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Trophic position and seasonal changes in the diet of the red wood ant *Formica aquilonia* as indicated by stable isotope analysis

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Abstract. 1. Red wood ants are among the most numerous generalist predators and strongly affect the composition of arthropod communities in forest ecosystems. However, their trophic position remains poorly understood. Stable isotope analysis was applied to study the trophic position of *Formica aquilonia* and reveal seasonal changes in its trophic links with both myrmecophilous aphids and other invertebrates in a mixed forest of western Siberia.

2. The δ^{15}N values of *F. aquilonia* exceeded those of herbivores and aphids by approximately 3.5‰. Despite obligate trophobiotic relationships with aphids, *F. aquilonia* occupied the trophic position of first-order predator. The higher content of ^{13}C in the worker ants, compared with members of grazing food chains, was explained by their consumption of ^{13}C-enriched aphid honeydew.

3. Myrmecophilous tree-dwelling aphids were enriched in ^{13}C and ^{15}N relative to grass-inhabiting species, and the honeydew of tree-dwelling aphids had higher δ^{13}C values than those of the honeydew of grass-inhabiting aphids.

4. The decrease in δ^{13}C values of the worker ants from spring and summer to autumn apparently reflected the transition from the collection of tree sap and feeding on the aphid honeydew from trees with high ^{13}C content in the spring and early summer to a more diverse liquid diet in late summer, which included ^{13}C-depleted honeydew of aphids from herbs.

5. The prevalence of the ^{15}N-depleted aphid honeydew in the ants' diet in the second half of the summer is discussed as one possible explanation for the seasonal decline in δ^{15}N values of the worker ants.

Key words. Ants, aphids, forest ecosystems, honeydew, prey, stable isotopes, trophic relations.

Introduction

Red wood ants (*Formica rufa* group) are among the most numerous generalist predators, strongly affecting the composition of arthropod communities in temperate forest ecosystems (Cherix & Bourne, 1980; Lenoir *et al.*, 2003; Reznikova & Dorosheva, 2004; Punttila *et al.*, 2004; Neuvonen *et al.*, 2012). These species are also herbivores, consuming honeydew of trophobiont insects (primarily aphids), plant sap, nectar and plant seeds (Horstmann, 1974; Skinner, 1980; Fowler & Macgarvin, 1985; Rosengren & Sundström, 1991; Domisch *et al.*, 2009). Therefore, the ecological impact of red wood ants extends over several trophic levels and affects both grazing and detrital food chains (Mahdi & Whittaker, 1993; Laakso & Setälä, 2000; Frouz *et al.*, 2008; Wardle *et al.*, 2011). Notably, despite many investigations of the diet of red wood ants, their trophic position within food webs remains unclear, possibly because of significant temporal dynamics and variability in their local food preferences (Rosengren & Sundström, 1991; Domisch *et al.*, 2009).

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Application of stable isotope analysis (SIA) can help to determine the trophic position because this method can obtain time-integrated information on animal nutrition (Gannes *et al.*, 1997; Post, 2002; Tiunov, 2007). The isotopic composition of carbon ($^{13}C/^{12}C$ ratio, usually expressed as $\delta^{13}C$) can be used to infer the $\delta^{13}C$ value of the primary sources of food because of the low trophic fractionation ($\Delta\delta = \delta_{consumer} - \delta_{food}$) of ^{13}C, which is estimated at approximately 0.5–1‰ (DeNiro & Epstein, 1978; Post, 2002; McCutchan *et al.*, 2003). $\delta^{13}C$ has been used in many ecological studies, in particular to discriminate between diets of different species based on plants with different photosynthetic pathways (C3 vs C4; Gratton & Forbes, 2006). By contrast, the isotopic composition of nitrogen ($^{15}N/^{14}N$ ratio, expressed as $\delta^{15}N$) can be used to infer the trophic level of the consumer because of the pronounced trophic fractionation of ^{15}N, which is estimated at approximately 2–4‰ with each trophic level (Minagawa & Wada, 1984; McCutchan *et al.*, 2003; Vanderklift & Ponsard, 2003).

Stable isotope analysis has been applied to several ant ecological studies in European and Mediterranean communities, but these studies do not include red wood ants (Ottonetti *et al.*, 2008; O'Grady *et al.*, 2010; Platner *et al.*, 2012). Data on the carbon isotopic composition of plants, arthropods and ants were used to reveal the contribution of different food sources derived from tree canopies and the herbaceous layer to the diets of ant species in Mediterranean communities (Ottonetti *et al.*, 2008; Platner *et al.*, 2012). The data on nitrogen isotopic composition show that most ant species in different ecosystems are omnivores and occupy an intermediate position between the specialised herbivorous and predatory groups of ant species (Davidson *et al.*, 2003; Blüthgen *et al.*, 2003; Platner *et al.*, 2012; Pfeiffer *et al.*, 2014). In central Europe, the $\delta^{15}N$ values of *Formica* species (combined data from eight species) place them into an intermediate trophic position between predominantly 'trophobiotic', i.e. herbivorous (e.g. *Camponotus*), and predatory (e.g. *Myrmica*) genera (Fiedler *et al.*, 2007).

The diet of red wood ants is complex, which leads to the hypothesis that species in this group also occupy an intermediate trophic position, below first-order predators and possibly close to herbivorous ('trophobiotic') ants. In examining this hypothesis, we should recognise that the trophic position and specific trophic relations of ants may vary seasonally throughout the period of foraging activity, depending on the changing requirements of the colony and the availability of food (Mooney & Tillberg, 2005; Menke *et al.*, 2010; Caut *et al.*, 2013).

We applied stable isotope analysis to study trophic relations among the red wood ant *F. aquilonia*, myrmecophilous aphids inhabiting trees and herbaceous plants, and invertebrates in the grazing and detrital food chains. The following questions were addressed: (i) what is the trophic position of *F. aquilonia*; and (ii) to what extent are the seasonal changes in food preferences of ants reflected in their isotopic signatures?

Materials and methods

Study site

The study was conducted in the Novosibirsk region, western Siberia, Russia (N 5500.586, E 8318.460), in a mixed birch forest with pine, spruce, willow, bird cherry, undergrowth of aspen and cover of herbaceous plants. Approximately 500 mounds of *F. aquilonia* occupied the entire forest territory. Four monodomous ant colonies of similar population size (approximately 10^6 individuals each) were selected. Neutral zones separated the foraging areas of the studied colonies, which were free of *F. aquilonia* workers and were from 50 to 200 m wide; no exchanges of brood, workers or food occurred among these colonies. Foraging areas of the studied colonies were approximately 0.2 ha each, with several dozens of trees visited by foragers. The vegetation cover was similar in all four foraging areas. All the materials were collected within the foraging areas of these four ant colonies.

Sampling

To study the trophic position of *F. aquilonia*, we collected ant foragers and various groups of organisms and substrates representing the primary sources of carbon and nitrogen in the diet of ants: plant litter, aphids and their host plants, aphid honeydew, birch sap and invertebrate prey items of ants. Seasonal variation was assessed by comparing samples collected in spring (18 April to 5 May), summer (7–27 July) and autumn (24 August to 5 September) (Tables S1 and S2).

Plant litter. First, to establish 'the isotopic baseline', four mixed samples of overwintered birch leaf litter were collected in the foraging area of each ant colony (see later).

Aphids and plants. Myrmecophilous aphids tended by *F. aquilonia* were collected with the parts of their host plants occupied by aphid colonies in summer and autumn. In total, data on 18 pairs of aphid–plant samples were collected from 93 aphid colonies (Table S1). To identify aphid species, a few specimens from each aphid colony were fixed in 70% alcohol. The aphids were studied on microscope slides prepared using Faure-Berlese fluid. The material was deposited at the Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences (Novosibirsk). The aphids were identified using Blackman and Eastop (2006). Aphid synonymy follows Favret (2016). A.V. Stekolshchikov verified the aphid determinations (Zoological Institute RAS, St Petersburg, Russia). To avoid the influence of ontogenetic factors on the isotopic signature, larvae and adult aphids were collected in equal proportions.

Honeydew. Honeydew was collected in autumn (Table S2) from the four most common species of aphids (*Symydobius oblongus* (von Heyden), *Chaitophorus populeti* (Panzer), *Chaitophorus* cf. *ramicola* (Börner) and *Aphis fabae* (Scopoli). Worker ants descending from the aphid colonies with swollen stomachs containing honeydew were carefully removed from the plant, their abdomens gently squeezed with soft plastic tweezers and the drop of honeydew was collected from their mouths using capillaries (Mooney & Tillberg, 2005).

Birch sap. Birch sap used by *F. aquilonia* as the carbohydrate food in early spring was collected on April 26 by drilling small

holes in the stems of *Betula pendula*. Ten millilitres of sap was collected from a single tree in the foraging area of each of the four ant colonies.

Invertebrate prey items. In the spring, the activity of *F. aquilonia* foragers was limited primarily to collecting birch sap and nest-building materials; therefore, the invertebrate prey of ants was collected only in summer and autumn (S2). Invertebrates or their parts were removed from foragers returning to their mound using soft tweezers (12–24 prey items per colony). Prey items were collected during the periods of maximal foraging activity of ants (from 10.00 to 12.00 hours or from 15.00 to 17.00 hours, depending on the weather). Invertebrate prey items were collected over a total of 8 h (for 1 h, once at each colony in summer and autumn).

Formica aquilonia foragers. Formica aquilonia foragers were collected in spring, summer and autumn (Table S2). From each colony, five workers were collected on foraging routes, with the same number collected from foraging trees (a total of 30 ants per colony).

All the samples intended for SIA, except for the prey items, were frozen at −20 °C within 6 h after collection. Prey items were stored in saturated NaCl solution until identification and were then thoroughly washed with water for 24 h and dried for SIA.

Stable isotope analysis

Sample preparation included drying at 50 °C for 2–3 days, weighing (400–600 µg for animal and 1500 µg for plant tissues) and packaging in tin capsules. Plant and litter samples were finely powdered using a ball mill (Retsch MM 200, Haan, Germany). Arthropods (but not aphids) were analysed individually. When possible (for species of Coleoptera, Diptera and Heteroptera), thoraxes and/or head capsules were analysed. Entire small arthropods were analysed. Aphid samples contained five to 20 specimens. In the preparation of worker ants for analysis, abdomens and head capsules were removed and only thoraxes with legs were analysed, which diminished the effects of lipid storage, undigested food in the gut or crop content on isotope measurements (Blüthgen *et al.*, 2003; Tillberg *et al.*, 2006). SIA was conducted using a Thermo Delta V Plus continuous-flow isotope ratio mass spectrometer coupled with an elemental analyser (Thermo Flash 1112, Milan, Italy) in the Joint Usage Centre at the Institute of Ecology and Evolution RAS. The isotopic composition of N and C was expressed in the δ-notation relative to the international standard (atmospheric nitrogen and Vienna Pee Dee Belemnite (VPDB), respectively): $\delta X(‰) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R is the ratio of the heavier isotope to the lighter isotope. Samples were analysed with reference gas calibrated against the IAEA reference materials USGS 40 and USGS 41 (glutamic acid). The drift was corrected using internal laboratory standards (glycine, acetanilide). The standard deviations of the δ^{15}N and δ^{13}C values of reference materials ($n = 8$) were <0.2‰. With the isotopic analyses, nitrogen and carbon contents (as mass %) were determined in all samples.

Data analyses

Local variations in δ^{13}C and δ^{15}N values of plant samples (leaves, leaf litter) are typically used as a baseline in isotopic studies of litter-dwelling macroinvertebrates, including ants (Woodcock *et al.*, 2012; Korobushkin *et al.*, 2014). The stable isotope composition of birch litter did not differ among foraging areas of the studied ant colonies [Kruskal–Wallis test: δ^{13}C (ranged from −29.8 to −29.1‰), $\chi^2(3) = 4.485$, $P = 0.214$; δ^{15}N (ranged from 0.7 to 1.1‰), $\chi^2(3) = 6.463$, $P = 0.091$]; therefore, the data from the four colonies were combined without additional baseline correction.

The ant prey contained 91 items, of which 16 were fragments of arthropods that could not be identified. The remaining 75 items were identified to species or a higher taxonomic rank. Based on published data on the feeding behaviour of invertebrates, the prey species were tentatively attributed to grazing or detrital food chains (Table S2). Dry-mass weighted mean δ^{13}C and δ^{15}N values of the selected groups of prey were calculated using the following equations:

$$\text{Weighted average } \delta\text{X of a group of prey} = \sum_{i=1}^{n} \text{w}_i.\delta\text{X}_i,$$

$$\text{where } \text{w}_i = \frac{\text{m}_i}{\sum_{i=1}^{n} \text{m}_i}, \text{m}_i - \text{dry mass of } i\text{th of } n$$

samples from a group of prey.

Statistical analyses were performed using the SPSS statistical software package v. 16.0. The Kruskal–Wallis ANOVA median test was used to compare variables for both δ^{13}C and δ^{15}N values. To reveal the differences between pairs of data, the Mann–Whitney U-test was used (with Bonferroni correction in cases of multiple comparisons). Linear regression and Spearman's correlation coefficients were used to determine the relationships between the isotope signatures of aphids and host plants. The Bray–Curtis similarity index was used to estimate the similarity in the composition of prey that ants collected in summer and autumn. The central tendency reported is the median, and measures of variability (25th and 75th percentiles) are presented in Tables S1 and S2.

Results

Plants, aphids and honeydew

In the investigated community, *F. aquilonia* maintained trophobiosis with 11 species of myrmecophilous aphids, and foragers of all four ant colonies collected honeydew from all these species. Tree-dwelling aphids inhabited three species of woody plants (*Betula pendula*, *Populus tremula*, and *Salix* sp.), and grass-inhabiting aphids were collected on eight species of herbaceous plants (*Anthriscus sylvestris*, *Crepis sibirica*, *Dactylis glomerata*, *Filipendula ulmaria*, *Heracleum sibiricum*, *Melilotus alba*, *Tripleurospermum perforatum*, and *Urtica dioica*). The δ^{13}C values of aphids ranged from −33.6 to

−27.0‰, and the δ^{15}N values ranged from −0.4 to 3.8‰ (Fig. 1; Table S1). The δ^{13}C and δ^{15}N values of the corresponding host plants ranged from −32.4 to −27.9‰ and from −1.2 to 2.6‰, respectively (Fig. 1; Table S1). Highly significant, positive correlations were found between the isotopic composition of aphids and their host plants (δ^{13}C, $\rho = 0.781$, $P < 0.0001$; δ^{15}N, $\rho = 0.688$, $P = 0.002$; Fig. 2).

The δ^{13}C and δ^{15}N values of aphids feeding on woody plants (−29.2‰ and 2.6‰, respectively) were significantly higher than those of aphids feeding on herbaceous plants (−30.9‰ and 1.2‰, respectively) [Kruskal–Wallis test: δ^{13}C, $\chi^2(1) = 8.265$, $P = 0.004$; δ^{15}N, $\chi^2(1) = 31.434$, $P < 0.0001$; Table S1]. However, no significant differences in isotopic composition were found between woody and herbaceous host plants [Kruskal–Wallis test: δ^{13}C, $\chi^2(1) = 1.026$, $P = 0.311$; δ^{15}N, $\chi^2(1) = 0.787$, $P = 0.375$; Table S1]. The total nitrogen content in herbaceous plants was two-fold that of woody plants [2.2% and 1.0%, respectively; Kruskal–Wallis test: $\chi^2(1) = 13.789$, $P < 0.0001$].

Most aphid colonies tended by *F. aquilonia* belonged to four species: *Symydobius oblongus* and *Chaitophorus populeti*, the predominant species from May to September, and *Chaitophorus* cf. ramicola and *Aphis fabae* from July to September. The honeydew collected from these four most common aphid species was probably a primary source of carbohydrate for *F. aquilonia* during the summer.

The grass-inhabiting aphid *A. fabae* colonising the herb *Crepis sibirica* had δ^{13}C and δ^{15}N values significantly lower than those of the three most common tree-dwelling aphid species [Kruskal–Wallis test: $\chi^2(3) = 13.204$, $P = 0.004$; Mann–Whitney test with Bonferroni correction for multiple comparisons: $U = 0$, $P < 0.0083$; Table S2; Fig. 3]. Similarly, honeydew produced by *A. fabae* was depleted in ^{13}C relative to the honeydew of the three tree-dwelling species [Kruskal–Wallis test: $\chi^2(3) = 18.658$, $P < 0.0001$]; however, pairwise comparisons between the δ^{13}C value of the honeydew of *A. fabae* and those of other species revealed no significant differences (Mann–Whitney test with Bonferroni correction, $P > 0.0083$; Fig. 3a).

Because of the very low N content, the δ^{15}N values could be determined for the honeydew of *S. oblongus* only. The honeydew was depleted in ^{15}N by 3.5‰ relative to that of aphids (Kruskal–Wallis test: $\chi^2(1) = 8.163$, $P = 0.004$) and by 1.6‰ relative to that of their host plant (Kruskal–Wallis test: $\chi^2(1) = 2.922$, $P = 0.087$; Fig. 3b).

The δ^{13}C value of birch sap was −26.6‰ and was thus enriched in ^{13}C compared with the honeydew of *S. oblongus* aphids inhabiting birches [Kruskal–Wallis test: $\chi^2(1) = 12.632$, $P < 0.0001$; Fig. 1]. The sap was also enriched in ^{15}N relative to that of the aphid honeydew [Kruskal–Wallis test: $\chi^2(1) = 6.429$, p = 0.011; Fig. 1]. The total nitrogen content in the birch sap was much higher than that in the honeydew of *S. oblongus* [1.5% and 0.1%, respectively; Kruskal–Wallis test: $\chi^2(1) = 13.714$, $P < 0.0001$]. The nitrogen content in the honeydew of *C.* cf. *ramicola* and *A. fabae* aphids was approximately 0.1%.

Trophic position of F. aquilonia

The isotopic composition of *F. aquilonia* prey items varied widely and was 7.6‰ for δ^{13}C and 13.0‰ for δ^{15}N (Fig. 1, Table S2). The prey included predominantly Hemiptera (37%), Diptera (14%) and fragments of arthropods (18%). Approximately 44% of the prey items were in the grazing food chain, 21% in the detrital food chain, and for 35% of prey items, the trophic links could not be identified (Table S2). Animals from the detrital food chain were enriched in ^{13}C in comparison with those from the grazing food chain. Dry-mass weighted mean values of δ^{13}C and δ^{15}N in the 'grazing', 'detrital' and 'unknown' prey groups were −29.2‰, −26.9‰, −27.1‰ and 1.9‰, and 6.3‰, and 5.1‰, respectively. The overall dry-mass weighted mean δ^{13}C and δ^{15}N values of prey items were −28.1‰ and 3.9‰, respectively.

The isotopic composition of nitrogen of many phytophagous insects such as Pseudococcidae (3.3‰), Cicadellidae (2.6‰), Heteroptera (2.0‰), and caterpillars of Geometridae (2.2‰) were within the range of δ^{15}N values of myrmecophilous aphids (Fig. 1). The nonmyrmecophilous aphid *Euceraphis betulae* (Koch) (2.3‰) was within the same range and formed approximately 23% of the total prey of *F. aquilonia*. The median δ^{15}N value of all herbivores, including myrmecophilous aphids, was 2.0‰.

The isotopic composition of *F. aquilonia* foragers differed significantly among the four colonies [Kruskal–Wallis test: δ^{13}C, $\chi^2(3) = 39.728$, $P < 0.0001$; δ^{15}N, $\chi^2(3) = 40.997$, $P < 0.0001$] and ranged in δ^{13}C from −26.4 to −25.7‰ and in δ^{15}N from 5.3 to 5.8‰ (Table S2). The median δ^{13}C values of *F. aquilonia* from all colonies and sampling events were the highest among δ^{13}C values for all invertebrates in our study (−26.0‰; Fig. 1). Foragers were enriched in ^{13}C by 2.1‰ relative to the dry-mass weighted average δ^{13}C value of invertebrate prey, by 0.6‰ relative to the birch sap and by 1.1–2.7‰ relative to the honeydew of aphids feeding on woody plants.

The median δ^{15}N value of red wood ants (5.5‰) was 3.5‰ higher than that of herbivores, including aphids, and 4.9‰ higher than that in plants. *Formica aquilonia* had a higher δ^{15}N value relative to that of litter-dwelling Linyphiidae spiders (4.4‰) but lower than the δ^{15}N value of predatory Geophilidae centipedes (6.7‰).

Seasonal changes in the diet of F. aquilonia

The stable isotope composition of worker ants differed significantly depending on the sampling time [Kruskal–Wallis test: δ^{13}C, $\chi^2(2) = 14.390$, $P = 0.001$; δ^{15}N, $\chi^2(2) = 7.382$, $P = 0.025$). The δ^{13}C and δ^{15}N values of worker ants were almost equal in spring (−25.9‰ and 5.5‰, respectively) and summer (−25.9‰ and 5.6‰, respectively) (Mann–Whitney test with Bonferroni correction: δ^{13}C, U = 776, $P = 0.817$; δ^{15}N, $U = 798$, $P = 0.985$). From summer to autumn, the δ^{13}C and δ^{15}N values of worker ants decreased significantly, by 0.3‰ (Mann–Whitney test with Bonferroni correction: δ^{13}C, $U = 415$, $P < 0.0001$) and 0.2‰ (Mann–Whitney test with Bonferroni correction: δ^{15}N, U = 552, $P = 0.017$), respectively (Fig. 4). From spring to autumn, isotopic signatures of worker

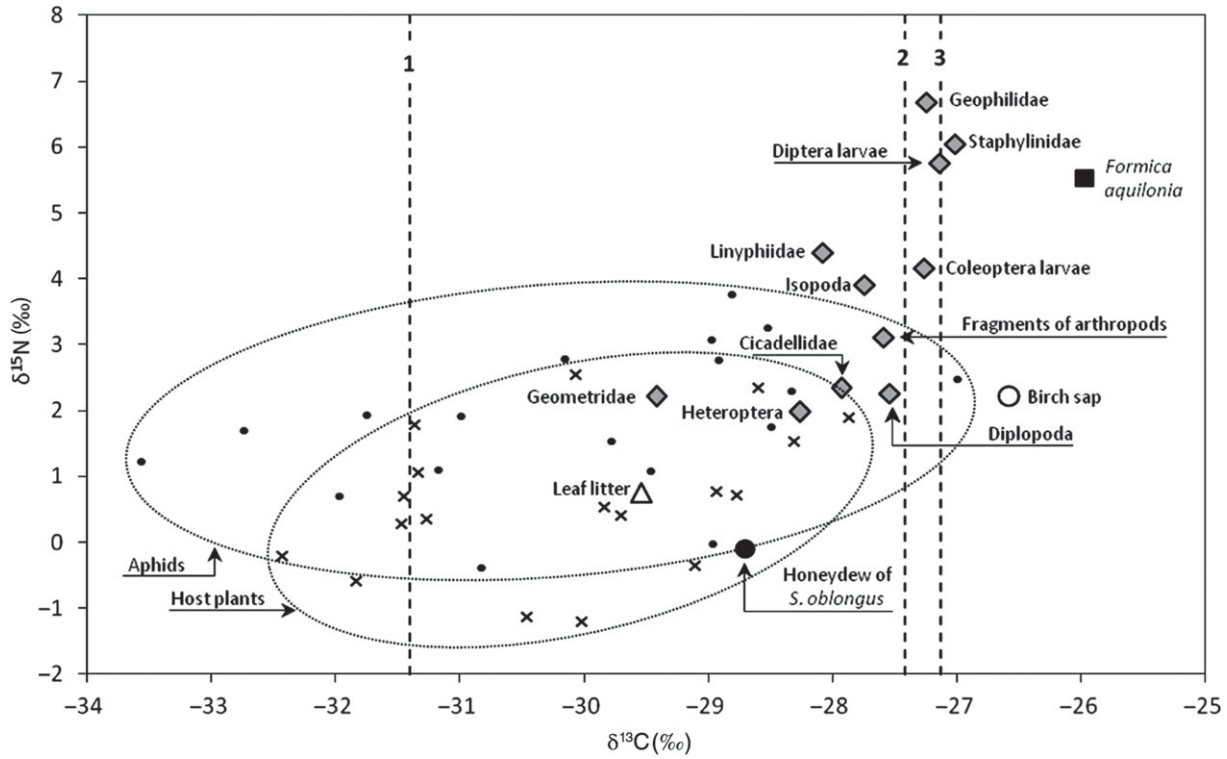

Fig. 1. Median δ^{13}C and δ^{15}N values of *Formica aquilonia* (black square), host plants (oblique crosses), ant-tended aphids (black dots), honeydew of *Symydobius oblongus* on *Betula pendula* (black circle), birch sap (white circle), birch leaf litter (white triangle) and prey of ants (grey diamonds). Vertical dashed lines depict δ^{13}C values of honeydew in case of failed measurements of δ^{15}N values: 1, honeydew of aphid *Aphis fabae* on the herb *Crepis sibirica*; 2, honeydew of *Chaitophorus* cf. *ramicola* on *Salix* sp.; and 3, honeydew of *Chaitophorus populeti* on *Populus tremula*. The ranges of δ^{13}C and δ^{15}N values of host plants and of ant-tended aphids are shown by ellipses. For further information, see Tables S1 and S2.

ants decreased significantly, by 0.3‰ (Mann–Whitney test with Bonferroni correction: δ^{13}C, $U = 504$, $P = 0.004$) and 0.1‰ (Mann–Whitney test with Bonferroni correction: δ^{15}N, $U = 560$, $P = 0.021$), respectively.

The time of sampling did not significantly affect the species composition of the prey of *F. aquilonia*, as indicated by a high Bray–Curtis similarity value (0.735) between the prey collected in summer and autumn. Additionally, no significant differences in isotopic signatures of prey samples were found between summer and autumn [Kruskal–Wallis test: δ^{13}C, $\chi^2(1) = 2.224$, $P = 0.136$; δ^{15}N, $\chi^2(1) = 0.026$, $P = 0.871$; Fig. 4]. Isotopic signatures of plants also did not change from summer to autumn [Kruskal–Wallis test: δ^{13}C, $\chi^2(1) = 0.989$, $P = 0.320$; δ^{15}N, $\chi^2(1) = 0.156$, $P = 0.193$; Fig. 4]. The median δ^{13}C value of myrmecophilous aphids was similar in summer and autumn samples [Kruskal–Wallis test: $\chi^2(1) = 3.108$, $P = 0.078$], whereas the median δ^{15}N values of aphids decreased by 0.1‰ [Kruskal–Wallis test: $\chi^2(1) = 4.457$, $P = 0.035$; Fig. 4].

Discussion

Plants, aphids and honeydew

The δ^{13}C values of aphids did not differ significantly from those of the host plants (Figs 1 and 2), similar to earlier observations (Spence & Rosenheim, 2005). By contrast, the honeydew produced by aphids was highly enriched in ^{13}C relative to the aphids and their host plants (Fig. 3). A similar ^{13}C enrichment of honeydew is reported in several other studies (Yoneyama *et al.*, 1997; Mooney & Tillberg, 2005; Sagers & Goggin, 2007), which may be related to the high δ^{13}C values of sugars in aphid excretions and to the low δ^{13}C values of the fat reserves in aphids (Gearing, 1991).

Tree-dwelling aphid species (*S. oblongus*, *C. populeti*, and *C.* cf. *ramicola*) had higher δ^{13}C values than the species inhabiting the herb layer (*A. fabae*). Most probably, this difference was a reflection of a well known 'canopy effect', i.e. the depletion of the ground vegetation in ^{13}C relative to canopy plants (van der Merwe & Medina, 1991; Brooks *et al.*, 1997). The pattern of δ^{13}C values in honeydew (Figs 1 and 3) suggested that high δ^{13}C values of *F. aquilonia* reflected a strong trophic dependence on tree-dwelling aphids, whereas aphid species colonising herbaceous plants were of much less importance to ants. Because of the high variability in the ^{13}C content of aphid honeydew, SIA could be used to study the vertical spatial organisation of honeydew foraging in ants (with canopy layers preferred for foraging, at least for honeydew collection).

Tree-dwelling aphids were enriched in ^{15}N relative to grass-inhabiting species, although the enrichment of host

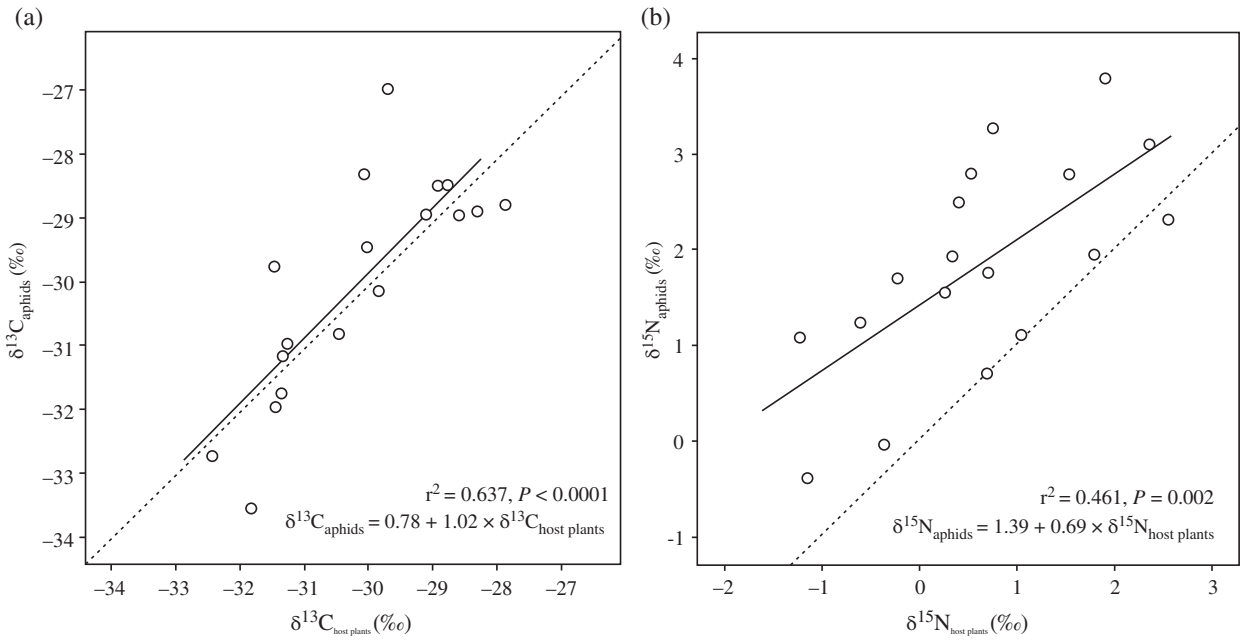

Fig. 2. Linear regressions between median δ^{13}C (a) and δ^{15}N (b) values of ant-tended aphids and their host plants. The dashed lines show identity lines.

plants was not different. Therefore, to explain the difference between tree-dwelling and grass-inhabiting aphids, the trophic fractionation of ^{15}N must have been somewhat higher in the aphids feeding on trees. This could be related to the differences in total N content in tissues of herbs and trees (2% and 1%, respectively), as trophic fractionation of ^{15}N in aphids is known to be affected by the dietary quality of phloem sap (Sagers & Goggin, 2007). The δ^{15}N value in the honeydew of *S. oblongus* aphids feeding on birch was approximately 4‰ lower than the δ^{15}N of aphid tissues, and the reasons for this depletion remain unclear.

Trophic position of F. aquilonia

According to earlier reports, depending on the forest type, the prey of red wood ants consists primarily of Lepidoptera, including larvae of Geometridae (Horstmann, 1974; Skinner, 1980; Punttila *et al.*, 2004) and Diptera (Rosengren & Sundström, 1991; Domisch *et al.*, 2009). In our study, the composition of prey of *F. aquilonia* displayed a wide taxonomic and trophic variety that is typical of generalist predators, with a predominance of phytophagous insects, mostly aphids.

Variation in δ^{15}N values among the four colonies of *F. aquilonia* (0.5‰) was lower than the intercolony variation in δ^{15}N values for five ant species (2–6‰; Tillberg *et al.*, 2006), which might be an indication of habitat similarity of the studied colonies.

The median δ^{15}N value of *F. aquilonia* exceeded the median δ^{15}N values of aphids and other herbivorous insects by approximately 3.5‰, which roughly corresponds to the level of trophic fractionation of ^{15}N/^{14}N in other ants (3.0‰; Feldhaar *et al.*, 2010) and other invertebrates (3.3‰; McCutchan *et al.*, 2003). Thus, in contrast to our expectations, these data indicated that *F. aquilonia* occupied the position of first-order predator in the studied community. The trophic position as a predator distinguished *F. aquilonia* from the combined data on eight species of the genus *Formica* (Fiedler *et al.*, 2007) and placed them closer to *Formica podzolica*, which is similarly enriched in ^{15}N relative to aphids, by 3.3‰ (Mooney & Tillberg, 2005). The close trophic positions of these two species can be explained by their ecological similarities because, similar to red wood ants, *F. podzolica* occurs in relatively large colonies (from 10^3 to 10^4 workers, up to 10^5 individuals), forages in the same layers of vegetation and the diet includes arthropods and sugar-rich liquids (Savolainen *et al.*, 1996; Mooney & Tillberg, 2005).

The δ^{15}N values of *F. aquilonia* were intermediate between litter-dwelling (linyphiid spiders) and soil-dwelling (geophilid centipedes) predators (Fig. 1). However, based on direct observations, *F. aquilonia* participated in the grazing food chain, with at least 40% of the total prey items being above-ground herbivores. Thus, red wood ants feed actively on both types of prey, linking detritus-based and plant-based energy pathways in the food web.

As noted previously, red wood ants are obligate trophobiotic species that obtain carbon primarily from aphid honeydew (Horstmann, 1974; Domisch *et al.*, 2009). Therefore, the high δ^{13}C values of *F. aquilonia* were probably primarily related to feeding on the ^{13}C-enriched honeydew of tree-dwelling aphids. However, because litter-dwelling arthropods are also consistently enriched in ^{13}C (~3–4‰) relative to plant litter due to the increase in ^{13}C content in saprotrophic microorganisms (Hyodo *et al.*, 2010; Potapov *et al.*, 2013), the high δ^{13}C values

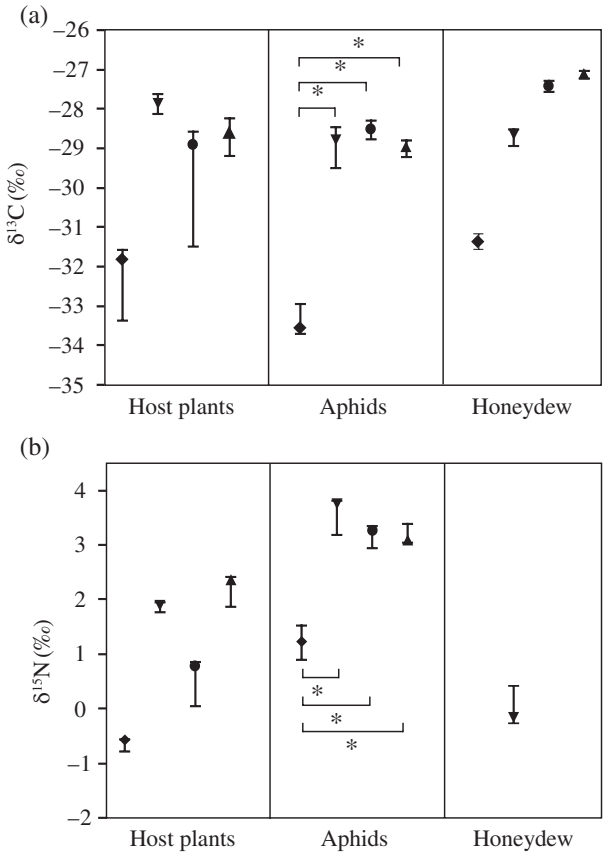

Fig. 3. δ^{13}C (a) and δ^{15}N (b) values (medians with 25th and 75th percentiles) of the four most abundant species of myrmecophilous aphids, their host plants and their honeydew: *Aphis fabae* on the herb *Crepis sibirica* (♦); *Symydobius oblongus* on *Betula pendula* (▼); *Chaitophorus* cf. *ramicola* on *Salix* sp. (●); and *Chaitophorus populeti* on *Populus tremula* (▲). δ^{15}N value of honeydew could be measured for *S. oblongus* only. Significant differences are marked with asterisks (Kruskal–Wallis test, $P < 0.05$; Mann–Whitney test with Bonferroni correction for multiple comparisons, $P < 0.0083$).

of *F. aquilonia* also suggested trophic links with the detrital food chain. Indeed, the δ^{13}C values of *F. aquilonia* (−26.0‰) were similar to those of invertebrates from the detrital food chain (−26.9‰).

To summarise, based on our analysis, the hypothesis that red wood ants occupied a low trophic position in the invertebrate community was rejected. Despite the major role of honeydew in *F. aquilonia* nutrition (Domisch *et al.*, 2009), we found this species was a first-order predator.

Seasonal changes in the diet of F. aquilonia

In red wood ants, the seasonal changes in the diet from spring to autumn included a reduction in the number of prey consumed, switching from tree sap collection in the spring to collection of aphid honeydew, and an increase in the volume of carbohydrate food required to feed adult workers (Dlussky, 1967; Horstmann, 1972; Skinner, 1980).

The δ^{13}C value of *F. aquilonia* decreased significantly from spring and summer to autumn (Fig. 4a). Because the carbon isotopic composition of plants, aphids and prey of ants in summer and autumn was similar, the seasonal variations detected in the carbon isotopic signatures of foragers could be associated primarily with changes in the consumption of liquid food substrates. The literature does not contain data on the time required for the assimilation of isotopes into ant tissues, and the extent to which carbon and nitrogen isotopic signatures of worker ants reflect their recent diet as adults or their diet as larvae is also unknown. In *F. aquilonia*, the development from an egg to a worker requires approximately 3–4 weeks, and 5–6 more weeks are required to become a forager (Otto, 1958). Hence, we suggest that 8–10 weeks are required to change the isotopic signatures of *F. aquilonia* foragers. Therefore, the 0.3‰ decrease in δ^{13}C values in *F. aquilonia* foragers from the end of April and mid-July to early September apparently reflected changes in the ant diet, which proceeded from consuming birch sap with high δ^{13}C (−26.6‰) in April and honeydew of the most common tree-dwelling aphids (δ^{13}C from −28.7 to −27.1‰) in June–July to a mix that included honeydew of grass-inhabiting aphids (δ^{13}C, −31.4‰), which became much more abundant in the second half of the summer. Platner *et al.* (2012) reported a seasonal decrease in δ^{13}C values of *F. rufibarbis* and *Lasius grandis* ants in citrus plantations due to the seasonal decrease in δ^{13}C of citrus trees, which provided the primary source of food for these species, rather than to changes in the composition of food substrates.

From spring and summer to autumn, the decrease in δ^{15}N in *F. aquilonia* was approximately 0.1–0.2‰. A similar seasonal decline in δ^{15}N values is recorded in *F. podzolica* workers, which was explained by an increase in the proportion of herbivore prey from early to late summer (Mooney & Tillberg, 2005). The autumnal decline in the δ^{15}N values of *F. aquilonia* foragers might reflect changes in their larval diet but this is difficult to determine. We did not find any significant differences in δ^{15}N values of plants or prey of ants or in the composition of prey between summer and autumn. The data on the composition of *F. aquilonia* prey were insufficient to answer the question whether the temporal change in δ^{15}N values was associated with a change in the trophic position of ants. In the second half of the summer, foragers probably obtained a higher proportion of nitrogen from aphid honeydew with relatively low δ^{15}N values compared with birch sap in the spring. The decrease in the δ^{15}N value of myrmecophilous aphids from the summer to autumn could also explain this decline in δ^{15}N values of foragers. Other studies also suggest that abundant feeding on honeydew and nectar leads to low δ^{15}N values in trophobiotic ant species (Blüthgen *et al.*, 2003; Davidson *et al.*, 2003; Menke *et al.*, 2010).

Conclusions

Based on the comparison of δ^{15}N values of *F. aquilonia*, its prey and myrmecophilous aphids, this species occupied the trophic position of a first-order predator. Despite obligate trophobiotic relationships with aphids, the δ^{15}N values of *F. aquilonia*

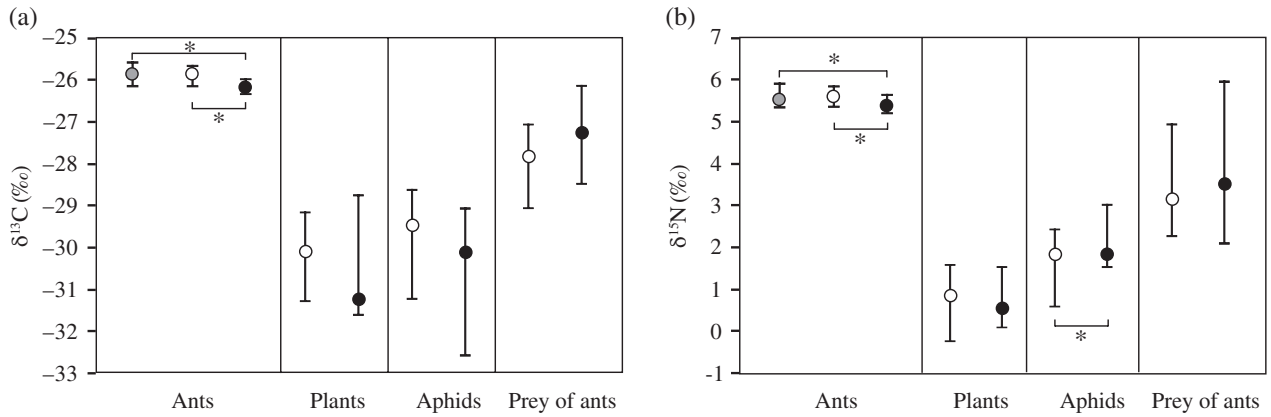

Fig. 4. Seasonal changes in δ^{13}C (a) and δ^{15}N (b) values of *Formica aquilonia*, host plants, ant-tended aphids and prey of ants. Grey points represent samples collected in spring, white points represent those collected in summer and black points represent those collected in autumn. Isotopic values represent medians with 25th and 75th percentiles. Significant differences are marked with asterisks (Kruskal–Wallis test, $P < 0.05$; Mann–Whitney test with Bonferroni correction for multiple comparisons, $P < 0.025$).

exceeded those of herbivores and aphids by approximately 3.5‰ in the studied community.

The red wood ant *F. aquilonia* had higher δ^{13}C values than the members of the grazing food chain, which was explained by the consumption of ^{13}C-enriched aphid honeydew. Tree-dwelling aphids tended by *F. aquilonia* were enriched in ^{13}C and ^{15}N relative to aphid species inhabiting herbaceous plants. Additionally, honeydew δ^{13}C values in tree-dwelling aphids were higher than those for grass-inhabiting species. Thus, the high δ^{13}C value of *F. aquilonia* showed that tree-dwelling aphids were more important for red wood ants than were grass-inhabiting aphid species. The decrease in δ^{13}C values of *F. aquilonia* during the period of foraging activity was attributed to the transition from the collection of tree sap and feeding on aphid honeydew from woody plants with high ^{13}C content in the spring and early summer to a more diverse liquid diet in late summer, which included ^{13}C-depleted honeydew of aphids from herbaceous plants. The prevalence of ^{15}N-depleted aphid honeydew in the ant diet during the second half of the summer is discussed as one possible explanation for the seasonal decline in δ^{15}N values of the worker ants.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12384

Table S1. Carbon and nitrogen isotopic signatures of ant-tended aphids, their host plants and plant–aphid trophic enrichment ($\Delta^{13}C_{plant\text{-}aphid}$, $\Delta^{15}N_{plant\text{-}aphid}$) and nitrogen content (as mass %) of host plants. The aphid species with analysed honeydew samples are indicated by asterisks. Values represent medians; 25th and 75th percentiles are shown in square brackets. Percentiles used only for the samples with $n > 2$; if $n < 3$, raw data are shown in round brackets.

Table S2. Food web type of prey of *Formica aquilonia*, taxonomic composition of prey, number of items collected (number of samples used for stable isotope analysis), δ^{13}C and δ^{15}N values of prey, *F. aquilonia* foragers, litter, birch sap and honeydews of aphids. Isotopic values represent medians; 25% and 75% percentiles are shown in square brackets. Percentiles used only for all samples with $n > 2$; if $n < 3$, raw data are shown in round brackets.

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