**ORIGINAL PAPER**

# Root aphid (*Aploneura lentisci*) population size on perennial ryegrass is determined by drought and endophyte strain

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**Abstract**

Climate change is anticipated to lead to an increase in the occurrence and intensity of drought and fluctuations in insect cycles that will challenge modern pasture systems. Feeding by root aphids such as *Aploneura lentisci* Pass. can be a significant challenge to pastures. These below-ground living aphids are commonly found in New Zealand and Australia, feeding year-round on the roots of graminaceous plants such as perennial ryegrass (*Lolium perenne* L.). Some strains of the fungal endophyte *Epichloë festucae* var. *lolii* in perennial ryegrass can provide protection against root aphids and greater resilience under drought, contributing to higher persistency and growth than endophyte-free plants. However, the interaction between insect pressure and drought is not understood. This study examined the effect of drought on root aphid populations and plant performance in perennial ryegrass plants relative to endophyte status ($\pm$) and endophyte strain (AR37, $NZ_{CT}$) in a glasshouse experiment. Plants were cloned across the drought and well-watered treatments, and half of the plants were inoculated with root aphids, whilst half of the plants were treated with insecticide. Endophyte infection with strain $NZ_{CT}$ and AR37 reduced root aphid numbers. Aphid populations were significantly higher in drought-stressed than in well-watered plants in both endophyte-infected and endophyte-free treatments. Under drought conditions, root aphid populations were increased 4-fold in $NZ_{CT}$ and 8-fold in AR37 and endophyte-free plants in comparison with their well-watered counterparts. Root aphids reduced shoot dry weight by 16% in drought-exposed and 26% in well-watered plants in comparison with their insecticide-treated counterparts whilst reducing root biomass by 49%. Our results suggest that root aphids are likely able to exploit the higher availability of amino acids in the plant sap of drought-exposed plants. This study provides evidence that climate change-mediated impacts of root aphids could reduce production in perennial ryegrass-dominant pastures. However, field trials would be necessary to determine whether this effect is seen in situ, where numerous additional factors will be operating at the same time.

**Keywords** Abiotic stress · Plant sap · Integrated pest management · Climate change

## Key message

- Under drought conditions, root aphid (*Aploneura lentisci*) populations were increased 4-fold in $NZ_{CT}$ and 8-fold in AR37 and endophyte-free plants in comparison with their well-watered counterparts
- Compared with endophyte-free plants, endophyte infection with *Epichloë festucae* var. *lolii*, strain $NZ_{CT}$ and AR37 reduced root aphid numbers
- Plants exposed to four weeks of drought had more root aphids than plants under an 8-week long drought and plants just reaching the permanent wilting point

- Root aphids reduced shoot dry weight by 16% in drought-exposed and 26% in well-watered plants in comparison with their insecticide-treated counterparts
- Root aphids reduced root biomass by 49% in endophyte-free plants in comparison with insecticide-treated plants

## Introduction

Perennial ryegrass (*Lolium perenne* L.) is one of the most important pasture species in the global pastoral farming industry (Saikkonen et al. 2010; Wilkins 1991). Fungal asexual *Epichloë* endophytes (Latch, M.J. Chr and Samuels, C.W. Bacon and Schardl) can form mutualistic relationships with Poaceae grasses, including perennial ryegrass. The seed-transmitted endophyte increases the plant's ability to tolerate insect herbivory through the production of a wide range of secondary metabolites. Four classes of alkaloids are produced by *Epichloë* endophytes: ergot alkaloids (ergovaline), indole diterpenoids (lolitrems, epoxyjanthitrems), lolines (e.g. N-formyl loline), and pyrrolopyrazines (peramine). All four alkaloid classes are involved in invertebrate herbivore deterrence and/or toxicity. The New Zealand common toxic endophyte strain (*Epichloë festucae* var. *lolii*, $NZ_{CT}$, also known as wild type or standard endophyte) is often found in old established pastures (Easton 1999) and produces lolitrems, ergovaline, and peramine. However, this strain is also associated with reduced livestock performance (Hume et al. 2016). Elimination of the endophyte is not an option as endophyte-free grasses are not viable in countries such as New Zealand and Australia which have high invertebrate pressure and a scarcity of natural predators allowing insect pests to flourish (Ferguson et al. 2019). More recently, endophyte strains with a favourable alkaloid profile have been identified and commercialised to provide host grasses with resistance/tolerance to insect herbivory, diseases, and abiotic stresses whilst minimising livestock health issues (Johnson et al. 2013). Novel endophyte-host associations, such as AR37 an epoxyjanthitrems producer, have become a fundamental management tool in New Zealand, Australia, and the USA to maintain and/or increase pasture persistence and production (Hume et al. 2020; Watson et al. 2004; Woodfield et al. 2019).

Root aphids (Hemiptera: Sternorrhyncha: Aphididae: *Aploneura lentisci* Pass.) originate from the Mediterranean region where they live on their primary host *Pistacia lentiscus* L. utilising temperate grasses as a secondary host. Since *P. lentiscus* does not grow in New Zealand, and rarely grows in Australia, root aphids permanently live on the roots of their secondary host and are believed to only reproduce asexually (Wool et al. 1986). Mature aphids are sedentary and live on roots where they surround themselves with self-produced flocculent white wax, which protects them from external stress such as extremes of soil moisture and predators. Root aphids

can be found throughout New Zealand (Popay et al. 2016) and Australia (Moate et al. 2012; Popay et al. 2021), where they are considered a major pest of perennial ryegrass, tall fescue (*Festuca arundinacea* Schreb.) (Popay et al. 2021), and young wheat plants (Mustafa et al. 1987), reducing plant growth and vigour (Pennell et al. 2005). Aphids suck nutrient-rich plant sap out of the phloem, reducing photograph assimilates needed by the plant for growth. In a pot trial, it was estimated that root aphids reduce ryegrass tiller survival by 35% with a 16–27% reduction in foliage (Popay et al. 2016). Furthermore, in a field trial, root aphids reduced perennial ryegrass growth by 38% (Popay et al. 2021).

Although fungal endophytes are only present in above-ground tissue (Christensen et al. 2008), some endophyte strains impair root aphid populations below ground and hence, contribute to superior growth of infected perennial ryegrass (Popay et al. 2016). The endophyte strain $NZ_{CT}$ can have some effect on root aphids, although these results are transitory (Popay et al. 2016; Popay et al. 2009). Commercially available endophyte strain AR1 has no negative effects on root aphid populations (Hume et al. 2007; Popay et al. 2007; Popay et al. 2009), whilst endophyte strain AR37 has a potent effect on root aphids, significantly reducing populations and hence, increasing plant growth and vigour (Pennell et al. 2005; Popay et al. 2016). This reduction in root aphids is caused by the translocation of certain fungal alkaloids into the roots (Patchett et al. 2011). The bioactive compounds responsible for the effects on aphids are not fully understood (Popay et al. 2016).

The pastoral industry is facing an increasingly complex operating environment through climate change. The frequency of drought has increased in some agricultural regions (Mullan et al. 2005; Strzepek et al. 2010), with major impacts on pastoral production and resilience (Rogers et al. 2022). Although climate models are highly variable between countries and regions, in temperate grass-producing countries such as New Zealand, Australia, and the USA, the likelihood of soil moisture depletion is increasing (King et al. 2020; Trenberth et al. 2014). *Epichloë* endophyte infection in tall fescue, a pasture grass often grown in the US, can improve the plant's ability to withstand soil water deficit (Arechavaleta et al. 1992), but results for perennial ryegrass are more variable (He et al. 2017). Reduced soil moisture also influences population dynamics, fitness, and phenology of herbivorous invertebrates (Aslam et al. 2013; Huberty et al. 2004). Low soil moisture content not only alters the physical properties of soils but can also affect their suitability as a food source and habitat for insects (Mattson 1980). The effect of drought on phloem-feeding insects has been variable. Drought has been reported to reduce the fitness metrics such as survivorship, fecundity, and density of phloem-feeding insects (Huberty et al. 2004; Leybourne et al. 2021), whilst other studies linked drought with an increase in pest abundance (Mattson et al. 1987; Pretorius et al. 2016). For example,

moderate drought events have promoted populations of the rice root aphid (*Rhopalosiphum rufiabdominalis* Sasaki), a pest of wheat (*Triticum aestivum* L.) (Kindler et al. 2004).

Despite the importance of fungal *Epichloë* endophytes and the abundance of root aphids in New Zealand pastures, little research has focussed on the extent to which endophyte-infected grasses can mediate drought tolerance and insect herbivory pressure simultaneously (Hewitt et al. 2021). Many drought trials are conducted without any awareness of insect pest pressure, yet this combination is often seen in the field. It is unclear to what extent the endophyte mediates grass-insect interactions and how water supply can affect this. In a previous field study, it was suggested that drought conditions may have benefited root aphids causing significant pasture losses (Popay et al. 2021). The questions examined in this research are:

I. How are root aphid populations affected when its host, *L. perenne* infected or free of *Epichloë* endophyte, is exposed to drought? Do *Epichloë* endophytes mitigate the effect of root aphid feeding under drought conditions?
II. How do multiple stress factors such as root aphid feeding and drought affect plant growth and production?

Here, we report the interactive effects of drought on root aphid population dynamics and its impact on plant growth and production, in endophyte-infected and endophyte-free perennial ryegrass. We hypothesised that *A. lentisci* populations would increase when their plant host is experiencing drought conditions. *Epichloë* infection with certain strains is expected to mitigate root aphid feeding in drought and well-watered conditions.

## Material and methods

In a glasshouse trial, a fully factorial design was used to compare two drought treatments (drought, well-watered), three endophyte-host associations (perennial ryegrass plants; cultivar One50, infected with *Epichloë festucae* var. *lolii* proprietary strains AR37, $NZ_{CT}$, endophyte-free), with simultaneous root aphid feeding (aphid, control), over five harvest time points with five replications ($n = 300$ experimental units). In this trial, root aphid populations, root, and herbage growth were quantified. The study took place over 12 months between May 2020 and April 2021 at the AgResearch Ruakura Agricultural Centre (lat: − 37° 46′ 17.23" S; lon: 175° 18′ 22.24" E).

### Plant preparation

All plants were grown from seeds sourced from the Margot Forde Germplasm Centre, AgResearch, Palmerston North. Individual plants were grown using a standard seed-raising mix (Daltons™). Once reaching the 4-tiller stage, each plant was tested for viable endophyte infection by the tissue immunoblot method (Simpson et al. 2012). Plants with ambiguous immunoblot results were checked by microscopy of leaf sheath material (Card et al. 2011). Plants with the appropriate endophyte status were split up to provide four clones, each consisting of three ramet tillers. Each of these genotype clones was used across the treatment combinations (e.g. drought-root aphid, drought-control, well-watered-root aphid, well-watered-control) to eliminate endophyte and plant genotype interactions. Tillers were planted into 100 cm long, 8 cm diameter PVC tubes filled with 3 levels of soil (0–25 cm New Plymouth brown loam topsoil (previously tested for soil nutrients and adjusted to optimum level accordingly), 25–60 cm 50/50 New Plymouth brown loam and washed river sand, 60–100 cm washed river sand. A 4 cm lip was left to allow appropriate watering (soil bulk density per tube 1.73 $g/cm^2$). Each tube was taped at the bottom with weed matting (Cosio Ultra-Pro Weedmat) to prevent soil loss. The tubes were placed in a framework supported by a floor made up of 25 mm pea gravel. A layer of 25 mm polyurethane foam was placed between the root tubes and pea gravel. Tubes were arranged in a full factorial design randomised to row triples within each block with each block containing one of each treatment. A full set of tubes (containing ryegrass plants) surrounded the experimental tubes to minimise the border effect. The framework was covered in insulation foil to reduce temperature fluctuation throughout the trial. Each tube weight was recorded enabling the calculation of per cent soil moisture per tube during the whole experiment. Plants were left to acclimatise for one month. A further set of 8 tubes without plants was saturated with water, and the soil was oven dried at 80 °C enabling the calculation of field capacity (FC, Eq. 1). The mean field capacity was applied to enable the calculation of the permanent wilting point of each tube (PWP, Eq. 2).

Equation (1) Field capacity calculation

$$FC\% = \frac{\text{weight wet (g) (after 24h saturation)} - \text{soil weight dry (g)}}{\text{soil weight dry (g)}} * 100 \quad (1)$$

Equation (2) Permanent wilting point calculation

$$PWP = \frac{\text{soil weight at field capacity (g)} - \text{soil weight at first sign of wilting (g)}}{\text{soil weight at first sign of wilting (g)}} * 100 \quad (2)$$

Mature and immature wingless root aphids were collected from roots of a different set of endophyte-free potted perennial ryegrass plants that were maintained in a screenhouse. In spring (September 2020), $2 \times 2$ cm infested root pieces were removed using a scalpel and

one piece/tube was added to the aphid treatment plants by excavating a small hole (1 cm) near the crown and burying the infested root piece. This step was repeated 4 weeks later to provide sufficient aphid loading per plant. Because of their fragile nature, root aphid numbers were estimated with the aim of adding at least 20 adult aphids per replicate plant. All control treatments, which had no aphids added to the plants, were treated monthly with an insecticide (liquid imidacloprid solution, applied as a soil drench at application rates according to manufacturer recommendations). An insecticide was chosen because young nymphs are highly mobile in the soil and easily spread between plants (Rasmussen et al. 2008b). Aphids were allowed to establish for 4 months before the first harvest was conducted.

Every 3 weeks throughout the whole experiment (May 2020–April 2021) plants were regularly trimmed to 5 cm above soil level using sterilised scissors to encourage vegetative growth, (dipped into 70% ethanol between plants). These shoot trimmings were independent of any harvests.

After every third trimming, each plant was fertilised with 30 mL nutrient solution (1.8 g/L Thrive™ and 1.3 g/L urea) to maintain vigorous growth. All trimmings were oven dried at 80 °C to determine dry weight. Temperature and relative humidity were recorded at 10 min intervals using a Digitech QP-6013 data logger (Jaycar Electronics, New Zealand).

## Root aphid trial and plant tube management

The effect of drought in conjunction with root aphid feeding was assessed by comparing the root aphid population size of plants infected with different endophyte strains and endophyte-free plants and drought versus well-watered plants. Root aphid populations and plant growth were measured on five occasions (harvest 1–5; Fig. 1). Drought treatments (Harvest 1-drought start, Harvest 2-four week long drought, Harvest 3-eight week long drought) were kept at 1% above PWP, whilst control plants were watered as required to meet 80% field capacity every 4 days (Fig. 1). This level of
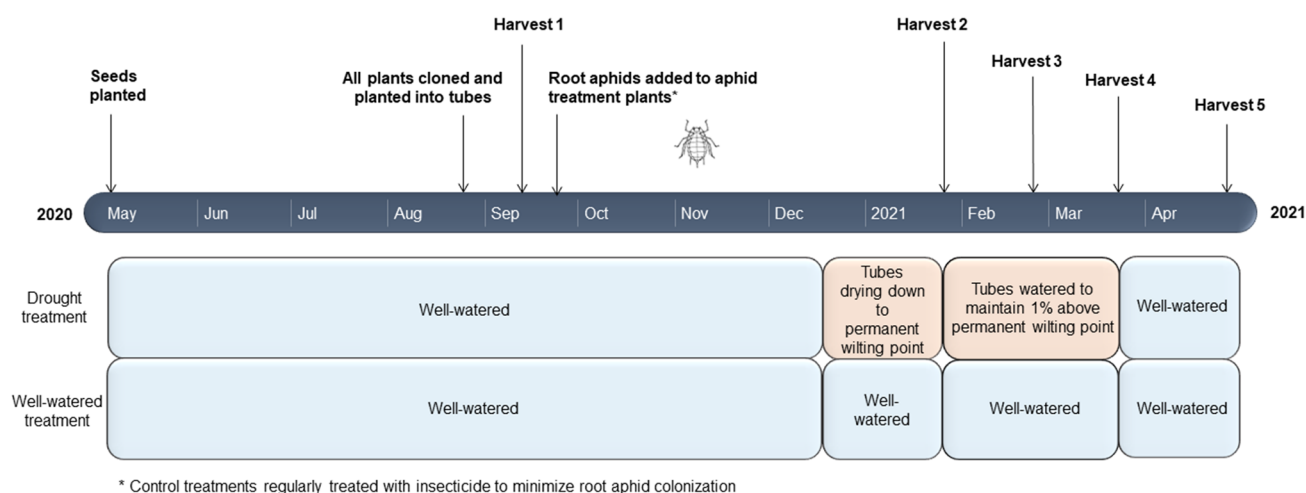


| Harvest | Description |
|---------|-------------|
| 1 | All plants are well watered and had no root aphid. Baseline |
| 2 | Droughted plants started to show drought (wilting and leaf rolling). Each tube was weighed allowing the calculation of water required to keep plants 1% above permanent wilting point |
| 3 | Droughted plants subjected to 4 weeks at 1% above permanent wilting point |
| 4 | Droughted plants subjected to 8 weeks at 1% above permanent wilting point |
| 5 | Previous droughted plants were able to recover and were well-watered for 4 weeks |

**Fig. 1** Timeline of the process to achieve drought and root aphid treatments in perennial ryegrass plants. All plants were grown under well-watered conditions for 7 months during which root aphids were able to establish for 4 months. Water was withheld from the "drought" plants until they started to show drought symptoms such as wilting and leaf rolling (permanent wilting point). Each tube was weighed allowing the calculation of water required to keep plants at 1% above the permanent wilting point. Well-watered treatments were watered as required. Plants were destructively assessed for root aphid populations and plant performance in five harvest time points: harvest 1—baseline, all plants were well-watered with no root aphids, Harvest 2—drought start, Harvest 3—four weeks at 1% above permanent wilting point, Harvest 4—eight weeks above permanent wilting point, Harvest 5—drought recovery

drought reflects a severe drought in late summer/early spring in the field (Hofmann et al. 2003).

At all harvest time points, plants were retested for endophyte presence using the immunoblot method and shoot dry matter, and tiller number was taken before each tube was destructively harvested to determine root dry matter (60 tubes/harvest). Two leaf laminas were randomly selected, and leaf length and leaf lamina area were determined using ImageJ analysis (Version 1.53r). The foliage was cut at the soil surface and immediately frozen to $-20\,°C°$ for later freeze drying and weighing. Each tube was opened, and the root column was divided into 4 Sections. (0–10 cm, 10–20 cm, 20–40 cm, and 40–100 cm). This was done to investigate if root aphid numbers differ between soil depths and how they may affect root growth. Each section was placed into a 10 L bucket filled with water and roots were thoroughly washed to separate the aphids from the root material. Roots from each section were bagged individually and frozen immediately to -20 °C. Aphids float on the surface due to their small size and the waxy layer they surround themselves with. The suspension of the floating layer in the wash bucket was decanted through two sieves (710 μm, 210 μm), and aphids were gently rinsed into a 70 mL specimen jar and stored at 4 °C for later counting. The number of aphids in each treatment replicate was determined firstly by transferring the sample into a beaker and diluting to 40 mL with tap water. When the sample was thoroughly mixed, a subsample of five 2 mL aliquots was transferred into a 9 cm Petri dish lined underneath with a 1 cm$^2$ grid. The number of aphids was counted and recorded using a stereo microscope at 8–60× magnification. The number of aphids in the subsample was multiplied by 4 to calculate total number of aphids in the 40 mL sample ($n*4$). This number reflects the total number of root aphids per tube.

## Chemical analysis

Endophyte-derived alkaloid concentrations and amino acid concentrations were measured on plants which were exposed to drought for 8 weeks. Whole tiller samples were freeze-dried at ambient temperature and $-0.4$ mbar vacuum (Christ, Alpha 1–2 LDplus, Germany) and ground to a fine powder to obtain homogenous samples. For the alkaloid analysis, 50 mg ($\pm 5$ mg) of freeze-dried plant material was weighed into 2 mL screw cap vials and further ground with a bead ruptor (FastPrep FP120, Savant Instruments Inc., Farmingdale, NY, USA) along with 3×3 mm stainless steel beads to increase the efficiency of alkaloid extraction. Alkaloids such as peramine, lolitrem B, ergovaline, and epoxyjanthitrems were analysed using the methods described in Miller et al. (2022). The limit of detection of each of the analytes was 0.1 μg g$^{-1}$.

For the total amino acid analysis, 400 mg of freeze-dried plant material from harvest 4 (8-week drought) was weighed into 2 mL screw cap vials and analysed using the official methods of AOAC International (AOAC 2016). Total amino acid concentration is the sum of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine.

## Statistical analysis

Response variables were analysed using an ANOVA with the treatment factors "moisture stress" (drought and well-watered), "aphid presence" (aphid and control), drought duration ("harvest"), and endophyte infection (AR37, NZ$_{CT,}$ Nil). The statistical model included all covariates, factors, and interactions, with stepwise removal of all terms that did not significantly improve the model. The total number of root aphids per plant was log (ln) transformed ($n+1$ where datasets included zeros) to stabilise the variance. Root aphid numbers for the different soil depths were analysed using "soil depth" as an additional factor. Where necessary to meet the statistical assumption of homogeneity of variance between moisture stress, aphid feeding, plant growth assessments, and endophyte infection, the data were square root or log-transformed prior to analysis. All transformed data were back-transformed. A principal component analysis (PCA) was performed to describe differences as well as similarities in the total amino acid concentration ($n=15$) with respect to the moisture status and root aphid feeding. Fisher's least significance tests were used to assess the significance of the relationship between insect population and moisture stress at $\alpha=0.05$. ANOVA residuals were checked for normality using graphs and the Shapiro–Wilk test and Bartlett`s test for homogeneity. PCA was carried out using the Data integration app (Donweng 2022). All other analyses were carried out using GenStat 21 statistical software package (VSN International 2021). All graphs were generated using SigmaPlot 14.0 (Systat Software Inc.)

## Results

The mean glasshouse temperature for the duration of the experiment was 21.4 °C ($\pm 0.06$ SE, min. 11 °C, max. 39 °C) with a mean relative humidity of 68% ($\pm 0.19$ SE, min. 25%, max. 97%). The first harvest was before aphid addition and drought treatments and was used as an initial comparison between endophyte strains. No differences in plant growth were detected (data not shown).
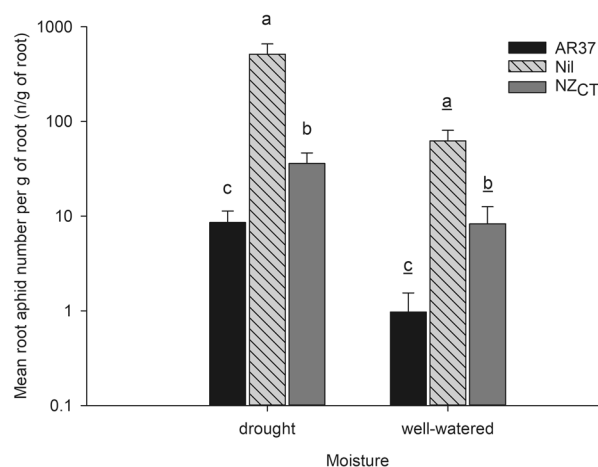
## Root aphid populations in drought conditions

Endophyte infection significantly reduced root aphid numbers ($F_{2,72} = 54.9$, $p < 0.001$). The observed interactions between mean root aphid number/g of root material and covariables are presented in Table 1. In an overall analysis including plants from harvests 2, 3, and 4, plants infected with AR37 had the lowest root aphid population followed by $NZ_{CT}$, whilst endophyte-free (Nil) had the highest populations (Fig. 2). Drought-exposed plants had higher root aphid populations than well-watered plants, which was apparent in all endophyte treatments ($F_{2,72} = 3.90$, $p < 0.05$). Drought increased root aphid numbers by fourfold in $NZ_{CT}$ and eightfold in AR37 and endophyte-free plants in comparison with their well-watered counterparts (Fig. 2). The highest root aphid population was counted in drought-exposed Nil plants with 512 aphids/plant and the lowest in well-watered AR37-infected plants with 1 aphid/plant.

Insecticide reduced aphid populations in all treatments ($F_{2,72} = 148.7$, $p < 0.001$). However, some aphids were found on insecticide-treated plants, more noticeably on the drought-exposed plants and hence, they were included in the total. Insecticide efficacy in control plants was 84% in droughted and 95% in well-watered plants.

In an overall analysis combining data from aphid-inoculated and insecticide-treated plants, the duration of drought to the plants significantly influenced root aphid numbers. Plants suffering a 4-week long drought had 3-fold more root aphids than plants under an 8-week long drought and
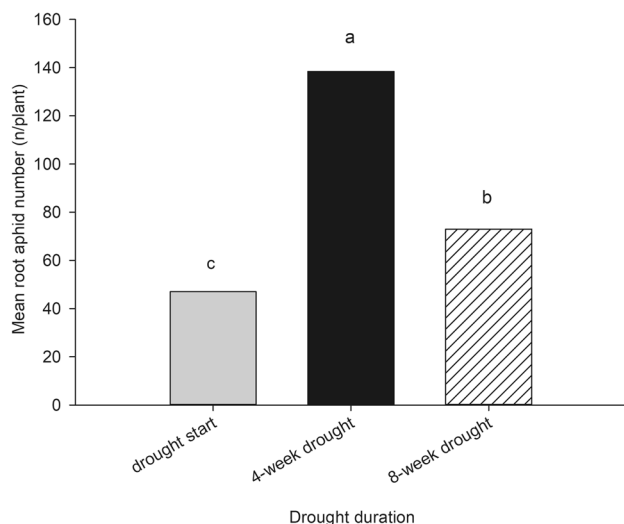


**Fig. 2** Root aphid populations in drought-exposed and well-watered perennial ryegrass plants infected with *Epichloë festucae* var. *lolii* strain AR37 and $NZ_{CT}$ and endophyte-free (Nil). The graph comprises mean of combined data from aphid-infested and insecticide-treated plants from harvests conducted throughout the experiment (i.e. at drought start, 4 week long drought; 8-week long drought). Error bars represent standard errors of the mean ($+1$ SEM). Values with the same letter compare means between endophyte strains at the same moisture status (underlined) and are not significantly different at $\alpha < 0.05$ (Fisher's Least Significance test)

1.9-fold more aphids than plants just reaching the permanent wilting point ($F_{2,72} = 4.08$, $p < 0.05$; Fig. 3).

Root aphids were observed in all four root sampling depths up to 100 cm in drought-exposed and well-watered plants. Root aphid numbers/g of root remained similar throughout the different sampling depths (Fig. 4). Drought increased root aphid numbers at all sampling depths in endophyte-free and plants infected with AR37 and $NZ_{CT}$ (Fig. 4).

## Plant performance

In endophyte-free plants, root aphids reduced shoot dry weight by 42% in comparison with plants treated with insecticide ($F_{2,72} = 12.89$, $p < 0.001$; Fig. 5a). Plants infected with $NZ_{CT}$ and AR37 had similar shoot dry weight in root aphid-inoculated plants and plants treated with insecticide. The reduction in shoot dry weight due to root aphids was apparent at all plant assessment stages (Harvest 2, 3, 4, 5), though the largest reduction of 30% was measured in plants that had recovered from drought ($F_{3,96} = 2.71$, $p < 0.05$; Fig. 5b). The moisture status of the plant affected shoot dry weight production. In general, drought reduced shoot dry weight. The lowest shoot dry weight was measured in plants that had simultaneous drought and root aphid feeding ($F_{1,72} = 7.11$, $p < 0.01$). Root aphids reduced shoot dry weight by 16% in drought-exposed and 26% in well-watered plants in comparison with insecticide-treated counterparts ($F_{1,72} = 7.11$, $p < 0.05$; Fig. 5c). In endophyte-free plants, root aphids

**Table 1** Interactions of total root aphid numbers/g of root material (dry weight) in droughted and well-watered perennial ryegrass plants (factor Moisture) infected with endophytes *Epichloë festucae* var. *lolii* (AR37, $NZ_{CT}$) and endophyte-free plants (factor Endophyte). The factor Harvest represents drought start, 4-week long drought, and 8-week long drought. The source of variations represents the lowest level of the ANOVA involving root aphid effects. The factor Insect represents root aphid presence and control (treated with insecticide). d.f. = degrees of freedom, m.v. = missing values, s.s. = sum of squares, m.s. = mean square, v.r. = variance ratio, F pr. = probability value

| Source of variation | d.f | m.s | v.r | F pr |
|---|---|---|---|---|
| Insect | 1 | 315.5 | 148.8 | < .001 |
| Harvest. Insect | 2 | 10.3 | 4.9 | 0.01 |
| Endophyte. Insect | 2 | 24.4 | 11.5 | < .001 |
| Insect. Moisture | 1 | 4.6 | 2.2 | 0.147 |
| Harvest. Endophyte. Insect | 4 | 3.0 | 1.4 | 0.245 |
| Harvest. Insect. Moisture | 2 | 1.8 | 0.9 | 0.429 |
| Endophyte. Insect. Moisture | 2 | 8.3 | 3.9 | 0.025 |
| Harvest. Endophyte. Insect. Moisture | 4 | 0.4 | 0.2 | 0.953 |
| Residual | 72 | 2.1 | | |
| Total | 179 | | | |

**Fig. 3** Root aphid populations in perennial ryegrass plants during drought. Graph comprises mean data from aphid-infested and insecticide-treated plants from harvest (i) drought start (ii) 4-week long drought (iii) 8-week long drought infected with *Epichloë festucae* var. *lolii* infected (AR37 and $NZ_{CT}$) and endophyte-free plants. "Drought start" represents when plants reached the permanent wilting point. Plants were kept at 1% above the permanent wilting point for 4 and 8 weeks. Values with the same letter are not significantly different at $\alpha < 0.05$ (Fisher's Least Significance test)

reduced leaf length by 26% ($F_{2,72} = 3.76$, $p < 0.05$; Fig. 5d) and tiller number by 36% ($F_{2,72} = 9.79$, $p < 0.001$; Fig. 5f). Such reduction was not seen in plants infected with AR37 and $NZ_{CT}$. Furthermore, root aphids significantly reduced leaf lamina area by 26%, but only in well-watered plants in comparison with plants treated with insecticide ($F_{1,72} = 4.99$, $p = 0.029$; Fig. 5e). Soil moisture did not affect total root biomass (data not shown). Root aphids reduced total root

biomass by 49% in endophyte-free in comparison with insecticide-treated plants ($F_{2,16} = 5.46$, $p < 0.05$; Fig. 5g). Although not significant root aphids also reduced total root dry weight in plants infected with AR37 and $NZ_{CT}$. This was apparent in all four root depth sections in drought-exposed and well-watered plants ($F_{3,504} = 3.39$, $p < 0.05$, Fig. 5h). This reduction in the root biomass created an increase in the shoot/root ratio in aphid-infested plants ($F_{1,72} = 11.09$, $p < 0.001$; data not shown).

## Amino acids and alkaloids

For well-watered but not drought-stressed $NZ_{CT}$ plants, there was a trend for root aphid feeding to increase ergovaline concentrations ($F_{1,19} = 5.13$, $p = 0.053$; data not shown) in the above-ground plant tissue. The total amino acid concentration was significantly higher in drought-exposed plants compared to those that were well-watered ($F_{1,23} = 15.82$, $p < 0.001$). Root aphid feeding reduced total amino acid concentration by 13%, but only in drought-exposed plants ($F_{1,23} = 15.82$, $p < 0.001$, Fig. 6). Drought increased three non-essential amino acids, aspartic acid, glutamic acid, and proline the most (Fig. 7).

For a graphical presentation of the data and to confirm the impact of moisture on amino acid concentration, we performed a principal component analysis (PCA; Fig. 8). The correlation structure of the traits is indicated by the directional vectors in the plot. The origin represents the average point of all 15 amino acids. The first and second principal components of the PCA explained 96.1% (PC1 = 91.2%; PC2 = 4.6%; Fig. 8) of the variation for the total amino acid concentration. The PCA shows a good separation between drought-exposed and well-watered plants but did not separate root aphid feeding from control plants.
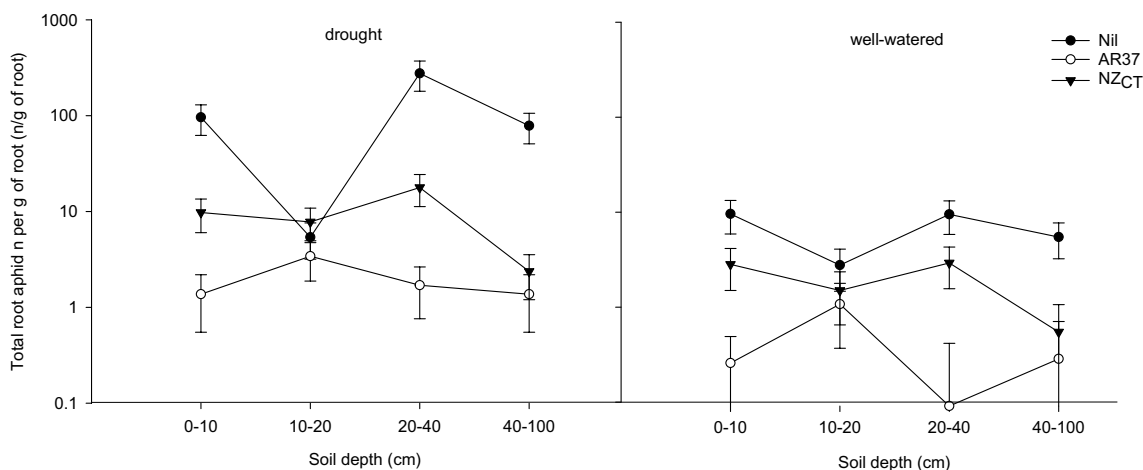


**Fig. 4** Mean root aphid number (log)/g of root in drought-exposed and well-watered perennial ryegrass plants infected with *Epichloë festucae* var. *lolii* strain AR37 or $NZ_{CT}$ or endophyte-free plants (Nil) at different soil depths. Error bars represent standard errors of the mean ($\pm 1$ SEM)
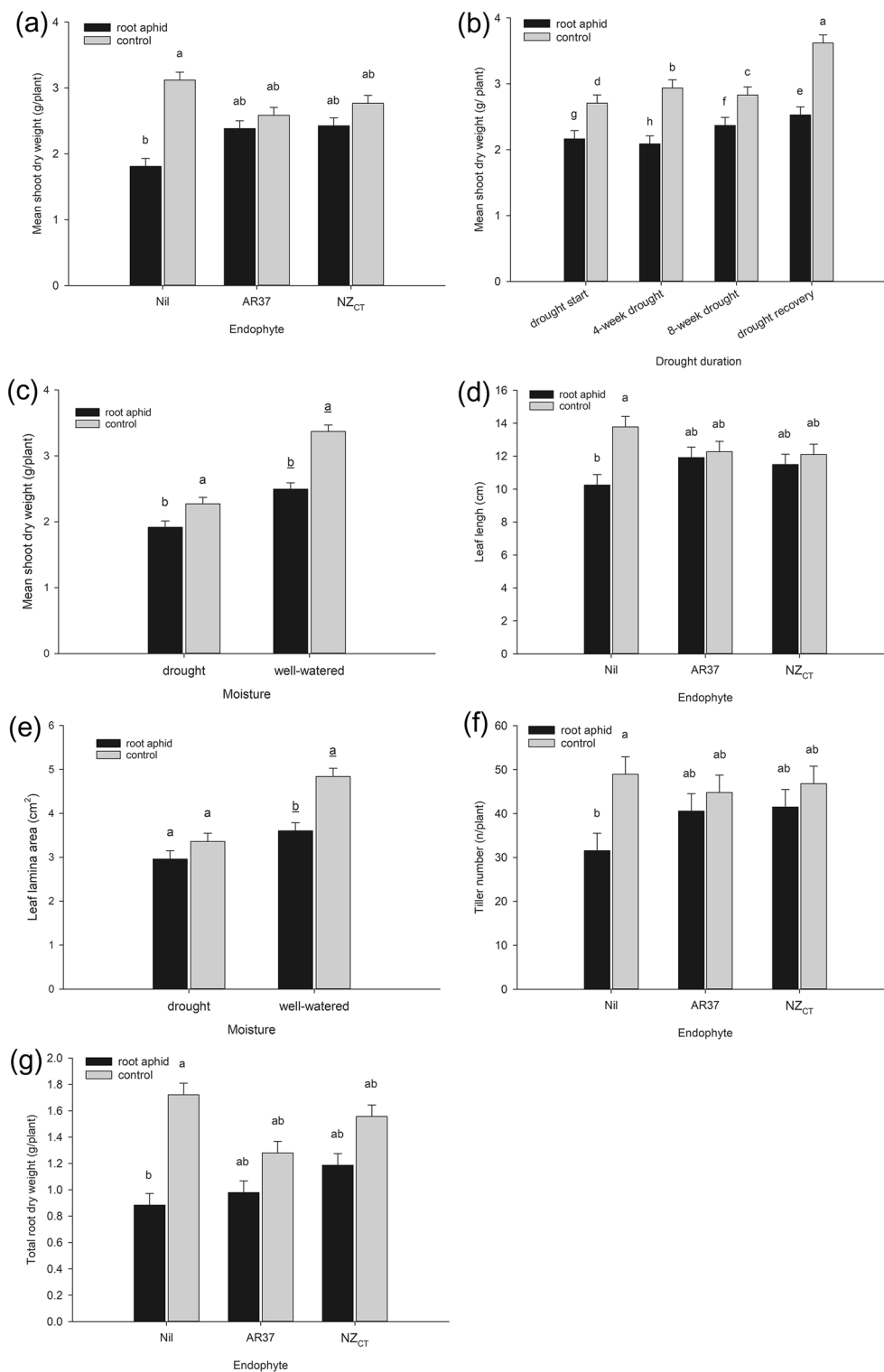
**Fig. 5** Effect of root aphid feeding on mean shoot dry weight g/plant **a**, **b**, **c**, total root dry weight g/plant **d**, **e**, leaf length cm **f**, tiller number/plant **g** and leaf lamina area cm2 **h** in drought-exposed and well-watered perennial ryegrass plants infected with *Epichloë festucae* var. *lolii* strain AR37 or NZ_{CT} or endophyte-free plants (Nil) with and without root aphid feeding. Control plants were treated with insecti-cide. Values with the same letter compare means between root aphid infected and control plants at the same moisture status (underlined) and are not significantly different at $\alpha < 0.05$ (Fisher's Least Significance test). Error bars represent standard errors of the mean ($+ 1$ SEM)
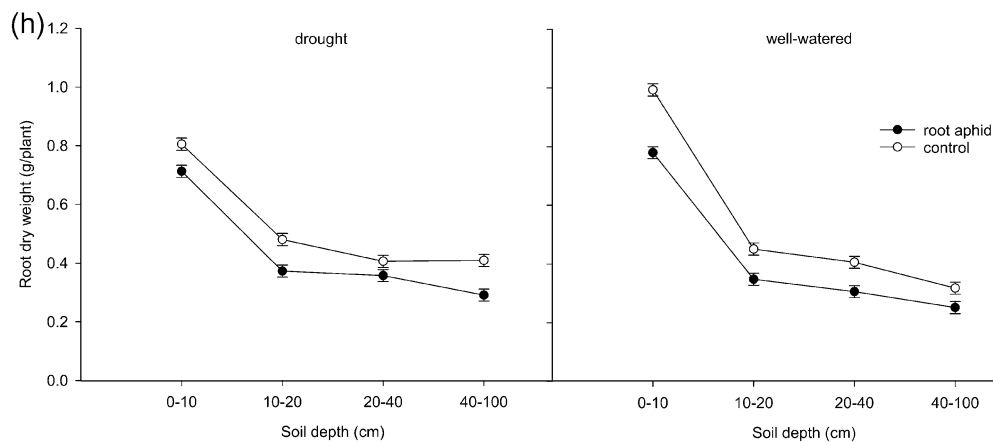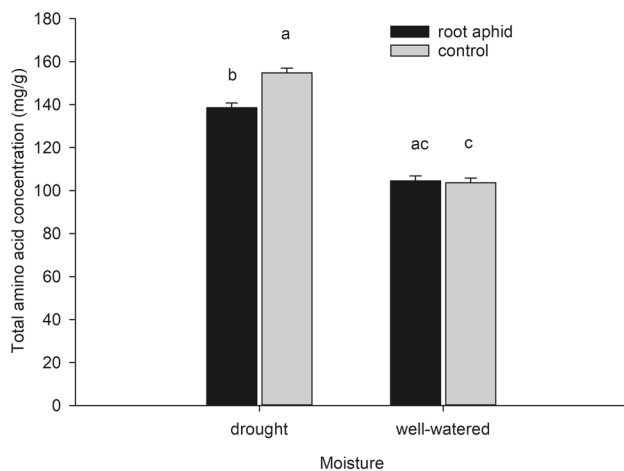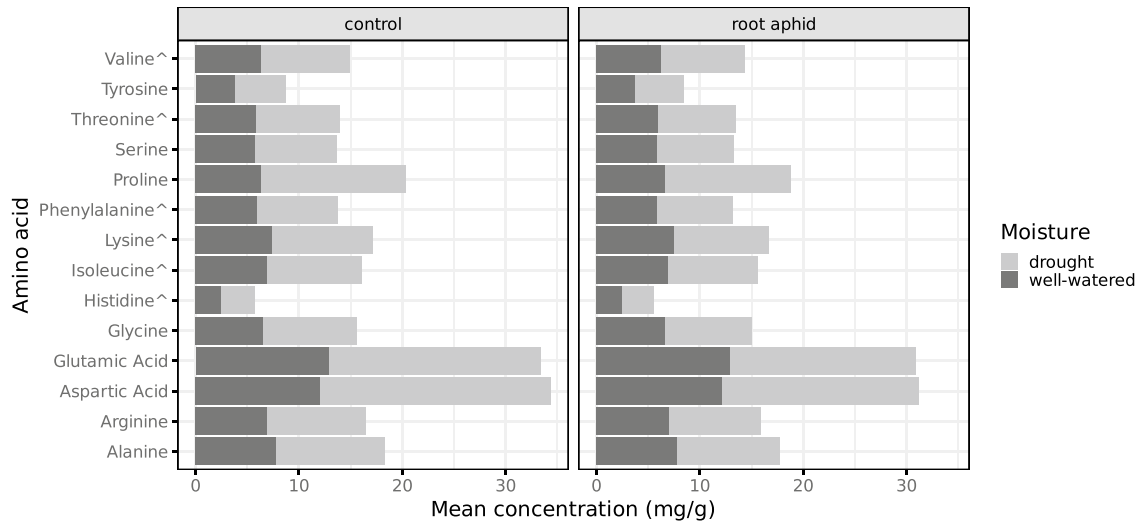
**Fig. 5** (continued)



**Fig. 6** Mean amino acid concentrations (mg/g) in drought-exposed and well-watered perennial ryegrass plants exposed to root aphid feeding. The graph comprises data from plants that were exposed to drought for 4 weeks and were infected with *Epichloë festucae* var. *lolii* strain AR37, $NZ_{CT}$, or endophyte-free plants (Nil). Values with the same are not significantly different at $\alpha < 0.05$ (Fisher's Least Significance test)

## Discussion

This study has provided the first data to show that root aphid populations are affected by the soil moisture status of their host plants. The interactions between host plants and insect herbivores depend on the quality of the host plant (Leather 2017). If a plant offers quality attributes (e.g. chemical, physical), herbivorous insects can utilise these to their advantage. Insect performance and populations are therefore governed by host plant availability, quality, and environmental conditions. These data confirm our hypothesis that root aphid numbers are higher in drought-stressed perennial ryegrass plants in comparison with their well-watered

counterparts. Contrasting observations have been made linking drought with fitness in other aphid species, reporting reduced aphid fitness (Huberty et al. 2004; Pineda et al. 2016), no effect (Banfield-Zanin et al. 2015; Mewis et al. 2012), or positive effects (Oswald et al. 1997; Tariq et al. 2012). An increase in sap-feeding insects has previously been linked with drought-stressed plants (Khan et al. 2010; White 1969). However, a recent meta-analysis encompassing 55 published studies on the effect of drought on sap-feeding insects, showed that, for Poaceae ($n = 24$ studies), aphid fitness is generally reduced under low soil moisture conditions (Leybourne et al. 2021). For example, the fitness of the foliage aphid *Rhopalosiphum padi* L. was reduced when feeding on drought-stressed wheat (*Triticum aestivum* L.) (Kansman et al. 2020). Multiple factors could explain the variation between these studies and our findings including aphid and/or host biology. Specific aphid-plant combinations may influence the response to drought. Various aphid species showed contrasting responses to drought on *Arabidopsis* plants (Mewis et al. 2012), whilst a single aphid species can display contrasting responses when feeding on closely related host plants (Hale et al. 2003). Furthermore, aphid species can influence the relationship between drought and aphid fitness and aphid-plant systems. Leybourne et al. (2021) analysed above-ground living aphids largely from the Aphidini and Macrosiphini tribes. However, *A. lentisci* lives below ground, leading to the conclusion that aphid biology may explain the contrasting response. Other below-ground living aphids can reproduce rapidly under drought conditions (Hein et al. 2005; Kindler et al. 2004; Pretorius et al. 2016). Low soil moisture conditions produce dry, friable soil with numerous airspaces, likely creating favourable conditions for root aphids. In these airspaces *A. lentisci* surround themselves with copious amounts of flocculent wax, protecting them from harsh environmental conditions (Popay et al. 2021).

**Fig. 7** Bar graph of the mean amino acid concentrations (mg/g) in drought-exposed and well-watered perennial ryegrass plants. Graph comprises data from plants that were exposed to drought for 4 weeks and were infected with *Epichloë festucae* var. *lolii* strain AR37, NZ$_{CT}$ or endophyte-free plants (Nil). Asterisk indicates outliers in the dataset. ^ essential amino acids as defined by Karley et al. (2002)

**Fig. 8** Biplot diagram of principal component analysis (PCA) of amino acids mg/g data **a** and total amino acid concentration mg/g **b** in drought-exposed and well-watered perennial ryegrass plants infected with *Epichloë festucae* var. *lolii* (AR37, NZ$_{CT}$) and endophyte-free plants (Nil) after root aphid feeding. Ellipses in PCA show 80% confidence limits. Sample scores on PC1 and PC2 explain 91.2% and 4.6%, respectively, of the variation in the dataset
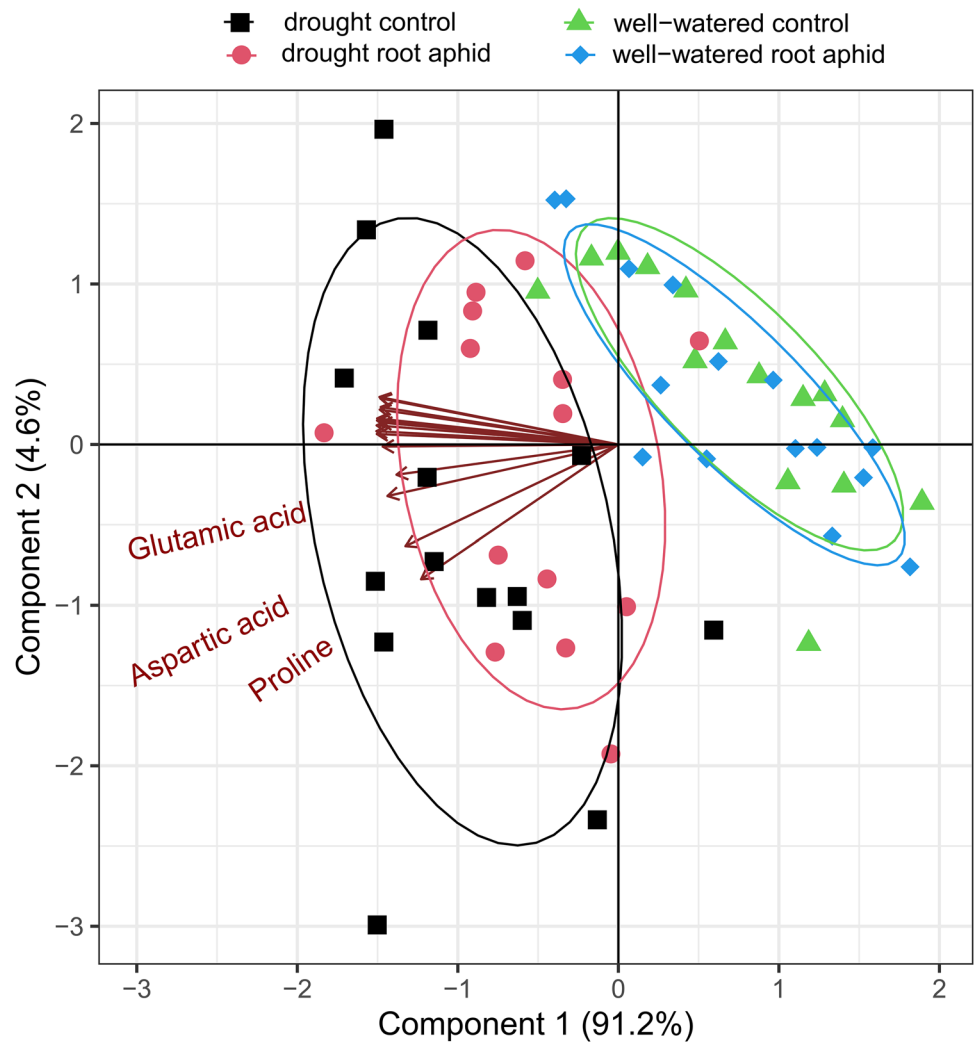
In general, aphids feed on plant sap that contains predominantly four non-essential amino acids: glutamine, glutamic acid/glutamate, asparagine/aspartic acid, and serine (Douglas 1993). These serve as a primary nitrogen source for the aphids. The composition and concentration of such amino acids in the phloem are therefore an important indicator of aphid performance and hence populations (Douglas 1993; Karley et al. 2002). During water deficit, the plant changes its protein metabolism and amino acid synthesis (Sun et al. 2020) which causes the existing proteins to hydrolyse, resulting in a higher concentration of free amino acids (Brodbeck et al. 1987). Although we did not measure amino acids in the phloem directly, this study shows the total amino acid concentration was unsurprisingly higher in drought-stressed plants (Fig. 8). The PCA analysis indicates a clear separation of the moisture status of the plants, indicating a shift of amino acid concentration in drought-exposed plants. Drought increased amino acid concentrations, such as aspartic acid, glutamic acid as well as proline, three non-essential amino acids the most, accounting for 35% of the total amino acid content. These amino acids are well known to be responsive to abiotic stress showing increased concentration in drought-stressed plants (Hayat et al. 2012; Nachappa et al. 2016). High levels of glutamic acid have been linked with reduced nutritional quality of phloem sap for aphids (Douglas 1993) and reduced aphid performance (Chen et al. 1997; Weibull 1988). However, all phloem-feeding hemipterans are nutritionally 'buffered' from phloem sap fluctuations because they obtain supplementary amino acids from their symbiotic bacteria *Buchnera* sp. (Shigenobu et al. 2000). Overall, drought-exposed plants offer relatively enriched essential amino acids, nutrients that insects cannot synthesis de novo, as well as non-essential amino acids. Thus, the phloem of drought-exposed plants could be considered more nutritious than that of well-watered plants. Aphids likely utilise the higher availability of amino acids to their advantage, promoting higher root aphid populations in drought-stressed plants. This is consistent with our observations that root aphid feeding reduced amino acid concentration in drought-exposed plants by 13% (Fig. 6), indicating that aphids deplete the plant of sap. However, our findings contrast with that of Leybourne et al. (2021), who reported that aphid fitness is linked with plant vigour and plant-derived defence compounds, rather than drought-induced altered amino acid concentration. Some aphids can remobilize plant nutrients (Sandström et al. 2000) or sequester plant-defence compounds (Kazana et al. 2007) when the host is suffering drought stress, improving their tolerance to abiotic stress. However, it is unknown if *A. lentisci* has similar attributes.

Despite the often reported deterrent attributes of *Epichloë* endophytes against insects (Caradus 2023), This study also showed that aphid numbers increased in *Epichloë*-infected plants, with drought-exposed $NZ_{CT}$ and AR37-infected plants harbouring fourfold and eightfold more aphids than well-watered plants (Fig. 2). *Epichloë* endophytes alter the quality of host plants by changing the chemistry (Rasmussen et al. 2008a, 2008b). Insects can respond to such changes in three ways (i) negative- alkaloid presence impairs insect performance (ii) neutral-insects are not affected (iii) positive-herbivorous insects' fitness improves when feeding on plants (Bultman et al. 2003; Saikkonen et al. 1999). Many studies have demonstrated that fungal *Epichloë* endophytes can reduce insect populations and hence improve plant performance (Caradus et al. 2020). Our results are consistent with previous studies that infection with certain strains of endophyte significantly reduces root aphid populations (Popay et al. 2016; Popay et al. 2021), even in drought conditions. In this study, drought-exposed plants infected with AR37 almost completely suppressed root aphid populations with a mean infestation of 9 aphids/g of root compared to 36 on $NZ_{CT}$ and 512 on endophyte-free. Similar results were found by Popay et al. (2016), who reported strong aphid suppression in AR37-infected plants as well as a toxic effect. These differences in root aphid populations on plants infected with different endophyte strains are likely attributed to the fungal chemistry. It has been hypothesised that ergovaline produced by the $NZ_{CT}$ endophyte strain has deterrent effects on root aphids (Popay et al. 2007). It is likely that higher alkaloid concentrations lead to increased exposure to defence compounds, causing reduced aphid fitness because of feeding deterrence and reduced sap ingestion, especially since ergovaline is a lipophilic compound occurring in the roots (Lane et al. 1997a). Ergovaline concentrations *in planta* are influenced by environmental conditions (Ball et al. 1995; Lane et al. 1997b). Although not significant, ergovaline concentrations in this study were highest in aphid-infested well-watered plants which generally had lower root aphid numbers than drought-exposed plants. In comparison with plants with the $NZ_{CT}$ endophyte, those with the AR37 endophyte have a greater deterrent effect on root aphids despite not producing ergovaline. The chemical compound produced by the AR37 endophyte responsible for this effect is unknown.

The plant-defence theory predicts that plants under abiotic stress, such as drought, will increase their alkaloid concentration (Arachevaleta et al. 1989; Hahn et al. 2008). Miranda et al. (2011) reported that endophyte infection in Italian ryegrass (*L. multiflorum*) reduced foliage aphids only in drought-stressed plants indicating that alkaloid concentrations may have been highest in plants suffering from the combined stress of insect feeding and drought. Similarly, endophyte infection in tall fescue (*Festuca arundinacea*) reduced the growth and development of fall armyworm (*Spodoptera frugiperda* Smith) in only drought-stressed herbage (Bultman et al. 2003). The reason for the effect between root aphid populations and alkaloid concentration in this study is unknown. It is likely that an unknown metabolite responsible

for root aphid deterrence may mask these effects since to date, the fungal secondary metabolite responsible for aphid deterrence/toxicity by AR37 has not been determined.

Plant performance is linked to the intensity of aphid pressure on the root system. The highest population of root aphids were found in the 0–10 cm section (data not shown). However, when considering the root mass, aphids did not show a preference for a particular soil depth. The root aphid presence in different soil depths would need to be confirmed in field experiments. However, root aphid feeding reduced root dry weight in all sampling depths in droughted and well-watered plants. Young nymphs are highly mobile in the soil (Rasmussen et al. 2008b). Large colonies were often found in areas with greater pore spaces in the soil structure as well as between the soil and planting container where there was prolific root growth. These colonies spread throughout the entire root system significantly reducing the above-ground shoot dry matter by up to 42% in endophyte-free plants (Fig. 5a), confirming previous research in which aphid feeding reduced foliar growth by up to 23% (Popay et al. 2016). Whilst drought did not significantly change the total root biomass, the combined effect of root aphid feeding and drought caused a significant reduction in root growth. This study has provided the first data to show the detrimental effects of root aphids on the total root biomass, reducing root growth in endophyte-free plants by 49% (Fig. 5g) in comparison with plants treated with insecticide. However, some aphids were found on insecticide-treated plants. The applied insecticide is classified as systemic which means that it gets taken up by the plant and circulated in the phloem system to achieve superior efficiency in all parts of the plant. It is believed that ongoing drought conditions may have decreased the plant's ability to fully take up the insecticide, which allowed a minor infestation of root aphids on insecticide-treated control plants. The reduced root growth compromises the plant's ability to access water under drought stress, and yet in the field, plants are often exposed to simultaneous drought and pest pressure. Root aphids prefer to feed on young roots (Popay et al. 2016), suggesting that root morphology plays a significant role in population dynamics. Although not measured in this experiment, it may be that drought-exposed plants had more young roots that are more efficient in water and nutrient uptake than old roots (Bouma et al. 2001; Eissenstat et al. 1997). Young roots contain less lignin making it easier for the aphid to insert its stylet into the root phloem (Whitham 1978). Plants in this experiment were kept at 1% above the permanent wilting point, which may have caused the development of new roots to maximise water uptake to secure plant survival, hence increasing root aphid populations.

Root aphids are phloem feeders requiring a positive plant turgor pressure to extract the available nitrogen-containing sap (Archer et al. 1995). To see an insect population increase, it is necessary that turgor pressure recovers periodically for the aphids to benefit from the increased nitrogen content (pulsed stress hypothesis by Huberty et al. (2004)). Therefore, the severity of drought plays an important role in predicting herbivore damage (He et al. 2014). In this study, the highest root aphid population was found in plants that were exposed to drought for 4-weeks, and the lowest amount in plants that had just reached the permanent wilting point (Fig. 3). In a similar study, above-ground aphid populations increased when plants were intermittently water-stressed, but not when plants were suffering from prolonged drought periods (Huberty et al. 2004) or were highly drought-stressed (Kansman et al. 2020). In this study, plants were exposed to drought for up to 8 weeks, during which intermittent drought was inflicted as often seen in natural situations. It may be that intermediate recovery of plant turgor pressure was sufficient for the aphids to take advantage of the stress-induced increase in nitrogen/amino acid content in the sap. Hence, if the turgor pressure falls below a certain threshold, it interferes with the aphid's ability to utilise the available nitrogen in the phloem (Huberty et al. 2004), possibly resulting in lower populations as seen in plants that reached a permanent wilting point.

## Pastoral farming in a changing environment

Climate change and extreme weather events, such as drought, are major drivers of pest populations as well as crop production (Skendžić et al. 2021). Despite the importance of the pastoral industry to New Zealand's economic well-being, few studies have investigated and predicted the impacts of climate change on pasture insects in New Zealand's farm systems (Dynes et al. 2010; Mansfield et al. 2021). The wide distribution of root aphids in New Zealand and the increasing severity and frequency of droughts make root aphids an increasingly important group of herbivorous insects. The population increases observed in this study will significantly challenge modern pasture systems and since root aphids are present year-round; they can constantly drain the plant resources resulting in reduced plant performance and vigour. With an increase in drought severity, aphids may be able to acclimatise to different environmental conditions utilising the stress-induced increase in nutrient content in the sap. With these changes, pasture systems will need to adapt to maintain production in a more variable and often drier climate. Root aphids are difficult to control due to their small size and below-ground habitat. In the field, the application of synthetic insecticides to prevent population build-up is not feasible. Therefore, an integrated pest management approach is necessary to minimise herbivore pressure. This study provides evidence that climate change-mediated impacts of root aphids can reduce pasture production. Field observations indicate that endophyte infection is most beneficial to plants

during simultaneous biotic and abiotic stresses (Popay et al. 2011). In New Zealand, such combined pressure occurs most often during late summer and autumn (Hume et al. 2007), when plants are the most vulnerable and fungal alkaloid concentrations are typically the highest (Fletcher et al. 2001). Our results have clearly demonstrated that the impact of simultaneous root aphid feeding and drought on plants can be ameliorated by the use of appropriate endophyte strains. Choosing the right endophyte strain is crucial to maximise pasture growth and is dependent on existing pest pressure and location (Caradus et al. 2021; Hewitt et al. 2021). Therefore, fungal *Epichloë* endophytes continue to be a critical constituent of intensively managed pastoral systems. However, their full potential under resource limitation and herbivorous pressure remains poorly understood, even though it is the combined pressure that can be terminal for pasture grasses. Further field trials are necessary to determine how climate change-mediated impacts of herbivorous insects affect pasture production.

## Conclusion

This study has shown that root aphids utilise intermittent drought conditions to their advantage causing an increase in population. Our findings suggest that the increase in root aphid population during drought may be mediated by the plant species response to drought by increasing available nutrients. Drought-stressed plants experience chemical changes leading to more viscous nutrient-rich phloem. Certain fungal endophytes not only reduce insect damage but continue to mediate root aphid feeding when its host is suffering from drought. Endophyte infection can ameliorate the damage to plants under combined resource limitation and herbivorous pressure. This experiment has illustrated the importance of *Epichloë* endophytes in managed pasture systems under simultaneous biotic and abiotic stress, a combination often seen in natural settings. Further research should investigate plant–insect interactions and insect population dynamics in high-intensity pasture systems under a changing climate.

## Author contributions

KGH, RWH, OJB and AJP designed study. KGH carried out conceptualization, data curation, formal analysis, and writing. RWH, OJB, and AJP provided valuable feedback on the manuscript. NC provided statistical expertise. All authors read and approved the manuscript.

## Declarations

**Conflict of interest** A.J.P is a patent holder for AR37 receiving research funding from IP owner Grasslanz Technology Limited and PGG Wrightson Seeds Limited. The research was conducted in the absence of any commercial interest.

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