Cover Crops in a Changing Climate:
Can Mixtures Reduce Water Stress?

by

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Abstract

Climate change models predict more extreme rain events and drought to occur in different areas of the mid-latitude region. Farmers and researchers have been looking for solutions to reduce negative impacts of these precipitation changes. Cover cropping is a promising practice as it minimizes erosion in extreme rain events and conserves water in drought years if used as mulch for the following crop. To adapt to climate change and achieve production goals at the same time, growing multispecies of cover crops is a potential approach as different species provide complementary ecosystem services. Despite a large volume of research on how cover crops support agriculture in a changing climate, understanding is limited of how climate change influences the growth of cover crops. I investigated the early development of two common cover crop species – crimson clover (*Trifolium incarnatum* L.) and rye (*Secale cereale* L.) – in response to water stress, and evaluated if a cover crop mixture is capable of ameliorating water stress. I hypothesized that rye and crimson clover grown in the mixture would have a better performance than those grown in monocultures. To examine the influence of water stress and diversity on plant growth, a one-month experiment was conducted, where the two factors were fully crossed in randomized blocks. To determine plant growth, I recorded survival and growth rate throughout the experiment; at the conclusion of the experiment, I collected total biomass and generated root traits using RhizoVision Explorer program. I found that water stress negatively influenced the growth of cover crops, and the impacts varied among species: crimson clover showed to be susceptible to drought, and rye performed poorly under waterlogging. In the face of these stresses, growing in mixtures showed potential to ameliorate water stress via sampling effect and niche complementarity.

1. Introduction

Climate change models project that more extreme rain events and droughts will occur across the mid-latitude region (IPCC 2013). This precipitation change will certainly affect farmers in achieving their production goals. According to Shortly et al. 2015, as precipitation variability increases, crop production systems will experience more negative influences. A longer dry period would result in dramatic losses to crop production, such as for soybean (Leng and Hall 2019) while a higher risk of flooding would delay planting (Shortly et al. 2015). To plants, water stress strongly reduces growth. Drought decreases soil moisture, causing a decline in nutrient availability in soil, thereby plant growth is suppressed due to a lack of water resource and nutrients (Studer et al. 2017). Meanwhile, waterlogging limits concentration of oxygen in soil, slowing root growth, which in turns reduces nutrient uptake and affects plant growth (Setter and Belford 1990).

Cover cropping is a promising practice that can help farmers mitigate and adapt to climate change while also providing ecosystem services for the growing season (Clark 2007; Finney and Kaye 2017). Indeed, cover crops can reduce the risk of soil erosion in extreme rainfall; after being terminated, if they are prepared as mulch, they can preserve water on fields in drought periods (Kaye and Quemada 2017). Normally, they provide benefits to improve soil health and water quality. Cover crops cover the ground, restrict the growth of weeds, and restore nutrients in the soil to support the next growing season (Clark 2007). There are several species that are used as cover crops across the US, and farmers have created different practices to manage cover crops, such as how to plant them.

A national survey conducted in 2019 among farmers across the US reported that improving soil structure and soil health is the leading motivation to adopt cover crops, and 58.3% (435 of

746 respondents) found positive changes in their fields after roughly two years of planting (CTIC 2020). In addition to growing one species, farmers are increasingly interested in cover crop mixes, particularly 45.5% (406 of 893 respondents) shared to have planted mixtures in 2019 and decided to continue using them in 2020. Moreover, the choice of how many species used in the blend varied widely, but mixing two species was a common recipe.

Plant biodiversity is expected to improve biomass productivity, which arises through two ecological mechanisms: (i) sampling effect and (ii) niche complementarity. The sampling effect suggests that the better performance of a mixture results from a higher chance of having productive species in a diverse community compared to a monoculture population (Heijden et al. 1999). On the other hand, the niche complementarity explains that a mixture's better performance is due to a more effective use of resources (Flombaum et al. 2014). Explaining the effective use proposed by the niche complementarity are two relationships: (a) resource partitioning in which species with different traits use resources differently, and (b) facilitation when one species alters the environment in a way that supports others (Flombaum et al. 2014). As productivity increases with diversity, ecosystem services are also expected to increase, benefiting the agricultural systems (Smith et al. 2014; Finney et al. 2016). Moreover, the productivity-diversity relationship becomes more apparent under environmental stress, specifically drought (Mulder et al. 2001).

While the practice of growing cover crops to address climate change is extensively investigated, the influence of climate change on cover crops is not substantially examined. In the Northeast US, total precipitation historically increased in warm seasons (June-October) which include the establishment period of cover crops (i.e., Aug-Sep) (Frei et al. 2015; Huang et al. 2017). In contrast to this historical pattern, climate projections predict that droughts will occur more often in the autumn by the mid-century (Shortly et al. 2015). Given the uncertainty of climate change, I wanted to study the impacts of water stress on the early development of cover crops, and investigate both directions of water stress: water scarcity and excess water. Considering the potential to improve performance under environmental disturbances with diversity, I further explored if growing in a mixture could ameliorate the influence of water stress on cover crops in their early development. I chose to conduct this research on two common cover crop species in Pennsylvania: crimson clover (*Trifolium incarnatum* L.) and rye (*Secale cereale* L.) (CTIC 2020). I hypothesized that the growth of cover crops would be affected by water stress, but the impacts would be alleviated when two species were grown together. As growth in ecology can be assessed by various parameters, I evaluated growth through one-time measurements of survival, total biomass, and root morphology and through long-term observations of growth rate. Results of growth under water stress and diversity treatments will be explained by the two mentioned ecological mechanisms: the sampling effect and niche complementarity.

2. Materials & Methods

2.1. Greenhouse Setting

To better understand the effects of water stress and diversity on cover crops' early development, I conducted a one-month experiment at Dr. Inge P. Stafford Greenhouse of Dickinson College (Carlisle PA, USA) in June 2021. The experiment was a randomized block design with a total of 4 replicates; in one replicate, two factors – water stress and diversity – were fully crossed (Figure 1). Two investigated cover crop species, crimson clover and rye, were cultivated from seeds in polyvinyl chloride (PVC) pots (18 cm in height and 16 cm in diameter) filled with coarse Turface® MVP clay (Profile Products LLC., Buffalo Grove, Ill).

This growing medium was mixed with 45g of organic fertilizer 3-4-4 (Garden-tone, The Espoma Company, Millville, NJ). Turface® had a bulk density of 577 ± 32 kg m^{-3} and was used as it was found to facilitate root morphology examination, even the delicate root hair (Goron et al. 2015).

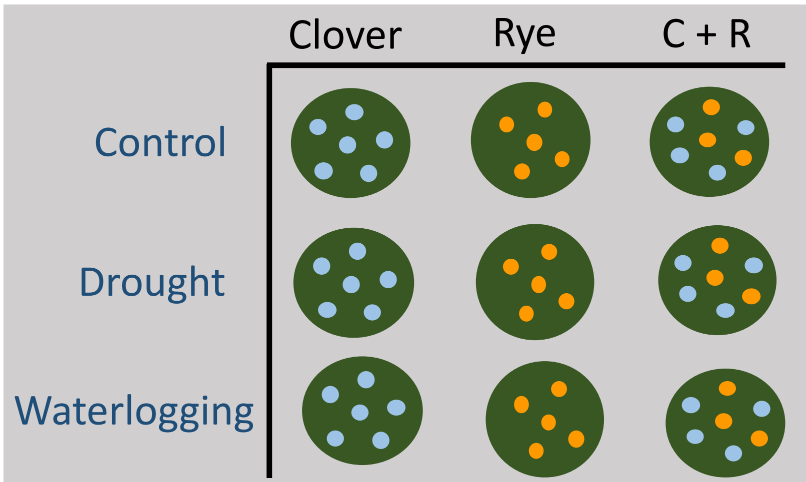

Figure 1. One replicate of the experiment's randomized block design.

The diversity treatments of my study were a mixture of crimson clover and rye, and their corresponding monocultures. Their seeding rates were determined according to recommendations by Clark (2007): (i) monoculture crimson clover (6 plants per pot; 15-20 lb./A), (ii) monoculture rye (5 plants per pot; 60-120 lb./A), and (iii) polyculture rye and crimson clover (3 rye plants and 4 crimson clover plants per pot). Rye seeds were seeded directly while crimson clover seeds were inoculated beforehand (N-Dure™ Alfalfa/True Clover, Verdesian, Cary, NC). To ensure successful germination, I sowed double the target number of seeds in every pot. On the 7th day after seeding, seedlings were thinned and transplanted among pots in order for all pots to obtain the desired number. Transplanting was carried out in early evening when the light intensity and air temperature were low; I also used the greenhouse roof cover for 24 hours after transplanting to reduce heat stress on transplanted seedlings.

The temperature of the greenhouse was set up as 27°C/16°C for day/night to represent the normal maximum and minimum conditions in Carlisle – a city in the Great Valley, PA, during the establishment period of cover crops – between August and September. The maximum and minimum temperature normals at Carlisle defined by the period of 1991-2020 for August and September were 29°C/16°C and 25°C/12°C, respectively (SC-ACIS 2022). The Turface® and ambient temperatures were logged daily using HOBO data loggers (Appendix A, Figure 7). The sensors measuring Turface® temperature were situated 4.5 cm below the surface; on the other hand, the sensors logging ambient temperature were situated 10 cm above the surface. The pots where those sensors were placed received the same water level as the controls.

2.2. Water Stress Imposition

Water stress imposed in my study consisted of three water levels: (i) control with 100% of field capacity, (ii) drought of no irrigation, and (iii) waterlogging where the water level was 2-5 mm above the surface to partly submerge the seedlings (Kaur et al. 2020; Yang et al. 2020). The field capacity of the Turface® was measured prior to the start of this experiment following Imakumbili (2019). Particularly, I watered 3 replicates containing the same amount of air-dried Turface® until the pots started leaking, then I covered the pots with plastic bags, allowing only downward drainage. After 3 days, I collected 100g of Turface® in the center of each pot. By comparing the percentage of water in saturated Turface®, I calculated the medium's field capacity to be 53.13% volumetric soil moisture, which was equivalent to 930g of water for 1750g of Turface® - the mass of Turface® in each pot of my experiment.

From seeding to harvest, the control pots were consistently watered to 100% field capacity. On the other hand, drought and waterlogging pots were maintained at 100% field capacity in the first two weeks. When the water treatments were initiated on the 13th day after seeding, drought pots were withheld water, and waterlogging pots were lined with a plastic bag to cover drainage holes and left flooded at about 5 mm above the surface. Following water stress experiments conducted by Kaur et al. (2020), Garcia et al. (2020), and Yang et al. (2020), I determined that the water treatment took place for one week and added a recovery phase. In detail, on the 20th day after seeding, the water levels of all pots were brought back to 100% field capacity, and the plants were allowed to recover for five days before being harvested. Throughout the experiment, I maintained the assigned water levels by weighing pots and irrigating daily (Ogbaga et al. 2020). Additionally, to avoid the influence of pot positions on the examined effects, pots were rotated daily within the block in the greenhouse (Bezuidenhout et al. 2012).

2.3. Growth Score & Survival Rate

To capture changes of seedlings under the water treatments and in the recovery from the stress without destroying samples, I recorded their growth every day during these periods by applying vegetative stages for clover and *Leaf Collar Method* for rye. For clover, early growth is generally categorized into three stages of emergence, leaf production, and branching (Mills 2017). Adapting these three main vegetative stages, I specified growth as the emergence of a new set of leaves to suit what I could observe in my one-month experiment. Thus, I set growth stages of clover as an emergence of cotyledons, first true leaf, first trifoliate leaf, second trifoliate leaf, etc., and each stage was equivalent to one point. For rye, according to the *Leaf Collar Method*, rye seedlings gained one point for a visible leaf collar (Abendroth et al. 2011). Growth points reflected the total number and/or types of leaves produced regardless of wilting.

2.4. *Root Trait Analysis*

On the 25th and 26th day after seeding, the experiment was terminated, and all plants were harvested. I carefully removed plants from the Turface® MVP and washed them with tap water. Their shoots and roots were separated and stored by replicates and species. The shoots were oven-dried at 60°C for 48 hours and then weighed. The roots were placed in a plastic bag and stored at 4°C while I processed the remaining roots. When all samples were completely processed, each group of roots were floated in 400 mL of water on an 8 x 10 in transparent tray and scanned with EPSON Pro V850 flatbed scanner at a resolution of 800 dpi (Sofi et al. 2018). Each group was first scanned with their intact root systems, then the roots were divided into smaller parts and spread for the second scan. Scanned roots were oven-dried at 60°C for 48 hours and weighed with their dry shoots to obtain a total dry biomass. The scanned images were analyzed by RhizoVision Explorer program under the Broken Root mode (Seethepalli and York, 2020). In the settings, I aimed to optimize the scanning of the root systems. To capture very fine roots, I created five root diameter ranges that distributed from 0 to 2 mm, and the last range was from 2 mm and above. For crimson clover, I excluded root hairs by setting image threshold to 200 and root pruning to 5. Since rye roots were highly dense and clumped, I further reduced the threshold to 196 and increased the pruning to 8.

2.5. *Statistical analysis*

All indicators of growth were analyzed by species with statistical tests in R 4.1.0 (R Core Team 2021). The general model that I employed was:

$$\text{Response} \sim \text{Water Stress} \times \text{Diversity Treatments}$$

Treatment effects were considered to be statistically significant at $P < 0.05$. Tukey's 'Honest Significant Difference' were computed to compare means among treatments.

Survival rate was the percentage of living seedlings in a particular pot at the end of experiment by its sowed seeds. In this study, I considered mushy or brittle stems that could not support the whole plant to stand up as dead seedlings. Since it was discrete data, I applied generalized linear models (GLM) with a quasi-binomial distribution (Zuur et al. 2007).

Growth rate – changes of points per day – was first calculated using linear regressions and then performed a statistical test with an analysis of variance (ANOVA). In detail, the average growth points of each pot recorded every day were distilled by replicates, species, and phases. I defined phases as the different periods when growth points were recorded, thus it indicated water treatment (day 13th to 19th after seeding) and recovery (day 19th to 26th after seeding). To estimate growth rate, I first fit linear regressions on the growth points where points were predicted by time:

$$\text{Growth Points} \sim \text{Date}$$

Since the slope of the model outcome indicated shifts in points per day, it was used to estimate growth rate, making up a total of 48 growth rates for one species. These rates became the response variable for a two-way ANOVA where they were predicted by water stress, diversity treatments, and phases with starting points added as a covariate:

$$\text{Growth Rate} \sim \text{Water Stress x Diversity Treatments x Phase + Starting Points}$$

The growth rates of rye were log-transformed to improve homoscedasticity.

Total dry biomass per plant for each species was determined by adding plant components (i.e., shoot and root) of the species in a particular pot, then dividing by its seedlings. This data was first fit with two-way ANOVA. When I removed one outlier in which half of the seedlings died before the water treatment, all assumptions were met for clover's total biomass. For rye, its total biomass violated the homogeneity assumption despite implementing general transformations such as log and square root, and I observed that the residuals spread more as the fitted values increased. To improve homoscedasticity of the fit, I applied generalized linear models (GLM), family Gamma (Zuur et al. 2007).

Root traits generated from the RhizoVision Explorer program were examined for correlations using the "corplot" package in R. Among strongly correlated traits, I selected certain traits to simplify the variables in further analysis and included other relevant growth indicators to conduct principal component analysis. The analyzed parameters were root:shoot mass ratio, average total biomass (grams per plant), number of root tips, total root length, branching frequency, average diameter, maximum diameter, root length of five diameter ranges, root length ratio of very fine to the entire root, and root length ratio of very fine to fine root. Adapting analyses performed by Sofi et al. 2018, I generated eigenvalues to assess what percentage of variance of the entire dataset was explained by each principal component. Within the first two principal components, I identified traits accounting for the maximum variability and designated them as the most influential traits. Then I mapped replicates of combined treatment groups on PCA based on the factor loading values of individuals.

3. Results

3.1. Survival Rate

The survival rate of crimson clover varied significantly under water stress (Table 1). Indeed, on average, only about 65% of germinated seedlings in the drought condition survived; in other words, two seedlings in every monoculture pot and one in every polyculture pot died under drought (Figure 1). In contrast, more than 80% of seedlings survived by the end of the experiment in the control and waterlogging conditions (Figure 2).

Contrary to clover, rye maintained the original number of seedlings under all treatments by the end of the experiment. Only two seedlings out of five died in one replicate under drought. The statistical test found no significant difference among treatments (Table 1).

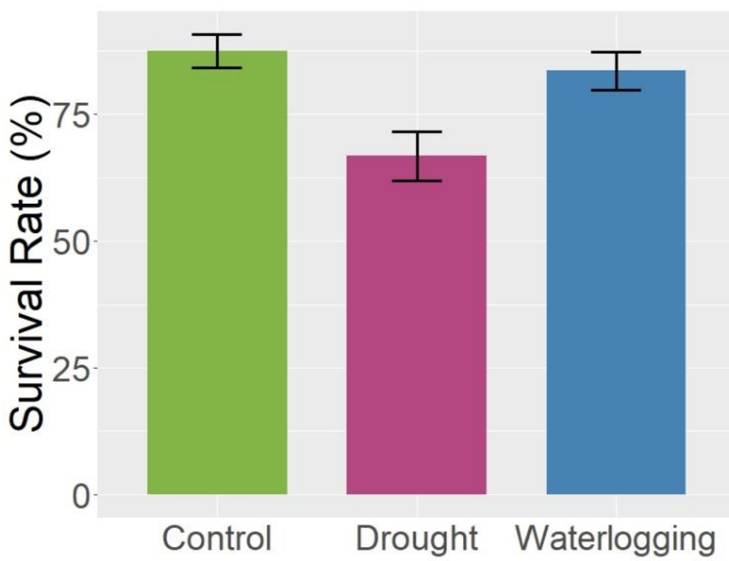

Figure 2. Survival rate in percentage of crimson clover seedlings under three water levels at the end of the experiment (N=8). When considering merely water stress, the monoculture and polyculture were combined, making up a total of 8 replicates for each water level.

3.2. Growth Rate

From the implementation of water stress to the end of the recovery, I found that the growth rate of crimson clover had statistically changed under two main effects of water stress and diversity treatments (Table 1). The seedlings took significantly longer to grow a new set of leaves under drought stress. Indeed, their average growth rate was suppressed by 46% and 60% compared to the control and the waterlogging, respectively (Figure 3A). Moreover, growing in a mixture increased clover's growth rate on average by roughly 40% compared to clover grown in monocultures.

Contrasting with crimson clover which was more susceptible to drought stress, rye was significantly impacted by waterlogging. A significant interaction between water stress and phase in its growth rate was exhibited (Table 1). After the seedlings were released from flooding, the growth rate in the recovery phase significantly declined by 34% (log-transformed) compared to that during the water treatment (Figure 3B). Furthermore, I also found significant main effects of diversity treatment and phase (Table 1). In detail, the species' growth rate in mixtures rose by 31%. Although there was a reduction in the growth rate of waterlogging seedlings during the transition from the water treatment back to the well-irrigated condition, rye seedlings overall, including the control plants, increased their growth rate by 55% in the recovery. Additionally, I statistically controlled for the starting points, and the analysis showed that it did influence the growth rate (Table 1).

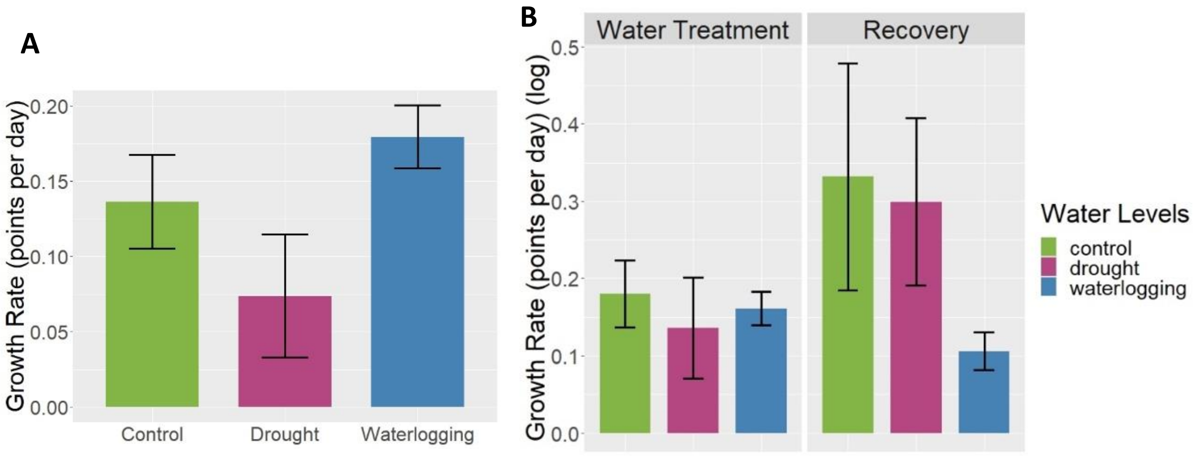

Figure 3. (A) Growth rate in points per day of crimson clover under water stress (N=8). When considering merely water stress, the monoculture and polyculture were combined, making up a total of 8 replicates for each water level. (B) Growth rate of rye under water stress in water treatment and recovery phases (N=8). When examining the interaction between water stress and phase, the monoculture and polyculture were combined, making up a total of 8 replicates for each water level. Moreover, since phase was two periods of time that all replicates of water stress experienced, the number of replicates for the interaction between water stress and phase remained 8.

3.3. Total Biomass

Crimson clover's dry total biomass was significantly influenced by an interaction between water stress and the diversity treatment. Specifically, under drought stress, the polyculture clover accumulated 2.4 times higher total dry mass than its corresponding monoculture did on average (Figure 4A). However, the polyculture clover produced as much total biomass (0.022 g per plant) as its monoculture (0.024 g per plant) under waterlogging (Figure 4A). For rye, I found that the mixture increased the total dry biomass by 50% compared to the monoculture (Figure 4B). This influence was statistically significant regardless of the water levels. In addition, I noticed a non-significant trend regarding water stress: rye seedlings in the waterlogging treatment had a total biomass that was 38% smaller than the control on average.

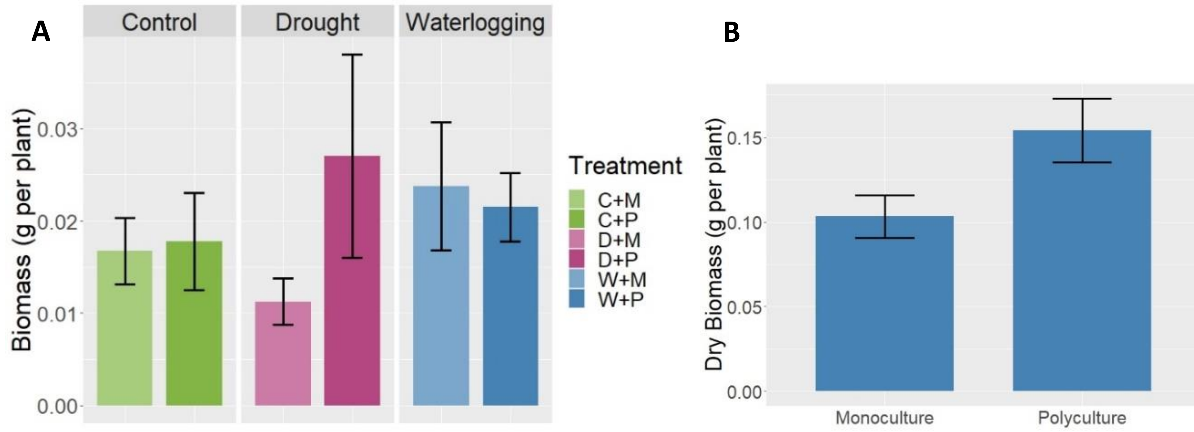

Figure 4. (A) Total dry biomass of crimson clover under diversity treatment (N=4). (B) Total dry biomass of rye under water stress by diversity treatment (N=12). When considering merely diversity, all three water levels were added up to a total of 12 replicates for each diversity treatment.

3.4. Root Analysis

Regardless of the water stress, scan images presented that crimson clover developed shorter roots relative to rye (Figure 5A). Moreover, crimson clover's roots were less dense and had less extensive root hairs compared to rye (Figure 5B & C). The scan images were then imported to the RhizoVision Explorer program to generate root traits which were later analyzed in PCA.

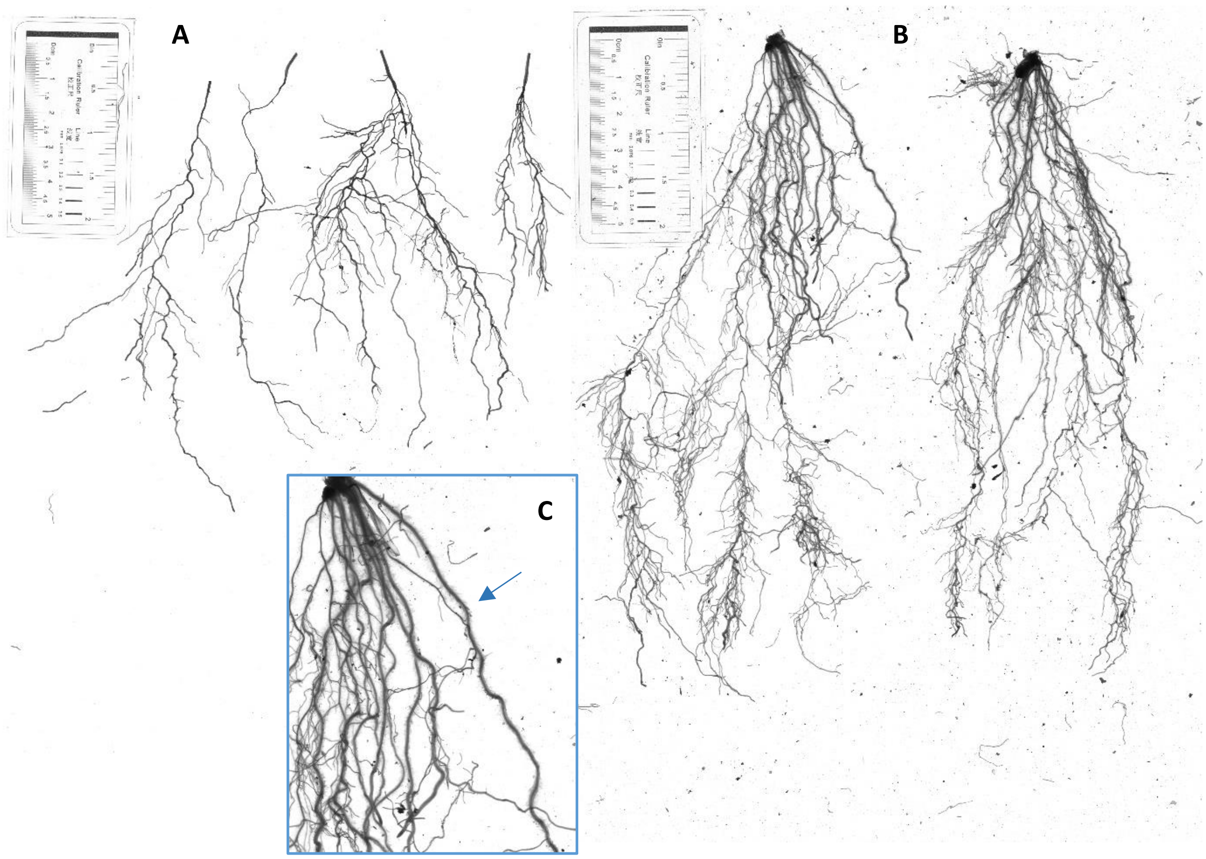

Figure **5.** (A & B) Intact root systems of a drought polyculture consisting of crimson clover and rye, respectively. A ruler is included in the intact root images (i.e., the first scan) as a standard scale to relatively compare the roots, but it was not included in the separated root images (i.e., the second scan) which were later formally analyzed in the RhizoVision Explorer. (C) Root hairs along the fine roots in rye.

The first two principal components (PC1 and PC2) accounted for 73.5% and 75.1% of the variability in crimson clover and rye root data, respectively (Figure 6). Among examined traits, the factor loading values showed that the total root length and the diameter explained the most variance of the first two principal components, respectively (Figure 6, A & B). Regarding the second principal component, the average diameter mainly contributed to variability in clover (Figure 6A), meanwhile it was the maximum diameter for rye (Figure 6B). Therefore, the most influential traits for the first two principal components for clover were the total root length and average diameter, and they were the total root length and maximum diameter for rye.

To put these results into context, I reviewed the literature on the relationship between the root system and water stress. Across multiple species, the root length and root diameter demonstrate greatly contradictory responses to water stress. Under drought, a synthesis of 128 published studies reported patterns of a decreasing root length and an increasing root diameter (Zhou et al. 2018). Meanwhile, Wijewardana et al. (2019) found a reduction in soybean's root length and thickness when the optimum irrigation level (100% field capacity) scaled down to the sub-optimal levels (66% and 33% field capacity). In a flooded environment, the root length increased while the average diameter decreased (Zhen et al. 2020). The diverse findings are perceived to have been caused by several factors, such as experimental duration, soil properties, and plant functional types (Zhou et al. 2018). Focusing on how plants adapt to water stress, Howell et al. (2019) found that plants exhibiting a resistance have a higher root length and a larger root diameter at a specific soil depth. Indeed, as these root traits increase, the root system can better explore moisture and nutrients in soil, which allows the plant to be more tolerant to drought and waterlogging stress (Howell et al. 2019). Additionally, an increasing average diameter strongly indicates a tolerance to drought in common bean performed in PCA by Sofi et al. (2018); the researchers also found positive correlations of a successful reproduction with the average diameter and total root length. Therefore, I decided to interpret the increasing total root length and root diameter as favorable characteristics, indicating an abundant root system in this study.

Depending on the direction where the most influential traits increased in the two principal components, I determined the most favorable quartile, in which seedlings had a longer total root length and a larger diameter (Figure 6C & D). As the major contributors decreased, the quartiles became less favorable; the opposite quartile characterized by a shorter total root length and a smaller diameter was perceived to be the least favorable one. Given the patterns of each quartile, I then mapped replicates of six combined treatments – the individuals – on PCA and computed ellipses to represent their confidence intervals at 95%.

For clover, I noticed that the waterlogging monoculture and polyculture resided in the favorable area, indicating that they had established highly abundant root systems (Figure 6C). The species' drought polyculture fell in the second most favorable quartile (+PC1, -PC2). Meanwhile, its drought monoculture occupied the least favorable quartile. For rye, abundant roots were mainly shown in the control monoculture and polyculture, followed by the drought polyculture which resided mainly in the second most favorable quartile (-PC1, +PC2) (Figure 6D). On the other hand, the waterlogging of both diversity treatments and the drought monoculture ellipses occupied the two least favorable areas.

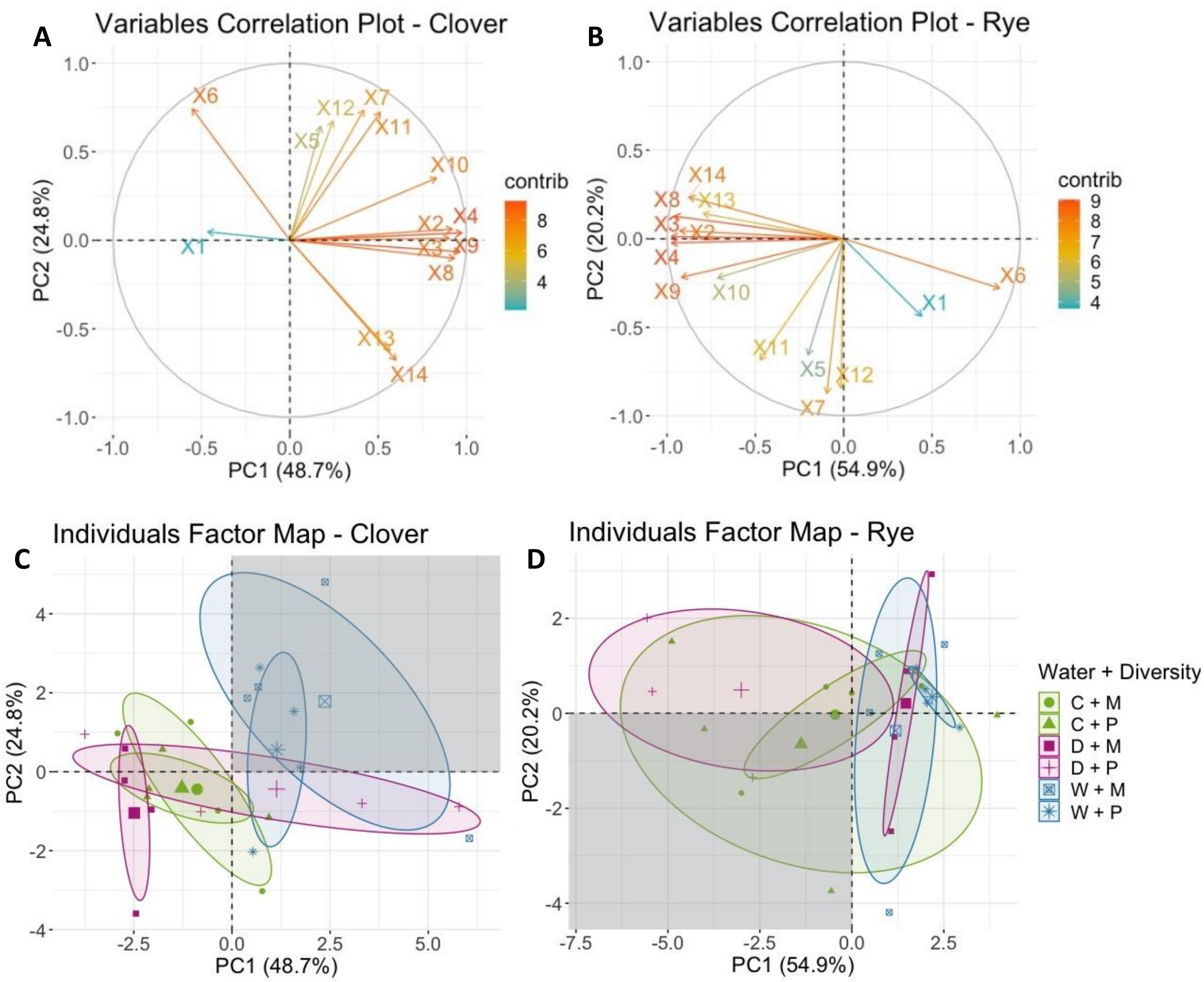

Figure 6. (A & B) Variable correlation plot for crimson clover and rye, respectively. X1: root:shoot mass ratio, X2: total biomass (gram per plant), X3: number of root tips, X4: total root length, X5: branching frequency, X6: average diameter, X7: maximum diameter, X8: root length of diameter range 0-0.25 mm, X9: root length of diameter range 0.25-0.5 mm, X10: root length of diameter range 0.5-1 mm, X11: root length of diameter range 1-2 mm, X12: root length of diameter that is greater than 2 mm, X13: root length ratio of very fine to the entire root, X14: root length ratio of very fine to fine root. (C & D) Individuals factor map for crimson clover and rye, respectively. Shaded quartiles are considered favorable characterizing increases in total root length and diameter. Each ellipse represents a combined treatment of water stress and diversity (N=4).

Table 1. Responses of plants in the greenhouse experiment under water stress and diversity treatments (with an addition of phase in the growth rate model)

Indicator	Treatment	Crimson Clover			Rye		
		df	***F***	***P****	**df**	***F***	***P****
Survival Rate (GLM, Quasi-binomial) †‡	Water	2	7.27	**0.010**	2	1.18	0.330
	Diversity	1	0.82	0.378	1	1.19	0.289
	Water x Diversity	2	0.71	0.506	2	1.07	0.365
Growth Rate (Two-way ANOVA) †‡	Water	2	13.05	**<0.001**	2	5.51	**0.008**
	Diversity	1	6.36	**0.016**	1	7.70	**0.009**
	Phase	1	1.86	0.182	1	6.26	**0.017**
	Start Stage	1	5.65	**0.023**	1	23.46	**<0.001**
	Water x Diversity	2	0.70	0.504	2	3.08	0.059
	Water x Phase	2	1.10	0.345	2	10.60	**<0.001**
	Diversity x Phase	1	0.77	0.721	1	0.01	0.928
	Water x Diversity x Phase	2	0.27	0.766	2	0.26	0.769
Total dry biomass (Two-way ANOVA†/GLM, Gamma‡)	Water	2	2.24	0.137	2	2.24	0.135
	Diversity	1	3.52	0.078	1	5.26	**0.034**
	Water x Diversity	2	5.56	**0.014**	2	2.14	0.146

*Bold values are significant at α = 0.05.
†Statistical tests performed on crimson clover's data
‡Statistical tests performed on rye's data

4. Discussion

The water deficit and excess water conditions both negatively influenced the growth of cover crops. Drought greatly increased crimson clover's mortality and decreased its growth rate. However, waterlogging did not affect its survival rate and total biomass, which agreed with the findings of Garcia et al. (2020) on the effects of a seven-day flooding on soybean seedlings. Considering rye, waterlogging inhibited its growth rate, specifically after being released from the stress, and reduced its total biomass accumulation, total root length and maximum diameter. I also observed leaf wilting in several replicates during the recovery phase, which could have resulted from waterlogging's inhibitory effect on photosynthesis found in Yang et al. 2020. Crimson clover and rye were negatively impacted by soil moisture stress and showed contradictory responses to each stress condition: crimson clover exhibited a tolerance to waterlogging; meanwhile, rye was more likely to endure drought. Their different tolerances to environmental disturbances give evidence for the sampling effect. Thus, if farmers grow crimson clover and rye together, the mixture is more likely to establish and perform more stably in an uncertain climate (Tribouillois et al. 2015).

My study found that regardless of water stress, rye in a mixture grew faster and accumulated a higher total dry biomass than the corresponding monoculture (Table 1). The consistently better performance of rye in polyculture corresponded to the overperformance patterns of grasses recorded by Murrell et al. (2017). This productivity of the mixture indicates niche complementarity. Furthermore, under water deficit condition, crimson clover exhibited a significantly higher total biomass production when grown in a mixture compared to its monoculture. Considering the same stress, crimson clover and rye both demonstrated a longer total root length and a larger diameter in the root system in the mixture compared to their corresponding monocultures. Although the confidence intervals between the two diversity treatments overlapped in clover, this pattern followed the species' total biomass results. For rye, the confidence intervals did not overlap, indicating a strong difference between its monoculture and polyculture under drought stress. The successful development of the mixture is potentially attributed to niche complementarity specifically under drought stress. This could have resulted from the species' positive interactions aboveground and belowground.

In this study, drought stress could have been alleviated via rye's canopy shade aboveground and via roots' reduced competition in water usage belowground. Since rye established fast and grew taller than crimson clover, the species could have ameliorated water deficit stress for crimson clover by creating shade that reduced heat stress on smaller plants – crimson clover – and decreased soil water evaporation (Whitford & Duval 2020). Belowground, crimson clover and rye have different root types and rooting depths (Kemper et al. 2020) (Figure 4). When growing together, two species could have occupied different parts of the soil profile, reducing competition for water and using this limited resource more effectively. Rye's alteration of environmental condition aboveground shows evidence for the facilitation, whereas the differences in root systems and water usage belowground demonstrate resource partitioning.

In summary, my hypothesis that growing a cover crop mixture can alleviate water stress on plant growth is confirmed for drought stress, but is not well-supported by the results for waterlogging. In a water deficit condition, niche complementarity influenced both species to perform better in a mixture. Meanwhile, under excess water, the sampling effect became more prominent. Indeed, crimson clover demonstrated a tolerance to waterlogging; thereby growing in a mixture or in a monoculture did not significantly change how it grew under this stress. For rye, although the species showed to be susceptible to waterlogging regarding the growth rate and root system, its total biomass generally increased in a mixture compared to in a monoculture. The interaction between waterlogging and diversity on rye's growth can be further investigated when growing the species longer.

The evidence for niche complementarity in this study further suggests no competition between the two examined species in their early development. Smith et al. (2014) found that in their cover crop mixture, one species becomes dominant; while it contributed to a high yield of the mixture, the dominant species can potentially limit functions of other species in the mix, which indicates possible competition among cover crop species. However, by comparing the species' performance in a mixture with its monoculture, I noticed that the growth in the mixture was either equal to or greater than that of the monoculture. Regardless, this research focused on the early development, so the interactions that I observed could change as I investigate cover crops' growth under a longer period.

Beyond the main scope of this research, I realize that there is an increase in studies on how diversity could contribute to a better yield in agricultural systems as farmers are generally interested in a higher total biomass production. Researchers have specifically examined overyielding (i.e., the average total biomass of the mixture is greater than that of monocultures) and transgressive yielding (i.e., the average total biomass of the mixture is greater than that of the most successful species in monoculture). It is worth to note that the biomass in this case is calculated by adding plant components of all plants in a replicate regardless of species. Smith et al. (2014) and Finney et al. (2016) both showed that mixtures could lead to an overyielding, but they did not observe a transgressive overyielding. While I did not intend to study diversity as a mechanism to increase yields, I noticed that in a well-irrigated condition, my mixture followed patterns found by Smith et al. (2014) and Finney et al. (2016) as the mixture's average total biomass was higher than that of component monocultures but did not surpass that of the most productive species' monoculture, which was rye (Appendix A, Table 2). However, my studied mixture exhibited different responses under water stress: the mixture could result in an overyielding and a transgressive yielding under drought, but I observed no evidence supporting both responses under waterlogging. In detail, under drought, the mixture accumulated a higher total biomass relative to the average total yield of component monocultures; even more, its biomass exceeded the average total biomass of rye, the most successful monoculture (Appendix A, Table 2). In contrast, under waterlogging, the mixture and the component monocultures on average produced relatively similar total yields; furthermore, the mixture's average total yield was, in fact, lower than that of the most productive monoculture (Appendix A, Table 2). When taking water stress into consideration, there arise interesting observations on the relationship between cover crop diversity and productivity, which is a potential direction for future research to develop more knowledge on biodiversity in agriculture.

In this study, the Turface® MVP was used as the growing medium. While the substrate is not common in agricultural systems, I decided to use it as it can be easily removed from the root systems without damaging the delicate structure, supporting visualization of the roots (Goron et al. 2015). Moreover, the Turface® MVP can address challenges of using field soil in containers. Field soil is slow to drain when used in pots and causes uneven distribution of water, which may introduce confounds to water stress experiments and reduce their reproducibility (Schnelle and Henderson 2017, Ogbaga et al. 2020).

Other limitations of this study are that it was a pot experiment, and it took place within one month. Greenhouse settings can have different influences on plant growth than an outdoor experiment due to several factors such as different wind conditions or growth spaces (Zhou et al. 2018). Moreover, a one-month duration is shorter than the actual growing period of cover crops as well as not long enough for them to fully perform their functions, for example the crimson clover in my research did not have enough time to develop its root nodules by the end of the experiment (Figure 4A). Additionally, given the wide range of species used as cover crops, there can be more unexplored benefits when growing a different mix and integrating more than two species. Therefore, future research can expand the scope (e.g. a field experiment) and the duration of this study as well as examine new cover crop mixtures. Such research can provide farmers with more concrete evidence that supports the adoption of cover cropping.

5. Conclusion

This study found that water stress did impact the growth of cover crops in their early development. Moreover, two species that I studied showed different responses to a specific stress: crimson clover was more tolerant to waterlogging, whereas rye demonstrated a tolerance to drought. This provides evidence for sampling effect, suggesting that given an uncertain weather, farmers will be more likely to gain stable yields on their fields when growing these cover crop species together. Additionally, under drought stress, both species exhibited a higher total biomass and a more abundant root system when they were grown in a mixture. Diversity influenced the growth of cover crops through the mechanism of niche complementarity which could have occurred aboveground and belowground. Furthermore, the evidence for niche complementarity did not suggest any potential competition between the two species as recorded in other cover crop mixes in Smith et al. 2014. Even more, my results supported potential overyielding and transgressive overyielding in the mixture in the early development of cover crops under drought. However, this study does have several limitations as well as unanswered questions that can be improved and explored in future research.

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Appendix A

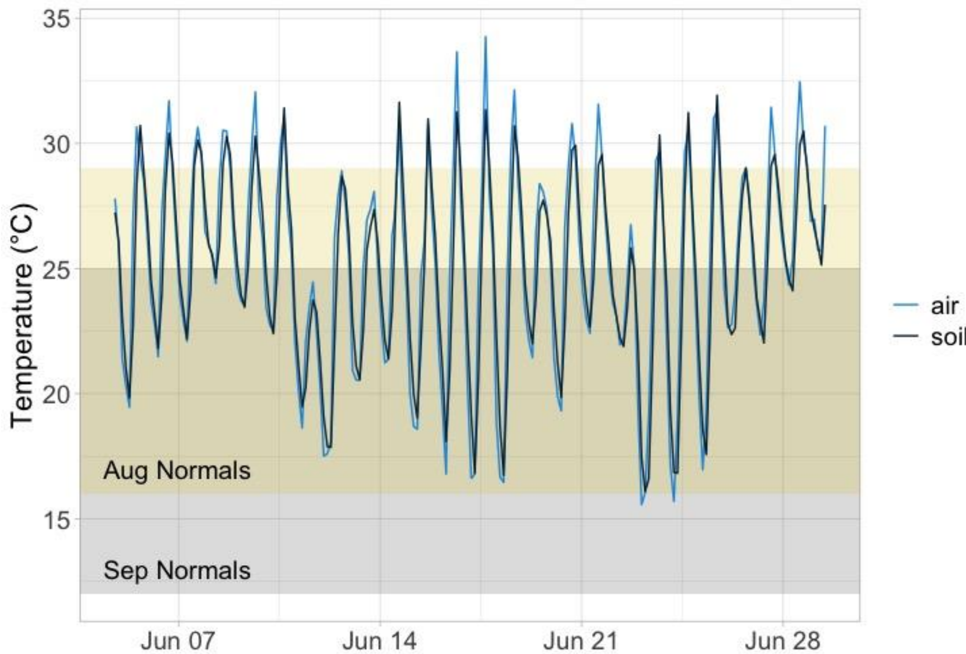

Figure 7. Hourly average ambient and Turface® MVP temperatures throughout the course of the experiment – June 4th to June 29th, 2021. Gray and yellow areas demonstrate ranges between the maximum and minimum temperature normals in Carlisle, PA in August and September, respectively. The values were derived from SC ACIS2 using the options: first Single-Station Products, then Daily/Monthly Normals.

Table 2. Average total biomass (in grams) of the two component monocultures, of the monocultures, and of the mixtures under three water levels. Standard errors of the mean were calculated.

Water Stress	Clover Monoculture	Rye Monoculture	Monoculture	Mixture
Control	0.10 ± 0.01	0.64 ± 0.11	0.37 ± 0.08	0.65 ± 0.18
Drought	0.04 ± 0.01	0.42 ± 0.08	0.23 ± 0.06	0.67 ± 0.12
Waterlogging	0.14 ± 0.02	0.50 ± 0.04	0.32 ± 0.03	0.37 ± 0.05

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