

# Influence of macronutrient imbalance on native ant foraging and interspecific interactions in the field

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**Abstract.** 1. Ants interact with a diversity of organisms. These interactions, coupled with their abundance, cause ants to have ecologically important effects across multiple trophic levels.

2. Empirical study of ant nutritional ecology has led to the prediction that a macronutrient imbalance will affect ant behaviour and interspecific interactions that underlie these broad-scale effects. Excess carbohydrate relative to protein is predicted to increase ant aggressiveness, predatory tendency and foraging activity, and to decrease collection of hemipteran honeydew and plant nectar.

3. In field experiments conducted in 2009 and 2010, captive colony fragments of a native ant, *Formica podzolica* (Hymenoptera: Formicidae), were provided with either simulated prey or carbohydrate solution *ad libitum*. Foraging behaviours and interactions with flowers, myrmecophilous aphids and aphid natural enemies on wild-grown plants were documented.

4. Strong effects of macronutrient imbalance on foraging manifested quickly and consistently across colonies; in accordance with predictions, prey-fed foragers collected both honeydew and floral nectar, whereas carbohydrate-fed ants ceased collecting these resources. Counter to predictions, carbohydrate-fed ants dramatically lowered their activity levels and did not prey upon aphids.

5. Ants had no effect on aphid enemies in 2009, when the latter were relatively rare, but decreased their abundance in 2010. Despite this protection, the net effect of ants on aphids was negative (measured only in 2009). Prey-fed ants demonstrated a strong preference for honeydew over floral nectar, thus demonstrating that a macronutrient imbalance may lead to different interactions with similar resources.

6. This study links ant nutrition and community ecology by demonstrating the rapid, asymmetric and multitrophic consequences of nutritionally mediated behaviour.

**Key words.** Ant–aphid interaction, ant behaviour, *Aphis valerianae*, *Formica podzolica*, nutrient imbalance, nutritional ecology, *Valeriana edulis*.

## Introduction

Ant protective associations with hemipterans, lepidopterans and plants are widely dispersed, both taxonomically and geographically (Pierce *et al.*, 2003; Stadler & Dixon, 2005; Rico-Gray & Oliveira, 2007). A large body of work has

clearly demonstrated that these interactions are not always mutualistic; rather, the outcomes of coevolved interactions with ants are labile and depend heavily on a variety of biotic and abiotic conditions (see reviews in Bronstein, 1994; Styrsky & Eubanks, 2007; Chamberlain & Holland, 2008; Palmer *et al.*, 2008). Additionally, determination of the net costs and benefits to each species may be complicated by indirect costs and benefits (e.g. Chamberlain & Holland, 2009). Accordingly, understanding the factors that mediate ant behaviour is critical

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to understanding their effects at higher levels of ecological organisation (Stradling, 1987).

Protection symbioses are contingent upon the attractiveness of insect and plant rewards to ants (Ness *et al.*, 2010) and ant nutritional ecology must therefore play a central role in these interactions. Ants balance their intake of carbohydrates and protein to meet species-specific nutritional needs (Kay, 2002) using complex foraging strategies that optimise colony performance (Traniello, 1989; Dussutour & Simpson, 2009). Maintaining a balance of these macronutrients is important for colony survival, growth and reproduction (Kay *et al.*, 2006). As a consequence of these balancing strategies, foraging preferences for carbohydrate or protein are increased when ant colonies are supplemented with the alternative resource (Kay, 2004; Wilder & Eubanks, 2010); that is, ants rapidly alter their foraging preference towards the limited resource. In addition to influencing the types of resources sought by ants, a macronutrient imbalance may also alter the behaviours employed in this pursuit. Surplus carbohydrate can increase both forager aggression (Grover *et al.*, 2007; but see Kay *et al.*, 2010) and predatory tendencies (Ness *et al.*, 2009), as well as foraging tempo and activity (Davidson, 1997; Portha *et al.*, 2002). These various perspectives are in no way contradictory and together offer an integrative set of predictions: relative to a diet of excess protein, a carbohydrate surplus is predicted to result in ants preferentially foraging for protein resources (Kay *et al.*, 2010; Wilder & Eubanks, 2010) with greater rates of forager activity and attack against other arthropods (Grover *et al.*, 2007; Ness *et al.*, 2009).

Several previous studies have experimentally manipulated macronutrient availability and documented effects on ant interactions with either hemipterans or plants. The availability of alternative carbohydrates reduced ant attendance of hemipterans in some cases (Addicott, 1978; Sudd & Sudd, 1985; Cushman & Addicott, 1989; Fiala, 1990; Engel *et al.*, 2001; Offenberg, 2001; Rico-Gray & Morais, 2006), but not others (Becerra & Venable, 1989; Del-Claro & Oliviera, 1993; Katayama & Suzuki, 2010), increased predation of hemipterans in some cases (Way, 1954; Rashbrook *et al.*, 1992; Sakata & Hashimoto, 2000; Engel *et al.*, 2001; Schumacher & Platner, 2009), but not others (Cushman & Addicott, 1989; Del-Claro & Oliviera, 1993; Offenberg, 2000; Katayama & Suzuki, 2010), and reduced ant visitation to floral nectaries (Wagner & Kay, 2002; Galen, 2005; Chamberlain & Holland, 2008). The inconsistencies in these results are difficult to interpret because of variations in methodologies that trade off between ecological realism and control of background variation. In field studies, the composition of ant colonies (queens, brood and workers) and their feeding history and access to non-experimental resources are unknown. However, laboratory studies exclude important ecological interactions known to mediate interactions with ants. In the case of ant–hemipteran interactions, aphid natural enemies (Stadler & Dixon, 2005), untended herbivores (Smith *et al.*, 2008) and natural variations in plant quality (Mooney & Agrawal, 2008) can strongly influence interactions with ants, but are largely absent from a laboratory setting. Finally, both field and laboratory studies have examined individual pairwise associations between ants and

either hemipterans or plants, and it is thus unknown whether macronutrients may differentially mediate these two types of interactions. Consequently, a full understanding of the implications of macronutrient availability for ant associates is still forthcoming.

We report on a field study documenting the effects of ant macronutrient imbalance on foraging behaviour, as well as the multitrophic consequences of such effects for interactions with plants, hemipteran herbivores and natural enemies. We split wild ant colonies into pairs of captive colony fragments, fed them either carbohydrate or simulated prey *ad libitum*, and provided foragers access to individual aphid-bearing plants in the field. In so doing, we controlled for ant nutritional demands and access to resources. With this approach, we sought to test the following predictions: relative to a prey diet, surplus carbohydrate should (i) reduce ant tending of aphids and collection of floral nectar (Kay, 2004), and (ii) increase overall ant activity (Portha *et al.*, 2002). We further sought to document the relative strength of ant interactions with hemipteran and floral resources, and to compare the effects of macronutrient imbalance on these two pairwise interactions. Finally, based upon such effects on ant behaviour, we sought to document the indirect consequences of ant macronutrient imbalance on aphid population growth, aphid natural enemies, and pollinator interference as indicated by ant attendance to floral nectaries. This study thus tests core predictions for the effects of ant macronutrient imbalance on a diverse set of interactions in an ecologically realistic setting.

## Materials and methods

### Study site and organisms

This study was conducted near the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, Colorado (38° 58' 01" N, 106° 59' 42" W) in a montane meadow at an elevation of 2900 m. Langenheim (1962) provided a detailed description of the plant communities in this area.

The aphid-tending native ant, *Formica podzolica* (Francoeur) (Hymenoptera: Formicidae), was used in this study. *Formica podzolica* is polygynous and omnivorous, and a single colony usually occupies multiple nests (Deslippe & Savolainen, 1995; DeHeer & Herbers, 2004). Satellite nests are frequently queenless; after exhaustively searching 28 nests at our field site, 23 were found to be queenless, containing only workers and brood. Foraging workers are active between 08.00 hours and 20.00 hours with peaks in late morning and mid-afternoon (W. K. Petry, personal observation, 2009).

We studied the interactions between these ants and the locally abundant *Aphis valerianae* Cowen (Hemiptera: Aphididae), a specialist that feeds on phloem sap from the stems and inflorescences of *Valeriana edulis* Nutt. Ex Torr. & A. Gray (Mooney *et al.*, In press). *Aphis valerianae* is holocyclic and has a single sexual generation each autumn, an egg stage in winter, and viviparous, parthenogenetic reproduction during the spring and summer (Blackman & Eastop, 2006). *Valeriana edulis* is a long-lived perennial herb with a primarily dioecious breeding system in which individual plants are typically

male or female and, although rare, hermaphrodites have been reported (Soule, 1981). A survey of 97 plants in our study area conducted during the course of our experiments found wild *F. podzolica* tending *A. valerianae* on 30% of aphid-bearing plants (W. K. Petry, K. I. Perry, S. K. Rudeen, M. Lopez, J. Dryburgh and K. A. Mooney, unpublished data, 2011).

#### Preparation of experimental ant colonies

Five satellite nests from separate wild colonies of *F. podzolica* (hereafter 'source colonies') were collected 3.5 km from our field site in 2009 (during 7–16 July) and seven source colonies were collected 1.9 km from our field site in 2010 (during 28–31 July). Each source colony was then sorted into two experimental colony fragments (hereafter 'experimental colonies') composed of 200 workers and 20 first- or second-instar larvae. Because the majority of *F. podzolica* mounds at our field site were queenless, queens were not included in experimental colonies for this species. Experimental colonies immediately engaged in apparently normal behaviours, including nest excavation, foraging at provided food sources (see below) and initial care for brood (W. K. Petry, personal observation, 2009).

Experimental colonies were maintained in 15-litre plastic containers (2009) or 19-litre plastic buckets (2010) rimmed with a sticky paste barrier (2009; Tanglefoot Company, Grand Rapids, Michigan) or a slippery fluoropolymer resin (2010; Bioquip Products, Rancho Dominguez, California). Containers were filled with soil collected near wild nests as a nesting substrate. The container lids were propped open to leave a space of 15–20 cm (2009) or had a 6-cm diameter hole drilled into them (2010) to prevent flooding by rain but to allow air circulation and ant passage to the plant. All colonies were regularly misted with water to prevent desiccation.

Prior to the initiation of the field trials, experimental ant colonies were maintained in an outdoor shadehouse on a diet of both honey solution (10% carbohydrate by mass) and tuna in oil (approximately 25–30% protein and 8–10% fat by mass). Preparation of the experimental colonies took several days and consequently the length of time for which each experimental colony was maintained on the full diet varied from 5 days to 11 days. Because each source colony was split into experimental colonies simultaneously, any variation in ant behaviours caused by differing durations on the full diet was accounted for by including source colony in statistical models (see below). Four days prior to field deployment, each experimental colony was switched to one of two treatments: the carbohydrate-rich diet of honey solution, or the protein-rich diet of tuna. The two experimental colonies derived from the same source colony were assigned to opposite treatments and all experimental colonies had access to their diet treatment throughout the experiment.

#### Effects of diet on ant behaviour: natural encounters

On 16 July 2009 and 9 August 2010, nest containers were moved to the field and placed on the ground adjacent to bolting female *V. edulis* plants with aphids. In 2009, plants

were stocked with a fixed density of aphids. Variation in immediate aphid mortality and dispersal before the experimental colonies were connected to the plants led to a range of 40–90 aphids on experimental plants, which corresponded to the mean natural aphid density of  $56.7 \pm 34.7$  [ $\pm 1$  standard error (SE);  $n = 15$ ]. Similarly in 2010, aphid densities varied between 100 and 430 aphids, corresponding to a higher mean natural aphid density of  $193.0 \pm 99.0$  ( $n = 16$ ) in that year. A paired *t*-test revealed no significant difference in initial aphid abundances between diet treatments (mean difference  $\pm 1$  SE; i.e. carbohydrate-fed to prey-fed for each pair of experimental colonies,  $9.0 \pm 8.9$ ;  $t = 1.01$ , d.f. = 11,  $P = 0.33$ ). After a 24-h settling period, a 25-cm copper wire was installed to form a bridge between a rock (2009) or bamboo pole (2010) within the nest container and an experimental plant stem, allowing the ants access to aphids on a single female flower-bearing plant stem. Sticky paste was applied to the base of the plant stem on a ring of tape to prevent foragers from foraging beyond this single stem, as well as to exclude non-experimental ants. The total sample size across both years comprised 12 treatment pairs of experimental colonies (2009: five pairs; 2010: seven pairs).

Observations of ant behaviour were made between 23 July and 3 August in 2009 and during 9–20 August in 2010 during 08.00–20.00 hours, corresponding to the foraging time of these ants. Observations were opportunistic with respect to weather and avoided rain and cold temperatures, during which ants remained in their nest boxes. For each observation (referred to as 'snapshot' observations), the number of ants on the associated plant was recorded for each experimental colony. Additionally, each ant was scored as tending (palpating an aphid with its antennae and collecting honeydew), scouting (roaming on the plant), visiting flowers (collecting nectar from *V. edulis* flowers) or preying upon aphids (holding an aphid in its mandibles). All experimental plants were consistently observed in the same order. The intervals between repeated snapshot observations were a minimum of 1-h and often longer, which allowed all observed foragers to return to the nest box. A total of 870 snapshot observations were made, including 52 for each of 10 experimental colonies between 23 July and 3 August 2009, and 25 for each of 14 *F. podzolica* experimental colonies during 13–20 August 2010. In both years, these observations were made on days when ants were foraging ( $n = 6$  days), avoiding cold and wet weather when foragers remained in their nest boxes.

To determine whether the overall activity of ants was consistent across the experiment, logistic regression was performed using the procedure GLIMMIX in SAS Version 9.2 (SAS Institute, Inc., Cary, North Carolina), in which the presence (or absence) of ants on a plant for each snapshot observation was modelled as dependent on diet treatment, days since initiation of diet treatment and their interaction. Source colony was included as a random effect to increase the power of the test for diet treatment effects by accounting for variation that was both natural (e.g. feeding history, genetic differences in nutrient needs) and caused by our experimental procedures (i.e. time maintained in captivity before the experiment). Interannual variation in ant activity was also accounted for by including year and its interaction with diet treatment in the model.

Because total ant activity was consistent over the course of the experiment (see below), data on ant behaviours were pooled over time. For each experimental colony, the proportion of all snapshot observations for which ants were observed tending, scouting or visiting flowers was calculated. Aphid predation was excluded because no such events were observed (see below). These multivariate data violated the multivariate normality assumption for multivariate analysis of variance (MANOVA) (Zar, 2010); therefore, a non-parametric Euclidean distance-based permutational MANOVA [perMANOVA sensu (Anderson, 2001)] was implemented in R 2.12.0 (R Development Core Team, 2010) using the `ADONIS` function in the `VEGAN` package (Dixon, 2003; Oksanen *et al.*, 2010). The multivariate response variables in this analysis were the proportions of total snapshots in which ants were noted as not active, tending, scouting and visiting flowers, respectively. Because these data are proportions (i.e. all behaviour variables sum to 1), 'not active' (81% of all snapshots) was excluded from the analysis *a priori* to allow the analysed behaviours to vary independently (Cisneros & Rosenheim, 1998). The independent factors in this analysis were diet treatment (carbohydrate- or prey-fed), year (2009 or 2010), and the interaction between diet treatment and year. Although 'source colony' should be treated as a random effect (Newman *et al.*, 1997), we are not aware of any reliable significance tests of random effects for perMANOVA. Accordingly, source colony was treated as a fixed effect, which results in a loss of power (Clark *et al.*, 2005; Bolker, 2008). Source colony was not significant (see below) and, on the basis of this conservative test, was ultimately excluded from analyses. Each *P*-value reported for these *F*-tests was based on one million permutations. Where the main or interactive effects of independent variables were found to be significant in the perMANOVA, 'protected' univariate analyses (*sensu* Scheiner, 2001) were conducted for each ant behaviour. Mann–Whitney *U*-tests were used in these univariate analyses as the residuals of these individual dependent variables were non-normally distributed and heteroscedastic, thus violating ANOVA assumptions.

#### *Effects of diet on ant behaviour: staged encounters*

Staged encounters were conducted during 13–17 August 2009. In staged encounters, ants on the nest surface of each experimental colony were individually coaxed onto blades of grass, transferred to a host plant to a position approximately 5 cm from the aphids and scored for their behaviour during their first interaction with aphids as tending, ignoring or preying upon aphids. The number of ants used varied between three and seven (mean:  $4.8 \pm 0.66$ ). As no ants preyed upon aphids, we simplified our analysis to a logistic regression of individual ant behaviour (i.e. tending or ignoring aphids), with diet treatment and source colony as fixed and random effects, respectively. One prey-fed experimental colony was excluded from this experiment because the ants escaped the nest container immediately prior to the assays.

#### *Indirect effects of ant diet*

Aphid population size was monitored for 20 days in 2009 (experimental period +9 days while experimental colonies remained attached but were not observed); this period was long enough to allow for three to four aphid generations. By contrast, study plants began senescing towards the end of the experiment in 2010 before sufficient time had elapsed to measure aphid population growth. Consequently, our test for the effects of ant diet on aphid population growth was restricted to observations made in 2009. The number of aphids per initial aphid (per capita population growth), *r*, was calculated as:

$$r = e^{\ln(N_{t_1}) - \ln(N_{t_0})} \quad (1)$$

where *e* is the base of the natural logarithm and  $N_{t_0}$  and  $N_{t_1}$  are aphid population sizes on 22 July and 10 August 2009, respectively. Thus, a population that remains constant in size would have *r* = 1, whereas populations that doubled or halved would have *r* = 2 and *r* = 0.5, respectively. The resulting variable, *r*, met the assumption of normality but was heteroscedastic; therefore, a Welch's *t*-test was used to test for an ant diet treatment effect on aphid per capita population growth. Because the production of winged (dispersal) morphs is very low in *A. valerianae* (W. K. Petry, personal observation, 2009), this measure is a relatively accurate metric for aphid colony growth.

Counts of aphid predators and parasitoids were taken simultaneously with observations of ant behaviour. Larval and adult ladybugs (Coccinellidae: Coleoptera), larval syrphids (Syrphidae: Diptera), lacewing larvae (Chrysopidae: Neuroptera), several genera of mirids (Miridae: Hemiptera) and parasitic hymenoptera were all classified as natural enemies based upon our own observations and published reports (Dixon, 1998; Wheeler, 2001). Because no single group was abundant enough to allow for individual analysis, all aphid enemies were grouped for analysis.

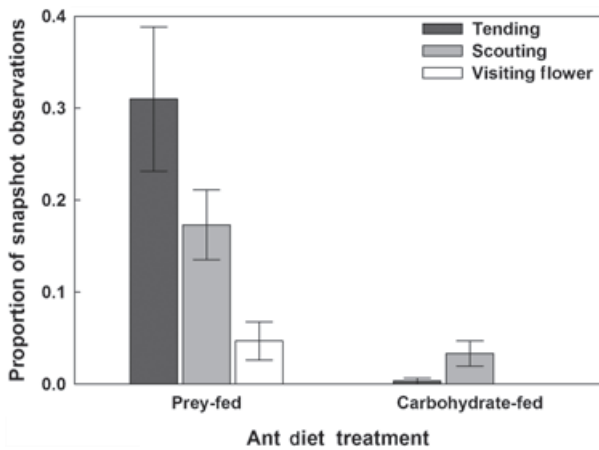
Aphid natural enemies were extremely rare in 2009 (see below) and therefore only data from 2010 were used to test for treatment effects on natural enemies. The abundance of natural enemies was summed across all snapshot observations (natural logarithm +1 transformed to meet assumption of normally distributed residuals) and tested for its dependence upon ant diet, with aphid population size included as a covariate. Source colony was not included in the model because ant behaviour, which mediates any diet treatment effects on aphid natural enemies, was not affected by source colony identity (see above). This analysis thus tests for the indirect effects of ant diet on predators mediated through colony foraging behaviours, controlling for any effects on predator abundance mediated through changes in aphid density and density-dependent enemy recruitment.

## Results

#### *Effects of diet on ant behaviour: natural encounters*

Foraging workers in both treatments were observed actively collecting their respective diet treatment on the soil surface





**Fig. 1.** Proportions of snapshot observations in which prey- and carbohydrate-fed *Formica podzolica* were seen tending *Aphis valerianae*, scouting on *Valeriana edulis* and visiting *V. edulis* flowers. These proportions do not sum to 1.0 as snapshots during which ants were inactive (overall means: prey-fed  $0.470 \pm 0.033$ , carbohydrate-fed  $0.963 \pm 0.004$ ) are not shown. Prey-fed ants were more active on *V. edulis* than carbohydrate-fed ants overall (permutational MANOVA,  $P = 2.2 \times 10^{-5}$ ), as well as more likely to be observed tending (ANOVA,  $P = 7.9 \times 10^{-5}$ ), scouting (ANOVA,  $P = 0.003$ ) and visiting flowers (ANOVA,  $P = 0.007$ ). No effects of source colony or year were found.

within nest containers, and most experimental colonies also foraged on *V. edulis* in one or more snapshot observations (seven of 10 in 2009; 12 of 14 in 2010). Averaged across all snapshot observations for all colonies active on *V. edulis* during one or more observations, a mean of  $0.58 \pm 0.15$  ants were observed on the plant. Predation of aphids by ants was never observed in either treatment in either year.

The logistic regression modelling ant presence or absence on a plant over time showed a significant influence of diet treatment (proportion of snapshots in which ants were present: prey = 0.355, carbohydrate = 0.028;  $F_{1,842} = 6.88$ ,  $P = 0.009$ ) and year (proportion of snapshots in which ants were present: 2009 = 0.142, 2010 = 0.264;  $F_{1,842} = 4.16$ ,  $P = 0.042$ ), but not of days since initiation of diet treatment ( $F_{1,842} = 0.11$ ,  $P = 0.740$ ) or the interactions of treatment with days since treatment initiation ( $F_{1,842} = 0.08$ ,  $P = 0.780$ ) or year ( $F_{1,842} = 1.77$ ,  $P = 0.184$ ). Because treatment effects on ants were strong and consistent from the initiation of the experiment, all subsequent analyses pooled observations for each experimental colony, taking the proportion of snapshot observations for which each behaviour was observed as the dependent variable.

Individual experimental colonies displayed variation in their foraging behaviour (Fig. S1), but the amount of variation attributable to the source colony was not significant in preliminary analyses ( $F_{1,20} = 3.24$ ,  $R^2 = 0.08$ ,  $P = 0.08$ ) and was excluded from subsequent analyses. The full model perMANOVA revealed no significant interaction between treatment and year ( $F_{1,20} = 2.22$ ,  $R^2 = 0.05$ ,  $P = 0.14$ ), nor an effect of year alone ( $F_{1,21} = 3.06$ ,  $R^2 = 0.08$ ,  $P = 0.083$ ), and these terms were removed from the model.

In the reduced model, there was a significant effect of diet treatment (Fig. 1) ( $F_{1,21} = 15.45$ ,  $R^2 = 0.39$ ,  $P = 2.2 \times 10^{-5}$ ). Subsequent univariate analyses showed that prey-fed ants were significantly more likely to be observed tending ( $U_{12,12} = 136.5$ ,  $P = 7.9 \times 10^{-5}$ ), scouting ( $U_{12,12} = 124.5$ ,  $P = 0.003$ ) and visiting flowers ( $U_{12,12} = 108.0$ ,  $P = 0.007$ ) than were carbohydrate-fed ants.

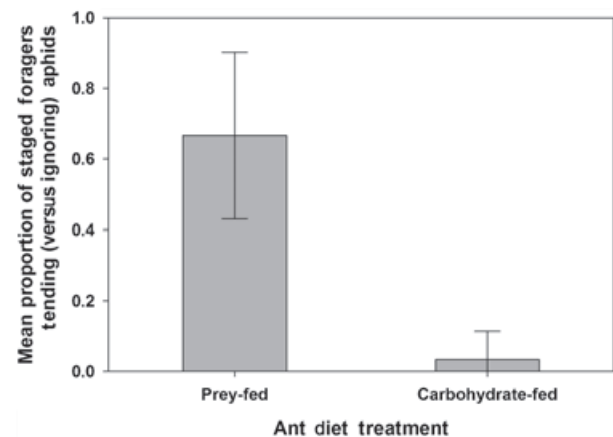
#### Effects of diet on ant behaviour: staged encounters

Staged encounters between *F. podzolica* and aphids showed a significant effect of diet treatment on ant–aphid interactions ( $P = 1.1 \times 10^{-6}$ ) when controlling for source colony that paralleled those of unstaged interactions: prey-fed ants tended aphids in 67% of encounters, whereas only one of 24 carbohydrate-fed ants tended aphids (Fig. 2).

#### Indirect effects of ant diet

Ant diet had a significant effect on aphid per capita population growth in 2009 (Welch's  $t = 2.59$ , d.f. = 5,  $P = 0.049$ ); aphid populations associated with prey-fed ant colonies decreased in size ( $r = 0.35 \pm 0.17$ ), whereas those associated with carbohydrate-fed ant colonies increased in size ( $r = 1.57 \pm 0.26$ ). Thus, prey-fed ants had a negative effect on aphid fitness both absolutely and in comparison with untended aphids attached to experimental colonies of carbohydrate-fed ants. Aphid performance was not measured in 2010.

Aphid natural enemies were rare in 2009 (eight were observed in 520 snapshot observations); therefore analyses were conducted using data from 2010 only (90 were observed in 350 snapshot observations). There was a significant positive effect of aphid abundance on natural enemies ( $F_{1,11} = 6.12$ ,  $P = 0.031$ ). Controlling for this variation in aphid abundance showed that four times as many natural enemies were



**Fig. 2.** Mean proportions of prey- and carbohydrate-fed *Formica podzolica* scored as tending (versus ignoring) aphids in staged interactions (see text). Ant diet treatment significantly affected ant interest in aphids ( $P = 1.1 \times 10^{-6}$ ) when source colony was controlled for.

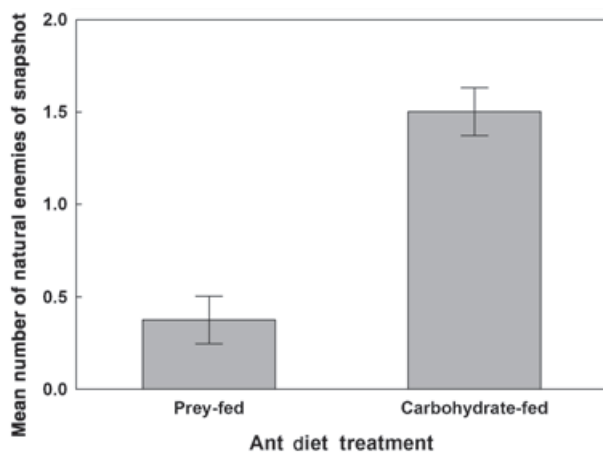
recruited to aphids associated with carbohydrate-fed ants as to aphids associated with prey-fed ants (Fig. 3) ( $F_{1,11} = 10.75$ ,  $P = 0.007$ ), which demonstrates that the increased activity of prey-fed ants effectively deterred aphid natural enemies.

## Discussion

This study shows that *F. podzolica* behaviour is conditional upon available dietary resources and that this response was consistent in two consecutive years. For both natural and staged encounters, prey-fed *F. podzolica* tended aphids and collected floral nectar, whereas carbohydrate-fed ants ignored aphids and were rarely observed on plants. This behavioural effect of ant diet manifested rapidly – within 4 days – and had indirect consequences for other arthropods, affecting aphid population growth and the abundance of aphid natural enemies. Accordingly, macronutrient imbalance had strong effects on both ant behaviour and indirectly on a diverse suite of ant associates. The testing of hypotheses about ant behavioural responses to diet manipulation in a field setting introduces many sources of variation. Despite this added variation, we detected strong ant behavioural responses to diet manipulation, supporting laboratory inferences that macronutrient availability is an important determinant of ant behaviour.

### Tests of predictions

The study results show partial support for the hypothesis that an imbalance in macronutrient availability will drive ant foraging preference towards the limited resource (Kay, 2004). Prey-fed ants were recruited to both aphid- and plant-based carbohydrates (Fig. 1), although carbohydrate-fed ants



**Fig. 3.** Least-square mean  $\pm$  1 SE number of aphid natural enemies per observation in 2010, controlling for aphid density. Plants connected to prey-fed *Formica podzolica* colonies had significantly fewer aphid natural enemies than plants connected to carbohydrate-fed colonies (ANCOVA,  $P = 0.007$ ). Data from 2009 are not shown and were not analysed because of the rarity of aphid natural enemies ( $0.015 \pm 0.006$  enemies per colony per snapshot).

did not prey upon aphids or aphid enemies (Figs 1–3). Two separate laboratory studies on *Lasius niger* (Hymenoptera: Formicidae) (Offenberg, 2001; Schumacher & Platner, 2009) similarly found that carbohydrate-deprived ants increased tending of the aphid *Aphis fabae* (Hemiptera: Aphididae). Yet, whereas Offenberg (2001) found that protein deprivation increased aphid predation, Schumacher and Platner (2009) did not. These differing results may reflect the fact that the former but not the latter study included queens and higher protein demands from young larvae (Schumacher & Platner, 2009). Although the *F. podzolica* colonies in our study were also queenless, they did include young brood and thus a demand for protein. An alternative, but not mutually exclusive, explanation is that *A. valerianae* may sequester host plant secondary metabolites (Opitz & Müller, 2009), rendering them unpalatable to ants. In support of this hypothesis, *V. edulis* contains high concentrations of iridoid glycosides in the inflorescences where *A. valerianae* feeds (K. I. Perry, W. K. Petry, M. D. Bowers and K. A. Mooney, unpublished data, 2009), and in other systems these compounds have been shown to be sequestered by aphids and to provide predator defence (Nishida & Fukami, 1989). A study conducted elsewhere in Colorado showed that *F. podzolica* simultaneously tends and preys upon *Cinara* spp. (Hemiptera: Aphididae) aphids on pine trees (Mooney & Tillberg, 2005); this suggests that the apparent lack of predation in the current study is likely to reflect, at least in part, aphid defence.

Our results do not support the hypothesis that carbohydrate supplementation should increase ant foraging activity (Portha *et al.*, 2002). In opposition to this prediction, carbohydrate-fed ants strongly reduced their activity and foraging on plants virtually ceased (Fig. 1). Whereas Portha *et al.* (2002) found increased activity in sucrose-fed ants (*L. niger*), Grover *et al.* (2007) found that activity levels increased when ants [*Linepithema humile* (Hymenoptera: Formicidae)] were fed intermediate levels of carbohydrates, but decreased at higher levels of carbohydrate availability. In the present study, we offered sucrose *ad libitum* and thus our findings lend support to those of Grover *et al.* (2007) in suggesting that the effects of carbohydrates on ant behaviour may be non-linear.

We also did not find support for the hypothesis that carbohydrates increase ant predation on aphids (Ness *et al.*, 2009), but our data are somewhat limited in this regard. Because of the lack of activity in these experimental colonies, it was not possible to compare the predatory tendencies of ants during naturally occurring foraging bouts across diet treatments. However, in staged encounters, carbohydrate-fed *F. podzolica* did not prey on *A. valerianae* (Fig. 2) as we had expected. These aphids may be chemically defended (see above) and it is uncertain whether carbohydrate-fed ants may have increased their attack of unambiguously palatable prey.

### Indirect effects of ant diet on associated insects and plants

The effects of dietary resources on ant behaviour had indirect effects on both aphids and their natural enemies. Previous work with this system has repeatedly documented that ant attendance

of *A. valerianae* reduces aphid population growth, despite the fact that ants reduce natural enemy abundance and that natural enemies have negative effects on aphid performance (W. K. Petry, K. I. Perry, S. K. Rudeen, M. Lopez, J. Dryburgh and K. A. Mooney, unpublished data, 2011). Our results are consistent with these findings; aphid population growth was lower in association with high levels of tending by prey-fed ants (Fig. 1), despite the fact that these ants also reduced natural enemy abundance (Fig. 3). Although it is not surprising that ant attendance carries a cost (Stadler & Dixon, 1998; Yao *et al.*, 2000), to our knowledge this is the first study to demonstrate a net negative effect of ant attendance on tended aphid performance in a field setting (i.e. the cost of ant attendance outweighed the benefits despite the presence of aphid natural enemies). These data warrant caution in that aphid enemy abundance was relatively low in 2009, although ant exclusion experiments over 3 years suggest the negative effect of ants exists over a wide range of aphid and enemy abundances (W. K. Petry, K. I. Perry, S. K. Rudeen, M. Lopez, J. Dryburgh and K. A. Mooney, unpublished data, 2011). With the caveat that ant tending has negative effects in this system, our results are thus consistent with past laboratory (Way, 1954; Engel *et al.*, 2001; Offenberg, 2001; Schumacher & Platner, 2009; but see Katayama & Suzuki, 2010) and field (Sudd & Sudd, 1985; Fiala, 1990; Rico-Gray & Morais, 2006; but see Del-Claro & Oliveira, 1993) studies; offering of alternative resources to ants had indirect effects on hemipteran populations as a result of a weakening of ant–hemipteran interactions.

Dietary resources also influenced ant visitation to flowers and collection of floral nectar (Fig. 1) and may thus have indirectly influenced pollinator services and plant fitness. Such effects may occur through the physical deterrence of pollinators (Ness, 2006) just as ants deterred natural enemy visitation to aphids (Fig. 3). However, prey-fed ants were only observed on flowers during  $4.7 \pm 2.1\%$  of snapshot observations (Fig. 1), which suggests that any such effects may be weak. By contrast, even occasional visitations by ants may influence pollinators through the collection of floral nectar (Wyatt, 1980; Irwin & Brody, 1998). Although floral larceny may benefit plants by encouraging outcrossing or deeper probing of flowers (Koopowitz & Marchant, 1998), *V. edulis* is unlikely to benefit from nectar thievery as it always outcrosses (is dioecious) and has shallow flowers.

## Conclusions

Ants are widely recognised for the ecologically important and trophically diverse roles they play, in which individual species and foragers simultaneously consume plant resources, herbivores and predators (Davidson *et al.*, 2003; Mooney & Tillberg, 2005). This study has built upon past laboratory studies by testing hypothesised predictions in a field setting. We show that ant diet has strong behavioural effects that cascade upward through trophic networks. Our study thus underscores the broad, multitrophic linkages between ant nutrition, resource environment and ecology, and the importance of studying such dynamics in a natural, community context.

## Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-2311.2012.01349.x

**Fig. S1.** Proportions of snapshot observations in which ants were observed tending *Aphis valerianae*, scouting on *Valeriana edulis* and visiting *V. edulis* flowers for (i) tuna-fed and (ii) honey-fed *Formica podzolica*. Squares represent the grand mean  $\pm 1$  SE for each behaviour and depict the same information shown in Fig. 1. Circles display the behaviours of individual experimental colonies, with adjacent letters denoting source colonies (a–e in 2009, f–l in 2010) from which pairs of experimental colonies were constructed. Several letters adjacent to a single circle indicate multiple experimental colonies with the same value. These proportions do not sum to 1.0 as snapshots during which ants were inactive (overall means: tuna-fed  $0.470 \pm 0.033$ , honey-fed  $0.963 \pm 0.004$ ) are not shown. Prey-fed ants were more active on *V. edulis* than carbohydrate-fed ants overall (permutational MANOVA,  $P = 2.2 \times 10^{-5}$ ), as well as more likely to be observed tending (ANOVA,  $P = 7.9 \times 10^{-5}$ ), scouting (ANOVA,  $P = 0.003$ ) and visiting flowers (ANOVA,  $P = 0.007$ ). No individual or interactive effects of source colony or year were found.

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