TEMPORAL AND SPATIAL VARIATION TO ANT OMNIVORY IN PINE FORESTS

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Abstract. To understand omnivore function in food webs, we must know the contributions of resources from different trophic levels and how resource use changes through space and time. We investigated the spatial and temporal dynamics of pine (*Pinus ponderosa*) food webs that included the omnivorous ant, *Formica podzolica*, using direct observation and stable isotopes. *Formica podzolica* is a predator of herbivorous and predatory arthropods, and a mutualist with some aphids. Observations in 2001 of foragers showed that in early summer (June) ants fed upon equal parts non-mutualist herbivores (31% prey biomass), mutualist aphids (27%), and predators (42%); ant trophic position was thus between that of primary and secondary predator (trophic level = 3.4). In late summer (September), ant feeding remained relatively constant upon non-mutualist herbivores (53%) and mutualist aphids (43%), but ant feeding upon predators fell (4%), thus shifting ant trophic position to that of a primary predator (trophic level = 3.0). Feeding on honeydew increased from 25% of ants in early summer to 55% in late summer. By increasing the frequency of their interactions with mutualist aphids, ants maintained a constant supply of arthropod prey through the summer, despite a two–thirds decline in arthropod biomass in pine canopies. Stable isotope analysis ($\delta^{15}N$, $\delta^{13}C$) of six pine food webs dispersed over 150 ha placed ant trophic level at 3.3 for early summer in 2002. There was significant variation among these trees in ant trophic position (range 3.2–3.6), but no indication of positive spatial autocorrelation. The combined results from this work shows that, across two years, *F. podzolica* fed, on average, at or slightly above the trophic position of primary predator, but trophic positioning varied both temporally and spatially by ~0.4 trophic levels.

Key words: aphid tending; canopy; Cinara; Essigella; *food web;* Formica podzolica; *intraguild predation; mutualism; omnivory;* Schizolachnus; *spatial autocorrelation; stable isotope.*

INTRODUCTION

Classic food web models (e.g., Hairston et al. 1960, Oksanen et al. 1981) assumed static systems of organisms that fit neatly into tiered trophic levels. The accumulating evidence that many species feed from multiple trophic levels (Polis et al. 1989, Rosenheim 1998) is posing an increasing challenge to these models. There is also a growing understanding that food web structure can vary through both space and time (Polis and Strong 1996, Polis et al. 1997). Synthesizing these emerging complexities into a revised body of theory is a work in progress. The success of this venture will be determined, in part, on the availability of empirical data documenting omnivore function in food webs, and the extent to which omnivore function varies temporally and spatially within a community.

Ants forage for arthropods on plants throughout the world, and classic food web models have predicted that ants play an important role in protecting plants from herbivores (Huxley and Cutler 1991). While many ants are indeed effective predators of herbivores (Skinner and Whittaker 1981, Warrington and Whittaker 1985*a*, *b*, Halaj et al. 1997), ants also frequently protect homopteran herbivores from their arthropod predators in return for carbohydrate-rich exudates (Way 1963, Buckley 1987, Dixon 1998). Thus, ants can simultaneously exhibit multiple trophic roles as primary predators (via feeding upon herbivores), secondary predators (via feeding on predatory arthropods), and as facilitators of mutualist herbivores.

Disentangling such a reticulate network of trophic connections is a challenging task, but it is also essential given the ecological importance of homopteran-tending ants in many systems. Exclusion studies reveal the *net* effect of ants on plants, but only impractical factorial manipulations of ants with all other actors (i.e., other predators, mutualist herbivores, non-mutualist herbivores, etc.) can reveal the contribution of each trophic pathway between ants and plants. Mesocosms are more tractable, but the dynamics revealed under these artificial conditions do not exactly mirror those occurring in nature. Path analysis allows for the inference of causation from nonexperimental data (Wootton 1994,

PLATE 1. The ant *Formica podzolica* (Francour) tending a colony of *Cinara schwarzii* (Wilson) feeding on ponderosa pine (*Pinus ponderosa* Laws. *scopulorum*) at the Manitou Experimental Forest, Woodland Park, Colorado, USA. Photo credit: K. A. Mooney.

Shipley 1999), yet the usefulness of this correlative approach has also been questioned (Smith et al. 1997).

We characterized the interactions between the ant *Formica podzolica* Francour and the arthropod community in the ponderosa pine (*Pinus ponderosa* Laws. *scopulorum*) canopy (see Plate 1). Past work has shown temperate *Formica* spp. ants to have profound effects on local arthropod communities (Fowler and Macgarvin 1985, Whittaker and Warrington 1985, Heads 1986, Ito and Higashi 1991, Sloggett and Majerus 2000) and plant fitness (Whittaker and Warrington 1985, Ito and Higashi 1991). *Formica* often occupy multiple trophic roles by feeding upon homopteran exudates, herbivores, and predators (Horstmann 1972, Skinner and Whittaker 1981, Warrington and Whittaker 1985, Ito and Higashi 1991). Furthermore, the balance of ant effects among these trophic roles varies as a function of multiple ecological factors including prey abundance, susceptibility of prey to ants, quality and quantity of homopteran exudates, and ant colony nutritional needs (Horstmann 1970, 1972, 1974, Rosengren and Sundstrom 1991, Portha et al. 2002, Blüthgen and Fiedler 2004). To the extent that the ecological factors affecting ant diet change over landscapes or seasonally, we can expect concomitant spatial and temporal variability in ant trophic position.

In the present study, we characterized the trophic roles of *F. podzolica* in the pine canopy food web. In addition to comparing the "average" trophic role of this ant to previously studied *Formica*, we also quan-titatively assessed the temporal (seasonal) and spatial (landscape scale) variability of *F. podzolica* trophic positioning. By directly observing ant foragers, we measured the contributions of honeydew, herbivores, and predators to the ant's diet, and how ant use of these various resources changed over the course of a single summer (2001). We then used stable-isotope analysis to evaluate how, at one point in time, pine canopy food web structure varied over a forested landscape (2002). By calculating precise trophic positions for replicate ant colonies through time and space, we compared the spatial and temporal variation in ant trophic position. Finally, we discuss the implications of our findings to the development of stable isotopes as an emerging methodology for the study of arthropod food webs.

METHODS

Field site and study system

We worked at the U.S. Department of Agriculture Forest Service Manitou Experimental Forest. Our sites were within mature stands of ponderosa pines at an elevation of ~2400 m (39°06′02″ N, 105°05′32″ W). This site receives 40 ± 21 cm of precipitation a year, with 19 ± 1 cm coming in the form of rain between June and September. We worked in a valley bottom of flat topography and coarse granitic soils. The trees were 10–50 cm in diameter at a height of 1.4 m, with partial canopy closure and frequent larger canopy gaps.

Formica podzolica builds gravel mounds in the forest understory and forages in pine canopies. The pine

arthropod community at Manitou includes at least 250 arthropod species (K. A. Mooney, *unpublished data*). *Formica podzolica* is a predator of most arthropods, but engages in a mutualism with several aphid species (Aphididae: Homoptera) in which it receives carbohydrate-rich honeydew, while protecting aphids from a number of specialist aphid predators (e.g., coccinellid beetles and neuropteran larvae) and parasitism (e.g., braconid wasps). Seibert (1992) and Bishop and Bristow (2001) described similar mutualist relationships between *Formica* species ants and aphids.

We divided arthropods into the following categories: (1) ant-tended aphids (*Cinara schwarzii* Wilson and *C. arizonica* Wilson [K. Mooney, *personal observation*]), (2) untended aphids (*C. solitaria* (Gillette & Palmer), *C. glabra* (Gillette & Palmer), *Essigella fusca* (Gillette & Palmer) [K. Mooney, *personal observation*], and *Schizolachnus piniradiatae* (Davidson) [Bishop and Bristow 2001; K. Mooney, *personal observation*]), (3) specialist aphid predators (larval and adult Coccinellidae beetles, larval Neuroptera [Dixon 1998], and five species of Miridae (Hemiptera) from three genera (*Daerocoris*, *Pilophoris*, *Phytochoris*) [Wheeler 2001]), (4) hunting spiders (Araneae; 15 species from five families), (5) hoppers (Homoptera, suborder Auchenorrhyncha; 36 species, none tended by ants [K. Mooney, *personal observation*]), (6) caterpillars (larval Lepidoptera; approximately five species from an unknown number of families), (7) thrips (Thysanoptera; one species), springtails (Collembola; one species) and barklice (Psocoptera; eight species), (8) herbivorous adult beetle (Coleoptera excluding Coccinellidae; 102 species), and (9) mites (Acari; at least three species).

Observation of ant diet

On 5 June 2001, we selected five ant mounds that were at the bases of pines ranging in trunk diameter from 30 cm to 50 cm and distributed over a 0.2-ha area. We observed colonies during the first seven days of June, July, August, and September during times of peak ant activity after 09:00 and before 14:00 on clear, warm days. Collection of all data (see next paragraph) required two to three hours of observation for each mound at each observation time.

At each observation time we observed 200 ants returning from the canopy and collected those carrying prey items in their mandibles. To compare the composition of ant prey with composition of the canopy arthropod community, we collected canopy arthropods from individual branches on a separate set of five pine trees during the same periods during which ant diet was measured. These trees were each associated with *F. podzolica* mounds and distributed over a 2-ha area surrounding the focal trees on which we observed ant foraging. We dislodged arthropods by repeatedly beating branches with a padded bat, and collected them from 1.5×1.5 m tub-design (0.5 m deep) beat sheets suspended around each branch (Appendix A). While highly agile fliers (adult flies [Diptera], bees and wasps [Hymenoptera]) were not adequately sampled, few arthropods from other groups evaded our collection; in total we estimate our collection technique captured over 97% of the less-mobile canopy arthropods (Appendix A), an estimate similar to others using beat sheeting to collect arthropods from tree canopies (e.g., Punttila et al. 2004). This set of arthropod collection trees was not completely interspersed with ant prey trees. Arthropod communities could potentially differ between the two sets of trees due to spatial variation in canopy arthropod composition. However, we do not believe this is the case because both sets of trees were within a relatively small area (2 ha) of uniform soil, topography, forest structure, and understory vegetation.

We measured the length of each ant prey item and canopy-collected arthropods to the nearest millimeter, and calculated arthropod dry biomass using a published length–dry biomass relationship (Rogers et al. 1976). At the conclusion of the experiment, we cut and weighed each branch and adjusted canopy biomass data to a per-kilogram pine branch scale. We calculated ant trophic position based on the proportion of herbivore and predator biomass captured by each colony at each month (i.e., 100% herbivore prey = primary predators = trophic level of 3.0, 100% predator prey = secondary predators = trophic level of 4.0).

In addition to collecting ant prey, we also measured the proportion of ants returning with honeydew in their crops to estimate (1) the contribution of aphid secretions to the ant's diet, and (2) aphid-tending activity by the ants. We randomly collected 30 foragers without prey, chilled them in a $-15°C$ freezer to the point of inactivity, compressed their abdomens with forceps, and observed whether droplets of honeydew were produced at each ant's mouth. In experimental trials, all ants that fed for 30 seconds or more on sugar water ($N = 20$) produced droplets of liquid when compressed, while unfed ants ($N = 20$) did not produce liquid. Prey hemolymph was the only other potential source of liquid available to ants in pine canopies besides honeydew. We only tested for crop liquid in ants returning without prey tissue in their mandibles, and it is unlikely that ants collected hemolymph from prey, but did not also return with prey tissue. In addition, the composition of crop liquid contained no detectable nitrogen (see *Results*); while the concentration of nitrogen in honeydew was likely below detectable limits, the much higher concentration of nitrogen in hemolymph (Jones 1977) would almost certainly have been detectable. For these reasons, we believe that most, if not all, of the liquid we observed from this methodology was honeydew. For each mound, at each month, we calculated the relationship between ant tending and predation on mutualist aphids (ants with honeydew per milligram of tended aphid captured as prey).

We tested whether there were significant effects of time (month) on (1) the percentage of ants with honeydew, (2) the percentage of ants with arthropod prey, (3) ant trophic position (range 3.0–4.0), (4) arthropod biomass in pine canopies (milligrams of arthropods per kilograms of branch), (5) the ratio of ant tending to predation of mutualist aphids (ants with honeydew per milligram tended aphid captured as prey), and ant capture (milligrams of prey per 100 returning ants) of (6) tended aphids, (7) other herbivore and detritivore arthropods, (8) predatory arthropods, and (9) total ant prey. In each analysis, we initially included ant mound as a fixed effect to control for the nonindependence of data among months, but then dropped mound for those tests in which there was no significant mound effect. For those variables where there were significant time effects, we performed post hoc tests for differences among months using Duncan's new multiple range test (Zar 1999).

To test whether ants preyed upon arthropods in proportion to their abundance in pine canopies, we calculated expected frequencies of ant prey in each arthropod group, and used a chi-square analysis to test whether observed prey composition deviated significantly from expected values. We only used those prey categories where the expected frequencies were >1.0 per the recommendations of Zar (1999) for tests of more than two categories.

Stable isotope analysis

In early August 2002, we selected six trees and associated *F. podzolica* mounds surrounding the area from which the observational data were collected in the preceding year. The polygon inscribing the trees was 150 ha, and the distances between the six trees (15 pairwise distances) ranged from 90 m to 3550 m with a mean (±1 SE) of 1570 ± 310 m. From each tree we collected five individuals each of *F. podzolica*, tended aphids, and specialist aphid predators. We clipped a pine needle from five separate branches on each tree and combined the tissues into a single sample. We collected honeydew by compressing the abdomens of ~20 returning foragers, and spreading any regurgitated liquid onto aluminum foil. We performed an experiment to determine if the honeydew chemistry was altered while in the ant crops; we allowed three ants to feed ad libitum on a 30% sugar solution, and then collected the crop contents to compare with the isotopic signature of the original sugar solution.

Stable isotope analysis measures the ratio of heavy to light isotopes of biologically relevant elements such as nitrogen and carbon. To calculate δ*X,* the heavy : light isotopic ratios of the samples are compared to an element-specific standard as follows: $\delta X = ((R_{smp}/R_{stn}) - 1) \times 1000$. R_{smp} and R_{stn} refer to the ratio of heavy to light isotopes of the sample and standard, respectively. The equation yields a ''per mil'' (‰) value; we report

per mil δ values for the $^{15}N/^{14}N$ ($\delta^{15}N$) and $^{13}C/^{12}C$ ($\delta^{13}C$) isotopic ratios in our analyses.

The $\delta^{15}N$ and $\delta^{13}C$ values of an animal's biomass reflect past diet and nutrient assimilation. We collected samples from 1 August to 3 August. If adult insects in our study have similar nitrogen turnover rates to adult insects studied elsewhere (Ostrom et al. 1997), then our analysis probably reflects food web structure in the previous July and perhaps late June (Ostrom et al. 1997). Having not performed controlled feeding trials, we do not know the precise feeding period reflected in early August ant signatures.

Nitrogen isotopes (^{15}N and ^{14}N) participate in physiological reactions at different rates and are thus fractionated. As a result, an animal's $\delta^{15}N$ typically is enriched by approximately +3‰ to +4‰ above that of its food resources (DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987). Values of $\delta^{13}C$ differ substantively between food webs based on C_3 and C_4 plants (DeNiro and Epstein 1978, Webb et al. 1998, Callaham et al. 2000), but show less trophic fractionation (approximately +0.5‰ to +1.0‰; Michener and Schell 1994). The relative contributions of an animal's dietary resources that differ in $\delta^{13}C$ and $\delta^{15}N$ can be inferred by comparing resource and consumer isotope signatures. Because $\delta^{15}N$ and (to a lesser extent) $\delta^{13}C$ values vary among trophic levels, they can discriminate the contributions of resources from differing trophic levels to omnivores (Blüthgen et al. 2003, Tillberg and Breed 2004).

We lyophilized, ground, and weighed the dried (60°C) samples into tin capsules; it was not necessary to lyophilize or grind the honeydew. Each insect constituted its own sample, providing $N = 30$ per group (aphids $N = 29$). We combined samples to generate a single sample each of honeydew and needle tissue per tree. Three of these honeydew samples were not usable due to processing errors ($N = 3$). The Stable Isotope Laboratory at the University of Georgia Institute of Ecology, Athens, Georgia, USA, performed the analyses using a Finnigan Delta Plus mass spectrometer (Finnigan, Waltham, Massachusetts, USA) coupled to an elemental analyzer (Costech, Valencia, California, USA) via the Finnigan Conflo III interface. The standard for carbon analysis was PeeDee Belemnite carbonate; atmospheric air was the standard for nitrogen analysis. In addition to $\delta^{13}C$ and $\delta^{15}N$ values, this analysis also measures the total percentage of carbon and nitrogen, and from these we calculated carbon to nitrogen ratios (C:N).

Because enrichment generates differences in both $\delta^{15}N$ and $\delta^{13}C$ among trophic levels, we first tested for differences among pine and arthropods in these two variables (i.e., different trophic positions) using the multivariate K nearest neighbor (KNN) randomization test (Rosing et al. 1998) with the S-PLUS programming language (Insightful Corporation 2003). We adjusted *P* values of post hoc pairwise comparisons using the se-

quential step-up Bonferroni method (Zar 1999). All other analyses were performed with SAS 8.1 (SAS Institute 2001). We followed the KNN randomization test with separate univariate tests for differences among arthropods, honeydew, and pine in $\delta^{13}C$, $\delta^{15}N$, and C:N. We used separate two-way ANOVAs with the pine tree from which the samples were collected as a block effect, and post hoc pairwise comparisons using Hochberg's GT2 method (SAS Institute 2001) because of unequal sample sizes. We also measured whether variability among the $\delta^{13}C$ and $\delta^{15}N$ signatures of each tree could be traced to honeydew and arthropods from that tree; we calculated separate Pearson product-moment correlation coefficients between (a) pine needles and (b) honeydew, aphids, predators, and ants. We performed these tests on the canopy mean for honeydew and arthropod groups (i.e., $N = 6$ per test).

To calculate a precise trophic position (i.e., primary predators = 3.0, secondary predators = 4.0) for ants, we used $\delta^{15}N$ alone because, as compared to $\delta^{13}C$, the greater variation in isotopic signatures among trophic levels provides more accurate estimates (Phillips and Koch 2002, Post 2002). We calculated trophic position separately for each tree using a modification of the single isotope, two-food source linear mixing model described by Post (2002). Post's model assumes that food resources from all tropic levels have the same nitrogen content, and that the contributions of resources to the isotopic signature of a focal animal are equal. Yet in most systems, the percentage nitrogen generally increases with increasing trophic level (Phillips and Koch 2002, Denno and Fagan 2003). We modified Post's equation (2002) to include variable nitrogen content in food resources. Our equation is based on the assumption that $\delta^{15}N$ of a focal animal is

$$\delta^{15}N_{fcl} = \frac{[\delta^{15}N_{src(1)} + \Delta_N]\rho_1 + [\delta^{15}N_{src(2)} + \Delta_N]\rho_1 N_{2:1}}{\rho_1 + (\rho_2 N_{2:1})} \quad (1)$$

where $\delta^{15}N_{src(1)}$ and $\delta^{15}N_{src(2)}$ are the signatures of food resources from trophic levels λ_1 and λ_2, $(\lambda_1 < \lambda_2)$, ρ_1 and ρ_2 are the proportions of the focal animal's diet coming from those two resources $(\rho_1 + \rho_2 = 1)$, $N_{2:1}$ is the ratio of the nitrogen content in the food resources from λ_2 and λ_1, respectively, and Δ_N is a system-specific standard for $\delta^{15}N$ enrichment with a trophic transfer. Substituting $\rho_1 - 1$ for ρ_2 and solving for ρ_1 in the above equation provides

$$\rho_1 = \{N_{2:1}[\delta^{15}N_{fcl} - \delta^{15}N_{src(2)} - \Delta_N]\}$$
$$\div \{N_{2:1}[\delta^{15}N_{fcl} - \delta^{15}N_{src(2)} - \Delta_N] + \delta^{15}N_{src(1)}$$
$$+ \Delta_N - \delta^{15}N_{fcl}\} \quad (2)$$

and the trophic position (TP) of the focal animal can be calculated with the following:

$$TP_{fcl} = \lambda_2 + 1 - (\lambda_2 - \lambda_1)\rho_1. \quad (3)$$

This approach is also analogous to that of Phillips and Koch (2002), which corrects for differences in elemental concentration in a three-source mixing model. In our calculations, we used the mean $\delta^{15}N$ fractionation between aphids and aphid specialist predators as Δ_N ($\Delta_N = 2.04$, see *Results*). Aphids ($\lambda_1 = 2$) and specialist aphid predators ($\lambda_2 = 3$) were the two food resources in question. $N_{2:1}$ (the ratio of percent nitrogen in specialist aphid predators to that of aphids) was 1.62 (see *Results*). The five ants, five aphids, and five specialist aphid predators from each tree provided five values each for $\delta^{15}N_{ant}$, $\delta^{15}N_{src(1)}$, and $\delta^{15}N_{src(2)}$ that in turn produced 125 separate estimates of TP_{ant} per tree. We used the average of these values to produce a point estimate of TP_{ant} for each tree, and their standard errors to calculate 95% confidence intervals.

We investigated whether there was positive spatial autocorrelation in TP_{ant}. The hypothesis being tested was that values for TP_{ant} would be more similar among closely situated trees than in distantly situated trees. The six experimental trees provided 15 pairs of trees upon which we based this analysis. From these tree pairs, we calculated the Pearson product-moment correlation coefficients between the tree pair distance and the absolute values of the difference in TP_{ant}. The 15 pairwise comparisons used to calculate the correlation coefficient were not independent, so we used a Mantel test to determine statistical significance based on 9999 randomized replications of our original data (Potvin and Roff 1993).

RESULTS

Observation of ant diet

We collected a total of 242 arthropod prey items from returning foragers. Canopy arthropod biomass varied significantly among months ($F_{3,12} = 8.34$, $P = 0.0029$), declining from a high in July of 45 ± 12 mg arthropod/ kg branch (mean $\pm$ 1 SE) to a low in September of 9 ± 3 mg/kg branch (Fig. 1). Despite this decline, there were no differences among months in ant foraging activity (overall mean = 2.2 ± 0.3 ants ascending and descending tree trunks per minute, $F_{3,12} = 1.50$, $P = 0.26$; Fig. 1), nor rate of ant prey capture (overall mean = 0.033 ± 0.004 mg prey per 100 returning ant foragers, $F_{3,16} = 2.11$, $P = 0.14$; Fig. 1), although the proportion of ants returning with prey was significantly higher in September than all other months ($F_{3,16} = 7.45$, $P = 0.0024$; Fig. 1).

The constitution of the ant's diet changed over the course of the summer (Figs. 1 and 2). From June to September, ant capture of predator biomass declined ($F_{3,16} = 4.02$, $P = 0.0262$), while honeydew consumption increased ($F_{3,16} = 4.63$, $P = 0.0162$). There was no detectable effect of month on ant capture of tended aphid biomass ($F_{3,16} = 0.45$, $P = 0.72$) or biomass of other herbivores ($F_{3,16} = 0.87$, $P = 0.48$). In August and September, the majority of biomass captured by
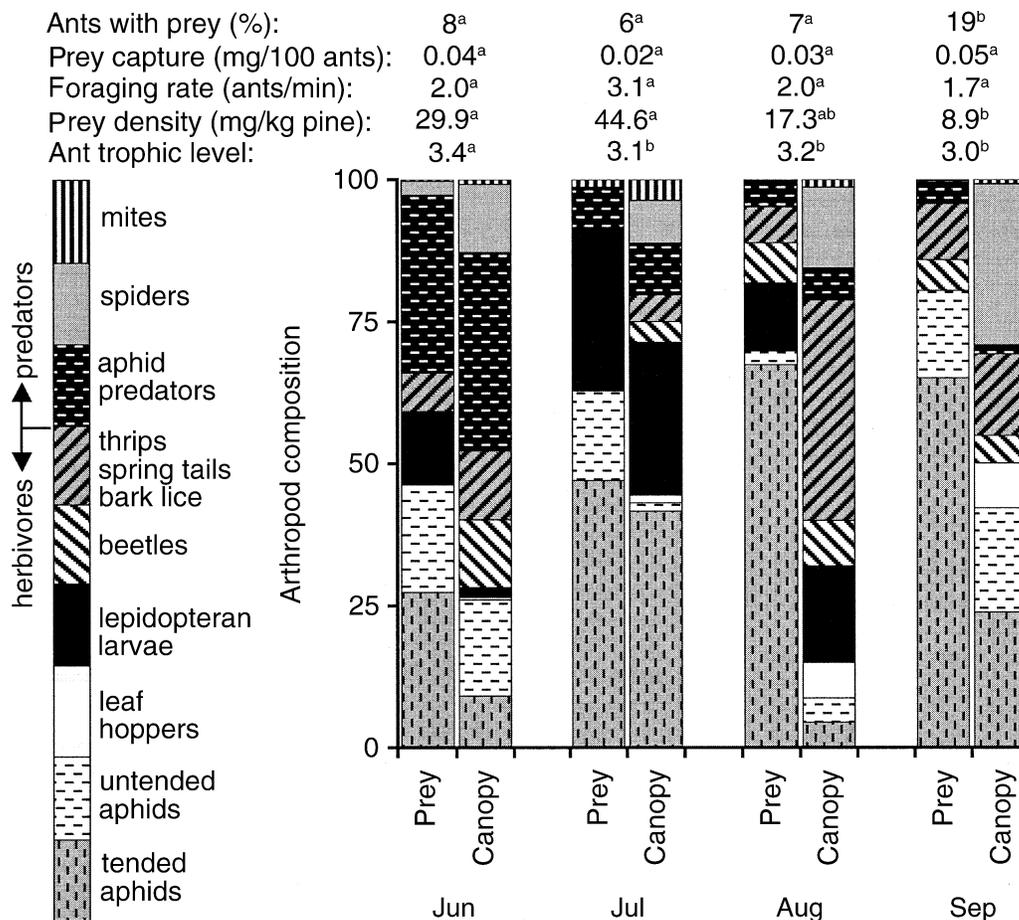
Ants with prey (%):	8[a]	6[a]	7[a]	19[b]
Prey capture (mg/100 ants):	0.04[a]	0.02[a]	0.03[a]	0.05[a]
Foraging rate (ants/min):	2.0[a]	3.1[a]	2.0[a]	1.7[a]
Prey density (mg/kg pine):	29.9[a]	44.6[a]	17.3[ab]	8.9[b]
Ant trophic level:	3.4[a]	3.1[b]	3.2[b]	3.0[b]

FIG. 1. Comparison of ant prey and canopy arthropod composition for 2001. "Prey" columns show composition of ant prey among nine arthropod groups. "Canopy" columns show the same groups for pine canopies. Prey and canopy composition differed in June, August, and September ($P < 0.05$), but not in July (see *Results; Observation of ant diet* for details). The percentage of ants with prey, rate of prey biomass captured, ant foraging activity, canopy arthropod density, and ant trophic level are shown above the columns for each month. Values for these variables not sharing superscript letters are significantly different ($\alpha = 0.05$) between months.

ants was tended aphids, despite the fact that they constituted <20% of canopy arthropod biomass during that time (Fig. 1). Tended aphids were not being moved between trees by ants; we never saw an ant ascending a tree with an aphid, and most aphids were crushed within the ants' mandibles. Despite these temporal changes in the intensity of ant–aphid interactions (i.e., increased tending in late summer), the ratio of ant tending : predation of mutualist aphids (honeydew-carrying ants per mg tended aphids captured as prey) was relatively constant among months (June = 37 ± 13, July = 31 ± 12, August = 42 ± 7, September = 28 ± 8) and did not differ significantly ($F_{3,16} = 0.42, P = 0.74$).

The constitution of prey biomass captured by ants differed significantly from that of the canopy arthropod community in June, August, and September (June χ^2 = 17, df = 5, $P = 0.0033$; August χ^2 = 222, df = 7, $P < 0.0001$; September χ^2 = 14.8, df = 4, $P = 0.0051$), and the majority of each total χ^2 value was due to

selective ant predation on tended aphids, with tended-aphid biomass contributing 62%, 94%, and 65% of the total χ^2 values, respectively. Ant prey did not differ significantly from the canopy community in July (χ^2 = 5.3, df = 6, $P = 0.50$). Ant trophic position differed significantly among months ($F_{3,16} = 4.37, P = 0.0198$); predators constituted 42% ± 11% of ant prey in June (trophic level = 3.42), 7% ± 2.5% in July (trophic level = 3.07), 15% ± 10% in August (trophic level = 3.15), and 4% ± 4% in September (trophic level = 3.04), and post hoc tests showed ant trophic position in June differed significantly from July, August, and September, but that the latter three months did not differ from each other (Fig. 1).

Stable isotope analysis

The multivariate test showed significant differences between pine, aphids, specialist aphid predators, and ants for $\delta^{13}C$ and $\delta^{15}N$ (KNN randomization test, $P <$
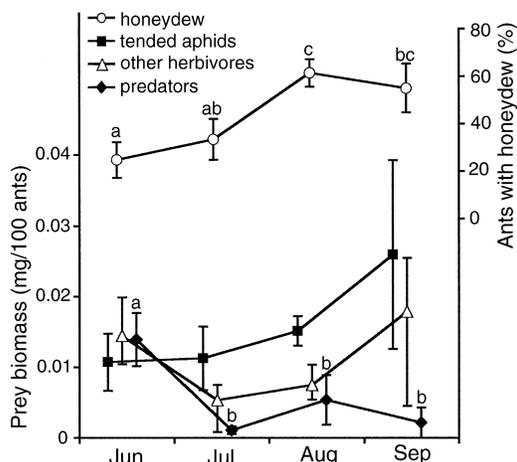
FIG. 2. Mean (±1 SE) composition of ant prey (bottom three data series, left-hand *y*-axis) and honeydew collection (top series, right-hand *y*-axis) for summer 2001. Honeydew and predator consumption differed among months ($P < 0.05$), and monthly means for those variables not sharing letters differed significantly ($\alpha = 0.05$). Consumption of tended aphids and other herbivores did not differ among months ($P > 0.05$). See *Results; Observation of ant diet* for detailed statistics.

0.05), and all post hoc pairwise comparisons were significant ($P < 0.05$). Honeydew did not contain detectable amounts of nitrogen and was omitted from this analysis. Because this multivariate test was significant, we did not adjust α for multiple comparisons in subsequent univariate tests for $\delta^{13}C$ and $\delta^{15}N$ (Johnson 1998).

The omnibus result for the univariate test of $\delta^{15}N$ showed highly significant differences between pine needles, aphids, specialist aphid predators, and ants ($F_{3,86} = 156$, $P < 0.0001$), and post hoc comparisons found significant differences between all pairwise comparisons (Fig. 3). There was no detectable nitrogen in our honeydew samples, but this is likely due to the mass of our samples (1.1–1.6 μg dried) not being sufficient for nitrogen detection by the equipment used in stable isotope analysis; if the C:N of phloem sap and honeydew were equal to pine needles, our samples were approximately half the size needed to provide detectable levels of nitrogen. Aphids were enriched by 1.067‰ over pine needles, and specialist aphid predators and ants by 2.042‰ and 3.105‰, respectively, compared to aphids. (We used the enrichment between aphids and specialist aphid predators [2.042‰] as Δ_N in ant trophic level calculations; see *Methods*.) The needle $\delta^{15}N$ of each tree was highly correlated with the $\delta^{15}N$ for aphids ($r = 0.93$, $P = 0.008$), aphid predators ($r = 0.94$, $P = 0.006$), and ants ($r = 0.88$, $P = 0.022$).

The omnibus result for the univariate test of $\delta^{13}C$ showed highly significant differences between pine needles, honeydew, aphids, aphid predators, and ants ($F_{2,81} = 37$, $P < 0.0001$), and post hoc comparisons found significant differences for all pairwise compar-

isons except between ants and aphid predators (Fig. 3). Aphids were enriched by 2.816‰ compared to pine needles, aphid predators and ants were enriched by 1.107‰ and 0.987‰, respectively, compared to aphids. Collecting honeydew from ant crops did not affect $\delta^{13}C$; the $\delta^{13}C$ for the sugar solution fed to ants was −23.60‰, and the mean $\delta^{13}C$ for the crop contents of ants fed on that solution was −23.49‰ ± 0.11‰ ($t_2 = 1.12$, $P = 0.38$). Despite the small sample size for this test, a power analysis for minimal detectable difference showed that a difference in $\delta^{13}C$ of 0.5‰ or greater would have been detected with 95% probability (Zar 1999). There were no significant correlations between the $\delta^{13}C$ of each tree's needle tissue and the $\delta^{13}C$ of the honeydew, aphids, aphid predators, and ants collected from that tree.

The omnibus result for differences in carbon to nitrogen ratio (C:N) among needles, aphids, aphid predators, and ants was significant ($F_{3,71} = 1116$, $P < 0.0001$). Post hoc comparisons showed differences for all pairwise comparisons except between ants and aphid predators (Fig. 3). The mean nitrogen content was 6.16% ± 0.14% in aphids and 10.0% ± 0.20% in predators. (These results produced the value of $N_{2:1} = 1.62$ used in ant trophic level calculations; see *Methods*.)

Estimated ant trophic position among the six replicate food webs ranged from a low of 3.17 (95% confidence interval = 3.15–3.20) to a high of 3.56 (95% confidence interval = 3.50–3.62), with an overall mean of 3.35 ($N = 6$, 95% confidence interval = 3.20–3.50) and differed significantly among trees (Appendix B). The test for spatial autocorrelation was not significant ($r = 0.08$, Mantel test $P = 0.38$), suggesting that spatial
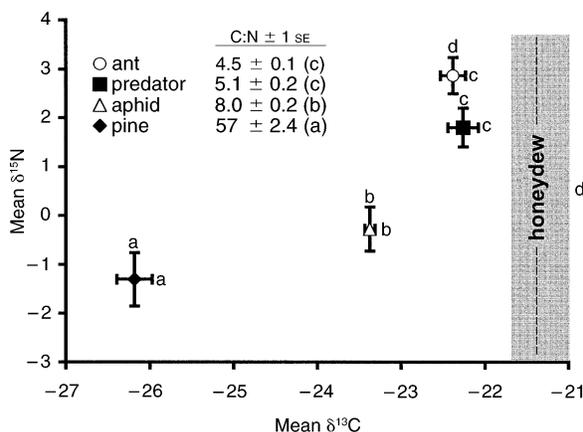

FIG. 3. Mean (±1 SE) $\delta^{13}C$ and $\delta^{15}N$ and C:N ratios for pine, honeydew, and insects. Nitrogen was below measurable levels for honeydew; the dashed line shows mean $\delta^{13}C$ and the shaded bar shows ±1 SE. The key includes C:N ratios (mean ± 1 SE). Different letters to the right of stable isotope means ($\delta^{13}C$), above the stable isotope means ($\delta^{15}N$), and next to the C:N values in the key denote significant differences ($P < 0.05$).

patterning in ecological processes was not responsible for variation in food web structure.

DISCUSSION

Food web structure through time

Our observation of *F. podzolica* diet (2001) provides a picture of temporally shifting consumer–resource dynamics from early to late summer. In June, predacious arthropods constituted 42% of ant prey and 25% of returning ants carried honeydew. By September, predators constituted only 4% of ant prey, and honeydew consumption increased to 55%. At the same time, there was no detectable change in ant consumption of mutualist aphids and non-mutualist herbivores. As a result of these diet changes, ant trophic position fell from 3.4 in June to 3.0 in September. Between years, ant trophic position appeared to be relatively constant; stable isotope ($\delta^{15}N$) analysis in 2002, which reflected ant feeding during June and July (exact period unknown), placed ants at a trophic position of 3.4. Thus, our study adds to past work showing temporally variable feeding in *Formica* spp. ants (e.g., Horstmann 1970, 1972, 1974, Skinner 1980, Rosengren and Sundstrom 1991), although there is as yet no consistent pattern to the precise nature of seasonal diet shifts in *Formica*.

Honeydew was an important component of the ant diet; averaged across the entire summer, only 10% of ants returned from pine canopies with arthropod prey, while 44% returned with detectable amounts of honeydew in their crops. While we did not measure nitrogen in honeydew, relatively low levels may have been present, but were simply undetectable with the equipment used for stable isotope analysis. Despite the fact that the $\delta^{13}C$ in honeydew sugars was highly enriched compared to other ant resources (Fig. 3), ant $\delta^{13}C$ was indistinguishable from predatory arthropods. Adult foragers, having completed their growth, may have metabolized honeydew sugars to fuel their foraging efforts, and consequently the honeydew-derived carbon was not incorporated into ant tissues.

Much of the *F. podzolica* foraging ecology in the late summer was apparently driven by its strong interactions with mutualist aphids. Averaged across August and September, tended aphids constituted only 10% of the arthropod community but 50% of ant prey (Fig. 1), and 60% of all returning ants carried honeydew (Fig. 2). While the outcome of interactions between ants and tended aphids (i.e., balance between tending and predation) in other systems has been shown to be contingent upon factors including the abundance of aphid enemies and other ant food resources (Cushman and Whitham 1989, Cushman and Addicott 1991, Breton and Addicott 1992, Sakata 1994), we did not find this to be the case; despite sharp declines in the abundance of aphid enemies and ant prey (Fig. 1), and a doubling of the frequency of ant–mutualist aphid interactions (Fig. 2), the balance between ant tending and predation of mutualists remained constant through time.

The increased dependence of ants on mutualist aphids may have occurred as a response to declining prey abundance; between June and September, the arthropod biomass in pine canopies fell by over two-thirds (Fig. 1). At the same time, ant foraging intensity (ant activity per minute) and prey capture (milligrams of prey per returning ant) remained constant (Fig. 1). Ants thus maintained a constant supply of arthropod prey despite a sharp decline in canopy arthropod abundance. While recent work has underscored the importance of homopteran exudates to ant feeding ecology (Davidson et al. 2003, Blüthgen and Fiedler 2004), our results suggest that predation on tended aphids plays an important and complementary role to exudate consumption.

Ant predation of non-mutualist herbivores in September remained constant as compared to the preceding months, despite a two-thirds decline in non-mutualist herbivore abundance (Fig. 1). This suggests an interaction between tended aphids, non-mutualist herbivores, and ants akin to apparent competition (Holt and Lawton 1994). Mutualist aphids attracted a population of predatory ants that continued to capture non-mutualist herbivores despite their declining abundance, and decoupled the interactions between ants and non-mutualist herbivores from density dependence. Mutualist aphids thus led ants to suppress non-mutualist herbivores to a lower level than would have occurred in the absence of an alternate food resource (Holt and Lawton 1994). Similar dynamics among other *Formica*, aphids, and untended herbivores have been shown elsewhere (Fowler and Macgarvin 1985, Ito and Higashi 1991).

Food web structure through space

While stable isotopes showed a mean trophic position for ants of 3.4 for early summer 2002, there were significant differences among our six spatially separate food webs. Ant trophic positioning ranged from a low of 3.2 to a high of 3.6 (Appendix B). This variation may have been driven by differences in arthropod community composition, in the nutritional needs of ant colonies of varying ages, or in the abiotic conditions within which colonies were situated (Portha et al. 2002, Sanders and Gordon 2002). These ecological and abiotic factors operated at a sufficiently localized scale such that we did not detect spatial autocorrelation in ant trophic positioning among replicate food webs; for instance, two trees that were separated by only 200 m differed in ant trophic positioning by 0.3 trophic levels, while another pairing of trees that were separated by 3.4 km differed by <0.1 trophic levels.

Implications for stable isotope methodology

The use of stable isotopes in the study of food web structure is still a relatively new application of the tech-

nique. The physiological dissimilarities among species means that isotopes likely behave differently among different consumers. In order to correctly interpret isotopic data, it is still necessary to scrutinize assumptions about the behavior of stable isotopes in trophic interactions (Gannes et al. 1997). Here we discuss how our data compare to fractionation of $\delta^{15}N$ and $\delta^{13}C$ in other studies, and indicate emerging trends in $\delta^{15}N$ and $\delta^{13}C$ enrichment in arthropod food webs.

Nitrogen enrichment in the aphids was only ~1‰ above their host plant, lower than the ~2‰ between aphids and their predators, and also lower than values reported in other studies measuring $\delta^{15}N$ enrichment of insect herbivores (reviewed by Vanderklift and Ponsard 2003). However, our finding of reduced enrichment agrees with other recent studies on phloem-feeding herbivores (Ostrom et al. 1997, Yoneyama et al. 1997, Oelbermann and Scheu 2002, Blüthgen et al. 2003, Davidson et al. 2003, McCutchan et al. 2003). Aphids feed upon a much poorer nitrogen source than predators (see C:N values in Fig. 3), and the imbalance between metabolic needs and nutrient availability for aphids may reduce nitrogen fractionation (Scrimgeour et al. 1995, Yoneyama et al. 1997, Oelbermann and Scheu 2002; but see Hobson et al. 1993, Webb et al. 1998, Adams and Sterner 2000, Vanderklift and Ponsard 2003).

Values of $\delta^{15}N$ of specialist aphid predators was enriched by ~2‰ compared to their prey and provides a baseline for $\delta^{15}N$ enrichment between the second and third trophic levels in this system. While these values are lower than those typically seen for vertebrate predators (Vanderklift and Ponsard 2003, and citations therein), this change in $\delta^{15}N$ is in agreement with other measures of fractionation between insectivores and their prey, including insectivorous bats (1.03‰, Herrera et al. 2001), coccinellid beetles (1.0‰, Scrimgeour et al. 1995), and the ant *Paraponera clavata* (1.2‰, Tillberg and Breed 2004). Reviews of multiple insectivores also report similar average values (1.4‰, McCutchan et al. 2003; 1.8‰, Vanderklift and Ponsard 2003). Our results bolster this emerging understanding that $\delta^{15}N$ fractionation for insectivores is in the range of 1–2‰, significantly less than the 3–4‰ that has been typically found for vertebrate carnivores (reviewed in Vanderklift and Ponsard 2003).

The $\delta^{15}N$ of each trophic level of the insect community was highly correlated with the $\delta^{15}N$ signature of the individual tree from which the insects were collected ($r > 0.88$ in all cases). Blüthgen et al. (2003) also found a correlation in $\delta^{15}N$ between phloem-feeding herbivores and their host plant, but this correlation did not persist to higher-order consumers. Furthermore, the $\delta^{15}N$ values for pines were highly variable, with a range of 3.7‰. These results have two implications. First, they suggest variability in nitrogen pools and/or the physiological mechanisms by which nitrogen is obtained by plants and their mycorrhizal symbionts. Sec-

ond, the tight correlations of animals with their host plants suggest that animal movement is at a scale significantly <90 m. Given the biology of the animals we studied, this finding is not surprising. Were the scale of $\delta^{15}N$ variation small, this variation could provide information about the connectedness of food webs among closely situated plants.

The results of the $\delta^{13}C$ comparisons among pine needles, aphids, and honeydew were somewhat surprising. Aphids were enriched by nearly 3‰ compared to the needles from which they fed, a value of $\delta^{13}C$ enrichment greatly exceeding previous reports for insect herbivores generally (McCutchan et al. 2003), and for phloem-feeding hemipterans in particular (McCutchan et al. 2003). Blüthgen et al. (2003) reported a mean enrichment of 1.7‰ (range −0.3‰ to 2.7‰) in membracids and cicadellids compared to host plants, but the aphids and coccids they measured were depleted in ^{13}C, with mean change in $\delta^{13}C$ of −1.1‰ (range −4.1‰ to 1.9‰), i.e., a shift in the opposite direction of what we observed. Furthermore, the honeydew collected in our study was enriched an additional 2‰ above the aphids, for a total $\delta^{13}C$ enrichment of almost 5‰ above the whole leaf tissue of the plant from which it was drawn. The physiological basis for this remarkable enrichment of aphids and honeydew was outside the scope of our study, but warrants further inquiry.

Conclusion

Our combination of behavioral and stable isotopic methods builds a picture of the feeding ecology of ants in this system. Tended aphids comprise a small proportion of the total pine arthropod community, but are an important resource to the ant as both prey and a source of honeydew. In the early months of summer, ants frequently prey upon predators and fed at a trophic level of 3.4. Later in the summer, aphids constitute a more important resource, both as prey and as a source of honeydew, and ants feed at the 3.0 trophic level. The ant mutualism with tended aphids facilitated a constant rate of ant predation upon non-mutualist herbivores in September, despite a sharp decline in non-mutualist herbivore abundance. Food web structure in early summer 2002 placed ants at an average trophic position of 3.4, but this value ranged from 3.2 to 3.6 among spatially disparate, replicated food webs. Thus, this ant exhibited both temporal and spatial variation in trophic position. In both cases, the magnitude of this variation was ~0.4 trophic levels. To the extent the spatial and temporal variation in *F. podzolica* diet is similar to other ants or generalist predators, future food web models must accommodate these complex dynamics.

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APPENDIX A

A description of arthropod collection methodology is available in ESA's Electronic Data Archive: *Ecological Archives* E086-068-A1.

APPENDIX B

A figure showing estimated trophic position of ants among six replicate food webs is available in ESA's Electronic Data Archive: *Ecological Archives* E086-068-A2.