




## Article

# Stable Isotope Analysis Supports Omnivory in Bank Voles in Apple Orchards

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**Abstract:** With only periodic and incomplete studies of its diet over time, all with differing methods and conclusions, the degree of omnivory in the bank vole (*Clethrionomys glareolus*) is not fully clear. We assessed the trophic niche of the species using isotopic ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) compositions from hair samples and analysed how *C. glareolus* shares trophic space with herbivores, granivores and insectivores living syntopically. According to the numbers trapped, *C. glareolus* was the fourth most numerous species in the apple orchards that we investigated, accounting for 14.4% of all trapped small mammals with a relative abundance of 1.12 individuals per 100 trap nights. The average values of both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in the hair of *C. glareolus* differed from the other trophic groups, with the average of  $\delta^{15}\text{N}$  in orchards and neighbouring habitats (6.55–6.95‰) being closest to that of insectivores. Intraspecific trophic niche differences (depending on age, gender and reproductive status) were not expressed, while correlations between body mass, body condition index and both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were not consistent. In comparison to analysed basal resources, isotopic signatures in the hair were closest to the values in invertebrates and apple seeds. The degree of omnivory in *C. glareolus* was not the same in different habitats. This may be an indication of ecological plasticity within the species, allowing its inclusion/success in multi-species small mammal communities.

**Keywords:** *Clethrionomys glareolus*; isotopic niche; abundance; apple orchards



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## 1. Introduction

Omnivory in small mammals can be understood as a strategy for surviving in communities involving several trophic groups. However, the significance of omnivory is not understood well, especially in terms of its stabilizing role, its influence on habitat selection and its role in the population dynamics of omnivorous species [1]. Based on the capacity of vertebrates to respond to habitat changes as a function of their ecological traits [2], omnivores show synchronous responses, and therefore could be more resistant to negative influences.

According to their teeth, rodents mostly originated as omnivores, but later, in the evolution of the order, radiated into several different trophic groups [3]. In small mammals, omnivores differ in their foods from herbivores, granivores and carnivores, but there may be seasonal shifts in the differences [4]. The proportion of omnivorous species in small mammal communities differs across continents, latitudes and habitats; they are best represented in tropical and desert habitats [5–7]. In desert habitats with limited availability of seed resources, granivory in small mammals is limited and replaced by omnivory [8].

Though deviation of the diet from the population average is not advantageous for an omnivore [9], seasonal shifts in the breadth of the isotopic niche depending on food resources is characteristic of tropical climates, abounding in generalist species [10]. In forest zones, clear-cutting can result in changes in the proportions of trophic groups, best expressed in granivore-omnivores and being characteristic of both coniferous and deciduous stands [11]. After canopy closure, the numbers of omnivores decrease and insectivores increase [12]. In addition to the influence of successional stage, the responses

of small mammals to herbicide treatment might be important to changes in the proportions of trophic groups [13]. This also should be kept in mind for small mammals in commercial apple orchards [14].

The bank vole (*Clethrionomys glareolus*), also known as *Myodes glareolus* [15], is a small mammal species widespread in the Palaearctic and inhabits various woodlands, shrubby areas and parklands [16]. In Lithuania, it is very widespread and numerous, found in all forest habitats, lakeshores, shrubby meadows and reedbeds [17,18], as well as agricultural and commensal habitats [14,19]. In orchards, *C. glareolus* can cause damage by gnawing bark [20] and, thus, knowledge of its diet might have practical significance.

The trophic position of *C. glareolus* has not been clearly defined, though an intermediate position between herbivorous and insectivorous/granivorous species was recognized recently [21]. It can survive on animal resources only [3] and shows preference for animal food when starving [22]. Other authors show various proportions of animal foods, seeds and other components in the diet of *C. glareolus*, thereby recognizing species omnivory [23,24] or terming the species omnivorous [25]. These studies included various methods of diet investigation, including stomach or faeces analyses [24,26], various cafeteria experiments [22,23,27], the studying the relative lengths of the parts of alimentary tract [4] and environmental niche modelling [28].

In *C. glareolus*, the content of nitrogenous substances in the stomachs and, consequently, in the food varied depending on the year and habitat [29]. This was in contrast to the findings of Palo and Olsson [30] about seasonal stability of nitrogenous substances in the stomachs. They also found that the nitrogen concentrations in stomachs were not related to the body masses of these voles.

Isotopic niches, as a substitute for trophic or dietary niches, of small mammals are currently used instead of direct investigations of the diet. Isotopic signatures in tissues or hair show dietary information, with a higher ratio of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) indicating consumption of animal foods [27]. This method allows diets to be related to body condition [31]. For *C. glareolus*, it was experimentally shown how big the trophic discrimination factor ( $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N}$ ) is, as measured between diet and hair, when fed on  $\text{C}_3$  plant-based diets and 30% animal matter based diets [32]. In Lithuania, isotopic analysis has been the only method used for dietary studies of small mammal herbivores [33] and granivores [34], while the diet of omnivorous small mammals, as a trophic group, had not been investigated so far.

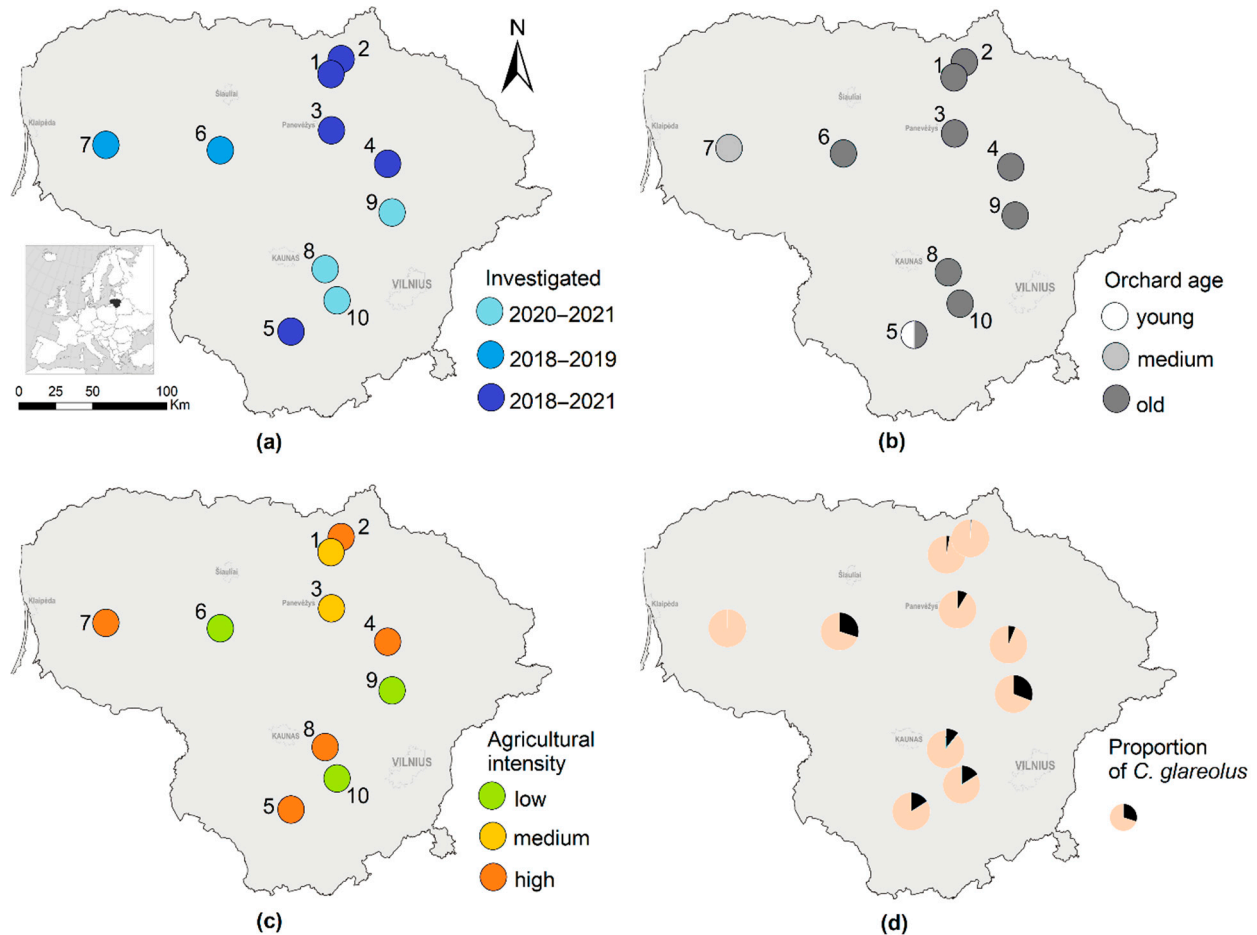
The aim of this investigation was to evaluate the trophic position of *C. glareolus* in commercial apple orchards along with proportion of the species in small mammal communities and its relative abundance. To assess its omnivory, we analysed whether the species position in the isotopic space differed from herbivorous and granivorous species according to carbon, and how close the species position was to insectivorous species according to nitrogen values. We tested two working hypotheses: (1) to demonstrate that *C. glareolus* is omnivorous, the values of stable carbon and nitrogen isotopes in their hair should differ from those in the other trophic groups; (2) concerning intraspecific patterns of diet, we tested whether higher nitrogen values were characteristic of females with forming embryos or in lactation and whether nitrogen values differed according body the mass/body condition index of individuals.

## 2. Materials and Methods

### 2.1. Study Sites

During 2018–2021, ten commercial apple orchards in Lithuania, characterised by different age and intensity of agricultural measures, were investigated (Figure 1). Every site had an adjacent control habitat: a mowed meadow, an unmowed meadow or a forest. The average size of an orchard was 63.7 ha. In the young and middle-aged apple orchards (Figure 1b) only one rodent individual was trapped during the first two years of study, therefore we expanded the number of old orchards in 2020 (sites 8–10), abandoning sites 6 and 7. The intensities of agricultural practices (Figure 1c) were defined as high when two

or more practices (application of plant protection agents, application of rodenticides, soil scarification, grass mowing, grass mulching) were applied frequently. Medium intensity meant applying two of the listed measures once or twice during the crop season, while low intensity meant only grass mowing [14,35].



**Figure 1.** Investigated apple orchards in Lithuania, 2018–2021: (a)—position of study sites with length of the study indicated; (b)—age of orchard; (c)—intensity of agricultural measures; (d)—proportion of *C. glareolus* in all trapped small mammals.

## 2.2. Small Mammal Trapping and Size of Bank Vole Samples

We used snap-traps, arranged in lines of 25 traps at 5 m intervals [36]. Traps were baited with brown bread and raw sunflower oil, exposed for three days, checked once per day in the morning. Bait was changed after rain or when consumed. Total trapping effort was 23,433 trap-days during 2018–2021 (15,458 for the orchards and 7965 for the control habitats). Due to the different representation of orchards and their control habitats, equal trapping effort was not achieved (Table S1).

Species of trapped small mammals were identified by their external features, and specimens of *Microtus* voles were identified by their teeth [17]. The age (adults, sub-adults, juveniles) and reproductive parameters of small mammals were identified under dissection based on body weight, the status of sex organs and atrophy of the thymus, the last of which decreases with animal age [37]. Individual fitness was calculated as body condition index *C* according to Moors [38], based on body weight in g (*Q*) and body length in mm (*L*). For pregnant females, the weight of the uterus with embryos was excluded [39]. In males, participation in reproduction was assessed by checking testes size and fullness of epididymis and additional glands. In females, participation in reproduction was assessed by checking

perforation and plug of vagina, counting the numbers of placental scars, corpora lutea and assessing the number and mass of embryos.

During the four years of study, 1270 small mammal individuals were trapped in orchards and non-orchard habitats (Table S2). Species were attributed to four groups according to [4,21–24,26–28,33,34]. These were insectivores—common shrews (*Sorex araneus*), pygmy shrews (*S. minutus*) and Mediterranean water shrews (*Neomys anomalus*); herbivores—field voles (*Microtus agrestis*), common voles (*M. arvalis*), root voles (*M. oeconomus*) and water voles (*Arvicola amphibius*); granivores—striped field mice (*Apodemus agrarius*), yellow-necked mice (*A. flavicollis*) and harvest mice (*Micromys minutus*). Omnivores were represented with *C. glareolus* and house mice (*Mus musculus*). This last species was trapped in the control habitats only (proportion 0.7%, CI = 0.3–1.7%) and, therefore, was not analysed further. According to the numbers trapped, *C. glareolus* was the fourth most numerous species in the apple orchards and the third in the adjacent control habitats.

During 2018–2021, 191 *C. glareolus* individuals were trapped. Excluding very dirty or blood covered individuals, as well as those affected by insects, this number was down-sampled to 141 individuals for the isotopic analysis. The distribution of the sampled *C. glareolus* by habitat, age and gender is presented in Table 1.

**Table 1.** *C. glareolus* sample sizes used for stable isotope analysis by habitat, age and gender.

Habitat	N	Males	Females	Adults	Subadults	Juveniles
Apple orchards	78	43	35	31	14	33
Mowed meadow	36	19	17	10	13	13
Unmowed meadow	22	11	11	2	4	16
Forest	5	2	3	1	1	3

### 2.3. Stable Isotope Analysis of Hair and Basal Resources

We analysed carbon and nitrogen stable isotopes in the hair of the trapped *C. glareolus*. About 5 mm of hair was clipped from the back of individuals between the shoulders. Dirty samples were washed in deionized water and methanol, then dried. Samples were stored dry in bags before analyses.

Basal resources, representing possible foods of *C. glareolus* in eight orchards, were collected in 2020–2021. They represented invertebrates, sedges (exclusively Poacea), forbs (other plants, such as *Taraxacum*, *Urtica*, *Trifolium*, *Plantago*, *Artemisia*, *Achillea*, *Chenopodium*), apple fruit, apple fruit seeds and leaves of the apple trees. Samples ( $n = 61$ ) of the basal resources were stored in a freezer at below  $-20\text{ }^{\circ}\text{C}$  prior to preparation and analysis. Samples were dried in an oven at  $60\text{ }^{\circ}\text{C}$  to a constant weight for 24–48 h and then homogenized to a fine powder (using mortar and pestle and a Retsch mixer mill MM 400).

Analyses were conducted using an elemental analyser (EA) (Flash EA1112) coupled to an isotope ratio mass spectrometer (IRMS) (Thermo Delta V Advantage) via a ConFlo III interface (EA-IRMS). Five percent of the samples were run in duplicates and the obtained results for these samples were averaged.

Caffeine IAEA-600 ( $\delta^{13}\text{C} = -27.771 \pm 0.043\text{‰}$ ,  $\delta^{15}\text{N} = 1 \pm 0.2\text{‰}$ ), Ammonium Sulfate IAEA-N-1 ( $\delta^{15}\text{N} = 0.4 \pm 0.2\text{‰}$ ), and Graphite USGS24 ( $\delta^{13}\text{C} = -16.049 \pm 0.035\text{‰}$ ), provided by the International Atomic Energy Agency (IAEA), were used as reference materials. We re-ran standards every 12 samples [34,35], obtaining SD = 0.06‰ for carbon and SD = 0.10‰ for nitrogen. Of the basal resources, invertebrates were analysed in a single run, and plant foods in duplicate. The obtained values were averaged.

### 2.4. Data Analyses

For each locality, the relative abundance (RA) of *C. glareolus* was expressed as the number of individuals trapped per 100 trap-days per trapping session [40]. We used standard statistics (average, SE, minimum and maximum in tables, 95% CI in figures) for RA and stable isotope values. The proportion of *C. glareolus* among all trapped small mammals was presented as average and the 95% CI was calculated with the Wilson method of the

score interval [41] using OpenEpi epidemiological software [42]. The Wilson confidence intervals have better coverage rates for small samples. Differences in the proportions were evaluated using the G test with an online calculator [43].

We used general linear models (GLM) in two analyses. In the first case, the RA of *C. glareolus* was used as the dependent variable, with habitat, orchard age, intensity of agricultural measures, year and season as categorical predictors, and trapping effort and abundances of three most numerous herbivore/granivore species as continuous predictors. In the second model, the values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were used as the dependent variables, with habitat, intensity of agricultural measures, season, year, animal age and animal gender as categorical predictors, and body mass and body condition index of an individual as continuous predictors. Hotelling's  $T^2$  was used to test the significance of the model and eta-squared for the influence of the categorical factors. We applied Tukey HSD with unequal N for post-hoc analysis. The minimum confidence level was set as  $p < 0.05$ . At the level  $p < 0.10$ , we supposed a trend, but not a difference, would exist.

Before running the GLM, we tested the normality of the distributions of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, body condition index, body mass and RA via Kolmogorov–Smirnov's D. The first three listed parameters conformed to normal distribution ( $D = 0.08, 0.09$  and  $0.07$  respectively, all  $p > 0.50$ ). The distributions of the body mass in adult, sub-adult and young *C. glareolus* also were normal ( $D = 0.12, p = 0.27$ ;  $D = 0.21, p = 0.06$ ; and  $D = 0.12, p = 0.15$  respectively). The distribution of RA was not normal due to the high number of trapping sessions when *C. glareolus* was not present, especially in summer.

The relationships between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and body mass, body condition index and reproduction parameters were assessed using ANOVA and Pearson correlation. In adult *C. glareolus*, we compared three male groups (non-breeding, spermatogenesis weak, spermatogenesis strong) and four female groups (inseminated, gravid with visible embryos, after normal gravidity and after gravidity when non-implantation occurred).

The isotopic signatures of the basal resources were analyzed by object group (invertebrates, sedges, forbs and apple). The reported values are the arithmetic means with SE of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for all basal resources mentioned above. Diet/hair trophic discrimination factors (TDF) were calculated as the difference between diet and hair, expressed as  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  [32,44].

Calculations were done in Statistica for Windows, version 6.0 (StatSoft, Inc., Tulsa, OK, USA), and biplots were drawn in SigmaPlot ver. 12.5 (Systat Software Inc., San Jose, CA, USA).

### 3. Results

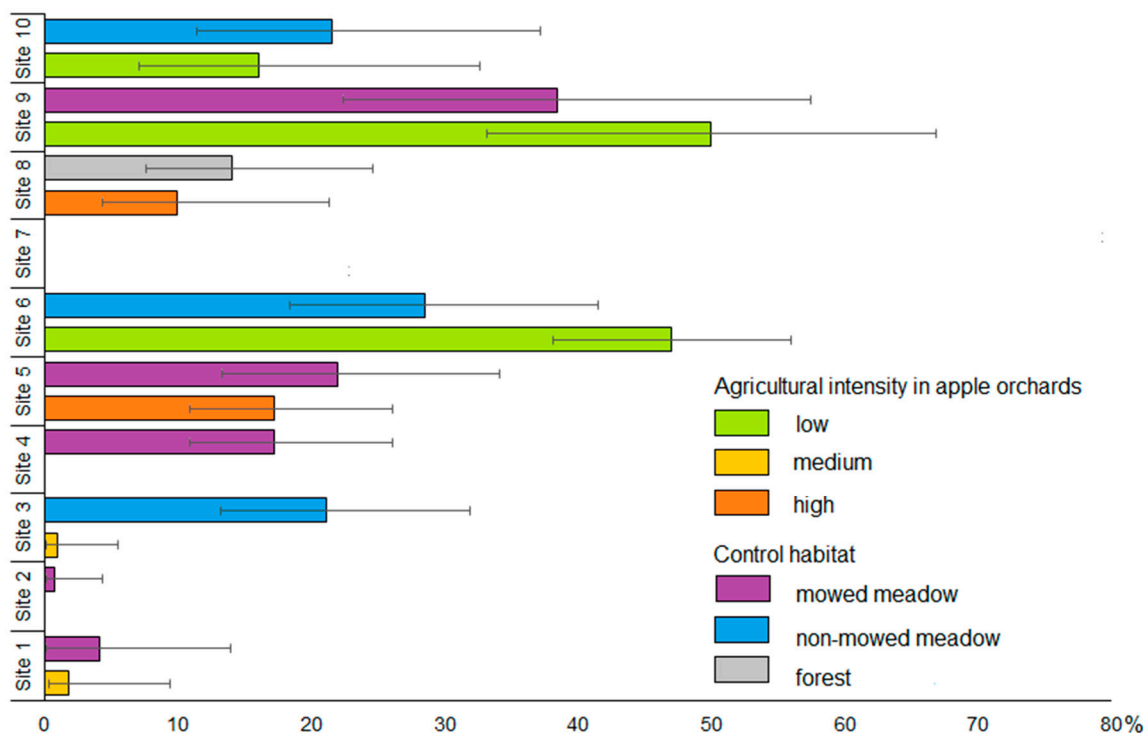
#### 3.1. Proportions of Bank Voles in Small Mammal Communities and Species Abundances in Apple Orchards and Control Habitats

Irrespective of habitat, the proportions of *C. glareolus* in the investigation sites varied considerably (Figure 1d). The proportions of *C. glareolus* in all trapped small mammals in sites 5, 8 and 10 varied in the range 10–20%, while in sites 1, 3 and 4 it was between 3–9% and in sites 2 and 7 between 0.6% and zero. These differences are significant ( $G = 186.7, p < 0.0001$ ).

The proportion of *C. glareolus* in small mammal communities varied depending on the habitat ( $G = 17.6, p < 0.005$ ). In total, the highest proportion was found in non-mowed meadows (29.8%, CI = 22.2–38.8%), followed by apple orchards (14.4%, CI = 12.0–17.3%) and forest (14.1%, CI = 7.6–24.6%), with smallest figure in mowed meadows, where *C. glareolus* comprised only 12.1% (CI = 9.3–15.6%).

In the orchards, the presence of *C. glareolus* was related to agricultural intensity (Figure 2). The proportion of the species at low intensity was 42.1% (CI = 35.1–49.5%), significantly exceeding that at medium (4.5%, CI = 2.2–9.1%) and high (6.1%, CI = 4.0–9.1%) intensity of treatment ( $G = 96.0, p < 0.0001$ ).





**Figure 2.** Proportion of *C. glareolus* in all trapped small mammals according to habitat (95% CI represented by error bars).

The average relative abundance (RA) of *C. glareolus* in the study period was  $1.2 \pm 0.2$  individuals per 100 trap-days, not very high. Out of 128 trapping sessions, the species was not trapped in 84 sessions (zero abundance), while the maximum abundance was 15.3 individuals per 100 trap-days. The dynamics of the RA are presented in Table S3. The general pattern (low RA and weak representation in summer across all sites, both increasing in autumn) remained the same throughout the study period.

The analysed GLM model was significant ( $F_{15,112} = 5.829$ ,  $p < 0.0001$ ) and explained 36% of the variation of RA of *C. glareolus*. The influence of the habitat ( $F_{2,112} = 5.53$ ,  $p < 0.0001$ ), the intensity of the agricultural treatment ( $F_{2,112} = 4.94$ ,  $p < 0.01$ ) and the abundance of *A. agrarius* ( $F_{1,112} = 4.59$ ,  $p < 0.05$ ) were the most expressed, with the partial  $\eta^2$  being 8.90, 8.11 and 3.94% respectively. These influences were not constrained by trapping effort ( $F_{1,112} = 0.021$ ,  $p = 0.88$ ).

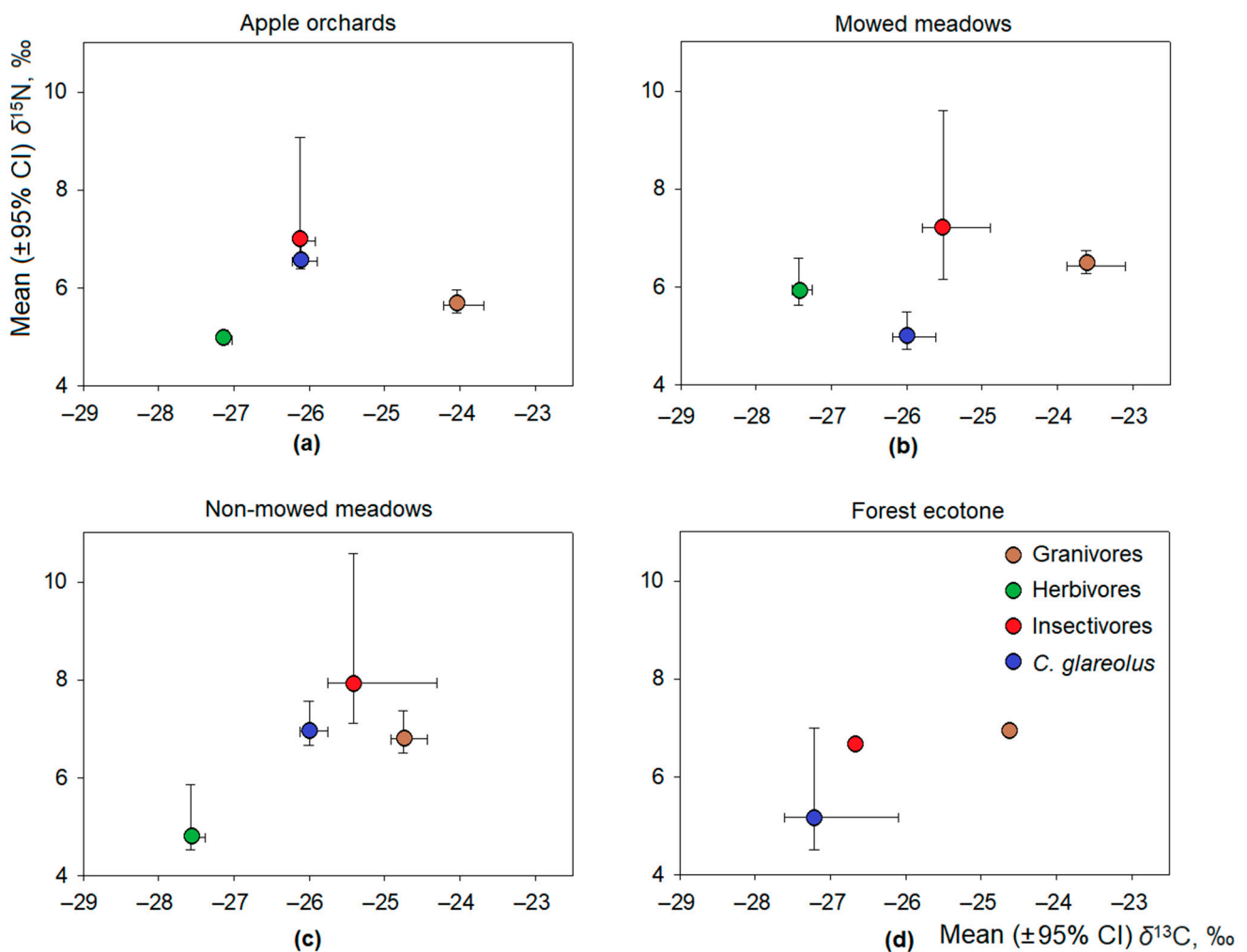
Post-hoc analysis confirmed significant differences of RA between seasons (0.84 individuals per 100 trap-days in summer versus 1.63 individuals per 100 trap-days in autumn, HSD,  $p < 0.05$ ). The highest average RA of the species were found in non-mowed meadows (3.39 individuals per 100 trap-days), significantly exceeding that in mowed meadows (0.83), but not significantly differing from those in apple orchards (1.12) and forest (1.50 individuals per 100 trap-days). The average RA of *C. glareolus* in the orchards with low intensity of treatment (5.28 individuals per 100 trap-days) exceeded those in meadows and forest (1.35), and orchards with medium (0.04) or high (0.21 individuals per 100 trap-days) intensity of treatment; all differences highly significant (HSD,  $p < 0.001$ ).

### 3.2. Stable Isotope Ratios of Omnivorous Bank Voles and Related Factors

The central positions and ranges of stable isotope ratios in the hair of *C. glareolus* irrespective of the habitat were:  $\delta^{13}\text{C} = -26.10 \pm 0.08$ , range  $-26.10$ – $-28.55\text{‰}$ ;  $\delta^{15}\text{N} = 6.17 \pm 0.14$ , range  $1.94$ – $11.30\text{‰}$ . Thus, variation in the nitrogen was quite wide. The cumulative influence of year, season, habitat, intensity of agricultural treatment, animal gender, age, body mass and body condition index explained 13.5% of  $\delta^{13}\text{C}$  variation (GLM,  $F_{11,126} = 2.94$ ,  $p < 0.002$ ) and 27.2% of  $\delta^{15}\text{N}$  variation ( $F_{11,126} = 5.64$ ,  $p < 0.0001$ ).

The most significant influences were by habitat (Hotelling's  $T^2 = 0.19$ ,  $p < 0.001$ , partial  $\eta^2 = 8.8\%$ ), intensity of treatment ( $T^2 = 0.08$ ,  $p < 0.05$ , partial  $\eta^2 = 7.5\%$ ) and season ( $T^2 = 0.05$ ,  $p < 0.05$ , partial  $\eta^2 = 5.0\%$ ). The influence of the animal's age was on a tendency level ( $T^2 = 0.08$ ,  $p = 0.054$ , partial  $\eta^2 = 3.7\%$ ), while the influences of the year, animal gender, body mass and body condition index were not significant.

In the apple orchards (Figure 3a), the differences in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  average values in the hair of co-occurring *C. glareolus* from the other trophic groups were significant ( $F_{3,384} = 87.5$  and  $F_{2,385} = 15.9$ , respectively, both  $p < 0.0001$ ). The average value of  $\delta^{13}\text{C}$  in *C. glareolus* was between those in granivores and herbivores, differing from both (Tukey HSD,  $p < 0.001$ ), but equalling that in insectivores. The average value of  $\delta^{15}\text{N}$  in *C. glareolus* was significantly above those in granivores and herbivores (HSD,  $p < 0.005$ ), but did not differ from that in insectivores (Figure 3a).

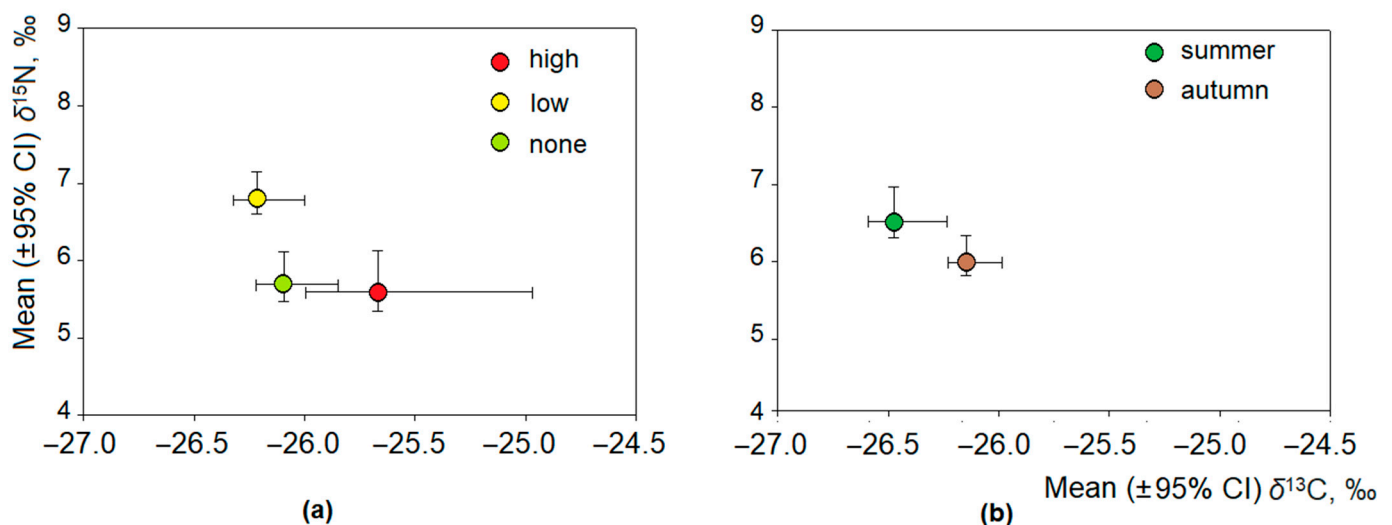


**Figure 3.** Position of *C. glareolus* and species of the other trophic groups according to stable isotope ratios: (a) in the apple orchards; (b) in mowed meadows; (c) in non-mowed meadows; and (d) in forests. Due to insignificant variation, SE is not visible for some groups. Sample size of herbivores and granivores in the forests was not sufficient.

In the mowed meadows (Figure 3b), significant differences in the hair of co-occurring *C. glareolus* from the other trophic groups were found in  $\delta^{13}\text{C}$  ( $F_{3,251} = 27.7$ ,  $p < 0.0001$ ), but not in  $\delta^{15}\text{N}$  ( $F_{3,251} = 1.3$ , NS). The average value of  $\delta^{13}\text{C}$  in *C. glareolus* was between those in granivores and herbivores, differing from both (Tukey HSD,  $p < 0.0001$ ), but not from that in insectivores. The average value of  $\delta^{15}\text{N}$  was the lowest, but did not differ significantly from other trophic groups (Figure 3b).

In the non-mowed meadows (Figure 3c), significant differences in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  average values in *C. glareolus* from the other trophic groups were found ( $F_{3,53} = 22.82$ ,  $p < 0.0001$  and  $F_{3,53} = 2.93$ ,  $p < 0.05$ , respectively). The average value of  $\delta^{13}\text{C}$  was similar to that in mowed meadows. This value was between those in granivores and herbivores, differing from both (Tukey HSD,  $p < 0.05$  and  $p < 0.001$ , respectively), but not from insectivores. The average value of  $\delta^{15}\text{N}$  did not differ from the other trophic groups (Figure 3c).

No significant differences were found comparing the  $\delta^{13}\text{C}$  values in *C. glareolus* in meadows/forests, apple orchards with high intensity of treatment and orchards with low intensity of treatment. The values of  $\delta^{15}\text{N}$  differed significantly, being highest (HSD,  $p < 0.0001$ ) in orchards with low intensity of treatment (Figure 4a). Note, no *C. glareolus* were trapped in apple orchards with medium intensity of treatment.



**Figure 4.** Position of *C. glareolus* according to stable isotope ratios: (a) depending on the intensity of agricultural treatment; (b) in different seasons.

As for the seasonal differences, there were no differences in  $\delta^{15}\text{N}$  (Figure 4b), but  $\delta^{13}\text{C}$  values were higher in the autumn (HSD,  $p < 0.02$ ).

Quite unexpectedly, nitrogen values were low in the basal resources of both animal and plant origin collected in the apple orchards (Table 2). Invertebrates were more enriched in  $^{15}\text{N}$  than in  $^{13}\text{C}$ , and the range of  $^{15}\text{N}$  was very wide in plants. The most negative value of nitrogen was found in a single sample of lichen (not readily present in other sites). The most positive value was found in apple seeds and was close to that in invertebrates. Average  $\delta^{13}\text{C}$  values were similar, with the range being wider in plants.

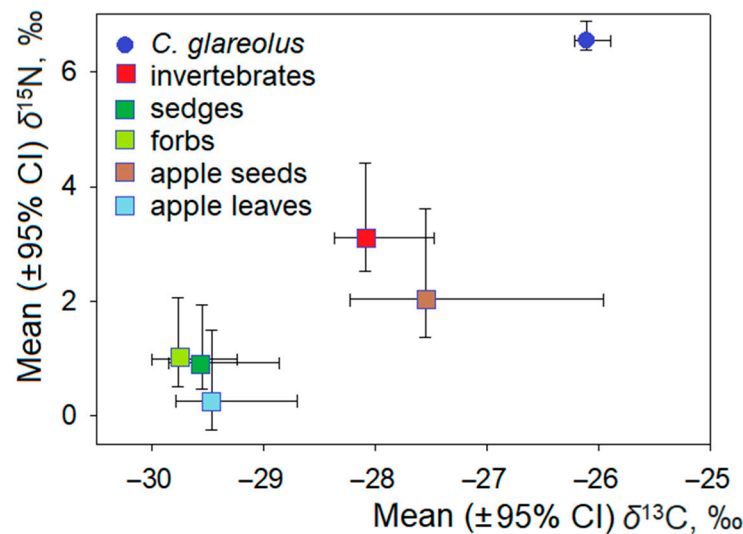
**Table 2.** Isotopic signatures of basal resources (invertebrates and plants, lichens not included) in the apple orchards.

Resources	<i>n</i>	$\delta^{13}\text{C}$ , ‰ ± SE	$\delta^{13}\text{C}$ , ‰ Min–Max	$\delta^{15}\text{N}$ , ‰ ± SE	$\delta^{15}\text{N}$ , ‰ Min–Max
Invertebrates	10	$-28.09 \pm 0.27$	$-29.41$ – $-26.77$	$3.10 \pm 0.57$	0.75–6.44
Plants	50	$-28.69 \pm 0.24$	$-31.63$ – $-24.95$	$0.76 \pm 0.41$	$-12.41$ – $5.38$

We compared the isotopic signatures in the hair of *C. glareolus* with the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the possible food sources. Both invertebrates and apple seeds were at the closest distance to the *C. glareolus* hair (Figure 5). As for the age-based differences, the highest amount of foods of animal origin was used by subadult and juvenile individuals (Figure S1b). As presented in Figure 3a, the isotopic composition of sedges, forbs and apple leaves were closest to the hair of herbivores, while the content of  $^{13}\text{C}$  in granivore hair is very different. The diet/hair TDF in *C. glareolus* from the apple orchards was 2.48‰/5.33‰



in a pooled sample (irrespective of age), with 2.10‰/4.85‰ in adult voles, 2.34‰/5.91‰ in subadult voles and 2.90‰/5.54‰ in juveniles.



**Figure 5.** Isotopic signatures of potential animal and plant foods compared to the isotopic signatures in the hair of *C. glareolus* from apple orchards.

### 3.3. Intraspecific Differences in Isotopic Ratios in Bank Voles

Irrespective of habitat, intraspecific differences of the isotopic niche of *C. glareolus* were significant depending on animals' age (Hotelling's  $T^2 = 0.12$ ,  $p < 0.005$ , partial  $\eta^2 = 5.8\%$ ), but not their gender ( $T^2 = 0.02$ ,  $p = 0.30$ ).

When analysed on the habitat basis, intraspecific differences of the isotopic niche of *C. glareolus* were less expressed (Figure S1). In the apple orchards, the influence of the animal age was significant ( $T^2 = 0.25$ ,  $p < 0.002$ , partial  $\eta^2 = 5.4\%$ ). The model worked for both  $\delta^{13}\text{C}$  ( $F_{2,75} = 6.43$ ,  $p < 0.003$ ) and  $\delta^{15}\text{N}$  ( $F_{2,75} = 3.41$ ,  $p < 0.05$ ). Juveniles were characterised by the highest average  $\delta^{13}\text{C}$ , significantly exceeding that of adults (HSD,  $p < 0.005$ ), but no differences in age groups were noted according to  $\delta^{15}\text{N}$  value (Figure S1a). Age-based differences in the other habitats were not significant (mowed meadows,  $T^2 = 0.11$ ,  $p = 0.48$ ; non-mowed meadows  $T^2 = 0.15$ ,  $p = 0.64$ ).

The influence of the animal gender was not significant in any of the habitats: apple orchards ( $T^2 = 0.02$ ,  $p = 0.47$ ), mowed meadows ( $T^2 = 0.04$ ,  $p = 0.54$ ) and non-mowed meadows ( $T^2 = 0.07$ ,  $p = 0.52$ ).

### 3.4. Relationship between Bank Vole Breeding Status, Body Mass, Body Condition Index and Their Isotopic Ratios

In *C. glareolus*, we did not find a significant influence of reproductive status on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the hair ( $F_{6,37} = 1.28$ ,  $p = 0.29$  and  $F_{6,37} = 0.51$ ,  $p = 0.80$ , respectively). Based on HSD, none of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the analysed groups of adult males and females differed according to reproductive status. Data on the central positions of the stable isotope ratios in the hair of *C. glareolus* according to their reproductive status are presented in Table S4.

We did not find regular and explainable correlation patterns between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and body mass or body condition index in *C. glareolus*. All significant correlations between body mass and  $\delta^{13}\text{C}$  were negative, and none of the correlations with  $\delta^{15}\text{N}$  were significant (Table S5). Scatterplots with distribution of raw values are presented in Figure S2.

## 4. Discussion

Our main goal was to demonstrate that *C. glareolus* is an omnivore small mammal species in apple orchards and surrounding habitats. The isotopic niche of *C. glareolus* was

separated from granivores and herbivores according to  $\delta^{13}\text{C}$  in apple orchards, mowed and non-mowed meadows, and according to  $\delta^{15}\text{N}$  in apple orchards and non-mowed meadows, thus confirming first research hypothesis. The average  $\delta^{15}\text{N}$  value in the hair of *C. glareolus*, being  $>6\%$ , clearly indicates diet peculiarities that place them close to carnivores/insectivores [45,46]. The similarity of  $\delta^{15}\text{N}$  values to insectivores allow us to suppose the importance of foods of animal origin to their diet, offering them some advantage in competition with herbivores and granivores when living syntopically. *C. glareolus* is known to use animal foods and even preferring them when starving and being able to survive on animal resources only [3,21–25]. It is quite possible that foods of animal origin (including small vertebrates and their carcasses is more widely used by *C. glareolus* as  $\delta^{15}\text{N}$  values in their hair were higher than in any of the dietary items we analysed.

Our second hypothesis was rejected—the  $^{15}\text{N}$  values in the hair of *C. glareolus* were not related to individual body mass (the same as in [30]), body condition index or reproduction patterns. Based on diet/hair TDS, increased amounts of animal-based foods were characteristic of non-adult voles, especially subadults. This finding might be related to the dispersion of the non-adult individuals [47,48], this being challenging to an individual, thereby changing its bioenergetics [49]. The movement of small mammals can be influenced by herbicide treatment [13], which was used in many of the investigated apple orchards [14]. We do not speculate on the influence of possibly scarcer food resources in the orchards due to agricultural treatments. In granivores, body condition decreases in lower quality habitats [50], while the influence of habitat richness on *C. glareolus* is not known. It has, however, been shown that *C. glaeolus* may be paradoxically less numerous in richer habitats [1]. We, therefore, interpret the values of TDS between the diet and hair of *C. glareolus*, exceeding those found in temperate forest [32], only as confirming species omnivory and as showing an increased share of animal food in the diet. Our data confirm seasonal stability of content of nitrogenous substances in the diet, as shown by [30], but are in contrast with [29].

As shown by [27], the diet of an individual is also dependent on individual preferences; niche differentiation in *C. glareolus* can be influenced by behavioural selection, supporting predatory behaviour. According to [27], predatory behaviour not only induces the consumption of more food items of animal origin, but also results in dietary niche heterogeneity and breadth of ecological niche. Therefore, assessments of the diet and the trophic position of species require estimates of TDS values derived from wild animals [51].

We understand that the registered  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values could be influenced not only by the factors assessed, but also (indirectly) by precipitation and land use, as found by [52] in California vole (*Microtus californicus*), as well as by the amount of fertilizers used [53], possible spatiotemporal variation [29,30,54], interactions with other species [55,56] and individual preferences [27].

Investigation of the use of apple seeds by *C. glareolus* could be of interest in the future, as (1) this resource is closest to invertebrates according to isotopic signatures (see Figure 5), and (2) they contain bioactive compounds and oils [57,58] and, therefore, might be preferred by these voles. As for the lichens, in our case they were very low in nitrogen ( $\delta^{15}\text{N} = -12.4\%$ ) and high in carbon ( $\delta^{13}\text{C} = -25.4\%$ ). Such values correspond to the published data [59] but, as shown by [60], lichens are unlikely to be used by *C. glareolus*. Consequently, we did not analyse lichens as a food source.

We found the central positions of the stable isotope ratios in the hair of *C. glareolus* in the apple orchards to not be fully in line with those found in other investigated habitats in the country [61–63]. Both carbon and nitrogen values in the orchards were similar to those in flooded meadows and mowed meadows (Table 3; Figure 3). However, the carbon and nitrogen values in *C. glareolus* from the forest and non-mowed meadows, and from forests in the colonies of great cormorants (*Phalacrocorax carbo*), differed significantly from the orchards (HSD,  $p < 0.001$ ).

**Table 3.** Central positions and ranges of stable isotope values in the hair of *C. glareolus* in various habitats of Lithuania. Great cormorant colonies 1, 2 and 3 are characterised with increasing numbers of breeding bird pairs, therefore, higher input of guano. Significance of differences from apple orchards: \*— $p < 0.01$ , \*\*— $p < 0.001$ .

Habitat	Reference	$\delta^{13}\text{C}$ Values, ‰		$\delta^{15}\text{N}$ Values, ‰	
		Mean $\pm$ SE	Min–Max	Mean $\pm$ SE	Min–Max
Apple orchards	This paper	$-26.11 \pm 0.11$	–28.28–25.22	$6.55 \pm 0.16$	3.03–11.30
Forest	[63]	$-27.91 \pm 0.17^{**}$	–29.14–27.91	$5.18 \pm 0.23^{**}$	3.32–7.96
Flooded meadow	[63]	$-26.22 \pm 0.14$	–27.53–25.46	$6.38 \pm 0.25$	4.77–8.21
Forest (colony 1)	[61]	$-26.73 \pm 0.12^*$	–28.51–24.93	$8.91 \pm 0.55^{**}$	3.12–15.80
Forest (colony 2)	[61]	$-24.78 \pm 0.17^{**}$	–26.15–23.12	$12.42 \pm 0.88^{**}$	5.04–19.24
Forest (colony 3)	[61]	$-25.72 \pm 0.17$	–28.88–24.28	$17.20 \pm 0.56^{**}$	5.79–20.55

Flooded meadows, mowed meadows and apple orchards have additional nitrogen input from floods or fertilizers. The differences in the stable isotope ratios in the hair of *C. glareolus* from forests in the cormorant colonies are a result of the extremely high enrichment of the basal resources in the colony environment with guano [62]: the  $\delta^{15}\text{N}$  values in the hair of *C. glareolus* were higher than normally found in carnivores [61].

Interspecific competing for limited resources could be limited if species compete for different foods [64]. The case of *C. glareolus* (this paper), as well as inter- and intraspecific competition of herbivorous [33] and granivorous [34] small mammals in the orchards confirm such a point of view. Omnivory of *C. glareolus* might be supported by a significant amount of animal-based foods in the diet, including frogs, bird nestlings and small mammal carcasses when starving, snails, earthworms, grubs, beetles, grasshoppers and other invertebrates [3,22]. Therefore, further studies on the diets of small mammals in agricultural habitats, assessing usage of fungi [65], pulses [66] and effects of mixed diets [67] are worthwhile.

Although we did not find a strong relationships between the diet of *C. glareolus* and body mass and body condition, the latter two parameters are important in defining the suitability of the habitat. For the wood mouse (*Apodemus sylvaticus*), a granivorous small mammal, physical condition is determined by two factors—the presence of food and the presence of shelter [68]. Consequently, body mass is one of the main factors defining migration in small mammals, based on risk taking decisions and on details of species-specific biology [69].

Other ecological parameters of *C. glareolus* in the apple orchards, such as the proportion of the species in the small mammal community and relative abundance, follow patterns already known from the habitats. According to [70], agricultural intensification results in a decrease in small mammal diversity, but might have a positive effect on the population abundance of surviving species. Challenges facing farmland small mammals may be solved by migration, which in the case of *C. glareolus* extend to an average of 135–240 m, with a maximum of 440–480 m [71]. In our context, this means that the populations of *C. glareolus* in the apple orchards might be augmented from the nearest habitats. However, as *C. glareolus* in orchards and commensal habitats are very close to human settlements [19], these aspects might be of importance in hantavirus transmission [72,73].

For *C. glareolus*, as a forest small mammal species, apple orchards might serve as secondary habitats fulfilling a gap between forest and farmland. Using secondary habitats, such as hedgerows, orchards and shrubby meadows, small mammals might survive successfully even in conditions of intensive agriculture [74–76]. In general, orchards provide long-term vegetation cover, therefore they are favourable habitats for small mammals [77]. However, it is presumed that under agricultural activities, the amount/diversity of foods available for small mammals in such habitats is limited, and there are sudden changes in food availability. To survive in such habitats, species must have certain degree of ecological plasticity. Varying degree of omnivory in *C. glareolus* is one of such traits, enabling species prosperity in the agrolandscape.

## 5. Conclusions

Isotopic analyses supported the hypothesis of omnivory in *C. glareolus* in the apple orchards, with the species being separated from granivores and herbivores according to  $\delta^{13}\text{C}$ , while being closest to insectivores according to  $\delta^{15}\text{N}$ . It is possible that the species is using foods with higher  $\delta^{15}\text{N}$  values, such as small vertebrates and their carcasses. In the apple orchards, foods of animal origin are preferred by non-adult individuals of *C. glareolus*.

The degree of omnivory in *C. glareolus* in the orchards differed from that in surrounding meadows and forests, indicating ecological plasticity of the species and allowing its inclusion/success in the multi-species small mammal communities.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/agriculture12091308/s1>, Figure S1: Intraspecific differences of the isotopic niches of *C. glareolus* in apple orchards, mowed and non-mowed meadows: (a) by gender, (b) by age; Figure S2: Correlation of *C. glareolus* body mass and body condition index as proxy of individual fitness with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (significant regression line shown in red, 95% CI represented with dotted line) and distribution of the raw values of these parameters: (a) in general, (b) in summer, (c) in autumn, (d) in apple orchards, and (e) in surrounding meadows or forests; Table S1: Trapping effort in 2018–2021 according to apple orchard age, intensity of agriculture, and control habitat type; Table S2: Numbers of small mammals trapped in commercial apple orchards and their control non-orchard habitats (mowed meadows, non-mowed meadows and forests) in 2018–2021, according to dietary groups; Table S3: Changes of relative abundances of *C. glareolus* (individuals per 100 trap-days) in 2018–2021 irrespective of the habitat; Table S4: Central positions and ranges of stable isotope ratios in the hair of adult *C. glareolus* males and females according to their reproductive status; Table S5: Pearson correlation coefficients of stable isotope values in the hair of *C. glareolus* with body mass (Q) and body condition index (BCI) of the individuals. Coefficients shown in bold are significant at  $p < 0.05$ .

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