaf angle) affected the interaction between plant phenotype and light environment, which had direct consequences for simulated PAR absorption and subsequently growth (Figs 6 and 7). Part of the model simulations were validated with a plant competition experiment that resulted in similar biomass accumulation in monocultures and mixtures for two Arabidopsis genotypes with similar petiole elongation response curves as used in the model simulations.

354 ***Model assumptions***

355 Before going on to the implications of our work we briefly reflect on the model assumptions,
356 such that our findings can be properly interpreted. For model simplicity, only touch and R:FR
357 ratio were the environmental cues that induced the studied SAS responses. It is however
358 known that additional canopy-related light cues, notably decrease in blue and PAR light
359 intensity, are involved in shade avoidance (e.g. Casal, 2012; Pierik & de Wit, 2014) and can
360 strengthen low R:FR responses (de Wit *et al.* 2016). In all scenarios, parameters related to leaf
361 optical properties and photosynthesis were set to be independent of light conditions or leaf
362 developmental stage. A decrease in potential photosynthesis with canopy depth (Anten *et al.*
363 1995) was not considered, as we assumed that such acclimations of photosynthetic parameters
364 would be negligible in relatively young and quickly developing Arabidopsis leaves compared
365 to the role of phenotypic change due to the SAS responses studied. In addition, we assumed
366 that chloroplasts in the petioles contributed to PAR absorption and photosynthesis, in contrast
367 to other light competition models which make a clear distinction between height growth
368 through investments in stems and branches that were considered to not contribute directly to
369 CO_2 fixation and light harvesting organs (leaves) that do fix carbon (Anten 2005; Dybzinski
370 *et al.* 2011). We checked the photosynthetic contribution of petioles, and concluded that even
371 without petiole photosynthesis plants with a slightly different plastic response curve have
372 different performances in mixture but equal performances in monocultures (Fig. S4).

373 Regarding plasticity costs, only two direct consequences of phenotypic changes were
374 considered: 1) substrates invested in petiole length were consequently not available for lamina
375 growth and 2) inclined leaf angles could potentially absorb less light than leaves with a
376 horizontal position. Other indirect costs, such as vulnerability of strongly hyponastic leaves
377 and long petioles to mechanical damage or hydraulic limitations, were not taken into account.
378 Overall, the model predicted the observed relative differences in biomass production between

379 genotypes with different petiole elongation responses well qualitatively (Fig. 5), suggesting
380 that costs and benefits of the petiole elongation response were reasonably well captured in the
381 current model regarding Arabidopsis responses. Modelling the induction of both SAS
382 responses was based on R:FR perception at the lamina (Kozuka *et al.* 2010). However, details
383 on site of perception versus site of response may differ between species, organs and responses
384 (Casal and Smith 1988a; b; Maddonni *et al.* 2002). The kind of organ-level plant modelling
385 presented in this paper makes it possible to explore the environmental context of R:FR
386 distributions and functional implications of localized signalling.

387

388 ***Tragedy of the commons***

389 Tragedy of the commons in light competition assumes that plants investing relatively more in
390 light harvesting compared to neighbour plants are the most successful competitors, but
391 because of the costs associated with this investment, such plants will perform less when
392 growing as monocultures (Falster and Westoby 2003; McNickle and Dybzinski 2013). This
393 conflict between individual-based selection and population performance has been proposed to
394 have major consequences for vegetation functioning and knowledge of this phenomenon may
395 provide input for crop management and breeding systems (Anten and Vermeulen 2016). Our
396 experimental results showed that the plant type with the stronger petiole response and thus a
397 higher petiole investment, outcompeted the individual with the weaker response in the
398 mixtures but had equal performance in monoculture (Fig. 5). This is in contrast to (mostly
399 theoretical) studies that evaluate tragedy of the commons in competition for light. Additional
400 model simulations also illustrated that although the competitiveness increased with stronger
401 plastic responses, the population-level performance decreased only marginally (Fig. 8 and
402 Fig. S3). These results suggest that selection on shade avoidance responses that favour light

competition does not necessarily result in strong decrease of population-level performance. The extent to which these results can be extrapolated to other plant types such as forest trees or crops that often have different growth forms and associated SAS responses than Arabidopsis, still needs to be explored. However, if the pattern that small difference in SAS responses affect competitive ability with limited or no impact on monoculture performance extends to crops, it could provide useful breeding targets.

Promising avenues

In this study we described plasticity as trait responses to a range of changing environmental conditions during the lifetime of the individual plant. Differences in degree of plasticity were described by different shapes of the response curves (Fig. 2), and these differences in response curves allowed quantification of how variation in trait responses would affect plant competitiveness. The sensitivity of plant competiveness to small differences in plastic responses due to mutations (i.e. use of Arabidopsis mutants like *hfr1-5* and *rot3-1*) suggest that selection on finely tuned signal transduction pathways is likely. Quantifying more contributors to the signal transduction pathway that influence plastic responses could be a next step in breeding programs that search for optimal plastic genotypes to deal with changing environments.

A next step with this model approach could be to analyse how natural selection could have acted on plastic responses in plants. Analysing how natural selection could have acted on trait values has often been approached by using game theoretical models (Falster & Westoby 2003; McNickle & Dybzinski 2013). However, analysing selection for plastic responses is challenging because a model system needs to consider i) the possibility of a single genotype to express multiple phenotypes, ii) the dynamic interaction between phenotypic changes and changes in environmental conditions and iii) variation in plasticity that is incorporated by a single parameter. The model system presented here complies with

these three requirements, because genotypes varied in their plastic responses due to different values of a single parameter. In that manner it extends on previous game theoretical studies (e.g. Dybzinski *et al.* 2013; Vermeulen 2015) by explicitly considering dynamic environmental trait responses rather than environment-dependent trait values. We thus argue that our approach provides a novel way to analyse natural selection for plasticity (Bongers *et al.*, 2014).

Conclusions

In this paper we illustrated that substantial difference in competitiveness may arise between phenotypes with slightly different SAS response levels, due to the amplification of plant growth differences by small changes in plant phenotype. These findings indicate that selection pressure could have played a role in fine-tuning the sensitive shade avoidance responses found in plants.

Supplementary Information

Material and Methods: Detailed information of model description.

Video: Visualization of Arabidopsis plants growing in low and high density vegetation stand, simulated by the functional-structural plant model.

Table S1: Overview of all used parameters in the FSP model of Arabidopsis, with parameter description, unit, value and source of parameter value.

Figure S1: Experimentally obtained petiole elongation response curves from five Arabidopsis genotypes.

Figure S2: Dynamically changing R:FR and Lamina Area Index (LAI) during the development of a high density Arabidopsis stand (1600 plants m^{-2})

Figure S3: Simulated aboveground biomass of an individual plant related to the plastic response value of the plants in the monoculture

Figure S4: Simulated total aboveground biomass of an individual plant growing in monoculture or mixture.

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Authors' Contributions

All authors designed the research; FJB performed experiments, model simulations and data analysis; All authors interpreted the data; FJB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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570

571

572 **Figure 1 Overview of the research design, in which three independent experiments**
573 **(bordered in green) are combined with functional-structural plant (FSP) modelling**
574 **(bordered in red) to address three questions (bordered in black).** Data of organ growth
575 and detailed plastic responses of Arabidopsis were used to develop an FSP model that
576 included two plastic responses of the shade avoidance syndrome (SAS); hyponasty and
577 petiole elongation. The model design was tested by comparing phenotypic and performance
578 data from plant experiment and model simulation (Scenario 1; bordered in grey). Additional
579 model simulations and plant experiments were performed to validate model output (Scenario
580 2 & 3) and answer the three research questions (Scenario 2 – 6). See **Supplementary Video**
581 for a visualisation of Arabidopsis plants growing in high and low population density.

582

583 **Figure 2 Petiole elongation response curves from three Arabidopsis genotypes.** (A)
584 Measured relative petiole elongation at different R:FR ratios for Col-0 (black - circle), *hfr1-5*
585 (red-square) and *rot3-1* (blue-triangle) with genotype specific fitted curves (equation 1).
586 Experimental data represents mean ± SD (n=12). (B) Petiole elongation response curves for
587 the corresponding Arabidopsis genotypes that were used in the model..

588

589

Figure 3 Experimentally and simulated obtained data of plant phenotype and performance. (A) Leaf angle change of plant growing in a high-density stand obtained from experimental data (square) and simulated for plant types that did (‘Col-0’- red line) or did not (‘NoHypo’ – black line) exhibit hyponastic responses. (B) Petiole length change of plants growing in low (open/dotted) and high (solid) density stands, from experimental data (symbols) and simulated for plant types that did not show petiole elongation (‘noPE’ – black line) or did show petiole elongation (‘Col-0’ – red line). Petiole rank number 12 was used as it was representative for other leaf ranks. (C) Total aboveground biomass of a plant growing in low (open/dotted) and high (solid) density stands, from experimental data (symbols) and simulated by the default plant type ‘Col-0’ (lines) that included both hyponastic and petiole elongation responses. Experimental data represent mean ± SD with n=10 for low and n=18 for high density). Simulated data represents mean (n=10).

Figure 4 Petiole lengths of all leaf ranks per plant after 46 days of growth of two Arabidopsis genotypes. (A) Petiole lengths of *hfr1-5* and (B) *rot3-1* plants from experimental data (symbols) or simulated by the model (lines) in low (dotted blue) and high (solid red) population density stands. Experimental data represent mean ± SD (with n=10 for low and n=18 for high density). Simulated data represents mean (n=10).

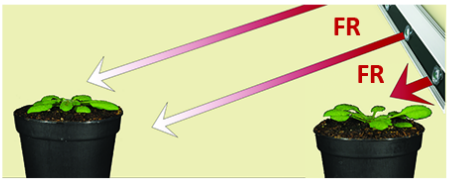
Figure 5 Total aboveground biomass of an individual Arabidopsis plant grown in a monoculture or mixture for 46 days. Plant biomass simulated by the model (A, Scenario 3) or obtained from experimental data (B). Simulated plant types 'Col-0' (dotted) and '*hfr1-5*' (solid) had 0.054 and 0.073 or their response curves, respectively. Simulated data represents mean ± SD (n=10). Experimental data represent mean ± SD (n=5) and ns; not significant and *; $P<0.05$.

Figure 6 Simulated leaf and plant characteristics during the development of Arabidopsis monocultures (black) or mixtures (red) existing of two genotypes with distinct petiole elongation response curves (Scenario 3). '*hfr1-5*' type (solid line) had a stronger petiole elongation response curve than 'Col-0' type (dotted line), shown in Fig. 2b. (A) Petiole length, (B) lamina absorbed PAR and (C) whole plant absorbed PAR during stand development. Leaf rank number 12 was used to visualise petiole length and lamina PAR absorption and was representative for other leaf ranks.

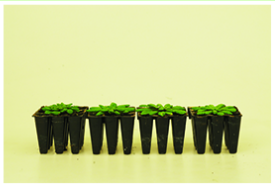
Figure 7 Simulated leaf specific and whole plant characteristics during the development of Arabidopsis monocultures (black) or mixtures (red) existing of two genotypes with distinct hyponastic responses (Scenario 4). The '15deg' plant type (solid line) had a stronger hyponastic response than the '10deg' plant type (dotted line). (A) Leaf angle, (B) lamina absorbed PAR and (C) whole plant absorbed PAR during stand development. Leaf rank number 12 was used to visualise petiole length and lamina PAR absorption and was representative for other leaf ranks.

Figure 8 Simulated performance difference related to the difference in plastic response values of 'wild-type' and 'competitor' plant types in high density mixtures (Scenario 6). Performance difference was calculated by the aboveground biomass of the 'competitor' minus the aboveground biomass of the 'wild-type' plant type. Performance difference related to the difference in (A) petiole elongation response curve value (Scenario 5) or (B) hyponastic response value. Also expressed the absolute petiole elongation and hyponastic response values for the two plant types. Data represents mean ± SD (n=10).

Organ-level growth



Plastic response curves



Plant phenotype and performance

Test model design

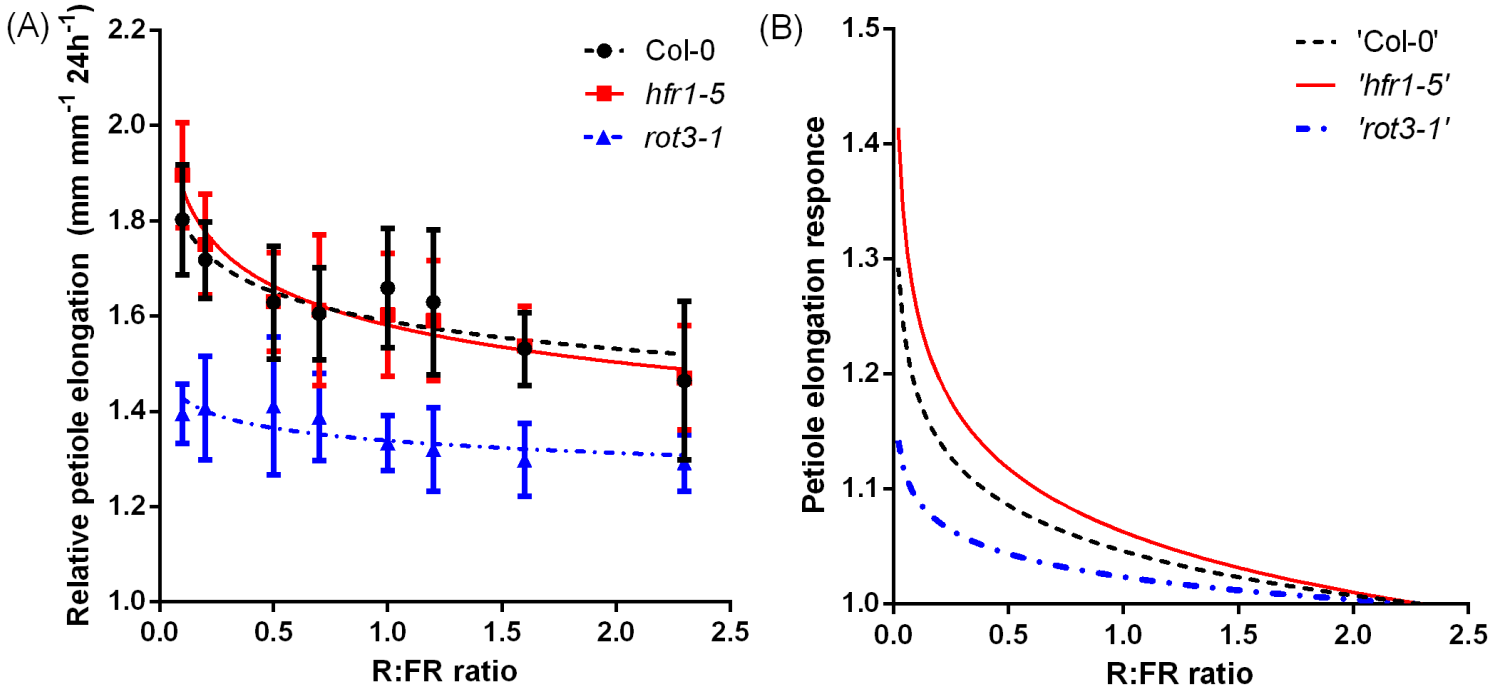
Arabidopsis FSP model + SAS responses

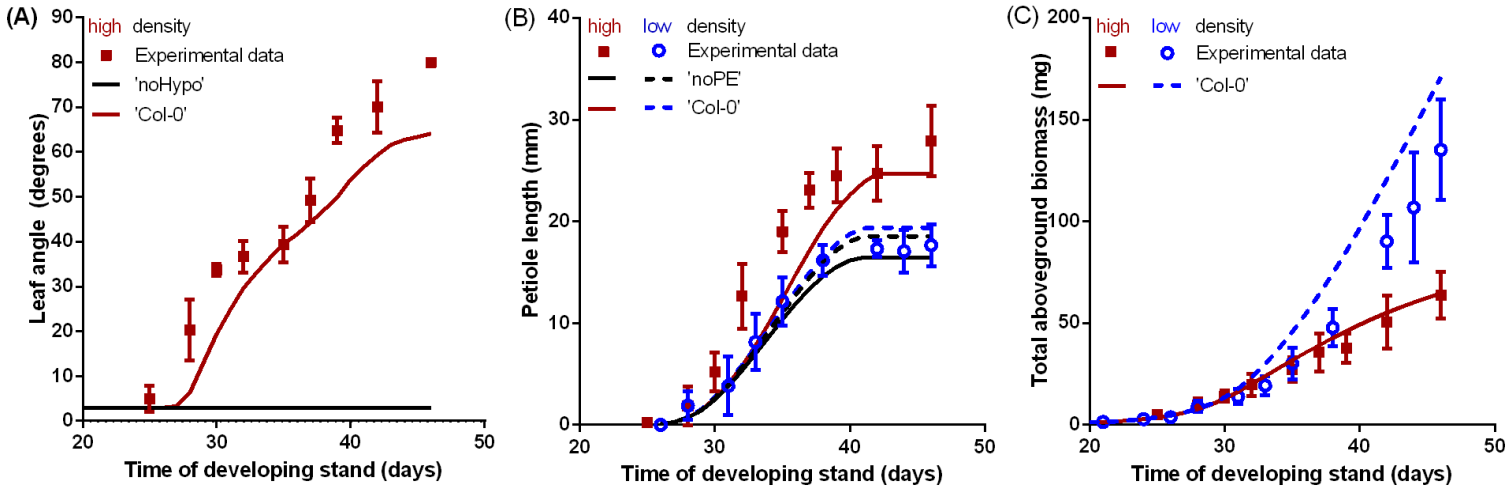
Does variation in plastic response curves result in petiole length differences at low and high density?

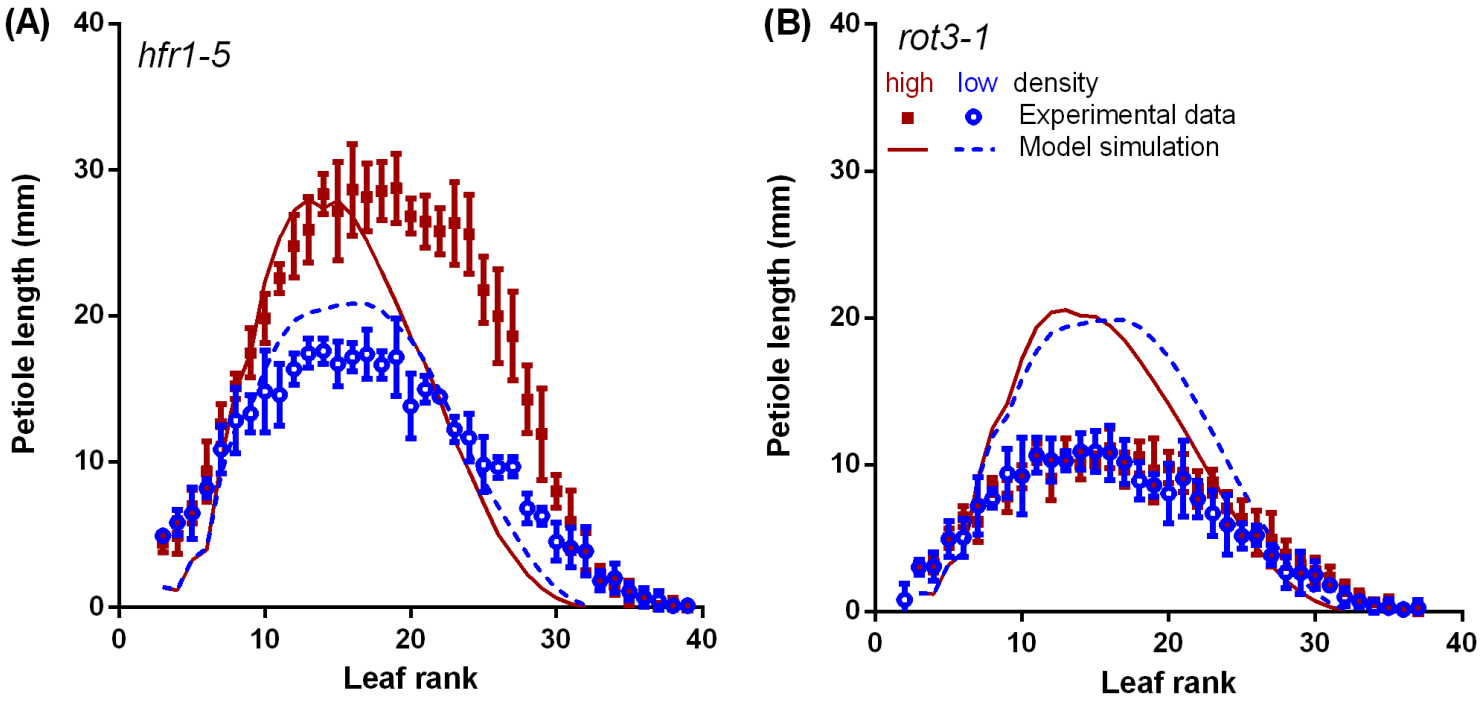
What is the impact of variation in plastic response curves on plant performance at high density?

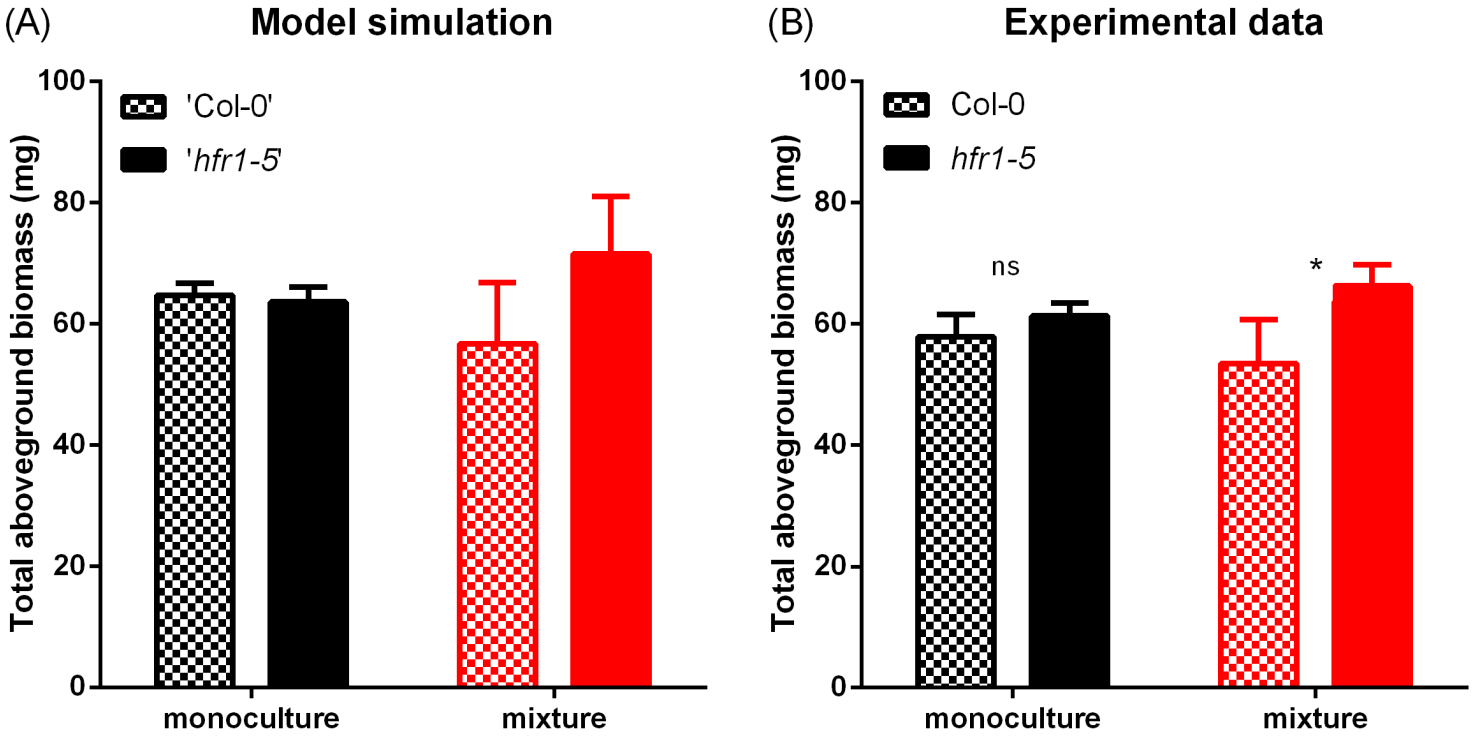
+

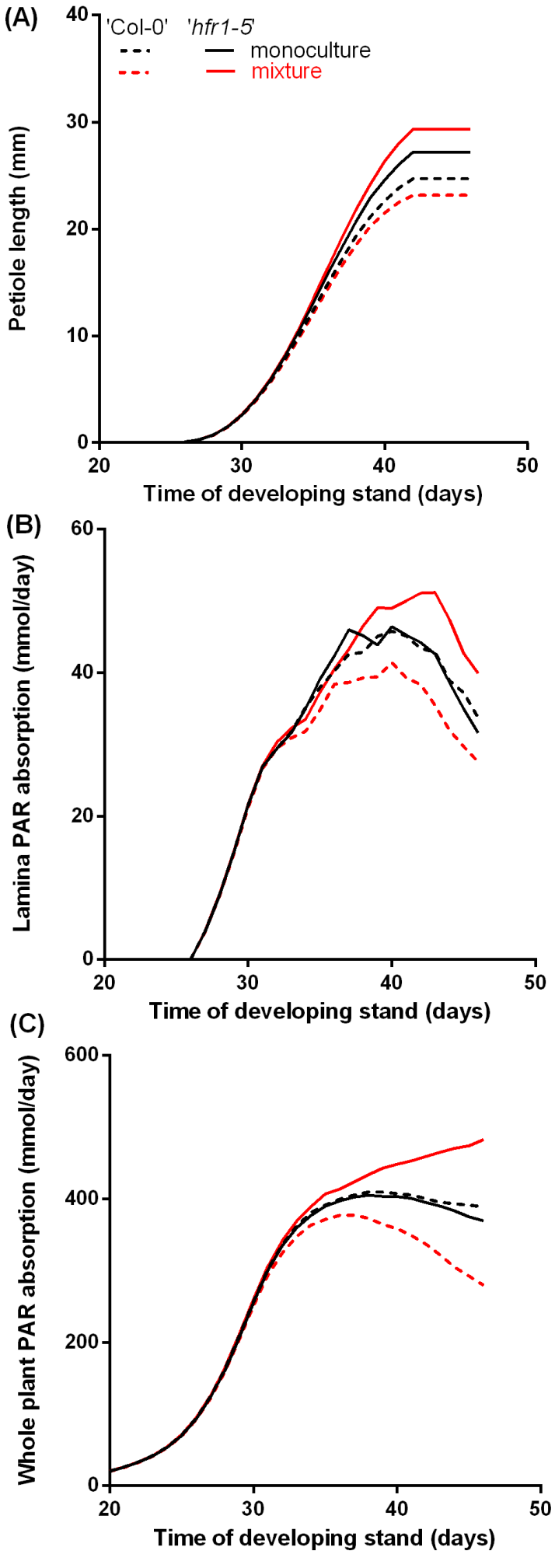
Does increasing the degree of the plastic response curve result in tragedy of the commons?

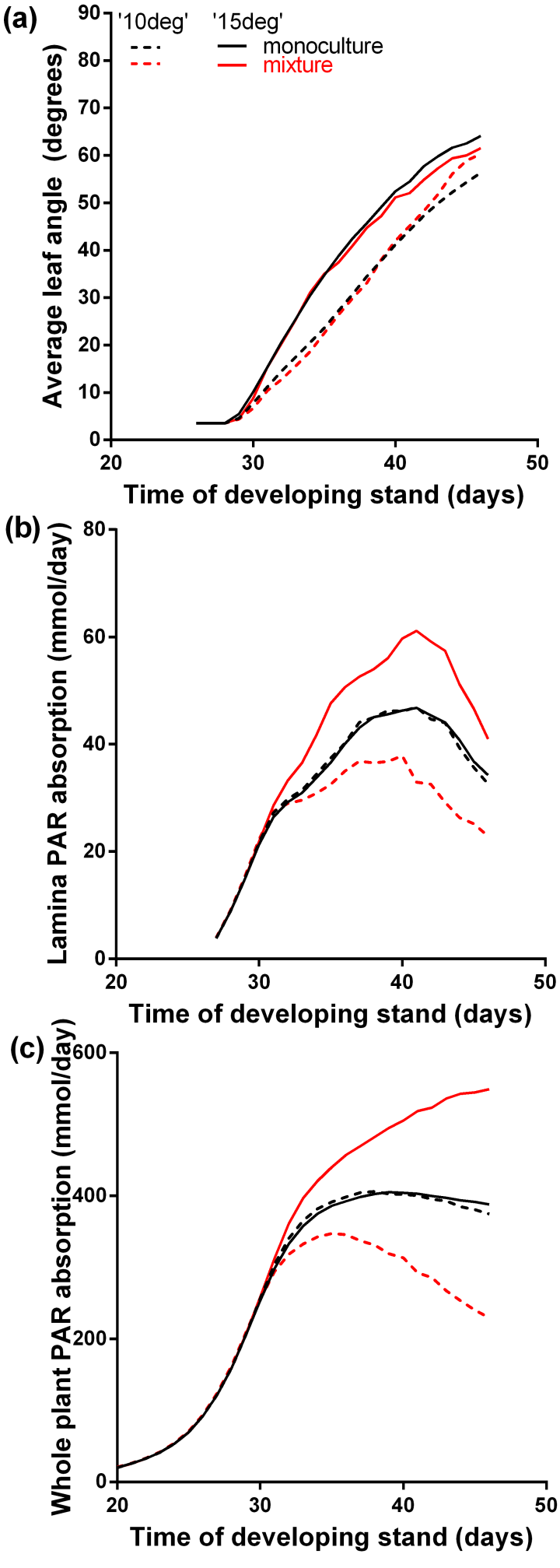

(A)
Relative petiole elongation (mm mm-1 24h-1)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.0
0.5
1.0
1.5
2.0
2.5
R:FR ratio
Col-0
hfr1-5
rot3-1
(B)
Petiole elongation responce
1.5
1.4
1.3
1.2
1.1
1.0
0.0
0.5
1.0
1.5
2.0
2.5
R:FR ratio
'Col-0'
'hfr1-5'
'rot3-1'

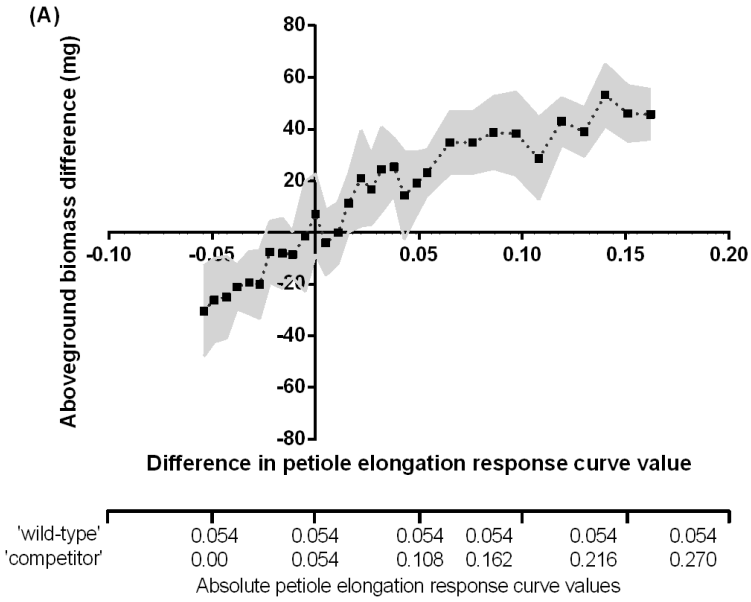

(A)
high density
Experimental data
'noHypo'
'Col-0'
Leaf angle (degrees)
Time of developing stand (days)
(B)
high low density
Experimental data
'noPE'
'Col-0'
Petiole length (mm)
Time of developing stand (days)
(C)
high low density
Experimental data
'Col-0'
Total aboveground biomass (mg)
Time of developing stand (days)

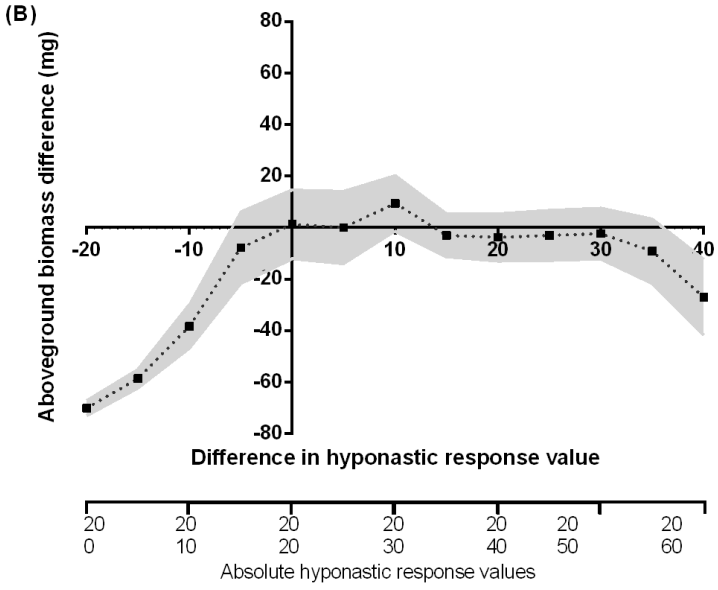

(A)
hfr1-5
Petiole length (mm)
Leaf rank
0
10
20
30
40
(B)
rot3-1
high low density
Experimental data
Model simulation
Petiole length (mm)
Leaf rank


(A)
Model simulation
'Col-0'
'hfr1-5'
Total aboveground biomass (mg)
100
80
60
40
20
0
monoculture
mixture
(B)
Experimental data
Col-0
hfr1-5
ns
*
Total aboveground biomass (mg)
100
80
60
40
20
0
monoculture
mixture


(A)
'Col-0' 'hfr1-5'
monoculture
mixture
Petiole length (mm)
0
10
20
30
40
20
30
40
50
Time of developing stand (days)
(B)
Lamina PAR absorption (mmol/day)
0
20
40
60
20
30
40
50
Time of developing stand (days)
(C)
Whole plant PAR absorption (mmol/day)
0
200
400
600
20
30
40
50
Time of developing stand (days)


(a)
'10deg'
'15deg'
monoculture
mixture
Average leaf angle (degrees)
0
10
20
30
40
50
60
70
80
90
20
30
40
50
Time of developing stand (days)
(b)
Lamina PAR absorption (mmol/day)
0
20
40
60
80
20
30
40
50
Time of developing stand (days)
(c)
Whole plant PAR absorption (mmol/day)
0
200
400
600
20
30
40
50
Time of developing stand (days)


(A)
Aboveground biomass difference (mg)
80
60
40
20
-20
-40
-60
-80
-0.10
-0.05
0.05
0.10
0.15
0.20
Difference in petiole elongation response curve value
'wild-type'
'competitor'
0.054 0.054 0.054 0.054 0.054 0.054
0.00 0.054 0.108 0.162 0.216 0.270
Absolute petiole elongation response curve values


(B)
Aboveground biomass difference (mg)
80
60
40
20
-20
-40
-60
-80
-20
-10
10
20
30
40
Difference in hyponastic response value
20 20 20 20 20 20 20
0 10 20 30 40 50 60
Absolute hyponastic response values