The Effect of Simulated Precipitation Change on Multi-trophic Interactions in a Cereal Crop

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Abstract

Climate models predict a 40% reduction in UK summer precipitation by 2080, together with increases in the frequency of drought and heavy rainfall events. These changes will have serious implications for UK agriculture as crop growth and yield as well as insect herbivores and their natural enemies will be affected by changes in soil moisture at a time when there is pressure to reduce inputs of chemical pesticides. To date, research has focused on the effect of continuous periods of drought on crop production, not considering the effect of changes in the frequency of rainfall events on plant growth and interactions between insect herbivores and their natural enemies in cereal ecosystems. The aim of this thesis was to investigate the impact of changes in the quantity and frequency of precipitation events on multi-trophic interactions in a barley ecosystem.

A 40% reduction in mean precipitation significantly reduced barley growth, yield, and changed plant chemical composition, whereas reductions in the frequency of precipitation events alleviated some of the negative effects of drought on barley plant growth, suggesting that changes in the quantity of rainfall may have larger negative effects on barley yield than changes in extreme rainfall events. Under future changes in precipitation patterns, below-ground insect herbivores, specifically wireworms, were found to have a smaller effect on plant biomass compared to under ambient watering regimes. In contrast, above-ground aphids increased in mass when feeding on plants grown under future precipitation patterns. Wireworm herbivory reduced the number of above-ground aphids on young barley plants but this interaction was removed under future precipitation patterns. These effects were evident in the third trophic level: Harlequin ladybirds increased in mass when feeding on aphids collected from plants subjected to future precipitation patterns.

This thesis provides the first experimental evidence that future reductions in mean precipitation and rainfall events can significantly affect multi-trophic interactions in a cereal ecosystem, with consequences for the abundance of pests and invasive species.

Table of Contents

List of Tables

List of figures

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Author's Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

Chapters 3 and 4 have been written as papers for publication and as such there is some unavoidable repetition with reoccurring methodologies throughout the thesis. Met office data presented in Fig. 5.2 was collected by a MET station position at The James Hutton Institute. Drawing of Fig. 3.1 and Fig. 5.3 was aided by Sarah Horne at The James Hutton Institute.

I would like to dedicate this thesis to my parents,

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for teaching me the greatest lesson of all…

……to never give up……

CHAPTER 1

General Introduction

1.1 Climate change and predicted changes in precipitation

Emissions of carbon dioxide (CO_2) and other greenhouse gases (GHG) through anthropogenic processes have altered, and will continue to alter, the properties of the Earth's atmosphere (Bouwer *et al.* 2014). Compared to pre-industrial levels, concentrations of greenhouse gases in the atmosphere have increased by at least 50% over the last two hundred years and are believed to have caused unprecedented successive global warming - the last three decades were warmer than any preceding decade since 1850 (Folland *et al.* 2001). Most models predict a $2^{o}C$ warming of global surface temperature by the end of the 21st century (Folland *et al.* 2001; Bates *et al.* 2008; Bouwer *et al.* 2014). Atmospheric temperature and radiation balance is intimately linked with the global hydrological cycle, where increases in atmospheric temperature and infrared radiation directed toward the earth's surface (downwelling) increases atmospheric water-holding capacity and rates of evaporation (Bates *et al.* 2008). Therefore rises in global temperature due to climate change are likely to intensify and accelerate global and local hydrological cycles. This will influence global precipitation patterns, snow cover, soil moisture and runoff (Fowler and Hennessy 1995; Mcguffie *et al.* 1999; Folland *et al.* 2001; Allen and Ingram 2002; Trenberth 2005; Huntington 2006; Bates *et al.* 2008; Allan and Soden 2008) and amplify the severity and frequency of rainfall events in the future whilst at the same time increasing the duration of drought periods (Fig. 1.1) (Fowler and Hennessy 1995; Allan and Soden 2008). The consequences of these changes are already apparent; extreme weather and climate events have been observed at increased frequency since at least the 1950s and are predicted to continue in the future (Folland *et al.* 2001; Osborn and Hulme 2002; Huntington 2006).

The majority of current climate models agree that continued global warming will intensify the rate at which certain aspects of the hydrological cycle undergo change, for example leading to greater rates of evaporation and precipitation, but with unequal distribution of this extra precipitation around the globe (Oldfield 2005; Ekstrom *et al.* 2005; Bates *et al.* 2008; Allan and Soden 2008). Areas at high latitude and with high levels of precipitation are anticipated to receive increased

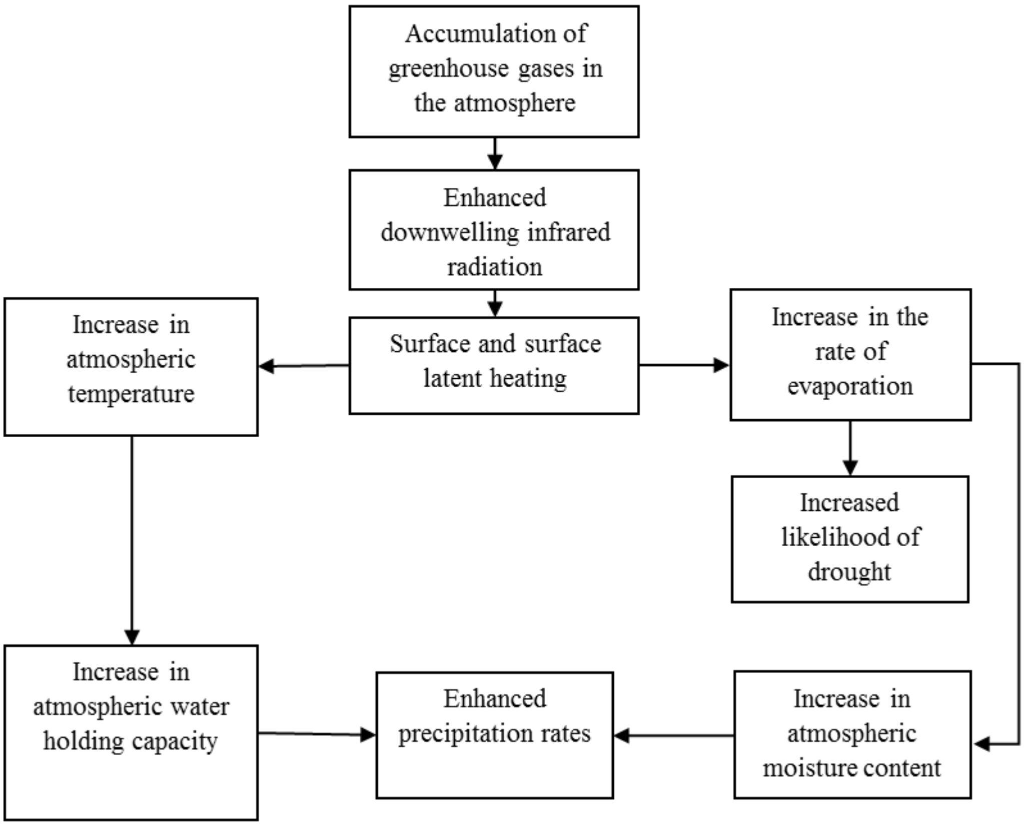

Fig. 1.1. A schematic of the likely ways in which accumulation of greenhouse gases in the atmosphere will affect global precipitation patterns (modified from Oldfield 2005).

precipitation, while dry regions with high evaporation rates are expected to experience decreased precipitation (Bates *et al.* 2008). Such changes in precipitation patterns are likely to be experienced primarily as an increased frequency of extreme events - namely floods and droughts - accompanied by shifts in the timing and severity of wet and dry seasons that are non-uniform both regionally and seasonally (Trenberth 2005; Bates *et al.* 2008; Allan and Soden 2008).

In the UK, major changes in precipitation are predicted in the next 50 years, with increases in the frequency and duration of drought periods as well as extreme rainfall events (Osborn and Hulme 2002; Fowler *et al.* 2005; Blenkinsop and Fowler 2007). The exact nature of these extreme weather events are difficult to model, although it is clear that there will be seasonal changes in UK precipitation with the most significant changes occurring in the south and east (Jones and Reid 2001; Fowler *et al.* 2005; Blenkinsop and Fowler 2007). High GHG emission scenarios predict a 50% reduction in summer precipitation in southeast UK and up to 30% increase in winter precipitation by 2080 (Bates *et al.* 2008). UK Climate Projections 2009 provide climate predictions based on a methodology designed by the Met Office and have reported that, by 2080, the volume of winter UK precipitation will increase by 33%, while summer precipitation will decrease by as much as 40% (Murphy *et al.* 2009). A drought severity index predicts that long- and short- lasting extreme rainfall events in the UK are estimated to increase by 10% and 30% respectively by 2070 (Fowler and Kilsby 2004). Predicted increases in the intensity of UK rainfall together with decreases in mean summer precipitation will result in a reduction in the total number of wet days (Hennessy, Gregory and Mitchell 1997). Therefore, in the future the UK is likely to experience more frequent periods of drought followed by heavy rainfall events.

1.2 Predicted changes in precipitation patterns and food security

The world's human population is predicted to reach nine billion by 2050 therefore future food security is critical in meeting the estimated 70% rise in global food demand expected during the next 50 years (Alexandratos 2009; Godfray *et al.* 2010; Tilman *et al.* 2011; FAO, IFAD and WFP 2014). However, future crop production is under threat from climate change, which is arguably the most significant challenge facing modern agriculture (Godfray *et al.* 2010; Wheeler and von Braun 2013). Crops growing in the UK will be affected by changes in the volume and pattern of precipitation events as well as changes in evapotranspiration as a result of interactive effects on the availability of soil water (Borken and Matzner 2009). Increases in the variability and unpredictability of precipitation in the UK, particularly during the growing season, will have important consequences for UK crop production (Hennessy *et al.* 1997; Fowler and Kilsby 2004; Ekstrom *et al.* 2005; Bates *et al.* 2008). Reductions in the volume of precipitation and increases in the incidence of extreme rainfall events will therefore result in two different stresses which have potentially different effects on soil conditions and plant physiology. As such, these predicted changes in precipitation represent a major challenge for managing water availability to the growing crop.

1.3 The effect of changes in water availability on cereal crops

Drought stress, including intermittent drought events at critical times during crop development, can cause significant yield losses in cereal crops (Buckland *et al.* 1997; Osborn *et al.* 2000; Morecroft *et al.* 2002; Marsh, Cole and Wilby 2010) as plant growth, development, morphology and physiology are all affected by changes in precipitation and soil water availability (Chaves, Maroco and Pereira 2003). Water deficit in plants is defined as “water content of a tissue or cell that is below the highest water content exhibited by the most hydrated state” (Taiz and Zeiger 2006). Under drought conditions, water flow is reduced and turgor pressure decreases, therefore cells are no longer in the most hydrated state. As turgor pressure decreases further cells begin to shrink, cell volume decreases and the concentration of solutes in the cell increases causing significant impacts on cellular processes (Chaves *et al.* 2003) as well as resulting in reductions in leaf expansion and root elongation which are usually the first signs of drought stress in plants (Blum 1996; Chaves *et al.* 2003).

Shortly after the onset of drought, plants begin to wilt, the stomata close and the leaf rolls as a means of reducing surface area and thus transpiration rates (Blum 1996).

While stomatal closure can allow leaf water potential to be stabilised (Mahdid *et al.* 2011), it also deprives leaves of CO_2 thus reducing photosynthetic carbon assimilation (Anjum *et al.* 2011). Reductions in leaf area can further reduce photosynthesis through reduction in light capture and gaseous exchange (Chaves *et al.* 2003). Drought stressed cereal crops can exhibit reductions in biomass, cell elongation, leaf growth, leaf number, specific leaf area, stem biomass, stem branching and yield, as well as delayed development (reviewed by (Blum 1996; Chaves and Oliveira 2004). At the molecular level, gene expression is also significantly altered by drought stress (Chaves *et al.* 2003). Genes controlling the light reaction in photosynthesis and the Calvin cycle, as well as genes controlling glycolysis, were down-regulated in barley under drought stress (Abebe *et al.* 2010).

In contrast, during times of extreme precipitation and flooding, soils become waterlogged as water fills the soil pores and limits gas diffusion; any remaining oxygen gas is consumed rapidly by aerobic processes in roots and soil organisms, leading to anaerobic soil conditions. Anaerobic soils favour microbial reduction of nitrate, nitrogen, manganese, sulphate and ferric oxides, resulting in the release of reduced ions which are toxic to plants (Blom 1996). Under waterlogged conditions, plant respiration and the uptake and transport of nutrient ions is reduced significantly, with negative effects on plant growth and development (Visser 2003; Yordanova and Popova 2007). Plants can become destabilised in flooded soils which increases the incidence of lodging and causes significant yield losses (Neenan and Spencer-Smith 1975; Visser 2003). Waterlogging can also change the physical structure of the soil by breaking down large soil aggregates (Emmett *et al.* 2004). After a flooding event, smaller soil aggregates are rearranged into a more dense structure with smaller soil pores, reducing gas diffusion and increasing resistance to root penetration (Blom 1996; Jin *et al.* 2013).

Research investigating the effect of water stress on plant growth and development is mainly focused on continuous, or extended, periods of with-holding water (Day *et al.* 1978; Blum 1996; Chaves *et al.* 2003; Abebe *et al.* 2010) or complete flooding (Visser 2003). In the natural environment, plants are likely to suffer periods of stress and recovery, with dry days followed by heavy rainfall events. Although this pattern is predicted to become more severe in the future, there is little published research attempting to simulate predicted changes in the frequency as well as the intensity of rainfall events and how this will effect crop growth and development (Fowler *et al.* 2005). Intermittent flooding and drought events may have more detrimental effects on plant growth and development due to the extremes in water stress and the potential additional stress caused by transitions between aerobic and anaerobic soil conditions (Setter and Waters 2003).

The few studies that do investigate predicted changes in frequency of precipitation focus on grassland systems. For example Fry *et al.* (2014) applied a “drought/ downpour treatment” which included long periods of no rainfall interspersed with downpours during the summer months, attempting to simulate future predicted changes in precipitation on a grassland system. They reported that this drought/ downpour treatment resulted in “mass dieback” of perennial plants with very little ability to recover once the treatment ceased to continue. However, ecosystem processes in this grassland system were reported to be fairly resistant to short term changes in extreme rainfall with available soil nitrogen unaffected by the different watering regimes (Fry *et al.* 2013, 2014). Furthermore tallgrass prairie and mixed grass prairie were found to have increased plant water stress under changes in extreme precipitation events. In contrast, semiarid steppe treated with extreme precipitation events had an increase in net primary productivity and reduced plant stress (Heisler-White *et al.* 2009). Grassland systems include vegetation types that are typical of contrasting climatic zones and are therefore more likely to be adapted to changes in precipitation patterns compared to modern cereal crops grown in the UK. Furthermore, high vegetation diversity in grassland is likely to increase the drought tolerance of this system (Tilman and Downing 1994). Tariq *et al.* (2012) measured the effect of different watering regimes on *Brassica* when determining the effects of drought on aphids. The watering regimes used included reductions in the quantity and frequency of watering and reported that these watering regimes affected foliar chemistry and reduce plant biomass.

Re-wetting studies, where plants are subjected to a drought and recovery phase, have shown contrasting results. Temperate grass species were found to have a reduced ability to accumulate plant biomass after re-wetting (Okamoto, Ishii and An 2011). Wheat crops that had been flooded for two, three or six days had fewer tillers, reduced plant height and yield (Sharma and Swarup 1988). Barley subjected to short term periods of flooding (2-7days) at different developmental stages exhibited reduced growth, slowed development and changes in tissue chemical composition (Leyshon and Sheard 1974). However, other studies suggest that plants become more resilient after a water stress event, and are able to cope better with future stress. An alfalfa (Lucerne) variety exhibited faster root growth and greater accumulation of osmolytes during recovery from a long-term drought event (Kang *et al.* 2011), and tissue concentrations of antioxidants in cotton and spurred anoda were higher during recovery after a drought event (Ratnayaka, Molin and Sterling 2012). A new irrigation technique known as partial root zone drying, where part of the root system is dry or drying and the rest of the root system is irrigated, has been used to investigate physiological responses in grain crops. Partial root zone drying in wheat and maize resulted in an increase in plant water use efficiency (WUE), where photosynthetic rate was maintained but transpiration rate was reduced (Du *et al.* 2010). These studies suggest that cereal crops may be able to adapt to changes in the frequency of precipitation events.

The effect of water stress on plant growth, development and yield depends on the length and severity of the water stress and the ability to access water and adapt to the intense periods of heavy rainfall and drought (reviews see Blum 1996; Chaves *et al.* 2003). For example, if dry conditions develop slowly, plants are better able to adapt developmental processes controlling and coordinating cell wall and membrane biosynthesis, cell division and protein synthesis (Chaves *et al.* 2003; Chaves and Oliveira 2004). Soil type and structure can also influence the magnitude of the drought stress on plants where water drains more quickly in sandy soils which hold less water compared to clay soils (Gupta and Larson 1979). However, dry compacted clay soils are much harder to re-wet and consequently the excess water is lost through runoff.

1.4 Plant adaptation to changing water availability

Certain plants are less sensitive to water stress than others and have adaptations which allow them to tolerate drought and waterlogged conditions (Blum 1996). Plants can escape water stress by completing their life cycles during the wet or dry season or before the onset of drought or flooding. Alternatively water stress can be delayed if plants maximise water uptake through mechanisms that maintain tissue water content. This can be achieved by investing in root growth to increase water absorption efficiency (Dhanda, Sethi and Behl 2004; Umezawa *et al.* 2006); reducing leaf area to reduce transpiration water loss under drought (Chaves and Oliveira 2004; Barnabás, Jäger and Fehér 2008); or, in the case of flooding, by reducing oxidative stress by morphological or biochemical features that facilitate the transport of oxygen into plant tissues (Blom 1996; Visser 2003).

Other plants can tolerate water stress through mechanisms involving osmotic adjustment including smaller cells with more rigid cell walls, smaller leaves, utilising sugars as osmoprotectants to minimising oxidative damage and partial plant dormancy (Chaves *et al.* 2003; Barnabás *et al.* 2008). Increased silicon (Si) uptake has been reported to improve the tolerance of plants to water stress by stimulating antioxidant systems, immobilising antioxidants avoiding cellular damage by reactive oxidative damage and providing cellular structural support to avoid lodging (Ma 2004; Gong *et al.* 2005, 2008; Liang *et al.* 2007). Plants that can maintain leaf turgor pressure and cellular water potential during drought show shorter turgor recovery times once re-wetting has occurred (Mahdid *et al.* 2011). Tissue abscisic acid (ABA) concentration is often recorded to be increased in drought stressed plants and it has been shown to improve drought tolerance (Mahdid *et al.* 2011). Although the role of ABA is not fully understood, it is believed to be involved in stomatal closure maintaining the turgor pressure of transpiring leaves, hydraulic conductivity from roots to growing zones and preventing a reduction in the water potential of cells in these growing zones (Mahdid *et al.* 2011). This allows a decrease in the turgor recovery time once re-wetting has occurred. The accumulation of free amino acids and nitrogen-containing osmoprotectants is often recorded in watered stressed plants (both drought and over-watering conditions), and is thought to be a mechanism to offset low osmotic pressure (Huberty and Denno 2004; Khan, Ulrichs and Mewis 2010).

Plant species and genotypes can differ in their tolerance to water stress depending on the selective forces that their ancestors were exposed to, or as a result of selective breeding for specific traits (Ceccarelli 1987; González, Martín and Ayerbe 1999; Mahdid *et al.* 2011; Vassileva *et al.* 2011). There is evidence that selective breeding for agriculturally important traits such as high yield may have led to reduced genetic variation in, or even loss of genes for, traits that confer resistance to abiotic stresses such as drought (Ellis *et al.* 2000; Purugganan and Fuller 2009). Ancestors of many of our modern crop varieties originated from the Fertile Crescent which is famous for its diverse habitats, consisting of ranges of soil types, temperatures, altitude and seasonal weather patterns including drought and flooding events (Purugganan and Fuller 2009; Newton *et al.* 2010). Large heterogeneity of barley landraces allows for stability under adverse climatic conditions and research is being conducted to identify genomic regions that may be valuable for combating drought stress in modern crop varieties (Newton *et al.* 2010; Dawson *et al.* 2015).

1.5 Changing rainfall patterns and insect herbivore threats to crops

Many studies report that drought stress promotes outbreaks of plant-eating insects. The range and distribution of many insects is correlated with water availability (Thacker, Thieme and Dixon 1997; Huberty and Denno 2004; Chown, Sørensen and Terblanche 2011; Gutbrodt, Mody and Dorn 2011; Tariq *et al.* 2012) which will have important consequences for crop production under future changes in precipitation. High temperatures usually associated with drought conditions benefit insects that have limited ability to thermoregulate (Mattson and Haak 1987). Insect growth, development and fecundity are increased under warmer environments (Bale *et al.* 2002). Furthermore, drought stressed plants may appear more attractive to insects with a larger number of yellowing leaves and the release of stress related cues (Chown *et al.* 2011).

Insect herbivores feeding on water stressed plants will also be affected by changes in precipitation through changes in the quality of their food source (Johnson, Hawes and Karley 2009). There are a number of hypotheses underpinning predictions on how insect herbivores respond to feeding on drought stressed plants. The Plant Stress Hypothesis suggests that drought stressed plants are better quality for insect herbivores (White 1969, 1984) as plant defence is often compromised under drought conditions (White 1984; Abebe *et al.* 2010). Any environmental stress that reduces plant growth has the potential to shift the allocation of resources to or away from defence strategies. As drought severity increases carbon becomes limited and secondary metabolite content declines. Drought conditions resulting in decreases in tissue concentrations of secondary metabolites, such as glucosinolates and isovitexin glucopyranoside in *Alliaria petiolata* (Gutbrodt *et al.* 2011). Increased damage to leaf discs collected from drought stressed *Alliaria petiolata* by *Lepidoptera* (specialist *Pieris brassicae* and generalist *Spodoptera littoralis)* correlated with decreased tissue concentrations of defensive metabolites (Gutbrodt *et al.* 2011). Other defence strategies, such as Si deposition as a physical defence to deter herbivores common in grasses, could be affected by reductions in transpiration in drought stressed plants as Si is mainly taken up passively through aquaporin-type transporters in the root (McNaughton and Tarrants 1983; Hartley *et al.* 2015). Si deposition as abrasive structures such as opaline phytoliths increases the abrasiveness of leaves, wearing down insect mandibles and reducing the insect's ability to absorb nutrients, an important anti-herbivore defence in grasses (Massey and Hartley 2006; Massey, Ennos and Hartley 2007). To date there is very little research investigating the effect of drought on Si concentration in plant leaf material and how this might influence the performance of insect herbivores, despite its important role in many crop species defence.

Increases in tissue concentrations of free amino acids common in drought stressed plants are also particularly beneficial to insect herbivores with nitrogen often limiting insect growth (Mattson 1980). Tariq *et al.* (2012) found an increase in aphid performance on continuously stressed plants with high nitrogen concentration. Aphids were also more successful on drought stressed plants which had higher leaf sugar content, although not on waterlogged plants which also exhibited increased leaf sugar content (Khan *et al.* 2010). This suggests that water stress can also influence other plant factors contributing to aphid success.

Other studies report that herbivorous insects perform better on healthy, rapidly growing plants. In response to this theory, Price (1991) developed the Plant Vigour Hypothesis. Rapidly growing plants move larger quantities of plant nutrients through the phloem which provides higher concentrations of nutrients to sap feeding insects (Inbar, Doostdar and Mayer 2001), and fast-growing plants provide a larger resource for feeding insects that minimises competition with other organisms attacking the plant (Huberty and Denno 2004). Choosing a healthy plant may therefore be an escape strategy for competitors (Gutbrodt *et al.* 2011).

Realistically, crop plants in the UK are most likely to experience pulsed water stress, caused by times of drought followed by heavy rainfall, resulting in periods of stress and recovery for the plant and associated organisms both above- and below-ground. This is likely to have different effects on insect herbivore growth, abundance and survival than continuous water stress. It has been suggested that plants experiencing intermediate levels of water stress may provide optimal food quality for insect herbivores (Mattson and Haak 1987; Huberty and Denno 2004). This would occur through increased nutritional quality (e.g. free amino acid concentrations) without resulting in the negative effects to plant growth and composition caused by severe and prolonged drought. However, Tariq *et al.* (2012) reported that fecundity of *Myzus persicae* feeding on *Brassica* species was reduced on pulsed stressed plants compared to those feeding on unstressed and moderately drought stressed plants.

There are numerous studies reporting both negative and positive effects of drought on the performance of insect herbivores. These contradictions in findings may be due to differences in watering regimes and the severity and/or timing of the drought stress. Interactions between drought and other environmental stresses such as shading and soil nutrient availability may also influence the experimental outcomes, as well as the methods used to impose and measure drought stress (Huberty and Denno 2004). The level of stress caused by changes in water availability and the plant's response to water stress differs between different species and varieties, influencing the quality of the food resource to herbivorous insects. Screening germplasm for aphid resistance has led to the discovery of resistant accessions with different resistant phenotypes (Dogimont *et al.* 2010). There is also evidence to suggest that selective breeding for modern cultivars, reducing genetic variation, has led to the loss of genes that confer resistance to biotic stresses (Ellis *et al.* 2000; Purugganan and Fuller 2009). A barley landrace collected from Afghanistan was found to exhibit strong resistance to Russian wheat aphid (*Diuraphisnxia* (Mordvilko)) and has been used to develop a resistant spring two-rowed barley variety ‘RWA 1758’ (Bregitzer *et al.* 2008). It could be that ancestral varieties of modern crops have a greater range of plant defences and may allocate resources to plant defences differently under water stress. Barley traditional landraces may provide genetic resources to improve the resistance of modern crops to abiotic and biotic stresses and therefore allow greater suitability for sustainable agriculture management practices (Newton *et al.* 2010; Dawson *et al.* 2015).

Different insect herbivores feeding guilds will also be differentially affected by drought (Huberty and Denno 2004) depending on the type of plant tissue they attack and their nutritional requirements. Phloem feeders are thought to be affected by changes in plant water status to a greater extent than chewing insects due to the significant effects of water stress on phloem sap viscosity, nutrient and allelochemical composition (Larsson 1989; Huberty and Denno 2004; Mody, Eichenberger and Dorn 2009). It is therefore very difficult to compare the different studies investigating the impact of water stress on insect performance.

1.6 The effect of water stress on above- and below- ground interactions

Organisms feeding on the same host plant can influence one another through changes mediated by the host plant. Insect herbivory can influence host plant growth, development and chemical composition, and, therefore, can alter plant quality for other organisms feeding on the same plant (Bezemer and van Dam 2005). Herbivores feeding on the same plant above- and below- ground can influence one another through competition (detrimental effects on each other), amensalism (only one is detrimentally affected), commensalism (only one is positively affected), contramenalism (one is positively affected and the other negatively affected) and mutualism (both are positively influenced by one another) (Arthur and Mitchell 1989). Bezemer *et al.* (2005) reported that a foliar feeding herbivore (*Spodoptera exigua*) was negatively affected by a root herbivore (*Agriotes lineatus*). This was suggested to be due to changes in the distribution of plant defence above-ground as well as a reduction in the ability of the plant to uptake water and nutrients from the soil due to root damage (Masters, Brown and Gange 1993; Bezemer and van Dam 2005). However, Johnson, Hawes and Karley (2009) found a positive bi-directional effect where wireworms (*Agriotes*) had a positive effect on the bird cherry oat aphid (*Ropalosiphum padi*), and *vice versa,* whilst feeding on barley. Many of the studies reporting above- and below- ground insect interactions report contrasting results supporting two separate hypotheses, the Stress Response Hypothesis (Masters *et al.* 1993; Bezemer and van Dam 2005) and the Defence Induction Hypothesis (Bezemer and van Dam 2005). The Stress Response Hypothesis suggests that the removal of roots by root herbivory impairs the plant's capacity for water and nutrient uptake leading to a drought response in the plant with accumulation of nitrogen compounds (White 1984) benefitting aboveground herbivores. In contrast, the Defence Induction Hypothesis, suggests that belowground herbivores induce a systemic plant defence response and the release of defence chemicals negatively affecting herbivore colonization aboveground (Bezemer and van Dam 2005). Depending on the feeding guild, different phytohormonal pathways will be activated, for example as root chewers can induce jasmonic acid defence response simultaneously reducing salicylic acid defence, impacting aphids (Masters *et al.* 1993; Bezemer and van Dam 2005; Mewis *et al.* 2012; Nabity, Zavala and DeLucia 2013).

Changes in precipitation patterns have the potential to influence the interaction between insect herbivores feeding above- and below- ground (Fig. 1.2) through changes in plant growth and chemical composition. However there are very few studies investigating the effect of drought stress on above- and below- ground insect interactions. One study reported that root chewing *Agriotes* sp. larvae reduced the abundance and performance of leaf-mining *Stephensia brunnichella* larvae but that this effect was negated by drought conditions (Staley *et al.* 2007a).

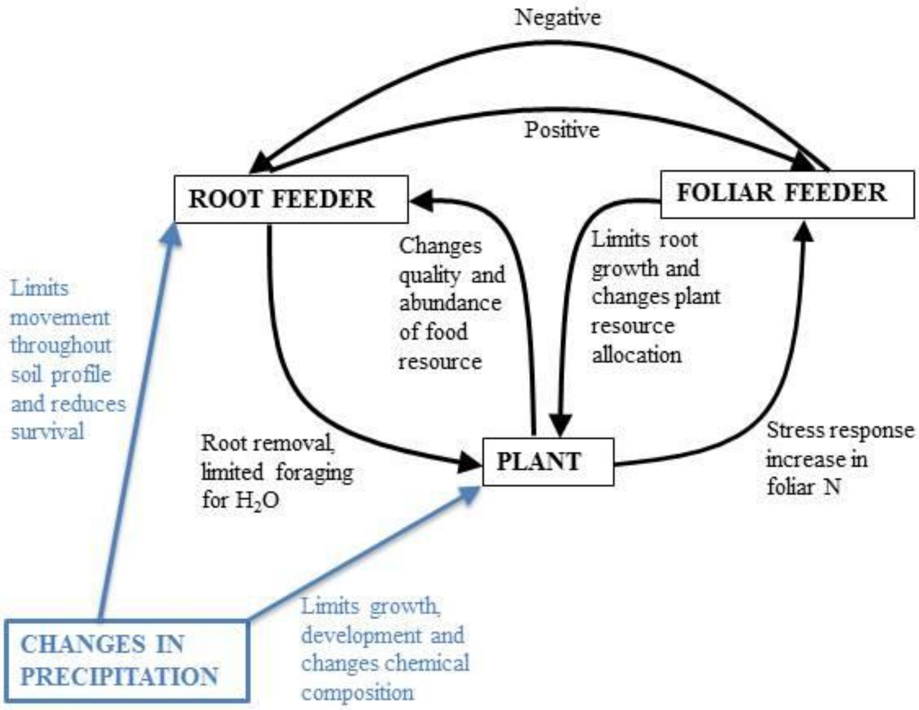

Fig. 1.2. A simple schematic demonstrating how above- and below- ground insect herbivores feeding on the same host plant have the potential to influence one another through plant mediated responses and how changes in precipitation can affect interactions between these herbivores (Image modified from Masters *et al.* 1993)

1.7 Effects of water stress on the third trophic level

Changes in rainfall have the potential to alter the outcome of plant-mediated interactions between insect herbivores as well as extend to the herbivore-natural enemy interaction. Changes in precipitation are predicted to destabilise and cause asynchrony between species interacting in an ecosystem by influencing development times and phenology (Watt and Woiwod 1999; Watt and McFarlane 2002; Emmerson *et al.* 2004; Hicks *et al.* 2007; De Lucia *et al.* 2012; McCluney *et al.* 2012) and allowing opportunities for invasive species to prosper (Olesen *et al.* 2011). Predators will be affected by changes in precipitation directly influencing insect predator development, as well as indirectly through changes in the quantity and quality of their food resource (Giles *et al.* 2002). It has been reported that higher trophic levels may be more sensitive to changes in climate (Voigt *et al.* 2003). There are very few studies investigating the effect of water stress on trophic interactions. Johnson *et al.* (2011) reported that aphids were negatively affected by drought and thatthe drought stress had a more severe effect on parasitoid wasps than their prey, aphids. It is also interesting to note that although not a root herbivore, the presence of earthworms in this study increased the negative effects of drought stress on the aphids by possibly reducing the suitability of the plant to the aphids. Drought conditions were also reported to remove the interaction between *Agriotes* species larvae, leaf-mining (*Stephensia brunnichella)* larvae and its associated parasitoid (Staley *et al.* 2007b). An outbreak of herbivorous moth larvae (*Hepialus californicus*) was suppressed under elevated rainfall due to an entomopathogenic nematode (*Heterorhabditis marelatus*) reducing the effects of the moth on the bush lupine (*Lupinus arboreus*) (Preisser and Strong 2004). The rate of parasitism attacks by the parasitoid wasp (*Aphidus ervi*) were reported to be lower on drought stressed plants compared to ambient plants, thought to be due to changes in aphid population demography, with a greater number of adult aphids recorded on drought stressed plants (Aslam, Johnson and Karley 2013). Furthermore levels of parasitism of caterpillar by parasitoid wasps (Hymenoptera) and tachinid flies (Tachinidae) decreased as climate variability increased (Stireman *et al.* 2005). As a result of potential increases in crop pests under future climate change and drives to increase the sustainability of farming (e.g. reducing the use of pesticides), future crop production will become more reliant on natural predators of crop pests to maintain insect herbivore populations (Henle *et al.* 2008; Gregory *et al.* 2009). Therefore any changes in trophic interactions due to the fluctuation of water availability will become particularly important for future crop production.

1.8 Aims of the thesis

Most current research on the effects of changes in precipitation on plant-herbivore-natural enemy interactions focuses on extended periods of drought. In a natural situation, however, crops grown in the UK are more likely to suffer from a pulsed water stress, with periods of drought followed by heavy rainfall, and this scenario is predicted to increase in severity in the future. This will result in periods of stress and recovery for plants, which is likely to affect plant suitability for, and performance of, organisms feeding on these plants both above- and below- ground. Thus, intermittent drought stress could have different effects on the growth, fecundity, survival and abundance of insect herbivores and their natural enemies compared to continuous drought stress.

The overall aim of this research is to investigate the effect of predicted changes in precipitation (in both water quantity and watering frequency) on a crop ecosystem, focussing on the interactions between the plant and representative functional groups of insect herbivores and their natural enemies.

Each chapter addresses a specific aspect of the general aim:

- Chapter 2 establishes methods to simulate predicted changes in the quantity and frequency of rainfall events and investigates the effect of these different watering regimes on the growth, development and chemical composition of two barley cultivars.

- Watering regimes are then finalised and used in Chapter 3 which investigates the effects of reductions in water quantity and watering frequency on the growth, development and chemical composition of a modern and traditional barley varieties.

- After gaining an understanding of how different barley varieties responded to the watering regimes, Chapter 4 investigates the effects of reductions in water quantity and watering frequency on the interactions between below-, above-ground insect herbivores and their natural enemies in controlled environment rooms. Plant growth and chemical composition, mass increase of an above- and below- ground insect herbivores, as well as mass increase and feeding preference of a predator were measured to gain an understanding of how these different organisms may respond to future changes in precipitation.

- The effects of reductions in water quantity and watering frequency on the interactions between above- and below- ground insect herbivores and their natural enemies is investigated in a field situation in Chapter 5. Measuring plant biomass, mass increase of below-ground as well as the abundance and mass of above-ground insect herbivores and their natural enemies provides information on how these different organisms may respond to future changes in precipitation in a field situation.

The research focuses on investigating two main hypotheses:

- The effects of reduced water availability on plant chemical composition, growth, physiology and development which will depend on plant variety and whether reductions in precipitation are continuous or interspersed with more extreme rainfall events.

- The effects of reduced water availability on plant-herbivore-natural enemy interactions will differ depending on whether reductions in precipitation are continuous or interspersed with more extreme rainfall events.

1.9 The study system

Organisms were chosen to represent a naturally occurring ecosystem which has important consequences for crop production in the UK with representative above- and below- ground insect herbivore and natural enemy interaction (Fig. 1.3). Barley (*Hordeum vulgare* L.) was chosen as the focus study organism as it is one of the main crops grown country wide in the UK, it is an important component of feed grain and it is used for malting and brewing (Ullrich 2011). In 2013 the UK produced c. 7 million tonnes of barley which was valued at £1.1 billion (DEFRA 2013a; b). Barley cultivars have been bred for strong, short straw, resistance to diseases and pathogens, as well as low grain nitrogen content for malting purposes (HGCA 2014). Barley growth, development and yield have been found to be negatively affected by drought and flooding (Day *et al.* 1978; Lawlor *et al.* 1981; Yordanova, Uzunova and Popova 2005; Abebe *et al.* 2010; de San Celedonio, Abeledo and Miralles 2014). Modern UK cultivars of barley have not been bred for their drought tolerance, despite the ability to cope with changes in precipitation becoming increasingly important with future predicted changes in rainfall and simultaneous demand for increases in yield. Compared to other cereal crops, barley is relatively drought tolerant and generally uses a water conservation strategy to cope with drought stress (Acevedo, Craufurd and Austin 1991). However, there is a significant lack of research investigating how UK barley cultivars will be affected by the predicted changes in rainfall particularly changes in the frequency of precipitation events.

Insect herbivores can significantly reduce barley crop production, reducing yields by as much as 30% (Oerke and Dehne 2004). The conversion of natural grassland to arable land, reductions in organochlorine insecticides, changes in agricultural practises and the increase of winter cropping has increased the recorded incidence of wireworm (*Agriotes* species) damage to crops (Jary 1942; Parker and Howard 2001). Wireworms, the larvae of click beetles, reside in the soil and bore into roots and stem of plants. They are one of the most destructive soil insect pests, with root feeding being particularly damaging by causing plant wilting and stunting (Jary 1942; Salt and Hollick 1944).

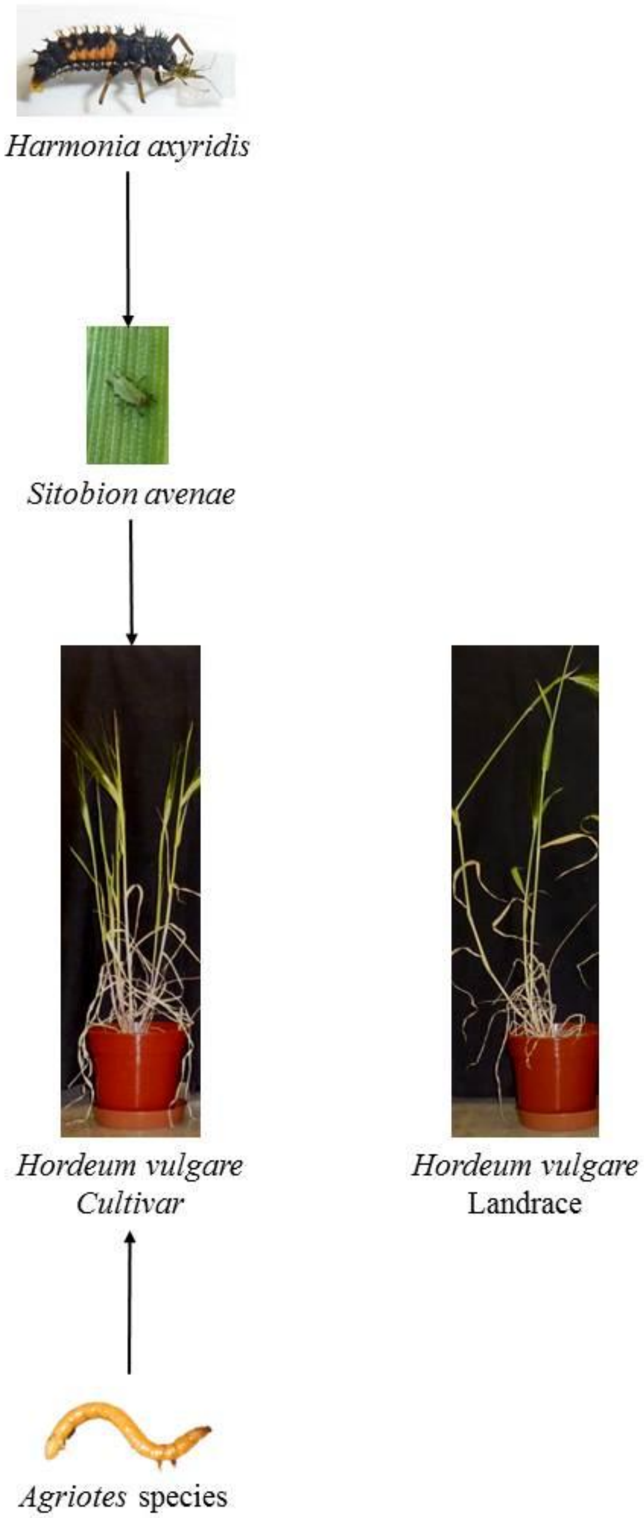

Fig. 1.3. Diagram of chosen study system (photographs taken by R Wade).

The control of wireworms in the soil can be very difficult without the use of harmful chemicals and farmers only become aware of wireworm infestation once the crop has been damaged (Parker and Howard 2001; Johnson *et al.* 2008a; Herk and Vernon 2013). Cereal aphids such as Bird-Cherry/Oat Aphid, *Rhopalosiphum padi* (L.) and English Grain Aphid, *Sitobion avenae* (Fab.) are phloem feeding insect which can cause direct damage to barley plants through the removal of nutrients and injection of salivary secretions but can also transmit disease (Giordanengo *et al.* 2010). Aphids are particularly difficult to control as they are very small in size and can reproduce very quickly asexually via parthenogenesis. Reductions in the quality of the host plant, aphid crowding, temperature, photoperiod and seasonality can result in aphids that produce wings to increase dispersal ability (Powell, Tosh and Hardie 2006). Barley plants can be simultaneously attacked below-ground by wireworms and above-ground by aphids, a scenario which is increasing, likely due to reductions in chemical insecticides and intensive farming practises (Parker and Howard 2001).

In the UK aphids are predated on by a number of different insect predators including ladybirds (coccinellid) and parasitoid wasps (*Aphidius colemani* and *A. ervi*) both of which are used as biocontrol agents (Brodeur and Rosenheim 2000). Harlequin ladybirds (*Harmonia axyridis*) are now one of the dominate species of coccinellid found in all habitats monitored in Europe (Roy and Brown 2015). It is an invasive species in the UK believed to be native to eastern Asia, and is a particularly voracious predator during its larval stage (Roy *et al.* 2008), having negative impacts on native ladybirds (Roy and Brown 2015). The effect of changes in precipitation on barley plant growth, development and yield as well as the effects on insect herbivores and their natural enemies will become more important in future barley production with drives in the UK to reduce agrochemical inputs and increase the sustainability of farming (McCracken and Stoate 2011).

CHAPTER 2

Simulating Changes in Precipitation: Infrequent Application of Water Mitigates the Negative Impacts of Drought on Barley Growth and Development.

2.1 Abstract

Climate change is predicted to alter UK rainfall patterns with changes in both the quantity of precipitation and the frequency of extreme rainfall events potentially affecting crops grown in the UK. Previous research focuses on the effects of imposing continuous severe reductions in soil moisture or extreme periods of water withholding on plant growth and yield. The aim here was to develop methods to investigate the effect of predicted changes in both the quantity and frequency of rainfall events on the growth and development of two barley (Hordeum vulgare L.) genotypes.

Five different watering regimes were established including 40% and 60% reduction in water quantity; the 40% reduction watering regime was delivered in different frequencies, three times per week, twice per week and once per week. Two spring barley genotypes, Optic and Quench, were grown in pots under greenhouse conditions and subjected to the different watering regimes. Plant growth, development and tissue elemental composition were measured to determine the physiological response of the barley genotypes to the different watering regimes.

Reducing water quantity significantly reduced barley plant growth and affected plant chemical composition, whereas reducing the watering frequency alleviated some of the negative effects of a 40% reduction in water quantity. Barley genotypes did not differ in the majority of growth parameters measured in this study and the growth and development of the barley genotypes was similarly affected by the different watering regimes.

The negative effects of future predicted reductions in the quantity of rainfall on barley growth could be partially mitigated by decreased frequency of rainfall events.

2.2 Introduction

Global climate models predict that with future anticipated rises in atmospheric temperatures, precipitation patterns are likely to be more variable and unpredictable with changes in rainfall quantity and frequency as well as changes in the rate of evapotranspiration (Fowler *et al.* 2005; Bates *et al.* 2008; Allan and Soden 2008). UK Climate Projections 2009 predicts that by 2080, the volume of UK precipitation will increase in winter by 33%, while summer precipitation will decrease by as much as 40% (Murphy *et al.* 2009). Long- and short- lasting extreme rainfall events are also estimated to increase in frequency by 10% and 30% respectively by 2070 (Fowler and Kilsby 2004). An increase in the frequency of flooding events together with a decrease in mean summer precipitation will result in a reduction in the total number of wet days (Hennessy *et al.* 1997). Therefore, as well as a decrease in rainfall quantity during the summer, plants grown in the UK are likely to suffer from drought and flooding events throughout the growing season under climate change. Previous research investigating the effect of changes in precipitation on crop production has focused on extremes of water stress and rewetting following extended periods of drought, with plant responses to changes in rainfall quantity reasonably well understood. However, despite the potential economic impact on cereal production, there are very few studies investigating the effect of changes in the frequency of precipitation events on crop production in the UK.

Research investigating the effect of drought on plant growth and development include treatments such as 50% and 60% reductions in water quantity or terminal drought, where plants are not watered at all for a specific duration of the experiment (Morgan and Riggs 1981; Jamieson *et al.* 1995; Chaves *et al.* 2003; Chaves and Oliveira 2004; Barnabás *et al.* 2008; Farooq *et al.* 2009; Legg *et al.* 2009). Other studies investigate the effect of severe drought events at different plant developmental stages (Savin and Nicolas 1996; Barnabás *et al.* 2008; Abebe *et al.* 2010). These studies use watering regimes that manipulate soil water content (determined gravimetrically or using theta probes) or which add fixed quantities of water based on MET station recorded rainfall data. In practical terms, adding a fixed quantity of water based on recorded rainfall data is easier to apply resulting in more accurate treatments, but it does not consider the conditions of the growth environment such as temperature, humidity and rate of evaporation which can significantly influence soil moisture. Alternatively determining soil water content gravimetrically can be inaccurate whilst plants are growing in the soil medium due to differences in plant growth and development as a result of the different watering regimes. Theta probes can be used to measure soil moisture but are expensive, invasive (can damage roots when pushing the probe into the soil) and can be inaccurate in sandy, dry soils, however, they provide a method of measuring soil moisture content throughout the duration of the experiment irrespective of plant development. Polyethylene glycol (PEG) has also been used to investigate controlled changes in osmotic pressures in hydroponic experiments particularly focussing on biochemistry and biomembranes (Premachandra and Shimada 1988; Blum 1996; Chaves *et al.* 2003), but these experiments do not use natural substrates which differ in particle size and aggregation, greatly influencing the water available to the plant.

Published studies report the impacts of reductions in precipitation on plant development, morphology, physiology and crop yield (summarised in Bates *et al.* 2008). Plant responses to drought can be split into two categories, 1. postpone water stress (minimizing water loss, maximising water uptake by mechanisms which maintain tissue water content such as increase root growth and regulation of transpiration by stomatal closure), and 2. tolerance of water stress (sustaining growth during water stress by maintaining cellular structure and photosynthesis with changes in cell wall properties and utilising sugars as osmoprotectants to minimising oxidative damage) (Barnabás *et al.* 2008). The effect of water stress on plant growth and development is influenced by species, genotype, plant development stage, and the length and severity of the drought stress (Blum 1996; Chaves *et al.* 2003), where different crop species and genotypes have been reported to exhibit differences in their tolerance to water stress (Chaves *et al.* 2003; Chaves and Oliveira 2004; Fry *et al.* 2013).

Other studies have investigated the effect of re-wetting on plant growth recovery reporting contrasting results where temperate grasses have been found to have a reduced ability to accumulate plant biomass after re-wetting (Okamoto *et al.* 2011) whereas other studies suggest that plants become more resilient after a drought stress events, able to cope better with future stress. Alfalfa plants had subsequently larger root systems and changes in the regulation of transcripts of amino acids after re-wetting (Kang *et al.* 2011) and the concentration of antioxidants were found to be higher during recovery after a drought event (Ratnayaka, Molin and Sterling 2003). Partial root drying experiments which dry part of the root system and irrigate the remaining root system simulate moisture resource patching in natural soils. These experiments resulted in maize and wheat plants with increased water use efficiency where photosynthetic rates were maintained with simultaneous reductions in transpiration (Du *et al.* 2010).

Flooding experiments have used methods such as completely submerging the root system for the duration of the experiment or at specific developmental stage (Sharma and Swarup 1988; Yordanova *et al.* 2005; Mommer *et al.* 2006; Yordanova and Popova 2007). Flooding has been reported to be particularly damaging to plant growth, development and yield, increasing lodging and disease susceptibility (Blom 1996; Visser 2003; Yordanova and Popova 2007).

Few studies have investigated the effect of changes in the frequency of rainfall events concurrent with changes in the quantity of rainfall on plant growth and development and those that have report contrasting results. Compared to continuous water stress, extreme rainfall events may be more damaging to plant productivity due to the fluctuation between the different extremes of water stress (Schmidhuber and Tubiello 2007) and the transitions between aerobic and anaerobic environments causing anoxic and aerobic shocks adding an additional stress (Setter and Waters 2003). This is likely to be more relevant to future scenarios which predict increases in the number of dry days followed by heavy rainfall events. Tariq *et al.* (2012) manipulated the watering regimes of *Brassica oleracea* L. to investigate the impact on aphid herbivory and reported a reduction in plant biomass and changes in foliar chemistry when reducing the frequency of watering events. A grassland system subjected to drought interspersed with downpour treatments was recorded to have reduced plant biomass and species richness (Fry *et al.* 2013, 2014). To date there is no published research investigating the effect of future changes in the frequency of precipitation on UK cereal crop production. Therefore the aim here was to develop a strategy for manipulating both the water quantity and watering frequency to simulate realistic future predicted changes in precipitation on a UK grown cereal crop.

Barley (*Hordeum vulgare* L. cv. Optic and Quench) was chosen as a model because it is one of the main crops grown country wide in the UK with 7.1 million tonnes produced in 2013 valued at £1.1 billion (DEFRA 2013b). Barley is mainly used for feed grain as well as malting and brewing (Ullrich 2011). Barley growth and development have been previously reported to be negatively affected by changes in water availability (Lawlor *et al.* 1981; Forster *et al.* 2004) and different genotypes of barley have been reported to differ in their response to abiotic and biotic stresses (Forster *et al.* 2004; HGCA 2014). Barley cultivars Optic and Quench have different parental lines (Syngenta Seeds Limited 2009) but both genotypes have been artificially selected for agriculturally important traits such as disease resistance, resistance to lodging and high yield (HGCA 2014) and therefore are likely to respond similarly to abiotic and biotic stresses. Optic was developed in 1990s from Chad x (Corniche x Force) (Syngenta Seeds Limited 2009). In 2010 Optic accounted for approximately 60% of the barley market (SASA 2010). Quench is one of the highest yielding spring malting barley variety across Europe with optimal yields gained in the East and West regions of the UK but with a smaller yield gained in North East UK (HGCA 2014; Syngenta 2013).

2.2.1 Aims

- Establish irrigation regimes which are appropriate in measuring the effects of predicted reductions in water quantity and watering frequency on barley growth and development in terms of non-lethal effects.

- Identify specific watering regimes that could be applied in subsequent experimental chapters conducted in growth chambers, controlled environment glasshouses and field situations.

- Identify how two different modern cultivars of barley respond to the different watering regimes and which is the most suitable to use in further experiments.

2.2.2 Hypotheses

- The developed watering regimes would be effective at testing the impact of reductions in both water quantity and watering frequency predicted by UKCP09 on barley plant growth, development and chemical composition.

- Reducing the water quantity and watering frequency would have a negative effect on barley plant growth and development with changes in the watering frequency having the largest effects.

- The two barley genotypes would not differ in their growth, development and would not respond differently to the watering regimes.

2.3 Materials and Methods

Spring barley cv. Optic and Quench (provided by Syngenta) were grown individually in 2.4L (18.5cm diameter) pots filled with 3kg topsoil (sourced from A1 Plant, Elvington) and mixed with washed sharp horticultural sand (Keith Singletans, UK) in a 3:1 ratio to give a sandy loam soil substrate. The saturation and desiccation (dried at 105°C for 7d) mass of the soil was measured and from this the total water holding capacity of the soil was calculated (0.293ml g^{-1} soil/ sand mix). Pots were initially watered to ensure soil moisture content was *c*. 50% of the total soil water holding capacity and air-filled porosity was $0.15cm^3$ cm^{-3}, with a substrate particle density of c. 2.65g cm^{-3}.

Pots were placed in a greenhouse with pre-set conditions of 16h daylight (c. 300µm m^{-2} s^{-1}) and day/ night temperatures of 20°C/15°C. Due to extremes in temperature outside the greenhouse and restricted ability to cool the temperature within the greenhouse, the air temperature was not controlled during the day and ranged from 20°C to 40°C throughout the growing period (Fig. 2.1).

Initially three seeds of either Optic or Quench were placed in the centre of the pot c. 2cm from the soil surface and watered from the top of the pot three times per week with 20ml deionised water for the first 14d. Pots were placed on saucers to capture any water loss and water remained in the saucer to allow the water to be taken up from the bottom of the pot over the growing period. Following germination, seedlings were removed to a single plant per pot of consistent height and development stage. The experiment comprised a randomised block design. Within each block, the position of each barley genotype (Optic or Quench) and water regime was assigned at random. Blocks were staggered temporally by two to three weeks to allow for complex harvest.

Watering regimes included reductions in water quantity; control and two drought treatments watered three times per week:

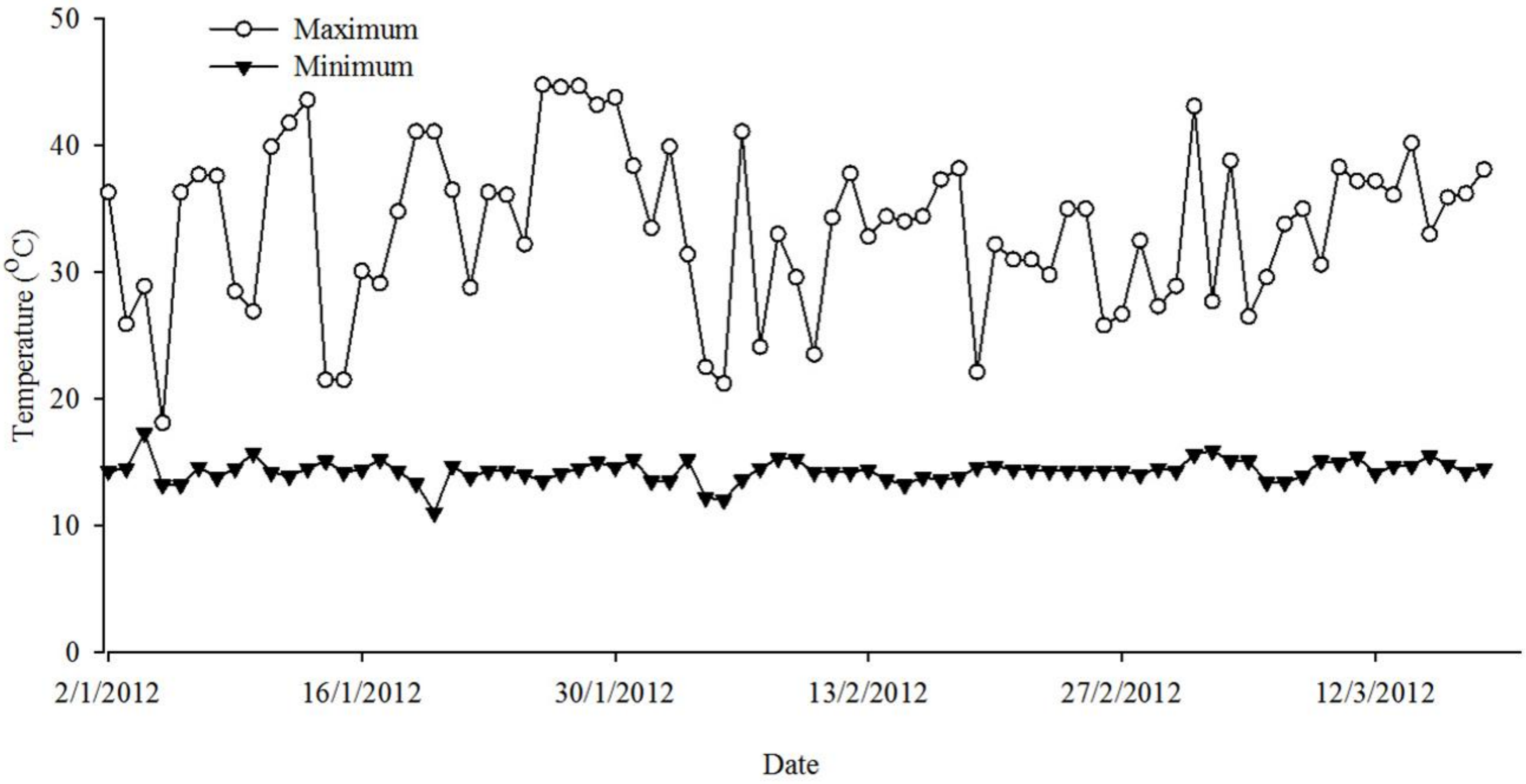

Fig. 2.1. Maximum and minimum air temperatures (^oC) in the greenhouse throughout the experimental period.

- Control plants were watered to reach c. 50% of the total soil water holding capacity (measured gravimetrically by weighing the pot) watered three times per week;

- Drought (D), 40% reduction in the volume of water added compared to control, watered three times per week;

- Severe Drought (SD), 60% reduction in the volume of water added compared to control, watered three times per week.

Two watering regimes were devised that reduced the watering frequency as well as reducing the water quantity by 40% compared to control plants:

- Drought with Reduced watering Frequency (DRF), watered twice per week;

- Drought with Infrequent Watering (DIF), watered once per week.

These different watering regimes allowed for comparison between reductions in water quantity (40% reduction as predicted by climate models and a more severe scenario 60% reduction compared to control) and reductions in watering frequency (c. 67% and 33% reduction the frequency of watering events compared to control) based on climate change predictions. The volume of water added at each watering event was greatly influenced by the temperature of the greenhouse and varied each week.

2.3.1 Plant growth and development measurements

Throughout the growing period, main stem height (cm) and the number of tillers were measured once per week. Nine weeks after sowing, number of leaves and av. tiller diameter (mm, 2 cm from the soil surface) were recorded and plants were harvested by cutting at the soil surface. Plants were separated into three fractions (roots, stem and leaves) and fresh mass was recorded. Harvested plant material was dried at 70°C for two weeks, then weighed to record biomass (g dry mass (DM)) and calculate water content (g plant^{-1}). Root: weight ratio was calculated using total root biomass and total plant biomass.

2.3.2 Si and C/N analysis

Elemental analysis was conducted on dried green leaf material that had been milled to a fine powder.

For silicon (Si) analysis, milled plant material was pressed at 11tons into 5mm thick cylindrical pellets with a manual hydraulic press using a 13mm die (Specac, Orpington, UK). Si concentration (%) was performed using a commercial P-XRF instrument (Niton XL3t900 GOLDD analyser: Thermo Scientific Winchester, UK) held in a test stand (SmartStand, Thermo Scientific, Winchester, UK) (Reidinger, Ramsey and Hartley 2012). Si analysis was performed on Control, D and DIF treated plants only.

The carbon (C) and nitrogen (N) concentrations of leaf (% dry mass) material was determined by flash combustion and chromatographic separation of ~ 1.5 mg milled leaf material using an elemental analyser (Elemental combustion system 4010 CHNS-O Analyser, Costech Analytical Technologies, Inc., Milan, Italy), calibrated against a standard ($C_{26}H_{26}N_2O_2S$). Percentage C and N of the leaves was used to calculate the C/N ratio.

2.3.3 Statistical analysis

Analyses were performed in R (version 3.0.2). Linear mixed effect models (*nlme* package '*nlme*') (Pinheiro *et al.* 2014) were used to test the main and interactive effects of barley genotype and watering regime (separately for changes in water quantity and watering frequency) on the measured variables. Block was included as a random term in the models. Proportion data were arcsine square root transformed and non-normal data was log (reduction in water quantity total plant biomass, stem diameter, stem height; reduction in watering frequency root biomass, stem height and C/N ratio; leaf Si concentration), squared (reduction in watering frequency stem biomass) or square root transformed (reduction in watering frequency total plant biomass) to meet the assumptions of the linear model and analysed using *nlme* from package '*nlme*' (Pinheiro *et al.* 2014). Count data were analysed using generalised linear mixed effect models (*glmer* package '*lme4'*) (Bates *et al.* 2014). Generalised linear mixed effect model chi-square and p-values were calculated using *Anova* function from package '*car*' (Fox *et al.* 2014). For repeated measures, week was included as a fixed term in the model and individual plant as a random term.

Models were simplified by stepwise elimination of non-significant terms using AIC values from *drop1* function to find the minimum adequate model (Chambers 1992). Data were checked for normality and homogeneity of variance by plotting Q-Q plots and residuals vs fitted values. Significance was set at $P<0.05$ for all analyses. *Post-hoc* Tukey contrast tests were performed using *ghlt* function from '*multcomp'* package (Hothorn *et al.* 2014).

All measured growth parameters were assessed on ten replicates (plants) for each watering regime apart from control Quench which had nine replicates due to a plant fatality. Leaf C/N ratio was assessed on six replicates (plants) and leaf Si on seven replicates (plants) selected at random.

2.4 Results

The key findings of plant responses to the different watering regimes are summarised in Table 2.1. Averages and standard errors of all measured parameters are presented in Appendix 1.1 and Appendix 1.2.

2.4.1 Growth and biomass allocation patterns

Regardless of watering regime total plant biomass did not differ significantly between Optic and Quench. Total plant biomass of both barley genotypes decreased significantly when water quantity was reduced by 40% (D) and 60% (SD) compared to the control watering regime (Fig. 2.2 (**A**)). SD treated plants suffered the largest decrease in total biomass (*c.* 61% reduction) compared to control plants. D treated plants exhibited *c.* 39% reduction in total plant biomass compared to control plants. Barley plants watered less frequently (once per week, DIF) accumulated more plant biomass (Fig. 2.2 (**A**)) than plants watered three times (D) and twice per week (DRF) despite receiving the same total quantity of water over the growing period. There were no interactive effects of barley genotype and watering regime on total plant biomass. Optic had a larger root: weight ratio than Quench regardless of watering regime (Fig. 2.2 (**B**)). Root: weight ratio declined in response to a decrease in water quantity but increased in response to a decrease in watering frequency (Fig. 2.2 (**B**)). There was no significant interaction between barley variety and water quantity on root: weight ratio.

The number of leaves per plant at harvest was similar for Optic and Quench. Leaf number declined in response to reduced water quantity (χ_2 = 63.13, $P<0.0001$), whereas reduced watering frequency had no effect on leaf number ($P<0.05$, see Appendix 2.1). Regardless of watering regime Quench developed more tillers than Optic throughout the growing period (Fig. 2.3, Table 2.1) and had thicker stems (Table 2.1), but there was no significant difference between the genotypes in main stem height (Fig. 2.4). Tiller number (Fig. 2.3 (**A**)), main stem height (Fig. 2.4 (**A**)) and width (Table 2.1) all decreased in response to a reduction in water quantity. By

Table 2.1. Output of linear models showing F statistic and degrees of freedom (subscript numbers) testing the effect of barley genotype, water quantity or watering frequency, and their interactions on barley growth and chemical composition.

	Quantity			**Frequency**		
	Genotype	**Treatment**	**Genotype x treatment**	**Genotype**	**Treatment**	**Genotype x treatment**
Growth and biomass allocation patterns						
Total plant biomass	NI	$213.7_{2,47}$***	NI	$2.40_{1,45}^{NS}$	$11.17_{2,45}$***	$2.75_{2,45}^{NS}$
Leaf biomass	NI	$339.2_{2,47}$***	NI	$15.10_{1,47}$***	$19.83_{2,47}$***	NI
Stem biomass	NI	$103.66_{2,47}$***	NI	NI	$3.43_{2,48}$*	NI
Root biomass	NI	$103.66_{2,47}$***	NI	$6.44_{1,47}$*	$10.68_{2,47}$***	NI
Root: weight ratio	$9.05_{1,46}$**	$4.63_{2,46}$*	NI	$5.57_{1,47}$*	$4.79_{2,47}$*	NI

Main stem width	$9.61_{1,46}$**	$15.72_{2,46}$***	NI	$12.76_{1,49}$***	NI	NI
Tissue chemical analysis						
Total plant water content	$12.92_{1,46}$***	$336.37_{2,46}$***	NI	$11.00_{1,47}$**	$12.89_{2,47}$***	NI
Leaf N	NI	$51.99_{2,28}$***	NI	$4.83_{1,27}$*	$4.02_{2,27}$*	NI
Leaf C/N ratio	NI	$50.63_{2,28}$***	NI	$3.29_{2,27}^{NS}$	$5.51_{2,27}$**	NI

NI non-significant and removed to improve the model. $^{NS}P>0.05$ *$P<0.05$, **$P<0.01$, ***$P<0.001$. Leaf C was removed from the table as there was no significant effect of water quantity, watering frequency or barley genotype.

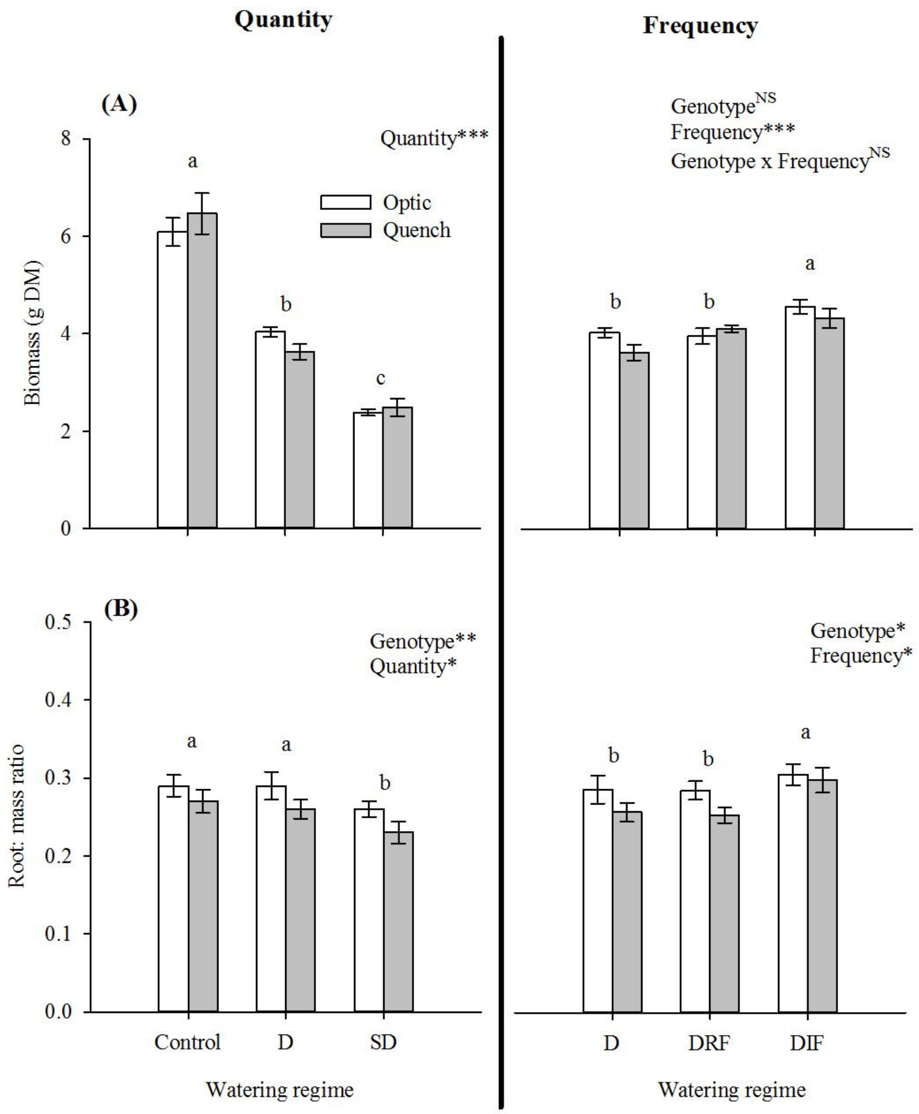

Fig. 2.2. (**A**) Total plant biomass (g dry mass (DM)) and (**B**) root: mass ratio of barley genotypes Optic and Quench treated in response to changes in water quantity or watering frequency. Values represent mean ± standard error bars of 10 replicates (except Quench in the Control watering regime, where n=9). Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrast for comparison of watering regimes.

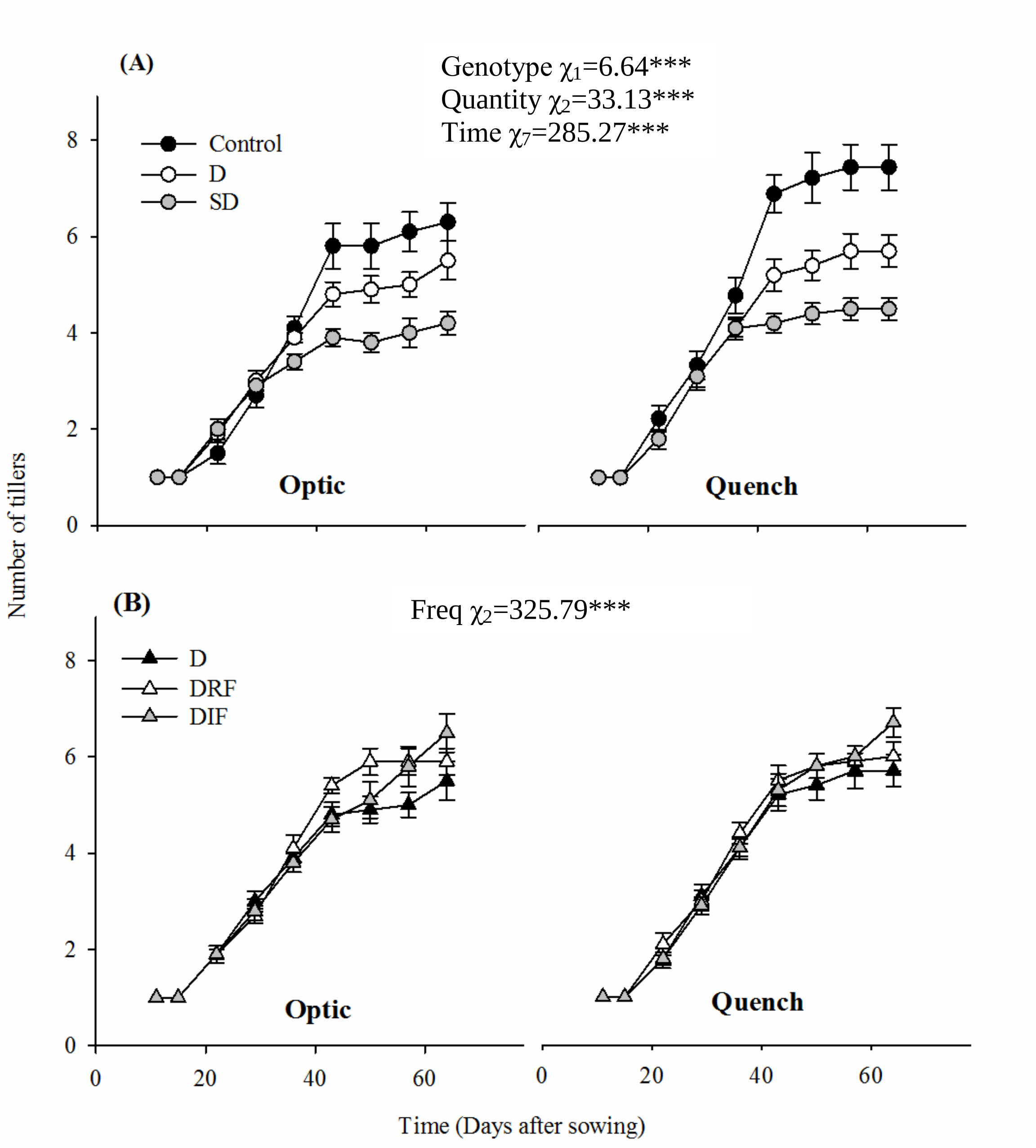

Fig. 2.3. Number of tillers of barley genotypes Optic and Quench treated with changes in the (**A**) water quantity or (**B**) watering frequency. Values represent mean ± standard error bars of 10 replicates (except Quench in the Control watering regime, where n=9).

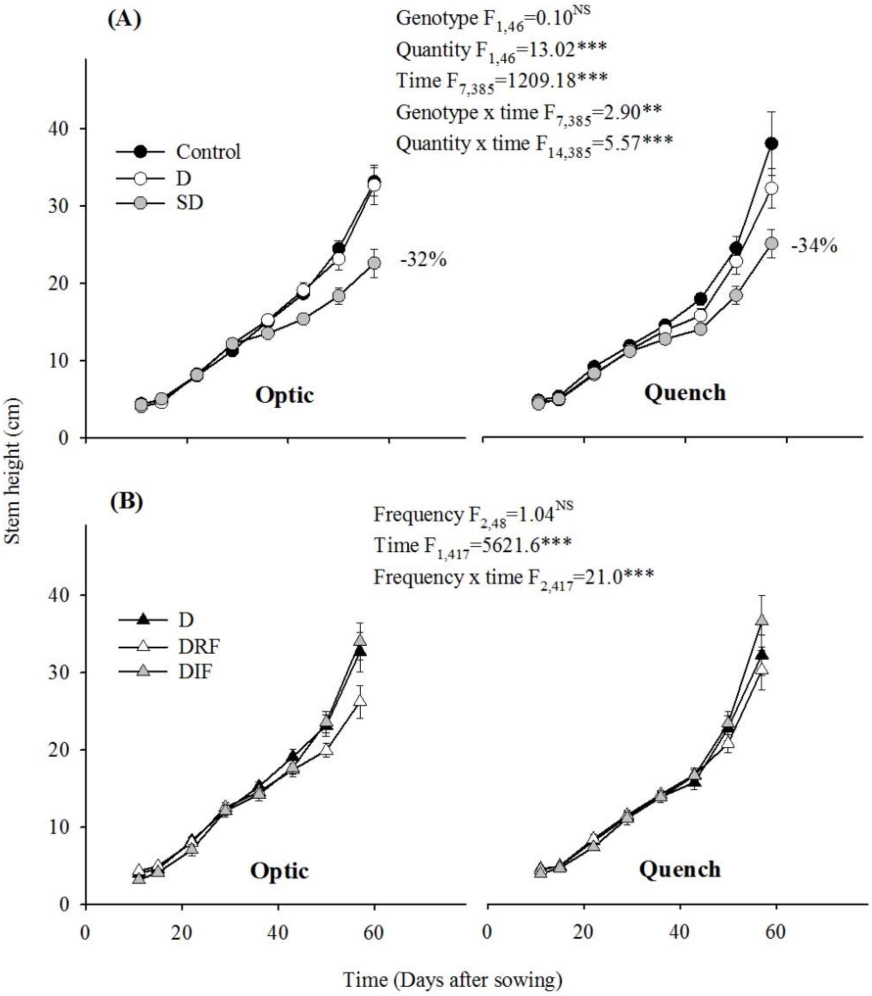

Fig. 2.4. Main stem height (cm) of barley genotypes Optic and Quench treated with changes in (**A**) water quantity or (**B**) watering frequency. Values represent mean ± standard error bars of 10 replicates (except Quench in the Control watering regime, where n=9). Percentages show the reduction in stem height of SD compared to Control treated plants.

contrast, reduced watering frequency increased main stem height (Fig. 2.4 (**B**)) but had no effect on main stem diameter or number of tillers (Fig. 2.3 (**B**)). There were significant interactive effects on main stem height between barley genotype and time (days after sowing), between water quantity and time, and between watering frequency and time (Table 2.1). The effects of reduced water quantity were observed at an earlier stage of growth in Optic compared to Quench, and these genotypic differences amplified as the experiment progressed (Fig. 2.4 (**A**)).

2.4.2 Tissue chemical composition

Tissue water content of plants was larger in Optic plants than Quench regardless of watering regime (Table 2.1). Tissue water content decreased in response to reduced water quantity, but increased in response to reduced watering frequency (Appendix 1.1 and Appendix 1.2). There was no significant interaction between barley genotype and watering regime on total plant water content (Table 2.1). There was no significant difference in leaf N concentration (% of dry mass) between the two barley genotypes, with the exception of a higher leaf N concentration in Quench compared to Optic when the watering frequency was decreased under 40% reduction in the water quantity (Fig. 2.5). Leaf N concentration increased in response to reductions in water quantity. Leaf N concentrations were generally similar across the watering frequency regimes, although plants watered twice a week had lower leaf N concentrations than those watered once a week (Fig. 2.5). Leaf C concentration was unaffected by barley genotype, watering regime and their interaction, with changes in C:N ratio reflecting the changes in leaf N concentrations (Table 2.1, Appendix 1.1 and Appendix 1.2.).

Regardless of watering regime leaf Si concentration did not differ between the two different barley genotypes (Fig. 2.6). Leaf Si decreased in response to the 40% and 60% reduction in water quantity (D), whereas with reduced watering frequency (DIF) leaf Si concentration was significantly increased. There was no significant interaction between barley genotype and watering regime in terms of Si concentration.

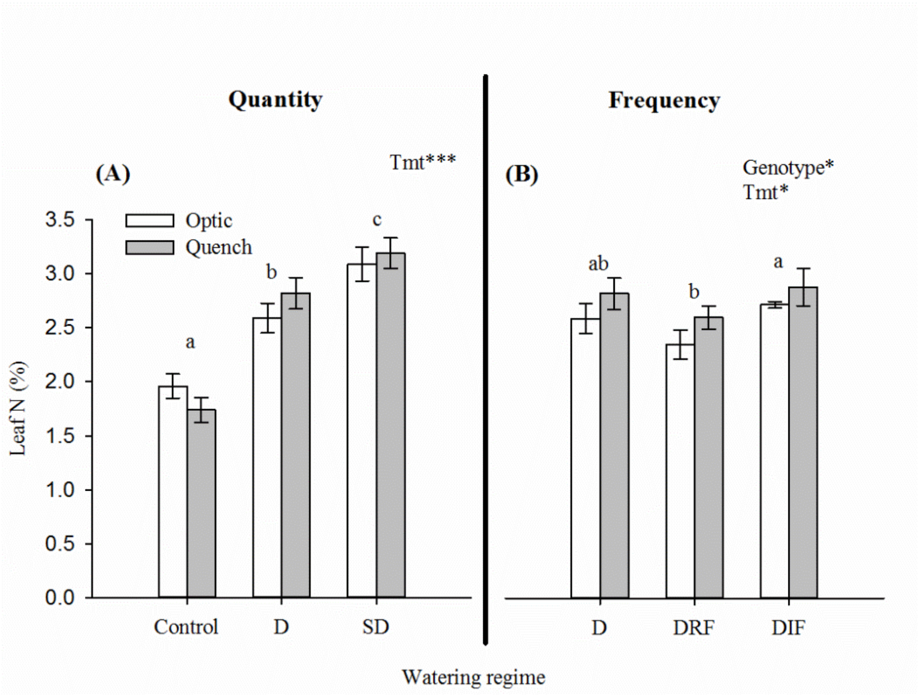

Fig. 2.5. Leaf N (%) concentration of barley genotypes Optic and Quench treated with changes in (A) water quantity or (B) watering frequency. Values represent mean ± standard error bars of 10 replicates (except Quench in the Control watering regime, where n=9). Bars sharing the same letter were not significantly different as determined by post-hoc Tukey contrast for comparison of water regime.

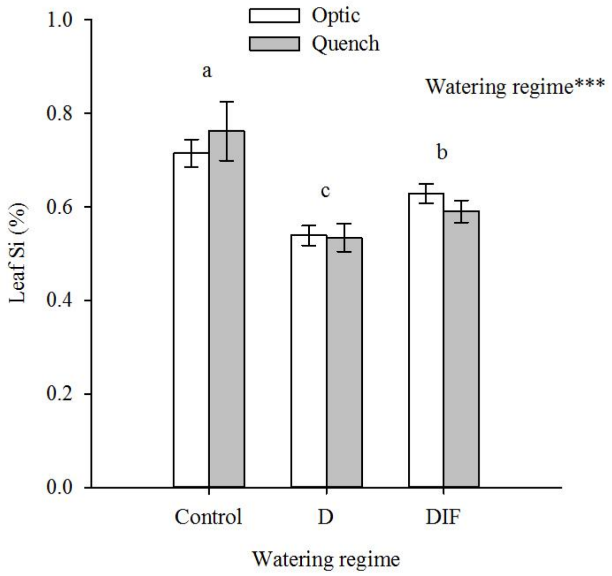

Fig. 2.6. Leaf silicon (% Si) concentration of barley genotypes Optic and Quench treated with changes in water quantity (D) and watering frequency (DIF). Values represent mean ± standard error bars of 10 replicates (except Quench in the Control watering regime, where n=9). Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrast for comparison of water regimes.

2.5 Discussion

2.5.1 Key findings

The selected watering regimes were sufficient to produce effects of changes in water quantity and watering frequency on both barley genotypes without lethal effects. Reductions in water quantity resulted in decreased barley growth, development, lower leaf Si concentration and higher leaf N concentration. In contrast, changes in watering frequency had either no effect or even alleviated some of the negative effects of the 40% reduction in water quantity on total pant biomass and leaf Si concentration. The growth and development of barley genotypes Optic and Quench were similar. However, Optic invested a greater proportion of total plant biomass in the roots compared to Quench and had a larger total plant water content. Despite this, the two barley genotypes were affected to a similar extent by changes in water quantity and watering frequency.

2.5.2 Were the developed watering regimes successful at testing the impact of predicted changes in precipitation on barley growth and development?

Watering regimes had significant effects on plant growth, development and chemical composition without causing lethal effects. Reductions in water quantity (40% reduction in water quantity) attempted to simulate predicted reductions in summer rainfall based on UK climate change projections (Murphy *et al.* 2009) and the severe drought watering regime (60% reduction in water quantity) represented a more extreme drought scenario. Whereas changing the number of watering events simulated predicted changes in the frequency of extreme rainfall events. Reduced water quantity had significant effects on plant growth and chemical composition, whereas changes in watering frequency had only minor effects on plant growth and chemical composition. UK MET office data show that during summer months in areas where barley crops are grown, watering events are less frequent than three

Table 2.2. Summary of established watering regimes and which chapters they feature in.

Watering regime		Reduction in water quantity compared to ambient or control	No. of watering events per fortnight	Chapter		
				Chp 2	Chp 3	Chp 4 & 5
Reduction in water quantity	D	40%	4 (6 for chp 2)	✓	✓	✓
	SD	60%	4 (6 for chp 2)	✓	✓	
Reduction in watering frequency	DRF	40%	4	✓		
	DIF or DMRF	40%	2	✓	✓	
	DSRF	40%	1		✓	✓

times a week (MET office 2015). With this in mind, future experiments investigating the effect of predicted changes in precipitation on barley growth and development should impose more severe reductions in watering frequency (Table 2.2). Extremes in air temperature of the greenhouse may have influenced barley plant responses to the different watering regimes. During periods of high temperature, rates of evapotranspiration and soil water evaporation would have increased, potentially amplifying the severity of the drought treatments (Shah and Paulsen 2003). Therefore, the response of the barley plants to the different watering regimes most likely reflected the interactive effects of changes in soil moisture with changes in air temperature. Moreover, measuring soil moisture using theta probes on the soil surface was unsuccessful due to the dry nature of the surface soil. Gravimetric measurements of total soil moisture of the pots were more successful but differed depending on the developmental stage and size of the barley plant growing in the pot. These experiments would be significantly improved and implemented more successfully in controlled environment cabinets with stabilised temperature control using watering regimes based on MET station rainfall data.

2.5.3 The effect of the watering regimes on barley growth, development and tissue chemical composition.

Both barley genotypes showed significant reductions in total plant biomass compared to the control plants when treated with a 40% (D) or 60% (SD) reduction in water quantity, with most severe effects observed under the SD regime. Plants treated with reduced water quantity had fewer tillers and a shorter, narrower main stem as well as a large reduction in the number of leaves and leaf biomass. Therefore future predicted reductions in precipitation have the potential to significantly reduce barley plant growth and development. Similar responses of barley plants to drought stress have been recorded previously, including reductions in stem height and tillering for example irrigated spring barley (cv. Julia) grown under rainshelters (Lawlor *et al.* 1981). Reductions in plant growth and development under drought stress is likely to be due to reductions in cell growth and expansion, and in rates of photosynthesis as a result of stomatal closure as a water saving strategy, which limits carbohydrate biosynthesis and leads to decreased leaf area, leaf senescence and reduced leaf production (Jamieson *et al.* 1995; Blum 1996). Drought-induced changes in plant growth and development could also be mediated by plant hormones such as abscisic acid (ABA) which is commonly elevated in the xylem sap of drought stressed plants (Popova *et al.* 1996; Chaves *et al.* 2003) and has been reported to influence stem development and leaf expansion by supressing ethylene production and, therefore, internode elongation (Hoffmann 2013).

Reduction in water quantity was also recorded to significantly affect plant chemical composition. Plants receiving reductions in water quantity exhibited higher leaf N and lower Si concentrations and tissue water content compared to control plants. The accumulation of carbohydrates, sugars and amino acids can also contribute to mechanisms for osmotic pressure adjustment (Setter and Waters 2003; Chaves and Oliveira 2004; Farooq *et al.* 2009). N content of barley grain is important for the distilling process; therefore potential changes in plant N concentration could have consequences for future barley production. Si is mainly taken up by plants passively through aquaporin-type transporters in the roots, so reductions in transpiration rates under drought conditions may explain the reductions in observed leaf Si (Ma and Yamaji 2006). However studies have shown that the relationship between transpiration and Si uptake is complex, with intra and inter species variability of Si uptake not fully explained by changes in water availability. Therefore differences in the density of transporters and possible active transport of Si in the root may also play a role in Si uptake (Hartley 2015). In grasses, Si plays an important role in plant defence against insect and mammalian herbivores (Massey, Ennos and Hartley 2006; Massey and Hartley 2006; Guntzer, Keller and Meunier 2012). Therefore, changes in leaf Si concentration under future predicted precipitation regimes could influence the resistance of barley to crop pests.

In contrast to the original hypothesis, reducing watering frequency alleviated some of the negative effects of the 40% reduction in water quantity. Total plant biomass and tissue water content of plants watered once per week (DIF) was greater than those plants watered three times per week (D). Although these plants received the same total quantity of water over the experimental period, it was supplied in fewer watering events. Therefore extreme rainfall events may alleviate some of the negative effects of drought on crops grown under future climate change. Studies investigating the effect of changes in precipitation on crop production may overestimate the detrimental effects of reductions in water quantity without considering changes in watering frequency. Plants receiving fewer watering events also maintained higher leaf Si concentration which may influence plant defence against insect herbivore as previously described. Furthermore Si can contribute to maintenance of cellular structure and biomechanical support to reduce lodging (Gong *et al.* 2005; Ma and Yamaji 2006; Balakhnina *et al.* 2012).

Infrequent applications of larger quantities of water could allow greater soil moisture at depth for longer, than frequent applications of small water quantities; this could mitigate the negative effects of reduced water supply on plant growth by providing water to deep roots that are also buffered from variation in air temperature and surface evaporation. Reducing watering frequency with periods of drought and recovery may have also improved the resilience of these plants to future stress events as there is evidence to suggest that once recovered from drought, plants can become more resilient to future water stress events (Ratnayaka *et al.* 2003; Kang *et al.* 2011). In contrast to the results reported here, Tariq *et al.*, (2012) reported significant reductions in *Brassica oleracea* biomass in response to pulse and drought treatments compared to the unstressed plants and biomass and species diversity declined in a grassland system under drought and deluge treatments (Fry *et al.* 2014). These conflicting results may reflect differences between studies such as the severity of the water stress and between plant species in their response to water availability.

2.5.4 The response of Optic and Quench to the different watering regimes

Optic and Quench did not differ in total plant biomass, but they were found to differ in biomass allocation, with Optic investing a larger proportion of dry mass in the roots. An increase in root: weight ratio is thought to be part of a water conserving strategy (Chaves and Oliveira 2004). In addition, plant water content was higher in Optic, suggesting that this cultivar was more effective at acquiring or conserving water than Quench. The differences between the growth and morphology of the two barley varieties is likely to be due to differences in their breeding history as the two genotypes were bred from different parent barley genotypes (Syngenta Seeds Limited 2009). Overall, the two barley genotypes showed similar responses to changes in water quantity and watering frequency for the majority of the measured physiological variables. An exception was stem height, which was affected at an earlier growth stage in Optic compared to Quench, suggesting that Quench has the ability to delay the effects of drought possibly by either an avoidance or tolerance strategy, although this did not translate into improved performance at harvest.

2.6 Conclusions

Changes in water quantity and watering frequency significantly impacted the growth, development and chemical composition of two barley genotypes. However, future experiments would be improved with more severe watering regimes to more accurately simulate predicted changes in precipitation. This study has demonstrated reducing water quantity and watering frequency has a significant effect on the growth, development and morphology of two barley genotypes, Optic and Quench. Reductions in water quantity caused a significant reduction in total plant biomass, stem height, number of leaves, tillers and leaf Si concentrations as well as increased leaf N concentrations, with strongest effects observed in the most severe watering regimes. Despite D, DRF and DIF treated plants all receiving the same total quantity of water weekly, delivering this water less frequently appeared to partially alleviate some of the effects of reduced water quantity on total plant biomass, leaf Si concentration and other measured growth parameters. Therefore reducing watering frequency decreases the severity of reductions in water quantity. The growth, development and chemical composition of the two barley genotypes and their responses to the watering regimes were very similar. Therefore under future precipitation barley growth could be significantly reduced but with changes in the frequency of rainfall events alleviating some of the negative effects of drought. Future studies investigating the effect of changes in precipitation on crop production may overestimate the impact of a reduction in precipitation without considering changes in the frequency of rainfall events.

CHAPTER 3

Drought Vs Deluge: Frequency of Rainfall Events Drives Changes in Growth, Physiology and Chemical Composition of Barley

3.1 Abstract

Extreme drought and heavy rainfall events are predicted to increase in the UK, with a 40% reduction in summer precipitation by 2080. Previous research addressing the responses of cereals to changes in precipitation has focused on sustained reductions in summer rainfall; detailed understanding of the responses of cereals to erratic rainfall events is currently lacking. This study examined the impact of alterations in both the quantity and frequency of simulated precipitation events on a modern spring cultivar and traditional landrace of barley grown in the UK.

A cultivar and a landrace of barley (Hordeum vulgare L.) were subjected to five precipitation scenarios that reduced water quantity by either 40% or 60% compared to a control; the 40% reduction was administered at three different frequencies, twice a week, once a week and once a fortnight. Barley growth, biomass allocation, yield and chemical composition were measured at harvest.

Reductions in water quantity resulted in smaller total plant and ear biomass, reduced leaf silicon concentrations, and increased leaf nitrogen concentrations. Reducing watering frequency either had no effect or even alleviated some of the negative effects of the 40% reduction; plants watered less frequently had increased plant biomass and leaf silicon concentration. Barley cultivar and landrace plants differed in growth, yield and chemical composition. Cultivar SLA, tiller diameter and leaf fructose concentration were more sensitive to reductions in water quantity compared to the landrace, whereas the landrace had a higher leaf phosphorus concentration which was affected by the different watering regimes.

Sustained reductions in water supply may be more damaging to barley growth and yield than intermittent drought followed by heavy rainfall. Hence studies focussing only on continuous reductions in water availability may overestimate the impact of climate change on plant growth.

3.2 Introduction

Climate change is one of the major challenges facing modern agriculture and future food security (Godfray *et al.* 2010). By 2080 the UK is predicted to experience reductions of up to 40% in the quantity of summer precipitation, concomitant with increases in the frequency of extreme drought and heavy rainfall events (Bates *et al.* 2008; Murphy *et al.* 2009). Crop plants grown in the UK are likely to suffer more extreme periods of stress and recovery in the future, with an increase in the number of dry days followed by heavy rainfall events (Fowler and Kilsby 2004; McCracken and Stoate 2011). This has the potential to severely impact crop production (Wheeler and von Braun 2013) as plant development, morphology and physiology, as well as nutrient uptake, are all influenced by soil water availability (Chaves *et al.* 2003; Farooq *et al.* 2009). The UK National Ecosystem Assessment highlights the need for UK agriculture to be more efficient and resilient to future climate conditions, including changes in water availability (McCracken and Stoate 2011). Most research to date focuses on the impact of extreme sustained reductions in soil water availability on plant growth and development, whereas in the natural environment plants are likely to be subject to periods of stress and recovery.

Barley (*Hordeum vulgare* L.) is one of the main cereal crops grown throughout the UK (DEFRA 2013). Barley growth and yield are vulnerable to both drought (Jamieson *et al.* 1995; Abebe *et al.* 2010; Lawlor *et al.* 1981) and flooding (Yordanova *et al.* 2003; de San Celedonio *et al.* 2014). Chapter 2 provided evidence to suggest that barley cultivar growth and development can be significantly affected by predicted changes in precipitation. However, this requires further investigation following the improvement and establishment of methods for investigating the effect of future precipitation patterns on barley plant growth, development and chemical composition.

Under water stress conditions cereals such as barley can exhibit a number of adaptive traits that mitigate or prevent damaging effects. These include mechanisms that increase water uptake and reduce water loss in the case of drought, such as increased root growth and reduced leaf area (Chaves *et al.* 2003; Barnabás *et al.*

2008). Plants can also tolerate water stress by utilising sugars such as sucrose and glucose for osmotic adjustment (Setter and Waters 2003; Chaves and Oliveira 2004; Farooq *et al.* 2009). Some cereals are also able to accumulate silicon (Si) for biomechanical maintenance of cellular structure and to reduce lodging under water stress (Gong *et al.* 2005; Ma and Yamaji, 2006; Balakhnina *et al.* 2012). While responses to drought are relatively well-characterised it is less clear how crop plants will respond to erratic heavy rainfall. Extreme drought and rainfall events would be more damaging to crop productivity than continuous reductions in the quantity of rainfall due to the extremes of water stress (Schmidhuber and Tubiello 2007) and the additional stress caused by transitions between aerobic and anaerobic environments (Setter and Waters 2003).

There is evidence to suggest that selective breeding for certain agriculturally important traits in modern crop cultivars has led to reduced genetic variation and loss of genes for key traits, such as resistance to biotic and abiotic stresses (Doebley *et al.* 2006; Purugganan and Fuller 2009). Barley landraces have been previously reported to exhibit greater yield stability under adverse abiotic conditions (Newton *et al.* 2010), suggesting that barley landraces might exhibit smaller changes in plant growth, development and yield in response to reductions in mean precipitation compared to modern cultivars. In this study, the effect of predicted changes in both the water quantity and watering frequency on the growth, morphology, chemical composition and yield of a modern cultivar of spring barley (Optic) was assessed and compared to responses of a traditional landrace (Bere). Cultivar Optic was chosen as it is one of the main modern cultivars grown countrywide in the UK (Syngenta Seeds Limited 2009). Bere is a very old barley variety once widely grown in the northern Britain but now grows on a small scale in the highlands and Islands of Scotland, particularly Orkney (O’Dell 1935; Scholten *et al.* 2009). The decline in Bere cultivation was most likely to be due to the breeding of higher yielding varieties with short straw which are better suited to mechanised agriculture. Bere is reported to be frost intolerant and as such it is sown in the spring but due to its rapid growth it is often harvested very early. It can also grow on more alkaline sand coastal soils derived from beach sands (O’Dell 1935; Scholten *et al.* 2009). It was hypothesised that (1) reducing water quantity and watering frequency would have negative effects on the growth, morphology and chemical composition of both the barley cultivar and landrace plants, with reductions in the watering frequency having the largest negative effect, and (2) there would be a greater negative effect of reductions in water quantity and watering frequency on the cultivar compared to the landrace.

3.3 Materials and Methods

3.3.1 Plants and growth conditions

Spring barley plants, cultivar Optic (supplied by Syngenta 2010) and the landrace, Bere (from the Orkney Isles, supplied by SASA 2010) were grown individually in 2.4L pots filled with 3kg of dried, sieved (10x10mm) topsoil (A1 Plant, Elvington) mixed in a 3:1 ratio with washed sharp horticultural sand (Keith Singleton, UK). Pots were maintained in three controlled environment cabinets with 16h daylight (av. light intensity across the three cabinets was 262.1±3.7µmol m^{-2} s^{-1} [mean ± standard error]) at 20^oC/ 18^oC day/ night and initially watered to ensure soil moisture content was 50% of the total soil water holding capacity. The saturation and desiccation (dried at 105^oC for 7d) mass of the soil was measured and from this the total water holding capacity was calculated (0.293ml g^{-1} soil/ sand mix).

The experiment comprised a randomised block design. Within each block, one replicate of barley variety (Optic or Bere) and watering regime were assigned at random to each pot. Nine blocks were staggered temporally by 2-3 weeks. Two seeds of either Optic or Bere barley were placed in the centre of each pot c. 2cm from the soil surface and watered from the top of the pot twice per week for two weeks with 150ml of deionised water. Saucers placed under each pot captured any water draining through the soil, which was left to be taken up through holes in the bottom of the pot. Following germination, seedlings were removed to a single plant per pot of consistent height and development stage. Cultivar or landrace plants in each experimental block were harvested when the ear on the main stem of the ambient watering regime for each variety reached Zadok's growth stage 71 (Zadoks *et al.* 1974), which defines the term 'harvest' in this study.

Plants were subjected to one of five watering regimes (Fig. 3.1) ambient: watered to ensure soil moisture reached 50% of total soil water holding capacity (measured by weighing the pot) until 5 weeks after sowing, and thereafter watering regimes were standardised and plants were watered 200ml per watering event (based on 10

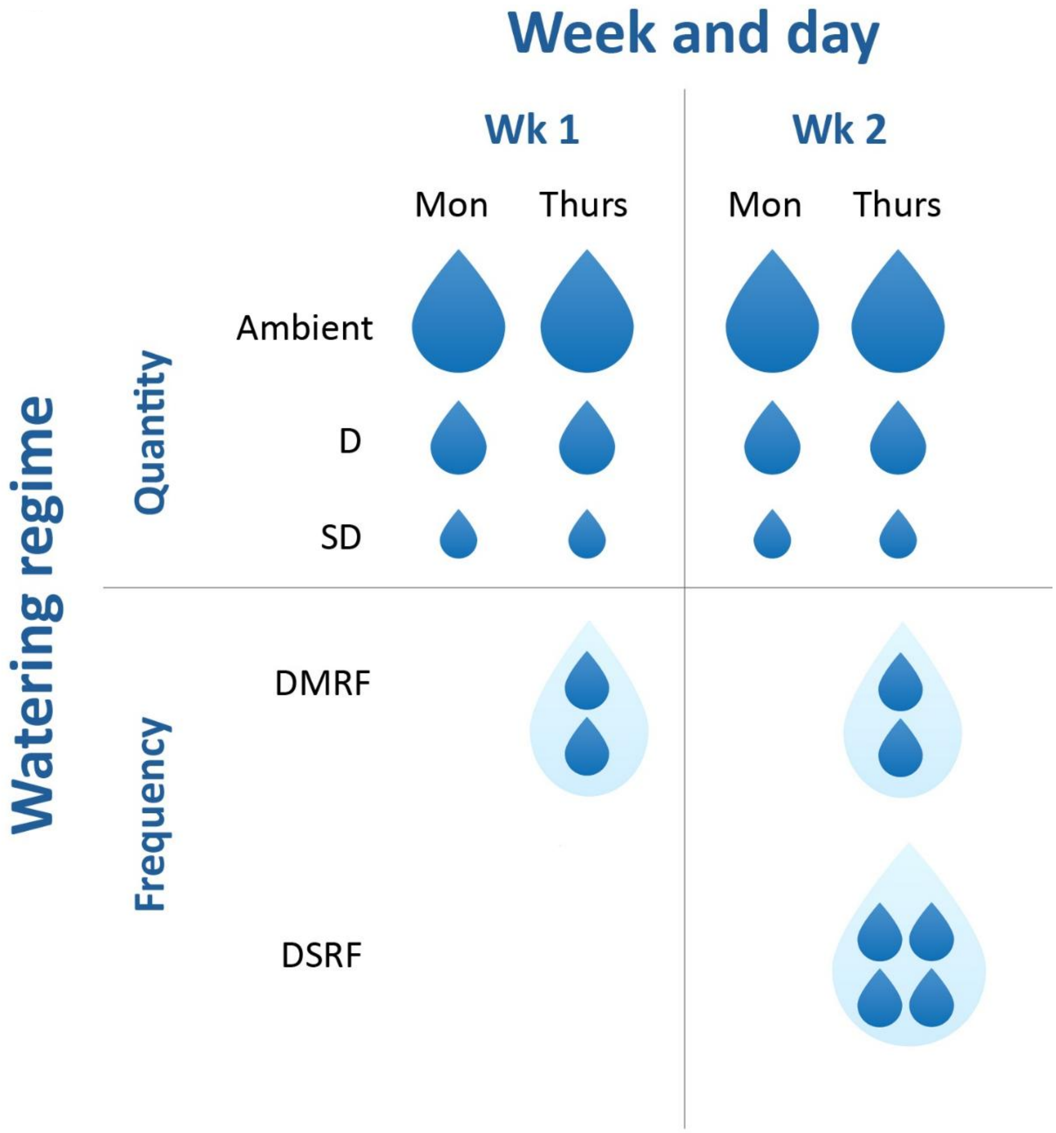

Fig. 3.1. Diagram representing the different watering regimes with changes in water quantity at each watering event and the watering frequency.

year av. weekly rainfall during June, July and August at The James Hutton Institute, Invergowrie, Dundee, Scotland) watered twice per week. Drought (D): 40% reduction in volume of water added compared to ambient, watered twice per week. Severe drought (SD): 60% reduction in volume of water added compared to ambient, watered twice per week. Drought with moderate reduced watering frequency (DMRF): 40% reduction in volume of water added compared to ambient, watered once per week i.e. 50% as frequently as ambient. Drought with severe reduced watering frequency (DSRF): 40% reduction in volume of water added compared to ambient, watered once per fortnight i.e. 25% as frequently as ambient.

These watering regimes allowed comparison of the effects of changes in water quantity (40% and 60% reduction in volume of water added compared to the ambient) and changes in the watering frequency under the 40% reduction regime (water provided twice per week, once per week or once per fortnight).

3.3.2 Plant growth and development measurements

At harvest, main stem height (cm), number of leaves, number of ears and av. tiller diameter (mm, 2cm from the soil surface) were measured and plant material separated into roots, stem, leaves and ears. The fourth fully expanded leaf on the main stem was removed for leaf area measurement (cm^2) using a portable area meter (Li-cor model LI-3000A). Harvested plant material was dried at 70^{o}C for c. four days and total dry biomass recorded (g dry mass (DM)). Root: mass ratio was calculated by dividing the dry mass of roots by total dry plant biomass. Specific leaf area (SLA) was calculated using dry mass of the individual leaf (cm^2 g^{-1}). Water content (g) of the plant material was calculated by subtracting the dry mass from the fresh mass. Grains were manually threshed, counted, dried and weighed. Harvest Index (HI) was calculated by dividing total ear mass by total above-ground dry mass.

3.3.3 Si, P and C/N analysis

Elemental analysis was conducted on dried milled green leaf material (c. four green leaves per plant) and grain from each plant. For Si and phosphorus (P) analysis, milled plant material was pressed at 11tons into 5mm thick cylindrical pellets with a manual hydraulic press using a 13mm die (Specac, Orpington, UK). Si and P concentration (% dry mass) was determined using a commercial P-XRF instrument (Niton XL3t900 GOLDD analyser: Thermo Scientific Winchester, UK) held in a test stand (SmartStand, Thermo Scientific, Winchester, UK) (Reidinger *et al.* 2012).

The carbon (C) and nitrogen (N) concentrations of leaf and grain (% dry mass) were determined by flash combustion and chromatographic separation of ~1.5mg milled leaf using an elemental analyser (Elemental combustion system 4010 CHNS-O Analyser, Costech Analytical Technologies, Inc., Milan, Italy), calibrated against a standard ($C_{26}H_{26}N_2O_2S$). Percentage C and N of the leaves was used to calculate the C/N ratio.

3.3.4 Sugar analysis

At harvest c. three green leaves per plant were snap frozen in liquid N and stored at -80^{o}C, then freeze-dried and milled. For sugar extraction, 500mg samples of freeze-dried milled leaf material was vigorously mixed with 500µl of 80% ethanol and placed in a pre-heated shaking water bath at 50^{o}C for 2h. Inositol (20µl of 1 mg ml^{-1}) internal standard was added to 80µl of the supernatant and evaporated under vacuum. Three drops of Tetramethylsilane $(CH_3)_4$ were added to each dried sample to derivatise the sugars into trimethylsilyl ethers increasing volatility. The sugar composition (mg g^{-1}) of each sample was quantified by GC-MS using a Hewlett Packard HP-6890 coupled to a HP 5973 mass selective detector as described by Doco *et al.* (2001) (methanolic HCL, SUPELCO, 1997 Sigma-Aldrich Co.), by comparison with a standard sugar solution (0.25 mg ml^{-1} of sucrose, glucose and fructose). Glucose, fructose and sucrose peaks were determined using Compass™ (Bruker Daltonics´s) software searching for pre-determined published retention times and ion mass (201 for glucose at c. 17.1min and 19.3min, and 437 for fructose at c. 15.3mins and sucrose at c. 33.8mins) (Kamerling *et al.* 1972; Doco *et al.* 2001; Mogosanu *et al.* 2011).

3.3.5 Statistical analysis

Analyses were performed in R (version 3.0.2). Linear mixed effect models (*nlme* package) (Pinheiro *et al.* 2014) or generalised linear mixed effect models (*lmer*) (Bates *et al.* 2014) were used to test the main and interactive effects of barley variety and watering regime on the measured variables. The effects of changes in water quantity and watering frequency were analysed separately. Block was included as a random term in the model. Data were checked for normality (Shapiro–Wilk normality test) and homogeneity of variance following Crawley (2007). Proportion data were arcsine square root transformed. Log transformation was performed on total plant biomass, root: mass ratio, ear biomass, leaf C/N and stem height (changes in water quantity only) data, and square root transformation was performed on grain mass data to meet the assumptions of the linear model. Significance was set at $P<0.05$ for all analyses. Models were simplified using AIC values (calculated using '*AIC'* function) to find the minimum adequate model (Crawley 2007). *Post-hoc* Tukey contrast tests were performed using '*ghlt*' function from '*multcomp*' package (Hothorn *et al.* 2014).

All measured growth parameters were assessed on nine replicates (plants) for each variety*watering regime combination. Leaf C/N ratio was assessed on seven replicates (plants), grain C/N on six replicates and leaf sugars concentrations on five to six replicates. Leaf Si and P concentrations were assessed on three to eight replicates depending on the availability of sufficient green plant material for analysis.

3.4 Results

The key findings of plant responses to the different precipitation regimes are summarised in Table 3.1 and Table 3.2. Averages and standard errors of all measured parameters are reported in Appendix 2.1 and 2.2.

3.4.1 Growth and biomass allocation patterns

Landrace plants subjected to ambient watering regime reached maturity on av. 22days earlier than the cultivar. Therefore landrace plants were harvested fewer days after sowing. Watering regime had no effect on the time to maturity of the ear on the main stem.

Cultivar plants had significantly larger (c. 30% larger) total plant biomass compared to landrace plants regardless of watering regime (Table 3.2). 40% (D) and 60% (SD) reductions in water quantity caused a significant reduction in total plant biomass (c. 30% and c. 60% reduction respectively) (Fig. 3.2). Reducing the watering frequency significantly affected total plant biomass. Cultivar and landrace plants watered once per week (DMRF) had significantly larger (c. 13%) total plant biomass compared to plants receiving the same total amount of water twice per week (D) (Fig. 3.2). Cultivar plants had a significantly larger leaf, stem and root biomass than the landrace regardless of watering regime (Table 3.2). Cultivar leaf, stem and root biomass were all significantly reduced by 40% (D) and 60% (SD) reduction in water quantity (Table 3.2). Cultivar and landrace plants watered once per fortnight (DSRF) had a significantly larger leaf biomass (c. 27% increase) than those watered twice per week (D). Landrace plants watered once per fortnight (DSRF) had significantly larger stem biomass than plants watered twice per week (D), whereas stem biomass of the DMRF-treated cultivar was significantly larger than plants watered twice per week (D) (Table 3.2) Landrace plants had a larger root: mass ratio than cultivar plants (Table 3.2). Root: mass ratio was unaffected by the watering regimes (Table 3.2).

Table 3.1. Summary of effects of 40% reduction in water quantity (D) and severe reduction in the watering frequency (DSRF) on different growth parameters of barley cultivar and landrace. Symbols represent increase (↑), decrease (↓) and no change (─) in plant response.

Plant response	D compared to ambient		DSRF compared to D	
	Cultivar	Landrace	Cultivar	Landrace
Growth and *biomass allocation patterns*				
Total plant biomass	↓	↓	↑	↑
Leaf biomass	─	─	↑	↑
Stem biomass	↓	↓	─	↑
Root biomass	↓	↓	─	─
No. leaves	─	─	↑	↑
Average tiller diameter	↓	─	─	─
Stem height	↓	↓	─	─
Yield and *grain nitrogen concentration*				
Ear biomass	↓	↓	─	─
No. grains	↓	↓	─	─
Grain mass	─	─	↓	↓
Grain N	─	↑	─	─
Chemical composition				
Plant water content	↓	↓	─	─
Leaf fructose	↑	─	─	─

Leaf glucose	↑	↑	–	–
Leaf Si	↓	↓	↑	↑
Leaf P	–	↓	↑	↑

No significant effect on the comparative watering regimes on the landrace and cultivar in this table on root: mass ratio, SLA, No. ears, HI, leaf C/N ratio and leaf sucrose concentration.

Table 3.2. Results of linear models showing F (or χ^2 where stated) statistic and degrees of freedom (subscript numbers) testing the effect of barley variety, changes in water quantity or watering frequency and their interactions on different physiological variables.

	Quantity			Frequency		
	Variety	Quantity	Variety x Quantity	Variety	Frequency	Variety x Frequency
Growth and biomass allocation patterns						
Total plant biomass	$68.90_{1,42}$***	$172.24_{2,42}$***	NS	$173.04_{1,42}$***	$6.54_{2,42}$**	NS
Leaf biomass	$5.23_{1,42}$*	$11.89_{2,42}$***	NS	$5.31_{1,42}$*	$5.46_{2,42}$**	NS
Stem biomass	$127.67_{1,40}$***	$137.45_{2,40}$***	$3.30_{2,40}$*	$186.54_{1,40}$***	$7.61_{2,40}$**	$4.40_{2,40}$*
Root biomass	$5.62_{1,42}$*	$59.85_{2,42}$***	NS	NS	NS	NS
Root: mass ratio	$96.59_{1,44}$***	NS	NS	$67.12_{1,44}$***	NS	NS
No. leaves	NS	$\chi^2=3.18_2$***	NS	$\chi^2=30.58_1$***	$\chi^2=8.20_2$*	NS

Average tiller diameter	$29.47_{1,40}$***	$10.96_{2,40}$***	$7.24_{2,40}$**	$17.13_{1,42}$***	$7.92_{2,42}$**	NS
Stem height	$12.49_{1,42}$*	$30.52_{2,42}$***	$3.69_{2,40}$*	$8.07_{1,42}$*	$3.32_{2,42}$*	NS
SLA	$208.16_{1,40}$***	$0.277_{2,40}$	$11.073_{2,40}$***	$70.73_{1,44}$***	NS	NS
Yield and grain nitrogen concentration						
Ear biomass	$30.12_{1,42}$***	$42.34_{2,42}$***	NS	$28.15_{1,44}$***	NS	NS
No. ears	$\chi^2=12.53_1$***	$\chi^2=20.49_2$***	NS	$\chi^2=28.51_1$***	NS	NS
No. grains	$\chi^2=7.04_1$**	$\chi^2=658.14_2$***	$\chi^2=10.13_2$**	$\chi^2=48.83_1$***	$\chi^2=46.45_2$***	NS
Grain mass	$26.34_{1,44}$***	$3.22_{2,42}$*	NS	$25.81_{1,42}$***	$3.252_{,42}$*	NS
HI	$21.16_{1,42}$***	$13.38_{2,42}$***	NS	$23.29_{1,44}$***	NS	NS
Grain [N]	$63.92_{1,30}$***	$28.19_{2,30}$***	NS	$82.27_{1,26}$***	NS	NS

Chemical analysis						
Total plant water content	$4.72_{1,42}$*	$261.88_{2,42}$***	NS	NS	$7.93_{2,43}$**	NS
Leaf C/N ratio	$54.70_{1,30}$***	$17.22_{2,30}$***	NS	$69.23_{1,32}$***	NS	NS
Leaf [fructose]	$30.40_{1,22}$***	$27.19_{2,22}$***	$6.90_{2,22}$**	$12.057_{1,21}$**	$0.20_{1,21}^{NS}$	$3.86_{2,21}$*
Leaf [glucose]	$6.02_{1,24}$*	$22.24_{2,24}$***	NS	NS	NS	NS
Leaf [Si]	$8.02_{1,21}$***	$15.01_{1,21}$***	NS	$7.33_{1,23}$*	$44.08_{2,23}$***	NS
Leaf [P]	55.351,19***	$4.96_{2,19}$*	$6.75_{2,19}$**	$72.85_{1,23}$***	$4.71_{2,23}$*	NS

NS non-significance and removed to improve the model, *$P<0.05$, **$P<0.01$, ***$P<0.001$. No significant effect of watering regime, barley variety or the interaction between watering regime and barley variety on No. tillers and leaf sucrose.

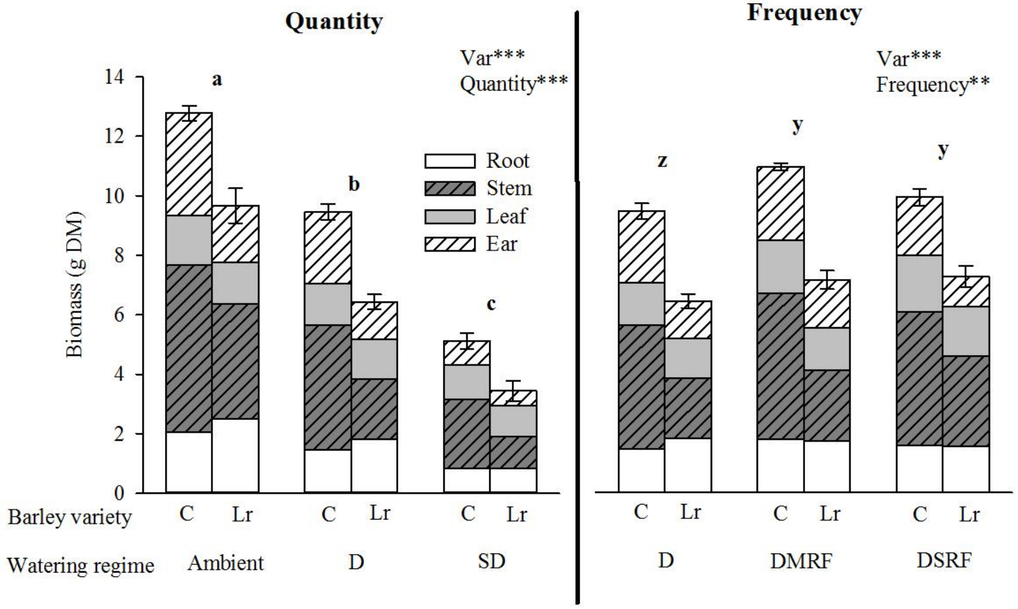

Fig. 3.2. Total plant biomass (g dry mass (DM)) of barley cultivar (C) and Landrace (Lr) plants treated with changes in water quantity or watering frequency. Values represent mean ± standard error bars of 9 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrast.

There was no significant difference in the number of leaves produced by the landrace and cultivar at harvest (Table 3.2). D and SD treated cultivar plants had significantly fewer leaves compared to ambient plants at harvest (Table 3.2). Plants that were watered with reduced watering frequency produced significantly more leaves (Table 3.2). The cultivar had a larger av. tiller diameter than the landrace (Fig. 3.3 (**A**)). D and SD treated cultivar plants had a significantly smaller av. tiller diameter compared to the ambient cultivar plants, but there was no effect of D and SD watering regimes on the av. tiller diameter of the landrace plants (Fig. 3.3 (**A**)). Changes in the watering frequency also had a significant effect on av. tiller diameter where plants watered once per week (DMRF) had a larger av. tiller diameter compared to those watered twice per week (Table 3.2). The landrace had a taller main stem compared to the cultivar (Table 3.2). Drought significantly reduced the stem height of the landrace and cultivar whereas changes in the frequency of watering events had no effect on stem height (Table 3.1 and Table 3.2). SLA of the landrace at harvest was significantly larger than the cultivar (Fig. 3.3 (**B**)). SLA of the landrace plants was unaffected by reductions in water quantity whereas SLA of the cultivar plants was found to increase (Fig. 3.3 (**B**)). There was no effect of changing the watering frequency on SLA (Table 3.2).

3.4.2 Yield and grain nitrogen concentration

HI of Cultivar plants under ambient watering regime (average 0.5026 ±0.0285 se) was similar to that reported in the field (51%) (Edwards *et al.* 2006). Cultivar plants had a significantly larger ear biomass, grain mass and HI, and produced more ears (Fig. 3.4 (**A**) and Table 3.2) and grain compared to the landrace regardless of watering regime. Reducing water quantity resulted in a reduction in ear biomass, number of ears, number of grains, grain mass and HI (Fig. 3.4 and Table 3.2). D and SD treated plants exhibited c. 30% and c. 70% decreases, respectively, in total ear biomass at harvest compared to ambient plants. Total ear biomass, number of ears and HI were unaffected by reduction in the watering frequency (Fig. 3.4 and Table 3.2). Cultivar plants watered once a week (DMRF) produced more grains at harvest than ambient-treated cultivar plants (Fig. 3.5 (**A**)). However, plants watered less frequently had smaller grain mass than ambient-treated plants (Table 3.2).

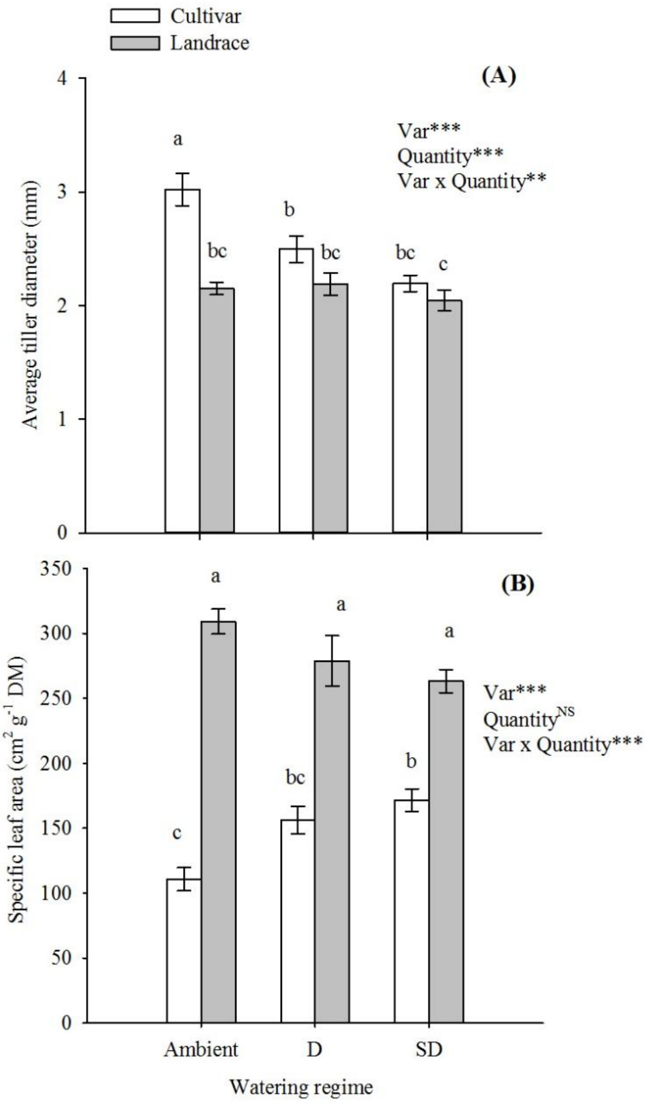

Fig. 3.3. (**A**) Average tiller diameter (mm) and (**B**) specific leaf area (cm^{-2} g^{-1} dry mass DM) of barley cultivar and landrace plants at harvest treated with changes in water quantity. Values represent mean ± standard error bars of 9 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrast

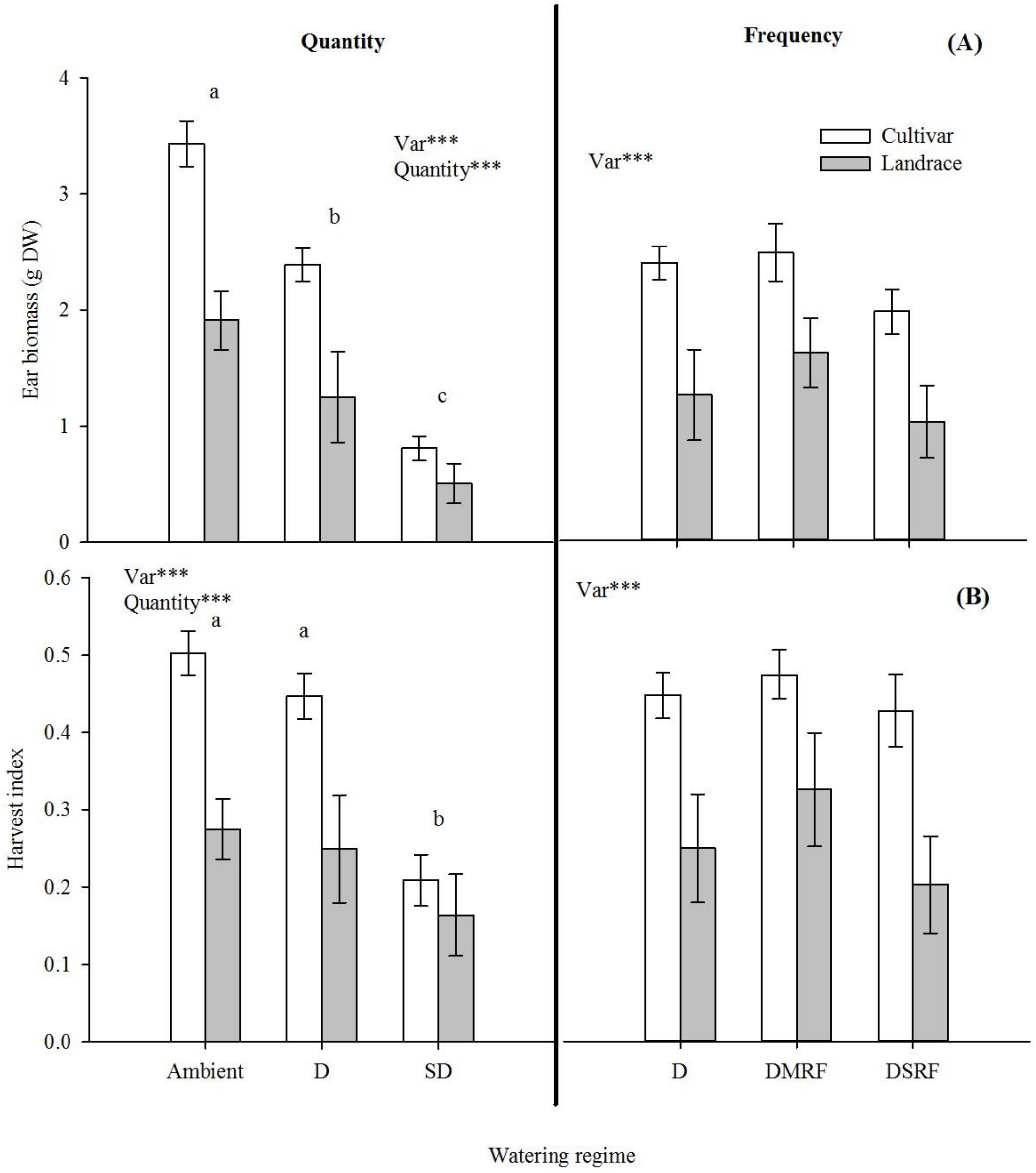

Fig. 3.4. (A) Ear biomass (g dry weight DW) and (B) harvest index of barley cultivar and landrace plants at harvest treated with changes in water quantity or watering frequency. Values represent mean ± standard error bars of 9 replicates for total number of grains and 6 replicates for grain N concentration. Bars sharing the same letter were not significantly different as determined by post-hoc Tukey contrast.

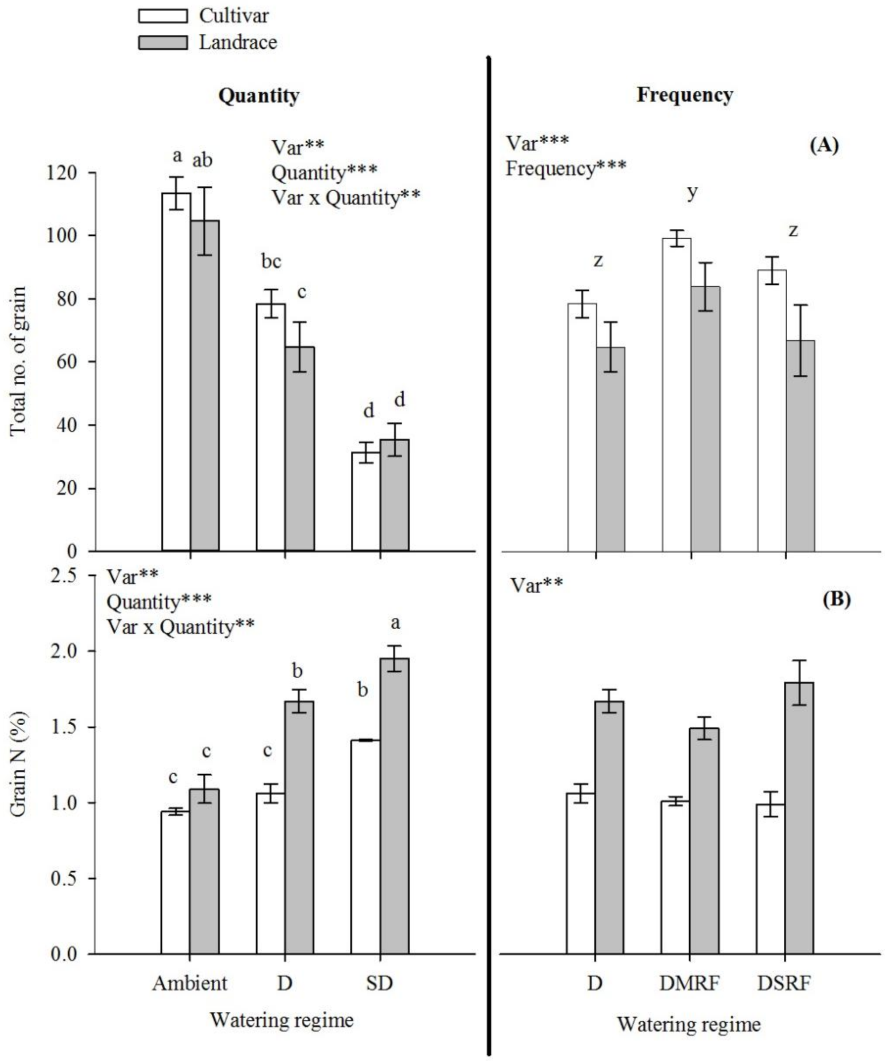

Fig. 3.5. (**A**) Total number of grains and (**B**) grain N (%) concentration of barley cultivar and landrace plants at harvest treated with changes in water quantity or watering frequency. Values represent mean ± standard error bars of 9 replicates for total number of grains and 6 replicates for grain N concentration. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrast.

Grain N concentration was lower in cultivar plants than landrace plants regardless of watering regime (Fig. 3.5 (**B**)). Grain N concentration increased significantly in response to reductions in water quantity (Fig. 3.5 (**B**)). There was a significant interactive effect of barley variety and water quantity on grain N concentration with to a larger effect of changes in water quantity on the landrace compared to the cultivar. There was no effect of changes in the watering frequency on grain N concentration (Fig. 3.5 (**B**)).

3.4.3 Chemical composition

Landrace plants had significantly larger total plant water content compared to cultivar plants regardless of watering regime (Fig. 3.6 (**A**)). Plants watered with reduced water quantity had significantly smaller total plant water contents (Fig. 3.6 (**A**)). Reducing the watering frequency had no effect on total plant water content (Fig. 3.6 (**A**)). Cultivar plants had a significantly higher leaf C/N ratio regardless of watering regime (Table 3.2). SD treated plants had a significantly smaller leaf C/N ratio compared to the other watering regimes (Table 3.2). There was no effect of reducing the watering frequency on leaf C/N ratio. Compared to the landrace, the cultivar had a significantly higher leaf fructose (Fig. 3.6 (**B**)) and glucose (Table 3.2) concentrations regardless of watering regime. Leaf fructose (Fig. 3.6 (**B**)) and glucose (Table 3.2) concentrations were significantly higher in D and SD treated plants; increase in leaf fructose concentration was larger in cultivar compared to landrace plants when treated with D and SD (Fig. 3.6 (**B**)). There was no overall effect of frequency of watering on leaf fructose and glucose of the landrace and cultivar. Leaf sucrose concentrations did not differ between the two barley varieties nor between watering regimes (Table 3.2). Landrace plants accumulated higher leaf concentrations of Si compared to the cultivar plants (Fig. 3.6 (**C**)). D and SD treated plants had significantly lower leaf concentrations of Si compared to ambient treated plants (Fig. 3.6 (**C**)). Leaf Si concentrations were higher in cultivar and landrace plants treated with reduced watering frequency (Fig. 3.6 (**C**)). Landrace plants also accumulated higher leaf P concentrations compared to cultivar plants (Fig. 3.6 (**D**)). There was a significant interactive effect of barley variety and water quantity on leaf P concentration due to lower leaf P concentrations in D and SD landrace plants

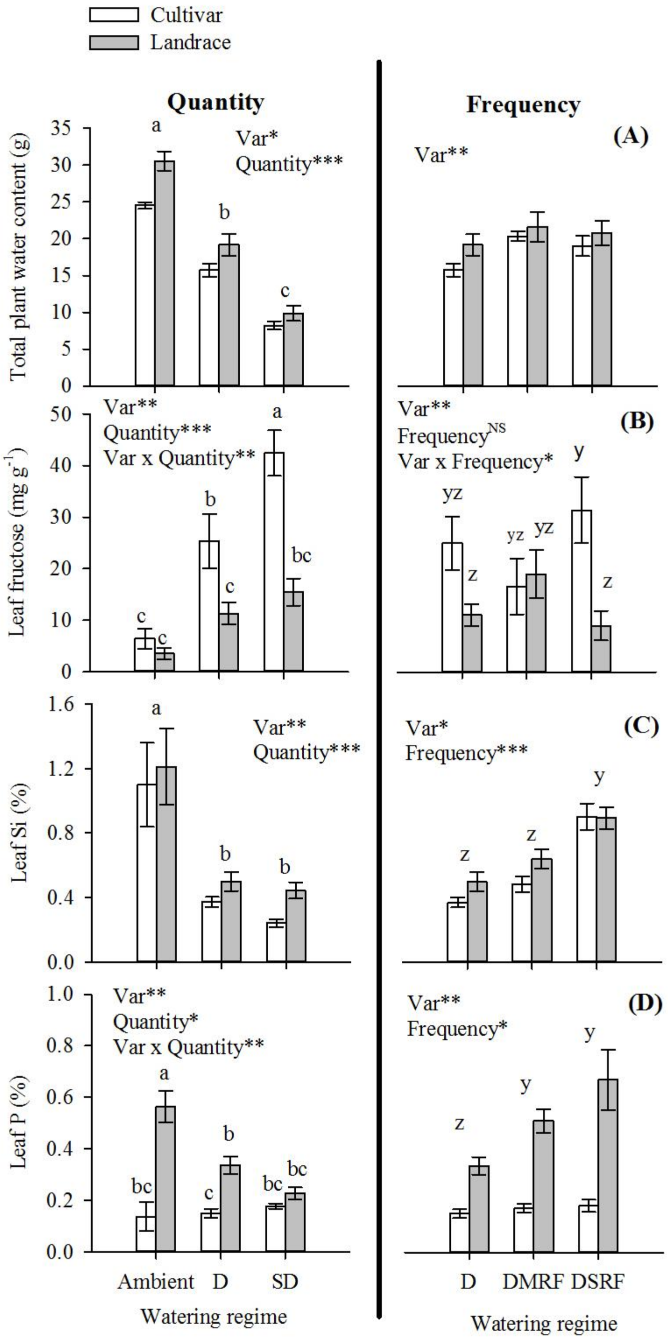

Fig. 3.6 (**A**) Total plant water content and leaf concentrations of (**B**) fructose (mg g^{-1} dry mass), (**C**) Si (% dry mass) and (**D**) P (% dry mass) of barley cultivar and landrace plants at harvest treated with changes in water quantity or watering frequency. Values represent mean ± standard error bars, 9 replicates for total plant water content, five to six replicates for leaf fructose concentration and three to eight replicates for leaf Si and P concentrations. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrast.

whereas cultivar leaf P concentrations were unaffected by these watering regimes (Fig. 3.6 (**D**)). Leaf P concentrations were higher in cultivar and landrace plants watered with reduced watering frequency (Fig. 3.6 (**D**)).

3.5 Discussion

3.5.1 Key findings

Reductions in water quantity resulted in reductions in growth, yield and leaf Si concentrations of the barley cultivar and landrace plants. By contrast, changes in the watering frequency had either no effect or even alleviated some of the negative effects of the same reductions in water quantity, exhibiting increased total plant biomass and higher leaf Si and P concentrations. Barley landrace and cultivar plants differed in the majority of parameters measured in the study including total plant biomass and yield. SLA, tiller diameter and leaf fructose concentration of the cultivar plants were more sensitive to reductions in water quantity compared to the landrace, whereas leaf P concentration of the landrace was higher than the cultivar but was affected by the different watering regimes.

3.5.2 The effect of the different watering regimes on barley plant growth, development, chemical composition and yield.

Reductions in water quantity caused significant negative effects on the growth, development and yield of the barley cultivar and landrace plants, affecting the majority of growth parameters measured. A 40% reduction in water quantity, the predicted decrease in UK summer precipitation by 2080 caused significant reductions in total plant biomass and yield, suggesting barley production under future climate change could suffer potential reductions in yield of 30% in ear biomass. Drought stressed plants accumulated sugars in leaves. Early after the onset of drought, photosynthesis often remains unaffected, whereas expansion growth reduces very quickly leading to the accumulation of photosynthetic products (Blum 1996). Sugars can play a role in maintaining osmotic balance and protection from antioxidants in water stressed environments. There were changes in the concentrations of fructose and glucose, both of which have been previously reported to be involved in osmotic adjustment (Chaves *et al.* 2003; Barnabás *et al.* 2008). Drought stressed plants also had a decrease in leaf C/N ratio. Nitrogen often accumulates during drought stress as a result of protein degradation and can play a role in osmotic adjustment (Blum 1996). Drought also significantly increased grain N concentration, potentially reducing grain quality for distilling under future climate change scenarios (Edwards *et al.* 2006).

In contrast to the adverse impacts of a reduction in water quantity, reduced watering frequency in most cases either had no effect or a positive effect on the growth parameters measured in this study. There were much smaller effects of reductions in watering frequency on landrace and cultivar plant growth and chemical composition compared to reductions in water quantity. At harvest, plants watered less frequently had larger total plant biomass and the cultivar had higher grain numbers, although ear biomass was unaffected due to a decrease in grain mass. The IPCC report published in 2014 highlighted that changes in the frequency of rainfall events in the UK will be more significant than reductions in quantity of precipitation (Bouwer *et al.* 2014). This will have consequences for barley productivity under climate change, with the potential for drought-induced decreases in plant biomass and yield to be partially ameliorated by reduced frequency of precipitation events. Plant responses to drought vary between species and with stress severity, and have been reported to differ in response to continuous and intermittent drought (Fry *et al.* 2013; Fry *et al.* 2014). This should be further evaluated as studies investigating continuous drought without considering changes in the frequency of rainfall events may overestimate the effects of climate change on crop productivity.

Improved growth and development of the barley plants in soils that receive water less frequently but in larger quantities is most likely due to the water penetrating deeper in the soil and therefore available to a different, deeper rooting zone. Furthermore precipitation events with less frequent rainfall but with larger quantities of water may result in the deeper parts of the soil profile remaining wetter for a longer period of time after the watering event has occurred enabling an increase in water uptake by roots (Heisler-White *et al.* 2009). However a new irrigation technique known as partial root zone drying, where part of the root system is dry or drying and the other is irrigated, results in an increase in water use efficiency where maize and wheat plants maintained similar photosynthetic rates with reductions in transpiration (Du *et al.* 2010), suggesting an alternative mechanism for plants performing better under less frequent watering. Other studies have suggested that plants can become more resilient after drought stress events, able to cope better with future stress. For example, alfalfa plants had subsequently larger root systems and changes in the regulation of transcripts of enzymes for the synthesis of amino acids for potential improved osmoprotection after re-wetting (Kang *et al.* 2011) and tissue concentrations of antioxidants were higher during recovery after a drought event in cotton and spurred anoda (Ratnayaka *et al.* 2003). Barley roots can also form aerenchyma which have been suggested to reduce the effects of waterlogging on shoot and root growth during a recovery phase (Pang *et al.* 2004). However, by contrast it has been reported that both intermittent and continuous drought treatments significantly reduced *Brassica oleracea* plant biomass compared to unstressed plants (Tariq *et al.* 2012), whilst mesotrophic grassland had reduced species richness and plant die back under simulated changes in precipitation (Fry *et al.* 2014) and temperate grasses showed reduced ability to accumulate plant biomass after re-wetting (Okamoto *et al.* 2011). Differences between studies are most likely to be due to differences in plant species ability to cope with water stress.

Reductions in transpiration in response to changing watering regime may explain the measured differences in leaf Si and P concentrations under different watering regimes. Phosphate uptake and grain P concentration have also been previously reported to decrease in barley grown under drought conditions (Day *et al.* 1978). Drought conditions may reduce the ability of the plants to take up and transport Si and P due to a reduction in transpiration rate in drought stressed plants. However the relationship between transpiration and Si uptake is complex, reflecting differences between species in accumulation of silicon which could relate to differences in the density of transporters in the root and the role of active anion transporters (Hartley, 2015). Although the distribution of silicon in the shoot is still thought to be largely controlled by transpiration (Hartley *et al.* 2015). Si accumulation provides an important physical defence against insect and mammalian herbivores particularly in grass species (Massey *et al.* 2006; Massey and Hartley 2009; Guntzer *et al.* 2012). Changes in the leaf Si concentration in response to altered precipitation could have significant consequences for barley which may be more vulnerable to pests under future climates, particularly given that the abundance of many crop pests is predicted to increase (Gregory *et al.* 2009)**.** Furthermore, drought is known to increase the vulnerability of crops to pests such as aphids (Johnson *et al.* 2011; Huberty and Denno 2004).

3.5.3 Differences in growth, development, chemical composition and yield of the landrace and cultivar plants.

Landrace and cultivar plants differed in the majority of growth parameters measured in this study. Cultivar plants had a larger total plant biomass at harvest compared to the landrace, with a larger av. tiller diameter and yield. In contrast, landrace plants had a taller main stem and a higher leaf concentrations of N, Si and P. Differences in the growth and development of cultivar and landrace plants have been previously reported including differences in plant height, days to maturity and number of seed (Assefa and Labuschagne 2004; Tondelli *et al.* 2013). The differences between the landrace and cultivar plants are likely to be a result of selection pressures for agriculturally important traits (Theobald *et al.* 2006; Martin, Chang and Wishart 2010). The cultivar Optic has been through a rigorous selection process for traits such as shorter stem for reduced lodging, high yield and malting qualities, whereas the UK landrace ‘Bere’ barley has not been subjected to the same intensity of artificial selection (Madic *et al.* 2009; HGCA 2014).

There is evidence to suggest that intense domestication of crops has led to the loss of plant natural defences (Massei and Hartley 2000). Si-containing structures such as trichomes is one of the main defences in grasses and the higher leaf Si concentration of landrace plants particularly under drought conditions could provide increased resistance to both mammalian and insect herbivory (Massey *et al.* 2006; Massey and Hartley 2006). Domestication of crops and the use of P rich fertilisers may have also reduced the efficiency of modern cultivars to take up nutrients (Purugganan and Fuller 2009; Newton *et al.* 2010), resulting in the higher leaf P concentrations in the landrace compared to the cultivar. The magnitude of difference in leaf P concentration between the landrace and cultivar plants is much larger than the difference in root biomass between the two barley varieties, suggesting that the landrace may be more efficient at P uptake than the cultivar. This might be linked to increased root phytase exudation by landraces (George *et al.* 2014) leading to improved soil P availability and uptake. Cereal landraces including barley landraces have also been previously reported to have higher nutrient use efficiency compared to cultivars (Górny 2001; Newton *et al.* 2010).

3.5.4 The response of the cultivar and landrace to the different watering regimes

Growth, yield and chemical composition of the cultivar and landrace plants responded differently to the watering regimes. For the majority of these growth parameters, changes in water quantity had a larger effect on the cultivar plants compared to the landrace plants, suggesting that the cultivar was more sensitive to changes in water availability. However the barley varieties responded similarly to changes in the frequency of watering events. Therefore the barley varieties responded differently to reduced water quantity, supporting hypothesis (2) but responded similarly to changes in watering frequency which in contrast does not support hypothesis (2). There is evidence to suggest that landraces have a higher water use efficiency compared to cultivars (Górny 2001; Newton *et al.* 2010). Landrace and cultivar leaf P concentrations also responded differently to reductions in water quantity. Cultivar leaf P concentration was much lower than landrace leaf P concentration under ambient watering regime. However, under 40% and 60% reduction in water quantity cultivar leaf P concentration was unaffected, whereas landrace leaf P concentration was reduced to such an extent that there was no difference in SD treated landrace and cultivar plants. This suggests that the landrace is more efficient at P uptake but that this efficiency is lost under severe drought conditions. This has implications for the nutrient use efficiency of crops if drought becomes more frequent, exacerbated by pressure to reduce fertiliser inputs under drives for more sustainable agriculture (Gregory and George 2011). Barley landraces better able to cope with future climate change may provide opportunities to improve modern crop varieties.

3.6 Conclusions

40% and 60% reduction in water quantity resulted in a reduction in total plant biomass, yield and leaf Si concentrations of both landrace and cultivar plants, as well as increasing leaf and grain N concentrations. In contrast, less frequent watering mitigated the negative effects of drought on total plant biomass and number of grains on the cultivar, although grain mass decreased and thus total ear biomass was unaffected. Leaf Si concentrations were significantly higher in barley plants watered less frequently. The landrace and cultivar plants differed significantly in chemical composition and the majority of growth parameters measured in this study; the landrace had a smaller total plant biomass and yield, but invested a larger proportion of total plant biomass in roots and had a higher total plant water content, and leaf Si and P concentrations. Some of the measured parameters of the cultivar plants were more sensitive to reductions in water quantity compared to the landrace plants, particularly tiller diameter, SLA and leaf fructose concentration. Whereas compared to the cultivar, landrace plants had higher leaf P concentrations; this was reduced when under continuous drought conditions but increased when watered less frequently.

These results suggest that future predicted changes in precipitation have the potential to significantly affect barley production, where both the changes in water quantity and watering frequency could have significant impacts on barley plant growth, yield, chemical composition and defence. It is clear that the nature of rainfall events can influence the impact of reductions in the quantity of precipitation for both the landrace and cultivar. Studies may overestimate the effect of predicted reductions in precipitation under climate change on plant growth and crop production if the impacts of reducing the frequency of extreme rainfall events are not considered.

CHAPTER 4

Differential Effects of Predicted Changes in Precipitation on Multi-trophic Interactions in a Barley System

4.1 Abstract

Predicted changes in the frequency and intensity of extreme rainfall events in the UK have the potential to affect terrestrial ecosystem function. However, responses of different trophic levels to changing rainfall patterns, and the underlying mechanisms, are not well characterised. The aim here was to investigate how changes in both the quantity and frequency of rainfall events will affect the outcome of barley-herbivore and herbivore-natural enemy interactions in a cereal system.

Barley (Hordeum vulgare L.) plants were grown in controlled environment conditions subjected to three precipitation scenarios that varied the quantity (ambient based on 10 year av. rainfall in Dundee and 40% reduction in quantity of water added) and frequency of precipitation events (25% as frequent as ambient under the 40% reduction regime). The effect of these different watering regimes on the performance of barley plants, below- ground insect herbivores (Agriotes lineatus and A. obscurus), above-ground insect herbivore (Sitobion avenae) and its natural enemy (Harmonia axyridis) was assessed from measurements of plant growth, insect mass and assays of feeding behaviour.

A 40% reduction in water quantity significantly reduced barley plant biomass by av. 23% whereas reducing the watering frequency had very little effect on plant growth. Wireworms reduced plant biomass and this effect was largest under ambient water supply (av. 18% reduction). Changes in barley plant growth and chemical composition due to reduced water quantity and watering frequency resulted in an increase in aphid mass; and ladybirds had a larger increase in mass when feeding on these aphids.

Predicted changes in both the frequency and intensity of precipitation events under climate change have the potential to limit barley plant growth as well as reduce wireworm herbivory but positively affect above-ground aphid mass, with these effects transferring to the third trophic level increasing ladybird mass. Therefore changes in rainfall patterns are particularly important in this system with significant consequences for future barley production.

4.2 Introduction

Climate models predict that by 2080 there will be increased frequency and intensity of drought and heavy rainfall events in the UK with overall reductions of up to 40% in summer precipitation volume (Murphy *et al.* 2009). The HadRM3H model estimates a 10% minimum increase in short duration (1-2d) and up to 30% increase in long duration (5-10d) extreme rainfall events in the next 25-50years (Ekstrom *et al.* 2005). Extreme precipitation events are predicted to destabilise terrestrial ecosystems (Knapp *et al.* 2008) through alterations in resources such as changes in plant growth and chemical composition as well as by disrupting interactions between plants and herbivores by causing asynchrony of development and life cycles between different trophic levels (Weltzin *et al.* 2003; Trotter, Cobb and Whitham 2008). The effect of changes in precipitation events on multi-trophic interactions is, however, largely undescribed and is of specific importance for agroecosytems which will be challenged directly by climate change and by changes in the population dynamics of insect pests and their natural enemies (Gregory *et al.* 2009). Climate change, particularly changes in precipitation may result in insect pest outbreaks which may not be maintained by natural predators due to asynchrony caused by changes in insect growth and fecundity. Potential increases in pest outbreaks will be detrimental to agriculture at a time when the use of many pesticides is being restricted (Pinstrup-Andersen and Pandya-Lorch 1998).

Drought and flooding events can affect plant morphology, physiology and chemical composition significantly reducing productivity (Blum 1996; Chaves *et al.* 2003) and influencing plant food quality for above- and below- ground arthropod herbivores, which in turn can affect herbivore performance (Huberty and Denno 2004; Chown *et al.* 2011). Previously published studies investigating the effect of water stress on insect herbivores mediated through changes in the host plant report contrasting results, which has led to the development of a number of hypotheses outlining the effect of water stress on insect herbivores. The ‘plant stress hypothesis’ (White 1974) states that the population growth of insect herbivores can benefit from increased concentrations of foliar nitrogen (Mattson and Haak 1987) and reduced plant defence (Rhoades 1985) as a result of drought stress. By contrast, the ‘plant vigour' hypothesis (Price 1991) suggests that insect herbivores are negatively affected by the loss of turgor pressure in drought stressed plants, which reduces the flux of phloem sap and nutrients, and as a result drought-stressed plants are a less suitable host for herbivores than unstressed plants (Price 1991). The 'pulsed stress' hypothesis (Huberty and Denno 2004) states that periods of plant stress and recovery could benefit herbivores due to increases in foliar nitrogen concentrations under drought accompanied by intermittent periods of turgor recovery (Huberty and Denno 2004; Mody *et al.* 2009; Tariq *et al.* 2013). However, differences in stress regime, plant species and developmental stage as well as insect species, feeding guild and life stage of the herbivores are likely to affect the precise outcome (Huberty and Denno 2004). For example below-ground root herbivore growth and development can be negatively affected by reduced soil moisture due to changes in plant carbon allocation and growth patterns (Lees 1943a; b; Masters *et al.* 1993; Awmack and Leather 2002). Furthermore the abundance and vertical distribution of below-ground insect herbivores has been reported previously to be significantly affected by soil moisture (Lees 1943a; Briones, Ineson and Piearce 1997; Staley *et al.* 2007a; Sinka, Jones and Hartley 2007). Severe water stress has been recorded to reduce plant tolerance to below-ground herbivores (Dunn and Frommelt 1998) suggesting that the severity of drought events may also be an important determinant of the outcome for root herbivores. For sap feeding insects such as aphids, 'pulsed water stress' arising from frequent drought and recovery events is thought to be beneficial due to increased foliar nitrogen availability and periods of turgor recovery (Larsson 1989; Huberty and Denno 2004; Mody *et al.* 2009). However this hypothesis was not supported by the study of Tariq *et al.* (2012), who reported an increase in aphid performance on continuously drought stressed plants compared to pulse-stressed plants with greatest nitrogen concentrations measured in high and moderately drought stressed plants. These studies confirm that soil moisture availability has the potential to influence the intensity and impact of both above- and below- ground herbivory.

Below-ground insect herbivores have also been reported to influence the abundance and performance of above-ground insect herbivores both positively and negatively, by inducing a plant stress response that leads to the accumulation of foliar free amino acids and carbohydrates, reducing plant growth and defence, as well as altering resource allocation and plant water status (Masters *et al.* 1993; Bezemer and van Dam 2005; Staley *et al.* 2007b; Johnson *et al.* 2011). Water stress may also affect the direction and intensity of interactions between above- and below- ground insect herbivores through changes in host plant growth and chemical composition (Staley *et al.* 2007b; Tariq *et al.* 2013). Drought stress in plants is often exacerbated by below-ground herbivory as the removal of root biomass can limit water foraging and uptake (Masters *et al.* 1993; Dunn and Frommelt 1998) which has been reported to both benefit and negatively affect above-ground insect herbivores feeding on the same host (Staley, Mortimer and Morecroft 2008; Johnson *et al.* 2013; Tariq *et al.* 2013). Staley *et al.*, (2007b) reported that summer drought removed the interaction between leaf-mining *Stephensia brunnichella* larvae and root-chewing *Agriotes* larvae on host plant *Clinopodium vulgare*.

Effects of water stress have also been reported to transfer into higher trophic levels (Johnson *et al.* 2011; Ledger *et al.* 2012; McCluney *et al.* 2012) influencing the fitness and abundance of natural enemies of insect herbivores as a result of changes in prey quality mediated by changes in the host plant (Ledger *et al.* 2012; McCluney *et al.* 2012). For example, Aslam, Johnson and Karley (2013) reported that aphid development rates on barley were altered by summer drought, and this was accompanied by reduced rates of aphid attack by the parasitoid *Aphidius ervi*; similarly, drought reduced the abundance of aphids and their parasitoid wasps in a model cereal ecosystem (Johnson *et al.* 2011). However, the majority of these studies focus on parasitoids and continuous reductions in the quantity of rainfall and do not consider predicted changes in the frequency of rainfall events and how this will influence insect herbivore quality as prey for natural enemies including insect predators.

Barley (*Hordeum vulgare* L.) is one of the main cereal crops grown in the UK; barley growth and yield are negatively affected by drought (Abebe *et al.* 2010), flooding (Yordanova *et al.* 2005; de San Celedonio *et al.* 2014) events and predicted changes in the quantity and frequency of precipitation (Chapter 3) as well as by insect pest feeding and disease transmission (Oerke and Dehne 2004). Barley plants can be attacked simultaneously by wireworms (*Agriotes* spp.) below-ground and by aphids (such as *Sitobion avenae*) above-ground, a scenario which is increasing in frequency due to the conversion of grassland to arable farming, the reduction in organochlorine insecticides, changes in agricultural practises and the increase of winter cropping (Parker and Howard 2001). Harlequin ladybirds (*Harmonia axyridis*) are an invasive species in the UK, first reported in Britain in 2004, after it was introduction in Europe as a very effective biocontrol agent for aphids (Roy and Brown 2015). The harlequin ladybird is now one of the most common ladybirds in the UK and plays a role in maintaining aphid populations (Majerus *et al*. 2006).

The aim of this chapter was to investigate the effect of changes in both the quantity and frequency of watering events on the interactions between barley, root feeding wireworms, phloem feeding aphids and their natural predator ladybird larvae. It was hypothesised that:

- Reduced water quantity and frequency of watering events would negatively affect the growth of barley plants and wireworms but would positively affect the development and fecundity of aphids;

- Root feeding by wireworms would positively affect the performance of aphids feeding above-ground, but this would be mitigated when combined with the effect of reduced water availability;

- Increased aphid performance (due to reduced water availability or wireworm feeding) would benefit the performance of a generalist natural enemy of aphids, the ladybird *Harmonia axyridis.*

4.3 Materials and Methods

4.3.1 Plant growth conditions

Spring barley cultivar Optic (James Hutton Institute) plants were grown individually in 2.4L (18.5cm diameter) pots filled with dried, sieved (10x10mm) topsoil (A1 Plant, Elvington) mixed in a 3:1 ratio with washed sharp horticultural sand (Keith Singletons, UK) resulting in a sandy loam soil substrate. Pots were placed in three controlled environment cabinets, maintained at 16h daylight (av. light intensity across the three rooms was 210.5µmol m^{-2} s^{-1} mean ± standard error 1.80); 23^oC ± 0.33 / 19^oC ± 0.21d/ night.

The experiment comprised a randomized block design with nine blocks that were also staggered temporally by two to three weeks to facilitate the final destructive harvest. Within each block, watering regime (three levels: see below) and herbivory (four levels: control (no herbivore), above- (aphids), below- (wireworms) ground herbivore or both) were assigned at random to each pot, resulting in one replicate plant per block of each watering regime x herbivory combination. Initially, two seeds were placed in the centre of each pot c. 2cm from the soil surface. All pots were watered with deionised water from the top of the pot to ensure soil water content reach 50% of total water holding capacity. To initiate germination, pots were watered from the top twice per week for two weeks with 200ml of deionised water. Saucers placed under each pot captured any water draining through the soil, which was left to be taken up through holes in the bottom of the pot. Following germination, the number of seedlings was reduced to a single plant per pot of consistent height and developmental stage. Barley plants in each experimental block were harvested seven weeks after sowing, at Zadoks growth stage 40 (Zadoks, Chang and Konzak 1974).

The saturation and desiccation (dried at 105^oC for 7d) mass of the soil was measured and from this the total water holding capacity was calculated (0.293ml g^{-1} soil/ sand mix). Soil water was estimated throughout a two week period by weighing three extra pots filled with the same volume of soil/ sand mix previously described and watered with the same quantity of water as the experimental pots but without a barley plant. These extra soil filled pots were weighed twice a week after watering on Monday and Thursday over a two week period (Fig. 4.1).

Watering regimes commenced two weeks after sowing and included ambient (A; based on 10 year av. rainfall at The James Hutton Institute, Invergowrie, Dundee, Scotland, 200ml delivered twice a week), drought (D; 40% reduction in water quantity 120ml delivered twice a week) and drought with severe reduced watering frequency (DSRF; 40% reduction in the quantity of water added with severe reduced watering frequency, watered 25% as frequent as ambient i.e. 480ml delivered every 14d). These watering regimes allowed comparison of the effects of changes in water quantity (40% reduction compared to the ambient) and in watering frequency under the 40% reduction regime (water provided weekly compared to fortnightly).

4.3.2 Plant growth, development and chemical analysis

At harvest leaves, stem, and roots in the top 7.5cm and bottom 7.5cm of the soil profile were collected, weighed for fresh mass, oven dried at 70°C for seven days and re-weighed for dry mass (g dry mass DM). Root: mass ratio was calculated by dividing the dry mass of roots by total dry plant biomass. Water content (g) of the plant material was calculated by subtracting the dry mass from the fresh mass.

Five weeks after sowing, stomatal conductance of the barley plants was measured on six replicate plants (six blocks) for each watering regime and herbivory treatment using a Porometer (AP4 Leaf Porometer, Delta-T Devices, UK). Porometer readings were taken between 9:00am and 10:00am.

Elemental analysis was conducted on dried milled green leaf material (c. four green leaves per plant). For silicon (Si) analysis, milled plant material was pressed at 11tons into 5mm thick cylindrical pellets with a manual hydraulic press using a 13mm die (Specac, Orpington, UK). Si concentration (% dry mass) was determined using a commercial P-XRF instrument (Niton XL3t900 GOLDD analyser: Thermo

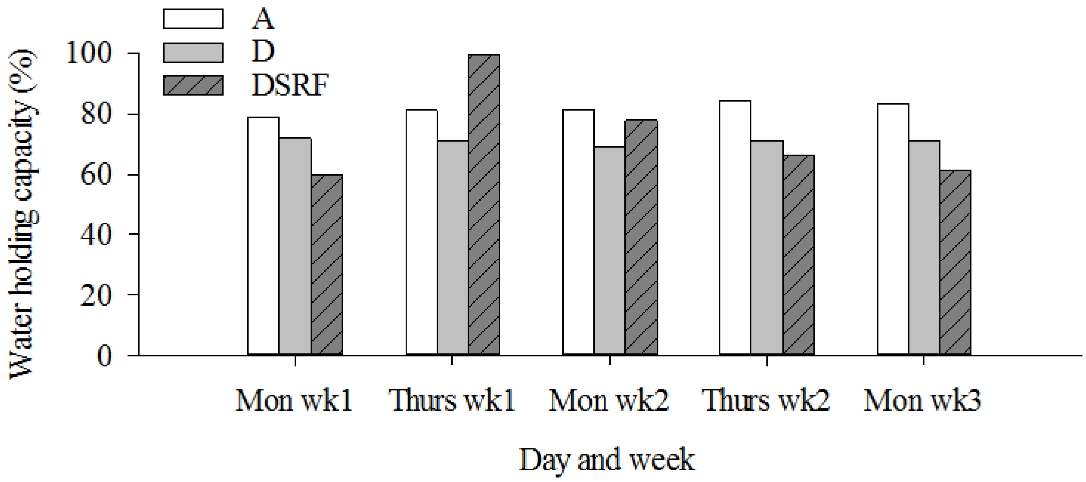

Fig. 4.1. Representative twice weekly measurements of soil moisture as a percentage of water holding capacity of soil treated with different watering regimes, ambient (A), drought (D) and drought with severe reduced watering frequency (DSRF) over a two week period.

Scientific Winchester, UK) held in a test stand (SmartStand, Thermo Scientific, Winchester, UK) (Reidinger *et al.* 2012).

The carbon (C) and nitrogen (N) concentrations of leaf (% dry mass) were determined by flash combustion and chromatographic separation of ~1.5mg milled leaf using an elemental analyser (Elemental combustion system 4010 CHNS-O Analyser, Costech Analytical Technologies, Inc., Milan, Italy), calibrated against a standard ($C_{26}H_{26}N_2O_2S$). Percentage C and N of the leaves was used to calculate the C/N ratio.

4.3.3 Below- ground wireworm herbivory

Prior to the start of the experiment wireworms (*Agriotes* spp. L. *Coleoptera: Elateridae*. A mix of *Agriotes* species, c. 60% *Agriotes lineatus* and 40% *A. obscurus* sourced from Praktijkonderzoek Plant and Omgeving / Applied Plant Research, Wageningen UR) were maintained on potato tubers (cv. Rooster; Albert Bartlett, UK) at 7^{o}C (Johnson *et al.* 2008a). For plants assigned the below-ground herbivory treatment, two weeks after sowing, three wireworms were weighed and buried c. 5cm from the soil surface in three different locations around the pot. A similar level of soil disturbance was inflicted on pots that were not assigned wireworm treatment. At harvest, wireworm location either in top or bottom 7.5cm of the soil profile was recorded. Wireworms were then collected, counted, re-weighed and mass gain calculated.

4.3.4 Above-ground aphid herbivory

Aphids (*Sitobion avenae*) were maintained on Optic barley plants in a controlled environment room, 15^{o}C, 16/8h day/night. Four weeks after sowing, three adult apterous aphids were caged (25mm internal diameter clip cages suspended from metal frames above the plant) to the second fully expanded leaf on the main stem of plants assigned above-ground herbivory treatment. After 24h, the adults and all but three nymphs were removed from each cage (cage 1). The three remaining nymphs were monitored every two days for four weeks to record aphid survival, the date of first reproduction and number of offspring production. Fecundity, the number of offspring produced in the same number of days it took for the adult to mature was calculated. Offspring were collected every two days and transferred to a second cage (cage 2) clipped onto on the same plant. After one week, all cages and aphids were transferred to a fully expanded leaf of similar age on a tiller due to senescence of the original main stem leaf. 14days after the first three aphids were added to cage 1, all aphids were individually weighed, counted, removed from the plants and stored in Petri-dishes (60mm diameter Nunc™) for <2h prior to the ladybird experiment (see below) or freezing.

4.3.5 Ladybird growth and prey choice

Ladybird growth: One second instar Harlequin ladybird (*Harmonia axyridis*) was weighed and presented with three pre-weighed apterous adult aphids and three first instar nymphs collected from cage 2 in a Petri-dish (90mm, Sterilin Ltd, UK). Ladybirds in Petri-dishes were left for 24h in a controlled environment room (12h daylight; c. 20^{o}C) and then removed, re-weighed and mass gain calculated.

Ladybird prey choice: Three live apterous adult aphids, one from a plant from each watering regime within the same block, were collected and positioned randomly in a 90 mm Petri-dish and equidistant from the dish centre. Aphids collected from plants with and without wireworm herbivory were kept separate and used in separate ladybird feeding experiments. The dorsal surface of each aphid was secured to the base of the dish with a small (c. 3mm x 4mm) piece of double sided sticky tape, leaving their legs free to defend from predator attack. One harlequin ladybird larvae (third or fourth instar) was placed in the middle of the arena. The ladybird larvae were monitored and the first aphid to be eaten was recorded. Aphid positioning in the Petri-dish was randomised.

4.3.6 Statistical analysis

Statistical analyses were performed in R (version 3.0.2) to test the main and interactive effects of watering regime, and above- and/ or below- ground herbivory treatment on the measured variables. Data were checked for normality and homogeneity of variance by plotting Q-Q plots and residuals vs fitted values. Significance was set at $P<0.05$ for all analyses. To meet the assumptions of the linear mixed effect model, proportion data were arcsine square root transformed (root: mass and Si data), total plant biomass and stem biomass data were squared, and aphid biomass and change in wireworm mass data were square root transformed.

Linear mixed-effects models (nlme package) (Pinheiro *et al.* 2014) or generalised linear mixed-effect models (*lmer*) (Bates *et al.* 2014) were used to analyse data with block as a random term in the model. Models were compared using AIC values and analysis of variance (ANOVA) to find the minimum adequate model (Crawley 2007) and the final models were checked for significance using 'car' package (Fox *et al.* 2014) to test the main and interactive effects of watering regime and insect herbivory on plant growth measurements. All measured growth parameters were assessed on nine replicates (plants) for each watering regime and herbivory treatment apart from ambient watered plants with no herbivory which was assessed on 8 plants due to a plant fatality. Leaf Si was assessed on seven replicates and aphid mass (g FM) was assessed on 14 to 16 replicates.

Ladybird mass gain was analysed using linear mixed effect models to test the main effects of watering regime, wireworm treatment with block and a category of aphid mass (FM) included as a random term. Ladybird prey choice was analysed using generalised linear mixed effects model (*glmer*) (Bates *et al.* 2014) to test the main effects of watering regime, wireworm treatment, and ladybird and aphid mass (FM) with arena included as a random term. Ladybird mass gain was assessed on five to seven replicates and ladybird prey choice was assessed on four to eight replicates.

Multiple comparison tests were performed using '*glht*' in '*multcomp*' package with *post-hoc* Tukey contrasts (Hothorn *et al.* 2014).

4.4 Results

4.4.1 Plant growth and biomass

D and DSRF treated plants had significantly smaller total plant biomass compared to ambient watered plants (*post-hoc* Tukey contrasts both $P<0.001$; Fig. 4.2). Changing the frequency of watering events had no effect on total plant biomass (*post-hoc* Tukey contrasts $P>0.05$). Wireworm herbivory significantly reduced total plant biomass under ambient watering regime, but not under D or DSRF (Fig. 4.2). Aphid herbivory had no effect on total plant biomass ($F_{1,87}=1.25$, $P=0.2671$) and these factors were removed from the statistical model when comparing AIC values and analysis of variance (ANOVA) to find the minimum adequate model (Crawley 2007)

D and DSRF treated plants had a significantly smaller root biomass compared to ambient watered plants (Fig. 4.3 (**A**)). Changing the frequency of watering events had no effect on root biomass (*post-hoc* Tukey contrasts $P>0.05$). Wireworm herbivory caused a significant reduction in total root biomass (Fig. 4.3 (**B**)) whereas those plants with aphid herbivory had a larger total root biomass (Fig. 4.3 (**C**)). Aphid herbivory significantly increased total root biomass collected from the bottom 7.5cm of the soil profile ($F_{1,93}=7.20$, $P<0.01$) but did not significantly affect root biomass in the top 7.5cm of the soil profile ($F_{1,87}=1.38$, $P=0.242$). There were no significant interactions between watering regime, aphid and wireworm herbivory on total root biomass. Root: mass ratio was unaffected by watering regimes and aphid herbivory treatments (Fig. 4.4).

Above ground biomass was significantly reduced by watering regime ($F_{2,93}=34.65$, $P<0.001$) and wireworm herbivory ($F_{1,93}=14.04$, $P<0.001$), and the effect of wireworms on above ground biomass was observed under ambient and D water regimes but had no effect under DSRF ($F_{1,93}=4.52$, $P<0.05$).

D and DSRF treated plants had a significant lower stomatal conductance compared to ambient watered plants ($F_{2,64}=29.41$, $P<0.001$; *post-hoc* Tukey contrasts $P<0.001$).Tissue water content (g) was significantly larger in ambient watered plants

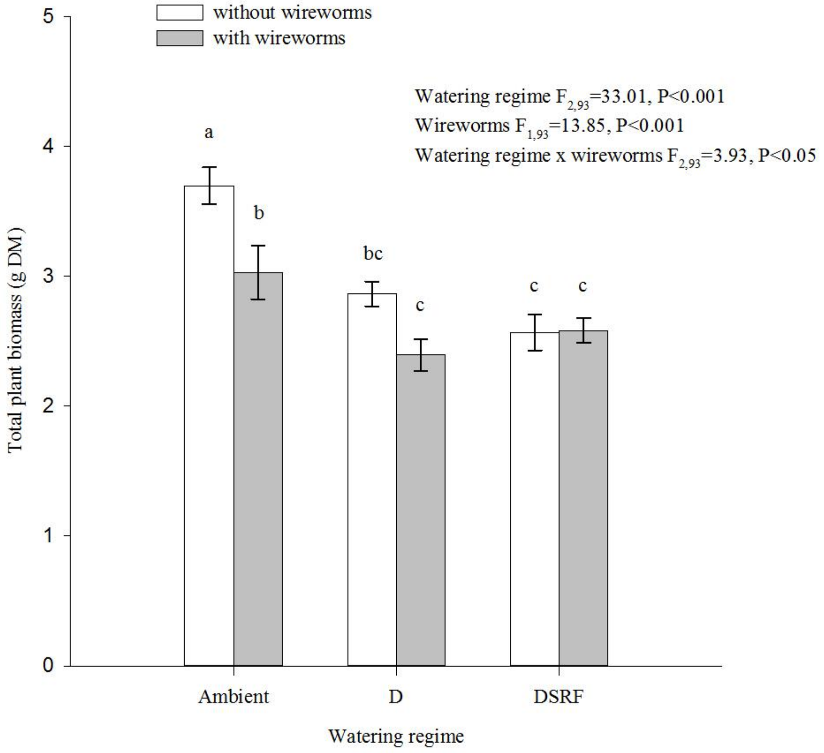

Fig. 4.2. Total plant biomass (g dry mass (DM)) of barley plants treated with different watering regimes, ambient, drought (D) and drought with severe reduced watering frequency (DSRF) with and without wireworm herbivory. Values represent mean ± standard error bars of 18 replicates for all watering regime and herbivory treatments apart from ambient watering regime without wireworms which represent 17 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

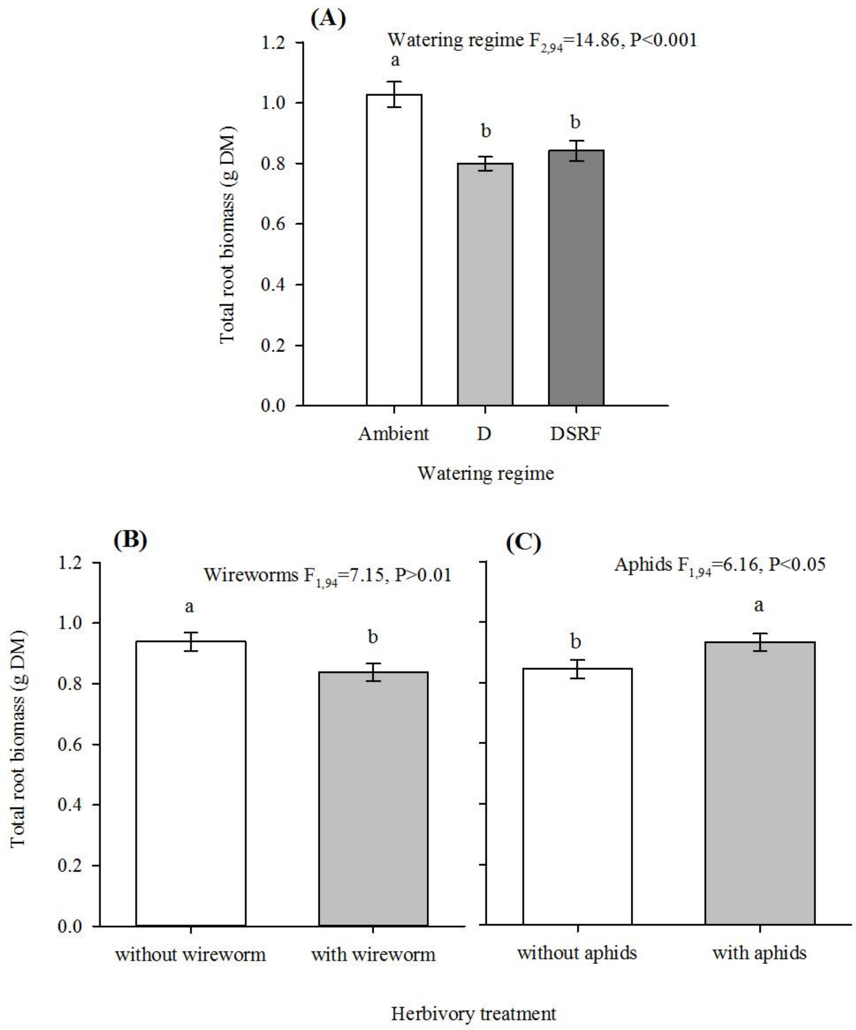

Fig. 4.3. (**A**). Total root biomass (g dry mass (DM)) of barley plants treated with watering regime ambient, drought (D) and drought with severe reduced watering frequency (DSRF), (**B**) wireworm herbivory, and (**C**) aphid herbivory. Values represent mean ± standard error bars of (**A**) 17-19, (**B**) and (**C**) 26-27 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

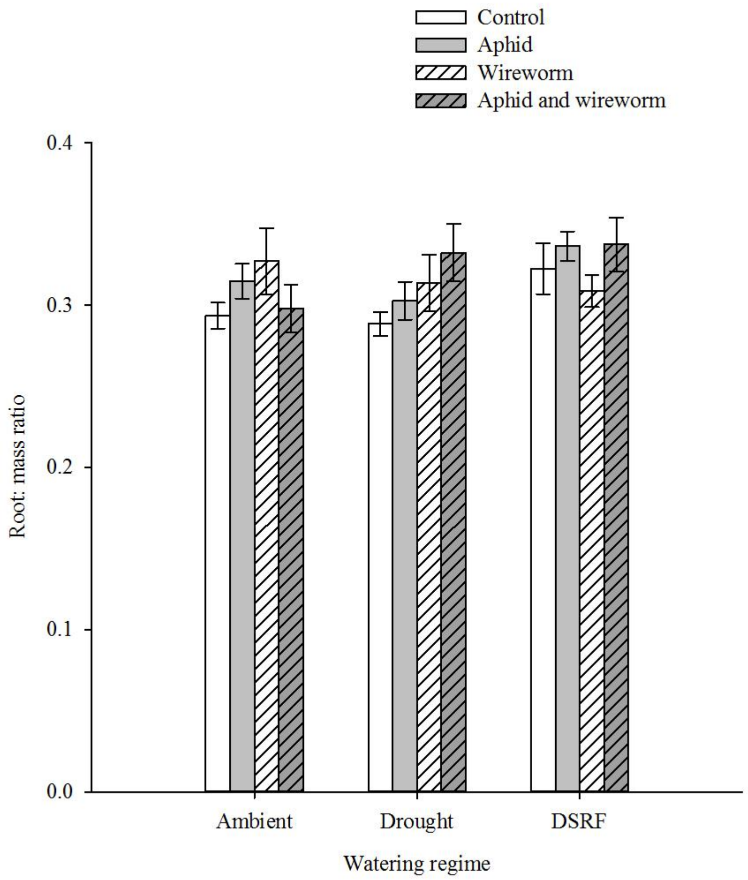

Fig. 4.4. Root: mass ratio of barley plants treated with different watering regimes, ambient, drought (D) and drought with severe reduced watering frequency (DSRF) with and without wireworm (hatched bars) and aphid (grey bars) herbivory. Values represent mean ± standard error bars of 9 replicates for all watering regime and herbivory treatments apart from ambient watering regime without herbivory which represent 8 replicates.

compared to D and DSRF ($F_{2,95}$=55.23, P<0.001) and smaller in plants subjected to wireworm herbivory ($F_{1,95}$=6.46, P<0.01) (Fig. 4.5). Aphids had no effect on plant water content (Fig. 4.5). There was a significant interaction found between watering regime and aphid herbivory on leaf C concentration (%), where aphids reduced leaf C under ambient and D treated plants but had no effect on leaf C concentration under DSRF watering regime (Fig. 4.6). Wireworms had no effect on leaf C concentration ($F_{1,66}$=0.10, P=0.732) and this factor was removed from the final statistical model when comparing AIC values and analysis of variance (ANOVA) to find the minimum adequate model (Crawley 2007). Regardless of herbivory treatment, plant grown under D and DSRF watering regime had a greater leaf N concentration compared to ambient watered plants (Fig. 4.6). Wireworms herbivory significantly increase leaf N concentration (%) (Fig. 4.6). There were no significant interactions between wireworms and watering regimes on leaf N concentration ($F_{2,66}$=1.756, P=0.1807) and aphids ($_{F1,66}$=0.204, P=6.532) had no effect on leaf N concentration therefore these factor were removed from the final statistical model when comparing AIC values and analysis of variance (ANOVA) to find the minimum adequate model (Crawley 2007). Plants grown under ambient watering regime had the greatest leaf Si concentration compared to DSRF and D treated plants, with D treated plants containing the smallest leaf Si concentrations (Fig. 4.7). Wireworm ($F_{1,65}$=0.009, P=0.93) and aphid herbivory ($F_{1,65}$=0.319, P=0.57) had no effect on leaf Si concentration and these factor were removed from the final statistical model when comparing AIC values and analysis of variance (ANOVA) to find the minimum adequate model (Crawley 2007)..

4.4.2 Wireworm distribution, survival and development

There was no effect of watering regime or aphid herbivory on survival, location or mass gain of wireworms (Table 4.1).

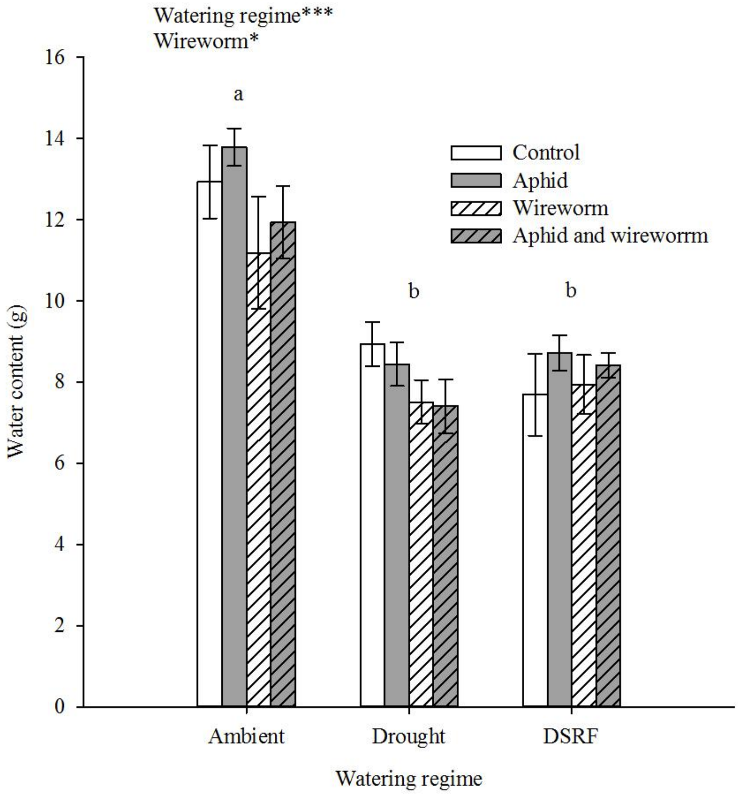

Fig. 4.5. Water content (g) of barley plants treated with different watering regimes, ambient, drought (D) and drought with severe reduced watering frequency (DSRF) with and without aphid (hatched bars) and wireworm (grey bars) herbivory. Values represent mean ± standard error bars of 9 replicates for all watering regime and herbivory treatments apart from ambient watering regime without herbivory which represent 8 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

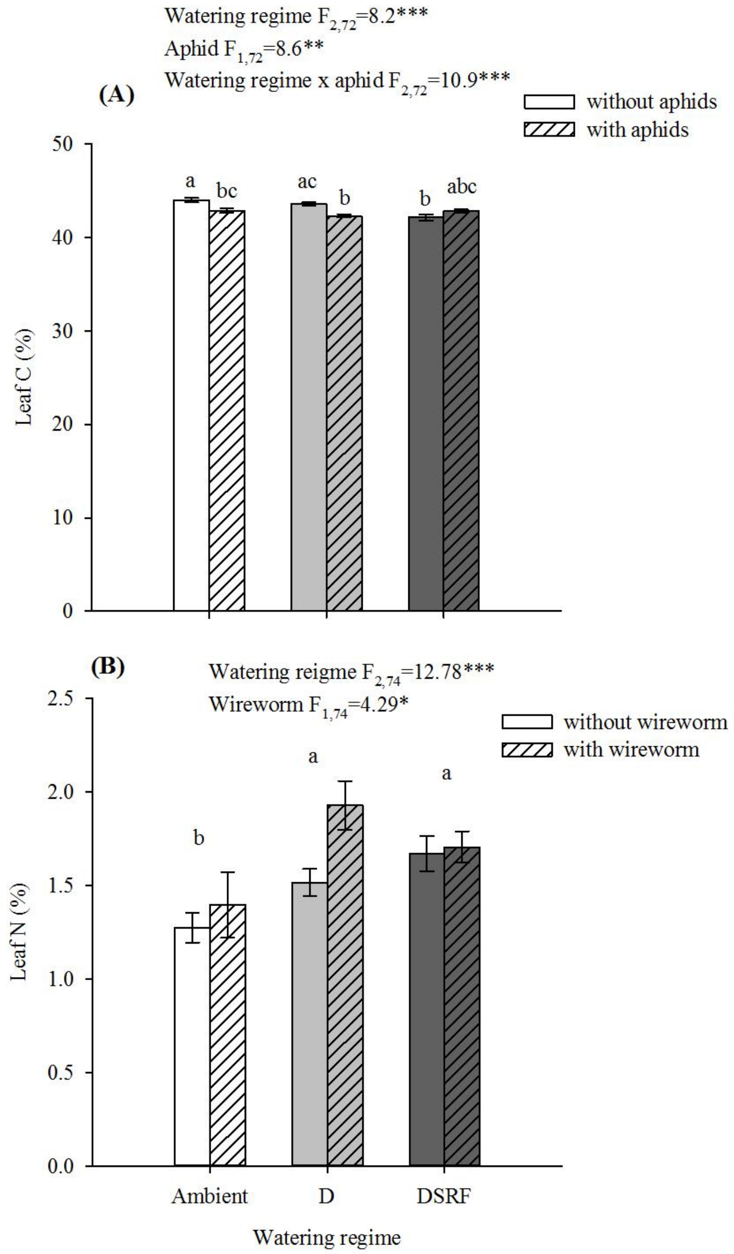

Fig. 4.6 (**A**) Leaf C and (**B**) N concentration of barley plants treated with different watering regimes ambient, drought (D) and drought with severe reduced watering frequency (DSRF), (**A**) with or without aphid or (**B**) wireworm herbivory. Values represent mean ± standard error bars of 14 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

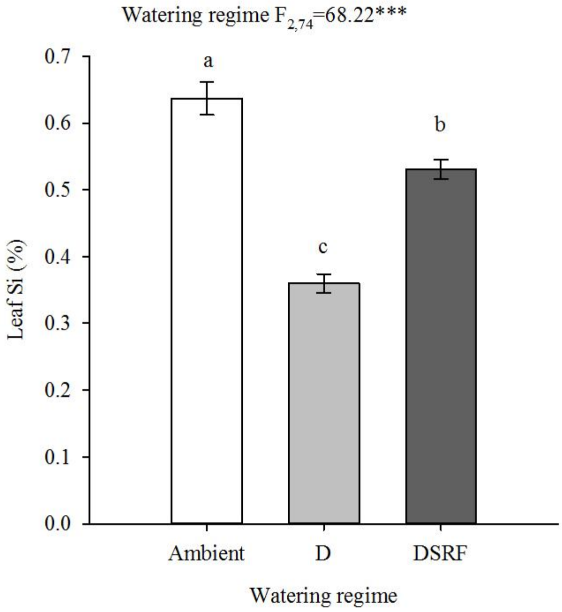

Fig. 4.7. Leaf Si concentration of barley plants treated with different watering regimes ambient, drought (D) and drought with severe reduced watering frequency (DSRF). Values represent mean ± standard error bars of 28 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

Table 4.1. The effect of different watering regimes and aphid herbivory on wireworm survival, location and mass gain.

	Watering regime						Statistics
	Ambient		D		DSRF		
	NA	Aphid	NA	Aphid	NA	Aphid	
Wireworms in the top of the pot (%)	48±17.0	33±11.1	57±13.4	59±22.1	18.3±5.8	44±13.6	Watering regime $F_{2,39}$=1.08, P=0.35 Aphid $F_{1,39}$=0.04, P=0.85 Watering regime x aphid $F_{2,39}$=0.71, P=0.50
Survival (%)	89±5.6	92±8.4	96±3.8	85±8.1	78±9.7	81±9.8	Watering regime $F_{2,39}$=1.38, P=0.26 Aphid $F_{1,39}$=0.02, P=0.90 Watering regime x Aphid $F_{2,39}$=0.89, P=0.42
Individual mass gain (mg FW)	0.0054±0.00163	0.0028±0.00163	-0.0022±0.00305	0.0037±0.00345	0.0054±0.00365	0.0017±0.00168	Watering regime $F_{2,39}$=0.37, P=0.69 Aphid $F_{1,39}$=0.67, P=0.42 Watering regime x Aphid $F_{2,39}$=0.58, P=0.57

Without aphid herbivory (NA), with aphid herbivory (A). Fresh weight (FW). Values represent mean ± standard error of 9 replicates for all treatments.

4.4.3 Aphid survival, development and reproduction

Aphid fecundity and survival was not affected by wireworms or watering regime (Table 4.2). Individual aphid total mass on DSRF treated plants was significantly heavier than those on ambient treated plants (Fig. 4.8 (**A**)).

4.4.4 Ladybird development and prey choice

Ladybird larvae gained significantly more mass when feeding on aphids collected from plants under DSRF watering regime (Fig. 4.8 (**B**)). Ladybird larvae choice was unaffected by the watering regimes or wireworm herbivory (Fig. 4.9).

Table 4.2. The effect of different watering regimes and wireworm herbivory on aphid fecundity and survival.

	Watering regime						Statistics
	Ambient		D		DSRF		
	NW	WW	NW	WW	NW	WW	
Fecundity	6.1±0.63	5.6±0.84	5.8±0.60	6.4±0.67	6.2±0.55	7.2±0.95	Watering regime χ^2_2=1.17, P=0.056 Wireworm χ^2_1=0.30, P=0.59 Watering regime x wireworm χ^2_2=0.94, P=0.63
Survival (%)	89±5.5	92±5.4	78±9.6	89±7.9	85±8.1	74±7.4	Watering regime $F_{2,39}$=0.93, P=0.40 Wireworm $F_{1,39}$=0.00, P=1.00 Watering regime x wireworm $F_{2,39}$=1.45, P=0.25

Without wireworm herbivory (NA), with wireworm herbivory (WW). Values represent mean ± standard error of 9 replicates for all treatments apart from Ambient watering regime without wireworms which represent 8 replicates.

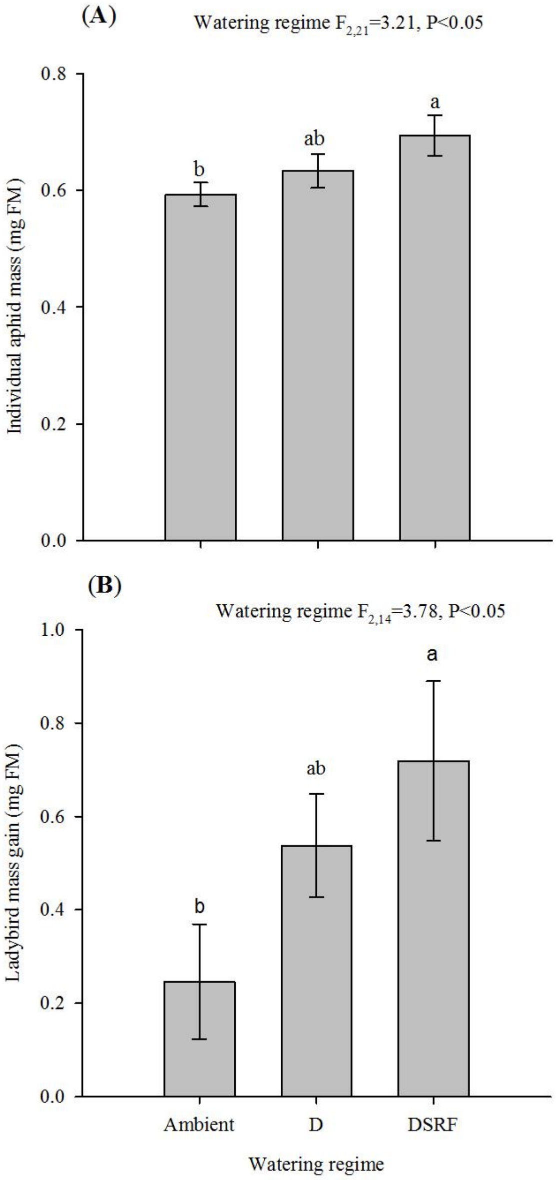

Fig. 4.8 (**A**) Individual aphid mass (mg fresh mass (FM) after 14d of feeding on barley plants treated with watering regimes ambient, drought (D) and drought with severe reduced watering frequency (DSRF). (**B**) Ladybird mass gain (mg fresh mass (FM) after feeding on aphids collected from barley plants treated with ambient, D and DSRF watering regimes. Values represent mean ± standard error bars of 30 to 31 replicates for aphid mass and 11 to 13 replicates for ladybird mass gain. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

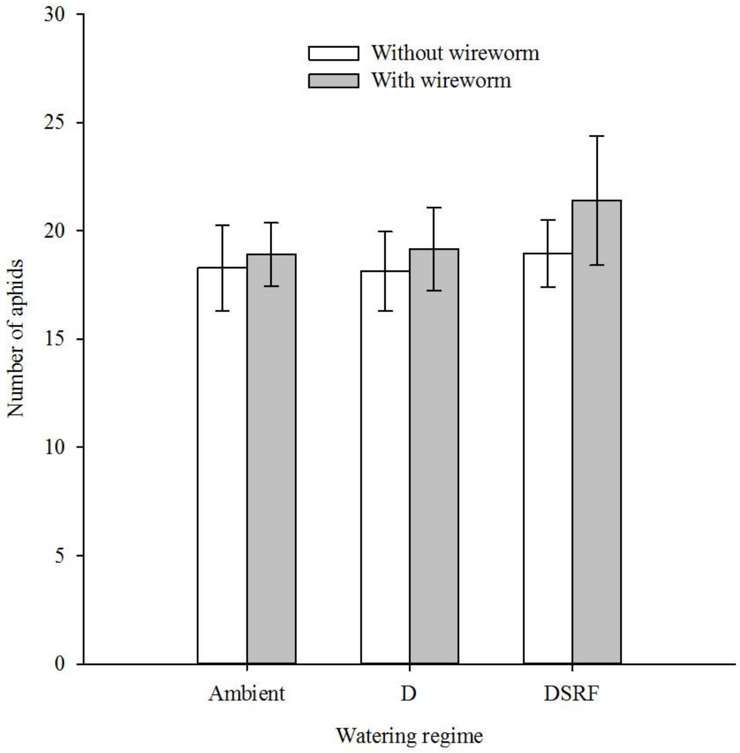

Fig. 4.9. The number of aphids from plants under different watering regimes chosen to be eaten first by a Harlequin ladybird larvae in an arena experiment. Values represent mean ± standard error bars of four to eight replicates.

4.5 Discussion

The effect of changes in both the quantity and frequency of watering events on tri-trophic interactions in a barley ecosystem were assessed. 40% reductions in water quantity reduced barley plant biomass, whereas reductions in watering frequency had no effect on plant biomass. Wireworms had a larger impact on barley plants grown under ambient watering regime but had no effect on plants under a 40% reduction regime regardless of watering frequency. In contrast aphids gained more mass on plants under 40% reduction regimes watered less frequently and ladybirds feeding on these aphids gained more weight. Therefore reductions in water quantity together with reductions in watering frequency could have significant consequences for barley production through changes in plant growth and pest dynamics.

4.5.1 The effect of the watering regime on barley growth and development, and insect performance

Reducing the quantity of water added by 40% significantly reduced total plant biomass including reductions in both above and below ground plant biomass as well as a reduced plant water content and stomatal conductance. Published studies also report reductions in total plant biomass and yield of drought stressed barley plants (Jamieson *et al.* 1995; Legg *et al.* 2009). Barley plants grown under the 40% reduction regime also had an increase in leaf N concentration and a decrease in leaf Si concentration supporting previously reported results in Chapter 3. Increases in leaf N and compatible solutes are commonly reported in drought stresses plants believed to play roles in maintaining turgor, protein stabilisation and cell structure (Chaves *et al.* 2003; Chaves and Oliveira 2004; Farooq *et al.* 2009). Changes in transpiration due to reductions in water availability could have resulted in reduced leaf Si uptake, however the relationship between leaf Si and transpiration is not fully understood with recorded intra- and interspecific differences in leaf Si concentrations suggesting that there may be other mechanisms involved in Si uptake (Hartley *et al.* 2015). Increased leaf N together with reduced leaf Si concentrations may influence the host suitability for pests under future precipitation as high leaf N can improve insect performance (Mattson and Haak 1987; Huberty and Denno 2004) and Si can reduce insect herbivory by increasing leaf surface abrasiveness in grass species (Massey *et al.* 2006). Significant reductions in total barley plant biomass will have important consequences for future barley production in the UK with predicted reductions of 40% in summer precipitation by 2080 (Murphy *et al.* 2009).

Seven weeks after sowing, reductions in the frequency of watering events had no effect on total plant biomass and water content. Chapter 2 and Chapter 3 reported that DSRF plants had an increase in biomass. Differences between the experiments are most likely due to differences in plant age and length of exposure to the different watering regimes. However, these results support previous conclusions that continuous reductions in the quantity of rainfall maybe more damaging to barley plant growth and development than changes in the frequency of extreme precipitation.

Wireworms caused a significant reduction in total plant biomass and water content only in ambient watered plants. This suggests that future changes in precipitation may reduce the incidence and impact of wireworm herbivory. Wireworms have been previously described as sensitive to temperature and soil moisture (Lees 1943a; Parker and Howard 2001). The difference in impact of wireworm herbivory under the watering regimes may also influence barley plant competitive abilities against weed species in the future when grown in the field (Johnson *et al.* 2011). Wireworms also caused a significant increase in barley plant leaf N concentration potentially due to the removal of root biomass resulting in reduced water uptake and a larger reduction in plant growth. Despite having a larger effect on barley plants under ambient watering regime, wireworms themselves appeared to be unaffected by the different watering regimes. Wireworm fresh mass is difficult to assess however, as moulting can significantly affect body mass (Parker and Howard 2001). The survival and location of the wireworms in this study was also unaffected by the different watering regimes at harvest. This may be due to the nature and severity of the drought stress, the soil moisture gradient and shallow depth of the soil profile in a confined pot experiment.

Aphid herbivory did not affect total plant biomass but was found to increase root biomass in the lower region of the soil profile. The relatively small effect of aphids on plant biomass is most likely due to the small numbers of aphids, short duration and confined nature of the aphid herbivory treatment. It has been previously reported that the number of aphids, plant growth stage and duration of herbivory influence plant growth and development (Giordanengo *et al.* 2010). Changes in root biomass could be due to aphid herbivory removing phloem sap and increasing transpiration resulting in an increased root growth for water uptake without the significant damaging effects of an aphid infestation. Aphid herbivory also significantly reduced leaf C concentrations under ambient and D watering regimes suggesting that either the aphids were removing larger quantities of C from these plants (Awmack and Leather 2002) or suggestive of a plant defensive response which was absent in plants watered less frequently (Giordanengo *et al.* 2010). Changes in the frequency of watering events affected the fresh mass of aphids. Heavier aphids were collected from plants watered less frequently under the 40% reduction regime, supporting the pulsed stress hypothesis (Huberty and Denno 2004). Insect body size has been reported to correlate with insect performance and fecundity (Honek 1993) however, in this study the number of offspring produced was unaffected by the watering regime of the adult aphids host plant. This is in contrast with the results reported by Tariq *et al.* (2012) where generalist and specialist aphid fecundity was greatest on medium drought stressed plants compared to pulsed water stress supporting the plant stress hypothesis.

4.5.2 The interaction between above and below ground herbivory

Despite the effect of wireworms on plant growth and chemical composition as well as previously published studies reporting the effects of wireworms on above-ground aphids (Masters *et al.* 1993; Tariq *et al.* 2013), there was no above- and below-ground interactions recorded in this experiment. Aphids were also found to have no effect on wireworms through the shared plant host. The differences in results may be due to different plant species, the severity of the stress and different species of root and phloem feeders (Gange and Brown 1989; Masters *et al.* 1993; Johnson *et al.* 2012).

4.5.3 The effect on the third trophic level

Ladybirds had a greater increase in mass when feeding on aphids collected from plants grown under reduced watering frequency. As insect mass is often correlated with increased insect fecundity and performance (Awmack and Leather 2002) it could be that ladybirds may have a greater fitness when feeding on aphids from plants grown under future predicted rainfall patterns. Despite the increase in mass when feeding on aphids collected from ambient treated plants, ladybird choice of aphid prey was unaffected by the different watering regimes. Previous research has also found that predator choice does not follow optimal prey diet (Sih and Christensen 2001) and this could influence ladybird success under future changes in precipitation (Hassel and Southwood 1978; Mayhew 2001).

4.6 Conclusions

40% reduction in water quantity caused significant reductions in plant biomass and water content whereas reducing watering frequency had no effect on plant growth. Wireworms had a larger effect on plant biomass and water content under ambient watering conditions and had no effect on plant biomass under future rainfall patterns. The different watering regimes had no effect on wireworm survival, distribution and growth. Whereas aphids collected from DSRF treated plants were heavier and ladybirds feeding on these aphids gained more mass. This suggests that aphids and ladybirds would benefit from future changes in the frequency of precipitation events. However, there was no effect of the different watering regimes on the survival and fecundity of the aphids. This study suggests that predicted changes in the frequency and intensity of extreme rainfall events will have significant impacts on barley production as well as above- and below- ground insect herbivores, and these effects can transfer to third trophic level impacting insect predators.

CHAPTER 5

Future Changes in Rainfall Removes Above- and Below- Ground Insect Herbivore Interactions in a Field Experiment

5.1 Abstract

The occurrence of extreme drought and heavy rainfall events in the UK is predicted to increase by 2080, with reductions in mean summer precipitation. Future changes in precipitation have the potential to destabilise terrestrial ecosystems mediated by changes in plant growth and chemical composition. The aim of this study was to investigate how predicted changes in the quantity and frequency of rainfall events will impact multi-trophic interactions in a barley ecosystem in a field situation.

Barley (Hordeum vulgare L. cv. Optic) plants were grown in pots under rain exclusion shelters, subjected to root herbivory (Agriotes wireworm spp) and three precipitation scenarios: ambient, drought (40% reduction in water quantity), and drought with reduced watering frequency (40% reduction in water quantity, delivered 25% as frequently as ambient and drought). Barley plants were exposed to natural establishment of aphids and their natural enemies. The effect of the different watering regimes was assessed by measuring the growth and yield of barley plants, the mass of wireworms and abundance of aphids and their natural enemies.

A 40% reduction in water quantity significantly reduced plant growth and yield, whereas reducing watering frequency alleviated some of the negative effects of drought on plant growth supporting previously reported results from controlled environment rooms. Despite the reduction in plant biomass, reductions in water quantity and watering frequency had no effect on above-ground aphid abundance. 40% reduction in water quantity removed the negative effect of wireworm herbivory on the number of aphids during early plant development. Natural enemy abundance was very low throughout the experiment most likely due to extreme temperatures with the number of mummified aphids unaffected by the different watering regimes.

Reductions in precipitation may be more detrimental to plant growth and yield under future climate scenarios than changes in precipitation frequency. Changes in the frequency of rainfall events could affect below- ground insect herbivore performance and influence above- and below- ground interactions during early plant development.

5.2 Introduction

Predicted changes in the frequency and intensity of UK precipitation have the potential to influence terrestrial ecosystems via changes in plant growth, development and chemical composition (Weltzin *et al.* 2003; Knapp *et al.* 2008; Trotter *et al.* 2008). The abundance and performance of insects feeding on water stressed plants can either be negatively affected by reductions in plant growth and thus reductions in food resource; or positively affected through reductions in host plant defence and increases in leaf nitrogen (Rhoades 1985; Mattson and Haak 1987; Huberty and Denno 2004). The impact of water stress on herbivorous arthropods is dependent on the severity and duration of the water stress, plant species and developmental stage as well as the species, age and feeding guild of the herbivore (Huberty and Denno 2004). The nature of the water stress can also be important for example aphids can benefit from pulsed water stress due to changes in plant defence, increases in free amino acids simultaneous with periods of phloem turgor recovery (Larsson 1989; Huberty and Denno 2004; Mody *et al.* 2009). Furthermore water stress can impact the behaviour of insect herbivores for example below-ground insect herbivore abundance and vertical distribution has been previously reported to be sensitive to soil moisture. Soil dwelling invertebrates have been recorded to move deeper in the soil profile under drought conditions (Lees 1943a; Briones *et al.* 1997; Staley *et al.* 2007a; Sinka *et al.* 2007). Attractants such as primary and secondary metabolites and chemical signals involved in plant defence can be influenced by drought stress affecting host preference of phytophagous herbivores such as aphids (Mody *et al.* 2009; Khan *et al.* 2010; Tariq *et al.* 2012) which often feed on more than one species of host plant and have the ability to disperse to a more suitable host if appropriate (Powell *et al.* 2006).

Root herbivory can cause a drought response in the host plant due to the removal of roots and consequently a reduction in water uptake from the soil. Therefore root herbivory can exacerbate the effects of drought stress on plant growth by reducing root surface area important in absorbing water under low soil moisture content, with serious consequences for crop production (Masters *et al.* 1993). Root herbivory has been reported to cause changes in host plant growth and chemical composition which can also influence above-ground herbivores through changes mediated by the shared plant host (Masters *et al.* 1993). For example aphids have been reported to be positively affected by root feeding coleoptera (Johnson *et al.* 2012). The effects of root feeding arthropods interacting with anticipated changes in precipitation on the abundance and performance of above-ground insect herbivores has the potential to affect the third trophic level through changes in their prey quality and quantity (Johnson *et al.* 2013). For example drought stress was reported to affect aphid population demography which consequently reduced parasitism rates (Aslam *et al.* 2013). However, there is very little understanding of how predicted changes in both the quantity and frequency of rainfall events in a field system will affect multi-trophic interactions including above- and below- ground interactions in a cereal ecosystem.

Barley is one of the main crops grown countrywide in the UK and barley growth, development and yield have been previously reported to be negatively affected by drought and flooding (Lawlor *et al.* 1981; Jamieson *et al.* 1995; Yordanova, Alexieva and Popova 2003; Forster *et al.* 2004; Abebe *et al.* 2010; de San Celedonio *et al.* 2014). Barley grown in the field can be simultaneously attacked by wireworms (*Agriotes* spp) below-ground and aphids (species such as *Rhopalosiphum padi, Sitobion avenae*) above-ground, significantly reducing barley yield (HGCA 2003; Gregory *et al.* 2009). Commonly occurring natural enemies of aphids in a barley ecosystem include ladybirds (such as *Adalia bipunctata*, *Harmonia axyridis* and *Coccinella septempunctata*) and parasitoid wasps (e.g. *Aphidius ervi*) which in the future will become even more important in controlling insect pests due to reductions in the use of chemicals such as pesticides to minimise the negative impacts of agriculture. Previous chapters have reported results from experiments manipulating the frequency and magnitude of rainfall events on barley growth and development as well as the effects on tri-trophic interactions under controlled environment conditions. Although controlled environment cabinet experiments provide fundamental understanding of this system, conditions in controlled environments might not reflect those of the field environment (Hughes 1959). Predicted changes in the quantity and frequency of rainfall events may affect plant growth and development differently in field conditions compared to controlled environment rooms due to differences in rates of soil drying, air flow, radiation, temperature and soil structure. It is therefore important to ensure that previously reported results are not an artefact of the controlled environment rooms (Rajan, Betteridge and Blackman 1971).

The aim of this chapter was to investigate the impact of root herbivory and changes in water quantity and watering frequency on plant growth and above- ground insect herbivores in a field system and to determine if the consequences of these treatments affect the third trophic level, i.e. predator and parasitoid abundance.

5.2.1 Hypotheses

- Reducing the quantity and frequency of watering events would negatively affect the growth of barley plants and wireworms but would positively affect the abundance and development of aphids.

- Below-ground herbivory would positively affect the abundance and mass of above-ground herbivores.

- The effects of the watering regimes and/ or wireworm herbivory would impact the third trophic level, affecting the abundance of natural enemies.

- The impact of the different watering regimes on multi-trophic interactions in a barley system would be similar to those reported in controlled environment rooms.

5.3 Materials and Methods

Nine spring barley (*Hordeum vulgare* L. cv. Optic) (supplied by James Hutton Institute) plants were grown in 15L pots (25.5cm × 25.5cm × 25.5cm) lined with plastic sheeting and filled with dried, sieved (10x10mm) topsoil (collected from barley arable farm land, High Pilmore, Invergowrie, Dundee) mixed in a 3:1 ratio with washed sharp horticultural sand (Keith Singletans, UK). Pots were wrapped in insulating material (Thermawrap loft insulation, 400mm x 5m, BandQ) and covered in Fine Mesh Garden Protection Net (2cm diameter netting, BandQ, UK) which was suspended c. 50cm above the pot and draped down the sides of the pots to prevent small mammalian herbivory but to allow small arthropod access to the plants. soil water holding capacity was calculated from the saturation and desiccation (soil dried at 105^{o}C for 7d) weight of a known volume of soil/ sand mix.

Plants were grown under rain exclusion shelters (Fig. 5.1) which consisted of a wooden frame with a 2100mm × 3500mm polycarbonate sheet (6mm thick polycarbonate sheeting, Polycarbonate Direct, Hull, UK) at an angle of 6^{o}. Total area under the rain exclusion shelter was 79200cm^{2}, 1176mm tallest height and 800mm shortest end. Compared to outside of the rain exclusion shelter, light intensity under the rain exclusion shelter (c. 900 µmol. m^{-2} sec^{-1}) was reduced by c. 60%. A Met station positioned in the same field provided meteorological data for the experiment. Soil temperature of the field was av. 14^{o}C ± 0.298 at 10cm and 14^{o}C ± 0.299 at 20cm. Soil temperature in the pots was unaffected by the rain exclusion shelter. Air temperature throughout the experiment was unusually high for the location. Maximum air temperatures were on av. 18.4^{o}C ± 0.351 with minimum temperatures averaging 9.4^{o}C ± 0.294 (Fig. 5.2).

The experiment comprised of a randomized block design with nine blocks (rain exclusion shelters). Initially, 18 pre-germinated seeds (soaked in deionised water at room temperature for c. 24hours and incubated on Petri-dishes lined with damp paper towelling at 15^{o}C for 3 days) were randomly sown across the surface of the each pot c. 2cm from the soil surface on 03/06/2013. To initiate germination, pots were watered to ensure soil water content reached 50% and from this point onwards

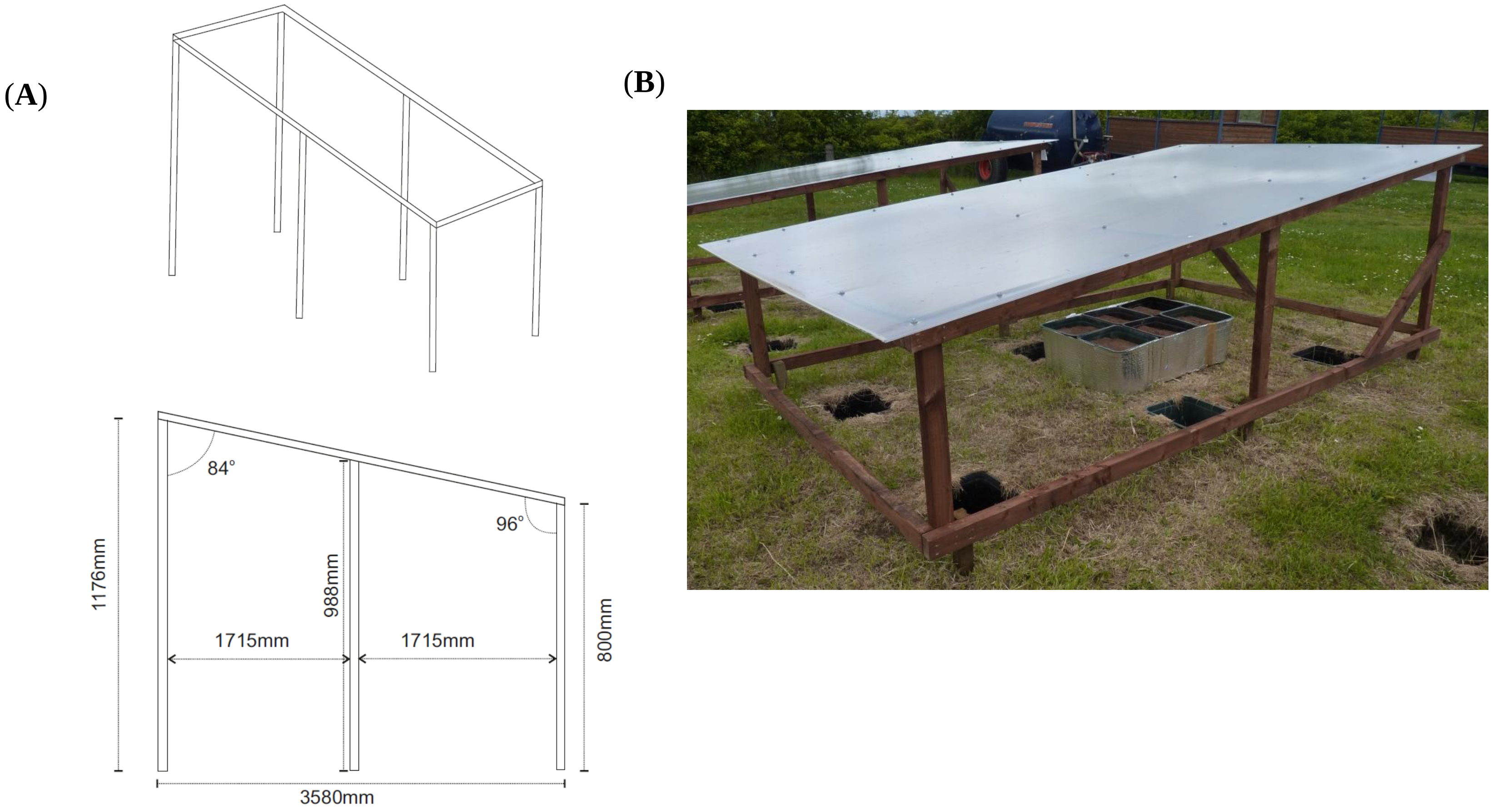

Fig. 5.1. (**A**) Diagram and dimensions of one rain exclusion shelter. Total area under rain exclusion shelter was 79200cm^2. (**B**) Photograph of a rain exclusion shelter *in situ* (photograph taken by R Wade)

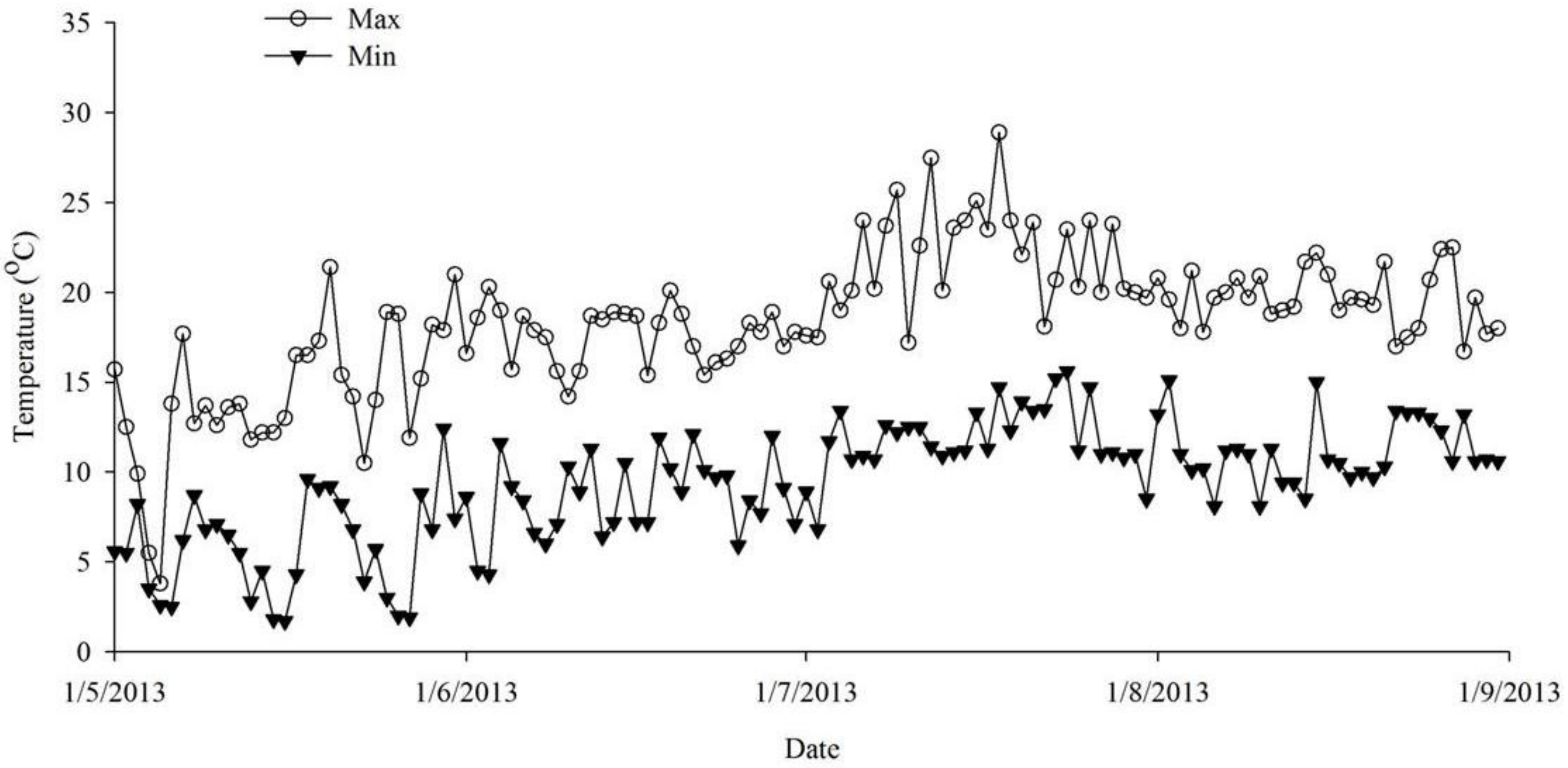

Fig. 5.2. Maximum and minimum air temperature (^{o}C) recorded during May to September 2013 by MET station positioned at Invergowrie, Dundee.

pots were watered from the top twice per week for two weeks with 500ml of deionised water. Following germination, seedlings were removed leaving nine plants per pot of consistent height and development stage randomly spread across the pot surface. Plants in five of the blocks were severely damaged by mammalian herbivores and these blocks had to be re-started. Any remaining plant material was removed, pots were re-weighed and if required water was added to ensure soil moisture in the pot was 50% of total soil water holding capacity. Fresh seedlings were then treated as previously described and sown on 24/06/2013. This meant that the plants in five blocks were staggered temporally by three weeks. Within each block, watering regime and root herbivory were assigned at random to each pot, one replicate pot per block of each watering regime × herbivory combination. There were six pots under each rain exclusion shelter and nine rain exclusion shelters in total.

5.3.1 Watering regimes

Two weeks after sowing, the following watering regimes commenced:

- Ambient: quantity of water added based on 10 year av. rainfall at The James Hutton Institute, Invergowrie, Scotland, watered twice per week (550ml delivered twice a week)

- Drought (D): 40% reduction in the quantity of water added, watered twice per week, (330ml delivered twice a week)

- Drought with Severe Reduced watering Frequency (DSRF): 40% reduction in the quantity of water added with severe reduced watering frequency, watered once per fortnight (1320ml delivered once every other week).

These different watering regimes allowed comparison of the effects of reductions in water quantity (40% reduction compared to ambient) and reductions in watering frequency under the 40% reduction regime (water provided once per fortnight) (Fig. 5.3).

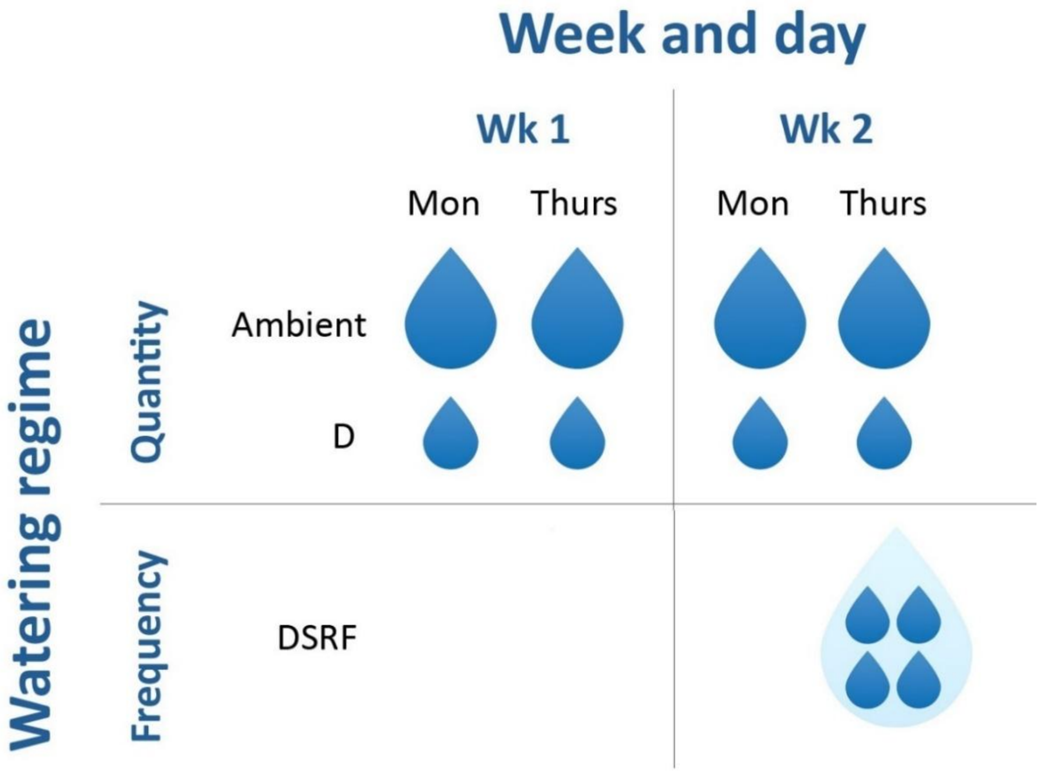

Fig. 5.3. Diagram representing the different watering regimes implemented with changes in water quantity at each watering event and the watering frequency.

Theta probes (Delta-T ML2 connected to a DL6 data logger and downloaded using DeltaLINK software) were buried horizontally 10 cm from the soil surface in all six pots under one rain exclusion shelter (one block) to measure soil moisture every minute throughout the experimental period (Fig. 5.4).

5.3.2 Below- ground herbivory

Prior to the experiment, wireworms (*Agriotes* spp. L. Coleoptera: Elateridae. A mix of *Agriotes* species, c. 60% *Agriotes lineatus* and 40% *A. obscurus* sourced from Praktijkonderzoek Plant and Omgeving / Applied Plant Research, Wageningen UR) were cultured and maintained on Rooster potatoes (Albert Bartlett) at 7^{o}C (Johnson *et al.* 2008a). Three weeks after sowing, three wireworms were added to those plants randomly assigned below-ground herbivory. Wireworms were weighed and then buried c. 5cm from the soil surface in three different locations around the plant. Soil disturbance was replicated for pots that were not assigned wireworm herbivory. Wireworms were then collected, counted and re-weighed and mass gain calculated.

5.3.3 Aphid and natural enemy abundance

Plants were open to natural establishment of above-ground herbivores and natural predators. Three barley plants in each pot were chosen at random and identified using a small piece of cotton tied very loosely around the main stem to measure insect herbivore and natural enemy abundance. Total number of aphids and natural enemy abundance including mummified aphids on these three plants per pot were recorded once a week throughout the growing period. At harvest, all aphids from all plants in each pot were collected and counted, then transferred to 1ml Eppendorf tubes, flash frozen in liquid nitrogen and stored at -20^{o}C. Frozen aphids were then freeze-dried and re-weighed, and individual aphid weight calculated by dividing total aphid weight per pot by the number of aphids collected.

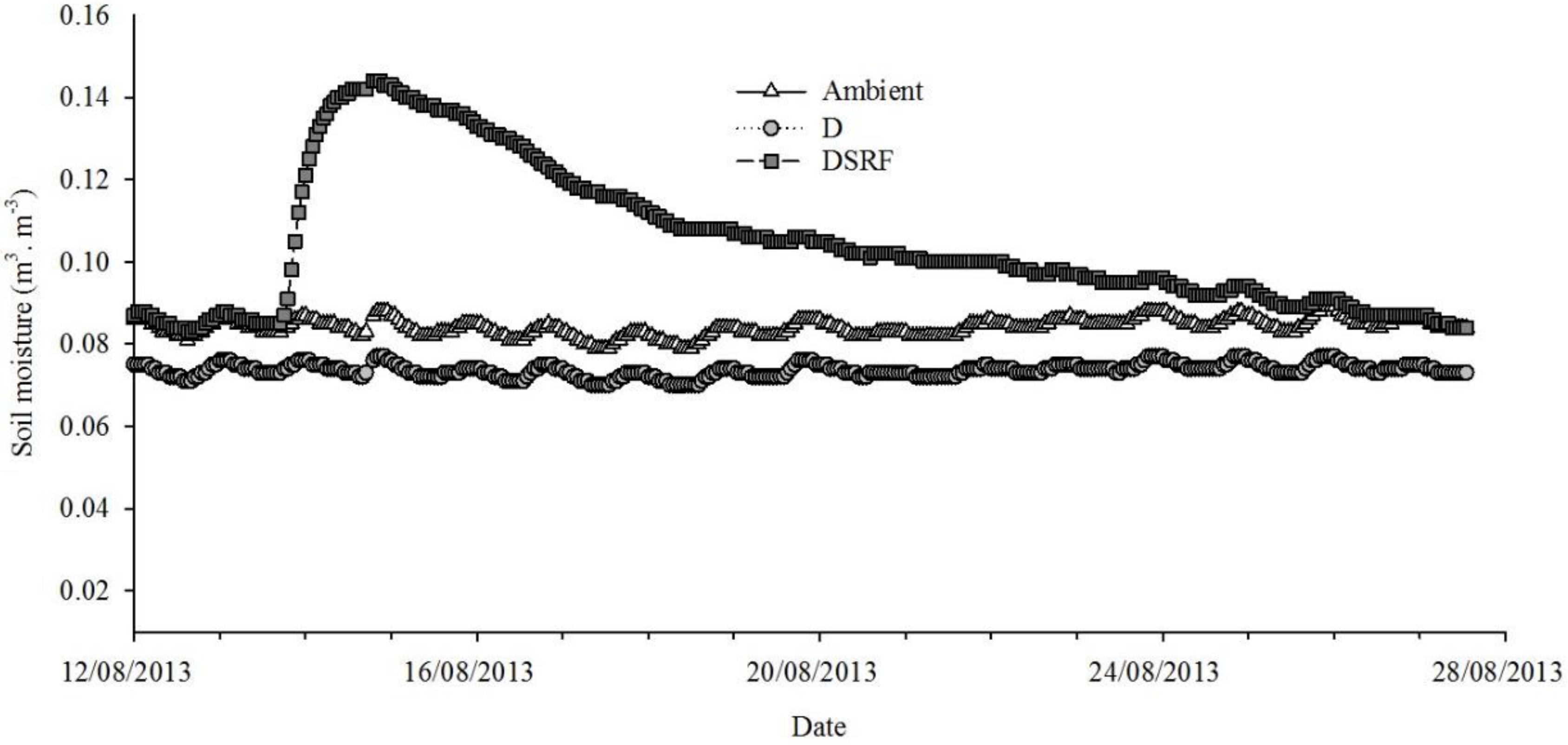

Fig. 5.4. Soil moisture (m^3. m^{-3}) measurements in pots under different watering regimes throughout a representative two weeks.

5.3.4 Plant growth and yield measurements

Barley plants in each experimental block were harvested 10 weeks after sowing (re-sown younger plants were harvested three weeks after older plants). The ear on main stem of all plants reached Zadok's growth stage 71 (Zadoks *et al.* 1974). Three barley plants randomly selected for herbivore and natural enemy abundance identified with a loosely tied piece of cotton (see above), were measured at harvest for percentage of plant infected with powdery mildew and relative chlorophyll content (CCM200 Chlorophyll Content Meter, Opti-sciences) of the third fully expanded leaf on the main stem. Ears, leaves, stem and roots in the top and bottom 12cm of the soil profile from all nine plants within the pot were separated, the number of ears and tillers counted and plant material weighed for fresh mass. Plant material was then dried at 70^{o}C for c. five days and re-weighed, and biomass, calculated. Water content (g) of the plant material was calculated by subtracting the dry mass from the fresh mass. Root: mass ratio was calculated by dividing the dry mass of roots by total dry plant biomass.

5.3.5 Statistical analysis

Statistical analyses were performed in R (version 3.1.0) to test the main and interactive effects of watering regime and wireworm herbivory on the measured variables. Data were checked for normality and homogeneity of variance by plotting Q-Q plots and residuals vs fitted values (Crawley 2007). Significance was set at $P<0.05$ for all analyses. Proportion data (powdery mildew infection and root: mass ratio) were arcsine square root transformed. Log transformation was performed on root biomass data and root biomass in top 10cm of pot data was cubed to meet the assumptions of the linear mixed effect model.

Linear mixed-effects models (*lme* from package *nlme*) (Pinheiro *et al.* 2014) were used to analyse continuous data with block included in the model as a random term. Generalised linear mixed-effect models (*glmer* from package *lme4*) (Bates *et al.* 2014) were used to analyse count data. For repeated measures data, time (date) was included in the model as a fixed effect and individual pot included as a random term.

Modes were compared using AIC values and analysis of variance (ANOVA) for stepwise elimination of non-significant terms to find the minimum adequate model (Crawley 2007) and the final models were then analysed using ‘*anova’* (F statistic) or *‘Anova’* (‘*car*’ package (Fox *et al.* 2014) chi-square statistic) to test the main and interactive effects of watering regime and insect herbivory on plant growth parameters. All measured growth parameters were assessed on nine replicates (pots) for each watering regime/ herbivory treatment. Multiple comparison tests were performed using ‘*glht’* in *multcomp* package with *post-hoc* Tukey contrasts (Hothorn *et al.* 2014).

5.4 Results

The effects of the watering regimes on barley growth and yield are summarised in Table 5.1.

5.4.1 Plant growth, development and yield

Wireworms had no effect on plant growth and developed of the barley plants and there was no significant interaction found between watering regime and wireworm herbivory on any of the plant growth parameters measured. Therefore wireworm herbivory was removed from statistical analysis of measured growth parameters by a stepwise elimination of non-significant terms to find the minimum adequate model (Crawley 2007).

Regardless of root herbivory, watering regimes significantly reduced total plant biomass per pot when compared to plants grown under ambient watering regime (Fig. 5.5 and Fig. 5.6 (**A**)). Plants watered with a 40% reduction in water quantity (D) had a decrease in ear, leaf, stem and root biomass as well as fewer ears and leaves compared to the ambient watered plants at harvest (Table 5.1). However, plants treated with a 40% in water quantity but watered once per fortnight (DSRF) had a larger total plant biomass compared to those watered twice a week under the 40% reduction regime (D). Plants watered once every fortnight (DSRF) also had a larger leaf and stem biomass as well as a greater number of ears and tillers compared to plants watered twice a week (D) (Table 5.1). Although DSRF plants had a greater number of ears than D treated plants there was no difference in ear biomass (Table 5.1). There was also no significant difference between root biomass of the D and DSRF treated plants ($F_{1,43}$=1.63, P=0.21). Root: mass ratio was unaffected by the watering regimes ($F_{2,40}$=1.59, P=0.22) or wireworm herbivory ($F_{1,40}$=0.97, P=0.33). However, D treated plants had a significantly smaller proportion of total roots in the top 12cm of the pot compared to ambient and DSRF treated plants (Fig. 5.6 (**B**)).

Regardless of root herbivory, D treated plants also had a significant reduction in relative leaf chlorophyll content compared to plants under ambient and DSRF

Table 5.1. The effects of the different watering regimes on measured parameters of barley plants at harvest.

Growth parameter	Watering regime			Statistics (df $_{2,43}$)	
	Ambient	D	DSRF	F	P
Total plant biomass (g DW)	34.83 ± 0.816^a	18.98 ± 0.381^b	21.44 ± 0.934^c	168.73	<0.001
Leaf biomass (g DM)	5.55 ± 0.090^a	3.21 ± 0.106^b	3.94 ± 0.195^c	102.56	<0.001
Stem biomass (g DM)	14.48 ± 0.309^a	7.57 ± 0.205^b	9.12 ± 0.413^c	179.32	<0.001
Root biomass (g DM)	6.91 ± 0.407^a	3.76 ± 0.162^b	3.95 ± 0.349^b	37.41	<0.001
Ear biomass (g DM)	7.89 ± 0.518^a	4.44 ± 0.315^b	4.43 ± 0.325^b	66.84	<0.001
No. of ears	20.8 ± 0.944^a	12.7 ± 0.866^b	14.5 ± 0.746^b	20.07	<0.001
Relative leaf chlorophyll content	33.6 ± 2.49^{ab}	24.9 ± 2.56^a	37.2 ± 3.72^b	5.10	=0.01
Total plant water content (g)	80.55 ± 1.906^a	39.90 ± 1.158^b	47.6 ± 1.945^c	184.75	<0.001
Powdery mildew infection (%)	24.4 ± 0.017^{ab}	20.0 ± 0.020^a	26.1 ± 0.030^b	4.51	<0.05

Mean ± standard error of 18 replicates. Means with the same letters were not significantly different as determined by *post-hoc* Tukey contrasts. No significant effect of watering regime, root herbivory or the interaction between watering regime and root herbivory on root: mass ratio.

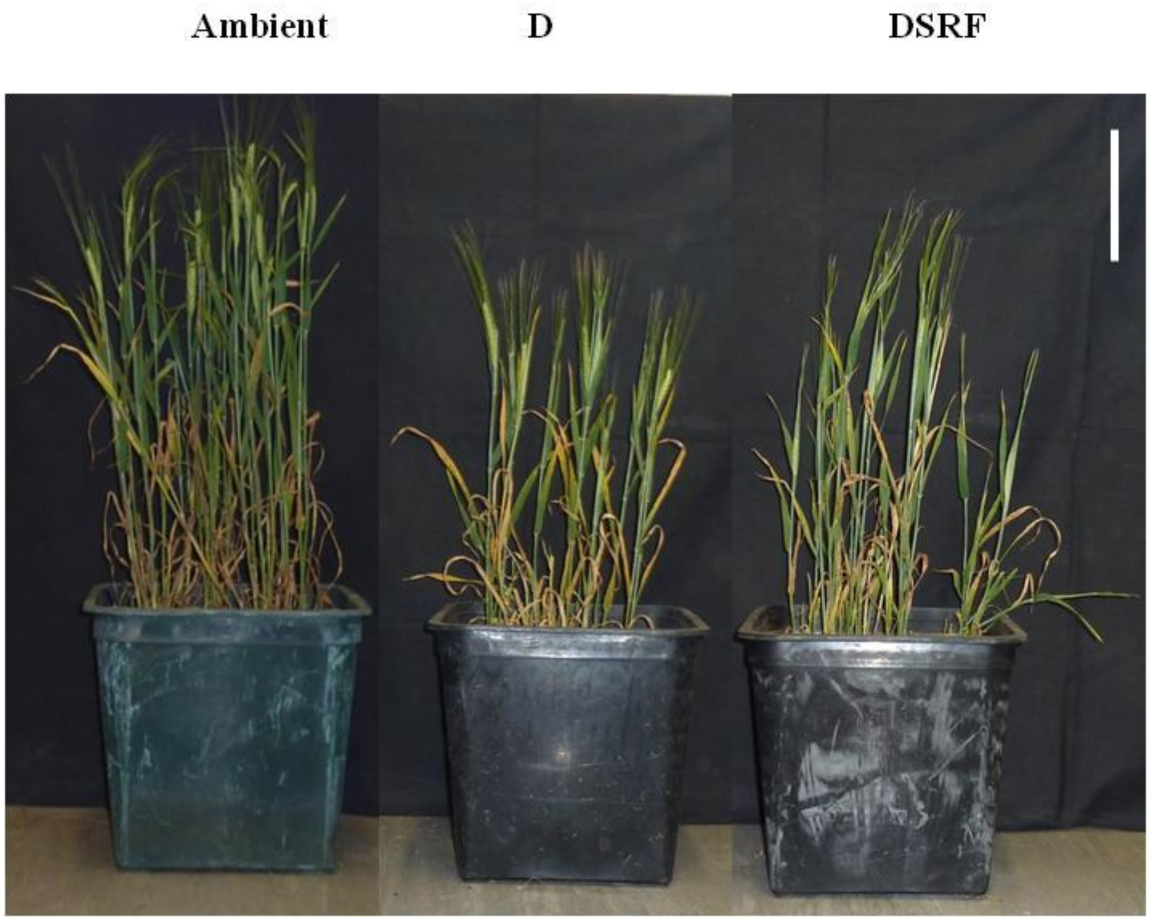

Fig. 5.5. Barley plants treated with different watering regimes at harvest (c. 65 days after sowing). Scale bar represents 12cm. (Photographs taken by R. Wade).

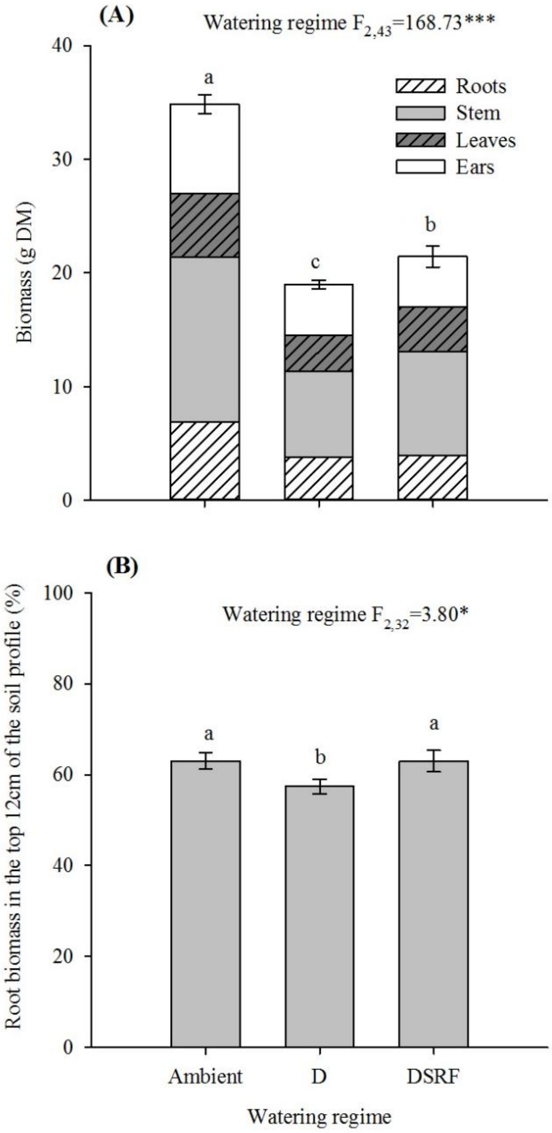

Fig. 5.6 (**A**) Total plant biomass (g dry mass (DM)) and (**B**) the proportion of root biomass in top 12cm of the pot (%) of barley plants treated with different watering regimes Values represent mean ± standard error bars of 18 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

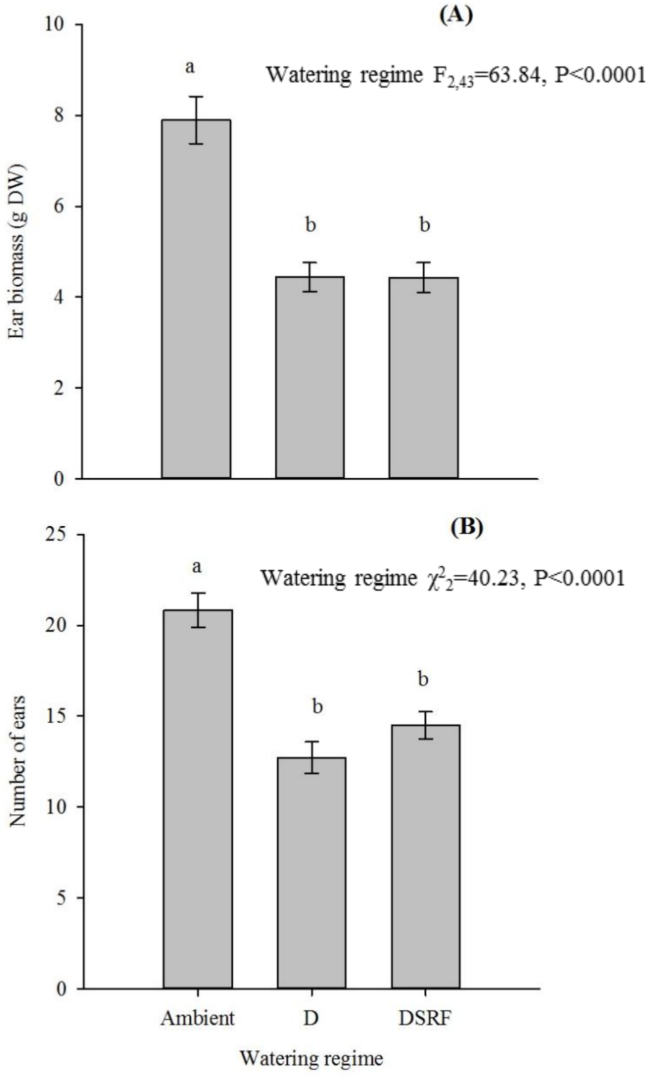

Fig. 5.7 (**A**) Ear biomass and (**B**) number of ears of barley plants treated with different watering regimes. Values represent mean ± standard error bars of 18 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

watering regime (Table 5.1). Percentage of leaf covered by powdery mildew was largest on DSRF treated plants and smallest on D treated plants (Table 5.1). Plants treated with D and DSRF had significantly smaller total plant water content compared to ambient watered plants (Fig. 5.8). Under a 40% reduction in water quantity regime, plants watered once every other week (DSRF) had significantly larger total plant water content than those watered once a week (D).

5.4.2 The effect of the different watering regimes on arthropod herbivores

On average, wireworms lost mass throughout the experiment (Fig. 5.9). Wireworms in pots watered under D or DSRF regime had a significantly larger reduction in mass compared to wireworms recovered from ambient watered pots (Fig. 5.9). There was no effect of watering regime on wireworm survival ($F_{2,16}$=2.43, P=0.12).

Regardless of plant age and root herbivory, watering regimes had no effect on the number of aphids (Table 5.2). There was however a significant interaction found between plant age, watering regime, wireworm herbivory and week on the number of aphids (Table 5.2). Eight weeks after sowing a greater number of aphids were counted on older plants compared to younger plants (Fig. 5.10). Wireworms had a significant effect on the number of aphids only on the re-sown younger plants, where they caused a significant reduction in total number of aphids on drought treated plants three and four weeks after sowing and on ambient watered plants four weeks after sowing (Fig. 5.10). Aphid biomass (g DW) was unaffected by changes in the watering regime or wireworm herbivory (Fig. 5.11 (**A**)).

5.4.3 Third trophic level

There were very few natural enemies recorded throughout the experiment with only one ladybird larvae recorded at the field site during the experiment and thus cannot be included in the data analysis. There were very few mummified aphids counted and collected from the experiment and total number of mummified aphids was unaffected by the watering regime and wireworm herbivory (Fig. 5.11 (**B**)).

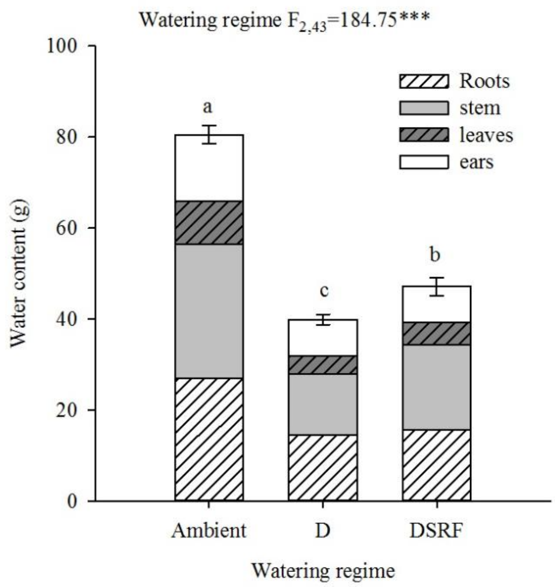

Fig. 5.8. Total plant water content of barley plants treated with three different watering regimes. Values represent mean ± standard error bars of 18 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts.

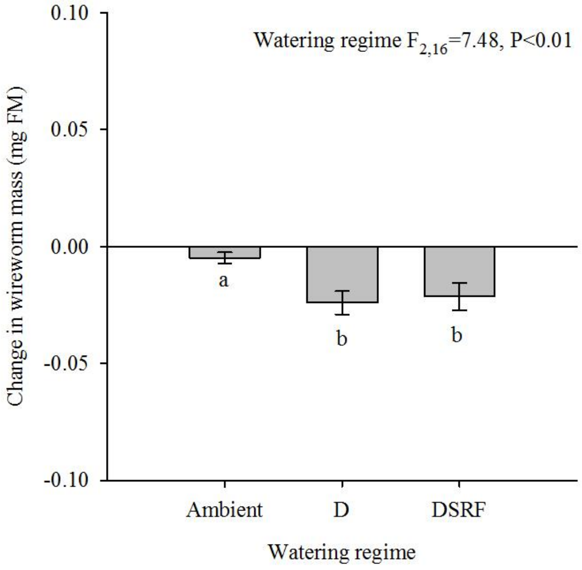

Fig. 5.9. Change in wireworm mass (mg fresh mass (FM)) when residing in soil supporting 9 barley plants treated with different watering regimes. Values represent mean ± standard error bars of 18 replicates. Bars sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts

Table 5.2. Generalised linear mixed effects model output investigating the interactive effects of plant age, watering regime, wireworm herbivory and time (week).

Factor	χ^2 value	DF	P value
Plant age	**8.42**	**1**	**P=0.004**
Watering regime	0.01	2	P>0.05
Wireworm herbivory	**8.06**	**1**	**P=0.005**
Time (date)	**153.16**	**1**	**P<0.001**
Plant age * Watering regime	1.26	2	P>0.05
Plant age * Wireworm herbivory	1.07	1	P>0.05
Watering regime * Wireworm herbivory	**7.81**	**2**	**P=0.020**
Plant age * Time (date)	**195.66**	**1**	**P<0.001**
Watering regime * Time (date)	**14.78**	**2**	**P<0.001**
Wireworm herbivory * Time (date)	**4.26**	**1**	**P=0.039**
Plant age * Watering regime * Wireworm herbivory	**9.57**	**2**	**P=0.008**
Plant age * Watering regime * Time (date)	**6.40**	**2**	**P=0.041**
Plant age * Wireworm * Time (date)	0.06	1	P>0.05
Watering regime * Wireworm herbivory * Time (date)	**8.37**	**2**	**P=0.015**
Plant age* Watering regime * Wireworm herbivory * Time (date)	**27.48**	**2**	**P<0.001**

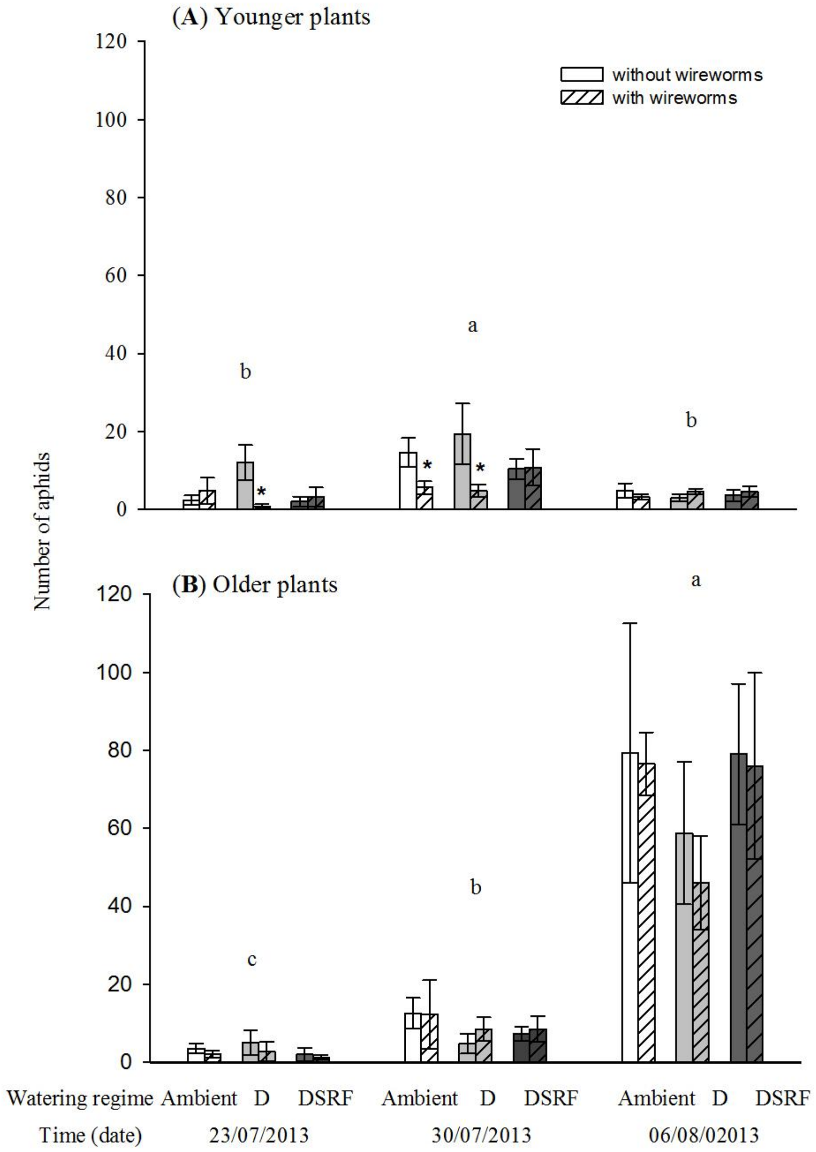

Fig. 5.10. Total number of aphids counted on three randomly selected (**A**) re-sown younger and (**B**) three week older barley plants within pots treated with different watering regimes and wireworm herbivory over three weeks. Graph shows the number of aphids on older plants 6weeks after sowing on 23/07/2013. Younger plants were sown 3weeks after older plants. Values represent mean ± standard error bars of nine replicates. Weeks sharing the same letter were not significantly different as determined by *post-hoc* Tukey contrasts. Stars represent significant effect of wireworm herbivory on number of aphids as determined by *post-hoc* Tukey contrasts.

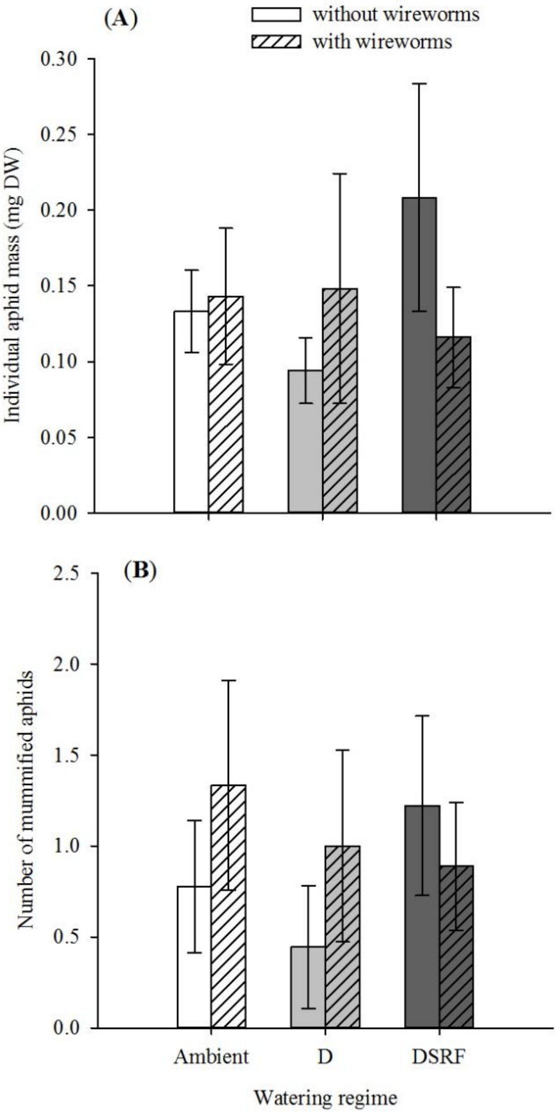

Fig. 5.11 **(A)** Individual mass (mg DM) of aphids collected from all nine barley plants treated with different watering regimes (ambient, D and DSRF) and wireworm herbivory. **(B)** Average total number of mummified aphids collected at harvest from nine barley plants treated with different watering regimes and wireworm herbivory. Values represent mean ± standard error bars of nine replicates. Watering regime and wireworm herbivory had no significant effect on individual aphid mass or number of mummified aphids.

5.5 Discussion

5.5.1 The effect of changes in the quantity and frequency of watering events on the growth of barley plants and herbivore performance.

In support of the first hypothesis, a 40% reduction in water quantity significantly reduced total plant biomass including root, stem, leaf and ear biomass. A reduction in plant growth and yield will have severe consequences for barley production in the UK under future changes in precipitation. Previous research has also reported negative effects of reductions in water availability on barley plant growth, development and yield (Lawlor *et al.* 1981; Jamieson *et al.* 1995; Popova *et al.* 1996; Legg *et al.* 2009). In contrast, reducing the frequency of watering events alleviated some of the negative effects of the 40% reduction in water quantity. Despite receiving the same quantity of water throughout the experiment, those plants watered less frequently had an increase in biomass and relative leaf chlorophyll content. Plants watered with reduced watering frequency may have adapted strategies to tolerate further water stress events as re-wetting studies suggest plants can become more resilient to future water stress after an initial water stress event through mechanisms such as up regulating stress related genes (Ratnayaka *et al.* 2003; Du *et al.* 2010; Okamoto *et al.* 2011). However, soil moisture measured by the theta probes buried half way down the soil profile demonstrated that although water quantity was reduced by 40%, reducing the watering frequency resulted in soil moisture in the deeper soil profile remaining wetter than pots watered more frequently. This suggests that an extreme rainfall event during periods of drought could facilitate water penetration to a deeper soil profile resulting in the bulk soil remaining wetter for longer and benefiting a deeper rooting zone enabling increased water uptake (Heisler-White *et al.* 2009). In comparison, regular, lighter precipitation events, may only penetrate the topsoil which is more exposed, and soil moisture is more likely to be lost due to evaporation. This is supported by the water content results, where DSRF plants had a larger total plant water content than plants watered more frequently. Similar soil moisture patterns were reported by Fry *et al.*, (2014) where a drought/ deluge treatment also resulted in soil remaining wetter for a longer duration.

Plant growth and development at harvest was unaffected by wireworm herbivory and is likely to be due to the low density of wireworms in each pot, possibly not sufficient to cause significant damage to the plants. Wireworm distribution is often uneven in natural grasslands and arable fields therefore one plant can be attacked by several wireworms at a given time (Lees 1943a; Parker 1996; Parker and Howard 2001). Wireworms have also been previously recorded to be sensitive to temperature as their activity and feeding rates reduce as they move deeper in the soil profile during the summer months to avoid high temperatures (Lees 1943a; Parker and Howard 2001). Throughout the experiment, air temperatures were recorded to be unusually high for the location during summer 2013 (Fig. 2) which will have affected the behaviour of the wireworms. Furthermore, wireworms were recorded to lose mass throughout the experiment suggesting that they were not thriving in this system. It is important to note however that wireworm fresh mass is difficult to accurately assess as it varies throughout wireworm life cycle and moulting can significantly affect body size (Parker and Howard 2001). However, wireworms were recorded to significantly lose more mass when feeding on plants receiving 40% reduction in water quantity. This may be due to a reduction in food resource as D treated plants had a significant reduction in root biomass. It is also possible that there were changes in feeding behaviour of the wireworms. Therefore under climate change increases in temperature and reductions in rainfall may affect the behaviour and feeding activity of wireworms, potentially reducing their impact on cereal crop growth.

Despite large differences in plant biomass, there was no effect of the different watering regimes on the abundance or mass of aphids. Different species of aphids were collected from the plants and it is possible that certain species of aphids were more abundant at different plant developmental stages. Different aphid species could have also been differently affected by the watering regimes due to preference in host plant species and developmental stage. Generalists and specialist aphids have been previously reported to respond differently to water stress (Khan *et al.* 2010). The extremes in temperature during the experiment would have also significantly affected aphid survival, abundance and fecundity (Bale *et al.* 2002; Nelson, Bjørnstad and Yamanaka 2013).

5.5.2 Interaction between below-ground and above-ground herbivores.

Despite the low density of wireworms and no effect on plant biomass at harvest, wireworms were found to reduce the number of aphids collected from ambient and drought treated plants early in development. This is in contrast to previous published results which report below-ground herbivory positively affects above-ground herbivory (Masters *et al.* 1993). However, plant development is clearly important in the interaction between above- and below- ground herbivory as the effect of wireworms on number of aphids was transient, only measured during early plant development on younger plants (three to four week old plants). Therefore contrasting results may be due to differences in plant age and development. Previous published studies have also reported that the interaction between above- and below- ground insect herbivores can change throughout the growing period and that the plant and insect species as well as the developmental stage and feeding guild of the insect herbivore can influence the interactions between above- and below- ground insect herbivores (Bezemer and van Dam 2005). It could also be possible that wireworms had a larger effect on the barley plants during early plant development influencing above-ground interactions to a greater extent and that by harvest there were no measurable effects of the wireworms. Increases in the number of aphids on young barley plants under future changes in precipitation patterns will have consequences for barley production in the future particularly with drives to reduce pesticides.

5.5.3 The effects of the watering regimes and wireworm herbivory on the third trophic level, natural enemies.

Throughout the experiment the number of natural enemies recorded was very low. This is likely to be due to extremes in temperature throughout the experiment possibly influencing development and survival of predators as well as impacting the synchrony between the different trophic levels (Hance *et al.* 2007). Parasitioid wasps were the most common natural enemy found on the barley plants throughout the experiment, despite this, there were relatively low numbers of mummified aphids recorded. Due to the low numbers of mummified aphids it is difficult to measure any effects of the different watering regimes on their abundance and as such there was no significant effect of the watering regimes recorded on the numbers of mummified aphids. In contrast to these results, published studies report significant effects of changes in plant water status on the population of mummified aphids (Aslam *et al.* 2013). This experiment was conducted in a controlled environment glasshouse and not in a field system open to extreme events such as high temperatures, and mummified aphid predation and/or hyper-parasitism, which would influence their abundance.

5.5.4 Field experiment results compared to controlled environment system

The response of barley plant growth, development and yield to the different watering regimes was very similar in the field and controlled environment rooms despite extreme events such as extremes in temperature, disease outbreaks, and mammalian herbivory suggesting that these results are robust. Despite the positive effects, plants watered less frequently had a larger leaf area covered in powdery mildew suggesting that these plants were more susceptible to this fungal infection. Water stressed plants are often reported to be more susceptible to disease due to reduced plant defence (Garrett *et al.* 2006). The positive effects of reducing watering frequency on plant growth and yield may be compromised if these plants are more susceptible to disease.

There were differences in the effects of the watering regimes on below- and above-ground arthropod herbivores and their natural enemies in controlled environment rooms and in the field system. This is most likely to be due to the extreme temperatures during the field experiment resulting in reduced wireworm activity and the low abundance of aphids, parasitoid wasps and ladybirds. Despite this, wireworms lost more mass when in pots under D and DSRF regimes in the field experiment and they also had a smaller effect on DSRF treated plants in the controlled environment rooms. This suggests that under future climate change scenarios wireworm performance and their impact on barley plants may be reduced.

Aphid mass was increased under DSRF watering regime in controlled environment rooms, whereas there was no effect of watering regime on aphid mass in the field experiment. Due to experimental constraints aphid fresh mass was recorded in the controlled environment rooms whereas aphid dry mass was recorded in the field experiment. Although not significant due to large variation, there was a general trend of a greater individual aphid mass recorded on DSRF plants without wireworm herbivory in the field supporting the increase in aphid fresh mass under DSRF watering regime recorded in the controlled environment rooms. Above- and below-ground interactions in this system are clearly sensitive to plant developmental stage and therefore interactions between the spatially separated herbivore in the controlled environment rooms may have occurred before measurements were taken. Due to powdery mildew infection, differences in plant age and extremes in temperature during the field experiment there is large variation in the number of aphids and mummified aphids data resulting in weak statistical analysis. Climate change is multi-faceted and this field experiment demonstrates that exposure to extreme events and other factors can influence the outcomes of one aspect of climate change, in this case changes in precipitation on tri-trophic interactions in a barley system. Therefore the interactions between different aspects of climate change and their affect on cereal ecosystems requires further investigation.

5.6 Conclusions

40% reduction in water quantity significantly reduced plant growth and yield, whereas reducing watering frequency alleviated some of the negative effects of the drought. Wireworm growth was more negatively affected in pots under drought watering regime; however wireworms did not affect plant growth and development at harvest. Wireworm herbivory reduced aphid abundance on five week old plants under ambient and drought watering regimes but this effect disappeared as the plants matured and throughout the growing period. The abundance of natural enemies in this experiment was very low, most likely to be due to the extremes in temperature during the experiment with no effects of watering regime or wireworm herbivory on the abundance of mummified aphids. The impact of the different watering regimes on barley plant growth and development in the field experiment supported findings reported in controlled environment rooms suggesting that this response is robust. However, the effect of watering regimes on arthropod herbivores, natural enemies and their interactions were recorded to differ in the field experiment with large variation in the field experimental data most likely due to extreme temperatures and powdery mildew infection.

Therefore, changes in the frequency of watering events may partially alleviate the impact of drought on crop growth and yield under future climate scenarios. Predicted changes in precipitation may negatively affect wireworm performance and impact on barley biomass reducing the incidence of wireworm herbivory in crops as well as removing the interaction between wireworms below-ground and aphids above-ground potentially increasing the number of aphids on barley plants early in development. Additional investigation is needed to gain further understanding of the effect of the different aspects of climate change on aphids and their natural enemies

CHAPTER 6

General Discussion

6.1 Introductory remarks

By 2080 the UK is predicted to experience significant changes in rainfall patterns with up to 40% reduction in mean summer precipitation simultaneous with increases in the frequency of drought and extreme rainfall events (Fowler and Kilsby 2004; Ekstrom *et al.* 2005; Bates *et al.* 2008; Murphy *et al.* 2009). The main aim of this thesis was to investigate the impact of predicted changes in both the quantity and frequency of precipitation events on tri-trophic interactions in a barley ecosystem. This chapter aims to summarise the main results of this thesis and to consider the importance of the findings in a wider context with suggestions of future work (for summary of main findings and future work see Table 3.1 and Fig. 6.1.)

6.2 Main findings of the thesis

Watering regimes were established based on ten year average rainfall data collected in Dundee and delivered in different quantities and frequencies throughout the growing period. Reductions in water quantity (40% and 60% reduction in the quantity of water added to the soil) caused a greater negative effect on plant growth and development than reducing the watering frequency (watered 50% and 25% as frequent as the control plants) (Fig. 2.2, Fig. 2.3, Fig. 2.4 and Fig. 3.2). Reductions in water quantity caused significant reductions in total plant biomass (Fig. 2.2, Fig. 3.2, Fig. 4.2 and Fig. 5.6), yield (Fig. 3.4, Fig. 3.5 and Fig. 5.7), leaf phosphorus (P) (Fig. 3.6), and increases in leaf nitrogen (N) (Fig. 2.5 and Fig. 4.6) concentration and sugar content (Fig. 3.6) which are commonly recorded responses of cereal crops to drought conditions (Blum 1996; Chaves *et al.* 2003). In contrast, barley plants receiving water less frequently either showed no change or an increase in total plant biomass (Fig. 2.2, Fig. 3.2, Fig. 4.2 and Fig. 5.6), as well as an increase in leaf silicon (Si) concentration (Fig. 2.6, Fig. 3.6 and Fig. 4.7), compared to plants receiving the same total quantity of water but in more frequent events. This is the first study to demonstrate that changes in the frequency of rainfall events may not be as damaging to future crop production as predicted

Table 6.1. The main findings of each chapter of the thesis and the implications for future cereal crop production

Chapter	Main findings	Implications
2	Watering regimes based on av. weekly rainfall data collected in Dundee were easily applied and provided a more accurate way of measuring the effect of predicted changes in both the quantity and frequency of rainfall on barley crop production. Results suggested that the quantity of precipitation is more important than the frequency of watering events but experiments needed to be repeated due to extremes in temperatures in the glasshouse during the experiment.	Experiments using av. rainfall data provide more accurate predictions of how future changes in precipitation will affect plant growth and development. Studies that do not consider changes in the frequency of rainfall events may overestimate the negative effects of future reductions in rainfall quantity on plant growth and yield.
3	Barley plants grown under future rainfall patterns had a reduction in total plant biomass, yield and changes in chemical composition. Reductions in water quantity resulted in larger reductions in plant growth and yield compared to reductions in watering frequency.	Reductions in yield due to changes in rainfall patterns will lead to financial loss to farmers, impacting the economy, and increase pressure to irrigate crops in the UK with consequences for water resources in the future.
4	Wireworms had a smaller effect on plant biomass under future rainfall patterns whereas aphids benefitted from changes in the host plant as a result of this watering regime. Harlequin	Crop production may benefit from reduced below-ground herbivory in the future. Whereas farmers may require further applications of insecticide to control possible increases in aphid

	ladybirds also benefitted from feeding on aphids collected from plants grown under future rainfall patterns but when given the choice, ladybirds had no preference for aphids despite differences in host plant watering regime.	herbivory which may not be controlled by natural predators such as ladybirds. An increase in harlequin ladybirds will have consequences for native ladybirds possibly resulting in a reduction in the number of native ladybirds. However increased barley leaf Si may negatively impact chewing insect herbivores reducing their impact on crop yield under future rainfall patterns.
5	The response of barley plant growth and yield was replicated in the field suggesting that the response of the barley plants to future rainfall patterns is robust. The interaction between below- and above- ground insect herbivores was removed under future changes in precipitation resulting in an increase in the number of aphids during early plant development. Responses of insects differed between controlled environment and field experiments suggesting that there are other factors involved in influencing pest dynamics.	Reductions in barley yield is inevitable under predicted changes in precipitation which may lead to the irrigation of UK crops in the future putting pressure on water sources. Predicted changes in rainfall patterns have the potential to disrupt species interactions within an ecosystem with consequences for agroecosystems. Plant responses between greenhouse and field experiments were robust but insect responses were more complex. It is therefore important to perform field experiments when predicting future changes in precipitation on insect herbivores and their natural enemies.

Main finding at each trophic level	**List of figures which support the main findings**
• Increased mass • No change in prey choice	Fig. 4.8
• Increased mass • Increase in numbers with reduced wireworm herbivory	Fig. 4.8 Fig. 5.10
• Reduced total plant biomass and yield • Increase in leaf N and Si	Fig. 2.2 Fig. 2.5 Fig. 2.6 Fig. 3.5 Fig. 3.6 Fig. 4.2 Fig. 4.6 Fig. 4.7 Fig. 5.6
• Reduced mass • Reduced effect on plant biomass	Fig. 5.9 Fig. 4.2

Fig. 6.1. Diagram representing multi-trophic interactions in a barley ecosystem together with the effect of future rainfall patterns on each trophic. Red arrows represent either an increase (upwards pointing arrow) or decrease (downwards pointing arrow) in growth and abundance of the different trophic levels under future predicted changes in precipitation (photographs taken by R Wade).

reductions in mean precipitation with consequences for Si plant defence. This is most likely to be due to heavier watering events enabling water to penetrate a deeper soil profile and rooting zone with less water lost to surface evaporation, resulting in the bulk soil remaining wetter for a longer period of time. Theta probes in the field experiment buried half way down the soil profile (described in Chapter 5, Fig. 5.4) provided evidence to support this, as the soil 12cm below the surface remained wettest in pots receiving the same total quantity of water but in fewer watering events. Furthermore barley plants watered less frequently also had higher water content (Fig. 3.6). Barley plant responses to the different watering regimes were very similar in the greenhouse (Chapter 2), controlled environment rooms Chapter 3) and in a field study (Chapter 5), suggesting that the response of the barley plants to the different watering regimes is robust under different growing environments.

Two barley cultivars responded similarly to the watering regimes (Chapter 2). As predicted due to differences in breeding histories, landrace and cultivated barley plants differed in growth and development. Cultivar plants were shorter (Table 3.2) and had a larger yield (Fig. 3.4) compared with landrace plants, whereas leaf P and Si concentrations were higher in landrace plants (Fig. 3.6). However, despite the differences between the landrace and cultivars, these barley varieties exhibited similar responses to the different watering regimes with the exception of certain measured parameters such as specific leaf area of the landrace was less sensitive to reductions in water quantity than the cultivar (Fig. 3.3). However these differences provided the landrace with no advantage under future precipitation scenarios compared to the cultivar.

Changes in watering regime significantly affected insect herbivores feeding on barley plants both above- and below- ground. Wireworm growth was reduced to a greater extent when feeding on plants watered with a 40% reduction in water quantity (Fig. 5.9). Wireworms also had a smaller negative effect on total barley plant biomass under the 40% reduction in water quantity regime (Fig. 5.6). It is not clear if this was due to changes in the host plant as a resource or if the behaviour of the wireworms was altered due to drier soil. However, in this thesis watering regimes were not recorded to have any effect on wireworm distribution in the soil profile

(Chapter 4), which might be expected if wireworms preferred to migrate into deeper, moister soil.

In contrast, above-ground aphid herbivores gained more mass when feeding on plants watered less frequently compared to when feeding on ambient watered plants (Fig. 4.8). Larger aphids also tend to produce higher numbers of offspring and exhibit greater fecundity (Honek 1993; Awmack and Leather 2002) but this was not recorded in this thesis (Chapter 4). However, studies in this thesis only measured effects of changes in precipitation on the first generation of aphids. It could be that further generations would be affected by changes in precipitation. Larger aphids may also be able to better defend and protect themselves from predators and parasitoids (Honek 1993) increasing survival rates. Climate change research has particularly focused on the effect of drought on insect population size (Mattson and Haak 1987; Hale *et al.* 2003; Chown *et al.* 2011), often overlooking population demography despite studies reporting that drought can significantly affect aphid population demography which influenced the number of parasitoid attacks on these aphids (Aslam *et al.* 2013).

Fewer aphids were recorded on plants with below-ground wireworm herbivory under ambient and drought watering regimes but this effect disappeared as the barley plants matured (Fig. 5.10). Reducing the frequency of watering events negated the interaction between the spatially separated herbivores most likely to be due to a reduction in the effect of wireworm herbivory on plant biomass under these watering regimes. Wireworms might have influenced the quality of the aphid’s food source by removing root biomass and thus increasing the severity of drought stress to the barley plants, further affecting plant growth and chemical composition (Johnson, Bezemer and Jones 2008b). The interaction between above- and below- ground insect herbivores has also been previously reported to differ due to plant development stage at the time of herbivory and change during plant development (Masters *et al.* 1993; Bezemer and van Dam 2005). Furthermore, drought has also been previously reported to remove interactions between root- and foliar feeding arthropods (Staley *et al.* 2007b).

The effects of reduced water quantity and watering frequency on aphid growth influenced the performance of Harlequin ladybirds. Harlequin ladybird larvae gained more mass when feeding on aphids collected from plants watered less frequently under the 40% reduction regime, compared to those feeding on aphids collected from ambient watered plants (Fig. 4.8). However, in a choice situation ladybirds did not select aphids that maximised ladybird growth, as such ladybird choice of prey did not match prey quality (Chapter 4). Therefore the benefits of future changes in precipitation on aphid mass may not benefit the ladybirds to the same magnitude. In contrast, there was no effect of the different watering regimes on the number of mummified aphids in the field (Chapter 5), although these findings should be interpreted with caution due to the low abundance of mummified aphids in the field experiment.

6.3 Implications of the findings for cereal crop production in the future with suggestions of further work

Potential areas for future research are summarised in Fig. 6.2.

6.3.1 Implications of future changes in precipitation on barley crop growth and yield

The findings of this thesis provide substantial evidence that predicted changes in precipitation will significantly affect barley plant growth, yield, and tissue chemical composition in the future. A 40% reduction in water quantity, the predicted reduction in rainfall by 2080 (Bates *et al.* 2008), resulted in a 30% reduction in barley yield (Fig. 3.4) which could cause significant financial losses for farmers in the UK (Oerke and Dehne 2004). Changes in plant chemical composition may also affect grain quality influencing the price at which the yield can be sold, for example grain N content affects the malting process (Edwards *et al.* 2006; Ullrich 2011). The severity of the reduction in mean precipitation in the future is dependent on the scenario which the model is based upon (Hennessy *et al.* 1997; Ekstrom *et al.* 2005; Bates *et al.* 2008). Scenarios which follow predictions of a future world of rapid economic and population growth result in greater impacts on global hydrological

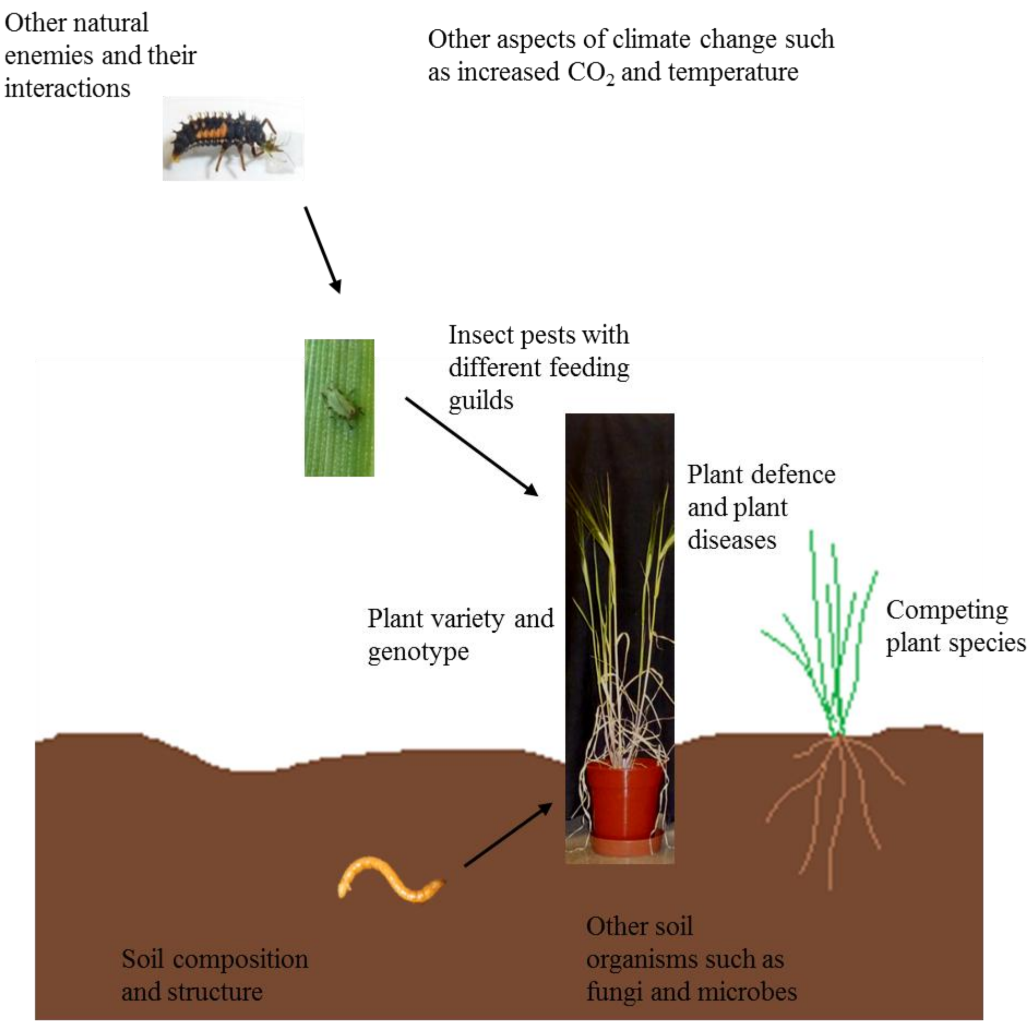

Fig. 6.2. Diagram representing areas for further research required to understand the effect of predicted changes in precipitation on a barley ecosystem (photographs taken by R Wade).

cycle compared to models based on future economic, social and environmental sustainability which predict smaller changes in precipitation (Folland *et al.* 2001). Larger reductions in mean precipitation will cause greater negative effects on barley production with 60% reduction in mean precipitation resulting in av. 60% and 70% reductions in barley yield and plant biomass respectively. However, predicted changes in the frequency of rainfall events resulting in less frequent but heavier rainfall events may alleviate some of the negative aspects of reductions in mean precipitation on barley growth and yield. Therefore future increases in extreme rainfall events may not be as damaging to future crop production as previously predicted and may temporarily increase soil water availability during drought periods increasing water use efficiency. Irrigating crops less frequently but with larger quantities of water in one watering event may be an improved irrigation method in the future. This type of irrigation could increase irrigation efficiency, reducing water usage by agriculture, which will be particularly important in the future as water becomes a commodity which needs to be conserved (Gregory *et al.* 2009). However, despite the positive effects of reducing watering frequency, barley plants grown under predicted rainfall patterns with both reduced water quantity and watering frequency still had a reduction in yield compared to plants grown under ambient watering. A consequence of this is that farmers may increase irrigation of crops growing in the UK to improve yields. This will increase the demand on water availability in the UK which will already be in short supply due to rising temperatures, reduced mean precipitation and increasing demand due to the rise in human population (Arnell 1999; Bates *et al.* 2008).

The majority of experimental work in this thesis was conducted in controlled environment rooms minimising environmental variation and ensuring that the detailed measured effects on plant and insect growth and development were solely due to changes in the watering regime. It is important to consider that barley grown in the field will be affected by varying temperatures, air movement, humidity and radiation which could all influence evaporation and soil moisture availability (Hughes 1959; Rajan, Betteridge and Blackman 1971). Despite this, the response of barley plant growth, development and yield to the different watering regimes was very similar in the field and controlled environment rooms therefore the responses were reproducible in different growing environments. This suggests that the results are highly robust and that reductions in barley crop production under predicted rainfall patterns is inevitable.

Soil type and structure will significantly impact soil water holding capacity influencing plant responses to future changes in precipitation. Considering soil type and organic matter content will be particularly important when predicting the effect of reductions in the frequency of extreme rainfall events on crop production, as sandy soils may not retain water during the drought periods increasing the negative impacts of reduced water quantity (Gupta and Larson 1979). Furthermore, spaces between particles in sandy soils may reduce water retention under extreme rainfall events, whilst a clay soil may retain water causing flooding. The composition of microbial communities and the rates of microbial processes are highly sensitive to aerobic and anaerobic environments (Van Gestel, Merckx and Vlassak 1993; Eisenhauer *et al.* 2011). Flooding and drying events can have significant effects on soil nutrient availability where a pulse of dissolved organic carbon and other nutrients can be released on rewetting (Van Gestel, Merckx and Vlassak 1993a; Hartmann *et al.* 2013). Further investigations into how future rainfall patterns will impact soil quality, composition and soil micro-organisms and how this may affect plant growth under future changes in precipitation are required.

Drought tolerant barley varieties could be developed to increase yield under future precipitation patterns and prevent increased pressure on water resources. However barley landraces and cultivars used in this thesis responded very similarly to the different watering regimes, despite evidence to show that domestication has led to the loss of genes that confer resistance to abiotic and biotic stresses (Massei and Hartley 2000; Ellis *et al.* 2000)**.** There is strong evidence in the literature to suggest that different genotypes of cereal crops are better adapted to cope with water stress than others (Fischer and Maurer 1978; Shakhatreh *et al.* 2001; Lukács *et al.* 2008; Annicchiarico, Pecetti and Tava 2013). Additional genotypes and landraces could be screened for resistance to both changes in the quantity and frequency of watering events. The ancestors of many of our modern crops originate from the Fertile Crescent, an area well known for its fertile soil (Ellis *et al.* 2000; Badr *et al.* 2000a;

b) suggested to be due to regular periods of drought and flooding (Ellis *et al.* 2000). Ancestral varieties and landraces from the Fertile Crescent, which is prone to periods of drought and flooding, could exhibit tolerance to extreme rainfall events and provide candidate genes that may have been lost during domestication (Tyagi *et al.* 2011) to improve the response of modern cereal crops to the changing climate.

In the field experiment (Chapter 5), powdery mildew infection was greatest in those plants watered less frequently suggesting that these plants may be also more susceptible to pathogens. This has serious consequences for future barley production particularly with pressures to increase the sustainability of farming by reducing chemical inputs (Birch, Begg and Squire 2011; Hillocks 2012). It is particularly important to gain an understanding of how future rainfall patterns may affect pathogen virulence as well as the tolerance or resistance of cereal crops to pathogens. Pathogens already cause significant yearly yield losses (Coakley, Scherm and Chakraborty 1999; Garrett *et al.* 2006) and any increase in pathogen incidence and virulence may result in further reductions in cereal crop production in the future. Periods of stress and recovery as a result of drought and flooding events could benefit many plant diseases and pathogens due to changes in environmental conditions and changes in plant physiology and chemical composition (Coakley *et al.* 1999; Garrett *et al.* 2006; Newton, Johnson and Gregory 2011b). The occurrence of these diseases is frequently recorded to be increased under drought stress and flooding (Parry *et al.* 2004; Li *et al.* 2008). There is also potential interactions between pathogens and insect herbivores for example aphids can be vectors of virus' (Ng and Perry 2004) and plant pathogens can influence aphids choice of host plant (White *et al.* 2000; Ingwell, Eigenbrode and Bosque-Pérez 2012). The positive effects of reducing watering frequency under drought on plant growth and yield, therefore, may be limited if these plants are more susceptible to diseases.

6.3.2 Compared to barley, will other cereal crops respond in the same way to future changes in UK precipitation?

In comparison with other cereals, barley has been described as relatively drought tolerant and is grown in a wide range of environments, with the immediate progenitor of cultivated barley (*H. vulgare* ssp. *Spontaneum*) able to grow in desert conditions (Nevo 1992; Zohary and Hopf 1998). Despite reportedly being relatively drought tolerant (Newton *et al.* 2011a), predicted changes in precipitation were found in this thesis to significantly reduce barley plant growth and yield. This is of particularly concern as other cereal crops grown in the UK which are not as drought tolerant may be affected by future precipitation to a greater extent. Wheat, for example, the most widely grown UK crop, is relatively sensitive to drought (Blum 1996; Barnabás *et al.* 2008) and may not perform as well as barley under future rainfall patterns. Reductions in the frequency of rainfall events at the same time as reductions in mean precipitation will result in increased occurrence of consecutive dry days. This could have significant consequences for more drought sensitive cereal crops resulting in yield and financial losses. Further investigation is required to assess how different varieties of cereal crops grown in the UK will respond to future changes in precipitation.

Reduced plant growth and development under future rainfall scenarios may also influence the competitive abilities of the crop plant. In the field, cereal plants are sown at close proximity to one another and will therefore be competing for water and nutrients with other crop plants and weeds. Competition between plants could also influence the effect of water stress on plant growth and development (Johnson *et al.* 2011). Changing precipitation patterns also have the potential to affect the ability of weed species to invade crop systems (Hellmann *et al.* 2008). Plant species considered as weeds, that are more drought tolerant may prosper under future precipitation patterns and outcompete cereal crops further reducing crop yields. This is particularly important as weed species growing in arable fields already results in extensive yield losses for example black grass in wheat fields (Hillocks 2012). Understanding the interacting effects of changes in precipitation and competition within crops and between crop species and weeds will be an important consideration for cereal crop production in the future.

6.3.3 Will changes in precipitation affect the quality of the host plant for above- and below- ground arthropod herbivores?

Plants watered with reduced watering frequency also had higher concentrations of leaf N (Fig. 4.6) which may benefit arthropod herbivores as many insects are N limited (Price 1991). Amino acid concentration and composition are known to be affected by drought (Blum 1996; Chaves *et al.* 2003; Chaves, Flexas and Pinheiro 2009; Seki *et al.* 2007; Witt *et al.* 2012) and can significantly influence aphid development (Douglas 1993; Mewis *et al.* 2012); therefore it is possible that the increase in aphid mass on barley plants watered less frequently was due, at least in part, to changes in amino acid content and composition. This could be further explored.

A decrease in water quantity was also found to reduce barley leaf Si concentrations (Fig. 2.6, Fig. 3.6 and Fig. 4.7), which may have important consequences for crop defence against insect herbivores, particularly chewing insects; Si increases the abrasiveness of plant tissue, wearing down insect mandibles and reducing nutrient absorption by insects and their growth rates (Massey *et al.* 2006). Chewing herbivores such as caterpillars were observed on barley plants growing in the field experiment and have the potential to reduce barley growth and yield. In addition to Si, the effect of changes in precipitation on other plant defences such as allelochemicals and secondary metabolites important for defence against plant diseases and pathogens could be examined. Changes in water availability have the potential to influence many plant defence pathways through changes in plant chemical composition and in resource allocation towards or away from plant defence strategies. Reduction in plant defences may result in increased susceptibility to, and outbreaks of, crop pests and diseases. Pulsed water stress could have important consequences for plant defence due to periods of stress that may “prime” plants for future stresses and improve plant defence (Conrath *et al.* 2006). ‘Primed’ plants have been previously exposed to an abiotic or biotic stress resulting in faster or stronger activation of defence strategies, enabling these plants to be more resistant to future exposure to the stress (Conrath *et al.* 2006; Bruce *et al.* 2007). For example, modulating ABA responsive gene expression by the histone deacetylase, AtHD2C, increased drought tolerance in *Arabidopsis* (Sridha and Wu 2006). Alternatively plant defences may be compromised under extremes in different water stresses due to resources allocated towards water conservation or drought/ flooding tolerance strategies (Newton *et al.* 2011b; Gutbrodt *et al.* 2011). Therefore research investigating the effect of predicted changes in precipitation on plant defence would provide important information for future crop protection and thus production.

6.3.4 What are the consequences of changes in the growth and abundance of soil dwelling organisms and above- ground insect herbivores under future changes precipitation?

Future precipitation patterns could mitigate against impacts of wireworm herbivory and reduce the incidence of below-ground damage to crop plants. This may benefit cereal crop production in the future due to reduced root damage. It was reported in Chapter 4 and Chapter 5 that wireworm movement was unaffected by future changes in precipitation patterns. However wireworm movement was only recorded at one time point, at the end of the experiment. Therefore improved monitoring of wireworm movement and feeding behaviour is required to better understand the effect of the different watering regimes on wireworm herbivory. The abundance of soil dwelling organisms has been previously reported to be affected by soil moisture availability (Briones *et al.* 1997). However, despite the negative impacts of root herbivory, root herbivores and other soil dwelling organisms for example earthworms, they can also provide an essential role in improving soil quality for example their movement through the soil improves soil aeration, reducing soil compaction, and they can be vectors for soil microorganisms such as mycorrihzal fungi (Crossley, Coleman and Hendrix 1989; Poveda *et al.* 2005). If indeed the movement of these important soil dwelling organisms is reduced, it could lead to poor soil quality (Crossley *et al.* 1989). Poor soil quality and structure may reduce crop growth particularly as a consequence of reduced available nutrients leading to increased use of fertilisers to improve crop yields.

Predicted changes in precipitation were also found to benefit above-ground herbivores. This is an important consideration for barley production in the future especially with drives for sustainable farming reducing chemical inputs such as pesticides because aphids are a particular problem for cereal crop production (HGCA 2003). Therefore increased aphid populations could significantly reduce cereal crop production in the future (Coakley *et al.* 1999; Gregory *et al.* 2009). An increase in aphid populations may also put farmers under pressure to use more insecticides to maintain high yields, this goes against the drives to increase the sustainability of farming. Many aphid species, particularly under stressed conditions, can produce winged offspring which have a greater ability to disperse to different host plants and thus have the potential to choose a host plant based on its quality as a resource (Mody *et al.* 2009; Łukasik *et al.* 2011). The effect of watering regimes on aphid host preference could be further explored. Furthermore, the responses of different insect herbivores to the watering regimes might vary between species and feeding guilds. Although these insects are regarded as pests to farmers and agriculture they play a significant role in an ecosystem and any changes in their abundance will have consequences for organisms interacting with these insects.

6.3.5 Implications of future predicted changes in precipitation on the third trophic level

Harlequin ladybird mass increased when feeding on aphids collected from plants watered less frequently under 40% reduction regime (Fig. 4.8). Larger ladybirds may be able to defend themselves better and could produce more offspring (Honek 1993). This could have beneficial effects for barley production as this introduced ladybird is frequently used as a biocontrol strategy (Dixon *et al.* 1997) for controlling aphid populations. Changes in precipitation could indirectly influence populations of insect herbivores, many of which are important agricultural pests such as aphids, which are maybe regulated by invertebrate predators (De Lucia *et al.* 2012). The effect of these changes in precipitation on natural enemies needs to be further explored including the effect on fecundity and further predator species. This is particularly important in the future due to the essential role these predators play in maintaining pest populations for future crop production (Landis, Wratten and Gurr 2000). Further work to elucidate the implications of this increase in mass for ladybird fecundity would provide information on how these watering regimes might affect ladybird population dynamics. If ladybirds are not choosing the larger aphids then it could exacerbate the issues of farmers wanting to use more pesticide due to crop losses.

Harlequin ladybirds are an invasive species in the UK, and any changes in population may also affect native ladybirds which compete for resources and can be cannibalised by the Harlequin ladybird (Majerus *et al.* 2006). With no change in ladybird feeding preference despite an increase in prey quality, native ladybirds could continue to be negatively affected by Harlequin ladybirds. The promotion of Harlequin ladybirds could also undermine ecosystem services provided by native ladybirds such as increased biodiversity and agricultural pest control (Majerus *et al.* 2006; Birch *et al.* 2011).

6.3.6 How will global changes in precipitation affect cereal crop production across the world?

Climate models predict changes in rainfall patterns across the globe with more severe changes in rainfall patterns at lower latitudes (Folland *et al.* 2001; Trenberth 2005; Bates *et al.* 2008). Significant reductions in barley yield production has been recorded in this thesis with predicted changes in UK rainfall patterns. This suggests that more severe changes in precipitation towards lower latitudes may have larger effects on crop production in these areas. The majority of the world’s food is supplied from areas predicted to suffer from significant changes in precipitation (Schmidhuber and Tubiello 2007; Aggarwal and Singh 2010). This has important consequences for future food security which is critical in meeting the estimated 70% rise in global food demand in the next 50 years due to increases in the world’s human population (Alexandratos 2009; Godfray *et al.* 2010; Tilman *et al.* 2011; FAO *et al.* 2014). Furthermore this will have particularly severe consequences for subsistent farmers in these areas who rely on their crop for food and face devastating yield losses due to future climate change (Bates *et al.* 2008; Newton *et al.* 2011a).

6.3.7 Further effects of climate change on agroecosystems

Other climate variables, in tandem with changes in precipitation, could also influence the response of insect herbivores and their natural enemies. This is particularly important as climate change is multi-faceted and the effect of changes in precipitation on crop production, insect pests and their natural enemies will also be influenced by changes in temperature, CO_2 and irradiation (Bates *et al.* 2008). As such, there are limitations to investigating the effect of one aspect of climate change alone, in this case investigating the effect of precipitation only. The interactive effects of temperature and precipitation can also influence multi-trophic interactions in cereal crop ecosystems (De Lucia *et al.* 2012). Insect growth, development and the number of insect generations per year are influenced by temperature (Bale *et al.* 2002; Jamieson *et al.* 2012). Therefore, elevated temperatures could generate asynchrony between insect phenology, timing of emergence and migration between plants, insect herbivores and their natural enemies which may be unable to adapt at the same rate as the rapidly changing environment (De Lucia *et al.* 2012; McCluney *et al.* 2012; Facey *et al.* 2014). The different responses of arthropod herbivores and their natural enemies differed between controlled environment rooms and the field experiment. This was most likely to be due to high air temperatures during the field experiment compared to the controlled environment experiments. Elevated temperatures have been previously reported to affect the growth, development and behaviour of wireworms, aphids and parasitoid wasps (van Herk and Vernon 2006; Hance *et al.* 2007). Increased temperatures and irradiation will result in increased evaporation and evapotranspiration and therefore further reduce soil moisture availability (Allan and Soden 2008). Increased CO_2 may also improve plant water use efficiency by stimulating stomatal closure and restricting transpiration rates (Lobell and Field 2007; Lobell and Gourdji 2012; Dias De Oliveira *et al.* 2013). Elevated CO_2 can also improve plant tissue quality as a food resource for insects by increasing leaf carbohydrate concentrations (De Lucia *et al.* 2012; Dray *et al.* 2014). Overall the negative effects of reductions in precipitation together with increased temperature on plant growth and development is likely to outweigh any positive effects of stomatal closure due to increases in CO_2 (Oerke and Dehne 2004; Godfray *et al.* 2010; Gregory and George 2011). Changes in plant chemical composition and growth as a result of the interacting aspects of climate change are also highly likely to benefit above-ground insect herbivores due to increases in the quality of their food resource and lead to increased development (Zvereva and Kozlov 2006; Gregory *et al.* 2009). Despite the challenges these experiments would pose for example large number of plants, space and high tech growing facilities, it is particularly important that future studies investigate the interactive effects of the different aspects of climate change on ecosystems to accurately predict the impacts on plants, insect herbivores and their natural enemies under future climate scenarios.

6.4 Summary

This thesis provides evidence that predicted changes in precipitation is likely to significantly reduce barley plant growth and yield with reductions in rainfall quantity having larger negative effects on plant growth than reductions in the frequency of precipitation events. The effect of changes in rainfall regimes on barley plant growth and development were consistent between controlled environment rooms and in the field suggesting that this response is robust. Reductions in barley production will result in potential financial losses for farmers and may increase irrigation with consequences for water supplies.

Predicted changes in precipitation were recorded to reduce below-ground wireworm herbivory but benefit above-ground aphids and natural enemy ladybirds. An invasive ladybird species was found to benefit from changes in precipitation due to changes in the quality of a food source, aphids. This could have negative consequences for native ladybird species and the ecosystem services they provide. Wireworm herbivory was recorded to decrease the number of aphids during early plant development under ambient watering. However predicted changes in precipitation removed this interaction between the above- and below- ground insect herbivores. Changes in pest dynamics will be particularly important potentially resulting in further reductions in crop losses at time where there are drives to reduce the use of pesticides. Arthropod responses to changes in rainfall patterns differed between controlled environment and field experiments suggesting that other climate factors are important in the response of arthropod herbivores. Further studies are required to gain an understanding on how different aspects of climate change will interact and affect the interactions between different trophic levels in cereal ecosystems.

Changes in the frequency of extreme rainfall events are particularly important as it may alleviate some of the negative effects of reductions in mean precipitation on barley plant growth, but may benefit major agricultural pests such as aphids potentially having significant consequences for future barley production, agroecosystems and farming procedures. Future studies investigating the effect of changes in precipitation on crop production and ecosystems may overestimate the effect of reductions in the quantity of rainfall if they do not consider future changes in the frequency of rainfall events.

Appendix 1

Appendix 1.1. The effect of changing water quantity on different growth parameters of barley genotypes, Optic and Quench, at harvest.

Barley genotype	Optic			Quench		
Watering regime	Control	D	SD	Control	D	SD
Growth and biomass allocation patterns						
Total plant biomass (g DM)	6.09±0.291	4.04±0.0971	2.38±0.0671	6.46±0.420	3.63±0.160	2.49±0.179
Leaf biomass (g DM)	1.71±0.052	1.21±0.027	0.81±0.024	1.71±0.064	1.11±0.021	0.82±0.011
Stem biomass (g DM)	2.50±0.100	1.53±0.064	0.86±0.054	2.45±0.146	1.35±0.061	0.98±0.181
Root biomass (g DM)	2.5±0.100	1.529±0.064	0.86±0.054	2.45±0.146	1.35±0.061	0.98±0.181
Root: mass ratio	0.29±0.014	0.29±0.018	0.26±0.010	0.27±0.015	0.26±0.012	0.23±0.014
No. leaves	34.9±1.178	28.6±1.579	20.9±0.823	36.8±1.331	27.8±1.114	23.3±1.193
Main stem diameter (mm)	5.27±0.309	4.58±0.158	3.90±0.175	4.77±0.418	3.86±0.138	3.60±0.148

Chemical composition						
Total plant water content (g)	26.43±1.546	12.79±1.072	6.47±0.311	22.11±1.514	11.04±0.693	6.07±0.498
Leaf C (% DM)	41.83±0.428	42.43±0.761	42.58±0.441	41.93±0.397	42.62±0.235	42.81±0.518
Leaf N (% DM)	1.96±0.112	2.59±0.138	3.09±0.157	1.74±0.115	2.82±0.144	3.19±0.141
Leaf C/N ratio	21.64±1.179	16.54±0.667	13.95±0.709	24.53±1.442	15.32±0.835	13.57±0.768

Dry mass (DM)

Appendix 1.2. The effect of changing watering frequency on different growth parameters of barley genotypes Optic and Quench at harvest.

Barley genotype	Optic			Quench		
Watering regime	D	DRF	DIF	D	DRF	DIF
Growth and biomass allocation patterns						
Total plant biomass (g DM)	4.04±0.097	3.97±0.166	4.56±0.145	3.63±0.160	4.1±0.071	4.33±0.203
Leaf biomass (g DM)	1.21±0.027	1.31±0.038	1.36±0.037	1.11±0.021	1.25±0.031	1.27±0.041
Stem biomass (g DM)	1.53±0.064	1.50±0.089	1.64±0.039	1.35±0.061	1.54±0.043	1.55±0.078
Root biomass (g DM)	1.16±0.088	1.14±0.083	1.40±0.098	0.94±0.068	1.05±0.047	1.31±0.119
Root: mass ratio	0.29±0.018	0.29±0.012	0.31±0.014	0.26±0.012	0.25±0.010	0.30±0.016
No. leaves	28.6±1.579	29.7±0.473	30.6±1.701	27.8±1.114	30.2±1.052	31.0±1.135

Main stem diameter (mm)	4.57±0.156	4.23±0.158	4.55±0.274	3.60±0.152	3.85±0.160	4.23±0.209
Chemical composition						
Total plant water content (g)	12.79±1.072	14.31±0.502	16.78±1.17	11.04±0.693	12.21±0.74	14.55±1.55
Leaf C (% DM)	42.43±0.761	41.84±0.808	41.50±0.218	42.62±0.235	42.89±0.355	42.84±0.525
Leaf N (% DM)	2.59±0.138	2.35±0.133	2.72±0.297	2.82±0.144	2.60±0.107	2.88±0.172
Leaf C/N ratio	16.54±0.667	17.98±0.756	15.28±0.198	15.32±0.835	16.62±0.712	15.13±0.900

Dry mass (DM)

Appendix 2

Appendix 2.1. The effect of changing water quantity on different growth parameters of barley cultivar and landrace plants at harvest.

Barley variety	Cultivar			Landrace		
Watering treatment	Ambient	D	SD	Ambient	D	SD
Growth and biomass allocation patterns						
Total plant biomass (g DM)	12.78±0.254	9.46±0.257	5.12±0.271	9.67±0.584	6.44±0.241	3.44±0.338
Leaf biomass (g DM)	1.66±0.079	1.42±0.078	1.17±0.078	1.40±0.073	1.34±0.143	1.01±0.133
Stem biomass (g DM)	5.62±0.185	4.17±0.126	2.30±0.143	3.87±0.306	2.02±0.103	1.09±0.201
Root biomass (g DM)	2.06±0.065	1.47±0.091	0.84±0.061	2.50±0.213	1.82±0.207	0.83±0.087
Root: mass ratio	0.162±0.0067	0.155±0.0073	0.163±0.0073	0.257±0.1335	0.283±0.2969	0.250±0.0222
No. leaves	43.8±1.706	40.3±1.756	29.3±1.803	40.1±2.632	36.8±2.493	27.8±0.925
No. tillers	5.8±0.147	6.3±0.553	5.1±0.484	5.8±0.547	6.2±0.547	5.0±0.333

No. tillers	5.8±0.147	6.3±0.553	5.1±0.484	5.8±0.547	6.2±0.547	5.0±0.333
Average tiller diameter (mm)	3.02±0.142	2.50±0.118	2.19±0.072	2.15±0.055	2.19±0.096	2.05±0.091
Stem height (cm)	58.71±0.956	51.16±1.569	38.33±1.845	61.16±7.078	42.19±4.468	24.69±3.323
SLA (cm^2 g^{-1})	110.70±8.981	156.14±10.681	171.59±8.746	309.05±9.569	278.12±19.400	263.16±8.848
Yield and grain nitrogen concentration						
Ear biomass (g DM)	3.44±0.196	2.39±0.144	0.81±0.104	1.91±0.255	1.25±0.391	0.51±0.173
No. ears	5.1±1.756	4.3±1.756	1.8±1.803	3.0±2.632	2.1±2.493	1.1±0.925
No. grains	113.4±5.140	78.4±4.397	31.2±3.286	104.7±10.686	64.8±7.935	35.3±5.228
Grain mass (mg DM)	30.78±2.343	31.87±3.661	26.03±3.111	19.87±3.444	18.72±4.898	11.86±3.453
HI	0.50±0.028	0.45±0.030	0.21±0.033	0.28±0.039	0.25±0.070	0.16±0.052
Grain N (%)	0.94±0.023	1.06±0.063	1.41±0.005	1.09±0.094	1.67±0.074	1.95±0.083

Chemical composition						
Total plant water content (g)	31.14±0.360	19.96±1.003	9.73±0.487	34.41±1.322	20.88±1.346	10.79±1.026
Leaf C/N ratio	40.72±2.382	42.50±7.364	27.932±2.950	26.74±0.485	26.02±2.357	16.57±1.302
Leaf [fructose]	6.42±2.007	25.34±5.218	42.42±4.412	3.58±1.106	11.25±2.133	15.44±2.635
Leaf [glucose]	4.41±1.084	18.91±6.138	41.61±4.614	4.36±1.708	11.26±1.515	23.62±5.754
Leaf [sucrose]	16.98±9.788	21.5±2.787	46.63±15.922	24.77±9.577	24.17±6.272	34.27±12.130
Leaf [Si] (%)	1.10±0.261	0.37±0.032	0.24±0.024	1.21±0.237	0.50±0.059	0.44±0.050
Leaf [P] (%)	0.137±0.055	0.150±0.018	0.178±0.010	0.564±0.062	0.336±0.034	0.227±0.023

Dry mass (DM)

Appendix 2. 2. The effect of changing the watering frequency on different growth parameters of barley cultivar and landrace plants at harvest.

Barley variety	Cultivar			Landrace		
Watering treatment	D	DMRF	DSRF	D	DMRF	DSRF
Growth and biomass allocation patterns						
Total plant biomass (g DM)	9.46±0.257	10.95±0.127	9.93±0.284	6.44±0.241	7.15±0.311	7.27±0.360
Leaf biomass (g)	1.42±0.078	1.77±0.109	1.88±0.116	1.34±0.143	1.43±0.176	1.65±0.111
Stem biomass (g)	4.17±0.126	4.92±0.128	4.51±0.204	2.02±0.103	2.37±0.122	3.04±0.341
Root biomass (g DM)	1.47±0.091	1.78±0.094	1.57±0.111	1.82±0.207	1.74±0.116	1.56±0.128
Root: mass ratio	0.155±0.007	0.163±0.008	0.158±0.010	0.283±0.2969	0.242±0.010	0.220±0.021
No. leaves	40.3±1.756	48.1±2.282	51.2±2.408	36.8±2.493	35.4±1.923	38.2±1.847
No. tillers	6.3±0.553	7.7±0.645	7.8±0.434	6.2±0.547	6.8±0.521	6.8±0.662

Average tiller diameter (mm)	2.50±0.118	2.86±0.112	2.74±0.093	2.19±0.096	2.60±0.135	2.33±0.065
Stem height (cm)	51.16±1.569	53.56±1.614	49.58±1.668	42.19±4.468	50.89±1.662	44.29±3.772
SLA (cm^2 g^{-1})	156.14±10.681	156.25±17.65	169.19±21.03	278.12±19.400	273.34±14.393	256.89±11.98
Yield and grain nitrogen concentration						
Ear biomass (g DM)	2.39±0.144	2.48±0.248	1.97±0.193	1.25±0.391	1.61±0.297	1.02±0.312
No. ears	4.3±0.408	5.3±0.333	5.9±.0.351	2.1±0.309	2.4±0.294	2.3±0.373
No. grains	78.4±4.397	99.2±2.488	89.0±4.419	64.8±7.667	83.9±7.667	66.8±11.241
Grain mass (mg DM)	31.87±3.661	29.98±2.41	22.32±2.054	18.72±4.900	18.44±2.700	13.07±2.529
HI	0.45±0.030	0.47±0.032	0.43±0.047	0.25±0.070	0.33±0.074	0.20±0.063
Grain N (%)	1.06±0.063	1.01±0.031	0.99±0.081	1.67±.074	1.49±0.074	1.79±0.147
Chemical composition						

Total plant water content (g)	19.96±1.003a	25.46±0.704b	22.85±1.220ab	20.88±1.346	24.42±1.875	22.80±1.287
Leaf C/N ratio	42.50±7.364	45.29±4.066	38.49±4.533	26.02±2.357	22.46±1.261	19.58±0.929
Leaf [fructose]	25.02±5.152	16.66±5.425	31.46±6.436	11.11±2.106	18.97±4.672	9.02±2.859
Leaf [glucose]	18.91±6.138	9.60±2.761	22.38±6.356	11.26±1.515	15.46±2.634	8.60±2.429
Leaf [sucrose]	21.75±2.787	22.28±8.872	18.12±5.026	24.17±6.272	16.99±6.311	17.72±4.680
Leaf [Si] (%)	0.37±0.032	0.48±0.051	0.90±0.082	0.50±0.059	0.64±0.059	0.89±0.068
Leaf [P] (%)	0.15±0.018	0.17±0.017	0.18±.024	0.34±0.034	0.51±0.045	0.67±0.116

Dry mass (DM)

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