Transgenerational Effects of Kin Recognition in Plants: Soil Conditioning by an Invasive Plant

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Title: Transgenerational Effects of Kin Recognition in Plants: Soil Conditioning by an Invasive Plant

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Abstract

Monospecific stands of invasive plant species are found in nearly all known ecosystems and can cause permanent lasting ecosystem damage via deleterious effects in soils. These deleterious soil effects are a proposed mechanism which drives invasions by plants and are known to be influenced by kin recognition in plants. Uncovering whether invasive species utilize kin recognition to facilitate their own ecological persistence via soil conditioning will allow us to better understand the drivers of plant invasions and help combat them. In my master's thesis, I examined the role of kin recognition and kin selection on soil effects. I grew groups of *Potentilla recta* in groups of maternal half-sibs or strangers to condition the soil. I then grew a second generation of plants in that conditioned soil to determine the impacts of soil conditioning effects on plant performance. I found soil conditioning by groups of plants affected the performance of a second generation of plants based on the relatedness of the conditioning plants. Further, these soil effects of conditioning selectively benefit future individuals of a subsequent generation based on their relatedness. Moreover, these soil effects only existed in soil that has not been sterilized, indicating these soil effects depended on soil microbes.

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Declaration of Academic Achievement

I declare this thesis to be an original report of our research, except where referenced. No part of this work has been submitted, in whole or in part, in any previous application or publications for a degree at another institution. All research conducted in this thesis will be prepared for a manuscript submission to a peer-reviewed journal.

Chapter 1: Introduction

1.1 Behavior and Impacts of Invasive Plants

Invasive plant species have established themselves in nearly all ecosystems on earth, resulting in the permanent loss of many species, ecosystem collapse, and damage to global and local economies. (Barney et al., 2015; Wardle et al., 2011). The total scope of the damage and complexity biological invasions entail have vastly exceeded prior estimates, leading to the development of invasion science (Simberloff et al., 2013). Especially troubling is the tendency for invasive species to form monospecific stands. It is well known invasive plants in introduced sites are often distributed at significantly higher densities than in native ones, more so than the co-occurring native plants(Iqbal et al., 2020; Zheng et al., 2021; Zheng et al., 2015). Once an invasive species has formed a monospecific stand, the occupied area becomes extremely difficult to restore. Even after a monospecific stand has been removed, restoration will often still be hampered due to persistent soil effects (Jordan et al., 2008; Nuñez & Paritsis, 2018).

1.2 Soil Conditioning

Soil conditioning, also called plant-soil feedbacks, refers to plants influencing soil conditions, altering soil microbe composition and nutrient content, resulting in soil effects affecting subsequent plant performance, and ecosystem composition(Bever, 2003; Kulmatiski et al., 2008; Perkins & Nowak, 2012; Perkins & Nowak, 2013). Often this is achieved through the utilization of root exudates, either directly influencing their plant neighbours or indirectly by influencing the rhizosphere microbes. Both the chemical composition and effects of root exudates varies drastically between species and environment. Root exudates are composed of amino acids, organic acids, sugars, phenolics and other secondary metabolites. Root exudates serve a variety of functions, including supporting mycorrhizal mutualists with carbon, signaling to trigger inducible defenses in conspecifics, manipulating soil pH, influencing microbe populations both to the benefit of the host and the detriment of competitors, as directly allelopathic compounds towards pathogens, herbivores, or competitors, for autotoxicity(allelopathy towards conspecifics), and other effects on soil chemistry (Bais et al., 2006; Haichar et al., 2014; Müller et al., 2016; Vives-Peris et al., 2020). These effects can persist even if the plants are removed, greatly inhibiting native restoration efforts (Davies & Sheley, 2011; Eviner & Hawkes, 2008; Jordan et al., 2008; Perkins & Nowak, 2012). Recent work has put forth the idea kin recognition may play a key role in plant invasions (Zheng et al., 2021), and can influence soil conditioning by plants (Jie Li et al., 2018; Semchenko et al., 2017). Therefore, understanding the effects of soil conditioning by invasive plants is of critical importance to understanding why invasive plants are so successful.

1.3 Kin Recognition in Plants

One potential mechanism that may assist the spread of invasive species into stressful environments and lead to the establishment of monospecific stands is kin recognition in plants (Zheng et al., 2021). Kin recognition can facilitate co-operation among kin, by reducing competition with closely related individuals. Kin recognition can facilitate co-operation among kin, by reducing competition with closely related individuals (Anten & Chen, 2021; Chen et al., 2012; Murphy et al., 2017; Platt & Bever, 2009). According to Hamilton's rule, altruism in organisms is dependent on the relatedness of the individuals. Thus, alleles which favor costly helping of relatives can be passed on through relatives. (Dudley, 2015; Hamilton, 1964; Smith et al., 2019). While well recognized in animals, the ability to recognize genetically related individuals and alter behavior in response is often ignored in plants. Plants will detect genetically related neighbors via volatile (Hussain et al., 2019; Rahman et al., 2019), light (Crepy & Casal, 2015; Zhang et al., 2021), or root *exudate cues (Rahman et al., 2019; Semchenko et al., 2014; Wang et al., 2020). Once neighbors have been detected, phenotypic plasticity in adaptive traits allow plants to modulat*e their competitive responses to the relatedness of neighbour (Cahill & McNickle, 2011; Dudley, 2004; Dudley et al., 2013; Sultan, 2003). To date, several invasive species have been reported to exhibit kin recognition responses, including *Alliaria petiolata, Aegilops triuncialis, and Eupatorium adenophorum* (Murphy, 2012; Smith et al., 2019; Zheng et al., 2021).

1.3.1 Influence of Kin Recognition in Plants on Soil Effects

Kin recognition in plants is known to be able to influence both biotic and abiotic soil effects (Bais, 2015, 2018; J. Li et al., 2018; Takigahira & Yamawo, 2019; Tangutur et al., 2017). Work done by Takigahira and Yamawo (2019) and J. Li et al. (2018) show *Sorghum vulgare* and *Fagus crenata* are capable of exhibiting kin recognition responses and reduce competition with kin neighbours by altering uptake of nutrients to decrease resource competition. In particular, J. Li et al. (2018) shows heavy metal stress can elicit a variety of reduced competition responses for plants grown with kin neighbors compared to strangers, depending on level of nutrient availability. Collectively, soil nutrient availability affects kin recognition responses in plants, and kin recognition responses in turn alter nutrient uptake in plants. Another possible mechanism by which plants can affect soil conditioning is via microbes (File et al., 2012; Y. Xu et al., 2021). Kin recognition in plants is known to affect soil microbe composition; groups of closely related *Oryza sativa* harbored greater relative abundances of soil bacteria, but decreased abundance of fungi and actinomycetes(Y. Xu et al., 2021). Kin recognition across a mycorrhizal network has been shown to increase resource allocation to mycorrhizae in *Ambrosia artemisiifolia* (File et al., 2012). Kin recognition is also known to influence root exudate secretion (Bais et al., 2006; Rahman et al., 2019; Wang et al., 2021; Wang et al., 2020).

1.3.2 Potential for Kin Recognition in Plants to Exert Transgenerational Effects

Although many plant kin recognition studies have examined the effects of kin recognition on nutrient uptake and signaling(Ehlers et al., 2016; J. Li et al., 2018; Wang et al., 2021; Y. Xu et al., 2021), only one study has examined the influences of kin recognition on subsequent generations of plants (Semchenko et al., 2017). Soil conditioning, i.e., plants growing in soil and leaving behind persistent soil effects (Fitzpatrick et al., 2019; Waring et al., 2015), offers a mechanism for multigenerational effects. (Semchenko et al., 2017) found that *Deschampsia cespitosa* plants were smaller when grown in soils conditioned by a previous generation compared to when grown in unconditioned soils. *Deschampsia cespitosa* grown in soils previously conditioned by kin are more resistant to the growth inhibition caused by soil microbes, compared to plants grown in soil conditioned by strangers (Semchenko et al., 2017). To date, Semchenko et al. (2017) is the only study to have ever studied kin recognition in plants across multiple generations. However, they did not ask whether transgenerational effects of kin recognition affected all conspecifics or exclusively benefitted future kin.

1.4 Hypotheses and Objectives

In this study, I aim to provide evidence kin recognition in plants may be a driver of plant invasions via the production of soil effects through soil conditioning. For this experiment I grew *Potentilla recta* L. Rosaceae in groups of kin or genetically diverse strangers, with either high or low water availability and measured both performance and adaptive traits. Following onset of root degradation, I grew a second generation in the same soil, measured performance traits, and observed significant performance responses to soil sterilization. In this study I hypothesized that: (I) the relatedness of neighbors influences adaptive and performance responses in groups of *Potentilla recta*; (II) the responses to relatedness depend on water availability; (III) performance of second generation plants depends on whether the soil they're grown in is sterilized or not; (IV) the relatedness of groups influences the performance of a second generation grown in the same soil; (V) the performance of second generation plants depends on its relatedness to the previous generation.

Chapter 2 - Materials and methods

2.1 Study System

For this experiment, sulfur cinquefoil (*Potentilla recta*) was chosen as the study species because it primarily reproduces sexually via seeding, produces large cohorts of seeds annually, individual plants may persist for over 10 years, and primarily distributes its seeds via gravity (Perkins et al., 2006; Werner & Soule, 1976). These abilities naturally give rise to multiple generations of sibling cohorts emerging in the same monospecific stand. Sulfur Cinquefoil was introduced to North America at the end of the 19th century (Britton and Brown 1897, as cited in Dwire et al. 2006; Dwire et al., 2006). Sulfur cinquefoil rapidly colonizes disturbed sites such as abandoned agricultural fields, meadows, and roadsides, but is capable of establishment in even relatively undisturbed habitats (Naylor et al., 2005). Sulfur cinquefoil is capable outcompeting other aggressive invasive species, such as spotted knapweed (Centaurea stoebe L.), yellow star thistle (Centaurea solstitialis L.), and leafy spurge (Euphorbia esula L.). Producing as an average of approximately 6000 seeds per plan when fully mature, sulfur cinquefoil has since spread to a variety of ecosystems, such as conifer, grassland, shrubland, and seasonal wetland ecosystems (Dwire et al., 2006; Frost & Mosley, 2012; Rice et al., 1999). Despite being capable of self fertilization, sulfur cinquefoil mostly reproduces through cross fertilization as self pollination results in far fewer seeds per flower (Werner & Soule, 1976).

2.2 Material Collection

Seeds were collected from 28 field-pollinated plants, which provided seed families with an unknown mix of maternal half sibs, self sibs and full sibs. Plants and soil for inoculum materials were collected from a roadside meadow in Burlington Ontario Canada (43°17'47.4"N 79°53'17.1"W). Individual plants over 20cm tall with full seed heads were randomly selected from each group of plants and harvested with the aid of a random number generator. A minimum of three meters was maintained between harvest points.

As bulk soil collection was not permitted by the landowners, a soil flora inoculum was created to transplant microbiota from the harvest site into the final soil medium . At each harvest point, mother plant roots and clay soil were collected and pooled. The inoculum was created by coarsely chopping roots of parent plants with gardening shears and mixing them with the high clay soil from the harvest site.

A similar high clay soil collected at McMaster Forest, Hamilton, Ontario, Canada (43°14'53.4"N 79°57'03.9"W) was used as the substrate for both phases. As the field soil was from a different site as the plant collection, the field soil was sterilized with four cycles of autoclaving at 121℃ for 30 minutes to remove the soil microbiota. The inoculum from the plant collection site was added to the field soil at 30g inoculum per liter field soil

2.3 General Experimental Design

This experiment was performed in two phases, a soil conditioning phase (phase 1), and a phase of second-generation plants responding to soil conditioning (phase 2). In phase 1, groups of 6 plants per tray were grown in inoculated field soil in groups of siblings or strangers (P1 relatedness) in either high or low water availability (P1 Water Availability). At 14 weeks aboveground plant biomass was harvested for morphology, allocation, and size measures. The roots could remain in the soil, and breakdown was encouraged with light weekly watering. After 2 weeks, the soil in each tray was chopped and mixed, and half of each tray was sterilized (P2 sterilization). Seedlings were transplanted into the soil conditioned either by at least one of the same family or all strangers (P2-P1 relatedness),

2.4 Phase 1: Soil Conditioning Phase

2.4.1 Experimental design

To determine whether sulfur cinquefoil displayed kin recognition capabilities, I examined plant shoot mass, leaf angle, leaf elongation, specific leaf area and leaf number responses to **P1 relatedness** of neighbours, **P1 water availability,** and plant **family**. Containers, each holding six plants, were grown at two relatedness levels: in groups of six *maternal half siblings*(kin), or six *different maternal lineages*(strangers), and in *high* or *low* water availability levels. The experiment featured two replicates across all treatment combinations, with 6 plants per replicate, for a total of 12 plants per treatment combination and 576 plants in total.

Seeds were germinated in sand for 14 days, using L 17.78 x W 13.335 x H 5.715 cm plastic tray inserts (PT2x3 Insert, Ancaster, ON, Canada), placed in standard nursery flats, and misted daily. The 12 most abundant families were used in the experiment. The positions of each insert on the bench were re-randomized weekly. 576 seedlings were transplanted into 96 of the same type of trays, filled with inoculated field soil. Each tray contained six seedlings arranged in a circle. Each individual seedling used in the experiment was denoted by its insert number, position in the insert, and maternal family number. All seedlings were grown for two months without fertilizer, followed by one month with weekly addition of 120ml of 160ppm 20-20-20 NPK (Plant-Prod 20-20-20 Classic, Ancaster, ON, Canada) fertilizer solution.

I harvested above ground biomass(shoots), leaving the roots in the soil. After harvesting, 3mm diameter aerating holes were punctured into the soil of each insert in a grid patten, with holes spaced approximately 2cm apart from each other. The pots with soil still containing roots were left in their original positions on the greenhouse bench for two weeks. Once a week, the soil was misted lightly to promote root degradation. The soil was then reserved for phase Two.

2.4.2 Treatments

2.4.2.1 P1 Relatedness

Each tray was planted with either six kin plants (from the same family) or six stranger plants (from six different families). Each of the 12 families was replicated four times to give 48 kin trays. There were 48 stranger trays that had equal representation of the 12 families.

2.4.2.2 P1 Water Availability

Half the trays within each relatedness level were watered four times a week (high water availability), and the other half were watered only three times a week (low water availability).

2.4.3 Measurements

Leaf number, largest leaf tip height and radius were measured one month prior to harvesting. Surface area and petiole length were measured from photograph taken during harvesting. While harvesting, the largest leaf and petiole of each plant were isolated, and pictures were taken using an iPhone X. Largest leaf surface area and petiole length were measured from these pictures using image analysis via the program image J (version:1.53; ImageJ, Bethesda, Maryland, USA). The remaining aboveground plant material from each plant was severed at the root and dried for a minimum of 48 hours in an oven at 50°C and weighed. Specific leaf area was calculated from leaf surface area and mass.

2.5 Phase 2: Response Phase

2.5.1 Experimental Design

In a second study (Phase 2), I examined the effects of soil conditioning from phase 1 on the performance responses of a second generation of sulfur cinquefoil. This experiment was a fully factorial design (2^4 combinations). The factors consisted of **P1**

relatedness of neighbours and **P1 water availability** from the first experiment, with the addition of **P2-1 relatedness,** and **P2 sterilization**. INDIVIDUAL plants were grown in soils conditioned in Phase 1. The soils were taken from phase 1 inserts conditioned by *kin* or *strangers* **(P1 relatedness)**, and in *high* or *low* water (**P1 water availability**). The soil from the previous experiment was either *sterilized* or *not sterilized* (**P2 sterilization**). The individual plant grown in phase 2 was either of the same (*kin*)or different (*stranger*) field-pollinated maternal family as at least one of the plants previously grown in the soil (**P2-1 relatedness)**. This results in 16 treatment conditions, each replicated three times, with one plant per replicate, for each of the 12 families(Table 1).

Seeds from the same families used in phase 1 were germinated under the same protocol as phase 1. Each container of soil reserved from phase 1 was collected, and coarsely chopped. Individual seedlings were transplanted into standard square 6.35cm pots, filled with specific soils conditioned by phase 1. Seedlings were watered every other day and left to grow for 31 days, before harvesting.

2.5.2 Treatments

2.5.2.1 Phase 2-1 Relatedness

Half of all seedlings transplanted were from the same maternal family as one of the plants that conditioned the soil, in phase 1. The other half consisted of seedlings from randomly selected families that did not condition the soil they were transplanted into. Seedlings were randomly assigned such that each family was equally represented across all treatment categories, whether the soil was conditioned by kin or strangers, subjected to low or high water availability in phase 1, sterilized or not, and whether or not the phase 2 seedling is related to one of the families that conditioned the soil in phase 1.

2.5.2.2 Sterilization

Half of each soil type conditioned in phase 1 was sterilized with 4 cycles of autoclaving at 121℃ for 30 minutes per cycle.

Table 1 All treatment combinations for Phase 2 soil conditions.

Phase 1 Kin Status	Phase 1 Water	Soil type	Phase 2 Sterilization	Phase 2-1 Relatedness
Kin	Drought	1	Yes/No	Kin/Stranger
Kin	Control	2	Yes/No	Kin/Stranger
Stranger	Drought	3	Yes/No	Kin/Stranger
Stranger	Control	4	Yes/No	Kin/Stranger
			Total:	16 Treatment Combinations

2.5.3 Measurements

Leaf number and leaf diameter were measured at two weeks and four weeks into the experiment. After the growth period was concluded, all plant shoots were harvested at the base of the stem and dried in an oven for 48 hours at 50°C and weighed. All surviving plants at the end of the experiment were measured.

2.6 Data Analysis

Phase 1

All data analysis was done using R statistical software (version 4.1.1: R Foundation for Statistical Computing, Vienna, Austria). Analyses of variance (ANOVA) and covariance (ANCOVA) were carried out using the LM command. Residual analysis was performed to check whether the residuals met the assumptions of the ANOVA, and, if necessary, natural logarithms (loge) were taken of raw data so that residuals were homoscedastic and normally distributed.

To test whether sulfur cinquefoil morphology and allocation traits varied by P1 relatedness and P1 water availability, and family, ANCOVA was performed for leaf angle, with leaf radius as the covariate; specific leaf area, with leaf mass; and petiole elongation, with petiole mass.

Similarly, to determine if shoot mass and leaf number responded to the relatedness of neighbours, I used ANOVA, and Poisson ANOVA to analyze shoot mass and leaf number, respectively. The independent variables were P1 relatedness, P1 water availability, and family.

Phase 2

To test if phase 1 soil conditioning affected the performance of phase 2 plants, I used analysis of variance to assess change in leaf number and diameter and shoot mass, with the independent variables P1 relatedness, P1 water availability, P2-1 relatedness, Family, and P2 soil sterilization.

To analyze the effect of P1 relatedness, P1 water availability, P2-1 relatedness, Family, and P2 soil sterilization on the survival rate of phase 2 plants, I used logistic regression on the survival status of phase 2 plants.

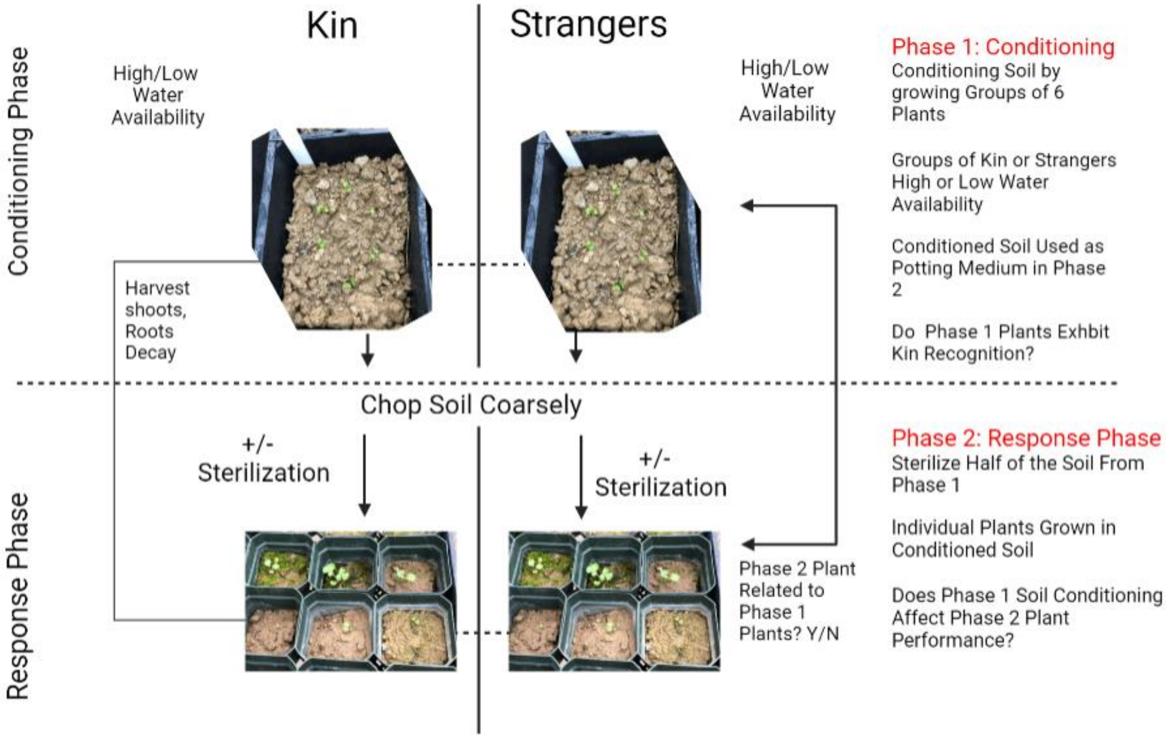

Figure 1: overview of both phases of the experiment. In phase 1 soils will be conditioned by groups of kin or strangers. Half each stranger and kin group will be subjected to drought, and the soil will be collected at the end of phase 1. Half of all the collected soils will be sterilized. All collected soils would then subsequently be used to grow individual plants in phase 2.

Chapter 3: Results

3.1 Phase 1

3.1.1 Responses to Relatedness

Neither shoot mass nor leaf number differed between kin and strangers (Table 3).

3.1.1.1 Leaf Angle Responses to Relatedness

Leaves of plants grown in groups of strangers were more vertical than leaves of plants grown in groups of kin (Table 2: Leaf angle: P1 Relatedness, Fig. 2). The effect of relatedness on the slope of leaf height on leaf radius differed among families (Table 2: Leaf angle: Covariate X P1 Relatedness X P1 Family). When grown with kin, there was an apparent decrease in among family variance in slopes (Fig. 3).

3.1.1.2 Petiole Elongation Responses to Relatedness

There was no significant difference in petiole elongation between kin and strangers (Table 2: Elongation: P1 Relatedness). However, there was a significant two-way interaction between relatedness and the covariate; petiole mass (Table 2: Elongation: P1 Relatedness X Covariate), where the effect of relatedness on petiole length depended on petiole mass. The slope of mean log transformed petiole height by log transformed petiole mass was steeper for plants grown among strangers than kin, and the y intercept for both slopes were not significantly different. Thus, at low petiole masses, there was no significant difference in mean petiole elongation between kin and stranger plants, but as petiole mass increased plants grown among strangers showed greater elongation than plants grown with kin (Fig. 4).

In another significant two-way interaction, families varied significantly in their average petiole elongation response to relatedness. Some families showed greater elongation in plants grown with kin, whereas other families exhibited more elongation in plants grown with strangers. Overall, there appeared to be less variation among families in petiole elongation for plants grown among strangers than kin (Fig. 5).

3.1.1.3 Specific Leaf Area Responses to Relatedness

For specific leaf area, no significant difference between levels of relatedness was observed (Table 2: P1 Relatedness). However, there was a significant two-way relatedness by family interaction (Table 2: Specific Leaf Area: P1 Relatedness X Family). Overall plants grown among kin exhibited greater among family variation in mean log transformed SLA than stranger plants (Fig. 6). There was also a significant three-way interaction between plant family, relatedness and the covariate, leaf mass. When grown with kin, there was greater among family variation in the slopes of log transformed leaf surface area to log transformed leaf mass than when grown with strangers (Fig. 7).

3.1.2 Responses to Water Availability

3.1.2.1 Leaf Angle Responses to Water Availability

Plants grown in low water availability had significantly lower leaf angles compared to plants grown in high water availability (Table 2: Leaf Angle: P1 Water Availability, Fig. 8). In a significant three-way interaction between water availability, family and the covariate; leaf radius. Plants grown with low water availability had significantly different slopes of leaf height by radius among families compared to plants grown in high water availability (Table 2; Fig. 9).

3.1.2.2 Petiole Elongation Responses to Water Availability

There was no significant effect of water availability on mean petiole elongation. However, petiole length demonstrated a significant interaction between water availability and the covariate, petiole mass (Table 2: Elongation: P1 Water Availability X Covariate). The slopes of mean log transformed petiole length by log transformed petiole mass differed significantly between plants grown with high and low water availability. Plants grown with low water availability had a lower y intercept and a steeper than that of high water plants, indicating plants grown in high water availability exhibited greater petiole elongation than stranger plants at low petiole masses, but the difference in petiole elongation decreased as petiole mass increased. There was no difference in mean log transformed petiole length between grown in either water availability level when the petiole mass is large (Fig. 10).

3.1.2.3 Specific Leaf Area Response to Water Availability

Water availability has a marginally significant (P=0.0919) effect on SLA, plants grown with high water availability exhibit greater SLA than plants grown with low water availability (Table 2: Specific Leaf Area: P1 Water Availability, Fig. 11).

3.1.2.4 Shoot Mass Responses to Water Availability

High water availability resulted in substantially greater shoot mass than low water availability plants (Table 3: Shoot Mass: P1 Water Availability, Fig. 12). Water availability had a marginally significant (p=0.0955) effect on leaf number (Table 3: Leaf Number: P1 Water Availability), with slightly more leaves for high water availability plants compared to low water availability plants (Fig. 13).

3.1.3 Interactions Between Relatedness and Water Availability

3.1.3.1 Petiole Elongation Response to an Interaction Relatedness and Water Availability

There was a significant three-way interaction of relatedness, water availability and family on petiole elongation (Table 2: Elongation: P1 Relatedness X P1 Water Availability X family). Across both high and low water availability, among family variation in petiole elongation was substantially lower in stranger plants than kin. In low water availability plants, the range of variation between kin and strangers was the same. However, family means of petiole elongation were clustered more densely in stranger plants; thus, family variation is reduced across all levels of water availability in groups of strangers (Fig. 14).

There was also a marginally (p=0.0720) significant three-way interaction of relatedness water availability, and the covariate on petiole elongation (Table 2: Elongation: P1 Relatedness X P1 Water Availability X Covariate). Nearly identical to the result shown in section **3.1.2.4,** when water availability is high, the slopes of mean log transformed petiole length by log transformed petiole mass are different from each other. In contrast, when water availability is low, there is no difference between plants grown with kin or strangers in the slopes of mean log transformed petiole length by log transformed petiole mass (Fig. 15). Among stranger plants, high water availability results in greater petiole elongation at low petiole mass, but no significant difference at high mass. There is no significant difference in petiole elongation between water availability levels when the plants are grown among kin.

3.1.3.2 Shoot Mass Response to an Interaction Relatedness and Water Availability

In a marginally significant (p= 0.0585) interaction, the effect of relatedness on shoot mass was dependent on water availability (Table 3: Shoot Mass: P1 Relatedness X P1 Water Availability). When grown under low water availability, there is no significant difference in shoot mass between kin and stranger plants (p = 0.5075). When grown under high water availability, stranger plants had greater shoot mass than kin plants (p = 0.0413) (Fig. 16).

Table 2 Analysis of covariance tables for phase 1 competition traits

		Leaf Angle (Leaf Height / Leaf Radius)**		**Elongation (Petiole Length/ Petiole Mass)**		**Specific Leaf Area (Leaf Surface Area/ Leaf Mass)**	
	Df	F	P	F	P	F	P
P1 Relatedness	1	6.9531	**0.0087**	0.0904	0.7639	0.0073	0.9319
P1 Water Availability	1	5.5168	**0.0193**	2.5050	0.1143	2.8545	0.0919†
P1 Family	11	0.6845	0.7537	2.1992	**0.0139**	0.7258	0.7139
Covariate X P1 Relatedness	1	0.9564	0.3286	6.2032	**0.0132**	0.1044	0.7468
Covariate X P1 Water Availability	1	2.4640	0.1172	4.3049	**0.0387**	1.6480	0.2000
P1 Relatedness X P1 Water Availability	1	1.0299	0.3107	0.2662	0.6062	2.4976	0.1148
Covariate X P1 Family	11	1.1594	0.3132	1.4640	0.1426	0.6945	0.7440
P1 Relatedness X P1 Family	11	1.5221	0.1201	1.8841	**0.0399**	1.8633	**0.0426**
P1 Water Availability X P1Family	11	0.4333	0.9410	1.0101	0.4365	1.2347	0.2616
Covariate X P1 Relatedness X P1 Water Availability	1	0.1576	0.6915	3.2547	0.0720†	2.6673	0.1032
Covariate X P1 Relatedness X P1 Family	11	2.3307	**0.0086**	1.4311	0.1563	2.1443	**0.0167**
Covariate X P1 Water Availability X P1 Family	11	1.8752	**0.0405**	0.8680	0.5720	1.2483	0.2529
P1 Relatedness X P1 Water Availability X P1 Family	11	0.6541	0.7819	1.8429	**0.0455**	0.7949	0.6452
Covariate X P1 Relatedness X P1 Water Availability X P1 Family	11	0.9619	0.4806	0.8489	0.5912	0.7379	0.7020
Covariate	1	432.0026	**<0.0001**	2022.9000	**<0.0001**	5845.0940	**< 0.0001**

Bolded and starred* numbers denote significant values.
† Denotes marginal significance
**Leaf radius and height were measured at the tip of the leaf
Bold numbers indicate significant values.

Table 3 Phase 1 analysis of variance tables for performance traits

	Shoot Mass	**Leaf Number**

	F	P	Chi-Sq	P
P1 Relatedness	1.0221	0.3125	0.1434	0.7049
P1 Water Availability	8.9799	**0.0029**	2.7798	0.0955†
P1 Family	2.5877	**0.0033**	8.2594	0.6899
P1 Relatedness X P1 Water Availability	3.5959	0.0585†	0.0011	0.9741

Bolded and starred* numbers denote significant values.
† Denotes marginal significance

3.2 Phase 2

3.2.1 Responses to Soil Sterilization

Sterilization significantly altered the effects of soil conditioning. Growing in sterilized soil reduced shoot mass for all combinations of soil conditions. Further, the survival rate of P2 plants depended on the phase 1(P1) relatedness of the plants that conditioned the soil.

3.2.1.1 Shoot Mass Responses to Sterilization

Soil sterilization had substantial effects P2 shoot mass (Table 4: Shoot Mass: P2 Sterilization). The average shoot mass of P2 plants grown in sterilized soil was less than half the average shoot mass in unsterilized soil (Fig. 17).

In a significant three-way interaction P2 plant mass was drastically lower when grown in sterilized soils compared to unsterilized soils. This result is consistent across all factor combinations of P1 relatedness and P1 water availability. For all combinations of soil conditions, the mean shoot mass of plants grown in sterilized soils did not significantly differ (Table 4: Shoot Mass: P1 Relatedness X P1 Water Availability X P2 Sterilization, Fig. 18).

In marginally significant (P=0.0693) four-way interaction between P1 relatedness, P1 water availability, P2-1 relatedness, and P2 soil sterilization on shoot mass, the mean shoot mass of P2 plants was substantially lower when grown in sterilized soils compared to unsterilized soils, for 7 out of the 8 combinations of soil conditions excluding soil sterilization. The only exception occurred for P2 kin plants grown in P1 stranger conditioned soil (see section 3.2.3 Fig. 19), in which P2 shoot mass did not differ significantly when grown in sterilized or unsterilized versions of the soil (Fig. 19).

3.2.1.2 Leaf Trait Responses to Sterilization

The change in P2 leaf number showed a significant response to soil sterilization (Table 4: Leaf Number: P2 Sterilization). In general, P2 plants lost leaves between weeks 2 and 4 of the experiment. However, the loss of leaves was significantly greater when grown in sterilized soils compared to unsterilized soils (Fig. 20).

Sterilization had a significant effect on the change in rosette diameter of P2 plants between weeks 2 and 4 of the experiment (Table 4: Rosette Diameter: P2 sterilization). Not only was the difference in leaf diameter change significant between phase 2 plants grown in sterilized and unsterilized soils, on average, there was growth in leaf diameter for P2 plants grown in unsterilized soils, and reduced size of P2 plants grown in sterilized soils (Fig. 21).

3.2.1.3 Survival Rate Responses to Sterilization

There was a marginally significant(P=0.0546) interaction between soil sterilization and P1 relatedness on survival rate of phase 2 plants. (Table 5: Survival: P1

Relatedness X P2 Sterilization). In P1 kin-conditioned soils, sterilization had no significant effect on the survival rate of P2 plants (p= 0.3948). In contrast, plants grown in stranger conditioned soils had higher survival rates when the soil was sterilized (p = 0.0572). In sterilized soil there was no significant difference in survival rate of P2 plants between P1 kin and P1 strangers conditioned soils (p = 0.6394). When the soil was not sterilized, phase 2 plants grown in P1 kin exhibited substantially greater survival rates than in P1 stranger conditioned soils (p = 0.0233) (Fig. 22).

3.2.2 Responses to P1 Soil Conditioning

P2 plants produced greater shoot mass when grown in P1 kin-conditioned soils than in P1 stranger soils (Table 4: Shoot Mass: P1 Relatedness, Fig. 22). Further P2 plants exhibited greater overall performance for both p2 plant mass and survival rate in soils conditioned by P1 kin under low p1 water availability.

3.2.2.1 Shoot Mass Responses to P1 Soil Conditioning and Water Availability

There was a marginally significant (p = 0.0938) interaction in soil conditioning by phase 1 relatedness and phase 1 water availability on P2 shoot mass (Table 4: Shoot Mass: P1 Relatedness X P1 Water Availability). P2 plants showed no significant difference in shoot mass between levels of P1 water availability when grown in P1 stranger conditioned soil. P2 plants grown in soils conditioned by P1 kin and low P1 water availability had substantially greater shoot mass than P2 plants grown in high P1 water availability kin-conditioned soils (p= 0.0303).

In soils conditioned with high P1 water availability, P2 shoot mass did not differ between P1 kin and P1 stranger conditioned soils. Thus, there was no significant difference in P2 shoot mass growing in soils conditioned by strangers of either P1 water availability level, or P1 kin-conditioned soil under high P1 water availability. With low P1 water availability, P2 shoot mass was greater in P1 kin-conditioned soil than P1 strangers ($p = 0.0650$) (Fig. 24).

3.2.2.2 Survival Responses to P1 Soil Conditioning and Water Availability

P2 plants demonstrated a marginally significant ($p = 0.0916$) interaction of phase 1 relatedness and phase 1 water availability on survival rate (Table 5: Survival Rate: P1 Relatedness X P1 Water Availability). When grown in P1 kin-conditioned soils, the number of surviving P2 plants was marginally greater ($p = 0.0942$) if the soil was conditioned in phase 1 with low water availability than in high water availability. P2 plants showed no difference in survival rates between levels of P1 water availability when grown in P1 stranger-conditioned soils. When grown in soils conditioned with low P1 water availability, the survival of P2 plants was significantly greater in P1 kin-conditioned soils than P1 stranger-conditioned soils ($p = 0.0443$). P1 relatedness had no effect on the survival rate of P2 plants grown in soils conditioned by plants with high P1 water availability. Collectively, the survival rates of P2 plants were not significantly different when grown in P1 high water soils, and P1 low water soils conditioned by strangers (Fig. 25).

3.2.3 Differential Effects of Soil Conditioning on P2 Plants in Response to P2-1 Relatedness

Phase 2-1 relatedness refers to whether a phase 2 plant was from the same maternal family (kin) as at least one of the phase 1 plants that conditioned the soil.

In a significant two-way interaction of P2-1 relatedness and P1 relatedness on shoot mass of P2 plants (Table 4: Shoot mass: P1 Relatedness X P2-1 Relatedness), P2 kin plants grew more mass in P1 kin conditioned soils than P2 strangers. Conversely, P2 stranger plants grew more mass in P1 stranger conditioned soils than P2 kin plants. Although the differences in mass between P2 kin and p2 strangers were not significant in either P1 kin or P1 stranger conditioned soils, the rank change exists. The shoot mass of P2 kin plants was marginally significantly($p=0.651$) higher when grown in P1 kin conditioned soils, compared to P1 stranger conditioned soils. By comparison, the shoot mass of P2 stranger plants did not differ significantly between p1 kin and p1 stranger conditioned soils (Fig. 26).

Examining the effects of P2-1 relatedness more specifically, in a significant three-way interaction between P2-1 relatedness, P1 relatedness and P1 water availability (Table 4: Shoot Mass: P2-1 Relatedness X P1 Relatedness X P1 Water Availability) exists. There was a similar rank change relationship with P2 kin plants growing marginally significantly($P=0.0979$) more mass in P1 kin conditioned soils, than P2 strangers, and vice versa($P=0.0846$), but only in soils conditioned in P1 under high water availability. In this interaction, P2 stranger plants grew significantly less ($p=0.0441$) mass when grown in P1 kin-conditioned soils, compared to P1 stranger-conditioned soils. Contrastingly, mass of P2 kin plants grown in P1 kin conditioned soil did not differ from those grown in P1 stranger conditioned soils. There were no significant differences in P2 shoot mass between any combination of P1 relatedness, P2-1 relatedness, when P1 water availability was low (Fig. 27).

Examining the effects of P2-1 relatedness even further, there is a marginally significant (P=0.0693) four-way interaction between P2-1 relatedness, P1 relatedness, P1 water availability, and P2 soil sterilization (Table 4: Shoot Mass: P2-1 Relatedness X P1 Relatedness X P1 Water Availability X P2 Soil Sterilization). Not only does the previously described rank change phenomenon not occur in soils conditioned in P1 with low water availability, it also does not occur sterilized soils (see section 3.2.1.1 for the effects of soil sterilization on P2 shoot mass). Specifically examining unsterilized soils with high P1 water availability, P2 kin plants gr3w more mass, but not significantly so, than p2 stranger plants in P1 kin-conditioned soils. Conversely, P2 stranger plants grew significantly (P=0.0167) more mass than P2 kin plants when grown in P1 stranger-conditioned soils. P2 kin plants grew significantly more mass when grown in P1 kin-conditioned soils than in P1stranger-conditioned soils (P=0.0439). P2 stranger plants grew marginally significantly (p=0.0614) more mass in P1 stranger-conditioned soils than in P1 kin-conditioned soils. The shoot mass of these P2 kin plants grown in P1 stranger-conditioned soils was not significantly different from the shoot mass of P2 kin plants grown in sterilized P1 stranger-conditioned soils (high P1 water availability) (Fig. 19).

Table 4 Analysis of variance tables for phase 2 performance traits

		Shoot Mass		**Rosette Diameter**		**Leaf Number**	
	Df	F	P	F	P	F	P
P1 Relatedness	1	4.1	**0.0439***	2.3235	0.1282	2.229	0.1362
P2-1 Relatedness	1	1.905	0.1687	0.0633	0.8015	0.1754	0.6756
P1 Water Availability	1	0.2066	0.6498	0.9345	0.3343	0.003	0.9561
P2 Sterilization	1	4.2908	**0.0393***	47.1688	**<0.0001 ***	17.2782	**<0.0001 ***
P2 Family	11	1.2437	0.2582	0.5535	0.8659	0.8323	0.6077
P1 Relatedness X P2-1 Relatedness	1	7.1274	**0.0081***	2.1555	0.1428	0.1402	0.7083
P1 Relatedness X P1 Water Availability	1	2.8284	0.0938†	0.9974	0.3185	0.0208	0.8855
P2-1 Relatedness X P1 Water Availability	1	2.6119	0.1073	0.1838	0.6684	0.0414	0.8388
P1 Relatedness X P2 Sterilization	1	1.6201	0.2042	0.1712	0.6793	0.2947	0.5875
P2-1 Relatedness X P2 Sterilization	1	0.1702	0.6803	0.0011	0.9738	0.6284	0.4284
P1 Water Availability X P2 Sterilization	1	1.2613	0.2624	0.0416	0.8385	0.0214	0.8839
P2 Sterilization X P2 Family	11	0.8816	0.5590	0.9909	0.4538	0.6203	0.8118
P1 Water Availability X P2 Family	11	1.0105	0.4372	0.6069	0.8233	0.7838	0.6564
P1 Relatedness X P2-1 Relatedness X P1 Water Availability	1	6.2504	**0.0130***	1.3259	0.2502	1.3761	0.2414
P1 Relatedness X P2-1 Relatedness X Sterilization	1	2.7148	0.1006	1.1204	0.2905	0.5651	0.4526
P1 Relatedness X P1 Water Availability X Sterilization	1	4.2246	**0.0408***	0.0007	0.9782	0.0157	0.9003
P2-1 Relatedness X P1 Water Availability X Sterilization	1	0.916	0.3394	1.2472	0.2647	0.4578	0.4990
P1 Relatedness X P2-1 Relatedness X P1 Water Availability X Sterilization	1	4.1	0.0693†	0.2093	0.6476	0.1012	0.7505

Bolded and starred* numbers denote significant values.
† Denotes marginal significance

Table 5 Logistic regression table for Phase 2 Survival status

		Survival	
	Df	Chi Sq	P
P1 Relatedness	1	1.6572	0.1980
P2-1 Relatedness	1	0.6947	0.4046
P1 Water Availability	1	1.0897	0.2965
P2 Sterilization	1	0.3032	0.5819
P2 Family	11	22.348	**0.0218***
P1 Relatedness X P2-1 Relatedness	1	0.6291	0.4277
P1 Relatedness X P1 Water Availability	1	2.8456	0.0916†
P2-1 Relatedness X P1 Water Availability	1	0.0582	0.8093
P1 Relatedness X P2 Sterilization	1	3.6931	0.0546†
P2-1 Relatedness X P2 Sterilization	1	2.1260	0.1448
P1 Water Availability X P2 Sterilization	1	0.7901	0.3741
P1Sterilization X P2 Family	11	10.1380	0.5180
P1 Water Availability X P2 Family	11	6.6296	0.8282
P1 Relatedness X P2-1 Relatedness X P2 Water Availability	1	0.0791	0.7786
P1 Relatedness X P2-1 Relatedness X P2 Sterilization	1	0.0510	0.8213
P1 Relatedness X P1 Water Availability X P2 Sterilization	1	1.0313	0.3099
P2-1 Relatedness X Water Availability X Sterilization	1	0.1216	0.7274
P1 Relatedness X P2-1 Relatedness X Water Availability X Sterilization	1	0.4288	0.5126

Bolded and starred* numbers denote significant values.
† Denotes marginal significance

3.3 Results Figures

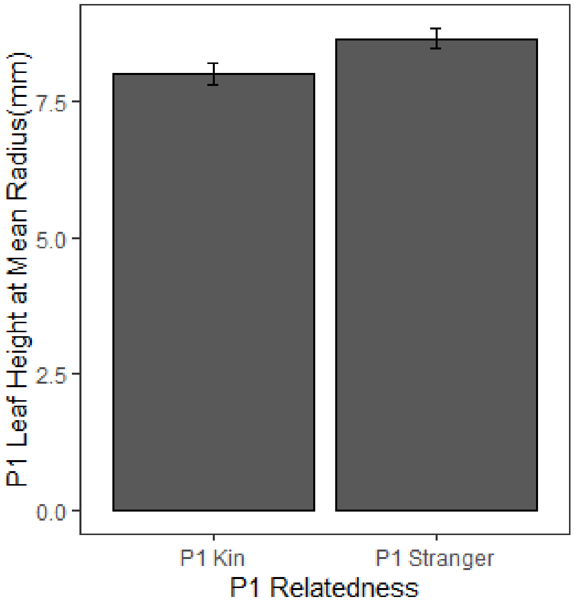

Figure 2 Plot of mean leaf angle (ratio of leaf height by leaf radius) response to relatedness of phase 1 neighbours in *Potentilla recta* grown in a greenhouse. Bars indicate 1 SE.

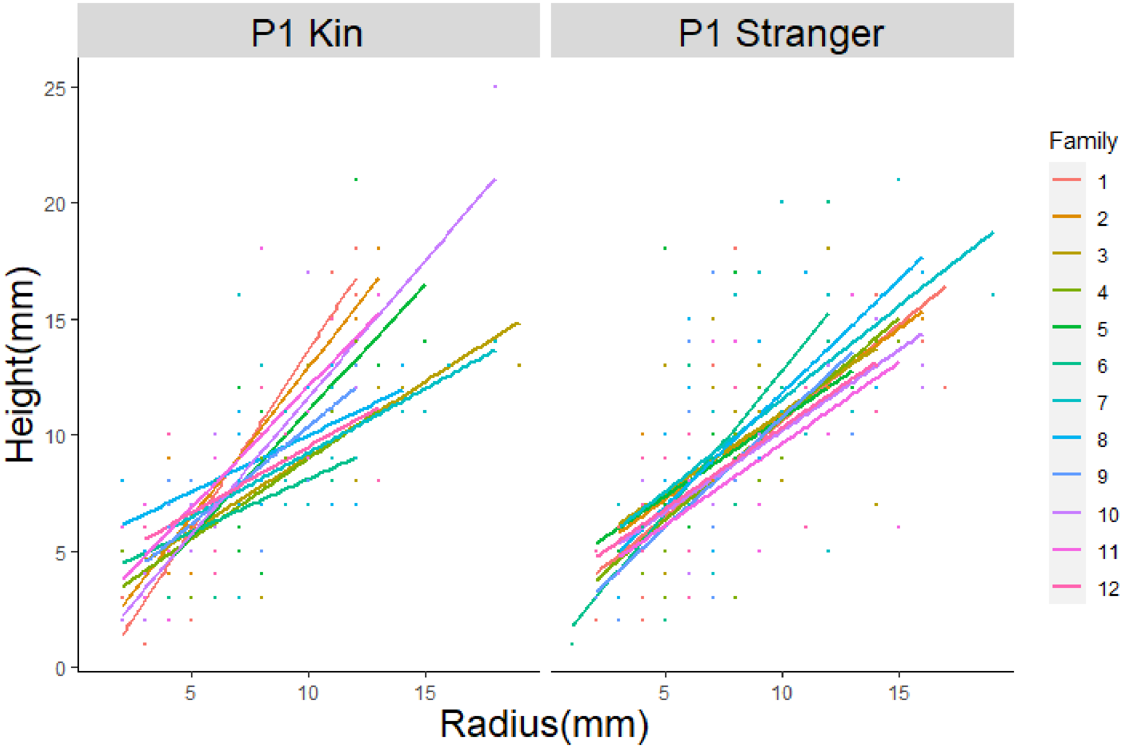

Figure 3 Phase 1 *Potentilla recta* height response to the interaction of relatedness, the covariate; leaf radius, and family. Lines represent mean height for each value of radius per family. Plants were grown in a greenhouse.

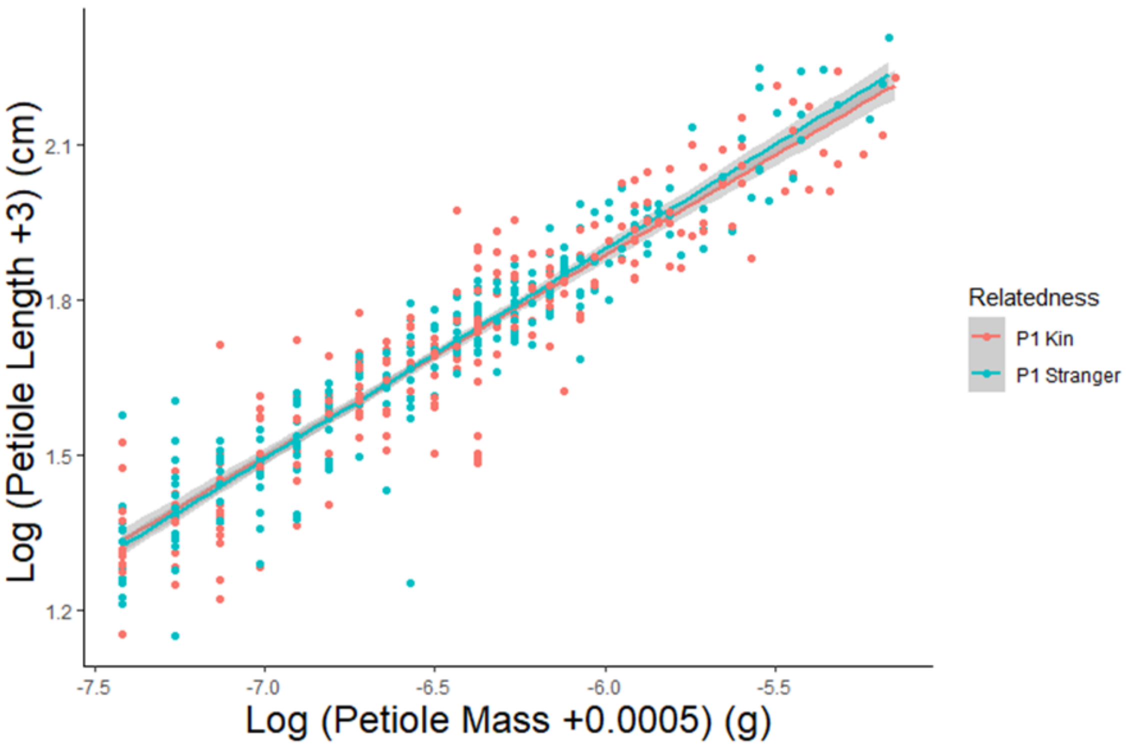

Figure 4 Phase 1 *Potentilla recta* log petiole length in response to relatedness and the covariate; log petiole mass. Plants were grown in a greenhouse. Lines represent mean log transformed petiole elongation for each value of log transformed petiole mass. Shaded area indicates 95% confidence interval.

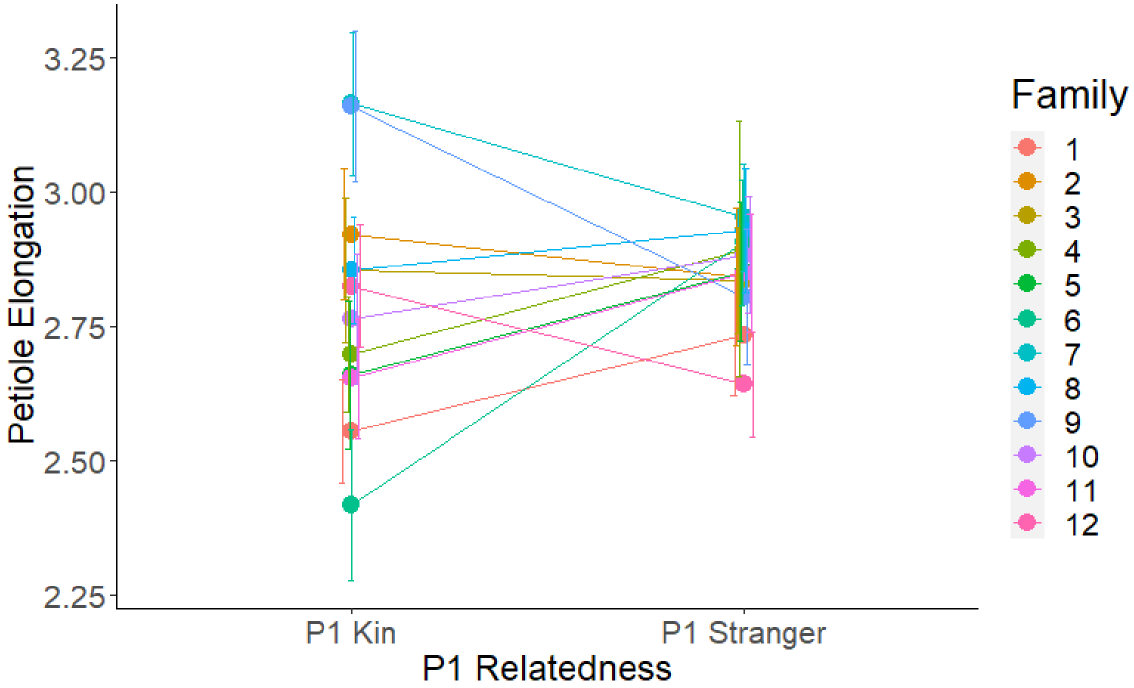

Figure 5 Interaction plot of petiole elongation in phase 1 plants in response to the interaction of family and relatedness for *Potentilla recta* grown in a greenhouse. Results are back transformed emmeans from the ANCOVA of log(petiole length+3) using log(petiole mass+0.0005) as the covariate. Bars indicate 1 SE.

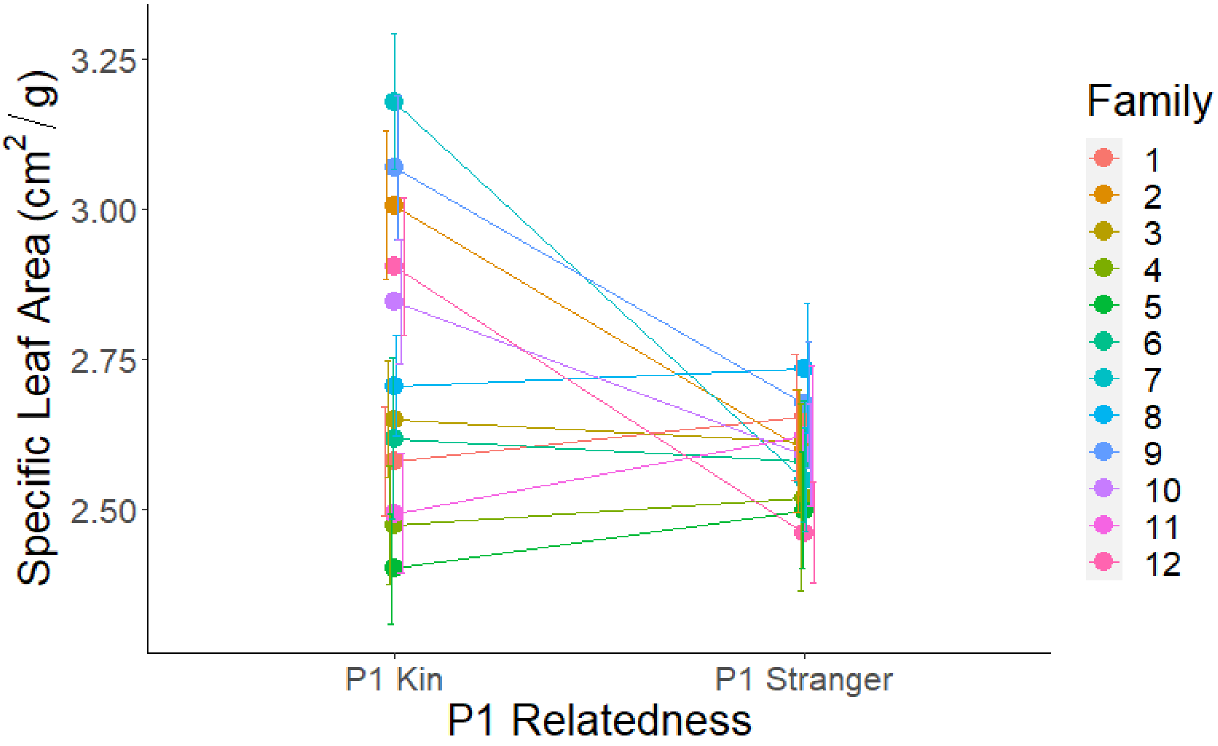

Figure 6 Interaction plot for specific leaf area of phase 1 *Potentilla recta* in response to the interaction of family and neighbour relatedness. Results are back transformed emmeans from the ANCOVA of log(leaf surface area+0.5) using the covariate log(leaf blade mass +0.001). Plants were grown in a greenhouse. Bars indicate 1 SE.

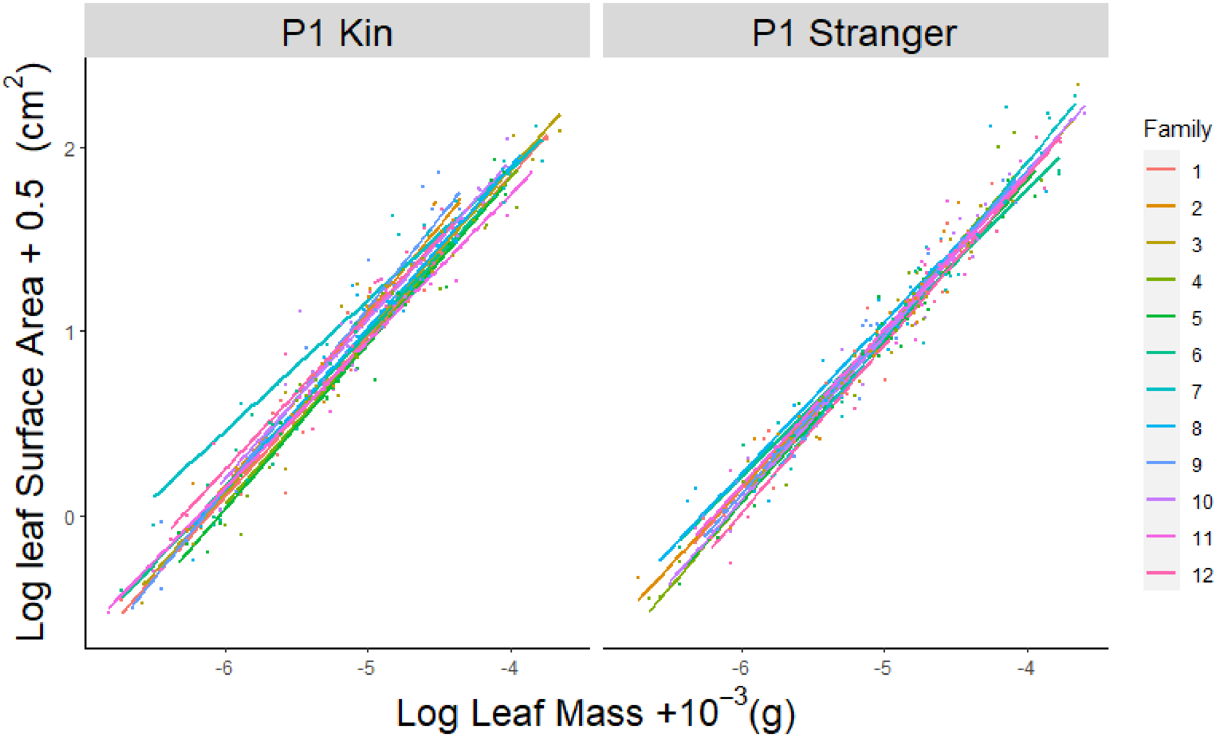

Figure 7 Phase 1 *Potentilla recta* log leaf surface area response to the interaction of relatedness, the covariate; leaf mass, and family. Lines represent mean log transformed specific leaf area for each value of log transformed leaf mass. Plants were grown in a greenhouse.

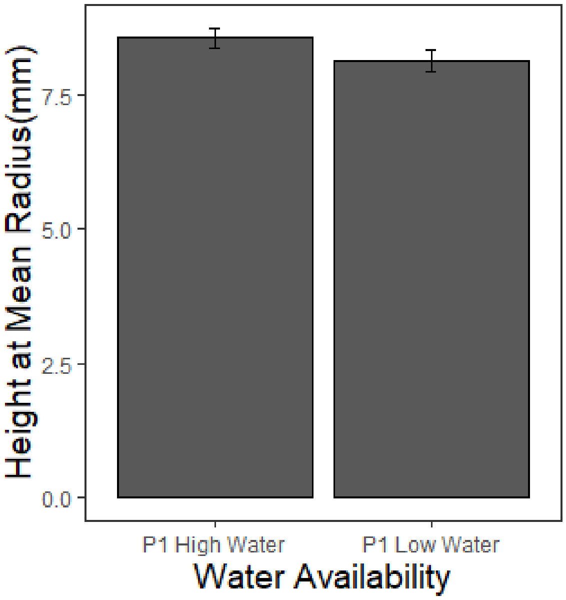

Figure 8 Plot of mean *Potentilla recta* leaf angle (ratio of leaf height by leaf radius) in response to water availability level in phase 1 plants, in a greenhouse. Bars indicate 1 SE.

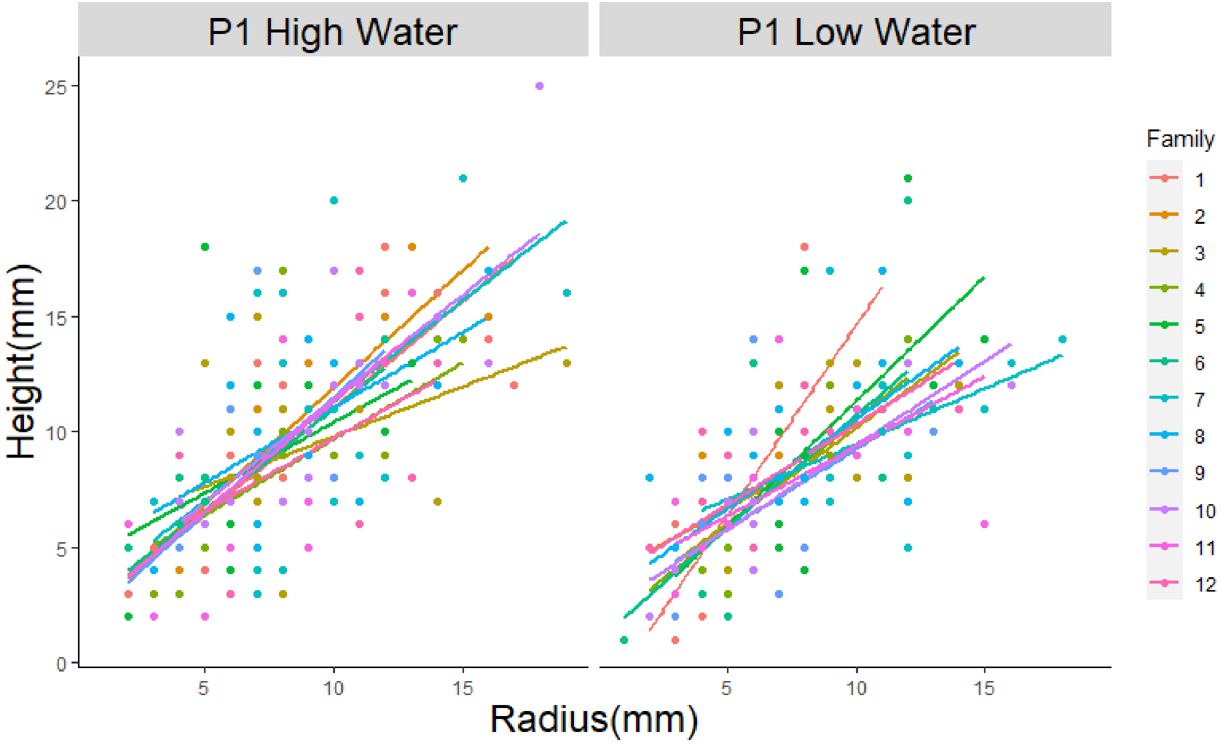

Figure 9 Phase 1 *Potentilla recta* height response to the interaction of water availability, and the covariate; leaf radius, by family. Lines represent mean height for each value of radius. Plants were grown in a greenhouse. Fig 4

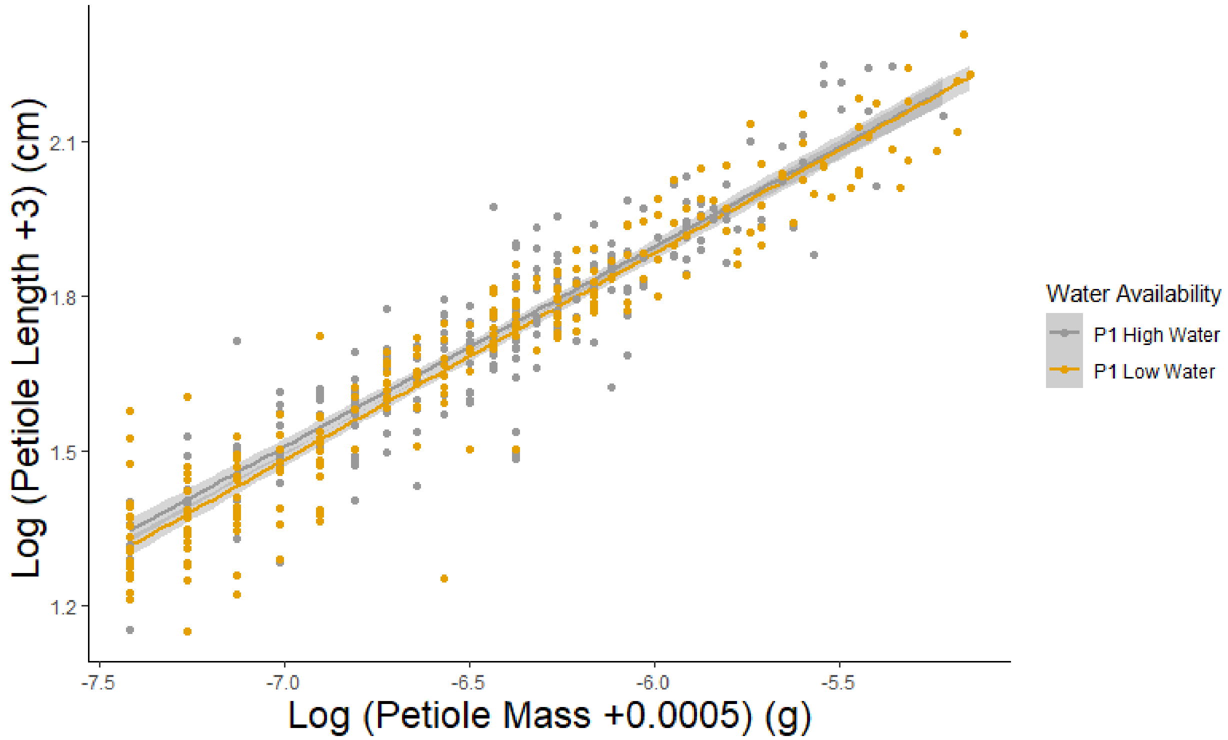

Figure 10 Phase 1 *Potentilla recta* log petiole elongation response to water availability and the covariate; petiole mass. Plants were grown in a greenhouse. Lines represent mean log transformed petiole elongation for each value of log transformed petiole mass. Shaded areas indicate 95% confidence interval.

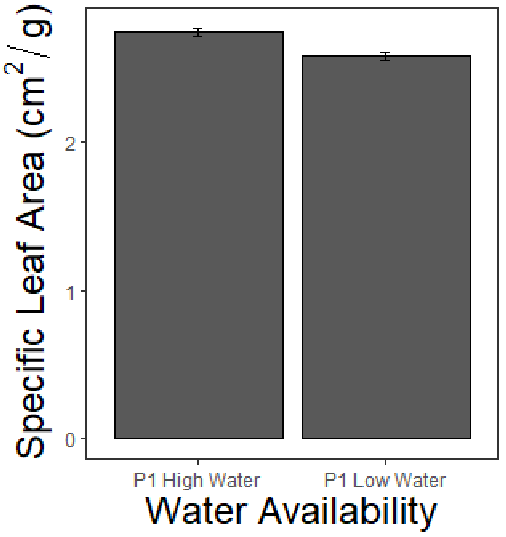

Figure 11 Plot of mean *Potentilla recta* specific leaf area (ratio of leaf surface area by leaf mass) in response to water availability in phase 1 plants grown in a greenhouse. Results are back transformed emmeans from the ANCOVA of log (leaf surface area+0.5) using the covariate log(leaf blade mass +0.001). Bars indicate 1 SE.

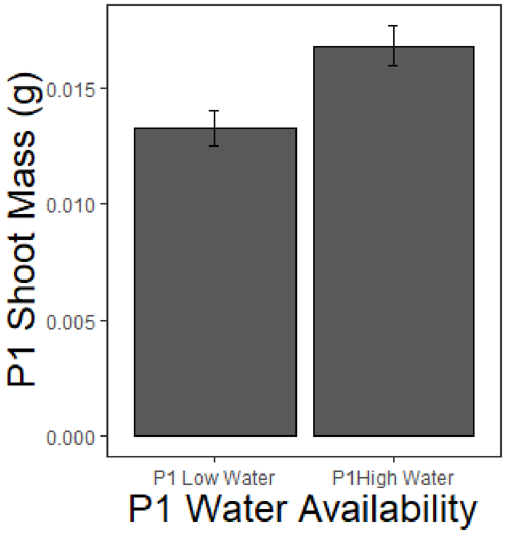

Figure 12 Plot of greenhouse grown *Potentilla recta* mean shoot mass response to phase 1 water availability. Results are back transformed emmeans from the ANOVA of log(shoot mass +0.005). Bars indicate 1 SE.

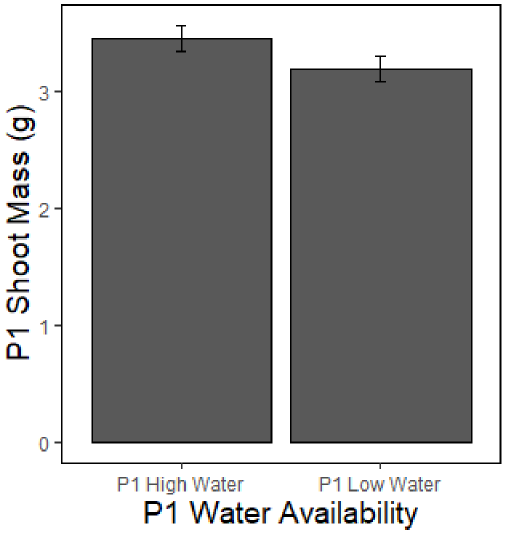

Figure 13 Mean leaf number of phase 1 greenhouse grown *Potentilla recta* plants in response to water availability. Results are emmeans from the Poisson ANOVA of leaf number. Bars indicate 1 SE.

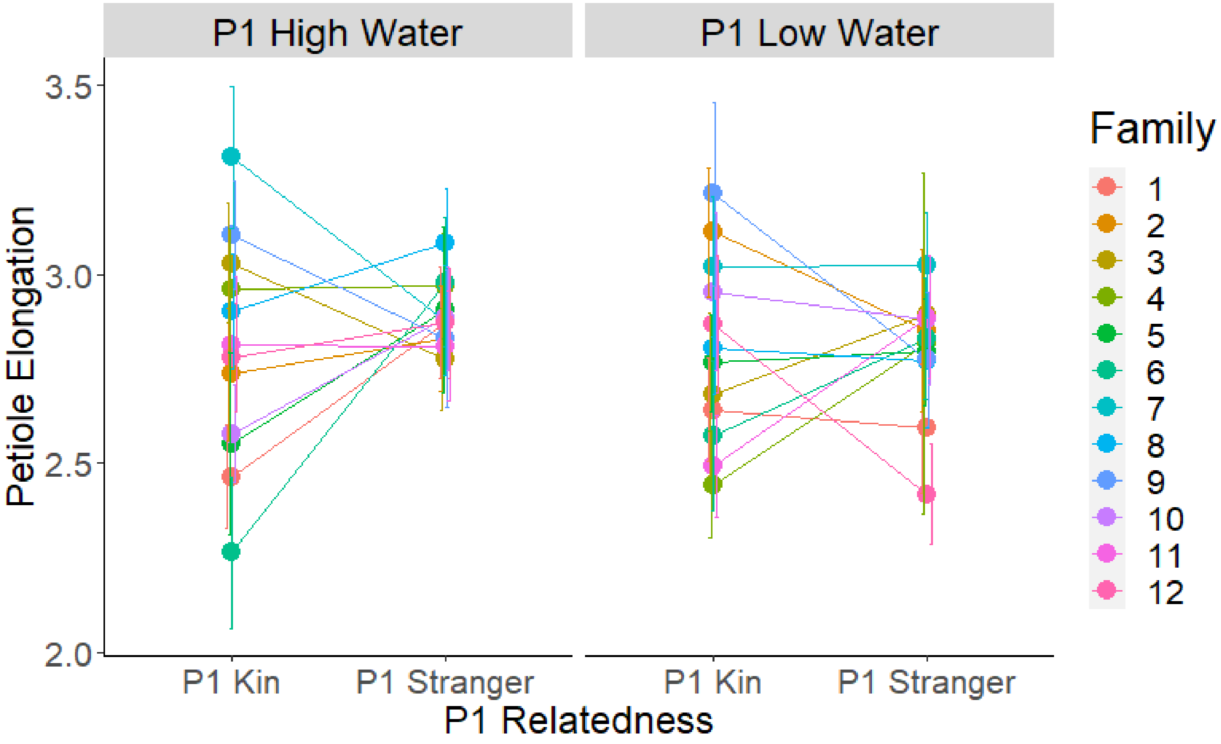

Figure 14 Interaction plot for petiole elongation of phase 1 plants in response to the interaction of family, neighbour relatedness, and water availability. Results are back transformed emmeans from the ANCOVA of log(petiole length +3) using the covariate log(petiole mass +0.0005) for greenhouse grown *Potentilla recta*. Bars indicate 1 SE.

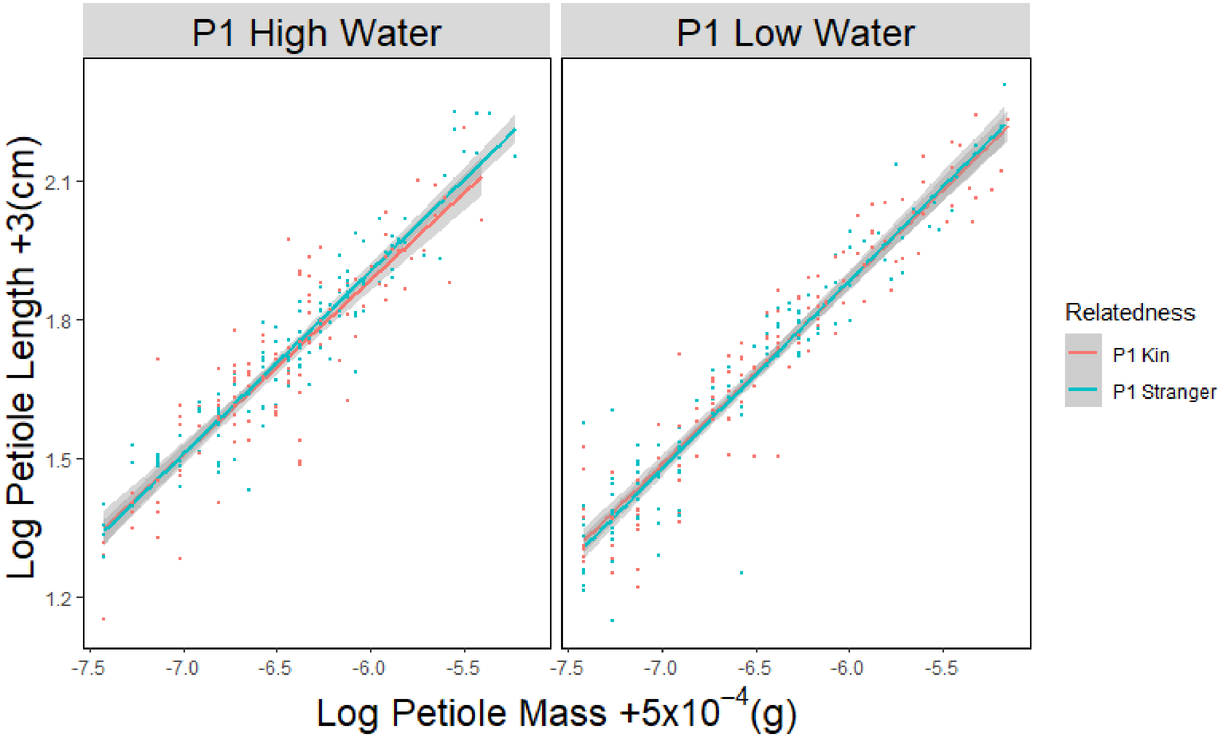

Figure 15 Interaction plot of phase 1 log petiole length in response to relatedness, water availability, and the covariate; log petiole mass, for greenhouse grown *Potentilla recta*. Graph is faceted by water availability. Lines represent mean log transformed petiole elongation for each value of log transformed petiole mass. Shaded areas indicate 95% confidence interval.

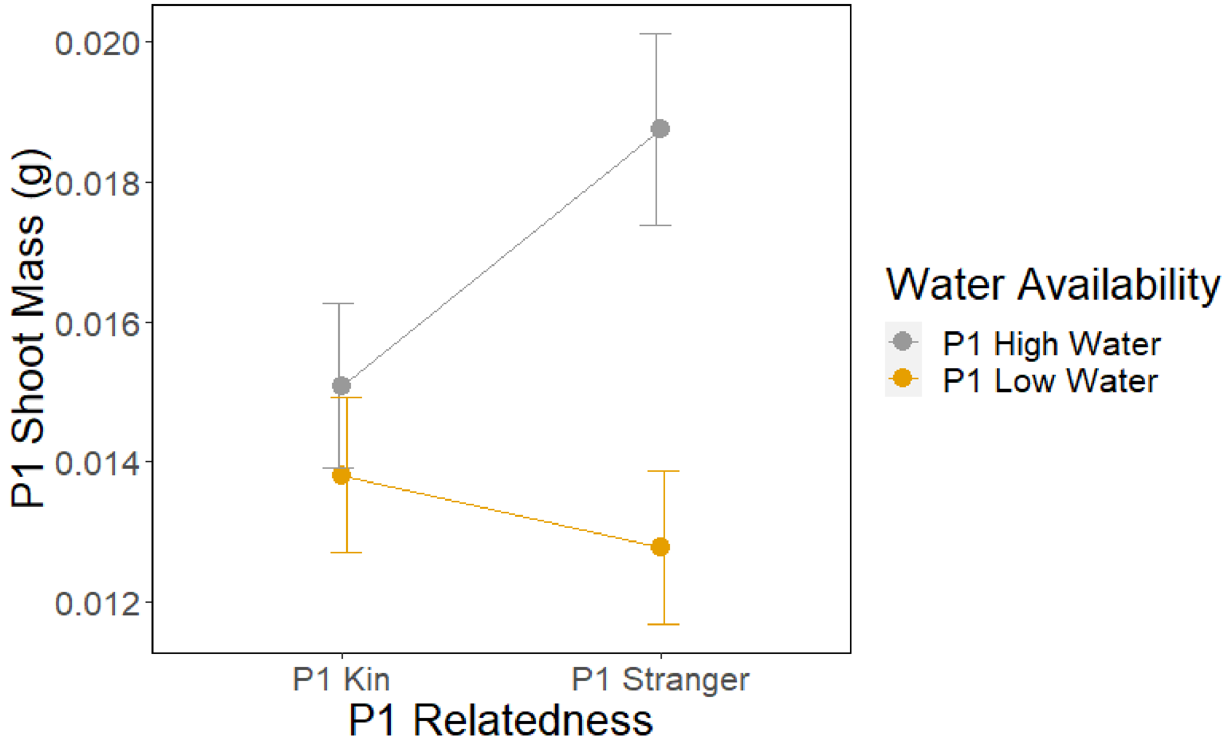

Figure 16 Interaction plot of phase 1 mean shoot mass in response to water availability and relatedness with neighbours for greenhouse grown *Potentilla recta*. Results are back transformed emmeans from the ANOVA of log(shoot mass +0.005). Bars Indicate 1 SE.

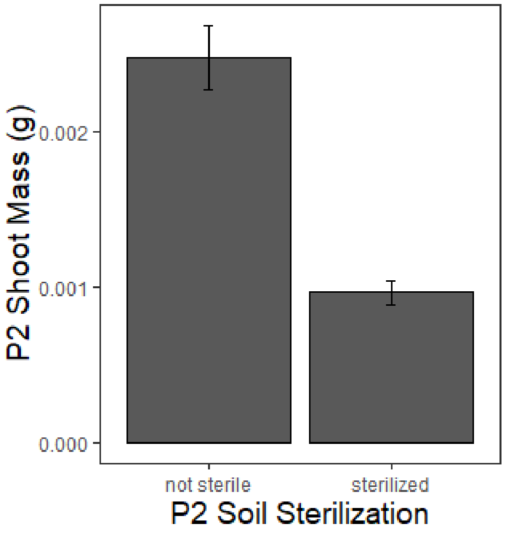

Figure 17 Mean shoot mass of phase 2 *Potentilla recta* plants in response to soil sterilization. Results are back transformed emmeans from the ANOVA of log (phase 2 shoot mass + 0.0001). Bars indicate 1 SE.

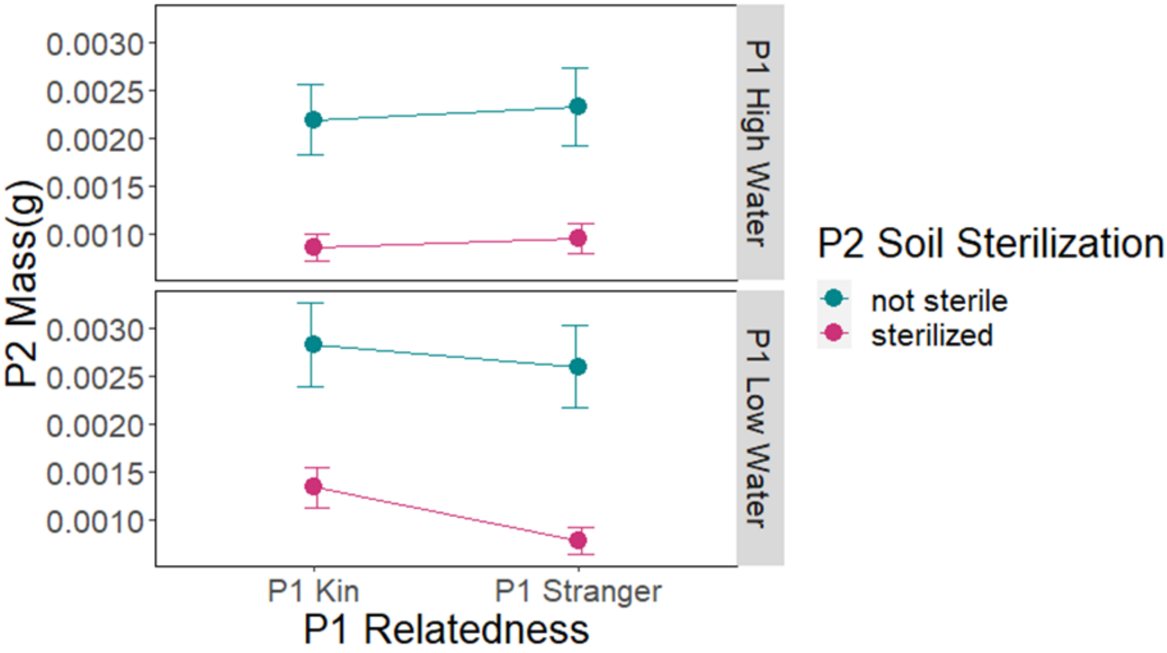

Figure 18 Interaction plot of phase 2 greenhouse grown *Potentilla recta* mean shoot mass in response to phase 1 relatedness, phase 1 water availability, and phase 2 soil sterilization. Results are back transformed emmeans from the ANOVA of log(mass +0.0001). Bars indicate 1 SE.

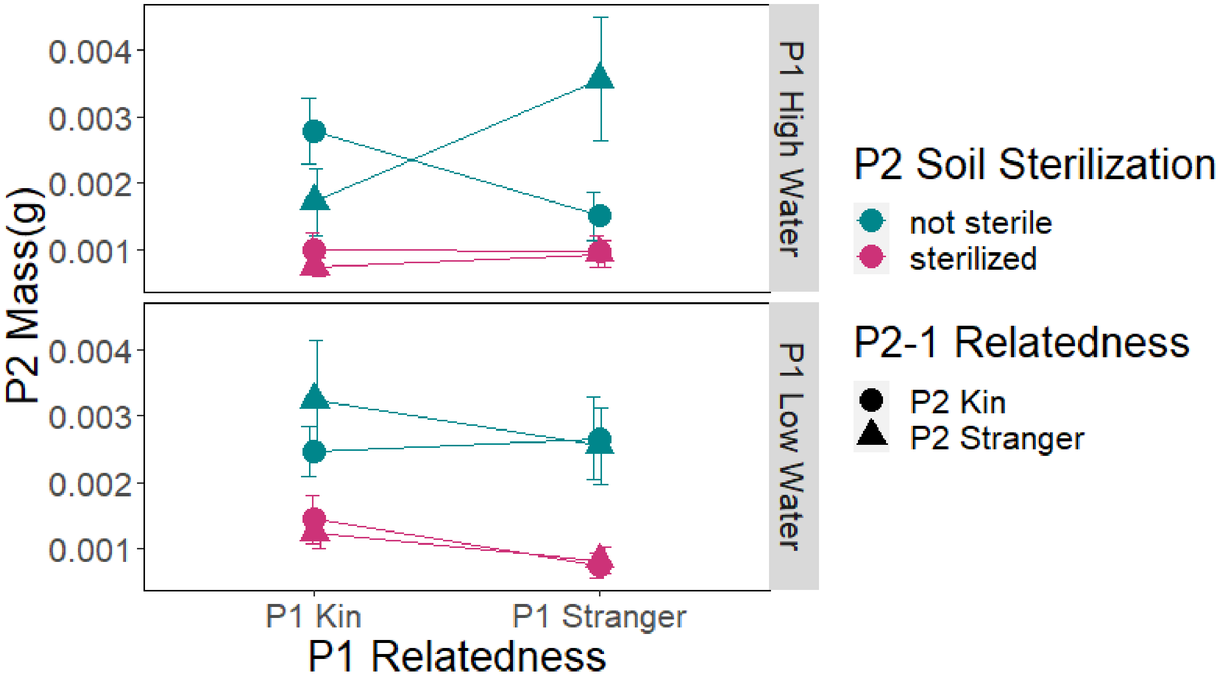

Figure 19 Interaction plot of phase 2 greenhouse grown Potentilla recta mean shoot in response to phase 1 relatedness, phase 1 water availability, phase 2-1 relatedness and phase 2 soil sterilization. Results are back transformed emmeans from the ANOVA of log (mass +0.0001). Bars indicate 1 SE.

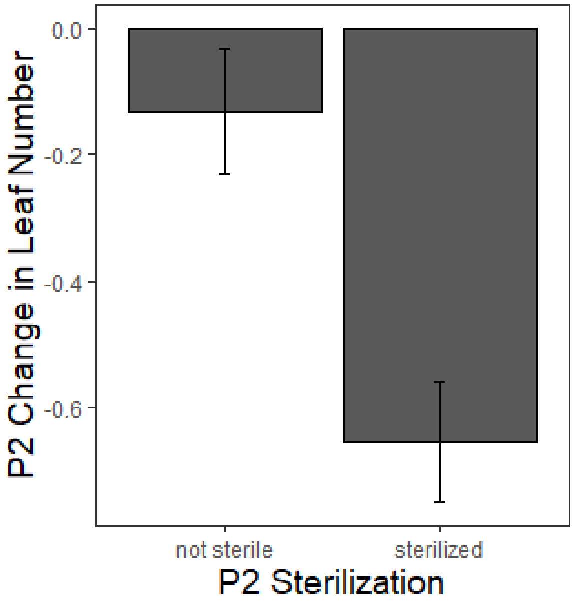

Figure 20 Mean change in leaf number over two weeks for phase 2 greenhouse grown *Potentilla recta* in response to phase 2 soil sterilization. Bars indicate 1 SE.

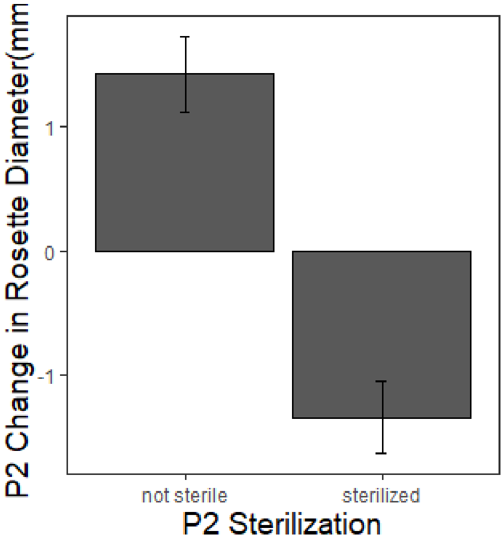

Figure 21 Mean change in leaf diameter over two weeks, for phase 2 greenhouse grown *Potentilla recta,* in response to phase 2 soil sterilization. Bars indicate 1 SE.

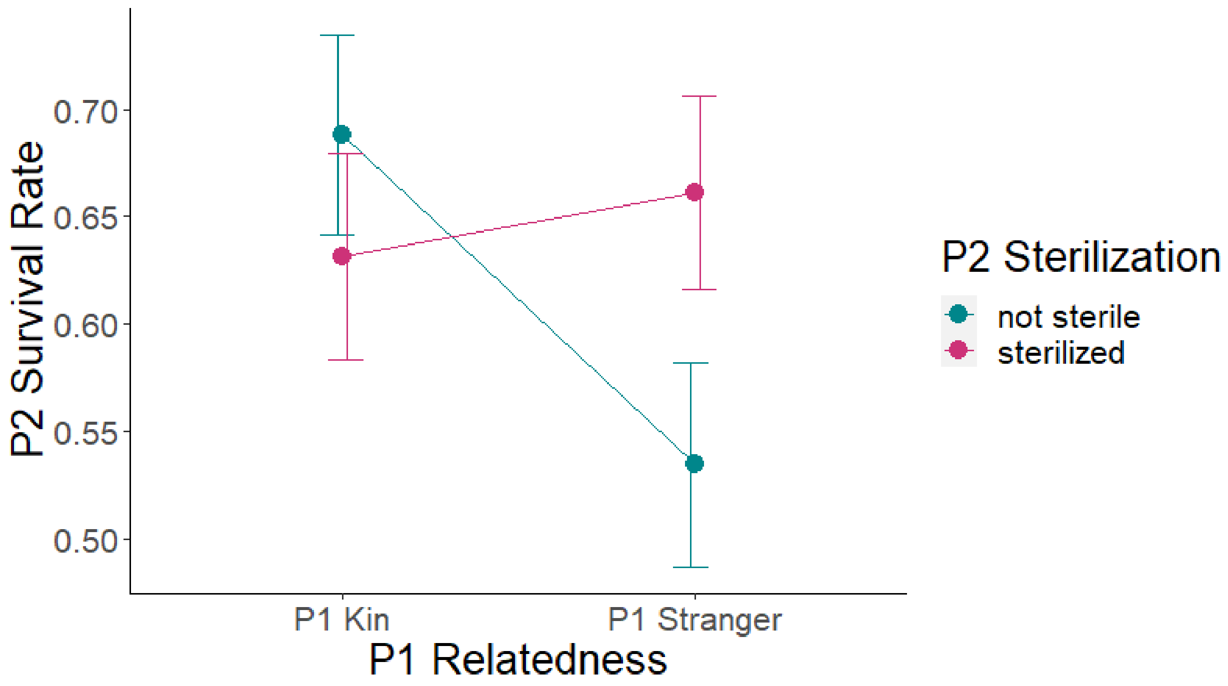

Figure 22 Interaction plot of survival rate of phase 2 seedlings in response to phase 1 relatedness and phase 2 sterilization for phase 2 *Potentilla recta* grown in a greenhouse. Results are emmeans from the quasibinomial logistic regression of survival status. Bars indicate 1 SE.

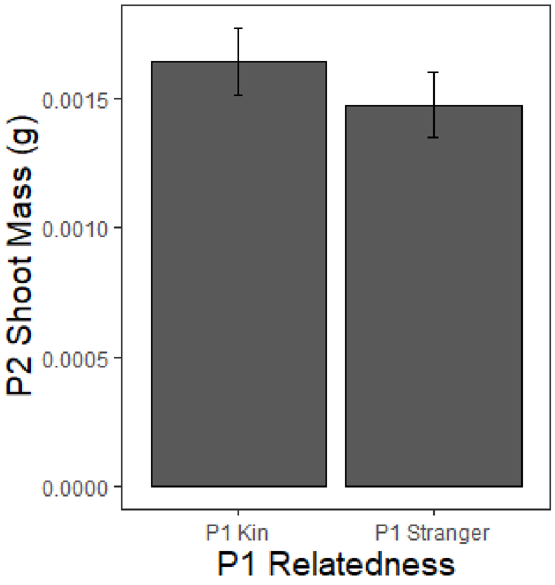

Figure 23 Mean shoot mass of phase 2 *Potentilla recta* plants in response to relatedness of phase 1 soil plants. Results are back transformed emmeans from the ANOVA of log (phase 2 shoot mass + 0.0001). Bars indicate 1 SE.

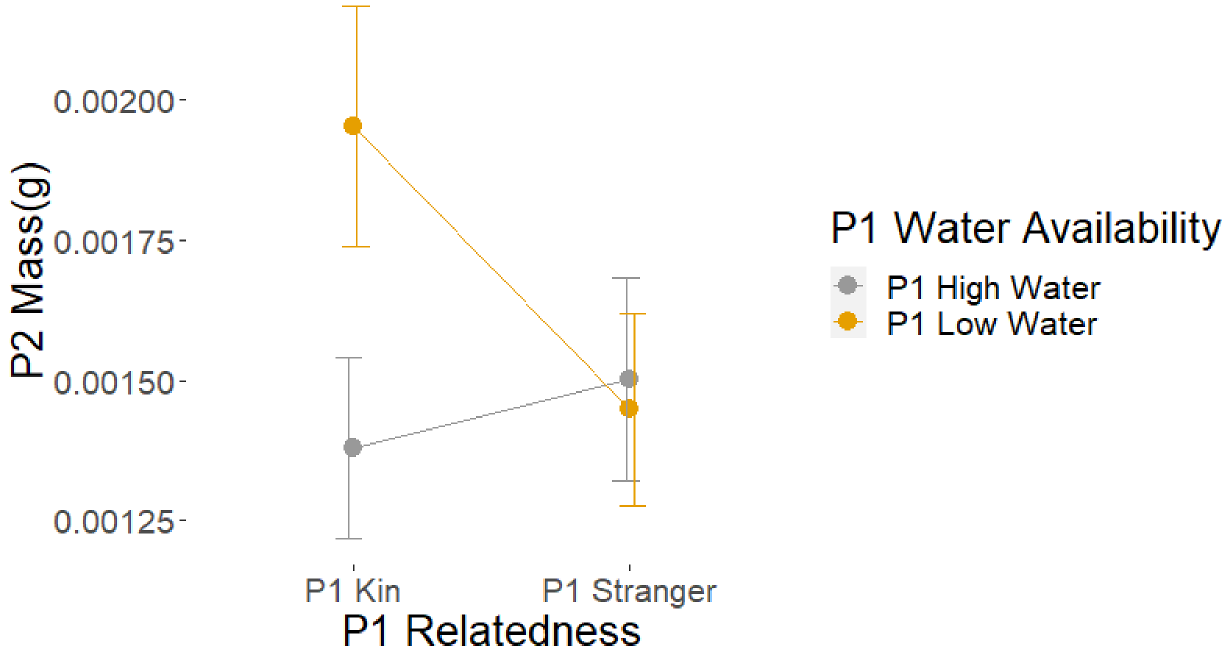

Figure 24 Interaction plot of phase 2 greenhouse grown *Potentilla recta* mean shoot mass in response to phase 1 relatedness and phase 1 water availability effects on phase 2 growth medium. Results are back transformed emmeans from the ANOVA of log(mass +0.0001). Bars indicate 1 SE.

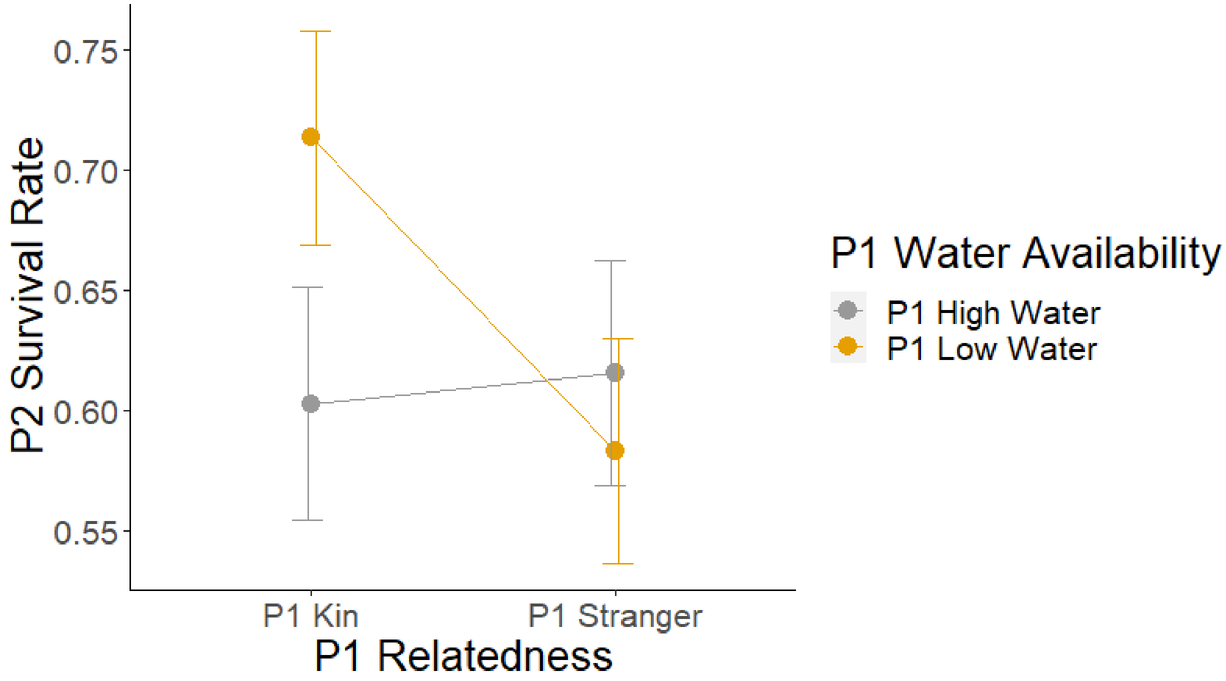

Figure 25 Interaction plot of survival rate in response to phase 1 relatedness and phase 1 water availability for phase 2 *Potentilla recta* grown in a greenhouse. Results are emmeans from the quasibinomial logistic regression of survival status. Bars indicate 1 SE.

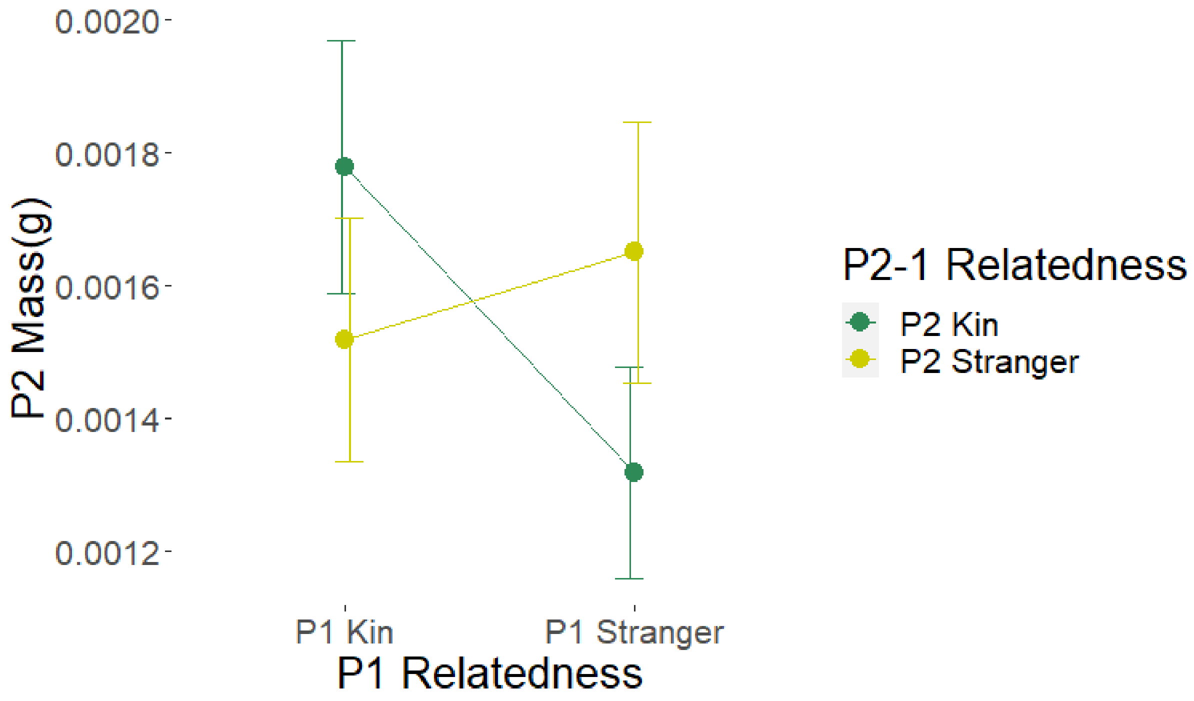

Figure 26 Interaction plot of phase 2 greenhouse grown *Potentilla recta* mean shoot in response to phase 1 relatedness and phase 2-1 relatedness. Results are back transformed emmeans from the ANOVA of log (mass +0.0001). Bars indicate 1 SE.

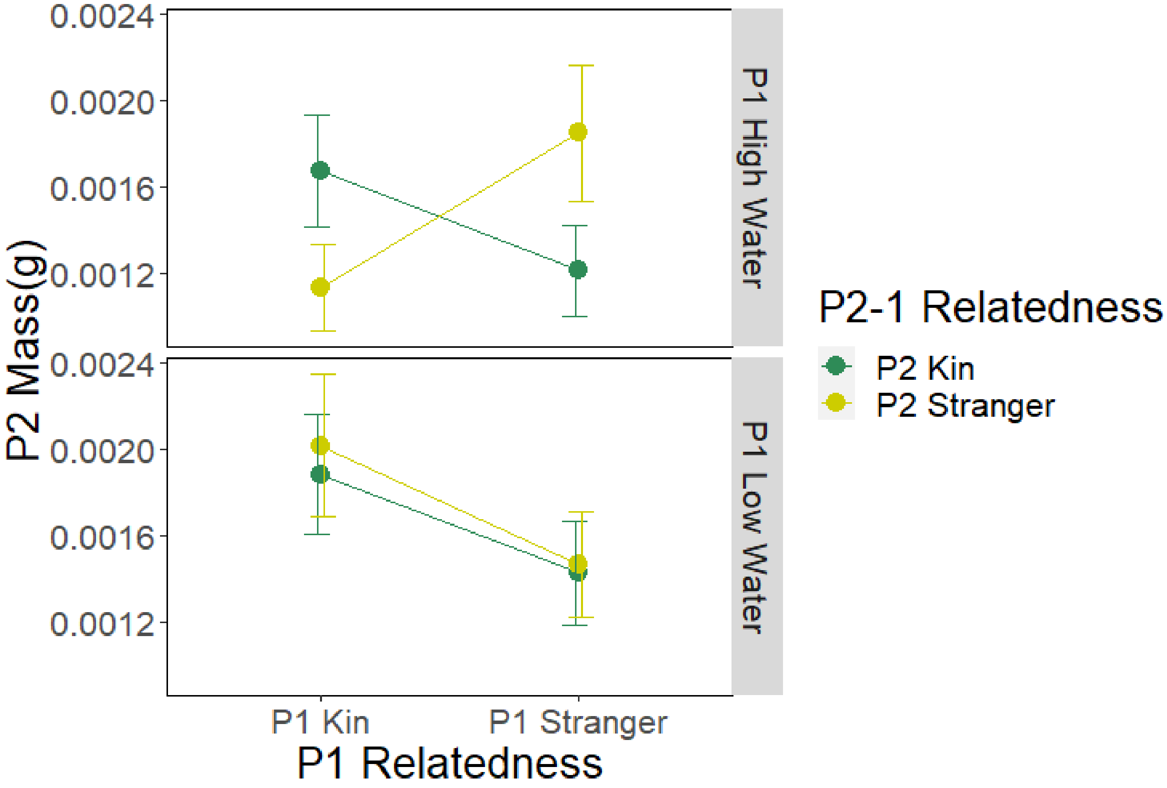

Figure 27 Interaction plot of phase 2 greenhouse grown Potentilla recta mean shoot mass in response to phase 1 relatedness, phase 1 water availability, and phase 2 plant relatedness to phase 1 plants. Results are back transformed emmeans from the ANOVA of log(mass +0.0001). Bars indicate 1 SE

Chapter 4: Discussion

In this study I asked whether the relatedness of the stand influences fitness of subsequent generations in the same area, and whether those influences are specific to whether the subsequent generations are related to the initial stand. In a study across two generations, I examined the performance of plants grown in soil conditioned by a monospecific stand of kin or strangers from a prior generation. In the soil condition generation (phase 1), sulfur cinquefoil did exhibit relatedness responses in adaptive and performance traits which depended on water availability. In the response to soil conditioning generation (phase 2), plant performance responded to the relatedness of phase 1 plants. The phase 2 performance responses to whether phase 1 stands were kin or stranger depended on the relatedness of plants in the two generations and phase 1 water availability. Surprisingly, soil sterilization reduced plant size but improved survival for plants growing in soil conditioned by strangers.

4.1 Phase 1

4.1.1 Kin Recognition in Sulfur Cinquefoil

The result that leaf angles were greater in plants grown in groups of strangers than kin is consistent with kin recognition in sulfur cinquefoil. There was also a significant but very small difference in petiole elongation between growing with kin and strangers. Both of these responses can affect competitive ability. Leaf angle is a complex adaptive trait in the context of plant competition. In plants with low self shading, horizontal leaves provide the greatest photosynthetic capacity, as it maximizes surface area for light interception (Hikosaka & Hirose, 1997). These responses indicated increased light competition in plants grown among strangers than kin, as higher leaf angles result in greater overall leaf height at the cost of photosynthetic rate (Fig. 2) (Ford, 2014; Van Zanten et al., 2010). One possible explanation for this phenomenon is cooperation in kin via reduced competition (Anten & Chen, 2021; Chen et al., 2012; Murphy et al., 2017; Platt & Bever, 2009). Previous work on *Arabidopsis thaliana*, another rosette species, has shown plants avoid vertical growth when grown with kin neighbors, supporting the reduced kin competition hypothesis (Crepy & Casal, 2015; Gruntman et al., 2017). Like leaf angle, petiole elongation is an adaptive trait which contributes to greater above ground competitive ability and is a well-known response to shade avoidance (Casal, 2012; Martínez-García et al., 2014; Roig-Villanova & Martinez-Garcia, 2016). I found greater petiole elongation in *Potentilla recta* grown among strangers than kin, but only when petiole mass was high (Fig. 5). The increased petiole elongation is a response to increased light competition and thus greater need for shade avoidance (Casal, 2012; Roig-Villanova & Martinez-Garcia, 2016). These results are consistent with decreased competitive ability among kin, as decreased light competition in kin plants leads to reduced need to avoid shade. Kin recognition in rosette plants can result in spatial reorientation of leaf petioles to decrease leaf overlap with related neighbours (Crepy & Casal, 2015). While no difference in petiole elongation exists between kin and strangers when petiole mass is small, plants with low petiole mass may not be large enough, and therefore not be in close enough proximity to compete with neighbours for light. Overall, these results show sulfur cinquefoil can recognize and responding to the relatedness of neighbours.

There was apparent among-family variation in the effect of relatedness on all three adaptive traits measured. Throughout, growing in groups of kin resulted in substantially greater among family variation than strangers, across all measured adaptive traits. To date, no study has reported such consistent effects of relatedness on family variation in adaptive traits. This result is interesting and should be followed up in future research.

4.1.2 General Responses to Water

As expected, low water availability resulted in reduced shoot mass and average leaf number compared to high water availability (Farooq et al., 2009; Seleiman et al., 2021). This indicates the water availability treatment successfully elicited more water stress from the low water availability plants compared to the high water availability plants. Unexpectedly, leaf angle was greater in plants grown with high water availability. Leaf angle was expected to be greater in low water availability plants as water use efficiency increases with higher leaf angles. Higher leaf angles result in decreased light interception by the leaf. This helps avoid overheating, leading to less water loss due to transpiration (Falster & Westoby, 2003; Van Zanten et al., 2010). One hypothesis is the plants were simply exhibiting wilting due to lowered turgor pressure (Calbo et al., 2010).

Water availability influenced petiole elongation, but only when petiole mass was small. At low petiole masses, high water availability results in greater petiole elongation

(Fig. 10). One possible hypothesis is plants are unlikely to be in contact with each other when they are small; small plants of low water availability can allocate fewer resources to shade avoidance when they're likely to not be in contact with neighbours. As plants get larger, they become better able to store and obtain water, and thus the impact of low water availability is minimized (Yoo et al., 2011; Zotz et al., 2001).

4.1.3 Influences of Water Availability on Kin Recognition Responses

Responses to relatedness in petiole elongation and shoot mass depended on levels of water availability. Shoot mass of sulfur cinquefoil was significantly greater when grown in groups of strangers, but only when water availability is high. While this result is consistent with kin selection theory (Dudley & File, 2007; Hamilton, 1964) this result also supports niche partitioning. According to the resource theory, closely related individuals share more similar resource requirements. Thus groups of strangers, with more varied resource requirements should outperform groups of kin (Zhang et al., 2016). However, when grown with low water availability, there is no significant difference in shoot mass between plants grown with kin or strangers. This contradicts niche partitioning, as the shoot mass of plants grown with strangers is not consistently greater than the mass of kin plants. Instead, this evidence suggests reduced competition among kin for water. Kin recognition is known to reduce water competition in groups of kin, so much so water uptake in pairs of shared pot kin *Fagus crenata* seedlings is comparable to the water uptake of individual seedlings (Takigahira & Yamawo, 2019). As shown previously in petiole elongation and leaf angle, kin are less competitive than strangers, which is consistent with kin selection theory. Thus, I argue that sulfur cinquefoil exhibits both kin recognition and niche partitioning in early shoot mass, depending on water availability.

4.2 Phase 2

4.2.1 Effects of Soil Sterilization on P2 Performance

The significant impacts of the sterilization imply there is a microbial effect. The finding that soil sterilization drastically reduced phase 2 plant biomass for all soil conditions (Fig. 18) is consistent with the enhanced mutualist hypothesis, where invasive plants can influence soil microbe communities in their invaded site to gain more benefit from microbial partners, for either the same or decreased cost (Dawson & Schrama, 2016; Sun & He, 2010; Z. W. Xu et al., 2021). As autoclaving increases the level of bioavailable nutrients in soil, such as Mn, N, P, S, and organic carbon (Berns et al., 2008; Liegel, 1986), the differences are not consistent with nutrient effects. Soil microbes can facilitate the invasion of non-native species (Dawson & Schrama, 2016; Reinhart & Callaway, 2004; Sun & He, 2010). Sulfur cinquefoil may be recruiting beneficial plant-growth-promoting bacteria via root exudates. Alternatively, sulfur cinquefoil may depend heavily on mycorrhizae, and its absence due to sterilization stunted growth rate in P2 plants.

While soil sterilization did not affect survival of P2 plants grown in kin-conditioned soils, soil sterilization increased survival of P2 plants grown in stranger soils to levels comparable to growing in kin-conditioned soils (Fig. 21). The difference in survival rate could be either increased microbial defense among groups of kin or allelopathy. Rice plants alter their chemical defenses in response to the relatedness of their neighbors(Y. Xu et al., 2021). While it is possible, albeit unlikely, some allelopathic exudates are reactive under the heat and pressure of the autoclave (Berns et al., 2008), sulfur cinquefoil is currently not known to be allelopathic (Powell, 1996). Instead, the effect of sterilization on P2 survival rate implies a second microbial effect resulting in different survival rates between P2 plants grown in kin or stranger-conditioned soils.

4.2.2 Effects of Soil Conditioning on Performance of P2 Plants

Soil conditioned by phase 1 kin resulted in greater shoot mass and survival, but only when the soil was conditioned by phase 1 plants grown under low water availability (Fig. 23). The mechanism for this is currently unknown. One possible mechanism behind these effects may have been groups of kin plants release nutrients such as potassium, into the soil to promote growth in subsequent generations. Potassium has been shown to increase drought resistance traits such as root mass and distribution (Q. Xu et al., 2021). Such traits could have been invaluable, given the plants were grown in clay soils, which are notorious for poor water retention and resistance to root proliferation (Amato & Ritchie, 2002; Colombi et al., 2021).

4.2.3 Differential Effects of Soil Conditioning Based on Relatedness of P2 Plants to P1 Soil Conditioning Plants

That phase 2 kin plants grew more mass in P1 kin-conditioned soil than in P1 stranger-conditioned soils and P2 stranger plants grew significantly more mass in soil conditioned by strangers than kin (Fig. 27) indicate strong specificity of kin recognition effects across generation. However, this interaction was driven by the responses of P2 plants grown in unsterilized soils conditioned by P1 plants grown with high P1 water availability (Fig. 25). That the interaction only occurs in unsterilized soil may indicate that this effect was also mediated by microbes while the dependence on P1 water availability suggests it occurred when P1 plants had more access to water. Increased defenses against deleterious microbes in groups of P1 kin would explain the benefit the difference in P1 kin-conditioned soils. According to this hypothesis, P2 kin would be more vulnerable in P1 stranger-conditioned soils due to microbes adapting to the specific chemical defenses of each P1 family. Allelopathy would explain the effect of P1 kin-conditioned soils benefitting only future generations of kin. However, to explain the effect of P1 stranger-conditioned soils selectively benefitting a P2 stranger with allelopathy, it would require the group of P1 strangers to selectively target its neighbours according to family. More work needs to be done to elucidate the underlying mechanics of these selective effects. I confirm soil conditioning by phase 1 plants results in soil effects which selectively benefit subsequent generations is conditional on the relatedness of phase 2 plants to the phase 1 conditioning plants. However, selective soil effects only exist when water availability in phase 1 is high. Soil conditioned by low water plants did not respond to phase 2-1 relatedness.

4.3 Conclusions

I have shown for the first time Sulfur Cinquefoil exhibits kin recognition behavior, specifically in size, morphology, and elongation. Further, I have shown sulfur cinquefoil exhibited some kin recognition responses that depended on water availability. In the second generation I found soil sterilization drastically inhibited growth, but improved survival rate of stranger-conditioned soils. Subsequently, I showed relatedness and water levels of the prior generation influenced the second generation growing in the same soil. Finally, found sulfur cinquefoil conditions soil to the benefit of subsequent generations of kin and detriment of subsequent generations of competitors. Taken together, my findings suggest that by influencing soil microbes, kin recognition in plants can exhibit transgenerational effects. These transgenerational effects could influence of soil for the benefit of future generations of kin, or the detriment of competing families.

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