

**Rooting with Neighbours:**  
**Detection Affects Growth and Reproductive Strategies of Plants**

**Bin Chen**

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## Rooting with neighbours:

Detection affects growth and reproductive strategies of plants

## Beworteling met buren:

Detectie bepaalt de groei- en voortplanting strategieën van planten  
(met een samenvatting in het Nederlands)

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# Chapter 1

## General introduction

## Chapter 1

One of the long-standing challenges in ecological research is how to scale up from basic physiological processes and functional traits of individuals to ecosystem structure and productivity (Reynolds *et al.* 1993; Grime 2001; Kerkhoff & Enquist 2006). This understanding is important for both natural and agricultural ecosystems, especially under the current context of global climate change. Although environmental impacts work directly at the physiological level, their consequences will ultimately be reflected at the individual, population and ecosystem levels (Ollinger *et al.* 2008; Knapp *et al.* 2014).

It has long been assumed that, after millions of years of evolution, plants have already optimized their performance (in terms of maximum seed production). Thus, traditionally, plant biologists have typically approached the scaling problem using simple optimization theory, which asserts that trait values are optimal when they lead to maximum seed production per unit area of vegetation stand (reviewed in Hikosaka & Hirose 1997; Anten 2005). This hypothesis has usually been addressed using individual plants in pots.

However, plants generally grow in vegetation stands, where they compete with their neighbours for both light and soil resources. Natural selection would thus favour traits that maximize competitive ability instead of reproductive ability of plants. Plants just have to do better than their direct neighbours (McNickle & Dybzinski 2013). In other words, the success of a strategy (i.e. trait) employed by a focal plant depends on the strategies employed by its neighbours (Heino *et al.* 1998). To investigate these frequency-dependent successes of traits in plants interacting (or competing) with neighbours, game theory provides a formal, logical framework (Riechert & Hammerstein 1983; Falster & Westoby 2003).

### 1.1 Game theory in plant ecology

To date, game theory has been applied in many aspects in plant ecology, including e.g. leaf area (Schieving & Poorter 1999; Anten 2002), canopy structure (Anten 2002), leaf turnover (Hikosaka & Anten 2012) height (Givnish 1982; Pronk *et al.* 2007), root growth (Riechert & Hammerstein 1983; Gersani *et al.* 2001), flowering time (Vincent & Brown 1984; Vermeulen 2015), pollen morphology (Till-bottraud *et al.* 1994), seed-

setting (Geritz 1995; Geritz 1998), seed germination and dormancy (Rees 1994), even pathogen and herbivore defence (Augner *et al.* 1991; Broom *et al.* 2005). Central to game theory in ecological research is the concept of the *evolutionarily stable strategy* (ESS, Maynard Smith & Price 1973). An ESS represents a strategy (i.e. trait) employed by all individuals of a population in dynamic equilibrium that cannot be invaded by a genetically different individual (e.g. a mutant or migrant from another population) employing a different strategy (Vincent & Brown 2005). It should also be noted that an ESS can be composed of multiple strategies in addition to a singular strategy (McNickle & Dybzinski 2013).

Game theoretical models predict that the strategy employed by an optimal plant population with maximum reproduction may not always be evolutionarily stable, i.e. the population can be invaded by individuals that grow more leaves, are taller or produce more roots. As a consequence, a population that is at an ESS is less than maximally productive (Falster & Westoby 2003; Anten 2005). Therefore, in the analyses of plastic traits in response to competition, game theoretical models predict that plants grown with neighbours should over-produce their vegetative (also competitive) organs with less seed production as compared to solitarily grown plants. This phenomenon has often been coined as a “Tragedy of the Commons” (Hardin 1968; Schieving & Poorter 1999; Zhang *et al.* 1999).

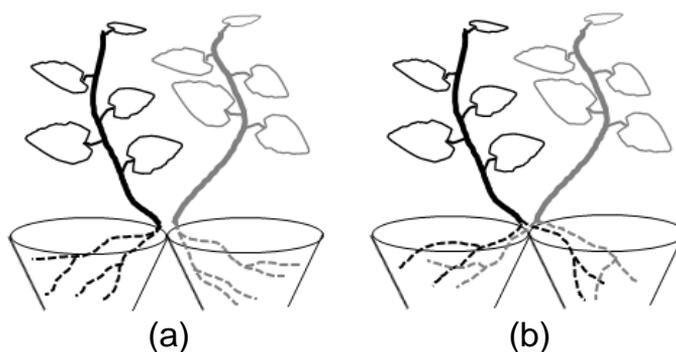
## 1.2 Tragedy of the commons in plants

The concept of a *tragedy of the commons* (TOC) was originally been proposed to describe the unsustainable social behaviour in natural resource over-exploration (Hardin 1968). Take commercial fishing for example. Fishermen share the resources in a given area of the sea. Each fisherman only gains benefits from his own catch, but everyone shares the cost that comes from reducing the amount of fish in the sea. When only driven by short-term economic self-interest, each fisherman will attempt to gain an advantage by trying to catch as many fish as possible. Thus, they will continue to catch more fish regardless of consequences for the sustainability of the fishery (i.e. the good of the group), eventually leading to a ‘tragedy’ that fish populations collapse in the sea (e.g. North Atlantic cod, Hutchings & Reynolds 2004). Thus, in short, TOC describes that, when sharing

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common or public resources, self-interests drive an individual to behave in a manner that is contrary to the best interests of the whole group (Rankin *et al.* 2007).

Intriguingly, game-theory-based TOC scenarios are not limited to human interactions but also occur in a wide variety of organisms including animals, microorganisms and plants (Rankin *et al.* 2007). For plants, the most obvious example of a TOC scenario involves plant height (Falster & Westoby 2003). A taller plant always has more access to light in competition. Selection thus favours plants growing taller and shading their shorter neighbours. However, any attempt to be taller than one's neighbours is a zero-sum game (i.e. no benefit to the fecundity of anyone), because its neighbours will also attempt to grow taller in order to avoid being shaded. Since growing taller requires more investment in the stem mass, the “arms race” in the height game will lead to a taller plant population with less reproduction (e.g. Givnish 1982). Therefore, the final height represents an ESS, and it is at the expense of plant reproductive performance.



**Fig. 1.1** Tragedy of the commons in root growth as shown by Gersani *et al.* (2001) using soybean plants with split-root (a) owners (plants grown alone) and (b) sharer (plants grown with neighbours) design.

Recent evidence suggests that TOC scenarios also exist at the level of roots. In a greenhouse study (Fig. 1.1), Gersani and his colleagues (2001) showed that, given some amount of nutrients, soybean plants that shared soil with a neighbour (i.e. sharers) engaged in an arms race in root growth for resource competition. Eventually, they produced 85% more roots but 30% less yield than those grown alone (i.e. owners). This suggests that natural selection has favoured soybean plants that increase root growth in the presence of neighbours to acquire a greater share of the common resource,

but that population of such plants would be less fit in terms of seed production. Similar phenomena have also been demonstrated for other plants such as Kenyan bean (Maina *et al.* 2002) and pea (O'Brien *et al.* 2005).

As noted above, inter-plant interaction is the basis of the occurrence of TOC scenarios in either above- or below-ground parts of plants. Game theoretical studies indicate that selection for investment in resource harvesting critically depends on the degree of self versus non-self interaction (Anten 2005). In other words, if plants predominantly interact with themselves, lower allocation to resource harvesting would be selected for. Thus, this suggests that plants must also have evolved some means of distinguishing between self and non-self.

### 1.3 Neighbour detection in plants

Indeed, without nerve system, i.e. no eyes, ears, or nose, plants are still able to sense their environment, including the detection of neighbours. The most well-known evidence is the shade-avoidance response (e.g. Vandenbussche *et al.* 2005). After the interception by leaves, light is rich in far-red but poor in red and blue light, leading to a lower red/far-red ratio. Therefore, by detecting the changed ratio of light reflected from neighbours, plants initiate an 'avoidance' reaction before they are actually shaded (see example in maize, Afifi & Swanton 2011). The syndrome associated with this response includes, e.g. stem elongation, leaf upward-orientation, branch reduction and early flowering (Vandenbussche *et al.* 2005). These responses are also what game theoretical models would predict (e.g. Hikosaka & Hirose 1997; Anten 2005). In addition to light signals, plants can also detect their neighbours via aerial volatiles (Ninkovic 2003), and even via direct physical contact between leaves (de Wit *et al.* 2012).

Interestingly, recent evidence suggests that plants can also detect neighbours below-ground. For instance, Mahall & Callaway (1991) found that root growth of the desert shrub *Ambrosia dumosa* was remarkably restricted after direct physical contact with the roots of another plant, but not influenced when its roots came into contact with other roots from the same plant. Biedrzycki *et al.* (2010) showed that root developmental processes of *Arabidopsis thaliana* seedlings were modified, in terms of shorter tap roots

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and higher number of lateral roots, when they were treated with root exudates from other plants. Particularly interesting results were obtained from clonal plants, which asexually produce numerous genetically identical shoot-root units (ramets). These genetically identical units tend to produce more roots towards each other when they lose their physical connection (e.g. Holzapfel & Alpert 2003).

In addition to the discrimination between self and non-self, evidence suggests that plant roots can even adjust their response in accordance with the relatedness of neighbours. Studies of, e.g. *Cakile edentula* (Dudley & File 2007), *Impatiens pallida* (Murphy & Dudley 2009) and *A. thaliana* (Biedrzycki *et al.* 2010) all found similar trends that plants produce more roots (e.g. Dudley & File 2007) or roots with more competitive morphological traits (e.g. Biedrzycki *et al.* 2010) when grown with less-related con-specifics than with siblings.

### 1.4 Knowledge gaps

Since the first publication that specifically focused on below-ground neighbour detection (Mahall & Callaway 1991), many relevant case studies have been conducted in the last two decades. These studies have generally been directed at two major questions: whether plants can differentiate neighbours' roots from self roots (i.e. self/non-self recognition) and whether plants can recognize the relatedness of neighbours' roots (i.e. kin/non-kin recognition). However, a coherent picture of the progress in this field is still lacking, especially for the underlying mechanisms by which plants would be able to distinguish between self/non-self and kin/non-kin.

As mentioned in the previous section, one of the outcomes of non-self root interactions predicted by evolutionary game theoretical models is that plants over-proliferate roots with less seed production, i.e. a TOC scenario (Callaway 2002). In addition to its ecological and evolutionary impacts in natural population, its potential influence on agriculture should not be neglected (Zhang *et al.* 1999; Anten 2005). It might be possible to improve crop yields by selecting crop cultivars that respond with a lower degree of root over-proliferation to neighbour presence (Zhang *et al.* 1999), or to genetically modify them to have this property. Some recent studies have claimed to find evidence of TOC scenarios using pot-based experiments (e.g.

Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005; Falik *et al.* 2006). However, others have argued that the results in such experiments have been at least partially confounded by the experimental set-ups used (Schenk 2006; Hess & de Kroon 2007). Therefore, unbiased experiments and analyses are needed to disentangle neighbour presence effects from potential confounding effects.

Regarding the mechanisms of self/non-self recognition, evidence shows a trend that both clonal (e.g. Gruntman & Novoplansky 2004) and non-clonal (e.g. Falik *et al.* 2003) plants tend to treat genetically identical but physically disconnected parts as non-self. This indicates that physiological coordination is needed for the recognition process (Gruntman & Novoplansky 2004). If it is true, at what spatial and temporal scale does this coordination persist within plants? At least for clonal plants, this knowledge is needed to determine the scale at which recognition and associated responses may occur.

To date, the focus on the responses of plant reproductive performance to neighbour presence is still limited to biomass partitioning. However, neighbour presence may work in other ways than simply through the amount of resources that can be transported to the seeds. Since seed size is important in determining a number of life history traits (Harper *et al.* 1970; Rees 1996; Westoby *et al.* 1996; Moles *et al.* 2005; Soriano *et al.* 2011) and is plastic to various abiotic environmental factors (e.g. Wulff 1986; Geritz 1995; Galloway 2001), it would be interesting and also necessary to investigate the potential responses of seed size of plants to neighbour presence.

## 1.5 Objectives

Given the above research needs, the main objective of this thesis is to use improved methodology to examine: *the extent to which there is evidence of below-ground neighbour detection and the occurrence of consequent game-theoretical scenarios*. The specific research questions are:

1. What can be deduced concerning mechanisms of belowground neighbour detection in plants from the literature published to date?
2. Does a TOC in root growth occur when confounding factors (e.g. rooting volume) have been corrected for in a split-root owner/sharer design?

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3. Does the loss of physiological coordination (by either disconnection or long distance) between ramets in clonal plants lead to a TOC in root growth?
4. To what extent does the presence of a belowground neighbour affect the reproductive traits of plants?

### 1.6 Thesis outline

In **Chapter 2**, I first critically assess the literature evidence for the existence of neighbour detection, in terms of self/non-self and kin recognition, in plants at the root level. Then, I specifically review and propose some plausible physiological mechanisms that may underlie these responses. Finally, I briefly discuss the relation between below- and above-ground interactions and the potential consequences of root identity recognition for agriculture, and conclude with several questions and research priorities for future studies.

In **Chapter 3**, I examine the extent to which plant investment in roots increases at the expense of reproduction in the presence of neighbours, when effects of rooting volume and impacts of plant size are considered. A greenhouse experiment was conducted, growing split-root *Pisum sativum* plants with or without a con-specific neighbour in a range of rooting volumes, but without changing the amount of nutrients available to each plant. I use path analyses to disentangle the effects of neighbour presence from the confounding effects of rooting volume and the impacts of plant size. The potential reasons for divergent findings of neighbour-induced root responses are also discussed.

In **Chapter 4**, I investigate the physiologically coordinated root interaction in clonal plants, using *Potentilla reptans* as the model species. I first conduct an experiment following the traditional “intact/twin ramet pair” design, in order to test to what extent the effects of severing connections are associated with root coordination. Then, I conduct a second experiment to investigate root coordination, using a novel experimental design that avoids the severance of connections and keeps ramets integrated with their maternal fragments. A potential distance alienation effect on root coordination was also examined.

In **Chapter 5**, I explicitly focus on the potential impacts of neighbour presence on reproduction of plants, but switching from the quantitative (biomass allocation) to qualitative (seed size) aspects. A greenhouse experiment was conducted, growing *Phaseolus vulgaris* plants with or without a con-specific neighbour in high or low soil nutrient conditions. To understand the changes of seed size distribution comprehensively, I use multiple descriptors to depict the range, frequency and hierarchy characteristics of the distributions. In addition, I also try to disentangle the resource and non-resource availability related effects of neighbours.

The thesis finishes with **Chapter 6**, in which the main findings of previous chapters are brought together in a general discussion.



# Chapter 2

## Detect thy neighbour: Identity recognition at the root level in plants

*with H.J. During & N.P.R. Anten*

Plant Science (2012) 159: 157-167

## Chapter 2

### **Abstract**

- Some plant species increase root allocation at the expense of reproduction in the presence of non-self and non-kin neighbours, indicating the capacity of neighbour-identity recognition at the root level. Yet in spite of the potential consequences of root identity recognition for the relationship between plant interactions and community structure and functioning, this phenomenon still remains poorly understood.
- We first critically assess the evidence for the existence of self/non-self and kin recognition at the root level in plants. While root identity recognition most likely exists to some degree, there remain valid points of criticism regarding experiments that have documented this, particularly concerning the effects of pot volume in self/non-self recognition experiments and the roles of size inequality and asymmetric competition in kin recognition studies.
- Subsequently we review and propose some plausible physiological mechanisms that may underlie these responses.
- Finally we briefly discuss the relation between under- and aboveground interactions and the potential consequences of root identity recognition for agriculture, and conclude with raising several questions for future studies.

**Key words:** game theory; root competition; signal transduction; volume effect; synchronized oscillation; trade-off

## 2.1 Introduction

Two levels of identity recognition in animals, i.e. self/non-self recognition (Boehm 2006) and kin recognition (Waldman 1988) are well documented. The former seems to be present in almost all forms of life (Boehm 2006), such as the immune system in vertebrates (Cooper 2010) and con-specific rejection or fusion in lower invertebrates (Grosberg 1988). The latter has already been found in almost all social animal species (West-Eberhard 1989): for instance, cuticular hydrocarbons mediated tolerance and inter-attraction in spider society (Grinsted *et al.* 2011), and odors mediated mate choice of mice (Penn & Potts 1998). The recognition mechanisms in all of these cases involve chemical signals and most of them are based on polymorphic genes (Grosberg & Hart 2000; Boehm 2006). Several forms of plant identity recognition have also been reported. Self-incompatibility preventing self-pollination is a well-documented form of self recognition (Nasrallah 2002). Recent studies have also demonstrated stronger herbivore resistance of sagebrush (*Artemisia tridentata*) responding to volatile cues from self cuttings than to those from non-self cuttings (Karban & Shiojiri 2009) and genotype-based changes in pollen competitive ability in heartsease (*Viola tricolor*) triggered by the presence of competitors (Lankinen 2008), providing evidence of other self/non-self recognition systems in plants. Several plant species have been claimed to respond to the identity of neighbour plants, with the ability to distinguish between con- and hetero-specific neighbours (Broz *et al.* 2010), con- and hetero-cultivar (Ninkovic 2003), self and non-self (Falik *et al.* 2003). Yet, so far identity recognition in plants has been poorly investigated compared to the situation in other life forms.

Plant self/non-self recognition may play an important role in processes linked to resource competition. Allocation to structures involved in competition (e.g., stems, leaves or roots) may strongly depend on the presence of competitors. Game-theoretical models predict that the pay-off associated with a given resource acquisition strategy depends on the identity and strategy of neighbour plants (Parker & Smith 1990). Populations of plants with traits optimal for maximum stand-level (e.g. height, amount of roots or leaf area) performance may not be evolutionarily stable (see review Anten & During 2011). They can be invaded by mutant individuals that

**Table 2.1** Summary of plant underground recognition studies. Besides the self/non-self recognition related studies, we only list some recent kin recognition studies. More could be found in ref. (File *et al.* 2012). In the table, we use reference number to indicate the specific study. For the property of "Growth form", a. = annual; b. = biennial; c. = clonal; p. = perennial. For the property of "Cultivation", RC = root growth observation chamber; P = pot; B = box; T = tray; F = field. For the property of "Substrate", A = agar medium; L = liquid; M = mixture of sand, soil, etc.; Sd = sand; Sl = Soil; V = vermiculite. For the property of "Nutrient", S = solution; S\* = saturated with solution; no = no complementary nutrient; C = contained in medium; NM = not mentioned in the article; PPF = plant-prod fertilizer. For the property of "Data collection", Sb = shoot biomass; St = shoot morphological traits; Rpb = reproductive biomass; Rpt = reproductive traits; Rb = root biomass; Rt = root morphological traits; Rg = root growth rate; Ge = gene expression.

Species	Growth form	Studies	Cultivation	Substrate	Nutrient	Treatment	Data collection	Root harvest level
<b>Self/non-self recognition</b>								
<i>Ambrosia dumosa</i>	shrub	Mahall & Callaway 1991	RC	Sd	S	intra- vs. inter-plant interaction; intra- vs. inter-specific interaction	Rg	no
		Mahall & Callaway 1992	RC	Sd	S	activated carbon; intra- vs. inter-plant interaction; intra- vs. inter-specific interaction	Rg	no
		Mahall & Callaway 1996	RC	Sd	S	intra- vs. inter-plant interaction; intra- vs. inter-population interaction; intra- vs. inter-clone interaction	Rg	no
<i>Andropogon gerardii</i>	p. b. grass	Marikham & Haiwas 2011	P	V	S	intra- vs. inter-plant interaction; volume level; nutrient level	Sb; Rb; Rt	individual
<i>Arabidopsis thaliana</i>	a. forb	Biedrzycki <i>et al.</i> 2010	in vitro	L	C	exposure to self, kin, non-kin exudates solution; secretion inhibitor; different genotypes	Rt	individual
		Caffaro <i>et al.</i> 2011	in vitro	L	C	exposure in self, non-self root exudates, activated carbon	Rb; Rt	individual
<i>Avena sativa</i> var. Dala	a. grass	Semchenko <i>et al.</i> 2007a	P	Sd	S	activated carbon; intra- vs. inter-plant interaction; volume level	Sb; Rb	individual
<i>Buchloe dactyloides</i>	p. c. grass	Gruntman & Novopliansky 2004	P	V	S	intact vs. disconnected; intra- vs. inter-genotype interaction; time alienation; ramets distance	Rb; Rt	pot
<i>Fragaria chiloensis</i>	p. c. forb	Holzappel & Alpert 2003	P	Sd	S*	intact vs. disconnected; underground separation	Sb; Rb	root core
<i>F. vesca</i>	p. c. forb	Semchenko <i>et al.</i> 2007b	T & RC	Sd	S	intact vs. disconnected; intra- vs. inter-species interaction	Rb; Rg	tray (in parts)
<i>Glechoma hederacea</i>	p. c. forb	Semchenko <i>et al.</i> 2007b	T & RC	Sd	S	intact vs. disconnected; intra- vs. inter-species interaction	Rnt; Rg	tray (in parts)
<i>Glycine max</i> var. William	a. forb	Gersani <i>et al.</i> 2001	P & B	V	S*	intra- vs. inter-plant interaction	Sb, Rb, Rpb; Rpt	pot

# Identity recognition at the root level in plants

<i>Larrea tridentata</i>	shrub	Mahall & Callaway 1991	RC	Sd	S	intra- vs. inter-plant interaction; intra- vs. inter-specific interaction	Rg	no	
<i>Phaseolus vulgaris</i> var. Kenya	a. forb	Maina et al. 2002	P	V	S*	intra- vs. inter-plant interaction; volume level; nutrient level	Sb; Rb; Rpb; Rpt	pot	
<i>Pisum sativum</i>	a. forb	Falik et al. 2003	P & RC	V	S	intra- vs. inter-plant interaction; volume level; nutrient level; underground separation	Rb; Rt;	individual	
<i>Trifolium repens</i>	p. c. forb	O'Brien et al. 2005	P	V	S*	intra- vs. inter-plant interaction; volume level; nutrient level; underground separation	Sb; Rb; Rpb; Rpt	pot	
		Falik et al. 2006	P	M	no	intact vs. disconnected; intra- vs. inter-genotype interaction; different genotypes	Sb; Rb; Rt; Rpt	individual	
<b>Kin recognition</b>									
<i>Arabidopsis thaliana</i>	a. forb	Masclaux et al. 2010	in vitro	A	C	self, kin and non-kin interaction	shoot fresh weight; Rpt; Ge	individual	
		Masclaux et al. 2010	P	SI	no	self, kin and non-kin interaction	shoot fresh weight; Rpt; Ge	individual	
		Bledrzycki et al. 2010	in vitro	L	C	exposure to self, kin, non-kin exudates solution; secretion inhibitor; different genotypes	Rt	individual	
		Bledrzycki et al. 2011a	in vitro	L	C	ABC transporter inhibitor; exposure to self, kin, non-kin exudates solution	Rt; Ge	individual	
		Bledrzycki et al. 2011b	in vitro	L	C	exposure to self, kin, non-kin exudates solution; pathogen infection	Ge; plant fresh weight	no	
<i>Cakile edentata</i> var. lacustris	a. forb	Donohue 2003	F	S	no	kin, non-kin and mixed interaction; two locations	St; Rpt	no	
		Dudley & File 2007	P	M	S	root interaction vs. solitary, kin vs. non-kin interaction	Sb; Rb; Rpb	pot	
		Bhatt et al. 2011	P	M	S	kin vs. non-kin interaction	Sb; St; Rb	pot	
<i>Ipomoea hederacea</i>	p. vine	Biernaskie 2011	P	SI	NM	shoot competition level; kin vs. non-kin interaction	St, Rb, Rpt	pot	
<i>Impatiens cf. pallida</i>	a. forb	Murphy & Dudley 2009	P	M	FFF	root interaction vs. solitary, kin- vs. non-kin interaction; R:FR ratio	Sb, St, Rb	pot	
<i>Lupinus angustifolius</i>	a. forb	Milla et al. 2009	P	M	NM	self, kin, half -kin and non-kin interaction	St; Rpt	individual	
<i>Triplaris purpurea</i>	a. grass	Cheplick & Kane 2004	P	M	no	self, kin and non-kin interaction	Sb; Rpb; Rpt	no	

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allocate more to resource harvesting (e.g. have larger leaf areas or rooting systems). This, in turn, indicates that natural selection may result in plant communities that are not maximally productive in terms of community-level growth or reproduction (Anten & During 2011). These game-theoretical studies also showed that pay-offs of allocation to resource harvesting and the consequences for community structure and performance critically depend on the degree of self/non-self interaction (Hikosaka & Hirose 1997; Anten 2002). That is, if plants predominantly interact with themselves, lower allocation to resource harvesting would be expected.

Kin recognition in plants could further affect allocation in resource harvesting, because individuals in a kin group with more similar characteristics are supposed to compete more strongly than individuals in a non-kin group would, in which mutual differences might lead to niche partitioning (“elbow-room model”, Young 1981). However, an individual can also indirectly benefit from the improved fitness or reproduction of its relatives (kin), as they share some of its genes (Hamilton 1964). Due to limitations of propagule dispersal, plants tend to grow in the proximity of other genetically-related plants (Vekemans & Hardy 2004). This would favour the evolution of kin recognition (Hamilton 1964), which may help plant individuals to save resource by cooperating with kin while competing with non-kin (Hamilton 1964), leading to less competitive traits as well as higher fitness. Hence, it could be expected that there is selection for traits that enable plants to discriminate between kin and non-kin.

Identity recognition at the level of roots and its consequences for underground competition and plant performance in the vegetation is receiving increasing attention (e.g. Callaway 2002; Callaway & Mahall 2007; de Kroon 2007; Hess & de Kroon 2007). Plant roots not only sense environmental cues on very fine-scale variation in soil conditions (Hodge 2009), but may also detect the presence of neighbour plants (Falik *et al.* 2003). Depending on species, both responses whereby plants increase root growth towards neighbours thus increasing competitive interactions (Gersani *et al.* 2001), and the reverse whereby they preferentially allocate roots away from neighbours, have been documented (Mahall & Callaway 1991). Roots also take part in communication with microbes, animals and the roots of other plants via exudates (Bais *et al.* 2004; Bais *et al.* 2006). These observations indicate that root interactions are much more sophisticated than were previously believed (Hodge 2009). Much of the

recent research on identity recognition in relation to resource competition has focused on roots (see Table 2.1).

There are many studies dealing with plant recognition at inter-specific level (e.g. HuberSannwald *et al.* 1996; Broz *et al.* 2010). However, in this review we mainly focus on intra-specific recognition. Firstly we critically discuss the ecological studies that have attempted to document self/non-self recognition and kin recognition at the level of roots. We then review and propose some plausible physiological mechanisms that may control these forms of identity recognition. Subsequently we briefly discuss the relation between under- and aboveground interactions and the potential consequences of root identity recognition for agriculture. We conclude with a set of questions that should help set the research agenda on plant identity recognition.

## 2.2 Self/non-self recognition

While self-incompatibility with respect to the avoidance of self-pollination is well documented, other forms of self/non-self recognition in plants, such as root recognition, have received much less attention and are still under debate. In this section we first discuss the evidence for self/non-self recognition at the root level and then discuss the physiological mechanisms thought to be involved in this recognition.

### 2.2.1 Ecological studies

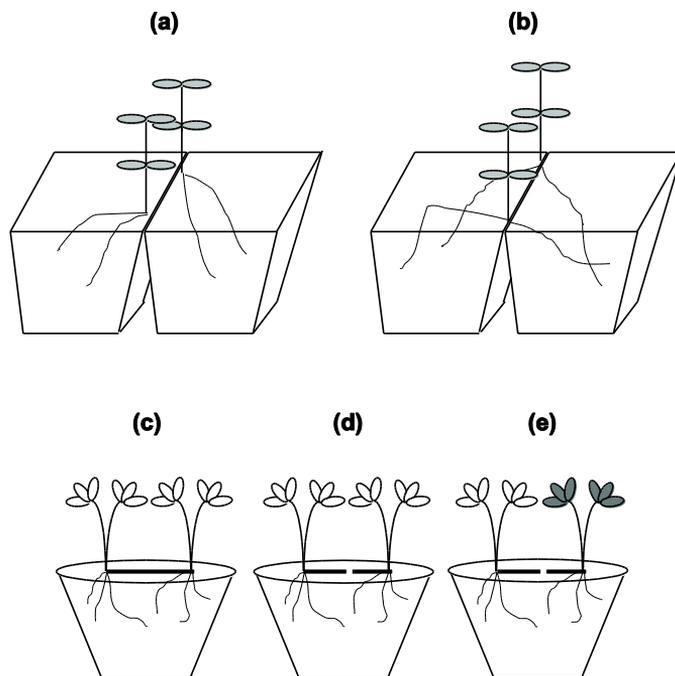
#### 2.2.1.1 *The evidence*

There is clear evidence that roots can respond to structures in their environment. Roots typically respond to obstacles (e.g. stones or impenetrable soil layers) by reducing growth (Falik *et al.* 2005; Semchenko *et al.* 2008) and changing directions (Falik *et al.* 2005). However, if the obstacles are the living roots of neighbours, the situation becomes different and roots behave in a more complicated way.

Early evidence for self/non-self recognition at the root level comes from a study of desert shrub *Ambrosia dumosa* (Mahall & Callaway 1991). Individuals of this species strongly reduced their root growth when these roots encountered roots of another *A. dumosa* individual, but showed no change when encountering their own roots. This reduction in root growth as

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a response to neighbours was believed to have a function in limiting intra-specific water competition (Mahall & Callaway 1992).



**Fig. 2.1** Classic experimental set-ups for underground self/non-self recognition in split-root system (a, b) and ramet-pair (c-e) studies. a, Intra-plant competition (owner scenario): two plants were planted in two adjacent pots, with each plant exclusively having two roots in one pot; b, inter-plant competition (sharer scenario): two plants were planted in two adjacent pots, with each plant having one root in each pot (redrawn from Gersani *et al.* 2001). c, sister ramets from the same mother plant connected with internodes; d, disconnected sister ramets; e, two ramets from different mother plants (redrawn from Falik *et al.* 2006).

Subsequent evidence for self/non-self recognition has come from so-called split-root experiments, whereby rooting systems were split into two parts that either both grew in one container thus interacting with each other (owners, i.e. self interaction, Fig. 2.1a) or were placed in different containers, where also roots of another split-root plant were present (sharers, i.e. non-self interaction, Fig. 2.1b). Soybean (*Glycine max*) total biomass was similar for both scenarios, but sharers produced 85% more root biomass, while owners had 30% more reproductive yield, higher harvest index and higher shoot/root ratio (Gersani *et al.* 2001). Similar results were

subsequently obtained with Kenya bean (*Phaseolus vulgaris* var. Kenya, Maina *et al.* 2002) and pea (*Pisum sativum*, O'Brien *et al.* 2005). These studies thus suggested that when plants grow with non-self neighbours, they respond by allocating more biomass to roots than when they grow alone, and that this may lead to a lower reproduction. These results have been considered evidence for self/non-self recognition at the root level (Callaway 2002). Pea plant root systems were split, but also the shoots of seedlings were split to create “twin plants” (Falik *et al.* 2003). Thus, competition between genetically-identical plants could be observed. Interestingly, genetically-identical disconnected twins produced more roots than connected twins (Falik *et al.* 2003), suggesting that plants react as strangers to disconnected parts.

Compared with unitary, non-clonal species, inter-plant interactions, especially cooperation, are thought to be stronger and more important in clonal plants (Holzapfel & Alpert 2003). Clonal plants asexually produce vegetative offspring (i.e. ramets, de Kroon & van Groenendael 1997). As a result, they may form patches of vegetation with genetically-identical ramets, each of which produces its own root system. Physical connections (e.g. stolons or rhizomes) among these ramets are often maintained for extended periods of time, which facilitate the exchange of information and substances via vascular transport (Hutchings & Mogie 1990). Clonal plants are thus highly suitable for research on self/non-self recognition. Studies so far clearly indicated that physical connections were a prerequisite for self/non-self recognition in clonal plants (Fig. 2.1c-e), such as beach strawberry (*Fragaria chiloensis*, Holzapfel & Alpert 2003), buffalo grass (*Buchloe dactyloides*, Gruntman & Novoplansky 2004) and white clover (*Trifolium repens*, Falik *et al.* 2006). All these studies showed stronger root growth in disconnected than in connected ramets, rooting in the same pot.

The change in root behaviour in buffalo grass induced by severing connections was time dependent. Cuttings that had been propagated separately for a longer period of time exhibited stronger increases in root growth when placed in the same container than cuttings that had been separated just before planting (Gruntman & Novoplansky 2004). The response of this species also appeared to depend on the distance between cuttings along the connecting stolons of the mother plant. Two cuttings originated three nodes apart showed more root growth when planted together in a pot than those originated from the same node (Gruntman & Novoplansky 2004). Overall these patterns have been interpreted as an

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“alienation” effect (Gruntman & Novoplansky 2004). Apparently, in some plant species the mechanism that coordinates root growth in connected ramets persists for some time after they have been disconnected (Gruntman & Novoplansky 2004).

The degree of self/non-self recognition in roots seems to differ between species. For example, root growth patterns in oat (*Avena sativa*, Semchenko *et al.* 2007a), big bluestem (*Andropogon gerardii*, Markham & Halwas 2011), ground ivy (*Glechoma hederacea*) and woodland strawberry (*Fragaria vesca*, Semchenko *et al.* 2007b) did not appear to respond to the presence of non-self neighbours.

### 2.2.1.2 *The criticism*

The methods that have been used to show self/non-self recognition at the root level in plants have been heavily criticized. Much of the criticism has focused on the extent to which these studies correctly controlled for pot volume effects (e.g. Hess & de Kroon 2007). Root growth responds to the available volume (McConnaughay & Bazzaz 1991), nutrient amount (O'Brien *et al.* 2005) and nutrient concentration (Maina *et al.* 2002). These effects are notoriously hard to correct for. In the series of split-root experiments from (Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005), both owner and sharer scenarios (Fig. 2.1a, b) were based on the concept that a plant has access to a certain volume  $V$ , independent of whether it has a pot with volume  $V$  for itself or shares two such pots with another plant, thus having access to  $2 \times (\frac{1}{2}V)$ . In other words, nutrients together with volume were assigned to two plants in both scenarios. Thus the only difference was assumed to be the presence of neighbouring roots from self or non-self.

It has been argued, however, that when plants share a pot they will both have access to the whole pot volume (e.g. Schenk 2006; Hess & de Kroon 2007; Semchenko *et al.* 2007a). Therefore, sharers had access to a two-fold larger volume than owners. The difference of root biomass between the two scenarios could thus be explained by sharer individuals having access to a larger pot volume (Schenk 2006; Hess & de Kroon 2007) with less self-inhibition (Hess & de Kroon 2007; Semchenko *et al.* 2007a). According to one hypothesis (Hess & de Kroon 2007), root biomass is mainly determined by the available volume, while whole-plant biomass is determined by the amount of nutrients plants get. Thus if volume increases but total nutrients remain constant, as in the case of sharers compared to owners in split-root

experiments, one could expect an increase in root allocation going at the expense of, e.g., reproduction (Hess & de Kroon 2007). This is a “maladaptive response” to soil volume available to plants growing in nature (O'Brien & Brown 2008). However, some results from split-root experiments can hardly be explained only by differences in pot volume or nutrient availability (Hess & de Kroon 2007), such as: the reported disproportionally larger root biomass of bean sharers compared to owners (Maina *et al.* 2002) and the increase in root biomass of pea plants with full nutrient solution with a competitor in comparison to that of plants receiving half the amount of nutrients without a competitor (O'Brien *et al.* 2005).

An elegant experiment addressed this issue by growing one plant in two pots (O'Brien *et al.* 2005). It either halved the size of one of the two pots, or halved the nutrient level of one of the two pots. There was no significant change of root biomass in the control pot, but nearly 50% root biomass reduction in the pot with half volume as well as in the pot with half nutrients. This result indicated that both the volume size and nutrient level are important factors in determining the growth of roots. It is evident from the above that in order to identify self/non-self recognition at the root level, it would be ideal to control for pot volume as well for the amount and concentration of nutrients. Unfortunately this seems impossible. In order to keep the amount and concentration of nutrients constant, the effect of volume was simply disregarded in the split-root experiments. Conversely, the effect of nutrient concentration is often ignored (e.g. Hess & de Kroon 2007). Not only pot volume but also pot shape may influence root growth of plants (Hanson *et al.* 1987) and the responses of plants to competition could vary with pot size (Gurevitch *et al.* 1990).

There are however some experimental set-ups that may have shown self/non-self recognition in roots independent of the above-mentioned confounding factors. For example, the direct observation that intact twin pea plants develop more roots towards non-self neighbours, while there was no difference of root growth of severed plant individual towards its severed twin and real non-self neighbour (Falik *et al.* 2003), cannot be explained by variation in volume or nutrients. Similarly, in an *in vitro* study, seedlings of *Arabidopsis thaliana* produced shorter roots when exposed to non-self root exudates than when exposed to root exudates from themselves (Biedrzycki *et al.* 2010). Another means of circumventing the problems of volume and nutrients in pots, is the use of clonal ramet pairs in experiments. This is because one can disconnect ramets without having to manipulate root

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distribution over pots as in split-root experiments, thus keeping volume and the amount and concentration of nutrients the same (Fig. 2.1c-e). This notion however was criticized (Hess & de Kroon 2007) by arguing that the connected scenario deals with a single two-root-system plant, while in the disconnected scenario there are two one-root-system plants. That is, the one connected plant has access to the full amount of nutrients whereas each of the disconnected plants would only get half of this amount. How this should inevitably lead to a larger total root biomass of the two-plant systems is not clear. The phenomenon that root biomass increases with the length of the alienation period of two disconnected sister cuttings of buffalo grass (Gruntman & Novoplansky 2004) suggested that self/non-self recognition in plant cannot be denied (Hess & de Kroon 2007).

Additionally it is possible that natural selection has already led to constitutive root over-production, as under natural conditions plants tend to be surrounded by neighbours (de Kroon 2007). Finally, the allocation trade-off between root growth and reproduction is not a zero-sum game: more roots bring greater resource acquisition, which could compensate for the extra cost of root growth (de Kroon 2007). In one resource-driven model “plants do not need to be aware of the presence of neighbours” (O'Brien & Brown 2008), because the ability of assessing net profits from increasing roots (so, including potential negative effects on the uptake by the plant's own neighbouring roots) might explain the larger root production in the sharer scenario of split-root experiments.

### 2.2.2 Possible mechanisms

Root growth responses associated with self/non-self interaction vary among species and also seem to depend on the overall growth conditions. For example, as noted, some species exhibit reductions in root growth when interacting with non-self neighbours (Mahall & Callaway 1991), while others respond with increases in root biomass (e.g. Gersani *et al.* 2001; Holzapfel & Alpert 2003; Gruntman & Novoplansky 2004). Increases in root growth in response to non-self neighbours become smaller at high nutrient availability in some (O'Brien *et al.* 2005) but not in all cases (Maina *et al.* 2002). In experiments with clonal plants, severance of the connection between sister ramets induced an immediate response in some species (Falik *et al.* 2006) but a delayed response (i.e. the above-mentioned alienation effect) in others (Gruntman & Novoplansky 2004). These results suggest

## Identity recognition at the root level in plants

that there is genetic variation in the regulatory mechanism controlling self/non-self recognition at the root level and that this mechanism may interact with the local environment in which plants grow. Yet to date there is no clear consensus about what this mechanism might be.

### 2.2.2.1 Possibility of chemical signals

Underground interactions of plants mainly happen in the rhizosphere, where plants produce and exude numerous chemical compounds (Bais *et al.* 2006). There is increasing evidence that root exudates play major roles in initiating and mediating these biological communications (Bais *et al.* 2004), thus having important effects on plant and soil communities (Perry *et al.* 2006). More than 100,000 different secondary metabolites have been documented in plants (Dixon 2001). Many of them are found in root exudates, and the biological functions of most of these are still unknown (Bais *et al.* 2006).

Some of the identified chemicals have toxic or growth-inhibiting effects on neighbour plants, and are categorized as allelochemicals (Inderjit *et al.* 2011). In low concentrations these allelochemicals may act as signals (Schenk & Seabloom 2010), and may sometimes even have positive effects, e.g. by inducing herbivore resistance of neighbours (Glinwood *et al.* 2003).

Some chemicals are signals for plant-microbe and host-parasite interactions (Bais *et al.* 2006). For instance, compounds called strigolactones are identified as the prerequisite signals for host recognition and root colonization by arbuscular mycorrhizal (AM) fungi to form AM symbiosis (Akiyama *et al.* 2005). They also induce germination in parasitic plants thus enabling parasitic plants to target host plants (Xie *et al.* 2010). Recently the para-benzoquinone products, haustorial inducers for *Striga*, are believed to play a broader role in inter-plant signalling by influencing the root development of non-parasitic plants (Palmer *et al.* 2009). Given this wide range of functions, a role of root exudates in mediating self/non-self recognition could be expected.

However, unlike quorum sensing, a density-dependent synchronized response in bacteria (Miller & Bassler 2001) and fungi (Albuquerque & Casadevall 2012) organized by group-shared chemical signals, the information carrier for self/non-self recognition must be individually unique and sensitively detected, just like ligands that regulate plant self-incompatibility in pollination (Boehm 2006). Unfortunately, little is known about the existence of individually-specific chemical compounds. Most

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studies have focused on species-specific compounds that may regulate the inter- and intra-specific interactions (e.g. Glinwood *et al.* 2003; Perry *et al.* 2005; Song *et al.* 2010). Moreover, so far, most of the identified compounds in root exudates are relatively small and simple (Badri & Vivanco 2009; Tsuchiya & McCourt 2012), and are thus unlikely to carry individually-specific information.

The above-mentioned *in vitro* study on *A. thaliana* not only found evidence for root exudates being involved in self/non-self recognition (see Section 2.2.1.2), but also showed that the secretion of non-self signals cannot be blocked by the addition of sodium orthovanadate, which inhibits the secretion of phenolic compounds by roots (Biedrzycki *et al.* 2010). Another *in vitro* study found that both self and non-self root exudates had a similar inhibitory effect on the root growth of *A. thaliana* (Caffaro *et al.* 2011), somewhat contradicting the former study (Biedrzycki *et al.* 2010). However, it also showed that only self- but not non-self root exudates inhibitory effects can be reversed by the addition of activated carbon (AC), which absorbs most organic chemicals (e.g. phenolic compounds) but not proteins of root exudates (Caffaro *et al.* 2011). This suggests that proteins or other AC-insensitive compounds might be involved in self/non-self recognition in *Ambrosia dumosa* (Mahall & Callaway 1992; Mahall & Callaway 1996).

Finally common mycorrhizal networks (CMNs) may provide another possible pathway for chemical signal transduction in self/non-self recognition. CMNs are ubiquitous networks linking the roots of different plants at the intra- and inter-specific level by common mycorrhizal mycelia (Simard *et al.* 2012). These networks not only bi-directionally transport nutrients (Philip *et al.* 2010), but also transfer defence signals from infected to healthy plants (Song *et al.* 2010). However, the role of CMNs in self/non-self root recognition has to date not been investigated.

The results from experiments with genetically-identical plants (in both clonal and non-clonal species) indicate that self/non-self recognition in plants may involve more than only chemical signalling. For example, ramets of clonal plants have shown to increase root production when they become disconnected from other ramets on the same clonal plant (e.g. Holzapfel & Alpert 2003; Falik *et al.* 2006), and this effect could become stronger with time after disconnection in some species (i.e., the so-called ‘alienation effect’, Gruntman & Novoplansky 2004). Similarly splitting pea seedlings into two genetically-identical but disconnected plants resulted in increased

root growth towards their severed half (Falik *et al.* 2003). These responses could not be induced by gene-dependent chemical compounds. Some other (undiscovered) signalling systems are probably involved (Gruntman & Novoplansky 2004) that we discuss in the next section.

### 2.2.2.2 *Oscillation hypothesis*

The discovery of AC-insensitive non-self root avoidance in the desert shrub *A. dumosa* stimulated speculation on a physiological mechanism for intra-specific self/non-self recognition (Mahall & Callaway 1992; Mahall & Callaway 1996). Results from studies with genetically-identical twin plants (or ramets, e.g. Falik *et al.* 2003; Holzapfel & Alpert 2003; Gruntman & Novoplansky 2004; Falik *et al.* 2006) further stimulated the discussion about the role of oscillatory signals (Falik *et al.* 2003; Gruntman & Novoplansky 2004) in the proposed physiological coordination process (Falik *et al.* 2003; Gruntman & Novoplansky 2004; Falik *et al.* 2006; Novoplansky 2009). The idea was that self-recognition among roots within plants is based on the “resonant amplification” of oscillatory signals, carried by internal hormone or electricity dynamics (Falik *et al.* 2003; Gruntman & Novoplansky 2004). A different oscillation pattern detected by the root from another root would be recognized as a non-self signal (Falik *et al.* 2003; Gruntman & Novoplansky 2004).

Thus, based on the previous research, we build a framework for interpreting underground self/non-self recognition in plants (Fig. 2.2), based on two speculative steps: 1) synchronizing oscillations in the plant, and 2) identifying neighbouring roots in the environment.

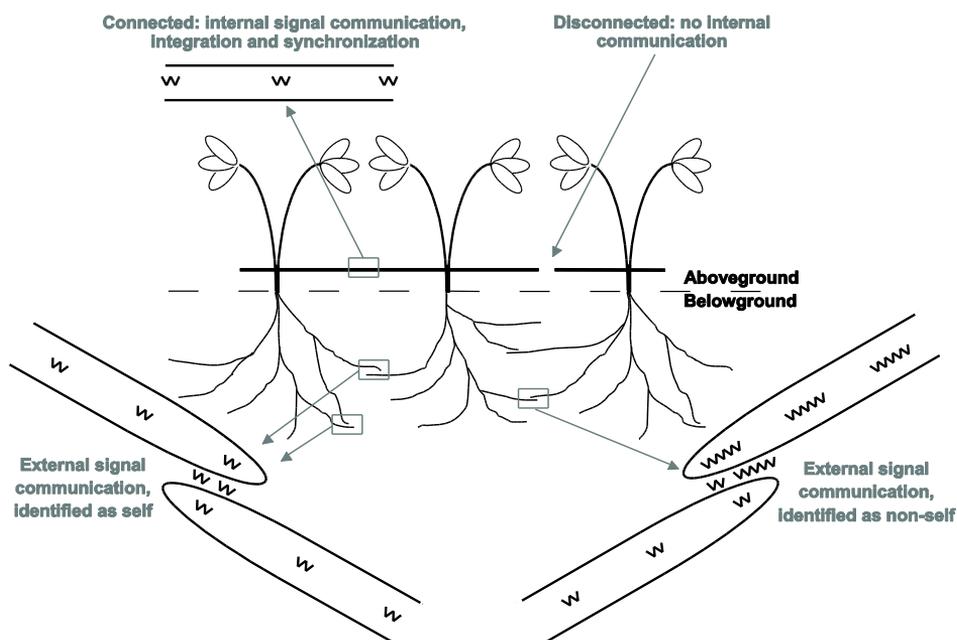
### 2.2.2.3 *Internal synchronization*

Plants lack a central nervous system but being modular organisms, nevertheless require a means of processing and integrating information within the whole system in order to sense and respond accurately to the environment. To achieve this, a synchronizing procedure with an information carrier is needed. The major phytohormones, such as Auxin, cytokinin, gibberellic acid, abscisic acid, brassinosteroids and ethylene, directly regulate root growth (see review Petricka *et al.* 2012). Some of them (e.g. auxin and cytokinin) are internally oscillatory (Ortuno *et al.* 1990). Recent studies showed that auxin contributes to oscillating gene expression in root branching (Moreno-Risueno *et al.* 2010), auxin gradients also regulate cell specification in roots (Dubrovsky *et al.* 2011), indicating

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the possible existence of an internal synchronized oscillation (ISO) involving dynamic chemical signals.

It has however been argued that chemical signals might be too slow to explain rapid responses of plants to stimuli (Davies 2004). Fast transmitting informative signals, i.e. electricity and pressure-concentration waves have thus also been proposed.



**Fig. 2.2** Illustration of the speculated mechanism for self/non-self recognition in plants, based on the oscillation hypothesis of previous studies (Falik *et al.* 2003; Gruntman & Novoplansky 2004). Waves in roots indicate internal synchronized oscillation (ISO); waves out of roots stand for external signals (ISO transported outside the roots, or new signals translated from ISO). As long as the ramets are connected, ISO remains intact and roots within the two ramets will be recognized as 'self'. Once ramets get disconnected, ISO may become out of phase with each other. Similarly, roots of different individual plants may emit external signals that are out of phase. Both cases would entail an indication of 'non-self'.

Electrical signals transmit information over long distances with high velocity. As such they not only enable timely responses to a fast-changing environment (Davies 2004; Fromm 2006), but also permit strong coordination, which contributes to accurate and appropriate responses

(Davies 2006; Mancuso & Mugnai 2006). Indeed electrical signals participate in a variety of plant physiological functions (see Fromm 2006; Trebacz *et al.* 2006; Fromm & Lautner 2007; Yan *et al.* 2009). Growth- and environment-related rhythmic electrical signals have been measured in several plant species (Fensom 1963; Fromm & Fei 1998), suggesting that oscillations, based on integration of electrical signals, do exist in plants.

Root apices are believed to play a central role in electrical signalling in plants. They possess a special cell-cell communication system based on endocytic structures and links between cytoskeletons of cells (Baluška *et al.* 2009), and have the capacity of propagating both spontaneous and stimulated synchronous electrical signals, which commonly carry information gathered from sensory organs (Masi *et al.* 2009). The synchronicity of electric waves in the apex may play an important role in controlling and coordinating developmental, physiological and metabolic processes in this region (Masi *et al.* 2009). Although the waves rapidly decline in amplitude and velocity (Masi *et al.* 2009), such signals may also be instrumental in coordinating physiological processes over the whole root system (e.g., Oyarce & Gurovich 2011; Shemesh *et al.* 2011). Each root apex continuously propagates electrical signals that may vary strongly depending on its actual condition and environment. Facilitated by plasmodesmata (van Bel & Ehlers 2007) and phloem (Fromm & Lautner 2006) based electrical transmission network (Fromm & Lautner 2007), electrical signal transmission and integration allow plant root systems to act as complicated communication systems (Baluška *et al.* 2009). Finally, local electrical waves from each root apex may integrate into a temporally-stable individually-specific ISO within the plant (Falik *et al.* 2003; Gruntman & Novoplansky 2004).

In addition to electrical signals, pressure-concentration waves (PCWs) generated by turgor pressure in the phloem may also act as a form of internal synchronization in plants. PCWs run much faster than the solutes themselves, and may thus rapidly transmit information (Thompson & Holbrook 2004; Thompson 2005; Starck 2006) on a similar timescale as electrical signals (Starck 2006). As the PCWs can be well conducted in different directions (Thompson 2005), they could also act as a reliable information carrier. Indeed, PCWs have been proposed to regulate photosynthesis and respiration and enable root-to-shoot communication (Mencuccini & Holttä 2010).

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### 2.2.2.4 External identification

All the above-mentioned signals could potentially play a role in self/non-self recognition between plants. However, as plant individuals are usually sectorial, any signal should be externally detectable. The internal synchronized oscillation (ISO) itself might act as external signal and be detected by other roots (Falik *et al.* 2003; Gruntman & Novoplansky 2004). Inner electrical currents could be transported outside the root surface of the plant (Weisenseel *et al.* 1992). Indeed, root growth has been shown to respond to electric fields as well as to pH gradients in the soil (Schenk *et al.* 1999). Oomycete plant pathogens target their host's root by these electrical fields (van West *et al.* 2002). It is unclear however over what distance electrical signals are detectable. Resonant amplification of signals may work somewhat differently as they can only be triggered within a plant. Thus, the appearance of resonant amplification could be detected as self recognition between two roots, while non-amplification would be indication of non-self (Falik *et al.* 2003; Gruntman & Novoplansky 2004).

Alternatively, special external signals with identity information translated from ISO inside the plant may be transported into the rhizosphere in the form of short pulses (in the case of an oscillatory signal) involving fast-decaying substances or signals, or in the form of a gradient (Biedrzycki *et al.* 2010), or even a “bouquet” (e.g. dynamic quality and quantity of root exudates, Badri & Vivanco 2009; Xie *et al.* 2010) of the signals to which other plants may respond.

Sometimes, self/non-self recognition requires physical contact of two roots (Mahall & Callaway 1996), while in other cases it just needs the presence of non-self root exudates (Biedrzycki *et al.* 2010; Caffaro *et al.* 2011). The difference may be due to the substrate: in soil the effective distance and life of the signals would be strongly limited, while in artificial solution the effects of signals may be prolonged.

Physiological functions of plants may involve several kinds of signals operating simultaneously. Responses of grapevine (*Vitis vinifera*) to soil water deficiency are mediated by hydraulic and chemical signals (Rodrigues *et al.* 2008), and electrical and chemical signals are involved in the responses of tobacco (*Nicotiana tabacum* cv. Samsun) to local burning (Hlavackova *et al.* 2006). Similarly, the possibility cannot be excluded, that multiple signals (e.g. electrical, chemical, hydraulic signals) operate in conjunction in self/non-self recognition.

### 2.3 Kin recognition

Self/non-self recognition is a discrete form of recognition in that it entails only two possible states: self or non-self. By contrast, kin recognition implies the recognition of the degree of genetic relatedness with many possible states. It is thus possible that kin recognition involves a different regulation system. In this section, we first discuss the evidence for kin recognition at the root level that has emerged from recent studies, and then we discuss potential physiological mechanisms that might regulate kin recognition.

#### 2.3.1 Ecological studies

##### 2.3.1.1 *The evidence*

The niche partitioning hypothesis predicts that more closely related and thus phenotypically more similar plants should exhibit more negatively competitive interactions than less related plants (File *et al.* 2012). This hypothesis has been tested in the studies of sibling competition (e.g. Schmitt & Ehrhardt 1987; Delesalle & Mazer 2002; Cheplick & Kane 2004). However, supporting evidence is limited (Cheplick 1992). Varying effects on reproductive performance have been found, including neutral effects in *Impatiens capensis* (Argyres & Schmitt 1992), transient positive effects in *Phytolacca* and *Solanum* (Willson *et al.* 1987) and positive effects in *Plantago lanceolata* (Tonsor 1989).

While most of these papers were primarily concerned with differences in fitness-related characteristics in different competitive scenarios, recent studies chose to pay special attention to allocation patterns (i.e. growth strategies). Thus in a study, the interactions in kin (sibling plants from the same mother plant) groups and non-kin (plants from different mothers) groups of Great Lake sea rocket (*Cakile edentula*) with or without root solitary treatment were compared (Dudley & File 2007). Plants interacting with kin allocated less biomass to fine roots than plants interacting with non-kin, indicating that plants have the ability to detect the relatedness of their neighbours (Dudley & File 2007) and that non-kin interaction induces a stronger allocation towards resource harvesting (Callaway & Mahall 2007). However, there was no difference in reproductive allocation between kin and non-kin groups (Dudley & File 2007). Yet this was considered to be evidence of root-mediated kin recognition in plants (Dudley & File 2007).

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Other examples include yellow jewelweed (*Impatiens pallida*, Murphy & Dudley 2009) and ivyleaf morning glory (*Ipomoea hederacea*, Biernaskie 2011), which produced more leaves and more roots, respectively, when grown with non-kin than with kin. Especially interestingly, the results from the yellow jewelweed experiment (Murphy & Dudley 2009) suggested that underground neighbour detection could have consequences for aboveground growth as well.

The degree of kin recognition as well as the associated responses in plants also seem to vary among species, often leading to positive effects (see above), but absence of any effect or even negative effects were also reported. For example, the performance of *I. capensis* was not influenced by relatedness (Argyres & Schmitt 1992) but just by the phenotypes of its neighbours (Kelly 1996), there was no influence on the reproduction of *A. thaliana* (Masclaux *et al.* 2010) and negative effects in *Lupinus angustifolius* (Milla *et al.* 2009) have been found when plants interacted with kin neighbours. Thus, it is suggested that one of the possible ways of understanding the reasons behind these varying results would be conducting trait-based approaches rather than direct fitness-based approaches (File *et al.* 2012).

### 2.3.1.2 *The criticism*

An important criticism on studies that have claimed to document kin recognition is associated with the combination of size inequality (Klemens 2008) and asymmetric competition (Klemens 2008; Masclaux *et al.* 2010). Because of the difficulties of separating underground parts from neighbour plants, roots are usually not collected individually but at the group level. The average root allocation and growth was calculated as the group-level root biomass divided by the number of individuals in the group (Klemens 2008). The problem with this method is that if there is significant size inequality among the plants, the summed root/shoot ratio would tend towards the value of the largest individual (Klemens 2008). Inherent size inequality tends to be larger among non-kin than among kin thus creating a methodological bias whereby non-kin interaction appears to result in enhanced root growth. If the reproductive growth is proportional to the vegetative growth, then the asymmetric competition driven by phenotypic variation might lead to the false conclusion that kin interaction results in higher reproductive growth than non-kin interaction (Klemens 2008; Masclaux *et al.* 2010). The performance (biomass, number of siliques) of *A.*

*thaliana* target plants surrounded by kin or non-kin plants was fully determined by the strength of the competitive abilities of the accessions (Masclaux *et al.* 2010). Whole-genome microarray analyses revealed that gene expression did not differ between kin and non-kin conditions, at the level of detection (Masclaux *et al.* 2010).

Non-kin vs. kin interaction was re-tested in Great Lakes sea rocket, but this time applying a reciprocal pair-wise experimental design, which contained all combinations of family pairs (Bhatt *et al.* 2011). This method bypassed the above-mentioned confounding effects of asymmetric competition (Masclaux *et al.* 2010) and could thus more clearly determine the extent to which shifts in root allocation are caused by the neighbour identity or by difference in competitive ability between neighbours. There was lower root allocation in kin pairs than in non-kin pairs (Bhatt *et al.* 2011), which indicated differential responses of roots towards kin and non-kin.

The evidence for increased reproduction of plants growing with kin compared to non-kin neighbours is not yet conclusive (Callaway & Mahall 2007; Klemens 2008; Milla *et al.* 2009; Masclaux *et al.* 2010). However, a field study showed that Great Lakes sea rocket individuals from some families had more reproduction in kin groups than in non-kin groups (Donohue 2003). Similarly, variation in height among climbing plants of ivyleaf morning glory was greater in mixed than in sibling groups (Biernaskie 2011), and the yield of mixed groups was less than that of sibling groups when adjusted to a common mean height (Biernaskie 2011). Overall, mixed groups tended to allocate more biomass to roots, and produced significantly fewer seeds per unit root biomass than kin groups (Biernaskie 2011).

### 2.3.2 Possible mechanisms

As noted above, contrary to self/non-self recognition that entails only two possible states: self or non-self, kin recognition implies the recognition of the degree of genetic relatedness with many possible states. A different mechanism of recognition would thus be expected.

Root exudates are possibly involved in kin recognition. There was more lateral root growth of *A. thaliana* seedlings when exposed to exudates from stranger roots than from sibling's, but this difference disappeared when a root secretion inhibitor was added to the exudates solution (Biedrzycki *et al.*

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2010). ABC transporter genes, which are involved in secretion of secondary metabolite compounds (Kato-Noguchi 2011), participate in the process (Biedrzycki *et al.* 2011a). Together this shows that kin recognition can be triggered by exudates without the presence of neighbour's roots, and blocked by the application of a root secretion inhibitor, suggesting that chemical signals are involved in kin-recognition at the root level (Biedrzycki *et al.* 2010). However, the self/non-self recognition root response in *A. thaliana* was found to be unaffected by secretion inhibitor (see section 2.2.2.1). Kin recognition in plants is thus unlikely to be “a by-product of self/non-self discrimination” (Milla *et al.* 2009). The sensory systems and mechanisms behind the two discrimination processes seem to be distinct (Biedrzycki *et al.* 2010).

### 2.3.3 Conflicting results

The findings of the absence of any difference in reproduction or gene expression between plants from kin- and non-kin groups in *A. thaliana* (Masclaux *et al.* 2010) are somewhat contrary to those discussed above (Biedrzycki *et al.* 2010; Biedrzycki *et al.* 2011a) on the same species. This was attributed to the use of liquid culture instead of soil (Biedrzycki *et al.* 2011b). Liquid culture allows rapid diffusion of chemical compounds and thus potentially a wider-reaching spatial signalling pattern than would be attainable in soil (Biedrzycki *et al.* 2011b). Moreover, one group focused on the morphological changes of the new-born seedlings (Biedrzycki & Bais 2010a; Biedrzycki & Bais 2010b; Biedrzycki *et al.* 2010), while the other focused on older plants (Masclaux *et al.* 2010).

Several studies produced conflicting results, perhaps due to differences in experimental set-up and focus. Moreover, there is a good possibility that both niche overlap (negative effect) and kin recognition (positive effect) have impacts on the growth of plants (File *et al.* 2012), and their relative effects depend on species, environmental conditions and plant developmental stages. Niche overlap would lead to more intense competition, while kin discrimination responses might reduce the degree of competition. The final reproductive performance of plants in kin groups would then be determined by the relative strength of these two effects.

### 2.3.4 Self/non-self recognition and kin recognition

Until now we have dealt with kin and self/non-self recognition as separate phenomena, and have argued that they are probably regulated by different mechanisms. Still, they may act simultaneously: a non-self neighbour can be a non-kin neighbour. Root growth could be stimulated by the presence of non-self roots, possibly induced by the internal synchronized oscillation system. This response in turn could be modified by the similarities of the family-specific chemical signals from neighbouring roots. Therefore, results from split-root experiments, clonal ramet pair experiments and kin/non-kin group experiments might already reflect a combination of responses induced by these two recognition systems. The non-self detection and root response of *Ambrosia dumosa* was only observed for individuals from the same population but not when plants from different populations were grown together (Mahall & Callaway 1996). This phenomenon might be explained by the significant influence of strong discriminatory competitive responses at kin recognition level.

## 2.4 Future directions

### 2.4.1 Aboveground and underground interactions

The signals and mechanisms underlying the underground identity recognition are still unclear, but a range of mechanisms has already been found for aboveground neighbour detection, based on red:far-red ratio of incident radiation, blue light intensity, ethylene, auxin and other signals (e.g. reviewed in Vandebussche *et al.* 2005). Most studies on identity recognition have focused on the interaction and associated responses in one compartment (above- or underground) without considering processes occurring in the other. Similarly game-theoretical studies on neighbour-dependent allocation responses have tended to focus on one aspect - e.g. height (Givnish 1982), leaf area production (Anten 2002) or root growth (Gersani *et al.* 2001) - without considering others.

Yet, in plants the functioning of above- and underground structures is strongly integrated and mutually dependent (Brouwer 1983). Assimilates produced by leaves are partly utilized to provide energy for the production and functioning of roots while leaf functioning depends on the water and nutrients acquired by roots. In theory, plants could sense the presence or identities of their neighbours simultaneously via aboveground and

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underground mechanisms. Changes in shoot growth were discovered in the inter-plant root-interaction scenario compared to those in an intra-plant scenario (e.g. Gersani *et al.* 2001; Falik *et al.* 2006), and in a kin root-interaction scenario in comparison with a non-kin scenario (Murphy & Dudley 2009), drought responses (stomatal closure) of a plant could be triggered by a drought-stressed plant when the rooting volumes were shared with their immediate neighbours (Falik *et al.* 2011), and genotype-based changes of pollen tube growth rate were observed with the presence of neighbour's roots (Lankinen 2008), indicating that underground interaction does influence aboveground performance. Such examples are rare, however, there is thus an urgent need for integrative studies that consider above- and underground identity recognition and responses simultaneously.

### **2.4.2 Modern approaches**

Much of the discussion has focused on the interpretation of results from identity recognition studies, but methods employed in these studies are also subject to debate. Most of the studies discussed above were conducted in pots, which influence the growth of roots (see section 2.2.1.2), and in greenhouses, where environmental factors are strictly controlled and highly artificial. The extent to which the results thus obtained also play a role under natural conditions in the fields remains to be seen. Moreover, it is notoriously hard to separate roots of individual plants from those of others, especially among con-specifics. Most studies therefore chose to average the root biomass of the group as an alternative way to present the root biomass at individual level, which is still problematic (see section 2.3.1.2). Modern approaches, such as using gene markers (e.g. Brunner *et al.* 2004; Lang *et al.* 2010) to observe the distribution pattern of individuals from different genotypes within one species, and injecting dye (e.g. Cahill *et al.* 2010) to discriminate the roots at individual level within one genotype, might provide opportunities to explore more detailed information in the studies of plant con-specific-neighbour detection.

### **2.4.3 Indications for agriculture**

In addition to the important implications of root recognition studies for pure ecological research, it is also worthwhile to note their potential importance for agriculture, where con-specific plants are generally grown

closely together in crop stands. Crop plants tend to be the result of an anthropogenic selection process aimed at obtaining higher yields per unit land area. Although root identity recognition has not been explicitly considered by crop breeders, it is possible that crop selection may unknowingly have led to reduce root sensitivity to neighbours. The results from split-root plant studies (Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005) however suggest that this is not the case. In 1968, the year in which the “Tragedy of the commons” theory (Hardin 1968) which was used as background for interpreting the results in the split-root plant experiments (e.g. Gersani *et al.* 2001) was published, the idea of “ideotype” in agronomic research was also proposed for crop breeding (Donald 1968). The key point of the “ideotype” concept was breeding a weakly-competitive crop (Donald 1968). Agronomists have already realized that the main improvements of crop yields are based on the trade-offs between competitive ability and productive ability (Weiner 2003). As noted in this paper, the ability of plants to respond to non-self neighbours tends to contribute to the increased competitive ability. This could imply that a reduction of the ability to recognize non-self in crops might be a potential way to increase crop yields.

### 2.5 Conclusion

Despite the methodological difficulties of studying roots, a growing number of ecological studies have provided substantial evidence that plants can recognize the identity of neighbours via root interactions. Many but not all species seem to distinguish self from non-self, and the degree of genetic relatedness (i.e. kin-ship). Neighbour-identity dependent responses in root growth may have important consequences for growth and reproduction of plants growing in vegetation stands. Although the mechanisms involved are still unclear, they seem to differ between the two recognition systems (Biedrzycki *et al.* 2010). Self/non-self recognition is possibly mediated at least in part by rhythmic signals (Falik *et al.* 2003; Gruntman & Novoplansky 2004), e.g. chemical, electrical or turgor-associated signals, while kin recognition is more likely to be controlled by the composition of chemical signals (Biedrzycki *et al.* 2010).

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Now that it is increasingly becoming clear that plants can recognize the identity of neighbouring roots, the challenge shifts to understanding the physiological regulation and ecological implications of the recognition. Several key questions need to be answered:

- 1) What are the signals involved in self/non-self and kin recognition systems?
- 2) What are the molecular mechanisms for perceiving signals outside the roots and what are the downstream responses?
- 3) To what extent do the root responses to neighbour-identity and consequences for plant performance, so far documented in highly controlled experiments, also occur under natural conditions?

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# Chapter 3

**Effects of neighbour presence  
on growth and reproduction in pea  
depend on rooting volume and plant size:**

**Implications for  
the tragedy of the commons  
hypothesis**

*with H.J. During, P.J. Vermeulen,  
H. de Kroon, H. Poorter & N.P.R. Anten*

**Submitted**

### Abstract

- A number of studies have indicated that plants produce more roots at the expense of reproduction when rooting with neighbours than when growing alone. This has been framed game-theoretically as a “Tragedy of the Commons”, and claimed to be evidence for belowground neighbour detection in plants. Others, however, argued that these results may at least partially be caused by a rooting volume effect associated with the experimental design.
- We grew split-root pea plants in the presence or absence of belowground neighbours at a range of rooting volumes, while providing equal amounts of nutrients per plant. Path analyses were used to disentangle the direct effects of belowground neighbour presence on allocation patterns from the confounding effects of rooting volume and plant size.
- Within the chosen range of rooting volumes, the presence of a belowground neighbour generally reduced plant root mass by 21% and total mass by 9%. A doubling of rooting volume on average increased plant root mass by 18% and total mass by 11%. Pod mass was only directly and positively correlated with vegetative mass.
- Belowground neighbour presence induced less root allocation but more pod allocation. Increasing rooting volume induced more allocation to roots and shoots but less to pods. A large part of these effects, however, was indirectly mediated through the influence on plant total mass. Nevertheless, there was a clear indication that neighbour presence directly induced higher shoot mass fractions at the expense of root mass fraction.
- Not considering the effects of rooting volume and plant size may lead to misinterpretations on neighbour presence effects. Accounting for these factors, we found evidence for neighbour-induced root responses but no evidence for a tragedy of the commons. More than expected, the results of such experiments in the literature seem to depend on the experimental set-ups in subtle ways, including the amount and spatiotemporal distribution of nutrients in the soil substrate.

**Key words:** biomass allocation, evolutionary game theory, neighbour detection, path analysis, *Pisum sativum*, plant-plant interactions, root competition, self/non-self recognition, split-root, tragedy of the commons

### 3.1 Introduction

In the last two decades, the subterranean aspects of plant life have received increasing attention. A growing body of research suggests that the responses of plants to their neighbours belowground are not only mediated by resource availability, but also directly through the perception of neighbour presence. This has been dubbed “belowground neighbour detection” (Callaway 2002; de Kroon 2007; Hodge 2009; Novoplansky 2009; Badri *et al.* 2012; Padilla *et al.* 2013; Semchenko *et al.* 2014). The detection may enable plants to discriminate between their own roots and roots from other plants, and even between the roots between kin and non-kin. It has subsequently been postulated that these responses may enhance the fitness of plants by adjusting their phenotypes depending on the identity of their neighbours (Callaway 2002; Callaway & Mahall 2007; de Kroon 2007; Dudley *et al.* 2013). However, the experimental evidence for this is still debated while the underlying mechanisms, which would produce such identity-dependent root responses, are still unclear (reviewed in **Chapter 2**).

Some studies showed that plants produced more roots but fewer seeds in the presence of neighbours (e.g. Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005). These findings have been considered in an evolutionarily game-theoretical context, which predicts that populations of plants with optimal traits for maximum population performance are not necessarily evolutionarily stable, and can be invaded by plants that overinvest in resource harvesting to outcompete neighbours (Schieving & Poorter 1999; Anten & During 2011; McNickle & Dybzinski 2013). This process inevitably results in a reduction of total plant performance, which has been coined as “Tragedy of the Commons” (Hardin 1968; Gersani *et al.* 2001), a situation in which competition results in reduced fitness of a group or population (Gersani *et al.* 2001; Rankin *et al.* 2007).

However, the methods employed in the above studies have been subjected to debate, mainly arising from concerns about the effects of rooting volume associated with containers (e.g. pots) in which plants were grown. Adopting the viewpoint that controlling the nutrient amount available for each plant is crucial for the research on neighbour presence effects (O'Brien & Brown 2008; Murphy *et al.* 2013), the studies mentioned above were designed in a way that the nutrient concentration was constant while total rooting volume increased proportionally with the number of

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plants present. This setup therefore allows testing for neighbour effects independent of a reduction in nutrient availability (McNickle & Brown 2014). Others, however, argued that the observed neighbour-induced root over-proliferation is at least partly due to a rooting volume effect associated with the experimental design of those studies. That is, sharers (i.e. plants with a neighbour) have access to a larger rooting volume than owners (i.e. plants without a neighbour) and will accordingly produce more root mass (Schenk 2006; Hess & de Kroon 2007; Semchenko *et al.* 2007a; Markham & Halwas 2011). These studies suggest that comparisons of root mass between owners and sharers should take the effects of rooting volume into account. This would entail conducting owner/sharer experiments at a range of volumes (Hess & de Kroon 2007). So far, almost no study has been conducted in this way (but see Nord *et al.* 2011).

In addition, root confinement studies have revealed that limiting rooting volume also restricts the development of aboveground organs (Körner *et al.* 1989; NeSmith & Duval 1998; Poorter *et al.* 2012a). Hence, simply enlarging rooting volume alone may influence the total mass of plants, even when the nutrient availability remains constant (e.g. McConnaughay & Bazzaz 1991; McConnaughay *et al.* 1993; Murphy *et al.* 2013). This implies that total mass might differ between sharers and owners, due to their different rooting volume sizes. It has long been known that biomass allocation patterns of plants change with plant size (Evans 1972; Coleman *et al.* 1994; Weiner 2004; Poorter *et al.* 2012b; Poorter & Sack 2012). Many changes in plant biomass allocation that are claimed to be caused by belowground competition, could be attributed to the differences in plant size (e.g. Cahill 2003). This indicates that when treatments induce differences in the absolute size of plants, interpretation of the difference in the mass fractional allocation of an organ, in terms of growth strategy, requires the consideration of size-related effects. In other words, plant size effects need to be included in the comparisons of allocation strategies between owners and sharers. To our knowledge this has so far not been done in studies examining effects of belowground neighbour presence.

Because the effects of rooting volume and plant size have not been taken into account so far, current results have not unambiguously demonstrated belowground neighbour detection or its effects on plant resource allocation. Here we present the results of an experiment in which we grew pea plants in the presence or absence of a con-specific belowground neighbour at a range of rooting volumes, using a split-root design and keeping nutrient amount

per plant constant. We examined the extent to which plants invest more in roots at the expense of pods in the presence of neighbours (in line with the above-mentioned tragedy of the commons scenario), when taking rooting volume effects and the potential differences in plant size explicitly into account using path analyses.

### 3.2 Materials and methods

#### 3.2.1 Plant material

For this study we used pea (*Pisum sativum* L. cv. Eminent) as the model experimental plant, since root responses to neighbour presence have previously been reported for this species (e.g. Falik *et al.* 2003; O'Brien *et al.* 2005; Meier *et al.* 2013). Split-root seedlings with *c.* 3 cm long lateral roots were created following the split-root protocol (Gersani *et al.* 2001), paired and transplanted into pots of different volumes (details in the next section).

#### 3.2.2 Experimental design and measurements

As a range of rooting volumes is crucial to our question, three pot sizes, i.e. small (9 cm × 9 cm × 9 cm in width × width × depth, hereafter), medium (11 cm × 11 cm × 11 cm) and large (13 cm × 13 cm × 13 cm), were used. For each pot size level, owner (i.e. plants growing in the absence of a belowground neighbour) and sharer (i.e. plants growing in the presence of a belowground neighbour) scenarios were implemented by using four planting regimes: each plant owns one pot, each plant owns two pots, two plants share one pot, and two plants share two pots (Table 3.1). Pots were filled with sterilized sand, which was mixed with nutrients in the form of slow-release pelletized fertilizer (16% N + 11% P<sub>2</sub>O<sub>5</sub> + 11% K<sub>2</sub>O + 3% MgO + micro nutrients, 3-4 months releasing period, Osmocote Exact, The Netherlands). In order to focus on the non-resource related effects of rooting volume and neighbour presence, the nutrient dose applied was 1.0 g fertilizer per plant throughout all the pot size × planting regime combinations (see details in Table 3.1). In each treatment combination, two seedlings were planted or positioned at a distance of 5 cm and supported by bamboo sticks in opposite directions to standardize and minimize

**Table 3.1** Experimental setup and rooting volumes for the belowground neighbour scenarios (i.e. owner and sharer) manipulated from the combinations of planting regimes and pot sizes

	Owner scenario		Sharer scenario	
	(a)	(b)	(c)	(d)
<b>Small pots</b>				
Total volume (L)	1.12	2.24	0.56	1.12
Rooting volume per plant (L)	0.56	1.12	0.56	1.12
Nutrients per pot (g)	1	0.5	2	1
Nutrients per plant (g)	1	1	1	1
<b>Medium pots</b>				
Total volume	2.2	4.4	1.1	2.2
Rooting volume per plant	1.1	2.2	1.1	2.2
Nutrients per pot	1	0.5	2	1
Nutrients per plant	1	1	1	1
<b>Large pots</b>				
Total volume	3.36	6.78	1.68	3.36
Rooting volume per plant	1.68	3.36	1.68	3.36
Nutrients per pot	1	0.5	2	1
Nutrients per plant	1	1	1	1

Planting regimes are: (a) each plant owns one pot; (b) each plant owns two pots; (c) two plants share one pot; (d) two plants share two pots. The three pot sizes are: small (0.56 L per pot); medium (1.10 L per pot) and large (1.68 L per pot).

aboveground interaction. That is, owners and sharers experienced very similar aboveground interactions and thus only differed significantly in terms of belowground interaction. Plants were grown in a plastic tunnel, where light availability was about 80% of natural sunlight, at the campus of Utrecht University, Utrecht, the Netherlands, from May to July in 2011. Depending on weather conditions, water was given up to four times per day in a way that sand remained moist. Twenty replicates with all combinations of pot sizes and planting regimes ( $n = 240$  in total) were arranged in five blocks on benches to take the micro-environmental variation into account.

Seven weeks after the start of the experiment, plants were harvested and divided into roots, vegetative shoots (shoot hereafter) and pods. At that stage, all plants had their first pods ripened, but vegetative plant parts did not yet show signs of senescence. During the harvesting, shoots and pods were assigned to individual plants, but roots from sharer pairs could not be separated and so were assigned to the pair. All plant material was weighed after 48 h oven-drying at a temperature of 70 °C. Then, the dry mass of roots, shoots and pods were averaged for each pair. Subsequently, total mass, vegetative mass, root mass fraction, shoot mass fraction and pod mass fraction were determined. For each treatment combination (pot size  $\times$  planting regime), rooting volume per plant was calculated assuming that each of the sharers had access to the entire volume of the pot or pots in which they were growing (Table 3.1).

### 3.2.3 Data analyses

We first examined the effects of neighbour presence and rooting volume on plant biomass and allocation (i.e. mass fractions). This was done using linear mixed models with belowground neighbour presence (i.e. owner vs. sharer), rooting volume per plant (covariate) and their interaction as explanatory variables, and with block as a random factor. Biomass data and rooting volumes were log-transformed before the analyses. Analyses were conducted in R v. 3.1.0 (R Core Team 2014) using the lme4 package (Bates *et al.* 2014).

We employed path models to further analyse the causal relationships between neighbour presence, root volume and plant size. Mechanistically, pods can be viewed as the product of roots and shoots (i.e., a large part of the assimilates and nutrients acquired by these latter organs are invested in pods). Many studies show that for annual species plant reproductive mass is

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often mainly determined by its vegetative mass (e.g. Samson & Werk 1986; Weiner 1988). Therefore, our path model was designed to examine the direct and indirect (via vegetative mass, i.e. the sum of root and shoot mass) effects of neighbour presence and rooting volume on plant reproductive performance (i.e. pod mass). In this path model, neighbour presence, rooting volume and their interaction were exogenous variables; plant vegetative mass (i.e. the mediator) and pod mass were endogenous variables (see the full model in Supporting Information Fig. S3.1).

Furthermore, due to the dependence of allocation on plant size (i.e. total mass), we conducted another path model to examine the direct and indirect (via total mass) effects of neighbour presence and rooting volume on plant biomass allocation patterns. In this model, neighbour presence, rooting volume and their interaction were exogenous variables; total mass (i.e. the mediator) and organ mass fraction were endogenous variables (see the full model in Supporting Information Fig. S3.2).

To avoid the multicollinearity problem caused by the correlation between the Neighbour×Volume product variable and its component variables, we used a residualized Neighbour×Volume product term (Lance 1988; Kline 1998) to represent the interaction between neighbour presence and volume in all path models. The final parsimonious models were achieved based on W-statistic and  $\chi^2$  test (Epstein *et al.* 1994). These analyses were conducted in R using the lavaan package (Rosseel 2012).

### 3.3 Results

#### 3.3.1 Effects on plant biomass

Linear mixed models, with only neighbour presence and rooting volume as independent variables, showed that increasing rooting volume significantly increased the mass of all plant organs (Table 3.2, Fig. 3.1a-e). Over the full range considered, a doubling of rooting volume increased plant root mass by on average 18% and total mass by 11%. The presence of a belowground neighbour, on the other hand, had significant negative effects on plant growth, in terms of all biomass measurements (Table 3.2, Fig. 3.1a-e). It reduced plant root mass by an average of 21% and total mass by 9%. However, the differences between owners and sharers in root and total mass declined as the rooting volume increased (Fig. 3.1a,c), and the difference in

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total mass seemed to disappear at the largest rooting volume, as indicated by the significant Neighbour  $\times$  Volume interactions (Table 3.2).

**Table 3.2** Summary of the effects of neighbour presence, rooting volume and their interaction on plant biomass and allocation (i.e. mass fraction) in linear mixed models

	Neighbour presence	Rooting volume	Neighbour $\times$ Volume
Total mass	22.54***	59.09***	5.37*
Vegetative mass	20.54***	57.78***	3.3
Root mass	47.22***	82.36***	5.55*
Shoot mass	6.61*	33.30***	1.82
Pod mass	9.40**	18.61***	4.37*
Root mass fraction	28.46***	36.91***	0.82
Shoot mass fraction	0.36	3.93*	< 0.01
Pod mass fraction	6.78**	23.88***	0.28

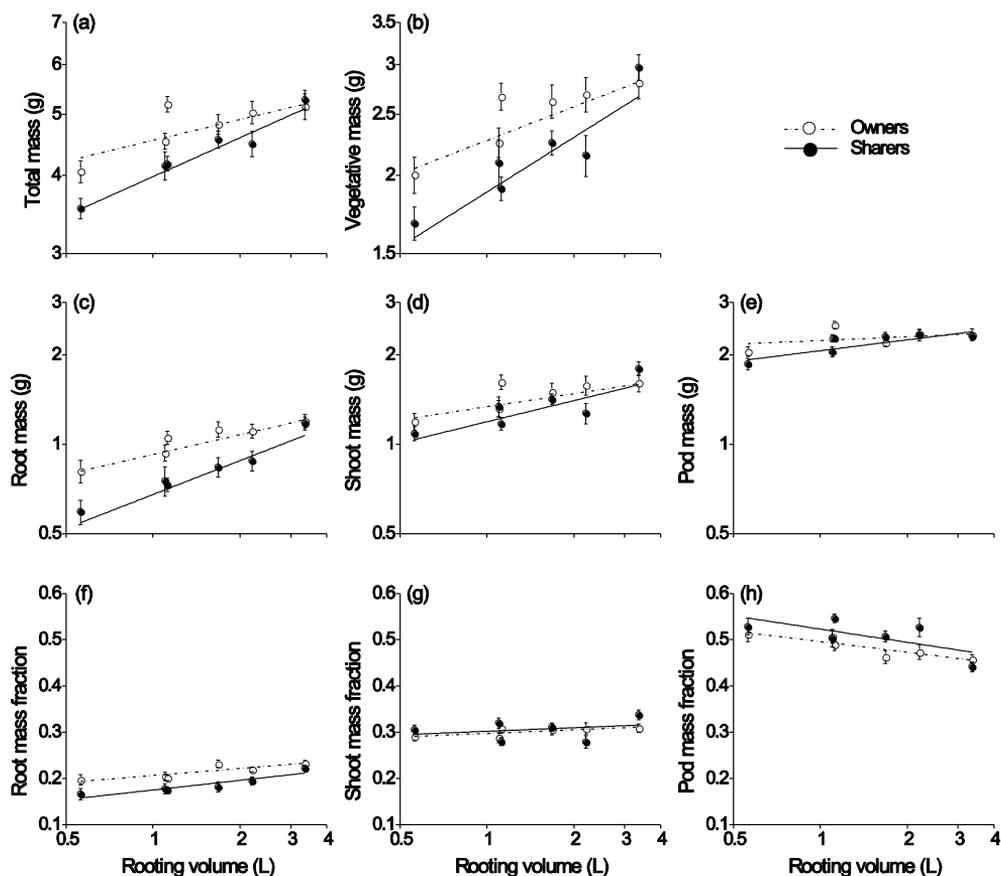
*F* values and significance levels (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ) are given, based on type III sums of squares and Kenward-Roger's approximation for the degrees of freedom.

Including plant vegetative mass in addition to rooting volume and neighbour presence in a path analysis (Fig. 3.2a), we found that pod mass was only directly and positively affected by plant vegetative mass, i.e. there were no significant direct effects of neighbour presence or rooting volume on pod mass. The negative effects of neighbour presence (standardized effect = -0.10,  $P < 0.001$ ) and positive effects of increasing rooting volume (standardized effect = 0.18,  $P < 0.001$ ) on pod mass were all indirectly mediated by their influences on plant vegetative mass. There was no significant Neighbour  $\times$  Volume interaction (standardized effect = 0.04,  $P = 0.078$ ) on pod mass.

### 3.3.2 Effects on allocation patterns

From the linear mixed models - where plant size effects were not considered - we found that root and shoot mass fractions increased with increasing rooting volume, while pod mass fraction decreased (Table 3.2, Fig. 3.1f-h). For a given volume, sharers consistently had significantly lower root mass fractions and higher pod mass fractions than owners (Table 3.2, Fig. 3.1f,h), while shoot mass fractions were not significantly different (Table 3.2, Fig. 3.1g). An analysis of the root mass fraction considered on

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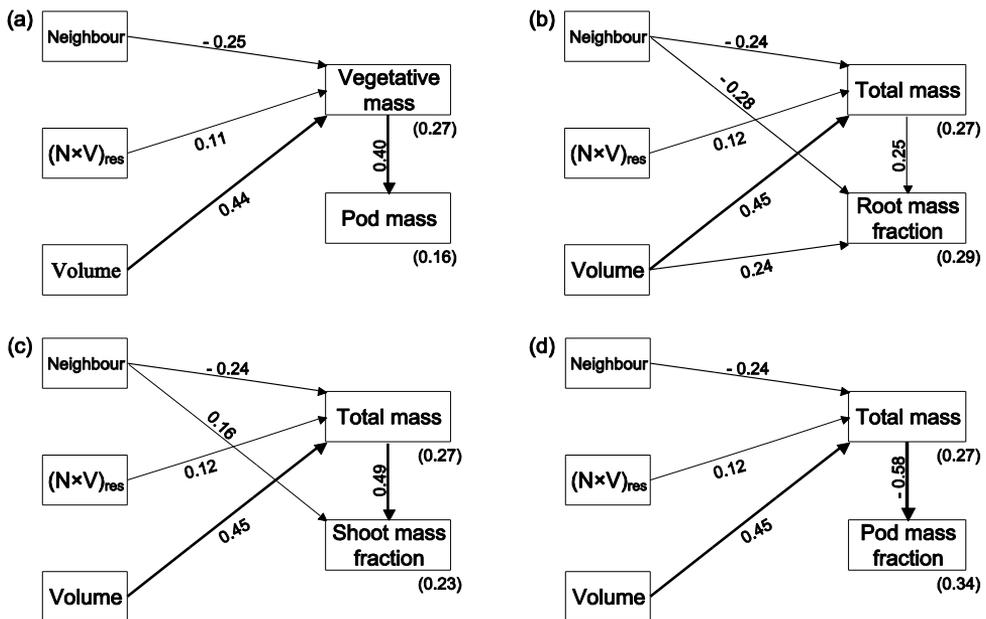


**Fig. 3.1** (a) Total mass, (b) vegetative mass, (c) root mass, (d) shoot mass, (e) pod mass, (f) root mass fraction, (g) shoot mass fraction and (h) pod mass fraction of owner and sharer plant individuals responding to the increase of rooting volume. The fitted lines are based on the results of linear mixed models. The error bars denote 1 s.e. above and below the means.

the basis of vegetative mass only basically showed the same result, with sharers allocating relatively less mass to roots than did owners (Supporting Information Fig. S3.3).

From the path analyses - where both plant total mass and volume were included - we found that total plant mass had direct positive effects on root and shoot mass fractions, but a negative effect on pod mass fraction (Table 3.3). Neighbour presence reduced root mass fraction both via its negative direct effect, and via its indirect effect by reducing total mass (Table 3.3, Fig.

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**Fig. 3.2** The final parsimonious path models for the effects of neighbour presence and rooting volume on (a) pod mass through vegetative mass (the sum of root and shoot mass); and on allocation patterns, in terms of (b) root, (c) shoot and (d) pod mass fractions, through total mass. Arrows indicate the direction of paths. For each path, standardized coefficient is given and the magnitude of the coefficient is also indicated by the thickness of the line. Values in brackets denote the goodness of fit (R<sup>2</sup>) for endogenous variables. The residualized Neighbour × Volume production term ((N×V)<sub>res</sub>) was used to represent the interaction between neighbour presence and rooting volume.

3.2b). The positive direct effect of neighbour presence on shoot mass fraction was cancelled out by the total mass mediated negative indirect effect, yielding a neutral total effect on shoot mass fraction (Table 3.3). Meanwhile, neighbour presence only had positive indirect effects on pod mass fraction via reducing total mass (Table 3.3, Fig 2d). Increasing rooting volume had both direct and indirect positive effects on root mass fraction (Table 3.3, Fig. 3.2b). Through the positive effects on total mass, increasing rooting volume indirectly increased shoot mass fraction, while reducing pod mass fraction (Table 3.3, Fig. 3.2c,d). The absence of significant direct or indirect effects of Neighbour×Volume interaction on any organ mass fraction (Table 3.3) suggested that neighbour presence and rooting volume affected mass fractions independently. Therefore, despite some differences, the results from linear mixed models and path models consolidated the

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conclusion that our pea plants invested relatively less mass in roots in the presence of a belowground neighbour.

**Table 3.3** Summary of the standardized direct, indirect and total effects of neighbour presence, rooting volume, their interaction and total mass on plant biomass allocation (i.e. mass fractions) in path analyses

Effects	Root mass fraction	Shoot mass fraction	Pod mass fraction	
Neighbour	direct	-0.28***	0.16**	–
	indirect	-0.06**	-0.12***	0.14***
	total	-0.34***	0.04	0.14***
Volume	direct	0.24***	–	–
	indirect	0.11**	0.22***	-0.26***
	total	0.35***	0.22***	-0.26***
$(N \times V)_{\text{res}}\dagger$	direct	–	–	–
	indirect	0.03	0.06	-0.07
	total	0.03	0.06	-0.07
Total mass	direct	0.25***	0.49***	-0.58***
	indirect	–	–	–
	total	0.25***	0.49***	-0.58***

Direct effects are equal to the standardized path coefficients shown in Fig. 3.2. Total effects are the sum of direct and indirect effects.

† Residualized Neighbour×Volume production term  $((N \times V)_{\text{res}})$  was used to represent the interaction between neighbour presence and rooting volume. – denotes that the effects were not included in the final parsimonious path models, due to their non-significance or non-existence. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

## 3.4 Discussion

Through a novel approach that combines a split-root experiment across a wide range of rooting volumes with path analyses, we found that our pea plants allocated less mass to roots when exposed to belowground neighbours and this result was independent of potential confounding effects of plant size and rooting volume. Neighbour presence reduced plant pod mass mainly through its negative effects on plant size. These results are

opposite in direction to the tragedy of the commons scenario proposed by Gersani *et al.* (2001), who showed that neighbour presence stimulated more allocation to roots at the expense of reproduction. Below, we discuss how we disentangled the neighbour presence effects on plant biomass and allocation patterns from the effects of rooting volume and the impacts of plant size. Then, we discuss the potential reasons for the discrepancies of neighbour-induced root responses from the literature.

### 3.4.1 The impacts of plant size

Our results confirmed that plant size has important impacts on biomass allocation patterns (Evans 1972; Coleman *et al.* 1994; Weiner 2004; Poorter *et al.* 2012b; Poorter & Sack 2012). Plant total mass was positively related to both root and shoot mass fractions, but negatively to pod mass fraction (Fig. 3.2b-d), although greater pod mass was associated with greater vegetative mass (Fig. 3.2a). This size-dependent reproductive effort might simply reflect the intrinsic relationship between vegetative and reproductive growth of the plants (Samson & Werk 1986; Weiner 1988). Such changes in allocation patterns due to size effects have consequences for the way changes in total root mass are interpreted. The current study revealed that the effects of neighbour presence and rooting volume on biomass allocation are at least partly mediated by their effects on plant size. Our data therefore shows that not including plant size could lead to biased conclusions, whereby plant size effects either are wrongly attributed to rooting volume or neighbour presence, or mask direct effects of these factors.

### 3.4.2 Rooting volume effects

In our study root growth increased with increasing rooting volume, which is consistent with various other studies (Gurevitch *et al.* 1990; McConnaughay & Bazzaz 1991; McConnaughay *et al.* 1993; Xu *et al.* 2001; Poorter *et al.* 2012a; but see Markham & Halwas 2011; Murphy *et al.* 2013). The path analysis also showed a direct positive effect on allocation to roots. However, contrary to the assumption that total mass is only determined by nutrient amount (e.g. Hess & de Kroon 2007; McNickle & Brown 2014), our study showed that it also increases with rooting volume (also found in e.g. McConnaughay & Bazzaz 1991; McConnaughay *et al.* 1993; but see Markham & Halwas 2011). A possible explanation is that photosynthetic

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carbon gain is more restricted in smaller pots, owing to less sink strength of smaller root system or greater temperature fluctuation in the pots (Poorter *et al.* 2012a and references therein).

It is noted that by keeping nutrient amount per plant constant, nutrient concentrations at very small volumes might reach toxic levels that inhibit plant growth. However, our calculations (nitrogen based) showed that in our experiment the highest concentration was similar to saturating pots daily with *c.* 3% standard Hoagland solution, which is much lower than the low nutrient treatment (10%) used in previous split-root experiments (Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005). We did not observe any signs of toxicity either. Thus, this suggests that the toxic effects due to small rooting volumes did not play a role in our study. At the other end, the positive response of root mass to increasing volume would eventually level off and even become reversed at extremely large volumes, simply because nutrient concentrations become so low that this imposes a limitation on root growth (O'Brien & Brown 2008). In our case, however, root growth and plant size continuously increased with volume indicating that this effect did not play a role either at the range of volumes that we used. Together, we conclude that our results were not seriously affected by the variation of nutrient concentration.

Therefore, our results do confirm the concerns that greater root mass or root allocation of sharer plants in previous split-root studies (Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005) could at least be partially attributed to their larger rooting volume (e.g. Schenk 2006; Hess & de Kroon 2007; Semchenko *et al.* 2007a), albeit in part due to a change in plant size. In general, this cautions against attributing changes in allocation to belowground interactions when part of the treatment effect is limiting the rooting volume.

### **3.4.3 Neighbour presence effects**

If the effect of a neighbour would be no more than a reduction of nutrient availability of plants, then keeping nutrient amount per plant constant in our experiment should have yielded a similar level of total mass in owners as in sharers when they are compared at the same rooting volume. However, our study clearly showed that sharers still had less total mass than owners, suggesting that neighbour presence can affect plant growth even when nutrient availability per plant remains unaltered. Apparently, this effect was

not caused by nutrient toxicity (as discussed above) or water limitation, as we supplied sufficient water throughout the experiment. Furthermore drought stress would have likely induced an increased root mass fraction (RMF) in sharers while we observed a reduction in this trait.

A possible explanation might be that pea plants growing with neighbours enhance root respiration and increase root secretion. In a study with pea Meier *et al.* (2013) observed a 29% increase on oxygen consumption by the root system pointing to a considerable increase in respiration when plants were exposed to neighbours. The cause of this increase in respiration remained unclear but it could be associated with increased activity of roots, i.e. the secretion of soluble carbon compounds or a neighbour-induced stress response (Meier *et al.* 2013). This could lead to extra carbon loss in sharers but not in owners, and consequently a lower total mass in sharers as was also observed by Meier *et al.* (2013). A second explanation could be associated with allelopathy: the production of substances by plants that inhibit growth of other plants (Muller 1969). Intra-specific allelopathy (also known as auto-toxicity) has been documented for several species (Putnam 1985; Perry *et al.* 2005; Wu *et al.* 2007). The difference in total plant mass between owners and sharers appeared to decrease with increasing rooting volume. This could be associated with the dilution of allelopathic substances. However, to our knowledge it is unknown whether intra-specific allelopathy plays a role in pea.

Explicitly taking rooting volume and plant size into account, our study reveals that our pea plants allocated *less* mass to roots when exposed to belowground neighbours. This is contrary to the tragedy of the commons (TOC) scenario, i.e. more root allocation in sharers than in owners, predicted by a game theoretical model (Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005). It is also different from the neutral response scenario, i.e. no difference in root allocation between sharers and owners, predicted by an ideal free distribution model (McNickle & Brown 2014).

### 3.4.4 Potential reasons for divergent findings of neighbour-induced root responses

We suggest that there might be no real difference in the observations of neighbour-induced root responses between our study and studies showing neutral response scenario. We showed that, in general, a doubling of rooting volume increased root mass by 18% and total mass by 11%; while the presence of a neighbour reduced root mass by 21% and total mass by 9%.

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This indicates that, in an experiment providing sharers with twice the rooting volume as owners, the effects of rooting volume and neighbour presence might counteract each other, yielding similar root mass and total mass between owners and sharers. Therefore, it might well explain the outcomes of studies finding a neutral response (e.g. Semchenko *et al.* 2007a; Lankinen 2008; Markham & Halwas 2011; Nord *et al.* 2011; McNickle & Brown 2014).

However, this ‘counteractive effects hypothesis’ cannot explain the discrepancies between our study and the studies showing a TOC scenario (e.g. Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005), especially the one using the same species (*P. sativum*) as ours from O'Brien and her colleagues. After carefully checking the methods employed in these TOC studies, we noticed that their nutrient levels (10%, 50% and 60% standard Hoagland solution) are much higher than ours (as discussed above). We suggest that nutrient application in their experiments might play an important role in stimulating these divergent outcomes.

Firstly, the level of nutrient application may affect rooting responses of plants to neighbours. For example, Cahill *et al.* (2010) showed that *Abutilon theophrasti* preferentially grows roots towards neighbours in resource-rich patches, but allocates roots away from neighbours in poor soil. Based on a cost-benefit balance assessment for nutrient uptake, a recent modelling study predicts that when plants grow with neighbours, more root production is favoured in resource-rich conditions while less root production is favoured in resource-poor conditions (McNickle & Brown 2012). Consequently, growth and allocation patterns of plants in response to belowground neighbours may also depend on nutrient level. This would suggest that the occurrence of a TOC scenario is limited to resource-rich environments. As noted, the fact that Gersani *et al.* and others did their experiments at very high nutrient availability fits this explanation. This might also explain why the observations of root segregation between neighbouring plants mainly come from resource-poor communities (e.g. Mahall & Callaway 1991; Schenk *et al.* 1999).

In addition, the way of nutrient application (e.g. see fertilizer-type effects in Murphy *et al.* 2013; substrate-type effects in Dalling *et al.* 2013) may also need to be considered. Studies showing TOC responses all utilized a procedure whereby potting substrates were frequently saturated and flushed with nutrient solutions and water (Gersani *et al.* 2001; Maina *et al.* 2002; O'Brien *et al.* 2005). On the other hand, studies finding no such responses

did not flush substrates (e.g. Semchenko *et al.* 2007a; Lankinen 2008; Markham & Halwas 2011; Nord *et al.* 2011; McNickle & Brown 2014 and the current one). Unlike field conditions where typically a 0 nutrient flush is followed by nutrient depletion as a result of uptake or immobilisation (Olf *et al.* 1994; Hodge *et al.* 2000), frequent saturation and flushing in the experiments likely result in leaching of water-soluble compounds (e.g. self-inhibitory signals, Sachs 1997; Falik *et al.* 2005; Semchenko *et al.* 2007a) and elimination of neighbour-induced nutrient depletion, and thus affect the balance of cues for root growth, which might subsequently change the growth responses to the presence of belowground neighbours. Thus in future experiments on neighbour induced root responses, the nutrient level and the way nutrients are applied should be taken into account.

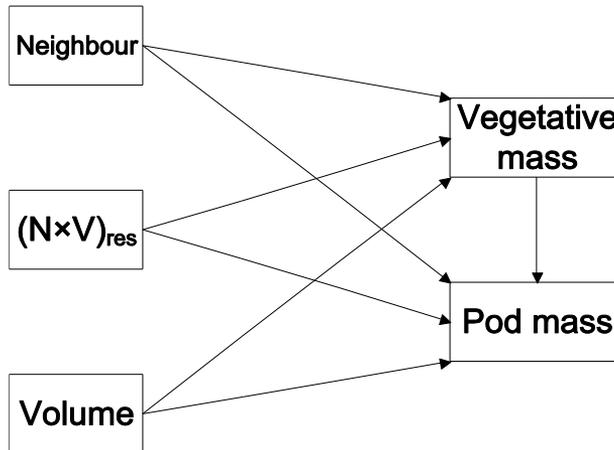
### 3.5 Conclusion

By including plant size, and explicitly including interactive effects with volume and neighbour presence, we have succeeded in unravelling the underlying mechanism of allocation changes in responses to neighbours. We demonstrate that the presence of a neighbour can directly reduce root allocation and plant size; enlarging rooting volume can directly increase root allocation and plant size; plant size is positively correlated with root allocation, but negatively associated with pod allocation. Together our results provide evidence for neighbour-induced root responses but no evidence for a tragedy of the commons. We further argue that neighbour-induced root responses may depend on the level and way nutrients applied, an issue that needs to be further investigated.

### Acknowledgements

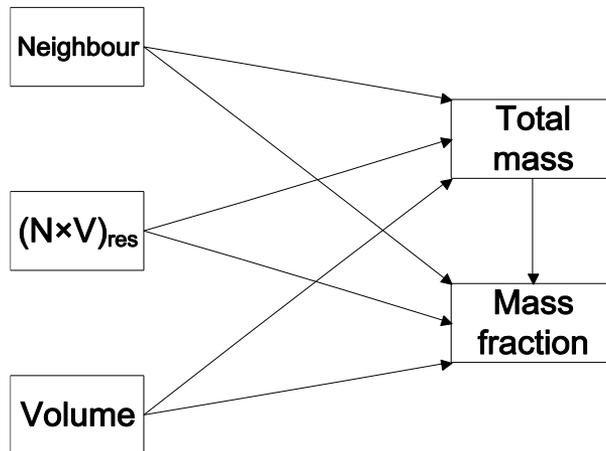
We are grateful to G.A. Kowalchuk and C. Xu for valuable comments. We thank J.C. Douma and Z.Y.X. Huang for statistical help. We appreciate F.H.J. Siesling, G.P. Verduyn and I.S. Roeling for the experimental assistance. Financial support was provided by a PhD fellowship (2010619022) from China Scholarship Council to B.C.

**Supporting information**



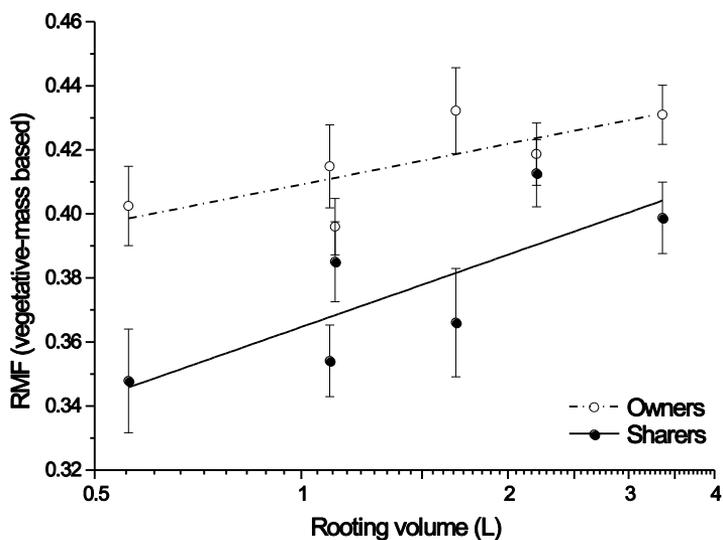
**Fig. S3.1** Full path model for the direct and indirect (via vegetative mass) effects of belowground neighbour presence and rooting volume on plant pod mass. Residualized Neighbour $\times$ Volume product term ( $(N \times V)_{res}$ ) represents the interaction between neighbour and volume.

## Neighbour and volume affect plant growth



**Fig. S3.2** Full model for the direct and indirect (via total mass) effects of belowground neighbour presence and rooting volume on plant allocation patterns, in terms of mass fraction of root, vegetative shoot or pod. Residualized Neighbour $\times$ Volume product term  $((N \times V)_{res})$  represents the interaction between neighbour and volume.

3



**Fig. S3.3** Vegetative-mass based root mass fractions (RMF) of owner and sharer plants as depending on rooting volume. The fitted lines are based on the results of a linear mixed model, which shows the vegetative-mass based RMF was significantly reduced by neighbour presence ( $F = 29.16$ ,  $P < 0.001$ ), significantly increased by enlarging rooting volume ( $F = 17.65$ ,  $P < 0.001$ ), but not affected by Neighbour $\times$ Volume interaction ( $F = 1.39$ ,  $P = 0.240$ ). The error bars denote 1 SE above and below the means.

# Chapter 4

**Physiological self-recognition  
persists  
over long distances  
within the clone of *Potentilla reptans***

*with P.J. Vermeulen, H.J. During & N.P.R. Anten*

Submitted

### Abstract

- Plant roots are able to detect the presence of their neighbours. This enables plants to engage in resource competition with non-self roots while avoiding self-competition. In clonal plants, self/non-self recognition even occurs between genetically identical individuals. It has been suggested that the responses to such self-recognition gradually disappear in disconnected sister ramet pairs, and also weaken with distance along the clone. However, these results may partly be due to side effects in the experimental design, caused by the severance of connections.
- We first tested for such side effects in an experiment where ramet pairs of *Potentilla reptans* were grown with their connections either intact or severed. Then we examined the existence of physiological self/non-self recognition and the effect of distance along the clone in another experiment that avoided severance effects. To this end, we grew ramets, remaining attached to clonal fragments, with a closely or remotely connected ramet from the same clonal fragment, or with a disconnected ramet from a different fragment of the same genotype.
- In the first experiment, severing connections mainly reduced the growth of younger ramets within the pairs. In the second experiment, ramets grown with a disconnected neighbour had similar amount of root mass but less vegetative propagation than those grown with a connected neighbour. Ramets grown with a closely or remotely connected neighbour had no significant difference in biomass allocation.
- Our first experiment suggests that the severance of connections strongly affects the growth and allocation of ramets in a way that is likely due to the disruption of source-sink relationships within the pairs. Our second experiment provides evidence of physiological self/non-self recognition, and suggests that self-recognition can persist over long distances within the clones of *P. reptans*.

**Key words:** evolutionary game theory; neighbour detection; *Potentilla reptans*; physiological coordination; physiological integration; root interaction; self/non-self recognition; severance effect; source-sink relationship; vegetative propagation

### 4.1 Introduction

One of the fascinating discoveries in plant biology in recent years is that plant roots are able to detect the presence and even recognize the relatedness of their neighbours (reviewed in **Chapter 2**), independently of soil nutrient status (Mommer *et al.* 2010; Padilla *et al.* 2013; Schmid *et al.* 2013). In an evolutionary game-theoretical context, it enables plants to over-proliferate roots for a greater capture of shared resources against non-self roots while avoiding unprofitable competition with self roots (e.g. Gersani *et al.* 2001; O'Brien *et al.* 2007). Interestingly, responses to non-self roots not only occur between genetically different plants (Dudley & File 2007; Biedrzycki *et al.* 2010; Fang *et al.* 2013), but also take place between genetically identical but physically independent individuals (Falik *et al.* 2003). The latter scenario might be particularly important for clonal plants. Such plants produce vegetative offspring (ramets) connected by stolons or rhizomes (de Kroon & van Groenendael 1997), and these connections in turn disintegrate over time or after disturbance (Hutchings & Mogie 1990). Thus, within clonal patches there are likely to be intensive root interactions between genetically identical ramets that are closely or remotely connected to the same clone, and that are located on different fragments which have been disintegrated from one clone.

Recent studies have shown that severing connections between sister ramets grown together in a pot will induce greater root growth at the expense of above-ground performance (e.g. Holzapfel & Alpert 2003; Gruntman & Novoplansky 2004; Falik *et al.* 2006; Roiloa *et al.* 2014). Their results were believed to show that these connected ramets exhibit self-recognition mediated by physiological coordination, which can be disrupted due to the loss of connections (Gruntman & Novoplansky 2004; Falik *et al.* 2006). Interestingly, a study of *Buchloe dactyloides* also showed that two ramet-halves originating several nodes apart on the stolon produced more root mass when grown together than two halves originating from the same node (Gruntman & Novoplansky 2004). From this, the authors suggested that physiological self-recognition can fade with the distance between two units along the clone. However, so far, other studies testing such physiologically based recognition including distance effect are still lacking.

Moreover, the procedure of comparing intact (i.e. connected) and twin (i.e. severed or disconnected) ramet pairs can be criticized. Severing

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connections between sister ramets may affect plant growth in more ways than just preventing the transduction of self-identity signals. It disrupts resource and hormone transportation (e.g. Ashmun *et al.* 1982; Hartnett & Bazzaz 1983; Alpert 1996; Hutchings 1999; van Kleunen & Stuefer 1999; van Kleunen *et al.* 2000; Alpert *et al.* 2002; Semchenko *et al.* 2007b), as well as the potential for division of labour (Roiloa *et al.* 2014). Therefore, experiments employing a sudden severance of connections to create twin pairs may induce effects that are not associated with physiologically based self-recognition. Furthermore, each pair always consists of a developmentally younger and an older ramet. Yet, the differences in growth and in responses to severing connections between the younger and older ramets have been seldom considered (but see Roiloa *et al.* 2014).

The objectives of our study are (1) to investigate the effects of severing connections on ramet growth with an “intact/twin pair” setup; (2) to examine the existence of physiological self/non-self recognition; and (3) to determine whether self-recognition depends on distance along the clone. We used the clonal species *Potentilla reptans* as our model plant. This species is highly plastic to both local and non-local environmental cues in above- and below-ground parts (e.g. Stuefer *et al.* 1994; He *et al.* 2011; Wang *et al.* 2013), suggesting that it is able to locally adjust below-ground allocation, making it suitable for our study.

For the first objective, we conducted an experiment whereby ramet pairs were grown in pots in the traditional way, i.e. their connections were either severed or kept intact. For the second and third objectives, we conducted another experiment whereby ramets, remaining attached to larger clonal fragments, were grown with closely connected ramets (adjacent sisters from the same stolon), remotely connected ramets (sisters from different stolons but from the same mother ramet), and disconnected ramets (from different mothers that share the same genotype). This second experimental setup avoids the severance of connections and keeps ramets integrated with their maternal fragments, which is more in line with the way root interaction may occur in natural vegetation. Based on the suggestion that physiological self-recognition between genetically identical ramets can be disrupted by separation (Holzapfel & Alpert 2003; Gruntman & Novoplansky 2004; Falik *et al.* 2006) and alienated by long distances (Gruntman & Novoplansky 2004) with a consequence of greater root production, we hypothesize that in our second experiment:

- I. Ramets have greater root mass when grown with disconnected neighbours than when grown with closely connected neighbours;
- II. Ramets have greater root mass when grown with remotely connected neighbours than when grown with closely connected neighbours.

## 4.2 Materials and methods

### 4.2.1 Plant material and propagation

The experiments were carried out with the stoloniferous perennial species *Potentilla reptans* (Rosaceae). Its common habitats include river and lake shores, moderately disturbed pastures, mown grasslands and road margins (van der Meijden 2005). The plant produces sympodially growing stolons with rooted rosette-forming ramets on the nodes. Without strong disturbance, the connections (i.e. internodes) between ramets will function throughout one growing season (Stuefer *et al.* 2002).

In April 2013, plants from one genotype were propagated in a greenhouse at Wageningen University, Wageningen, the Netherlands. After two months, new-grown rootless ramets were individually pinned in pots (1.0 L, with potting soil). Two weeks later, when root systems were initiated, these ramets were severed from the stock plants and designated as mother ramets. They were then propagated for another month. To promote growth, each pot (i.e. each mother ramet) received nutrient solution (7.79 mM NO<sub>3</sub><sup>-</sup>, 1.1 mM NH<sub>4</sub><sup>+</sup>, 1.5 mM PO<sub>4</sub><sup>3-</sup>, 5.11 mM K<sup>+</sup>, 3 mM Ca<sup>2+</sup>, 1.0 mM SO<sub>4</sub><sup>2-</sup>, 0.87 mM Mg<sup>2+</sup> and micronutrients) three times (60 ml per occasion) in this period. One month later, when mother ramets were well developed and had produced several stolons bearing rootless daughter ramets without access to soil, these mother ramets were used in the two following experiments.

### 4.2.2 Expt I: the effects of severing connections

This experiment, which was started on 29 July 2013, followed the traditional setup in which root recognition has been studied in the past, whereby connections in ramet pairs are severed (e.g. Holzapfel & Alpert 2003; Falik *et al.* 2006; Roiloa *et al.* 2014). From each mother ramet, we selected stolons that bore eight to ten rootless daughter ramets. On each selected stolon, the third (younger) and fourth (older) rootless daughter

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ramets counting from the apex were chosen when both of them had three newly formed leaves and had not produced their own stolons yet, in order to standardize the developmental stages throughout all replicates.

Then, the chosen pairs were pinned in pots (1.0 L) that were filled with a sand-soil mixture (river sand and sieved nutrient-poor arable soil in 1:1 volume ratio). One week later, these newly rooted ramet pairs were severed from their maternal stolons. Subsequently, for half of these pairs the connections were left intact (intact pair treatment, Fig. 4.1A), while for the other half their connections were severed (twin pair treatment, Fig. 4.1B). Plants were grown under ambient light conditions (c. 80% of full sunlight) and watered daily in a plastic-roof-only tunnel, from August to September in 2013. Each treatment consisted of 20 replicates. To promote root competition, no additional nutrients were given in this period. During the experiment, root production on new ramets was prevented.

After 40 days, all plants were harvested and divided into roots, leaves and stolons (including attached new ramets). New ramets were subsequently separated from the stolons. During the harvesting, the two root systems within each pair were carefully separated, washed and assigned to individual ramets (i.e. the younger and the older) together with leaves and stolons. All materials were weighed after three days of oven-drying at a temperature of 70 °C.

We first examined the effects of severing connections (i.e. intact and twin) on the biomass of ramet pairs at pot level, using linear models. Then we examined the severance effect on individual ramets, taking age (i.e. younger and older) into account. The tests were done using linear mixed models with severance, age and their interaction as fixed factors and with pot as a random factor.

### **4.2.3 *Expt II*: physiological self/non-self recognition**

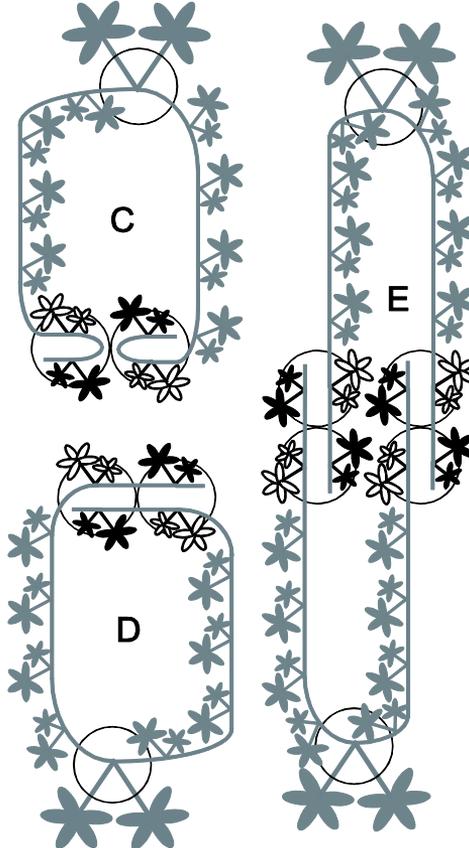
This experiment was started on 7 August 2013. For each mother ramet, we only kept two stolons that bore eight to ten rootless daughter ramets (the same standard as in *Expt I*) with all the other stolons removed. On each selected stolon, the sixth (younger) and fifth (older) rootless daughter ramets counting from the mother ramet were selected when both of them had three leaves but had not produced stolons, in order to standardize the distances between mothers and daughters in all replicates, and also to be comparable

## Physiological self-recognition in clonal plants

*Expt I:*



*Expt II:*



**Fig. 4.1** Illustrations for *Expt I* (the effects of severing connections) and *Expt II* (physiological self/non-self recognition) using *Potentilla reptans*. In *Expt I*, younger (black leaves) and older (white leaves) ramets were grown in (A) intact and (B) twin pairs. In *Expt II*, younger (black leaves) and older (white leaves) ramets, remaining attached on the stolons of mother ramets, were grown in (C) closely connected, (D) remotely connected and (E) disconnected interactions. Circles represent pots. The grey parts in *Expt II* stand for maternal size (including the mother ramet and its two stolons attached with the rest of the ramets). For clarity, the secondary structures of younger and older ramets are not shown.

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with the developmental stages of selected daughter ramets in *Expt I*, considering their positions on the maternal stolons.

Subsequently, we introduced three types of root interactions between genetically identical ramets: (1) closely connected interaction (CCI), whereby a pair of adjacent younger and older daughter ramets from the same maternal stolon were pinned in one pot (Fig. 4.1C); (2) remotely connected interaction (RCI), whereby one younger and one older daughter ramet from different stolons of the same mother were pinned in one pot (Fig. 4.1D), representing a distance effect; (3) disconnected interaction (DCI), whereby one younger and one older daughter ramet from different mothers with the same genotype were pinned in one pot (Fig. 4.1E), representing a separation effect. In addition, before the start of the experiment, the first to fourth daughter ramets on each maternal stolon were left untouched but were not placed in pots to prevent rooting, while daughter ramets distal to the sixth (i.e. the younger one) on each maternal stolon were removed. With this setup, we were able to standardize the internal growth conditions (i.e. physiological integration within one clonal fragment) for all younger and all older ramets throughout the whole experiment. Plants were grown in the same tunnel with the same kind of pots filled with the same type of sand-soil mixture as used in *Expt I*. Each type of treatment consisted of 18 replicates.

After 40 days, all clonal fragments were harvested. During the harvesting, younger and older daughter ramets rooted in pots were first marked and severed from the fragments, and were then separated and treated following the same protocol as in *Expt I*. The remaining parts of the clonal fragment (i.e. the mother ramet and its two stolons attached with the rest of the ramets) were collected and assigned together as maternal size. All materials were weighed after three days of oven-drying at a temperature of 70 °C.

We first examined the effects of root interaction treatments on the total mass and maternal size of clonal fragments, using linear mixed models with root interaction (i.e. CCI, RCI and DCI) as a fixed factor and treatment unit as a random factor. Subsequently, we examined whether the pot-level biomass measures of root-interacting ramet pairs (i.e. two ramets in one pot) differed among root interaction treatments. Since the growth of a ramet also depends on the size of the clonal fragment to which it is attached (Birch & Hutchings 1999), the effect of maternal size was also included in the analyses as a covariate. The tests were done using linear mixed models with root interaction, maternal size and their interactive effect as fixed factors,

and treatment unit as a random factor. Each CCI and RCI treatment unit consisted of one clonal fragment, while each DCI treatment unit comprised two clonal fragments (see Fig. 4.1C-E). Hence, for every DCI treatment unit, the averaged maternal size of the two interacting clonal fragments was used. Finally, we examined the effects of root interaction treatments on the performance of younger and older ramets. Each target ramet grew in one pot with another ramet, but at the same time remained attached to a clonal fragment which included three other target ramets. Thus, the tests were done using linear mixed models with root interaction, age, maternal size and their interactive effects as fixed factors, with pot and clonal fragment as random factors.

For the analyses of both experiments, the best fitted models were selected using backward selection procedures based on full models, where the Akaike information criterion test was applied for difference in the fit of the nested models (Bozdogan 1987). Data were transformed when necessary. All statistical analyses were performed using lme4 (Bates *et al.* 2014) and lmerTest (Kuznetsova *et al.* 2014) packages in R version 3.1.0 (R Core Team 2014).

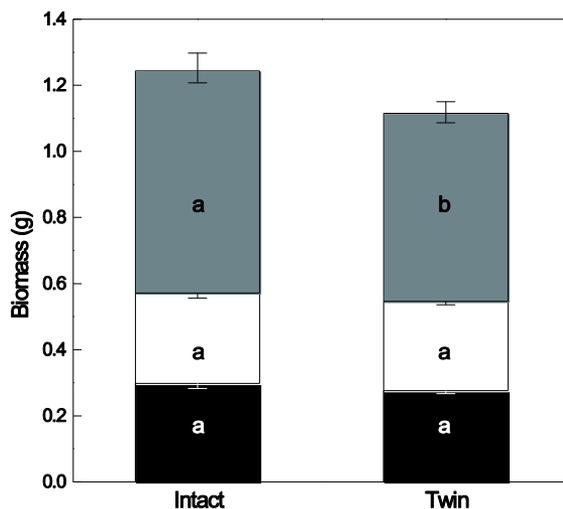
## 4.3 Results

### 4.3.1 *Expt 1*: the effects of severing connections

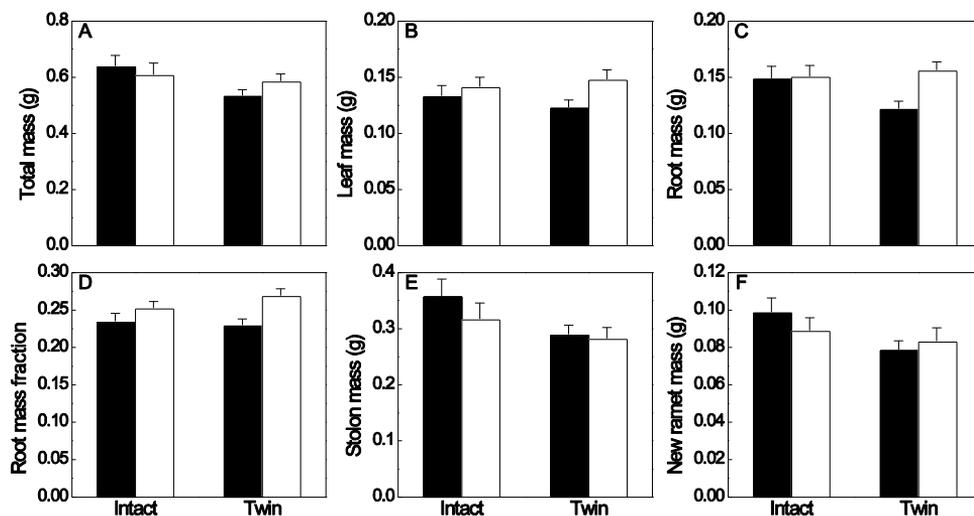
Severance significantly reduced the stolon (including both internodes and new ramets) mass of ramet pairs at pot-level, but had no effect on root or leaf mass (Table 4.1, Fig. 4.2). As a result, it marginally reduced the total mass of ramet pairs (Table 4.1, Fig. 4.2).

When taking age into account, older ramets in general had greater leaf mass, greater root mass and higher root mass fractions than younger ramets (Table 4.1, Fig. 4.3B-D). Severance of connections significantly reduced root mass of younger ramets but had no effect on that of older ramets, as indicated by the significant severance  $\times$  age interaction (Table 4.1, Fig. 4.3C). Severance also significantly reduced stolon mass of ramets (Table 4.1, Fig. 4.3E). This was especially pronounced for the younger ones, though no significant severance  $\times$  age interaction was found. Overall, there was a trend that severance-induced biomass reductions mainly occurred in younger ramets (Fig. 4.3).

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**Fig. 4.2** The pot-level mean root (black bars), leaf (white bars) and stolon (grey bars) mass, and thus total mass of intact and twin ramet pairs in *Expt 1*. Error bars give 1 s.e. of component (downwards) and total (upwards) means. A difference in letter denotes a significant difference in root, leaf or stolon mass between treatments.



**Fig. 4.3** The individual-level mean (A) total mass, (B) leaf mass, (C) root mass, (D) root mass fraction, (E) stolon mass and (F) new ramet mass of younger (black bars) and older (white bars) ramets in intact and twin pairs in *Expt 1*. Error bars denote 1 s.e.

## Physiological self-recognition in clonal plants

**Table 4.1** Summary of statistics for the effects of severance, ramet age and their interaction in *Expt I*

Variables	(a) <i>Pot level tests</i>		(b) <i>Individual level tests</i>	
	Severance	Severance	Age	Severance×Age
Total mass	0.055	0.085	0.649	—
Root mass	0.283	0.243	<b>0.014</b>	<b>0.049</b>
Root mass fraction	0.314	0.551	<b>0.003</b>	—
Leaf mass	0.928	0.825	<b>0.026</b>	—
Stolon mass	<b>0.029</b>	<b>0.038</b>	0.339	—
new ramet mass	0.052	0.059	0.743	—
new ramet number	0.507	0.112	0.411	—

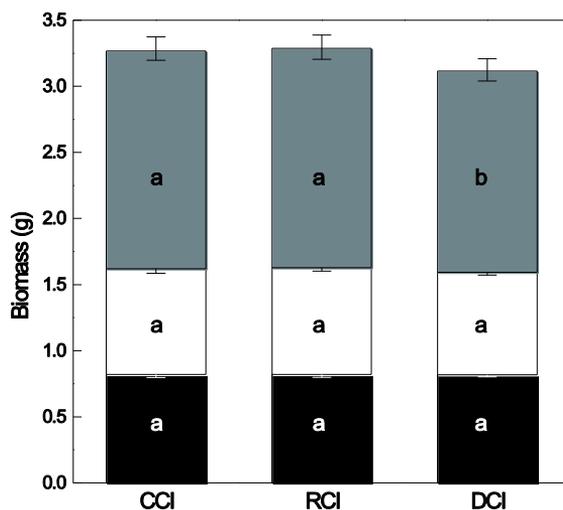
(a) Tests were done at pot (i.e. pair) level, using linear models. (b) Tests were done at ramet individual level when age effect was considered, using linear mixed models. “—” indicates that the interaction term, due to its non-significance and based on the Akaike information criterion test, was excluded in the selected model. *P* values (in bold when  $P < 0.05$ ) are given, based on type III sums of squares and Kenward-Roger’s approximation for the degree of freedom (in linear mixed models).

### 4.3.2 *Expt II*: physiological self/non-self recognition

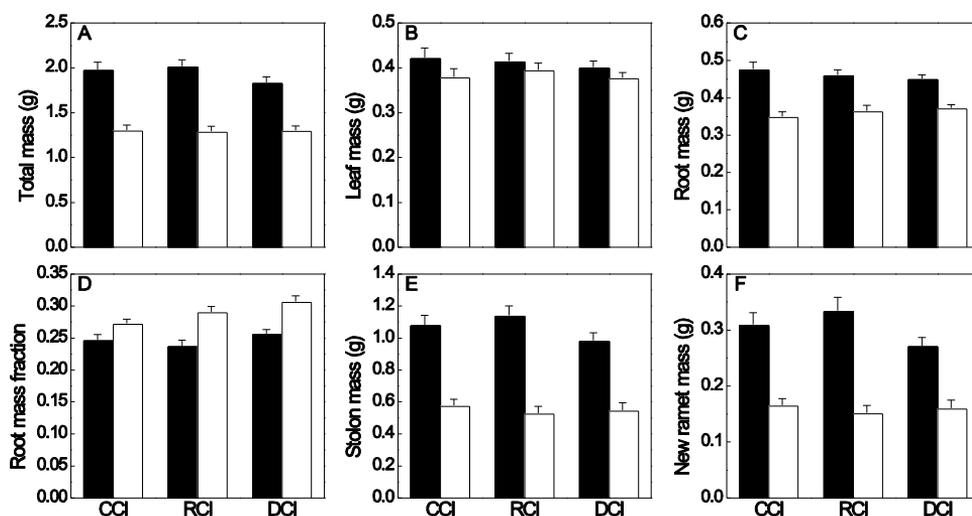
There was no significant difference in the total mass ( $F = 0.831$ ,  $P = 0.442$ ) or maternal size ( $F = 0.717$ ,  $P = 0.493$ ) at the level of the whole clonal fragments among three root interaction treatments. Stolon and total mass of root-interacting ramet pairs at pot-level, however, were significantly lower in the disconnected interaction than in the closely or remotely connected interaction (Table 4.2, Fig. 4.4). Root mass of ramet pairs in all treatments was only and positively correlated with maternal size (Table 4.2).

When age was considered, we found that younger ramets performed much better than older ones in all growth and fitness measures (Table 4.2, Fig. 4.5). Older ramets had significantly higher root mass fractions in all treatments (Table 4.2, Fig. 4.5D). The effects of root interaction treatments found at ramet individual-level in general were similar as those analysed at pot-level (Table 4.2). Although there were no significant interactive effects between root interaction and age, the effects of root interaction treatments tended to be stronger on younger ramets (Fig. 4.5). In addition, the stolon mass, new ramet production and total mass of both younger and older ramets were significantly and positively correlated with their maternal size only in disconnected interaction treatment, but not in other treatments (Fig. 4.6, Table 4.2).

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**Fig. 4.4** The pot-level mean root (black bars), leaf (white bars) and stolon (grey bars) mass, and thus total mass of root-interacting ramet pairs in closely connected (CCI), remotely connected (RCI) and disconnected interactions (DCI) in *Expt II*. Error bars give 1 s.e. of component (downwards) and total (upwards) means. A difference in letter denotes a significant difference in root, leaf or stolon mass between treatments.



**Fig. 4.5** The individual-level mean (A) total mass, (B) leaf mass, (C) root mass, (D) root mass fraction, (E) stolon mass and (F) new ramet mass of younger (black bars) and older (white bars) ramets in closely connected (CCI), remotely connected (RCI) and disconnected interactions (DCI) in *Expt II*. Error bars denote 1 s.e.

**Table 4.2** Summary of statistics for the effects of root interaction treatment (I), ramet age (A), maternal size (S) and their interactions in Expt II

Variables	(a) Pot level tests			(b) Individual level tests				
	I	S	I×S	I	A	S	I×S	A×S
Total mass	<b>0.027</b>	0.172	<b>0.033</b>	<b>0.026</b>	< <b>0.001</b>	0.167	<b>0.032</b>	<b>0.010</b>
Root mass	0.839	< <b>0.001</b>	—	0.757	< <b>0.001</b>	< <b>0.001</b>	—	—
Root mass fraction	<b>0.008</b>	0.357	<b>0.011</b>	<b>0.024</b>	< <b>0.001</b>	0.160	<b>0.035</b>	<b>0.006</b>
Leaf mass	0.741	0.831	—	0.688	<b>0.004</b>	0.669	—	—
Stolon mass	<b>0.006</b>	0.254	<b>0.008</b>	<b>0.018</b>	< <b>0.001</b>	0.444	<b>0.023</b>	<b>0.010</b>
new ramet mass	<b>0.025</b>	0.537	<b>0.031</b>	0.052	< <b>0.001</b>	0.760	0.066	<b>0.010</b>
new ramet number	<b>0.019</b>	0.775	<b>0.023</b>	<b>0.034</b>	< <b>0.001</b>	0.944	<b>0.042</b>	<b>0.002</b>

(a) Tests were done at pot (i.e. pair) level, using linear mixed models. (b) Tests were done at ramet individual level when age effect was considered, using linear mixed models. "—" indicates that the interaction term, due to its non-significance and based on the Akaike information criterion test, was excluded in the selected model. *P* values (in bold when *P* < 0.05) are given, based on type III sums of squares and Kenward-Roger's approximation for the degree of freedom.



### 4.4 Discussion

In *Expt I* we showed that the severance of connections between ramets in *Potentilla reptans* mainly reduced root and stolon mass of younger ramets. In *Expt II* where severance effects were excluded, we showed that ramets had significantly less stolon mass but a similar root mass when grown with disconnected genetically identical neighbours than with closely or remotely connected neighbours. These differences between connected and disconnected interaction treatments provide evidence of physiological self/non-self recognition. On the other hand, the similarity between closely and remotely connected interaction treatments indicates that this capacity for self-recognition is not affected by distance between ramets within the same clone of *P. reptans* in our study.

#### 4.4.1 Side effects of severing connections

As also found for other clonal species (e.g. *Fragaria chiloensis*, Holzapfel & Alpert 2003), twin pairs of *P. reptans* tended to have less total mass than intact pairs in *Expt I*. This was mainly due to a significant reduction in stolon mass (c.15%, Fig. 4.2). There was a trend that the negative effects of severance on biomass production mainly occurred in younger ramets (Fig. 4.3). For instance, severance reduced root mass of younger ramets but not that of older ones. These effects might reflect that if physiological integration mainly entails acropetal movement of resources (e.g. Chapman *et al.* 1991; Price & Hutchings 1992; Alpert 1996; D'Hertefeldt & Jónsdóttir 1999), younger ramets would suffer from the loss of support from the older ones. This could be further aggravated through competition effects. On the other hand, severance did not increase biomass of older ramets (Fig. 4.3), indicating that partial support to a younger ramet did not limit the growth of the older (also observed in *F. chiloensis*, Alpert & Mooney 1986). This suggests that, by importing assimilates from older ramets (Alpert *et al.* 2002; Semchenko *et al.* 2007b), younger ones act as sinks that may in turn stimulate the photosynthetic activities of older ramets (Roiloa & Retuerto 2005; Roiloa & Retuerto 2006; You *et al.* 2014). Together these results indicate that less stolon mass in twin pairs was likely to be the consequence of a reduction in growth rate caused by the disruption of physiological integration (e.g. Alpert & Mooney 1986; Birch & Hutchings 1999; van Kleunen & Stuefer 1999).

Our results also showed that biomass partitioning between connected younger and older ramets depended on whether they had been separated from the rest of clone systems (i.e. intact pairs in *Expt I*) or not (i.e. sister pairs with closely connected interaction treatment in *Expt II*). The total mass of younger ramets was similar to that of older ones in the former (Fig. 4.3A), but was remarkably greater in the latter (Fig. 4.5A). This difference might reflect the source-sink relationships at the level of physiological integration: in a separated pair the older ramet is the source and the younger is the sink, but when this pair is still attached to a clonal fragment, both older and younger ramets act more as sinks with their mother ramet being the major source. By remaining attached to a clonal fragment the sink strength of a younger ramet may have been stronger than that of the older one, which may explain the greater mass of the younger (Fig. 4.5A).

Together, the results indicate that separating ramets within the pairs or isolating ramet pairs from the rest of the clone through the severance of connections can both strongly affect the growth and allocation of ramets in a way that is not necessarily associated with physiological non-self interaction. Thus, it suggests that the results of previous studies, which claimed evidence of physiological self/non-self recognition using an intact/twin pair design, could be potentially confounded by the unintended effects of disrupting source-sink relationships in their plants.

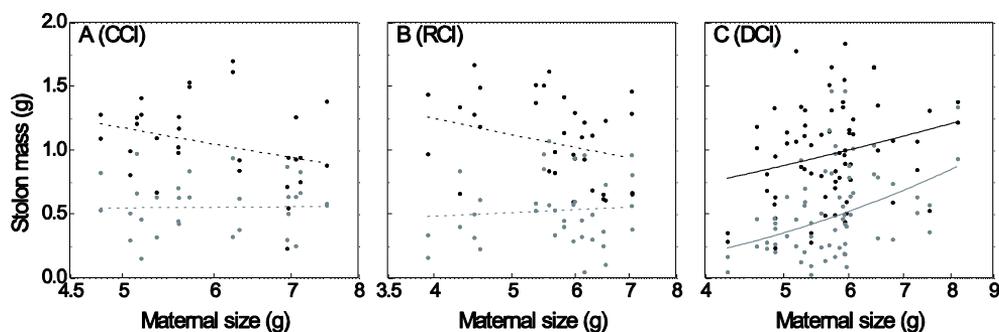
### 4.4.2 Evidence of physiological self/non-self recognition

In contrast to our hypotheses that ramets should produce greater root mass when they interact with disconnected or remotely connected neighbours than with closely connected neighbours, results from our *Expt II* clearly showed that root growth of *P. reptans* was not affected by any of these treatments, but was mainly determined by ramet age and maternal size within the clonal system. Interestingly, however, we did find significant responses to the treatments at the level of vegetative propagation. Only when interacting with disconnected neighbours, stolon growth (also new ramet production, as indicated in Table 4.2) was limited by the maternal size (Fig. 4.6). This led to less stolon mass (a reduction of *c.* 10%) than those in the other two treatments with connected neighbours (Fig. 4.4). Therefore, our results provide evidence of physiological self/non-self recognition within the clones of *P. reptans*. However, the fact that no difference was found between the closely and remotely connected interaction treatments

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suggests that self-recognition was maintained over quite a long distance (more than 100 cm, B. Chen pers. obs.) within the clone.

So far, the underlying mechanism for this type of self/non-self recognition is still unclear. Since genetically identical plants would be expected to produce the same biochemical substances, it can be hardly attributed to genetically-based allogeneic recognition (see **Chapter 2**). Other non-allogeneic mechanisms, however, have been proposed, e.g. an oscillatory signalling system that relies on physical connection (see Falik *et al.* 2003; Gruntman & Novoplansky 2004). If this is indeed a mechanism by which clonal plants can distinguish self from non-self ramets, the fact that we did not find a distance effect would suggest that this oscillatory signalling can persist over long distances in *P. reptans*, in contrast to what Gruntman and Novoplansky (2004) concluded for *Buchloe dactyloides*.



**Fig. 4.6** The dependence of stolon mass of younger (black dots) and older (grey dots) ramets of *Potentilla reptans* on their maternal sizes in (A) closely connected (CCI), (B) remotely connected (RCI) and (C) disconnected interactions (DCI) in Expt II. Regression lines are based on the results of linear mixed models. Lines in dash denote non-significant correlations.

Interestingly, when ramets were interacting with other ramets to whom they were not connected, they had less stolon mass but similar root mass as ramets that interacted with connected neighbours. In *Glechoma hederacea*, ramets had no more root mass but lower degrees of root segregation in twin pairs than in intact pairs (Semchenko *et al.* 2007b). In the non-clonal species *Pisum sativum*, plants exposed to roots of neighbours had similar root mass but higher metabolic activities, thus higher respiration rates, in roots than

plants exposed to their own roots (Meier *et al.* 2013). This further led to a lower shoot mass (Meier *et al.* 2013). In *Deschampsia caespitosa*, a plant had roots with similar levels of biomass but more branches and higher specific lengths when exposed to root exudates from unrelated con-specifics than when exposed to the exudates from siblings (Semchenko *et al.* 2014). Roots with higher metabolic activities or with finer diameters can have higher maintenance costs (Eissenstat & Yanai 1997; Meier *et al.* 2013). However, more detailed physiological and morphological measurements are needed to determine the extent to which these mechanisms played a role in our study as well.

Our study together with previous ones (e.g. Holzapfel & Alpert 2003; Gruntman & Novoplansky 2004; Falik *et al.* 2006) have shown a complicated type of neighbour recognition, including both internal (within-plant) communication via physical connections and external communication via root interaction (**Chapter 2**). Our study also clearly demonstrates the reproductive benefits from physiological self-recognition, and further indicates that such a process may work potentially throughout large clones, thus could contribute to the performance of clonal plants at least in terms of vegetative propagation. Self-recognition, hence, may affect the success of clonal plants in many ways and should be included in the study of, e.g., patch expansion (e.g. Liu *et al.* 2007; Herben & Novoplansky 2008), environmental heterogeneity adaptation (e.g. van Kleunen & Fischer 2001; Luo *et al.* 2014) and new habitat invasion (e.g. Liu *et al.* 2006; Wang *et al.* 2008).

Finally, a potential rooting volume effect should be considered (Hess & de Kroon 2007; **Chapter 3**). In our *Expt II* each clonal fragment had two pots (in addition to the pot for the mother ramet) for four daughter ramets growing roots in closely (CCI) and remotely connected interaction (DCI) treatments but four pots in the disconnected interaction (DCI) treatment to exploit the same amount of resources. Plants commonly produce more roots in larger rooting volumes (reviewed in Poorter *et al.* 2012a; also see **Chapter 3**) and it could go at the expense of investments to other organs (Hess & de Kroon 2007). If this rooting volume effect would have been dominant in our study, it would have likely led to greater root mass of ramets in DCI treatment than in CCI and RCI treatments. This is, however, contrary to the lack of difference in root mass among three treatments found in our study. Therefore, less stolon mass of plants in DCI treatment in *Expt II* is unlikely to be associated with their larger total rooting volumes.

### 4.5 Conclusions

In contrast to previous research showing that a sudden loss of connections between sister ramets led to the loss of physiological self-recognition, our first experiment suggests that such loss in *P. reptans* acts more likely as a disruption of source-sink relationships. By applying a novel design that avoids the side effects of the severance of connections, our second experiment provides evidence of physiological self/non-self recognition in *P. reptans*. It also suggests that such self-recognition can persist over a relatively long distance within a clone of *P. reptans*, thus potentially regulating the development of the clone. It has been estimated that *c.* 80% of angiosperms species possess the ability of clonal growth (Klimeš *et al.* 1997). Thus, the phenomenon of physiological self/non-self recognition may be widespread in the plant kingdom (Gruntman & Novoplansky 2004) and potentially plays an important role in the network of interactions within plant communities. For a better understanding of its evolutionary and ecological impacts, the next important step is to investigate the underlying mechanisms.

### Acknowledgments

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# Chapter 5

The presence of  
a belowground neighbour  
alters within-plant seed size distribution  
in *Phaseolus vulgaris*

*with H.J. During, P.J. Vermeulen & N.P.R. Anten*

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### Abstract

- Considerable variation of seed sizes commonly exists within plants, and is believed to be favoured under natural selection. This study aims to examine the extent to which the seed size distribution depends on the presence of competing neighbour plants.
- *Phaseolus vulgaris* plants rooting with or without a con-specific neighbour were grown in soil with high or low nutrient availability. Seeds were harvested at the end of the growth cycle, the total nitrogen and phosphorus invested in seed production were measured and within-plant seed size distribution was quantified using a set of statistical descriptors.
- Exposure to neighbours' roots induced significant changes in the seed size distribution. Plants produced proportionally more large seeds and fewer small ones, as reflected by significant increases in minimal seed size, mean seed size, skewness and Lorenz asymmetry coefficient. These effects were different from, even opposite to, the responses when soil nutrient level was reduced, and were significant after correction for the amount of resource investment in seed production.
- Belowground neighbour presence affects within-plant seed size distribution in *P. vulgaris*. This effect appears to be non-resource mediated, i.e. acting independent of neighbour-induced effects on resource availability. It implies that based on current environmental cues plants can anticipatorily adjust their investment strategies in offspring as an adaptation to the local environment in the future.

**Key words:** anticipatory maternal effect; bet-hedging; game theory; neighbour detection; *Phaseolus vulgaris*; root competition; seed-setting; seed size variation; size inequality; skewness

## 5.1 Introduction

A considerable degree of variation in seed size within plants is commonly observed (Michaels *et al.* 1988; Silvertown 1989; Ruiz de Clavijo 2002; Voller *et al.* 2012). Such variation is often interpreted as an adaptive bet-hedging strategy (Harper *et al.* 1970; McGinley *et al.* 1987; McGinley & Charnov 1988; Venable & Brown 1988; Geritz 1995). Many studies also reveal that plants modify the patterns of the variation (i.e. distribution) to cope with their abiotic environmental conditions (e.g. temperature, Wulff 1986; light, Galloway 2001; nutrients, Galloway 2001; water, Parciak 2002). Here we demonstrate that seed size distribution may also be modified in response to the presence of a belowground neighbour.

Within a species, seed size (following common practise, seed size refers to seed weight in this paper) often positively correlates with the competitiveness of the offspring (e.g. Houssard & Escarre 1991; Eriksson 1999; Lehtila & Ehrlen 2005; Dubois & Cheptou 2012). Based on a trade-off, induced by resource limitation in plants, between competition (favours large seeds) and colonization (favours a great number of small seeds), Geritz (1995) extended an optimal offspring size model (Smith & Fretwell 1974) by considering seedling competition and using evolutionary game theory. He assumed that (i) seedlings from larger seeds always outcompete seedlings from smaller seeds within a micro-site, and (ii) seeds are randomly dispersed over all micro-sites. He concluded that due to the uncertainty of seed density (i.e. seedling competition intensity) in different micro-sites, plants producing seeds of various sizes would be favoured by natural selection. Meanwhile, they should adjust their seed size distributions in response to nutrient availability and the probability of seedling competition in micro-sites.

The most obvious impact of a belowground neighbour is the reduction of soil resource availability (de Kroon *et al.* 2003). Thus, one might intuitively assume that introducing belowground neighbours will have similar effects on the within-plant seed size distribution as limiting nutrients. The consequences often include a smaller mean seed size (e.g. in *Desmodium paniculatum*, Wulff 1986; *Campanula americana*, Galloway 2001; *Sarcobatus vermiculatus*, Breen & Richards 2008) and a narrower seed size range that caused by a decline of maximal seed size (Geritz 1995).

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However, the presence of a neighbour not only indicates reductions in resource availability, but, when seed dispersal is limited, also future competition in next generation (Geritz 1995). That is, offspring of plants that grow close to neighbours are more likely to experience competition. A modelling study predicts that adaptiveness of plant responses to environmental cues increases with the extent which these cues accurately indicate future environmental conditions (Wong & Ackerly 2005). Recent evidence shows that plant roots can detect their neighbours independent of resource status (reviewed in **Chapter 2**). These would leave open the possibility that plants can use the neighbour cues to adjust their reproductive strategy to the probability of seedling density in next seasons. Indeed, the model of Geritz (1995) predicts that as the total seed density within the seed shadow of a plant increases with its neighbour density, its seeds will have a higher probability of dispersal to a micro-site with several other seeds, and then the production of larger seeds owing to their advantage in competition should be favoured over the production of smaller ones. Empirical evidence shows that *Bromus madritensis* has fewer small seeds with more equal seed provisioning in response to higher competition intensity, but not to lower soil nutrient level (Violle *et al.* 2009). Similarly, in marine animal *Bugula neritina*, mothers experiencing competition produce larger offspring (Allen *et al.* 2008).

Therefore, we hypothesize that (i) if plants only respond to resource reduction effects of belowground neighbours, they should produce seeds with a smaller mean size and a smaller maximal size. On the other hand, (ii) if they respond to non-resource effects of belowground neighbours which indicate a higher likelihood of seedling competition in the future, they should invest proportionally more in large seeds within the size distribution. This would imply that perception of current environmental cues enables plants to adjust their offspring investment as an adaptation to the future environment.

To test these hypotheses, we performed an experiment using *Phaseolus vulgaris* L. plants. This species has been shown to detect its con-specific neighbours (Maina *et al.* 2002). Its limited seed dispersal distance, caused by the relatively large seed size, implies that seeds from such competing plants have a high chance of experiencing seedling competition with seeds from their neighbours. For this species, plants from large seeds outcompete plants from small seeds via faster seedling growth (Cipollini & Stiles 1991).

## Neighbour presence alters within-plant seed size distribution

The experiment was conducted with plants rooting either with or without a con-specific neighbour in soil with high or low nutrient level. In addition to mean and CV, which cannot specify the changes of investments in the productions of small and large seeds, we applied a set of descriptors to comprehensively characterize the within-plant seed size distribution. Furthermore, we used the total amount of nitrogen and phosphorus in seed production as covariates in the analyses to disentangle neighbour-induced non-resource effects from neighbour-induced resource reduction effects.

### 5.2 Materials and methods

#### 5.2.1 Experimental design

The experiment was conducted at a plastic greenhouse facility of Utrecht University, Utrecht, the Netherlands. Commercially available seeds of *P. vulgaris* (red kidney bean, variety ‘Canadian wonder’) with similar size were selected and sown solitarily in small pots (0.25 L) with moist sand. Seven days later, seedlings with a height of c. 10 cm and having two healthy leaves were selected and transplanted into round plastic pots (19.5 cm height × 25 cm diameter, c. 7.5 L) filled with a mixture of potting soil and sand (1:2 in volume). These seedlings were randomly subjected to one of four treatments involving belowground neighbour (‘presence’, two plants in one pot, or ‘absence’, two plants in two adjacent pots) and soil nutrient level (‘high’ or ‘low’, manipulated by 1.0 g L<sup>-1</sup> or 0.3 g L<sup>-1</sup> nutrient solution with 19% N + 6% P<sub>2</sub>O<sub>5</sub> + 20% K<sub>2</sub>O + 3% MgO + micronutrients fertilizer: Kristalon Blauwmerk, Yara Benelux B.V., the Netherlands). To standardise aboveground interactions, the seedling pairs were planted so that the distance between the two (measured from the stem) was 12.5 cm in both treatments (i.e. either within the pot or between the plants in their own pots). During the cultivation period, every pot received 240 ml nutrient solution (either high or low) twice a week. Plants were grown in a plastic-roofed greenhouse where light availability was about 50% of natural day light, and watered daily (except the days when nutrient solutions were applied) from June to October. Each treatment combination consisted of 24 plants (i.e. 12 pairs), which were arranged in two blocks on benches to take micro-environmental variation in the plastic greenhouse into account.

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### 5.2.2 Harvest and measurements

On 10 October 2012, at the end of the growth cycle when most of the leaves were yellow and dry, pods were harvested (c. 114 days of growth). Most plants had many ripe pods and some aborted small pods, but on several plants we also found a few unripe ones. Hence, pods were divided into ripe, aborted and unripe groups, oven-dried at 70 °C for four days and weighed separately. We summed the mass of the three pod groups to determine total pod mass for each plant. We did not harvest the vegetative parts of the plants, as these were largely gone by the time of harvesting.

As seeds in aborted pods were undeveloped and seeds in unripe pods were still at intermediate developmental stages, they could not be treated in the same way as the mature seeds in ripe pods and included equally in the seed pool for the next generation. Consequently, only viable seeds (easily distinguishable from aborted ones by appearance and weight) from ripe pods (more than 95% of the harvested pods) were weighed individually and used for subsequent analyses. After measuring the dry mass of individual seeds, all viable seeds of each mother plant were ground to a powder. Then total nitrogen content and total phosphorous content were determined with a continuous flow analyser (SKALAR Breda, the Netherlands) after Kjeldahl digestion.

To comprehensively characterize the within-plant seed size distribution for each plant, we not only calculated the frequently used mean and CV, but also applied additional descriptors, including maximal (i.e. heaviest) and minimal (i.e. lightest) sizes, which define the absolute range of the distribution; skewness, which quantifies the degree of number-related asymmetry of the distribution (Sokal & Rohlf 1995); Gini coefficient, which describes the degree of resource investment bias (i.e. size inequality or hierarchy) in the distribution (Weiner & Solbrig 1984; also see the bias adjustment in Deltas 2003), and Lorenz asymmetry coefficient, which differentiates the major contributor (large seeds vs. small seeds) to the resource investment bias (Damgaard & Weiner 2000).

### 5.2.3 Data analysis

In the experimental set-up, two adjacent plants were nested in each plant-pair, and all plant-pairs were nested in two blocks. We examined the overall effect of a belowground neighbour on within-plant seed size distribution

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using linear mixed models (LMMs) with belowground neighbour (presence vs. absence), soil nutrient level (high vs. low) and their interaction as fixed factors, with block and plant-pair as random factors.

Since the amount of resource invested in seed production accurately represents the effects of plant nutrient availability (i.e. both soil nutrient level and neighbour-induced resource reduction), the neighbour-induced non-resource effect on seed size distribution can be examined by correcting for the level of resource investment in seed production in the analyses. This was done with LMMs, in which belowground neighbour (presence vs. absence) was introduced as a fixed factor, resource investment (i.e. total seed mass, and total amount of nitrogen or phosphorus in seed production, respectively) as covariate, with block and plant-pair as random factors. In other words, the detection of a significant non-resource effect of a belowground neighbour provides evidence that neighbour presence can affect the seed size distribution irrespective of its effect on resource availability for seed production, as represented by the level of resource invested in seeds.

Total pod mass, total seed mass, total nitrogen and total phosphorus content were log-transformed, seed number was square-root-transformed. For all the analyses, the interaction term was excluded from the full model if it did not show significant effects. All statistical analyses were performed using lme4 (Bates *et al.* 2014) and lmerTest (Kuznetsova *et al.* 2014) packages in R version 3.1.0 (R Core Team 2014).

5

### 5.3 Results

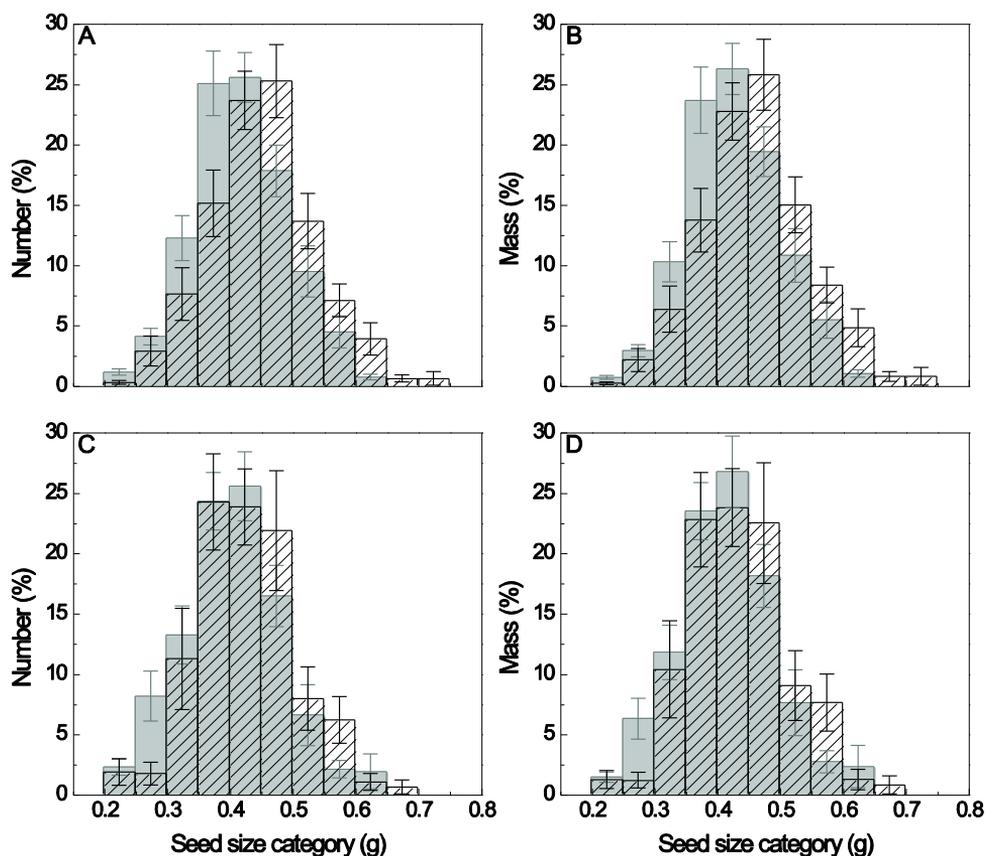
In total, 3001 mature seeds were collected and analysed. For each treatment combination, within-plant seed size distribution (relative numbers and masses of seeds in different size categories) of *P. vulgaris* is presented in Fig. 5.1.

#### 5.3.1 Overall effect of a belowground neighbour

Total pod mass, total seed mass, seed number, as well as total amount of nitrogen and phosphorus in the seed production of individual plants were significantly reduced by a belowground neighbour and low soil nutrient

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level, but only for seed number there was a significant neighbour  $\times$  nutrient interactive effect (Table 5.1, Fig. 5.2).

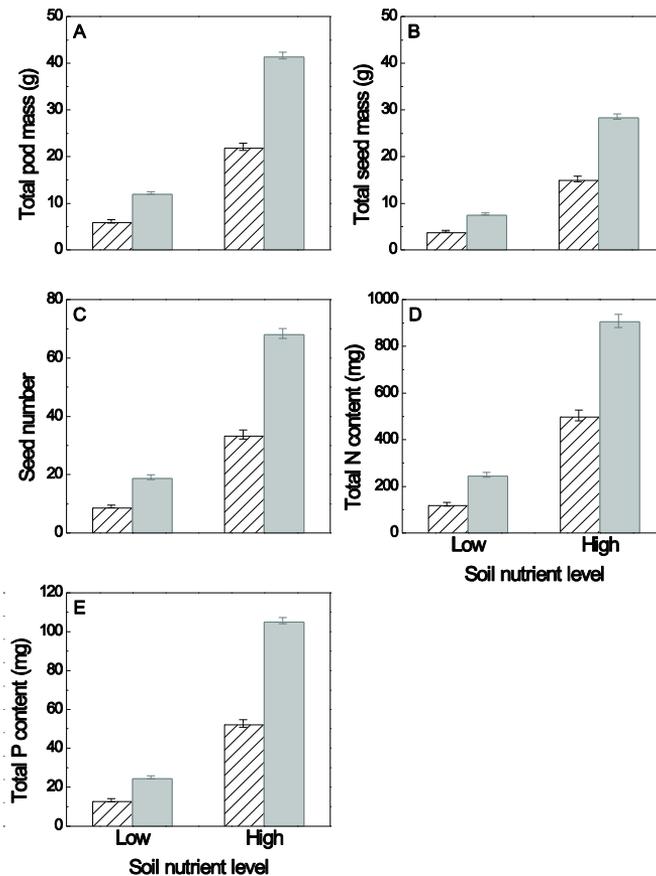


**Fig. 5.1** The relative numbers and masses of seeds in different size categories of *Phaseolus vulgaris* plants growing in the presence (open bars with cross-hatching) and absence (grey bars) of a belowground neighbour at (A, B) high and (C, D) low nutrient levels. Data were first standardized for each plant (i.e. in percentage), and then averaged to represent the individual level. Data are means  $\pm 1$  s.e.

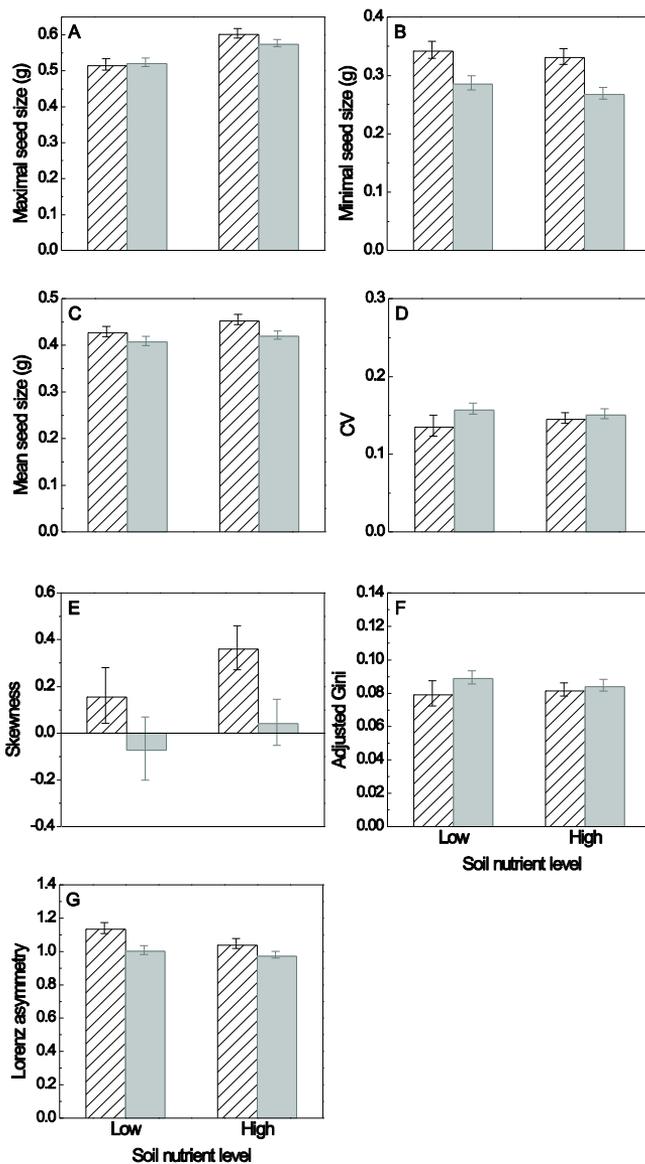
Lowering nutrient level in the soil significantly reduced maximal seed size while leaving minimal seed size unchanged. In contrast, growing with a belowground neighbour had no effect on maximal seed size, but significantly increased minimal seed size (Table 5.1, Fig. 5.3A,B). Mean seed size and skewness were significantly increased by the presence of a

## Neighbour presence alters within-plant seed size distribution

belowground neighbour, but were not affected by lowering soil nutrient level (Table 5.1, Fig. 5.3C,E). Neither a belowground neighbour nor soil nutrient level had an effect on CV or adjusted Gini coefficient (Table 5.1), but they both significantly increased the Lorenz asymmetry coefficient (Table 5.1, Fig. 5.3G). The neighbour  $\times$  nutrient interaction was not significant for any of the listed distribution descriptors (Table 5.1).



**Fig. 5.2** Total reproductive output, i.e. (A) total pod mass, (B) total seed mass, (C) seed number, and resource investment in seed production, i.e. total (D) nitrogen and (E) phosphorus content, of *Phaseolus vulgaris* plants growing in the presence (open bars with cross-hatching) and absence (grey bars) of a belowground neighbour in high and low nutrient levels. Data are means  $\pm 1$  s.e.



**Fig. 5.3** The descriptors of within-plant seed size distribution, i.e. (A) maximal seed size, (B) minimal seed size, (C) mean seed size, (D) coefficient of variation (CV), (E) skewness, (F) adjusted Gini coefficient and (G) Lorenz asymmetry coefficient, of *Phaseolus vulgaris* plants growing in the presence (open bars with cross-hatching) and absence (grey bars) of a belowground neighbour in high and low nutrient levels. Data are means  $\pm$  SE.

## Neighbour presence alters within-plant seed size distribution

### 5.3.2 Non-resource effect of a belowground neighbour

When the level of resource investment in seed production (i.e. total seed mass, total nitrogen content or total phosphorus content) was included in the analyses as a covariate to represent the effects of nutrient availability (i.e. both soil nutrient level and resource reduction caused by the neighbour), the non-resource effect of a belowground neighbour was still significant (Table 5.2). Similar to the analyses of the overall effects of neighbours (Table 5.1), such non-resource effects entailed significant increases in minimal size, mean size, skewness and Lorenz asymmetry coefficient of the distribution (Table 5.2). On the other hand, the non-resource effects on maximal seed size depended on the level of resource investment in seeds (Table 5.2). Mean seed size was also significantly reduced by limiting resource investment level (Table 5.2).

## 5.4 Discussion

Our results show that the greatest plasticity in plant seed production in response to reduced resource availability occurred in terms of number reduction (Fig. 5.2C, also see Harper *et al.* 1970; Venable 1992). However, a negative response of mean seed size to a lower resource investment level was also found (Table 5.2), which is consistent with many studies (e.g. Wulff 1986; Galloway 2001; Breen & Richards 2008). This reduction was associated with a decline in maximal seed size, which is too costly to be maintained at high values (Geritz 1995). Together these results indicate that the quantity of seeds (i.e. seed number) tends to be favoured over their quality (i.e. size) when resources are limited. The maintenance of minimal seed size at low nutrient availability might imply that a basic level of resource storage should be fulfilled for seedling germination and survival at early developmental stage (Geritz 1995).

As neighbour presence typically entails a reduction in resource availability and thus a lower resource investment level in seeds (Fig. 5.2D, E), neighbour presence effects on seed size distribution could be mediated through reduced resource availability. However in our study: (i) the overall effects of a belowground neighbour on various descriptors of the size distribution were different from, and in several cases opposite to, those of

**Table 5.1** The overall effects of a belowground neighbour (BN, i.e. joint effects of resource reduction and non-resource related cues from neighbours) and soil nutrient level (NL) on total reproductive output and within-plant seed size distribution of *Phaseolus vulgaris* plants

Total reproductive output	Fixed effects			Descriptors of distribution	Fixed effects		
	BN	NL	BN×NL		BN	NL	BN×NL
Total pod mass	< <b>0.001</b>	< <b>0.001</b>	—	Maximal size	0.363	< <b>0.001</b>	—
Total seed mass	< <b>0.001</b>	< <b>0.001</b>	—	Minimal size	< <b>0.001</b>	0.292	—
Seed number	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>	Mean size	<b>0.013</b>	0.077	—
total N content	< <b>0.001</b>	< <b>0.001</b>	—	CV	0.180	0.914	—
total P content	< <b>0.001</b>	< <b>0.001</b>	—	Skewness	<b>0.025</b>	0.189	—
				Adjusted Gini	0.289	0.812	—
				Lorenz asymmetry	<b>0.001</b>	<b>0.043</b>	—

*P* values (in bold indicates when  $P < 0.05$ ) are given, based on type III sums of squares and Kenward-Roger's approximation.

— denotes that the interaction term was excluded from the full model when its effect was not significant.

## Neighbour presence alters within-plant seed size distribution

lowering nutrient availability in soil; (ii) the non-resource effects of a belowground neighbour were significant when plants were compared at the same level of resource investment in seed production; and (iii) significant interactive effects were seldom found. These clearly indicate that the presence of a belowground neighbour had effects that were independent of resource availability.

The responses of within-plant seed size distribution to non-resource effects of neighbours can be mainly interpreted as a maternal adaptation (Burgess & Marshall 2014) to the future environment conditioned on current environmental cues (Wong & Ackerly 2005). Due to the competitive advantage of large seeds (especially in dense populations, e.g. Houssard & Escarré 1991; Eriksson 1999), the presence of a neighbour, which indicates higher likelihood of intensive seedling competition, would favour the production of large seeds. Indeed, in our study we found plants with neighbours to produce relatively fewer seeds in small size categories as reflected by the increases of both skewness and minimal seed size (as shown in Fig. 5.1A,C). The larger values of the Lorenz asymmetry coefficient with an unchanged Gini coefficient suggested that plants with neighbours produced relatively less very small seeds and relatively more very large seeds. Together this resulted in larger mean seed size in plants with neighbours, consistent with our second hypothesis.

The findings of non-resource effects also provide evidence of root-mediated neighbour detection in *P. vulgaris* plants. In the last decade, considerable research has shown that plants are capable of discriminating self/non-self (e.g. Falik *et al.* 2003) and even the relatedness (e.g. Dudley & File 2007; Fang *et al.* 2013) of a con-specific neighbour via root interaction, independently of nutrient availability. However, the mechanisms underlying this detection are still unclear (**Chapter 2**). So far, most of these studies mainly focused on root growth (e.g. Falik *et al.* 2003; Fang *et al.* 2013), although some also paid attention to the responses in biomass allocation to reproduction (e.g. Gersani *et al.* 2001; Dudley & File 2007). Our study extends current understanding by demonstrating that root-mediated neighbour detection can also influence plant reproductive strategy in terms of seed size distribution. Another example of the neighbour-presence effect on developmental processes in plant reproduction is provided by *Viola tricolor*, in which rooting with neighbours increases pollen tube growth rate much more than the availability of nutrients did (Lankinen *et al.* 2013).

**Table 5.2** The non-resource effects of a belowground neighbour (BN') on the within-plant seed size distribution of *Phaseolus vulgaris* plants, correcting for the amount of resource investment in seed production (in forms of total mass of seeds, total nitrogen content and total phosphorus content, respectively)

Descriptors of distribution	The form of resource investment in seed production											
	Total seed mass (TSM)			Total nitrogen content (TN)			Total phosphorus content (TP)					
	BN'	TSM	BN'×TSM	BN'	TN	BN'×TN	BN'	TP	BN'×TP			
Maximal size	0.418	< <b>0.001</b>	<b>0.038</b>	0.108	< <b>0.001</b>	<b>0.034</b>	0.162	< <b>0.001</b>	<b>0.026</b>			
Minimal size	<b>0.001</b>	0.246	—	< <b>0.001</b>	0.345	—	<b>0.001</b>	0.251	—			
Mean size	<b>0.002</b>	<b>0.045</b>	—	<b>0.001</b>	<b>0.021</b>	—	<b>0.002</b>	<b>0.042</b>	—			
CV	0.421	0.320	—	0.425	0.286	—	0.424	0.293	—			
Skewness	<b>0.008</b>	0.127	—	<b>0.008</b>	0.120	—	<b>0.009</b>	0.142	—			
Adjusted Gini	0.489	0.522	—	0.498	0.473	—	0.500	0.473	—			
Lorenz asymmetry	<b>0.045</b>	<b>0.020</b>	—	<b>0.038</b>	<b>0.024</b>	—	<b>0.033</b>	<b>0.037</b>	—			

P values (in bold indicates when  $P < 0.05$ ) are given, based on type III sums of squares and Kenward-Roger's approximation. — denotes that the interaction term was excluded from the full model when its effect was not significant.

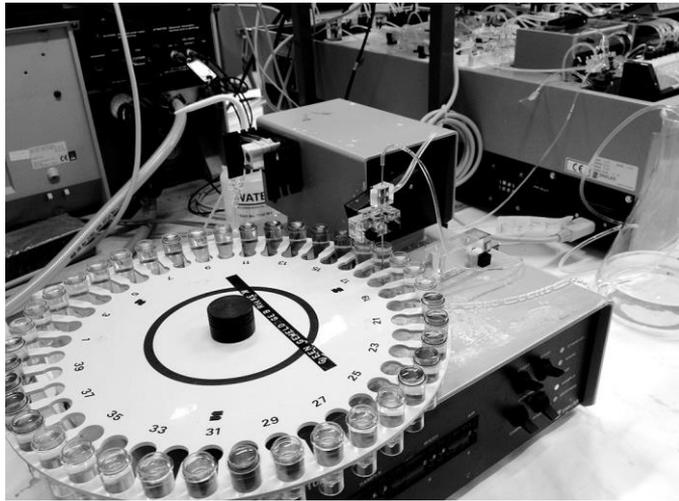
## Neighbour presence alters within-plant seed size distribution

In contrast to our findings that competition with belowground neighbours increased mean seed size but left CV unchanged, some studies using other species have shown that mean seed size stabilizes (e.g. Weiner *et al.* 1997; Halpern 2005; Violle *et al.* 2009) yet CV varies in response to neighbour density (e.g. Halpern 2005; Violle *et al.* 2009). This may simply indicate that plant species differ in their adaptive traits related to seed size distribution (Harper *et al.* 1970). More importantly, however, our results suggest that mean or CV of seed size is just one element of a complex in the distribution, which may not be sufficient by itself to describe the changes. More descriptors that specify the range (maximal and minimal size), the degrees of asymmetry (skewness) and the size hierarchy structure (Gini and Lorenz asymmetry coefficients) of the distribution are needed to get more insight into the offspring investment strategy of plants.

To our knowledge, the current study is the first to demonstrate that the presence of a belowground neighbour alters seed size distribution within plants. It provides an example of anticipatory maternal effects (Burgess & Marshall 2014). That is, a neighbour in the current generation carries a reliable cue which increases the predictability of next generation's density in the future, and plants can use it to adjust their bet-hedging (i.e. seed size distribution) accordingly (Wong & Ackerly 2005). It also evokes the need for further studies of the mechanisms of belowground neighbour detection, as well as the subsequent crosstalk between below- and aboveground plant parts in influencing reproductive strategy. Insight into such neighbour induced responses may also be important for agriculture, as crops tend to grow in close proximity to con-specific neighbours, and seed size and uniformity therein are important quality measures in crops.

### Acknowledgements

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# Chapter 6

## General discussion

## Chapter 6

In this thesis, I report the results of one literature-based study (**Chapter 2**) and three experiments (**Chapter 3, 4 and 5**) that designed to investigate the growth and reproductive responses of plants to the presence of their below-ground neighbours. The main objective is to better understand below-ground neighbour detection and to analyse the extent and conditions under which a tragedy-of-the-commons scenario in plant-plant root interaction may occur. By synthesizing the results, in this final chapter, I first illustrate how the divergent concepts of volume affect the interpretations of plant root responses to neighbours in container-based studies. I then reemphasize the importance of plant size in the analyses of environmental effects on plant growth strategies. Next, I assess the progress and limitations in the research field of below-ground neighbour detection and the consequent tragedy-of-the-commons scenarios. Finally, I discuss the potential implications for agricultural research.

### 6.1 How much soil volume does a sharer have access to?

The debate regarding the interpretation of root over-proliferation at the expense of reproduction in split-root experiments (e.g. one-pot-owner versus two-pot-sharer in Gersani *et al.* 2001) mentioned in **Chapter 3** is conceptually muddled by the differences in how concept of volume has been defined. In the neighbour effect hypothesis (neighbour presence stimulates root growth), *volume* is treated as a proxy of space-linked soil resources that are available for plants (see Gersani *et al.* 2001). So, the pot volume, just like nutrients, is conceived as being partitioned among the plants sharing a pot. This leads to the assertion that plants exclusively growing in one pot (one-pot-owner) have access to the same volume as two plants sharing two pots (two-pot-sharer plants). On the other hand, in the volume effect hypothesis (larger rooting volume promotes more root growth), *volume* is a three-dimensional physical space bounded by obstacles (pot walls in this case) that will eventually inhibit root growth (see Falik *et al.* 2005; Semchenko *et al.* 2007a). Thus, pot volume is independent of soil nutrients. Plants in principle have access to the entire pot volume, even if there is also a neighbour with roots in this pot. Accordingly, in an experiment using pots of uniform size (e.g. Gersani *et al.* 2001), for two-pot-sharers the available volume per plant was twice that of one-pot-owners.

This discrepancy regarding the concept of volume not only occurs in the aforementioned debate, but extends across most container-based experiments. For instance, 65 studies of pot volume effects on plant growth selected in a meta-analysis all implicitly designate volume as a nutrient carrier, since its size was associated with total nutrient content (Poorter *et al.* 2012a). On the other hand, several empirical (e.g. Semchenko *et al.* 2007a; Markham & Halwas 2011; Nord *et al.* 2011) and modelling studies (O'Brien & Brown 2008; McNickle & Brown 2012) analysed the effects of below-ground neighbours by explicitly designating volume as a common space resource independently of nutrients. Therefore, there is a need to investigate to what extent the volume concept affects the results and conclusions in the research.

To clarify this debate, I introduce (i) the resource-related volume concept as **exclusive volume** ( $EV = \frac{\text{total volume}}{\text{plant number}}$ ), i.e. total volume is divided for each plant (e.g. Gersani *et al.* 2001); and (ii) the space-only volume concept as **inclusive volume** ( $IV = \text{total volume}$ ), i.e. a plant has access to the full volume, regardless of the number of plants it is sharing with (e.g. Hess & de Kroon 2007). Here, I also assume that the organ (e.g. root) mass of an owner plant is a function of its available volume (as found in **Chapter 3**):

$$\log(\text{organ mass of owner}) = M_O(V) = \alpha + \beta \times \log(V) \quad \text{Function 6.1}$$

where  $\alpha$  is the intercept of the mass-volume regression,  $\beta$  is the slope of the regression, and  $V$  is the size of the available volume (e.g. one pot).

Therefore, in a one-pot-owner and two-pot-sharer experiment using pots with uniform size and giving the same amount of nutrients per plant, the available volume for an owner plant is the same in both IV and EV; while the available volume for a sharer plant assuming IV is twice as large as when EV is assumed. With the aforementioned mass-volume relationship assumption, under the null hypothesis (i.e. no effect of neighbour presence), the  $\log(\text{organ mass})$  of a sharer in EV will be:

$$M_S = M_O(V) = \alpha + \beta \times \log(V)$$

While in IV will be:

$$M_S = M_O(2V) = \alpha + \beta \times (\log(V) + \log(2)) = M_O(V) + \log(2^\beta)$$

Apparently,  $\log(\text{organ mass})$  of a sharer plant in IV predicted by the null hypothesis is  $|\log(2^\beta)|$  units higher (when  $\beta > 0$ ) or lower (when  $\beta < 0$ ) than

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$\log(\text{organ mass})$  of an owner plant in IV (also in EV), thus also compared to predicted  $\log(\text{organ mass})$  of sharer plants in EV.

Therefore, the choice of the EV or the IV concept in the data analyses can lead to different predictions on the effects of neighbour presence. We define  $M_{\text{obs}}$  as the log-transformed observed organ mass of sharer plants. It should be noted that:

When plant  $\log(\text{organ mass})$  is positively ( $\beta > 0$ ) correlated with  $\log(\text{volume})$ , and when

- (a).  $M_{\text{O}}(\mathbf{V}) < M_{\text{O}}(2\mathbf{V}) < M_{\text{obs}}$ , both EV and IV agree that neighbour presence has a positive effect on the organ growth (Fig. 6.1a);
- (b).  $M_{\text{O}}(\mathbf{V}) < M_{\text{obs}} = M_{\text{O}}(2\mathbf{V})$ , EV suggests positive neighbour presence effect, while IV suggests no effect (Fig. 6.1b);
- (c).  $M_{\text{O}}(\mathbf{V}) < M_{\text{obs}} < M_{\text{O}}(2\mathbf{V})$ , EV suggests a positive effect, while IV suggests a negative effect (Fig. 6.1c);
- (d).  $M_{\text{O}}(\mathbf{V}) = M_{\text{obs}} < M_{\text{O}}(2\mathbf{V})$ , EV suggests no effect, while IV suggests a negative effect (Fig. 6.1d);
- (e).  $M_{\text{obs}} < M_{\text{O}}(\mathbf{V}) < M_{\text{O}}(2\mathbf{V})$ , both EV and IV agree that neighbour presence has negative effect on the organ growth (Fig. 6.1e).

Similarly, the analyse can be made exactly if  $\log(\text{organ mass})$  is negatively ( $\beta < 0$ ) related to  $\log(\text{volume})$ . In short, we can conclude that: when

$$M_{\text{obs}} \notin [M_{\text{O}}(\mathbf{V}), M_{\text{O}}(\mathbf{V}) + \log(2^\beta)] \quad (\text{for both } \beta > 0 \text{ and } \beta < 0)$$

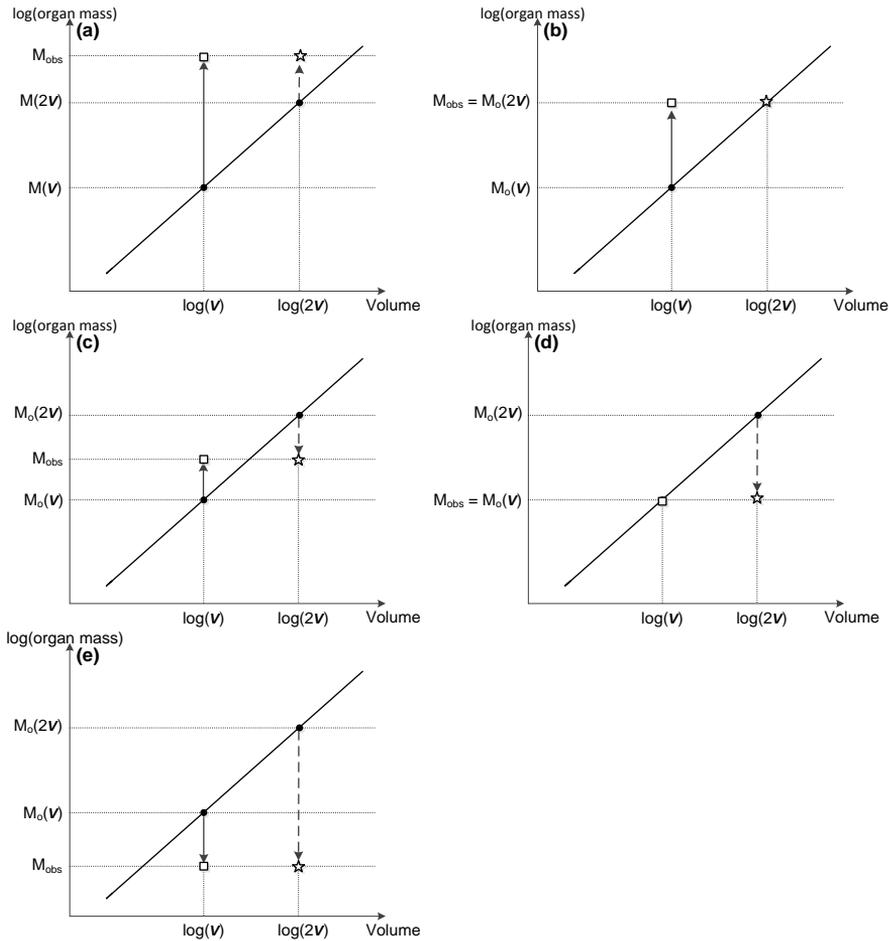
the direction of neighbour effects is the same in both EV and IV; however only when

$$M_{\text{obs}} \in [M_{\text{O}}(\mathbf{V}), M_{\text{O}}(\mathbf{V}) + \log(2^\beta)]$$

the direction of neighbour effect is opposite (or different) between EV and IV, and leading to a discrepancy in interpreting plant growth response to the presence of a neighbour.

We also can examine to what extent the discrepancy in the volume concept can affect the interpretation of neighbour presence effect in an experiment with  $n$  ( $n \geq 2$ ) plants grown together sharing a full volume size  $V_S$  and given nutrients per plant constant. If the  $\log(\text{organ mass})$  of an owner plant grown with volume level  $V_S$  still follows **Function 6.1**, we can follow the same deduction and conclude that: when

$$M_{\text{obs}} \notin [M_{\text{O}}\left(\frac{V_S}{n}\right), M_{\text{O}}\left(\frac{V_S}{n}\right) + \log(n^\beta)] \quad \textbf{Situation 1}$$



**Fig. 6.1** Illustration for the impacts of discrepancy in volume concept on the interpretation of neighbour presence effect. Here, we assume a positive linear relationship between  $\log(\text{organ mass})$  and  $\log(\text{volume})$  for owner plants. The filled circles are  $\log(\text{organ mass})$  of owner plants ( $\bullet$ ) growing in the volume of one pot ( $M_O(V)$ ) or two pots ( $M_O(2V)$ ). The open square and star represent the  $\log(\text{observed organ mass})$  of a two-pot-sharer ( $M_{obs}$ ) in EV concept (“□”, assuming it only has  $1V$ ) and in IV concept (“☆”, assuming it has  $2V$ ), respectively. The arrows with solid and dashed lines represent the  $\log(\text{organ mass})$  difference between owners and sharers compared in EV concept (“—”, two-pot-sharer versus one-pot-owner) and in IV concept (“- -”, two-pot-sharer versus two-pot-owner), respectively. In other words, the presence of an arrow indicates a significant effect of neighbour presence. Arrows upwards or downwards denote more ( $\uparrow$ ) or less ( $\downarrow$ ) organ mass in two-pot-sharers than in owner plants, i.e. positive ( $\uparrow$ ) or negative ( $\downarrow$ ) effect of neighbour presence. Five possible scenarios are (a)  $M_O(V) < M_O(2V) < M_{obs}$ ; (b)  $M_O(V) < M_{obs} = M_O(2V)$ ; (c)  $M_O(V) < M_{obs} < M_O(2V)$ ; (d)  $M_O(V) = M_{obs} < M_O(2V)$  and (e)  $M_{obs} < M_O(V) < M_O(2V)$ .

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there is no discrepancy in interpreting neighbour effects between EV and IV; and when

$$M_{\text{obs}} \in [M_{\text{O}}(\frac{V_s}{n}), M_{\text{O}}(\frac{V_s}{n}) + \log(n^\beta)]$$

### Situation 2

the choice of using either EV or IV can substantially affect the understanding of neighbour effects.

In addition, it is also noted that, when the organ mass of an owner plant follows the function:

$$\text{organ mass of owner} = M'_{\text{O}}(V) = \alpha + \beta \times \log(V)$$

the aforementioned conclusions also hold true. We only need to designate  $M'_{\text{obs}}$  to be the measure of original rather than log-transformed organ mass of sharers.

When applying such methods to the analysis of root mass data in **Chapter 3**, the results (see Table 6.1) reflect the **Situation 2**, showing that  $M_{\text{obs}}$  fell in the closed interval between  $M_{\text{O}}(V)$  and  $M_{\text{O}}(V) + 2 \times \log(\beta)$ . In other words, when comparing log(root mass) between one-pot-owners and two-pot-sharers, EV suggests null or positive (in small pots) neighbour effects, while IV suggests negative neighbour effects.

**Table 6.1** Summary of re-analyses for testing neighbour presence effect on the root mass of two-pot-sharer plants using data in **Chapter 3**

Pot size (V)	one-pot-owner		two-pot-sharer		two-pot-owner
small pot (0.56L)	0.618	<	0.721 ± 0.037	<	0.882
medium pot (1.10L)	0.874	≈	0.880 ± 0.067	<	1.246
large pot (1.68L)	1.085	≈	1.175 ± 0.058	<	1.547

Root mass of owner plants listed here is calculated based on the log(mass) - log(volume) regression function. Root mass of sharer plants listed here is the observed values expressed by mean ± SE. “<” means significantly ( $P < 0.05$ ) less than; “≈” means not significantly ( $P < 0.05$ ) different from. Comparisons are based on one-sample t test.

As I argued, in **Situation 2** the choice between EV and IV can substantially affect the understanding of neighbour effects. Below, I explain why I use the **inclusive volume** concept in **Chapter 3**.

In nature, the limitation of physical space on plant root growth seldom happens, except for rare habitats such as gaps in cliffs and between stones. This might be one of the most influential differences between field studies and container-based greenhouse studies. Therefore, we can find a

fundamental difference of volume definition between field and container studies: *in the field, volume is only an evaluation for the size of a certain physical space that is conceptually isolated from the outside world for mathematical calculation; but in the containers, volume is a combination of both the size of physical space and the limitation of physical space by barriers (e.g. pot wall).*

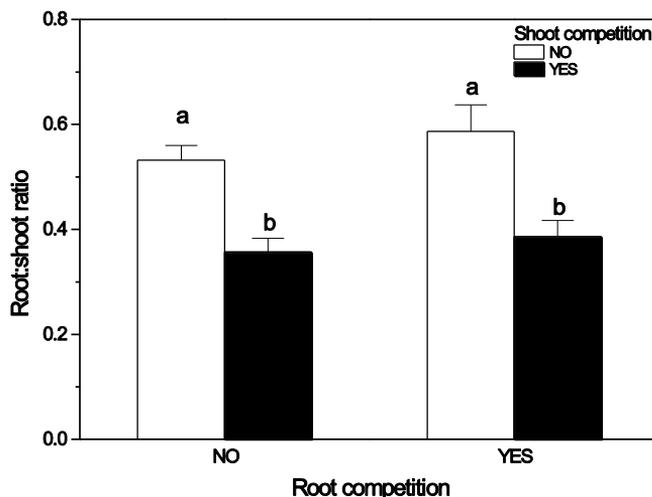
Therefore, the functioning of volume in the fields is more related with the amounts of nutrients it holds, since nutrient limitation is the key element for root growth of plants in the fields and the most obvious impact from a below-ground neighbour is a reduction of nutrient availability (de Kroon *et al.* 2003). This is also how the concept of exclusive volume originates in the studies of, e.g. Gersani *et al.* (2001) and Maina *et al.* (2002). However, they disregarded the additional effects of the volume in the containers, i.e. roots cannot penetrate the real walls of the containers, but they can pass through the conceptual barriers of the volume in the fields. On the other hand, the remarkable and significant non-nutrient-availability-related volume effects on the growth and allocation of owner plants in **Chapter 3**, and in other studies (e.g. McConnaughay & Bazzaz 1991; McConnaughay *et al.* 1993; Murphy *et al.* 2013), provide robust support for my arguments for the effects of physical space limitation of volume in a container. Hence, I suggest that the analyses for the container-based experiment should use the concept of inclusive volume.

## 6.2 Impacts of plant size

It is well accepted that biomass allocation in plants is strongly related to plant size (Evans 1972; Coleman *et al.* 1994; Cahill 2003; Weiner 2004; Poorter *et al.* 2012b; Poorter & Sack 2012). Using path analysis in **Chapter 3**, I explicitly show that a large part of the neighbour presence and rooting volume effects on plant allocation strategy was indirectly mediated through their influence on plant total mass. Hence, not including plant size could lead to biased conclusions, whereby plant size effects are either wrongly attributed to rooting volume or neighbour presence, or whereby the influence of these factors is dismissed because plant size masks their direct effects. To stress the importance of plant size, here, I present another example.

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I conducted a root and shoot competition experiment, in which four scenarios were established, i.e. no competition, shoot competition only, root competition only and full competition, using pea (*Pisum sativum*). Regarding the impacts of volume as discussed before, I grew one plant in one pot with a size of 1.0 L as the “no root competition” treatment, and two plants in one pot with a size of 1.0 L as the “root competition” treatment. Furthermore, following the classic definition, competition in this experiment refers to the full effects of a neighbour, including the reduction of nutrient availability and the non-resource related effects (Schenk 2006). In other words, I provided the same amount of nutrients per pot, irrespectively of the number of plants in the pot. After roughly two months of cultivation, plants were harvested, and their root mass and shoot mass were determined.



**Fig. 6.2** The mean root:shoot ratio of *Pisum sativum* in response to root and shoot competition. Bars that share the same superscript are not significantly different ( $P > 0.05$ ). The error bars denote 1 *SE* above and below the means.  $N = 76$ .

Based on the results from a two-way ANOVA test, it was clear that root:shoot ratios (RSR) of plants were significantly reduced by shoot competition ( $F = 11.56$ ,  $P = 0.001$ ), but not affected by root competition ( $F = 1.18$ ,  $P = 0.281$ , see Fig. 6.2). This could lead one to conclude that root vs. shoot allocation only responds to shoot competition, i.e. when competing for light, pea plants proportionally invest more in shoots; while when only

competing for nutrients, pea plants do not invest more in roots. However, this direct interpretation ignores the differences in plant size. Hence, the aforementioned interpretation could be biased.

Having realized the size difference issue, numerous studies have taken plant size as a covariate in their analyses (e.g. Müller *et al.* 2000; Cahill 2003). Following their methods using an ANCOVA test, the results of my experiment are altered: while shoot competition still significantly reduced RSR ( $F = 6.52$ ,  $P = 0.013$ ), root competition significantly increased RSR ( $F = 8.02$ ,  $P = 0.006$ ) and plant size (vegetative mass) was significantly positively correlated with RSR ( $F = 14.62$ ,  $P < 0.001$ ). Therefore, the interpretation then becomes: pea plants adjusted their allocation patterns correspondingly to resource competition. When competing for light, plants proportionally invested more in shoots; when competing for nutrients, plants proportionally invested more in roots. Apparently, inclusion of plant size in the analyses had a strong influence on the interpretation of the results and the consequent understanding of the phenomenon.

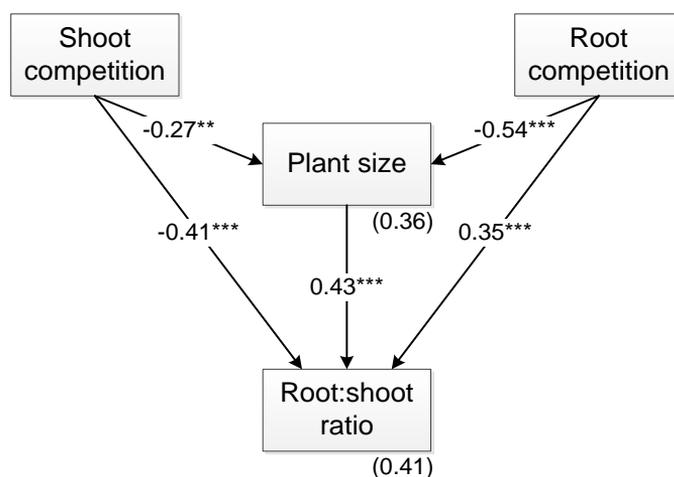
Once the role of plant size is established, one may also want to know to what extent shoot and root competition affect plant RSR via their effects on plant size. More importantly, to what extent are their effects on RSR independent of plant size?

To answer these questions, path analysis can be used. From a path model (Fig. 6.3) including plant size (vegetative mass), the underlying processes are revealed in detail. Going back to the above-mentioned pea experiment, root competition had a direct and significant positive effect on RSR (Fig. 6.3). However, it was counteracted by an indirect and significant negative effect (via reducing plant size, Fig. 6.3), resulting in a neutral total effect (standardized effect = 0.12,  $P = 0.227$ ), as we found from the ANOVA test. On the other hand, the direct and significant negative effect of shoot competition on RSR was further aggravated through its indirect and significant negative effect (via reducing plant size, Fig. 6.3), leading to a negative total effect (standardized effect = -0.52,  $P < 0.001$ ). Therefore, we also can see the difference between the path analysis and the ANCOVA test: the former designated plant size as a mediator that was simultaneously affected by competition and affecting RSR; while the latter simply treated plant size as an independent factor (covariate).

So far, the analysis above has only considered plant size in relation to biomass allocation pattern. However, there is evidence that other traits of plants also correlate with their sizes. For instance, specific leaf area and

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specific ectomycorrhizal root area decreased with the size of *Betula pendula* (Rosenvald *et al.* 2012), and leaf area ratios (whole plant leaf area/plant total mass) of *Abutilon theophrasti*, *Chenopodium album* and *Polygonum pennsylvanicum* had quadratic relationships with their sizes (McConnaughay & Coleman 1999). Apparently, these traits all share a common characteristic: they are calculated based on ratios between size-related traits, and so, absolute size (or mass) differences are cancelled. Therefore, this implicitly suggests that the studies of responses in *ratio type* traits of plants to environmental factors need to consider the potential impacts of plant size.



**Fig. 6.3** The path model for the direct and indirect (via plant size, i.e. vegetative mass) effects of root and shoot competition on root:shoot ratio of *Pisum sativum*. Arrows indicate the direction of paths. For each path, standardized coefficient and its significant level (\*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ) are given. Values in brackets denote the goodness of fit ( $R^2$ ) for endogenous variables.

### 6.3 Evidence for below-ground neighbour detection?

In the past two decades, numerous studies have shown evidence of root-mediated neighbour detection in plants (reviewed in **Chapter 2**). In this thesis, I show that: given equal amount of nutrients, *Pisum sativum* sharers allocated less biomass to roots than owners (**Chapter 3**); *Potentilla reptans* had less vegetative propagation when rooting with disconnected neighbours than with connected neighbours (**Chapter 4**); and *Phaseolus vulgaris*

produced proportionally more large seeds in the treatment that allowed root competition (**Chapter 5**). Thus, this thesis presents robust evidence that the presence of below-ground neighbours has direct effects on biomass increment, biomass allocation and reproductive strategy of plants.

In addition to recent studies that show morphological changes in roots in response to neighbours (e.g. Fang *et al.* 2013; Semchenko *et al.* 2014), some studies also provided support for neighbour detection at the molecular level. In a competition experiment where *Arabidopsis thaliana* plants were at a density of 20 plants per pot, transcriptional analysis showed that the genes expressed in competing plants were mainly related to nutrient deficiency and pathogen defence as compared to plants grown alone (Masclaux *et al.* 2012). Since the pathogen defence genes can be simply activated by nutrient starvation (Masclaux *et al.* 2012 and references therein), it is possible that competition-induced defensive responses may just be the consequences of nutrient deficiency. However, in another competition experiment where one *A. thaliana* plant was grown with one plant of a weak competitor *Hieracium pilosella*, only pathogen defence related gene expressions were revealed (Schmid *et al.* 2013). It is expected that competition intensity (approximately the number of competitors) is highly related to the nutrient status of the target plants. Therefore, the discovery that nutrient-deficiency-related gene expressions only appeared in severe competition while pathogen-defence-related gene expressions occurred in both severe and mild competition implied that the defensive responses of gene expressions to competition are at least partially independent of nutrient status (Schmid *et al.* 2013). It further suggests a possible process of neighbour detection. In an ecological or evolutionary context, the results were also interpreted as a preparation for potential interactions (Schmid *et al.* 2013), e.g. against higher chance of pathogen infection carried over from neighbours.

However, it is also noted that, so far, the existence of below-ground neighbour detection is based on the comparison of traits between plants growing with neighbours and plants growing alone. Unlike the well-established mechanistic understanding of above-ground neighbour detection (Pierik *et al.* 2013; Gundel *et al.* 2014) or below-ground water (Bao *et al.* 2014) and nutrient foraging (Forde 2014; Kellermeier *et al.* 2014; Talboys *et al.* 2014; Tian *et al.* 2014), we are still far from having a coherent picture of how below-ground neighbour detection is regulated.

In **Chapter 2** I attempted to derive such a picture from the existing literature, and I noted that there are probably multiple mechanisms involved.

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To date, root exudates have frequently been proposed as the signals that mediate allogeneic recognition processes, e.g. kin versus non-kin and con-versus hetero-specifics (Biedrzycki *et al.* 2010; Caffaro *et al.* 2013; Fang *et al.* 2013; Schmid *et al.* 2013; Semchenko *et al.* 2014). However, to what extent they also can be applicable to physiological self/non-self recognition, e.g. between clonal ramets (**Chapter 4**), is doubtful, because in this situation plants are genetically identical and thus they would be expected to produce the same biochemical substances. For such kind of recognition, some hypotheses have been proposed, e.g. an oscillatory signalling system that relies on physical connection (Falik *et al.* 2003; Gruntman & Novoplansky 2004; and reviewed in **Chapter 2**). Empirical evidence, however, is still generally lacking.

Therefore, to have a better understanding of belowground neighbour detection, especially its ecological and evolutionary impacts, one must take an additional important step - identifying the physiological and even molecular processes of detection rather than biomass- or trait-based response (Pierik *et al.* 2012; Gundel *et al.* 2014).

### 6.4 Evidence for a tragedy of the commons in root growth?

In **Chapter 3** I showed that pea sharers allocated less mass to roots than pea owners. In **Chapter 4** I showed that ramets of *P. reptans* produced no more roots when grown with disconnected neighbours than grown with connected neighbours. Apparently, I found no evidence of a classical tragedy-of-the-commons (TOC) scenario associated with neighbour-induced root over-proliferation. Furthermore, I demonstrated that the methodologies in the studies that did find a TOC scenario can be challenged: (i) TOC results in the studies using an ‘one-pot-owner and two-pot-sharer’ design (e.g. Gersani *et al.* 2001; Maina *et al.* 2002) can be confounded by the difference in rooting volume and plant size between owners and sharers (**Chapter 3**); (ii) TOC results in the studies using an ‘intact and twin pair’ design with clonal ramets (e.g. Falik *et al.* 2006) can be confounded by the side effects of severing connections (**Chapter 4**).

Based upon a thorough study of the literature, in **Chapter 3**, I argue that root responses to below-ground neighbours in plants might depend on the environmental context, especially related to soil resource conditions. The

original game-theoretical model which predicts TOC scenarios from Gersani *et al.* (2001) only includes the mass cost of root production, and the model implicitly assumes that root distributions of competing plants are completely overlapping in the whole soil volume. However, in reality, the distance cost of root production also needs to be considered, and root distributions of plants are more likely to be only partially overlapping. A subsequent spatially explicit model from O'Brien *et al.* (2007) shows that the degree of root overlapping is strongly determines the extent to which a TOC scenario may evolve, because root over-proliferation is only beneficial in the parts or the habitat where roots of neighbouring plants overlap (see also Anten & During 2011 for a more general discussion in this issue). It also predicts that the degree of root overlapping positively correlates with soil resource levels. A newer model from McNickle & Brown (2012) further indicates that the growth strategy of roots in response to the presence of neighbours relies on soil nutrient conditions: natural selection should favour plants that over-proliferate roots in nutrient-rich conditions, but produce less root biomass in nutrient-poor conditions. This is because the production cost for per unit mass of roots is low, and the uptake ability is high in fertile soil. The production cost is high while rewards are low in infertile soils. Together, this suggests that TOC scenarios in root competition are more likely to occur in dense plant populations (i.e. high degree of root overlapping) with high resource conditions (i.e. high benefit and low cost for root proliferation in competition).

Root proliferation and distribution of plants competing with each other also highly depend on the type and the spatiotemporal dynamics of the resource (Hutchings *et al.* 2003; Hodge 2004). For above-ground competition in nature, light is the main limiting resource. It arrives continuously during the day, and it does not lead to consistently heterogeneous conditions above the canopy. However, this does not apply to below-ground resources, which are composed of several kinds of nutrient elements and tend to be depleted when taken up by plants (Schenk 2006). The distribution of different resources in the soil also differs considerably, because of their physical (e.g. mobility) and chemical characteristics, their heterogeneous distributions, as well as their interaction with the soil properties and substances. Based on the assumption that root growth is only determined by the local nutrient concentration, a modelling study (de Vries 2013) showed that root growth strategy in competition also varies with the characteristics of the limiting resource. For example, plants tend to have a

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lower degree of overlapping roots when competing for more mobile resources (e.g. nitrogen) than when competing for less mobile resources (e.g. phosphorus). Therefore, this indicates that the extent to which a TOC in growth occurs also depends on the type and the distribution of the key soil resource for which plants are competing.

It is interesting to note that the TOC scenarios described in the literature are mainly based on the argument that more root mass comes at the expense of reproductive mass of plants in root interaction. However, competitive responses to neighbours may involve other costs besides mass. In **Chapter 4**, I found that although interacting with disconnected neighbours did not lead to greater root mass in *P. reptans* ramets, a significant reduction in vegetative propagation was observed. It is possible that such reduction might be due to a higher maintenance cost per unit mass of roots. This line of thought is based on the findings that plants grown with neighbours may also modify the morphological or physiological traits of roots with e.g. more root branches, thinner root diameters and more root secretion without changes in root mass (e.g. Meier *et al.* 2013; Semchenko *et al.* 2014). These more active root traits may have higher resource uptake efficiency or more inter-plant communications, but also have a higher maintenance cost, i.e. higher respiration rates (Eissenstat & Yanai 1997; Meier *et al.* 2013). Such responses could also be interpreted as a TOC phenomenon. This further suggests that it is also important to measure respiration, secretion and other aspects of root growth in addition to the biomass.

Thus, more experiments are needed to determine the extent to and conditions under which TOCs in root may occur. The associated experiments should be preferentially done in fields or big containers that do not limit root growth. Plants should be harvested sequentially in several developmental stages rather than one final event. Plant size difference need to be considered. Systematic experiments at different nutrient levels and possibly distribution patterns should also be conducted. Finally, other costs of belowground resource uptake, in addition to mass, should be considered.

### **6.5 Cross-talk between above- and below-ground plant parts**

As argued in **Chapter 2**, most neighbour detection or game theoretical studies to date have focused on the interaction and associated responses in

one compartment (above- or below-ground) without considering processes occurring in the other (see Givnish 1982; Anten 2002; Falster & Westoby 2003; Vermeulen 2015, for examples of aboveground studies). In most cases, the relationship between above- and below-ground parts of plants in plant-plant interactions are simply thought to be a consequence of within-plant resource partitioning, e.g. in the concept of the TOC in root growth, the production of seeds depends on the proportion of resources taken up by roots, which can then be transported to seeds.

However, in **Chapter 5** I explicitly show that, when rooting with neighbours, *P. vulgaris* produced proportionally more large seeds independently of the amount of resources transferred from roots to seeds. I interpret this result as a maternal preparation for intensive competition in the offspring generation. Interestingly, similar neighbour effects seem to operate at other stages of the reproductive cycle. Falik *et al.* (2014) showed that the exposure of root exudates from early flowering plants accelerated the flowering time of late flowering plants in *Brassica rapa*. The authors suggested that the acceleration is a response for synchronizing flowering time to increase pollination and seed dispersal. Lankinen (2008) found that a con-specific below-ground neighbour stimulated *Viola tricolor* to produce more competitive pollen, in terms of higher pollen tube growth, independently of nutrient availability. Her results can also be seen as evidence for competition in terms of pollination opportunities, when several pollens of different plants fall on the same stigma. Together, these findings suggest that there are also some non-resource-related regulation systems that mediate above- and below-ground communication, which may play important roles in the adaptation of plants. Therefore, there is an urgent need for integrative studies that consider above- and below-ground interactions and responses (both resource and non-resource related) simultaneously.

## 6.6 Implications for agriculture

In the same year when the concept of the *tragedy of the commons* (TOC) came to the public, another influential concept was developed in agricultural research: the *ideotype* (Donald 1968). It refers to a cultivar that makes a minimum demand on resources per unit of yield produced. It was proposed that the key element for designing such an ideotype is to create a *weak*

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*competitor* (Donald 1968). In this way, competition within the crop community will be reduced to a minimum, and resources can be channelled into yields (Donald 1981; Weiner 2003). More recently, scientists have begun to formalize the link between the concept of TOC defined in plant ecological research and the ideotype defined in agricultural research (see Zhang *et al.* 1999). From an evolutionary game-theoretical context, the former focuses on the cause and impacts of the over-production of vegetative organs in natural selection; while the latter aims to minimize such over-production in the crop fields against natural selection (Zhang *et al.* 1999; Denison *et al.* 2003). Therefore, a better understanding of the TOC phenomenon and its underlying mechanisms (e.g. neighbour detection) will also benefit agricultural research, at least for crop breeding.

Since the beginning of last century, especially during the *green revolution* (see Evenson & Gollin 2003), the above-ground parts of crops, e.g. cereals, have been consciously and continuously selected against evolutionarily stable traits, e.g. shorter and stronger stems, erect leaves and less tillers (e.g. Austin *et al.* 1980; Mäkelä *et al.* 2008; Peng *et al.* 2008; and see Anten & During 2011), in order to obtain a higher harvest index, thus approaching the ideotype. However, root traits have seldom been similarly considered (Gregory 2006). For instance, Ma *et al.* (2009) showed that root pruning significantly reduced water consumption at the expense of root competitive ability in winter wheat, but had no effect on final yields. The results reflected a classical TOC scenario in water competition, implicitly suggesting that their crop plants produced more root mass than would be optimal for maximization of yields, i.e., a TOC scenario. Therefore, it indicates that there are needs but also opportunities for the improvement of root traits towards an ideotype.

Some evidence suggests that, in the course of breeding high yielding crops, breeders may have unintentionally selected cultivars that grow smaller root systems. Take wheat for example: Waines and Ehdiaie (2007) found that some modern high yielding wheat cultivars only have 30 ~ 40% of the root biomass typical of their landrace ancestors. As a consequence, the modern wheat cultivars are often inferior in root competition with landraces (e.g. Reynolds *et al.* 1994; Fang *et al.* 2011).

In a pot-based study of competition between an old low-yielding (landrace) and a modern high-yielding wheat cultivar with a replacement series design, Zhu and Zhang (2013) found that root mass of the old cultivar was 2.5 times larger than that of the modern cultivar when grown in mono-

stands. More interestingly, the authors also found that root growth of the old cultivar remained at a high level regardless of the cultivar composition in mixed stands, while root growth of the modern cultivar significantly increased as the frequency of the old cultivar in the mixed stands increased. Based on these results, the authors proposed that the old cultivar adopted a growth strategy that is close to an evolutionary stable strategy, while the modern one behaved more ‘cooperatively’, resembling of kin recognition in ecological studies (see Dudley *et al.* 2013). However, to what extent their arguments hold true is still unclear.

The claim of kin recognition also occurs in the study of rice. Fang *et al.* (2013) showed that, by growing rice seedlings in transparent gel medium, the degree of root overlap in intra-genotypic interaction was significantly higher than that in inter-genotypic interaction. This was mainly due to an avoidance response of root growth between hetero-genotypic seedlings. Such findings are in contrast to the kinship regulated responses in other species (e.g. *Arabidopsis thaliana*, Biedrzycki *et al.* 2010; *Cakile edentula*, Bhatt *et al.* 2011) that tend to avoid severe competition with kin. However, it is also noted that auto-toxic phenomena are often discovered in rice fields (e.g. Chou 1995). Ghahari and Miransari (2009) showed that the allelopathic effects of rice hull extracts in most cases can significantly inhibit the root growth of rice seedlings from other cultivars. Therefore, a question related to the results of Fang *et al.* (2013) can be raised: is the discovered root avoidance in inter-genotypic interaction caused by kin recognition or simply by toxicity?

In short, the above-mentioned findings and the possible alternative interpretations emphasize the need for further exploration of the underlying mechanisms of below-ground neighbour detection and plant-plant interaction. Only then, can we identify the right directions for future crop breeding: selecting either more kin-friendly, or less nutrient-sensitive, or less auto-toxic cultivars.



# Bibliography

- Afifi M & Swanton C. (2011) Maize seed and stem roots differ in response to neighbouring weeds. *Weed Res.*, 51, 442-450.
- Akiyama K, Matsuzaki K & Hayashi H. (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*, 435, 824-827.
- Albuquerque P & Casadevall A. (2012) Quorum sensing in fungi - a review. *Med. Mycol.*, 50, 337-345.
- Allen RM, Buckley YM & Marshall DJ. (2008) Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *Am. Nat.*, 171, 225-237.
- Alpert P. (1996) Nutrient sharing in natural clonal fragments of *Fragaria Chiloensis*. *J. Ecol.*, 84, 395-406.
- Alpert P, Holzapfel C & Benson JM. (2002) Hormonal modification of resource sharing in the clonal plant *Fragaria chiloensis*. *Funct. Ecol.*, 16, 191-197.
- Alpert P & Mooney HA. (1986) Resource sharing among ramets in the clonal herb, *Fragaria chiloensis*. *Oecologia*, 70, 227-233.
- Anten NPR. (2002) Evolutionarily stable leaf area production in plant populations. *J. Theor. Biol.*, 217, 15-32.
- Anten NPR. (2005) Optimal photosynthetic characteristics of individual plants in vegetation stands and implications for species coexistence. *Ann. Bot.*, 95, 495-506.
- Anten NPR & During HJ. (2011) Is analysing the nitrogen use at the plant canopy level a matter of choosing the right optimization criterion? *Oecologia*, 167, 293-303.
- Argyres AZ & Schmitt J. (1992) Neighbor relatedness and competitive performance in *Impatiens capensis* (Balsaminaceae) - a test of the resource partitioning hypothesis. *Am. J. Bot.*, 79, 181-185.
- Ashmun JW, Thomas RJ & Pitelka LF. (1982) Translocation of photoassimilates between sister ramets in two rhizomatous forest herbs. *Ann. Bot.*, 49, 403-415.
- Augner M, Fagerström T & Tuomi J. (1991) Competition, defense and games between plants. *Behav. Ecol. Sociobiol.*, 29, 231-234.
- Austin RB, Bingham J, Blackwell RD, Evans LT, Ford MA, Morgan CL & Taylor M. (1980) Genetic improvements in winter wheat yields since 1900 and associated physiological changes. *J. Agric. Sci.*, 94, 675-689.
- Badri DV, De-la-Peña C, Lei Z, Manter DK, Chaparro JM, Guimarães RL, Sumner LW & Vivanco JM. (2012) Root secreted metabolites and proteins are involved in the early events of plant-plant recognition prior to competition. *PLoS ONE*, 7, e46640.
- Badri DV & Vivanco JM. (2009) Regulation and function of root exudates. *Plant Cell Environ.*, 32, 666-681.
- Bais HP, Park SW, Weir TL, Callaway RM & Vivanco JM. (2004) How plants communicate using the underground information superhighway. *Trends Plant Sci.*, 9, 26-32.

## Bibliography

- Bais HP, Weir TL, Perry LG, Gilroy S & Vivanco JM. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.*, 57, 233-266.
- Baluška F, Mancuso S, Volkmann D & Barlow PW. (2009) The 'root-brain' hypothesis of Charles and Francis Darwin: revival after more than 125 years. *Plant Signal. Behav.*, 4, 1121-1127.
- Bao Y, Aggarwal P, Robbins II NE, Sturrock CJ, Thompson MC, Tan HQ, Tham C, Duan L, Rodriguez PL, Vernoux T, Mooney SJ, Bennett MJ & Dinneny JR. (2014) Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proc. Natl. Acad. Sci. U.S.A.*, 111, 9319-9324.
- Bates D, Maechler M, Bolker B & Walker S. (2014) *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-6. <http://CRAN.R-project.org/package=lme4>
- Bhatt MV, Khandelwal A & Dudley SA. (2011) Kin recognition, not competitive interactions, predicts root allocation in young *Cakile edentula* seedling pairs. *New Phytol.*, 189, 1135-1142.
- Biedrzycki ML & Bais HP. (2010a) Kin recognition in plants: a mysterious behaviour unsolved. *J. Exp. Bot.*, 61, 4123-4128.
- Biedrzycki ML & Bais HP. (2010b) Kin recognition: another biological function for root secretions. *Plant Signal. Behav.*, 5, 401-402.
- Biedrzycki ML, Jilany TA, Dudley SA & Bais HP. (2010) Root exudates mediate kin recognition in plants. *Commun. Integr. Biol.*, 3, 28-35.
- Biedrzycki ML, L V & Bais HP. (2011a) The role of ABC transporters in kin recognition in *Arabidopsis thaliana*. *Plant Signal. Behav.*, 6, 1154-1161.
- Biedrzycki ML, Venkatachalam L & Bais HP. (2011b) Transcriptome analysis of *Arabidopsis thaliana* plants in response to kin and stranger recognition. *Plant Signal. Behav.*, 6, 1515-1524.
- Biernaskie JM. (2011) Evidence for competition and cooperation among climbing plants. *Proc. R. Soc. B*, 278, 1989-1996.
- Birch CPD & Hutchings MJ. (1999) Clonal segmentation - the development of physiological independence within stolons of *Glechoma hederacea* L. (Lamiaceae). *Plant Ecol.*, 141, 21-31.
- Boehm T. (2006) Quality control in self/nonself discrimination. *Cell*, 125, 845-858.
- Bozdogan H. (1987) Model selection and Akaike's information criterion (AIC): the general theory and its analytical extensions. *Psychometrika*, 52, 345-370.
- Breen AN & Richards JH. (2008) Irrigation and fertilization effects on seed number, size, germination and seedling growth: implications for desert shrub establishment. *Oecologia*, 157, 13-19.
- Broom M, Speed MP & Ruxton GD. (2005) Evolutionarily stable investment in secondary defences. *Funct. Ecol.*, 19, 836-843.
- Brouwer R. (1983) Functional equilibrium - sense or nonsense. *Neth. J. Agr. Sci.*, 31, 335-348.
- Broz AK, Broeckling CD, De-la-Pena C, Lewis MR, Greene E, Callaway RM, Sumner LW & Vivanco JM. (2010) Plant neighbor identity influences plant biochemistry and physiology related to defense. *BMC Plant Biol.*, 10, 115.

## Bibliography

- Brunner I, Ruf M, Luscher P & Sperisen C. (2004) Molecular markers reveal extensive intraspecific below-ground overlap of silver fir fine roots. *Mol. Ecol.*, 13, 3595-3600.
- Burgess SC & Marshall DJ. (2014) Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos*, 123, 769-776.
- Caffaro MM, Vivanco JM, Boem FHG & Rubio G. (2011) The effect of root exudates on root architecture in *Arabidopsis thaliana*. *Plant Growth Regul.*, 64, 241-249.
- Caffaro MM, Vivanco JM, Botto J & Rubio G. (2013) Root architecture of *Arabidopsis* is affected by competition with neighbouring plants. *Plant Growth Regul.*, 70, 141-147.
- Cahill JF. (2003) Lack of relationship between below-ground competition and allocation to roots in 10 grassland species. *J. Ecol.*, 91, 532-540.
- Cahill JF, McNickle GG, Haag JJ, Lamb EG, Nyanumba SM & St Clair CC. (2010) Plants integrate information about nutrients and neighbors. *Science*, 328, 1657.
- Callaway RM. (2002) The detection of neighbors by plants. *Trends Ecol. Evol.*, 17, 104-105.
- Callaway RM & Mahall BE. (2007) Plant ecology: family roots. *Nature*, 448, 145-147.
- Chapman DF, Robson MJ & Snaydon RW. (1991) Quantitative carbon distribution in clonal plants of white clover (*Trifolium repens*): source-sink relationships during undisturbed growth. *J. Agric. Sci.*, 116, 229-238.
- Cheplick GP. (1992) Sibling competition in plants. *J. Ecol.*, 80, 567-575.
- Cheplick GP & Kane KH. (2004) Genetic relatedness and competition in *Triplasis purpurea* (Poaceae): resource partitioning or kin selection? *Int. J. Plant Sci.*, 165, 623-630.
- Chou CH. (1995) Allelopathy and sustainable agriculture. In: Inderjit, Dakshini KMM, Einhellig FA, eds. *Allelopathy: Organisms, Processes, and Applications*. Washington, DC: American Chemical Society, 211-223.
- Cipollini ML & Stiles EW. (1991) Seed predation by the bean weevil *Acanthoscelides obtectus* on *Phaseolus* species: consequences for seed size, early growth and reproduction. *Oikos*, 60, 205-214.
- Coleman JS, McConnaughay KDM & Ackerly DD. (1994) Interpreting phenotypic variation in plants. *Trends Ecol. Evol.*, 9, 187-191.
- Cooper EL. (2010) Evolution of immune systems from self/not self to danger to artificial immune systems (AIS). *Phys. Life Rev.*, 7, 55-78.
- D'Hertefeldt T & Jónsdóttir IS. (1999) Extensive physiological integration in intact clonal systems of *Carex arenaria*. *J. Ecol.*, 87, 258-264.
- Dalling JW, Winter K, Andersen KM & Turner BL. (2013) Artefacts of the pot environment on soil nutrient availability: implications for the interpretation of ecological studies. *Plant Ecol.*, 214, 329-338.
- Damgaard C & Weiner J. (2000) Describing inequality in plant size or fecundity. *Ecology*, 81, 1139-1142.
- Davies E. (2004) New functions for electrical signals in plants. *New Phytol.*, 161, 607-610.
- Davies E. (2006) Electrical signals in plants: facts and hypotheses. In: Volkov AG, ed. *Plant Electrophysiology - Theory & Methods*. Berlin Heidelberg: Springer-Verlag, 407-422.
- de Kroon H. (2007) Ecology: how do roots interact? *Science*, 318, 1562-1563.

## Bibliography

- de Kroon H, Mommer L & Nishiwaki A. (2003) Root competition: Towards a mechanistic understanding. In: de Kroon H, Visser EJW, eds. *Root Ecology*. Berlin Heidelberg: Springer-Verlag, 215-234.
- de Kroon H & van Groenendael J. (1997) *The Ecology and Evolution of Clonal Plants*. Leiden: Backhuys.
- de Vries J. (2013) *Modelling the effect of competition through soil resources on root architecture*. MSc Thesis, Utrecht University, The Netherlands.
- de Wit M, Kegge W, Evers JB, Vergeer-van Eijk MH, Gankema P, Voeselek LACJ & Pierik R. (2012) Plant neighbor detection through touching leaf tips precedes phytochrome signals. *Proc. Natl. Acad. Sci. U.S.A.*, 109, 14705-14710.
- Delesalle VA & Mazer SJ. (2002) The neighborhood matters: effects of neighbor number and sibling (or kin) competition on floral traits in *Spergularia marina* (Caryophyllaceae). *Evolution*, 56, 2406-2413.
- Deltas G. (2003) The small-sample bias of the Gini coefficient: results and implications for empirical research. *Rev. Econ. Stat.*, 85, 226-234.
- Denison RF, Kiers ET & West SA. (2003) Darwinian agriculture: when can humans find solutions beyond the reach of natural selection? *Q. Rev. Biol.*, 78, 145-168.
- Dixon RA. (2001) Natural products and plant disease resistance. *Nature*, 411, 843-847.
- Donald CM. (1968) The breeding of crop ideotypes. *Euphytica*, 17, 385-403.
- Donald CM. (1981) Competitive plants, communal plants, and yield in wheat crops. In: Evans LT, Peacock WJ, eds. *Wheat Science - Today and Tomorrow*. Cambridge: Cambridge University Press, 223-247.
- Donohue K. (2003) The influence of neighbor relatedness on multilevel selection in the Great Lakes sea rocket. *Am. Nat.*, 162, 77-92.
- Dubois J & Cheptou P-O. (2012) Competition/colonization syndrome mediated by early germination in non-dispersing achenes in the heteromorphic species *Crepis sancta*. *Ann. Bot.*, 110, 1245-1251.
- Dubrovsky JG, Napsucially-Mendivil S, Duclercq J, Cheng Y, Shishkova S, Ivanchenko MG, Friml J, Murphy AS & Benkova E. (2011) Auxin minimum defines a developmental window for lateral root initiation. *New Phytol.*, 191, 970-983.
- Dudley SA & File AL. (2007) Kin recognition in an annual plant. *Biol. Lett.*, 3, 435-438.
- Dudley SA, Murphy GP & File AL. (2013) Kin recognition and competition in plants. *Funct. Ecol.*, 27, 898-906.
- Eissenstat DM & Yanai RD. (1997) The ecology of root lifespan. *Adv. Ecol. Res.*, 27, 1-60.
- Epstein LH, Wisniewski L & Weng R. (1994) Child and parent psychological problems influence child weight control. *Obes. Res.*, 2, 509-515.
- Eriksson O. (1999) Seed size variation and its effect on germination and seedling performance in the clonal herb *Convallaria majalis*. *Acta Oecol.*, 20, 61-66.
- Evans GC. (1972) *The Quantitative Analysis of Plant Growth*. Berkeley and Los Angeles: University of California Press.
- Evenson RE & Gollin D. (2003) Assessing the impact of the green revolution, 1960 to 2000. *Science*, 300, 758-762.
- Falik O, de Kroon H & Novoplansky A. (2006) Physiologically-mediated self/non-self root discrimination in *Trifolium repens* has mixed effects on plant performance. *Plant Signal. Behav.*, 1, 116-121.
- Falik O, Hoffmann I & Novoplansky A. (2014) Say it with flowers: flowering acceleration by root communication. *Plant Signal. Behav.*, 9, e28258.

## Bibliography

- Falik O, Mordoch Y, Quansah L, Fait A & Novoplansky A. (2011) Rumor has it ... : relay communication of stress cues in plants. *PLoS ONE*, 6, e23625.
- Falik O, Reides P, Gersani M & Novoplansky A. (2003) Self/non-self discrimination in roots. *J. Ecol.*, 91, 525-531.
- Falik O, Reides P, Gersani M & Novoplansky A. (2005) Root navigation by self inhibition. *Plant Cell Environ.*, 28, 562-569.
- Falster DS & Westoby M. (2003) Plant height and evolutionary games. *Trends Ecol. Evol.*, 18, 337-343.
- Fang S, Clark R, Zheng Y, Iyer-Pascuzzi A, Weitz J, Kochian L, Edelsbrunner H, Liao H & Benfey P. (2013) Genotypic recognition and spatial responses by rice roots. *Proc. Natl. Acad. Sci. U.S.A.*, 110, 2670-2675.
- Fang Y, Liu L, Xu B-C & Li F-M. (2011) The relationship between competitive ability and yield stability in an old and a modern winter wheat cultivar. *Plant Soil*, 347, 7-23.
- Fensom DS. (1963) The bioelectric potentials of plants and their functional significance: V. some daily and seasonal changes in the electrical potential and resistance of living trees. *Can. J. Bot.*, 41, 831-851.
- File AL, Murphy GP & Dudley SA. (2012) Fitness consequences of plants growing with siblings: reconciling kin selection, niche partitioning and competitive ability. *Proc. R. Soc. B*, 279, 209-218.
- Forde BG. (2014) Nitrogen signalling pathways shaping root system architecture: an update. *Curr. Opin. Plant Biol.*, 21, 30-36.
- Fromm J. (2006) Long-distance electrical signaling and physiological functions in higher plants. In: Volkov AG, ed. *Plant Electrophysiology - Theory & Methods*. Berlin Heidelberg: Springer-Verlag, 269-285.
- Fromm J & Fei HM. (1998) Electrical signaling and gas exchange in maize plants of drying soil. *Plant Sci.*, 132, 203-213.
- Fromm J & Lautner S. (2006) Characteristics and functions of phloem-transmitted electrical signals in higher plants. In: Baluška F, Mancuso D, Volkmann D, eds. *Communication in Plants*. Berlin Heidelberg: Springer-Verlag, 321-332.
- Fromm J & Lautner S. (2007) Electrical signals and their physiological significance in plants. *Plant Cell Environ.*, 30, 249-257.
- Galloway LF. (2001) The effect of maternal and paternal environments on seed characters in the herbaceous plant *Campanula americana* (Campanulaceae). *Am. J. Bot.*, 88, 832-840.
- Geritz SAH. (1995) Evolutionarily stable seed polymorphism and small-scale spatial variation in seedling density. *Am. Nat.*, 146, 685-707.
- Geritz SAH. (1998) Co-evolution of seed size and seed predation. *Evol. Ecol.*, 12, 891-911.
- Gersani M, Brown JS, O'Brien EE, Maina GM & Abramsky Z. (2001) Tragedy of the commons as a result of root competition. *J. Ecol.*, 89, 660-669.
- Ghahari S & Miransari M. (2009) Allelopathic effects of rice cultivars on the growth parameters of different rice cultivars. *Int. J. Biol. Chem.*, 3, 56-70.
- Givnish TJ. (1982) On the adaptive significance of leaf height in forest herbs. *Am. Nat.*, 120, 353-381.
- Glinwood R, Pettersson J, Ahmed E, Ninkovic V, Birkett M & Pickett J. (2003) Change in acceptability of barley plants to aphids after exposure to allelochemicals from couch-grass (*Elytrigia repens*). *J. Chem. Ecol.*, 29, 261-274.

## Bibliography

- Gregory PJ. (2006) *Plant Root: Growth, Activity and Interactions with the Soil*. Oxford: Blackwell Publishing.
- Grime JP. (2001) *Plant Strategies, Vegetation Processes, and Ecosystem Properties*. New York: Wiley.
- Grinsted L, Bilde T & D'Etorre P. (2011) Cuticular hydrocarbons as potential kin recognition cues in a subsocial spider. *Behav. Ecol.*, 22, 1187-1194.
- Grosberg RK. (1988) The evolution of allorecognition specificity in clonal invertebrates. *Q. Rev. Biol.*, 63, 377-412.
- Grosberg RK & Hart MW. (2000) Mate selection and the evolution of highly polymorphic self/nonself recognition genes. *Science*, 289, 2111-2114.
- Gruntman M & Novoplansky A. (2004) Physiologically mediated self/non-self discrimination in roots. *Proc. Natl. Acad. Sci. U.S.A.*, 101, 3863-3867.
- Gundel PE, Pierik R, Mommer L & Ballaré CL. (2014) Competing neighbors: light perception and root function. *Oecologia*, 176, 1-10.
- Gurevitch J, Wilson P, Stone JL, Teese P & Stoutenburgh RJ. (1990) Competition among old-field perennials at different levels of soil fertility and available space. *J. Ecol.*, 78, 727-744.
- Halpern SL. (2005) Sources and consequences of seed size variation in *Lupinus perennis* (Fabaceae): adaptive and non-adaptive hypotheses. *Am. J. Bot.*, 92, 205-213.
- Hamilton WD. (1964) The genetical evolution of social behaviour. I. *J. Theor. Biol.*, 7, 1-16.
- Hanson PJ, Dixon RK & Dickson RE. (1987) Effect of container size and shape on the growth of northern red oak seedlings. *HortScience*, 22, 1293-1295.
- Hardin G. (1968) The tragedy of the commons. *Science*, 162, 1243-1248.
- Harper J, Lovell P & Moore K. (1970) The shapes and sizes of seeds. *Annu. Rev. Ecol. Syst.*, 1, 327-356.
- Hartnett DC & Bazzaz FA. (1983) Physiological integration among intraclonal ramets in *Solidago Canadensis*. *Ecology*, 64, 779-788.
- He WM, Alpert P, Yu FH, Zhang LL & Dong M. (2011) Reciprocal and coincident patchiness of multiple resources differentially affect benefits of clonal integration in two perennial plants. *J. Ecol.*, 99, 1202-1210.
- Heino M, Metz JA & Kaitala V. (1998) The enigma of frequency-dependent selection. *Trends Ecol. Evol.*, 13, 367-370.
- Herben T & Novoplansky A. (2008) Implications of self/non-self discrimination for spatial patterning of clonal plants. *Evol. Ecol.*, 22, 337-350.
- Hess L & de Kroon H. (2007) Effects of rooting volume and nutrient availability as an alternative explanation for root self/non-self discrimination. *J. Ecol.*, 95, 241-251.
- Hikosaka K & Anten NPR. (2012) An evolutionary game of leaf dynamics and its consequences for canopy structure. *Funct. Ecol.*, 26, 1024-1032.
- Hikosaka K & Hirose T. (1997) Leaf angle as a strategy for light competition: optimal and evolutionarily stable light-extinction coefficient within a leaf canopy. *Écoscience*, 4, 501-507.
- Hlavackova V, Krchnak P, Naus J, Novak O, Spundova M & Strnad M. (2006) Electrical and chemical signals involved in short-term systemic photosynthetic responses of tobacco plants to local burning. *Planta*, 225, 235-244.

## Bibliography

- Hodge A. (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol.*, 162, 9-24.
- Hodge A. (2009) Root decisions. *Plant Cell Environ.*, 32, 628-640.
- Hodge A, Robinson D & Fitter A. (2000) Are microorganisms more effective than plants at competing for nitrogen? *Trends Plant Sci.*, 5, 304-308.
- Holzzapfel C & Alpert P. (2003) Root cooperation in a clonal plant: connected strawberries segregate roots. *Oecologia*, 134, 72-77.
- Houssard C & Escarré J. (1991) The effects of seed weight on growth and competitive ability of *Rumex acetosella* from two successional old-fields. *Oecologia*, 86, 236-242.
- HuberSannwald E, Pyke DA & Caldwell MM. (1996) Morphological plasticity following species-specific recognition and competition in two perennial grasses. *Am. J. Bot.*, 83, 919-931.
- Hutchings JA & Reynolds JD. (2004) Marine fish population collapses: consequences for recovery and extinction risk. *BioScience*, 54, 297-309.
- Hutchings MJ. (1999) Clonal plants as cooperative systems: benefits in heterogeneous environments. *Plant Spec. Biol.*, 14, 1-10.
- Hutchings MJ, John EA & Wijesinghe DK. (2003) Toward understanding the consequences of soil heterogeneity for plant populations and communities. 84, 2322-2334.
- Hutchings MJ & Mogie M. (1990) The spatial structure of clonal plants: control and consequences. In: van Groenendael J, de Kroon H, eds. *Clonal Growth in Plants: Regulation and Function*. The Hague: SPB Academic, 57-76.
- Inderjit, Wardle DA, Karban R & Callaway RM. (2011) The ecosystem and evolutionary contexts of allelopathy. *Trends Ecol. Evol.*, 26, 655-662.
- Körner C, Menendez-Riedl SP & John PCL. (1989) Why are bonsai plants small? A consideration of cell size. *Aust. J. Plant Physiol.*, 16, 443-448.
- Karban R & Shiojiri K. (2009) Self-recognition affects plant communication and defense. *Ecol. Lett.*, 12, 502-506.
- Kato-Noguchi H. (2011) Barnyard grass-induced rice allelopathy and momilactone B. *J. Plant Physiol.*, 168, 1016-1020.
- Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE & Amtmann A. (2014) Analysis of the root system architecture of *Arabidopsis* provides a quantitative readout of crosstalk between nutritional signals. *Plant Cell*, 26, 1480-1496.
- Kelly JK. (1996) Kin selection in the annual plant *Impatiens capensis*. *Am. Nat.*, 147, 899-918.
- Kerkhoff AJ & Enquist BJ. (2006) Ecosystem allometry: the scaling of nutrient stocks and primary productivity across plant communities. *Ecol. Lett.*, 9, 419-427.
- Klemens JA. (2008) Kin recognition in plants? *Biol. Lett.*, 4, 67-68.
- Klimeš L, Klimešová J, Hendriks R & van Groenendael J. (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H, van Groenendael J, eds. *The Ecology and Evolution of Clonal Plants*. Leiden: Backhuys, 1-29.
- Kline RB. (1998) *Principles and Practice of Structural Equation Modeling*. New York: Guilford Press.
- Knapp AK, Carroll CJW & Fahey TJ. (2014) Patterns and controls of terrestrial primary production in a changing world. In: Monson RK, ed. *Ecology and the Environment*. New York: Springer, 205-246.

## Bibliography

- Kuznetsova A, Brockhoff B & Christensen RHB. (2014) *lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package)*. R package version 2.0-6. <http://CRAN.R-project.org/package=lmerTest>
- Lance CE. (1988) Residual centering, exploratory and confirmatory moderator analysis, and decomposition of effects in path models containing interactions. *Appl. Psych. Meas.*, 12, 163-175.
- Lang C, Dolynska A, Finkeldey R & Polle A. (2010) Are beech (*Fagus sylvatica*) roots territorial? *Forest Ecol. Manag.*, 260, 1212-1217.
- Lankinen Å. (2008) Root competition influences pollen competitive ability in *Viola tricolor*: effects of presence of a competitor beyond resource availability? *J. Ecol.*, 96, 756-765.
- Lankinen Å, Larsson MC & Fransson A-M. (2013) Allocation to pollen competitive ability versus seed production in *Viola tricolor* an effect of plant size, soil nutrients and presence of a root competitor. *Oikos*, 122, 779-789.
- Lehtilä K & Ehrlén J. (2005) Seed size as an indicator of seed quality: a case study of *Primula veris*. *Acta Oecol.*, 28, 207-212.
- Liu FH, Yu FH, Liu WS, Krüsi BO, Cai XH, Schneller JJ & Dong M. (2007) Large clones on cliff faces: expanding by rhizomes through crevices. *Ann. Bot.*, 100, 51-54.
- Liu J, Dong M, Miao SL, Li ZY, Song MH & Wang RQ. (2006) Invasive alien plants in China: role of clonality and geographical origin. *Biol. Invasions*, 8, 1461-1470.
- Luo FL, Chen Y, Huang L, Wang A, Zhang MX & Yu FH. (2014) Shifting effects of physiological integration on performance of a clonal plant during submergence and de-submergence. *Ann. Bot.*, 113, 1265-1274.
- Mäkelä P, Muurinen S & Peltonen-Sainio P. (2008) Spring cereals: from dynamic ideotypes to cultivars in northern latitudes. *Agric. Food Sci.*, 17, 289-306.
- Müller I, Schmid B & Weiner J. (2000) The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspect. Plant Ecol. Evol. Syst.*, 3, 115-127.
- Ma S-C, Li F-M & Xu B-C. (2009) Effects of root pruning on the growth and water use efficiency of winter wheat. *Plant Growth Regul.*, 57, 233-241.
- Mahall BE & Callaway RM. (1991) Root communication among desert shrubs. *Proc. Natl. Acad. Sci. U.S.A.*, 88, 874-876.
- Mahall BE & Callaway RM. (1992) Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. *Ecology*, 73, 2145-2151.
- Mahall BE & Callaway RM. (1996) Effects of regional origin and genotype on intraspecific root communication in the desert shrub *Ambrosia dumosa* (Asteraceae). *Am. J. Bot.*, 83, 93-98.
- Maina GG, Brown JS & Gersani M. (2002) Intra-plant versus inter-plant root competition in beans: avoidance, resource matching or tragedy of the commons. *Plant Ecol.*, 160, 235-247.
- Mancuso S & Mugnai S. (2006) Long-distance signal transmission in trees. In: Baluška F, Mancuso D, Volkmann D, eds. *Communication in Plants*. Berlin Heidelberg: Springer-Verlag, 333-349.
- Markham J & Halwas S. (2011) Effect of neighbour presence and soil volume on the growth of *Andropogon gerardii* Vitman. *Plant Ecol. Divers.*, 4, 265-268.

## Bibliography

- Masclaux F, Hammond RL, Meunier J, Gouhier-Darimont C, Keller L & Reymond P. (2010) Competitive ability not kinship affects growth of *Arabidopsis thaliana* accessions. *New Phytol.*, 185, 322-331.
- Masclaux FG, Bruessow F, Schweizer F, Gouhier-Darimont C, Keller L & Reymond P. (2012) Transcriptome analysis of intraspecific competition in *Arabidopsis thaliana* reveals organ-specific signatures related to nutrient acquisition and general stress response pathways. *BMC Plant Biol.*, 12, 227.
- Masi E, Ciszak M, Stefano G, Renna L, Azzarello E, Pandolfi C, Mugnai S, Baluska F, Arecchi FT & Mancuso S. (2009) Spatiotemporal dynamics of the electrical network activity in the root apex. *Proc. Natl. Acad. Sci. U.S.A.*, 106, 4048-4053.
- Maynard Smith J & Price GR. (1973) The logic of animal conflict. *Nature*, 246, 15-18.
- McConnaughay KDM & Bazzaz FA. (1991) Is physical space a soil resource? *Ecology*, 72, 94-103.
- McConnaughay KDM, Berntson GM & Bazzaz FA. (1993) Limitations to CO<sub>2</sub>-induced growth enhancement in pot studies. *Oecologia*, 94, 550-557.
- McConnaughay KDM & Coleman JS. (1999) Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. *Ecology*, 80, 2581-2593.
- McGinley MA & Charnov EL. (1988) Multiple resources and the optimal balance between size and number of offspring. *Evol. Ecol.*, 2, 77-84.
- McGinley MA, Temme DH & Geber MA. (1987) Parental investment in offspring in variable environments: theoretical and empirical considerations. *Am. Nat.*, 130, 370-398.
- McNickle GG & Brown JS. (2012) Evolutionarily stable strategies for nutrient foraging and below-ground competition in plants. *Evol. Ecol. Res.*, 14, 667-687.
- McNickle GG & Brown JS. (2014) An ideal free distribution explains the root production of plants that do not engage in a tragedy of the commons game. *J. Ecol.*, 102, 963-971.
- McNickle GG & Dybzinski R. (2013) Game theory and plant ecology. *Ecol. Lett.*, 16, 545-555.
- Meier IC, Angert A, Falik O, Shelef O & Rachmilevitch S. (2013) Increased root oxygen uptake in pea plants responding to non-self neighbors. *Planta*, 238, 577-586.
- Mencuccini M & Holttä T. (2010) The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. *New Phytol.*, 185, 189-203.
- Michaels HJ, Benner B, Hartgerink AP, Lee TD, Rice S, Willson MF & Bertin RI. (1988) Seed size variation: magnitude, distribution, and ecological correlates. *Evol. Ecol.*, 2, 157-166.
- Milla R, Forero DM, Escudero A & Iriondo JM. (2009) Growing with siblings: a common ground for cooperation or for fiercer competition among plants? *Proc. R. Soc. B*, 276, 2531-2540.
- Miller MB & Bassler BL. (2001) Quorum sensing in bacteria. *Annu. Rev. Microbiol.*, 55, 165-199.
- Moles AT, Ackerly DD, Webb CO, Tweddle JC, Dickie JB, Pitman AJ & Westoby M. (2005) Factors that shape seed mass evolution. *Proc. Natl. Acad. Sci. U.S.A.*, 102, 10540-10544.
- Mommer L, van Ruijven J, de Caluwe H, Smit-Tiekstra AE, Wagemaker CAM, Ouborg NJ, Bögemann GM, van der Weerden GM, Berendse F & de Kroon H. (2010) Unveiling below-ground species abundance in a biodiversity experiment: a test

## Bibliography

- of vertical niche differentiation among grassland species. *J. Ecol.*, 98, 1117-1127.
- Moreno-Risueno MA, Van Norman JM, Moreno A, Zhang JY, Ahnert SE & Benfey PN. (2010) Oscillating gene expression determines competence for periodic *Arabidopsis* root branching. *Science*, 329, 1306-1311.
- Muller CH. (1969) Allelopathy as a factor in ecological process. *Vegetatio*, 18, 348-357.
- Murphy GP & Dudley SA. (2009) Kin recognition: Competition and cooperation in *Impatiens* (Balsaminaceae). *Am. J. Bot.*, 96, 1990-1996.
- Murphy GP, File AL & Dudley SA. (2013) Differentiating the effects of pot size and nutrient availability on plant biomass and allocation. *Botany*, 91, 799-803.
- Nasrallah JB. (2002) Recognition and rejection of self in plant reproduction. *Science*, 296, 305-308.
- NeSmith DS & Duval JR. (1998) The effect of container size. *HortTechnology*, 8, 495-498.
- Ninkovic V. (2003) Volatile communication between barley plants affects biomass allocation. *J. Exp. Bot.*, 54, 1931-1939.
- Nord EA, Zhang C & Lynch JP. (2011) Root responses to neighbouring plants in common bean are mediated by nutrient concentration rather than self/non-self recognition. *Funct. Plant Biol.*, 38, 941-952.
- Novoplansky A. (2009) Picking battles wisely: plant behaviour under competition. *Plant Cell Environ.*, 32, 726-741.
- O'Brien EE & Brown JS. (2008) Games roots play: effects of soil volume and nutrients. *J. Ecol.*, 96, 438-446.
- O'Brien EE, Brown JS & Moll JD. (2007) Roots in space: a spatially explicit model for below-ground competition in plants. *Proc. R. Soc. B*, 274, 929-934.
- O'Brien EE, Gersani M & Brown JS. (2005) Root proliferation and seed yield in response to spatial heterogeneity of below-ground competition. *New Phytol.*, 168, 401-412.
- Olf H, Berendse F & de Visser W. (1994) Changes in nitrogen mineralization, tissue nutrient concentrations and biomass compartmentation after cessation of fertilizer application to mown grassland. *J. Ecol.*, 82, 611-620.
- Ollinger SV, Richardson AD, Martin ME, Hollinger DY, Froking SE, Reich PB, Plourde LC, Katul GG, Munger JW, Oren R, Smith M-L, Paw U KT, Bolstad PV, Cook BD, Day MC, Martin TA, Monson RK & Schmid HP. (2008) Canopy nitrogen, carbon assimilation, and albedo in temperate and boreal forests: Functional relations and potential climate feedbacks. *Proc. Natl. Acad. Sci. U.S.A.*, 105, 19336-19341.
- Ortuno A, Sanchezbravo J, Moral JR, Acosta M & Sabater F. (1990) Changes in the concentration of indole-3-acetic-acid during the growth of etiolated lupin hypocotyls. *Physiol. Plant.*, 78, 211-217.
- Oyarce P & Gurovich L. (2011) Evidence for the transmission of information through electric potentials in injured avocado trees. *J. Plant Physiol.*, 168, 103-108.
- Padilla FM, Mommer L, de Caluwe H, Smit-Tiekstra AE, Wagemaker CAM, Ouborg NJ & de Kroon H. (2013) Early root overproduction not triggered by nutrients decisive for competitive success belowground. *PLoS ONE*, 8, e55805.
- Palmer AG, Chen MC, Kingler NP & Lynn DG. (2009) Parasitic angiosperms, semagenesis and general strategies for plant-plant signaling in the rhizosphere. *Pest Manag. Sci.*, 65, 512-519.

- Parciak W. (2002) Environmental variation in seed number, size, and dispersal of a fleshy-fruited plant. *Ecology*, 83, 780-793.
- Parker GA & Smith JM. (1990) Optimality theory in evolutionary biology. *Nature*, 348, 27-33.
- Peng S, Khush GS, Virk P, Tang Q & Zou Y. (2008) Progress in ideotype breeding to increase rice yield potential. *Field Crops Res.*, 108, 32-38.
- Penn D & Potts WK. (1998) Untrained mice discriminate MHC-determined odors. *Physiol. Behav.*, 64, 235-243.
- Perry LG, Thelen GC, Ridenour WM, Weir TL, Callaway RM, Paschke MW & Vivanco JM. (2005) Dual role for an allelochemical: (+/-)-catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. *J. Ecol.*, 93, 1126-1135.
- Perry LG, Weir TL, Prithiviraj B, Paschke MW & Vivanco JM. (2006) Root exudation and rhizosphere biology: multiple functions of a plant secondary metabolite. In: Baluška F, Mancuso S, Volkmann D, eds. *Communication in Plants*. Berlin Heidelberg: Springer-Verlag, 403-420.
- Petricka JJ, Winter CM & Benfey PN. (2012) Control of *Arabidopsis* root development. *Annu. Rev. Plant Biol.*, 63, 563-590.
- Philip L, Simard S & Jones M. (2010) Pathways for below-ground carbon transfer between paper birch and Douglas-fir seedlings. *Plant Ecol. Divers.*, 3, 221-233.
- Pierik R, Mommer L, Voeselek LACJ & Robinson D. (2013) Molecular mechanisms of plant competition: neighbour detection and response strategies. *Funct. Ecol.*, 27, 841-853.
- Poorter H, Bühler J, van Dusschoten D, Climent J & Postma JA. (2012a) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct. Plant Biol.*, 39, 839-850.
- Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P & Mommer L. (2012b) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.*, 193, 30-50.
- Poorter H & Sack L. (2012) Pitfalls and possibilities in the analysis of biomass allocation patterns in plants. *Front. Plant Sci.*, 3, 259.
- Price EAC & Hutchings MJ. (1992) The causes and developmental effects of integration and independence between different parts of *Glechoma hederacea* clones. *Oikos*, 63, 376-386.
- Pronk TE, During HJ & Schieving F. (2007) Coexistence by temporal partitioning of the available light in plants with different height and leaf investments. *Ecol. Model.*, 204, 349-358.
- Putnam AR. (1985) Allelopathic research in agriculture: past highlights and potential. In: Thompson AC, ed. *The Chemistry of Allelopathy: Biochemical Interactions among Plants*. Washington, D.C.: American Chemical Society, 1-8.
- R Core Team. (2014) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org/>
- Rankin DJ, Bargum K & Kokko H. (2007) The tragedy of the commons in evolutionary biology. *Trends Ecol. Evol.*, 22, 643-651.
- Rees M. (1994) Delayed germination of seeds: a look at the effects of adult longevity, the timing of reproduction, and population age/stage structure. *Am. Nat.*, 144, 43-64.

## Bibliography

- Rees M. (1996) Evolutionary ecology of seed dormancy and seed size. *Phil. Trans. R. Soc. Lond. B*, 351, 1299-1308.
- Reynolds JF, Hilbert DW & Kemp PR. (1993) Scaling ecophysiology from the plant to the ecosystem: a conceptual framework. In: Ehleringer JR, Field CB, eds. *Scaling Physiological Processes: Leaf to Globe*. San Diego: Academic Press, 127-140.
- Reynolds MP, Acevedo E, Sayre KD & Fischer RA. (1994) Yield potential in modern wheat varieties: its association with a less competitive ideotype. *Field Crops Res.*, 37, 149-160.
- Riechert SE & Hammerstein P. (1983) Game theory in the ecological context. *Annu. Rev. Ecol. Syst.*, 14, 377-409.
- Rodrigues ML, Santos TP, Rodrigues AP, de Souza CR, Lopes CM, Maroco JP, Pereira JS & Chaves MM. (2008) Hydraulic and chemical signalling in the regulation of stomatal conductance and plant water use in field grapevines growing under deficit irrigation. *Funct. Plant Biol.*, 35, 565-579.
- Roiloa SR & Retuerto R. (2005) Presence of developing ramets of *Fragaria vesca* L. increases photochemical efficiency in parent ramets. *Int. J. Plant Sci.*, 166, 795-803.
- Roiloa SR & Retuerto R. (2006) Physiological integration ameliorates effects of serpentine soils in the clonal herb *Fragaria vesca*. *Physiol. Plant.*, 128, 662-676.
- Roiloa SR, Rodríguez-Echeverría S & Freitas H. (2014) Effect of physiological integration in self/non-self genotype recognition on the clonal invader *Carpobrotus edulis*. *J. Plant Ecol.*, 7, 413-418.
- Rosenvald K, Ostonen I, Uri V, Varik M, Tedersoo L & Lõhmus K. (2012) Tree age effect on fine-root and leaf morphology in a silver birch forest chronosequence. *Eur. J. For. Res.*, 132, 219-230.
- Rosseel Y. (2012) lavaan: An R package for structural equation modeling. *J. Stat. Softw.*, 48, 1-36.
- Ruiz de Clavijo E. (2002) Role of within-individual variation in capitulum size and achene mass in the adaptation of the annual *Centaurea eriophora* to varying water supply in a Mediterranean environment. *Ann. Bot.*, 90, 279-286.
- Sachs T. (1997) Mosses as petri dish models for underground foraging. In: Altman A, Waisel Y, eds. *Biology of Root Formation and Development*. New York: Springer, 341-346.
- Samson DA & Werk KS. (1986) Size-dependent effects in the analysis of reproductive effort in plants. *Am. Nat.*, 127, 667-680.
- Schenk HJ. (2006) Root competition: beyond resource depletion. *J. Ecol.*, 94, 725-739.
- Schenk HJ, Callaway RM & Mahall BE. (1999) Spatial root segregation: are plants territorial? *Adv. Ecol. Res.*, 28, 145-180.
- Schenk HJ & Seabloom FW. (2010) Evolutionary ecology of plant signals and toxins: a conceptual framework. In: Baluška F, Ninkovic V, eds. *Plant Communication from an Ecological Perspective*. Berlin Heidelberg: Springer-Verlag, 1-19.
- Schieving F & Poorter H. (1999) Carbon gain in a multispecies canopy: the role of specific leaf area and photosynthetic nitrogen-use efficiency in the tragedy of the commons. *New Phytol.*, 143, 201-211.

## Bibliography

- Schmid C, Bauer S, Muller B & Bartelheimer M. (2013) Belowground neighbor perception in *Arabidopsis thaliana* studied by transcriptome analysis: roots of *Hieracium pilosella* cause biotic stress. *Front. Plant Sci.*, 4, 296.
- Schmitt J & Ehrhardt DW. (1987) A test of the sib-competition hypothesis for outcrossing advantage in *Impatiens capensis*. *Evolution*, 41, 579-590.
- Semchenko M, Hutchings MJ & John EA. (2007a) Challenging the tragedy of the commons in root competition: confounding effects of neighbour presence and substrate volume. *J. Ecol.*, 95, 252-260.
- Semchenko M, John EA & Hutchings MJ. (2007b) Effects of physical connection and genetic identity of neighbouring ramets on root-placement patterns in two clonal species. *New Phytol.*, 176, 644-654.
- Semchenko M, Saar S & Lepik A. (2014) Plant root exudates mediate neighbour recognition and trigger complex behavioural changes. *New Phytol.*, 204, 631-637.
- Semchenko M, Zobel K, Heinemeyer A & Hutchings MJ. (2008) Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. *New Phytol.*, 179, 1162-1170.
- Shemesh H, Rosen R, Eshel G, Novoplansky A & Ovadia O. (2011) The effect of steepness of temporal resource gradients on spatial root allocation. *Plant Signal. Behav.*, 6, 1356-1360.
- Silvertown J. (1989) The paradox of seed size and adaptation. *Trends Ecol. Evol.*, 4, 24-26.
- Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ & Teste FP. (2012) Mycorrhizal networks: mechanisms, ecology and modelling. *Fungal Biol. Rev.*, 26, 39-60.
- Smith CC & Fretwell SD. (1974) The optimal balance between size and number of offspring. *Am. Nat.*, 108, 499-506.
- Sokal RR & Rohlf FJ. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. New York: Freeman.
- Song YY, Zeng RS, Xu JAF, Li J, Shen XA & Yihdego WG. (2010) Interplant communication of tomato plants through underground common mycorrhizal networks. *PLoS ONE*, 5, e13324.
- Soriano D, Orozco-Segovia A, Márquez-Guzmán J, Kitajima K, Gamboa-de Buen A & Huante P. (2011) Seed reserve composition in 19 tree species of a tropical deciduous forest in Mexico and its relationship to seed germination and seedling growth. *Ann. Bot.*, 107, 939-951.
- Starck Z. (2006) Role of conducting systems in the transduction of long-distance stress signals. *Acta Physiol. Plant.*, 28, 289-301.
- Stuefer JF, During HJ & De Kroon H. (1994) High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments. *J. Ecol.*, 82, 511-518.
- Stuefer JF, van Hulzen JB & During HJ. (2002) A genotypic trade-off between the number and size of clonal offspring in the stoloniferous herb *Potentilla reptans*. *J. Exp. Bot.*, 15, 880-884.
- Talboys PJ, Healey JR, Withers PJ & Jones DL. (2014) Phosphate depletion modulates auxin transport in *Triticum aestivum* leading to altered root branching. *J. Exp. Bot.*, 65, 5023-5032.

## Bibliography

- Thompson MV. (2005) Scaling phloem transport: elasticity and pressure-concentration waves. *J. Theor. Biol.*, 236, 229-241.
- Thompson MV & Holbrook NM. (2004) Scaling phloem transport: information transmission. *Plant Cell Environ.*, 27, 509-519.
- Tian H, De Smet I & Ding Z. (2014) Shaping a root system: regulating lateral versus primary root growth. *Trends Plant Sci.*, 19, 426-431.
- Till-bottraud I, Venable DL, Dajoz I & Gouyon PH. (1994) Selection on pollen morphology: a game theory model. *Am. Nat.*, 144, 395-411.
- Tonsor SJ. (1989) Relatedness and intraspecific competition in *Plantago lanceolata*. *Am. Nat.*, 134, 897-906.
- Trebacz K, Dziubinska H & Krol E. (2006) Electrical signals in long-distance communication in plants. In: Baluška F, Mancuso D, Volkmann D, eds. *Communication in Plants*. Berlin Heidelberg: Springer-Verlag, 277-290.
- Tsuchiya Y & McCourt P. (2012) Strigolactones as small molecule communicators. *Mol. Biosyst.*, 8, 464-469.
- Völler E, Auge H, Prati D, Fischer M, Hemp A & Bossdorf O. (2012) Geographical and land-use effects on seed-mass variation in common grassland plants. *Basic Appl. Ecol.*, 13, 395-404.
- van Bel AJE & Ehlers K. (2007) Electrical signalling via plasmodesmata. In: Oparka KJ, ed. *Plasmodesmata*. Oxford: Blackwell Publishing, doi: 10.1002/9780470988572.ch12.
- van der Meijden R. (2005) *Heukels' Flora van Nederland*. Groningen: Wolters-Noordhoff.
- van Kleunen M & Fischer M. (2001) Adaptive evolution of plastic foraging responses in a clonal plant. *Ecology*, 82, 3309-3319.
- van Kleunen M, Fischer M & Schmid B. (2000) Clonal integration in *Ranunculus reptans*: by-product or adaptation? *J. Exp. Bot.*, 13, 237-248.
- van Kleunen M & Stuefer JF. (1999) Quantifying the effects of reciprocal assimilate and water translocation in a clonal plant by the use of steam-girdling. *Oikos*, 85, 135-145.
- van West P, Morris BM, Reid B, Appiah AA, Osborne MC, Campbell TA, Shepherd SJ & Gow NAR. (2002) Oomycete plant pathogens use electric fields to target roots. *Mol. Plant Microbe Interact.*, 15, 790-798.
- Vandenbussche F, Pierik R, Millenaar FF, Voeselek LA & Van Der Straeten D. (2005) Reaching out of the shade. *Curr. Opin. Plant Biol.*, 8, 462-468.
- Vekemans X & Hardy OJ. (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol. Ecol.*, 13, 921-935.
- Venable DL. (1992) Size-number trade-offs and the variation of seed size with plant resource status. *Am. Nat.*, 140, 287-304.
- Venable DL & Brown JS. (1988) The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *Am. Nat.*, 131, 360-384.
- Vermeulen PJ. (2015) On selection for flowering time plasticity in response to density. *New Phytol.*, 205, 429-439.
- Vincent TL & Brown JS. (1984) The effects of competition on flowering time of annual plants. In: Levin SA, Hallam TG, eds. *Lecture Notes in Biomathematics, Vol. 54*. Heidelberg: Springer-Verlag, 42-54.

## Bibliography

- Vincent TL & Brown JS. (2005) *Evolutionary Game Theory, Natural Selection, and Darwinian Dynamics*. New York: Cambridge University Press.
- Violle C, Castro H, Richarte J & Navas M-L. (2009) Intraspecific seed trait variations and competition: passive or adaptive response? *Funct. Ecol.*, 23, 612-620.
- Waines JG & Ehdaie B. (2007) Domestication and crop physiology: roots of green-revolution wheat. *Ann. Bot.*, 100, 991-998.
- Waldman B. (1988) The ecology of kin recognition. *Annu. Rev. Ecol. Syst.*, 19, 543-571.
- Wang N, Yu FH, Li PX, He WM, Liu FH, Liu JM & Dong M. (2008) Clonal integration affects growth, photosynthetic efficiency and biomass allocation, but not the competitive ability, of the alien invasive *Alternanthera philoxeroides* under severe stress. *Ann. Bot.*, 101, 671-678.
- Wang ZY, van Kleunen M, Dalling HJ & Werger MJA. (2013) Root foraging increases performance of the clonal plant *Potentilla reptans* in heterogeneous nutrient environments. *PLoS ONE*, 8, e58602.
- Weiner J. (1988) The influence of competition on plant reproduction. In: Lovett Doust J, Lovett Doust L, eds. *Plant Reproductive Ecology: Patterns and Strategies*. Oxford: Oxford University Press, 228-244.
- Weiner J. (2003) Ecology - the science of agriculture in the 21st century. *J. Agric. Sci.*, 141, 371-377.
- Weiner J. (2004) Allocation, plasticity and allometry in plants. *Perspect. Plant Ecol. Evol. Syst.*, 6, 207-215.
- Weiner J, Martinez S, Müller-Schärer H, Stoll P & Schmid B. (1997) How important are environmental maternal effects in plants? A study with *Centaurea maculosa*. *J. Ecol.*, 85, 133-142.
- Weiner J & Solbrig OT. (1984) The meaning and measurement of size hierarchies in plant populations. *Oecologia*, 61, 334-336.
- Weisenseel MH, Becker HF & Ehlgötz JG. (1992) Growth, gravitropism, and endogenous ion currents of cress roots (*Lepidium sativum* L.): measurements using a novel three-dimensional recording probe. *Plant Physiol.*, 100, 16-25.
- West-Eberhard MJ. (1989) Kin recognition in animals. *Evolution*, 43, 703-705.
- Westoby M, Leishman M, Lord J, Poorter H & J. Schoen DJ. (1996) Comparative ecology of seed size and dispersal [and discussion]. *Phil. Trans. R. Soc. Lond. B*, 351, 1309-1318.
- Willson MF, Thomas PA, Hoppes WG, Katusicmalmberg PL, Goldman DA & Bothwell JL. (1987) Sibling competition in plants - an experimental study. *Am. Nat.*, 129, 304-311.
- Wong TG & Ackerly DD. (2005) Optimal reproductive allocation in annuals and an informational constraint on plasticity. *New Phytol.*, 166, 159-171.
- Wu H, Pratley J, Lemerle D, An M & Liu DL. (2007) Autotoxicity of wheat (*Triticum aestivum* L.) as determined by laboratory bioassays. *Plant Soil*, 296, 85-93.
- Wulff RD. (1986) Seed size variation in *Desmodium paniculatum*: I. Factors affecting seed size. *J. Ecol.*, 74, 87-97.
- Xie XN, Yoneyama K & Yoneyama K. (2010) The strigolactone story. *Annu. Rev. Phytopathol.*, 48, 93-117.
- Xu G, Wolf S & Kafkafi U. (2001) Interactive effect of nutrient concentration and container volume on flowering, fruiting, and nutrient uptake of sweet pepper. *J. Plant Nutr.*, 24, 479-501.

## Bibliography

- Yan XF, Wang ZY, Huang L, Wang C, Hou RF, Xu ZL & Qiao XJ. (2009) Research progress on electrical signals in higher plants. *Prog. Nat. Sci.*, 19, 531-541.
- You W, Fan S, Yu D, Xie D & Liu C. (2014) An invasive clonal plant benefits from clonal integration more than a co-occurring native plant in nutrient-patchy and competitive environments. *PLoS ONE*, 9, e97246.
- Young JPW. (1981) Sib competition can favour sex in two ways. *J. Theor. Biol.*, 88, 755-756.
- Zhang D-Y, Sun G-J & Jiang X-H. (1999) Donald's ideotype and growth redundancy: a game theoretical analysis. *Field Crops Res.*, 61, 179-187.
- Zhu L & Zhang D-Y. (2013) Donald's Ideotype and growth redundancy: a pot experimental test using an old and a modern spring wheat cultivar. *PLoS ONE*, 8, e70006.

# Summary

A major challenge in plant ecology is how to scale from basic physiological processes and plant functional traits to ecosystem structure and productivity. In recent years, plant biologists have been using evolutionary game theory to approach this scaling problem. Game theoretical modelling studies have indicated that plant populations with maximum seed production per unit area can be invaded by a mutant or intruder that grows more leaves, is taller or produces more roots, and that an evolutionarily stable vegetation (one that cannot be invaded) is therefore less than maximally productive. This phenomenon has been dubbed a “Tragedy of the Commons”, which is important from a fundamental ecological perspective, but also in agriculture (i.e. crop yields could potentially be increased by creating cultivars that are less competitive but more productive than those with evolutionarily stable responses).

In the last twenty years, one of the most fascinating discoveries in plant biology is that plant roots are able to detect the presence and even recognize the relatedness of their belowground neighbours. A growing body of research has shown that root morphological traits and distribution patterns can be modified by the presence of a below-ground neighbour, independently of nutrient status. Such responses often lead to an over-proliferation in roots at the expense of investments in reproduction. In line with evolutionary game theory, it is argued that neighbour detection enables plants to engage in resource competition with non-self roots for a greater capture of shared resources, but at the same time to avoid non-profitable competition with self roots.

However, the mechanisms underlying such detection are still unclear. More importantly, the evidence of neighbour detection and its consequent tragedy-of-the-commons scenarios in some studies have been subjected to debate, mainly arising from concerns about the methods employed in those studies. Therefore, the central topic addressed in this thesis is to determine the extent to which there is evidence of belowground neighbour detection with improved methodology and examine the possible occurrence of the consequent game-theoretical scenarios.

After a general introduction in **Chapter 1**, **Chapter 2** provides an

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extensive review that summarizes the results of numerous ecological studies dealing with self/non-self and kin/non-kin root interactions. Also by extensively reviewing the physiological literature on this issue it proposes putative mechanisms for both self/non-self and kin/non-kin recognition suggesting that these forms of recognition are differentially regulated. In addition, the debate, challenges as well as opportunities in this field are discussed. This chapter also serves as a knowledge pool for the following empirical studies.

In **Chapter 3**, I explicitly focus on one of the major methodological concerns, i.e. rooting volume difference between neighbour presence and absence treatments. After correcting for the differences in rooting volume and plant size, I show that *Pisum sativum* plants allocated less mass to roots in the presence of a con-specific neighbour, which in contrary to what one would expect based on a tragedy-of-the-commons scenario. A major part of neighbour presence and rooting volume effects on plant growth was indirectly mediated through the influence on plant size. Integrating the findings with relevant studies in the literature, I propose that neighbour-induced root responses depend on experimental set-ups in subtle ways, especially the amount and spatiotemporal distribution of nutrients in the soil substrate.

Another concern is about the side effects of connection severance in the studies of physiological self/non-self recognition with clonal plants. Using *Potentilla reptans* in **Chapter 4**, I explicitly show that growth differences between twin (connection severed) ramet pairs and intact ramet pairs were more likely to be caused by the disruption of source-sink relationship within the pairs than to be the result of loss of physiological self-recognition. Furthermore, with a novel design that avoids severing connections, I show that ramets grown with genetically identical disconnected neighbours produced similar amounts of root mass but less stolon mass than those grown with connected neighbours, irrespective of the distance along the clone. I argue that the results show evidence of physiological self/non-self recognition, and suggest that root coordination can be maintained over relatively long distances within the clones. But as in **Chapter 3**, the results are not consistent with a classical tragedy-of-the-commons scenario.

In **Chapter 5**, I specifically pay attention to non-resource effects of a belowground neighbour on within-plant seed size distribution. I show that *Phaseolus vulgaris* plants produced proportionally more large seeds when exposed to the roots of a con-specific neighbour, independently of the status

of resource availability in seed production. It is known that, in this species, seedlings from large seeds commonly outcompete seedlings from small seeds. With an assumption that neighbour presence entails cues implying higher likelihood of seedling competition in the next generation, I argue that plants can adjust their investment strategy in offspring to adapt the future environments based on current environmental cues.

In **Chapter 6**, I integrate the results from previous chapters, and conclude that (1) rooting volume affects plant growth far more than the amount of resources it contains, therefore container-based experiments must carefully control plant rooting volume; (2) the size of a plant is highly related with the growth strategy of the plant, so, research on plant responses to environmental factors also need to take plant size into account; (3) there is ample evidence that plants respond to the presence of belowground neighbours, and that it is time now to focus on the underlying mechanisms of the detection; (4) the responses of root growth to the presence of belowground neighbours seem to be environmentally dependent, a tragedy-of-the-commons scenario is more likely to occur in dense populations under resource-rich conditions; (5) the effect of belowground neighbour presence is not only limited to root growth or within-plant biomass partitioning, it also can influence plant reproductive strategy that relates to the adaptation of offspring.

# Samenvatting

Eén van de grote uitdagingen in de planteneecologie betreft de vraag, hoe op te schalen van basale fysiologische processen en functionele eigenschappen van planten naar de structuur en productiviteit van ecosystemen. In de afgelopen jaren hebben plantenbiologen evolutionaire speltheorie gebruikt om dit schalingsprobleem aan te pakken. Speltheoretische modelstudies lieten zien, dat plantenpopulaties met maximale zaadproductie per eenheid bodemoppervlak binnengedrongen kunnen worden door een mutant of indringer die meer bladeren maakt, hoger wordt of meer wortels produceert, en dat een evolutionair stabiele vegetatie (eentje die niet binnengedrongen kan worden) daarom minder dan maximaal productief is. Dit fenomeen wordt een ‘Tragedie van de Meent’ genoemd, een belangrijk concept in de fundamentele ecologie maar ook in de landbouw (d.w.z., oogsten in de landbouw zouden verhoogd kunnen worden door cultivars te kweken die minder concurrentiekrachtig zijn maar productiever dan variëteiten met evolutionair stabiele reacties op hun milieu).

Eén van de meest fascinerende ontdekkingen in de plantenbiologie van de afgelopen twintig jaar is, dat plantenwortels in staat zijn om ondergrondse burenen te detecteren en zelfs te kunnen herkennen in hoeverre deze burenen genetisch verwant zijn. Steeds meer onderzoek laat zien, dat de morfologische eigenschappen van wortels en de ondergrondse patronen in beworteling kunnen veranderen als gevolg van de aanwezigheid van een ondergrondse buurplant, onafhankelijk van de voedingstoestand in de bodem. Zulke reacties van de plant leiden vaak tot overproductie van wortels, die ten koste gaat van investeringen in voortplanting. In overeenstemming met de evolutionaire speltheorie is het argument daarbij, dat buurplant-detectie het planten mogelijk maakt om te investeren in concurrentie om hulpbronnen met wortels van andere planten en zo een groter deel van die, voor alle planten bereikbare, hulpbronnen te bemachtigen, maar wel een vruchteloze concurrentie met de eigen wortels te vermijden.

Welke mechanismen aan deze detectie ten grondslag liggen is echter nog onduidelijk. Belangrijker nog, de aanwijzingen voor buurplant-detectie en het daarop volgende ‘Tragedie van de Meent’ scenario die in sommige

studies gepresenteerd werden zijn onderwerp geworden van discussie, vooral voortkomend uit zorgen over de methoden die bij deze studies gebruikt zijn. Het centrale onderwerp van dit proefschrift is dan ook om te bepalen, in hoeverre er evidentie is voor ondergrondse buurplant-detectie met behulp van verbeterde onderzoeksmethoden, en te onderzoeken in hoeverre de daaraan gekoppelde speltheoretische scenario's inderdaad voorkomen.

Na een algemene inleiding in **hoofdstuk 1** verschaft **hoofdstuk 2** een uitvoerig literatuuroverzicht dat de resultaten samenvat van talrijke ecologische studies naar interacties tussen wortels van de plant zelf met elkaar en met wortels van andere planten, en tussen wortels van genetisch verwante (bijv. uit zaden van dezelfde moederplant) of niet verwante planten. Mede op basis van een uitgebreide bespreking van de fysiologische literatuur hierover doet dit hoofdstuk daarna voorstellen voor mogelijke mechanismen voor herkenning van wortels van niet-eigen of niet-verwante planten. Verder wordt gesuggereerd, dat deze twee vormen van herkenning verschillend gereguleerd worden. Bovendien worden het debat zelf, en de uitdagingen en de kansen in dit veld van onderzoek bediscussieerd. Dit hoofdstuk dient ook als bron van kennis voor de hierna volgende empirische studies.

In **hoofdstuk 3** richt ik me expliciet op een van de grote methodologische punten van zorg, namelijk het verschil in bewortelingsvolume tussen behandelingen met en zonder buurplant. Ik laat zien, dat na correctie voor de verschillen in bewortelingsvolume en plantgrootte, planten van *Pisum sativum* (Erwt) minder massa in de wortels investeren als ze de pot delen met een buurplant van dezelfde soort, hetgeen ingaat tegen wat men zou verwachten op basis van een 'Tragedie van de Meent' scenario. Een groot deel van de effecten van de aanwezigheid van buurplanten en van het verschil in bewortelingsvolume op de groei van de plant blijkt indirect het gevolg te zijn van hun invloed op de grootte van de planten. Ik integreer deze resultaten met data van relevante onderzoeken in de literatuur, en stel voor dat deze door wortels van buurplanten geïnduceerde reacties in beworteling op subtiele wijze afhangen van de opzet van het experiment, waarbij vooral de hoeveelheid en verdeling in ruimte en tijd van de voedingsstoffen in de bodem een rol spelen.

Een ander punt van zorg betreft de neveneffecten van het verbreken van verbindingen tussen plantendelen in de studies van fysiologische eigen/niet-eigen herkenning bij clonale planten. In **hoofdstuk 4** laat ik expliciet zien,

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dat de verschillen in groei tussen tweelingparen (verbinding verbroken) en intacte, nog steeds verbonden paren van ramets (rozetten) van *Potentilla reptans* (Vijfvingerkruid) eerder veroorzaakt lijken te zijn door het verbreken van de bron-put relaties binnen de paren dan door het verlies van fysiologische herkenning van wortels als ‘eigen’. Met een nieuwe proefopzet die het verbreken van de verbindingen vermijdt laat ik verder zien, dat ramets die opgekweekt worden met genetisch identieke niet-verbonden ramets net zoveel wortelmassa, maar minder stolonmassa maken dan ramets die fysiek verbonden zijn met de ernaast groeiende ramet, onafhankelijk van de afstand tussen de ramets langs de uitlopers van de plant. Ik betoog, dat de resultaten aanwijzingen laten zien voor fysiologische eigen/niet-eigen herkenning, en ik suggereer dat wortelcoördinatie in stand gehouden kan worden over grote afstanden binnen de klonen. Net als in **hoofdstuk 3** zijn de resultaten echter niet in overeenstemming met een klassiek ‘Tragedie van de Meent’ scenario.

In **hoofdstuk 5** besteed ik specifiek aandacht aan niet aan hulpbronnen gerelateerde effecten van een ondergrondse buurplant op de grootte-verdelingen van zaden binnen de plant. Ik laat zien, dat planten van *Phaseolus vulgaris* (Boon) naar verhouding meer grote zaden maken als ze blootgesteld zijn aan de wortels van een andere plant van dezelfde soort, onafhankelijk van de hoeveelheid voedingsstoffen die beschikbaar zijn voor de zaadproductie. Het is bekend, dat kiemplanten van deze soort die uit grote zaden komen doorgaans de concurrentie winnen van kiemplanten uit kleinere zaden. Aannemend dat de aanwezigheid van buurplanten informatie oplevert die een grotere kans op concurrentie in de volgende generatie aannemelijk maakt, stel ik dat planten hun strategie van investeringen in nakomelingen kunnen bijstellen om aangepast te zijn aan toekomstige milieu-omstandigheden, gebaseerd op informatie over de huidige situatie.

In **hoofdstuk 6** inntegreer ik de resultaten van de vorige hoofdstukken, en ik concludeer 1) dat het bewortelingsvolume veel meer invloed heeft op de groei van planten dan de hoeveelheid hulpbronnen erin, en dat daarom experimenten met planten in potten het volume dat beschikbaar is voor beworteling door de planten zorgvuldig onder controle moeten houden; 2) dat de grootte van een plant sterk gerelateerd is aan de groeistrategie van de plant, en dat daarom onderzoek naar reacties van planten op milieufactoren ook plantgrootte in de beschouwing moeten betrekken; 3) dat er ruimschoots aanwijzingen zijn dat planten reageren op de aanwezigheid van ondergrondse buurplanten, en dat het nodig is om de aandacht verder te

richten op de onderliggende mechanismen van die herkenning; 4) dat de reacties van wortelgroei op de aanwezigheid van ondergrondse buurplanten afhankelijk lijken te zijn van het milieu, waarbij een ‘Tragedie van de Meent’ scenario vooral lijkt op te treden in populaties van dicht op elkaar groeiende planten onder voedselrijke omstandigheden; 5) dat het effect van de aanwezigheid van ondergrondse buurplanten niet beperkt is tot wortelgroei van of biomassa-verdeling binnen de plant, maar dat ook de voortplantingsstrategie van planten beïnvloed kan worden, gerelateerd aan de aanpassing van de volgende generatie.

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# Curriculum vitae

Bin Chen was born on 1985 June 25 in Nanjing, the capital of Jiangsu Province, Eastern China. He graduated in 2007 from Nanjing University with a Bachelor degree in biological science. Then, he continued his education in ecology as a master student at the same place in the following three years. During that period, he focused on aridity-conditioned plant adaptation and belowground interaction



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In 2010, Bin completed his Master degree and obtained a four-year scholarship from China Scholarship Council. Then, he moved to Utrecht University for his PhD research in Ecology & Biodiversity Group under the supervision of Dr. Niels Anten, Dr. George Kowalchuk and Dr. Heinjo During. From 2013 to 2014, Bin spent most time working in Crop & Weed Ecology Group in Wageningen University. His PhD project specialized in plant belowground recognition and the associated responses in growth and reproductive strategies of plants under evolutionary game-theoretical context.

# Publications

*Peer reviewed only*

- Chen, B.J.W.**, During, H.J., Vermeulen, P.J. & Anten, N. P. R. (2014) The presence of a below-ground neighbour alters within-plant seed size distribution in *Phaseolus vulgaris*. *Annals of Botany*, 114: 937-943.
- Xu, C., Huang, Z.Y.X., Chi, T., **Chen, B.J.W.**, Zhang, M. & Liu, M. (2014) Can local landscape attributes explain species richness patterns at macroecological scales? *Global Ecology and Biogeography*, 24: 436-445.
- Chen, B.J.W.**, During, H.J. & Anten, N.P.R. (2012) Detect thy neighbor: Identity recognition at the root level in plants. *Plant Science*, 195: 157-167.
- Xu, C., Liu, M., Zhang, M., **Chen, B.**, Huang, Z., Uriankhai, T. & Sheng, S. (2011) The spatial pattern of grasses in relation to tree effects in an arid savannah community: Inferring the relative importance of canopy and root effect. *Journal of Arid Environments*, 75: 953-959.
- Huang, Z., Liu, M., **Chen, B.**, Uriankhai, T., Xu, C. & Zhang M. (2010) Distribution and interspecific correlation of root biomass density in an arid *Elaeagnus angustifolia*-*Achnatherum splendens* community. *Acta Ecologica Sinica*, 30: 45-49.

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