



**THE UNIVERSITY OF EDINBURGH**

**SCHOOL OF GEOSCIENCES**

**Biomass allocation patterns of three herbaceous species in  
response to the combined drought effect of low irrigation  
and high soil porosity**

*BY*

**LOUISE LITRICO**

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# ABSTRACT

There is a pressing need to understand how plants will respond to extreme climate events in the future. The response of herbaceous species to drought stress has been studied extensively over the last century. Herbaceous plants are known to display characteristics of drought resistance by increasing root biomass under limited water availability for example. However, the combined effect of low irrigation levels and soil composition has yet to be the focus of research on biomass allocation patterns in aromatic herbs. This dissertation looks at this combined effect on three species: Basil, Dill and Parsley. By using a fully crossed experimental design between three soil types and three irrigation levels, this study was able to describe the biomass allocation patterns of these plants under different drought intensities. The root:shoot ratio, leaf area, total biomass production and allometric relationship between root and shoots of the plant individuals were compared between treatments. Ontogenetic drift explained all the variation in leaf area and total biomass but little of the variation in resource allocation between roots and shoots. The plants responded to higher drought by increasing root:shoot ratios under lower irrigation and higher soil porosity. The plant individuals also showed changes in allometric regressions in a way consistent with the optimal partitioning theory that combined true and apparent plasticity. These results stress the importance of drought on biomass allocation patterns of aromatic herbs which can change greatly in the early growth stages after germination.

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## LIST OF ABBREVIATIONS

|        |                             |
|--------|-----------------------------|
| VWC    | Volumetric Water Content    |
| BAP    | Biomass Allocation Patterns |
| ANCOVA | Analysis of Covariance      |
| ANOVA  | Analysis of Variance        |
| LM     | Linear Model                |

# 1-INTRODUCTION

Understanding the way that plants respond to the availability of resources in their environment is a major question in ecology (McConnaughay and Coleman, 1999). Knowing if the plant will change and how this will manifest can help us predict the effects of future environmental conditions. This interest is not only restricted to plant ecologists since agricultural producers and everyday consumers are also concerned by these changes that could impact their livelihood or their consumption in the future. Environmental conditions can have high impacts on agricultural harvests and food prices around the world since changes in temperature, water availability, light availability or essential nutrients can greatly change the way plants grow and develop (Gedroc, McConnaughay and Coleman, 1996). Extreme climate events such as intense rainfalls, flooding, drought, and heat stress are expected to increase in frequency and amplitude in the next few decades because of climate change (IPCC, 2019). Knowing how plants will change under those future conditions can help us prepare for the effects and provide useful insight for agricultural purposes in the future.

Plants generally respond to changes in their environment by adapting the way they grow through variations in morphology (Coleman, McConnaughay and Ackerly, 1994). Similar to the way a tree will grow taller under limited canopy light by increasing resources allocated to the branches and the trunk, other plants can change their resource allocation to adapt to their environment. Although several changes can occur in the partitioning of resources in plants, the main variable of interest has historically been biomass (Bazzaz et al., 1987). Biomass is an easy way to look at resource partitioning since it can be measured simply through the weight of the different parts of the plant (Bazzaz et al., 1987, Eziz et al., 2017).

## 1-2 BIOMASS ALLOCATION IN THEORY

The changes in biomass in the plant throughout its growth are called biomass allocation patterns (BAP)(Coleman, McConnaughay and Ackerly, 1994). BAP in plants have historically been conceptualized in two ways. First, as a time-dependent phenomenon, where a plant of a certain age or at a certain point in time will have a finite quantity of resources to allocate to its various structures (Bloom, Chapin, and Mooney, 1985). This is the ratio partitioning perspective where the biomass allocation is akin to a ratio between the different structures of the plant. From this concept stems the optimal partitioning theory or functional equilibrium (Poorter et al., 2012a), an idea adapted from economics according to which the ratio of resources allocated to different structures of a plant will change in response to environmental changes in order to maximize biomass production (Bloom, Chapin, and Mooney, 1985). In the case of a shortage of essential

resources (such as CO<sub>2</sub> or water), the plant will enhance the growth of the organ that absorbs these resources (leaves or roots) to increase uptake of the limited resource and maximize total growth. By doing so, the plant reduces the biomass allocated to non-resource-limited organs, making the acquisition of all resources equally limiting (Gedroc, McConnaughay and Coleman, 1996). These changes in biomass allocation allow the plant to keep a constant level of various resources and maximize growth by reaching what is termed “optimal behavior” (Bloom, Chapin, and Mooney, 1985). Ingestad and Ågren (1991) added to this theory by conceptualizing the ‘steady state’ of a plant when its environmental needs are equally limiting already (steady environment), and the same resources are allocated to each organ.

Secondly, biomass allocation has been described as a size-dependent phenomenon, where the plant of a certain size will change BAP as it continues to grow (Weiner, 2004). This theory argues that the relative growth rate of all the organs of the plants is related to the total size through a power function and unrelated to the surrounding environmental conditions (Eqn 1).

$$Y = aX^b \quad (\text{Eqn 1})$$

Where  $Y$  is the mass of an organ of the plant,  $X$  is the whole mass of the plant,  $a$  is the allometric constant and  $b$  is the scaling component (Poorter et al., 2015). This is called the allometric growth or trajectory of the plant individual (Weiner, 2004). This allometric relationship can also be seen between different organs of the plant and is stable throughout the growth of the plant. This has been proven by Pearsall (1927) who showed that throughout the development of the plant, the ratio of the relative growth of two organs is stable and equal to the scaling component  $b$  for the allometric trajectory of this same plant.

The allometric partitioning theory predicts that under similar environmental conditions, small or young plants will have different BAP than bigger plants, usually showing larger root proportions compared to shoots at the beginning stages of growth and reducing this root proportion as the plant grows to increase shoot biomass (Geng et al., 2007). Biologists theorized that this stable allometric trajectory exists because of natural selection which increases fitness of the organisms on the long-term, creating a plant generally most adapted to its environment rather than boosting biomass allocation changes during short-term environmental stress (Weiner, 2004). In response to variation in environmental conditions, these BAP can still change depending on the plasticity of the plant individuals, but the general allometric relationship will stay constant. Weiner (2004) explained that this idea of plasticity is a “flexible strategy which allows a genotype to give rise to different phenotypes depending on the biotic as well as abiotic environment in which the plant grows and develops”. Therefore, the BAP changes of the ratio-partitioning theory have been explained by some researchers as a type of plastic response that can still be explained by the allometric partitioning theory (Eziz et al., 2017).

Evans (1972) added to this theory by defining ontogenetic drift, the phenomenon visible in plant individuals, where what might seem like a plastic response to the environment is only due to variability in the allometric trajectory of the plant's growth. For example, the plants grown under a certain environmental stress might be smaller than those grown under normal conditions and display different BAP. But these changes could be explained by ontogenetic differences since the plant had a slower development and displays the BAP of young plants, rather than changes due to an adaptation to the environmental conditions. In comparison, true plasticity was defined as the changes in the allometric trajectory of the plant in response to their environment (Weiner, 2004). Therefore, the interest in the study of plant plasticity is to decipher which BAP are due to ontogenetic drift (akin to apparent plasticity), or true plasticity, or even complex plasticity (both at the same time) (Geng et al., 2007). To avoid mistaking an apparent plasticity pattern as true plasticity when studying the response of plants to an environmental treatment, the response variable of the plant individuals must be compared between same-size individuals (Evans, 1972, Coleman, McConnaughay and Ackerly, 1994). Indeed, since the ontogenetic trajectory of the plants changes with increased growth, individuals of the same size should display similar BAP, and the differences between those same-size groups can then be explained by true plasticity in response to the environmental treatments.

In addition to this, McConnaughay and Coleman (1999) created a model to help visualize the presence or absence of ontogenetic drift in a population. They theorize that if the shoot and root contents of plant individuals are plotted against each other, the curve of this allometric relationship should be linear with a scaling exponent of 1 in the absence of ontogenetic drift. This means that at the scale of a population of plants of different sizes grown under similar conditions, the amount of biomass allocated to roots and shoots should be equal (Fig. 1, f(a)). When another group of plants grown in different conditions is plotted on this same graph, the scaling exponent should still be equal to 1, but the intercept should change because of true plasticity of the population in response to their environmental conditions (Fig. 1, f(b) f(c)). Indeed, if true plasticity was in action, most of the individuals of the population would have changed their BAP in similar ways to reach a 'steady state', regardless of their developmental stage, resulting in a lower or higher intercept, but no change in scaling exponent (Fig. 1, f(b), f(c)). However, if the scaling exponent is not equal to 1 or if the relationship is not linear, then the BAP cannot be differentiated from the effects of ontogenetic drift, and the differences between groups cannot be attributed to true plasticity only (Fig. 1, f(d)). If we look at f(d), we have an example of complex plasticity where both apparent and true plasticity are in action. In this case, the only way to describe the effect of the environment on the biomass allocation will be by comparing individuals of similar sizes together, as explained above.



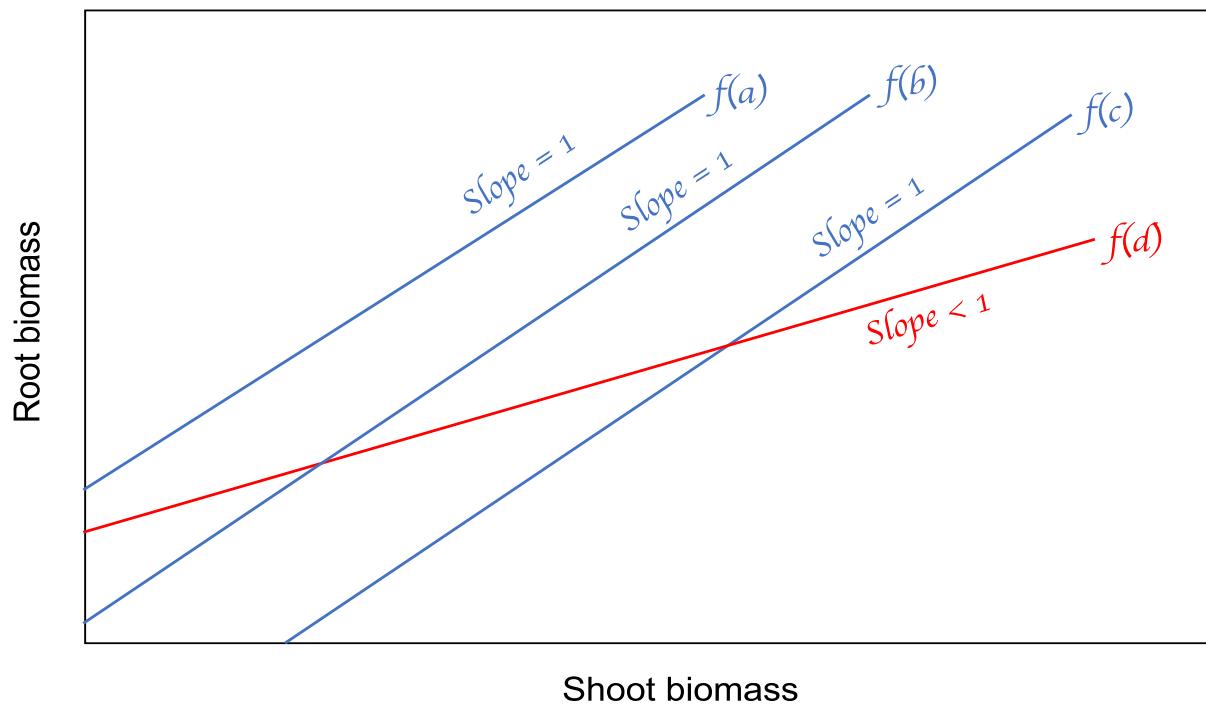


Fig. 1: Theoretical graph of biomass allocation in roots and shoots under the influence of true or apparent plasticity, adapted from McConnaughay and Coleman (1999).

### 1-3 BIOMASS ALLOCATION PATTERNS IN HERBS

Aromatic herbs are good plants to focus on for biomass allocation studies. They are often annual or biennial plants and grow quickly to reach their adult stage faster than other plants. This means that short-term experiments can be used to look at the effect of environmental changes on their BAP. Biomass allocation in herbaceous plant species has been shown to follow the predictions set out by the allometric partitioning theory (Poorter et al., 2012a, Eziz et al., 2017). Several studies on this topic found that herbs changed BAP along environmental gradients but that the general allometric scaling parameters stayed stable throughout those changes (Zhou et al., 2014, Guo et al., 2016). This is consistent with the plasticity idea explained by Weiner (2004).

The plastic biomass allocation to roots or shoots in herbaceous species has been found to be driven by below-ground resource availability mostly. In a meta-analysis, Poorter et al (2012a) found that the BAP along above-ground resource gradients disappeared when individuals were compared at similar sizes, showing the effect of apparent plasticity. In comparison, they found that same-size comparisons enhanced the effect of below-ground resource limitation on BAP. Similarly, Zhou et al. (2020) looked at 129 multi-factor studies and found that the relative mass of root to shoot (root:shoot ratio) varied only along below-ground environmental gradients such as irrigation, drought, and nutrient availability and showed no response to elevated  $\text{CO}_2$  or increase in temperatures.

In addition, differences have been found between the BAP of annual and perennial herbaceous plants (Zhou et al., 2020). Annual herbs have been found to be more ontogenetically constrained

than perennial herbs (Geng et al. 2007). The general ontogenetic trajectory of annual herbaceous species shows that root:shoot ratios will decrease as the size of the plant increases whereas the opposite trend is true for perennial herbs (McConnaughay and Coleman, 1999, Poorter et al., 2012a). This means that under environmental stress coming from below-ground, the annual plants will reduce total growth and their ontogenetic trajectory will naturally increase biomass allocation to roots. Therefore, they won't need to activate true plasticity as well. McConnaughay and Coleman (1991) predicted that under below-ground resource limitations, annual herbs would only show apparent plasticity since ontogenetic drift would be enough to adapt the plant to its environment. In comparison, if the environmental stress is coming from above-ground resources, such as light or carbon dioxide, the annual herbs are expected to show true plasticity patterns (McConnaughay and Coleman, 1999). Regarding perennial herbs, Geng et al (2007) found that the opposite predictions were true: under above-ground resource limitation, apparent plasticity accounted for all the changes in BAP whereas complex and true plasticity was found for plants under below-ground resource limitations. The papers cited previously either grouped annual and biennial herbs together (Zhou et al., 2020) or excluded biennials from their meta-analysis (Poorter et al., 2012a). This could be because biennial herbs are closer to annual than to perennial herbs in terms of growth speed, and only small differences should be found when comparing their growth and BAP.

#### 1-4 EFFECT OF DROUGHT

As explained previously, below-ground resources have been shown to affect herbaceous species more than above-ground ones. A major environmental stress related to this is drought. Drought is defined as a water stress caused by lower water availability compared to the plant's needs (Volaire, 2018). Drought stress has been shown to affect both the physiological and morphological characteristics of plants. Indeed, drought can cause changes in osmotic potential in plants but also decrease the leaf area to limit loss of water through transpiration (Edler et al., 2015). Drought conditions can also reduce the plant-soil biota feedbacks and decrease total growth in plants (Wilschut and Kleunen, 2021, Geng et al., 2007). Some plants have evolved to display drought resistance characteristics to maintain growth of leaves and plant productivity under moderate levels of drought stress (Volaire (2018). These drought resistant plants usually increase root biomass to increase the water uptake and absorb enough water to sustain their growth (Padilla et al., 2009). Van Wijk (2011) found that root:shoot ratios in plants usually increase with increased aridity and the same pattern is found under low nutrient conditions. Eziz et al. (2017) carried out a meta-analysis of 164 studies and found that root biomass generally increased under drought at the expense of stem, leaves and reproductive tissues. They also found that annual herbs had a more intense response than perennial herbs by increasing roots almost two times more and reducing leaf mass whereas perennial leaves increased in mass. This is not necessarily contradictory with the previous theory regarding the differences between annual and perennial

herbs since this study did not control for ontogenetic drift. They concluded that the differences could be due to ontogenetic processes (Eziz et al., 2017).

## 1-5 EFFECT OF SOIL COMPOSITION

Soil composition differences have also been found to affect plant species. Soils with high nutrient contents are related to higher above-ground biomass and lower root:shoot ratios (van Wijk, 2011). The proportion of sand in the soil can also influence the resource allocation in plants by lowering the water retention potential and creating a drought effect. This effect can increase root:shoot ratios and root length at greater soil depths (van Wijk, 2011). In addition, soil types with lower water holding potentials will dry faster when precipitation events are rare and the interaction between those two effects can increase the drought stress (Edler et al., 2015). Indeed, Edler et al. (2015) found that the interaction between moisture level and soil type had a strong effect on the biomass production, the root:shoot ratio, and the leaf area of three weed species. Their study used a fully crossed experimental design between species, moisture levels, temperature levels and soil types which enabled them to reach this conclusion. Even though the individual effects of soil type and moisture have been shown to affect plant growth and BAP separately in herbaceous species relevant for agricultural production (Farouk and Omar, 2020, Petropoulos et al., 2008, van Wijk, 2011), the combined effect of those variables on aromatic herbs has not yet been studied in the way that Edler et al. (2015) did on crop-weed species.

## 1-6 STUDY OBJECTIVES

Although the effect of drought on herb biomass production and allocation is well known, its effect when combined with different soil types has not yet been studied. In this experiment, the combined effect was studied in three different herb species in a fully crossed setup with several soil compositions and drought intensities. Based on previous assessment of the effect of drought stress on BAP in herbaceous species, I predict that total biomass will decrease under higher drought and that biomass allocation will change with an increase in relative root biomass. However, this could be due to apparent plasticity as explained by McConnaughay and Coleman (1999). In addition, I hypothesize that coarser soils with low nutrient contents will create harsher drought stress and lead to further adaptation from the plants. In comparison, soils with higher nutrient contents or with high water holding capacities closer to steady environments should lessen the drought stress (Ingestad and Ågren, 1991). Finally, since species have different allometric trajectories, and since stark differences have already been described between annual and perennial species, I expect the response of different plants to differ when compared under similar environmental conditions.

Therefore, the research questions to be answered are as follow:

- How do aromatic herb species change their biomass allocation patterns in response to drought stress?

- Is this response consistent with the allometric partitioning theory and do plants still display adaptation through true plasticity?
- Does this response differ depending on the intensity of the drought and on the growth medium?

## 2-METHODS

### 2-1 SPECIES AND SOIL TYPE SELECTION

The species used for this experiment were chosen based on speed of germination and growth to ensure higher biomass harvest at the end of the treatment.

To find the species and soil type combinations that germinated the quickest, a pilot study was carried out in June in Paris, France. Four herbaceous plant species: *Coriandrum sativum* (Coriander), *Anethum graveolens* (Dill), *Petroselinum crispum* (Parsley), and *Ocimum basilicum* (Basil), were planted from seed in ten different soil mixes (Fig. 2). Within this mix, only Parsley is biennial whereas the other three are annual herbs. Three seeds of each species were planted in each pot. The pots were left outside and watered every other day.

The soil mixes were made from four base elements: compost, vermiculite, sand and gravel (Appendix A). The soil types were mixed in a way to get a wide variation of nutrient content and particle size. Compost is the only nutrient rich element in those four bases. Vermiculite is made of crushed mica and while it is known to be relatively infertile, it reduces the soil compaction and increases the oxygenation and water retention of the soil (Padilla et al., 2009). In comparison, sand and gravel have very low water retention abilities and are expected to increase the drought stress.



*Fig. 2: Pilot study set up (from left to right, soils 1 to 10, and every 2 rows from top to bottom were planted with each species). The yellow arrow represents 6cm, the diameter at the top of the pots.*

After 15 days, all Basil seeds had germinated and already showed 2 non-cotyledon leaves, Dill had germinated in almost all pots and already reached about 2cm in height, and Parsley had germinated in most pots although it was still only showing cotyledon leaves. Only two pots out of 20 showed signs of Coriander sprouts. Therefore, only Basil, Dill and Parsley were chosen for the main experiment. Soils 1, 2 and 4 showed most success (germination) and were chosen for the main experiment.

## 2-2 EXPERIMENT SET-UP

The three soil types were mixed according to pot volume as follows:

- Soil 1: only compost
- Soil 2: ½ compost and ½ vermiculite
- Soil 3: 1/3 compost, 1/3 vermiculite and 1/3 sand

9 propagator pots were filled with each soil type (3 pots for each). Two seeds of each species were then sowed in each sub-unit of the pots (Fig. 3).

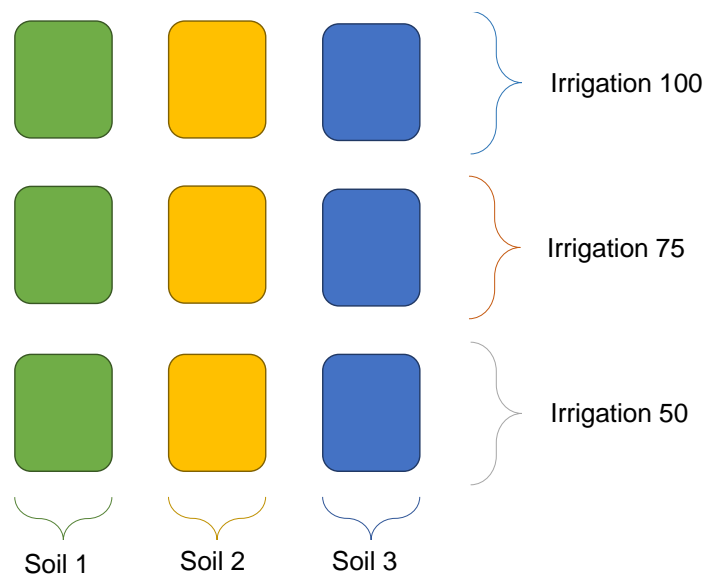
The pots were kept inside and were watered equally for the first 15 days after sowing to allow germination of a maximum of seeds before starting the irrigation treatment. After 15 days, all the pots were transferred in a greenhouse.



*Fig. 3: Experiment set-up with propagator pots in green containing 12 sub-units. Each column of subunits was sowed with one plant species. Labels show the first letter of the species and the number of the soil type.*

## 2-3 TREATMENT

The plants were kept in the greenhouse for 3 weeks. During this time, the soil moisture of each propagation unit was measured every Monday, Wednesday, and Friday with the ML3 Theta Probe Soil Moisture Sensor from Delta-T services, measuring Volumetric water content (VWC) with 0.01 cm<sup>3</sup>/cm<sup>3</sup> accuracy. The probe was calibrated to organic soil prior to the first measurement. From these volumetric water content measures, a mean moisture measure was obtained for each pot to reduce errors of measurement and any variability due to the plants or other variables. After taking probe measures, all the pots were watered according to their irrigation level (Fig. 4). Since the propagation units in each pot were linked together at the bottom, only one irrigation treatment was applied to each pot (Fig. 4).



*Fig. 4: Experiment set-up, each small rectangle is a propagation pot.*

The drought effect was created by decreasing the irrigation volume progressively over the course of the experiment and three levels of irrigation were created to study the effect of drought intensity (Fig. 5). The three irrigation levels were set up as follows:

- 100 = maximum irrigation treatment (in volume of water added each time)
- 75 = 75% of the first volume
- 50 = 50% of the first volume



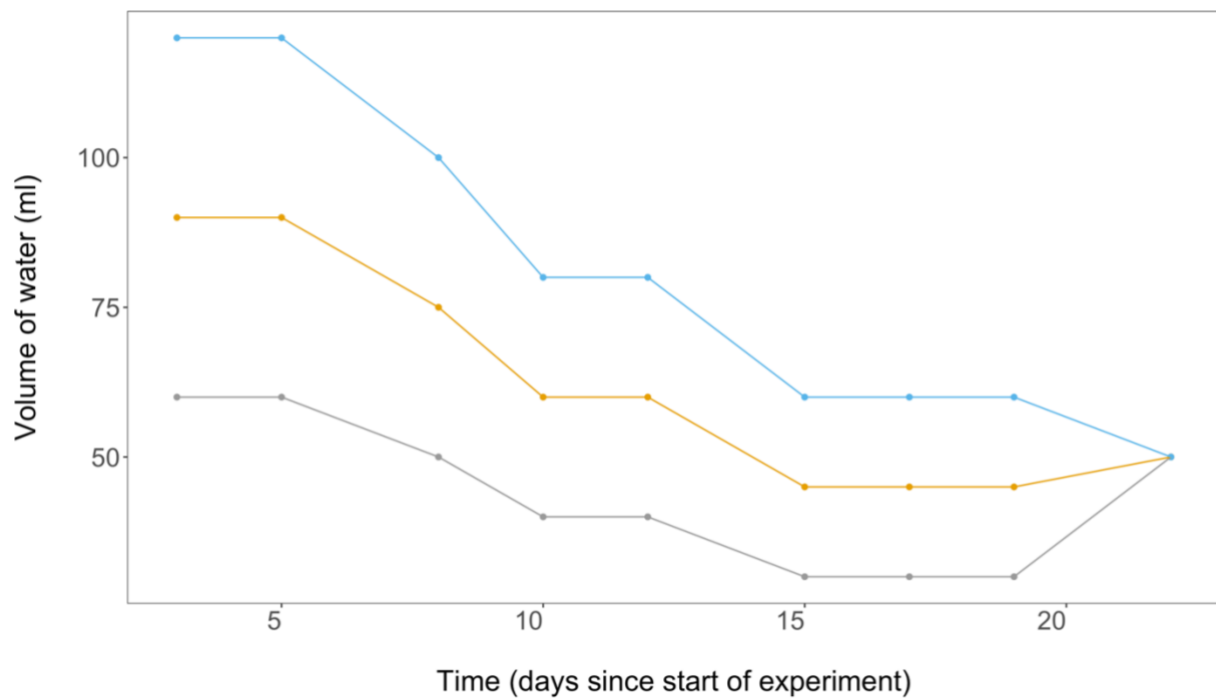


Fig. 5: Volume of water added each time according to the irrigation treatment. Colors correspond to irrigation level: — 50 — 75 — 100

## 2-4 HARVEST AND MEASUREMENT

On day 22, all pots were watered equally (Fig. 5). The plants were then harvested by carefully removing the roots from the soil and cleaning them under running water. In most units of propagation, both seeds had germinated and grown, increasing the sample count. However, in some units, no seeds had germinated, and these were deleted from the dataset. The sample sizes and germination rates of all species, soil type and irrigation level combination can be found in Appendix B.

A picture of all individuals was taken with an iPhone SE (version iOS 13.1.3), and a measure of mean leaf area ( $\text{cm}^2$ ) for each individual was obtained with the ImageJ2 software version 2.3.0 (Rueden et al., 2017). The images were analyzed by turning them into black and white images, reducing the threshold, and making corrections by hand to get an accurate measurement of the leaf area. Only the non-cotyledon leaves were taken into account in this process. Some Basil leaves were highly curved-in which would have led to inaccurate measurements. Therefore, only the flattest leaf for each Basil individual was used instead of a mean. This was considered acceptable since most plants only had 2 grown leaves which had similar sizes. All the pictures of plant individuals and of grown plants in pots are available on my GitHub account (<https://github.com/louise-litrico/dissertation-work>).

All plant individuals were weighted immediately after harvest (roots and shoots separately) to obtain the fresh weight of plants. The plant material was then dried at  $50^\circ\text{C}$  for 2 days and then weighted again to obtain the dry weight values. The first batch of plant material placed in the oven

for this length of time showed no change in weight after further drying, therefore, it was assumed that all water had evaporated and that 2 days was enough for other batches as well. Afterwards, dry root and shoot weights were added together to create the total biomass values.

Root:shoot ratio values were obtained by dividing dry root biomass with dry shoot biomass values for each plant individual. High root:shoot ratio values correspond to a higher percentage of roots in the plants and lower values correspond to higher percentage of shoots in the plants.

## 2-5 DATA ANALYSIS AND VISUALIZATION

All data manipulation, analysis, and visualization were done in R version 4.1.1 (R Core Team, 2021) through RStudio version 2021.09.0.351 (RStudio Team, 2021). All plots were created with the ggplot2 package (Wickham, 2009).

### 2-5-1 DROUGHT EFFECT

The successful creation of a drought effect was verified by testing the influence of the different irrigation levels on the VWC measured throughout the experiment. Since soil types could have had an influence on the VWC, an ANCOVA was carried out instead of an ANOVA by adding time and soil type as covariates (Appendix C). The assumptions of independence between covariates and equal variance across samples were tested beforehand with Pearson's Chi-squared test and Levene's test for homogeneity of variance. The data passed both tests. The mean VWC was then expressed as a percentage (cm<sup>3</sup>/cm<sup>3</sup>) and plotted against time for each soil type and differentiated between irrigation treatments.

### 2-5-2 BIOMASS VARIABLES AND LEAF AREA

Since all treatments were crossed and pots were treated the same throughout the experiment, it was chosen not to include random effects in a mixed linear model but to use linear models instead to study the effect of all treatments (species, irrigation level and soil type) on total biomass, root:shoot ratios, and leaf area. The linear models analyzing the variation of root:shoot ratio and leaf area also included total biomass as an explanatory variable to account for the variation due to ontogenetic drift. To achieve better fit, an interaction term between the main variables was included only when the adjusted R<sup>2</sup> value was higher than for the simpler model (Appendix C).

The assumptions of independence of points, homogeneity of variance, linear relationship between variables and normal distribution of residuals were verified beforehand with the gvlma package (Pena and Slate, 2006) and the plot function in base R. The total biomass fulfilled these assumptions, but the root:shoot ratio and leaf area data did not. Using the logarithmic values of the main variables did not help. However, taking out outliers solved this issue, bringing the total sample size down (Appendix B and C). This does not reduce the value of my results since very few outliers were taken out compared to the total sample size (max 4/96). However, it implies that the results and interpretation presented later will only cover the data that fit the assumptions of the statistical model. I tried to find out why those data points didn't fit the assumptions and noted



that there was no pattern between irrigation treatment, but that there were no outliers from Soil 1, and most outliers (4/6) were Parsley individuals. Some possible explanations for these outliers are covered in the discussion.

The total biomass measures were plotted in a bar plot against irrigation levels for each species. For root:shoot ratios and leaf area, the variables were plotted against total biomass for each irrigation treatment and colored by soil type. This plotting methods enabled the comparison of individuals of the same size and reduced the risk of misinterpreting effects due to ontogenetic drift as effects of treatments. High values on the y-axis represent a large proportion of leaves in the plant individual and low values represent a low proportion of leaves compared to the total size of the plant. Points placed low on the x-axis can be used to represent plants at the early stage of growth, not long after germination and points placed higher on the y-axis can be used to represent older plants. Separately, a boxplot was used to show the differences in leaf area and root:shoot ratio between species.

### 2-5-3 ALLOMETRIC REGRESSIONS

As explained previously, the relationship between the roots and shoots of the plant should be allometrically related and deviation from this trajectory can tell us what BAP the plants display and if these results are due to true or apparent plasticity. This trajectory can be expressed by the following equation (Poorter et al., 2012a):

$$\log(\text{Root dry mass}) = \log(\mathbf{b}) + \mathbf{a} \log(\text{Shoot dry mass}) \quad (\text{Eqn 2})$$

To study the BAP of the plants and their response to the treatments, linear regressions were plotted between root and shoot biomass in each species following McConnaughay and Coleman (1999). The logarithmic values of each variable were used. A linear model was used to estimate the scaling exponent of these regressions and to test whether they differed between treatments. The assumptions of this model were tested in the same way as the other previous ones (*Biomass variables and leaf area*). In this case, four outliers had to be taken out to fit the assumptions (Appendix B).

Coleman, McConnaughay and Ackerly (1994) argued that using a standardized major axis regression would be better for these types of data. Indeed, the variables used here are not independent since they are part of the same plant individuals and the linear regressions obtained cannot be used to create predictions. They argue that using type 2 regressions would be more appropriate in this case. However, I have not found an R package that carries out type 2 regressions that are able to compare the regressions between several groups the way that a linear model can (Appendix C). Since the treatments in this experiment are all crossed, using a linear model with multiple treatments seemed more sensible, even if the model does not carry any predictive value.

## 3-RESULTS

### 3-1 TREATMENTS

The volumetric water content measures taken throughout the experiment decreased significantly over the course of the experiment (ANCOVA:  $F_{1,66}=437.121$ ;  $p<0.01$ , Fig. 6) and were significantly affected by both irrigation levels (ANCOVA:  $F_{2,63}=115.59$ ;  $p<0.01$ , Fig. 6) and soil types (ANCOVA:  $F_{2,66}=19.94$ ;  $p<0.01$ , Fig. 6). A posthoc Tukey test showed that the VWC measures were highest in the pots that were under a higher irrigation treatment ( $p<0.01$ ). Another posthoc Tukey test showed that the decrease over time was greater in Soil 3 than in the other two soils ( $p<0.01$ ) and that Soils 1 and 2 showed no significant differences ( $p>0.05$ ).

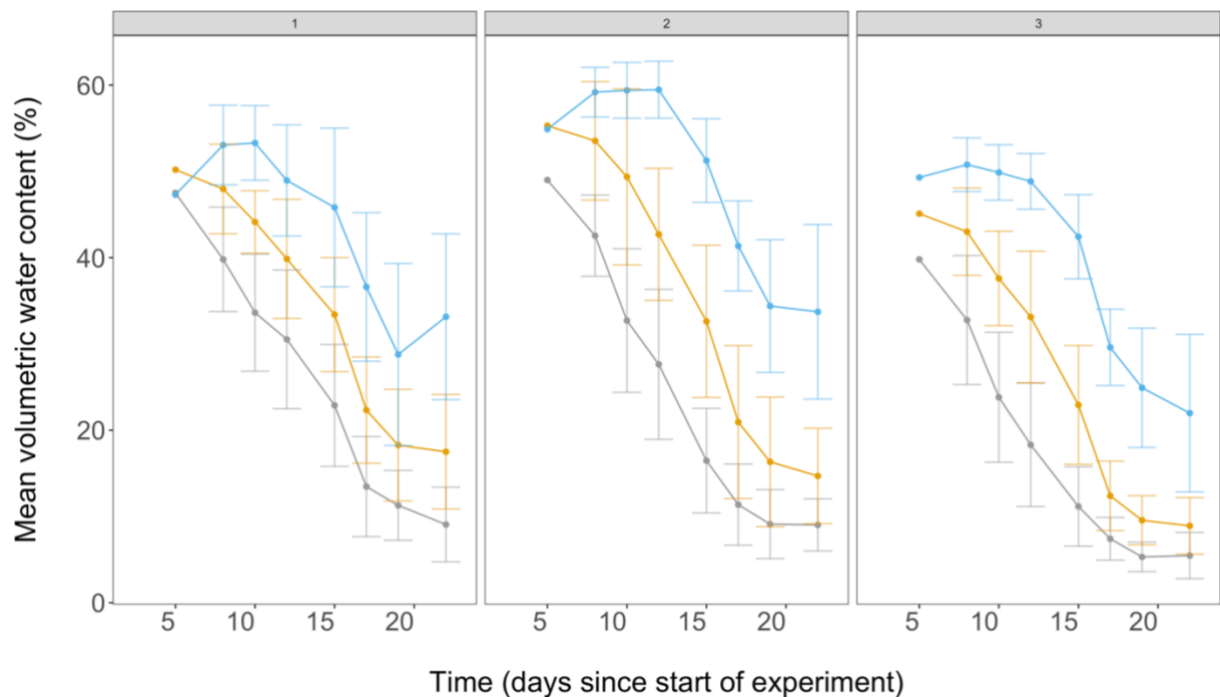


Fig. 6: Variation of the mean volumetric water content (%) over time in different soil types and under different irrigation levels. Error bars represent standard deviation. Colors correspond to irrigation levels: —●— 50 —●— 75 —●— 100

### 3-2 TOTAL BIOMASS

The total dry weight of each plant individual was significantly different between species with Basil plants being significantly bigger than Dill and Parsley (LM:  $F_{10,85}=10.59$ ;  $p<0.01$ , Fig. 7). Plants grown under 100% irrigation had significantly lower biomass than those grown under 50% irrigation (LM:  $F_{10,85}=10.59$ ;  $p<0.01$ , Fig. 7) and no difference was found between the 75% and 50% treatment groups (LM:  $F_{10,85}=10.59$ ;  $p>0.05$ , Fig. 7). In addition to this, higher biomass was found in plants that grew in Soil 3 and with 100% irrigation than others (LM:  $F_{10,85}=10.59$ ;  $p<0.01$ ). Finally, no differences were found between the biomass produced in the different soil types (LM:  $F_{10,85}=10.59$ ;  $p>0.05$ ).

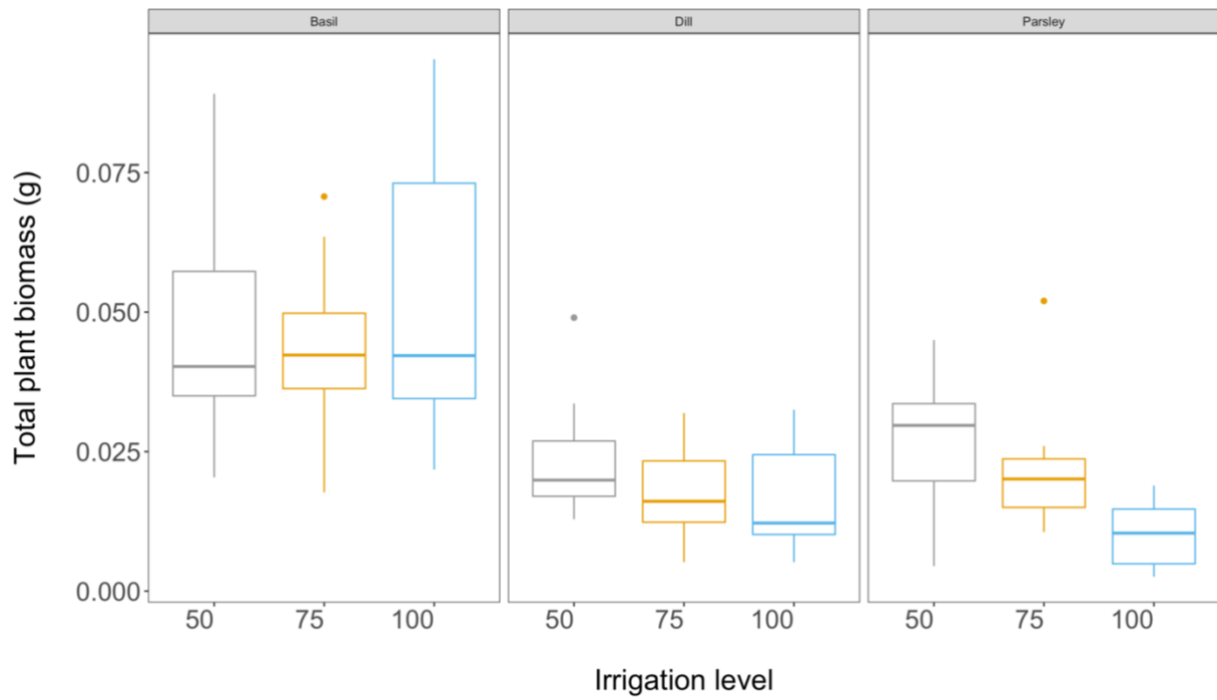


Fig. 7: Total biomass (total dry weight in g) associated to each plant species growing under each irrigation level ( $n_{Basil}=38$ ,  $n_{Dill}=33$ ,  $n_{Parsley}=25$ ,  $n_{50}=35$ ,  $n_{75}=32$ ,  $n_{100}=29$ , see Appendix B for details).

Colors correspond to irrigation levels: 50 75 100

### 3-3 ROOT TO SHOOT RATIO

The root:shoot ratio values did not change significantly between plant sizes (LM:  $F_{11,82}=10.82$ ;  $p>0.05$ , Fig. 8). Plants grown under 100% irrigation had significantly lower root:shoot ratios than those grown under 50% irrigation (LM:  $F_{11,82}=10.82$ ;  $p<0.01$ , Fig. 8) but there were no significant differences between the 50% and 75% irrigation groups (LM:  $F_{11,82}=10.82$ ;  $p>0.05$ , Fig. 8). The linear model also revealed that plants grown in Soil 3 had significantly higher root:shoot values than the other two soil groups (LM:  $F_{11,82}=10.82$ ;  $p<0.01$ , Fig. 8). No differences in root:shoot ratio values were found between species (LM:  $F_{11,82}=10.82$ ;  $p>0.05$ , Fig. 9).

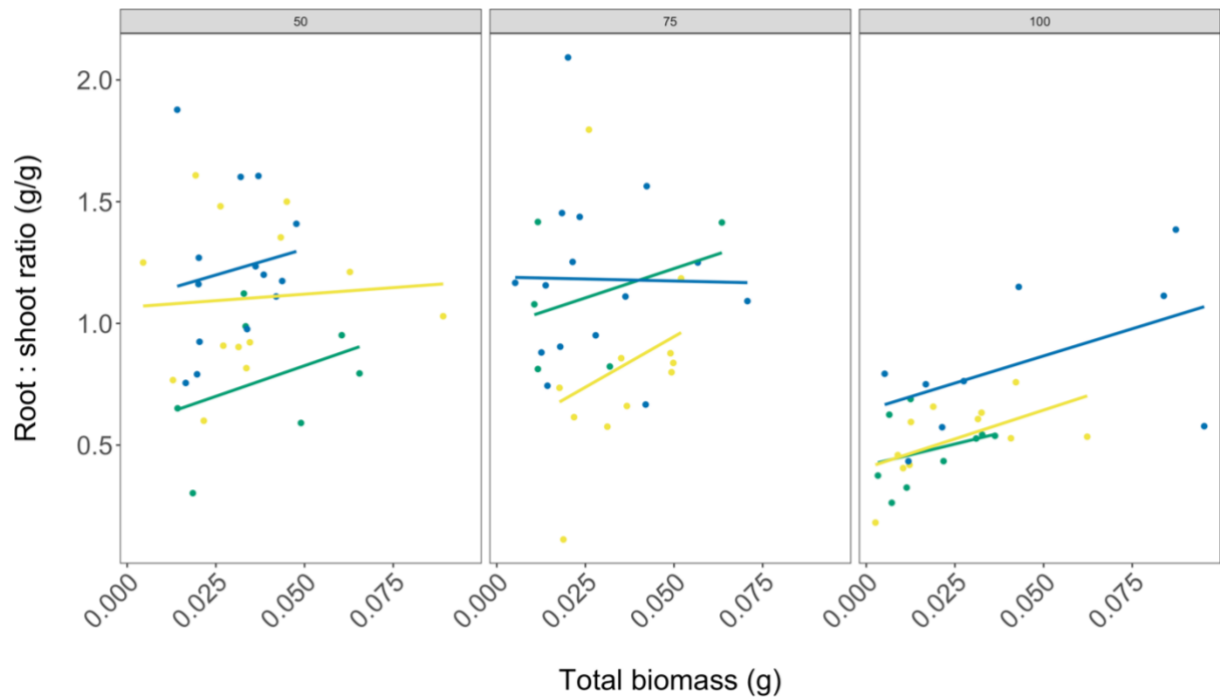


Fig. 8: Linear regression equations fitted to describe the allometric relationship between root:shoot ratio and total biomass under each irrigation level and colored by soil type. ( $n_1=21$ ,  $n_2=35$ ,  $n_3=40$ ,  $n_{50}=35$ ,  $n_{75}=32$ ,  $n_{100}=29$ , see Appendix B for details). Colors correspond to soil types: ● 1 ● 2 ● 3

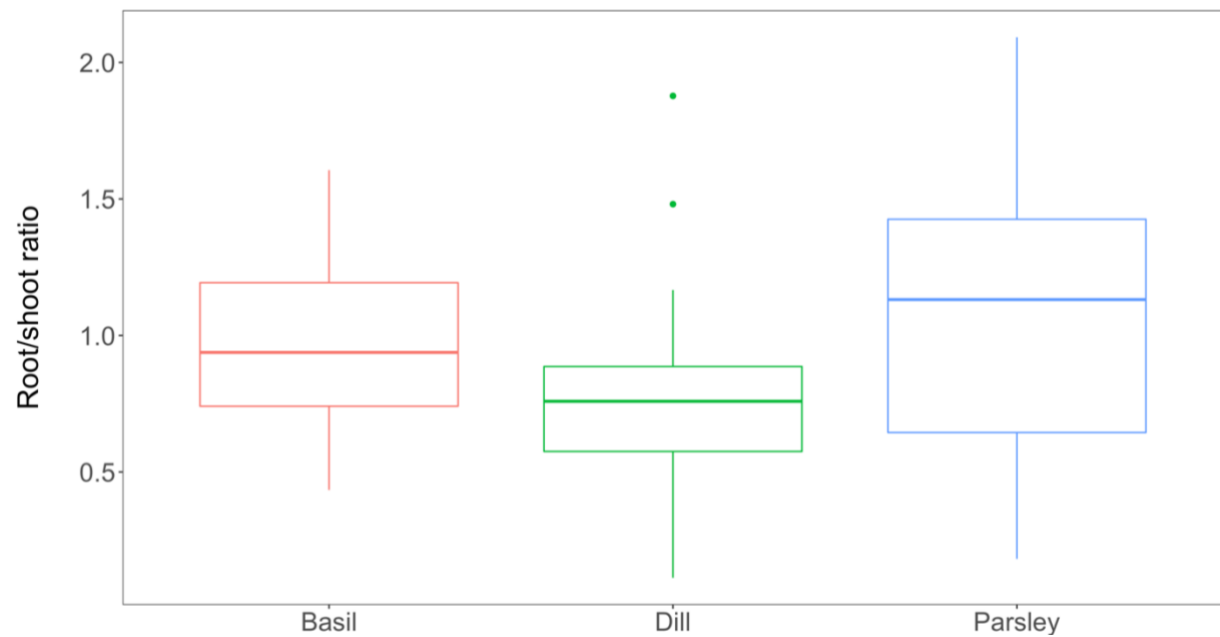


Fig. 9: Root:shoot ratio values from each species ( $n_{Basil}=38$ ,  $n_{Dill}=33$ ,  $n_{Parsley}=25$ ). Colors correspond to species.

### 3-4 LEAF AREA

The leaf area measures in Basil leaves were significantly bigger than Dill leaves (LM:  $F_{7,85}=35.66$ ;  $p<0.01$ , Fig. 10) but showed were no differences with Parsley leaves (LM:  $F_{7,85}=35.66$ ;  $p>0.05$ , Fig. 10). Leaf area measures significantly increased with increased total biomass (LM:

$F_{7,85}=35.66$ ;  $p<0.01$ , Fig. 11). There was no significant effect of irrigation levels on leaf area (LM:  $F_{7,85}=35.66$ ;  $p<0.05$ , Fig. 11). Plants grown in Soil 1 had larger leaves than those from Soil 2, although the difference was marginally significant (LM:  $F_{7,85}=35.66$ ;  $p<0.05$ , Fig. 11) and no differences were found between Soil 2 and 3 (Fig. 11).

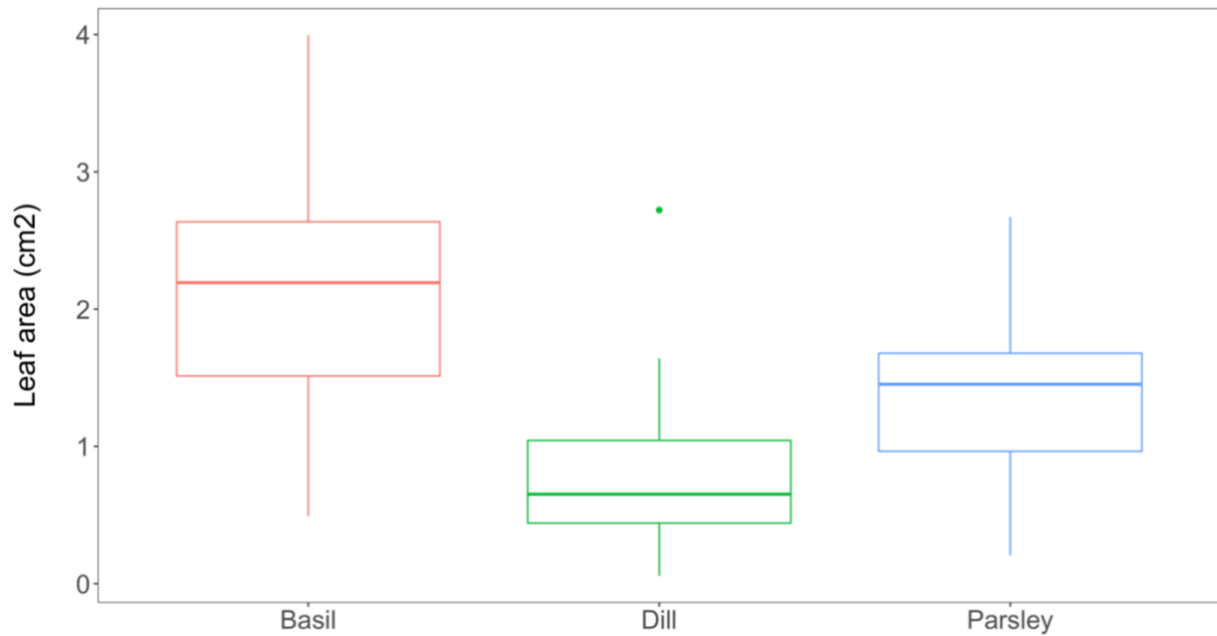


Fig. 10: Leaf area measures from each species grown in different soil types. ( $n_{Basil}=38$ ,  $n_{Dill}=32$ ,  $n_{Parsley}=24$ ,  $n_1=21$ ,  $n_2=33$ ,  $n_3=40$ , see Appendix B for details).

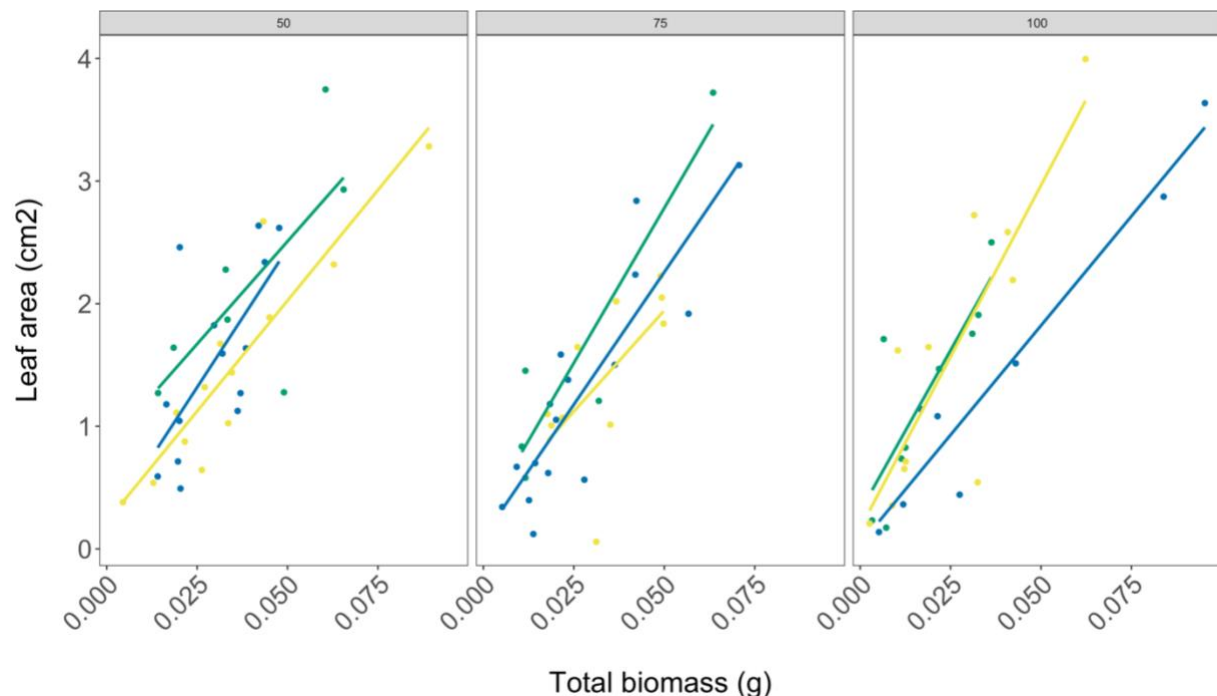


Fig. 11: Linear regression equations fitted to describe the allometric relationship between leaf area and total biomass under each irrigation level and colored by soil type. ( $n_1=21$ ,  $n_2=35$ ,  $n_3=40$ ,  $n_{50}=35$ ,  $n_{75}=32$ ,  $n_{100}=29$ , see Appendix B for details). Colors correspond to soil types:   
● 1 ● 2 ● 3

### 3-5 ALLOMETRIC LINEAR REGRESSIONS

The linear model revealed that the regression between root and shoot measures was lower in Dill individuals than in Basil (LM:  $F_{7,84}=87.46$ ;  $p<0.01$ , Fig. 12 and 13) but showed no significant difference between Dill and Parsley (LM:  $F_{7,84}=87.46$ ;  $p>0.05$ , Fig. 12 and 13). Root biomass significantly increased with increased plant shoot biomass with an average slope of  $0.703\pm0.062$  (LM:  $F_{7,84}=87.46$ ;  $p<0.01$ , Fig. 12 and 13). Plants under the highest irrigation had significantly lower estimates than the others (LM:  $F_{7,84}=87.46$ ;  $p<0.01$ , Fig. 12). There were no significant differences between the estimates of irrigation levels 75% and 50% (LM:  $F_{7,84}=87.46$ ;  $p>0.05$ , Fig. 12). A similar pattern was found between soil types where Soil 3 had a significantly higher estimate (LM:  $F_{7,84}=87.46$ ;  $p<0.01$ , Fig. 13) whereas no difference was found between the other two (LM:  $F_{7,84}=87.46$ ;  $p>0.05$ , Fig. 13). The coefficients and  $R^2$  values for each regression can be found in Appendix D.

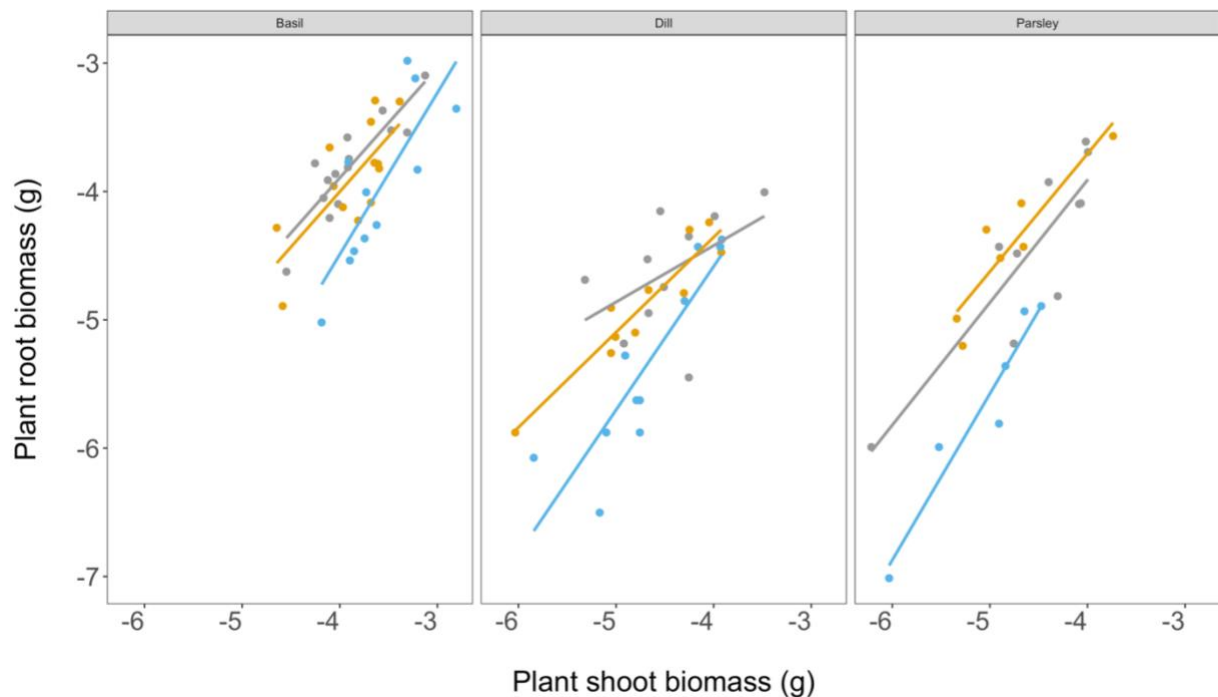


Fig. 12: Allometric relationship between  $\text{Log}(\text{shoot dry weight})$  and  $\text{Log}(\text{root dry weight})$  of each species grown with varied amounts of irrigation. ( $n_{\text{Basil}}=38$ ,  $n_{\text{Dill}}=33$ ,  $n_{\text{Parsley}}=25$ ,  $n_{50}=35$ ,  $n_{75}=31$ ,  $n_{100}=28$ , see Appendix B for details). Colors correspond to irrigation levels: 50 75 100

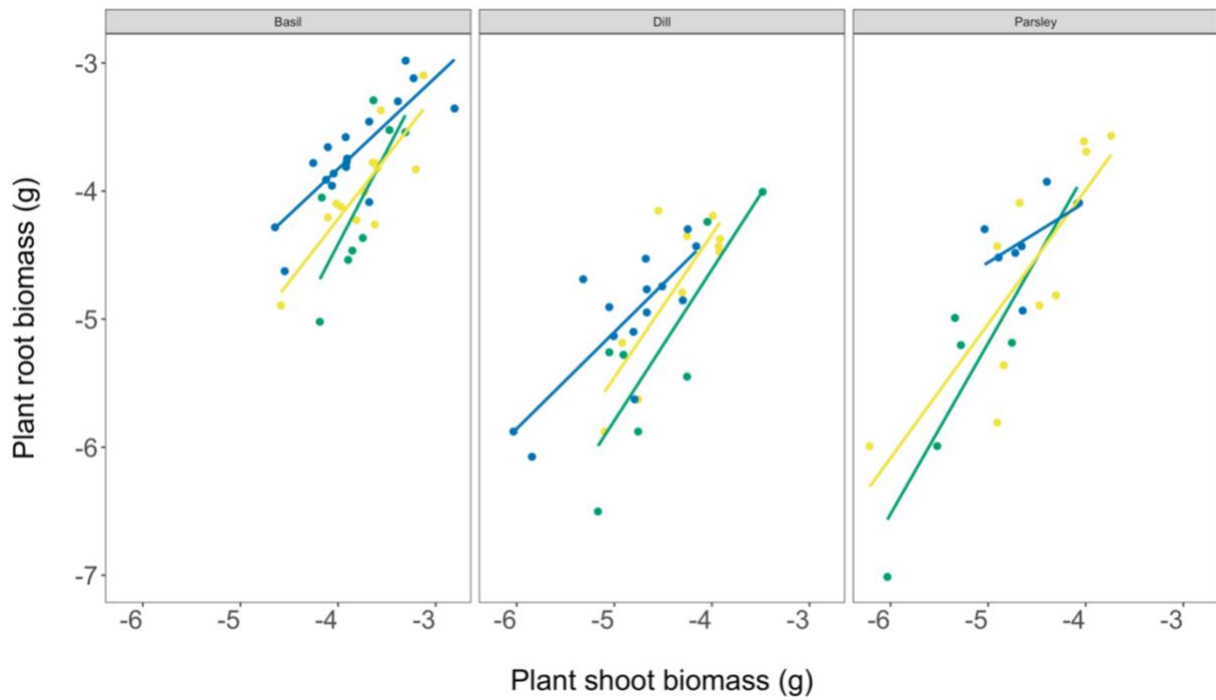


Fig. 13: Allometric relationship between  $\text{Log}(\text{shoot dry weight})$  and  $\text{Log}(\text{root dry weight})$  of each species grown in different soil types (b). ( $n_{\text{Basil}}=38$ ,  $n_{\text{Dill}}=31$ ,  $n_{\text{Parsley}}=23$ ,  $n_1=21$ ,  $n_2=33$ ,  $n_3=38$ , see Appendix B for details). Colors correspond to soil type: — 1 — 2 — 3

## 4-DISCUSSION

The experiment was successful in creating a drought effect of two different intensities. Contrary to expectation, the total growth of the plants was not affected by the treatments. The biomass of the plant and the leaf area measures varied between species according to ontogenetic trajectory and only small differences were found between treatments. However, the BAP of each species did show some changes. As was expected, root:shoot ratios increased with drought intensity and when plants grew in Soil 3, reflecting the increase in root percentage in plants subjected to drought stress. The allometric relationships between shoot and root organs revealed that plants growing under higher irrigation seemed to reflect a steady state as defined by Ingestad and Ågren (1991) and followed a normal ontogenetic growth trajectory. In comparison, the individuals growing under drought conditions showed complex plasticity.

### 4-1 EFFECT OF IRRIGATION LEVELS AND SOIL TYPES

As was expected, the plants growing in Soil 1 and under 100% irrigation displayed the growth and BAP of a simulated 'steady state' (Ingestad and Ågren, 1991). They showed good fit to a linear regression and a scaling exponent relatively equal to one, in the way that plants following the ontogenetic trajectory of a normal or steady environment would (Fig. 12 and Appendix D). In comparison, the plants grown under 75% and 50% irrigation displayed changes in biomass allocation (Fig. 8 and 12). This indicated that the plants' drought response was activated only below a certain level of water deficit. This level was between the 75% and 100% irrigation

treatments in this experiment since the root:shoot ratios decreased between those two levels only (Fig. 8). The exact volumetric water content related to this response activation cannot be determined in this experiment since it would require separating the effect of the irrigation levels, soil types and the length of the experiment (time). Indeed, the volumetric water content of the soils decreased significantly over time and was significantly different between irrigation levels and between soil types (Fig. 6). Therefore, only the combined effect of these variables can be used as the cause of the stress response of the plants. Quantifying the relative effect of each of these variables would require another experiment with a larger variation of soil compositions, both constant irrigation levels and decreasing ones similar to this experiment, additional irrigation levels between the current 75% and 100%, and samples taken at regular intervals throughout the experiment. If the root:shoot ratio of the plants do not change over time or between soil types but only because of irrigation, we would then be able to determine which volumetric water content of the soil represents the threshold of activation of the drought response of these plants.

Although the volumetric water content of the pots significantly decreased throughout the experiment and I expected the drought effect to be different between the three irrigation treatments (Fig. 5), only two levels of drought intensity were successfully created. Indeed, the plants only responded to differences between levels 75% and 100% and showed no change between levels 50% and 75% (Fig. 7, 8 and 12). This could be because the drought intensity required for an increase of the drought response of the plants was not reached within this experiment. Wilschut and Kleunen (2021) found that geranium plants didn't increase root biomass when under drought stress and hypothesized that this was due to a lack of actual stress because the pots were too small to limit the amount of water and create a drought response in the plants. The propagation units used in this experiment were relatively small since they could only contain approximately 15mL of soil. Therefore, this lack of increased drought intensity between 50% and 75% could be related to the shallowness of the pots.

In addition to this drought stress from irrigation levels, the particular composition of Soil 3 also participated in increasing the intensity of this drought since VWC was lower for this soil type, and the significantly higher root:shoot ratios and allometric relationships showed that the plants responded to this difference as well (Fig. 8 and 13). This was expected as Soil 3 was the only one to contain sand and a minimum of compost, therefore having a higher porosity and lower water content. This means that the specific composition of Soil 3 enhanced the drought effect already created by the irrigation treatments. This result is in line with previous findings of the effect of soil composition on the drought response of plants (van Wijk, 2011).

There seemed to be no effect of nutrient availability on biomass production. However, leaf area values seemed to be affected since larger leaves were found in Soil 1 (Fig. 11). Unfortunately, the experimental set-up did not allow me to determine whether this result is an effect of lower drought stress or higher nutrient availability. It could be hypothesized that this result reflects lower



drought stress in Soil 1, but this is not likely since no differences were found between Soil 1 and 2 in the other variables (Fig. 8 and 13). Similarly, nutrient availability could be higher in Soil 1 due to the higher percentage of compost, but this did not reflect in lower root:shoot ratios or higher biomass production. One way to further study this would be to add nutrients to the irrigation water for Soil 2 and 3 and see the response of plants compared to Soil 1 afterwards.

To relate back to my research questions, these results show that plants respond to drought differently depending on the intensity of the drought, increasing root:shoot ratio and deviating from their usual allometric trajectory to survive. In addition, using sandy soil in combination to lower irrigation levels increased the drought stress on plants and further intensified the response of the plants.

#### 4-2 BIOMASS ALLOCATION PATTERNS

The results of root:shoot ratios and the allometric regressions revealed that the drought stress created a change in BAP (Fig. 8 and 12). The lower root:shoot values found in plants that grew under higher irrigation levels and their lower allometric curves showed that more biomass was allocated to shoots than to roots for these plants whereas the opposite trend was found for the plants under higher drought stress (50% and 75%). Similar results were found by Poorter et al. (2012a) where root biomass significantly increased under intense drought but showed no difference under low drought pressure. This is an example of dehydration avoidance and drought resistance under low stress as defined by Voltaire (2018). Indeed, the total plant growth was not reduced under drought, and the plants showed a fast response by increasing root biomass to increase the water uptake and avoid desiccation.

Contrary to expectations, the total plant biomass did not reduce under higher drought intensity and did not change significantly between soil types (Fig. 7). Similar results were found in other studies. Wilschut and Kleunen (2021) looked at drought response of geranium species and found no effect of drought stress on total biomass. Padilla et al. (2009) also found that biomass of arid shrub species changed very little under drought conditions. However, many more studies found that drought stress significantly reduced total biomass production for the herb species used in this experiment (Petropoulos et al., 2008, Selmar et al., 2017 Farouk and Omar, 2020).

Surprisingly, the opposite trend was found as total biomass was larger in pots with less irrigation (Fig. 7). This could be due to low biomass production in 100% irrigation pots which could be a result of waterlogging of the pots at the beginning of the experiment (Fig. 7). Indeed, most pots under 100% irrigation retained water between watering days and could have suffered from waterlogging, reducing the total growth of the plants under those conditions. However, this is only a hypothesized reason since the roots of these same plants did not have significantly lower biomass, even though they should have been the first to show dying tissue (Poorter et al., 2012a).

The leaves of the plants also showed no significant variation between any of the treatments and only differed between species (Fig. 10 and 11). Padilla et al. (2009) found the same pattern in arid shrubs. They hypothesized that the lack of significant response from leaves and total biomass was not due to a lack of drought effect, but rather due to their small sample size (ranging 6-14 for each treatment and species combination). In my experiment, the sample sizes of each irrigation level, soil type and species combination were even smaller than that, ranging between 1 and 7 after outliers were taken out to fit the assumptions of the linear model (Appendix B). Therefore, the lack of response in leaf area could be due to a lack of data rather than a lack of drought stress.

These results relate to my first research question and show that the species used in this experiment displayed characteristics of drought resistance through increases in root biomass which allowed them to keep a constant growth under higher drought intensity.

#### 4-3 ALLOMETRIC OR OPTIMAL PARTITIONING THEORY

The results gathered show that the plants did not follow the response predicted by the allometric partitioning theory. Indeed, the allometric regression curves significantly changed under higher drought levels (Fig 12 and 13). This means that the plants responded differently to the varied amounts of resources, optimally changing the biomass allocation to their roots and shoots. In addition, the root:shoot ratio was not affected by total plant size at all (Fig. 8), showing minimal effect of allometry on the BAP of the plants. If the plants had responded according to the allometric partitioning theory, no change would have been seen between the curves of the different treatments, demonstrating how the plant's biomass allocation is pre-determined by a stable allometric relationship (Eziz et al., 2017). This result contrasts with general literature on the topic. Indeed, a meta-analysis carried out by Eziz et al. (2017) found that most herbaceous species responded according to the allometric partitioning theory. Similarly, McConnaughay and Coleman (1999) found that three species of annual plants showed no change in biomass partitioning in response to limited water availability.

However, the plants did not perfectly follow the prediction of the optimal partitioning theory either. Indeed, the root biomass did not seem to increase at the expense of shoot biomass since leaf area did not change and it did not reduce total growth either (Fig. 7 and 11). For this to be possible, the plants could have reduced the stem biomass allocation. Poorter et al. (2012a) found that under drought stress, total biomass did not reduce, and root biomass increased at the expense of stem biomass. However, the stems and leaves were measured together in this experiment and this hypothesis cannot be tested. The leaves and stems of plants were measured together to avoid measurement errors when distinguishing between stem, petiole, and leaf in Dill plants. Indeed, although those organs were easily distinguishable in Parsley and Basil plants, this was harder in Dill and could have led to false conclusions. Therefore, special attention should be given to the stem biomass if this experiment were to be reproduced to figure out whether these plants follow the BAP predicted by the optimal partitioning theory.

#### 4-4 TRUE AND APPARENT PLASTICITY

Interestingly, features of all types of plasticity (apparent, true, and complex) were visible in this experiment. As explained earlier, McConnaughay and Coleman (1999) theorized that only when the allometric curve of root dry mass and shoot dry mass has a scaling exponent equal to one, and when the relationship is linear, can we be certain that ontogenetic drift has no effect on the BAP. If we choose  $R^2$  values above 0.6 to represent good fit of a linear regression, we can see that the Parsley individuals' response to drought was mostly driven by true plasticity and minimal ontogenetic drift (Fig. 12, 13 and Appendix D). In comparison, Dill BAP varied mostly because of ontogenetic drift. Parsley was the only perennial species used in this experiment whereas Basil and Dill are both annual herbaceous species. Similar results were found by Geng et al. (2007) and reflect the predictions of McConnaughay and Coleman (1999). Indeed, they predicted that annual species would show apparent plasticity for a resource limitation below-ground whereas perennial herbs would show true plasticity in a similar scenario.

As mentioned previously, the plants growing in Soil 1 and under 100% irrigation followed the ontogenetic growth of plants in a 'steady environment' where all resources are equally limiting (Ingestad and Ågren, 1991). In comparison, the plants growing under drought stress (irrigation 50% and 75% and Soil 3), showed very low  $R^2$  values or scaling exponents very different from 1. This means that most plants growing in those stress conditions displayed complex plasticity, with their BAP reflecting the effects of ontogenetic drift along with true plasticity. This could also explain the fact that all outliers were from Soils 2 and 3, which grew under non-steady conditions and did not fit the assumptions of linear models.

By comparing plant individuals' plant traits between same-size individuals, we can break down this complex plasticity and only focus on the true plasticity of the individuals. That way, we can see that the leaf area values were not affected by any treatment and increased only because of total biomass (Fig. 11). This shows that the growth of the leaves was only affected by apparent plasticity or ontogenetic drift. In comparison, root:shoot ratio values were not affected by total biomass and varied significantly between treatments, showing the true plasticity of the plants in response to the drought stress (Fig. 8). The combination of these results is interesting because it shows that the plants' plasticity can be locally activated. In this experiment, the stress was localized in the soil, and no stress was imposed on the shoots of the plants, and this is reflected in the adaptation of these plants to their environment through true plasticity of the roots.

To refer back to my research questions and predictions, these results show that the ontogenetic trajectory varied between species, with Dill being more ontogenetically constrained than Parsley. In addition, the biomass allocation to roots displayed by plants under higher drought intensities was activated by localized true plasticity, whereas the leaf area of those plants was predetermined by ontogenetic drift.

#### 4-4 STUDY LIMITATIONS

The main pitfall of this study can be found in the shortcut used to estimate the plants' response to resource limitation. As in several other studies on this topic, plant organ mass was used to represent organ functionality instead of measuring this variable directly. Indeed, it was assumed that an increase in plant root mass must be proportionally linked with an increase in plant water uptake, and any change in leaf area must result in a change in photosynthetic rate of the plant. However, this is not always the case. This issue was discussed by Poorter et al. (2012a) who pointed out that this is a major pitfall in root:shoot ratio studies. Koyama and Kikuzawa (2009) found that specific leaf area was not proportional to photosynthetic rate of the whole plant. Researchers also found that adaptations in response to resource limitation below-ground can result in changes in specific root length (Aerts and Chapin, 2000) or root architecture (Fitter and Stickland, 1991) rather than root mass only. In the case of this experiment where the presence or absence of a response to drought was the focus, the issue is less problematic. Indeed, the limitation arises in the quantification of the response. If the plants responded to drought by increasing their water uptake in other ways than by increasing root mass, it would not be visible in this experiment, and the drought response would be underestimated compared to reality. To quantify this, measures of specific root length, root architecture, turgor pressure and total water potential (as defined by Begg and Turner, 1976) would be required to fully understand the extent of the changes engendered by the drought in the plants.

Another pitfall concerns Parsley individuals which showed very different results than the other two species. By the end of the growth period of the experiment, very few individuals had germinated as reflected in the sample size for this species which was almost a third smaller than the others (Appendix B). The total biomass of this species showed no significant difference with Dill, and there was no difference of mean leaf area with Dill even though large differences were expected (Fig. 7 and 10). These results could be explained by the fact that this variety of Parsley was not adapted to the growing conditions created during the experiment which caused it to have a very low germination success. Since it took longer to germinate, most individuals were at a very early growth stage by the time of the harvest, as expressed by the low biomass content of the whole plants (Fig. 7) and the low leaf area values (Fig. 10). In addition, the high root:shoot ratio values indicate a higher proportion of roots in most individuals compared to shoots (Fig. 9), which is characteristic of herbaceous species in early growth states (Coleman, McConnaughay and Ackerly, 1994). Finally, the fact that most of the outliers taken out were Parsley individuals also shows that they displayed very different BAP from other plants. This means that the interspecific differences observed between Parsley and the other two species could be due to the failure of the experiment rather than the effects of the treatments.

Finally, the relevance of these results could be limited because of the specific methods used here. Several studies found large variation in drought response due to the specifics of the experimental

design (Sinclair et al., 2017, Poorter et al., 2012b, Begg and Turner, 1976). Poorter et al. (2012b) noted that the size of pots used could have a very large effect on the response of the plants. Indeed, increasing the size of pots used in drought response experiments can increase the total plant biomass, leaf area, and water uptake of the roots (Ismail, Hall, and Bray, 1994, Poorter et al., 2012b). Although this does not undermine the results of this study since all pots had the same size, it limits the relevance of the conclusions on a broader scale.

This lack of relevance also comes from the fact that the plants only grew for three weeks, and most did not grow more than 2 leaves, whereas the usual harvest time for agricultural production is much later and at higher biomass levels. To make sure that these results are relevant in economic and agricultural production settings, a similar experiment looking at the effect of treatments at later growth stages is needed.

## 5- CONCLUSIONS

By creating two levels of drought intensity, the drought resistant response of Dill, Parsley and Basil were described. The species showed characteristics of true plasticity by changing biomass allocation patterns under drought stress. This response seemed to follow the predictions of the optimal partitioning theory with an increase in root percentage. It is also worth noting that several surprising results were found. Moderate drought significantly increased root biomass but this was not at the expense of leaves. Regarding plasticity, Dill plants seemed to be more ontogenetically constrained than Parsley individuals. In addition, most individuals activated drought resistance characteristics through true or complex plasticity very shortly after germination. The exact level of irrigation required to activate this drought response was not determined, but the bracket of irrigation level within which it is found was described. In addition, I showed that the effect of both irrigation and soil composition could significantly increase the drought stress and the response of herbaceous species. However, these results stem from very low sample sizes and could have been influenced by the limitations of the study discussed previously. Therefore, longer lasting studies looking at the combined response of biomass allocation and organ functionality might be required to predict the changes taking place in the later stages of development of the plants, closer to the harvest time in agricultural production sites. In addition, increasing the number of drought level and gaining a clear understanding of the effect of pot size would scale up the results and help further determine the cause of these phenomenon.

*(8,783 words including abstract and excluding figure captions)*

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## 7-APPENDICES

### 7-1 APPENDIX A: COMPOSITION OF SOIL MIXES IN PILOT STUDY.

The soil fractions were made according to the volume of a single pot.

| Soil mix n° | Sand | Vermiculite | Gravel | Compost |
|-------------|------|-------------|--------|---------|
| 1           |      |             |        | 1       |
| 2           |      | 1/2         |        | 1/2     |
| 3           |      | 2/3         |        | 1/3     |
| 4           | 1/3  | 1/3         |        | 1/3     |
| 5           | 1/4  | 1/4         |        | 1/2     |
| 6           | 1/2  |             |        | 1/2     |
| 7           | 2/3  |             |        | 1/3     |
| 8           | 1/4  |             | 1/4    | 1/2     |
| 9           |      |             | 1/2    | 1/2     |
| 10          | 1/4  | 1/4         | 1/4    | 1/4     |

### 7-2 APPENDIX B: SAMPLE SIZES AND GERMINATION RATES

The germination rate was calculated as the percentage of planted seeds that germinated. Regarding the sample sizes after taking out outliers, the numbers correspond to the analysis of root:shoot ratio / allometric regression / leaf area. X or blank squares are used when no outliers were taken out of the dataset.

| Soil type | Species | Irrigation level | Sample size | Germination rate | Sample size without outliers |
|-----------|---------|------------------|-------------|------------------|------------------------------|
| 1         | Basil   | 50               | 3           | 37,5             |                              |
| 1         | Basil   | 75               | 1           | 12,5             |                              |
| 1         | Basil   | 100              | 4           | 50               |                              |
| 1         | Dill    | 50               | 2           | 25               |                              |
| 1         | Dill    | 75               | 2           | 25               |                              |
| 1         | Dill    | 100              | 3           | 37,5             |                              |
| 1         | Parsley | 50               | 2           | 25               |                              |

|   |         |     |   |      |           |
|---|---------|-----|---|------|-----------|
| 1 | Parsley | 75  | 2 | 25   |           |
| 1 | Parsley | 100 | 2 | 25   |           |
| 2 | Basil   | 50  | 4 | 50   |           |
| 2 | Basil   | 75  | 6 | 75   |           |
| 2 | Basil   | 100 | 3 | 37,5 |           |
| 2 | Dill    | 50  | 4 | 50   |           |
| 2 | Dill    | 75  | 3 | 37,5 | X / 2 / X |
| 2 | Dill    | 100 | 4 | 50   |           |
| 2 | Parsley | 50  | 5 | 62,5 | 4 / 4 / X |
| 2 | Parsley | 75  | 2 | 25   | X / X / 1 |
| 2 | Parsley | 100 | 4 | 50   | X / 3 / X |
| 3 | Basil   | 50  | 7 | 87,5 |           |
| 3 | Basil   | 75  | 6 | 75   |           |
| 3 | Basil   | 100 | 4 | 50   | X / X / 3 |
| 3 | Dill    | 50  | 4 | 50   |           |
| 3 | Dill    | 75  | 7 | 87,5 | 6 / 6 / X |
| 3 | Dill    | 100 | 4 | 50   |           |
| 3 | Parsley | 50  | 4 | 50   | X / X / 3 |
| 3 | Parsley | 75  | 3 | 37,5 |           |
| 3 | Parsley | 100 | 1 | 12,5 |           |

### 7-3 APPENDIX C: R SCRIPT FOR DATA ANALYSIS

The complete code used for the data manipulation, analysis and visualization can be found on my GitHub account (<https://github.com/louise-litrico/dissertation-work>). This script only includes the code used for statistical models and to tests their assumptions.

#### 7-3-1 MOISTURE ANALYSIS

ANCOVA carried out to test the effect of time, soil type and irrigation level on the volumetric water content of the soil throughout the experiment.

```
# Assumptions with chi-squared test and Levene's test
explanatory_table = table(moisture$day, moisture$soil_type)
print(explanatory_table)
print(chisq.test(explanatory_table))
# p-value > 0.05 so covariates are independent
```

```

leveneTest(mean_moisture ~ irrigation_level, data = moisture)
# p-value > 0.05 so variance is equal
# then actually fitting the model
ancova_model <- aov(mean_moisture ~ irrigation_level + soil_type + day, data
= moisture)
Anova(ancova_model, type="III")
# posthoc test
postHocs <- glht(ancova_model, linfct = mcp(soil_type = "Tukey"))
summary(postHocs)
postHocs1 <- glht(ancova_model, linfct = mcp(irrigation_level = "Tukey"))
summary(postHocs1)

```

### 7-3-2 ROOT AND SHOOT ANALYSES

Linear model carried out to test the effect of species, soil type and irrigation level on the root:shoot ratio values while controlling for total biomass of the individuals.

```

ratio1 <- ratio %>% filter(!count %in% c(67,90)) # taking out outliers
ratio_model <- lm(root_shoot ~ irrigation_level*species + soil + Dry_weight_t
otal, data = ratio1)
summary(ratio_model)
plot(ratio_model)
summary(gvlma(ratio_model))

```

Linear model carried out to test the effect of dry shoot weight, irrigation level, soil type and species, on the dry root weight of the plants.

```

ratio2 <- ratio %>% filter(!count %in% c(50,67,87,90)) # taking out outliers
ratio_model1 <- lm(log(Dry_weight_root) ~ log(Dry_weight_shoot) + irrigation_
level + soil + species, data = ratio2)
summary(ratio_model1)
plot(ratio_model1)
summary(gvlma(ratio_model1))

```

### 7-3-3 LEAF AREA ANALYSIS

Linear model carried out to test the effect of species, soil type and irrigation level on the leaf area values while controlling for total biomass of the individuals.

```

ratio3 <- ratio %>% filter(!count %in% c(38,84,92)) # taking out outliers
leaf_area_model <- lm(Leaf_area ~ species + soil + irrigation_level + Dry_wei
ght_total, data = ratio3)
summary(leaf_area_model)

```

```
plot(leaf_area_model)
summary(gvlma(leaf_area_model))
```

#### 7-3-4 TOTAL BIOMASS ANALYSIS

Linear model carried out to test the effect of species, soil type and irrigation level on the total biomass of the individuals.

```
total_biomass_model <- lm(Dry_weight_total ~ irrigation_level*soil + species,
  data = ratio)
summary(total_biomass_model)
plot(total_biomass_model)
summary(gvlma(total_biomass_model))
```

#### 7-4 APPENDIX D: PARAMETERS AND $R^2$ VALUES FROM ALLOMETRIC REGRESSIONS

In red are the individuals that showed good fit to a linear regression ( $R^2 > 0.6$ ) and a scaling exponent ( $a$ )  $\approx 1$ .

| Species | Irrigation level /<br>Soil type | a    | b     | $R^2$ | Figure |
|---------|---------------------------------|------|-------|-------|--------|
| Basil   | 50%                             | 0.86 | -0.46 | 0.77  | 11     |
| „       | 75%                             | 0.86 | -0.56 | 0.56  | „      |
| „       | 100%                            | 1.3  | 0.55  | 0.64  | „      |
| „       | 1                               | 1.5  | 1.4   | 0.56  | 12     |
| „       | 2                               | 0.98 | -0.3  | 0.71  | „      |
| „       | 3                               | 0.72 | -0.96 | 0.67  | „      |
| Dill    | 50%                             | 0.44 | -2.7  | 0.23  | 11     |
| „       | 75%                             | 0.74 | -1.4  | 0.88  | „      |
| „       | 100%                            | 1.1  | -0.11 | 0.78  | „      |
| „       | 1                               | 1.2  | 0.11  | 0.7   | 12     |
| „       | 2                               | 1.1  | 0.13  | 0.66  | „      |
| „       | 3                               | 0.75 | -1.3  | 0.63  | „      |
| Parsley | 50%                             | 0.96 | -0.09 | 0.77  | 11     |

|   |      |      |        |      |    |
|---|------|------|--------|------|----|
| „ | 75%  | 0.92 | -0.031 | 0.82 | „  |
| „ | 100% | 1.3  | 0.91   | 0.93 | „  |
| „ | 1    | 1.3  | 1.5    | 0.82 | 12 |
| „ | 2    | 1    | 0.2    | 0.67 | „  |
| „ | 3    | 0.47 | -2.2   | 0.22 | „  |