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Task-specific and seasonal differences in individual nutritional status of red wood ant workers

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Abstract

1. Diet plays a crucial role in caste differentiation and division of labour in ants, but relationships between behavioural specialization and the nutritional status of workers in monomorphic species remain understudied.
2. We used the red wood ant *Formica aquilonia*, a key species of forest communities in North Eurasia, to examine whether (i) nurses are fatter than foragers; (ii) task groups collected on the nest surface, ground surface and trees have different fat reserves; and (iii) task groups differ in the diet and trophic level.
3. Higher fat content in nurses including sunbathing workers compared to foragers was shown by abdomen: thorax dry mass, C:N ratio and $\delta^{13}C$ values in the abdomen and thorax. Colony fat reserves were minimal in summer and maximum in autumn, especially in nurses.
4. Ground foragers restoring the foraging territory in spring showed signs of starvation according to the thoracic $\delta^{15}N$ values. Nest guards and foragers were similarly lean in summer. Tree foragers collecting honeydew were leaner than ground foragers (hunters) in autumn.
5. Workers were of similar trophic level. Differences in crop content between nurses and foragers were indicated by the $\delta^{15}N$ value in the abdomen.
6. Our study suggests that the seasonal dynamics of energy reserves and nutritional differences between task groups are linked to the annual life cycle of the colony, contributing to its reproductive success.

KEYWORDS
colony life cycle, fat storage, Formicidae, seasonality, stable isotopes, task specialization

INTRODUCTION

Division of labour, a key trait of eusocial Hymenoptera, is represented by differences in form and function between reproductive (queens) and non-reproductive (workers) castes, as well as between workers of different age and morphology performing task-specific behaviours (Hölldobler & Wilson, 2009). Further worker task specialization unrelated to differences in worker morphology and age may also exist, especially in large-colony species with sophisticated social organization (Page Jr et al., 2006; Reznikova, 2021; Robson & Traniello, 2002). Understanding proximate mechanisms underlying task specialization and social resilience can suggest how worker division of labour and colony organization emerged.

Caste differentiation and division of labour are affected by environmental conditions (nutrition, temperature and social influence; Corona et al., 2016) and intrinsic factors (maternal and genetic effects; Anderson et al., 2008; Klein et al., 2016) whose interaction varies widely among social insects. In particular, diet composition is a major factor controlling queen/worker and major/minor worker differentiation in diverse ant species via hormonal cascades (e.g., juvenile hormone (JH); Abouheif, 2021), signalling molecules flow (Chandra et al., 2018), regulation of differential gene expression (Friedman &

Gordon, 2016), including epigenetic mechanisms (Yan et al., 2014). More food or higher-quality food causes the larva to develop into a queen, whereas less or lower-quality food leads to worker development (Psalti & Libbrecht, 2020).

The relationships between nutritional status and worker division of labour in polymorphic ant species with morphologically distinct worker subcastes are relatively well studied. In particular, for polymorphic *Pheidole* and *Pogonomyrmex* ants, minor and major worker subcastes are determined by a JH-mediated threshold during the larval stage in response to food, when soldiers receive a highly proteinaceous diet (Abouheif, 2021; Smith & Suarez, 2010).

Still, almost nothing is known about the role of nutritional factors affecting worker division of labour within colonies of monomorphic species lacking morphologically distinct subcastes. An exception is the widespread pattern of age-related lipid loss in workers during the transition from inside to outside tasks ('corpulent nurse-lean forager' pattern; Blanchard et al., 2000; Porter & Jorgensen, 1981). This pattern has been observed in species from different subfamilies with diverse food preferences (Porter & Jorgensen, 1981; Silberman et al., 2016; Tschinkel, 1998). Furthermore, in monomorphic *Pogonomyrmex owheeyi*, defenders and refuse workers exhibited intermediate energy reserves between those of corpulent nurses and lean foragers, with a shortened life expectancy in the latter, presumably due to starvation mortality and environmental stress (Porter & Jorgensen, 1981). The accumulation of lipid reserves by the ant colony varies during the year and precedes periods of limited feeding (e.g., overwintering) and producing sexual alates (Kwapich & Tschinkel, 2013; Tschinkel, 1998). It remains poorly understood whether monomorphic workers belonging to different task groups can vary seasonally in lipid balance, trophic position and diet. In this study, we focus on the links between task-specific behaviour and the nutritional status of individual workers in monomorphic ant species with sophisticated social organization.

Red wood ants (*Formica rufa* group) are among the most functionally important and well-studied keystone species of forest ecosystems in North Eurasia (Stockan & Robinson, 2016). Colonies of these ants, numbering up to 1–2 millions nestmates, display a highly social mode of life and worker specialization based primarily on their behavioural traits (Reznikova, 2008, 2020; Rosengren & Sundström, 1987; Stockan & Robinson, 2016) but the considerable variation in worker size may be linked to their task specialization, for example, honeydew foragers are typically smaller than protein foragers and nest builders (Véle & Modlinger, 2019; West & Purcell, 2020). Deep task specialization is associated with behavioural and cognitive traits (Iakovlev & Reznikova, 2019; Reznikova & Ryabko, 2011). Workers of red wood ants display spatial fidelity and temporal consistency in task preference. Inner-nest task groups are represented by nurses, nest builders and reserve foragers, while out-nest task groups include hunters and collectors of nest material acting on the ground, honeydew collectors within the tree crowns, carriers of brood/workers and guards on the nest surface (Dlussky, 1967; Dobrzanska, 1958; Horstmann, 1972; Otto, 1958; Rosengren & Sundström, 1987). Age polyethism is common when young workers perform inner-nest tasks and adult workers specialize in out-nest tasks (Rosengren, 1977). For ants of the genus *Formica*, there is contradictory data on the age-related 'corpulent nurse-lean forager' pattern. A study on *Formica fusca* showed that foragers and nurses have a similar fat content (Silberman et al., 2016). However, in an early study by Kirchner (1964) on *Formica polyctena*, young workers (apparently nurses) showed a higher fat content than older workers (apparently foragers). This is most pronounced before and after overwintering in spring (Kirchner, 1964), when ants are seen sunbathing on the nest (Kadochová & Frouz, 2013). It remains unclear which workers perform sunbathing behaviour and how consistently, although there are indications that nest heating may be performed by corpulent young workers (Martin, 1980; Rosengren et al., 1987). The hypothesis that sunbathing workers in wood ants belong to inner-nest task group of nurses should be tested. Beyond that, there is no data on the differences in nutritional status, for example, abdominal fat content, between out-nest task groups in *Formica* ants.

Like many other *Formica*, red wood ants are omnivores, collecting a variety of arthropods along with vast amounts of sugar secretions (Skinner, 1980; Stockan & Robinson, 2016). These ant species occupy the trophic position of a first-order predator, as estimated by δ^{15}N values (Iakovlev et al., 2017; Mooney & Tillberg, 2005). Seasonal variations in δ^{13}C and δ^{15}N values of *Formica aquilonia* workers are associated with the dietary switching from tree sap collection in the spring to the consumption of aphid honeydew in late summer (Iakovlev et al., 2017). Excess food is stored as fat reserves in the bodies of workers in autumn that are used during brood production in spring, being at their minimum in summer (Kirchner, 1964; Kondoh, 1968). It remains unclear to what extent the seasonal life cycle of wood ant colonies is reflected in the nutritional status of different task groups of workers.

In this study, the following questions were addressed to examine trophic factors related to the task specialization of *F. aquilonia* workers: (i) Is there a 'corpulent nurse-lean forager' pattern in *F. aquilonia*? (ii) Do task groups collected on the nest mound, ground surface and foraging trees have different fat reserves? (iii) Do task groups differ in the diet and trophic level? As red wood ants exhibit seasonal dynamics in feeding and nutrient storage, these assumptions were tested using data collected in spring, summer and autumn. We collected samples from four *F. aquilonia* Yarrow colonies in a mixed forest in Western Siberia. We compared the nutritional status of workers from four spatial zones of colony activity: inside the nest (nursing), on the nest surface (thermoregulation in spring and guarding in summer and autumn), on the ground surface (foraging for protein food) and on the trees (foraging for carbohydrate food).

Elemental and stable isotope carbon and nitrogen composition of ant tissues is commonly used as a proxy of temporal and spatial variation in the diet, trophic position, protein and fat content between and within ant species (Balzani et al., 2021; Davidson, 2005; Kay et al., 2006; Smith & Suarez, 2010). The following indicators of nutritional status were used to answer the research questions. In the ant abdomen, the fat body is well developed, and the high C:N ratio and low δ^{13}C value imply high content of lipids (fat) (Feldhaar et al., 2010). Additionally, abdomen:thorax dry mass ratio indicates corpulence in ant workers (Blanchard et al., 2000). The isotopic composition of the ant thorax, which mostly consists of muscles, indicates food sources (δ^{13}C value), the trophic level (higher δ^{15}N value with a more predatory diet; Menke et al., 2010; Tillberg et al., 2006) and possible starvation (higher $\delta^{15}N$ values in starving insects; Doi et al., 2017).

MATERIALS AND METHODS

Study site

The study was conducted in the Novosibirsk region, Western Siberia, Russia, in 2011. The study site (N 5500.586, E 8318.460) was a mixed birch forest with pine, spruce, willow, bird cherry, undergrowth of aspen. Herbaceous layer was composed mainly of *Aegopodium podograria*, *Dactylis glomerata*, *Maianthemum bifolium* and *Crepis sibirica*. Some myrmecochorus plants (*Viola* sp.) occasionally occur at the edges of the studied forest area, but they were not observed in the foraging territory of the studied ant colonies. Four monodomous ant colonies of similar population size (approximately 10^6 individuals each) and territory size (approximately 0.2 ha each) were selected. The vegetation cover was similar in all four foraging areas. Most aphid colonies tended by *F. aquilonia* belonged to four species: *Symydobius oblongus* on *Betula pendula* and *Chaitophorus populeti* on *Populus tremula* from May to September, and *Chaitophorus* cf. *ramicola* on *Salix* sp. and *Aphis fabae* on *C. sibirica* from July to September. Diptera, Coleoptera, spiders, aphids and other hemipterans dominated ant prey. See Iakovlev et al. (2017) for detailed data on the trophic relationships of the studied *F. aquilonia* colonies.

Collection of task groups

Based on data on the division of labour and annual life cycle in red wood ants (Dlussky, 1967; Kadochová & Frouz, 2013; Rosengren & Sundström, 1987; Stockan & Robinson, 2016; Zakharov & Zakharov, 2022), we identified four spatial zones of worker activity on the territory of *F. aquilonia* colonies: inside the nest, on the nest surface, on the foraging ground and on foraging trees. We then selected one of the most abundant task groups predominantly present in a given spatial zone at a given time of year (e.g., on the ground surface in summer, hunters were selected, but not carriers of nestmates). Task groups may not always be strictly bound to a single spatial zone, and members of different task groups may be present in the same zone simultaneously (e.g., honeydew foragers and hunters follow the same trails to the nest). However, only in a specific spatial zone can the specialization of workers be determined with certainty before sampling (e.g., nest guards and sunbathing workers on the mound). At different times, ants sampled in the same spatial zone may belong to different task groups due to seasonal differences in *F. aquilonia* colony life. The groups of workers collected in the spring differed from those collected in the summer and autumn (see below). This influence was accounted for in the data analysis by including the two main effects, spatial zone and season, and the effect of their interaction in the statistical models. Thus, the study focused on comparing workers from different spatial zones within each season and comparing seasons across all data, rather than examining data averaged across all seasons for individual spatial zones.

For each ant colony, five workers were collected in each of the four spatial zones three times during the growing season: in spring (18–26 April), summer (19–27 July) and autumn (5 September). In total, 60 workers per colony were collected. All samples intended for stable isotope analysis were frozen at −20°C within 6 h of collection.

Spatial zones of worker activity:

1. Inside the nest. Inner-nest workers were collected in the nest mound at a depth of about 30 cm from the surface. In summer and autumn, ants with pupae and nest material were placed in a container with artificial shelters, and after 6 h, only workers that carried brood into the shelters when disturbed (hereafter referred to as ‘nurses’) were sampled. In spring, when no brood was observed in the samples, inner-nest workers were randomly selected. Recently emerging workers with light-coloured bodies (callows) were excluded to avoid significant age differences.
2. Surface of the nest. In summer and autumn, we selected workers actively guarding the nest (hereafter, ‘guards’). This was done by presenting a needle to the ants above the mound surface and selecting individuals that performed a mortal grip for more than 30 s. In spring, workers forming sunbathing warming spots on the mound surface (hereafter, ‘sunbathing workers’) were selected. To test the additional hypothesis that sunbathing workers belong to inner-nest nurse workers, and in line with the study design, we analysed data on sunbathing workers separately from data on workers collected inside the nest (presumably nurses) in spring samples.
3. Ground surface. Ground foragers were individuals engaged in foraging and transporting prey to the nest. In summer and autumn, ants were collected from those carrying prey to the nest along foraging trails at a distance of 3–5 m from the nest (hereafter ‘hunters’). In spring, when prey was scarce and foraging trails were not yet established, workers were collected from individuals actively moving on the ground 3–5 m from the nest.
4. Foraging trees. Tree foragers were sampled from workers engaged in collecting carbohydrate excretions. In summer and autumn, ants were collected from colonies of the aphids *Symydobius oblongus* on birch and *Chaitophorus populeti* on aspen (hereafter ‘honeydew foragers’). For this purpose, twigs of forage trees with aphids were cut, and all ants were removed. Workers were randomly selected without separation into those that carry honeydew, milking aphids and guarding aphids (Novgorodova, 2015; Reznikova & Novgorodova, 1998). In spring, when aphid colonies had not yet emerged and birch sap was an available carbohydrate source, we collected foragers descending from sap-producing birch trees with enlarged abdomens. The foraging trees from which ants were collected were located 5–10 m from the nest.

Stable isotopic and elemental composition

To identify different indicators of the nutritional status of the ants, the isotopic and elemental composition of C and N were determined separately for the thoracic and abdominal body parts (Table 1). The thorax of workers, including the legs, was analysed for four colonies of *F. aquilonia* ($n = 240$, five individuals from four spatial zones of the colony three times per year), and the abdomen was analysed for two colonies ($n = 120$). Ant tissues were analysed individually, except for honeydew foragers belonging to two colonies. Due to their small mass, these samples consisted of the thoraxes of two specimens.

It was assumed that apart from the integuments, muscles are the main tissues of the thorax in ant workers, and that the fat body is the main tissue of the abdomen. To a lesser extent, haemolymph, tracheae and the parietal fat body are represented in the thorax, and the crop content, gut and ovaries are presented in the abdomen (Chapman, 1998).

High abdominal fat content in workers is indicated by a high abdominal C:N ratio and a low abdominal $\delta^{13}C$ value (Tillberg et al., 2006), as lipids are depleted in ^{13}C (DeNiro & Epstein, 1978; Potapov et al., 2019). Additionally, the abdomen:thorax dry mass ratio was considered an indicator of fat reserves in worker ants, as a major component of abdominal dry mass is lipids stored in the fat body (Blanchard et al., 2000). In ants, the $\delta^{15}N$ values of the abdomen are lower than those of the thorax, due to feeding on ^{15}N-depleted carbohydrate liquid food (Blüthgen et al., 2003; Iakovlev et al., 2017). The variability in $\delta^{15}N$ values of the abdomen of *F. aquilonia* workers also reflects the isotopic composition of undigested food in the crop.

Isotopic composition of the thorax was used to assess consistent differences in food sources and the trophic level of workers (Table 1). The carbon isotopic signature of ants can be used to infer the $\delta^{13}C$ value of primary food sources due to the low trophic fractionation of ^{13}C, estimated to be 1.1‰ in ants (Feldhaar et al., 2010). In addition, variation in thoracic $\delta^{13}C$ values in ants may be associated with variation in parietal fat content (Tillberg et al., 2006). Thus, thoracic $\delta^{13}C$ values and C:N ratios are expected to be negatively correlated.

Thoracic $\delta^{15}N$ values were used to assess the trophic level because of pronounced trophic fractionation of ^{15}N, estimated to be 3.0‰ per each trophic level in ants (Feldhaar et al., 2010). Furthermore, ants show seasonal and caste-specific variation in $\delta^{15}N$ value, indicating temporal variation in the diet, with lower $\delta^{15}N$ values in ant colonies with access to honeydew (Menke et al., 2010; Tillberg et al., 2006). In addition, we considered the possible effect of starvation on $\delta^{15}N$ values in ants. Starvation increases $\delta^{15}N$ values in insect tissues, indicating a dependence on internal nitrogen recycling during prolonged fasting (Doi et al., 2017; Scrimgeour et al., 1995). Due to incomplete data on the effect of starvation on nitrogen isotope composition in invertebrates (Doi et al., 2017; Martinez del Rio et al., 2009), high $\delta^{15}N$ combined with a low fat content (low C:N ratio, high $\delta^{13}C$ value, low abdomen:thorax dry mass ratio) can be considered a more reliable sign of starvation in ant workers (Table 1).

TABLE 1 Stoichiometric and isotopic indicators used for assessing nutritional status in ant workers.

Body parts	Indicators	Nutritional traits
Abdomen	High C:N ratio Low $\delta^{13}C$ value High abdomen:thorax dry mass ratio	High fat content
	Variation in $\delta^{15}N$ values	Variations in crop content (undigested food)
Thorax	High $\delta^{15}N$ value	High trophic level, Starvation
	Variation in $\delta^{13}C$ values Low $\delta^{13}C$ value High C:N ratio	Variation in food resources, High fat content
Pupa	$\delta^{15}N$ value $\delta^{13}C$ value C:N ratio	Larval diet

Sample preparation included drying at 50°C for 2–3 days, weighing (400–600 μg) and packing in tin capsules. Stable isotope and elemental analyses were conducted using a Thermo Delta V Plus continuous-flow IRMS coupled with an elemental analyser (Thermo Flash 1112) at the Joint Usage Center at the Institute of Ecology and Evolution RAS (Moscow). The isotopic composition of N and C was expressed in a δ-notation relative to the international standard (atmospheric nitrogen and VPDB, respectively): $\delta X(‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$, 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 $\delta^{15}N$ and $\delta^{13}C$ values of the reference materials ($n = 8$) were <0.2‰. Along with isotopic analyses, nitrogen and carbon contents (as mass%) and C:N ratio were determined in all samples.

Effects of larval diet

Data on the time required for achieving isotopic equilibrium in ant tissues and the extent to which $\delta^{13}C$ and $\delta^{15}N$ values of workers reflect their adult or larval diet are scarce. The shift in $\delta^{15}N$ values of workers in response to the experimental diet occurred within 10 weeks in *Linepithema humile* (Menke et al., 2010), whereas the shift in $\delta^{15}N$ and $\delta^{13}C$ values of *Solenopsis invicta* brood and workers in response to the dietary changes was observed within 2 weeks (Barriga et al., 2013).

To assess the influence of larval feeding, the isotopic signatures of mature workers and pupae of *F. aquilonia* were compared. Based on data for *Lasius alienus* and *Aphaenogaster rudis* (Tillberg et al., 2006), we suggested that the isotopic composition of worker pupae would be intermediate between that of the thorax and abdomen of mature workers. To test this, a comparison was performed on a single colony of *F. aquilonia*. Pupae were collected in the summer (21 July). Considering it takes 1–3 weeks to develop from pupa to nurse and 6–9 weeks from pupa to forager (Otto, 1958), workers were collected in summer (21 July) and autumn (5 September): five individuals each of nurses, nest guards, hunters and honeydew foragers. Whole pupae were analysed (head, thorax with legs and abdomen together) without indusium ($n = 12$). For workers, the thorax ($n = 40$) and abdomen ($n = 40$) were analysed separately.

Effects of body size

The wide variation in worker size in red wood ants may be associated with task preferences (Batchelor et al., 2012; Véle & Modlinger, 2019). For example, foragers for proteins are larger than foragers for carbohydrates (Dlussky, 1967; Véle & Modlinger, 2019). To assess the possible influence of worker size on their specialization and nutritional status, we estimated worker body size from the dry mass of the thorax (with legs), based on the known correlation between dry body mass (whole body, thorax only or thorax with head) and linear body size in different ant species (Blanchard et al., 2000; Helms & Kaspari, 2015; Kaspari & Weiser, 1999), including the genus *Formica* (Kondoh, 1968, 1969). The same samples of workers from four *F. aquilonia* colonies were used for stable isotope analysis. Body parts were dried at 50°C for 2–3 days and then weighed on a Mettler Toledo XP6 microbalance with an accuracy of 2 μg.

Data analysis

Mean δ^{13}C and δ^{15}N values of the most abundant plant species (leaves, leaf litter) are typically used as a baseline in isotopic studies of litter-dwelling macroinvertebrates, including ants (Korobushkin et al., 2014). To establish the isotopic baseline, four mixed samples of overwintered birch leaf litter were collected from the foraging area of each ant colony. The stable isotope composition of birch litter did not differ among 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.

Variation in δ^{13}C, δ^{15}N, C:N ratio and dry mass was compared across four spatial zones and three seasons using a multivariate ANOVA accounting for interactions between these two main effects, separately for abdomen and thorax samples. Outliers lying beyond the 1.5 interquartile range of the first or third quartiles were excluded from the analysis. Tukey's HSD test was used for post hoc comparisons. Differences between ant colonies in the isotopic, elemental and mass parameters were insignificant except for the δ^{15}N value of thorax (Table S1). Interactions between the colony and main effects (season, spatial zone) were insignificant or marginally significant and were therefore not included in the analysis.

Linear regression and Pearson's correlation coefficients were used to determine the relationships between dry mass, isotopic and elemental composition. Means and 95% confidence intervals are reported. All analyses were performed using SPSS 22.0 (IBM, Armonk, NY, USA).

RESULTS

C:N ratio, δ^{13}C and δ^{15}N values and dry mass of abdomen

As expected, the C:N ratio of abdominal tissues was negatively correlated with abdomen δ^{13}C value ($r = -0.84$, $p < 0.0001$, $n = 120$; Figure S1) and positively correlated with abdomen:thorax dry mass ratio ($r = 0.85$, $p < 0.0001$, $n = 120$; Figure S1). These three indicators of fat reserves in *F. aqilonia* ants varied significantly depending on the season (C:N ratio: $F_{2,119} = 37.0$, $p < 0.0001$; δ^{13}C value: $F_{2,119} = 21.1$, $p < 0.0001$; abdomen: thorax dry mass: $F_{2,237} = 43.8$, $p < 0.0001$) and spatial zone of workers (C:N ratio: $F_{3,119} = 35.2$, $p < 0.0001$; δ^{13}C value: $F_{3,119} = 21.0$, $p < 0.0001$; abdomen: thorax dry mass: $F_{3,237} = 58.8$, $p < 0.0001$).

The lowest fat reserves of workers, that is, the lowest C:N and mass abdomen:thorax ratios and the highest δ^{13}C values, were observed in summer, being higher in spring, and highest in autumn (Figure 1). The abdomen:thorax dry mass was twofold higher in the inner-nest workers compared to foragers, while this ratio for workers sunbathing on the nest was similar to that of inner-nest workers in spring but for nest guards this ratio was similar to that of foragers in summer and autumn (interaction between season and spatial zone of task group for the abdomen:thorax dry mass ratio: $F_{6,237} = 7.9$, $p < 0.0001$; Figures 1c and S2). In spring, the C:N ratio was higher in inner-nest workers compared to foragers (Figure 1a), and the δ^{13}C value of inner-nest and sunbathing workers was lower than that of sap foragers (Figure 1b). In summer, the only difference between the task groups was the lower δ^{13}C values in the nurses compared to honeydew foragers (Figure 1). In autumn, nurses differed from other task groups with a doubled C:N ratio and lower δ^{13}C values (Figure 1). In autumn, honeydew foragers had higher δ^{13}C values than hunters (Figure 1b; interaction between season and spatial zone of task group: C:N ratio, $F_{6,119} = 12.3$, $p < 0.0001$; δ^{13}C value, $F_{6,119} = 3.5$, $p = 0.004$).

Abdominal δ^{15}N values were 0.6‰ lower than thoracic δ^{15}N values (4.9 ± 0.1 vs. 5.5 ± 0.1; $p < 0.0001$). Higher δ^{15}N values in the abdomen were found in spring compared to autumn, with intermediate values in summer ($F_{2,119} = 8.5$, $p < 0.0001$; Figure S3a). Tree and ground foragers differed from inner-nest workers on average by a higher abdominal δ^{15}N value (5.1 ± 0.2 and 5.0 ± 0.2 vs. 4.7 ± 0.2; $F_{3,119} = 4.2$, $p = 0.007$), with intermediate values in guards and sunbathing workers (4.9 ± 0.2) (Figure S3b). Differences between spatial zones of task groups were independent of season ($F_{6,119} = 1.1$, $p = 0.3$).

δ^{15}N and δ^{13}C values and C:N ratio of thorax

Regarding intercolonial differences ($F_{3,225} = 63.0$, $p < 0.0001$; Table S1, Figure S4), the mean thorax δ^{15}N value was similar between seasons ($F_{2,225} = 1.9$, $p = 0.15$; Figure 2a). In spring, ground foragers differed from inner-nest workers in their higher thorax δ^{15}N value, but in summer and autumn, the δ^{15}N values of all worker groups were similar (interaction between the season and spatial zone of task group: $F_{6,225} = 3.1$, $p < 0.01$; Figure 2a). In spring, thoracic δ^{15}N values were negatively correlated with the abdomen:thorax dry mass ratio ($r = -0.46$, $p < 0.005$, $n = 80$), and the C:N ratio of the abdomen ($r = -0.66$, $p < 0.0001$, $n = 40$) and thorax ($r = -0.29$, $p < 0.01$, $n = 80$).

Mean thoracic δ^{13}C values were higher in spring and summer than in autumn ($F_{2,225} = 10.9$, $p < 0.0001$; Figure 2b). Foragers had,

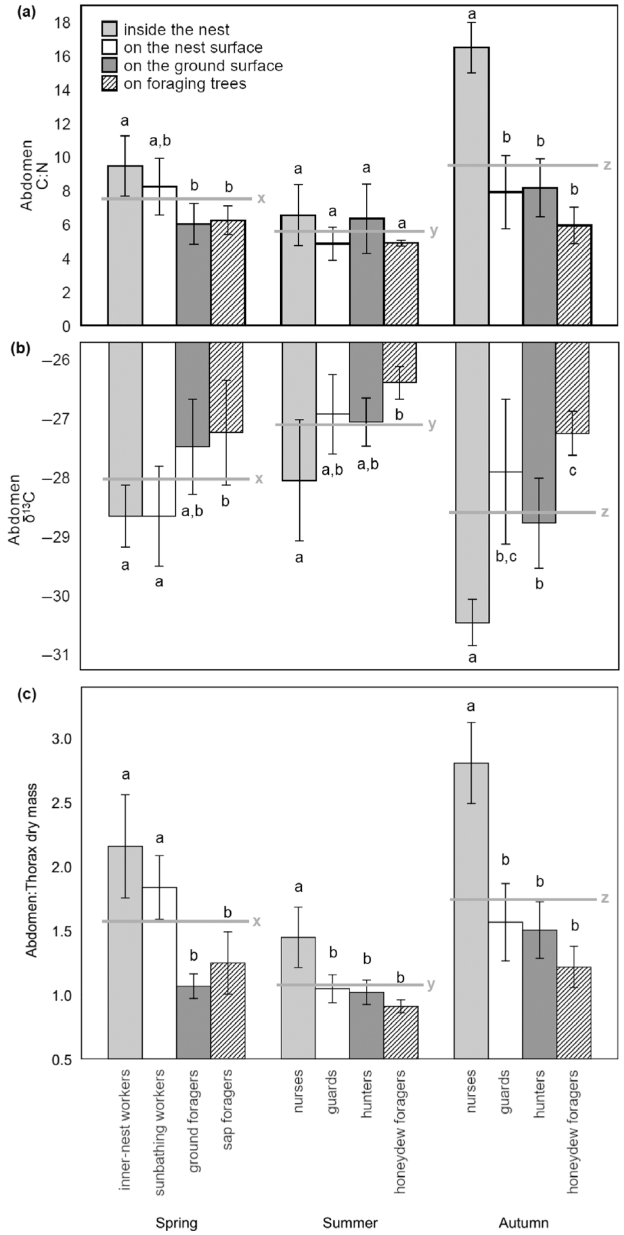

FIGURE 1 Seasonal variation (mean and 95% CI) in the abdominal C:N ratio (a), carbon isotope composition (b), and abdomen: thorax dry mass ratio (c) of *Formica aquilonia* task groups from four spatial zones. Different letters indicate significant differences between worker groups within each season (Tukey's HSD, $p < 0.05$, $n = 10$ for C:N and $\delta^{13}C$, $n = 20$ for abdomen: thorax dry mass). The grey lines depict mean seasonal values, while grey letters indicate a significant difference between seasons (Tukey's HSD, $p < 0.05$, $n = 40$ for C:N and $\delta^{13}C$, $n = 80$ for abdomen: thorax dry mass).

on average, higher thoracic $\delta^{13}C$ values than inner-nest workers ($F_{3,225} = 37.6$, $p < 0.0001$). Sunbathing workers were similar to inner-nest workers in spring, whereas guards were closer to foragers in summer and autumn (interaction between season and spatial zone of task group: $F_{6,225} = 8.9$, $p < 0.0001$, Figure 2b). Thoracic $\delta^{13}C$ value correlated with thorax C:N ratio ($r = -0.94$, $p < 0.0001$, $n = 239$), abdominal $\delta^{13}C$ value ($r = 0.75$, $p < 0.0001$, $n = 120$) and abdomen:thorax dry mass ratio ($r = -0.47$, $p < 0.0001$, $n = 120$).

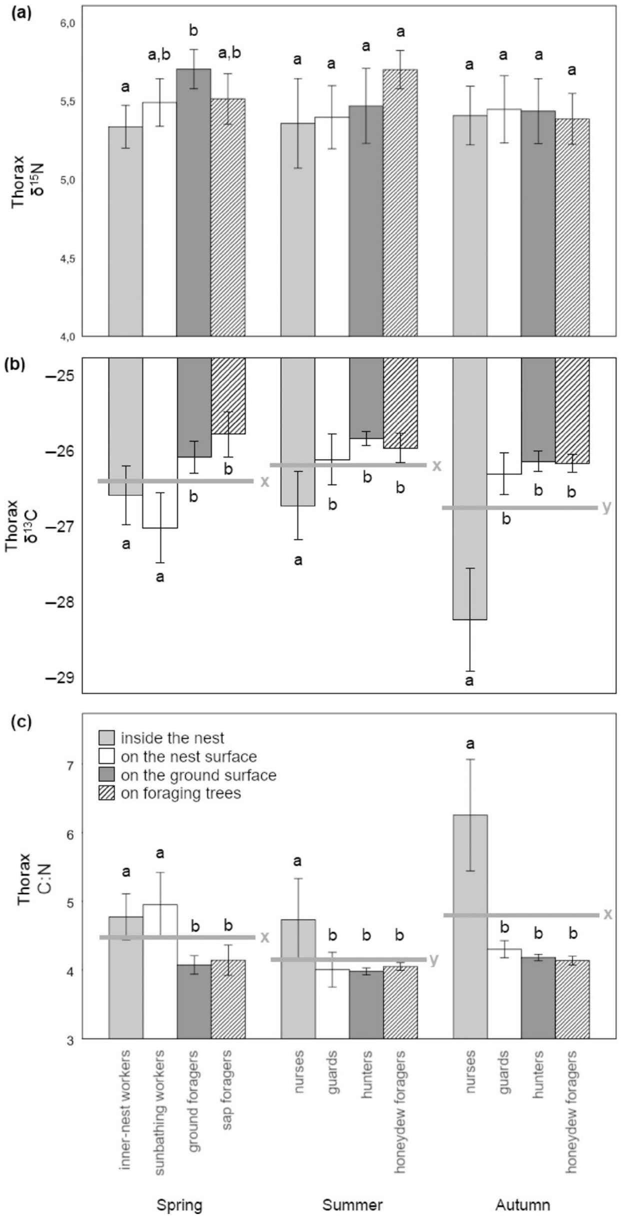

FIGURE 2 Seasonal variation (mean and 95% CI) in the thoracic nitrogen (a) and carbon (b) isotope composition and (c) thorax C:N ratio of task groups from four spatial zones. Different letters indicate significant differences between worker groups within each season (Tukey's HSD, $p < 0.05$, $n = 20$). The grey lines depict mean seasonal values, while grey letters indicate a difference between seasons (Tukey's HSD, $p < 0.05$, $n = 80$).

The C:N ratio in thoracic tissues was higher in inner-nest workers than in other task groups ($F_{3,225} = 32.2$, $p < 0.0001$), and on average higher in spring and autumn than in summer ($F_{2,225} = 9.2$, $p < 0.0001$; Figure 2c). The thorax C:N ratio of workers sunbathing on the nest was similar to that of inner-nest workers in spring, whereas the thorax C:N ratio of nest guards was similar to that of foragers in summer and autumn (interaction between season and spatial zone of task group: $F_{6,225} = 4.2$, $p < 0.0001$, Figure 2c). Thoracic C:N ratio correlated with abdominal C:N ratio ($r = 0.73$, $p < 0.0001$, $n = 120$), abdomen $\delta^{13}C$

value ($r = -0.66$, $p < 0.0001$, $n = 120$) and abdomen:thorax dry mass ratio ($r = 0.49$, $p < 0.0001$, $n = 120$).

Comparison of pupae and mature workers

Worker pupae had lower mean δ^{15}N values compared to the body parts of mature workers (4.1 ± 0.1, 4.5 ± 0.2, and 5.1 ± 0.1‰ for pupae, abdomen and thorax, respectively; $F_{2,89} = 39.7$, $p < 0.0001$; Figure 3a, Figure S5). The δ^{15}N values of the thorax and abdomen of honeydew foragers were higher than those of pupae ($F_{8,83} = 12.7$, $p < 0.0001$; Figure 3a). For nurses, guards and hunters, abdominal δ^{15}N values were similar to those of pupae, with higher values in the thorax (Figure 3a).

The δ^{13}C values of pupae were similar to those of the thorax and abdomen of mature workers, with lower values in the abdomen than in the thorax (−27.2 ± 0.2, −28.0 ± 0.6 and −26.5 ± 0.4‰, respectively; $F_{2,89} = 10.9$, $p < 0.0001$; Figures S5 and 3b). The δ^{13}C values of the thorax and abdomen of nurses were lower than those of pupae ($F_{8,83} = 25.2$, $p < 0.0001$; Figure 3b). In other task groups, the δ^{13}C values in the abdomen and thorax were similar to those of pupae (Figure 3b).

Pupae and thoracic tissues of mature workers had lower C:N ratios compared to abdominal tissues (5.6 ± 0.4, 4.8 ± 0.4, and 7.9 ± 1.3‰, respectively; $F_{2,89} = 12.0$, $p < 0.0001$). Among the task groups, nurses differed from pupae by a higher abdomen C:N ratio ($F_{8,83} = 17.1$, $p < 0.0001$; Figure 3c).

Effects of body size

F. aquilonia workers had a sevenfold difference in thorax dry mass (range 184–1341 μg, mean 590 ± 30 μg). The thorax dry mass depended on the season ($F_{2,223} = 40.3$, $p < 0.0001$) and spatial zone of task group ($F_{3,223} = 4.4$, $p = 0.005$). In general, workers were larger in spring than in summer and autumn, while the difference between spatial zones depended on the season ($F_{6,223} = 7.5$, $p < 0.0001$; Figure 4). In spring, sunbathing workers and tree foragers were larger than inner-nest workers. In summer, hunters were larger than honeydew foragers. In autumn, nurses and guards were larger than honeydew foragers (Figure 4). To examine the interaction of body size and task effects on the fat reserves of honeydew foragers in the autumn (Figure 1b), hunters of small size (thorax dry mass ranged from 204 to 484 μg) were used for comparison. In autumn, honeydew foragers had higher abdomen δ^{13}C values than small-sized hunters (−27.1 [−27.7; −26.8]‰ ($n = 10$) and − 28.5 [−28.9; −27.7] ($n = 6$), respectively; $U = 8$, $p = 0.017$, Mann–Whitney *U* test) and, at the tendency level, lower abdomen C:N ratios (5.5 [5.0; 6.5] μg ($n = 10$) and 6.8 [6.1; 8.5] ($n = 6$), respectively; $U = 14$, $p = 0.083$, Mann–Whitney *U* test), although differences in body size were expressed (347 [306; 408] μg ($n = 20$) and 411 [379; 447] μg ($n = 10$), respectively; $U = 44$, $p = 0.013$, Mann–Whitney U test).

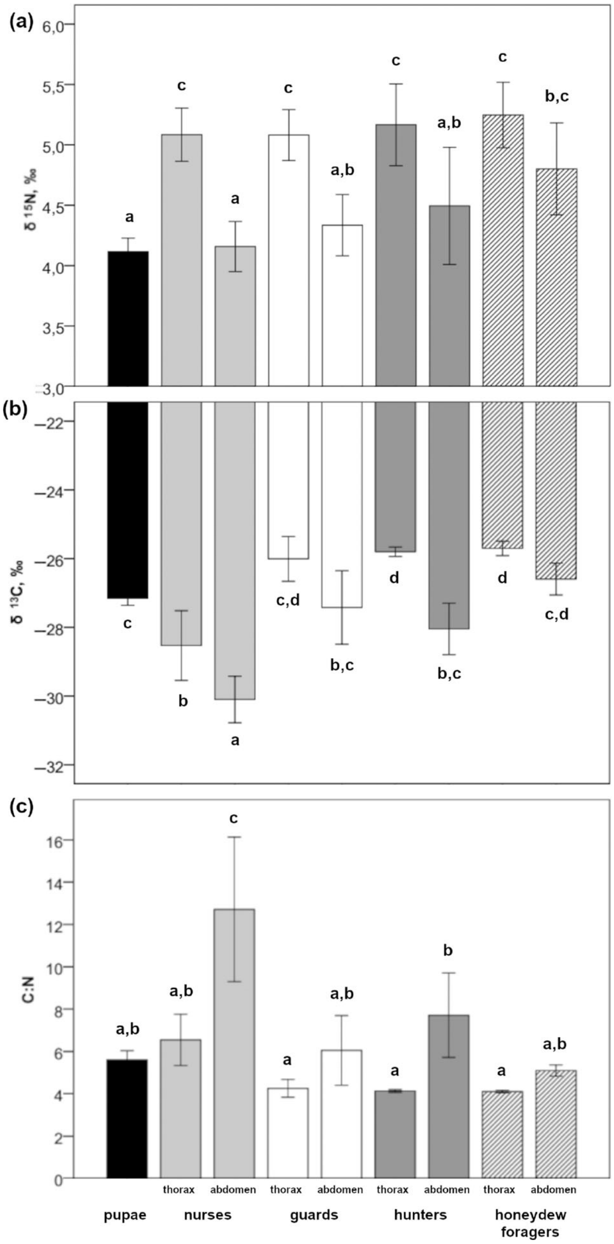

FIGURE 3 Comparison of worker pupae and adult workers of different task groups by the nitrogen (a) and carbon (b) isotope composition and (c) C:N ratio. Whole pupae (collected in summer) were analysed (without indusium). Thorax (with legs) and abdomen of adult workers (collected in summer and autumn) were analysed separately. Different letters indicate significant differences across all samples (Tukey's HSD, $p < 0.05$, $n = 12$ for pupae, $n = 10$ for body parts of adult workers). Mean and 95% CI are shown.

Significant correlations were found between thoracic dry mass and C:N ratios of the abdomen and thorax ($r = 0.23$, $p < 0.05$, $n = 117$ and $r = 0.30$, $p < 0.0001$, $n = 227$, respectively), and δ^{13}C values of abdomen and thorax ($r = -0.32$, $p < 0.05$, $n = 117$ and $r = -0.29$, $p < 0.0001$, $n = 227$, respectively). Although the correlation coefficients were small, there was a tendency for fat content to be higher in larger workers.

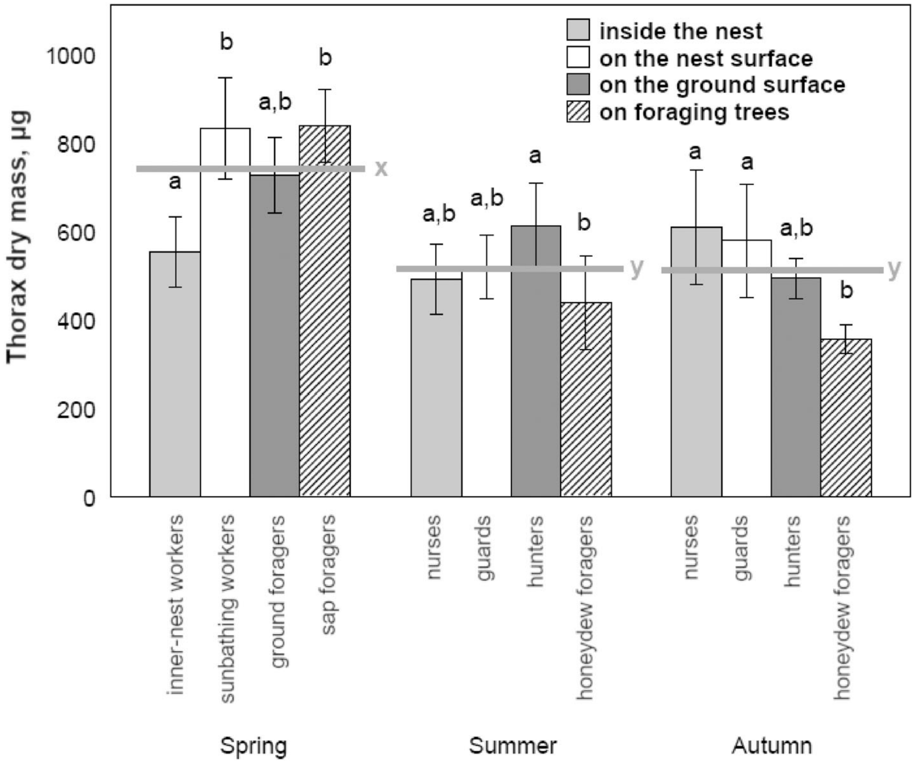

FIGURE 4 Seasonal variation (mean and 95% CI) in the dry mass of the thoraxes of various task groups from four spatial zones. Different letters indicate significant differences between groups within each season (Tukey HSD, $p < 0.05$). The grey lines depict mean seasonal values, while grey letters indicate a difference between seasons (Tukey HSD, $p < 0.05$).

DISCUSSION

Based on the elemental and isotopic composition of *F. aquilonia* ant tissues, the following task-specific differences in worker nutritional traits were identified: (1) The presence of 'corpulent nurse-lean forager' pattern in red wood ants was confirmed. Indicators of fat content in the abdomen and thorax showed that this pattern persisted throughout the season, with a decrease in colony energy reserves in the summer. The maximum differences observed in these indicators between workers in autumn are explained by the fat accumulation prior to overwintering, mainly in the bodies of nurses. In addition, differences in crop content between nurses and foragers were indicated by abdominal $\delta^{15}N$ values. (2) Workers sunbathing on the nest surface in spring are as corpulent as workers inside the nest (presumably nurses), and later in the year, guards located on the nest surface are as lean as foragers. (3) Fat reserves were lower in honeydew foragers than in ground foragers (hunters) in autumn. (4) The task groups had similar thoracic $\delta^{15}N$ values, that is, occupied a similar trophic level, except for higher thoracic $\delta^{15}N$ values in spring in ground foragers. Together with low fat reserves, this may indicate starvation in the active workers restoring the foraging territory of the colony after wintering. This suggests that workers may store fat and starve to varying degrees, depending on the season and task specialization.

Limitations of the study design include differences in the collection technique of ants in late April compared to collections in mid-July and early September. This is due to the early onset of activity and the seasonal change in the composition of task groups in red wood ants, which occurs after nest repair and heating and the production of sexual offspring (from early April to mid-May) when colony status and environmental conditions (scarcity of prey and aphids) do not require the allocation of workers to 'summer' task groups (nest guards, honeydew collectors and hunters; Kirchner, 1964; Rosengren & Sundström, 1987; Zakharov & Zakharov, 2022). Therefore, the data analysis primarily focused on comparing groups of workers within each season. Adding the interaction of season and spatial zone effects to the MANOVA allowed us to account for seasonal differences in ant collection methods. This interaction was significant for almost all fat content-related variables. It was driven by different workers presented on the mound surface in spring (corpulent sunbathing ants) versus summer and autumn (lean guards). Although we are not entirely sure whether the workers randomly collected inside the nest in late April were engaged in brood care, as were the nurses collected in the summer and autumn. The high fat content of the former rules out the possibility that foragers were collected by mistake, but allows for similarities in tasks with sunbathing workers (see discussion below). The temporal stability of *F. aquilonia* forager behaviour appears to be high, both on the ground surface and in trees. In red wood ants, two distinct task groups are responsible for foraging for spatially and temporally stable carbohydrate food (honeydew of aphids on trees) and unpredictable protein food (prey randomly distributed mainly on the forest floor). There are indications that these differences may persist in spring and summer (Rosengren &

Sundström, 1987; Sundström, 1993; Zakharov & Zakharov, 2022). It remains to be studied whether tree foragers switch from sap collection in April to honeydew collection in May and whether active ground workers, that re-establish foraging territory and recruit nestmates in April, switch to prey foraging on the ground later.

A 'corpulent nurse-lean forager' pattern in *F. aquilonia*

The high abdominal C:N ratio, low abdominal $\delta^{13}C$ value and high abdomen:thorax dry mass ratio in inner-nest workers randomly collected inside the mound in spring and those acting as nurses of brood in summer and early autumn indicate their higher fat reserves than those of foragers (Figure 1). The differences in abdominal fat content between nurses and foragers in *F. aquilonia* are observed in spring, summer and autumn. This suggests a common ontogenetic mechanism for the reduction of fat reserves in more mature workers (Blanchard et al., 2000; Tschinkel, 1998) that transit from nest tasks to foraging at about 40 days of age (Otto, 1958; Rosengren, 1977). This is consistent with the higher abdominal fat content of young workers than old workers of *F. polyctena* (Kirchner, 1964) and supports the existence of a 'corpulent nurse-lean forager' pattern in red wood ants.

Furthermore, based on the isotopic composition of pupae, which reflects the larval diet of workers, evidence was obtained for an ontogenetic increase in fat stores in nurses of *F. aquilonia*. As expected, pupal $\delta^{13}C$ values were between thoracic and abdominal values of workers, except nurses, which had 1.3 and 2.8‰ lower thoracic and abdominal $\delta^{13}C$ values, respectively, compared to pupae (Figure 3). This indicates higher fat reserves in nurses than in younger callow workers. Similarly, in *Leptothorax albipennis*, callows had intermediate lipid reserves, on average higher than foragers and lower than corpulent nurses (Blanchard et al., 2000). In red wood ants, the high fat content in nurses is probably related to brood feeding via the production of trophic eggs and secretions from the labial and postpharyngeal glands (Billen, 1982).

A decrease in fat reserves is an intrinsic mechanism that triggers the transition of workers to foraging in eusocial Hymenoptera (Bernadou et al., 2020; Toth & Robinson, 2005; Tschinkel, 1998). The accumulation of colony energy stores in the bodies of inner-nest workers appears to be an adaptive trait, as resources important for brood rearing remain safe within the nest, while more risky activities outside the nest are performed by workers lacking fat reserves, leading to high mortality among foragers (Moroń et al., 2012; Porter & Jorgensen, 1981). The 'corpulent nurse-lean forager' pattern has mainly been described in the ant subfamily Myrmicinae (Blanchard et al., 2000; Silberman et al., 2016; Tschinkel, 1993, 1998). There is much less data available for the subfamily Formicinae and, in particular, for the genus *Formica*. In *Nylanderia fulva* (Formicinae, Lasiini), as in *F. aquilonia*, nurses have a higher fat (and carbohydrate) content than foragers, but in *F. fusca* (Serviformica), in contrast, nurses and foragers have similar fat content (Silberman et al., 2016). These differences within the genus *Formica* may be related to differences in food storage strategy and timing of sexual brood production. Species of the *Formica fusca* group rear sexual offspring later than species of the *Formica rufa* group, and they depend on foragers for food rather than on fat reserves accumulated in the bodies of corpulent nurses in autumn, as in wood ants (Savolainen & Deslippe, 1996). The effect of environmental factors on colony condition provides an additional explanation. The *F. fusca* colonies studied by Silberman et al. (2016) were from the northern part of its range in North America (42^{0}N). In this region, relatively small colony sizes and high intraspecific competition have been observed in *F. fusca* (Tuzzolino & Brown, 2010). Ant colonies of smaller size (*S. invicta*: Tschinkel, 1993) and at the latitudinal extremes of the range (*Myrmica rubra*: Elmes et al., 1999) may have the lowest fat content and, as a consequence, may presumably exhibit a reduced 'corpulent nurse-lean forager' pattern.

Do task groups collected on the nest mound, ground surface and foraging trees have different fat reserves?

Differences in abdominal fat reserves between workers collected on the nest, ground surface and foraging trees depended on the season and task specialization (Figure 1). In spring, the fat content of workers sunbathing on the mound surface was as high as that of inner-nest workers. In summer and autumn, the fat content of guards on the nest was similar to that of foragers and lower than that of nurses, except for honeydew foragers, which had a lower fat content than hunters in autumn.

As hypothesized, sunbathing workers were as corpulent as inner-nest workers in spring, indicating a common origin from young nurse workers (Martin, 1980; Rosengren et al., 1987). The maintenance of a stable temperature (25–30°C) for brood rearing in red wood ant mounds during spring and summer is widely known and was explained by the collection of solar radiation due to nest architecture and worker activity, and metabolic heat production by ants and microflora (Kadochová & Frouz, 2013). Our data on high fat content in sunbathing workers of *F. aquilonia* forming mass clusters on the mound surface in spring supported the heat production hypothesis (Kneitz, 1964; Rosengren et al., 1987). It has been argued that nest heating can start early in spring, even under snow cover, as corpulent young workers utilize lipid reserves and sunbathe in surface clusters (Martin, 1980; Rosengren et al., 1987). In turn, the raising of the sexual brood in early spring is based on the transformation of lipid reserves into nutrient glandular secretions by young corpulent workers (Kneitz, 1964; Rosengren et al., 1987). Since ants periodically rise to the surface and descend inside the nest during heating (Kadochová & Frouz, 2013), it is suggested that corpulent workers of *F. aquilonia* collected at a depth of about 30 cm and from warming spots on the nest surface may belong to the same group of workers. However, sunbathing workers differed from workers collected inside the nest in larger body size and correspondingly higher fat stores, indicating their likely independent task specialization. This new fact may be explained by higher heat tolerance in larger ant workers (Roeder et al., 2021), as the body temperature of red wood ant workers in sunbathing clusters can reach up to 40–45°C (Kadochová et al., 2019). On the other hand, our field data are not consistent with the results of laboratory experiments with *F. polyctena*, in which sunbathing and non-sunbathing workers did not differ in body size or respiration rate (Kadochová et al., 2017). More field data are needed to verify the effects of body size variation on nest thermoregulation efficiency and task allocation in inner-nest workers of red wood ants.

To our knowledge, the study by Porter and Jorgensen (1981) on *P. owyheei* is the only study of monomorphic ants to show differences in nutritional status between out-nest workers beyond the 'corpulent nurse-lean forager' pattern. In this species, defenders and refuse workers have energy reserves and starvation mortality intermediate between those of nurses and foragers, presumably due to age polyethism with a transition from tasks inside the nest to tasks near nest entrances and then to foraging (Porter & Jorgensen, 1981). *P. owyheei* foragers, as workers exposed to the greatest risk of predation, are considered a 'disposable caste' (Porter & Jorgensen, 1981). In contrast, in *F. aquilonia*, we found no differences in nutritional status between guards and hunters in summer and autumn. The similarities between hunters and guards may be due to their close ages (Otto, 1958) and the fact that older workers tend to be recruited for more risky tasks in social insects (Giraldo & Traniello, 2014). In red wood ants, workers guarding nest mound and feeding territory may take high risks in the context of colony defence against predators and competitors (Mabelis, 1983; Swenson et al., 1999).

In autumn, when colony fat reserves were highest, the lowest fat content among workers was observed in honeydew foragers, as indicated by the higher abdominal δ^{13}C value (Figure 1b). Moreover, in summer, honeydew foragers were the only out-nest task group that differed from nurses in their lower fat content. In summer and autumn, honeydew foragers had higher thoracic and abdominal δ^{15}N values than worker pupae (Figure 3), probably due to starvation (Doi et al., 2017). This further suggests low fat reserves in honeydew foragers in early September. In part, this may be explained by the size effect due to the smaller body size of honeydew foragers (Figure 4), which is related to task efficiency (smaller workers collect honeydew more efficiently, while larger workers hunt larger prey over longer distances and better defend territories in *Formica*; Rosengren & Sundström, 1987; Kay & Rissing, 2005; Tanner, 2008). In many polymorphic ants, there is a positive correlation between fat content and body size expressed as lean minors and replete majors (Hasegawa, 1993; Silberman et al., 2016; Yang, 2006). This correlation is, however, much less pronounced within the minor worker subcaste (positive correlation in *Camponotus* (Hahn, 2006); no correlation in *Pogonomyrmex badius* (Tschinkel, 1998)) and, to our knowledge, there are no data for monomorphic ants. We found a weak positive correlation between the size and fat content of *F. aquilonia* workers. When the size effect was taken into account, *F. aquilonia* small hunters were fatter than honeydew foragers in early autumn (although the former were still larger than the latter). It is difficult to establish whether the low fat content of honeydew foragers is due solely to their small body size or to the interaction of task and size effects. As red wood ants are characterized by a wide variation in worker body size (Véle & Modlinger, 2019), it remains to be studied which out-nest tasks other than honeydew collection can be performed by such small workers and to what extent their fat reserves are determined by body size.

Do task groups differ in the trophic level?

According to thoracic δ^{15}N values, *F. aquilonia* task groups occupied a similar trophic level of first-order predators that persisted throughout the season (Iakovlev et al., 2017). The exception was the 0.4‰ higher thoracic δ^{15}N values of ground foragers collected in late April after 6–7 months of overwintering, compared to inner-nest workers (Figure 2a). A meta-analysis showed that the δ^{15}N values of most consumers (including nine invertebrate taxa) increased with the length of the starvation period (mean increase 0.5‰; Doi et al., 2017), but five of eight studies on invertebrates found a significant increase in δ^{15}N value during fasting and three found no effect (Martinez del Rio et al., 2009). Although there are no experimental data on the influence of prolonged starvation or diapause on the δ^{15}N value of ants, we assume that the higher δ^{15}N value of *F. aquilonia* foragers collected on the ground surface in late April is not due to their more predatory diet but to starvation effect. This is indicated by the depleted fat reserves of the foragers (Figure 1) and by the negative correlation between the thoracic δ^{15}N value and the fat content as measured by the abdomen:thorax dry mass ratio and the C:N ratio in the abdomen and thorax in spring. A possible explanation for the high δ^{15}N value and the presumed starvation of *F. aquilonia* ground foragers in spring could be their older age, high locomotion and prolonged activity during winter and spring (from October to April;Rosengren, 1977; Zakharov & Zakharov, 2022).

Do task groups differ in diet?

Carbon isotope composition allows us to assess dietary differences in ants, but only when there are significant differences in δ^{13}C values between food sources (Feldhaar et al., 2010; Rico-Gray & Sternberg, 1991). We found that thoracic δ^{13}C values in foragers are consistently higher than in nurses by 0.5‰–2.0‰. This probably stems from the difference in fat content in the thoracic parietal fat body and/or hemolymph rather than differences in diet. This is evidenced by strong correlations between thoracic δ^{13}C values and C:N ratios, as well as between these values and indicators of abdominal fat reserves, with similar seasonal and task-specific differences (Figures 1 and 2). In the clonal ant *Platythyrea punctata*, fat content in the abdomen, thorax and head of ants decreased during their ageing and transition to foraging (Bernadou et al., 2020).

We used the abdominal δ^{15}N value to estimate the variation of undigested food in the crop of workers. *F. aquilonia* showed 0.3‰–0.4‰ lower abdominal δ^{15}N values in nurses than in foragers (Figure S3b). These differences persisted throughout the season, although mean abdomen δ^{15}N values were higher in spring than in autumn, which may be related to the consumption of ^{15}N-enriched birch sap by the colonies in spring (Iakovlev et al., 2017). Lower $\delta^{15}N$ values in the abdomen than in the thorax of worker ants were associated with nitrogen-poor honeydew and plant nectar/sap in the gut (Feldhaar et al., 2010). Task-specific variation in abdominal $\delta^{15}N$ values may be due to high levels of ^{15}N-depleted honeydew in the crop of nurses (Iakovlev et al., 2017) or high levels of ^{15}N-rich protein fluids from prey, such as caterpillars, in the crop of *F. aquilonia* foragers (Horstmann, 1972).

Annual cycle of colony life in red wood ants

The annual life cycle of red wood ant colonies in the south part of Western Siberia includes periods of activity (from April to October) and hibernation. In spring, the nest is heated up, the foraging territory is re-established and the generation of sexual individuals is produced using accumulated fat reserves, followed by the mating flight in late spring. In summer, there is a peak in hunting activity during the rearing of 3–5 generations of workers. From mid-summer to late autumn, the honeydew collection is at a high level. In autumn, preparation of the wintering nest and accumulation of fat reserves based on processed honeydew takes place (Dlussky, 1967; Kirchner, 1964; Stockan & Robinson, 2016; Zakharov & Zakharov, 2022).

Data from this study suggest that there are seasonal dynamics and differences between nurses and foragers in fat reserves in *Formica*, with the maximum fat content in inner-nest workers in autumn (Kirchner, 1964; Silberman et al., 2016). This scheme of the annual cycle of red wood ants can now be supplemented with new data on the nutritional status of the workers that may differ depending on task specialization and time of the year.

First, we obtained indications of a possible size-dependent division of labour among corpulent inner-nest workers in late April into larger sunbathing workers engaged in thermoregulation and smaller workers engaged in other tasks inside the nest. Similarly, in *F. yessensis*, nursing was observed to be performed by smaller workers and, moreover, in summer among young workers, nurses were smaller than inner-nest builders, although both groups were smaller than on-nest builders and hunters (Higashi, 1974). We relate the size differences among corpulent workers of *F. aquilonia* after overwintering to higher heat tolerance and higher heat production in the core of the nest mound during fat decomposition by larger workers (Kadochová & Frouz, 2013). This may serve for earlier production of sexual brood by queens and for early brood development (from April to mid-May) (Rosengren et al., 1987). It remains to be investigated to what extent these size differences between inner-nest workers are common in the *F. aquilonia* range and whether they are related to colony size, colony condition and reproductive success.

Second, there are nutritional differences between foragers. Ground foragers differed from tree foragers collecting sap and other task groups in showing signs of starvation in the spring. In red wood ants, old foragers, called ‘veteran foragers’ by Rosenren (1977), perform colony reactivation, nest rebuilding and re-establishment of foraging territory in early spring; they may also remain active in the wintering nest (Rosengren, 1977; Zakharov & Zakharov, 2022). Later, these active foragers recruit younger reserve foragers, called ‘pioneer foragers’ by Rosengren (1977), from the nest to foraging areas and trees, thus maintaining route fidelity at the colony level (Rosengren, 1977). It may explain the lack of signs of starvation in foragers collecting birch sap in spring, being presumably younger than ground foragers. Interestingly, both groups of foragers in spring (as well as sunbathing workers) are characterized by the largest sizes in the whole dataset. In different ant species, larger workers withstand environmental stress, for example, starvation and desiccation, longer than their nestmates (Calabi & Porter, 1989; Hölldobler & Wilson, 2009). This could probably explain the increase in body size of *F. aquilonia* workers from autumn to spring. We hypothesize that in *F. aquilonia*, the decrease in worker body size from spring to summer is due to the high mortality of overwintered larger and older workers and the production of new generations of workers (Rosengren, 1977).

Third, despite slight differences in body size, nest guards had similar nutritional status to hunters, which may be explained by the close age of these task groups in red wood ants (Otto, 1958).

Fourthly, in early autumn, tree foragers collecting aphid honeydew had lower fat reserves than hunting ground foragers and nurses. Honeydew collection can continue until mid-autumn, when the majority of foragers are already in the nest, and ceases with the onset of cold weather (Zakharov & Zakharov, 2022). The prolonged performance of these functions by small workers with low energy reserves may allow the colony to accumulate maximum energy reserves in the bodies of larger individuals within the nest before hibernation. However, smaller workers with lower energy reserves tend to have a lower life expectancy (Calabi & Porter, 1989; Porter & Jorgensen, 1981). Field marking and recapture experiments are required to evaluate their role in restoring trails to forage trees with aphids the following year (Rosengren, 1977). It can be assumed that this task is performed by less numerous and more active foragers (scouts; Novgorodova, 2015; Reznikova & Novgorodova, 1998), presumably larger in size and not caught in autumn collections, which recruit workers to forage for honeydew.

CONCLUSION

Our study confirms the important role of fat reserve dynamics in the division of labour in social insects and extends the understanding of the proximal mechanisms of worker behavioural specialization and adaptations of the colony to seasonal environments. In a monomorphic ant species with a sophisticated social organization, *F. aquilonia*, higher fat content was found in nurses compared to foragers. Differences in fat content and the effects of starvation between ground foragers and tree foragers, as well as the dynamics of the fat content of nurses, are timed to the stages of the annual life cycle of the colony, the most important event of which in red wood ants is the preparation for overwintering and the subsequent production of the sexual generation. Thus, the allocation of energy resources among workers performing different tasks can contribute to the reproductive success of the ant colony.

AUTHOR CONTRIBUTIONS

Ivan K. Iakovlev: Conceptualization; investigation; methodology; data curation; formal analysis; writing – review and editing; writing – original draft. **Zhanna I. Reznikova:** Conceptualization; methodology; writing – review and editing; supervision. **Alexei V. Tiunov:** Conceptualization; methodology; investigation; formal analysis; writing – review and editing; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in DRYAD at https://doi.org/10.5061/dryad.hx3ffbgps.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Full ANOVA results on the effects of colony, season, and spatial zone of task group on the thoracic $\delta^{15}N$ values.

FIGURE S1. The relationships between indicators of fat content in *F. aquilonia* workers. The correlation of (a) $\delta^{13}C$ value and C:N ratio in the abdomen, and (b) abdomen:thorax dry mass ratio and the abdominal C:N ratio. Different colors reflect spatial zones of task groups (red for workers collected inside the nest, rose for workers on the nest surface, blue for workers foraging on the ground, and green for workers foraging on trees).

FIGURE S2. The relationship between thoracic dry mass and abdominal dry mass in workers collected (a) inside the nest, (b) on the nest surface, (c) on the foraging ground, and (d) on foraging trees. Colors reflect the time of sample collection (white for spring, grey for summer, black for autumn).

FIGURE S3. Seasonal (a) and task-specific (b) differences in abdominal $\delta^{15}N$ values of *F. aquilonia* workers. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$, $n = 120$). Mean and 95% CI are shown.

FIGURE S4. Differences between four colonies of *F. aquilonia* in thoracic $\delta^{15}N$ values. Different letters indicate significant differences between colonies (Tukey's HSD test, $p < 0.05$, $n = 60$). Mean and 95% CI are shown.

FIGURE S5. The relationship between carbon and nitrogen isotope composition of individual worker pupae and adult workers collected in different spatial zones of colony activity. Whole pupae were collected in summer and analyzed (without indusium). Thorax (with legs) and abdomen of adult workers collected in summer and autumn were analyzed separately.

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