

Exploring above- and belowground behavioural responses to nutrients and neighbours in wheat (*Triticum aestivum* L.)

by

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ABSTRACT

Taking in environmental information and responding in ways that maximize their ability to thrive and reproduce, plants exhibit behaviour through constant changes in biomass development and spatial distribution. Studies aiming to map this behaviour often use model plants like sunflowers and *Arabidopsis* spp. that may behave differently than high-density crop plants. Recent evidence indicates that plants alter root development and spatial distribution when dealing with the interplay of nutrient density and neighbour presence. This is especially relevant in agriculture where monoculture crops of a single genetic makeup force familial competition, potentially reducing yields through decreased competitive effort. However, there is a gap in the research looking at the prevalence of these behavioural traits within wheat, *Triticum aestivum* L., since most studies emphasize morphological characteristics.

In this study, I explored the above- and belowground behavioural responses of *T. aestivum* to soil fertility, nutrient distribution, and neighbour presence and identity. We conducted a mesocosm experiment on five Canadian Red Spring Wheat varieties: CDC Titanium, Carberry, Glenn, Go Early, and Villian. These cultivars were either grown alone or with a neighbour: either the same cultivar as a kin treatment or in a pairwise combination with another cultivar as a stranger treatment. The pair-wise combinations of cultivars were fully-factorial, and we planted these treatments in heavily or lightly fertilized soil using slow-release 14:14:14 NPK fertilizer. The fertilizer was either homogeneously mixed throughout the pot or placed in a heterogeneous patch equidistant from both plants.

We found that aboveground biomass shifted growth towards shared aerial space in the pots when a neighbouring plant was absent, but there was no impact of neighbour identity, contrary to what we would have expected with kin selection theory. When looking at belowground factors,

the soil nutrient level significantly impacted the shift of biomass, increasing neighbour avoidance when under low nutrient conditions. In addition, homogeneous soil resulted in more growth towards the neighbouring plant. This indicates that belowground environmental changes impact aboveground biomass architecture in *T. aestivum*. We also looked at the impacts of these factors on fitness outcomes as measured through reproductive effort. Interestingly, none of the soil structure elements or social interactions we investigated had any effect.

Last, we evaluated the effects on belowground plant behaviour by looking at the distribution of roots in five locations in the pots, focusing on the areas between the plants and the edge of the pot and the shared soil space in the centre. Our study shows that soil heterogeneity affected root growth throughout the mesocosms, with homogeneous soil resulting in more roots, with one exception. In heterogeneous treatments, the nutrient patch contained significantly more roots, indicating foraging precision. The effects of nutrient level suggest that the central shared soil space was predominantly affected, with more roots in less fertile soil due to increased foraging. Curiously, our study did not reveal any direct effects of neighbour identity, with neighbour presence only affecting the space opposite the nutrient patch interacting with soil heterogeneity. With fewer roots found here in the heterogeneous treatments with a neighbour, plants allocated more roots in the area when they were not facing resource competition in a small space with a nearby neighbour.

Altogether, these findings provide evidence that *T. aestivum* responds to changes in its social and soil environments. However, the impact of these factors varies, and more research is required to determine why the neighbour's presence and identity prompted a lack of results. For example, a highly relevant future study would use intra- and inter-specific competition to determine the genetic distance necessary for a kin/stranger effect within wheat.

PREFACE

This thesis is an original, collaborative work between me, Dr. James Cahill, and Dr. Dean Spaner at the University of Alberta. I was responsible for developing the methods, collecting and processing data, conducting and interpreting statistical analyses, and writing the manuscript. Dr. James Cahill was involved in concept development and manuscript development. Dr. Dean Spaner was responsible for providing guidance and agricultural expertise throughout the experiments and providing seeds for both sets of experiments. We have not yet submitted this data for publication in a peer-reviewed journal.

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1. INTRODUCTION

Though plant and animal biology differ significantly, both have remarkable similarities in overcoming life challenges. Some of the most prevalent challenges include obtaining food and resources, coping with intra- and interspecific neighbours, and fending off pests (Trewavas, 2014; Gagliano, 2015). In both plants and animals, resource capture and social interactions are inherently spatially explicit, with these processes occurring in specific locations on the landscape or in the soil. For many animals, the primary mechanism for foraging and social decisions involves movement. Movement is critical for foundation theories such as the marginal value theorem, which models the foraging of organisms in an environment with patchy resource distribution, and ideal free distribution, which models the dispersal of individuals among those landscapes to minimize resource competition while maximizing fitness. In addition, animals are typically motile creatures, so they influence their interactions with competitors and predators (Bilas *et al.*, 2021). In contrast, plants cannot move about their environment so that they may be stuck in unfavourable conditions, such as next to problematic neighbours, for their entire lifetime.

Compared to animals, we have traditionally seen plants as passive due to their sessile state. They have been thought to simply react to neighbours' indirect effects, including shading and resource depletion (Pierik *et al.*, 2013). However, current research shows that plants constantly take in environmental information and respond in ways that maximize their fitness through alterations in biomass development and spatial distribution (Hodge, 2004; Semchenko *et al.*, 2007; Vicherová *et al.*, 2020). The most common mechanisms behind behavioural decisions in a patchy landscape include differential root production and stem/leaf orientation shifts in response to local conditions. Therefore, behavioural reactions of plants to standard spatially explicit features of landscapes involve both localized movement and changes in overall development and growth.

Although these processes occur both aboveground and belowground, it is unknown whether plants exhibit overall coordination in their responses.

Competition is a prevalent ecological and evolutionary driver (Aschehoug *et al.*, 2016). Consequently, a principal example of biomass manipulation in response to environmental conditions includes changes in behavioural shoot placement in response to neighbours (Li *et al.*, 2014; Takigahira & Yamawo, 2019; Yamawo & Mukai, 2020). The term "crown shyness" has been used to describe the tendency of some species to avoid leaf overlap with intraspecific neighbours (Bilas *et al.*, 2021). This plastic reaction is a dynamic attempt to optimize photosynthesis (Goudie *et al.*, 2009; Uria-Diez & Pommerening, 2017), impacting plant growth and fitness. For example, individuals in dense habitats like crop fields could be victims of density-dependent mortality, but they attempt to prevent this by shifting their crowns strategically (Getzin & Wiegand, 2007; Uria-Diez & Pommerening, 2017).

Studies focusing on root growth show that plants exhibit plasticity in root development and spatial distribution when dealing with the interplay of nutrient density or neighbour presence. Studies illustrating dynamic decision-making processes and subsequent plant responses include earlier work by Brady *et al.*, 1993, McNickle & Cahill Jr, 2009, and VanVuuren *et al.*, 1996 and more recent papers by Anten & Chen, 2021 and Bilas *et al.*, 2021. They indicate that plants shift root biomass towards soil with high nutrient content or away from other roots to avoid neighbours and direct competition. Thus, plants produce notably more roots in high-nutrient soil patches than in low-nutrient soil (Gersani *et al.*, 2001; Semchenko *et al.*, 2007; Cahill & McNickle, 2011). Furthermore, with plants favouring high-yield patches over background soil, many studies note a considerable shift in root placement (Fransen *et al.*, 1999; Wijesinghe *et al.*, 2001).

Kin selection theory ties together the concepts of inclusive fitness and altruism between close relatives, stating that many plant species will demonstrate competitive restraint when interacting and competing locally with other highly related individuals (Hamilton, 1964a,b; Rankin *et al.*, 2007). Many species have developed mechanisms to detect and determine both the neighbours' presence and identity to establish if neighbours are family or foe. These mechanisms involve detecting changes in aboveground environmental conditions, including light quality or shading (Roig-Villanova & Martínez-García, 2016), touch (Anten *et al.*, 2010; Markovic *et al.*, 2016), and volatile organic compounds (Heil & Karban, 2010; Baldwin, 2010; Bilas *et al.*, 2021). Plants will then alter aboveground biomass to facilitate or hinder neighbour development (Bilas *et al.*, 2021).

For example, when looking at reproduction in plants, Donohue's influential 2003 paper showed that some species might have higher reproductive success when grown with siblings than unrelated plants, supporting the kin selection hypothesis. Subsequent studies have corroborated this (Dudley, 2015; Yamawo & Mukai, 2020). However, other studies have illustrated the opposite, showing marked decreases in seed yields when plants are grown with neighbours, especially relatives (Cheplick & Kane, 2004; Milla *et al.*, 2009; Masclaux *et al.*, 2010; Postma *et al.*, 2021). Some studies demonstrate no difference in reproduction (Masclaux *et al.*, 2010).

Studies also state that neighbour presence may only impact seed production when we sow plants at specific densities, with increasing density causing decreases in seed yield (Li *et al.*, 2015). Some research has shown that behavioural responses to varying soil nutrient levels include the plants sacrificing collective yield to steal nutrients from neighbours belowground (Maina *et al.*, 2002; Zhu *et al.*, 2019). These plants are engaging in a tragedy of the commons, where plants will allocate more mass to roots rather than reproduction to ensure maximum nutrient capture at

the expense of their competitors (Gersani *et al.*, 2001; O'Brien *et al.*, 2005; Semchenko *et al.*, 2007). However, studies like Parise *et al.*'s 2021 paper found no evidence for competition under various environmental conditions, supporting the stress-gradient hypothesis. The hypothesis states that facilitation may occur when plants undergo stressful environments together and shows that we cannot generalize the effects of neighbours on reproduction.

Early work on neighbour recognition through plant root systems showed that many plant species could distinguish whether neighbouring roots are from the same plant body or a different one, showing self versus non-self recognition was occurring (Gersani *et al.*, 2001; Holzapfel & Alpert, 2003; Gruntman & Novoplansky, 2004). Subsequent studies looking at root-mediated kin recognition showed shifts in biomass allocation (Dudley & File, 2007; Murphy & Dudley, 2009; Chen *et al.*, 2015; Palmer *et al.*, 2016), lateral root density (Palmer *et al.*, 2016), root branching intensity and specific root length (Semchenko *et al.*, 2014), and resource uptake rate (Zhang *et al.*, 2016; Takigahira & Yamawo, 2019). Additionally, recent studies on wild and domesticated species across traits have shown that plants can distinguish between neighbours based on relatedness, indicating kin/non-kin recognition and selection (Chen *et al.*, 2012; Bilas *et al.*, 2021). This has significant consequences for both plant ecology and evolutionary biology.

Kin selection may affect population structure and diversity since it may lower group genetic diversity (Platt & Bever, 2009), while cooperation between kin plants may lead to better population-level outcomes (Anten & During, 2011; Anten & Chen, 2021). This would lead to significant implications for agricultural crop plants (Denison, 2010; Anten & Vermeulen, 2016; Murphy *et al.*, 2017a). There is evidence for changes in population-level outcomes, particularly grain yields, in crops we cultivate in single-genotype cultures. For example, this would include species like barley (Ninkovic, 2003), rice (Yang *et al.*, 2018), soybean (Murphy *et al.*, 2017a),

sorghum (Zhang *et al.*, 2016), and wheat (Zhu & Zhang, 2013; Fréville *et al.*, 2019). In conventional agriculture, we typically cultivate genetic monocultures in intensive agrosystems, focusing on crop-level yields rather than individual plant outputs. Still, even small changes in the grain output of a single plant would cause massive shifts in the overall production of fields that span hundreds of acres and thousands of plants.

Whether plants coordinate above and belowground behavioural responses to nutrients and neighbours or whether root and shoot systems respond individually to local conditions remains unknown. While some studies have explored the effects of belowground environmental conditions and neighbour presence on aboveground biomass development and distribution (Aphalo *et al.*, 1999; Hautier *et al.*, 2009; Brown *et al.*, 2019), direct connections between changes belowground and their effects aboveground remain unclear.

Studies on plant behaviour in patchy environments have been concerned with changes in a single plant's above- or belowground biomass distribution, leaving significant literature gaps. In addition, practically no studies have examined the combined effects of intraspecific kin recognition, soil fertility, and nutrient placement both above- and belowground. This study is unique because it looks for coordination between shoot and root responses by a non-model crop plant, wheat (*Triticum aestivum* L), which has far-reaching implications for human use. We used a mesocosm experiment on different wheat varieties where we manipulated soil nutrient amounts, soil heterogeneity, and neighbour identity to test whether *T. aestivum* 1) altered the distribution of aboveground biomass, 2) altered fitness outcomes as measured through reproductive effort, and 3) altered the development and distribution of roots throughout the experimental arenas.

2. METHODS

Experimental Design

We used five wheat genotypes, or cultivars, commonly cultivated in North America as our test varieties. We created experimental mesocosms in round plastic pots (15.2 cm diameter) filled with low nutrient soil (Figure 1). Each had a single plant, used as a control, or two plants of the same or different wheat varieties. Using a full-factorial design for the treatments with a neighbour present, we created 15 pairwise combinations. Each pot received one of four soil nutrient treatments (high vs low fertility x heterogeneous vs homogeneous distribution). The soil treatments were replicated five times for each planting treatment, creating five blocks. The pots with two plants had one replicate per block, while singular plants had three replicates per block. Hence, we had a total of 600 experimental arenas [(15 pairwise combinations x 4 soil treatments x 5 blocks) + (5 cultivars x 3 replicates x 4 soil treatments x 5 blocks)] grown on the roof of the University of Alberta Biotron in a randomized design.

Study Species

We used wheat, *Triticum aestivum* L., for this experiment because of its extreme global importance as a food crop. Due to this significance, and its cultivation internationally in various environments, there are hundreds of varieties, or cultivars, readily available. Though many studies have investigated the morphological traits of these numerous cultivars, few have delved into their behaviour despite how essential wheat is for modern agriculture and human existence.

Some prior work indicates that wheat alters root and shoot proliferation in the presence of neighbours (Fréville *et al.*, 2019; Zhu *et al.*, 2019) and resource patches (Hackett, 1972; Bhatt & Derera, 1975; Zhu & Zhang, 2013). However, no studies have explored the effects of both factors simultaneously, making it an ideal choice for this study.

We chose five cultivars of Canada Western Red Spring (CWRS) wheat: Carberry, C.D.C. Titanium, Glenn, Go Early, and Lillian. They have been studied extensively for various morphological traits, especially traits related to disease resistance, including wheat rusts (Randhawa *et al.*, 2013; Chen *et al.*, 2016, 2019), head blight (Hucl, 2016), and common bunt (Spaner, 2017). However, none of these commonly cultivated Canadian varieties have been the specific focus of any behavioural studies. Thus, we attained seeds for all cultivars from the Spaner Research Lab at the University of Alberta in Edmonton, AB.

Soil treatments, neighbour treatments, and plant growth

The soil in all pots consisted of a low nutrient soil mix (3:1 sand to topsoil mixture (Canar Rock Products Ltd.)), which has been used extensively in other root foraging experiments (Karst *et al.*, 2012; Martinková *et al.*, 2018). We added two levels of fertilization (0.551g/L and 4.403g/L) using slow-release 14:14:14 NPK fertilizer to create low and high soil fertility treatments. This fertilizer was either mixed evenly by hand in large batches, making a homogeneous treatment or in a single 1cm diameter patch for the entire depth of the pot for the heterogeneous treatment. The patch was placed equidistant from both plants, creating the heterogeneous treatment. In treatments with two plants, the patches were placed 5.4cm from each seed location while being 3.8 cm from both the centre and edge of the pot (Figure 1). We assigned to each pot five focal soil locations, which we would subsequently sample for root growth. The total nutrients added per pot were identical between the homogenous and heterogeneous treatments within a soil fertility treatment, either high or low. The locations of the soil patches in both the alone and neighbour treatments were also the same for consistency.

Seeds were directly planted in the pots without initial germination and transplantation since a pilot study with the same batch of seeds indicated exceptionally high germination rates. We

placed three seeds at each planting location and then thinned them down to one plant per location within three days, selecting the fastest-growing individual. Treatments comprising of two plants had each planted halfway between the edge and centre of the pot such that each plant was 3.8 cm from both the centre and edge while being 7.6cm from the other plant (Figure 1). We planted the seeds in the same manner for the plants grown alone, with one of the planting locations in the pot remaining empty (Figure 1).

We placed the pots on the roof of the University of Alberta Biotron in Edmonton, Canada, in a completely randomized block design, with blocks arranged randomly, created through Microsoft Excel to account for spatial variation. The plants grew for an average of 43 days, depending on each plant's initial thinning and final harvest (June 7th to July 20th, 2018). Water was provided twice daily throughout the growth period through an oscillating sprinkler system to all pots.

Harvest

Once the wheat had gone to seed and roots became pot-bound, we clipped each plant at the soil surface. We separated the biomass into two categories: growth towards or away from the centre of the pot by marking the initial planting location and collecting biomass on either side of this starting point. We used this separation to quantify aboveground shifts resulting from a neighbour's presence. These two categories were further split into reproductive and non-reproductive biomass for each plant and then dried at 65°C for a minimum of 48 hours and weighed.

We also took five root cores per pot, using a template to ensure consistency (Figure 1). With the nutrient patch at the top of the pot from a top-down perspective, we took core 1 between the left plant left and the pot edge. For both soil fertility treatments, we took core 2 directly where we had inserted the heterogeneous nutrient patch. Next, we placed core 3 in the centre of the pot

and core 4 opposite of core 2. Finally, we took core 5 between the plant on the right and the pot's edge for both alone and neighbour treatments. We also collected all remaining root fragments in the pots. The roots were washed over a 1mm sieve, dried at 65°C for 48 hours, and weighed. Unfortunately, due to a lack of visual differentiation between the roots of the wheat plants, especially those with kin neighbours of the same genotype, cultivar determination and separation were not possible for the cores or root fragments.

Statistical Analysis

We took the natural log of the data, both above- and belowground, to normalize it and ensure homogeneity of variances. Then we used IBM SPSS Statistics 27 to perform statistical analyses including generalized linear mixed models (GLMMs) to assess biomass allocation, fitness metrics, and root proliferation. For each GLMM conducted, we also included all two- and three-way interaction terms between the variables as fixed effects. In addition, we had blocks as random effects for all GLMMs. Since cultivar type in the models created a lot of noise and the cultivars displayed no difference in the behavioural responses, we included them as random effects for some GLMMs. They were coded as dummy variables whenever included. Due to the random effects in the GLMMs, we used two-tailed Satterthwaite's F-tests to determine the significance of fixed effects.

To determine aboveground biomass asymmetry, we calculated the total biomass ratio, including both reproductive and non-reproductive parts, grown towards the centre of the pot over the total biomass produced away. To prevent dividing by zero where there was no biomass growing away from the centre, we added 0.005g to all biomass values. Since the data is log-transformed, zero indicates a neutral response with no distinct growth towards or away from the centre of the experimental arena. A value less than zero represents crown shyness, meaning the wheat is shifting

biomass away from the shared aerial space. Finally, a value greater than zero signifies bold aboveground distribution, with the *T. aestivum* showing increased growth towards the central area. The first GLMM, with aboveground biomass asymmetry as the response variable, had the nutrient level, heterogeneity, neighbour presence as fixed effects (Table 1). The second GLMM retained the same response variable and fixed effects but included cultivars as random effects (Appendix Table 1.1). To determine the impact of neighbour identity on the biomass asymmetry, the third GLMM had the nutrient level, heterogeneity, and neighbour identity (kin neighbour of the same cultivar variety or stranger neighbour of a different cultivar variety) as fixed effects (Table 2). We again included the cultivar as a random effect with plants grown alone removed to look at the direct differences in growth when comparing neighbour type.

We calculated reproductive effort by taking the proportion of biomass containing reproductive structures out of the total aboveground biomass. Of the 900 plants in the experiment, two had extremely low results: 14.6 times smaller than the 3rd smallest number. Therefore, they were removed from further analyses as extreme outliers, leaving 898 plants. Since the data is log-transformed, a zero indicates that 100 percent of the aboveground biomass was reproductive. All the values less than zero represent plants where we designated less than 100 percent of the total biomass as reproductive; the closer the negative value is to zero, the higher the reproductive effort. Finally, values greater than zero are impossible since the wheat cannot allocate more than 100 percent of its aboveground biomass to reproduction. The first GLMM with reproductive effort as the response variable has the nutrient level, heterogeneity, and neighbour presence as fixed effects and cultivar included as random effects (Table 3). The second GLMM looks at the impact of neighbour identity on reproductive effort had the nutrient level, heterogeneity, and neighbour identity as fixed effects with cultivar coded as a random effect (Table 4) and plants grown alone removed.

We took the proportion of roots placed at each core location relative to overall roots produced in the pot to look at root proliferation, creating a pot-level measure. The data is log-transformed, so a zero response indicates that all the belowground biomass was present in that coring location in the pot. All the values less than zero represent plants where less than the total belowground biomass developed in that region. Hence, the closer the negative value is to zero, the greater the proliferation of roots in the area. Values greater than zero are impossible since the wheat cannot develop more than 100 percent of the belowground biomass in a particular spot. Next, we ran GLMMs for each of the five cores, with the first set containing nutrient level, heterogeneity, and neighbour presence as fixed effects (Table 5). In the second GLMMs run, the fixed factors remained the same, with cultivars included as random effects (Appendix Table 1.2). Finally, we made the third set of GLMMs to determine if neighbour identity impacted root proliferation. It had the nutrient level, heterogeneity, and neighbour identity (alone plant, kin neighbour of the same cultivar variety, or stranger neighbour of a different cultivar variety) as fixed effects, including blocks and cultivars as random factors (Table 6).

3. RESULTS

In the first biomass asymmetry GLMM (Table 1), plants that grew in fertile ($df = 1,893$, $F = 12.739$, $p < 0.0001$) or homogeneous soil ($df = 1,893$, $F = 4.668$, $p = 0.031$) or without competition ($df = 1,893$, $F = 12.739$, $p = 0.007$) showed significantly higher levels of growth towards the potential neighbour than away (Figure 2a). None of the interactions between fixed factors impacted the asymmetry. In the second GLMM (Appendix Table 1.1) with cultivars included as random effects, neighbour presence was no longer significant ($df = 1,892$, $F = 0.050$, $p = 0.824$, Figure 2a). Only plants grown with high nutrients ($df = 1,892$, $F = 12.690$, $p < 0.0001$) or homogenous soil ($df = 1,892$, $F = 4.661$, $p = 0.031$) illustrated asymmetry. Finally, with the last GLMM (Table 2), only the nutrient level had a significant impact ($df = 1, 596$, $F = 7.458$, $p = 0.007$, Figure 2b). Hence, plants grown in soil with higher fertility had more considerable growth bias towards the central area.

Nutrient level ($df = 1,893$, $F = 1.976$, $p = 0.160$), heterogeneity ($df = 1,893$, $F = 1.036$, $p = 0.309$), or neighbour presence ($df = 1,893$, $F = 0.000$, $p = 0.993$) did not impact reproductive effort in the first GLMM (Table 3, Figure 3a). Similarly, when investigating the effect of neighbour identity in the second GLMM, there was no effect on reproductive effort ($df = 1,889$, $F = 0.075$, $p = 0.928$, Table 4, Figure 3b).

In the first set of belowground GLMMS (Table 5, Figure 4a), the nutrient level affected root growth in cores 1 ($df = 1,591$, $F = 6.716$, $p = 0.010$), 2 ($df = 1,590$, $F = 6.596$, $p = 0.010$), and 4 ($df = 1,591$, $F = 14.259$, $p < 0.0001$). More roots were found in highly fertile soil for cores 1 and 2, with the opposite being true for core 4. Soil heterogeneity affected all five cores, with more roots present in homogeneous soil for cores 1 ($df = 1,591$, $F = 81.091$, $p < 0.0001$), 3 ($df = 1,591$, $F = 128.38$, $p < 0.0001$), 4 ($df = 1,591$, $F = 188.314$, $p < 0.0001$), and 5 ($df = 1,591$, $F = 133.639$,

$p < 0.0001$). However, patchy soil proved more lucrative for roots in core 2 ($df = 1,591, F = 128.38, p < 0.0001$), where we had added fertilizer. The nutrient level and heterogeneity interaction affected cores 1 ($df = 1,591, F = 4.049, p = 0.045$) and 5 ($df = 1,591, F = 10.270, p = 0.001$). More root biomass was placed there when the soil was highly nutritious and homogeneous. Finally, the interaction of heterogeneity and neighbour presence only affected core 4 ($df = 1,591, F = 5.184, p = 0.023$) with more roots in homogenous soil without a neighbour present.

In the second set of GLMMS (Appendix Table 1.2, Figure 4a), nutrient level affected cores 2 ($df = 1,589, F = 6.210, p = 0.013$), 3 ($df = 1,591, F = 6.232, p = 0.013$), and 4 ($df = 1,590, F = 14.762, p < 0.0001$). More roots were found in the more fertile soil for cores 2 and 3, with core 4 illustrating the opposite. Nutrient heterogeneity impacted all five cores. Hence, cores 1 ($df = 1,591, F = 130.137, p < 0.0001$), 2 ($df = 1,589, F = 633.345, p < 0.0001$), 3 ($df = 1,591, F = 83.185, p < 0.0001$), 4 ($df = 1,590, F = 188.999, p < 0.0001$), and 5 ($df = 1,591, F = 120.774, p < 0.0001$) showed the same trends as the first set of belowground GLMMs (Table 5, Figure 4a). However, the interaction of nutrient level and heterogeneity affected only core 5 ($df = 1,591, F = 8.844, p = 0.003$), with more root biomass placed in highly nutritious, homogeneous soil. Lastly, only core 4 was impacted by the interaction of heterogeneity and neighbour presence ($df = 1,590, F = 5.194, p = 0.023$), with more roots were found in homogenous soil without a neighbour present.

With the final set of GLMMs (Table 6, Figure 4b), the nutrient level only impacted core 4 ($df = 1,587, F = 12.064, p = 0.001$), with roots more prominent in low nutrient soil. Soil heterogeneity influenced all five cores again. The proportion of roots at cores 1 ($df = 1,587, F = 68.564, p < 0.0001$), 2 ($df = 1,586, F = 503.422, p < 0.0001$), 3 ($df = 1,587, F = 113.383, p < 0.0001$), 4 ($df = 1,587, F = 138.834, p < 0.0001$), and 5 ($df = 1,587, F = 108.144, p < 0.0001$) were

the same as the first two sets of GLMMs (Table 5 and Appendix Table 1.2, Figure 4a). Finally, the interaction of nutrient level and heterogeneity only affected core 5 ($df = 1,587$, $F = 10.959$, $p = 0.001$), with greater root biomass located in fertile, homogeneously mixed soil.

4. DISCUSSION

Our results indicate significant differences in wheat's above- and belowground behavioural responses to neighbours and nutrients when looking across all investigated cultivars. When looking at the changes in overall aboveground biomass distribution, it is apparent that soil fertility, nutrient level, and neighbour presence have a significant impact (Figure 2a). However, neighbour identity did not matter (Figure 2b). Also, the reproductive effort had drastically different results, as our findings indicate that none of the fixed effects had any significant impact (Figure 3). Finally, the belowground biomass was affected by the nutrient level, soil heterogeneity, and neighbour presence and identity throughout the pot. Shared and fertilized areas showed different responses than areas closer to a single plant (Figure 4).

Aboveground

Aboveground, our results show that the wheat plants increased growth towards the centre of the pot when a neighbouring plant was absent (Figure 2). Some studies have suggested that plants may over-proliferate shoots when grown with a neighbour (Gersani *et al.*, 2001). Over-proliferation would prove wasteful, however, if the neighbour similarly responds. Both plants would increase resource allocation to growth, effectively cancelling out a potential net gain in resource capture, or they would create a tragedy of the commons, collectively exhausting the resources (Novoplansky, 2009; Smyeka & Herben, 2017). To determine if a neighbour is present, many plant species interpret environmental cues such as shading, volatile organic compounds, touch, and root exudates (Bilas *et al.*, 2021). In our study, the lack of these above- and belowground signals indicating a neighbour would allow the plant to spread out and maximize photosynthesis without fear of competition. This unconstrained growth due to a lack of neighbours enables solitary plants to organize their development solely concerning resource availability, modelling Ideal Free Distribution (IFD) (Bilas *et al.*, 2021).

A review of the literature suggests that belowground kin recognition may result in less competitive growth aboveground. *Impatiens pallida*, an understory species, developed more belowground biomass in the presence of kin compared to stranger groups (Murphy & Dudley, 2009). As a result, the *I. pallida* had smaller shoots correlating to reduced aboveground competition. These plants also built more branches, likely to minimize mutual shading. *Fagus crenata* seedlings also seemingly reduced competition for light with the production of leaves with reduced chlorophyll when facing the same social conditions when these plants had root interactions with a relative rather than with a stranger (Takigahira & Yamawo, 2019).

Our study, however, did not show any impact of neighbour identity on the distribution of aboveground biomass (Figure 2b). This is because wheat may consider all the neighbours related rather than segregating them into stranger and kin categories. If the 'stranger' neighbours are not genetically distant enough, the wheat may not classify them as non-kin. Hence, there would not be a significant effect of neighbour identity, which we observe for all our aboveground and belowground measures. This lack of ability could be attributed to similar genetic backgrounds and close genetic relatedness within the crop (Murphy *et al.*, 2017a; Yang *et al.*, 2018)).

When looking at shifts in aboveground biomass, heterogeneity of the soil also had a significant impact (Figure 2). Homogeneous soil resulted in more growth towards shared aerial space and the potential neighbouring plant due to a potentially decreased need for expansive root proliferation belowground. The lack of patches in the soil would allow for relatively uniform root distribution without extensive foraging. Plants could then maximize aboveground resource capture, both with and without a neighbour present, by decreasing energy expended in root

development. There is a marked lack of literature directly looking at the effects of heterogeneous versus homogenous soil on aboveground biomass development, so there is a possibility that other significant factors are at play here. However, a study looking at the responses of native Albertan grassland species to size-asymmetric competition indicates that aboveground architecture changes with belowground changes to the environment (Brown *et al.*, 2019). This study considered nutrient heterogeneity and soil fertility variation and found that nutrient heterogeneity, namely high nutrient patches, tended to reduce competitive size asymmetry compared to homogeneous soil. Conversely, low nutrient patches increased competitive size asymmetry relative to the homogeneous treatments.

In our study, the soil nutrient level significantly impacted the shift of biomass towards the shared area in the pot, increasing neighbour avoidance if the plants are under low nutrient conditions (Figure 2). The plants may be following IFD when under low nutrient conditions, minimizing overlap of shared aerial space when belowground resources are already limited, but abandoning IFD when belowground resources are high enough for them to compete aggressively. The decrease in shading at low nutrient levels could be due to a decreased importance of chlorophyll production when plants must conserve nutrients due to limited availability. A greenhouse pot experiment conducted on wheat showed that decreasing fertilizer reduced chlorophyll content in the leaves (Shah *et al.*, 2017). This nutrient conservation would also matter when the plant assesses trade-offs between allocating resources into shoot and root development. With a greater need to place roots belowground due to an enhanced necessity for foraging and structural integrity considering the competition, plants would be far less free-handed with their allocation of resources towards aboveground competition.

The effect of different soil nutrient levels on aboveground development is also seen elsewhere in the literature. For example, a study found that increasing homogeneous soil fertility under homogeneous conditions decreased aboveground size-asymmetric competition (Brown *et al.*, 2019). These results, however, were contrary to expectations. Increasing nutrient levels in the soil can inadvertently increase size-asymmetric competition by prompting shoot growth, effectively altering the competition to aboveground from belowground (Cahill, 1999; Hautier *et al.*, 2009; Lamb *et al.*, 2009), which is more akin to what we observed in our study (Figure 2).

Beyond the statistical results, visual inspection of the graphs (Figure 2) indicates that the interaction between neighbour presence and nutrient level could influence aboveground biomass placement, even if it is insignificant in the models made. The statistically negligible results could be due to low power, or the power could be high enough and is not significant. Further studies investigating this interplay of conditions could shed light on this. Regardless, it is apparent from our research that aboveground architecture changes in direct response to alterations in belowground environmental conditions.

Reproduction

When looking at the reproductive effort, we found that none of the soil structure elements or social interactions we investigated, including soil fertility, nutrient homogeneity, neighbour presence, or neighbour identity, had any impact (Figure 3). This could be due to a variety of reasons. For example, Li *et al.*'s 2015 paper studying the effects of plant density on maize found that yield was stable across a wide range of plant densities (Tollenaar, 1992; Echarte & Andrade, 2003). However, output linearly declined when the plant density, ranging from 2 to 13 plants per meter, was above an optimum level set by the species (Tollenaar, 1992). This trend could be true

for crop plants other than maize since wheat displayed consistent reproductive effort across plants grown singularly or in pairs in our experiment.

Over time, the extensive breeding of wheat may be causing this lack of impact that soil structure and social interactions had on the reproductive effort. Crop plants are often bred intentionally and unintentionally for traits that emphasize group fitness over individual performance (Donald, 1968; Denison, 2010; Weiner *et al.*, 2010; Anten & Vermeulen, 2016; Anten & Chen, 2021). Hence, past selection for inclusive fitness may have favoured constitutively more cooperative plant genotypes with traits like shorter stems, erect leaves, and restrained roots (Kiers & Denison, 2014). This artificial selection is akin to the selection occurring in natural environments where dispersal is predominantly local. In these environments, when the negative consequences of competition between kin, including clones, full-siblings, and half-siblings, are minimized, overall fitness is improved (Cheplick & Kane, 2004). So, the ability for crop species to recognize kin may increase yield by reducing competitive effects (Chen *et al.*, 2012; Kiers & Denison, 2014; Murphy *et al.*, 2017a; Yang *et al.*, 2018). Accordingly, in a study on rice, the data showed that cultivars in mixed cultures with kin recognition increased grain yields, but interestingly, not all the cultivars possessed this ability (Yang *et al.*, 2018)

However, in another study, eight wild species were tested for resource allocation to reproduction considering neighbour presence (Lepik *et al.*, 2012). Only one clonal species, *Trifolium repens*, had increased allocation when crowded by kin but not strangers. Furthermore, numerous studies have shown that cooperation based on kin may decrease the prevalence of competitive traits (Cahill *et al.*, 2010; Bhatt *et al.*, 2011; Fang *et al.*, 2013). This would also allow for optimized above- and belowground resource capture (Lepik *et al.*, 2012) and subsequent increases in overall fitness (Donohue, 2003; Biernaskie, 2011; Torices *et al.*, 2018).

However, Kiers & Denison (2014) refute the emphasis on group fitness by crop plants. They state that high genetic relatedness, particularly siblings or clones, does not necessarily select for cooperation. This indicates that single-genotype crop fields would not necessarily mean more significant reproductive effort or yield (Taylor, 1992; Kiers & Denison, 2014). Milla et al.'s 2009 study supports this since *Lupinus angustifolius* plants made significantly more flowers and seeds when grown with unrelated neighbours than siblings.

These differences in kinship effects could be because of specific biotic or abiotic environments (Goodnight, 1985; Masclaux *et al.*, 2010). For example, in a 1984 field study by Antonovics & Ellstrand, *Anthoxanthum odoratum*, a sweet pasture grass, had higher fitness when grown with siblings than unrelated neighbours. However, another study showed that the same species, *A. odoratum*, survived post-aphid attacks better when growing with unrelated neighbours than siblings (Schmitt & Antonovics, 1986). This indicates that different environmental stressors may elicit various behaviours from the plants.

Thus, though many studies illustrate that the genotype of neighbours influences growth and fitness, our study does not indicate this for any above- or belowground measure we examined (Figure 3). Hence, the effect of the relatedness between neighbours is difficult to predict, especially when it comes to crop species, and we lack conclusive evidence of the impacts of kin recognition.

Belowground

When looking at belowground plant behaviour, our study indicates that soil heterogeneity affected root growth at each of the five focal locations in the pot across all the GLMMs (Figure 4). However, the effects of nutrient level were more sporadic across the coring locations, and

neighbour presence only affected belowground growth when interacting with soil heterogeneity (Figure 4).

We saw more roots in all locations in the pots when the soil was homogeneous instead of heterogenous, except for where we placed the nutrient patch in the heterogeneous treatments (Figure 4). There were more roots present in the heterogenous pots than the homogenous ones in the nutrient patch location. Since the plants decreased root growth in all the core locations without a nutrient patch, they likely reallocated this energy to grow more roots in the patch of highly nutritive soil. Hence, we observed a cascading effect throughout the pot. Our plants did not keep consistent root growth throughout the pot across the homogenous and heterogenous soil treatments. They just increased root proliferation in the nutrient patch in the heterogeneous treatment. Instead, they decreased the number of roots throughout the pot to use that energy for maximum foraging in the nutrient patch.

A substantial body of evidence supports our results, illustrating the significant effect of soil heterogeneity. Many different plants species use environmental information about resources and competition to optimize root foraging behaviour (Mahall & Callaway, 1991a; Schenk, 2006; Cahill & McNickle, 2011; Belter & Cahill, 2015; Ljubotina & Cahill, 2019). For example, an older study by Jennings & DeJesus in 1968 also demonstrates this root foraging behaviour. *Achillea millefolium* roots exhibited foraging precision when they found high-nutrient patches and aggregated roots within them rather than continuing to investigate the soil. This could be explained by plants trading off between maximizing resource intake from the high-quality soil patch or prolonged exploration.

Bilas et al.'s 2021 study looking at sorghum, another crop species, investigated the impact of the interaction between nutrient availability and the identity of neighbouring plants. In this

study, only when they subjected plants to low nutrient availability did they reduce root activity and nutrient uptake when next to a sibling neighbour than a non-sibling (Li & Xu, 2018). Likewise, in a 2020 study by Pezzola et al., low nutrient availability triggered cultivars experiencing competition with the same genotype to produce more seeds than those competing with different cultivars. Finally, a study looking at *A. thaliana* seedlings found that plants paired with strangers had noticeably more lateral root development than those paired with a sibling or more distant relative only when nutrient availability was considerably lower than the standard solution (Palmer *et al.*, 2016).

We observed that the interaction between nutrient heterogeneity in the soil and neighbour absence or presence only significantly impacted the roots found in core 4, the space opposite the nutrient patch in the heterogeneous treatments. With more roots found in homogenous soil without a neighbour present (Figure 4), plants allocated more roots in the area opposite the nutrient patch when they were not facing competition for resources in a limited space with a proximate neighbour. This ties into our earlier findings on the effects of nutrient heterogeneity in the soil. When dealing with a low nutrient environment and a singular high nutrient patch equidistant to a neighbour, the plant will expend energy in maximizing resource capture, engaging in a tragedy of the commons. When the plants are free of these restraints and are alone in homogenous soil, they are free to explore the entirety of the soil. Opposite to this, plants grown with neighbours are less prone to exploring the soil thoroughly. They may be partly discouraged through the presence of physical and chemical indicators of neighbour presence (Ljubotina & Cahill, 2019; Bilas *et al.*, 2021).

Studies have observed plants making decisions belowground based on detecting other plant roots and the depletion of nutrients near these roots (Falik *et al.*, 2005; Semchenko *et al.*, 2008). They have been shown to respond to close kin by reducing root growth and maintaining spatial

segregation (Biedrzycki & Bais, 2010; Fang *et al.*, 2013; Semchenko *et al.*, 2014). For example, pea plants have shown increased root growth towards non-self roots, which likely reduces over-exploration of the soil and prevents careless root development (Falik *et al.*, 2003). However, soya bean plants have notably more roots in pots shared with a neighbour (Gersani *et al.*, 2001; Kiers & Denison, 2014). Our study, however, does not indicate any direct effects of neighbour presence or identity on belowground biomass placement (Figure 4). Though this could be due to several reasons, it is likely that wheat either cannot recognize kin or has been bred to disregard familial connections when placing roots belowground.

The lack of over-proliferation in the presence of neighbours, especially strangers, has been seen in some studies before (Lankinen, 2008; Markham & Halwas, 2011; Meier *et al.*, 2013). In addition, plants have been observed disregarding neighbour presence and only using nutrient availability to determine root proliferation (Nord *et al.*, 2011; McNickle & Brown, 2014). So, while our results do not fully support previous studies illustrating that plants may base belowground foraging decisions on social information (Cahill *et al.*, 2010; Mommer *et al.*, 2012; McNickle *et al.*, 2016; Ljubotina & Cahill, 2019), there is a body of literature that lends credence to our findings.

The lack of response to neighbours belowground could also be due to the spatial restraints placed on the wheat roots. Closer to harvest, we observed the roots starting to grow out of the bottom of the pots, indicating that they were becoming pot-bound. Though we still observed effects for some of the factors we manipulated belowground, some confounding effects could be present when looking at the impact of neighbour presence alone. This could also be affecting the influence of the interaction of neighbour presence or identity and nutrient level on root placement. The interaction term was not significant for any root locations, even though nutrient level alone was

highly influential on root placement.

Conversely, the interaction of nutrient level and soil heterogeneity was impactful to root growth, but only in core locations 1 and 5, so between the plants and the edge of the pots (Figure 4). More root biomass was placed in these locations when the soil was highly nutritious and homogeneous. This finding indicates that the plants more readily utilized the soil they had first access to when not facing direct competition for a patch equally accessible to both plants, and the soil was fertile. This foraging strategy would reduce the need to search the rest of the pot extensively for a potential nutrient patch or higher quality soil. Plants experience a trade-off between exploration of the environment and exploitation of resources (Semchenko *et al.*, 2008; Peng *et al.*, 2012), which is comparable to animals as they move and forage across landscapes (Stephens, 1987; Nimmo *et al.*, 2019). Thus, they may invest more energy in pre-empting resources within a high-nutrient patch they have direct access to by increasing root growth (Gersani *et al.*, 2001; Ljubotina & Cahill, 2019).

When looking at the impact of just nutrient level on the belowground biomass distribution, the results are more sporadic across the GLMMs. However, various combinations of the central shared soil space, including cores 2, 3, and 4, were impacted, with core 4, the space opposite to the nutrient patch in the heterogeneous treatments, consistently affected (Figure 4). More roots were found in these locations when the overall amount of nutrients in the pot were lower. This result indicates that the plants were more prone to exploring the soil space when in need of nutrients and not readily finding them nearby.

5. CONCLUSION

Overall, our results showed different responses aboveground, both with general biomass placement and reproductive effort, and belowground with root placement, when we alter soil fertility, nutrient placement, neighbour presence, and neighbour identity. However, what remained consistent was that there were no discernable effects of cultivar type on the different metrics.

Aboveground, we found that nutrient level, heterogeneity, and neighbour presence significantly impacted biomass distribution. There is a clear indication that belowground changes in the context affected aboveground shifts in placement. With the nutrient levels, the plants exhibited an increase in neighbour avoidance when experiencing low nutrient conditions and greater boldness when the soil was highly fertile. In addition, homogeneous soil also resulted in more growth towards shared aerial space and the potential neighbouring plant. Regarding neighbour presence, the wheat shifted aboveground biomass away from neighbours. Finally, our study did not show an impact of neighbour identity on aboveground biomass.

The reproductive effort was not affected by any of the manipulations we did to the pots. Furthermore, neither soil nor social treatment affected the reproductive effort, indicating that it remained stable across various conditions.

Finally, belowground, nutrient level, heterogeneity, and neighbour presence had varying effects throughout the pot. The results indicate that the nutrient level predominantly affected central, shared soil space, especially the area opposite the nutrient patch in the heterogeneous treatments. These locations had more roots in less fertile soil. When looking at the effects of heterogeneity, we saw more roots in the whole pot when the soil was homogeneous, except for where we placed the nutrient patch in the heterogeneous treatments, where the opposite was true. However, our study did not indicate any direct effects of neighbour presence or identity on

belowground biomass placement. We did observe a significant interaction between nutrient heterogeneity in the soil and neighbour presence, but only in the area across from the heterogeneous treatments' nutrient patch. Here, pots with homogeneous soil and no neighbour had the highest number of roots. Finally, the interaction of nutrient level and soil heterogeneity was impactful to root growth, but only between the plants and the edge of the pots, where we found more roots when the soil was highly fertile and homogeneous.

An interesting finding is that neighbour identity had no impact either above- or belowground such that *Triticum aestivum* did not exhibit any kin/non-kin discrimination. However, there is heavy evidence for this behaviour across other plant species. This evidence mainly comes from studies focussing on root interactions, though the phenomenon is also applicable to shoot systems (Murphy & Dudley, 2009; Karban *et al.*, 2013; Crepy & Casal, 2016). This ties into another fascinating observation: there were no effects of neighbour presence belowground, but distinct responses were evident in the aboveground biomass placement.

Our study did not dive into how the wheat plants determined neighbour presence and location to alter aboveground behaviour. However, there is a plethora of evidence stating the relevance of chemical cues to above- and belowground neighbour detection (Mahall & Callaway, 1991b; Falik *et al.*, 2005; Semchenko *et al.*, 2014; Biedrzycki *et al.*, 2017). A potential cue at play here could be nutritive (O'Brien & Brown, 2008). The wheat plants may be determining nutrient distributions across the soil, along with any changes in the landscapes like pot boundaries and neighbour roots, to determine where to proliferate (Gersani & Sachs, 1992; Fang *et al.*, 2013). In some studies, plant responses to social and soil contexts occurred early in the growth period (Ljubotina & Cahill, 2019). So, a potential avenue of future research could include

harvesting and analyzing above- and belowground biomass metrics in shorter intervals to establish possible trends along a timescale. This study could also combat the issue of pot-bound roots.

Crop systems are drastically different from most natural biological systems, specifically due to their organization in closely planted monoculture fields to promote maximum yield from minimum space on the landscape. The dynamics of plant-plant interactions in these synthetically constructed plant communities have been prone to drastic change through intentional and unintentional artificial selection, fundamentally altering crop species over time. For example, inadvertent selection for traits was not uncommon during the green revolution, especially in cereal crops like wheat (Khush, 1999; Anten & Chen, 2021). As Anten & Chen, 2021, state, another avenue of exploration could involve looking at past crop selection and the extent to which plants select for or against kin. Through comparison of wild ancestors and domesticated cultivars, future studies could apply an evolutionary lens.

Additionally, most research conducted on the effect of kin/non-kin on spatial distribution and fitness has been genetically limited. Most studies look at half to full siblings versus strangers without quantification or scale of genetic relatedness. Further testing would be needed to fill this gap in the literature and determine the genetic distance required for a kin/stranger effect within wheat. Perhaps future studies should look at wheat, or other crop species, responses in the presence of intra- and inter-specific neighbours with increasing genetic distance. These studies would be especially pertinent since kin selection can lead to better group fitness outcomes, directly tied to increases in grain production (Kiers & Denison, 2014; Zhang *et al.*, 2016; Murphy *et al.*, 2017b; Yang *et al.*, 2018). This has important implications for ecosystem functioning, and in turn, agriculture. Its potential use in crop breeding should be emphasized. There appears to be untapped potential for improving community resource-use efficiency via enhancing cooperation among crop

individuals. We need to understand the underlying mechanisms better if we are to effectively apply this and enhance crop performance.

6. TABLES AND FIGURES

Table 1. A generalized linear mixed model testing the effect of soil fertility, nutrient distribution, and neighbour presence on the ratio of the total aboveground biomass grown towards the neighbouring plant versus away in wheat, *Triticum aestivum*. Fixed effects included nutrient level (high or low nutrients), heterogeneity (homogenous or heterogenous patch), neighbour absence or presence, and their interactions. We included blocks as a random effect.

source	biomass asymmetry	
	F-value	p-value
nutrient level	12.739	0.000
heterogeneity	4.668	0.031
neighbour presence	7.370	0.007
nutrient level x heterogeneity	0.688	0.407
nutrient level x neighbour presence	0.058	0.809
heterogeneity x neighbour presence	1.004	0.316
nutrient level x heterogeneity x neighbour presence	0.028	0.867

Table 2. A general linear mixed model testing the effect of soil fertility, nutrient distribution, and neighbour identity on the ratio of the total aboveground biomass grown towards the neighbouring plant versus away in wheat, *Triticum aestivum*. Fixed effects included nutrient level (high or low nutrients), heterogeneity (homogenous or heterogenous patch), neighbour identity (kin or stranger), and their interactions. We included blocks and cultivars as random effects.

source	biomass asymmetry		
	d.f	F-value	p-value
nutrient level	1, 596	7.458	0.007
heterogeneity	1, 596	0.150	0.698
neighbour identity	1, 596	1.881	0.171
nutrient level x heterogeneity	1, 596	0.256	0.613
nutrient level x neighbour identity	1, 596	0.241	0.624
heterogeneity x neighbour identity	1, 596	1.927	0.166
nutrient level x heterogeneity x neighbour identity	1, 596	0.645	0.422

Table 3. A general linear mixed model testing the effect of soil fertility, nutrient distribution, and neighbour presence on reproductive effort, as measured through the proportion of aboveground biomass comprised of reproductive structures. Fixed effects included nutrient level (high or low nutrients), heterogeneity (homogenous or heterogenous patch), neighbour absence or presence, and their interactions. We included blocks and cultivars as random effects.

source	reproductive effort		
	d.f	F-value	p-value
nutrient level	1, 893	1.976	0.160
heterogeneity	1, 893	1.036	0.309
neighbour presence	1, 893	0.000	0.993
nutrient level x heterogeneity	1, 893	0.023	0.880
nutrient level x neighbour presence	1, 893	0.163	0.686
heterogeneity x neighbour presence	1, 893	0.793	0.373
nutrient level x heterogeneity x neighbour presence	1, 893	0.002	0.963

Table 4. A general linear mixed model testing the effect of soil fertility, nutrient distribution, and neighbour identity on reproductive effort, as measured through the proportion of aboveground biomass comprised of reproductive structures. Fixed effects included nutrient level (high or low nutrients), heterogeneity (homogenous or heterogenous patch), neighbour identity (kin or stranger), and their interactions. We included blocks and cultivars as random effects.

source	reproductive effort		
	d.f	F-value	p-value
nutrient level	1, 889	1.988	0.159
heterogeneity	1, 889	0.809	0.369
neighbour identity	2, 889	0.075	0.928
nutrient level x heterogeneity	1, 889	0.041	0.840
nutrient level x neighbour identity	2, 889	0.000	0.818
heterogeneity x neighbour identity	2, 889	1.763	0.172
nutrient level x heterogeneity x neighbour identity	2, 889	0.006	0.994

Table 5. A general linear mixed model testing the effect of soil fertility, nutrient distribution, and neighbour presence on the proportion of total roots grown at each core location (1, 2, 3, 4, and 5). Fixed effects included nutrient level (high or low nutrients), heterogeneity (homogenous or heterogenous patch), neighbour absence or presence, and their interactions. We had blocks as a random effect.

source	core 1 proportion			core 2 proportion			core 3 proportion		
	d.f	F-value	p-value	d.f	F-value	p-value	d.f	F-value	p-value
nutrient level	1, 591	6.716	0.010	1, 590	6.596	0.010	1, 591	0.134	0.715
heterogeneity	1, 591	81.091	0.000	1, 590	631.021	0.000	1, 591	128.380	0.000
neighbour presence	1, 591	2.480	0.116	1, 590	0.127	0.721	1, 591	0.001	0.976
nutrient level x heterogeneity	1, 591	4.049	0.045	1, 590	0.462	0.497	1, 591	1.253	0.263
nutrient level x neighbour presence	1, 591	1.443	0.230	1, 590	0.078	0.780	1, 591	0.014	0.907
heterogeneity x neighbour presence	1, 591	0.008	0.929	1, 590	0.429	0.513	1, 591	1.664	0.198
nutrient level x heterogeneity x neighbour presence	1, 591	0.294	0.588	1, 590	0.530	0.467	1, 591	0.155	0.694

source	core 4 proportion			core 5 proportion		
	d.f	F-value	p-value	d.f	F-value	p-value
nutrient level	1, 591	14.259	0.000	1, 591	0.420	0.517
heterogeneity	1, 591	188.314	0.000	1, 591	133.639	0.000
neighbour presence	1, 591	0.573	0.449	1, 591	0.842	0.359
nutrient level x heterogeneity	1, 591	2.160	0.142	1, 591	10.270	0.001
nutrient level x neighbour presence	1, 591	0.003	0.953	1, 591	0.011	0.918
heterogeneity x neighbour presence	1, 591	5.184	0.023	1, 591	1.223	0.269
nutrient level x heterogeneity x neighbour presence	1, 591	0.044	0.835	1, 591	0.447	0.504

Table 6. A general linear mixed model testing the effect of soil fertility, nutrient distribution, and neighbour identity on the proportion of total roots grown at each core location (1, 2, 3, 4, and 5). Fixed effects included nutrient level (high or low nutrients), heterogeneity (homogenous or heterogenous patch), neighbour identity (kin or stranger), and their interactions. We included blocks and cultivars as random effects.

source	core 1 proportion			core 2 proportion			core 3 proportion		
	d.f	F-value	p-value	d.f	F-value	p-value	d.f	F-value	p-value
nutrient level	1, 587	2.488	0.115	1, 586	3.410	0.065	1, 587	0.607	0.436
heterogeneity	1, 587	68.564	0.000	1, 586	506.551	0.000	1, 587	115.604	0.000
neighbour identity	2, 587	1.248	0.288	2, 586	0.710	0.492	2, 587	0.005	0.995
nutrient level x heterogeneity	1, 587	1.735	0.188	1, 586	0.051	0.821	1, 587	3.078	0.080
nutrient level x neighbour identity	2, 587	1.660	0.191	2, 586	0.659	0.518	2, 587	0.643	0.526
heterogeneity x neighbour identity	2, 587	0.137	0.872	2, 586	0.434	0.648	2, 587	0.904	0.405
nutrient level x heterogeneity x neighbour identity	2, 587	0.740	0.478	2, 586	0.397	0.673	2, 587	1.584	0.206

source	core 4 proportion			core 5 proportion		
	d.f	F-value	p-value	d.f	F-value	p-value
nutrient level	1, 587	12.540	0.000	1, 587	0.100	0.752
heterogeneity	1, 587	140.711	0.000	1, 587	109.715	0.000
neighbour identity	2, 587	0.248	0.780	2, 587	1.765	0.172
nutrient level x heterogeneity	1, 587	1.451	0.229	1, 587	10.606	0.001
nutrient level x neighbour identity	2, 587	0.023	0.977	2, 587	0.275	0.759
heterogeneity x neighbour identity	2, 587	2.556	0.078	2, 587	0.783	0.458
nutrient level x heterogeneity x neighbour identity	2, 587	0.137	0.872	2, 587	0.451	0.637

Figure 1. Planting design for heterogenous and homogenous soil treatments in 15.2 cm pots. The nutrient patch (depicted by the green circle with dashed line border) was 1 cm in diameter and placed equidistant from both plants at 5.4 cm. In treatments with a neighbour, each plant was 3.8 cm from both the centre and edge of the pot while 7.6 cm from the other plant. Control treatments had a single plant placed in one of these two locations. We took five root cores at harvest, depicted by the numbered brown circles with dashed-line borders. The numbers correspond to the numbering of coring locations in the statistical analyses and subsequent discussion.

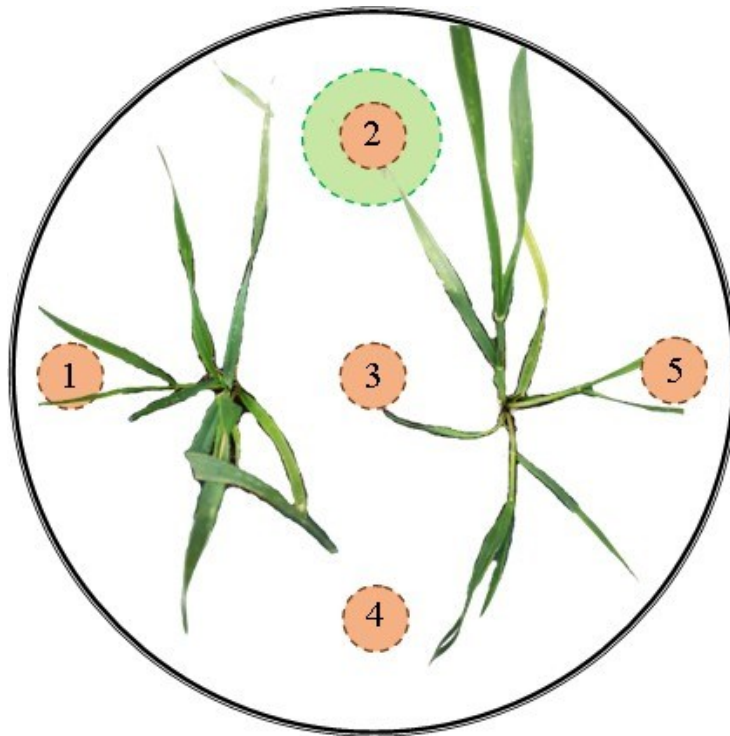
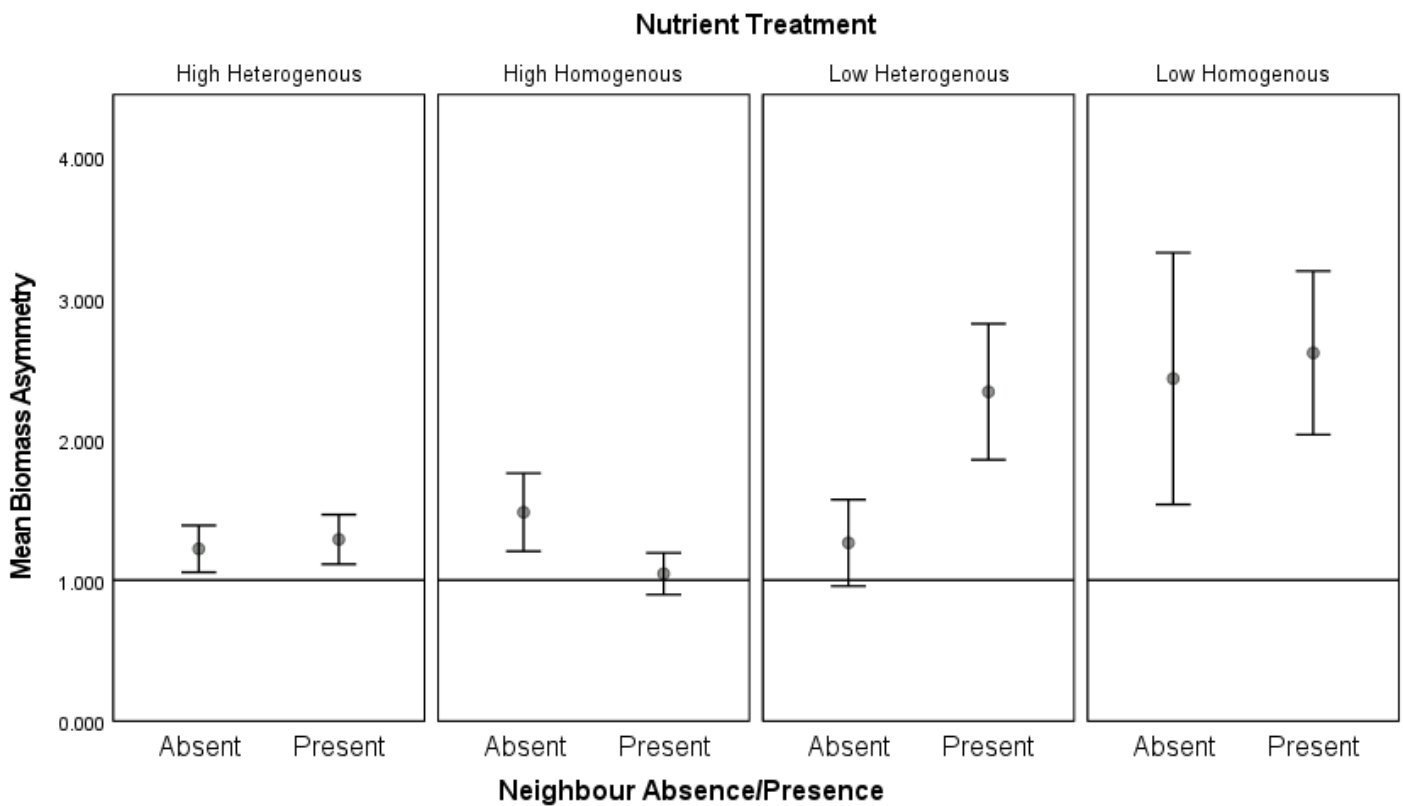


Figure 2. Average of the total aboveground biomass grown towards the centre of the pot versus away from the shared aerial space (mean \pm standard error) of each soil treatment: high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous. The means are separated by a) neighbour absence or presence and b) neighbour identity, either none, kin, or stranger, for each soil treatment along the x-axis.

a)



b)

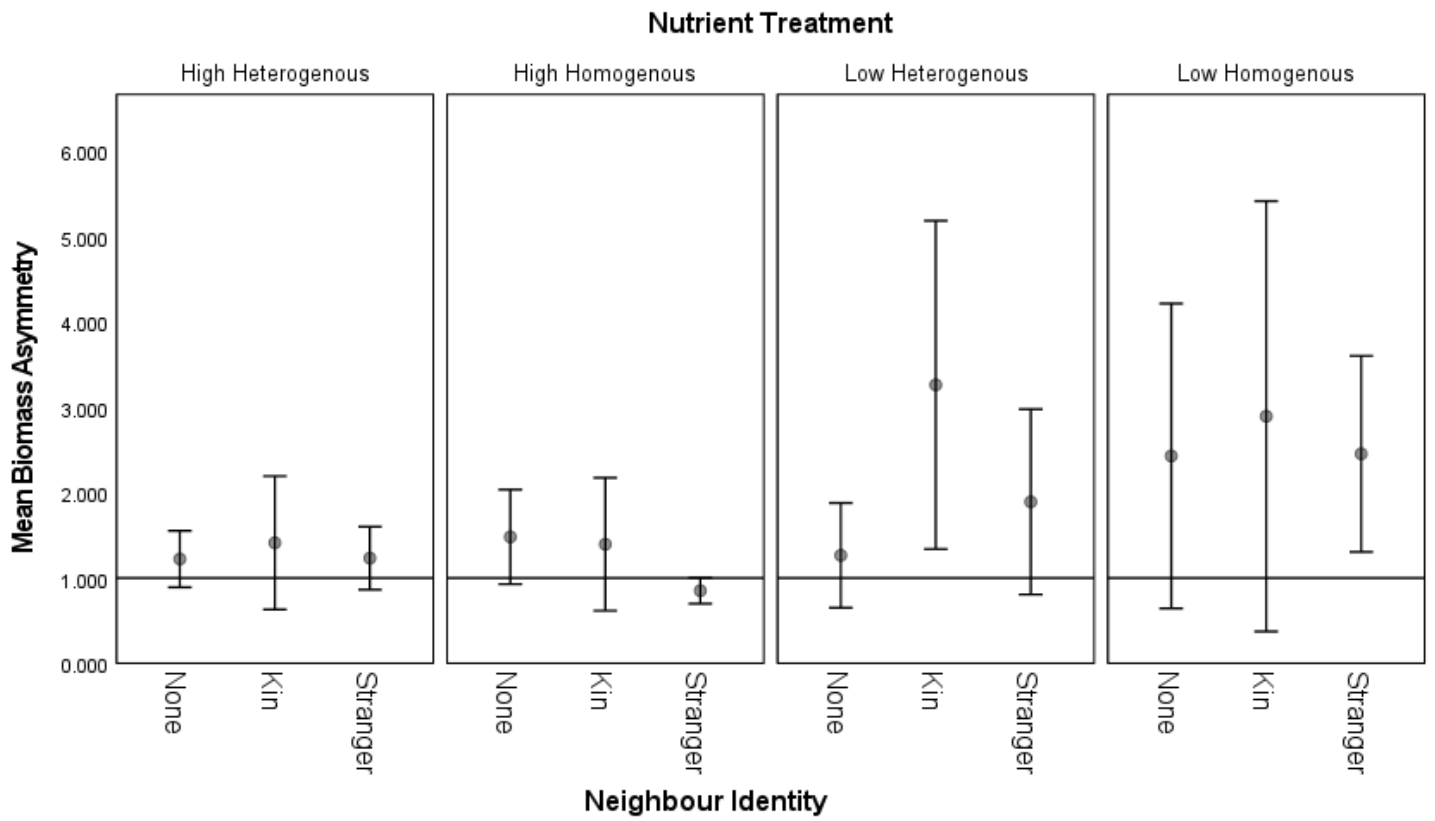
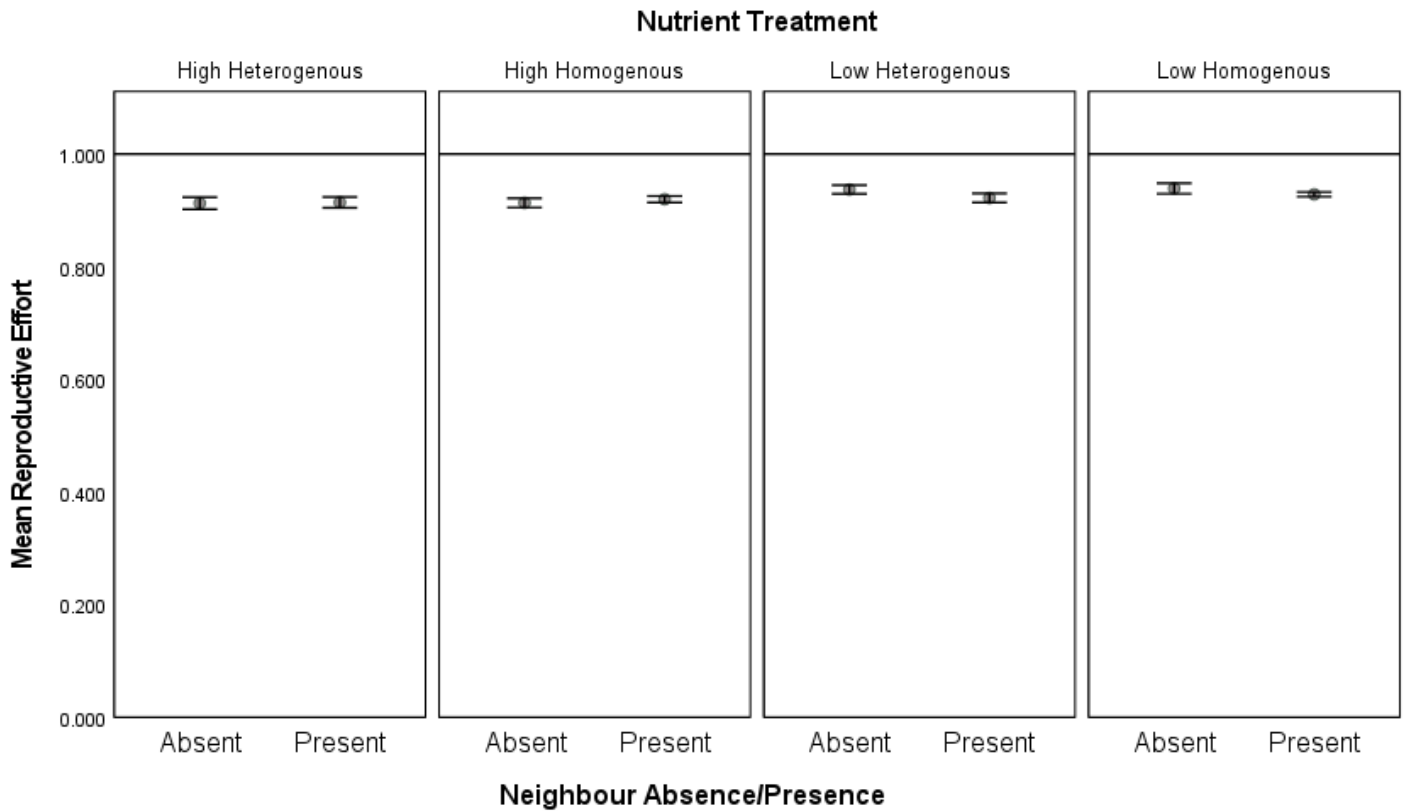


Figure 3. Average of the aboveground reproductive effort as calculated by taking the proportion of aboveground biomass consisting of reproductive structures over the total aboveground biomass (mean \pm standard error) of each soil treatment: high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous. The means are separated by a) neighbour absence or presence and b) neighbour identity, either none, kin, or stranger, for each soil treatment along the x-axis.

a)



b)

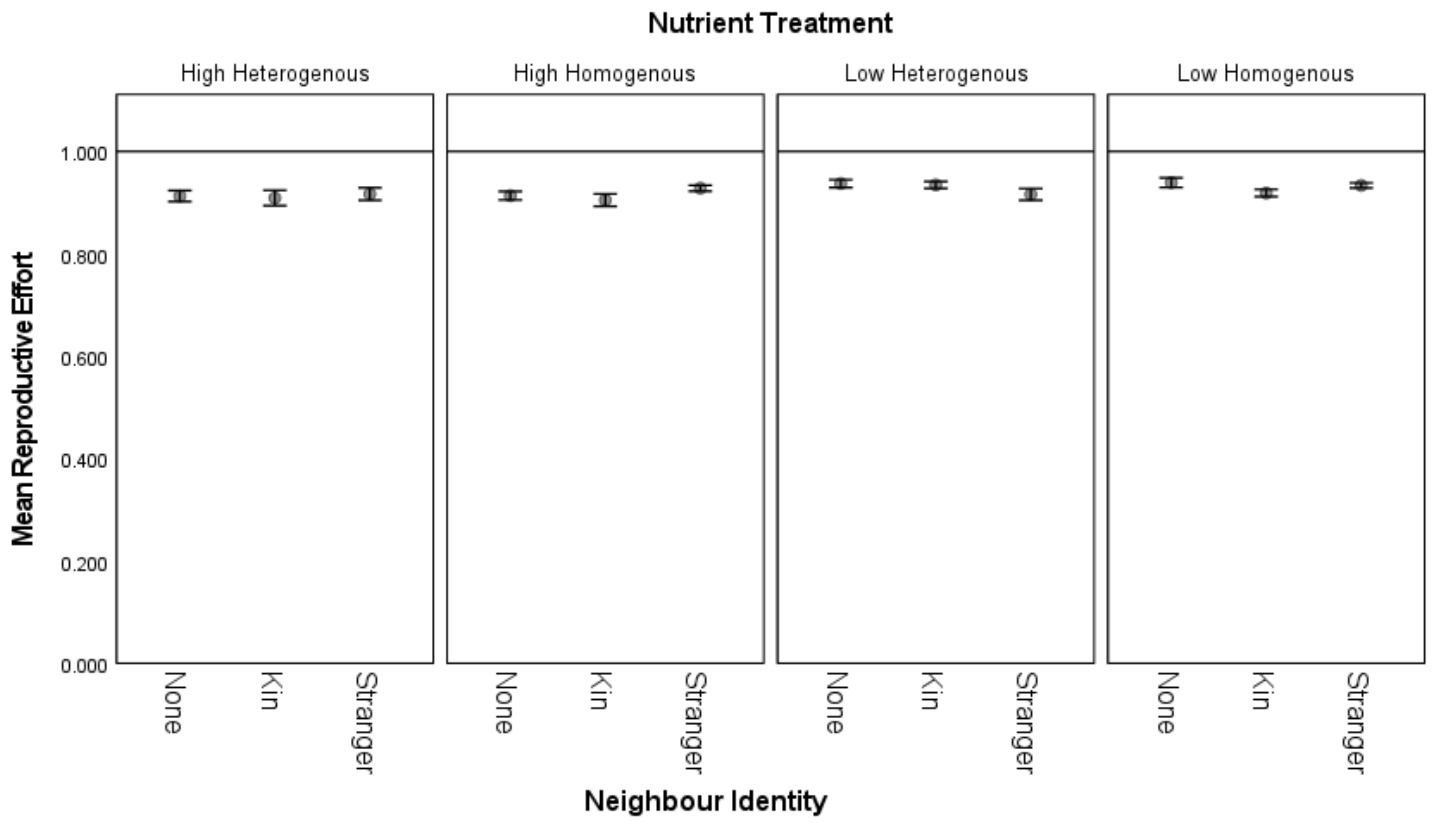
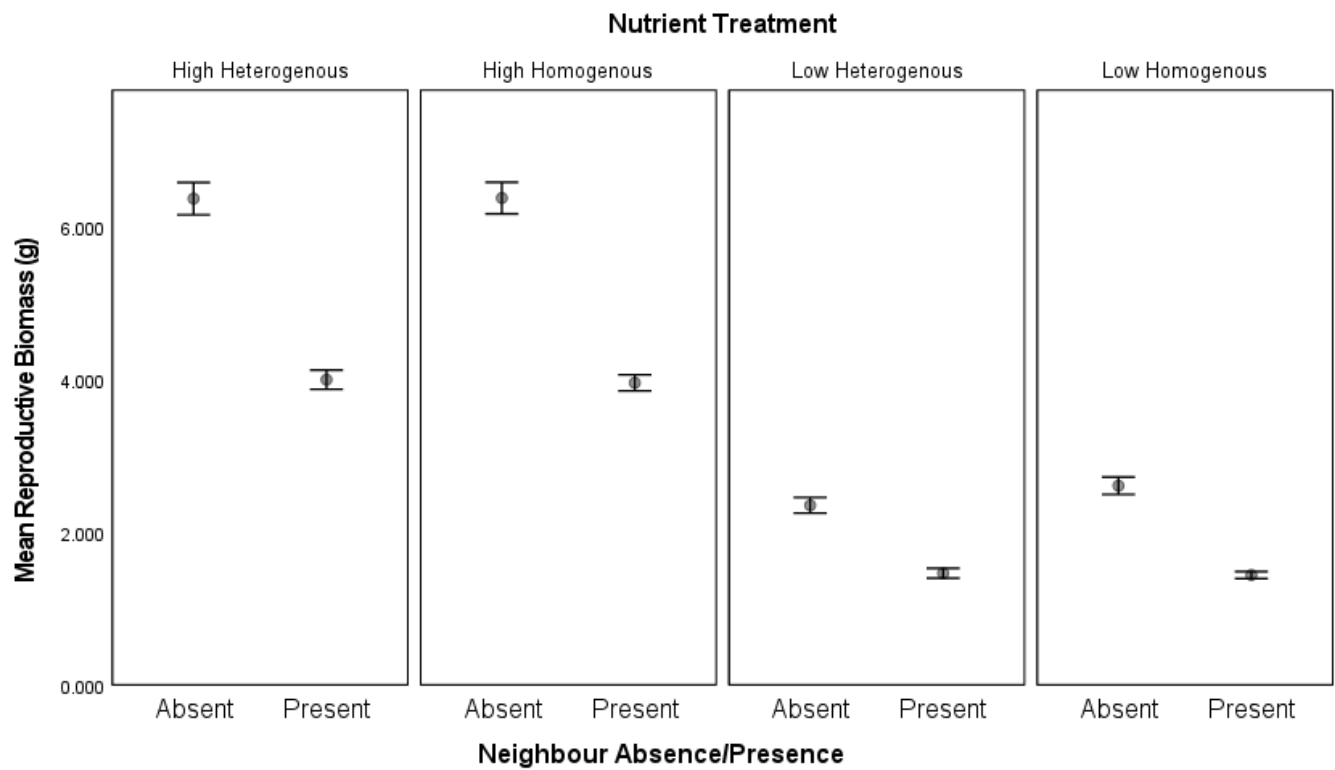


Figure 4. Average of the aboveground reproductive biomass (mean \pm standard error) of each soil treatment: high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous. The means are separated by a) neighbour absence or presence and b) neighbour identity, either none, kin, or stranger, for each soil treatment along the x-axis.

a)



b)

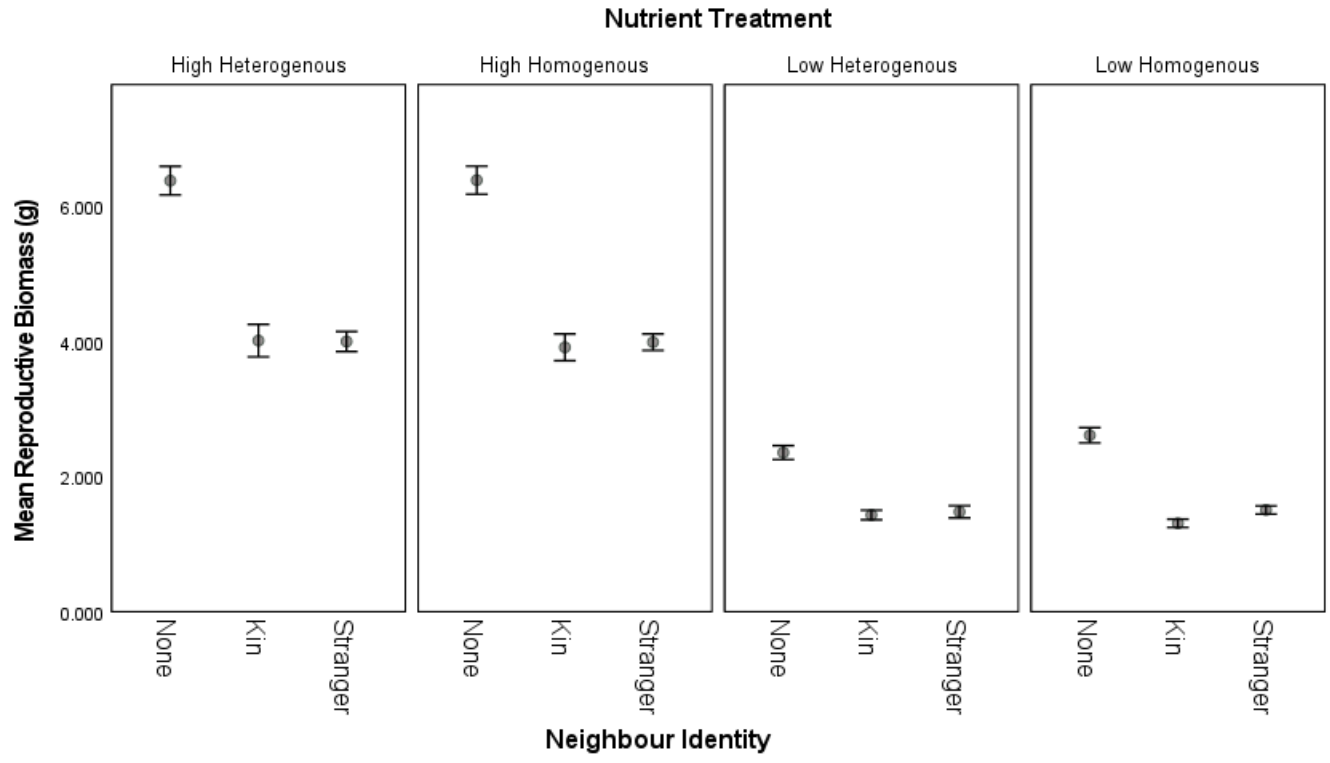
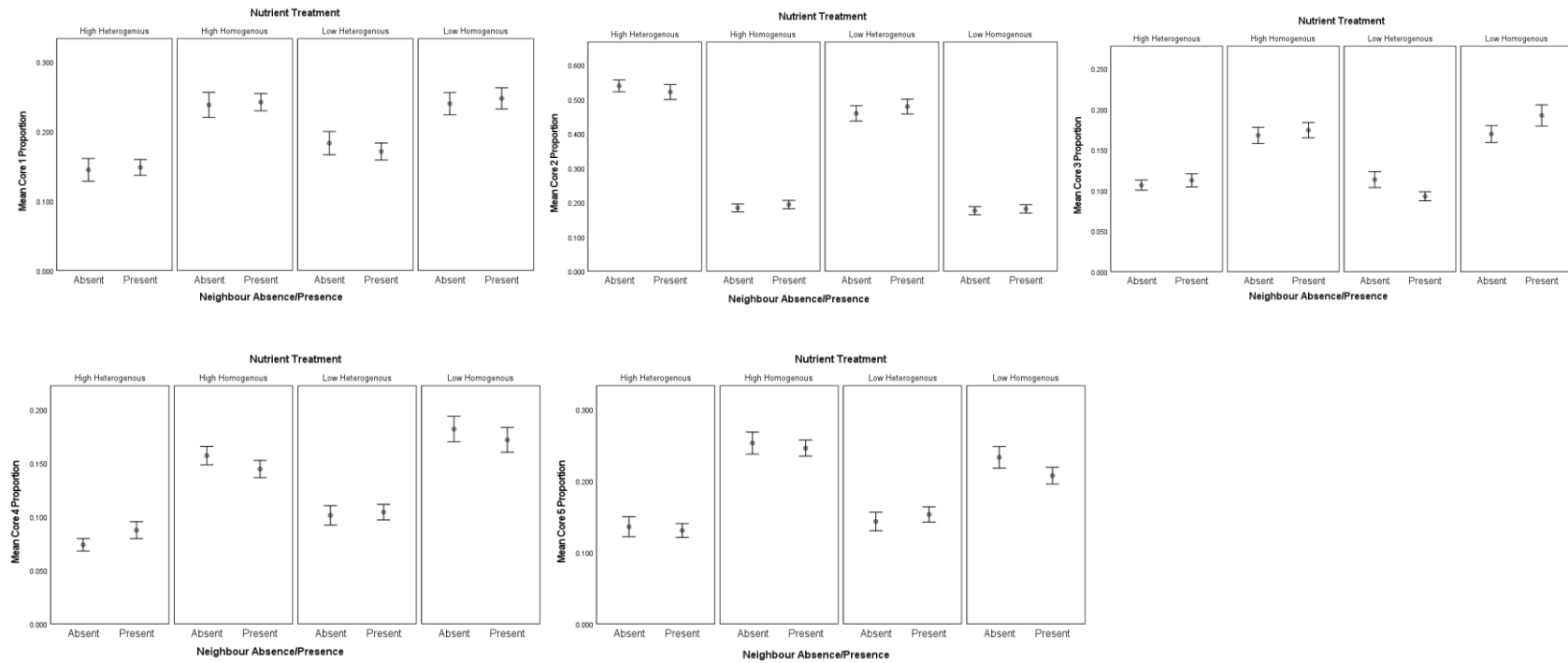
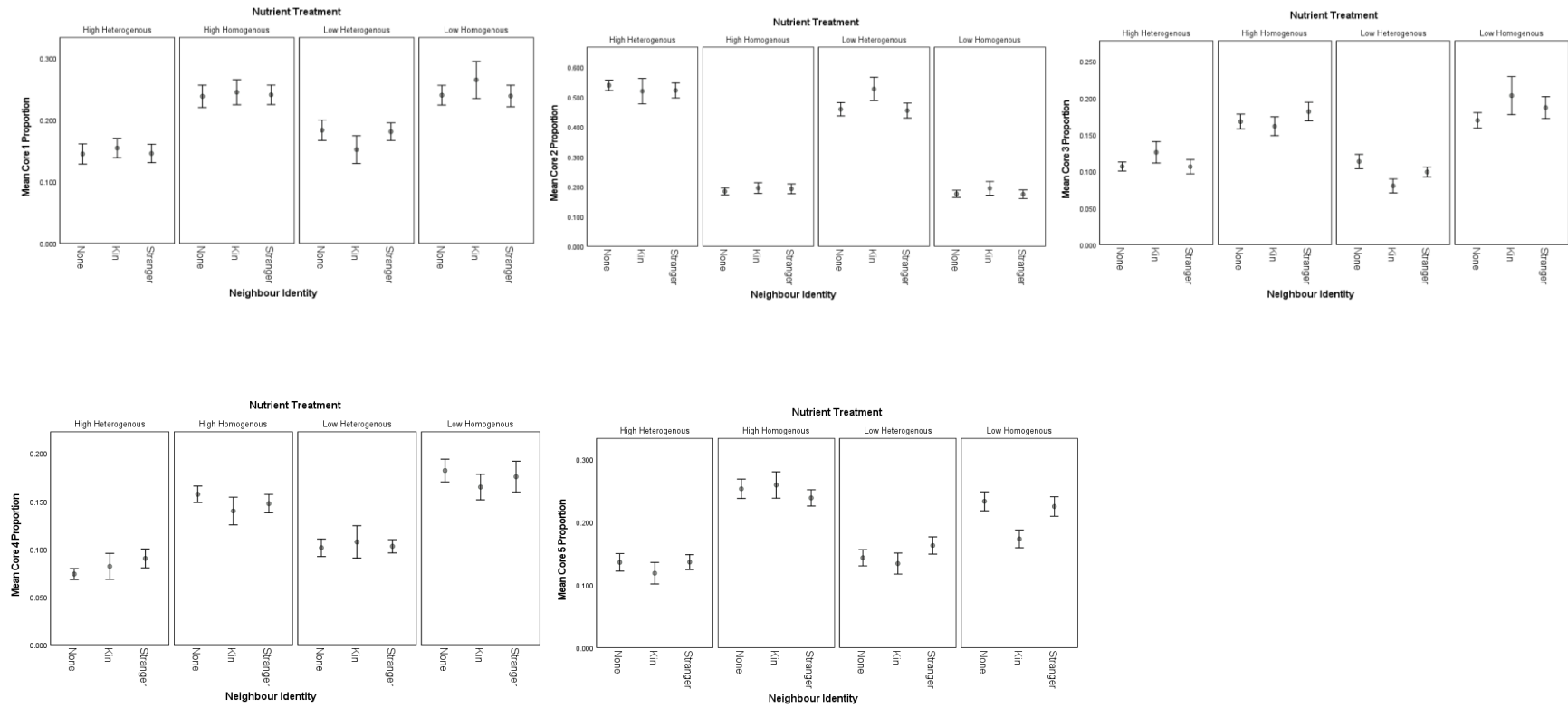


Figure 5. Average of root biomass grown in each coring location, 1 to 5, relative to the total root biomass found across all cores in the pot (mean \pm standard error) of each soil treatment: high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous. Along the x-axis, the means are separated by a) neighbour absence or presence and b) neighbour identity, either none, kin, or stranger, for each of the soil treatments.

a)



b)



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APPENDIX 1 – SUPPLEMENTARY MATERIAL

Appendix Table 1.1. A generalized linear mixed model testing the effect of soil fertility, nutrient distribution, and neighbour presence on the ratio of the total aboveground biomass grown towards the neighbouring plant versus away in wheat, *Triticum aestivum*. Fixed effects included nutrient level (high or low nutrients), heterogeneity (homogenous or heterogenous patch), neighbour absence or presence, and their interactions. We included blocks (1, 2, 3, 4, and 5) and cultivars (Carberry, Titanium, Go Early, Glenn, and Villian) as random effects.

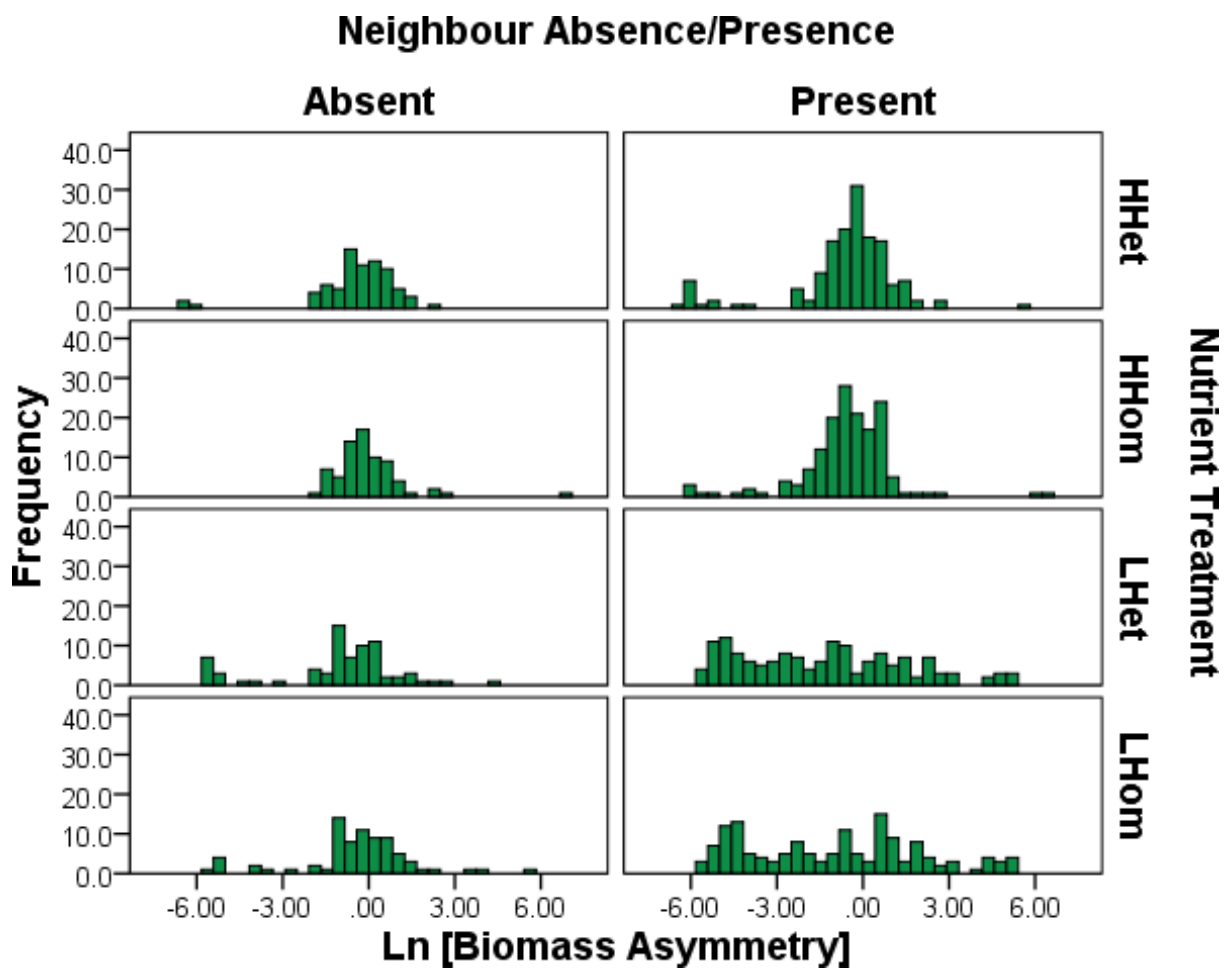
source	biomass asymmetry	
	F-value	p-value
nutrient level	12.690	0.000
heterogeneity	4.661	0.031
neighbour presence	0.050	0.824
nutrient level x heterogeneity	0.693	0.405
nutrient level x neighbour presence	0.058	0.809
heterogeneity x neighbour presence	0.985	0.321
nutrient level x heterogeneity x neighbour presence	0.025	0.875

Appendix Table 1.2. A general linear mixed model testing the effect of soil fertility, nutrient distribution, and neighbour presence on the proportion of total roots grown at each core location (1, 2, 3, 4, and 5). Fixed effects included nutrient level (high or low nutrients), heterogeneity (homogenous or heterogenous patch), neighbour absence or presence, and their interactions. We included blocks (1, 2, 3, 4, and 5) and cultivars (Carberry, Titanium, Go Early, Glenn, and Villian).

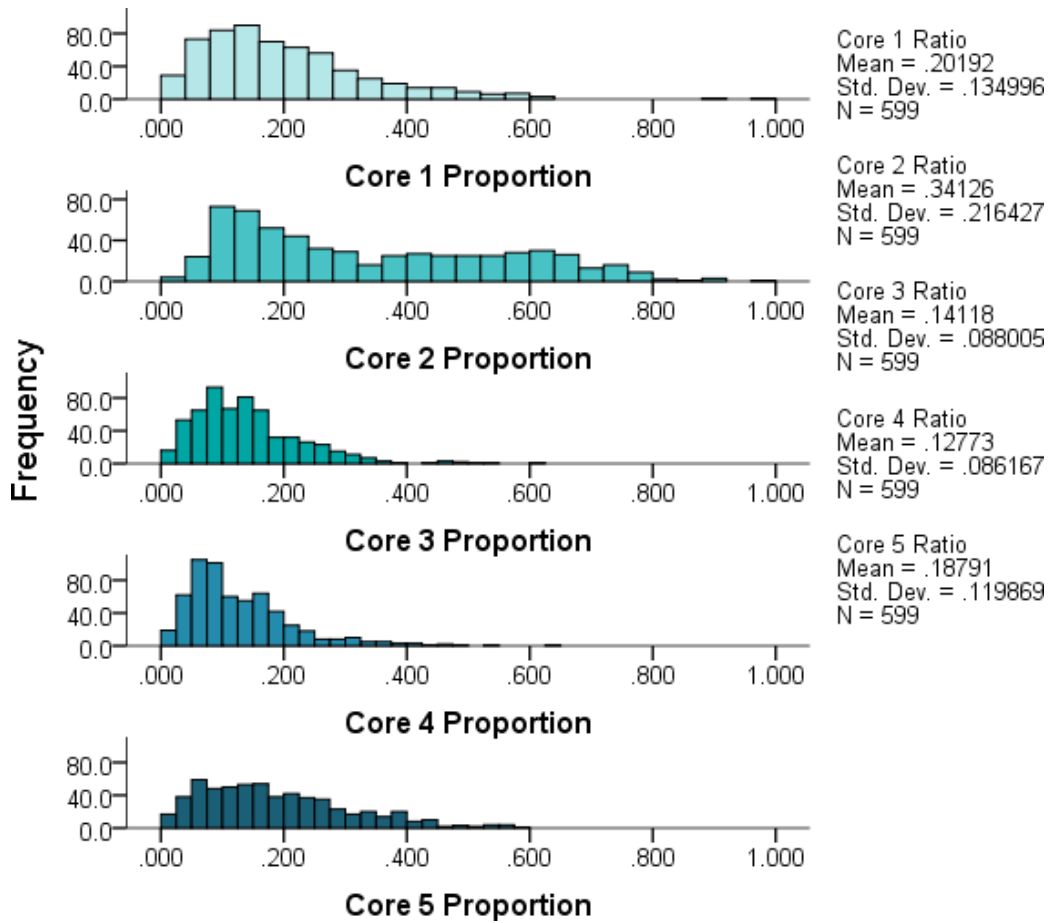
source	core 1 proportion			core 2 proportion			core 3 proportion		
	d.f	F-value	p-value	d.f	F-value	p-value	d.f	F-value	p-value
nutrient level	1, 591	6.232	0.013	1, 589	<u>6.210</u>	0.013	1, 591	0.175	0.676
heterogeneity	1, 591	83.185	0.000	1, 589	<u>633.345</u>	0.000	1, 591	<u>130.137</u>	0.000
neighbour presence	1, 591	0.017	0.896	1, 589	<u>0.003</u>	<u>0.960</u>	1, 591	0.001	0.980
nutrient level x heterogeneity	1, 591	3.601	0.058	1, 589	<u>0.409</u>	<u>0.523</u>	1, 591	1.443	0.230
nutrient level x neighbour presence	1, 591	1.677	0.196	1, 589	<u>0.124</u>	<u>0.725</u>	1, 591	0.032	0.859
heterogeneity x neighbour presence	1, 591	0.001	0.981	1, 589	<u>0.409</u>	<u>0.523</u>	1, 591	1.807	0.179
nutrient level x heterogeneity x neighbour presence	1, 591	0.450	0.503	1, 589	<u>0.561</u>	<u>0.454</u>	1, 591	0.223	0.637

source	core 4 proportion			core 5 proportion		
	d.f	F-value	p-value	d.f	F-value	p-value
nutrient level	1, 590	14.762	0.000	1, 591	<u>0.365</u>	<u>0.546</u>
heterogeneity	1, 590	188.999	0.000	1, 591	<u>120.774</u>	0.000
neighbour presence	1, 590	0.009	0.926	1, 591	<u>0.011</u>	<u>0.917</u>
nutrient level x heterogeneity	1, 590	2.336	0.127	1, 591	<u>8.844</u>	0.003
nutrient level x neighbour presence	1, 590	0.000	0.999	1, 591	<u>0.014</u>	<u>0.906</u>
heterogeneity x neighbour presence	1, 590	5.194	0.023	1, 591	<u>1.026</u>	<u>0.312</u>
nutrient level x heterogeneity x neighbour presence	1, 590	0.010	0.918	1, 591	<u>0.332</u>	<u>0.565</u>

Appendix Figure 1.1. Histograms depicting the distribution of the natural log of the ratio of total aboveground biomass grown towards the centre of the pot versus away from the shared aerial space. The samples are separated on the x-axis by the presence or absence of a neighbouring plant. We split the y-axis by nutrient treatment. The first letter indicates the nutrient level (H = high nutrient levels; L = low nutrient levels) while the suffix indicates the nutrient distribution (Het = heterogeneous patch in the soil; Hom = homogenous nutrient placement).

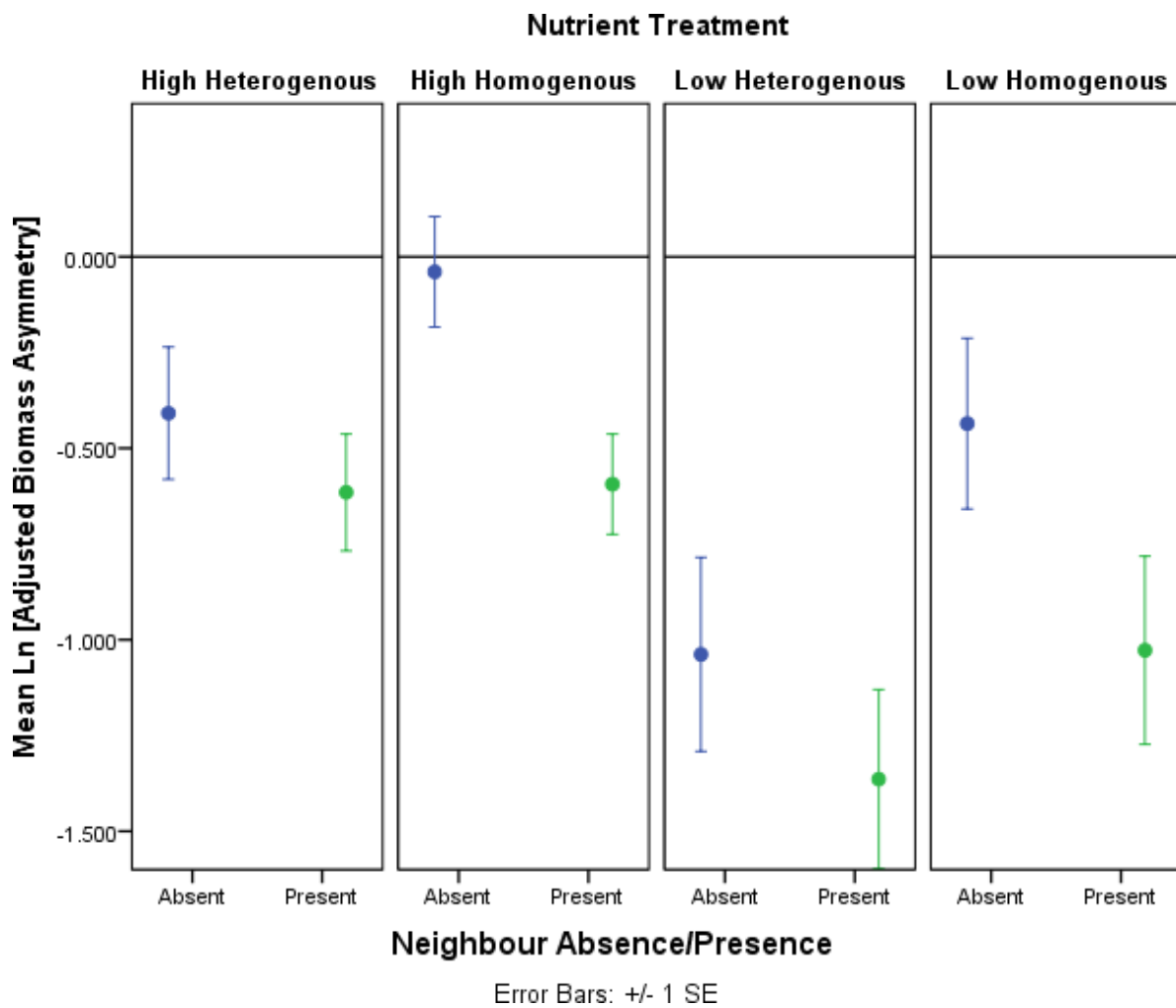


Appendix Figure 1.2. Histograms depicting the distribution of the belowground biomass collected from each of the five coring locations within the pots. The proportion at each core location is the amount of root biomass found at that root location relative to the total root biomass in each pot. We did not transform this data.

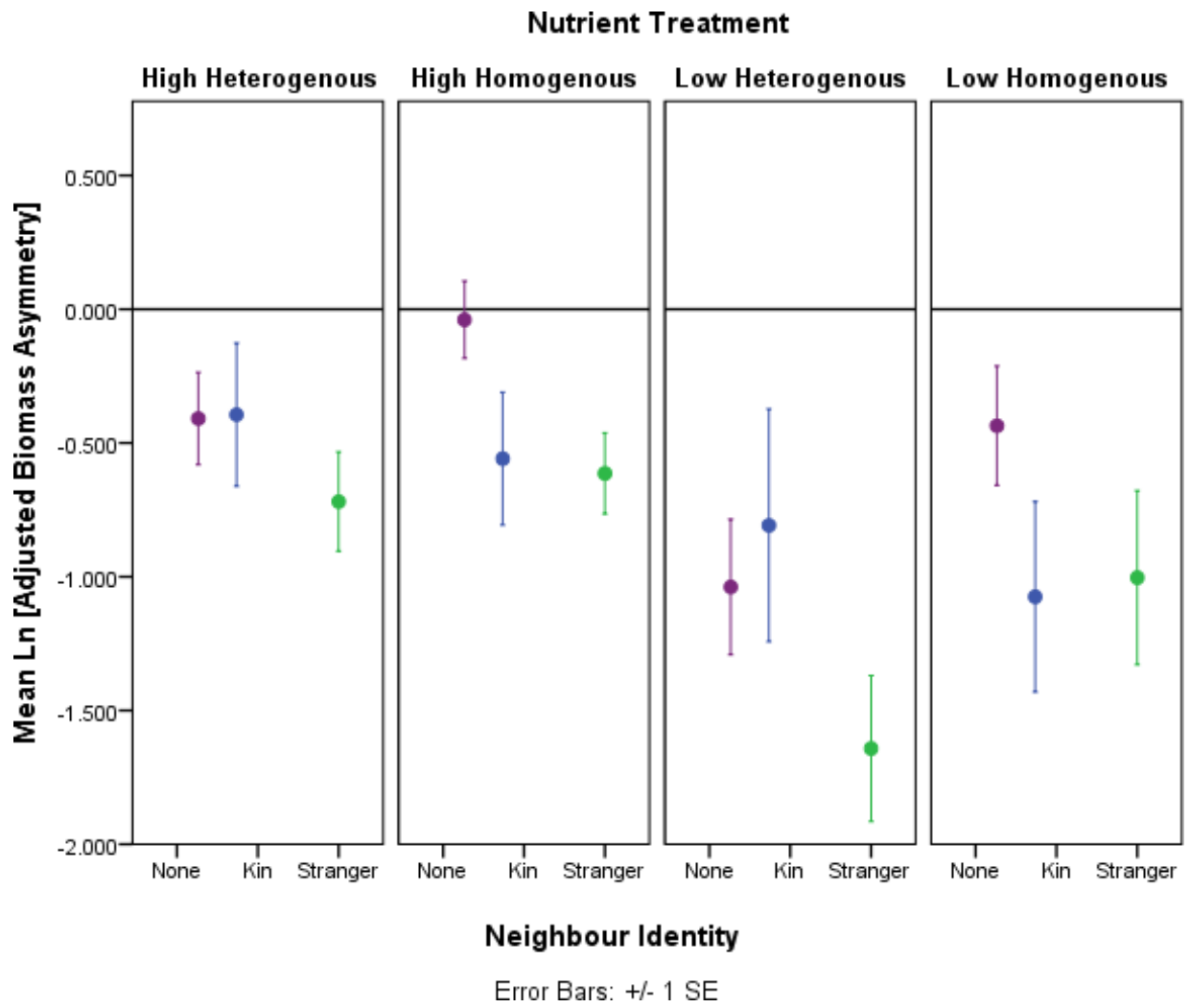


Appendix Figure 1.3. Average of the natural log of total aboveground biomass grown towards the centre of the pot versus away from the shared aerial space (mean \pm standard error) of each soil treatment. The means are separated by a) neighbour absence or presence and b) neighbour identity, either none, kin, or stranger, for each soil treatment (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous) along the x-axis.

a)

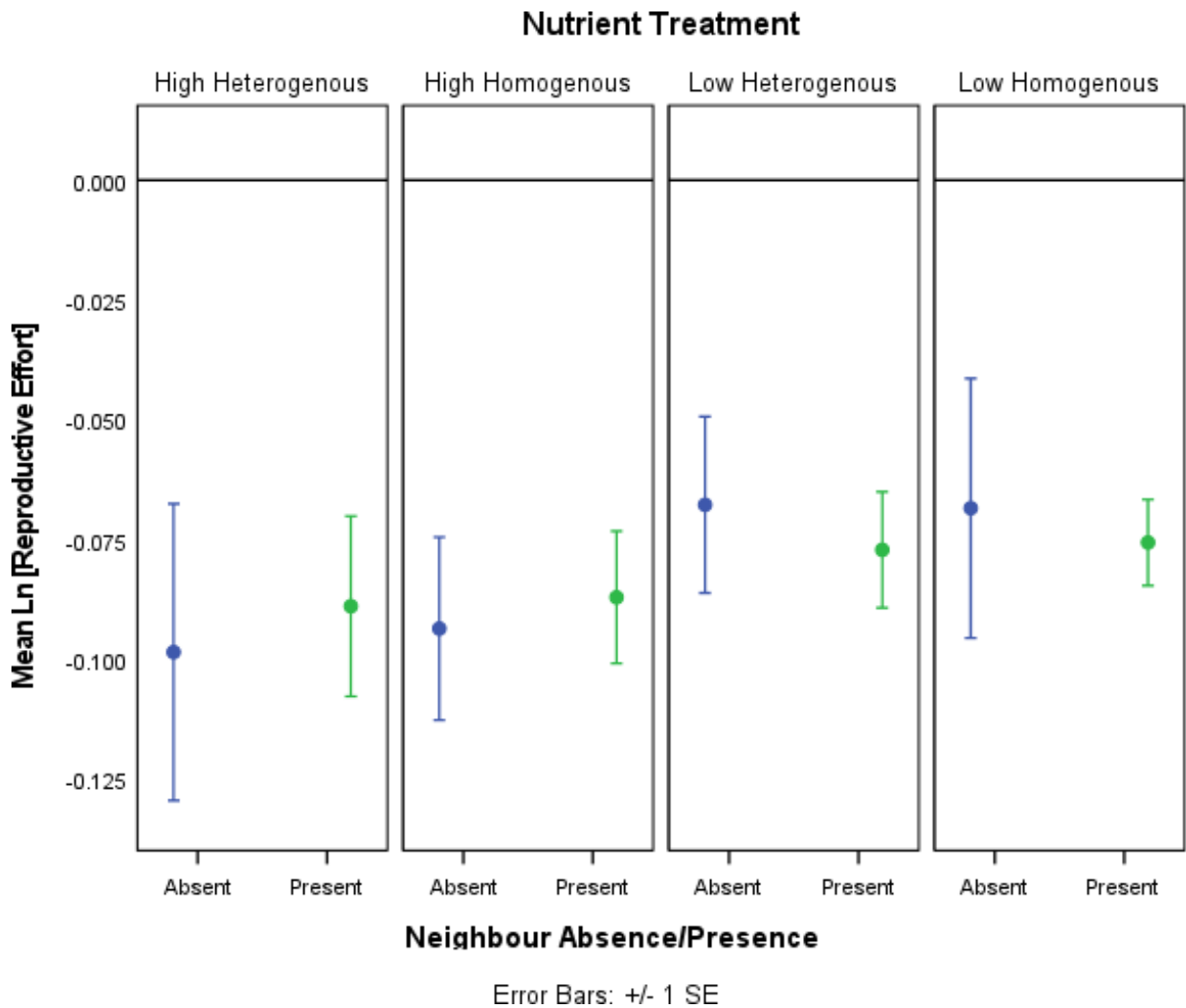


b)

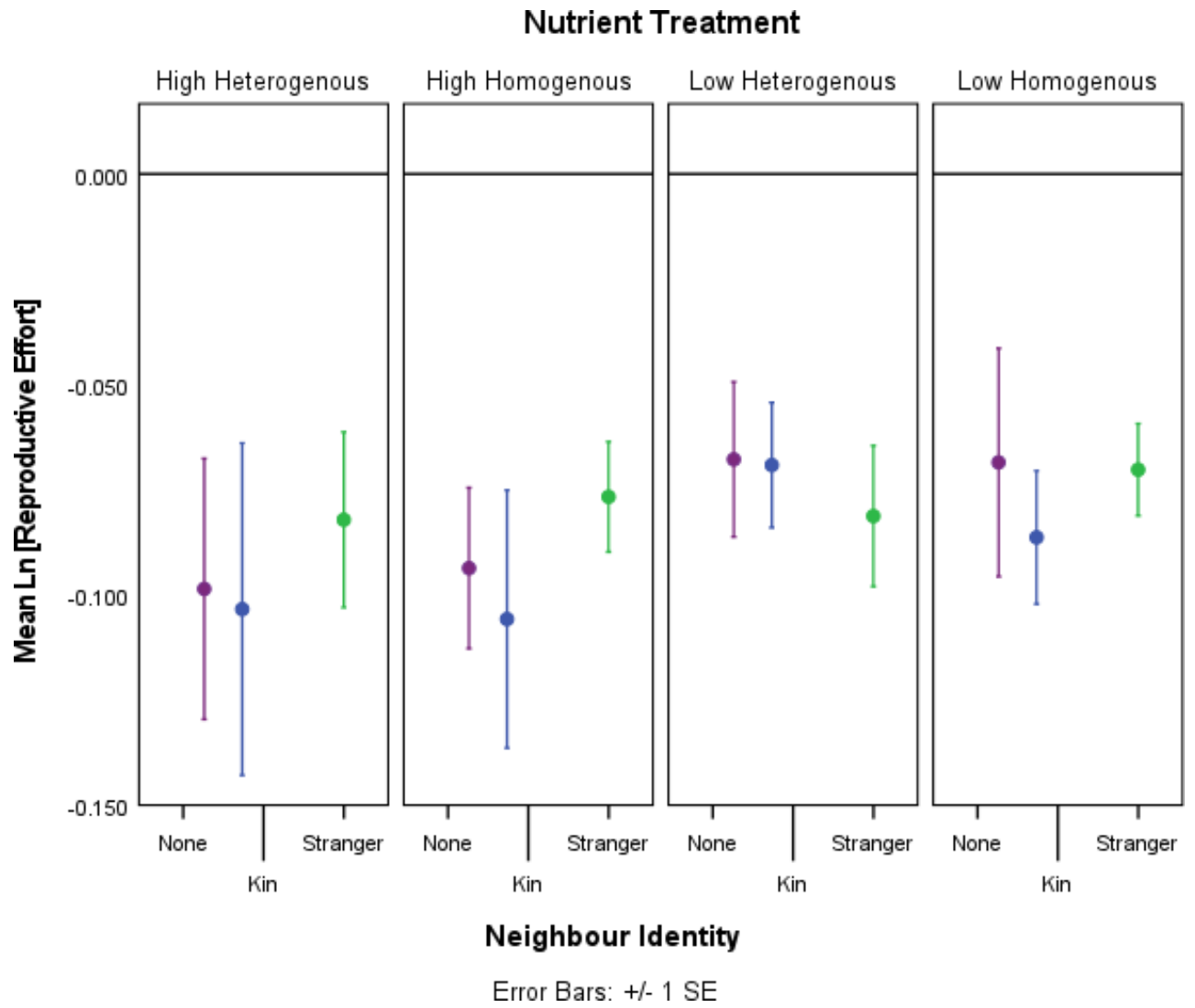


Appendix Figure 1.4. Average of the natural log of aboveground reproductive effort as calculated by taking the proportion of aboveground biomass consisting of reproductive structures over the total aboveground biomass (mean \pm standard error) of each soil treatment. The means are separated by a) neighbour absence or presence and b) neighbour identity, either none, kin, or stranger, for each soil treatment (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous) along the x-axis.

a)

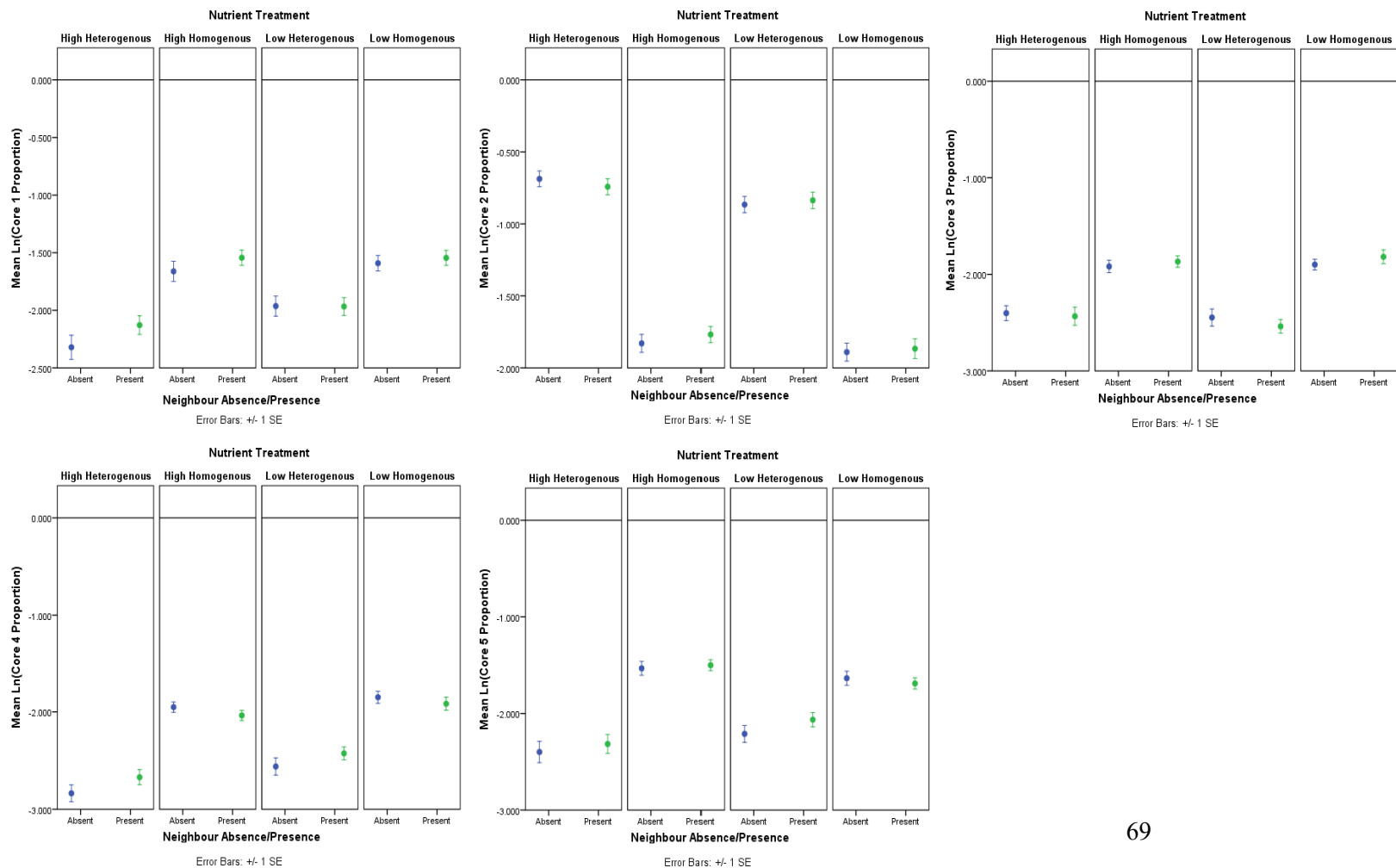


b)

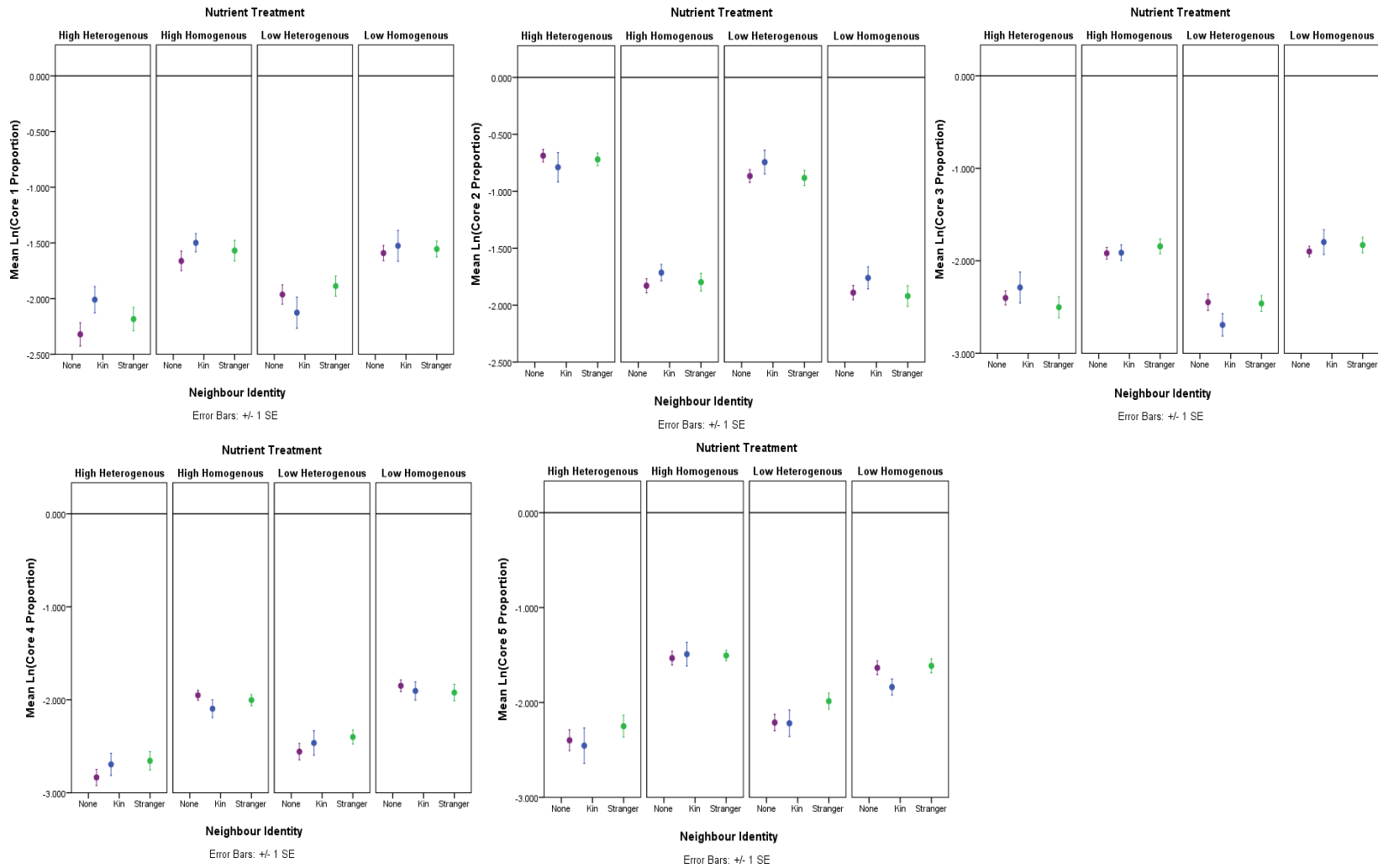


Appendix Figure 1.5. Average of the natural log of root biomass grown in each coring location, 1 to 5, relative to the total root biomass found across all cores in the pot (mean \pm standard error) of each soil treatment. Along the x-axis, the means are separated by a) neighbour absence or presence and b) neighbour identity, either none, kin, or stranger, for each of the soil treatments (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous).

a)

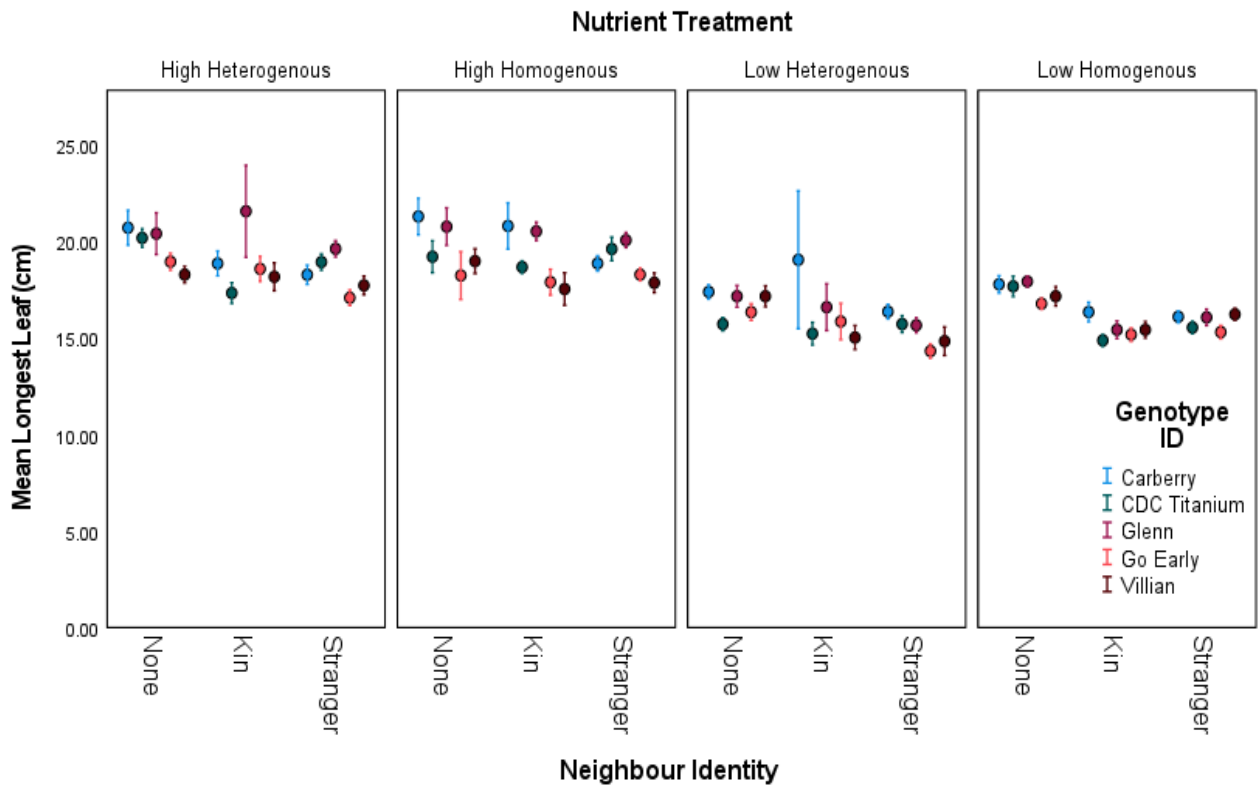


b)

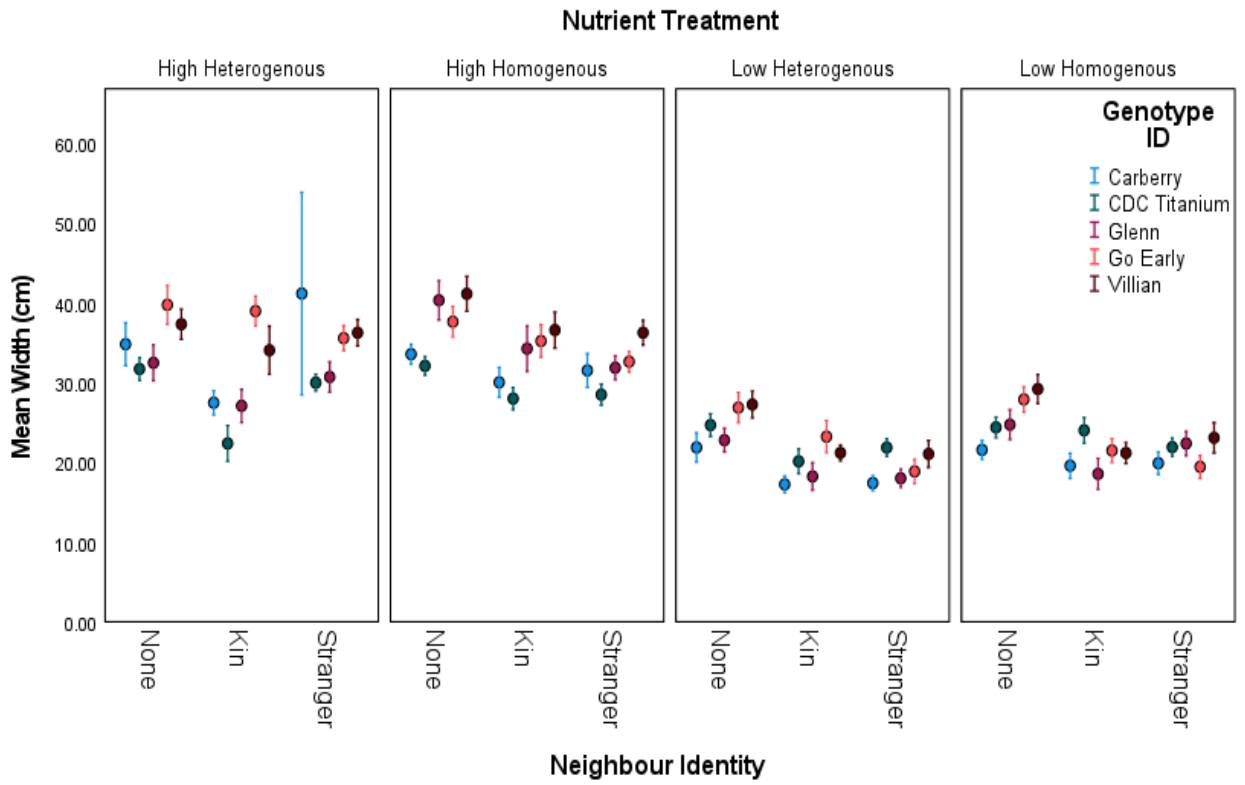


Appendix Figure 1.6. Average (mean \pm standard error) of the three aboveground interim measures a) longest leaf length (cm), b) width of the plant (cm), and c) height of plant (cm) for each soil treatment (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous). The means are separated along the x-axis by neighbour identity: either none, kin, or stranger.

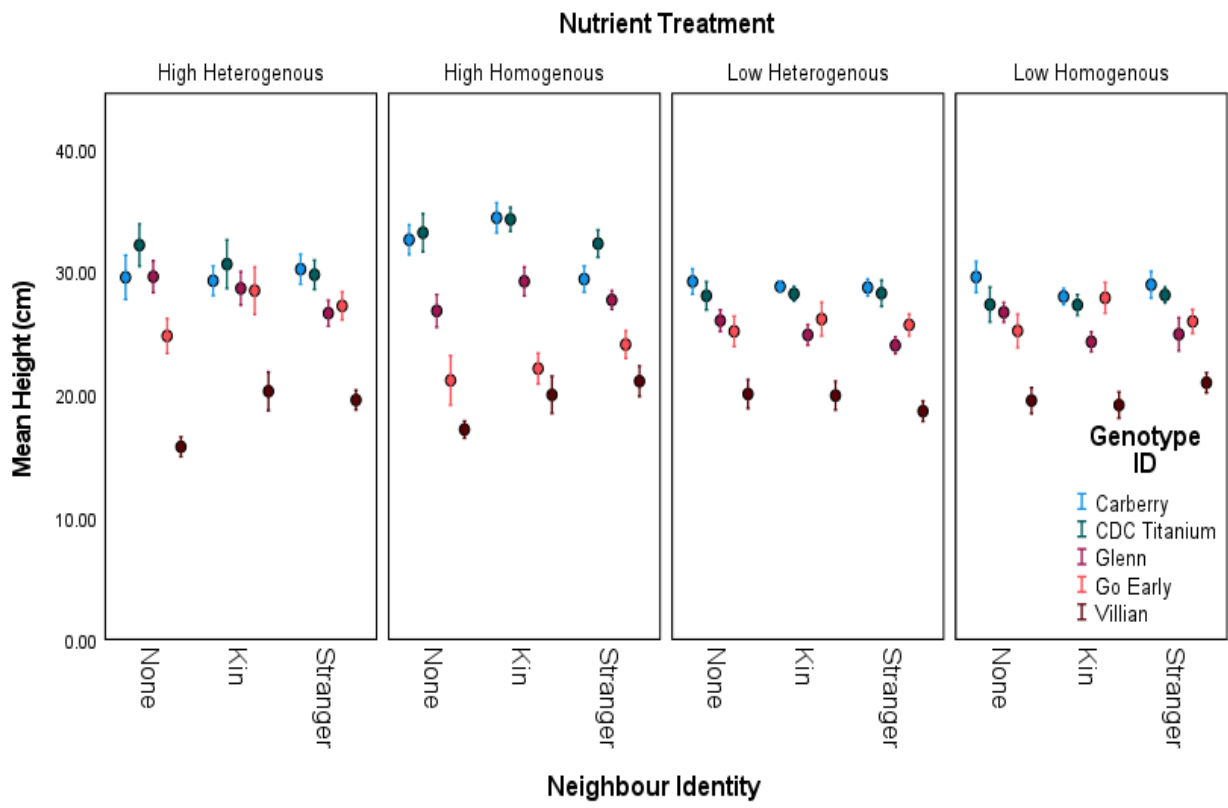
a)



b)

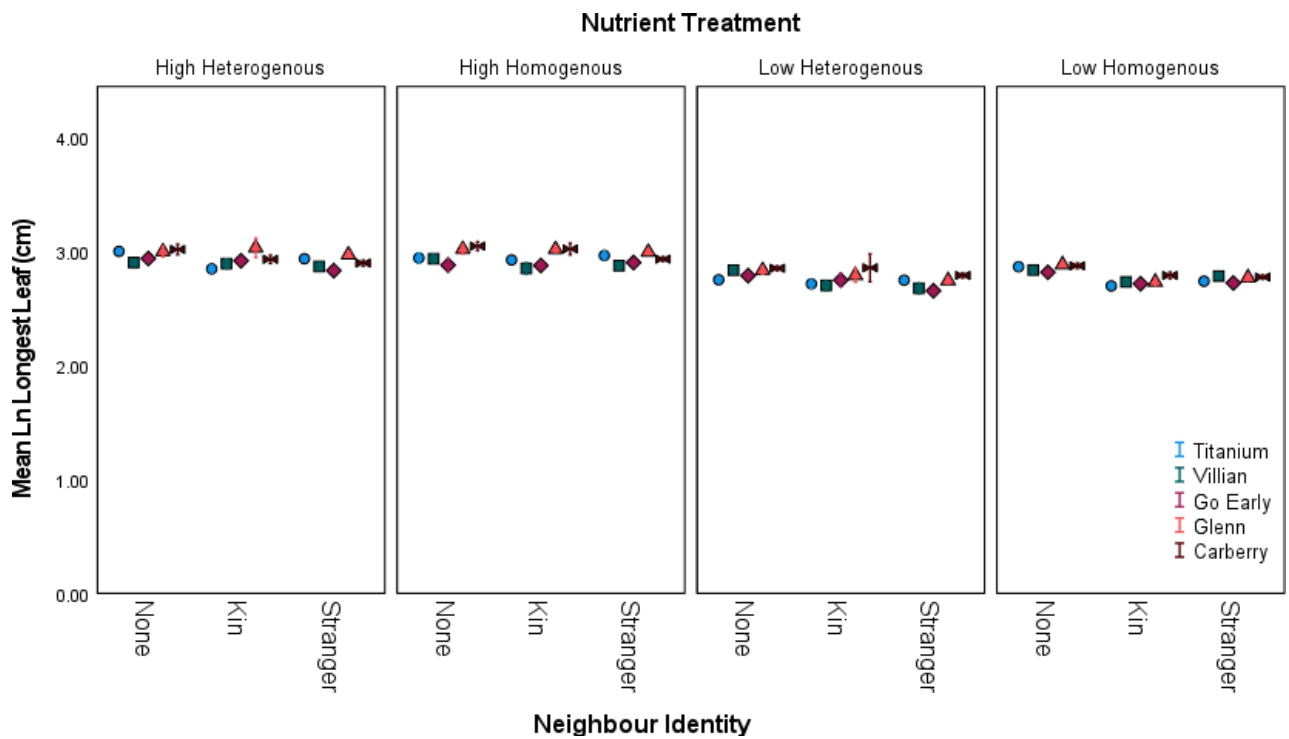


c)

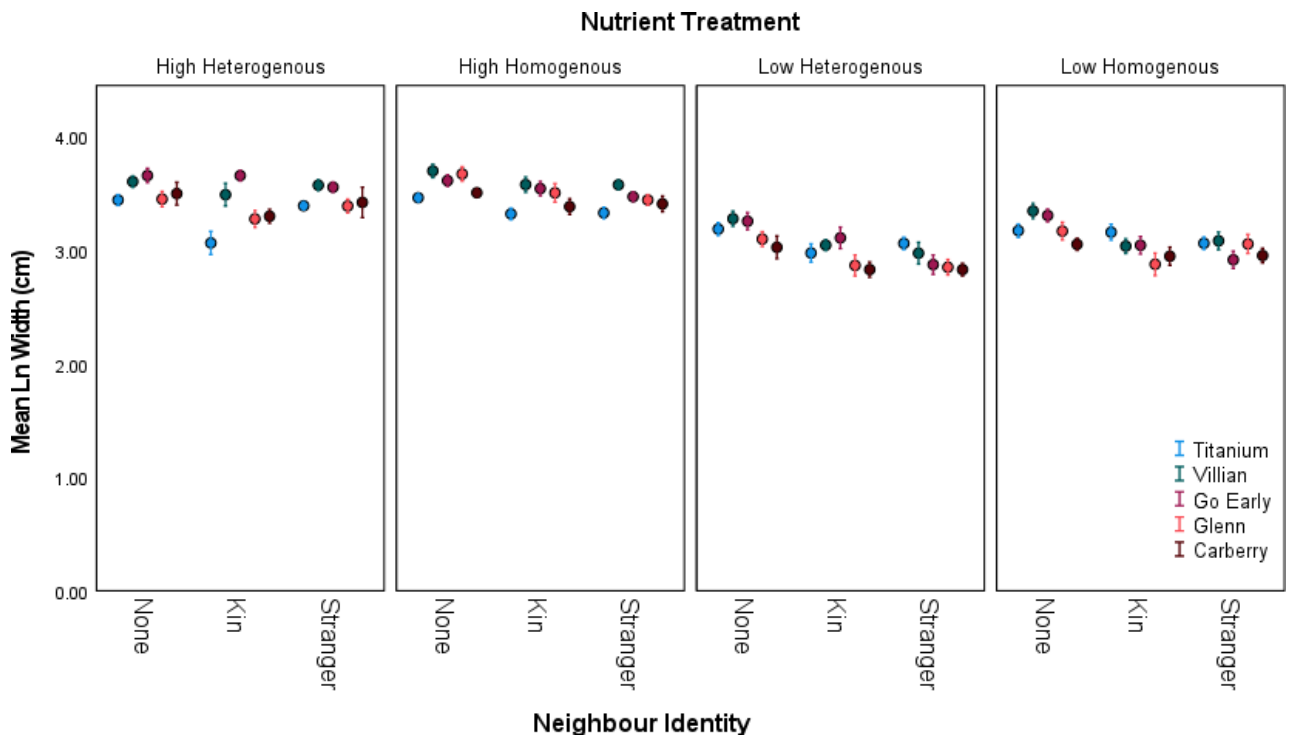


Appendix Figure 1.7. Average (mean \pm standard error) of the natural log of three aboveground interim measures a) longest leaf length (cm), b) width of the plant (cm), and c) height of plant (cm) for each soil treatment (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous). The means are separated along the x-axis by neighbour identity: either none, kin, or stranger.

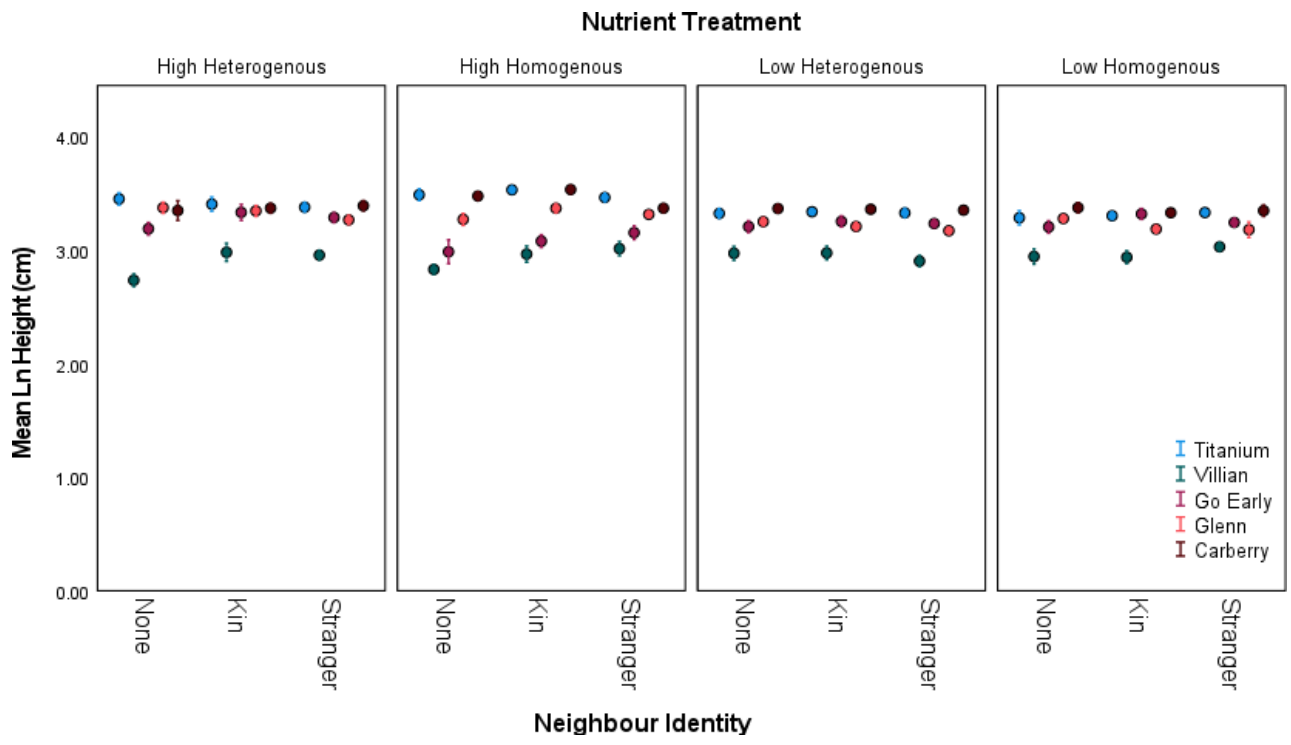
a)



b)

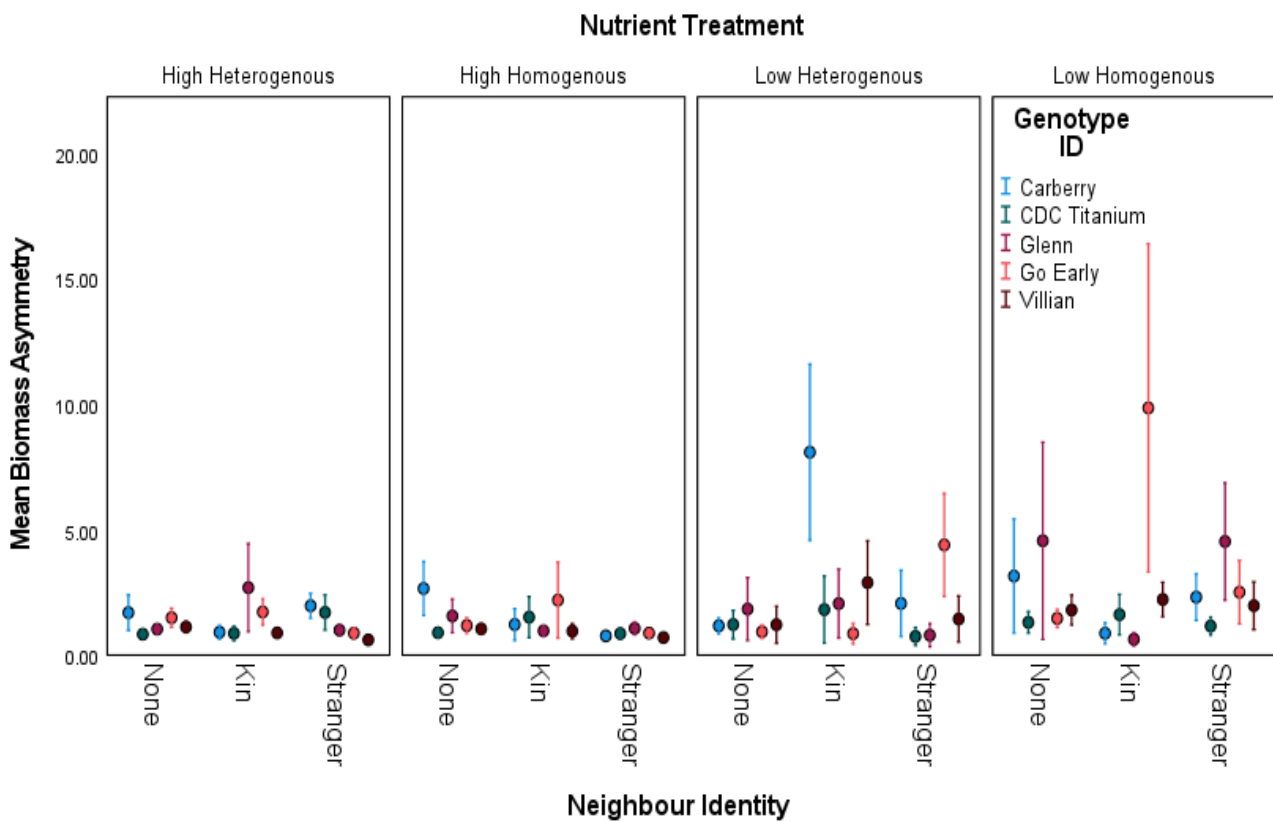


c)

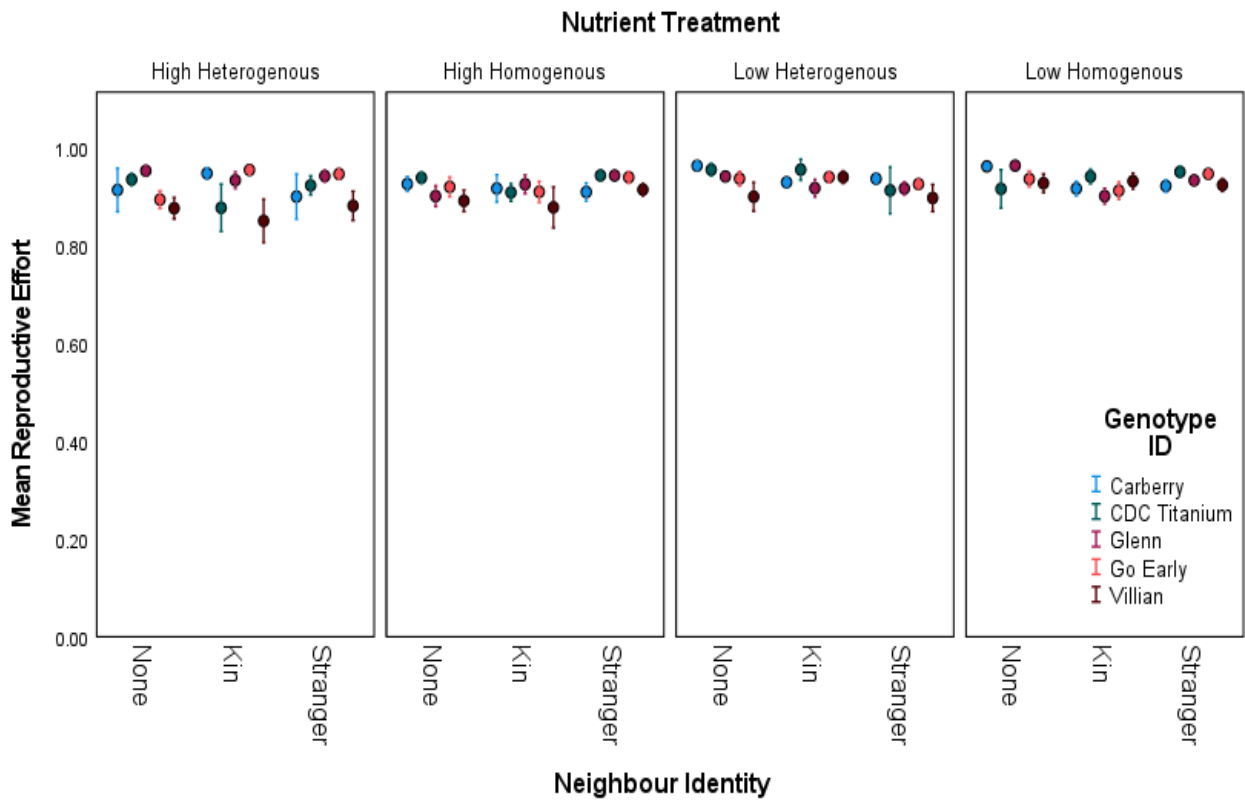


Appendix Figure 1.8. Average (mean \pm standard error) of three aboveground measures a) biomass asymmetry [total aboveground biomass grown towards the centre of the pot/biomass grown away], b) reproductive effort [aboveground biomass consisting of reproductive structures/total aboveground biomass], and c) reproductive biomass across the four soil treatments (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous). The means are separated by neighbour identity, either none, kin, or stranger, for each of the five cultivars.

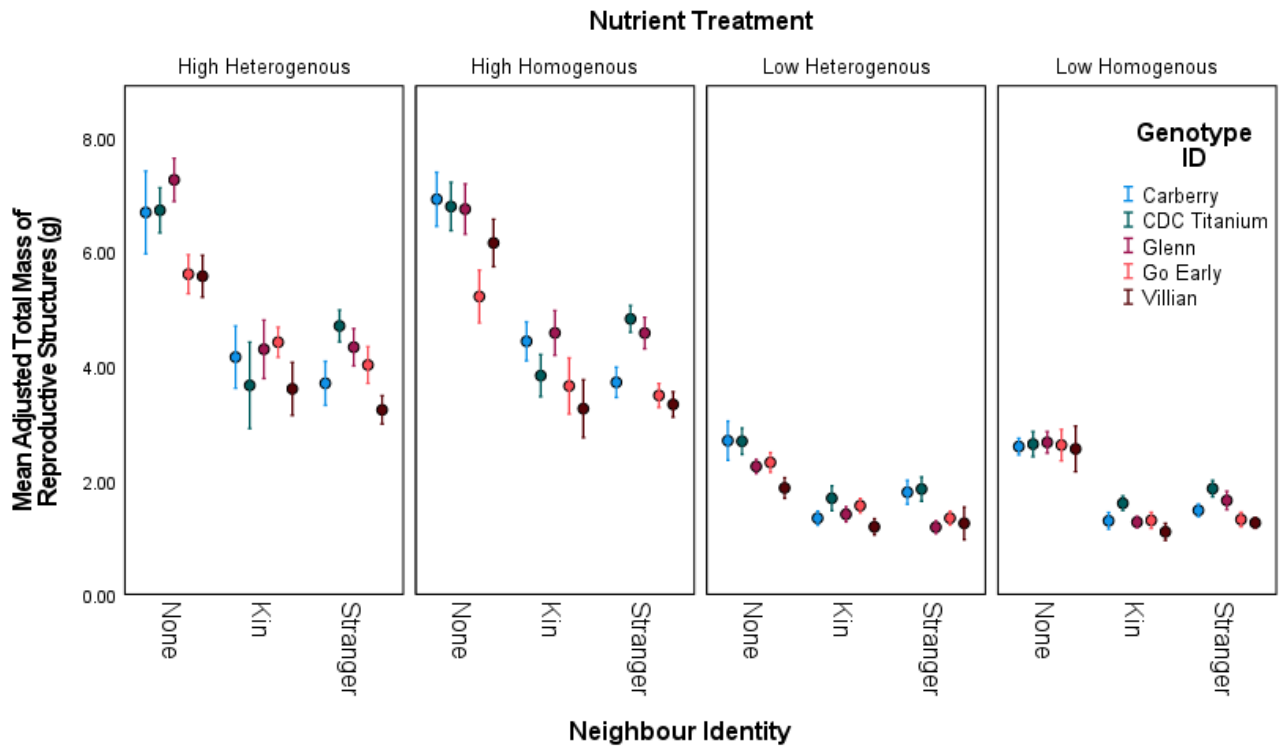
a)



b)

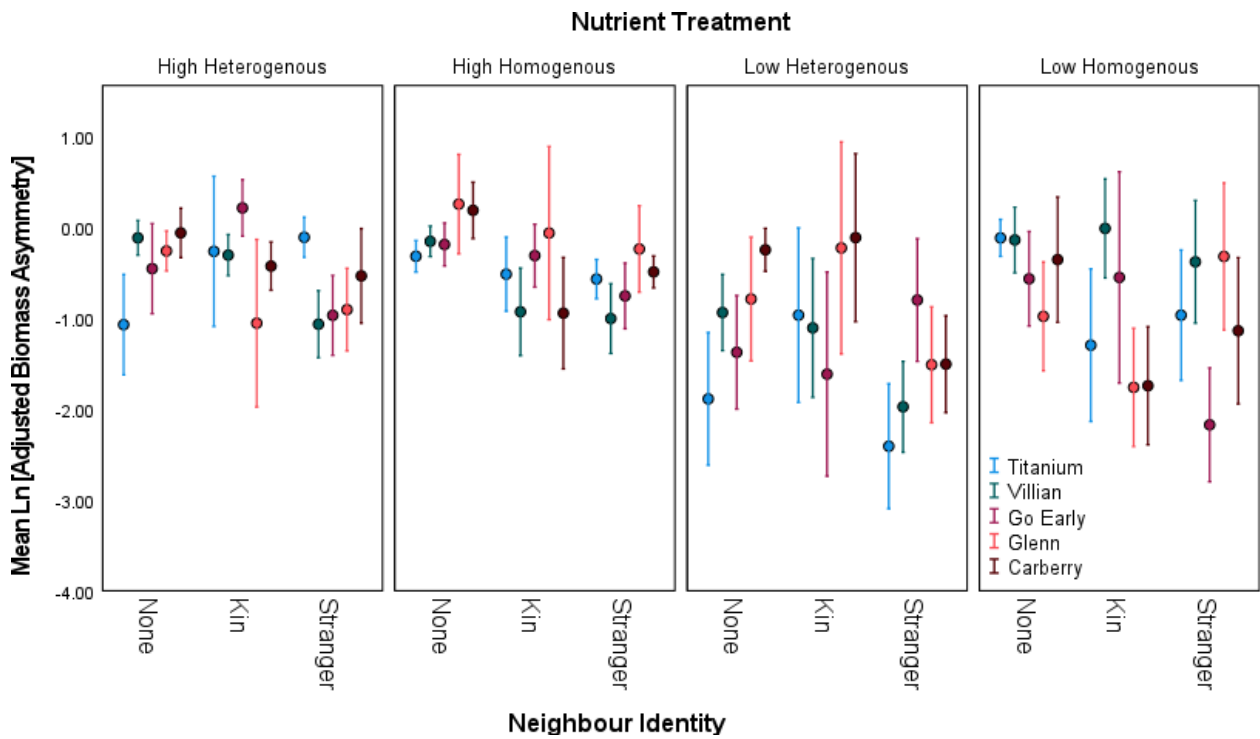


c)

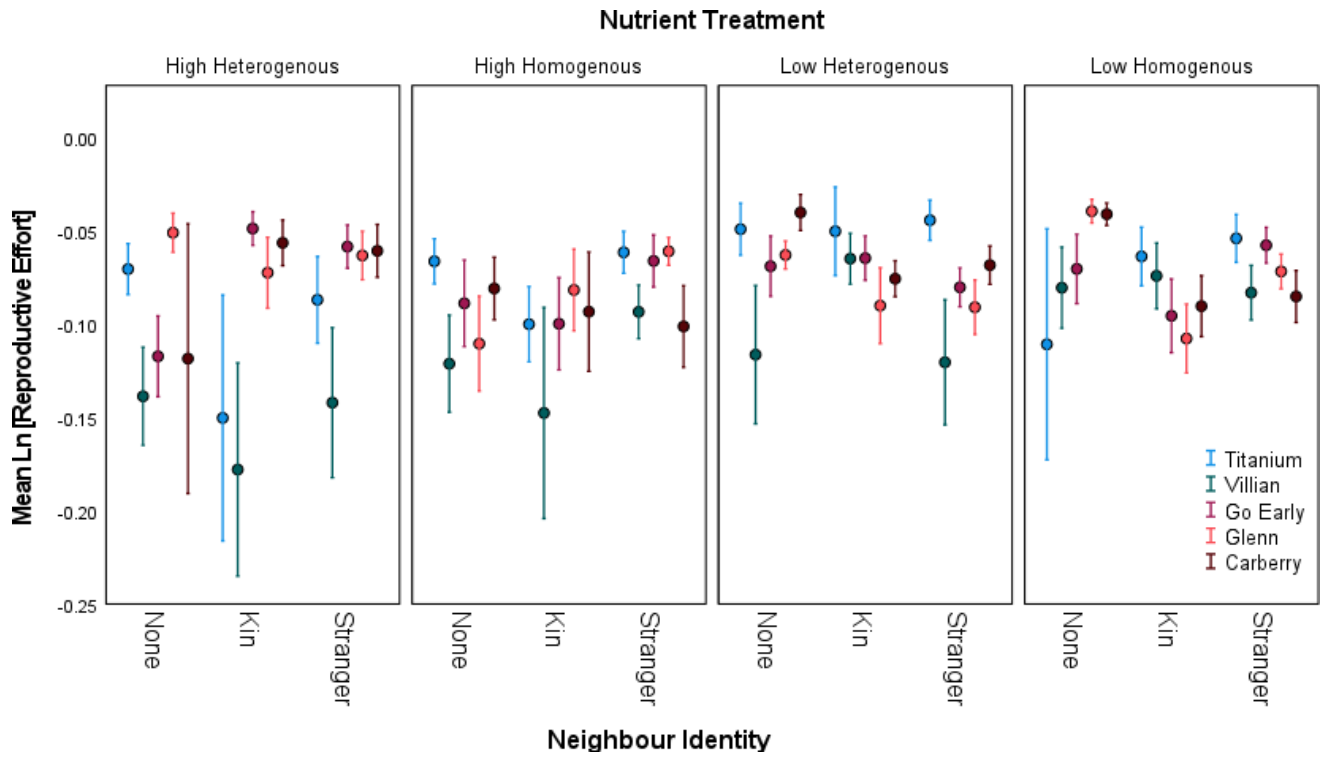


Appendix Figure 1.9. Average (mean \pm standard error) of the natural log of two aboveground measures a) biomass asymmetry [total aboveground biomass grown towards the centre of the pot/biomass grown away] and b) reproductive effort [aboveground biomass consisting of reproductive structures/total aboveground biomass] across the four soil treatments (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous). The means are separated by neighbour identity, either none, kin, or stranger, for each of the five cultivars.

a)

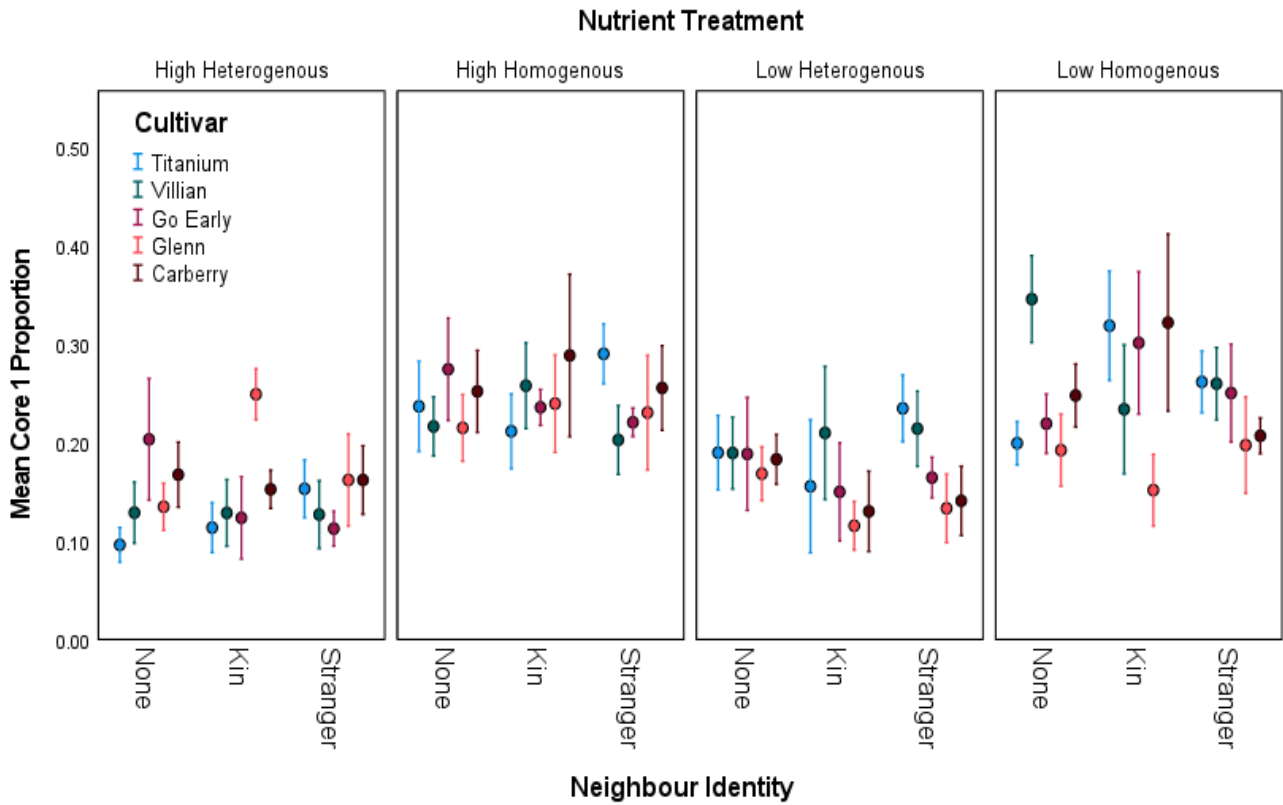


b)

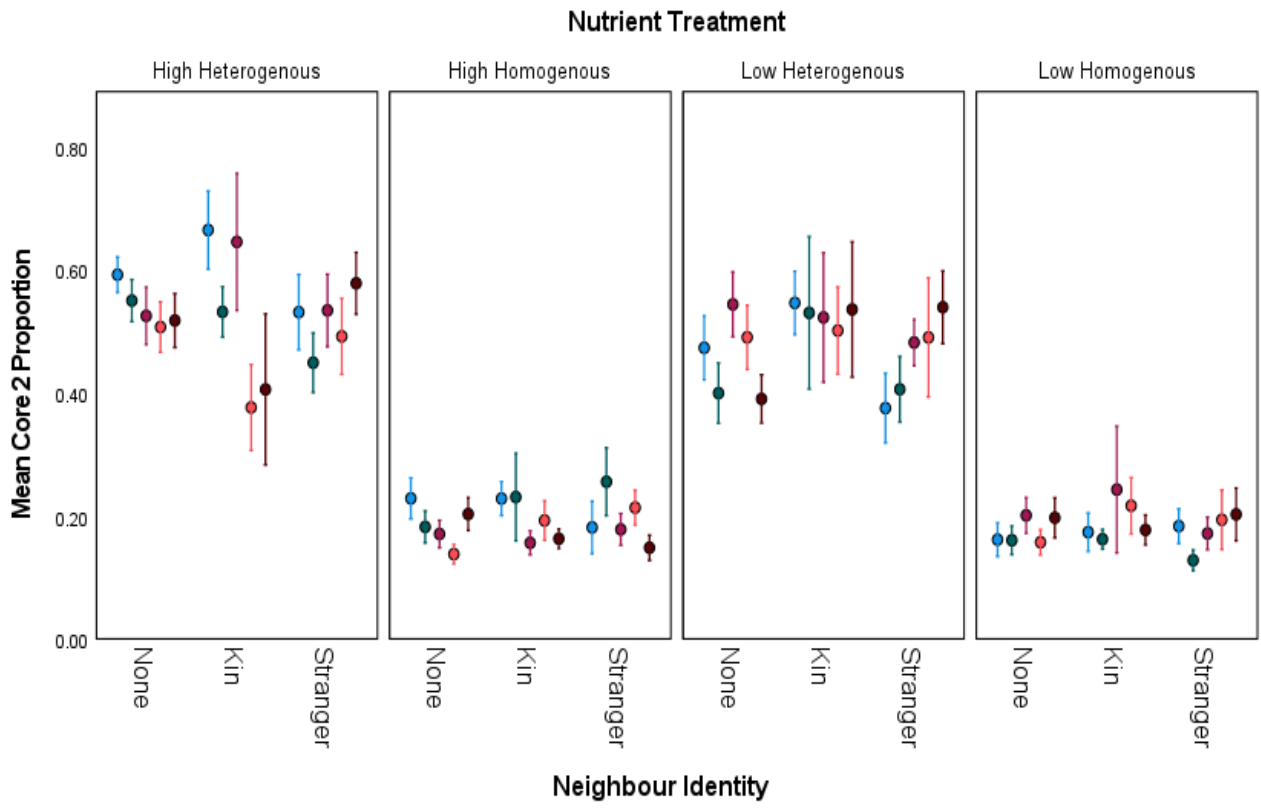


Appendix Figure 1.10. Average of the root biomass grown in each coring location relative to the total root biomass found across all cores in the pot (mean \pm standard error). The graphs, a) core 1, b) core 2, c) core 3, d) core 4, and e) core 5, are broken down by soil treatment (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous). Along the x-axis is the neighbour identity, either none, kin, or stranger, for each of the five cultivars.

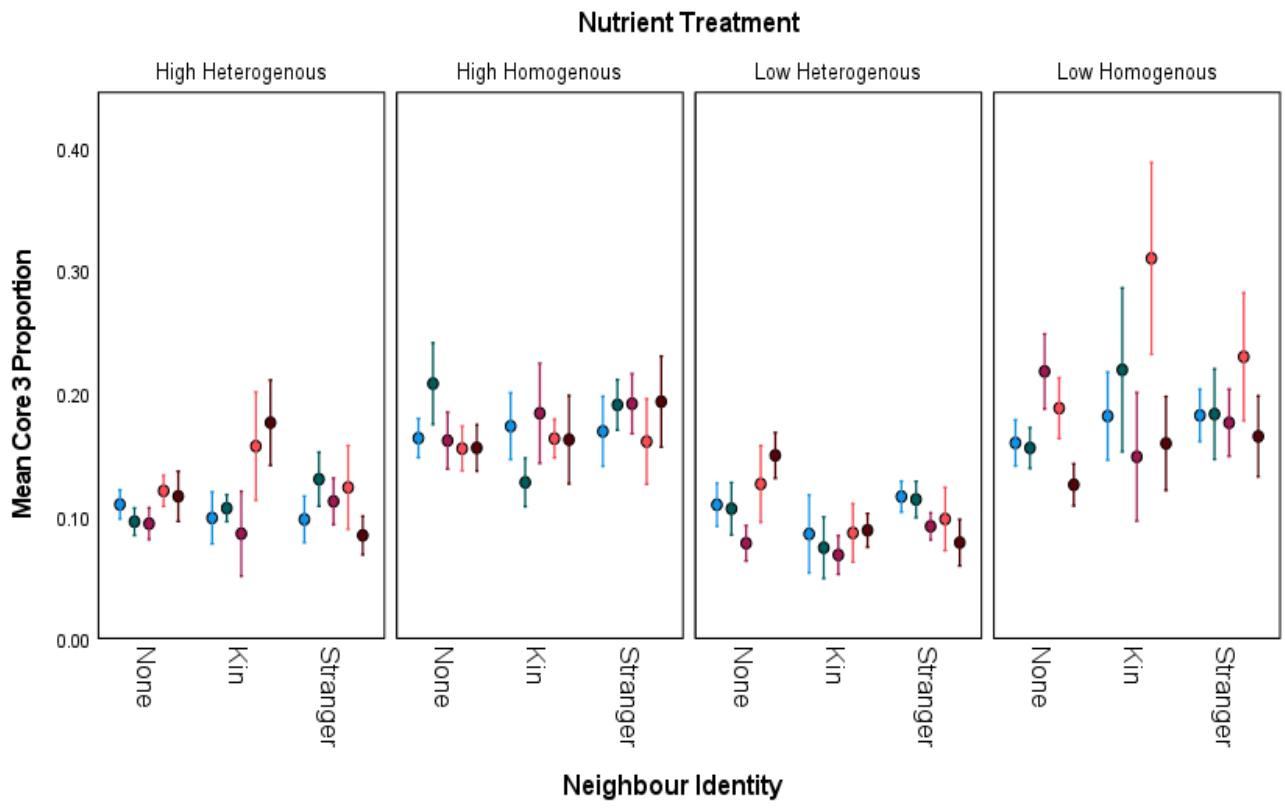
a)



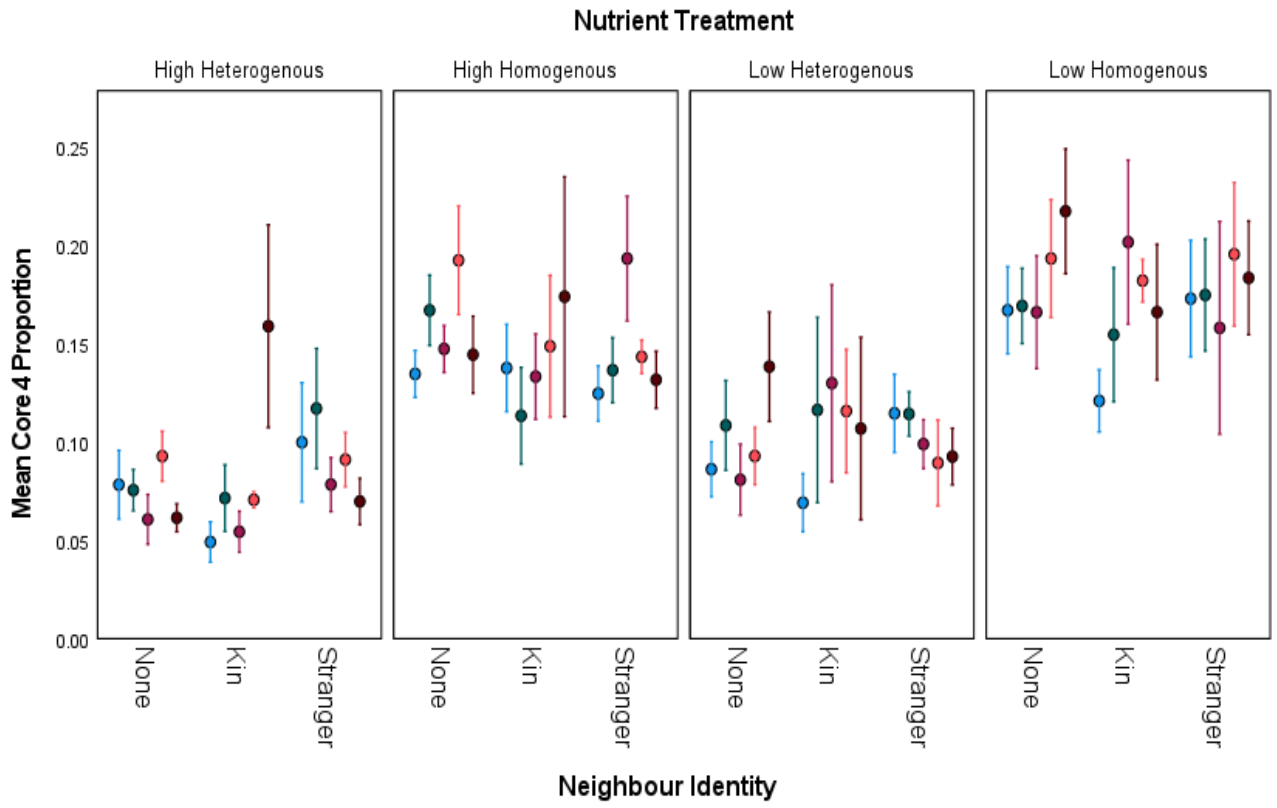
b)



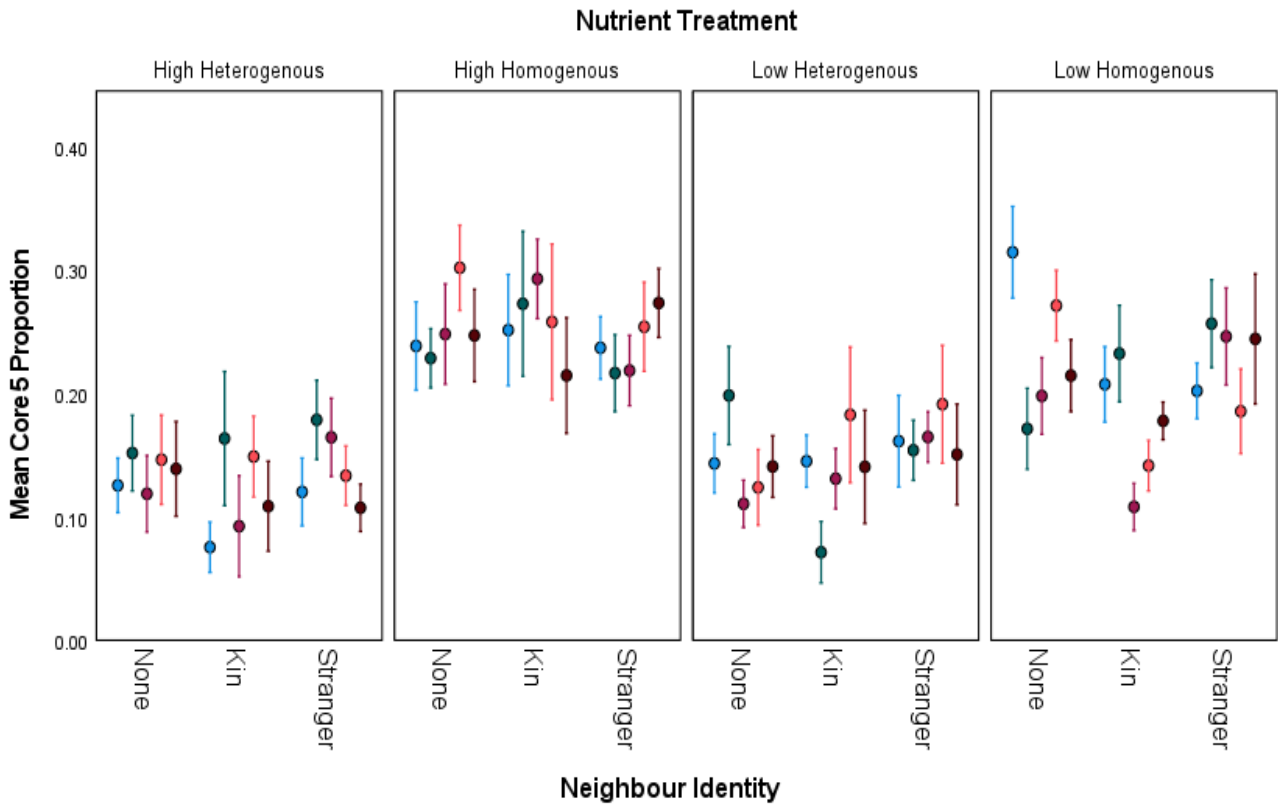
c)



d)

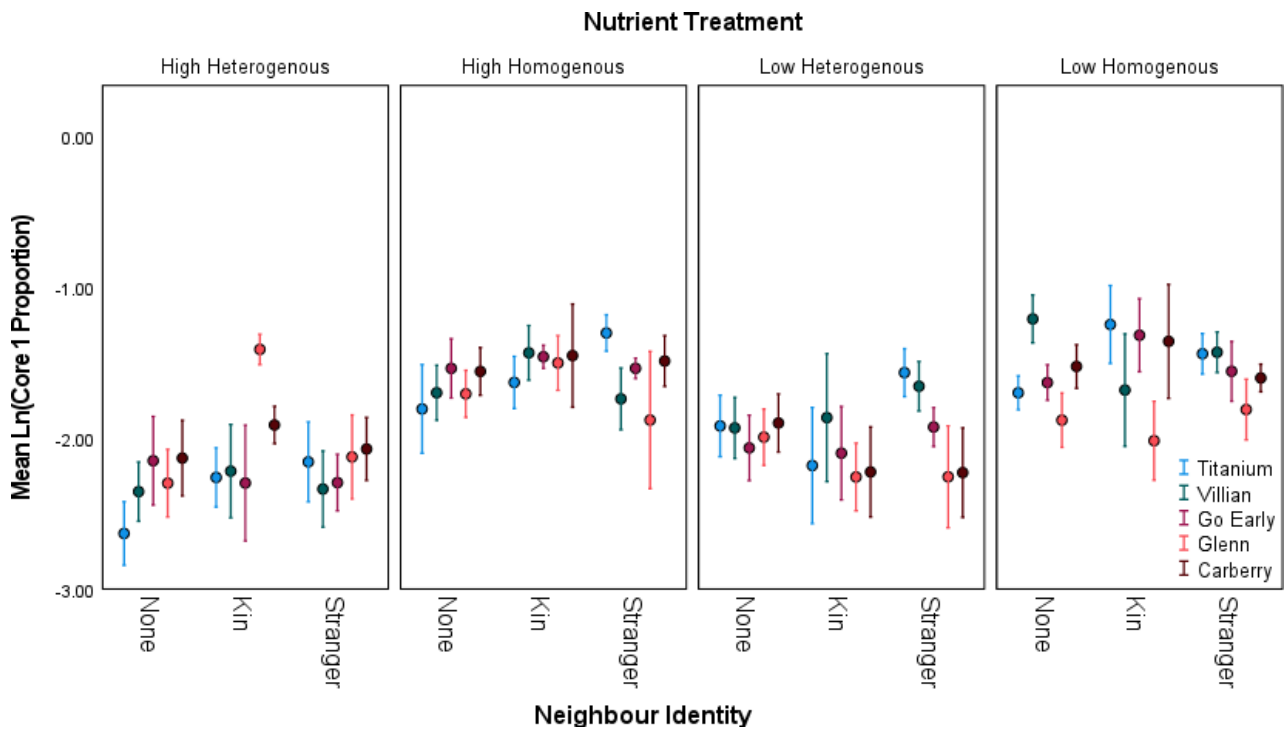


e)

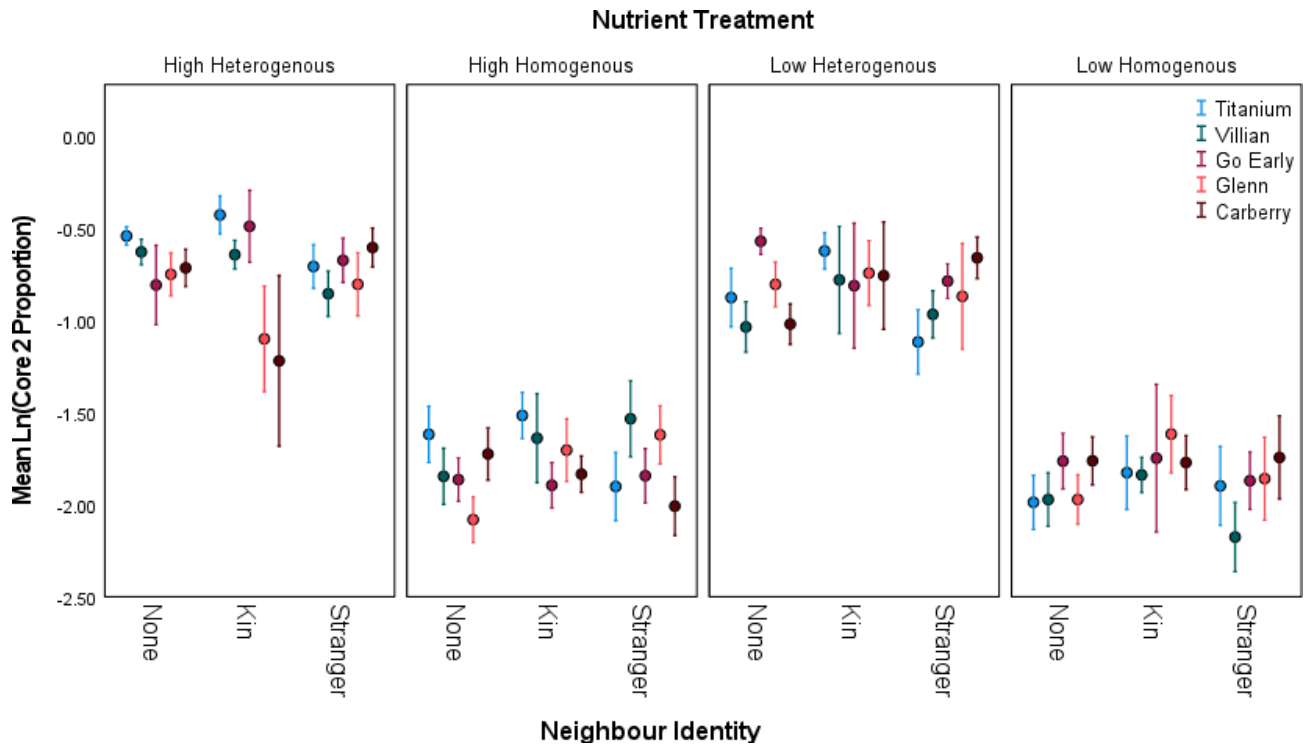


Appendix Figure 1.11. Average of the natural log of root biomass grown in each coring location relative to the total root biomass found across all cores in the pot (mean \pm standard error). The graphs, a) core 1, b) core 2, c) core 3, d) core 4, and e) core 5, are broken down by soil treatment (high heterogeneous, high homogeneous, low heterogeneous, and low homogeneous). Along the x-axis is the neighbour identity, either none, kin, or stranger, for each of the five cultivars.

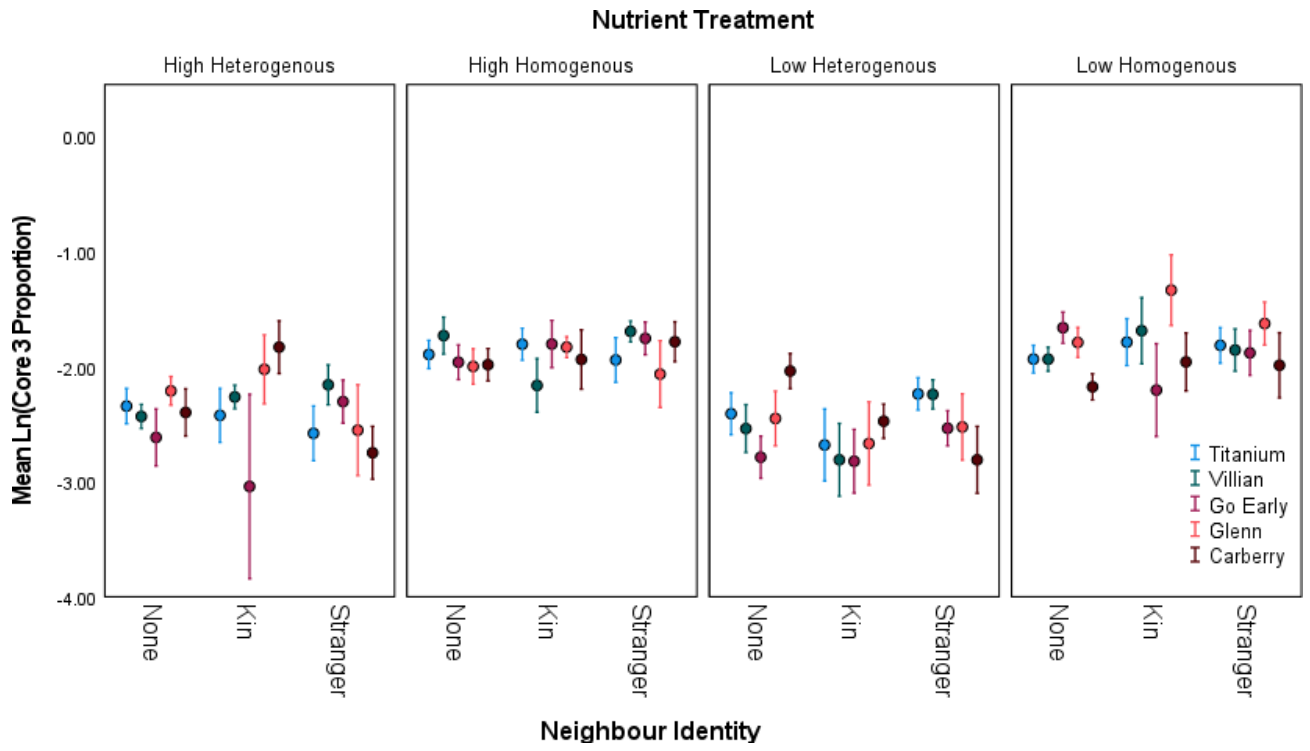
a)



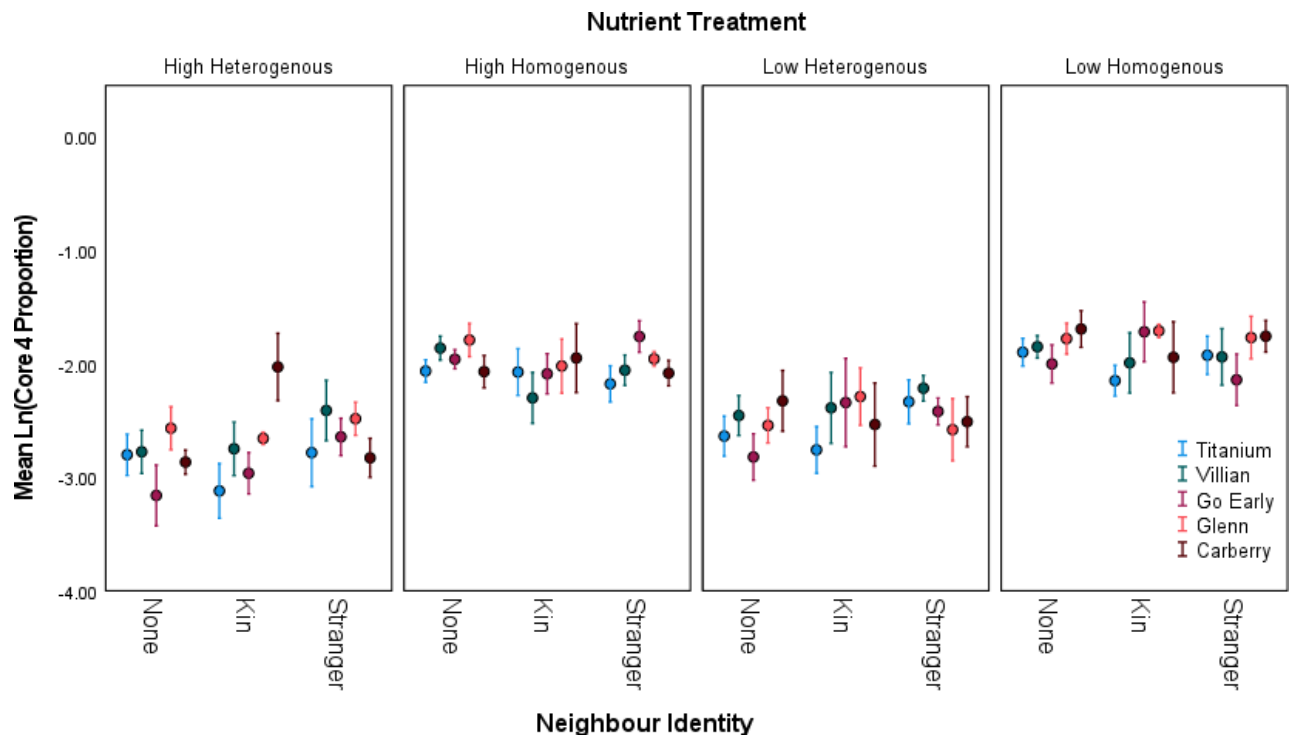
b)



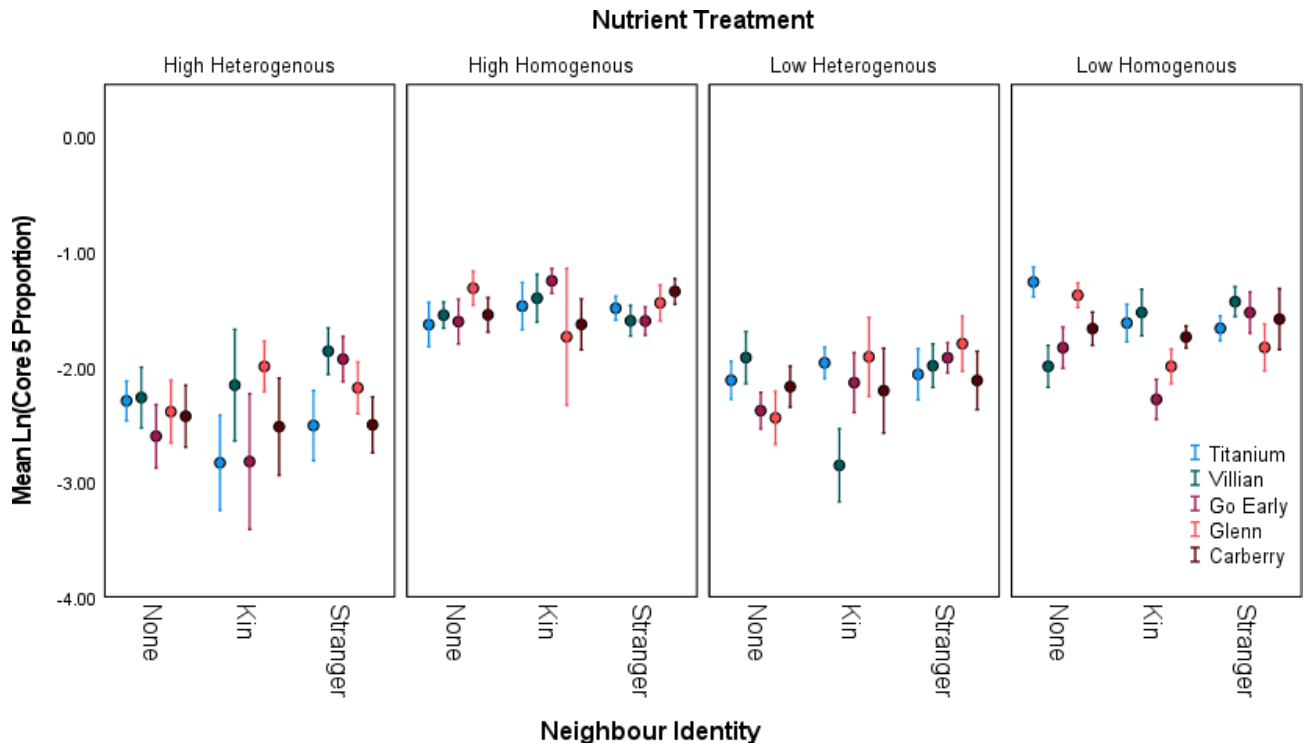
c)



d)



e)



APPENDIX 2 – LACK OF WHEAT, *TRITICUM AESTIVUM*, RESPONSE TO FERTILIZER ADDITION: A CONSEQUENCE OF TOXICITY?

Introduction

As discussed earlier with our rooftop experiment, plants are constantly competing for resources in both natural and agronomic systems, and this often limits fitness, either for the individual or the group. As a result, many plant species have naturally developed strategies to combat this battle for space and resources. However, since these strategies often aim to increase individual fitness in the presence of competition, they could potentially reduce overall population performance (Zhang *et al.*, 1999; Denison *et al.*, 2003; Zhu *et al.*, 2019), which could be detrimental in agriculture.

Interestingly, our rooftop study on five wheat cultivars did not indicate belowground behavioural changes in response to neighbour presence or identity. Yet, our plants exhibited increased root proliferation in highly fertile soil, with decreased growth in less lucrative soil regions. In addition, the plants showed shifts in aboveground biomass in response to nutrient level, soil heterogeneity, and neighbour presence but not identity. However, our rooftop study did not highlight any differences in the reproductive effort as a function of neighbour presence/identity, nutrient addition, or soil heterogeneity. The variation in these responses shows that making predictions on energetic allocation and avoidance or aggression is highly nuanced. Producing accurate estimates for a species would require behavioural assays encompassing a variety of genotypes to account for intraspecific genetic variations in behavioural traits.

Our preliminary study addressed this knowledge gap, but it only looked at a handful of wheat cultivars. This limited cultivar selection allowed for a full-factorial combination of pairwise interactions and four different nutrient treatments with high or low nutrient amounts placed either

homogeneously or heterogeneously in the soil. However, it prevented the ability to look at above- and belowground behaviours for a broad range of *Triticum aestivum* cultivars. Also, since we conducted the study in pots with a maximum of two plants per pot, it did not resemble typical growing conditions for wheat. As a result, we conducted a second study to analyze wheat behaviours in conventional agronomic conditions. For this study, we set up a broad field experiment screening 220 different cultivars of wheat (Appendix Table 2.1), *Triticum aestivum* L. for above- and belowground responses to nutrient addition when grown in a field and subjected to natural light and precipitation cycles. We tested whether the cultivars 1) altered the development of aboveground biomass, 2) altered fitness outcomes as measured through reproductive effort and biomass, and 3) altered the development and distribution of roots towards and away from fertilized soil areas.

Methods

Experimental Design

We planted 220 wheat cultivars (Appendix Table 2.1) in rectangular plots at the University of Alberta South Campus Research Station at 52 Ave NW, Edmonton, AB. The field had been uniformly fertilized via machine before seeding, and staff at the research station seeded each plot with a single cultivar type planted in 6 rows. We obtained seeds for the cultivars from the Spaner Research Lab at the University of Alberta. Detailed information on the pre-experiment nutrient levels and the seeding have been described in a previous study (Chen *et al.*, 2020a). With two replicates per cultivar, we had a total of 440 plots organized in a completely randomized design to account for spatial variation. All these plots were subjected to typical agronomic conditions, with water and light dependent on natural conditions.

Within each plot, we selected two rows of wheat as the focus of our study, one as the experimental row and one as a control. For both, we chose rows of wheat that were the second row from the outside of the plot, such that we could consider the experimental group as row 2 and the control group as row 5, from a top-down perspective (Appendix Figure 2.1). This layout ensured that they would not be experiencing direct edge effects and would allow both to respond to neighbour presence above- and belowground without overlapping in the plot's centre.

Soil treatments and plant growth

We added one level of fertilization (15.67g/L) using slow-release 14:14:14 NPK fertilizer to create a high soil fertility treatment relative to the pre-fertilized background soil. This fertilizer was applied in a single band (2 cm wide by 3 cm deep) equidistant to the experimental row of wheat and the neighbouring row closest to the edge of the plot (Appendix Figure 2.1). We used a precision vegetable planter right after the wheat was planted to ensure consistent application and even mixing with the soil. We did not apply fertilizer to the control group.

Interim Measures

Aboveground, we selected three plants from both the experimental and control rows of wheat for a total of 6 plants per plot. In both rows, one plant was chosen near the beginning, the second was in the middle (1.5 m away from the first plants), and the third was at the other end (1.5 m away from the central plants) (Appendix Figure 2.1). Thus, with six plants per plot and 440 plots, we had a total of 2640 plants. Unfortunately, some of these plants experienced mortality during the study due to natural causes and accidental herbicide placement, resulting in 2614 plants used in our analyses.

We took measures twice before harvest: first from June 24th to the 28th, 2018 and then from July 15th to 19th, 2018, three weeks after taking the previous set of measures. Our measurements

included height (cm) and greenness along a scale of 1 to 3, with 1 indicating extreme brown/yellow plants, 2 displaying some loss of colour, and 3 meaning total greenness.

Harvest

After the wheat grew for 13 weeks (from May 13th to August 12th, 2019), we clipped all the plants used for interim measures at the soil surface. Thus, we collected three plants in both the experimental and control rows. Separating the biomass for each specimen into two categories, we harvested both the reproductive and non-reproductive elements of each plant and then dried them at 65°C and weighed them.

We also took root samples in the plots using sledgehammers and metal cores 2.5 inches in diameter. We took three cores on either side of the row with the same spacing as the aboveground samples for both the experimental and control rows. Hence, the first core was taken near the beginning, the second was in the middle, and the third was at the other end (Appendix Figure 2.1). We then pooled the root samples, combining the soil from the fertilized area into one larger sample in the experimental row. Then we did the same with the unfertilized soil from the other side of the aboveground samples. Pooling the samples created a total of two soil samples for the experimental row. The samples from the control group were similarly pooled, resulting in 4 overall root samples per plot. With 440 plots, this created 1760 soil samples to process that were washed over a 1mm sieve, dried at 65°C, and weighed.

Field Experiment

We assessed biomass development, fitness metrics, and root proliferation using IBM SPSS Statistics 27. Due to the low number of replicates, only two, our analyses combined all the 220 cultivars when conducting the independent sample t-tests for each response variable.

To look at biomass development aboveground, we analyzed the mean total biomass present in the experimental versus the control rows. The reproductive effort was calculated to determine the allocation to reproduction: [biomass of reproductive structures/total aboveground biomass produced]. The higher this value, the greater the allocation towards reproduction and fitness by the plant. We also looked at reproductive yield, so we analyzed the mean reproductive biomass in the experimental and control rows.

Belowground, we looked at the allocation of roots in the pooled fertilized soil sample compared to the pooled unfertilized soil sample. We calculated this as a ratio: [biomass of roots in fertilized soil/ un-fertilized soil]. The greater this ratio, the greater the proliferation of roots in the fertile ground over the background, lower-nutrient soil. A value of 1 would indicate equal growth in both soil types, while a value less than 1 would reflect under-proliferation in the fertilized soil.

Study Comparison

To determine differences in cultivar behaviour between our rooftop experiment with arenas containing combinations of neighbours, soil fertility, and nutrient homogeneity and this experiment, we had to ensure we were evaluating comparable plants across the two studies. Of the five cultivars present in the rooftop study, CDC Titanium, Carberry, Go Early, Glenn, and Villian, only four were used in the field study. Hence, we excluded Villian from the comparisons made. Additionally, the plants in the field study were monoculture plots, and kin neighbours surrounded

them, so we only included pots from the rooftop experiment with a kin neighbour. Also, since nutrient placement was heterogeneous in the field experiment, we only used rooftop pots with a heterogeneous treatment (i.e., a patch).

Though we did not conduct any statistical analyses, we did produce a series of bar graphs for height, total aboveground biomass, reproductive effort, reproductive biomass, and root placement in fertilized versus unfertilized soil with standard error bars.

Results

Field Experiment

When looking at scatterplots of all the cultivars for the aboveground measures, height and total aboveground biomass (Appendix Figure 2.2 a, b), it is evident that the cultivars fall across a spectrum with only a few falling outside of the typical range. Looking at height, the cultivars Alikat, Biggar, and Oslo were shorter than the rest, while Chester and CDC Alsask were taller (Appendix Figure 2.2 a). With biomass, only AAC Connery was drastically different, with a much higher biomass (Appendix Figure 2.2 b). Interestingly, our results indicated that the aboveground biomass was not affected by the application of fertilizer. As seen in Appendix Figure 2.3 a, there are no differences in the means between the control and fertilized experimental rows. The figure also shows more variation in the biomass produced in the control group relative to the experimental one. The independent sample t-test confirms this ($df = 2614$, $t = -0.942$, $p = 0.346$, Appendix Table 2.2).

No cultivars exhibited relatively high results in the scatterplot for reproductive effort, but Alikat, CDC Bradwell, Somerset all had low effort (Appendix Figure 2.2 d). With reproductive biomass, again, only AAC Connery was substantially larger than the rest (Appendix Figure 2.2 c).

This cultivar was removed from the statistical analysis for reproductive biomass as it was over 78 times larger than the next largest reproductive biomass, leaving 2613 samples. When looking at reproduction, neither reproductive effort nor biomass appeared to be affected by fertilizer addition (Appendix Figure 2.3 b, c). The means and standard error between the two groups were similar for both variables. In addition, the independent sample t-tests (Appendix Table 2.2) for both metrics confirmed that there was no difference between the fertilized and non-fertilized wheat for either (reproductive effort: $df = 2614$, $t = -1.451$, $p = 0.147$; reproductive yield: $df = 2613$, $t = 0.770$, $p = 0.442$).

Finally, when analyzing the belowground proliferation, our results indicate a difference between the control and experimental groups. However, as Appendix Figure 2.3 d shows, the row without the fertilizer applied had greater growth in the 'fertilized' area relative to the 'unfertilized' area compared to the experimental treatment, where it was more evenly split. This is significantly significant, as seen in Appendix Table 2.2 ($df = 878$, $t = -2.032$, $p = 0.042$). The scatterplot for the ratio of roots placed in fertilized versus unfertilized soil shows that AC Abbey had the least root proliferation in the fertile soil, indicating avoidance (Appendix Figure 2.2 f). On the other hand, AAC Iceberg, Sumai, AAC Whitefox, and BW970 Pro had substantial over-proliferation in the fertilized soil (Appendix Figure 2.2 f). Again, when looking at root biomass, AC Abbey was smaller than the other cultivars (Appendix Figure 2.2 e). At the same time, Glenavon, Leader, Snowwhite 476, AC Meena developed more than average root biomass (Appendix Figure 2.2 e).

Study Comparison

With average height, the cultivars were consistently far larger in the field setting than in the pot experiment (Appendix Figure 2.4a). The figure also shows slight differences in the mean heights across the cultivars. The field study yielded more biomass than the rooftop study

(Appendix Figure 2.4b). When making inter-cultivar comparisons, Go Early produced less biomass than the other cultivars when in the field, but it and Glenn produced more biomass than the others in the rooftop experiment, which is interesting.

The trend continues in terms of reproductive biomass, with the field study producing more biomass than the pot study (Appendix Figure 2.4c). When comparing across cultivars, Go Early has considerably less biomass than the others in the field study, but like the total biomass, Go Early and Glenn produced more than Carberry and CDC Titanium in our first study. Then with reproductive effort, the two studies are very similar, though the field study is slightly higher for all the cultivars except Go Early; this could be due to its high variance (Appendix Figure 2.4d). Between the cultivars, differences in the reproductive effort are practically negligible.

Finally, looking at belowground measures, the ratio of root placement in fertile over unfertilized background soil was strikingly higher in the rooftop experiment (Appendix Figure 2.4e). With 1 indicative of no response, or equal root distribution in both soil types, the field study shows that the plants were apathetic in their root placement. However, the rooftop experiment with the same cultivars shows considerable over-proliferation in the fertilized soil, even with sizeable standard error bars. Due to significant variances, however, it does not appear that there are substantial differences between the cultivars for either experiment.

Discussion

Field Experiment

When looking at the aboveground biomass, reproductive biomass, and reproductive effort, we found that changes in the soil structure impacted none. Hence, increasing soil fertility did not cause any discernable effects aboveground. However, belowground, this is not the case. There was

an even spread between the fertilized and unfertilized soil for the experimental group when looking at proliferation. In contrast, the control group had more growth towards the centre of the plot. Prior literature (Brady *et al.*, 1993; VanVuuren *et al.*, 1996; McNickle & Cahill Jr, 2009) and the *T. aestivum* in our first study have shown distinct above- and belowground responses to nutrient addition, making this finding quite interesting.

This lack of response could be due to a toxicity effect from the over-application of fertilizer. The plots were uniformly fertilized prior to seeding and this, in combination with the nutrient treatment, appears to have over-saturated the soil space, causing potential soil acidification through the Nitrogen excess (Raza *et al.*, 2020). As a result, the attraction of the fertile soil would have been counter-acted by the toxicity of excess nutrients. As a result, the roots would not over- or under-proliferate in the region, creating a neutral response overall.

Therefore, the total aboveground and reproductive biomass development and allocation were unaffected because the wheat cultivars were not maximizing the fertilizer use belowground, treating it like background soil. If the wheat had recognized the fertilized soil without the adverse effects of over-fertilization, we would have anticipated results akin to our first study for all aboveground metrics. Hence, we lack conclusive evidence of the impacts of nutrient addition to *Triticum aestivum* cultivars grown in standard agronomic conditions.

Study Comparison

When comparing both studies, the field study yielded larger results than the rooftop experiment for aboveground biomass, height, and reproductive biomass (Appendix Figure 2.4). This difference is likely due to increased space for the plants in the field experiment and a longer growing season. The field study plants grew for 13 weeks, while we ran the rooftop experiment for six weeks before the wheat roots became pot-bound. The ability to grow without the spatial

constraints of the pot study for double the amount of time would allow for larger plants, impacting all the aboveground metrics. For reproductive effort, however, the results between the two studies are more similar, which may be because the metric is not an absolute value but the proportion of aboveground biomass constituting reproductive elements. So, while our results show that the cultivars increased energy allocation to reproduction in the field, this difference is not as stark as the other metrics, likely due to the nature of the variable.

When looking at root proliferation, the toxicity effects of over-fertilization are evident in the comparisons between the two studies. Here, the trend flips so that the ratio of root placement in fertile over unfertilized background soil was strikingly higher in the rooftop experiment for all four cultivars.

Between the cultivars, Go Early was consistently smaller than the others when grown in the field. Yet, it, along with Glenn, was larger when grown in the pots. This result could indicate early germination and rapid growth followed by slower growth until reaching maturity earlier than the other cultivars (Chen *et al.*, 2020b). Conversely, CDC Titanium and Carberry are exhibiting the opposite trend, with potential lag in growth for at least the first six weeks post-seeding and then faster development, resulting in overall larger plants. Nevertheless, CDC Titanium and Carberry have exhibited the same trends regarding growth and yield compared to Glenn and Go Early, in a 2020 paper by Chen *et al.*, suggesting the trends are consistent for the genotypes.

These cultivar differences could also be due to the spatial constraints the pot study imposed on the plants. The lack of belowground room may have hindered Go Early to a lesser extent than the other cultivars. Thus, though overall smaller in the pots than in the field study, this cultivar may prove to be slightly better adapted to constrained spaces. When looking for maximal growth

and output, particularly in a field setting, Glenn, CDC Titanium, and Carberry had comparable results in our studies.

Conclusion

This study aimed to provide a large-scale picture of the behavioural traits of *T. aestivum* above- and belowground when faced with heterogeneous nutrient addition while in an agronomic setting. By adding fertilizer in already-fertilized soil, we may have inadvertently created soil toxicity, potentially through acidification from excess Nitrogen. This error resulted in no discernable behavioural responses above- or belowground, with plants appearing apathetic to the nutrient addition. When comparing the results to our preliminary study, we found that the field study produced larger plants overall, most likely due to more space and a longer growing period. However, when looking at cultivar-specific patterns between the studies, there were shifts in where the cultivars fell along the spectrum of traits. For example, Go Early was typically on the larger side during the pot mesocosm study but was considerably smaller than the other cultivars when subjected to field conditions.

Though the field study and subsequent comparisons provide some insight into the growth patterns and results for an array of *T. aestivum* cultivars, there are areas where the field study could be improved, which would open avenues for future research. For example, increasing the number of replicates from two per cultivar and then applying various nutrient amounts would increase statistical power while potentially solving the toxicity issue we created here. Furthermore, looking at behavioural responses along a gradient of nutrient addition would provide us with more nuanced information on competition and kin selection within wheat. In addition, future research could look at comparisons between modern cultivars and traditional landraces. This comparison could point at differences in behaviour, particularly regarding kin recognition and selection, that have evolved

due to natural and artificial selection. Similarly, using other types of wheat, such as durum (*Triticum durum*), or other crops typically sown in monocultures, such as rice, barley, and canola, could provide insight into the evolutionary development of behavioural traits and guide our ever-evolving understanding of plant behaviour.

Tables and Figures

Appendix Table 2.1. Cultivars used in the field study. Placement in the field, as indicated by plot number, was randomly assigned to each cultivar. Thousand kernel weight, which is the weight in grams of 1,000 seeds, is also included for seed size reference.

Plots	Cultivar	Thousand Kernel Weight
160, 255	25SAWYT_329(WAXWING/2*ROLF07//BORL14)	40
189, 377	25SAWYT_340(FRANCOLIN #1//WBLL1*2/BRAMBLING*2/3/COPIO)	40
44, 311	25SAWYT_348(MEX94.27.1.20/3/SOKOLL//ATTILA /3*BCN/5/GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92)	40
52, 318	25SAWYT_350(SUP152/6/OASIS/5*BORL95/5/CNDO /R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI)	40
5, 378	5602HR	38.6
54, 405	5603HR	36.9
11, 329	5604HR CL	34.2
177, 249	5605HR CL	36.1
201, 334	5700PR	47.2
37, 437	5701PR	46.8
211, 258	5702PR	47.6
200, 252	AAC Bailey	41
154, 263	AAC Brandon	40.4

130, 416	AAC Chiffon	33.6
73, 254	AAC Connery	40
193, 352	AAC Crossfield	43.2
168, 261	AAC Crusader	51.2
147, 394	AAC Elie	37.8
62, 320	AAC Entice	42.6
208, 300	AAC Foray	45.8
123, 294	AAC Iceburg	35.6
155, 425	AAC Innova	48.6
61, 337	AAC Penhold	45
102, 365	AAC Proclaim	38.6
159, 316	AAC Redwater	35.6
121, 381	AAC Ryley	33.9
59, 433	AAC Tenacious	38.7
167, 324	AAC Viewfield	38.1
187, 307	AAC Whitefox	37.1
94, 436	AC Abbey	41
2, 400	AC Andrew	38.8
38, 350	AC Barrie	39.2
76, 340	AC Cadillac	41
127, 315	AC Cora	37.3
50, 238	AC Corinne	46.4
157, 229	AC Crystal	45.8

136, 346	AC Domain	35.5
116, 234	AC Eatonia	37.5
67, 251	AC Elsa	37.4
41, 344	AC Foremost	50.7
150, 242	AC Intrepid	41
183, 317	AC Karma	44.1
17, 358	AC Majestic	37.1
28, 292	AC Meena	36.4
165, 333	AC Michael	35.1
156, 331	AC Minto	48.2
75, 223	AC Nanda	40.6
105, 391	AC Phil	39.6
220, 222	AC Reed	40.9
64, 302	AC Splendor	38.4
72, 420	AC Taber	45.4
181, 227	AC Vista	47.8
86, 259	AC2000	44.2
202, 374	Alikat	36.9
112, 306	Alvena	39.4
212, 266	Amazon	48.8
213, 314	Benito	32.8
68, 369	Bhishaj	40.1
34, 265	Biggar	46.2

184, 293	Bluesky	34.7
95, 269	Burnside	45.6
91, 232	BW1039	44.3
120, 260	BW278	32.5
109, 368	BW488	37.5
30, 275	BW493	47.7
8, 355	BW496	36.6
178, 379	BW5005	38.7
128, 297	BW5018	40.3
198, 295	BW5020	34.6
3, 421	BW963	39.8
24, 319	BW966	37.4
96, 367	BW968	43.1
118, 383	BW970 Pro	36
129, 370	BW971	42.4
164, 278	BW986	42.3
207, 231	BYT13-18	34.6
27, 408	BYT13-23	41.7
194, 362	BYT14-11	37.4
180, 360	BYT14-19	34.1
114, 385	Canthatch	36.8
15, 312	Canuck	42
175, 288	Carberry	38.8

195, 276	Cardale	34.9
182, 349	CDC Abound	37.7
87, 228	CDC Alsask	38.8
131, 237	CDC Bounty	39.9
148, 247	CDC Bradwell	35.4
14, 271	CDC Go	45.8
74, 412	CDC Imagine	38.6
70, 235	CDC Kernen	43.4
48, 430	CDC Makwa	36
171, 264	CDC Merlin	39.1
51, 429	CDC NRG003	46
204, 396	CDC Osler	35.7
45, 354	CDC Plentiful	37.4
92, 399	CDC Primepurple	45.8
43, 305	CDC Rama	51.3
25, 270	CDC Stanley	36.4
29, 384	CDC Teal	38.2
81, 382	CDC Thrive	36.6
33, 413	CDC Titanium	43
69, 375	CDC Utmost	34.7
110, 440	CDC VR Morris	36.2
6, 268	CDC Walrus	44.4
82, 335	CDC Whitewood	38.4

143, 321	CDN Bison	48.7
192, 325	Chester	39.4
23, 285	Coleman	37.2
57, 253	Columbus	42.1
119, 244	Conquer	44.6
107, 423	Conway	37.2
106, 273	Cutler	41.6
176, 419	Cypress	35.8
132, 427	Enchant	51.4
133, 415	Faller	44.6
215, 438	Fielder	46.4
88, 388	Fieldstar	34.4
55, 418	FL62R1	36
13, 409	Garnet	31.1
42, 392	Glenavon	47.1
117, 290	Glencross	44.4
93, 291	Glenlea	48
1, 313	Glenn	43
161, 274	Go Early	39.9
170, 310	Goodeve	38.1
173, 281	GP112	36.6
19, 283	GP122	42.1
78, 328	GP131	51.2

100, 239	GP146	39.6
124, 299	GP151	41.6
65, 356	GP168	40.1
219, 323	GP183	37.1
210, 366	GP184	42.8
36, 364	GP193	42.1
108, 389	Grandin	42.6
138, 422	Harvest	38.2
63, 332	Helios	41
60, 233	HY2003	45.7
135, 428	HY2013	33.9
115, 395	HY2021	50.1
9, 411	HY320	50.2
206, 225	HY355	43.7
140, 426	HY537	45.8
199, 341	Infinity	35.6
49, 424	Invader	47.6
216, 363	Journey	35.6
101, 338	Kanata	37.4
217, 336	Kane	37.6
139, 303	Katepwa	36.6
31, 372	Kenyon	34.4
185, 326	Lancer	38.2

16, 347	Laser	46.8
162, 345	Laura	37.4
97, 301	Leader	35.6
151, 417	Lillian	40.3
18, 236	Lovitt	37.7
142, 267	Manitou	34.6
4, 327	Marquis	36.9
174, 403	McKenzie	36.4
90, 279	Minnedosa	46.4
85, 434	Muchmore	42.7
188, 230	Napayo	35.5
113, 431	Neepawa	35.4
152, 296	NH004	38
196, 402	Oslo	40.4
104, 406	Owens	37.5
79, 262	Parata	37.4
145, 309	Park	39.1
214, 289	Pasqua	36.2
111, 246	Pasteur	37.8
103, 376	Peace	41.3
10, 308	Pembina	34.8
40, 380	Pitic 62	45.3
22, 342	Prodigy	35.1

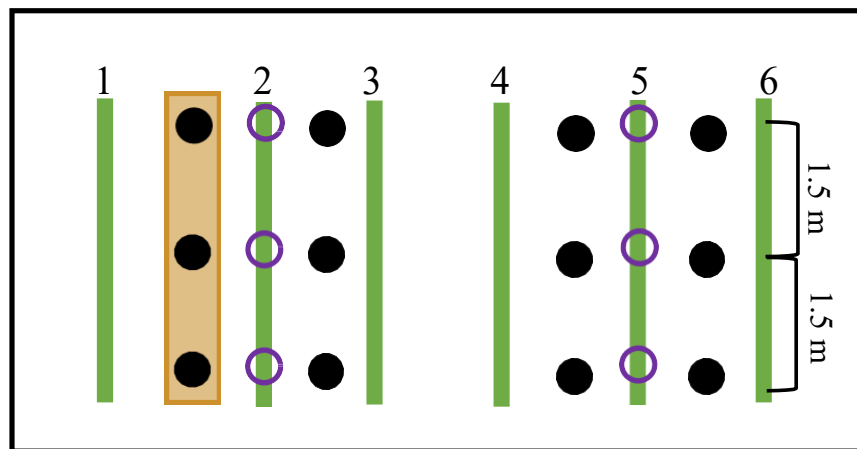
12, 414	Prosper	50.1
137, 397	PT250	41.8
126, 386	PT472	40.1
141, 240	PT479	34.8
53, 245	PT485	40.8
98, 353	PT588	45.4
58, 357	PT595	46.5
134, 439	PT650	37.2
205, 284	PT771	32.1
203, 287	PT778	45.6
80, 435	PT780	39.5
83, 371	PT781	41.1
190, 243	PT782	37.8
163, 330	PT783	35.3
209, 280	PT784	36.1
191, 277	PT785	34.6
32, 361	Red Bobs	37.6
149, 432	RL6077	32.1
122, 398	Roblin	39.2
158, 410	SAAR	51.4
21, 404	Sadash	43.2
47, 359	Shaw	41.2
71, 250	Sinton	42.6

20, 373	Snowbird	38.1
77, 322	Snowwhite 475	46
56, 387	Snowwhite 476	47
89, 224	Snowstar	33.4
197, 221	Somerset	40.4
7, 298	Springfield	34
46, 226	Stettler	36.6
172, 351	Sumai-3	35.1
39, 286	Superb	45.4
66, 393	SWS52	48.1
186, 257	SY087	40.3
179, 339	SY433	33.6
153, 343	SY479	36.6
35, 304	SY637	36.8
146, 282	SY985	46.4
99, 256	SY995	45.2
84, 348	Thorsby	39.3
26, 390	Unity (PURE SM1)	38.4
144, 407	Vesper	41.6
166, 241	Waskada	36.8
218, 272	Whitehawk	33.9
125, 248	Wildcat	37.5
169, 401	WR859CL	37.1






Appendix Table 2.2. Results of independent sample two-tailed t-tests looking at the effect of nutrient addition on aboveground and reproductive biomass production, reproductive effort [aboveground biomass consisting of reproductive structures/total aboveground biomass], and belowground biomass placement [root biomass in fertilized soil/root biomass in unfertilized soil]. Only belowground biomass placement came out significant.

	d.f	t-value	p-value
aboveground biomass	2614	-0.942	0.346
reproductive biomass	2613	0.770	0.442
reproductive effort	2614	-1.451	0.147
belowground biomass placement	878	-2.032	0.042

Appendix Figure 2.1. Field design illustrating the two rows of wheat used in the field study, row 2 (the experimental group) and row 5 (the control). Fertilizer was applied in a band to one side of the experimental group, with none applied to the control. We selected three plants from each row to measure throughout the growing season and harvest at the end. We took belowground root samples via coring at harvest, with three cores taken on either side of the focal rows of wheat and then pooled these cores for analyses.

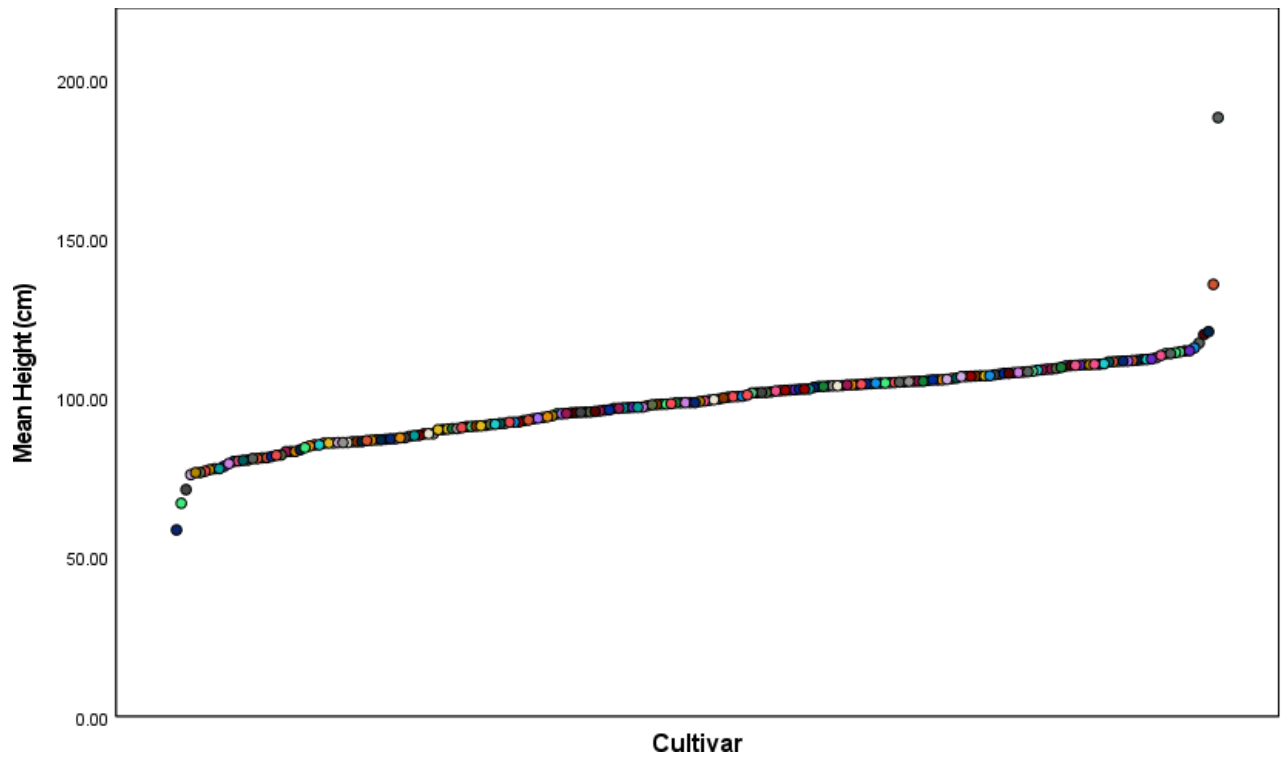


Legend:

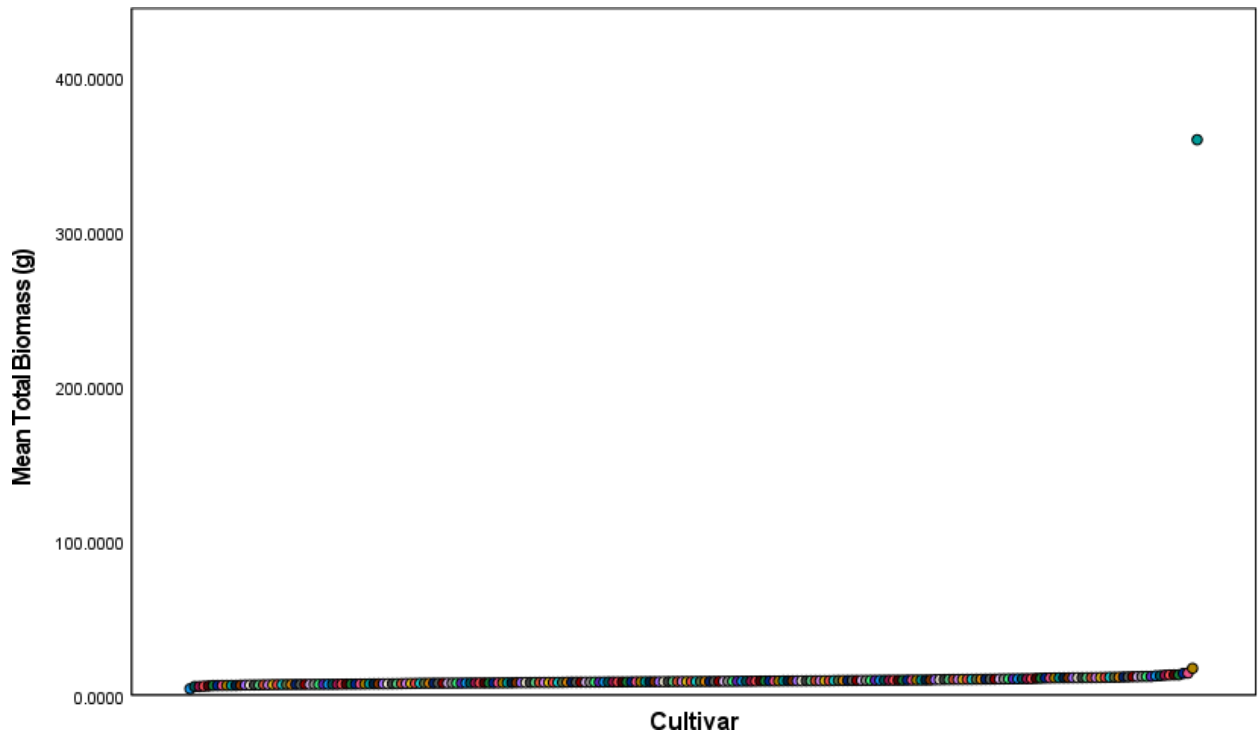
-  = one plot
-  = wheat row
-  = nutrient band
- # = row number
-  = plant measured and harvested
-  = root core taken at harvest

Appendix Figure 2.2. Scatterplots of the a) height, b) total aboveground biomass, c) reproductive biomass, d) reproductive effort, e) total belowground biomass, and f) belowground root distribution in fertilized over unfertilized soil.

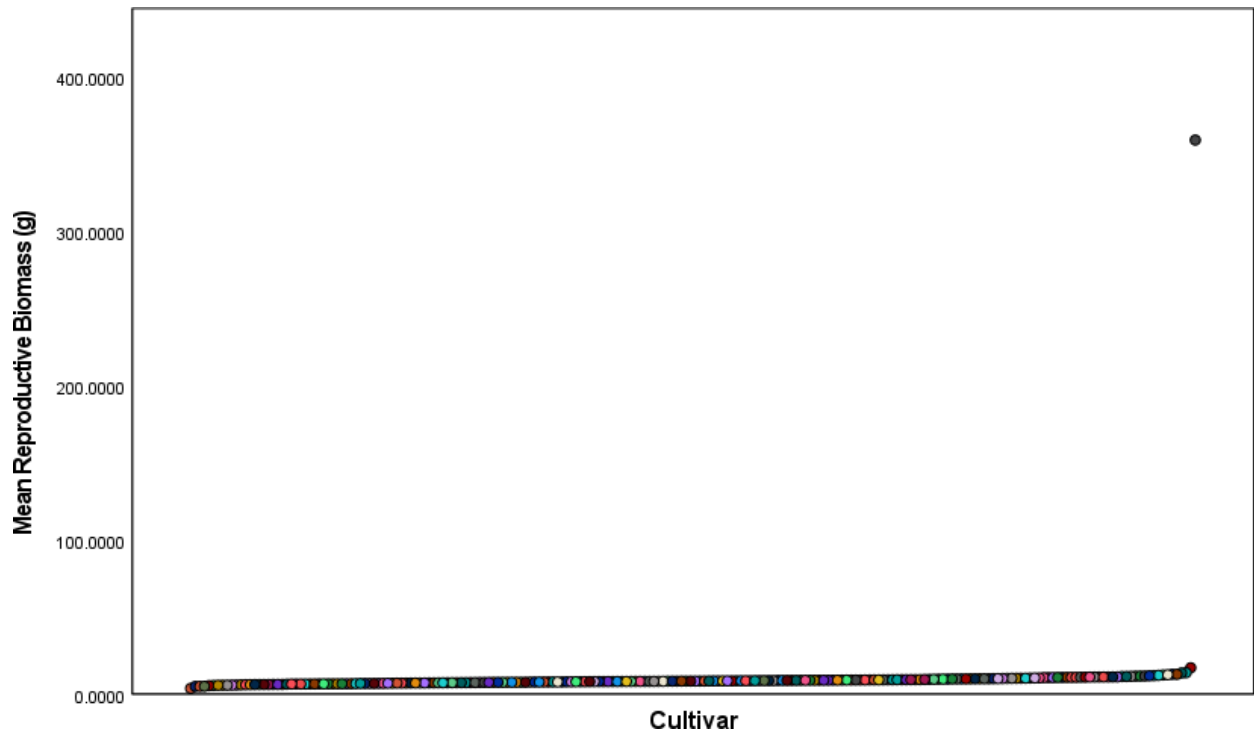
a)



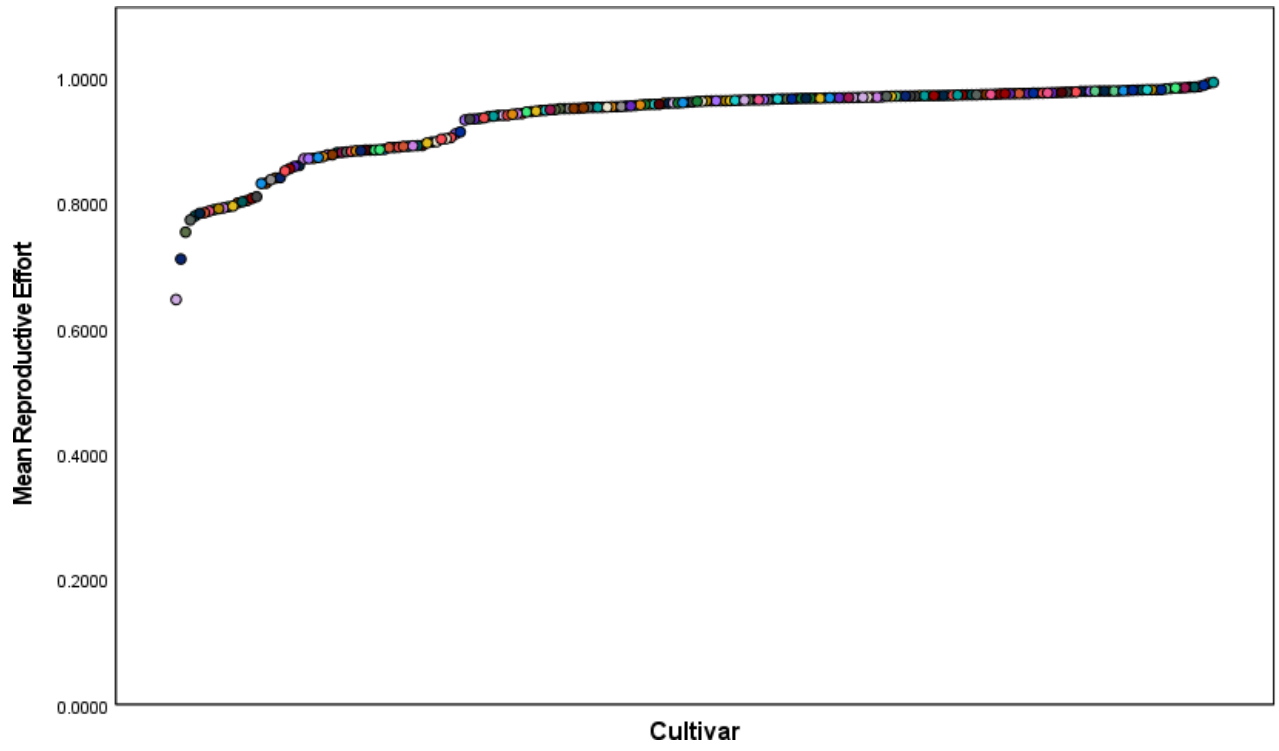
b)



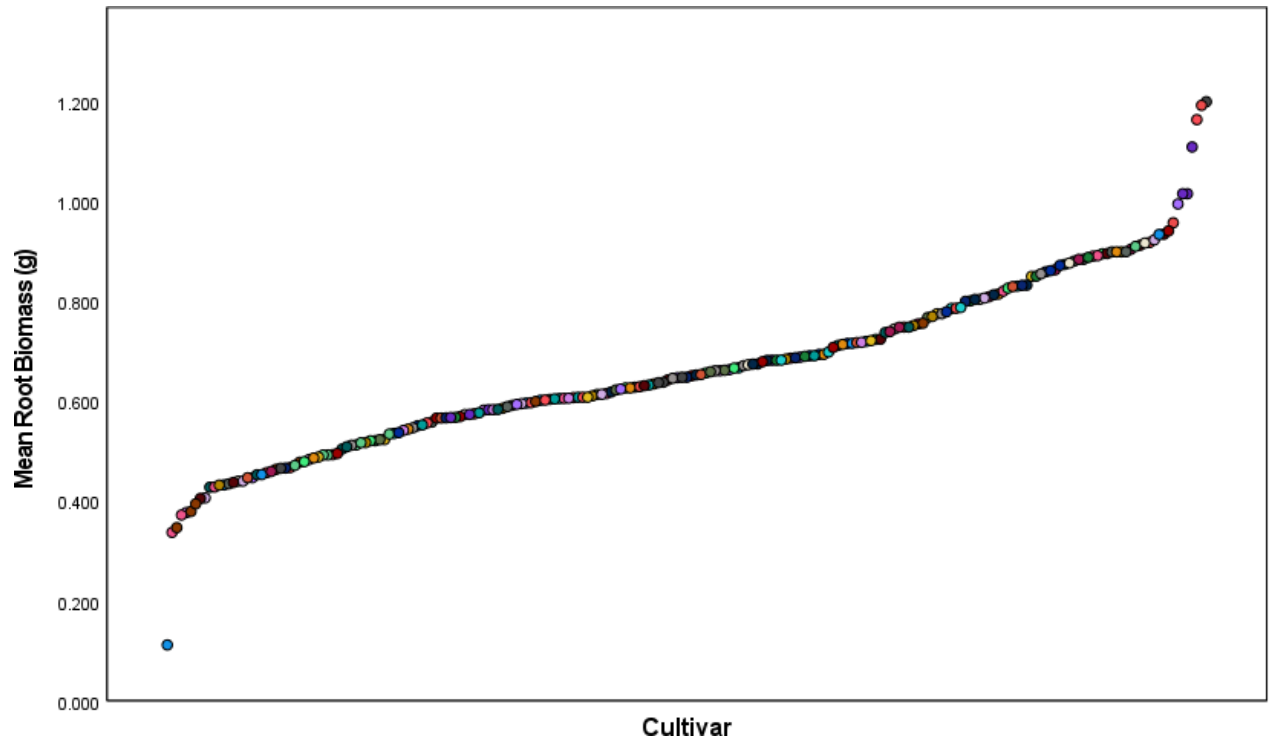
c)



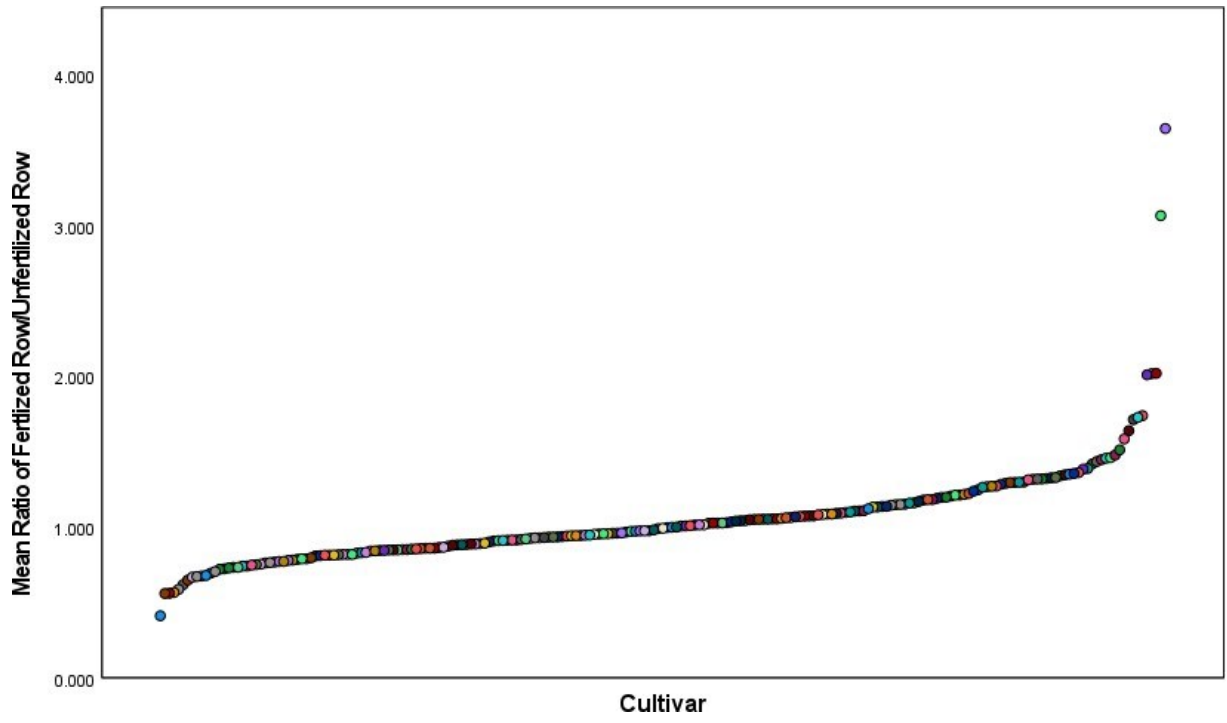
d)



e)

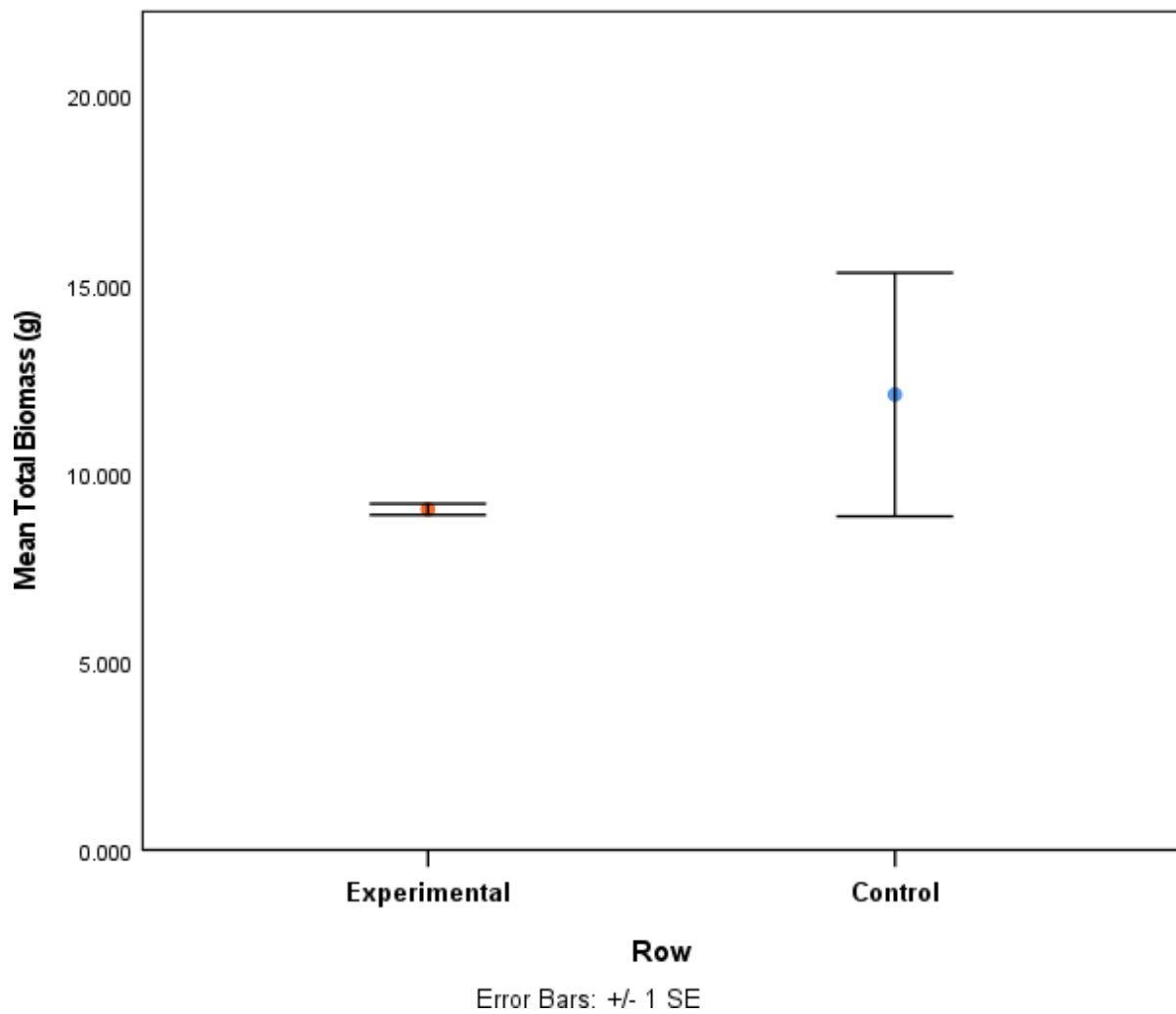


f)

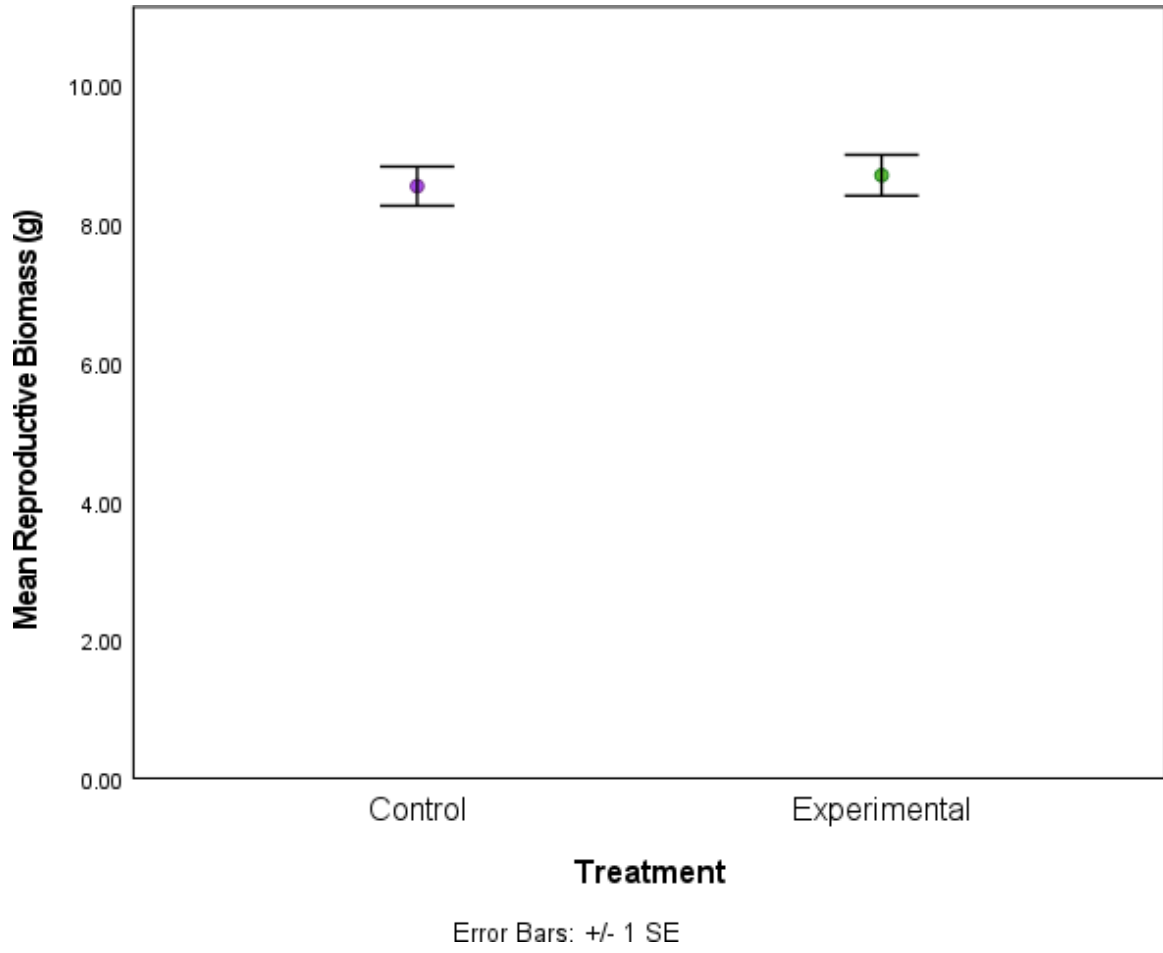


Appendix Figure 2.3. Average (mean \pm standard error) of the a) total aboveground biomass, b) reproductive biomass, c) reproductive effort, and d) belowground root distribution in fertilized over unfertilized soil. Results are taken across all cultivars and separated by experimental (fertilizer applied) and control (no fertilizer applied) groups.

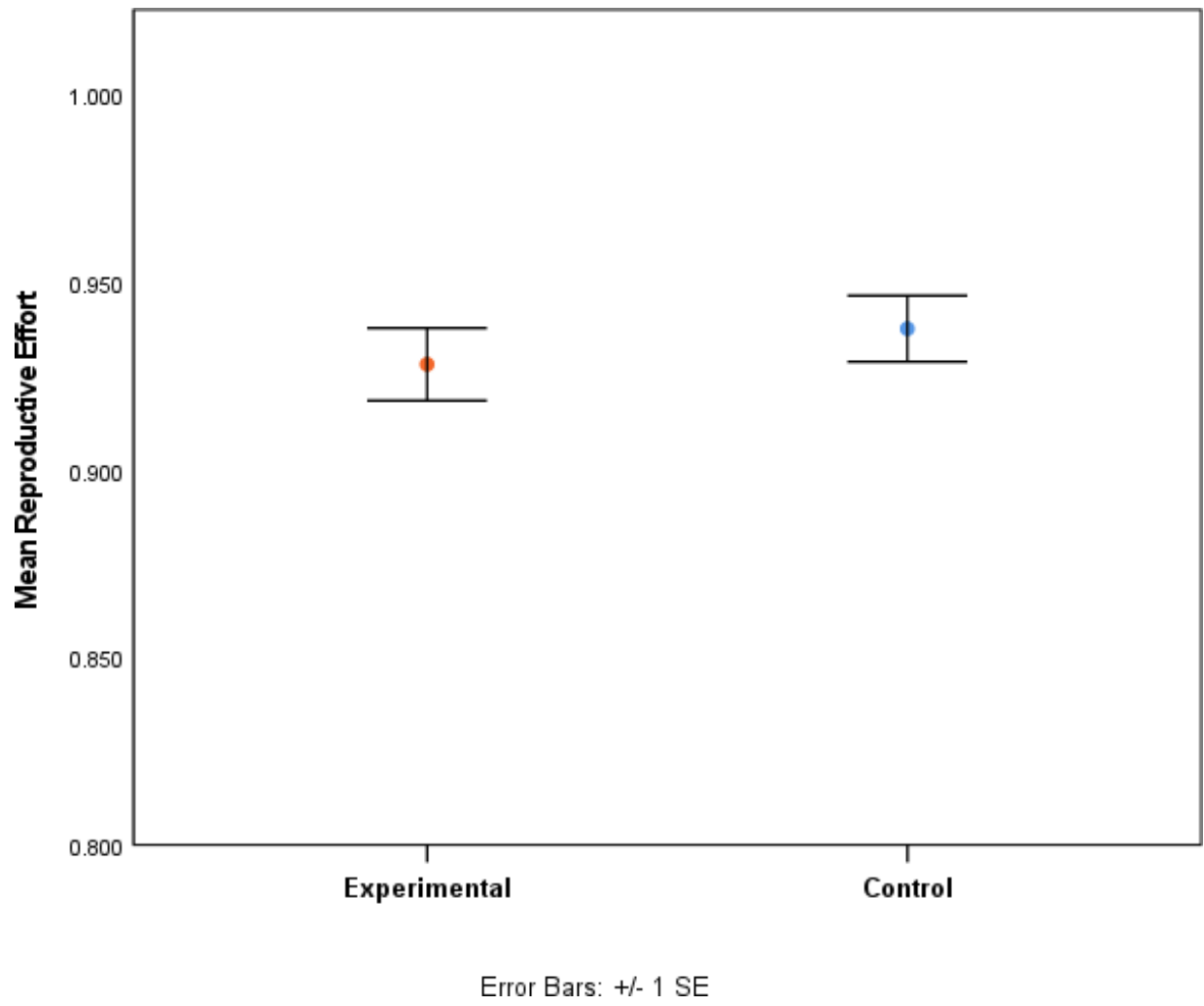
a)



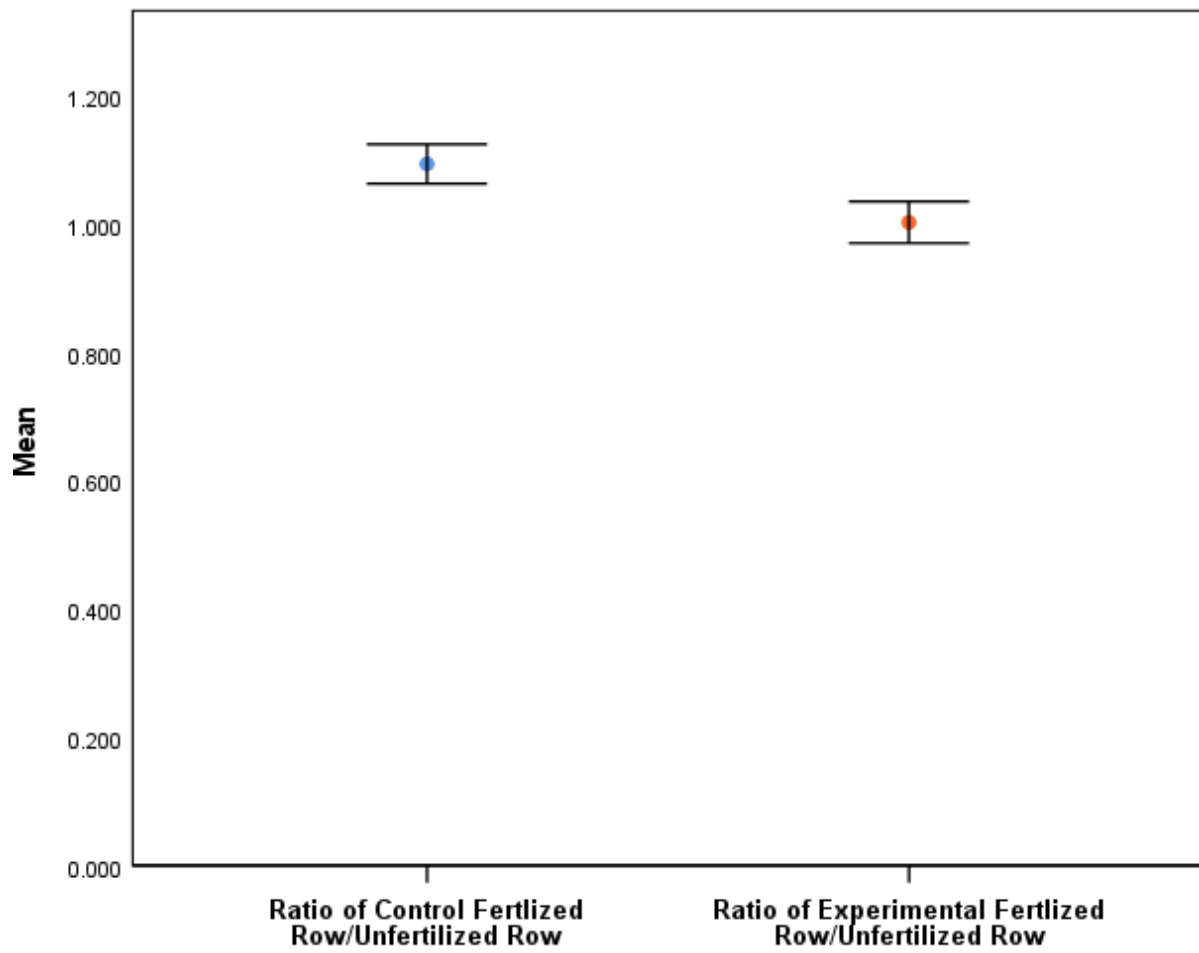
b)



c)



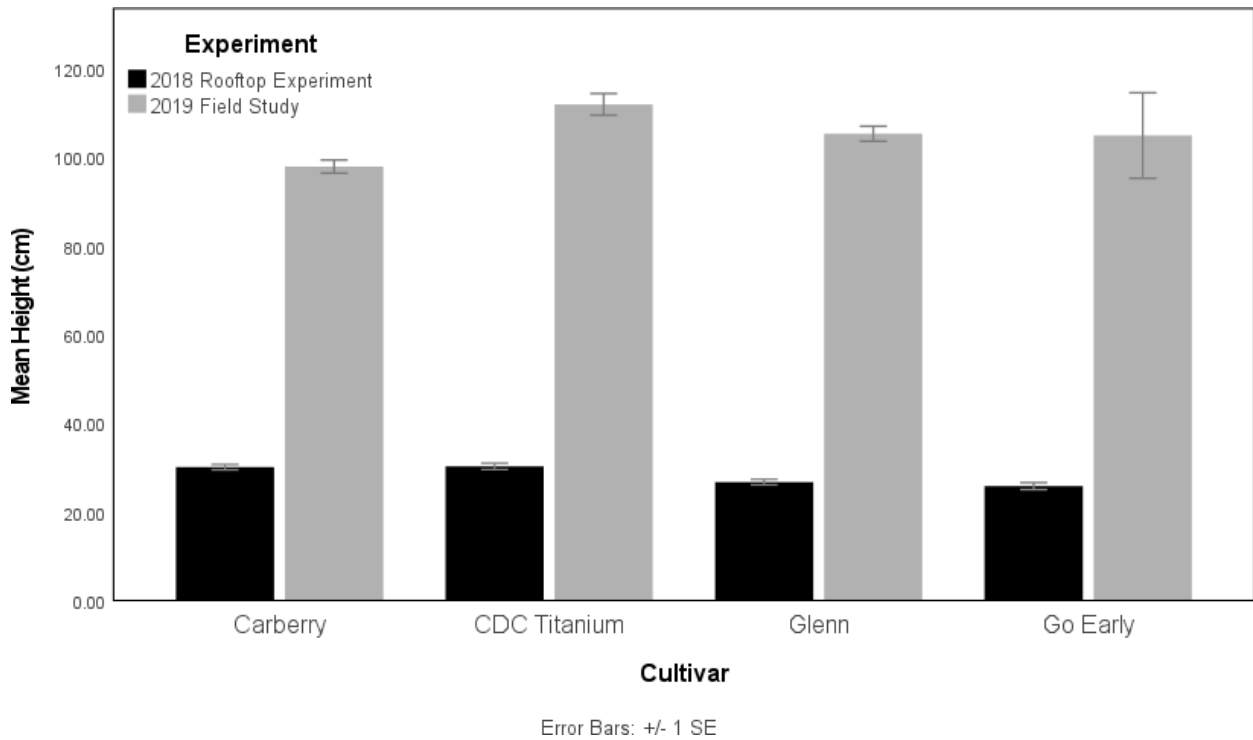
d)



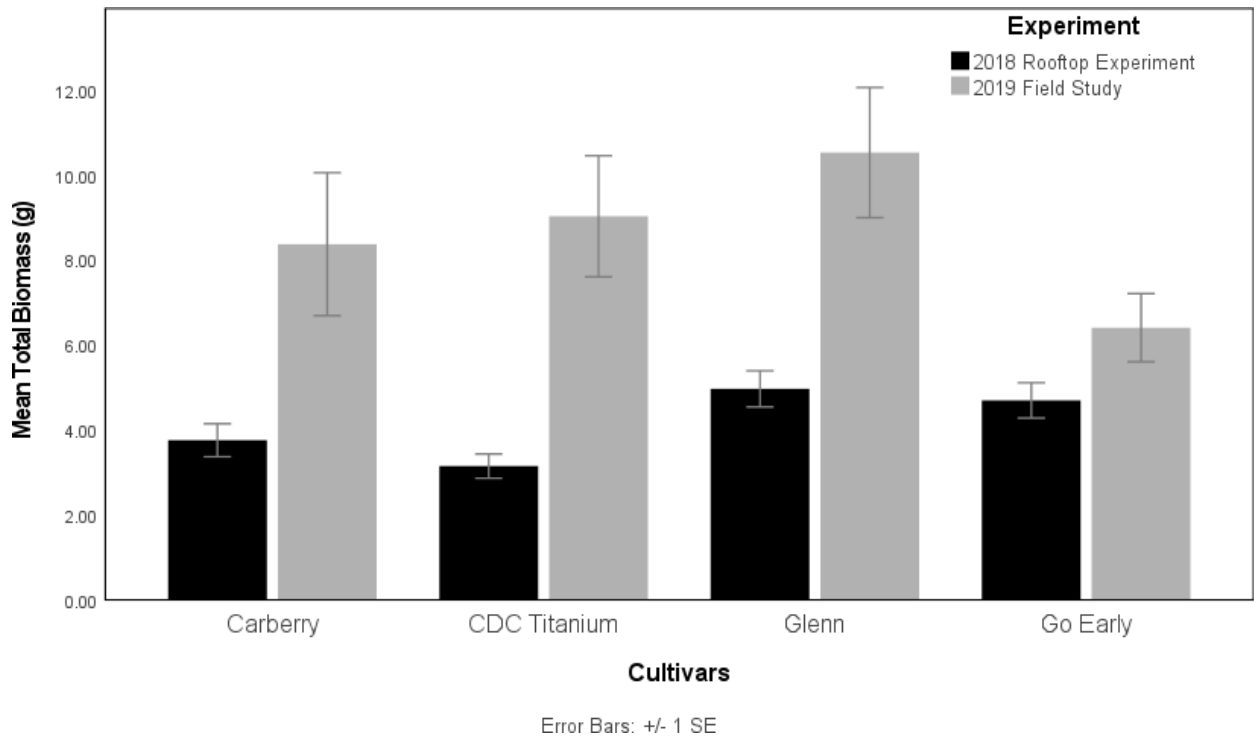
Error Bars: +/- 1 SE

Appendix Figure 2.4. Average (mean \pm standard error) of the a) height (cm), b) total aboveground biomass (g), c) reproductive biomass (g), d) reproductive effort, and e) belowground root distribution in fertilized over unfertilized soil. The rooftop pot experiment and the field study are included, with comparisons of four cultivars used in both studies: Carberry, CDC Titanium, Glenn, and Go Early.

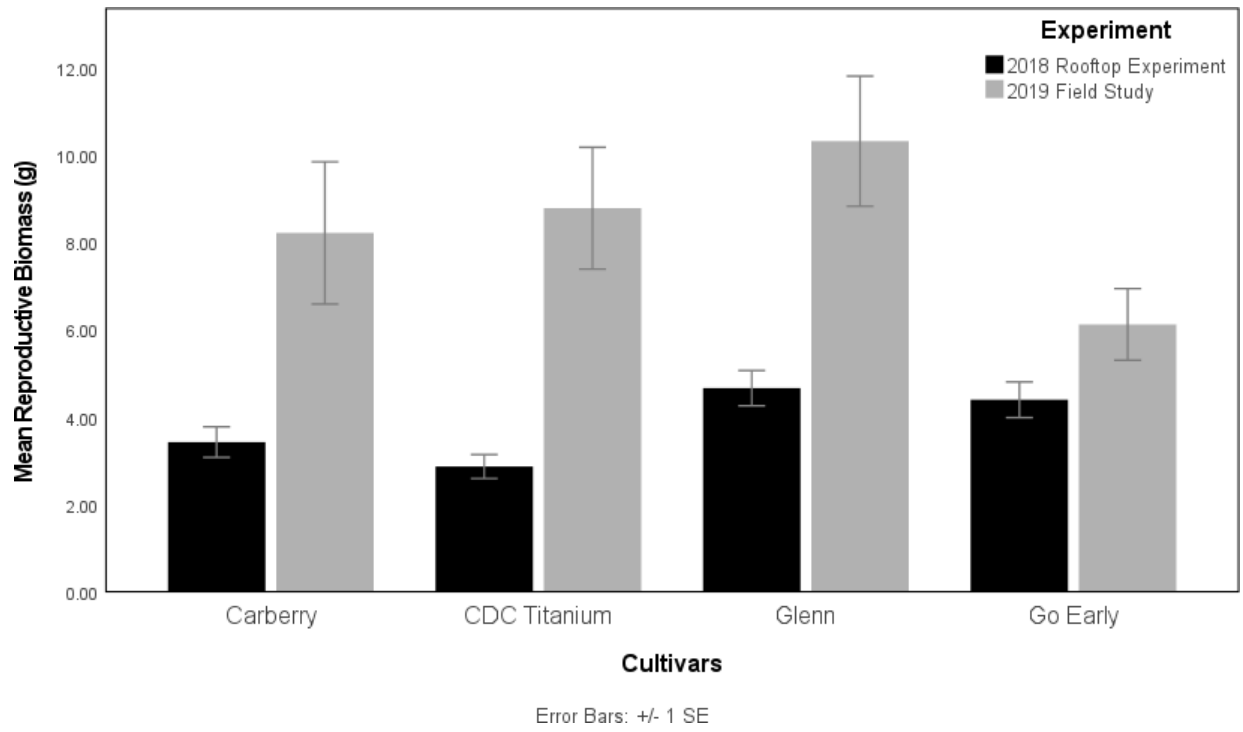
a)

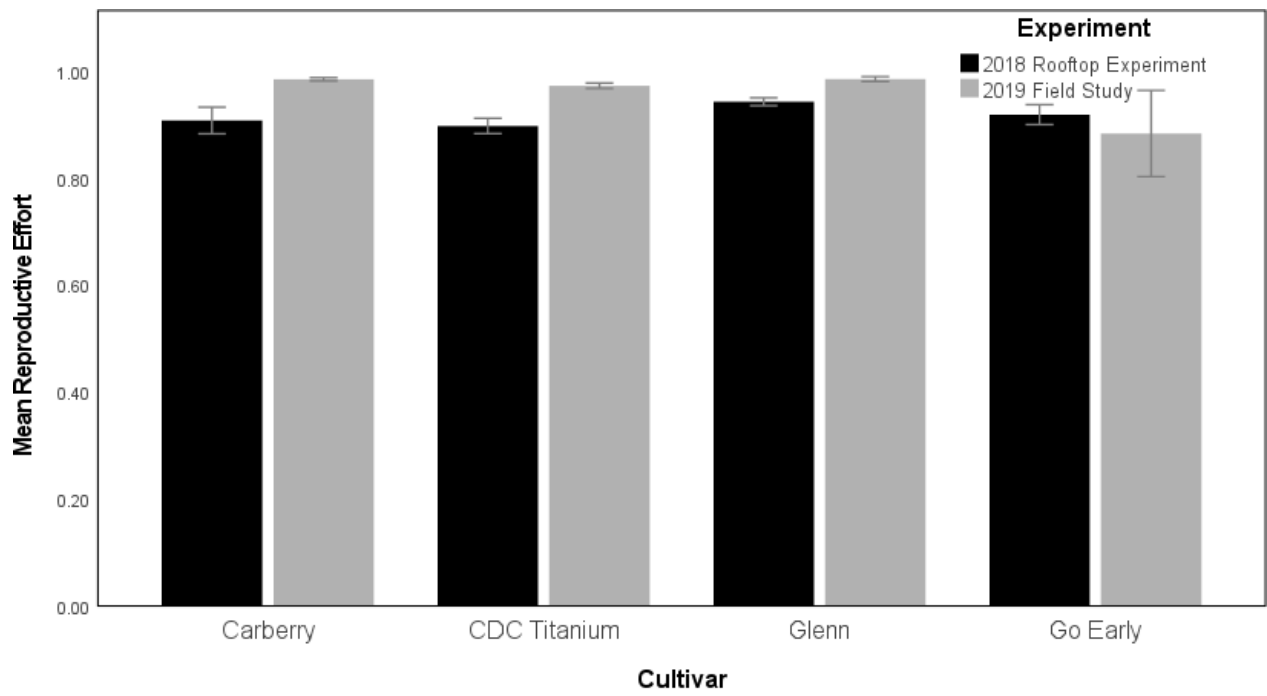


b)



c)





Error Bars: +/- 1 SE

