Ecological Entomology (2008), DOI: 10.1111/j.1365-2311.2008.00993.x

Insect herbivore stoichiometry: the relative importance of host plants and ant mutualists

PATRICK ABBOT[1], JOSH GRINATH[2,3], JULIA BROWN[1,4], EMILY PEEDEN[1], DAN ERICKSON[1,2] and IAN BILLICK[2]

[1]Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee, U.S.A., [2]The Rocky Mountain Biological Laboratory, Crested Butte, Colorado, U.S.A., [3]Department of Biological Science, Florida State University, Tallahassee, Florida, U.S.A. and [4]Department of Ecology and Evolution, Yale University, New Haven, Connecticut, U.S.A.

Abstract. 1. Mutualistic associations can vary over spatial and ecological gradients. For herbivorous insects that engage in mutualisms with ants, plant quality can be a particularly important source of variation, because of the upward transfer of nutrients from plants to herbivores to ants.

2. A previous study demonstrated that mutualistic ants, *Formica obscuripes*, exert a top-down effect on the carbon and nitrogen concentrations (stoichiometry) in an herbivorous membracid, *Publilia modesta*. We characterised the consequences of mutualism for carbon and nitrogen stoichiometry between the same species pair, yet on an alternative, geographically-distinct host plant.

3. We found no top-down effect of ants on the carbon or nitrogen in the herbivore, but a strong, bottom-up effect of individual plants on membracid nitrogen concentration.

4. These results suggests that spatial heterogeneity in host plant traits, and ultimately the diet breadth of herbivore mutualists, may be important factors mediating stoichiometric patterns in mutualistic associations.

Key words. Ants, bottom-up, herbivore, mutualism, nitrogen, spatial scale, stoichiometry, top-down.

Introduction

A prevailing theme of the study of mutualisms in the past decade has been how mutualistic associations vary along spatial and ecological dimensions (Parker, 1999; Thompson, 2005; Bronstein *et al.*, 2006). For example, positive species interactions may be more likely to occur in poor-quality environments (Brooker & Callaghan, 1998), or may be less susceptible to cheaters when dispersal is restricted (Yamamura *et al.*, 2004). For phytophagous insects that engage in mutualisms with or on plants, such ecological and spatial variation may be particularly pronounced (Bronstein *et al.*, 2006). All phytophagous insect populations are affected by the quality of their host plants (*bottom-up effects*), as well as by the communities of natural enemies that prey upon them (*top-down effects*; Price *et al.*, 1980; Hunter & Price, 1992; Rosenheim, 1998; Denno *et al.*, 2002). Spatial and ecological heterogeneity in the relative strength of these effects are likely key factors that can determine the strength, specificity, and stability (or *conditionality*) of plant-associated mutualisms (Yu *et al.*, 2001; Wilson *et al.*, 2003; Bronstein *et al.*, 2006; Gove & Rico-Gray, 2006).

A difficulty in characterising ecological heterogeneity in mutualistic associations is defining a common currency that readily scales across communities in which the interactions occur. One avenue is to consider how the availability or proportionate allocation of limiting nutrients and elements in mutualistic species varies across ecological gradients, an approach that draws from the emerging field of biological or ecological stoichiometry (Elser & Hamilton, 2007). Despite the successful application of ecological stoichiometry to disparate problems in ecology (Elser & Hamilton, 2007), it has only rarely been applied to the study of mutualisms. However, in mutualisms that involve the exchange of resources (Ollerton, 2006), there are obvious relationships between dietary nutrient limitation, resource exchange, and mutualism function. In one of the few studies, Kay *et al.* (2004) used a stoichiometric approach to characterise the effect of

Correspondence: Patrick Abbot, Department Of Biological Sciences, Vanderbilt University, Nashville, TN 37235, U.S.A. E-mail: patrick.abbot@vanderbilt.edu

© 2008 The Authors
Journal compilation © 2008 The Royal Entomological Society

mutualism with ants on carbon/nitrogen budgets of herbivorous membracids (*treehoppers*; *Publilia modesta* Say) on giant goldenrod (*Solidago gigantea*). Like many hemipterans, the phloem-feeding *P. modesta* reward ants with their sugary excretions (*honeydew*) in return for ants' services, such as protection from predators (Buckley, 1987; Ollerton, 2006). These ant–herbivore mutualisms are variously described as *food-for-service*, *ant-tending*, or *ant-protection* mutualisms. In the presence of ants, membracids exhibited a reduced concentration of nitrogen, indicating that ants may be exerting a top-down effect on membracid elemental composition. An implication of the results of Kay *et al.* (2004) is that a major component of ecological variability in ant mutualisms may be the degree to which ants alter the ability of herbivores to balance nutritional budgets on their host plants (Kersch & Fonseca, 2005; Kagata & Ohgushi, 2006a).

An unanswered question, however, is whether carbon/nitrogen stoichiometry in ant–herbivore mutualisms exhibits ecological and spatial variation across different communities in which the interactions may occur. In addition to variation associated with different ant mutualists or herbivore metabolism, the nitrogen effect of ant mutualism likely depends upon the plant substrate on which the interaction occurs. Host plant variation is especially important for ant-protection mutualisms, because the composition of the exudates herbivores use to recruit ants can depend on the quality of the substrate on which the insects feed (Pierce, 1985; Cushman, 1991; Douglas, 1993; Fischer *et al.*, 2005). Nevertheless, homeostatic mechanisms may maintain relative constancy in carbon/nitrogen stoichiometry in herbivores across host plants (Fagan *et al.*, 2002; Kagata & Ohgushi, 2006b). Here, we investigated the degree to which the effect of an ant mutualism on herbivore stoichiometry can vary within a herbivore species by considering a geographically-distinct population of *P. modesta* and *Formica obscuripes* mutualists (the same species examined previously (Kay *et al.*, 2004) on an alternative host plant (*Chyrsothamnus viscidiflorus* Nuttall).

Methods

Biological background

This study was conducted in the Almont Triangle management area of the Gunnison National Forest approximately 25 km north of Gunnison, Colorado. The site is an open meadow at 2700 m elevation on a northeast facing slope that is heavily grazed by elk in the winter and grazed by cattle for several weeks in late May or early June. The site is relatively arid and the dominant plants are the woody perennials *Artemisia tridentata* Nuttall (Asteraceae) and *Chyrsothamnus viscidiflorus* Nuttall (Asteraceae). The remaining vegetation consists primarily of grasses and herbaceous perennials.

Publilia modesta Uhler (Homoptera: Membracidae) occurs primarily in the western United States (Kopp & Yonke, 1973). It is a host plant generalist (Kopp & Yonke, 1973) and patterns of host plant use have been well documented at the site (Reithel, 2003). Seventy-five per cent of the host plants belong to three species, *C. viscidiflorus*, *Wyethia spp*. (a complex hybrid zone involving *W. arizonica* and *W. amplexicaulis*), and *Balsamorhiza sagittata* Nuttall (Asteraceae) (Reithel, 2003). *Chyrsothamnus viscidiflorus* is locally the important host plant and our manipulations of host-plant quality focused on this plant. At the Almont Triangle the primary tending ant is *F. obscuripes* Forel (Formicidae).

Pilot study – 2005

In 2005, we conducted a small pilot study which provided information on the feasibility of the general experiment. We excluded ants from some *C. viscidiflorus* with membracids, but did not exclude them from others. We collected adults and nymphs on 15 August 2005. Approximately one adult membracid and one nymph from each plant were haphazardly chosen. The membracids were weighed and packaged whole using a standard protocol for micro-Dumas combustion elemental analysis (J. Shifflette, pers. comm.). All packaged samples were sent to the Stable Isotope/Soil Biology Laboratory at the University of Georgia for measurement of total carbon and nitrogen content by dry micro-Dumas combustion analysis using a NA1500 C/H/N Analyzer (Carlo Erba Strumentazione, Milan, Italy). The results of the pilot study suggested no effect of ants on nitrogen or carbon concentration in nymphal membracids; however, the exclusion of ants resulted in a marked reduction of larger nymphs (Fig. 1), suggesting that the absence of ant protection was biasing the nymphal sizes between the treatments (Billick & Tonkel, 2003). Consequently, we repeated the study with a larger sample size, particularly for the *no-ant* treatment.

Main study – 2006

Stoichiometry. To determine the effect of ants on nitrogen concentrations in nymphs, we randomly assigned 60 plants for which oviposition scars were present to two treatments, ants and no ants, in late June of 2006. Because of the concern that treehopper survivorship would be lower in the absence of ants, we assigned two plants to the no-ant treatments for every one plant

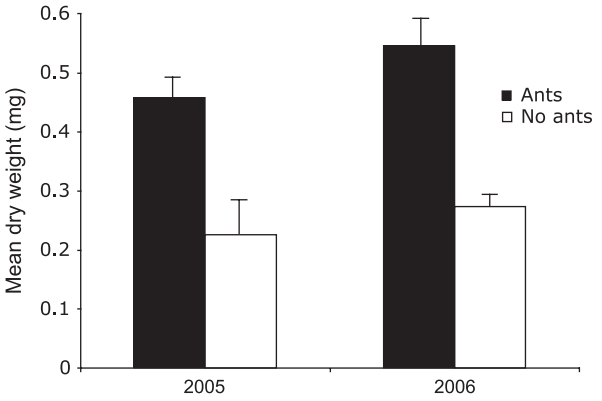

Fig. 1. Mean dry weight (mg) of *Publilia modesta* nymphs in the presence and absence of ants in 2005 and 2006. Data from 2005 are the results from an initial pilot study. Our analysis focuses on data from 2006. While we analyse the datasets separately, we show both years to make it clear that we were able to repeat the finding that ants increase the size of nymphs.

© 2008 The Authors

assigned to the ant treatment. We trimmed plants and placed duct tape on the base of the stem of each plant. We covered the duct tape in the ant-exclusion treatment with Tanglefoot© on 29 June and removed all vegetation touching or close to all plants to prevent access via surrounding vegetation.

We collected nymphs on plants 4 weeks later, on 25 July. All samples were mailed to Vanderbilt University and preserved in an ultracold freezer. Each nymph was then weighed to the nearest hundred thousandth of a gram and measured from the tip of the head to the tip of the abdomen under a dissecting microscope to the nearest millimetre. Instars for each individual were estimated by comparing the sizes of each nymph to the ranges determined previously. They were subsequently transferred to a clear glass vial and dried at 60 °C for 48 h. Nymphs were again weighed to the nearest hundred thousandth of a gram on the same scale. After obtaining a dry weight, we carefully packaged each membracid in a tin capsule, and mailed them to the Laboratory for Biotechnology and Bioanalysis Stable Isotope Core at Washington State University in Pullman, Washington. For each individual, we obtained total carbon and nitrogen values by dry micro-Dumas combustion analysis on a GC-MS, with a 15-point normalization using acetanilide and atropine.

Statistical analysis

Because we had four dependent variables (carbon, nitrogen, dry weight, and length) with strong correlations among them, we used an approach rooted in multivariate analysis to statistically detect the effects of treatments on the response variables (Scheiner, 1993). We used the correlation matrix to generate principal components as the dependent variables for the analysis and then rotated the principal components to facilitate interpretation. We performed a MANOVA (Wilks-Lambda) on the rotated components with treatment (ants vs. no ants) and plant (nested within treatment) as the independent variables and proceeded to univariate analyses if the MANOVA was significant. Visual examination of the residuals indicated that they were approximately normally distributed. To minimise measurement error, we dropped from the analysis nymphs for which the dry weight was less than 0.00001 g (about seven individuals), which was below the detection limits of our scales, and thus resulted in unambiguously unrealistic carbon and nitrogen results. We also performed univariate analyses for nitrogen and carbon content via a nested ANOVA, with nitrogen and carbon as response variables against treatment (ants present, ants absent) and plants nested within treatments. The statistical program JMP (SAS Institute Inc., Cary, NC) was used for all analyses.

Results

Because many plants contained no membracids by the end of the experiment, we had complete data on 122 individuals from 30 plants. Mean nitrogen content of all membracids was about 10% higher than that in the study of Kay *et al.* (2004), and carbon content was slightly lower. There were strong correlations among the response variables. Per cent carbon and per cent nitrogen displayed a strong, positive correlation, as did dry mass and length (Table 1).

Table 1. The correlation matrix among the four independent variables ($n = 122$). We show significant pairwise correlations (*<0.05, **<.001, ***<0.0001).

	%C	%N	Length
Dry weight	0.09	–0.12	0.82***
%C		0.86***	0.36***
%N			0.12

When we generated the rotated principal components, the first and second components explained 47.4% and 46.2% of the variance, respectively. The first component (PC1) consisted primarily of %C and %N, with loadings of 0.95 and 0.97, respectively (weight and length were –0.08 and 0.21). The second component (PC2) consisted primarily of weight and length, with loadings of 0.96 and 0.94 respectively (%C and %N were 0.19 and –0.07).

When a MANOVA was performed on the rotated components, the whole model was highly significant using Wilks Lambda ($F_{58,182} = 3.76$, $P < 0.0001$). When we proceeded to ANOVA using PC1 and PC2 as the response variables we found the ant treatment had a strong effect on PC2, the size principal component (dry weight and length) ($F_{1,92} = 29.7$, $P < 0.0001$). Nymphs experiencing ants had an average weight of 0.452 mg compared with 0.304 mg for which ants were excluded (Fig. 1). Nymphs with ants averaged 2.56 mm in length, compared with 2.34 mm for nymphs without ants. However, there was no ant treatment effect on PC1 (%C and %N). Nymphs with ants averaged 52% C and 10% N relative to 47% and 10%, respectively, for nymphs without ants, differences that were not statistically significant (Fig. 2) ($F_{1,92} = 0.41$, $P = 0.53$).

Plants nested within treatment had a strong effect on PC1 ($F_{28,92} = 2.06$, $P = 0.005$). Mean per cent nitrogen and carbon was strongly heterogeneous across host plants (Fig. 3) (nitrogen: $F_{28,92} = 2.07$, $P = 0.005$; carbon: $F_{28,92} = 2.3$, $P = 0.001$ when each variable was analysed separately). Plants also had a strong effect on PC2 (dry weight and length) ($F_{28,92} = 5.7$, $P < 0.00501$).

Discussion

Ecological and spatial variation in the relative impact of bottom-up and top-down interactions is an important and often overlooked feature of herbivore populations (Stadler, 2004; Underwood, 2004; Denno *et al.*, 2005; Gripenberg & Roslin, 2007). Habitats are often fragmented and patchy, herbivore populations often exhibit considerable ecological and genetic structure, and the biotic communities with which herbivores interact may differ dramatically along spatial gradients (Gripenberg & Roslin, 2007). Such patterns are equally true of insect mutualisms, many of which involve herbivores, yet studies of ecological variation in the bottom-up and top-down factors affecting such species remain rare (Kersch & Fonseca, 2005). The potential hazard is that overlooking ecological heterogeneity may result in conclusions regarding the mechanisms that govern herbivore populations that apply locally, rather than globally (Helms & Hunter, 2005; Inouye, 2005; Hewitt *et al.*, 2007).

© 2008 The Authors

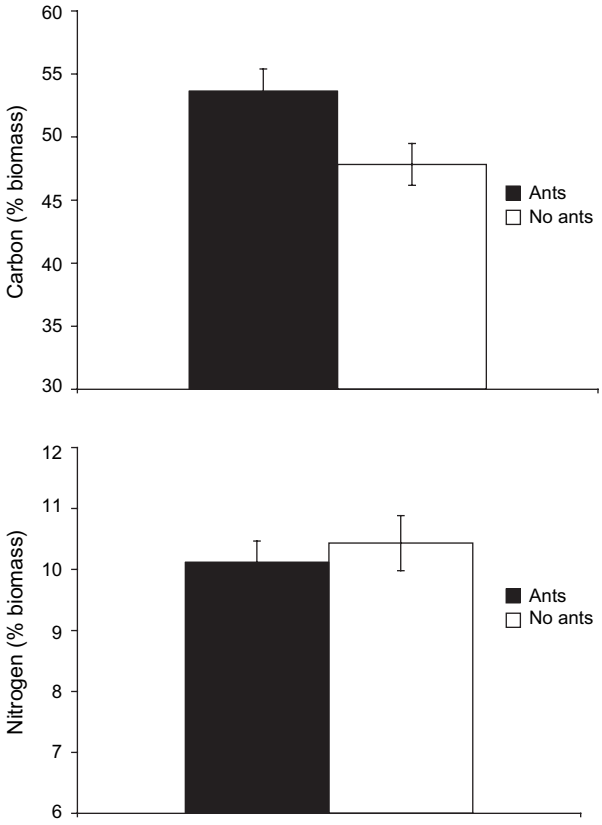

Fig. 2. Mean per cent nitrogen and carbon (±SE) in *Publilia modesta* nymphs in the presence and absence of ants over a 4- week period.

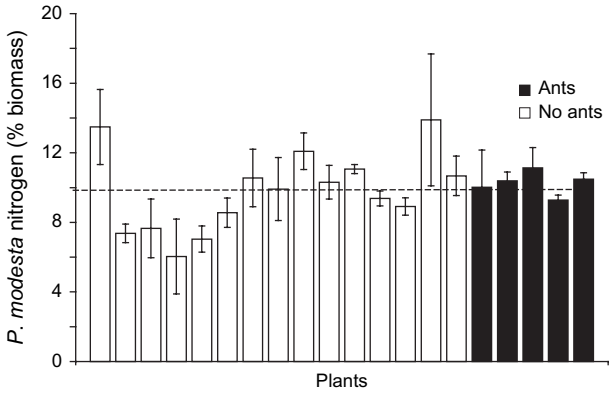

Fig. 3. Mean per cent nitrogen (±SE) in *Publilia modesta* nymphs on individual plants and across both ant and no-ant treatments (dark bars = plants with ants present; light bars = plants with ants absent). Not all plants surveyed in the experiment for membracids are presented in the figure because of insufficient replication on those plants ($n = 1$ or 2). The dashed line indicates the mean per cent nitrogen in *P. modesta* across plants. The MANOVA indicates that ants do not have an effect on per cent carbon. A univariate analysis supports this ($F_{1,92} = 3.0$, $P = 0.09$), although there is a statistically insignificant trend towards more carbon in the presence of ants. The strong effect of ants on size, and the correlation between length and carbon, suggests any effect on carbon is driven by the effect of the ants on size.

We characterised the effect of ant attendance on nitrogen and carbon concentration in membracids on *Chrysothamnus viscidiflorus*, in which previous work indicated the mutualism results in a reduction in nitrogen on an alternative host plant (Kay *et al.*, 2004). We found no effect of ant attendance on nitrogen or carbon concentration in *P. modesta* on *C. viscidiflorus*. However, we found a strong effect of individual plants on membracid stoichiometry (Fig. 3). Phloem feeders generally face a particularly severe imbalance between metabolic requirements and nutrient availability (Douglas, 2006). Ant recruitment and/or appeasement may thus further limit the available nutrients for tissue construction and growth because ant mutualism represents a direct transfer of often enriched nutrients (Yao & Akimoto, 2002). However, the contrast between the two host plant-associated populations (this study vs. Kay *et al.*, 2004) suggests that the metabolic consequences of ant mutualism on one host plant may not scale across host plant species or across geographically-isolated populations on the same host plants. In the study of Kay *et al.* (2004), the magnitude of the effect of ants on membracid nitrogen on *S. gigantea* was clearly large. Ant attendance resulted in a ∼14% reduction in nitrogen concentration, relative to those without ants. The effect was transmitted downward onto the plants themselves: leaves with ant-tended membracids exhibited a ∼34% reduction in nitrogen concentration relative to those without. If the effect of ants on membracid stoichiometry on *C. viscidiflorus* was of equal magnitude, it would have been readily detectable in this population.

Rather, either *F. obscuripes* has no effect on *P. modesta* stoichiometry on *C. viscidiflorus*, or *C. viscidiflorus* exerts an effect on membracid stoichiometry that renders similar effects by ants small or undetectable at the population level (i.e. across host plants). Previous work suggests strong bottom-up control by *C. viscidiflorus* on *P. modesta* (Billick & Tonkel, 2003). The plant may act to limit the abundance of membracids, thereby exerting a density-dependent reduction in the positive, protective benefits of ants. Likewise, individual *Chrysothamnus* may vary in the availability of consumable nitrogen, both within and between plants (Drenovsky & Richards, 2006). The availability of nitrogen and phosphorus in the soil in the semi-arid environments that *Chrysothamnus* favours can exhibit pronounced spatial and temporal fluxes, even within a few metres and over a growing season (Jackson & Caldwell, 1989; James & Richards, 2006). Thus, to *P. modesta*, individual *C. viscidiflorus* may present strongly unequal dietary resources; in any population on this host, mosaic patterns of plant quality may exist that do not overlap with patterns of ant abundance.

In accord with the hypothesis that *C. viscidiflorus* varies substantially in dietary quality, we found an effect of both host plants and ant exclusion on the size of nymphs. In both the pilot study of 2005 and in 2006, untended treatments were composed of nymphs of smaller mass (Fig. 1). Herbivore survival rates tend to be higher in the presence of ants (Baylis & Pierce, 1991; Cushman & Whitham, 1991; Del Claro & Oliveira, 2000; Billick & Tonkel, 2003; Morales & Beal, 2006), and by extension, membracids are probably short lived in the absence of ants. The small size overall of the membracids may account for the

© 2008 The Authors

approximately 10% higher nitrogen content than that reported by Kay *et al.* (2004). In both 2005 and 2006, untended treatments were composed of nymphs of smaller mass than tended treatments. Kay *et al.* (2004) found no effect of ant exclusion on membracid mass. However, if nitrogen concentration varies allometrically with body size in insects (Fagan *et al.*, 2002), then habitat or seasonally-dependent variation in the effects of mutualisms on body size could be one mechanism that directly mediates herbivore stoichiometry. However, it remains to be determined how the *P. modesta/F. obscuripes* mutualism varies across *S. gigantea* genets. *Solidago gigantea* favours mesic habitats (Abrahamson *et al.*, 2005), and given its habit of clonal propagation, may present relatively large patches of uniformly high-quality plants (Hartnett & Bazzaz, 1983; Saitoh *et al.*, 2006). If so, a hypothesis for the top-down effects of ants on *S. gigantea* is that, on host plants of relatively high quality, the effects are manifested in a density-dependent manner, where constraints on aggregation sizes are reduced and ant recruitment is facilitated (Kay *et al.*, 2004). On *C. viscidiflorus*, by contrast, host and oviposition choice may be at a premium. Reithel (2003) and Reithel and Billick (2006) showed that, on various alternative host plants in Colorado, performance is consistently unequal and hosts differ strongly in quality. In these populations, and unlike those on *S. gigantea*, the spatial distribution of membracids may be driven largely by membracid host choices, and ultimately by host plant quality.

In sum, the generalist herbivore *P. modesta* experiences a top-down effect of ant mutualism on one host plant, *S. gigantea* (Kay *et al.*, 2004), but not on another. The effects of mutualism on herbivore stoichiometry therefore exhibit local patterns and heterogeneity across host plants, likely depending on the diet breadth of the herbivore, the degree of variation in host plant quality, and patterns of dispersal within and between host plant species (Helms & Hunter, 2005; Kagata & Ohgushi, 2006a). There are two additional implications. The first relates to the potential insights ecological stoichiometry offers the study of mutualisms, particularly ant-herbivore mutualisms. Physiological approaches may be particularly useful for identifying the metabolic currencies that form the basis for ecological variation in many types of co-evolved interactions (Ollerton, 2006). For example, in *nutrition-for-service* mutualisms, like those between ants and various exudate-producing herbivorous insects, the production and transfer of nutritious materials can have negative effects on fitness-related traits such as development and fecundity, although the net effect of the interaction is positive (Stadler & Dixon, 1998; Stadler *et al.*, 2002; Morales & Beal, 2006). However, the metabolic basis of these effects for either herbivore donor or ant consumers are only beginning to be understood (Pierce *et al.*, 2002; Cook & Davidson, 2006). As described here, equally little is known about the spatial or ecological variation in the relative effects of top-down and bottom-up forces on mutualisms. Stoichiometric approaches have great potential in this regard because of what they reveal about the effects of interactions on limiting elements, and the relative ease with which they can provide a standard measure of the metabolic consequences of interactions on plants at multiple scales (Kay *et al.*, 2005; Gripenberg & Roslin, 2007). The second relates to the role that ant-hemipteran mutualisms are thought to play in structuring the larger communities in which they occur. Styrsky & Eubanks (2007) argue that ant-hemipteran mutualisms may function as *keystone interactions* in arthropod communities because of the effect that hemipterans can have on their host plants, and the effects that ants have on numerous predators and parasitoids. If ant-hemipteran mutualisms exhibit strong spatial heterogeneity in the relative importance of top-down and bottom-up control, then the degree to which these mutualisms influence arthropod community structure may depend upon the scale across which these mutualisms vary.

Acknowledgements

We thank Adam Kay, Art Woods, James Grover, two anonymous reviewers, and the associate editor for helpful suggestions, and Tom Maddox at the University of Georgia and Ben Harlow at Washington State University for assistance with the elemental analysis.

References

Abrahamson, W.G., Dobley, K.B., Houseknecht, H.R. & Pecone, C.A. (2005) Ecological divergence among five co-occurring species of old-field goldenrods. *Plant Ecology*, **177**, 43–56.

Baylis, M. & Pierce, N.E. (1991) The effect of host-plant quality on the survival of larvae and oviposition by adults of an ant-tended lycaenid butterfly, *Jalmenus evagoras*. *Ecological Entomology*, **16**, 1–9.

Billick, I. & Tonkel, K. (2003) The relative importance of spatial vs. temporal variability in generating a conditional mutualism. *Ecology*, **84**, 289–295.

Bronstein, J.L., Alarcon, R. & Geber, M. (2006) The evolution of plant-insect mutualisms. *New Phytologist*, **172**, 412–428.

Brooker, R.W. & Callaghan, T.V. (1998) The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos*, **81**, 196–207.

Buckley, R.C. (1987) Interactions involving plants, Homoptera, and ants. *Annual Review of Ecology and Systematics*, **18**, 111–135.

Cook, S.C. & Davidson, D.W. (2006) Nutritional and functional biology of exudate-feeding ants. *Entomologia Experimentalis Et Applicata*, **118**, 1–10.

Cushman, J.H. (1991) Host-plant mediation of insect mutualisms – variable outcomes in herbivore-ant interactions. *Oikos*, **61**, 138–142.

Cushman, J.H. & Whitham, T.G. (1991) Competition mediating the outcome of a mutualism – protective services of ants as a limiting resource for membracids. *American Naturalist*, **138**, 851–865.

Del Claro, K. & Oliveira, P.S. (2000) Conditional outcomes in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. *Oecologia*, **124**, 156–165.

Denno, R.F., Gratton, C., Peterson, M.A., Langellotto, G.A., Finke, D.L. & Huberty, A.F. (2002) Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. *Ecology*, **83**, 1443–1458.

Denno, R.F., Lewis, D. & Gratton, C. (2005) Spatial variation in the relative strength of top-down and bottom-up forces: causes and consequences for phytophagous insect populations. *Annales Zoologici Fennici*, **42**, 295–311.

Douglas, A.E. (1993) The nutritional quality of phloem sap utilized by natural aphid populations. *Ecological Entomology*, **18**, 31–38.

© 2008 The Authors

Douglas, A.E. (2006) Phloem sap feeding by animals: problems and solutions. *Journal of Experimental Botany*, **57**, 744–754.

Drenovsky, R.E. & Richards, J.H. (2006) Low leaf N and P resorption contributes to nutrient limitation in two desert shrubs. *Plant Ecology*, **183**, 305–314.

Elser, J.J. & Hamilton, A. (2007) Stoichiometry and the new biology: the future is now. *PLOS Biology*, **5**, e181. doi: 10.1371/journal.pbio.0050181.

Fagan, W.F., Siemann, E., Mitter, C., Denno, R.F., Huberty, A.F., Woods, H.A. *et al*. (2002) Nitrogen in insects: implications for trophic complexity and species diversification. *American Naturalist*, **160**, 784–802.

Fischer, M.K., Volkl, W. & Hoffmann, K.H. (2005) Honeydew production and honeydew sugar composition of polyphagous black bean aphid, Aphis fabae (Hemiptera: Aphididae) on various host plants and implications for ant-attendance. *European Journal of Entomology*, **102**, 155–160.

Gove, A.D. & Rico-Gray, V. (2006) What determines conditionality in ant-Hemiptera interactions? Hemiptera habitat preference and the role of local ant activity. *Ecological Entomology*, **31**, 568–574.

Gripenberg, S. & Roslin, T. (2007) Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos*, **116**, 181–188.

Hartnett, D.C. & Bazzaz, F.A. (1983) Physiological integration among intraclonal ramets in *Solidago canadensis*. *Ecology*, **64**, 779–788.

Helms, S.E. & Hunter, M.D. (2005) Variation in plant quality and the population dynamics of herbivores: there is nothing average about aphids. *Oecologia*, **145**, 197–204.

Hewitt, J.E., Thrush, S.F., Dayton, P.K. & Bonsdorff, E. (2007) The effect of spatial and temporal heterogeneity on the design and analysis of empirical studies of scale-dependent systems. *American Naturalist*, **169**, 398–408.

Hunter, M.D. & Price, P.W. (1992) Playing chutes and ladders - heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology*, **73**, 724–732.

Inouye, B.D. (2005) Scaling up from local competition to regional coexistence across two scales of spatial heterogeneity: insect larvae in the fruits of *Apeiba membranacea*. *Oecologia*, **145**, 188–196.

Jackson, R.B. & Caldwell, M.M. (1989) The timing and degree of root proliferation in fertile-soil microsites for 3 cold-desert perennials. *Oecologia*, **81**, 149–153.

James, J.J. & Richards, J.H. (2006) Plant nitrogen capture in pulse-driven systems: interactions between root responses and soil processes. *Journal of Ecology*, **94**, 765–777.

Kagata, H. & Ohgushi, T. (2006a) Bottom-up trophic cascades and material transfer in terrestrial food webs. *Ecological Research*, **21**, 26–34.

Kagata, H. & Ohgushi, T. (2006b) Nitrogen homeostasis in a willow leaf beetle, *Plagiodera versicolora*, is independent of host plant quality. *Entomologia Experimentalis Et Applicata*, **118**, 105–110.

Kay, A.D., Ashton, I.W., Gorokhova, E., Kerkhoff, A.J., Liess, A. & Litchman, E. (2005) Toward a stoichiometric framework for evolutionary biology. *Oikos*, **109**, 6–17.

Kay, A.D., Scott, S.E., Schade, J.D. & Hobbie, S.E. (2004) Stoichiometric relations in an ant-treehopper mutualism. *Ecology Letters*, **7**, 1024–1028.

Kersch, M.F. & Fonseca, C.R. (2005) Abiotic factors and the conditional outcome of an ant-plant mutualism. *Ecology*, **86**, 2117–2126.

Kopp, D.D. & Yonke, T.R. (1973) The treehoppers of Missouri. Part 2. Subfamily Smilinae; Tribes Acutalini, Ceresini, and Polyglyptini (Homoptera: Membracidae). *Journal of the Kansas Entomological Society*, **46**, 233–277.

Morales, M.A. & Beal, A.L.H. (2006) Effects of host plant quality and ant tending for treehopper *Publilia concava*. *Annals of the Entomological Society of America*, **99**, 545–552.

Parker, M.A. (1999) Mutualism in metapopulations of legumes and rhizobia. *American Naturalist*, **153**, S48–S60.

Pierce, N.E. (1985) Lycaenid butterflies and ants - selection for nitrogen-fixing and other protein-rich food plants. *American Naturalist*, **125**, 888–895.

Pierce, N.E., Braby, M.F., Heath, A., Lohman, D.J., Mathew, J., Rand, D.B. *et al*. (2002) The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology*, **47**, 733–771.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. (1980) Interactions among 3 trophic levels - influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41–65.

Ollerton, J. (2006) "Biological barter": patterns of specialization compared across different mutualisms. *Plant-pollinator Interactions: From Specialization to Generalization* (ed. by N. M. Waser and J. Ollerton), pp. 411–435. University of Chicago Press, Chicago, Illinois.

Reithel, J.S. (2003) *The ecology and evolution of host plant use by the generalist membracid*, Publilia modesta. Dissertation, University of California, Irvine, California.

Reithel, J.S. & Billick, I. (2006) Bottom-up mediation of an ant-membracid mutualism: effects from different host plants. *Evolutionary Ecology*, **20**, 27–38.

Rosenheim, J.A. (1998) Higher-order predators and the regulation of insect herbivore populations. *Annual Review of Entomology*, **43**, 421–447.

Saitoh, T., Seiwa, K. & Nishiwaki, A. (2006) Effects of resource heterogeneity on nitrogen translocation within clonal fragments of *Sasa palmata*: an isotopic (N-15) assessment. *Annals of Botany*, **98**, 657–663.

Scheiner, S.M. (1993) MANOVA: multiple response variables and multiple species interactions. *Design and Analysis of Ecology Experiments* (ed. by S. M. Scheiner and J. Gurevitch), pp. 94–112. Chapman and Hall, New York.

Stadler, B. (2004) Wedged between bottom-up and top-down processes: aphids on tansy. *Ecological Entomology*, **29**, 106–116.

Stadler, B. & Dixon, A.F.G. (1998) Costs of ant attendance for aphids. *Journal of Animal Ecology*, **67**, 454–459.

Stadler, B., Dixon, A.F.G. & Kindlmann, P. (2002) Relative fitness of aphids: effects of plant quality and ants. *Ecology Letters*, **5**, 216–222.

Styrsky, J.D. & Eubanks, M.D. (2007) Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **274**, 151–164.

Thompson, J.N. (2005) *The Geographic Mosaic of Coevolution*. University of Chicago Press, Chicago, Illinois.

Underwood, N. (2004) Variance and skew of the distribution of plant quality influence herbivore population dynamics. *Ecology*, **85**, 686–693.

Wilson, W.G., Morris, W.F. & Bronstein, J.L. (2003) Coexistence of mutualists and exploiters on spatial landscapes. *Ecological Monographs*, **73**, 397–413.

Yamamura, N., Higashi, M., Behera, N. & Wakano, J.Y. (2004) Evolution of mutualism through spatial effects. *Journal of Theoretical Biology*, **226**, 421–428.

Yao, I. & Akimoto, S.I. (2002) Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. *Ecological Entomology*, **27**, 745–752.

Yu, D.W., Wilson, H.B. & Pierce, N.E. (2001) An empirical model of species coexistence in a spatially structured environment. *Ecology*, **82**, 1761–1771.

Accepted 19 December 2007

© 2008 The Authors
Journal compilation © 2008 The Royal Entomological Society,