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## Impact of predicted precipitation scenarios on multitrophic interactions

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Impact of predicted precipitation scenarios on multitrophic interactions.

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14 Summary

1. Predicted changes in the frequency and intensity of extreme rainfall events in the UK have the potential to disrupt terrestrial ecosystem function. However, responses of different trophic levels to these changes in rainfall patterns, and the underlying mechanisms, are not well characterised.

2. This study aimed to investigate how changes in both the quantity and frequency of rainfall events will affect the outcome of interactions between plants, insect herbivores (above- and below-ground) and natural enemies.

3. *Hordeum vulgare* L. plants were grown in controlled conditions and in the field, and subjected to three precipitation scenarios: ambient (based on a local 10 year average rainfall); continuous drought (40% reduction compared to ambient); drought/deluge (40% reduction compared to ambient at a reduced frequency). The effects of these watering regimes and wireworm (*Agriotes species*) root herbivory on the performance of the plants, aphid herbivores above-ground (*Sitobion avenae, Metapophium dirhodum* and *Rhopalosiphum padi*), and natural enemies of aphids including ladybirds (*Harmonia axyridis*) were assessed from measurements of plant growth, insect abundance and mass, and assays of feeding behaviour.

4. Continuous drought decreased plant biomass, whereas reducing the frequency of watering events did not affect plant biomass but did alter plant chemical composition. In controlled conditions, continuous drought ameliorated the negative impact of wireworms on plant biomass.

5. Compared to the ambient treatment, aphid mass was increased by 15% when feeding on plants subjected to drought/deluge; and ladybirds were 66%
heavier when feeding on these aphids but this did not affect ladybird prey choice. In field conditions, wireworms feeding below-ground reduced the number of shoot-feeding aphids under ambient and continuous drought conditions but not under drought/deluge.

6. Predicted changes in both the frequency and intensity of precipitation events under climate change have the potential to limit plant growth, but reduce wireworm herbivory, while simultaneously promoting above-ground aphid numbers and mass, with these effects transferring to the third trophic level. Understanding the effect of future changes in precipitation on species interactions is critical for determining their potential impact on ecosystem functioning and constructing accurate predictions under global change scenarios.

Keywords *Agriotes*, climate change, extreme events, *Harmonia axyridis*, herbivory, *Hordeum vulgare*, *Sitobion avenae*.
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; Bouwer et al. 2014). 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, and by disrupting interactions between plants and herbivores. This can result in asynchrony between the development, behaviour and life cycles of different trophic levels (Weltzin et al. 2003; Trotter, Cobb & Whitham 2008). Multi-trophic interactions are critical in ecosystem structure and function (Hellmann et al. 2008), and understanding the effects of future changes in precipitation on such interactions is important for food security, pest management and constructing more accurate predictions of global change impacts (van der Putten et al. 2004). Despite a large amount of evidence predicting changes in precipitation patterns and their potential to disrupt ecosystems, there is very little published research attempting to simulate changes in the frequency as well as the intensity of rainfall events and test how this will impact multi-trophic interactions (Weltzin et al. 2003; Facey et al. 2014).

Some plants can tolerate or adapt to water stress through a number of mechanisms such as changes in resource allocation to growth and development (Blum 1996; Chaves, Maroco & Pereira 2003) and osmotic adjustment including utilising sugars as osmoprotectants to minimising oxidative damage (Chaves, Maroco & Pereira 2003; Barnabás, Jäger & Fehér 2008). Increased silicon (Si) uptake has also been reported to improve the tolerance of plants to water stress by stimulating antioxidant
systems, immobilising antioxidants avoiding cellular damage by reactive oxidative
damage (Gong et al. 2005, 2008; Pei et al. 2010) and providing cellular structural
support to avoid lodging (Ma 2004; Cooke & Leishman 2011; Balakhnina et al.
2012). These changes in plant morphology, physiology and chemical composition
due to water stress can influence plant food quality for above- and below- ground
arthropod herbivores, which in turn can affect herbivore performance (Huberty &
Denno 2004; Chown, Sørensen & Terblanche 2011). To date, most current research
focuses on the impact of continuous drought or complete water withholding events
on insect herbivore populations, despite evidence to suggest that the severity of
drought events maybe an important determinant of the outcome for root and foliar
feeding herbivores (Mody, Eichenberger & Dorn 2009; Jamieson et al. 2012; Tariq
et al. 2012; Rosenblatt & Schmitz 2014). For example, 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 & Denno 2004; Mody et al. 2009). The
timing of these extreme rainfall events within the growing seasons is particularly
important (Griffin & Hoffmann 2011; de San Celedonio, Abeledo & Miralles 2014).
However, there is currently a lack of research investigating how changes in the
frequency of rainfall events throughout the growing season would affect crop growth
and whether these effects transfer to higher trophic levels.

Water stress can also affect the direction and intensity of interactions between above-
and below- ground insect herbivores through changes in root herbivore behaviour
and host plant growth and chemical composition (Staley et al. 2007). The severity
and number of days of a drought treatment or level of reduction in soil moisture has
also been reported to influence the abundance and vertical distribution of below-ground insect herbivores (Lees 1943a; Briones, Ineson & Pierce 1997; Sinka, Jones & Hartley 2007), potentially impacting their feeding behaviour. Root herbivores themselves can influence host plant growth, development and chemical composition (Johnson, Erb & Hartley 2016) and can also increase the severity of a drought event to plants due to the removal of roots, thereby altering the quality of the plant as a food source for the other organisms feeding on the same plant (Bezemer & van Dam 2005; Tariq et al. 2013a). Therefore, any changes in below-ground herbivore feeding intensity due to changes in soil moisture availability have the potential to also impact above-ground herbivores. The impact of herbivory below-ground on above-ground herbivores has specific importance as there is evidence to suggest that above-, below-ground interactions can affect the third trophic level (Barnett & Johnson 2013; Johnson et al. 2013), with potential to influence ecosystem functions associated with the wider insect community.

Effects of water stress have also been previously reported to transfer into higher trophic levels (Johnson et al. 2011) 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). However, the very few studies investigating the impact on the third trophic level focus on parasitoids (e.g. Johnson et al. 2011; Aslam, Johnson & Karley 2013; Tariq et al. 2013b) and do not consider how this will influence insect herbivore quality as prey for insect predators. Moreover, the majority of research is conducted in controlled environment systems. Although controlled environment experiments provide fundamental understanding of species interactions within complex systems,
conditions in these environments might not reflect those of the field environment (Hughes 1959) which differ in rates of soil drying, air flow, radiation, temperature and soil structure. Very few studies use a combination of controlled environment conditions and field conditions to determine how relevant their findings are to the natural environment. Research measuring the impact of realistic rainfall patterns in field conditions and determining if studies conducted in controlled environments produce reliable results compared to field conditions is particularly lacking. Therefore, here we aim to increase fundamental understanding of how changes in rainfall quantity and pattern can interact with different herbivore guilds and transfer to a third trophic level. To our knowledge, this is the first study to achieve this trophic complexity in both field and controlled conditions.

This study investigates the effect of predicted changes in precipitation (in both rainfall quantity and frequency) using realistic water stress scenarios on plant-herbivore interactions above- and below-ground. We used root- (wireworm *Agriotes* spp.) and shoot-feeding herbivores (aphid *Sitobion avenae* F., *Metopolophium dirhodum* Walker and *Rhopalosiphum padi* L.) commonly found attacking barley as a model system (Johnson, Hawes & Karley 2009), and examined the effect of water stress on the performance of common natural enemies of this herbivore, including the invasive Harlequin ladybird (*Harmonia axyridis*) (Majerus, Strawson & Roy 2006) and parasitoid wasps such as *Aphidius ervi*. Experiments were conducted in controlled environment conditions and in field mesocosms, to assess if plant and insect herbivore responses are consistent between different experimental conditions. It was hypothesised that (i) continuous drought and drought/ deluge would reduce barley growth but positively affect aphid development and fecundity, with drought/
deluge having larger effects on both plants and aphids; (ii) wireworm root feeding would positively affect performance of aphids above-ground, but reduced water availability would mitigate this interaction and (iii) increased aphid performance (due to changes in water availability or wireworm herbivory) would benefit the performance of natural enemies.

**Materials and Methods**

*Plant growth conditions*

Two separate experiments were performed, one with plants grown in pots in controlled environment conditions and the other with plants grown in pots positioned in a field situated at the James Hutton Institute, Dundee, UK. For both experiments *H. vulgare* spring barley cultivar Optic (seeds supplied by The James Hutton Institute, Dundee, UK) plants were grown in pots (see below for details) filled with dried, sieved (10 mm x 10 mm aperture sieve) topsoil (A1 Plant, Elvington, UK) mixed in a 3:1 ratio with washed sharp horticultural sand (Keith Singletons, Egremont, UK) to give a sandy loam soil substrate. Prior to the experiment all pots were watered with deionised water from the top of the pot to ensure soil water content reached 50% of total water holding capacity and soil water content was maintained at 50% total water holding capacity for the first two weeks to ensure seedling establishment (see Appendix S1 in Supporting Information).

Three different watering regimes were applied to the plants:
Ambient = a quantity of water added based on 10 year weekly average rainfall at the James Hutton Institute, Invergowrie, Scotland. Half of the weekly average was provided twice per week;

Drought = a 40% reduction in the quantity of water added, also provided twice per week;

Drought/deluge = a 40% reduction in the quantity of water added with severe reduced watering frequency, provided once per fortnight.

Quantities of water added to each pot were calculated based on pot surface area (see Appendix S1). These different watering regimes allowed comparison of the effects of reductions in rainfall quantity (a 40% reduction compared to ambient) and reductions in rainfall frequency under the 40% reduction regime (water provided once per fortnight compared with twice per week).

For plants assigned the below-ground herbivory treatment, two weeks (controlled environment experiment) or three weeks (field experiment; to account for slower plant development in the field) after sowing, three wireworms (a mixture of Agriotes spp. L. Coleoptera: Elateridae: 60% A. lineatus and 40% A. obscurus sourced from Praktijkonderzoek Plant and Omgeving / Applied Plant Research, Wageningen, UR) were buried c. 5cm from the soil surface in three different locations within the pot. A similar level of soil disturbance was imposed on pots that were not assigned wireworm treatment.

At harvest, plant ear, leaf, stem and root fractions were collected separately, weighed for fresh mass, dried at 70 °C for c. five days and re-weighed for dry mass (g dry
mass DM). One exception to this was the leaf fraction of plants grown in the
controlled environment experiment; prior to weighing, two green leaves were
removed for amino acid analysis as described below. The dry mass of these leaves
was calculated based on the fresh mass: dry mass ratio of the rest of the leaf fraction,
which was processed for oven drying in the same way as the other plant fractions.
Root: mass ratio and water content (g) of the plant material was calculated (see
Appendix S1).

Controlled environment experiment

The experiment comprised a randomised block design to account for spatial variation
within the controlled environment space. The experiment was conducted in three
growth rooms, with each room divided equally into three spatial blocks (nine blocks
in total). Within each block (12 plants), each combination of watering regime (three
levels; see above) and herbivory (four levels: control (no herbivore), above- (aphids),
below- (wireworms) ground herbivore or both) were assigned at random to each pot
location. This resulted in one replicate plant per block of each watering regime x
herbivory combination, with nine replicate blocks giving a total of 108 plants. Plants
were grown individually in 2.4 L pots (see Appendix S1) maintained at 16 h daylight
(average light intensity across the three rooms was 210.5 µmol m⁻² s⁻¹ mean ± 1.80
standard error); 23 °C ± 0.33 / 19 °C ± 0.21, day / night. 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, Cambridge UK). Porometer readings were taken
between 0900 h and 1000 h (BST).
Three aphid (S. avenae) nymphs (born on the plant see Appendix S1) were monitored daily for c. four weeks to record aphid survival, the date of first reproduction and number of offspring produced. Fecundity was calculated as the number of offspring produced in the same number of days it took for the adult to mature. Offspring were collected every two days and transferred to a second cage (cage 2) clipped onto on the same plant. After four weeks of aphid herbivory, all aphids were individually counted, removed from the plants and weighed, before using in the ladybird performance assays (see below).

Ladybird responses to the water treatments were assessed using a combination of feeding and choice assays: one second instar Harlequin ladybird (H. axyridis) was weighed, and then presented with three pre-weighed apterous adult aphids and three first instar nymphs (collected from cage 2; see above), in a Petri-dish (90 mm, Sterilin Ltd, Mid Glamorgan, UK). Assays were conducted over 24 h in a controlled environment room (12 h daylight; c. 20 °C), after which the ladybird was removed, re-weighed and mass gain calculated (11 to 13 replicates). For the ladybird prey choice assay: three live apterous adult aphids, one from a plant treated with each watering regime within the same block, were collected and positioned randomly in a 90 mm Petri-dish (Sterilin Ltd, Mid Glamorgan, UK) and equidistant from the dish centre. The ventral surface of each aphid was secured to the base of the dish with a small (c. 3 mm x 4 mm) piece of double sided sticky tape, leaving their legs free to defend from predator attack, a common form of defence for aphids (Roy et al. 2013). One harlequin ladybird larva (third or fourth instar) was placed in the middle of the
arena. The ladybird larvae were monitored and the first aphid to be consumed was recorded.

Barley plants in each experimental block were harvested seven weeks after sowing, at Zadoks growth stage 40 (Zadoks, Chang & Konzak 1974). Elemental analysis was conducted on dried milled green leaf material (c. four oven-dried green leaves per plant) (see Appendix S1). Si 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 (% dry mass) were determined by flash combustion and chromatographic separation of ~1.5 mg 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$_{36}$N$_{2}$O$_{2}$S).

Total amino acids were extracted (see Appendix S1) from 25 mg of freeze-dried, milled green leaf material using 1 mL solution of 49% methanol, 49% milli-Q water and 2% glacial acetic acid (adapted from Matsuda et al. 2005; Noctor et al. 2007) and were analysed by HPLC to quantify amino acid composition. Amino acids were separated by reverse-phase HPLC and quantified as described by (Johnson et al. 2009).

Field experiment
Nine spring barley plants were grown in 15 L pots (25.5 cm × 25.5 cm × 25.5 cm) lined with plastic sheeting. Pots were wrapped in insulating material (Thermawrap loft insulation, 400 mm x 5 m, B&Q, UK) and covered in Fine Mesh Garden Protection Net (2 cm diameter netting, B&Q, UK) which was suspended c. 50 cm above the pot and draped down the sides of the pots to prevent small mammalian herbivory but to allow arthropod access to the plants. Theta probes (Delta-T ML2, connected to a DL6 data logger, and downloaded using DeltaLINK software, Delta-T, Cambridge, UK) 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 min throughout the experimental period. All plants were grown under rain exclusion shelters including the ambient treatment. A Met station positioned in the same field provided meteorological data for the experiment. Maximum air temperatures were on average 18.4 °C ± 0.3 with minimum temperatures averaging 9.4 °C ± 0.3.

The experiment comprised a randomized block design with five blocks (rain exclusion shelters). Within each block, watering regime and root herbivory were assigned at random to each pot, with one replicate per block of each watering regime × herbivory combination (six pots under each rainshelter).

Plants were open to natural establishment of above-ground herbivores and natural predators. Three barley plants in each pot selected at random and identified using a small piece of cotton tied very loosely around the main stem were used for routine monitoring of insect herbivore and natural enemy abundance. Total numbers of aphids (*Sitobion avenae, Metapophium dirhodum* and *Rhopalosiphum padi*) on
these plants were recorded weekly throughout the growing period as well as the
number of mummified aphids (parasitised aphids) to assess the combination of
acceptance of aphids for oviposition and the suitability of aphids for parasitoid
survival. Barley plants in each experimental block were harvested 10 weeks after
sowing, by which time the ear on the main stem of all plants had reached Zadok’s
growth stage 71 (Zadoks et al. 1974). Material from all nine plants within the pot
was pooled. At harvest, all aphids were collected from all the plants in each pot and
counted, then transferred to 1 mL Eppendorf tubes, flash frozen in liquid nitrogen
and stored at -20 °C. Frozen aphids were then freeze-dried and re-weighed, and
individual aphid mass calculated by dividing total aphid mass per pot by the number
of aphids collected.

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, Si data) and controlled environment experiment total plant biomass data were
squared, amino acid data were transformed using natural log and aphid biomass data
were square root transformed. 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. 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) $\chi^2$ statistic). Multiple comparison tests were performed using ‘glht’ in multcomp package with post hoc Tukey contrasts (Hothorn et al. 2014). For controlled environment experimental data, 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 eight 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. The field experiment was assessed on five replicates for each watering regime and herbivory treatment.

For controlled environment experiment, aphid mass gain was analysed using linear mixed effect models to test the main effects of watering regime, wireworm treatment with block and clip cage included as a random term. Ladybird mass gain was also 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.
Amino acid concentrations were converted to µmol/g leaf dry mass prior to analysis.

Variation in plant amino acid concentration (µmol/g) and composition was explored by principal components analysis (PCA) performed on Minitab 17. Pearson product-moment correlation coefficient was used to measure if there was a linear correlation between aphid mass and N or total amino acids.

Results

Impact of the different watering regimes on wireworm herbivory and plant growth and development.

Regardless of herbivory treatment, a 40% reduction in water quantity significantly reduced total plant biomass, whereas reducing the frequency of watering events had no effect on total plant biomass (Fig. 1). In controlled environment conditions, total plant biomass was significantly reduced by wireworm herbivory under ambient watering regime (Post-hoc Tukey test =P<0.001) (Fig. 1a), but wireworms had no effect on total plant biomass under continuous drought and drought/deluge watering regime. In contrast, wireworms had no effect on plant biomass in the field experiment (Fig. 1b). There was also no effect of wireworm herbivory (F_{1,20}=0.52, P=0.48) or watering regime (F_{2,20}=2.76, P=0.087) on root: mass ratio. There was no effect of aphid herbivory recorded on plant biomass (F_{1,92}=1.301, P=0.257).

In the field, soil moisture in the continuous drought treatment was lower than that in the ambient treatment (see Figure S1 in Supporting Information). Soil in the drought/deluge treatment showed a large increase in moisture immediately after a watering event which slowly declined over the next two weeks until the next watering event.
Aphid survival, development and reproduction

In the controlled environment, the number of days to aphid reproduction, aphid fecundity and aphid survival were not affected by either wireworms or watering regime or the interaction between these factors (see Table S1 in Supporting Information). Individual mass of aphids collected from drought/deluge treated plants was significantly heavier than those collected from ambient treated plants (Fig. 2a) but was unaffected by wireworm herbivory ($F_{1,36}=0.019, P=0.89$).

In the field, wireworms caused a significant reduction in total number of aphids on drought treated plants five and six weeks after sowing and on ambient watered plants six weeks after sowing (Fig. 3). Total aphid biomass (g DW) was unaffected by changes in the watering regime ($F_{2,23}=1.78, P=0.19$), or by wireworm herbivory ($F_{2,23}=0.79, P=0.47$).

Third trophic level

Ladybird larvae in the controlled environment experiment gained significantly more mass when feeding on aphids collected from plants under drought/deluge watering regime compared to when feeding on aphids collected from ambient watered plants (Fig. 2b). Wireworm herbivory of the aphid’s host plant had no effect on ladybird mass gain ($F_{1,11}=0.241, P=0.63$). Ladybird larvae choice was unaffected by the watering regimes ($\chi^2=1.379, df=2, P=0.502$), or by wireworm herbivory ($\chi^2=0.000, df=1, P=0.995$).
There were very few natural enemies recorded throughout the field experiment. Total number of mummified aphids was unaffected by the watering regime ($\chi^2=2.519$, df=2, $P>0.05$) and wireworm herbivory ($\chi^2=0.850$, df=1, $P>0.05$).

*Plant chemical composition*

In the controlled environment, tissue water content (g) was significantly greater in ambient watered plants compared to drought and drought/deluge (F$_{2,94}=67.841$, $P<0.0001$) and smaller in plants subjected to wireworm herbivory (F$_{1,95}=6.474$, $P=0.0126$) (see Figure S2a in Supporting Information). Aphids had no effect on plant water content (F$_{1,94}=0.655$, $P=0.4203$). Drought and drought/deluge treated plants had a significantly lower stomatal conductance compared to ambient watered plants (F$_{2,62}=29.064$, $P<0.001$; *Post-hoc* Tukey contrasts $P<0.001$) (see Figure S2b).

Wireworms (F$_{1,62}=0.086$, $P=0.770$) and aphids (F$_{1,62}=1.097$, $P=0.299$) were found to have no impact on stomatal conductance. Plants grown under ambient watering regime had the highest leaf Si concentration compared to drought/deluge and drought treated plants, with drought treated plants containing the lowest leaf Si concentrations (Fig. 4a). Neither wireworm (F$_{1,65}=0.009$, $P=0.93$) nor aphid (F$_{1,65}=0.319$, $P=0.57$) herbivory had any effect on leaf Si concentration.

Regardless of herbivory treatment, plants grown under drought and drought/deluge watering regimes had a higher leaf N concentration than ambient watered plants.

Wireworm herbivory significantly increased leaf N concentration (Fig. 4b). Aphids (F$_{1,66}=0.204$, $P=0.6532$) had no effect on leaf N concentration. Drought/deluge plants had higher concentration of amino acids compared to ambient treated plants.
with the concentration of amino acids in drought treated plants intermediate (but not significantly different) from ambient or drought/deluge treated plants (Fig. 4c).

Drought treated plants had a significantly higher concentration of essential amino acids compared to plants under the ambient watering regime ($F_{2,88}=4.701, P<0.05$; Post-hoc Tukey test $P<0.01$). Foliar essential amino acid concentrations were significantly increased by aphid herbivory ($F_{1,88}=5.436, P=0.022$), but wireworms had no effect on essential amino acids ($F_{1,88}=2.245, P=0.138$). Total amino acids comprised 17% essential amino acids under drought conditions compared to 14% essential amino acids under ambient watering. Therefore the drought treatment increased the proportion of essential amino acids ($F_{2,81}=8.051, P<0.001$). Visual exploration of the amino acid data by PCA revealed that PC1 (accounting for 68.8% of the variation in the data set) separated glutamate, tryptophan and methionine from all other amino acids. Tryptophan, glutamine and glutamate were separated along PC2 (22% of the variation; Fig. 5a). A plot of the score values indicated that these two axes separated ambient plants from the other watering regimes suggesting that quantity of water has a greater effect than changes in the frequency of watering events on amino acid composition (Fig. 5b). There was no correlation between aphid mass and total amino acid concentration ($R=-0.0036, P=1.00$) or leaf N concentration ($R=0.304, P=0.060$).

**Discussion**

This study shows that predicted changes in both the frequency and intensity of precipitation events can have significant impacts on above-ground multi-trophic interactions, reducing plant growth but also reducing the impact of wireworm
herbivory below-ground whilst potentially increasing aphid and ladybird

performance above-ground. Plant responses to the watering regimes were similar in
the controlled environment and the field mesocosm experiment but insect herbivore
responses differed between the two experiments.

The impact of changes in precipitation on plant growth and chemical composition

Plant growth was reduced under predicted rainfall scenarios, but changes in the
frequency of rainfall events had very little impact on plant biomass. This was
surprising as it was originally predicted that changes from dry conditions to flooding
would increase the level of stress for plants. However, soil moisture measured by the
theta probes buried half way down the soil profile demonstrated that although water
quantity was reduced by 40% under the drought/deluge watering regime, reducing
the watering frequency resulting in deluge events caused soil moisture in the deeper
soil profile to remain 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 leading to the bulk soil remaining wetter for longer, benefiting a
deeper rooting zone and enabling water uptake during periods of drought (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. However, barley plants have been recorded to root deeper
than 25 cm (the depth of the pot) (Lampurlanés, Angás & Cantero-Martínez 2001),
which would potentially enable these plants to have access to water stores deeper in
the soil profile in a field system. Similar soil moisture patterns were reported by Fry
et al. (2014) where a drought/deluge treatment in a grassland system also resulted in
the soil remaining wetter for a longer duration, but this treatment was reported to
reduce plant biomass and species richness.

Drought/deluge treated plants exhibited lower Si concentrations and tissue water but
higher leaf N and amino acid concentrations compared to ambient watered plants.
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 & Denno 2004; Khan, Ulrichs & Mewis 2010). The relative changes in
amino acids tryptophan, methionine, glutamine and glutamate explain a large
proportion of the impacts of drought on total amino acids. Tryptophan particularly is
classed as an essential amino acid and can play a role in reducing the effects of
drought in maize when applied as a treatment (Rao et al. 2012) and has been found
to increase in drought stress wheat plants (Bowne et al. 2012). Silicon 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 & Yamaji 2006). However, despite receiving the same
quantity of water over the growing period, reducing the frequency of watering events
significantly increased leaf Si concentrations. Therefore, changing the frequency of
rainfall events could have increased the ability of the plant to take up Si, possibly
due to the deeper soil profile remaining wetter for longer affecting plant transpiration
rates and thus Si uptake (Hartley et al. 2015). In grasses, Si plays an important role
in plant defence against insect and mammalian herbivores (Massey, Ennos & Hartley
2006; Guntzer, Keller & Meunier 2012). Therefore, changes in leaf Si concentration
under future predicted precipitation regimes could influence the resistance of barley
to crop pests. Silicon levels were not found to affect aphid performance in this study, supporting some previous evidence to suggest that Si physical defence may be more effective against chewing insects rather than phloem feeders (Massey et al. 2006; Reynolds, Keeping & Meyer 2009; Reynolds et al. 2016).

**The impact of changes in precipitation on aphids**

Under future precipitation patterns, aphid biomass was increased in the controlled environment experiment. In the controlled environment experiment, heavier aphids were collected from plants watered less frequently under the 40% reduction regime most likely due to changes in plant nitrogen and amino acid concentration (White 1984). Insects cannot synthesize amino acids and research demonstrates that one of the functions of the aphid obligate bacterial endosymbiont *Buchnera aphidicola* is to synthesize tryptophan (Rouhbakhsh et al. 1996), confirmed by the fact that aphids treated with antibiotic to disrupt the symbiosis exhibit high nymph mortality when feeding on a synthetic diet lacking tryptophan (Douglas & Prosser 1992). It is particularly interesting to note that drought had a large impact on the amino acid tryptophan in the controlled environment experiment reported here. However, there was no significant correlation found between aphid mass and nitrogen or amino acid concentration which suggests that there were other factors, such as changes in turgor pressure, influencing the impact of changes in N and amino acid availability on aphid performance (Huberty & Denno 2004; Mody et al. 2009).

Insect body size has been reported to correlate with insect performance and fecundity (Honek 1993), but 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 highest on medium drought stressed plants compared to pulsed water stress. In the field experiment however, despite large differences in plant biomass, there was no effect of the different watering regimes on the abundance or mass of aphids, suggesting that variable conditions in the field such as changes in temperature during the experiment could have larger impacts on aphid survival, abundance and fecundity (Bale et al. 2002; Nelson, Bjørnstad & Yamanaka 2013) masking any effects of changes in precipitation.

The impact of below-ground wireworm root feeding on above-ground aphid performance

Wireworms reduced aphid abundance on ambient and drought treated plants during early plant development in the field experiment. Wireworms could have reduced the number of aphids through a number of different mechanisms such as altering the attractiveness of the host plant, increasing concentrations of defence compounds and/or reducing the nutritional quality of the host plant (Bezemer & van Dam 2005; Johnson et al. 2013). This is in contrast to previous published results which report that below-ground herbivory positively affects above-ground herbivory (Johnson et al. 2012). However, in the controlled environment there were no above- and below-ground interactions recorded, despite the effect of wireworms on plant growth and chemical composition. Plant development is clearly important in the interaction between above- and below-ground herbivory, as the effect of wireworms on the number of aphids in the field experiment was transient, only measured during early plant development. In the field experiment, plant growth and development at harvest
was unaffected by wireworm herbivory; the low density of wireworms in each large pot may have been insufficient to cause significant damage to fast growing, well established, matured plants. Older plants are not as severely affected by wireworms and plants in natural grasslands are often attacked by several wireworms at once (Lees 1943b; Parker 1996; Parker & Howard 2001), so might be expected to be resilient to the low experimental densities. Therefore contrasting results between the two experiments may be due to differences in plant age and differences between controlled environment condition and field environment which differ in rates of soil drying, air flow, radiation, temperature and soil structure (Hughes 1959). 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 (Poveda et al. 2005; Johnson et al. 2012, 2013; Barnett & Johnson 2013).

Do the effects of changes in precipitation impact the third trophic level?

Changes in water availability and below-ground herbivory were found to affect the potential fitness of a predator mediated by the plant and herbivore. To date, very few studies have investigated the impact of water stress on multi-trophic interactions encompassing above- and below-ground interactions particularly in agroecosystems (Hentley & Wade 2017) despite reports that higher trophic levels may be more sensitive to changes in climate (Voigt et al. 2003). Ladybirds were reported here to have a greater increase in mass when feeding on aphids collected from plants grown under reduced watering frequency. The increase in mass gain is likely to have been
due to differences in aphid mass. However, water regime may have also affected handling time (e.g. larger aphids are better able to defend themselves), which might have influenced final ladybird mass. Insect mass is often correlated with increased insect fecundity and performance (Awmack & Leather 2002), therefore ladybird fitness maybe higher when feeding on aphids from plants grown under future predicted rainfall patterns. However, ladybird choice of aphid prey was unaffected by the different watering regimes, despite the increase in mass when feeding on aphids collected from ambient treated plants. This has consequences for the performance of predators in this system and could impact their effectiveness as natural enemies of crop pests. Previous research also reports that predator choice does not follow optimal prey diet (Sih & Christensen 2001). This could influence ladybird success under future changes in precipitation (Hassel & Southwood 1978; Mayhew 2001). Nitrogen availability often limits insect growth (Mattson 1980) therefore the reduction in mass gain of the ladybirds is potentially due to changes in nitrogen (e.g. amino acid) concentration and composition of their prey mediated by the host plant. Predators who feed on chewing herbivores maybe further affected by changes in precipitation patterns mediated by the host plant due to potentially larger reductions in chewing herbivore quality and quantity as a food source because of increased leaf Si concentration of the host plant (Massey & Hartley 2009).

Parasitioid wasps were the most common natural enemy found on the plants throughout the field experiment, but there was no significant effect of the watering regimes on the numbers of mummified aphids. In contrast, published studies report significant effects of changes in plant water status on the population of mummified aphids (Aslam et al. 2013; Tariq et al. 2013b). However, these studies were
conducted in a controlled environment glasshouse. A field system may differ to controlled environment due to varying temperatures, mummified aphid predation and/or hyper-parasitism, which could influence mummified aphid abundance.

Conclusions

Barley plant growth was reduced under continuous drought whereas changes in the frequency of precipitation events did not affect plant growth. However, changes in the frequency of watering events removed the effects of wireworm herbivory on plant growth and aphid abundance suggesting that the effect of wireworm herbivory on this ecosystem will be reduced under future precipitation patterns. The drought/deluge watering regime also caused significant changes in plant chemical composition, as well as increased aphid mass and the mass of the ladybirds feeding on these aphids. This study provides the first evidence that predicted changes in the frequency as well as the intensity of rainfall events can affect plant growth and chemical composition significantly as well as above- and below-ground insect herbivores and their interactions, with these effects transferring to a third trophic level, insect predators. Future research investigating the impact of predicted changes in precipitation needs to consider changes in both the frequency and intensity of precipitation events, as well as the experimental setting. These factors were shown to affect the response of different trophic levels to changes in water availability and will therefore influence the predicted outcomes of global change scenarios.
Author contributions

RW, AK, SJ and SH designed the study. RW generated and analysed the data. RW wrote the paper with the help of all the authors. Seeds were kindly provided by Syngenta and The James Hutton Institute. RW was funded by a studentship from University of York and The James Hutton Institute.

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Data Accessibility

All data are available in public archive Dryad. doi:10.5061/dryad.t6m9m
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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1 Supplementary Methods

Figure S1 Soil moisture measurements throughout a representative two weeks.

Figure S2 (a) Water content of barley plants treated with different herbivory and watering regimes. (b) Stomatal conductance of barley plants treated with the different watering regimes.

Table S1 Results of linear models, testing the effect of the treatments and their interactions on different measures of aphid performance.

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Figures

Fig. 1. Total plant biomass (g dry mass (DM)) per pot of barley plants grown under controlled conditions treated with different watering regimes (ambient, drought and drought/deluge) with and without wireworm herbivory in (a) controlled environment and (b) field conditions. 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 for controlled environment, and 5 replicates for all watering regime and herbivory treatments for the field experiment. Bars sharing the same letter were not significantly different as determined by Post-hoc Tukey contrasts. Statistical analysis: (a) Controlled environment, watering regime $F_{2,93}=33.01$, P<0.001, wireworms $F_{1,93}=13.85$, P<0.001, watering regime x wireworms $F_{2,93}=3.93$, P<0.05. (b) Field mesocosm, watering regime $F_{2,20}=61.33$, P<0.001, wireworms $F_{1,20}=0.11$, P>0.05, watering regime x wireworms $F_{2,20}=0.03$, P>0.05

Fig. 2. Controlled environment: (a) Aphid mass (mg fresh mass (FM)) after 4 weeks of feeding on barley plants treated with three watering regimes (ambient, drought and drought/deluge). (b) Ladybird mass gain (mg fresh mass (FM)) after feeding on aphids collected from barley plants treated with ambient, drought and drought/deluge 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. Statistical analysis: (a) Aphid mass, watering regime $F_{2,39}=3.49$, P<0.05. (b) Ladybird mass, watering regime $F_{2,14}=3.78$, P<0.05
Fig. 3. Total number of aphids counted on three randomly selected barley plants per pot in field mesocosm with three different watering regimes in the presence or absence of wireworm herbivory over three weeks. Values represent mean ± standard error bars of five replicates. Stars represent significant effect of wireworm herbivory on the number of aphids as determined by Post-hoc Tukey contrasts $P<0.001^{***}$. Statistical analysis, watering regime $\chi^2=0.547$, df=2, $P>0.05$, wireworms $\chi^2=17.74$, df=1, $P<0.001$, week $\chi^2=105.92$, df=1, $P<0.001$, watering regime x wireworms $\chi^2=27.45$, df=2, $P<0.001$, watering regime x week $\chi^2=14.58$, df=4, $P<0.01$, wireworm x week $\chi^2=15.22$, df=2, $P<0.001$, watering regime x wireworm x week $\chi^2=34.4307$, df=4, $P<0.001$.

Fig. 4. Leaf concentrations of (a) Si (b) nitrogen (N), and (c) amino acids for barley plants grown under controlled conditions treated with different watering regimes (ambient, drought and drought/ deluge) and wireworm herbivory (b only). Values represent mean ± standard error bars of 14 replicates (c) or 28 replicates (a and b). Bars sharing the same letter were not significantly different as determined by Post-hoc Tukey contrasts. Statistical analysis: (a) Leaf Si, watering regime $F_{2,74}=68.22$, $P<0.0001$. (b) Leaf N, watering regime $F_{2,74}=12.78$, $P<0.0001$, wireworm $F_{1,74}=4.29$, $P<0.05$. (c) Amino acids, watering regime $F_{2,90}=3.36$, $P<0.05$.

Fig. 5. Principal component analysis of amino acid mol% data in green leaf material sampled from plants grown under controlled conditions at harvest treated with different watering regimes (ambient, drought and drought/ deluge). (a) The mean sample scores plotted onto PC1 and PC2, which explain 68.8% and 22.0% of the variation in the data set, respectively. (b) Attribute loadings on the first two components PC1 and PC2. Standard abbreviations are:
Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Glu, glutamate; Gln, glutamine;
Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe,
phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.
(a) Aphid mass (mg FM)

(b) Ladybird mass gain (mg FM)

Watering regime:

- Ambient
- Drought
- Drought/deluge
(a)

(b)

- ▲ Ambient
- ○ Drought
- ▼ Drought/ deluge
Appendix S1 Supplementary methods

Insect culturing

Prior to both experiments, wireworms were maintained on potato tubers (cv. Rooster; Albert Bartlett, UK) at 7 °C (Johnson et al. 2008). For the controlled environment experiment, aphids (S. avenae) were maintained on H. vulgare cv. Optic plants in a controlled environment room at 15 °C and with 16/8 h day/night. Four weeks after sowing, three adult apterous aphids were caged (25 mm internal diameter clip cages suspended from metal frames above the plant) to a fully expanded leaf on the main stem of plants assigned the above-ground herbivory treatment. Cages without aphids were also placed on plants not assigned the above-ground herbivory treatment. After 24 h, the adults and all but three nymphs were removed from each cage (cage 1). 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 leaf.

Plant growth measurements and experimental design

The controlled environment experiment comprised of a randomized block design with nine blocks that were staggered temporally by two to three weeks to facilitate the final destructive harvest. Plants were grown in 2.4 L pots with 18.5 cm diameter top of the pot and 13 cm diameter at the bottom. Initially, two seeds were placed equidistant from the other seed and the centre of each pot and buried c. 2 cm from the soil surface. To initiate germination, pots were watered from the top twice per week for two weeks with 200 mL of deionised water. Shortly after germination the number of seedlings was reduced to a single plant per pot of consistent height and developmental stage. Plants were sufficiently spaced to allow this to happen without disturbing neighbouring plants.
For the field experiment, plants were grown under rain exclusion shelters which consisted of a wooden frame supporting a 2100 mm × 3500 mm polycarbonate sheet (6 mm thick polycarbonate sheeting, Polycarbonate Direct, Hull, UK) at an angle of 6° from a maximum height of 1176 mm to the minimum height of 800 mm. Total area under the rain exclusion shelter was 7.92 m². Initially, 18 pre-germinated seeds (soaked in deionised water at room temperature for c. 24 h and incubated on Petri-dishes lined with damp paper towelling at 15 °C for 3 days) were sown randomly across the surface of the each pot c. 2 cm from the soil surface on 24th June 2013. Pots were watered from the top twice per week for two weeks with 500 ml of deionised water. Following germination, seedling number was reduced to nine plants per pot of consistent height and development stage randomly spread across the pot surface to represent typical barley high sowing densities.

The saturation and desiccation (dried at 105 °C for 7 days) mass of the soil used in both experiments was measured and from this the total water holding capacity was calculated. When harvesting the plants, root: mass ratio was calculated by dividing the dry mass of roots by total dry plant biomass.

Plant chemical analysis
Water content (g) of the plant material was calculated by subtracting the dry mass from the fresh mass.

For silicon (Si) analysis, milled plant material was pressed at 11 tons into 5 mm thick cylindrical pellets with a manual hydraulic press using a 13 mm die (Specac, Orpington, UK)
prior to XRF analysis. C/N ratio was calculated using percentage C and N of the leaves from flash combustion and chromatographic separation.

To analyse leaf amino acids for the controlled environment experiment, two green leaves from each plant were removed from the leaf fraction before oven-drying and rapidly frozen in liquid nitrogen for amino acid analysis. After 15 min extraction with gentle agitation (Bulker shaker, MM 400, Retsch, Hope Valley, UK), samples were centrifuged first at 10 000 g for 15 min and the supernatant transferred to a clean tube. The remaining pellet was re-extracted in 1 mL extraction solution following the same procedure. The pooled supernatant was centrifuged at 15 000 g for 15 min to pellet any remaining leaf powder and aliquots of supernatant were dried to a residue using a speedvac followed by freeze-drying (Freeze Dryer Modulyo, Edwards, Apeldoorn, the Netherlands). β-Aminobutyric acid was used as an internal control. Samples were stored at -20°C prior to analysis, when they were re-dissolved in 1 mL ultra-pure water and aliquots (10 µl) prior to reverse-phase HPLC analysis.
Table S1. Results of linear models showing $F$ or $\chi^2$ statistic, degrees of freedom (df) and p value (P) testing the effect of watering regime, wireworm herbivory and their interactions on different measures of aphid performance.

<table>
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<tr>
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<th>Watering regime</th>
<th>Wireworm herbivory</th>
<th>Watering regime x wireworm herbivory</th>
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<td></td>
<td>$\chi^2$ or $F$</td>
<td>df</td>
<td>$\chi^2$ or $F$</td>
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<td>1.77</td>
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<td>Aphid fecundity</td>
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<td>Aphid survival</td>
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Figure S1. Soil moisture (m$^3$ m$^{-3}$) measurements in pots under different watering regimes throughout a representative two weeks.
Figure S2. (a) Water content (g) of barley plants treated with different watering regimes, ambient, drought and drought/deluge with (hatched bars) and without aphid and wireworm herbivory (grey bars). (b) Stomatal conductance of barley plants treated with different watering regimes (ambient, drought and drought/deluge). Values represent mean ± standard error bars of 9-8 replicates for water content and six replicated for stomatal conductance. Bars sharing the same letter were not significantly different as determined by Post-hoc Tukey contrasts. Statistical analysis, (a) watering regime $F_{2,95}=55.23$, $P<0.001$, wireworm $F_{1,95}=6.46$, $P<0.001$, (b) watering regime $F_{2,64}=29.41$, $P<0.001$. 