Climate-warming alters the structure of farmland tri-trophic ecological networks and reduces crop yield

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Abstract. It is unclear how sustained increases in temperature and changes in precipitation, as a result of climate-change, will affect crops and their interactions with agricultural weeds, insect pests and predators, due to the difficulties in quantifying changes in such complex relationships. We simulated the combined effects of increasing temperature (by 1.4°C over a growing season) and applying additional rainwater (10% extra per week) using a replicated, randomized block experiment within a wheat crop. We examined how this affected the structure of 24 quantitative replicate plant-aphid-parasitoid networks constructed using DNA-based methods. Simulated climate-warming affected species richness, significantly altered consumer-resource asymmetries and reduced network complexity. Increased temperature induced an aphid outbreak but the parasitism rates of aphids by parasitoid wasps remained unchanged. It also drove changes in the crop, altering in particular the phenology of the wheat as well as its quality (*i.e.* fewer, lighter seeds). We discuss the importance of considering the wider impacts of climate change on interacting-species across trophic levels in agro-ecosystems.

Introduction

Climate change is expected to have profound impacts on food production systems over the coming decades (Lobell *et al.* 2008). Crops will be adversely affected by a combination of both abiotic (e.g. heat, drought, salinity and submergence in water) and biotic (e.g. pests and pathogens) stresses (Baulcombe *et al*. 2009; Maxmen 2013; Bebber *et al.* 2014; Lesk *et al.* 2016), posing significant threats to food security (Godfray *et al.* 2010). Despite the growing research demonstrating the impacts of climate change on species abundances and distributions, community composition and organismal physiology (Sala *et al.* 2000; Parmesan 2006; Garcia *et al.* 2014), climate change effects on the networks of interactions among species are poorly understood (Tylianakis *et al.*, 2008), particularly in agro-ecosystems. This is largely due to the difficulties in quantifying changes in interactions compared with changes in biodiversity (McCann 2007). Yet, complex networks of biotic interactions, such as insect pollination and parasitism, play an important role in the maintenance of biodiversity (Bascompte *et al.* 2006), provide valuable ecosystem services (Pocock *et al.*, 2012), and can mediate ecosystem responses to environmental change (Sydes & Miller 1988; Brooker 2006). Species-interactions may, however, be more susceptible to climate change, as they are sensitive to the phenology, behaviour, physiology and relative abundances of multiple species (Memmott *et al.* 2007; Suttle *et al.* 2007; Tylianakis *et al.* 2007).

Combining advances in both network theory and molecular ecology offers unprecedented opportunities to describe interactions between species, the structure of communities and the function and stability of ecosystems (Evans *et al.* 2016). Ecological networks provide a quantitative framework to unify the study of biodiversity and ecosystem function (Thompson *et al.* 2012) and have been successfully used to quantity the ecosystem-level consequences of global environmental change (Tylianakis *et al.* 2010). There is growing interest in developing

these approaches to provide a more holistic, systems-based understanding of agro-ecosystems that could be used to maximise the ecosystem services provided by farmland biodiversity, as well as for anticipating and mitigating future scenarios (Bohan *et al.* 2013). For example, Macfadyen *et al.* (2009) constructed quantitative plant-herbivore-parasitoid networks on paired organic and conventional farms and showed that the organic farms had more species across the three trophic levels and significantly different network structure. However, such networks take considerable effort to construct and can be subject to bias because of the limitations of taxonomically selective rearing success as well as the reliance on accurate morphological identification (Evans *et al.* 2016). Advances in DNA sequencing technologies provide enormous potential to determine hitherto difficult to observe species interactions and thus to produce highly-resolved ecological networks (Wirta *et al.* 2014; Derocles *et al.* 2018; Evans *et al.* 2016). An accurate and cost-effective PCR diagnostics approach has recently been developed to allow the rapid construction of quantitative ecological networks of farmland aphid-parasitoid interactions (Derocles *et al*. 2012a, 2014) providing new opportunities to examine the impacts of environmental change on network structure and complexity.

In northern Europe, climate models predict significant warming and an increase in both precipitation (mainly in winter) and the frequency of extreme weather events (IPCC 2014), which are likely to cause significant damage to agro-ecosystems (Olesen *et al.* 2011). With increasing evidence that present climate change is altering geographical ranges, population dynamics and phenologies of some insects (Altermatt 2010; Morris *et al.* 2015), there is growing concern that global food security is threatened by the emergence and spread of crop pests and pathogens (Maxmen 2013). Given the ecological and economic importance of phytophagous insects and their natural enemies, a greater understanding of their direct and indirect interactions and how these respond to experimental manipulation is needed (van Veen

101 *et al.* 2006), particularly in the context of climate-warming.

102

103 Experimental manipulations of temperature and precipitation have provided important insights
104 into the responses of terrestrial ecosystems, with climate-warming generally stimulating total
105 net primary productivity, increasing ecosystem photosynthesis and respiration (see Wu *et al.*
106 2011 for a review). Real-world experimental climate manipulations can help to fill the
107 knowledge gap between highly controlled, closed-system laboratory studies (e.g. Le Lann et
108 al. 2014) that tend to focus on a small number of species, and large scale open-field
109 experiments that rely on variations in temperature along environmental gradients (see de Sassi
110 & Tylianakis 2012; Romo & Tylianakis 2013). To date, most field-based simulated-warming
111 experiments have used infrared heating devices (see de Sassi et al. (2012) who used
112 underground heating cables) but have mainly focused on plant responses to elevated
113 temperatures. To our knowledge, none have examined the impacts on networks of interacting
114 species across multiple trophic levels. Within grasslands, de Sassi & Tylianakis (2012)
115 demonstrated that in a tri-trophic system of plants, herbivores and parasitoids, each trophic
116 level responded differently to warming and overall the community was increasingly dominated
117 by herbivores. Within arable crops, a small number of individual simulated climate-warming
118 studies have demonstrated a reduction in wheat yield (Fang *et al.* 2013) and increases in aphid
119 pests (Dong *et al.* 2013) and insect predators (Berthe *et al.* 2015). Thus it is unlikely that
120 climate-warming will affect species richness within arable crops, rather it will alter network
121 structure and complexity, in particular consumer-resource asymmetries (e.g. network
122 'generality' - the mean effective number of lower trophic level species per higher trophic level
123 species) and interaction evenness, driven by changes in the abundances and frequency of
124 interactions between plants, aphids and parasitoids. However, predicting the specific impacts
125 on the complex pattern of interactions among species in a community remains a pressing

challenge (Staniczenko et al. 2017).

Here, we experimentally increase temperature and rainwater within farmland plots consisting of spring-sown wheat and common uncultivated plant (weed) species. The study is framed in the context of understanding climate change implications as it relates to policy targets (e.g., limiting warming to 2°C) within North European agriculture (Olesen et al. 2011). We examine the responses of quantitative plant-aphid-parasitoid networks, constructed using DNA-based methods, as well as the impacts on crop yield. Although predicting the direct and indirect responses of plants, phytophagous insects and their natural enemies to perturbation is a major challenge, quantitative ecological networks are particularly well suited for assessing direct and indirect interactions in the first instance (van Veen *et al.*, 2006). Our objectives are threefold. (1) To construct replicated, quantitative tri-partite food-webs describing the interactions between crop and non-crop plants, aphids and parasitoids. We apply a DNA-barcoding approach to accurately and cost-effectively quantify the interactions of Aphidiinae endoparasitoids with their aphid hosts. (2) To examine the combined effects of a 1.4°C temperature elevation and increase in rainwater on measures of network structure and complexity. We use suspended infrared heaters, which have been effectively applied in other habitats for climate change simulation experiments (Price & Waser 2000; Wan *et al.* 2002; Harte *et al.* 2015) to warm farmland plots *in situ*, and apply extra rainwater following established protocols (Rollinson & Kaye 2012). We predict no impacts on total species richness, but significant increases in aphid abundances in warmed plots due to a positive direct effect on population growth rate (Barton & Ives 2014) and a corresponding increase in the frequency of parasitoid interactions, potentially leading to changes in network consumer-resource asymmetries and interaction evenness. As aphids and parasitoids are highly specialized in agro-ecosystems (Le Ralec *et al.* 2011; Derocles *et al.* 2014), we do not expect

an increase in network connectance (a measure involving the number of interactions) in the short-term, as this would indicate an expansion of generalism of the species involved. We test this for both bipartite and tripartite networks. (3) To investigate the overall effects of warming on crop yield and whether any changes can be mediated by an increase in rainwater (either as precipitation or as added irrigation).

MATERIALS AND METHODS

Experimental layout

The study was conducted in 2013 at Stockbridge Technology Centre (STC), North Yorkshire, UK (53°49' N -1°9' W), a conventional farm consisting of meadows and cereal crops used for field experiments. The climate is temperate oceanic, with a mean minimum and maximum annual temperature and precipitation of 5.5–14°C (8.6–19.1 °C during the experiment) and 537.7 mm (156.6 mm during the experiment) respectively. We established a replicated, randomized block open-field experiment consisting of six replicates of four simulated climate change treatments in a field of spring wheat (*Triticum aestivum* cultivar Tybalt) (see Berthe *et al.* 2015, Figure S1, Supporting information). The four treatments consisted of: (W) 1.4°C increase in temperature; (P) 10% increase in precipitation/rainwater per week, based on historic records; (WP) warming and precipitation treatments combined; and (C) control (ambient conditions). We refer to "climate-warming" when reporting the effect of warmed treatments and "precipitation" when reporting the effect of additional rainwater treatments. Treatments were randomly allocated to 2×2 m experimental plots that were each separated by 2 m of wheat to provide a buffer and allow the free movement of insects. The W and WP treatments involved suspending 240 V infrared heaters 1.5 m above each plot (following Rollinson & Kaye 2012), consistently heating throughout the day and night: this primarily drives plant phenology rather than heating the column of air (Kimball 2005; White *et al.* 2011). A 'dummy' heater of the

same size and shape was suspended in the non-heated plots to account for any possible effects of shading/shelter.

A real-time proportional-integrative-derivative feedback system ensured constant temperature plot warming through infrared radiometer (IRR) monitoring of surface temperatures in warmed plots. Soil-surface temperatures were monitored by 6 Infrared Remote Temperature Sensors (IR120; Campbell Scientific; Loughborough, UK), positioned 1.10 m above the plots and directed to the middle of the plot and connected to a data logger (Campbell Scientific; Loughborough, UK) to record the temperatures every 10s and to control the constant output of the infrared heaters. Their positions were selected randomly, three within a heated plot (W, WP) and three within an unheated plot (C, P). Our aim was for the system to increase the temperature in the warmed plots by 2°C. Over the course of the experiment, temperatures were raised, on average, by 2.2°C (standard deviation 0.6) in block 1; 1.1°C in block 2 (standard deviation 0.6) and 1.1°C in block 3 (standard deviation 0.8) that most likely reflected subtle microclimate differences within the field. This provided a mean temperature increase of 1.4°C (standard deviation 0.9) across all the plots. Increased rainwater was simulated in the P and WP plots by manually adding 10% extra collected rainwater each week based on STC mean monthly rainfall data collected between 2002 and 2012. This can either be interpreted as representing weather conditions in a warm and wet summer, or a farmer increasing irrigation to mitigate the effects of a warm and drier summer. We added the following water each week: 13 L in April; 19 L in May; 24 L in June; 26 L in July and 30 L in August, amounting to 407 L in total for each plot. During the course of the experiment, just 156.6 mm of rainfall was measured at Stockbridge Technology Centre, well below the annual average. Thus we actually increased precipitation/rainwater by 65% that year. The experimental area in which the plots were located received herbicide applications on 2nd April and 13th May (pendimethalin;

metsulfuron-methyl and thifensulfuron-methyl); our aim was to allow some weed growth without out-competing the wheat. Experimental treatments commenced immediately after the sowing of spring wheat on 13th April and stopped with the harvest of the crop on 16th August.

Plant surveys and crop yield

Plants were identified to the species level, with a small proportion to the genus or family, and the percentage cover of each was recorded weekly (18 surveys) in each plot. The date of emergence of the first leaf and the date of emergence of the first ear for *T. aestivum* was recorded in each plot and converted into Julian date for statistical analysis. At harvest, a 0.5×0.5m quadrat was placed in the area directly below the heaters/dummy heaters (we selected this area because the heating pattern is likely to be more consistent; Kimball 2005) and the number of *T. aestivum* ears counted. The density of wheat (number of wheat ears m^{-2}) was then calculated for each plot. We also harvested five ears randomly from each plot, which were dried in an oven at 80°C for 48 hours in the laboratory. The seeds were counted and the total seed weight was measured for each ear. For each plot, crop yield (g/m^2) was calculated as: (total seed weight / ear) x density of wheat.

Insect surveys

Plant-aphid interactions were recorded by systematically searching each plot and counting the total number of aphids and visibly parasitized aphids ('mummies') on each plant species every week (18 surveys) throughout the sampling period. We collected up to 30 aphid individuals per colony and placed them in a 1.5ml tube filled with 95% ethanol and then stored at -20 °C in the laboratory for later identification (see below). All aphid mummies were collected and stored in 1.5ml tubes, but without 95% ethanol. Instead, these were stored under laboratory conditions and observed for 10 days for the emergence of adult parasitoids. Adult parasitoids and aphid

mummies where parasitoids did not emerge were then stored individually in a 1.5ml tube filled with 95% ethanol at -20 °C.

Insect identification

Aphids were first identified morphologically following Blackman & Eastop (1994, 2000, 2006). We extracted the DNA of all the aphids collected using a hotshot DNA extraction (Montero-Pau *et al.*, 2008). Aphid identification was confirmed with DNA barcoding: a fragment 658 bp from Cytochrome C oxidase subunit I [COI] was amplified and sequenced with the PCR conditions described by Derocles *et al.* (2012b) and the following primer pairs: LCO1490 (5'- GGTCAACAAATCATAAAGATATTGG-3'; Folmer *et al.* 1994) and the degenerate reverse primer HCO2198-puc (5'-TAAACTTCWGGRTGWCCAAARAATC-3'; Cruaud *et al.* 2010). Adult parasitoids and non-emerged parasitoids from the mummies (n = 181) were identified using the DNA barcoding tool described by Derocles *et al.* (2012b): a fragment 658 bp from COI was amplified and sequenced to identify these parasitoids. Aphid-parasitoid interactions and parasitism rates were determined using two different molecular methods based on the extracted DNA of aphids. First, we used a multiplex PCR approach developed by Traugott *et al.* (2008) on the aphid species collected on *T. aestivum* (*Sitobion avenae* and *Metopolophium dirhodum*) to detect both primary and secondary parasitoids. We used nine primary parasitoid and two hyperparasitoid species-specific primer pairs to detect and identify immature primary and secondary parasitoids within cereal aphid hosts. Second, for all the other aphid species, we used the approach developed by Derocles *et al.* (2012a) that uses the sequences of a 210 bp fragment from the 16S gene to identify to species-level (in most cases) the immature Aphidiinae parasitoids within an aphid host. To improve the reliability, we added an 'in tube control' to determine if an absence of parasitoid detection is due to either a true absence of parasitism or a technical problem during DNA extraction or PCR

amplification. For this, we followed the PCR protocol for parasitoid detection described by Derocles *et al.* (2012a) and we added in the PCR-mix the aphid COI barcode assay described above. A detection of a parasitoid within an aphid host is characterized by two bands on a 1.5% agarose electrophoresis gel: a band of 658 bp (COI, aphid and parsitoid) and a band of 210 bp (16S parasitoid); an unparasitized aphid is characterised only by the band of 658 bp, from the aphid DNA. An absence of band indicates a failure from either the DNA extraction or the PCR amplification. In this case, the PCR amplification is performed a second time. If after a second PCR amplification a failure is observed, the individual is removed from the analysis. Sixteen aphids were removed from the analysis following two PCR failures. We used two hyperparasitoid species-specific primer pairs (Traugott *et al.* 2008) to detect the secondary parasitoids in non-crop aphid species. We compared parasitism rate determined using this method versus the conventional approach (i.e. the number of aphid mummies collected) / (number of aphid counted).

Insect abundance, species richness and parasitism rates

For each plot, we pooled data across the sampling period to calculate:

1) percentage of aphids sampled: number of aphids sampled / number of aphids counted
2) aphid abundance: total number of aphids counted on each plant species throughout the sampling period
3) total species richness
4) species richness per trophic level (i.e. plants, aphids, parasitoids)
5) parasitism rate using the DNA-based method: (the number of aphid mummies collected + number of parasitised aphids detected) / (number of aphid mummies collected + number of aphids collected);
6) parasitism rate using the conventional method (no DNA-based method): (the number

of aphid mummies collected) / (number of aphid counted);

7) multiparasitism rate using the DNA-based method: (number of aphids parasitised by at least two detected primary parasitoid species / number of aphids collected + number of mummies collected)

8) hyperparasitism rate using the DNA-based method: (number of aphids parasitised by a secondary parasitoid + number of secondary parasitoids identified in mummies / number of aphids collected + number of mummies collected).

Ecological network construction, visualisation and description

Plant-aphid-parasitoid quantitative networks were constructed for each plot by pooling data collected during the course of the experiment. We visualised the tripartite interactions for each of the four treatments (by pooling replicate data from replicate plot) using the "HiveR" package (Krzywinski *et al.* 2011) in R 3.3.1 (R Core Team, 2016). We were particularly interested in how the experimental treatments affects consumer-resource asymmetries, classically described in network ecology as vulnerability and generality (i.e. the mean number of consumers per prey, and the mean number of prey per consumer, respectively), as well as standard measures of complexity (Bersier *et al.* 2002). They are well suited for describing antagonistic interactions and the extent to which consumers are specialized to the resource and how the resource is attacked by the higher trophic level (Wirta *et al.* 2014). For each of the 24 tripartite networks, we calculated the following qualitative, unweighted quantitative and weighted quantitative network descriptors described by Bersier *et al.* (2002) using 'cheddar' (Hudson et *al.* 2013) and 'bipartite' packages in R 3.3.1 (Dormann *et al.* 2009): Link density (average number of links per species: LD, LD'q, LDq); Connectance (proportion of possible links between species that are realized: C, C'q, Cq); Vulnerability (mean effective number of higher trophic level species per lower level species: V, V'q, Vq) and Generality (mean effective number of lower

trophic level species per higher trophic level species: G, G'q, Gq). We based our analysis on weighted quantitative network descriptors (LDq, Cq, Vq and Gq) to specifically examine changes in network complexity and consumer-resource asymmetries as they are commonly used in ecological network studies and less prone to sampling biases (Tylianakis *et al.* 2007; Macfadyen *et al.*, 2009; Wirta *et al.* 2014). As interaction evenness may be ecologically important, and that these network descriptors are relatively insensitive to differences in the evenness of the distribution of link magnitude, we calculated the quantitative tri-partite interaction evenness (IEq) following Albrecht *et al.* (2007). To examine whether plant-herbivore and herbivore-parasitoid interactions react differently to climate change, we also calculated network descriptors for the plant-aphid and aphid-parasitoid bipartite networks separately.

Statistical analysis

Statistical analysis was performed in R 3.3.1 (R Core Team, 2016). The effects of treatment on the plants (including yield), insects, parasitism rates and network descriptor response variables were examined using Generalized Linear Models (GLM) with a Gaussian family (except for aphid abundance data, where a Poisson family was used). To account for the intercorrelation between the network descriptors, and to reduce the probability of a type I error, we used a Bonferroni-corrected α of 0.01 to assess the level of significance for the five network descriptors (*i.e.* LDq, Cq, Vq, Gq and IEq, following Tylianakis *et al.* 2007). This correction was used when assessing the effect of treatments on tri-partite network descriptors and on bipartite (plant-aphid and aphid-parasitoid) network descriptors.

The effects of treatment on the crop phenology (Julian dates of emergences of first leaf and first ear) were examined with Mann-Whitney tests. The effects of treatment on the crop (i.e.

number of seeds / ear, the seed weight / ear, the density of wheat and yield) were examined using GLM with a Gaussian family. In addition to the climatic treatments, biological interactions may also affect the crop yield (see Gagic *et al.* 2016). Non-crop plants are competitors for space and resources with the crop (Fahad *et al.* 2015). *Sitobion avenae* and *Metopolophium dirhodum* are aphid species feeding on the wheat which may alter the yield (van Emden & Harrington 2007). In order to examine the potential impact of uncultivated plants on the density of the wheat, a second GLM was performed with the percent cover of non-crop plants included as a covariate. Similarly, when analysing the yield data, we included the abundance of wheat aphids *S. avenae* and *M. dirhodum* as a covariate in a separate model and compared the model fit with and without the covariates using Akaike Information Criteria (AIC). In summary, we compared the AIC of the following models:

a) the effect of precipitation and increased temperatures on the density of the wheat versus the effect of precipitation, increased temperatures and percent cover of non-crop plants on the density of the wheat;
b) the effect of precipitation and increased temperatures on the crop yield versus the effect of precipitation, increased temperatures and the abundance of wheat aphids on the crop yield.

In addition, ANOVA was performed on these two model comparisons to test whether the inclusion of covariates provided a significantly better fit to the model.

Results

We quantified 2836 interactions between eight plant species (6 plants identified to species level, 1 to the genus level and 1 to the family level), 1946 aphids (1765 living aphids and 181 aphid mummies) belonging to six species, 761 primary parasitoids from 13 species and 129 secondary parasitoids from two species. Of the 129 secondary parasitoids identified, only 41

primary parasitoid – secondary parasitoid interactions were recovered. Consequently, primary and secondary parasitoids were considered as belonging to the same trophic level and separate primary parasitoid – secondary parasitoid interactions were not examined (Figure 1). Overall, the 1946 aphids included in the ecological network analysis represented 56.3% of the total aphids counted in the experimental plots.

Plant cover and richness

We found no effect of treatment on plant species richness (GLM, warming: $F = 1.577$, $df = 1$, $p = 0.223$; precipitation: $F = 3.09$, $df = 1$, $p = 0.093$; Table 1), but climate-warming significantly reduced crop percentage cover (GLM, warming: $F = 11.746$, $df = 1$, $p = 0.003$; precipitation: $F = 1.043$, $df = 1$, $p = 0.319$). The overall non-crop species cover was significantly increased in the warmed plots (GLM, warming: $F = 4.78$, $df = 1$, $p = 0.04$; precipitation: $F = 1.519$, $df = 1$, $p = 0.231$).

Aphid abundance and parasitism rates

We found no effect of treatment on aphid species richness, but climate-warming resulted in significant aphid outbreaks (GLM, $df = 1$, $p < 0.001$; Table 1), with four times as many aphids in the warmed plots compared to control plots. The abundance of the wheat aphids *S. avenae* and *M. dirhodum* doubled as a result of warming (GLM, $df = 1$, $p = 0.009$; Figure 1, Table 1). Molecular analyses revealed high rates of parasitism (based on parasitoid detection within aphids and mummies sampled; mean 36 ± 1.7%) compared to the conventional 'mummy' collection/rearing method (based solely only on mummies sampled; mean 9.9 ± 1.8%). Climate-warming did not significantly change parasitoid species richness, although we did detect a trend (GLM, $df = 1$, $F = 4.247$, $p = 0.052$). There were no significant effects of treatment on parasitism rates nor multiparasitism (two primary parasitoids within a single

aphid) and hyperparasitism rates (aphids parasitised by secondary parasitoids), which were relatively low across the treatments (3.77 ± 0.01% and 7.38 ± 0.01% respectively; Table 1).

Tripartite ecological network structure

We found a significant effect of climate-warming on total species richness across trophic levels (Table 1, Figure 1). Precipitation did not affect quantitative tripartite network descriptors Vq (GLM, $F = 0.003$, $df = 1$, $p = 0.959$), IEq (GLM, $F = 0.274$, $df = 1$, $p = 0.606$; Figure 2), LDq, Cq and Gq (Table 2). Simulated climate-warming did not affect qualitative network descriptors (Table S1), however it did significantly decrease quantitative tripartite Vq (GLM, $F = 10.063$, $df = 1$, $p = 0.005$; Figure 2) and LDq, but did not affect Cq, Gq (Table 2) and IEq (GLM, $F = 0.362$, $df = 1$, $p = 0.554$; Figure 2). Within the 24 networks, both aphids and parasitoids never consumed more than three different species from the lower trophic level (Figure 1).

Plant-aphid bipartite network structure

Precipitation did not affect plant-aphid quantitative network descriptors Vq (GLM, $F = 0.425$, $df = 1$, $p = 0.522$), IEq (GLM, $F = 0.0001$, $df = 1$, $p = 0.991$; Figure 2), LDq, Cq and Gq (Table 2). Likewise, climate-warming did not affect plant-aphid quantitative network descriptors Vq (GLM, $F = 0.753$, $df = 1$, $p = 0.395$), IEq (GLM, $F = 5.574$, $df = 1$, $p < 0.029$; Figure 2), LDq, Cq and Gq (Table 2)

Aphid-parasitoid ecological network structure

Precipitation did not affect aphid-parasitoid quantitative network descriptors Vq (GLM, $F = 0.005$, $df = 1$, $p = 0.944$), IEq (GLM, $F = 1.091$, $df = 1$, $p = 0.308$; Figure 2), LDq, Cq and Gq (Table 2). However, climate-warming significantly decreased aphid-parasitoid quantitative network descriptors Vq (GLM, $F = 18.456$, $df = 1$, $p < 0.001$) and LDq, but did not affect Cq

401 and Gq. Climate-warming negatively affected aphid-parasitoid IEq (GLM, $F = 37.599$, $df = 1$,
402 $p < 0.0001$). This suggests that higher trophic interactions are more sensitive in our system and
403 was likely caused by an increase in the frequency of interactions between wheat aphids and
404 two primary parasitoid species: *Aphidius rhopalosiphi* and *Aphidius ervi*.
405
406 *Wheat phenology*
407 First leaves emerged three days earlier in the warmed plots (Mann-Whitney, $W = 118$, $p =$
408 0.002). First ears emerged eleven days earlier on average in the warmed plots (Mann-Whitney
409 $W = 144$, $p < 0.001$). Precipitation affected neither the emergence of the first leaves (Mann-
410 Whitney, $W = 63.5$, $p = 0.586$) nor the first ears (Mann-Whitney, $W = 72$, $p = 1$).
411
412 *Crop yield*
413 Climate-warming significantly reduced the seed number (GLM, $F = 4.272$, $df = 1$, $p = 0.041$,
414 Table 3), the seed weight (GLM, $F = 3.049$, $df = 1$, $p = 0.012$; Table 3) but not the density of
415 the wheat (GLM, $F = 2.109$ $df = 1$; $p = 0.161$; Table 3), resulting in no overall reduction in crop
416 yield (GLM, $F = 3.835$, $df = 1$, $p = 0.064$; Table 3). However, when including the detrimental
417 effect of non-crop plants or wheat aphid abundance in the models, we found a significant
418 decrease in wheat density (GLM, $F = 11.606$, $df = 1$, $p = 0.003$; Table 4) and crop yield (GLM,
419 $F = 6.33$, $p = 0.021$, $df = 1$; Figure 3; Table 4). Yield loss was not compensated by increased
420 rainfall (GLM, $F = 0.066$, $df = 1$; $p = 0.8$; Table 4). These models, which including wheat aphid
421 abundances and non-crop cover, provided a significantly better fit to the data than the models
422 considering experimental treatment alone (ANOVA; wheat density: $F = 95.582$, $p < 0.001$;
423 crop yield: $F = 14.663$, $df = 1$, $p = 0.001$) Table 5).
424
425

Discussion

We provide the first experimental evidence, to our knowledge, of the impacts of climate-warming on the structure of tripartite ecological networks, constructed using a DNA-barcoding approach. Experimental warming altered total species richness across trophic levels (but not plant and aphid species richness respectively), it significantly reduced crop percentage cover and substantially increased aphid abundance (the abundance of the economically important aphids *S. avenae* and *M. dirhodum* doubled as a result of warming). This affected quantitative network structure and complexity, including aphid-parasitoid interaction evenness. Molecular analyses revealed much higher rates of parasitism compared to traditional rearing/identification methods, with generally fewer natural predator species in the warmed plots. However there were no significant effects of treatment on parasitism rates, nor multiparasitism and hyperparasitism. Thus, in the short-term at least, natural pest control (assessed here using a molecular approach to determine parasitism rate) provided by parasitoids appears unaffected, although studies of aphid and parasitoid population dynamics over the long-term are needed. Overall, we show that wheat grown 1.4°C above ambient temperature produced significantly fewer and lighter seeds resulting in a reduction in crop yield, with the best fitting model including aphid abundance and non-crop cover as covariates. We found no statistically significant effect of increased rainwater on any of our response variables, despite it being a very low rainfall season.

Study limitations

Despite the advances made by our study, there are important limitations to our experiment. First, the 4 m^2 plots sampled are not directly comparable to a large cereal crop field. Our results may instead reflect how agricultural communities at field edges respond to climate change. However, because insect herbivory and parasitism rate are edge-dependent (Maron & Crone

2006; Reeve & Cronin 2010), our results might nevertheless be indicative of the direction of change for plant and animal populations and communities at larger spatial scales as a result of climate-warming, although more research is necessary. Although all simulated climate-warming methods have limitations (Sassi *et al.* 2012), they are nevertheless one of the few tools available in empirically testing how ecosystems response to climate change and provide much-needed data for predictive network models (Staniczenko *et al.* 2017). In the future, complementary approaches including large-scale field experiments and small-scale mesocosms or laboratory experiments (see Romo & Tylianakis 2013) might give a more comprehensive view of the ecosystem response to climate change. Second, we did not control non-crop plant or insect communities as we wished to quantify how they interact. Thus, conventionally managed cereal crops are likely to have responded differently to the experimental treatments. Third, we only examined the effects of treatment over a single growing season. Further temporal replicates would determine whether the response observed is year-dependent and the extent of interaction turnover (Kemp *et al.* 2017). Fourth, we did not consider other organisms potentially affecting the plant-aphid-parasitoid networks, such as ants interacting with aphids or predators consuming both aphids and parasitoids (Traugott *et al.* 2011; Barton & Ives 2014; Raso *et al.* 2014). Future studies should examine a more exhaustive range of species interactions (e.g. following Pocock *et al.* 2012; Evans *et al.* 2013), which are increasingly possible using the molecular approaches described here and/or Next Generation Sequencing technologies (Kitson *et al*. 2018). Fifth, we could not disentangle whether the insect responses were mainly due to foraging decisions of organisms (*i.e.* dispersal between the plots) or their demographic response to climatic manipulation (*i.e.* treatments affecting population growth rates), although it is likely that the observed aphid species responses were demographic. Further experimental manipulations at larger-spatial scales (and including other important factors such as elevated CO_2) are urgently required as well as more detailed observations of host-parasitoid

and other predator-prey interactions, although this would need to involve much larger controlled enclosures than are currently available. Finally, we conducted a relatively exhausting sampling where all aphid mummies and more than 50% of aphids were collected for further molecular analyses. Such intensive sampling may certainly affect the aphid and parasitoid population dynamics at the plot-level and could have potentially affected our results. However, adequate network analysis is very dependent on sampling completeness (Blüthgen et al. 2006; Rivera-Hutinel et al. 2012; Jordano 2016). Consequently, such intensive sampling is well established in studying host-parasitoid interactions (see Traugott et al. 2008) and ecological networks more generally (e.g. Macfadyen *et al.* 2009) and was therefore necessary for the purposes of this study. Assessing the effect of climate change on aphid and parasitoid dynamics, while also a major issue, would then require a different experimental design.

Trophic level to network level responses

When considering each trophic level separately, climate-warming promoted weed growth (especially *Chenopodium album* and *Cirsium arvense*), which increased competition with the crop and contributed to a reduction in crop percentage cover. At the second trophic level, there was a fourfold increase in aphid abundance in the warmed plots, as we predicted, mostly driven by aphids associated with *T. aestivum*. At the third trophic level, contrary to our predictions, parasitism rates remained unchanged. However, a decrease in aphid-parasitoid interaction evenness in the warmed plots suggests that climate-warming might benefit some parasitoid species at the expense of others. Whilst both the reduction in crop yield and the aphid pest outbreak followed the general patterns observed in other recent studies (Maxmen 2013; Dong *et al.*,2013; Bebber *et al.* 2014; Liu *et al.* 2016), the significant effects on network structure observed in this study provides new insights into how climate-warming affects entire communities of interacting species. First, we found evidence that climate-warming affects tri-

501 partite consumer-prey asymmetries, with significantly lower network vulnerability and linkage
502 density. Second, connectance was not affected, most likely due to the high trophic
503 specialization for both aphids and associated parasitoid wasps (Le Ralec *et al.* 2011; Derocles
504 *et al.* 2014). Third, although there was no effect of treatment on tripartite interaction evenness,
505 climate-warming negatively affected bipartite aphid-parasitoid interaction evenness,
506 suggesting that higher trophic interactions might be more sensitive in our system. Indeed,
507 changes in tri-partite network structure are essentially driven by aphid-parasitoid interactions:
508 plant-aphid networks were not affected by simulated-warming while aphid-parasitoid linkage
509 density and vulnerability decreased. Overall, our results support the findings from de Sassi *et*
510 *al.* (2012) showing that climate-warming may have bottom-up effects (on host density and
511 body size) which can in turn affect the structure of host-parasitoid networks.
512
513 *Parasitism*
514 We found no effect of climate-warming on the parasitism rate and species richness of parasitoid
515 wasps (although precipitation and warming treatment tended to decrease parasitoid richness)
516 which are intimately linked to the ecosystem service of natural pest control (Traugott *et al.*
517 2008; Derocles *et al.* 2014). In Northern European agricultural habitats, the most abundant
518 parasitoid species appear more specialized, with reduced attack rates on alternative hosts
519 (Derocles *et al.* 2014). Macfadyen *et al.* (2009) showed significant differences in network
520 structure between organic and conventional farms with more species at three trophic levels
521 (plant, herbivore and parasitoid) on organic farms. Despite herbivores on organic farms being
522 attacked by more parasitoid species, differences in network structure did not affect parasitism
523 rate across a variety of host species. In our study, climate-warming mainly influenced two
524 parasitoid species, *A. rhopalosiphi* (the main natural enemy of *Sitobion avenae*) and *A. ervi*,
525 driving a decrease in aphid-parasitoid interaction evenness. These species differ in their trophic

specialization: *A. rhopalosiphi* is a specialist and *A. ervi* is a generalist (Kavallieratos *et al.* 2004; Starý 2006). Previous work by Le Lann *et al.* (2014) under laboratory conditions showed a decrease in the attack rate of *A. rhopalosiphi* on *S. avenae* as a result of warming, whereas aphid defense rate increased. Under more realistic field-based scenarios, which include a greater range of interacting species, we found the opposite effect. This not only suggests that the degree of specialization may not necessarily explain which species will be more adaptable to environmental changes (as hypothesized by Rand & Tscharntke 2007; Tylianakis *et al.* 2008; Jeffs & Lewis 2013) but that other factors, such as changes in apparent competition (Morris *et al.* 2004), might be important considerations within a food-web context. Overall, an accurate assessment of natural pest control cannot be undertaken by the single measure of parasitism rate, but would require a careful examination of host and parasitoid population dynamics through further study and a different experimental design. These results, together with a recent study by Berthe *et al.* (2015) at the same study site that showed significant increases in Coleoptera activity-densities but a reduction in community diversity as a result of climate-warming, demonstrate the short-term impact of climate-warming on higher trophic levels (*i.e.* predators and parasitoids) in particular. Given the potential top-down effects driven by these organisms, we expect that climate-warming will result in long-term changes to the structure of the ecological network and consequently in natural pest control. Thus long-term climate-manipulation studies across spatial-scales are necessary to better understand the effects of environmental change on agricultural plant-aphid-parasitoid interactions and the ecosystem service of natural pest control (Cardinale *et al.* 2003; Tylianakis e*t al.* 2006; Macfadyen *et al.* 2011; Peralta *et al.* 2014).

Impacts of climate-warming on crop yields within an ecological network context

We found significant effects of climate-warming not only on ecological network structure, but

551 also on crop yield. Experimental-warming has been shown to advance flowering and fruiting
552 phenology for a range of plant species (Sherry *et al.* 2007; Hovenden *et al.* 2008; Dong *et al.*
553 2013) and in our study, first wheat leaves emerged three days earlier on average and ears
554 emerged at least a week earlier in the warmed plots. Wheat grown under experimental-warming
555 produced fewer and lighter grain, resulting in a significant impact on crop yield. There was no
556 significant effect of increased rainwater, which was perhaps surprising given the study was
557 conducted during a very low rainfall season. The yield data from the experiment is nevertheless
558 not directly comparable with commercial agricultural wheat yields. It should be emphasized
559 that it was not our intention to simulate conventional farming methods, where routine spraying
560 would have removed most of the weed species within our plots. Rather we wished to study the
561 community wide response of interacting species across trophic levels. In this context, the
562 reduction in crop yield was primarily driven by a combination of the wheat producing fewer,
563 smaller grain as well as increased competition with weed species, rather than significant insect
564 damage (aphid load was very low: 0.14±0.09 aphids per wheat ear across all plots). Despite
565 this, warming did trigger a fourfold increase in aphid abundance and this is likely to cause
566 significant damage to crops in years when fluctuating aphid numbers are higher. As
567 demonstrated recently by Gagic *et al.* (2016), attacks by several pests can have both positive
568 and negative impacts on crop yield. In our study, crop yield models produced a better fit when
569 pest aphid abundance was included as a co-variate. However, it still remains unclear how crop
570 (yield in particular) and non-crop plants are affected both directly and indirectly by changes in
571 aphid-parasitoid interactions. By pioneering new molecular methods to construct highly-
572 resolved species-interaction networks, we have provided new, cost-effective tools to examine
573 the response of communities of interacting agricultural species to environmental change.
574
575 *Merging DNA-based methods with ecological network analysis*

576 Merging molecular methods and ecological network analysis (ENA) provides new tools for
577 understanding ecology and evolution (Raimundo *et al.* 2018). Here we showed that the
578 detection-rate of aphid parasitism was more than three times higher using molecular assays
579 than by conventional insect-rearing approaches. Plant-aphid-parasitoid networks, constructed
580 using molecular methods, were more highly-resolved than traditional rearing methods, with
581 significant implications for host-parasitoid network-level analyses (Condon *et al.* 2014; Hrček
582 & Godfray 2015). Traditional approaches based on insect rearing and morphological
583 identification would have failed to detect changes in species interactions mediated by the
584 increase in temperature. Indeed, such approaches rely on the collection of aphid mummies and
585 rearing adults for identification. In our study, only 181 aphid mummies were collected while
586 709 parasitoids were detected and identified within their living aphid hosts. Parasitism cases
587 using molecular methods were therefore able to capture a more exhaustively range of host-
588 parasitoid interactions. These parasitism cases however still need to be considered cautiously:
589 parasitoid eggs or larvae detected do not always achieve their development to the adult stage
590 (Starý *et al.* 1989). Consequently, molecular tools could potentially overestimate parasitism
591 (Traugott et al. 2008). Moreover, aphids collected on the same plant were placed together in a
592 single tube. This may lead to a potential risk of contamination between aphids with parasitoid
593 DNA. But this risk is low because in the same tubes we found both unparasitised and parasitised
594 aphids (from different parasitoid species). Completely eliminating this risk would require each
595 collected aphid to be separately stored but might also result in unrealistic, time-consuming
596 sampling protocols.
597
598 In summary, this study provides the first evidence, to our knowledge, of the impact of climate
599 change on farmland tri-partite ecological networks, ecosystem services and agricultural output.
600 In the short-term, we highlight the potential winners (*i.e.* pests) and losers (*i.e.* pest natural

enemies) of agro-ecosystems in a warmer world. Overall, our study provides insights into the potential threat of global warming on both farmland biodiversity and food production. Despite limited changes to biodiversity *per se*, climate-warming affects the frequency of interactions between species, ultimately affecting network structure, although the long-term consequences of altered network structure on ecosystem functioning warrants further study. The detrimental impact of climate-warming on wheat suggests the need for adapting future agricultural methods of cropping in response to a climate change (Asseng *et al.*, 2013); cropping methods which in turn can also have cascading effects on agro-ecosystems and their networks of interactions. Considering the effects of environmental changes on ecological networks in dynamic models rather than snapshots of communities is essential (Säterberg *et al.* 2013) as well as taking into account a more complete range of interactions (*i.e.* 'networks of ecological networks'; Pocock *et al.* 2012; Evans *et al.* 2013). Future studies should consider the combined effects of climate-warming and elevated CO_2 as the latter also affects wheat growth and grain yield in particular (O'Leary *et al.* 2015). Such changes in plants may also induce bottom-up effects on high trophic levels (*e.g.* pest arthropods feeding on the crop). Finally, increased rainwater did not affect the ecological networks and the crop yield in the present study, suggesting that extra water (either as increased precipitation or irrigation) might not mitigate the effects of increased temperature. Further considerations are nevertheless needed to understand the predicted changes in rainfall on agroecosystems. Consequently, future climate change experiments need to simulate more realistic climate change scenarios and consider increases of temperatures, precipitation and CO_2 combined. A more exhaustive examination of climate change consequences on agricultural ecosystems through a combined approach using ENA and DNA-based methods is the fundamental first step to predict the impact of global changes on food production.

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Data accessibility

DNA sequences were assigned Genbank accession numbers: MF154009 – MF154409

Plot level plant, insect and network data is available on Dryad: doi:10.5061/dryad.80vd7q6

Author contributions

DME designed the project. SAPD and SCFB performed the field sampling. SAPD and DHL developed the molecular methodology. SAPD, SCFB and PCN performed the molecular work in the laboratory. EDM processed the crop yield data. SAPD and DME analysed the data. SAPD and DME wrote the first draft of the manuscript, all authors contributed substantially to revisions.

Supporting information

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

Figure S1 Experimental layout of the simulated climate change experiment at Stockbridge Technology Centre, North Yorkshire, UK.

Table S1 Qualitative network descriptors (LD, C, G, V) and unweighted quantitative network descriptors (LD'q, C'q, G'q, V'q) for each treatment (mean ± standard deviation).

Tables and figure captions

Table 1. Trophic-level descriptors for each treatment (mean ± standard deviation). Descriptors were calculated for each of the 24 experimental plots and then compared between treatments. Effects of treatments on descriptors were tested with GLM (Family Gaussian, F-test; except for Aphid abundances: family Poisson; Chi-square test). W+P: warming and precipitation; ↑P: increase in precipitation; ↑T°: increase in temperature. Significant effects at α of 0.05 are in bold.

	Plant richness	Weed Cover	Wheat Cover	Aphid richness	Aphid abundance	Wheat aphid abundance	Parasitism rate	Multiparasitism rate	Hyperparasitism rate	Parasitoid richness	Total species richness
Control	2 ± 0.632	20.5 ± 4.806	77.167 ± 4.215	2.667 ± 1.033	46.167 ± 19.682	31.167 ± 7.494	0.339 ± 0.077	0.037 ± 0.038	0.082 ± 0.072	8.333 ± 2.16	12.833 ± 3.488
Precipitation	1.667 ± 0.816	19 ± 4.195	78.833 ± 1.602	2.167 ± 0.753	59.667 ± 55.479	36 ± 13.387	0.347 ± 0.111	0.042 ± 0.028	0.063 ± 0.053	7.833 ± 1.472	11.667 ± 2.066
Warming	2.667 ± 1.033	33.333 ± 16.525	62.5 ± 14.053	2.833 ± 0.753	183.333 ± 109.485	86.333 ± 49.443	0.39 ± 0.093	0.041 ± 0.014	0.067 ± 0.033	7.833 ± 1.941	13.333 ± 3.077
W+P	1.833 ± 0.753	24.5 ± 10.635	68.333 ± 10.801	2.667 ± 0.816	286.167 ± 267.655	97.167 ± 55.315	0.362 ± 0.067	0.031 ± 0.023	0.084 ± 0.044	5.5 ± 0.548	10 ± 1.265
↑P F-value	3.09	1.519	1.043	0.966			0.079	0.082	0.044	4.247	4.413
↑P p-value	0.093	0.231	0.319	0.337	**<0.001**	0.609	0.782	0.777	0.948	0.052	**0.048**
↑T° F-value	1.577	4.78	11.746	0.966			0.851	0.091	0.02	4.247	0.297
↑T° p-value	0.223	**0.04**	**0.003**	0.337	**<0.001**	**0.009**	0.367	0.766	0.889	0.052	0.592

Table 2. Weighted quantitative network descriptors (LDq, Cq, Gq) of tri-partite networks and bipartite (plant-aphid and aphid-parasitoid) networks for each treatment (mean ± standard deviation). Vq and IEq are presented in Figure 2. Network descriptors were calculated for each of the 24 networks and then compared to each other. Effects of treatments on network descriptors were tested with GLM (Family Gaussian; F-test). W+P: warming and precipitation; ↑P: increase in precipitation; ↑T°: increase in temperature; sd: standard deviation. Significant effects at a Bonferroni-corrected α of 0.001 are in bold.

	Plant-aphid-parasitoid networks			Plant-aphid networks			Aphid-parasitoid networks		
	LDq	Cq	Gq	LDq	Cq	Gq	LDq	Cq	Gq
Control	1.945 ± 0.452	0.157 ± 0.045	1.142 ± 0.1	1.284 ± 0.175	0.297 ± 0.085	1.111 ± 0.183	3.305 ± 0.828	0.317 ± 0.095	1.231 ± 0.163
Precipitation	1.978 ± 0.22	0.175 ± 0.039	1.197 ± 0.201	1.322 ± 0.197	0.380 ± 0.104	1.164 ± 0.234	3.445 ± 0.504	0.354 ± 0.072	1.266 ± 0.174
Warming	1.593 ± 0.208	0.125 ± 0.035	1.131 ± 0.093	1.223 ± 0.116	0.245 ± 0.096	1.113 ± 0.123	2.369 ± 0.579	0.228 ± 0.056	1.170 ± 0.124
Warming+Precipitation	1.589 ± 0.136	0.162 ± 0.028	1.159 ± 0.128	1.251 ± 0.11	0.306 ± 0.094	1.066 ± 0.148	2.307 ± 0.221	0.285 ± 0.042	1.361 ± 0.185
↑P F-value	0.015	2.789	0.474	0.239	3.018	0.002	0.024	2.409	2.373
↑P p-value	0.905	0.11	0.499	0.63	0.097	0.966	0.878	0.136	0.138
↑T° F-value	9.168	1.859	0.161	0.944	2.284	0.37	16.985	6.724	0.053
↑T° p-value	**0.006**	0.187	0.693	0.342	0.146	0.549	< **0.001**	0.017	0.821

Table 3. Impact of the simulated climate-change on the wheat: number of seeds / ear, total seed weight per ear (g), density of wheat (number of wheat plants / m^2) and crop yield (g / m^2) for each treatment (mean ± standard error). Measurements of *T. aestivum* were calculated for each of the 24 experimental plots and then compared to each other. Effects of treatments were tested with GLM (Family Gaussian; F-test; non-crop cover and pest aphid abundance were not included in these models). Significant effects ($p < 0.05$) are in bold.

	Number of seeds	Seed weight / ear (g)	Density of wheat	Yield (g/m2)
Control mean ± se	46.67 ± 1.72	2.46 ± 0.11	534.7 ± 57.5	1323.7 ± 192.4
Precipitation mean ± se	44.5 ± 2.18	2.37 ± 0.12	519.3 ± 27.5	1239.3 ± 97
Warming mean ± se	40.77 ± 1.7	2.1 ± 0.11	401.3 ± 82.4	872.5 ± 209.7
W+P mean ± se	42.7 ± 1.81	2.14 ± 0.12	478.7 ± 61.5	1024.8 ± 167.7
↑Precipitation F-value	0.0039	0.0166	0.2677	0.0399
↑Precipitation p-value	0.9502	0.8509	0.6103	0.8435
↑Warming F-value	4.2722	3.0485	2.1088	3.8351
↑Warming p-value	**0.0410**	**0.0119**	0.1612	0.0636

Table 4. Effect of treatments and covariates on the density of wheat (number of wheat plants / m^2) and on the crop yield (g/m^2). Warming treatment, precipitation treatment and non-crop cover were included in a single GLM (Family Gaussian; F-test) when assessing their effects on wheat density. Warming treatment, precipitation treatment and pest aphid abundance were included in a single GLM (Family Gaussian; F-test) when assessing their effects on crop yield. Significant effects ($p < 0.05$) are in bold.

		Density of wheat		Crop yield (g/m^2)	
	d.f.	F-value	P-value	F-value	p-value
Warming	1	11.6064	**0.002797**	6.3304	**0.020518**
Precipitation	1	1.4736	0.238918	0.0659	0.799986
Non-crop cover	1	95.5818	**<0.0001**	not included in this GLM	
Wheat aphid abundance	1	not included in this GLM		14.6634	0.001048

Table 5. Generalized linear models comparisons for the density of the wheat (number of wheat plants / m^2) and the crop yield (g/m^2). AIC were calculated for GLMs including only the warming and the precipitation treatments (GLM1, models presented in Table 3) and for GLMs including warming and precipitation treatments, non-crop cover or wheat aphid abundance as covariate (GLM2, models presented in Table 4).

	AIC GLM1: Treatments only	AIC GLM2: Treatments with covariate
Density of wheat	312.3637	272.2618
Crop yield (g/m^2)	362.4188	351.2199

Figure 1. Impact of simulated climate-change on quantitative ecological plant–aphid–parasitoid networks. All interactions detected during the sampling season were pooled across all the plots sharing the same treatment to draw the networks. Interactions related to the crop are represented in green. Connectivity represents the number of species with which each species interacts.

Figure 2. Impact of climate-change on weighted quantitative vulnerability Vq and interaction evenness IEq for tri-partite and bipartite networks. Different letters indicated a significant difference at a Bonferroni-corrected α of 0.01. Impact of treatments on the network descriptors was tested in distinct Generalized Linear models.

Figure 3. Impact of the simulated climate-change on the crop yield (g/m^2). The effects of the treatments on the crop yield were tested with a Generalized Linear model (pest aphid abundance included as covariate in the model).