THE IMPACT OF ELEVATED CO_2 AND SILICON FERTILISATION ON ANT–APHID MUTUALISMS: TURNING BODYGUARDS INTO ASSASSINS?

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Introduction

In the natural world, ants often form mutualisms with aphids, receiving nutritious honeydew in exchange for protection from natural enemies (Way, 1963; Ness et al., 2010). In general, tending by ants increases the population size of aphids on a plant, due to protection from natural enemies. Despite increases in aphid damage, this association can be positive for the plant as the ants also provide protection from other herbivorous insects (Way, 1963; Dixon, 1998; Styrsky and Eubanks, 2007). The formation and continuation of mutualisms between ants and aphids is dependent on a number of factors, and is most favourable when honeydew quality and quantity is high, alternate sources of sugar are scarce, ant colony protein demand is low and aphid colonies are located near the ants nest (Way, 1963; Cushman, 1991; Offenberg, 2001; Oliver et al., 2012).

As is well established, atmospheric CO_2 concentrations are rising (IPCC, 2014). Under future CO_2 concentrations, alterations in plant quality may change the quality and/or quantity of honeydew produced by aphids. A reduction in amino acid concentration induces compensatory feeding in aphids, increasing honeydew output (Sun et al., 2009; Fu et al., 2010; Prasannakumar et al., 2012), which could have the potential to disrupt ant–aphid mutualisms. Silicon fertilisation has also been shown to have a positive effect on plant growth in lucerne (Guo et al., 2006) and a mixed effect on aphid population growth in other plant species (Reynolds et al., 2009; Dias et al., 2014; Keeping et al., 2014). Acting individually, and in combination, alterations in CO_2 and silicon may therefore change the nature, frequency of formation and strength of aphid mutualisms with tending ants. In particular, we hypothesise that decreases in plant amino acid concentrations (a marker of plant quality) will increase the reward value of honeydew which will stimulate ant tending (more bodyguards) whereas deterioration in reward value to ants would reduce tending behaviour and may increase aggression towards aphids (more assassins).

Methods

We investigated the effect of both elevated CO_2 (eCO_2) (640 ppm) and silicon fertilisation (25 mg sodium silicate in 50 mL water, twice weekly) in a fully factorial experiment on the pasture species lucerne (*Medicago sativa*) (cultivar 'Hunter River') and the cowpea aphid (*Aphis craccivora*). Plants were grown for six weeks before being supplied with two aphids per plant two weeks before being harvested, at which point aphids were removed and plants removed from the soil and snap frozen. Plant biomass and aphid population growth under eCO_2 (640 ppm) and Si fertilisation were measured. Glasshouse raised lucerne plants with colonies of cowpea aphid (as above, however aphids were added after five weeks) were placed in open top chambers (OTC's) with CO_2 concentration of 181 ± 63 ppm above the ambient CO_2 concentration (400 ± 30 ppm) with silicon fertilised plants in a split-plot design to expose them to natural populations of ants (primarily *Iridomyrmex* spp.). Plants were observed for 1 minute each three times a day, twice a week for two weeks, during which time the total number of ants visiting the aphid colonies on the plant were counted. Amino acid concentrations of the freeze-dried, milled glasshouse raised plants were analysed using high performance liquid chromatography as described by Ryalls et al. (2015). Data were analysed using linear models in R, with CO_2 and silicon as fixed effects.

Results

Aphid population size was not impacted by silicon fertilisation ($\chi^2_1 = 0.388$, $P = 0.534$) or eCO_2 ($\chi^2_1 = 0.025$, $P = 0.873$), although dry mass increased with both silicon fertilisation ($\chi^2_1 = 2.071$, $P = 0.018$) and eCO_2 ($\chi^2_1 = 2.718$, $P = 0.007$). Total amino acid concentrations increased with silicon fertilisation and decreased under eCO_2 (Figure 1). These differences were driven by changes in the concentration of non-essential amino acids, while essential amino acids were unaffected by eCO_2 and decreased by silicon fertilisation. Moreover, eCO_2 increased the frequency with which aphids were tended by ants, while silicon decreased the frequency of ant tending in the OTC's (Figure 2).

Figure 1.
Effects of elevated CO_2 and silicon fertilisation on amino acid profile of lucerne plants. Mean values shown (n = 20).

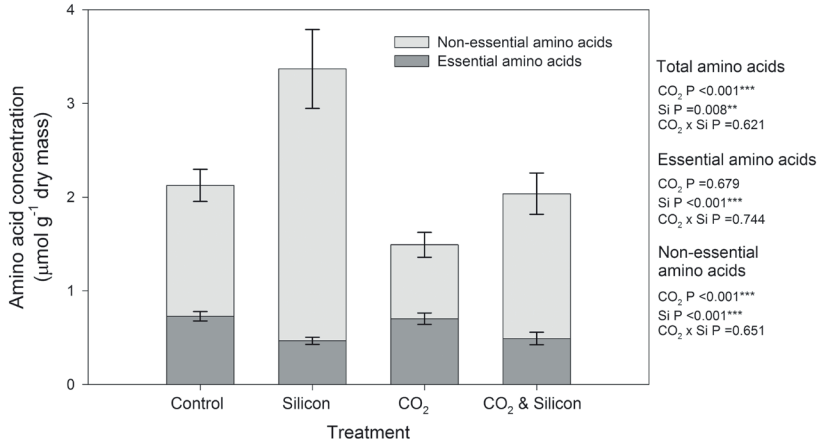
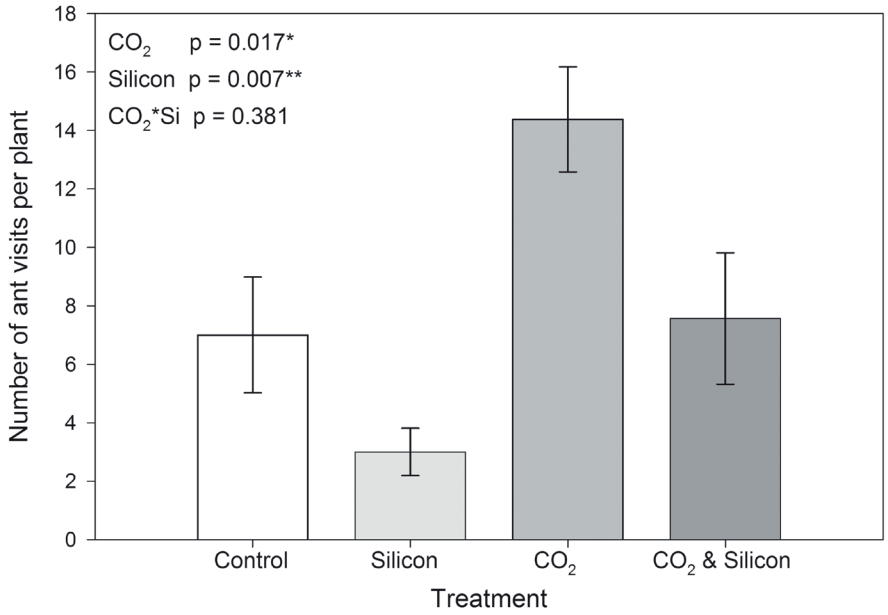


Figure 2.
Effects of elevated CO_2 and silicon fertilisation on ant visits in the OTC's during run 1. Mean values (± SE) are shown (n = 4).



Discussion

Despite the cowpea aphid not showing a population response to the change in amino acid concentration in lucerne, a strong response was demonstrated by tending *Iridomyrmex* spp. ants to both eCO_2 and silicon fertilisation. The means by which they were presumably impacted is via honeydew quantity, as this was likely impacted by the change in the amino acid profile of the plant. Other authors have observed that eCO_2, through its effect on plant amino acids, induces an increase or decrease in honeydew quantity. When plant amino acid concentration is decreased under eCO_2, aphids need to process a greater quantity of plant phloem to obtain the same amount of amino acids, and produce more honeydew as a result through a process known as compensatory feeding (Sun et al., 2009; Sun and Ge, 2011). It has also been found that the reverse is true; when plant amino acid concentration increases, feeding and honeydew production decreases (Fu et al., 2010). Ants are also known to preferentially tend those aphids which are the most abundant honeydew producers. Völkl et al. (1999) found that, of three species of myrmecophilous (ant tended) aphids, the species most frequently tended by ants was the one which produced the most honeydew, with the second highest concentration of sugars. Further to this, Woodring et al. (2004) calculated a score for honeydew 'richness' which took into account both honeydew quantity and sugar concentration, and found that, of eight species of aphids tested, those with the richest honeydew were preferentially tended by ants. In line with what was observed in this experiment, it seems likely that the observed changes in the frequency of ant tending were the result of an increase (in the case of eCO_2) or a decrease (in the case of silicon fertilisation) in honeydew production.

It is likely that the cowpea aphid, currently considered a minor pest of lucerne (Ryalls et al., 2013), will retain the same pest status under future eCO_2 conditions or silicon fertilisation given that aphid abundance was unaffected by either treatment. However, it is worth considering that ant–aphid mutualisms, potentially important factors within the agro-ecosystem (Styrsky and Eubanks, 2007; Layman and Lundgren, 2015) may change in strength and frequency of formation under both eCO_2, causing agricultural managers to reassess current insect management strategies. The results of this study also suggest that silicon fertilisation may have the potential to reduce the incidence of ant tending when this is having a net negative effect on plants.

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