UNIVERSITY OF LEEDS

Coordination of shoot growth with the soil environment by long-distance signalling

Cara Dee Wheeldon

Submitted in accordance with the requirements for the degree of Master of Science by Research in Plant Science

The University of Leeds

School of Biology, Centre for Plant Science

September 2019

Intellectual property and publication statements

Work presented in this thesis is my own and includes data presented in a jointly authored bioRxiv preprint. The data presented in this thesis is solely experimental work I have carried out myself. No work by Catriona Walker (second author on Wheeldon et al, 2019) is presented in this thesis. In all cases, except Figure 2.0, any data presented in Wheeldon et al has been presented visually differently to this thesis.

The bioRxiv pre-print is:

Wheeldon, C.D., Walker, C.H., Bennett, T.A. 2019. Root volume restriction causes pro-active modulation of shoot growth to avoid future resource limitation. Biorxiv. https://www.biorxiv.org/content/10.1101/539726v1

The chapters in this thesis which include data presented in Wheeldon et al, 2019 are:

Chapter 2

2.0: Figure 1 from Wheeldon et al is presented in the chapter introduction to give context.

2.1: Figure 3A from Wheeldon et al (branch number data).

Chapter 3

3.1: Figure 2B from Wheeldon et al (only branch number data).

3.2: No data is presented in Wheeldon et al but this experiment was briefly mentioned in line.

Chapter 4

4.1A: Figure 8F in Wheeldon et al

4.3: Figure 6 in Wheeldon et al, Figure 6A, D and E are the same pictures as presented in this thesis.

4.4: Figure 7 C-H in Wheeldon et al features the same pictures as this thesis.

Discussion

Themes in the discussion are also mentioned in the preprint

All work presented in Wheeldon et al, 2019 was carried out by myself with the exception of: Figure 4D+E, Figure 8A-E which was carried out by Catriona Walker and Tom Bennett.

Acknowledgements

Firstly, I would like to thank Dr Tom Bennett for all his support and guidance this year. I would like to thank the members of the Bennett lab for all their support and kindness this past year. I am grateful for Catriona Walker's assistance with watering and Mary McKay for technical support. I would like to thank Maxime Josse for his help setting up the hydroponic system and assistance with pea plant care.

Abstract

Plants constantly integrate information about their environment to control their growth and development. However, the mechanisms by which plants detect and respond to the physical components of their soil environment, such as soil volume, soil depth and the presence of other plants remains widely unknown. Understanding these mechanisms would be highly valuable for generating improvements in crops and perhaps lead to yield enhancements. Soil compaction is a large issue in agricultural contexts, and results in global crop losses. Crops subjected to soil compaction have a reduced soil volume and soil depth. I show that mechanical impedance of the pot wall is unlikely to contribute to the reduced growth seen in plants grown under soil limiting conditions, and instead I propose that a root density sensing mechanism explains reduced plant growth in response to both crowding and limited soil volume. I propose that strigolactones are an exuded signal involved in root density sensing, and show that they modulate shoot growth of neighbouring plants. I describe the ability of plants to make proactive decisions on growth by assessing their soil volume and nutrient availability throughout their lifecycle. These findings have important implications on agricultural practice regarding yield and nutrient use efficiency.

Contents

Intellectual property and publication statements 2
Acknowledgements 3
Abstract 4
Materials and Methods 8
1.1 Plant growth conditions 8
1.2 Fertiliser treatments 10
1.3 Plant materials 11
1.4 Phenotypic assessments 11
1.5 Statistical analysis 12
Chapter 1: Introduction 13
1.1 Plant responses to the soil 14
1.2 Volume restriction 15
1.3 Volume restriction and nutrient availability 17
1.4 Volume restriction and mechanical impedance 18
1.5 Crowding and plant growth 18
1.6 Aims 19
Chapter 2: Physical aspects of the rhizosphere directly affect plant growth 20
2.0 Introduction 21
2.1 Soil volume results in a saturable limitation on plant growth 24
2.2 Shallow soil constrains plant growth 26
2.3 Shoot growth does not linearly scale with increased soil depth 28
Chapter 3: Soil volume and nutrients separately influence plant growth 32
3.0 Introduction 33
3.1 Soil volume and nutrient availability determine plant growth 33
3.2 Soil volume and nutrient availability in wheat 36
Chapter 4: Root density sensing links volume restriction and crowding 40
4.1 Shoot growth is affected by soil volume and crowding 41
4.2 The effect of shading in plant crowding 44
4.3 The effects of soil volume are unlikely to be due to mechanical impedance 47
4.4 Root density sensing could explain soil volume responses 49
4.6 Strigolactones contribute to neighbour detection and root density sensing in pea 53
4.8 Neighbour detection and ‘soil’ volume responses do not require soil 59
Chapter 5: Discussion 61
5.1 Soil volume can be used to predict nutrient availability over the plants lifecycle 62
5.2 Soil volume could be a predictor for water availability 62

5.3 Soil volume and soil depth in the environment....63
5.4 Soil volume and plant neighbour detection....64
5.5 Strigolactones and plant-plant detection....66
5.6 Crowding in the field....67
5.7 The overall model....68
Bibliography....70

Figures

Methods and Materials

M1.0 Cartoon depicting hydroponic growth conditions……..……..9

Chapter 1: Introduction

1.0 Soil volume restriction results in root and shoot biomass reduction……16

Chapter 2: Physical aspects of the soil rhizosphere directly affect plant growth

2.0 Soil volume results in a non-nutritional limitation on yield, seed set and shoot growth……...23
2.1 Shoot growth response to soil volume is saturable……25
2.2 Effect of soil depth on plant growth…..……27
2.3 Shallow soil limits plant growth…….……...30
2.4 Wheat growth is limited by shallow soil……31

Chapter 3: Soil volume and nutrients separately influence plant growth

3.1 Soil volume and N concentration separately influence Arabidopsis growth…..35
3.2 Growth of wheat plants is determined by nutrient availability not soil volume...38
3.3 Nutrient availability determines shoot growth in wheat……..……...39

Chapter 4: Root density sensing links volume restriction and crowding

4.1 Crowding effects are interchangeable with soil volume……...43
4.2 The effect of belowground crowding is stronger than the effect of shading.…46
4.3 Inhibition of shoot growth under volume restricting conditions is not the result of mechanical stimulus....48
4.4 End of life shoot growth is determined by soil volume....51
4.5 Shoot growth temporally correlates with effective root density…….……..52
4.6 Strigolactone exudates modulate neighbouring plant growth....55
4.7 Strigolactone exudates modulate neighbouring plant growth....58
4.8 Neighbour detection is not limited to soil based systems....60

Chapter 5: Discussion

5.0 Soil compaction on root growth.....64

5.1 Strigolactone exudates are mobile and influence neighbouring plant growth...67

5.2 Model describing how root density sensing links soil volume and neighbour detection responses.....69

Tables

Methods and Materials

M1.0 Arabidopsis Thaliana Salts (ATS) reagents……10

Materials and Methods

1.1 Plant growth conditions

All Arabidopsis plants were grown in controlled environment growth rooms under standard 16 hour day / 8 hour night conditions (20°C/ 16°C), with light intensity of ~120µmol/m^2s^{-1} from white fluorescent tubes. Pea and wheat plants were grown in glasshouses in 16 hour day / 8 hour night conditions (22°C) with LED lights at an average light intensity of ~250 µmol/m^2s^{-1}. All plants were grown on Petersfield No.2 compost with the exception of the experiments outlined in chapter 3 where wheat and Arabidopsis were grown on sand / perlite (1:1) (3.1, 3.2, 3.3), and chapter 4.8 where peas were grown in a hydroponic system.

For sections 2.2 and 2.3, 500ml of compost was used for all depth conditions. In 2.2, soil depths of 1.5, 9 and 16cm were used. The containers used were filled with compost to the depth required. The container widths for these depth conditions were as follows: 1.5cm depth container: width 15.5 x 21.5cm, 9cm depth container: sloping container which had a width of 8 x 8cm at the top and 6.5 x 6.5cm at the base, 16cm depth container: the slightly concave container had a maximum diameter of 6cm.

For section 2.3, soil depths 3, 6, 11, 16 and 20cm were used. All containers were made of clear plastic to allow root growth to be monitored visually. Once again, the containers were not filled to the top, only to the required depth and 500ml of compost was used for all depths. The container widths for these were as follows; 3cm depth container: 17.5 x 13cm wide, 6cm depth container: 10 x 10cm wide, 11cm depth container: the plastic container had sloping sides with a diameter of 6.5cm at the base and 8cm at the top, 16cm depth: the slightly concave container had a maximum diameter of 6cm, 20cm depth container: cylinder with a diameter of 5cm.

For the hydroponic experiment in 4.8, I grew L77 background peas in 1L pots. There was either 1 plant per pot (1/pot) or 5 plants per pot (5/pot). The lids of the pots were drilled to allow either 1 or 5 falcon tubes to sit inside. The falcon tubes were unlidded and shortened to remove the last centimetre of the closed tube; producing a plastic open-ended cylinder (Fig. M1.0). A foam bung was placed around the shoot-root junction of each pea plant, and the roots were subsequently

placed inside the open-ended falcon tube. The falcon tube acted as a support for the plants, allowing the plants to be held in the same position. Each black plastic pot was filled with a litre of water plus standard ATS solution. The water level was topped up daily, and standard ATS nutrient solution was added weekly. In order to provide water aeration, an aquatic pump (All Pond Solutions, AP-12-Kit pump) was connected to each pot using tubes and air stones (Fig. M1.0). After 3 weeks, the water was disposed of and fresh water and nutrient solution was added.

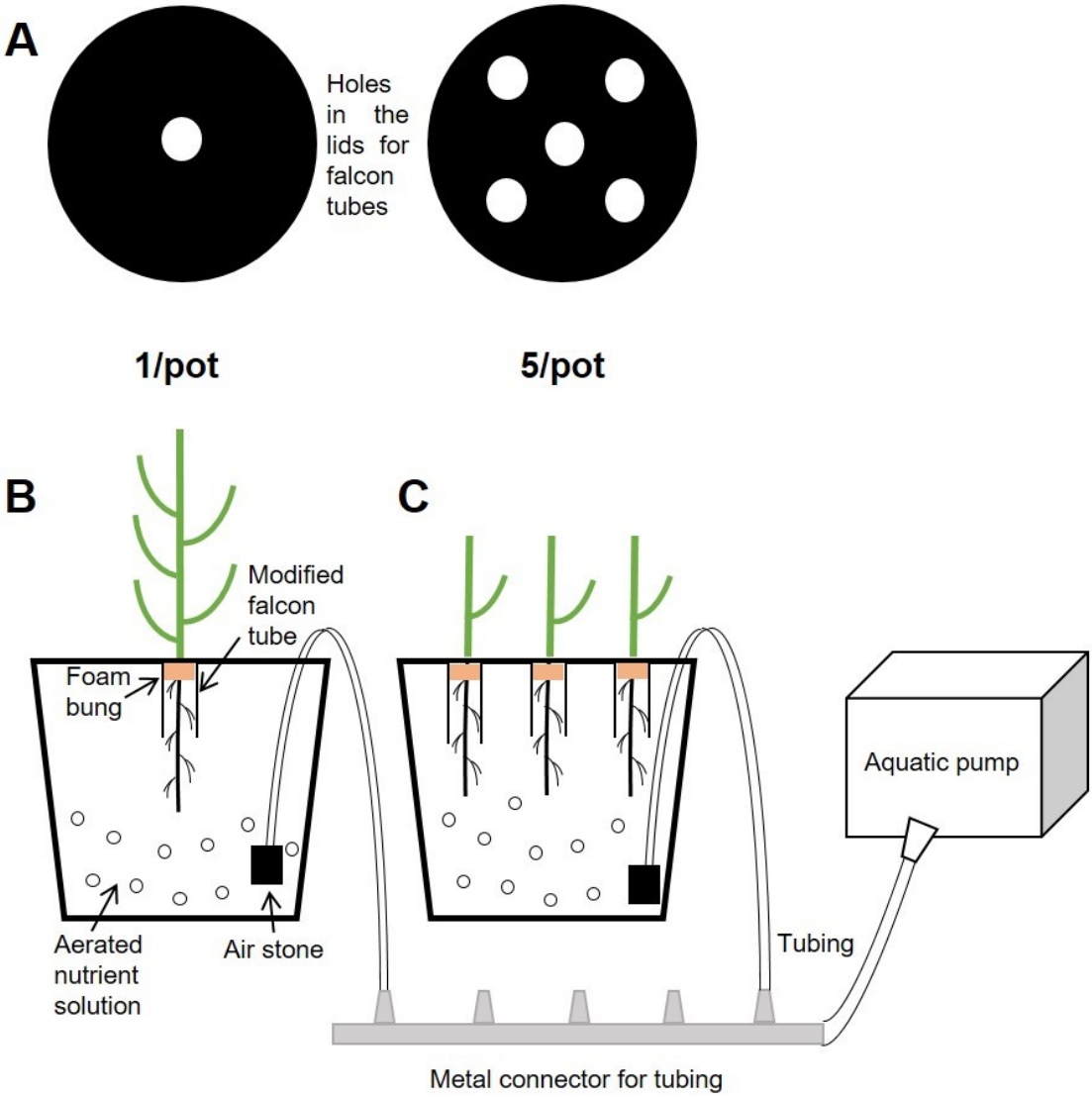


Figure M1.0: Cartoon depicting hydroponic growth conditions

A) Cartoon representing lids for 500ml pots with either 1 or 5 holes drilled to allow falcon tubes to be inserted.

B) Cartoon showing a cross section of the 1/pot condition.

C) Cartoon showing a cross section of the 5/pot condition. 3 plants have been shown due to the other 2 being further back in the plane hence not visible.

1.2 Fertiliser treatments

Arabidopsis Thaliana Salts (ATS) (Wilson et al., 1990), was used as a standard fertiliser (Table M1.0).

Reagents	mM
Potassium dihydrogen orthophosphate (KH_2PO_4)*	2.5
Potassium Nitrate (KNO_3)	5
Calcium Nitrate ($Ca(NO_3)_2$)	2
Magnesium sulphate ($MgSO_4$)	2
Micronutrients	**µM**
Iron-ethylenediaminetetraacetic acid (Fe-EDTA)	50
Orthoboric acid (H_3BO_4)	70
Manganese Chloride ($MnCl_2$)	14
Sodium Chloride (NaCl)	10
Copper Sulphate ($CuSO_4$)	0.5
Zinc Sulphate ($ZnSO_4$)	1
Sodium Molybdenate ($NaMoO_4$)	0.2
Cobalt Chloride ($CoCl_2$)	0.01

*Buffered to pH 5.5 using K_2HPO_4

Table M1.0: Arabidopsis Thaliana Salts (ATS).

Initially described in Wilson et al., 1990, this table describes standard ATS.

Nitrate concentration was varied for the fertiliser experiments outlined in Chapter 3, however concentrations of the other ATS components remained constant. Standard N fertiliser has 0.005M nitrate (Table M1.0), low N fertiliser had 0.0015M, medium low N had 0.00375M, high N had 0.015M, and super high N had 0.015M with the addition of 0.06M N from 60mM KNO3. Plants grown on sand/perlite, received 5ml water + 5ml ATS (of the required N concentration) once weekly instead of standard watering.

Arabidopsis plants grown on compost received 5ml of water and 5ml standard ATS (Fig. 4.1), weekly in place of watering, where stated. Wheat and oilseed plants were provided with 10ml standard ATS solution instead of watering once weekly (Fig. 2.0).

Pea plants in the hydroponic system were provided with standard ATS solution once weekly for 2 weeks whilst germinating on hydrated perlite. Following transfer

to the hydroponic system, each pot had standard ATS added weekly until 7 weeks post germination when the plants were assessed.

1.3 Plant materials

All *Arabidopsis thaliana* was wild type Col-0. Spring wheat varieties were Mulika and Willow (for all wheat experiments Mulika was used, Fig 2.0 additionally used Willow). Spring oilseed (*Brassica napus*) rape variety was Heros (2.0). For the pea (*Pisum sativum* L.) experiments, two backgrounds (L77 and Torsdag) were used. This is because *rms1* is in the L77 background and *rms3* is in the Torsdag background. L77 wild type and *rms1* pea was used in 4.6 and 4.8, L77 *rms1* and the Torsdag wild type and *rms3-2*, was used in 4.7.

1.4 Phenotypic assessments

All biomass measurements were of dry biomass and were taken at end-of-life, with the exception of pea plants that were harvested 7 weeks post germination (when flowering initiated). Dry shoot biomass was measured for Arabidopsis, wheat and pea whereas fresh biomass was assessed in oilseed rape. Sand and perlite was carefully washed off the wheat roots before they were dried and weighed (3.2D). Pea roots were separated from the shoots at the shoot-root junction and were subsequently dried then weighed (4.8).

Where stated, wheat seed number was counted by threshing the ears to remove all chaff (3.2B). Wheat spikelet number was counted for each ear, including all productive and unproductive spikelets and presented as total spikelet number. Plant height and main stem length for all species was measured from the shoot-root junction to the top of the plant.

Wheat tiller number was counted weekly from 3 weeks post germination. For the soil-based pea experiments, total branch number was counted (all classes) (4.6, 4.7) when flowering initiated. For the hydroponic pea experiment, secondary branches were counted (4.8). For Arabidopsis, in all cases, the total number of branches were counted (all classes: primary, secondary, tertiary and quaternary).

For all experiments using pea (4.6, 4.7, 4.8), and Arabidopsis plants in 2.1, shoots were cut at the shoot- root junction and main stem height was measured from this

point to the apex of the plant. Dry biomass measurements were taken for each plant, not per pot.

1.5 Statistical analysis

Data sets with more than two groups were tested for normality, if the data were normally distributed ANOVA with Tukey post hoc test was carried out. If data were not normally distributed, a pairwise Kruskal-Wallis test was used. Tests used are stated in the figure legends.

Where more than one parameter is presented within a graph, each parameter was statistically assessed separately. For example, Fig. 3.2D shows data for ear, root and shoot biomass. Each type of biomass was separately tested for normality and then a statistical test was carried out for each type of biomass. For crowding experiments involving more than one genotype (4.6-4.8), where WT, *rms1* and *rms3* were compared in different co-sown treatments, normality tests and statistical tests were carried out separately for wild type and each mutant genotype.

Statistical test outcomes are depicted by letters on the graphs. The same letter depicts no statistical difference.

Independent samples T-test was used for 4.2 and 4.8. For Fig. 4.2D, biomass of medial and outside plants for Treatment B were compared using an Independent samples T-test. Total biomass per pot for Treatment A and B were compared using an additional independent samples T-test.

Independent samples T-tests were used separately for WT and *rms1* in Fig. 4.8. For example, 1x *rms1* and 5x *rms1* were compared using an Independent samples T-test, then a separate independent samples T-test was used to compare the 1x WT and 5x WT data in each panel.

Chapter 1: Introduction

1.1 Plant responses to the soil

As plants are sessile organisms, their ability to respond to aspects of their immediate environment is crucial for survival and growth. The responses to water, nutrient availability, CO_2 concentration and light have been intensely researched, however plant responses to physical aspects of the soil environment are understudied. In the rhizosphere, there are several physical stimuli which plants must respond to by adapting their growth. These include available soil volume and/or soil depth, neighbouring plant roots, discreet obstacles (e.g. rocks), and soil compaction.

These stimuli can cause a non- nutritional limitation on root growth, known as 'root restriction'. Root restriction can occur when soil volume is limited ('volume restriction'), perhaps by pot or container, or limited space in the field, and this root system confinement leads to restrained root growth (Poorter et al., 2012). Root growth reduction under volume restricted conditions has also been associated with decreased shoot growth in a variety of species such as tomato (*Lycopersicon esculentum Mill*) (Bar-Tal and Pressman, 1996), cucumber (*Cucumis sativus L.*) (Kharkina et al., 1999) cotton (*Gossypium hirsutum*) (Yong et al., 2010) and bean (*Phaseolus vulgaris L.*) (Carmi and Heuer, 1981) (Figure 1.0). However, the mechanism by which plants detect and integrate volume restriction is currently unclear.

Volume restriction is highly relevant to gardeners, farmers and plant researchers in a range of scenarios. For instance, plant research often involves growing plants in containers or pots due to the high demand for space within growth facilities. Using small pots is a simple way to allow for more replicates and experiments at any one time (Poorter et al., 2012). Despite the space saving advantage of small pots, this can lead to biological constraints. Small pots invariably contain a small volume of substrate and consequently a reduction in nutrient and water availability (Poorter et al., 2012). It is not uncommon for plants to produce long roots; often more than 1m in length (Jackson et al., 1996) and so this can lead to large plants becoming 'pot-bound' as the small pots impede their growth (Herold and McNeil, 1979). Volume restriction is also relevant in agriculture. For instance, root growth can be reduced by rocks or compacted soils caused by heavy machinery (Correa et al., 2019). In addition, in agricultural contexts, crops are

often densely sown (Hecht et al., 2016) hence this crowding causes an effective reduction in the available soil volume per plant.

1.2 Volume restriction

Poorter et al (2012) carried out a large meta-analysis of 65 publications reporting plant growth responses in different pot sizes, in addition to 10 publications of hydroponic root restriction. Pot sizes ranged between 5ml to 1700L, and a variety of species were assessed including woody and herbaceous plants. An increase in pot volume leads, in majority of reports, to increased biomass. They found biomass to on average increase by 43% when pot volume was doubled and when plants were grown in 2L pots, they are three times larger than those grown in 0.2L pots (Poorter et al., 2012). Simply by the nature of plant growth, when plants develop from seed to fully grown, their root and shoot mass is expected to increase overtime. It is interesting in these studies, that even when root volume is restricted, biomass still increases throughout the life cycle, suggesting plants pro-actively 'plan out' their growth to match their pot size. Another interesting observation is that in most studies, the influence of pot size on plant growth did not plateau, suggesting that an 'unrestricted' root volume was not reached (Poorter et al., 2012).

Figure 1.0: Soil volume restriction results in root and shoot biomass reduction.

Plants with small soil volumes (left) produce less shoot and root biomass than plants grown in large pots (right).

Original figure produced to reflect the findings from Carmi and Heuer (1989) Bar-Tal and Pressman (1996), Kharkina et al (1999), Yong et al (2010).

It would be expected that plants have mechanism(s) that allow them to cope with volume restriction, due to the constant competition for space in nature. However, despite the negative effects prolonged root restriction has on plant growth in agriculture, forestry and horticulture (Carlson and Endean, 1976; Kharkina et al., 1999; Hecht et al., 2016), little research has been conducted into such mechanisms. Furthermore, there have been few suggestions made in the literature previously as to how plants perceive limited soil volume itself (Falik et al., 2005), despite clear evidence of growth changes as a result of such conditions.

Following perception of available soil volume, it is unclear how such information is communicated to the shoots. Yong et al (2010) suggested that the reduction in plant growth seen when restricting soil volume is through root-shoot signalling changes leading to associated physiological changes. They suggest the potential signalling events to be hydraulic and non-hydraulic. Non-hydraulic refers to endogenous phytohormones such as abscisic acid (ABA) (Ternesi et al., 1994; Liu and Latimer, 1995; Ismail and Davies, 1998), cytokinin (Yong et al., 2010) and ethylene (Peterson et al., 1991; Haver and Schuch, 2001), low oxygen

availability (Shi et al., 2007; Shi, Fu, et al., 2008) and uptake of nutrients (Carmi and Heuer, 1981; Zhu et al., 2006) while hydraulic refers to uptake water.

1.3 Volume restriction and nutrient availability

Growth changes in volume restriction may be the result of decreased nutrient supply due to the inherent reduction in soil in small pots. Photosynthesis is known to be decreased under low phosphorous (P) and nitrogen (N) conditions (Sinclair and Horie, 1989; Lynch et al., 1991). However, there have been many studies which have shown that reduced nutrient uptake does not account for the growth differences in volume restricted plants. One such example assessed the nitrogen concentration in leaves of plants grown in different pot sizes (Poorter et al., 2012). They found a small, non-significant increase in nitrogen concentration when pot size was increased, suggesting lower nutrient resources in small pots cannot be the main factor to explain growth differences under root restricting conditions (Poorter et al., 2012). Another study assessed leaf phosphorous concentrations and the findings further support that decreased nutrient availability does not play a major role in growth changes when root volume restricted (Krizek et al., 1985).

Under hydroponic conditions where nutrients are not limited, there is still strong evidence that plants become volume restricted. In one study, tomatoes were grown in an aero hydroponic system with two root restriction conditions (0.4L or 1.0L) and with high and low N concentrations (1.0 and 9.0 mmol l^{-1} respectively) (Bar-Tal et al., 1995). To allow the plants to access the nutrient solution, different sized bags were used which were impermeable to the roots ensuring the volume accessible to the roots was independently manipulated to that of the amount of nutrients present. The root mass of tomato plants grown in 1.0L bags was greater (69g) than those grown in the smaller rooting volume (37g in low N and 38g in high N) (Bar-Tal et al., 1995). These findings therefore suggest that root mass is not affected by the available nutrients, instead it is by the volume accessible for root exploration (Bar-Tal et al., 1995). Other studies using hydroponic systems have also provided evidence that root dry weight decreases when root volume decreases (Hameed et al., 1987; Kharkina et al., 1999).

1.4 Volume restriction and mechanical impedance

If neither reduced water or nutrient availability can provide explanations for the growth changes seen with volume restriction, one alternative explanation might be the mechanical impedance of roots by the pot itself (Young et al., 1997). It is widely recognised that roots are able to detect physical obstacles in their soil environment and redirect their growth to other areas (Kozlowski, 1999; Clark et al., 2003). However, little research has been conducted into these mechanisms other than the suggestion they involve chemical signalling responses (Goss and Russell, 1980; Falik et al., 2005).

1.5 Crowding and plant growth

Another key way in which root systems can become restricted is by the presence of other plants in their environment. As with limiting soil volume, there are many negative changes to shoot growth when plants are grown in high-density (crowded) conditions (Misyura et al., 2014; Hecht et al., 2016). This has been researched mostly from the perspective of agriculture yield, with a strong emphasis on physiological changes. Chen et al (2015) assessed pea plant biomass following crowding in three different pot sizes. To ensure effects seen were non-nutritional, plants were grown in sterilized sand and each plant was provided with 1.0g of slow release fertiliser in all crowding conditions (Chen et al., 2015). They discovered that when one plant occupied the pot, root mass was significantly higher than in conditions where two plants shared a pot, regardless of pot volume. When pea plants had below ground neighbours this lead to a direct reduction in root allocation and plant size as total biomass was reduced by 9% (Chen et al., 2015). In rice (*Oryza sativa*) grown under high-density sowing conditions, shoot fresh biomass, seed yield, tiller number, reproductive tiller length and chlorophyll content were reduced when compared to plants grown without crowding, irrespective of nitrogen availability (Misyura et al., 2014).

There have been wide range of suggestions as to how plants are able to recognise neighbouring plants, but the recognition of root exudates has had particular research interest in recent years (Bais et al., 2006; Biedrzycki et al., 2010). However, research identifying individual exudates responsible for growth

changes is limited. Furthermore, how such information is integrated into shoot growth is understudied.

1.6 Aims

This thesis will explore plant growth responses to physical aspects of the soil rhizosphere where the following aims will be explored:

Chapter 2: To assess plant phenotypic responses to physical factors of the soil rhizosphere such as soil volume and soil depth.

Chapter 3: To better understand the interplay of nitrate availability and soil volume on plant growth and identify scenarios where soil volume effects may not be apparent.

Chapter 4: To develop ideas for a unifying mechanism that explains plant responses to crowding and soil volume, and to uncover a putative rhizosphere-signalling molecule exuded by plants into the soil which is involved in such a mechanism.

Chapter 2: Physical aspects of the rhizosphere directly affect plant growth

2.0 Introduction

In this chapter, I assess how the physical environment of the rhizosphere influences plant growth. This work builds on research I performed previously, which I will discuss here to provide context for the following results.

In my previous research, I examined how root restriction (in the form of soil volume) affected plant growth. I examined how plants respond to different soil volumes and whether the addition of mineral nutrients could overcome any negative effects of limited soil. One key experiment involved spring wheat (*Triticum aestivum*; varieties Mulika and Willow), spring oilseed rape (*Brassica napus*; variety Heros) and spring barley (*Hordeum vulgare*; varieties Charon and Propino) grown in compost in 3 soil volumes (100, 500 and 2000ml) until end-of-life (wheat and barley: 16 weeks, oilseed rape: 14 weeks). For each pot size, half of the plants were provided with additional fertiliser (Fert) (10ml/ week standard ATS solution at comparable rates to field practices ~80-200kg/ha of N) and the other half were not. At the end-of-life, total branches, fresh shoot biomass, total flowers and total fruit number was assessed for oilseed rape (Fig. 2.0 C,D,G,H). For wheat and barley, peak tiller number, dry straw biomass, total spikelets and total seed were assessed at the end-of-life (Fig. 2.0 E,F,I,J).

For all the species, ‘shoot size’ was directly proportional to pot size over the tested range, regardless of the parameter measured, and regardless of the availability of mineral nutrients (Fig. 2.0). Along with previous work (Hameed et al., 1987; Bar-Tal and Pressman, 1996; Poorter et al., 2012), these data suggest that plants are able to sense and respond to available soil volume and modulate their growth accordingly, in a manner that is not dependent on mineral nutrient availability. These observations motivated my current research, and form the basis for the questions posed in this, and following chapters.

Despite research previously carried out into the phenotypic responses to different pot volumes, relating the effects of soil volume and depth on plant growth is understudied. Soil compaction is a common issue in agriculture caused primarily by heavy machinery (reviewed by Correa et al., 2019). Heavily compacted soils are difficult for plant roots to penetrate through, which ultimately means that plants growing in heavily compacted soils are likely to experience reductions in their available soil depth and soil volume. Moreover, soil types can vary across

agricultural land, meaning root exploration may be hindered if there is underlying bedrock, soils with high clay or stone content (reviewed by: Correa et al., 2019). If soil depth is found to be a key limitation on plant growth, methods to overcome this may ultimately contribute to improved yield.

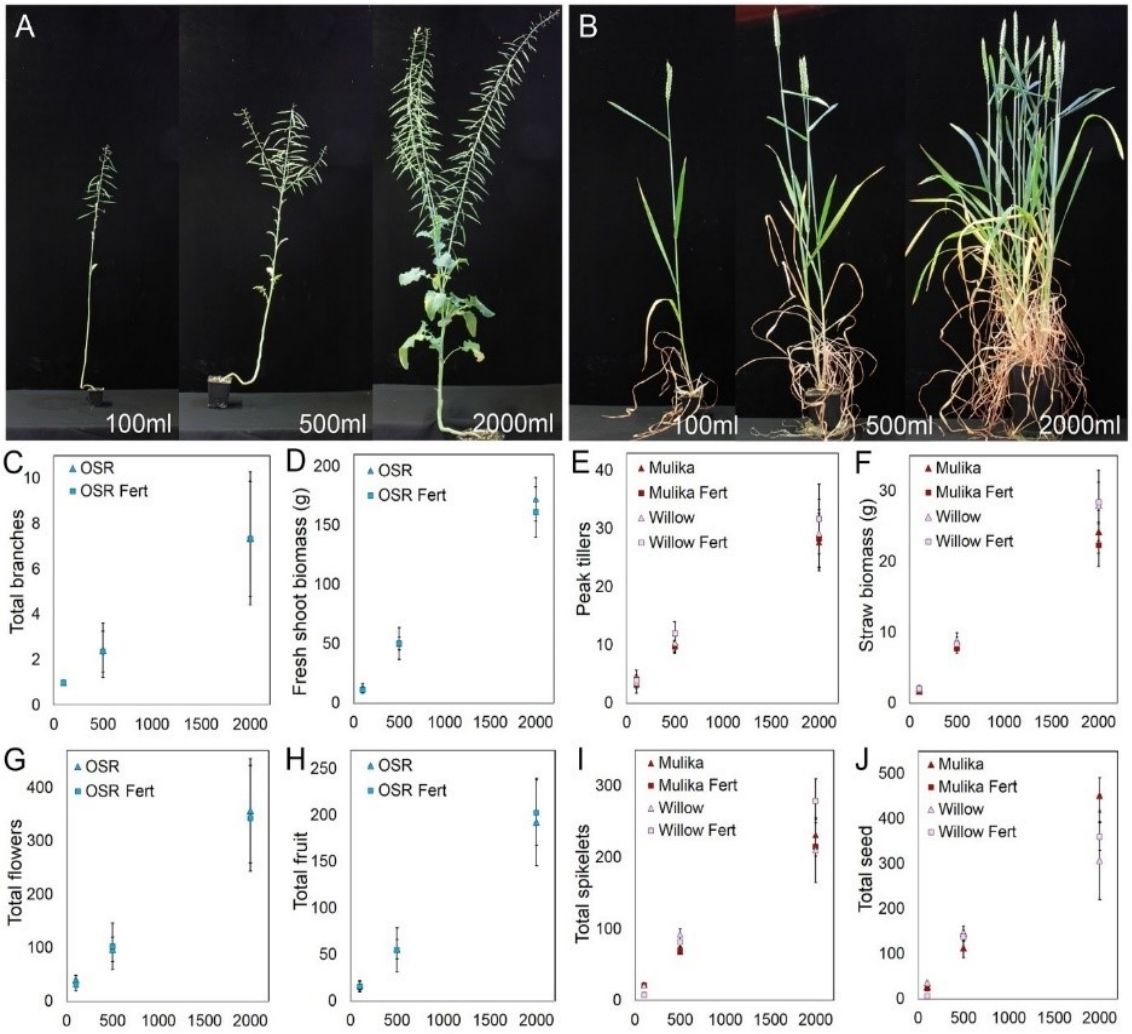


Figure 2.0 Soil volume results in a non-nutritional limitation on yield, seed set and shoot growth.

Figure taken from Wheeldon et al, 2019, https://doi.org/10.1101/539726

A, B) End of life plant size in spring oilseed rape (Heros) (**A**) and spring wheat (Mulika) (**B**) grown in 3 soil volumes (100, 500 and 2000ml).

C, D, G, H) Graphs showing mean total branch number (**C**), mean fresh shoot biomass (g) (**D**), mean total flower number (**G**), mean total fruit number (**H**) in spring oilseed rape (Heros), grown in 3 soil volumes (100, 500 and 2000ml). Half the plants in each soil volume were provided with additional fertiliser ('Fert') or without additional fertiliser. Error bars indicate s.e.m, n=6-12.

E, F, I, J) Graphs showing mean peak tiller number (**E**), mean dry shoot biomass (g) (**F**), mean total spikelet number (**I**) and mean total seed number (**J**) in spring wheat varieties Mulika and Willow grown in 3 soil volumes (100, 500 and 2000ml). Half of the plants in each pot size were provided with additional fertiliser ('Fert') or without additional fertiliser. Error bars indicate s.e.m, n=6-12.

2.1 Soil volume results in a saturable limitation on plant growth

The results in Fig 2.0 show that plant growth responds linearly to soil volume over the tested range. However, an interesting question is whether this scaling is unlimited, or whether there is a point at which plants no longer respond to additional soil volume.

I hypothesised that above a particular soil volume, for a given species, there is an inherent limit on plant growth. I therefore correspondingly hypothesised that above a particular soil volume, an increase in soil volume would no longer result in an increase in branch number and biomass. This point would define the maximum growth capacity of the plant. To test these hypotheses, *Arabidopsis thaliana* (hereafter 'Arabidopsis') was grown in 100, 500, 1000 and 2000ml pots until end-of-life (~7 weeks post germination) under controlled growth chamber conditions (Fig 2.1D). At end-of-life, the total number of branches produced by each plant was measured, along with shoot dry biomass.

Between 100 and 500ml, Arabidopsis plants showed a strong increase in growth; plants grown on 500ml of soil were ~3.5 times larger than those grown on 100ml of soil (Fig. 2.1A,B). However, above 500ml, the increase in growth with increasing soil volume was much smaller (Fig. 2.1A,B). Despite the twofold increase in the soil volume, plants grown in 1000ml pots had a slight but statistically insignificant increase in branch number relative to 500ml pots (Fig. 2.1B); the same was also observed between 1000ml and 2000ml pots. This trend was the same for shoot biomass (Fig. 2.1A). The main stem height of the plants did not increase linearly with soil volume and above 100ml there was no significant difference in height (Fig. 2.1C). Thus, consistent with my hypothesis, there appears to be an inherent limit on the size of Arabidopsis plants, such that increasing soil volume has a saturable effect on their growth.

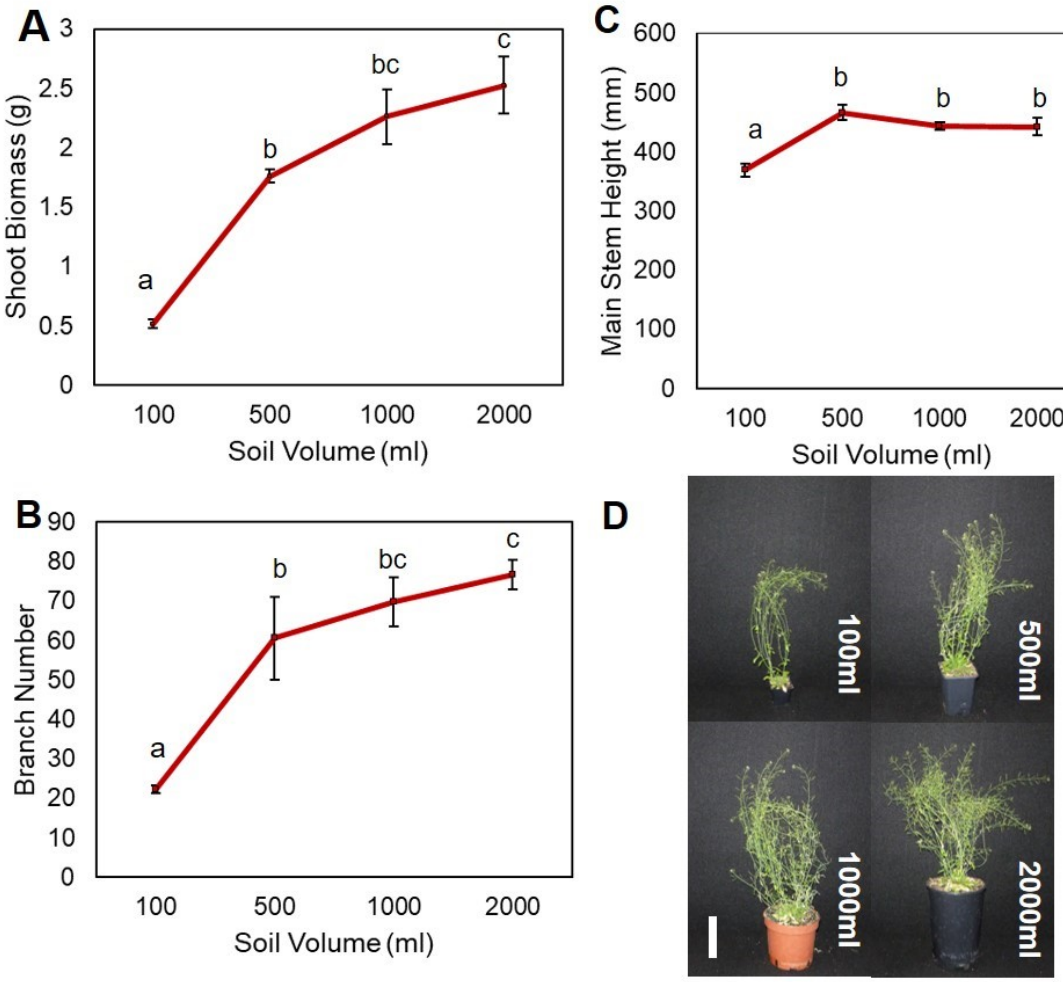


Figure 2.1: Shoot growth response to soil volume is saturable.

Figure showing end-of-life parameters for wild type *Arabidopsis thaliana* (Col-0) grown in 100, 500, 1000, 2000ml pots on compost.

A) Line graph showing the mean dry shoot biomass (g). Error bars depict s.e.m. The same letter indicates results that are not statistically different from each other (ANOVA with Tukey HSD), $p>0.05$, n=8-12.

B) Line graph showing mean total branch number and mean main stem height (mm). Error bars depict s.e.m. The same letter indicates results that are not statistically different from each other, ANOVA with Tukey HSD, $p>0.05$, n=8-12.

C) Line graph showing mean main stem height (mm). Error bars depict s.e.m, n=8-12. The same letter indicates results that are not statistically different from each other, ANOVA with Tukey HSD, $p>0.05$, n=8-12.

D) Pictures showing shoot size of Arabidopsis grown in 100, 500, 1000, 2000ml pots.

2.2 Shallow soil constrains plant growth

As pot depth typically increases when overall pot volume increases, an intriguing possibility was that soil depth and not soil volume might be the critical factor limiting plant growth. However, I hypothesised that plant growth would be primarily determined by soil volume, not depth. To examine this, I conducted an experiment in which I kept soil volume constant (500ml), but altered soil depth (and by extension soil width) by using different shaped containers. Soil depths of 1.5, 9 and 16cm were used. Wheat plants were grown in these containers for 16 weeks under standard glasshouse conditions. Tiller number was counted from week 3-9. This time period was chosen as the first tiller emerged at 3 weeks and changes in tiller number ceased at week 9. Once the plants reached the end-of-life (16 weeks post germination), the maximum height of the tallest tiller of each plant, flag leaf length (the leaf below the ear on the tallest tiller), dry shoot and ear biomass, and ear length and spikelet number of each individual ear from all plants were recorded.

Contrary to my prediction, there was a clear effect of soil depth in this experiment. Until week 4, the plants grown on 1.5cm soil depth initially grew like the other plants (Fig. 2.2A), but afterwards displayed a significant reduction in tiller number, ear number, spikelet number, shoot biomass, maximum tiller height, and flag leaf length (Fig. 2.2A-H). However, there were no significant differences in these parameters between plants grown on 9 and 16cm soil depths. This tentatively suggested that, below a certain threshold, soil depth might strongly affect plant growth, but above this threshold, soil volume is the key factor.

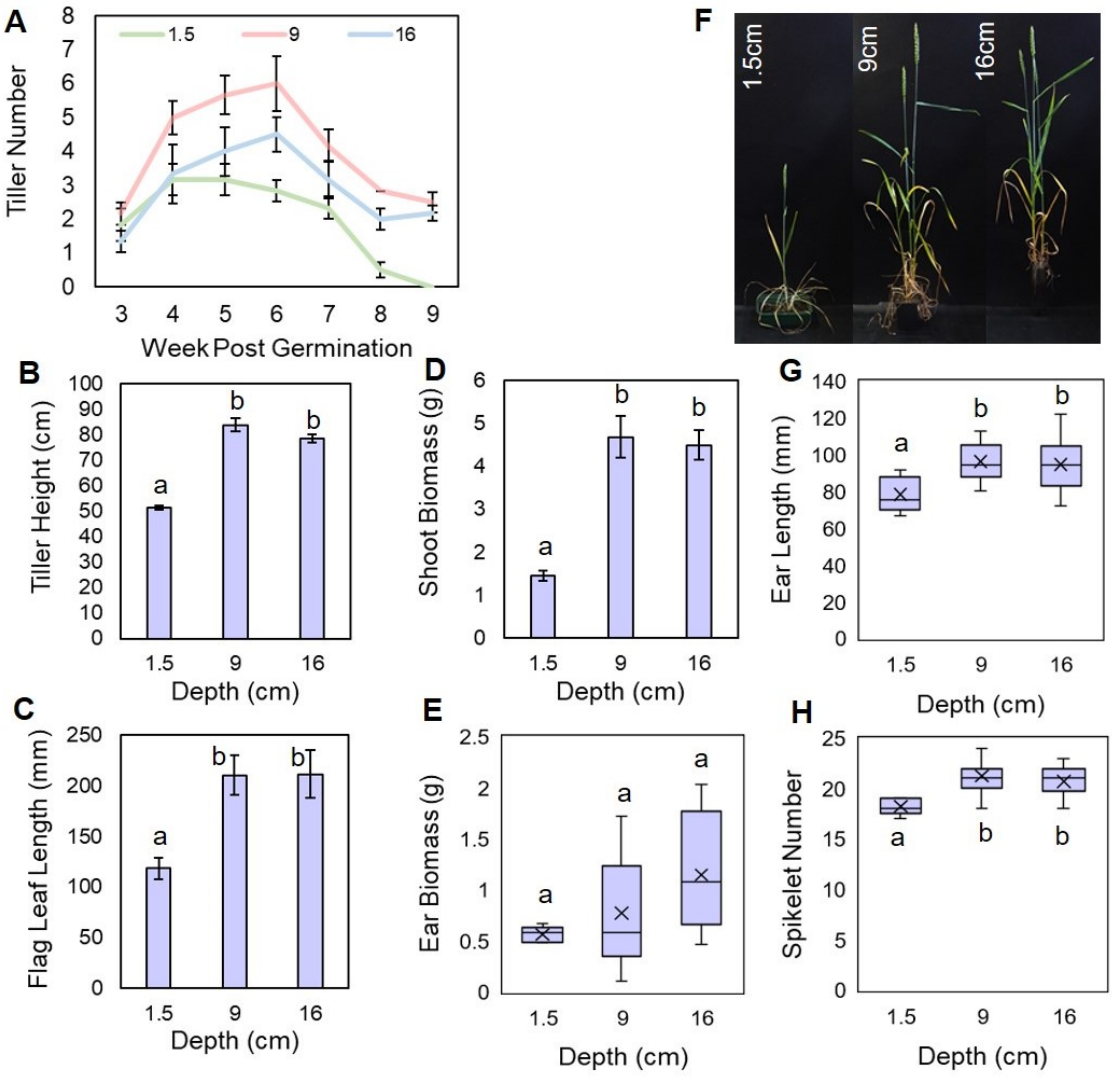


Figure 2.2: Effect of soil depth on plant growth

Figure to showing spring wheat (Mulika) grown in 3 soil depths (1.5, 9 and 16cm), each with 500ml of compost.

A) Line graph showing tiller number between week 3 and 9 post germination for each soil depth. Error bars indicate s.e.m, n=6.

B,C) Bar graphs showing height of the tallest tiller (**B**) and flag leaf length **(C)** 16 weeks post germination for each soil depth**.** Error bars depict s.e.m. Statistical analysis was carried out separately for each graph. Bars with the same letter indicate no statistical difference, ANOVA with Tukey HSD, p>0.05, n=6.

D) Bar graph showing dry shoot biomass (g), 16 weeks post germination for each soil depth. Error bars depict s.e.m. Bars with the same letter indicate no statistical difference, Kruskal-Wallis pairwise comparison, p>0.05, n=6.

E,G,H) Box plots showing end-of-life measurements taken at 16 weeks post germination for each soil depth. Dry ear biomass (recorded for each ear from each plant) (**E**)**,** ear length (recorded for each ear from each plant) (**F**) and total spikelet number (recorded for each from each plant) (**H**). Statistical analysis was carried out separately for each graph. Bars with the same letter indicate no statistical difference, ANOVA with Tukey HSD, p>0.05, n= 5-14.

F) Picture showing the experimental set up at 14 weeks post germination.

2.3 Shoot growth does not linearly scale with increased soil depth

In reflection of the findings from the preliminary experiment (Fig. 2.2), I wanted to explore plant growth responses to soil depth over a larger range of depths. I hypothesised that increasing soil depth, above a point, would not result in increased plant growth when soil volume remained the same. To test this, wheat was grown in 5 soil depths (3, 6, 11, 16 and 20cm), in 500ml of compost and the same parameters were measured as above.

Tiller number was unaffected by soil depth; all treatments followed the same trend over time, and peak tiller number reached around 7 for all soil depths (Fig. 2.3A). However, for all other parameters, this was not the case (Fig. 2.3B-D). Instead there was an increase with soil depth of all parameters peaking at the 11cm depth, but then a decrease in all parameters as soil depth increased further, with the exception of flag leaf length (Fig. 2.3B,D-G).

In the preliminary experiment (Fig. 2.2), I observed that plants grown in the 1.5cm depth pots had majority of roots on the base of the pot, with no roots visible on the sides. However, in the other two soil depths (9 and 16cm), roots grew along the sides of the pot and on the base. To look at this in more detail all pots used for this experiment were made of clear plastic. Once again, the shallowest pots did not have roots along the sides of the pot, instead they were tightly aggregated at the base of the pot (Fig. 2.4B).

As in the first soil-depth experiment (Fig. 2.2), there was a strong effect of shallow soil, especially in the shallowest pots (3cm). I theorise that this is due to these plants only colonising the base of the soil volume with their roots (Fig. 2.3B). They are unable to detect they have more soil volume available to them, resulting in reduced biomass allocation and other shoot parameters. The angle of the seminal roots could play a crucial role in this response. If the seminal roots hit the base of the pot and send mechanical stimuli information back to the plant to ‘’warn’’ of limited soil depth, this could explain the reduced biomass, ear length, height and spikelet number compared to the 11cm deep pots.

The progressive reduction in growth in the deeper pots was unexpected. An obvious explanation for this is that due to soil volume being constant, pot width decreases as pot depth increases. Therefore, this data suggests soil width can

also affect plant growth. As roots hit the side of the narrow pots this could also, like with shallow soil, send mechanical stimuli back to warn the plant of limited soil width.

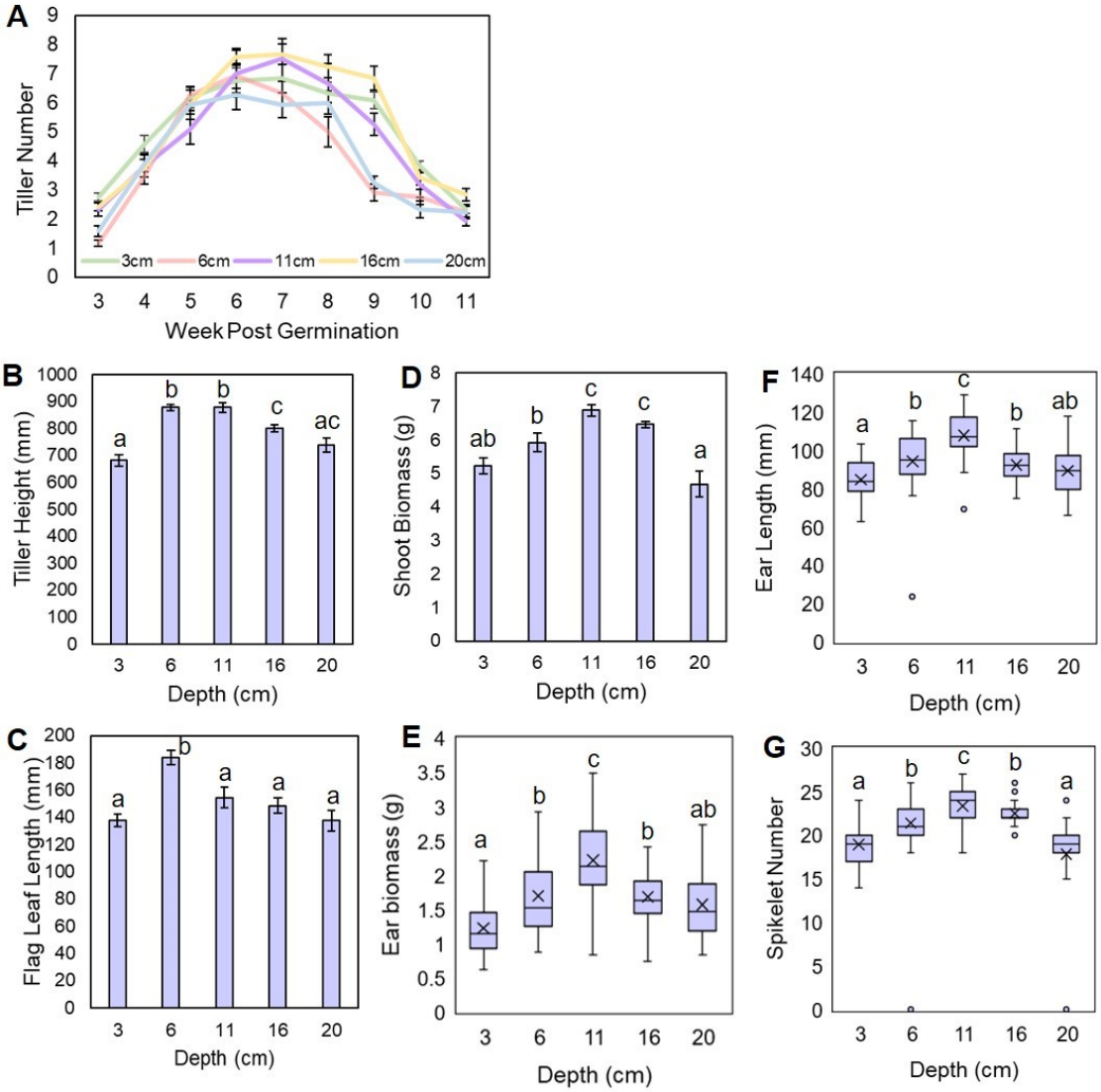


Figure 2.3: Shallow soil limits plant growth

Figure showing spring wheat (Mulika) grown in 500ml of compost in 5 soil depths: 3, 6, 11, 16 and 20cm until end-of-life (16 weeks post germination).

A) Line graph showing tiller number from 3-11 weeks post germination of wheat grown 5 soil depths. Bars indicate s.e.m, n=12.

B-D) Bar graphs showing the mean tallest tiller height (**B**), mean flag leaf length (**C**) and mean dry shoot biomass of wheat grown in 5 soil depths at 16 weeks post germination. Error bars depict s.e.m. Statistical tests were carried out separately for all graphs. Bars with the same letter are not statistically different, ANOVA with Tukey HSD, $p>0.05$, n=12.

E-G) Box plots showing mean individual dry ear biomass (**E**), mean individual ear length (**F**) and mean individual spikelet number (**G**) at 16 weeks post germination. x indicates the mean, the midline represents the median, dots above and below the whiskers represent outliers and the box depicts the interquartile range. Whiskers show the minimum and maximum values. Boxes with the same letter are not statistically different, ANOVA with Tukey HSD, $p>0.05$, n=35-43.

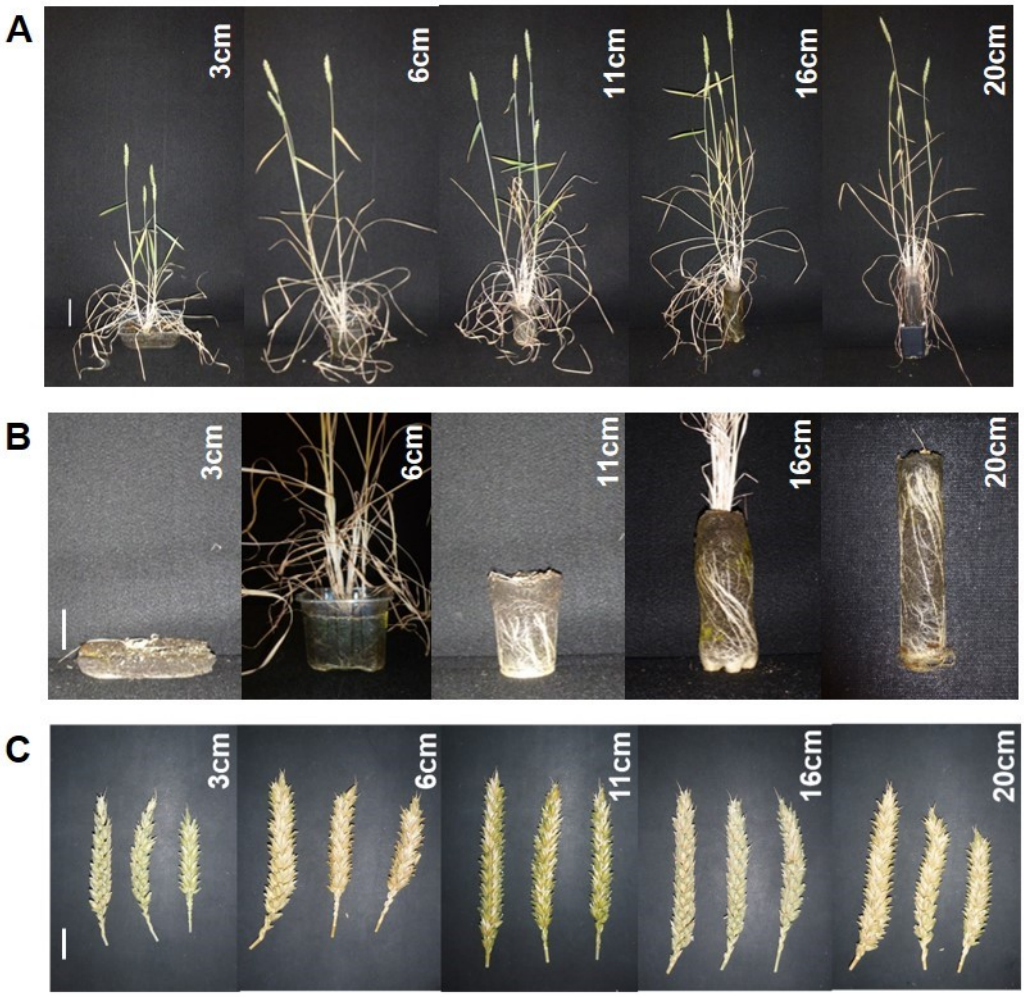


Figure 2.4: Wheat growth is limited by shallow soil

Pictures to support the findings of Fig 2.3.

A) Spring wheat plants (Mulika) at 14 weeks post germination, grown in 5 pot sizes (3, 6, 11, 16, 20cm). Scale bar depicts 5cm.

B) Side profiles of the soil showing the distribution of the roots on the outside of the soil masses at 16 weeks post germination, in one plant for each of the 5 soil depths. Scale bar represents 5cm.

C) Ears from one plant for each of the 5 soil depths. Scale bar depicts 2cm.

Chapter 3: Soil volume and nutrients separately influence plant growth

3.0 Introduction

A possible explanation for the findings presented in Figure 2.0 is that small soil volumes inherently contain less nutrients such as nitrate (N) and phosphate (P). However, as discussed in chapter 2, there was no significant difference in biomass, branching or yield in plants provided with additional mineral nutrients compared to untreated plants (Fig. 2.0). Therefore, as indicated by previous studies (Hameed et al, 1987; Bar-Tal & Pressman, 1996; Poorter et al, 2012), the effect of soil volume on plant growth is non-nutritional, and instead must result from a different stimulus which acts separately to nutrient availability.

In many contexts, particularly agricultural ones, plant growth is highly sensitive to mineral nutrient application. It is therefore intriguing that in root-restricted plants, growth seems completely insensitive to mineral nutrient application. The research in this chapter was motivated by wanting to understand this apparent paradox, and the interplay between soil volume and nutrient availability.

3.1 Soil volume and nutrient availability determine plant growth

One possible explanation for the observations in Fig. 2.0 could be that for a particular soil volume, there is a maximum capacity (or need) for nutrient uptake by plants. Thus, perhaps the compost used for that experiment already exceeded the maximum nutrient use level, such that additional nutrients had no effect on plant growth. I reasoned that if plants were grown with a much lower starting level of nutrient, the effect of additional nutrients would be more obvious. I also questioned whether responses to soil volume would be reduced under nutrient limited conditions. I hypothesised that under strongly nutrient limited conditions, soil volume effects are less visible, and the addition of nutrients would be the primary factor influencing plant growth.

To test these hypotheses, I firstly grew wild type Arabidopsis (Col-0) for 7 weeks (until end-of-life) in a nutrient free system of sand/perlite (1:1), allowing me to precisely control the levels of nutrients each plant received through the addition of a nutrient solution. As the major nutrient limiting plant growth, I focussed my attention on the availability of nitrogen, in the chemical form of nitrate (N). The plants were grown in 100 or 500ml pots, and half the plants in each pot size were provided with ‘low N’ fertiliser (7.5µmol of N/week) and the other half were

provided with ‘high N’ fertiliser (75µmol of N/week). It is important to note that all the plants, irrespective of N concentration, were provided with the same standard concentration of all other essential plant nutrients, and the different pot volumes were provided with the same total amount of fertiliser each week.

Irrespective of pot size, plants provided with high N had a greater primary inflorescence height, shoot biomass and silique number, compared to plants in the low N treatment (Fig. 3.1A-C). Thus, under these conditions, nutrient availability strongly influences plant growth.

It was also clear that in this system, soil volume influences growth. Under low N conditions, primary inflorescence height, shoot biomass and silique number was greatest when grown in the 500ml pots compared to plants in the same fertiliser treatment grown in 100ml pots (Fig. 3.1A-C). This suggests that soil volume alone can influence plant growth, even under identical nutrient levels. Similarly, under high N conditions, shoot biomass and silique number were greater in 500ml pots (Fig. 3.1B-C), however primary inflorescence height remained constant between the pot sizes (Fig. 3.1A).

Collectively, these data suggest that in Arabidopsis, both soil volume and nutrient availability separately regulate shoot growth.

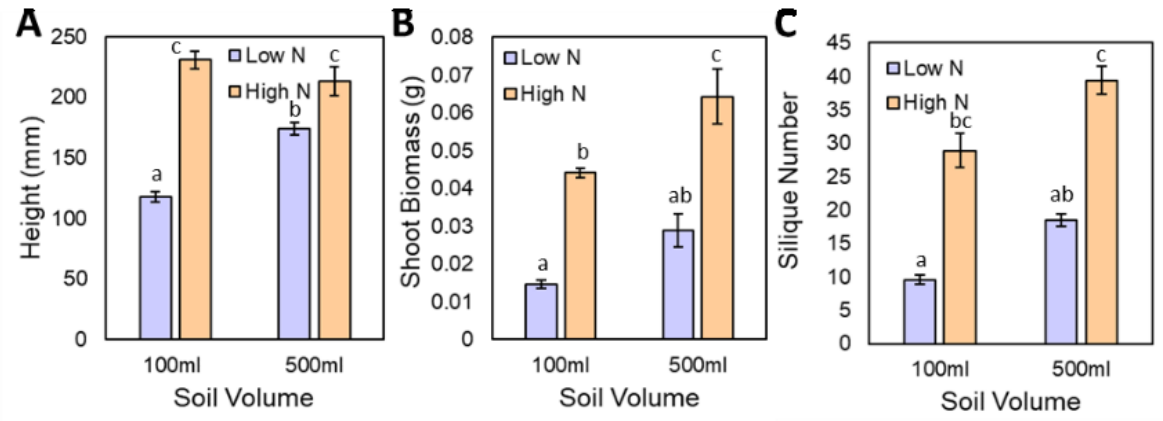


Figure 3.1: Soil volume and N concentration separately influence Arabidopsis growth

Graphs showing Arabidopsis (Col-0) at end-of-life (7 weeks post germination) grown in 50 and 100ml pots, on a sand/perlite mix provided with low nitrate (low N 7.5µmol/week) or high nitrate (high N 75µmol/week) fertiliser weekly.

A-C) Primary inflorescence height (mm) (**A**), mean shoot biomass (g) (**B**), mean silique number (**C**) at end-of-life. Errors bars indicate s.e.m. Bars with the same letters are not statistically different from each other, (**A,B**) ANOVA with Tukey HSD, $p>0.05$, $n=10$. Bars with the same letters are not statistically different from each other, (**C**) Kruskal-Wallis pairwise comparison, $p>0.05$, $n=10$.

3.2 Soil volume and nutrient availability in wheat

Arabidopsis is a small plant with a low nutrient requirement, and even under low N conditions responded to soil volume. I reasoned that larger plants, with higher nutrient demands, may not be sensitive to soil volume under low nutrient conditions, and would only become sensitive to soil volume at higher nutrient levels. To test this, I grew spring wheat (Mulika) on a sand/perlite mix (1:1), in either 100ml or 500ml pots and provided with low N (7.5µmol) or high N fertiliser (75µmol) weekly until end of life (14 weeks post germination). At the end-of-life, tiller height, seed number, shoot, root and ear biomass, ear length and spikelet number were assessed.

For all the parameters assessed, plants in the high N condition were significantly larger than those in the low N condition, irrespective of soil volume (Fig. 3.2A-F). There was no effect of soil volume in any of the measured parameters, except for tiller height where there was a slight but significant increase in height in 500ml pots under the low N condition (Fig. 3.2A).

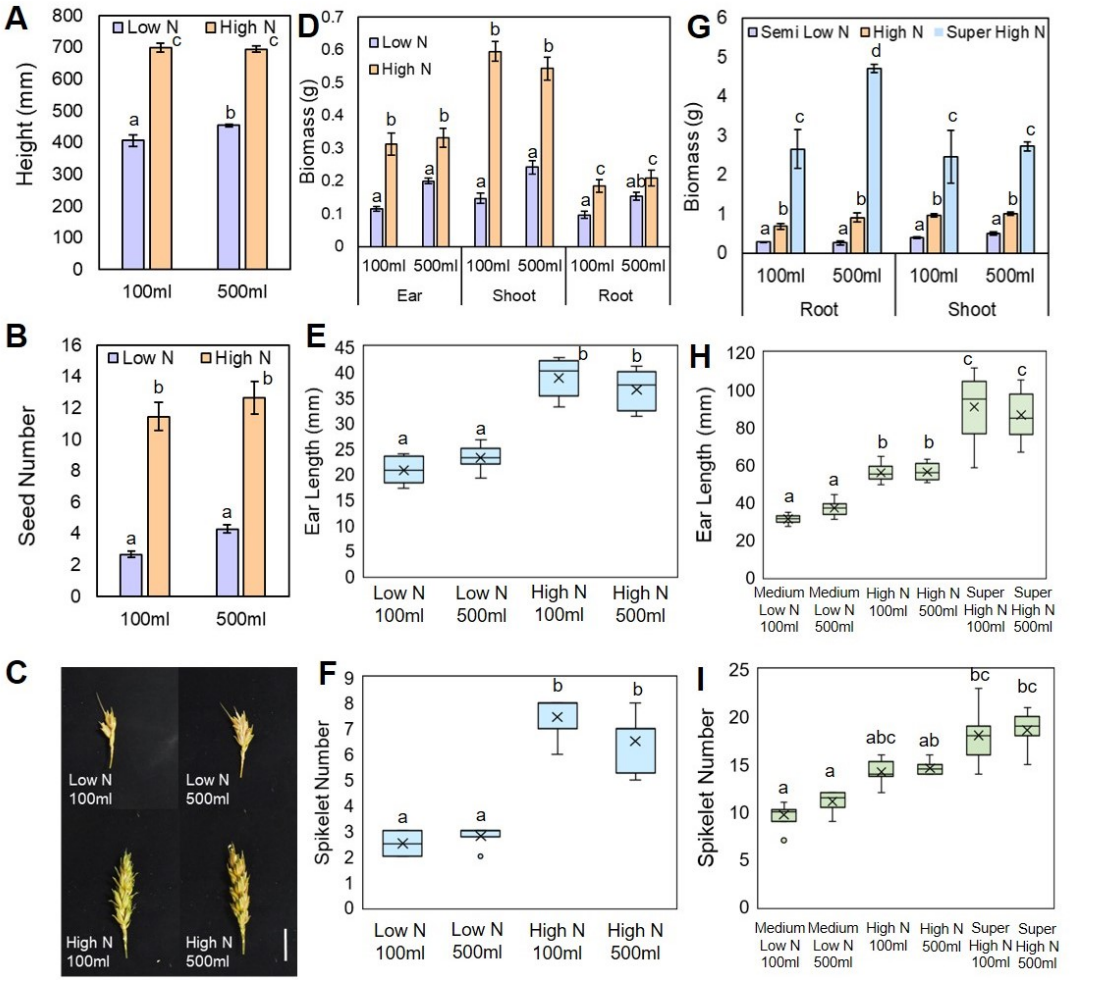
Since very few soil volume effects were seen in this experiment, increasing nitrate levels might be necessary to observe responses to different soil volumes. To assess this, the same experimental set up as above was used with a variation in fertiliser regime. I grew spring wheat (Mulika) on a sand/perlite mix (1:1), in either 100ml or 500ml pots and provided with ‘medium low N’ (18.75µmol of N), ‘high N’ (75µmol of N) or ‘super high N’ (300µmol of N) nutrient solution weekly until end-of-life (14 weeks post germination). At the end-of-life, root and shoot biomass, ear length and spikelet number were assessed.

As in the previous experiment, the greater the N concentration, the greater the root and shoot biomass, ear length and spikelet number (Fig. 3.2G-I). For ear and spikelet number, there are clear numerical differences between each nutrient condition, however statistically for spikelet number this is less clear (Fig. 3.2H-I)

As with the previous study, plants did not display any response to increasing soil volume, under any nutrient condition. Therefore, the effect of soil volume seen in Arabidopsis under low nutrient conditions (Fig. 3.1) was not seen in wheat. This could be due to inherent size differences between the two species, with Arabidopsis having a much lower N demand than wheat. Additionally, the lack of response of wheat to soil volume here could be due to wheat being highly

sensitive to nutrient availability and the concentrations of N used in this experiment could still be below the required levels to show a soil volume response. However, it is more likely that, since all the plants were provided with the same concentration of other essential nutrients, that the wheat plants were limited by another nutrient. This is likely to be phosphate, especially since branching/ tillering (which was absent in all plants in these experiments) is known to be highly responsive to phosphate concentration (Umehara et al., 2008; Umehara et al., 2010). Therefore, perhaps soil volume effects would have been visible if phosphate availability had also been increased.

To conclude this chapter, the results from Arabidopsis suggest that for this given plant size and nutrient requirement, when nitrate conditions are very low the growth of plants is completely limited by the availability of nutrients, whereas at very high nitrate conditions plant growth is limited completely by soil volume. For wheat, growth was entirely determined by nitrate availability not soil volume, but limitations in other essential nutrients may prevent soil volume effects from being seen in these experiments. In summary, the separable effects of nutrient availability and soil volume presented in this chapter further reinforce that the effects of volume restriction on shoot growth is non-nutritional.



3.2: Growth of wheat plants is determined by nutrient availability not soil volume

Graphs showing spring wheat (Mulika) grown for 14 weeks (to end-of-life) on a sand/perlite (1:1) mix in two pot sizes (100ml and 500ml), provided weekly with either low nitrate (Low N 7.5µmol/week) or high nitrate (High N 75µmol/week) nutrient solution (**A-F**), medium low nitrate (Medium Low N 18.75µmol/week), high nitrate (High N 75µmol/week) or super high nitrate (Super High N 300µmol/week) nutrient solution (**G-H**).

A-B) Graphs showing mean tiller height (mm) (**A**) and mean seed number (**B**) at end-of-life. Error bars represent s.e.m. Bars with the same letter are not statistically different from each other, (**A**) ANOVA +Tukey HSD, p>0.05, n=6-10. (**B**) Kruskal-Wallis pairwise comparison, p>0.05, n=6-10 .

C) Pictures to illustrate a single ear produced by an example plant from each condition. Scale bar= 1cm.

D) Graph to show end-of-life dry mean ear, shoot and root biomass (g). Error bars represent s.e.m, n=6-10. Statistical tests were run separately for each type of biomass. Bars with the same letter show no statistical difference, ANOVA + Tukey HSD (ear and root biomass), p>0.05, n=6-10. Kruskal-Wallis pairwise comparison (shoot biomass), p>0.05, n=6-10.

E,F) Box plots to show ear length and spikelet number per ear from each plant. x indicates the mean, the midline represents the median and the box depicts the interquartile range. Whiskers show the minimum and maximum values. Boxes with the same letter are not statistically different. (**E**) ANOVA + Tukey HSD, p>0.05, n=6-10. (**F**) Kruskal-Wallis pairwise comparison, p>0.05, n= 6-10.

G) Graph to show end-of- life mean dry shoot and root biomass (g). Error bars represent s.e.m, n=10. Statistical tests were run separately for each type of biomass. Bars with the same letter show no statistical difference, ANOVA + Tukey HSD (shoot biomass) p>0.05, n=10. Kruskal-Wallis pairwise comparison (root biomass), p>0.05, n=10.

H-I) Box plots to show mean ear length (mm) (**H**) and mean spikelet number (**I**), per ear per plant. x indicates the mean, the midline represents the median and the box depicts the interquartile range. Whiskers show the minimum and maximum values. Boxes with the same letter are not statistically different. (**H**) ANOVA + Tukey HSD, p>0.05, n=10-19. (**I**) Kruskal-Wallis pairwise comparison, p>0.05, n=10-19.

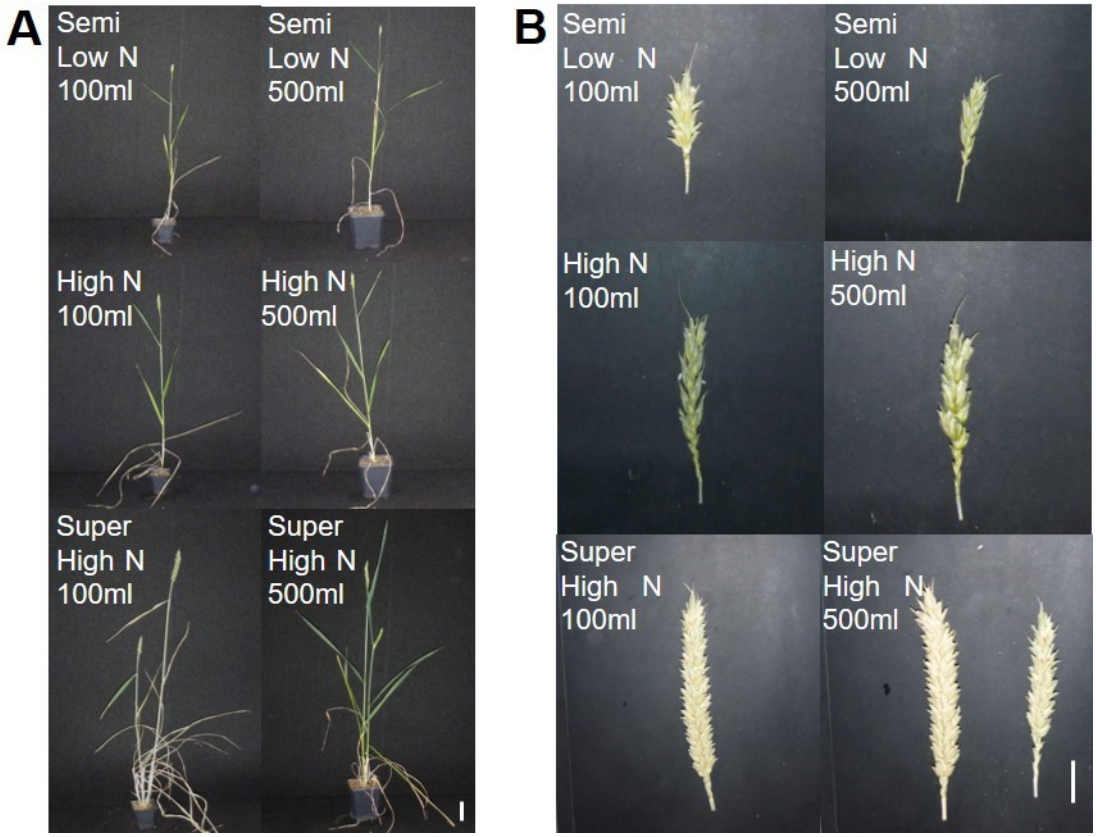


Figure 3.3: Nutrient availability determines shoot growth in wheat

Supplementary pictures to support Fig. 3.2G-H. Pictures showing spring wheat (Mulika) at 14 weeks post germination (end-of-life) on a sand/perlite (1:1) mix in 2 pot sizes (100ml and 500ml), provided with medium low nitrate (Medium Low N 18.75µmol/week), high nitrate (High N 75µmol/week) or super high nitrate (Super High N 300µmol/week)

A) Pictures of spring wheat (Mulika) at 12 weeks post germination. Scale bar: 5cm.

B) Pictures of the ears from one plant for each condition at end-of-life (14 weeks post germination). Scale bar: 1cm.

Chapter 4: Root density sensing links volume restriction and crowding

4.1 Shoot growth is affected by soil volume and crowding

The previous chapter discounted nutrient levels as a method by which plants detect their available soil volume. In this chapter, I explore alternative mechanisms by which plants detect soil volume.

In my previous research, I found that shoot growth is affected by plant crowding in a comparable manner to soil volume. I therefore hypothesised that these responses might be related to each other, and if so, that this might provide insights into the mechanism for soil volume sensing.

I hypothesised that crowding effects would be reduced when a larger soil volume is available and that the addition of fertiliser would be unable to alleviate the effect of crowding. To test these ideas, Arabidopsis (Col-0) was grown either 1 plant per pot (1/pot hereafter) or 4 plants per pot (4/pot hereafter), and in either 100 or 500ml pots. In the 500ml pots, a template was used to ensure the crowded plants had occupied the same surface area as those in the 100ml pots, to rule out shading as a causative factor for changes in growth. Half of each treatment were provided with supplementary nutrients, to rule out lack of nutrients as a causative factor.

The 1/pot plants responded to soil volume as expected, and those in the 500ml pots were larger than the 100ml pot plants (Fig. 4.1A). For both the 100ml and 500ml grown plants, the 4/pot treatment resulted in much smaller plants with fewer branches than the 1/pot plants in the same soil volume. Nevertheless, 4/pot plants grown in 500ml pots were proportionally larger and produced more branches than the crowded plants in 100ml pots (Fig. 4.1A). Intriguingly, the 4/pot 500ml plants produced a similar number of branches to the 1/pot plants grown in 100ml pots. The addition of nutrients did not have an effect in any of the treatments (Fig. 4.1A).

Using this experimental design, I also measured shoot biomass. Individual crowded plants were ~4 times smaller, than the 1/pot plants grown in the same soil volume (with 500ml -Fert as an exception). In 100ml pots, the total biomass per pot is the same in the 4/pot condition as the 1/pot condition (Fig. 4.1B). The addition of supplementary nutrients for plants grown in 100ml pots, irrespective of sowing rate, did not result in an increase in biomass. However, nutrient addition

did allow 500ml 1/pot plants to grow larger than their non-fertilised counterparts (Fig. 4.1B).

The interchangeable responses to soil volume and crowding suggests that these responses might have a related mechanism. The most obvious connection between these two treatments seemed to be that changing both soil volume and plant density would affect the density of roots within the pot. These data therefore suggest that plants might use the mean root density in their environment to modulate shoot growth, and to avoid nutrient limitation. This is the key hypothesis I will explore during this chapter.

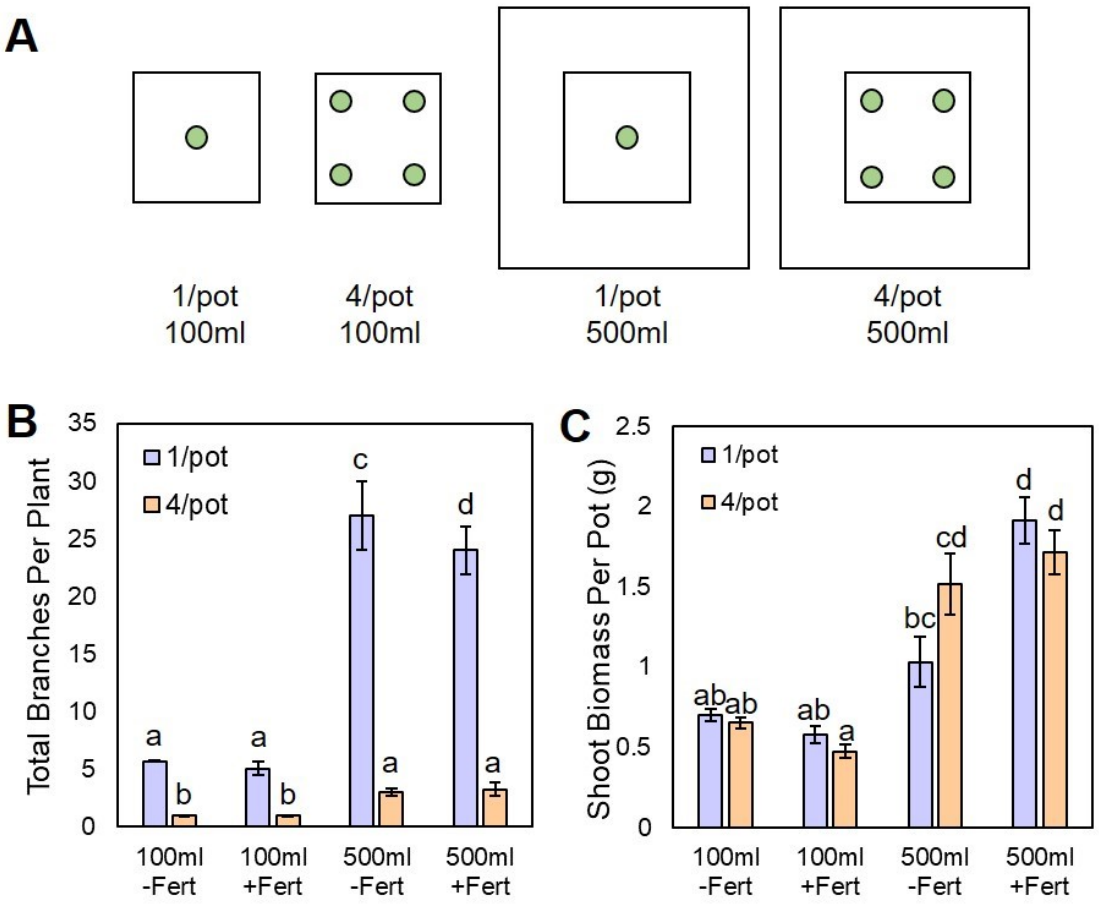


Figure 4.1: Crowding effects are interchangeable with soil volume

A) Cartoon depicting the use of a template to ensure sowing distance in the 500ml pots was equal to the 100ml pots.

B) Bar chart to show total number of branches per Arabidopsis (Col-0) plant at end-of-life (7 weeks post germination). The plants were grown in either 1 plant per pot or 4 plants per pot (1/pot, 4/pot), provided with supplementary fertiliser (+Fert) or not (-Fert), grown in 100ml or 500ml of compost. For the 4/pot treatment, the graph depicts each plant within the treatment not the total number of branches per pot. Bars with the same letter show no statistical difference, ANOVA + Tukey HSD, $p>0.05$, $n=5$-48. Error bars indicate s.e.m.

C) Bar chart showing the average biomass of Arabidopsis (Col-0) plants at end-of-life (7 weeks post germination) per treatment. The plants were grown in either 1 plant per pot or 4 plants per pot (1/pot, 4/pot), provided with supplementary fertiliser (+Fert) or not (-Fert), grown in 100ml or 500ml of compost. For the 4/pot treatment, the biomass of all 4 plants was weighed at once, not each individual plant. Bars with the same letter are not statistically different from each other, ANOVA + Tukey HSD, $p>0.05$, $n=12$. Error bars indicate s.e.m.

4.2 The effect of shading in plant crowding

Shading is considered one of the major ways in which neighbouring plants influence each other's growth. Although controlled for in the previous experiments, I wanted to further examine the role of shading in crowding responses. I postulated that if shading was the major factor responsible for crowding responses shown in Fig. 4.1, altering the spacing of plants in the same soil volume should result in larger plants. However, I hypothesised that shading would cause a weak effect on growth compared to soil volume and that the crowding response was more likely due to below ground root crowding.

To test this, Arabidopsis (Col-0) was grown for 7 weeks (until end-of-life) in two treatments, under normal growth cabinet conditions. As previously described in Chapter 1, soil volume strongly influences plant growth, therefore, soil volume was controlled across the two treatments. In both treatments, there were two intact 500ml pots, filled with compost and secured together for the duration of the experiment. In the midpoint of the joined 500ml pots, a 5cm x 5cm template was placed within the soil as a guide for sowing, indicated by the central square in Figure 4.2 A+B. Both treatments had two plants within each 500ml pot, and thus the soil volume available to each plant was the same. However, the spacing of the plants, and hence degree of shading was different. Treatment A had 4 plants within the template (Fig. 4.2A), while, Treatment B had 2 plants within the template and one on the opposite side of each pot (Fig. 4.2B). When the plants reached end-of-life, total branch number of each plant was assessed, and dry biomass measurements were taken. The 4 plants in Treatment A, were weighed together, whereas, for Treatment B, the 2 medial plants were weighed together, and the 2 outside plants were weighed together (shown as a stacked bar in Fig. 4.2D).

There was no difference in the number of branches per plant between the two treatments (Figure 4.2C). The total number of branches and biomass per pot was the same in both treatments (total biomass per pot: Independent samples T-test, $p= 0.525$). Regarding treatment B, there was no statistical difference between the biomass of the medial and outside plants (Independent samples T-test, $p=0.941$) (Fig. 4.2D).

The findings of Figure 4.2 therefore indicate that shading has no observable role in the crowding response tested here and my previous experiments, since despite the increased shading in Treatment A, there was no difference in the branching or biomass phenotypes of the plants grown in Treatments A and B. As root density was the same in all conditions (as each 500ml pot contained 2 plants, only their distance apart was changed), these findings strongly suggest that below ground root sensing has a larger impact on plant growth than shading.

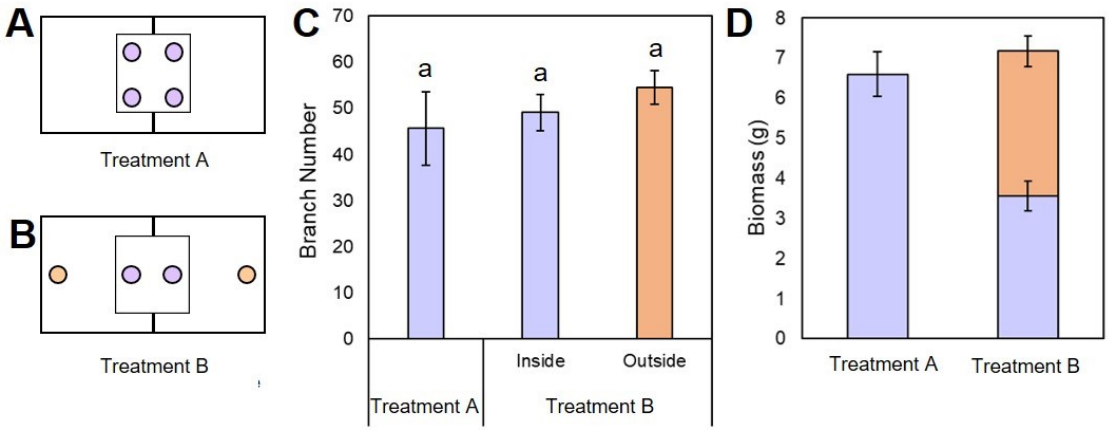


Figure 4.2: The effect of belowground crowding is stronger than the effect of shading

A) Diagram to show the experimental set up for the Treatment A. The large squares represent 500ml pots secured together with all their walls intact. The smaller square is a thin plastic 5cm x 5cm template placed across the two pots. The purple dots denote four plants within the template, two in each 500ml pot.

B) Diagram to show the experimental set up for Treatment B. The large squares represent 500ml pots secured together with all their walls intact. The smaller square is a thin plastic 5cm x 5cm template placed across the two pots. The purple dots denote two plants within the template, One in each 500ml pot, while the orange dots denote two plants grown outside the template, one in each pot.

C) Bar chart showing total branch number per plant. For Treatment B, the purple bar represents the average branch number of the plants inside the template, the orange bar represents average branch number in plants grown outside the template. Bars with the same letter are not statistically different from each other, ANOVA + Tukey HSD, $p>0.05$, n=4-8. Error bars depict s.e.m.

D) Bar chart to show the total dry shoot biomass (g) per pot. For Treatment A, all 4 plants were weighed together. For Treatment B, the two plants within the template were weighed separately to the two plants outside the template, this data is presented as a stacked bar (the purple section represents the two plants inside, the peach section represents the two plants outside). Error bars depict s.e.m, n= 4-8

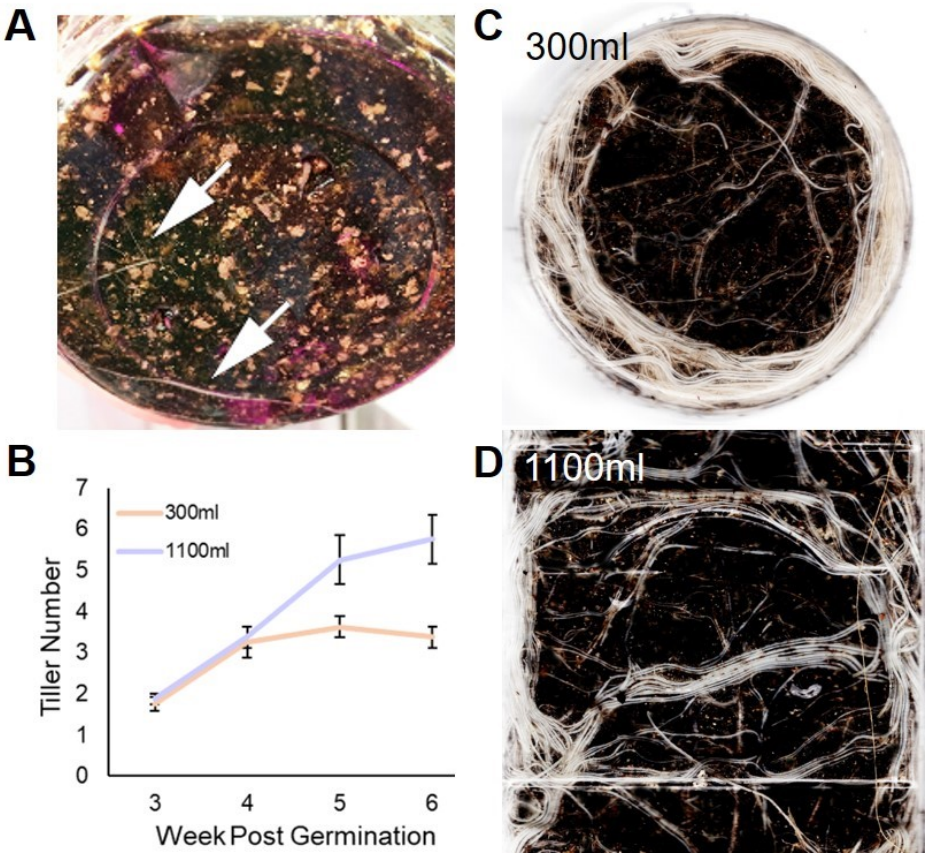
4.3 The effects of soil volume are unlikely to be due to mechanical impedance

The apparent ability of plants to sense their soil volume might arise because of mechanical impedance caused by the pot walls. However, it is difficult to see how mechanical impedance could explain crowding responses. Nevertheless, it was important to assess whether mechanical impedance played a role in soil volume responses.

To assess whether mechanical impedance plays a role in the soil volume response, spring wheat (Mulika) plants were grown in clear walled pots (300 and 1100ml) in a compost/vermiculite mix (1:1) to allow visualisation of root growth.

Less than a week after germination, roots were observed to have hit the walls of the pot and had grown along them in both the 300 and 1100ml pots (Fig. 4.3A). By three weeks after germination, tiller formation had begun and was identical in both container sizes, until week 4-5 when tiller formation slowed in the 300ml pots compared to the 1100ml pots. Peak tiller number was seen in the small pots at week 5 post germination, while tiller number continued to increase in the 1100ml pots through week 6 after germination (Fig. 4.3B). In both pot sizes, large numbers of roots were in contact with the pot walls 6 weeks after germination, and this did not appear to differ between the pot sizes. However, root density was much greater in the smaller pots, most notably along the base of the pot (Fig 4.3C).

These observations suggest that the mechanical impedance of roots hitting the pot walls does not correlate with shoot growth changes, but further supports the idea that root density may be a critical parameter determining shoot growth responses.



4.3: Inhibition of shoot growth under volume restricting conditions is not the result of mechanical stimulus

A) Image showing spring wheat (Mulika) roots on the base of a 300ml clear pot, 1 week post germination

B) Line graph showing mean tiller number from week 3 to week 6 post germination in spring wheat (Mulika) grown on a compost/ vermiculite 1:1 mix in 300ml and 1100ml pots. Error bars depict s.e.m, n=8.

C-D) Scans of the base of clear pots (300ml and 1100ml) showing spring wheat (Mulika) roots at 6 weeks post germination.

4.4 Root density sensing could explain soil volume responses

Since I determined that soil volume effects are unlikely to be the result of mechanical impedance, I attempted to directly test the effect of root density on shoot growth.

To do this, I conducted an experiment in which plants were subjected to spatial and temporal variation in root density. Spring wheat (Mulika) plants were grown in 100ml pots, for 4 weeks until the tillering had initiated. At this point the plants were divided into different experimental treatments (Fig. 4.4A). A quarter of the plants (Treatment A) remained in the 100ml pots. Another quarter (Treatment B) were taken out of the 100ml pot (with an intact soil ball) and transplanted into 500ml pots full of compost. The remaining half of the plants stayed in the original 100ml pots but a $1cm^2$ hole was carefully cut on each of the 4 lateral faces (the base of the pot already contained drainage holes). These modified 100ml pots with additional holes were placed in either 500ml (Treatment C) or 2000ml (Treatment D) pots filled with compost (Fig. 4.4A). The A, C and D plants all began the second stage of the experiment with the same absolute root density, while the C and D plants had the same mechanical impedance as each other. However, A, C and D plants had greatly different access to total soil volume. I thus assessed the growth of these plants over the remainder of their life-cycle to assess which factors might influence shoot growth.

All end-of-life growth parameters (tiller number, spikelet number, dry shoot and ear biomass), were clearly determined by the total soil volume. Plants with the same initial soil volume, but different total volumes (A,C,D) were very different sizes, as were plants with the same mechanical stimulus but different soil volumes (C,D) (Fig. 4.4B-D). Thus ultimately, the key parameter controlling shoot growth is the total soil volume.

However, looking at tillering over the course of the experiment, this reveals interesting effects of the different treatments. The initial rate of tiller production after transfer was the same in Treatments B, C and D, until week 7 when tiller production increased greatly in Treatment D (Fig. 4.5A).

The delay seen in C and D plants could be caused by the roots of these plants being at high density until some of the roots are able to ‘’escape’’ the 100ml pot into the outer soil jacket of the larger pots. In individual plants when tiller number

remained low, this suggests that roots of these plants may have struggled to colonise the outer soil layer of the larger pot. To assess whether this was the case, root system growth was examined in treatments C and D 6 weeks after transfer (10 weeks post germination) in plants which had low tiller production, and compared these to high tillering plants from the same treatments (Fig. 4.5D-G). Root system growth was also visually assessed in the A and B control plants, in which extensive root growth was seen, and the soil was bound tightly by the roots; in both pot sizes the root density appeared similar (Fig. 4.5B-C). In the low tillering C plants (Fig. 4.5E), the soil was much less tightly bound than in the controls (A and B), and in the low tillering D plants (Fig. 4.5G), the outer soil layer was completely unbound. However, in the high tillering C and D plants (Fig. 4.5D+F), the outer soil layer was much more tightly bound than in the low tillering plants, and this was comparable to the A and B control plants (Fig. 4.5B-C).

These results suggest that the number of tillers produced by the transferred plants reflects the soil volume they were able to utilise during the tillering window. Ultimately all transferred plants were able to make roots in the outer soil layer but in the case of the low tillering plants, this colonisation was much slower, missing some crucial time in the tiller formation window, and so tillering was reduced. When root density was reassessed at 9 weeks after transfer, there was more root colonisation visible in the low tillering plants but this failed to completely restore tiller production.

These results suggest that tillering is determined by effective root density- the average density of roots present in the utilised soil volume at any given time. Plants do not instantaneously detect the total soil volume; they have to explore the soil volume in order to respond to it’s availability. In the A plants, their effective root density increases more rapidly than the B plants, resulting in faster tiller inhibition. Tillering stopped in both A and B treatments when they reached a similar degree of root density. If the transferred C and D plants are able to efficiently colonise the outer soil layer, then effective root density is decreased, at least temporarily, resulting in increased tiller production. However, in the plants which are unable to efficiently ‘escape’ the inner 100ml pot and colonise the outer soil layer, root density remains high, resulting in partially inhibited tiller production. These data thus support the idea of root density sensing in plants.

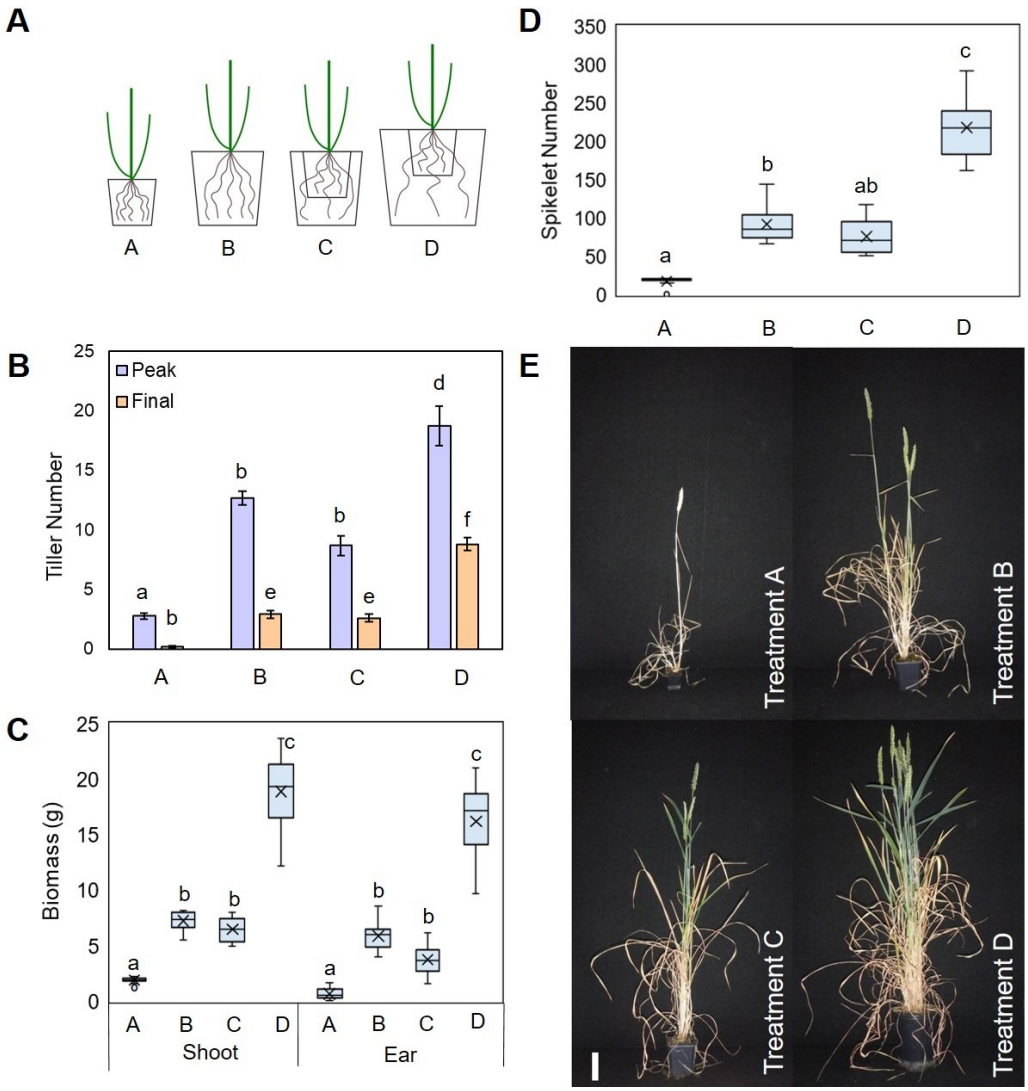


Figure 4.4: End of life shoot growth is determined by soil volume

A) Cartoon showing the four root density treatments. Spring wheat was grown for 4 weeks in 100ml pots and then transferred to the following conditions A: remained in a 100ml pot. B: transferred without 100ml pot to a 500ml pot. C+D: original 100ml pot had 1cm^2 holes cut in the sides and placed in a 500ml (C) or 2000ml (D) pot.

B) Final and peak tiller number in spring wheat (Mulika) in the four root density treatments. Error bars represent s.e.m. Bars with the same letter are not statistically different, ANOVA + Tukey HSD, p>0.05, n=12.

C) Boxplot showing dry shoot and total ear biomass of plants grown in four root density conditions. Shoot biomass refers to all the aboveground tissues except for ears, which were removed. Ear biomass was measured by weighing all ears from each plant. x indicates the mean, the midline represents the median, dots below whiskers indicate outliers and the box depicts the interquartile range. Whiskers show the minimum and maximum values. Boxes with the same letter depict no statistical difference, ANOVA +Tukey HSD, p>0.05, n=11-12. Statistical analysis was run for each type of biomass separately.

D) Boxplot showing total spikelet number of each plant from the four root density conditions. x indicates the mean, dots below whiskers indicate outliers, the midline represents the median and the box depicts the interquartile range. Whiskers show the minimum and maximum values. Boxes with the same letter show no statistical difference, Kruskal-Wallis pairwise comparison, p>0.05, n= 11-12.

E) Pictures of example wheat plants from each condition at 15 weeks post germination. Scale: 8cm.

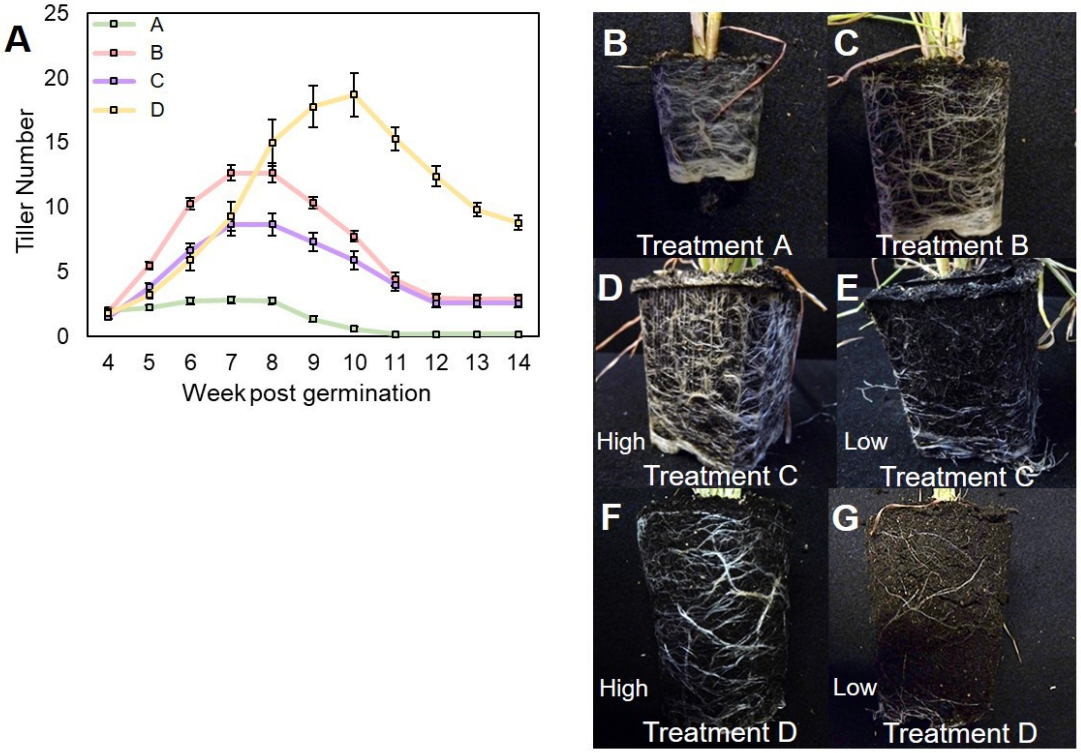


Figure 4.5: Shoot growth temporally correlates with effective root density

A) Line graph showing tiller number from week 4 to week 14 for the four root density treatments. Error bars represent s.e.m, n=12.

B-G) Examples of soil masses from each treatment, 10 weeks post germination. Treatment A (**B**), Treatment B (**C**), high tillering Treatment C (**D**), low tillering Treatment C (**E**), High tillering Treatment D (**F**), low tillering Treatment D (**G**).

4.6 Strigolactones contribute to neighbour detection and root density sensing in pea

The previous findings have suggested root density sensing as the mechanism behind the crowding and soil volume responses. However, the signal behind this root density sensing is unknown. There have been many suggestions in the literature that root exudates play a role in neighbour detection in the rhizosphere (Biedrzycki et al., 2010; Caffaro et al., 2011; Semchenko et al., 2014). Strigolactones regulate shoot development and are known to be exuded into the soil by many plant species, to stimulate mycorrhizal associations (Akiyama et al., 2005; Besserer et al., 2006; Besserer et al., 2008). However, little is known about other functions strigolactones may have in the rhizosphere. Research by Proust et al (2011) suggests strigolactones allow *Physcomitrella patens* colonies to detect each other and prevent colonies from growing over each other. Therefore, I was intrigued whether strigolactone exudates might play a role in neighbour detection, and by extension contribute to shoot growth modulation by root density sensing. If a plant can detect strigolactone exudates this could mean it can sense if there is another plant in close proximity and then make decisions about shoot growth as a result. Therefore, I hypothesised that detection of strigolactone exudates would allow plants to sense neighbouring plants and/or soil volume.

Pea plants are known to exude strigolactone and this regulates nodule formation (Foo and Davies, 2011), and since there are several strigolactone mutants available, in both biosynthesis and signalling, pea was chosen for the following experiments. *rms1* (*ccd8*) is a strigolactone synthesis mutant which cannot produce strigolactones but can still detect them (Sorefan et al., 2003; Gomez-Roldan et al., 2008), *rms3* is a signalling mutant that is able to synthesise strigolactone but is unable to respond to them (Beveridge et al., 2009; De Saint Germain et al., 2016).

To assess the role of strigolactone in neighbour detection, I grew combinations of wild-type (WT) and *rms1* mutants together. I grew plants in 500ml of compost under normal glasshouse conditions, containing either 1x WT, 1x *rms1*, 4x WT, 4x *rms1*, 3x WT / 1x *rms1* or 3x *rms1* / 1x WT (Fig. 4.6A+E). At 7 weeks post germination, total branch number, main stem length and dry shoot biomass were measured.

The strigolactone mutant grew as expected, producing a much higher branch number in *rms1* mutants than WT plants grown in the same conditions (Fig. 4.6B) Unsurprisingly, the 4x WT and 4x *rms1* plants had a reduced number of branches compared to their 1x counterparts, showing that pea plants respond as expected to soil volume. However, branch number in WT plants grown with *rms1* plants showed a significant increase in branch number compared to 4x WT plants (Fig. 4.6B). Conversely, *rms1* plants grown with WT plants showed a decrease in branch number relative to 4x *rms1* plants (Fig. 4.6B)

For WT plants, main stem length was the greatest in the 1x WT condition, but reduced under crowded 4x WT conditions (Figure 4.6C). However, as with branching, the reduction in stem length in WT plants was less in the 3x WT/1x *rms1* condition and especially in the 3x *rms1* / 1x WT condition (Fig. 4.6C). The main stem length of *rms1* did not vary between treatments.

Biomass was increased in WT plants grown in the presence of neighbouring *rms1* plants compared to WT plants grown together (4x WT); most noticeably in the 1x WT / 3x *rms1* condition (Fig. 4.6D). Conversely, *rms1* biomass decreased when grown in the presence of neighbouring WT plants, especially in the 1x *rms1* / 3x WT condition (Fig. 4.6D).

The increase in WT branch number, biomass and main stem height, relative to 4x WT controls, when grown in the presence of *rms1* suggests that WT plants are partially blind to the *rms1* plants. Conversely, the reduction in branch number and biomass, relative to 4x *rms1* plants, in *rms1* plants grown in in the presence of WT plants is consistent with the idea that *rms1* plants are partially blind to each other, but can be inhibited by the strigolactone exuded by WT plants. This data suggests that strigolactone plays an important role in neighbour detection.

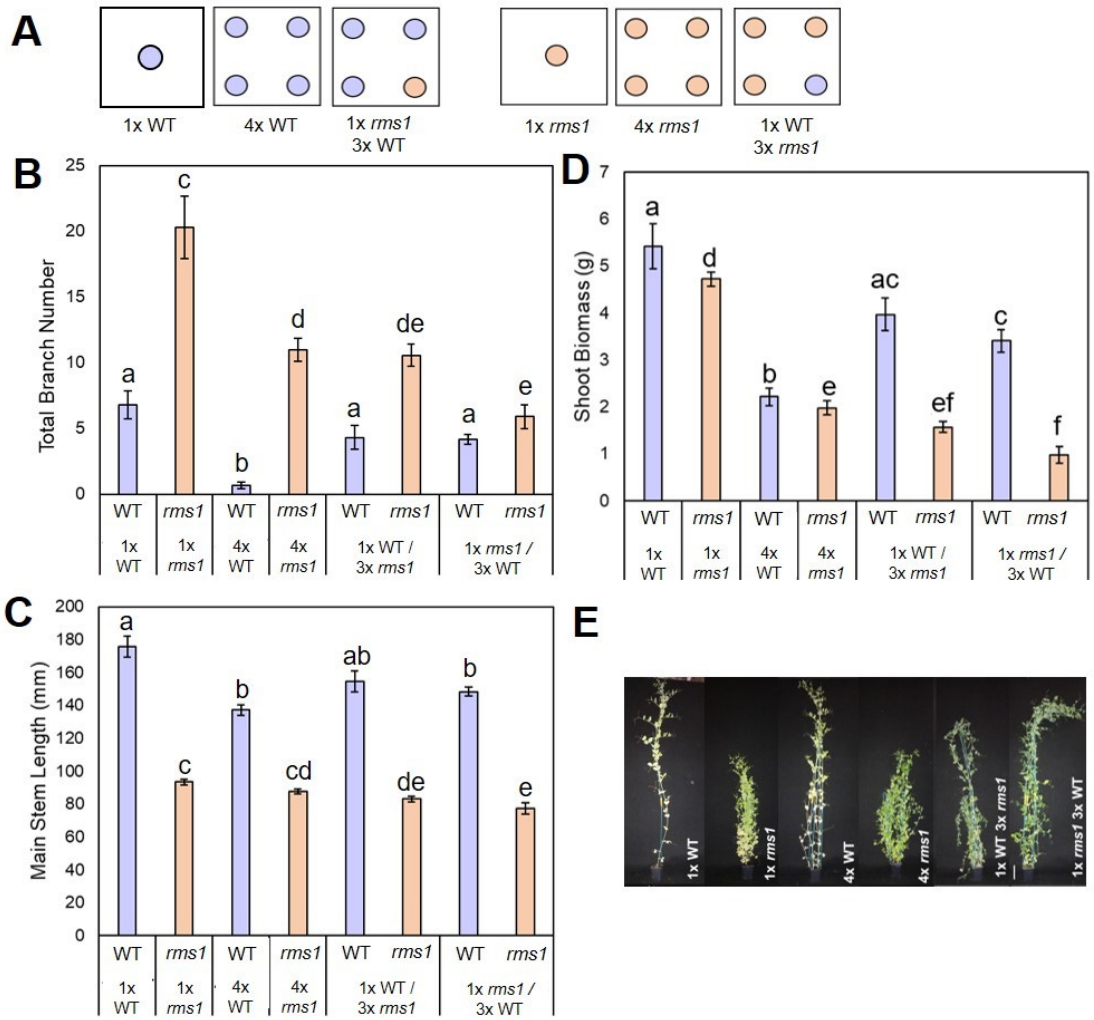


Figure 4.6: Strigolactone exudates modulate neighbouring plant growth

A) Cartoon showing how the peas were sown for each condition. Purple dots represent each WT plant and peach dots represent each *rms1* plant.

B) Bar graph showing mean total branches per pea plant in the 6 conditions. For the combination conditions (1x WT / 3x *rms1*, 1x *rms1* / 3x WT) each genotype is shown as a separate bar. Bars with the same letter are not statistically different, WT data: Kruskal-Wallis pairwise comparison, p>0.05 n=10-40. Bars with the same letter are not statistically different, *rms1* data: ANOVA with Tukey HSD, p>0.05, n=10-40. Error bars depict s.e.m.

C) Bar graph showing mean main stem length (mm) per pea plant in the 6 conditions. For the combination conditions (1x WT / 3x *rms1*, 1x *rms1* / 3x WT), each genotype is shown as a separate bar. Bars with the same letter are not statistically different. Kruskal-Wallis pairwise comparison was ran separately for each genotype, p>0.05, n=10-40. Error bars depict s.e.m.

D) Bar graph showing mean shoot biomass (g) per pea plant in the 6 conditions. For the combination conditions (1x WT / 3x *rms1*, 1x *rms1* / 3x WT), each genotype is shown as a separate bar. Bars with the same letter are not statistically different from each other, Kruskal-Wallis pairwise comparison was ran separately for each genotype, n=10-40, p>0.05. Error bars depict s.e.m.

E) Pictures of example pots from each condition. Scale bar= 10cm.

One possible issue with these results is the difference in growth habit between *rms1* and WT; WT is much taller, and effects of WT on *rms1* could also arise from shading. Comparing the effects of *rms1* and *rms3* on each other's growth should allow these problems to be overcome, because the plants have the same basic growth habit (unlike WT), but opposite strigolactone synthesis and perception profiles. Growing *rms1* and *rms3* together in a similar experimental design as this (Fig. 4.6), would allow the effects of growth habit to be controlled for, and a clearer association between strigolactone exudation and shoot growth to be demonstrated.

I reasoned that *rms3* would be able to inhibit the growth of *rms1* and WT, and would not be inhibited when grown with WT.

To test these ideas, three pea genotypes (WT, *rms1* and *rms3*) were grown in 500ml pots in 10 different treatments (Fig. 4.7A) for 5 weeks in normal glasshouse conditions. The conditions were as follows; 1x WT, 1x *rms1*, 1x *rms3*, 4x WT, 4x *rms1*, 4x *rms3*, 3x *rms3* / 1x WT, 3x WT / 1x *rms3*, 3x *rms1* / 1x *rms3*, 3x *rms3* / 1x *rms1*. At 5 weeks post germination, main stem height and total branch number for all individual plants was measured.

As expected, branch number in 1x *rms1* and 1x *rms3* was the same, with 1x WT producing many fewer branches (Fig. 4.7B). Also as expected, this pattern is mirrored in the crowded controls as 4x *rms1* and 4x *rms3* produced the same number of branches and WT plants produced many fewer; all genotypes produced fewer branches than in 1x treatments (Fig. 4.7B). As predicted, *rms3* branch number remained largely consistent in all crowding conditions except in the 3x WT / 1x *rms3* condition where branch number was reduced slightly. Conversely, *rms1* branch number, was strongly (although not statistically significantly) reduced when grown with *rms3* plants, especially in the 1x *rms1* / 3x *rms3* treatment. Branching was also reduced in WT plants grown with *rms3*, again, in proportion to the number of *rms3* plants present in the pot (Fig. 4.7B).

As with the previous results, there was little difference in main stem length in *rms1* or *rms3* plants across the treatments (Fig. 4.7C). There was a small reduction in *rms3* height in the 3x WT / 1x *rms3* treatment, but this could be the result of *rms3* plants being slower to germinate than in the other treatments, allowing the WT plants to become established sooner than the *rms3* plants. As expected from

previous findings, when WT plants were crowded together (4x WT), the main stem length per plant was reduced. WT main stem length increased slightly in the 3x WT / 1x *rms3* condition compared to the 4x WT control, and more strongly in 3x *rms3* / 1x WT plants (Fig. 4.7C). This is the opposite pattern to that seen in WT branching in *rms3* co-grown conditions, which was more strongly inhibited in the presence of an increased number of *rms3* plants. The effects of crowding on stem length might therefore be caused primarily by shading, rather than strigolactone exudation.

As predicted *rms3* plants had a similar effect to WT on neighbouring *rms1* plants. As *rms3* plants are able to exude strigolactone, like WT plants, this further supports the role of strigolactone exudates as a plant-plant detection stimuli. However, intriguingly *rms3* plants are able to inhibit WT growth, as well as *rms1*. This might be because *rms3* produces higher levels of strigolactones; a common effect of hormone insensitivity in increased hormone synthesis (Arite et al., 2009). The ability to recognise strigolactone exudates released from neighbouring plants, allows plants to make informed decisions about shoot growth by assessing the presence of neighbouring plants. It remains unknown however, what type of strigolactone is exuded in crowded conditions. Research into strigolactone exudates involved in parasitic plant germination suggests more than one type of strigolactone is present in root exudates, and there are differences in these between varieties of a single species (Awad et al., 2006). Furthermore, we do not know the concentration of strigolactone is exuded into the soil and whether this is reduced or increased in responses to crowded conditions.

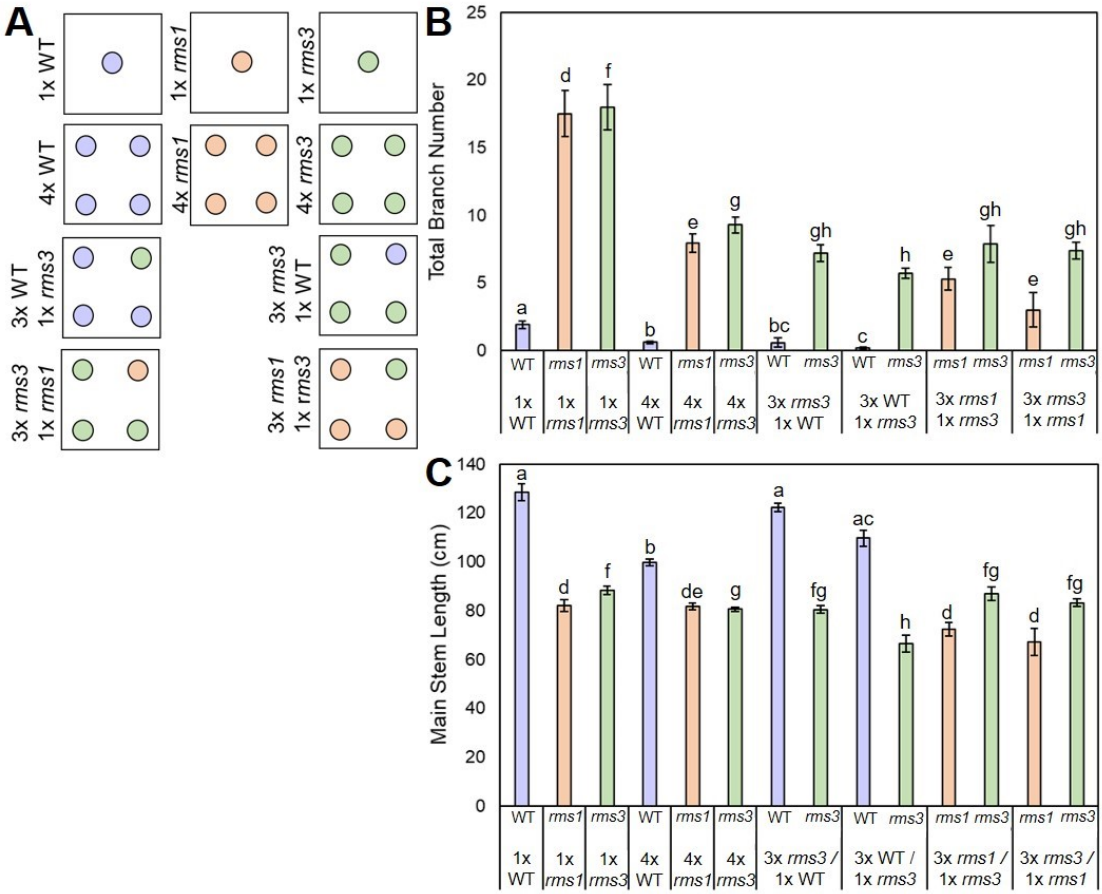


Figure 4.7: Strigolactone exudates modulate shoot growth of neighbouring plants

Pea plants (WT, *rms1* and *rms3*) were grown in 10 different combinations for 5 weeks.

A) Cartoon depicting the control (1x WT, 1x *rms1*, 1x *rms3*, 4x WT, 4x *rms1* and 4x *rms3*) and combination (3x *rms3* / 1x WT, 3x WT / 1x *rms3*, 3x *rms1* / 1x *rms3*, 3x *rms1* / 1x *rms3*) plant growth conditions. Each dot symbolises one plant. Purple dots represent WT, orange dots represent *rms1* and green dots represent *rms3* plants.

B) Graph showing the mean total branch number per condition. For the combination conditions (3x *rms3* / 1x WT, 3x WT / 1x *rms3*, 3x *rms1* / 1x WT, 3x *rms3* / 1x*rms1*), mean total branch number per genotype is shown. Bars with the same letter are not statistically different, Kruskal-Wallis pairwise comparison was used separately for each genotype, p>0.05, n=9-40 (1x conditions n=8-10, 4x conditions n= 40, for combination conditions the 1x plant n=8-10 and the 3x plant n=24-30). Error bars depict s.e.m.

C) Graph showing the mean main stem length per condition. For the combination conditions (3x *rms3* / 1x WT, 3x WT / 1x *rms3*, 3x *rms1* / 1x WT, 3x *rms3* / 1x *rms1*) mean stem length per genotype is shown. Bars with the same letter are not statistically different from each other, Kruskal-Wallis pairwise comparison was used separately for each genotype, p>0.05, n=9-40 (1x conditions n=8-10, 4x conditions n= 40, combinational conditions the 1x plant n=8-10 and the 3x plant n=24-30). Error bars depict s.e.m.

4.8 Neighbour detection and 'soil' volume responses do not require soil

A final intriguing question is whether neighbour detection and soil volume responses require the physical presence of soil or whether chemical exudates are sufficient for these responses. To examine this, I developed a hydroponic system, and examined neighbour detection in peas using it.

The 4 treatments I used were: 1x WT, 1x *rms1*, 5x WT and 5x *rms1* (Figure 4.8D). WT and *rms1* seeds were germinated on hydrated perlite for 2 weeks, provided with standard nutrient solution weekly and were grown under standard glasshouse conditions. Once 2 weeks old, seedlings of equal size for each genotype were selected and transplanted into the hydroponic system. 1L pots were used for all conditions to control for any container volume effects. At 7 weeks post germination, primary branch number, main stem height, dry root and dry shoot biomass were measured.

As expected *rms1* plants produced more branches than WT, and for both genotypes branch number was greatest in the 1x conditions compared to the 5x plants (Independent samples T-test, WT: p=0.004, *rms1*: p=0.002) (Fig 4.8A). Main stem length remained the same in both 1x *rms1* and 5x *rms1*, however main stem length was reduced slightly, but not significantly in crowded WT plants (Independent samples T-test, WT: p=0.147, *rms1*: p=0.105) (Fig. 4.8B). Shoot and root biomass per plant was reduced in the crowded conditions (Independent samples T-test, Shoot biomass: WT: p=0.000, *rms1*: p= 0.000, Root Biomass: WT: p=0.028, *rms1*: p=0.000) (Fig. 4.8C).

As all the measured parameters were reduced in the crowding conditions, except main stem length, as with soil-based experiments (Fig. 4.6 and 4.7); this suggests that the effects of crowding and root density sensing are not limited to soil based environments. Furthermore, the findings from this hydroponic study indicate that the signal involved in root density sensing is able to modulate shoot growth before degrading in the growth solution, which suggests that plants are highly sensitive to this signal and perhaps do not require large quantities in order to undergo a subsequent growth response. For future experiments, having this hydroponic system will allow exudates to be collected, and strigolactone exudate levels to be quantified to allow further understanding of their role in root density sensing.

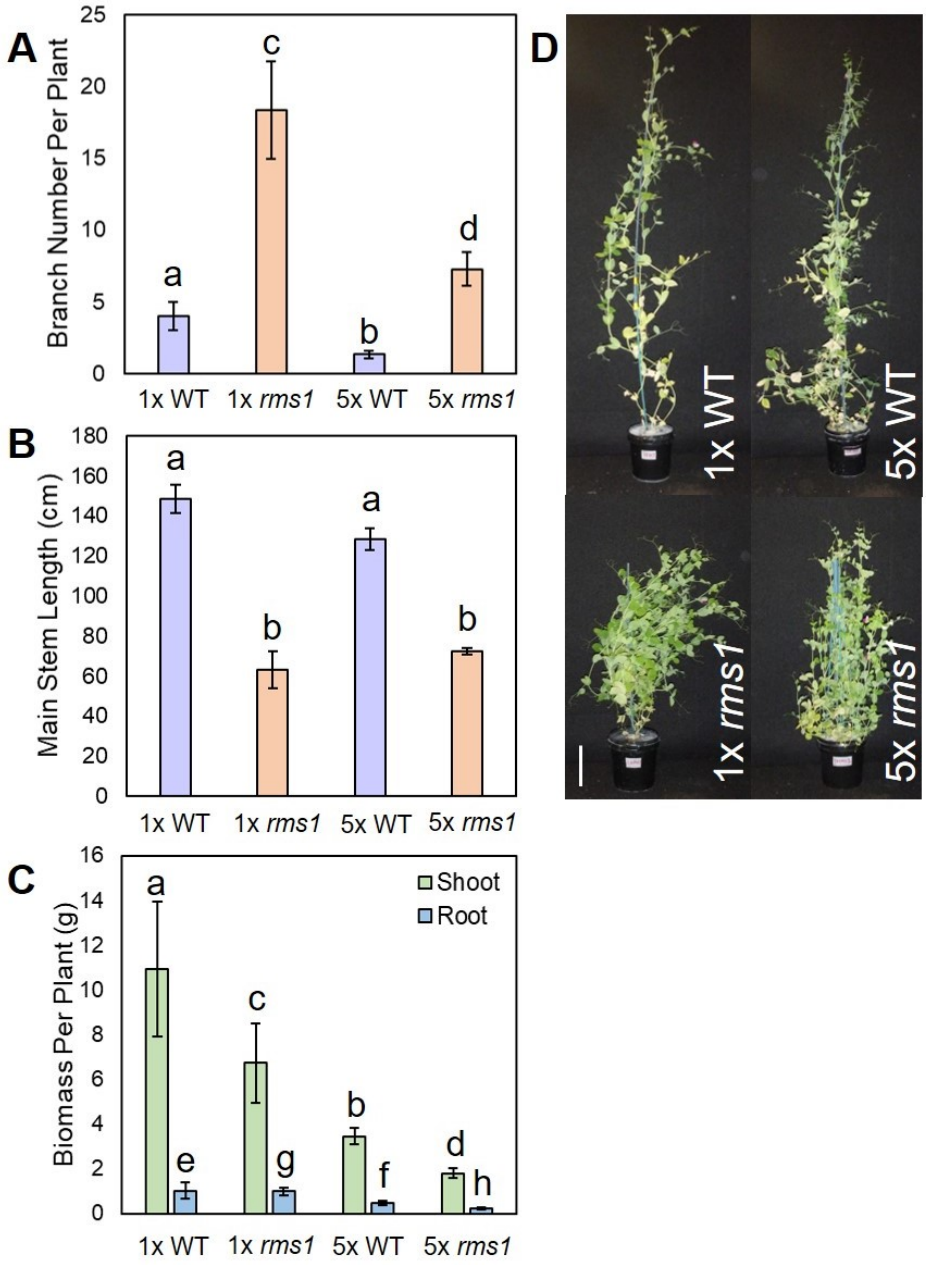


Figure 4.8: Neighbour detection is not limited to soil-based systems

Figure showing WT and *rms1* pea plants grown in either 1 plant per pot or 5 plants per pot (1x, 5x) conditions in a hydroponic system.

A) Bar chart showing mean primary branch number per plant. Error bars are s.e.m, n= 3-15. Independent samples T-test was ran for each genotype, WT: p=0.004, *rms1:* p=0.002. Bars with the same letter show no statistical difference.

B) Bar chart showing mean main stem length (cm) per plant. Error bars indicate s.e.m, n=3-15. Independent samples T-test was ran for each genotype. WT: p=0.147, *rms1*: p=0.105. Bars with the same letter show no statistical difference.

C) Bar graph showing mean dry shoot and mean dry root biomass per plant. Error bars indicate s.e.m, n=3-15. Independent samples T-test was ran for each genotype and each type of biomass. Shoot biomass: WT: p=0.000, *rms1*: p= 0.000, Root Biomass: WT: p=0.028, *rms1*: p=0.000. Bars with the same letter show no statistical difference.

D) Pictures showing example pots for each condition. Scale bar depicts 10cm.

Chapter 5: Discussion

The detrimental effect of growing plants in small pots is well-known by gardeners but little is understood about the underlying mechanisms behind it (Poorter et al, 2012). Research shows that the effects of volume restriction are not directly a result of lack of nutrients, however the mechanism involved in producing such phenotypic differences and the adaptive advantage of these is unknown (Poorter et al, 2012). The data presented in this thesis provide an outline for understanding the developmental biology behind the volume restriction and associated neighbour detection phenomena. Plants can determine the volume of soil or below-ground space in which they reside by sensing their root density, together with that of neighbouring plants.

5.1 Soil volume can be used to predict nutrient availability over the plants lifecycle

As indicated in previous literature (Hameed et al., 1987; Bar-Tal and Pressman, 1996; Poorter et al., 2012), soil volume responses are non-nutritional. Data presented in this thesis suggests plants might detect the nutritional content of the soil together with its volume, and use this to predict the total amount of nutrients which they may acquire throughout their life, allowing a more useful guideline for growth than nutrient availability alone. An example of this system is that even if a high nutrient concentration is available early in the plant's life cycle, but soil volume is very small, the total amount of nutrients available over their life may be low and hence growth should be restricted to prevent the plant from exhausting those nutrients.

5.2 Soil volume could be a predictor for water availability

A factor worth noting is water availability. Within this thesis, soil volume was a defined limiting factor on growth, and I believe in addition to predicting nutrient availability over life, soil volume could also be a predictor of water availability. As water uptake is a primary role of roots, suggestions have been made that a reduction in their uptake is the cause of altered growth rates in volume restricted plants due to the reduced water holding capacity of small pots resulting in a higher risk of drying out (Tschaplinski and Blake, 1985). However, in research on soybean (Krizek et al., 1985) and pepper (Ismail and Davies, 1998) and indeed

a multitude of hydroponic studies where water is not limited (Ternesi et al., 1994; Bar-Tal et al., 1995; Bar-Tal and Pressman, 1996; Ismail and Noor, 1996; Shi et al., 2007; Shi, Ding, et al., 2008), volume restriction still results in reductions in shoot growth. Within a particular soil volume, the roots grow and occupy the space; this occupancy of the entire free soil volume can allow the plant to determine the maximum amount of water available any given time. This ability to predict future water availability consequently allows plants to keep shoot growth at a level that can be supported.

5.3 Soil volume and soil depth in the environment

Plants are not grown in pots in nature, but there are several scenarios where plants may encounter physical barriers. This could be in areas where the soil layer is thin or underlying bedrock could prevent root growth, both of which could cause volume restriction. In addition, trees with large root systems could become volume restricted even in areas of deep soil due to their large inherent size. In agricultural practice, soil compaction is a large issue and is a major cause of yield losses in crops across the globe (Oldeman, 1992), with estimated losses to be between 20-25% (Barken et al., 1987; Arvidsson, 1999). Soil compaction is primarily caused by the use of heavy machinery in agricultural practices, which compresses soil over time resulting in highly dense soil with reduced porosity and permeability (Correa et al., 2019). Heavily compacted soils are difficult for plant roots to penetrate through (Correa et al., 2019) meaning that these plants experience a reduction in their available soil volume through restricted soil depth.

Sadras et al (2001) showed that in a field context, wheat, soybean, sunflower and maize had reduced grain yield and shoot biomass when grown in shallow soil (Sadras and Calviño, 2001). Soil compaction effectively results in a shallower area for root exploration (Fig. 5B), and hence this could be a contributing factor to the yield reductions seen in soil compacted fields. The low yields of plants subjected to soil compaction have been linked to the mechanical impedance of the compacted soil on plant roots, and the resultant limitation on nutrient and water uptake (Håkansson et al., 1988). My results show that shallow soil has strong negative effects on plant growth independently of soil volume (Fig. 2.2-2.3) and may explain some proportion of compaction responses. As soil depth is reduced, roots may attempt to change their root system architecture by allocating

more resources to non-vertical root growth (such as lateral root formation), cease vertical root growth or attempt to penetrate the compacted soil (Dexter and Chant, 1991; Clark et al., 2003). Crop fields are often densely sown, so horizontal root growth may not be possible or could be hindered by neighbouring plants. These factors would ultimately reduce each plant's available soil volume as it is heavily shared by neighbouring plants. Thus, crop responses to soil compaction are likely, multi-factorial.

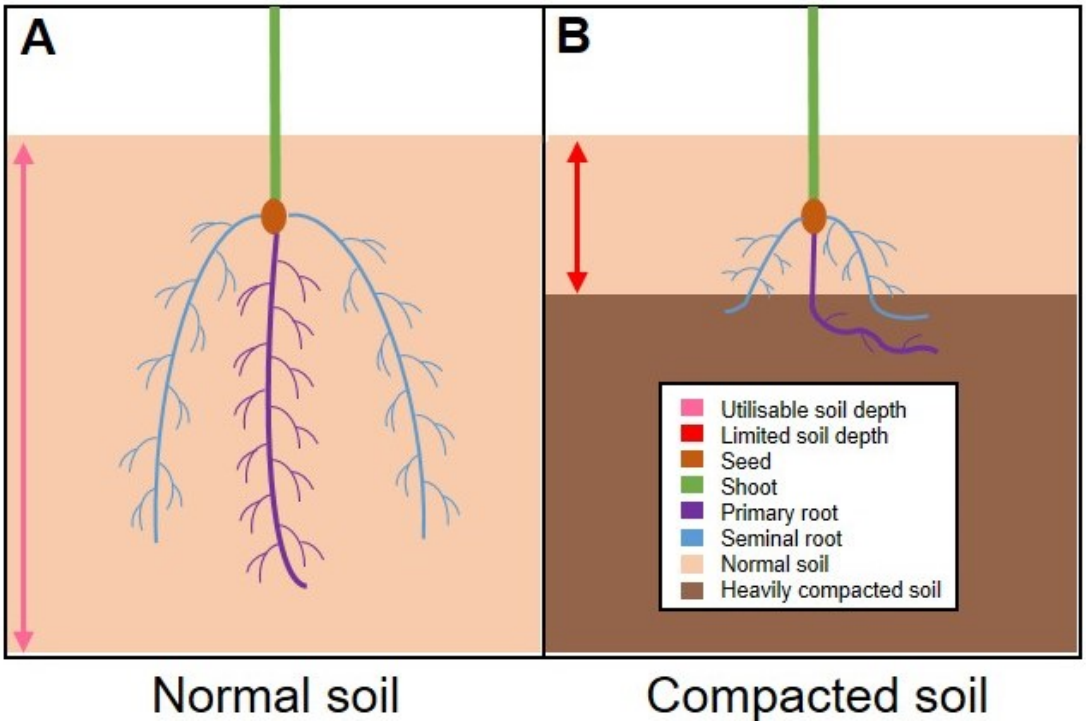


Figure 5.0: Soil compaction on root growth

Adapted from Correa et al (2019).

A) Depicts normal root growth in perfect soil conditions with the optimum soil density and soil depth to allow unhindered root exploration.

B) Depicts roots growing through normal quality soil until the second soil layer which is dense and heavily compacted. The roots are unable to penetrate through this layer so begin to grow more horizontally. The compacted soil restricts rooting depth and limits the total soil volume available for water and nutrient exploration.

5.4 Soil volume and plant neighbour detection

There is much phenotypic evidence to support the detrimental effects on plant growth when under root restricting conditions, however elements of neighbour detection are still elusive. It is clear that plants are able to use their root systems to detect and respond to neighbouring plants in their soil environment, despite this, the literature is highly complicated and often confusing to decipher (Depuydt,

2014). Soil volume is a variable which often is not controlled for in neighbour detection and crowding studies and this can lead to data being misconstrued (Hess and De Kroon, 2007; Semchenko et al., 2008; Chen et al., 2012). Crowding experiments in this thesis have supported the need to control soil volume in neighbour detection research (Fig. 4.1, 4.6, 4.7, 4.8). Generally, early research into this demonstrated how plants (often unrelated and not accounting for plant size) can recognise neighbouring plants in a generic way with little explanation of how this is done (Mahall and Callaway, 1992). Developing from this, the self/non-self discrimination concept has been reported in several studies where plants have the ability to distinguish their own roots (self-roots) from those of other plants (non-self) in the rhizosphere (Gersani et al., 2001; Falik et al., 2003; Gruntman and Novoplansky, 2004). However, this concept does not shed light on whether these plants are responding to genetic relatedness or simply another plant in their soil volume.

The focus has shifted in recent years with reports of plants having the ability to identify different levels of genetic relatedness and respond with growth changes accordingly (Biedrzycki et al., 2010; Crepy and Casal, 2016; Yang et al., 2018). Cooperative behaviour is often reported between plants which are closely related (close-kin) and as the genetic relatedness increases there is decrease in competitive root growth (Yang et al., 2018). The majority of research in this area has looked at naturally occurring plants with little focus on highly bred, intensely selected crop lines (Yang et al., 2018). Understanding plant-plant detection in an agricultural context could allow for less competition between densely sown crops and instead more cooperative growth with the hope of increasing yields (Chen et al., 2012; Kiers and Denison, 2014; Murphy et al., 2017). Kin recognition research has focused mainly on early plant growth rather than growth and yield outcomes in agricultural settings (Yang et al., 2018). The mechanism behind neighbour detection still remains unclear, with communication methods suggested to involve, mechanical or electrical signals, root exudates or hormones (Depuydt, 2014).

5.5 Strigolactones and plant-plant detection

There is increasing evidence that root exudates play a large role in the communication and detection of neighbouring plants (Biedrzycki et al., 2010; Caffaro et al., 2011; Semchenko et al., 2014). Plants are known to exude a wide range of exudates, including over 100,000 secondary metabolites into the rhizosphere (Dixon, 2001). However, the identity and function of many of these is still largely unknown (Bais et al., 2006) and most identified compounds are simple and small (Badri and Vivanco, 2008; Tsuchiya and McCourt, 2012). Some chemicals found as exudates have growth-inhibiting or toxic effects on neighbouring plants and are referred to as allelochemicals (Inderjit et al., 2011). Suggestions have been made that these allelochemicals, when in low concentrations may act as signalling molecules (Schenk and Seabloom, 2010). The role of exudated (-)-loliolide and jasmonic acid has been suggested to play a role in neighbour recognition (Kong et al., 2018). However, these are small and simple chemicals and so are unlikely to be able to 'encode' enough information to allow plants to detect the relatedness of neighbours.

Strigolactones are phytohormones that regulate shoot development and are known to be exuded into the soil by some plant species where they can act as prerequisite signals for arbuscular mycorrhizal symbiosis (Akiyama et al., 2005). The role of strigolactones in flowering plant neighbour detection has not been explored, but it is highly plausible that this phytohormone could be acting as a neighbour-detection signal that modulates growth.

Prior to this thesis, to my knowledge, no research into the role of strigolactone root exudates in neighbour detection has been carried out in flowering plants. However, previous work in the model organism *Physcomitrella patens* indicates that moss strigolactone exudates act as a signalling factor for moss colony detection. Moss *ccd8* (carotenoid cleavage dioxygenase 8) mutants, have reduced strigolactone synthesis and highly branched filaments compared to wild type (WT) (Proust et al., 2011). Interestingly, the growth of *ccd8* mutant moss in medium surrounded by WT moss colonies resulted in reduced colony diameter and WT filament branching. This suggests a signal is secreted from WT moss which allows complementation of the *ccd8* phenotype. Expressing *CCD8* from pea (*RMS1*) in the moss *ccd8* mutant rescued the phenotype, which suggests the biochemical activity of moss and pea *CCD8* is a conserved trait (Proust et al.,

2011). When a WT colony was surrounded by moss *ccd8* colonies, the side of the *ccd8* colonies nearest the central WT colony showed reduced colony extension and the WT colony showed an increase in diameter. These findings suggest that when the signal involved in recognition of neighbouring colonies reaches a particular threshold, colony extension ceases (Proust et al., 2011). Thereby, this thesis presents a consistent finding in flowering plants that strigolactone can modulate neighbouring plant growth (visualised in Fig. 5.1).

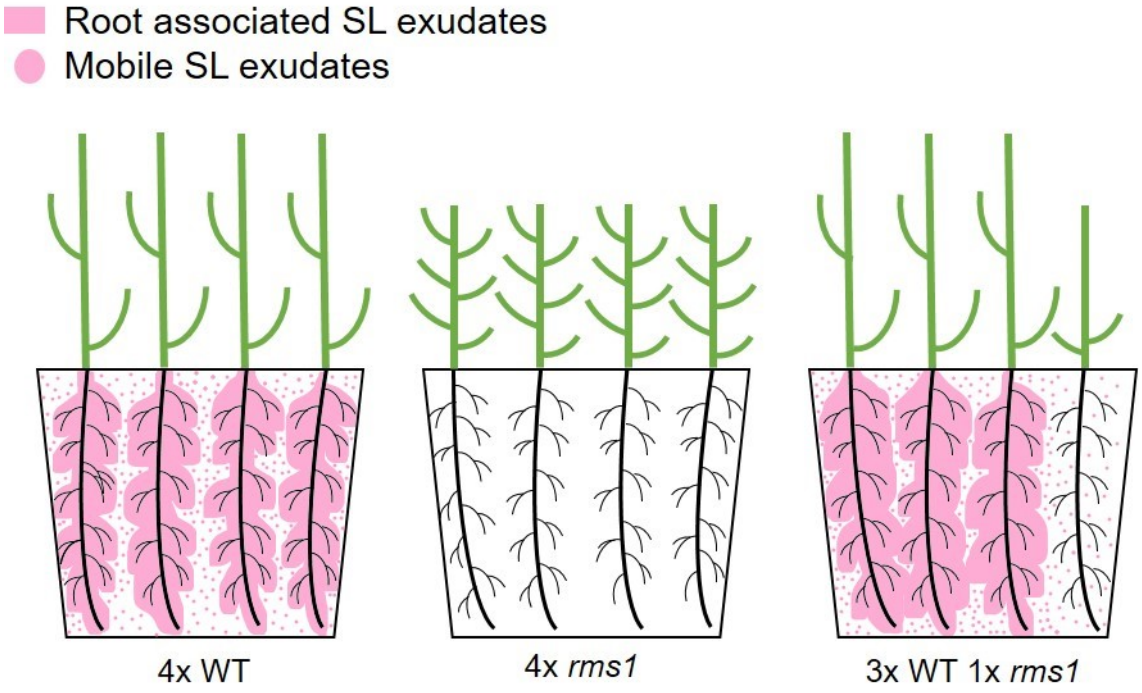


Figure 5.1: Strigolactone exudates are mobile and influence neighbouring plant growth

Figure visualising the findings from 4.6.

Areas close to WT roots have a high strigolactone (SL) concentration (blocks of colour), further away from these areas the concentration of strigolactone is reduced (dots). *rms1* plants are unable to produce strigolactone hence no strigolactone is present in the soil (centre). When WT plants are crowded with *rms1* plants, the concentration of strigolactone around the WT roots is greater than the *rms1* plant (right). Mobile strigolactone exudates, exuded from WT plants (dots), spread through the soil and are recognised by *rms1* plants resulting in shoot modulation in the form of reduced branch number and shoot biomass.

5.6 Crowding in the field

Crowding is a common occurrence in nature and indeed in commercial or agricultural settings. Farmers often sow crops at high densities with the aim of maximising yield within a given space, effectively reducing the soil volume each plant has access to. However, research shows that with increasing sowing

density, yield plateaus and when sowing density is increased beyond a point, yield increases are no longer seen and can even result in yield reductions at too high density (Villalobos et al., 1994). New inbred crop lines have the ability to withstand high-density conditions by maintaining higher yields as a result of more efficient nutrient and resource acquisition (Tollenaar and Wu, 1999). Thus, by understanding the mechanisms of neighbour detection, further progress could be made in breeding crops for high-density sowing.

Furthermore, as space is an expensive commodity in farming, understanding the interplay between fertiliser application and soil volume-limited plants could provide important information when deciding sowing density regimes. For example, soil volume could be one of the key limiting factors for poor fertiliser use seen in UK farming (Dungait et al., 2012). If plants limit their growth based on their available soil volume they may not 'need' the fertiliser that is applied to the field as they are 'cautious' about growing larger. Understanding this interplay could have key implications to farming practices.

5.7 The overall model

Data presented in this thesis strongly suggests that the mechanism plants use to detect their root density is interchangeable with a mechanism by which plants detect neighbouring plants (Fig. 5.2). In crowded conditions, plants did not discriminate between their own roots and the roots of the other plants in the scenarios, instead their shoot growth was affected by the total root density, even when additional nutrients were supplied.

As with other recent reports in this area, the data gathered in this thesis strongly supports the theory that neighbour detection involves root exudates. These exudates therefore may also function in generic root density- sensing, and allow plants to detect their soil volume. Strigolactone is proposed as a key root exudate involved in this root density sensing mechanism.

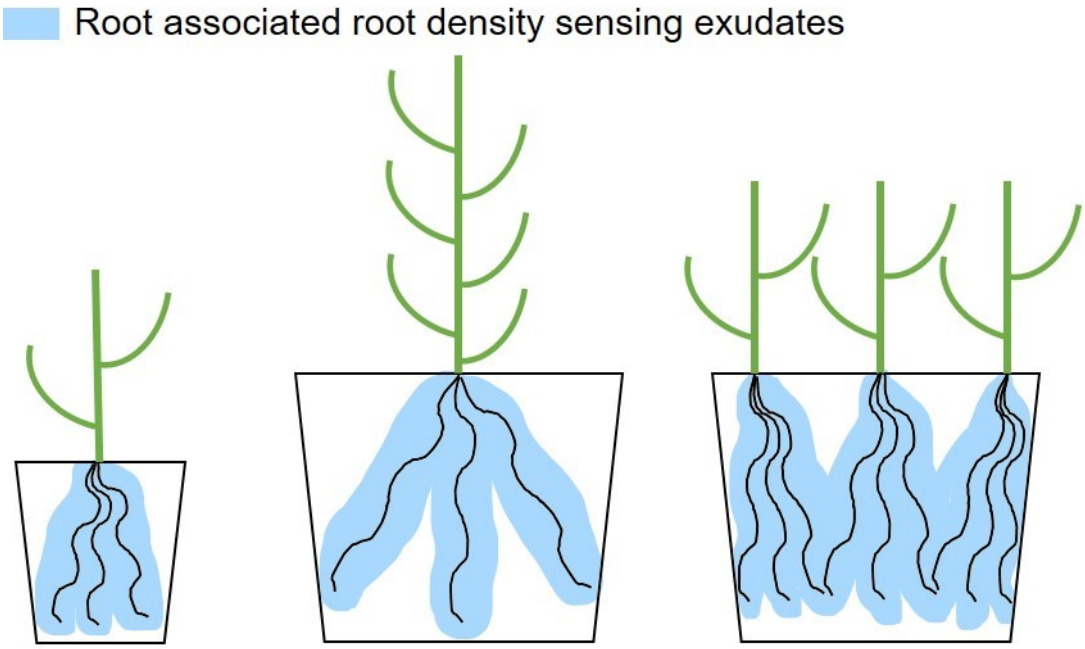


Figure 5.2: Model describing how root density sensing links the soil volume and neighbour detection responses

Early in life, each plant exudes a similar amount of root associated root density sensing exudate into the soil (blue solid colour around the roots). When plants are grown in small soil volumes (left) or in high-density crowded scenarios (right), the density of root exudates rises quickly. This warns plants of future nutrient and water limitations. Larger plants overtime exude larger quantities of the self-recognition signal than those grown in small pots. Before root growth ceases, root to shoot signalling in crowded and small soil volume plants is decreased to reduce branch production and biomass allocation to the shoot before any resource limitation. Plants grown in larger pots (centre) do not reach the threshold level until further on in development, hence produce a greater number of branches and have a higher shoot biomass.

Original figure based on Figure 9 in Wheeldon et al (2019)

Bibliography

Akiyama, K., Matsuzaki, K. and Hayashi, H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*. **435**(7043), pp.824–827.

Arite, T., Umehara, M., Ishikawa, S., Hanada, A., Maekawa, M., Yamaguchi, S. and Kyozuka, J. 2009. D14, a strigolactone-Insensitive mutant of rice, shows an accelerated outgrowth of tillers. *Plant and Cell Physiology*. **50**(8), pp.1416–1424.

Arvidsson, J. 1999. Nutrient uptake and growth of barley as affected by soil compaction. *Plant and Soil.* **208**, pp.9-19.

Awad, A.A., Sato, D., Kusumoto, D., Kamioka, H., Takeuchi, Y. and Yoneyama, K. 2006. Characterization of strigolactones, germination stimulants for the root parasitic plants Striga and Orobanche, produced by maize, millet and sorghum. *Plant Growth Regulation*. **48**(3), pp.221–227.

Badri, D. V. and Vivanco, J.M. 2008. Regulation and function of root exudates. *Plant, Cell & Environment*. **32**(6), pp.666–681.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. and Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*. **57**(1), pp.233–266.

Bar-Tal, A., Feigin, A., Sheinfeld, S., Rosenberg, R., Sternbaum, B., Rylski, I. and Pressman, E. 1995. Root restriction and N-NO3 solution concentration effects on nutrient uptake, transpiration and dry matter production of tomato. *Scientia Horticulturae*. **63**(3–4), pp.195–208.

Bar-Tal, A. and Pressman, E. 1996. Root restriction and potassium and calcium solution concentrations affect dry-matter production, cation uptake, and blossom-end rot in greenhouse tomato. *Journal of the American Society for Horticultural Science*. **121**(4), pp.649–655.

Barken, L.R., BØsrresen, T. and NjØss, A. 1987. Effect of soil compaction by tractor traffic on soil structure, denitrification, and yield of wheat (Triticum aestivum L.). *Journal of Soil Science*. **38**(3), pp.541–552.

Besserer, A., Bécard, G., Jauneau, A., Roux, C. and Séjalon-Delmas, N. 2008. GR24, a synthetic analog of strigolactones, stimulates the mitosis and growth of the arbuscular mycorrhizal fungus Gigaspora rosea by boosting its energy metabolism. *Plant Physiology*. **148**(1), pp.402–413.

Besserer, A., Puech-Pagè, V., Kiefer, P., Gomez-Roldan, V., Jauneau, A., Bastien Roy,

S., Portais, J.-C., Roux, C., Bé Card, G. and Sé Jalon-Delmas, N. 2006. Strigolactones Stimulate Arbuscular Mycorrhizal Fungi by Activating Mitochondria. *PLoS Biology*. **4**(7), pp.1240–1247.

Beveridge, C.A., Dun, E.A. and Rameau, C. 2009. Pea has its tendrils in branching discoveries spanning a century from auxin to strigolactones. *Plant Physiology*. **151**(3), pp.985–990.

Biedrzycki, M.L., Jilany, T.A., Dudley, S.A. and Bais, H.P. 2010. Root exudates mediate kin recognition in plants. *Communicative & integrative biology*. **3**(1), pp.28–35.

Caffaro, M.M., Vivanco, J.M., Gutierrez Boem, F.H. and Rubio, G. 2011. The effect of root exudates on root architecture in Arabidopsis thaliana. *Plant Growth Regulation*. **64**(3), pp.241–249.

Carlson, L.W. and Endean, F. 1976. The effect of rooting volume and container configuration on the early growth of white spruce seedlings. *Canadian Journal of Forest Research*. **6**(2), pp.221–224.

Carmi, A. and Heuer, B. 1981. The role of roots in control of bean shoot growth. *Annals of Botany*. **48**, pp.519–527.

Chen, B.J.W., During, H.J. and Anten, N.P.R. 2012. Detect thy neighbor: Identity recognition at the root level in plants. *Plant Science*. **195**, pp.157–167.

Chen, B.J.W., During, H.J., Vermeulen, P.J., de Kroon, H., Poorter, H. and Anten, N.P.R. 2015. Corrections for rooting volume and plant size reveal negative effects of neighbour presence on root allocation in pea J. Schweitzer, ed. *Functional Ecology*. **29**(11), pp.1383–1391.

Clark, L.J., Whalley, W.R. and Barraclough, P.B. 2003. How do roots penetrate strong soil? *Plant and Soil*. **255**(1), pp.93–104.

Correa, J., Postma, J.A., Watt, M., Wojciechowski, T., Jülich, F. and Straße, W. 2019. Root system architectural plasticity and soil compaction: a review. *Journal of Experimental Botany*.

Crepy, M.A. and Casal, J.J. 2016. Kin recognition by self-referent phenotype matching in plants. *New Phytologist*. **209**(1), pp.15–16.

Depuydt, S. 2014. Arguments for and against self and non-self root recognition in plants. *Frontiers in Plant Science*. **5**.

Dexter, A.R. and Chant, K.Y. 1991. Soil mechanical properties as influenced by

exchangeable cations. *Journal of Soil Science*. **42**(2), pp.219–226.

Dixon, R.A. 2001. Natural products and plant disease resistance. *Nature*. **411**(6839), pp.843–847.

Dungait, J.A.J., Cardenas, L.M., Blackwell, M.S.A., Wu, L., Withers, P.J.A., Chadwick, D.R., Bol, R., Murray, P.J., Macdonald, A.J., Whitmore, A.P. and Goulding, K.W.T. 2012. Advances in the understanding of nutrient dynamics and management in UK agriculture. *Science of The Total Environment*. **434**, pp.39–50.

Falik, O., Reides, P., Gersani, M. and Novoplansky, A. 2005. Root navigation by self inhibition. *Plant, Cell and Environment*. **28**(4), pp.562–569.

Falik, O., Reides, P., Gersani, M. and Novoplansky, A. 2003. Self/Non-Self discrimination in roots. *Journal of Ecology*. **91**, pp.525–531.

Foo, E. and Davies, N.W. 2011. Strigolactones promote nodulation in pea. *Planta*. **234**(5), pp.1073–1081.

Gersani, M., Brown, J. s., O'Brien, E.E., Maina, G.M. and Abramsky, Z. 2001. Tragedy of the commons as a result of root competition. *Journal of Ecology*. **89**(4), pp.660–669.

Gomez-Roldan, V., Fermas, S., Brewer, P.B., Puech-Pagès, V., Dun, E.A., Pillot, J.P., Letisse, F., Matusova, R., Danoun, S., Portais, J.C., Bouwmeester, H., Bécard, G., Beveridge, C.A., Rameau, C. and Rochange, S.F. 2008. Strigolactone inhibition of shoot branching. *Nature*. **455**(7210), pp.189–194.

Goss, M.J. and Russell, R.S. 1980. Effects of mechanical impedance on root growth in Barley (Hordeum vulgare L.). *Journal of Experimental Botany*. **31**(2), pp.577–588.

Gruntman, M. and Novoplansky, A. 2004. Physiologically mediated self/non-self discrimination in roots. *Proceedings of the National Academy of Sciences of the United States of America*. **101**(11), pp.3863–3867.

Håkansson, I., Voorhees, W.B. and Riley, H. 1988. Vehicle and wheel factors influencing soil compaction and crop response in different traffic regimes. *Soil and Tillage Research*. **11**(3–4), pp.239–282.

Hameed, M.A., Reid, J.B. and Rowe, R.N. 1987. Root Confinement and its Effects on the Water Relations, Growth and Assimilate Partitioning of Tomato (Lycopersicon esculentum Mill). *Annals of Botany*. **59**(6), pp.685–692.

Haver, D. and Schuch, U. 2001. Influence of root restriction and ethylene exposure on apicaldominance of petunia (Petunia xhybrida Hort. Vilm.-Andr.). *Plant Growth*

Regulation. **35**(2), pp.187–196.

Hecht, V.L., Temperton, V.M., Nagel, K.A., Rascher, U. and Postma, J.A. 2016. Sowing Density: A Neglected Factor Fundamentally Affecting Root Distribution and Biomass Allocation of Field Grown Spring Barley (Hordeum Vulgare L.). *Frontiers in plant science*. **7**, p.944.

Herold, A. and McNeil, P.H. 1979. Restoration of Photosynthesis in Pot-Bound Tobacco Plants. *Journal of Experimental Botany*. **30**(6), pp.1187–1194.

Hess, L. and De Kroon, H. 2007. Effects of rooting volume and nutrient availability as an alternative explanation for root self/non-self discrimination. *Journal of Ecology*. **95**(2), pp.241–251.

Inderjit, Wardle, D.A., Karban, R. and Callaway, R.M. 2011. The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology & Evolution*. **26**(12), pp.655–662.

Ismail, M.R. and Davies, W.J. 1998. Root restriction affects leaf growth and stomatal response: the role of xylem sap ABA. *Scientia Horticulturae*. **74**(4), pp.257–268.

Ismail, M.R. and Noor, K.M. 1996. Growth, water relations and physiological processes of starfruit (Averrhoa carambola L) plants under root growth restriction. *Scientia Horticulturae*. **66**(1–2), pp.51–58.

Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E. and Schulze, E.D. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia*. **108**(3), pp.389–411.

Kharkina, T.G., Ottosen, C.-O. and Rosenqvist, E. 1999. Effects of root restriction on the growth and physiology of cucumber plants. *Physiologia Plantarum*. **105**(3), pp.434–441.

Kiers, E.T. and Denison, R.F. 2014. Inclusive fitness in agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences*. **369**(1642).

Kozlowski, T.T. 1999. Soil compaction and growth of woody plants. *Scandinavian Journal of Forest Research*. **14**(6), pp.596–619.

Krizek, D.T., Carmi, A., Mirecki, R.M., Snyder, F.W. and Bunce, J.A. 1985. Comparative effects of soil moisture stress and restricted root zone volume on morphogenetic and physiological responses of Soybean [Glycine max (L.) Merr.]. *Journal of Experimental Botany*. **36**(1), pp.25–38.

Liu, A. and Latimer, J.G. 1995. Water relations and abscisic acid levels of watermelon

as affected by rooting volume restriction. *Journal of Experimental Botany*. **46**, pp.1011–1015.

Lynch, J., Läuchli, A. and Epstein, E. 1991. Vegetative Growth of the Common Bean in Response to Phosphorus Nutrition. *Crop Science*. **31**(2), p.380.

Mahall, B.E. and Callaway, R.M. 1992. Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. *Ecology*. **73**(6), pp.2145–2151.

Misyura, M., Guevara, D., Subedi, S., Hudson, D., McNicholas, P.D., Colasanti, J. and Rothstein, S.J. 2014. Nitrogen limitation and high density responses in rice suggest a role for ethylene under high density stress. *BMC Genomics*. **15**(1), p.681.

Murphy, G.P., van Acker, R., Rajcan, I. and Swanton, C.J. 2017. Identity recognition in response to different levels of genetic relatedness in commercial soya bean. *Royal Society Open Science*. **4**(1).

Oldeman, L.R. 1992. *Global Extent of Soil Degradation*.

Peterson, T.A., REINSEL, M.D. and KRIZEK, D.T. 1991. Tomato (Lycopersicon esculentum Mill., cv. 'Better Bush') Plant Response to Root Restriction. *Journal of Experimental Botany*. **42**(10), pp.1241–1249.

Poorter, H., Bühler, J., Van Dusschoten, D., Climent, J. and Postma, J.A. 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*., pp.839–850.

Proust, H., Hoffmann, B., Xie, X., Yoneyama, Kaori, Schaefer, D.G., Yoneyama, Koichi, Nogué, F. and Rameau, C. 2011. Strigolactones regulate protonema branching and act as a quorum sensing-like signal in the moss Physcomitrella patens. *Development (Cambridge, England)*. **138**(8), pp.1531–1539.

Sadras, V.O. and Calviño, P.A. 2001. Quantification of grain yield response to soil depth in Soybean, Maize, Sunflower, and Wheat. *Agronomy Journal*. **93**(3), pp.577–583.

De Saint Germain, A., Clavé, G., Badet-Denisot, M.A., Pillot, J.P., Cornu, D., Le Caer, J.P., Burger, M., Pelissier, F., Retailleau, P., Turnbull, C., Bonhomme, S., Chory, J., Rameau, C. and Boyer, F.D. 2016. An histidine covalent receptor and butenolide complex mediates strigolactone perception. *Nature Chemical Biology*. **12**(10), pp.787–794.

Schenk, H.J. and Seabloom, E.W. 2010. Evolutionary ecology of plant signals and toxins: a conceptual framework. In: Baluška F., Ninkovic V. (eds) Plant communication from an ecological perspective. *In*: *Signaling and Communication in Plants*. Springer, Berlin, Heidelberg, pp.1–19.

Semchenko, M., Saar, S. and Lepik, A. 2014. Plant root exudates mediate neighbour recognition and trigger complex behavioural changes. *New Phytologist*. **204**(3), pp.631–637.

Semchenko, M., Zobel, K., Heinemeyer, A. and Hutchings, M.J. 2008. Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. *New Phytologist*. **179**(4), pp.1162–1170.

Shi, K., Ding, X.-T., Dong, D.-K., Zhou, Y.-H. and Yu, J.-Q. 2008. Root restriction-induced limitation to photosynthesis in tomato (Lycopersicon esculentum Mill.) leaves. *Scientia Horticulturae*. **117**(3), pp.197–202.

Shi, K., Fu, L.-J., Dong, D.-K., Zhou, Y.-H. and Yu, J.-Q. 2008. Decreased energy synthesis is partially compensated by a switch to sucrose synthase pathway of sucrose degradation in restricted root of tomato plants. *Plant Physiology and Biochemistry*. **46**(12), pp.1040–1044.

Shi, K., Hu, W.-H., Dong, D.-K., Zhou, Y.-H. and Yu, J.-Q. 2007. Low O2 supply is involved in the poor growth in root-restricted plants of tomato (Lycopersicon esculentum Mill.). *Environmental and Experimental Botany*. **61**(2), pp.181–189.

Sinclair, T.R. and Horie, T. 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. *Crop Science*. **29**(1), pp.90–98.

Sorefan, K., Booker, J., Haurogné, K., Goussot, M., Bainbridge, K., Foo, E., Chatfield, S., Ward, S., Beveridge, C., Rameau, C. and Leyser, O. 2003. MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. *Genes and Development*. **17**(12), pp.1469–1474.

Ternesi, M., Andrade, A.P., Jorrin, J. and Benlloch, M. 1994. Root-shoot signalling in sunflower plants with confined root systems. *Plant and Soil*. **166**(1), pp.31–36.

Tollenaar, M. and Wu, J. 1999. Yield Improvement in Temperate Maize is Attributable to Greater Stress Tolerance. *Crop Science*. **39**(6), pp.1597–1604.

Tschaplinski, T.J. and Blake, T.J. 1985. Effects of root restriction on growth correlations, water relations and senescence of alder seedlings. *Physiologia Plantarum*. **64**(2), pp.167–176.

Tsuchiya, Y. and McCourt, P. 2012. Strigolactones as small molecule communicators. *Molecular BioSystems*. **8**(2), pp.464–469.

Umehara, M., Hanada, A., Magome, H., Takeda-Kamiya, N. and Yamaguchi, S. 2010. Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice. *Plant and Cell Physiology*. **51**(7), pp.1118–1126.

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., Kyozuka, J. and Yamaguchi, S. 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature*. **455**(7210), pp.195–200.

Villalobos, F.J., Sadras, V.O., Soriano, A. and Fereres, E. 1994. Planting density effects on dry matter partitioning and productivity of sunflower hybrids. *Field Crops Research*. **36**(1), pp.1–11.

Wheeldon, C.D., Walker, C.H., Bennett, T.A. 2019. Root volume restriction causes pro-active modulation of shoot growth to avoid future resource limitation. *Biorxiv*.

Wilson, A.K., Pickett, F.B., Turner, J.C. and Estelle, M. 1990. A dominant mutation in Arabidopsis confers resistance to auxin, ethylene and abscisic acid. *MGG Molecular & General Genetics*. **222**(2–3), pp.377–383.

Yang, X.F., Li, L.L., Xu, Y. and Kong, C.H. 2018. Kin recognition in rice (Oryza sativa) lines. *New Phytologist*. **220**(2), pp.567–578.

Yong, J.W.H., Letham, D.S., Wong, S.C. and Farquhar, G.D. 2010. Effects of root restriction on growth and associated cytokinin levels in cotton (Gossypium hirsutum). *Functional Plant Biology*. **37**(10), p.974.

Young, I.M., Montagu, K., Conroy, J. and Bengough, A.G. 1997. Mechanical impedance of root growth directly reduces leaf elongation rates of cereals. *New Phytologist*. **135**(4), pp.613–619.

Zhu, L., Wang, S., Yang, T., Zhang, C. and Xu, W. 2006. Vine growth and nitrogen metabolism of 'Fujiminori' grapevines in response to root restriction. *Scientia Horticulturae*. **107**(2), pp.143–149.