

ARTICLE

Animal Ecology

Does water vole diet meet the prerequisites of the “plant hypothesis” for explaining population cycles?

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Abstract

Rodent population cycles are observed in highly seasonal environments. As most rodents are herbivorous, the availability and the quality of their food resources vary greatly across seasons. Furthermore, it is well documented that herbivore densities have a measurable effect on vegetation and, conversely, that vegetation dynamics can influence herbivore population dynamics. Many previous studies have investigated whether rodent population cycles could be induced by bottom-up regulation. A recent review summarized several sub-hypotheses regarding rodent population cycles: cycles may be due to inherent inter-annual variations of plant quantity, to overshoot of carrying capacity by overgrazing (i.e., lack of quantity), or to changes in quality of food (decrease in quality of preferred food or switch towards lower quality food) in response to rodent grazing (e.g., plant defenses). While some sub-hypothesis seems to be more important than others, there is currently a prerequisite to construct scientific consensus: dietary description is still overlooked in many systems and should be more investigated. The fossorial water vole shows contrasted population dynamics depending on its geographical location and exhibits large outbreaks in grasslands in highly seasonal climate. It is thus a good model species to investigate plant hypotheses. In this study, the diet of water vole was investigated in and out of an outbreak area with a combination of approaches in the field, in different sampling sites and across seasons. We demonstrated that voles have a very large fundamental trophic niche, but strong behavioral selection, inducing a narrower realized niche, especially during winter. Using an experimental device based on camera traps and cafeteria tests, we observed a strong preference for dandelion (*Taraxacum officinale*) in wild water voles, resulting in exclusive selection during winter for food stores. These preferences were constant across seasons, in control and outbreak areas

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and independent of vole density on plots. When dandelions were overabundant on plots, this preference was less marked, with voles favoring white clover (*Trifolium repens*). We concluded that dandelion is a winter key resource for water vole. It thus might be interesting to investigate the role of dandelion in vole population dynamics.

KEYWORDS

Arvicola amphibius, Campascope, DNA sampling, feeding preferences, field study, grassland pest, trophic niche

INTRODUCTION

Rodents are an important model for studying population dynamics. Numerous studies have explored the mechanisms underlying fluctuations in their numbers, particularly in the context of cyclical dynamics. Several reference books have been regularly published on the subject (e.g., Berryman, 2002; Elton, 1942; Krebs, 2013; Royama, 1992; Turchin, 2003), but it is clear that further work is needed to fully understand the relative importance of the different hypotheses in the general pattern of cycles (Andreassen et al., 2021; Oli, 2019; Soininen & Neby, 2023). There is, however, a scientific consensus that trophic interactions, as well as intrinsic interactions, are important drivers of cycles (Andreassen et al., 2021; Berryman, 2002), that cycles occur in seasonal environments (Kendall et al., 1998; Tkadlec & Stenseth, 2001), and that delayed density dependence is necessary to observe long-phase cycles (Hornfeldt, 1994; Stenseth, 1999).

Seasonal environments are typically characterized by simplified trophic chains. This can result in the establishment of prey–specialist predator relationships, favored by the presence of snow cover (Hansson & Henttonen, 1985). The delay in prey regulation is induced by the generation time of specialist predators (Hanski et al., 1991). Seasonality also implies significant variation in food resource throughout the year. Rodent–plant interactions may also be highly detrimental to plants during winter, when they cannot regrow; yet, rodents still need to feed. As a result, rodents can significantly impact the quantity (e.g., Huitu et al., 2003) or quality (through silicon, e.g., Massey et al., 2007) of their resources. The time required for the regeneration of preferred resources after depletion, or for the restoration of food quality following plant defenses, can theoretically induce cycles (e.g., Massey et al., 2008; Turchin et al., 2000, for a review, see also Soininen & Neby, 2023). In both cases, rodents depend on a preferred food and the absence of this resource must impact their survival, reproduction, or dispersal (Soininen & Neby, 2023). Plant–rodent interactions have been proposed to explain certain rodent cycles in

several different contexts (e.g., *Lemmus*, mosses: Turchin et al., 2000, *Lemmus*, carex: Seldal et al., 1994, *Microtus*, graminoids: Massey et al., 2008, *Myodes*, blueberry: Selås, 2020). However, scientific consensus has not yet been completely achieved in these systems because of data that are often difficult to access. Further research is needed to determine the extent to which observed cycles are driven by plant–rodent relationships.

To achieve a cycle driven by plant–rodent interactions, candidate systems must meet the three following points: (1) the plants exhibit strong seasonality in their growth period, (2) the rodents have strong food preference or are dependent on a limited number of plant species, and (3) after the rodents have affected their preferred food source, a recovery time is required to restore its quality or quantity.

In the French mid-mountains (Jura and Massif Central), water voles exhibit some prerequisites for plant-based hypotheses. Firstly, outbreaks occur only above 750 m above sea level (asl) (Fichet-Calvet et al., 2000) in areas where there is significant seasonality in grassland growth. In outbreak areas, maximum densities of voles are different across plots, which shows a wide diversity of grasslands in the Massif Central mountains due to gradients in altitude, productivity, soil hygrometry, and type of harvesting (for a full description, see Gaillot et al., 2020). Moreover, outbreaks significantly change plant communities (Nicod et al., 2020), highlighting a close link between plants and water vole populations. However, our understanding of the water vole's diet remains limited, mainly due to the challenges of studying the feeding habits of subterranean animals within a complex environment.

In this context, our study aimed to investigate seasonal variation in voles' diets and to document their feeding preferences (points 1 and 2), building on our understanding of whether plant–vole interactions could drive population cycles in France. While it is scientifically agreed that the fossorial form of water vole is strictly herbivorous, its precise diet has not been clearly described. Airolidi (1976) and Potapov et al. (2004) found voles' winter food stores. It was composed of roots from various

species in the former study. In both cases, the mass of these food stores was substantial, reaching hundreds of grams or even kilograms, and was season-dependent (i.e., mainly observed in fall and winter).

Several other studies have indicated that water voles exhibit food preferences (Kopp, 1988; Saucy et al., 1999; Viette et al., 2000). Viette et al. (2000) and Saucy et al. (1999) found that these preferences occur at the intraspecific scale in response to variations in plant chemical traits, particularly plant defenses. Kopp (1988) observed that food preferences among grassland species influenced the voles' foraging behavior and space use, with a notable preference for dandelions and clovers over Poaceae. However, while the study design was relevant, its conclusions were constrained by the limited number of plant species considered and the lack of replication. A more recent study did a large investigation about the diet of the aquatic form of water vole in the wild; it revealed that

voles have a diverse diet with Poaceae being dominant, exhibit strong site-specific variations, and exhibit a slight seasonal pattern (Østby, 2019). Furthermore, Neyland (2011) showed that water voles (aquatic form) make food choices likely linked to the chemical traits of plants, particularly their nitrogen content. All these studies suggest that the water voles' diet results from a complex interplay of factors, including habitat and seasonal variations. They also showed that water voles are sensitive to food chemical traits and make choices.

In this study, the trophic niche was investigated as part of the ecological niche including potentiality of voles' diet and behavioral preferences for some aliments in the natural environment. We used definitions from the theoretical framework described by Shipley et al. (2009; see Figure 1). The term "fundamental trophic niche" hereafter in this paper describes voles' eating possibilities regardless of habitat or season. In contrast, the realized

DATA COLLECTION

THEORETICAL FRAMEWORK

(adapted from Shipley et al., 2009)

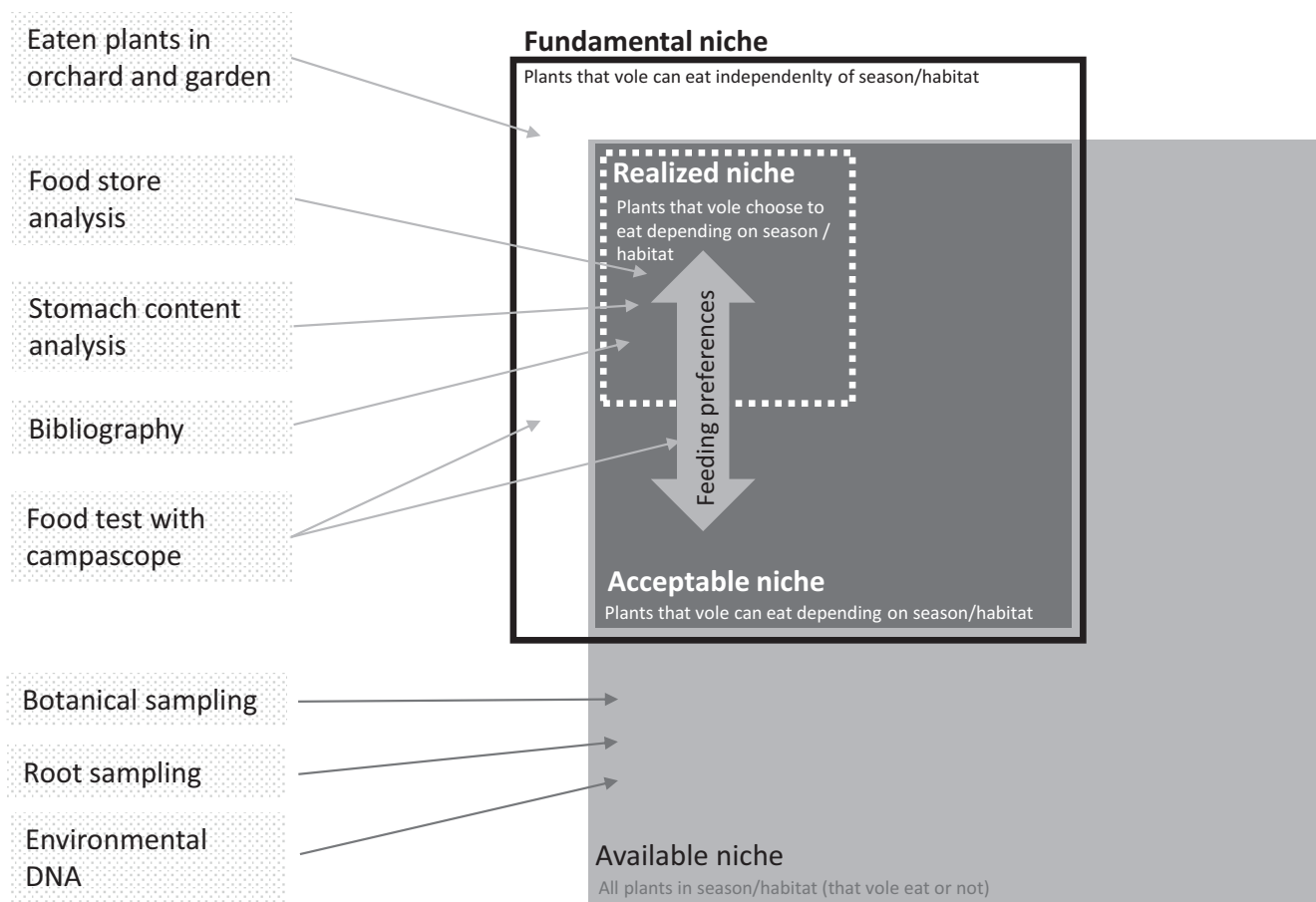


FIGURE 1 Overview of data collection and use. To investigate interactions between plants and the fossorial form of water vole, different types of data were collected from natural habitat and from bibliography. Arrows highlight the links between the collected data and the theoretical framework, which was inspired by Shipley et al. (2009).

trophic niche defines the part of the fundamental trophic niche that is determined by habitat and feeding behavior under natural living conditions. The realized niche obviously depends on the available niche (plants that the vole encounters in its habitat) and also on the vole's preferences (plants that the vole chooses to eat). Indeed, feeding behavior *in natura* might reveal differences between realized niche and acceptable niche. This results from strict crossing between available and fundamental niche and might not reflect exactly voles' behavior in the study context.

Formerly, many designs were explored in dietary studies to assess the trophic niche of rodents. Authors usually used direct observations of vole stomachs or feces contents with species determination under the microscope (Hansson, 1970), such as Cole and Batzli (1979) on the prairie vole, Ashby and Vincent (1976) on the aquatic form of water vole, Pinter et al. (1992) on the montane vole, and Rothstein and Tamarin (1977) on the insular beach vole. More recently, DNA amplification and sequencing for species determination in stomachs or fecal contents have been used for comparison with DNA bases (Taberlet et al., 2012), giving good results for dietary study in small herbivores and rodents (Soininen et al., 2009), such as for the fossorial form of water vole in Norway (Østby, 2019), the Amargosa vole in California (Castle et al., 2020), the Norwegian lemming in Norway (Soininen et al., 2017), or the bank and tundra vole in Norway (Neby et al., 2024). Moreover, voles are known to have consistent food stores, mainly composed of roots. Food stores contents analysis also provided information on the plants of interest for voles (Airoldi, 1976). Other studies focused on indirect observations, such as resource depletion, to assess voles' foraging behavior in semi-captivity experimentation on prairie voles (Cole & Batzli, 1979; Giban & Spitz, 1967; Kopp, 1988; Saucy et al., 1999) and common voles (Spitz, 1968). All methods have shown their own advantages and limitations in diet interpretation.

To investigate the trophic niche of water voles, we also used several complementary methods based on DNA stomach contents analysis, determination of the species contained in food stores, and behavioral observations. Due to the lack of methods to investigate the food preferences of subterranean species, we developed an original device with a camera trap to record the feeding behavior of wild animals (Lisse & Pinot, 2024) for which the first ecological results are presented in this study. The main objectives of our study were (1) to document the available and realized trophic niches of voles in spring and winter, under natural conditions and in outbreak areas and (2) to compare feeding choices across seasons, between outbreak and non-outbreak areas, and across different vole densities and different levels of plant availability.

MATERIALS AND METHODS

Fossorial water voles' biology in the studied area

The water vole is known for its diversity in mass and body length, as well as its ecotypes (Strachan et al., 2011), ranging from a strictly aquatic form to a strictly fossorial form, with an intermediate form in between. Our study focused exclusively on the fossorial form of the water vole, *Arvicola amphibius*, in the Massif Central mountains in France. In this area, water voles inhabit exclusively natural grasslands used for farming production. The fossorial form of the water vole is characterized by its subterranean lifestyle, predominantly living in burrows and galleries, rarely surfacing except during sexual dispersal (Airoldi, 1978; Delattre et al., 2006; Saucy & Beat, 1997).

In the study area, the body mass of adult (i.e., reproductive) animals ranged from 71.2 to 137.6 g (quantiles 0.05–0.95, $N = 2102$ individuals; our data) and body length ranged from 140 to 175 mm as measured from the nose to the base of the tail.

The populations of the fossorial form of water vole exhibit strong amplitude cycles (Pascal & Boujard, 1987; Saucy, 1988), with periodicity ranging between 6 and 8 years (Fichet-Calvet et al., 2000). In our study area, vole outbreaks have been monitored by the government since the 1980s. These surveys have revealed regular cycles, synchronous over a very large scale (Note & Poix, 2006), with periods of 6–8 years.

Methodological approach

The spatial and temporal structure of vegetation is crucial for understanding herbivore–plant interactions (Soininen & Neby, 2023). We therefore monitored the available niche (as defined by Shipley et al., 2009) accounting for seasonality and plant morphology (i.e., green parts vs. roots).

To assess water voles' diet, we described three levels.

First, we defined the fundamental trophic niche of the fossorial form of the water vole. This was achieved using all available data on plant consumption: bibliography (focused on fossorial form in Western Europe), single food tests using Campascopes (Lisse & Pinot, 2024), roots found in food stores, and DNA barcoding of vole stomach contents. We also added information on species eaten in market gardens and orchards when we were able to catch voles actively eating plants. This part of the study provided results on the diversity of potential diet at the species scale.

Second, we assessed the realized trophic niche in the meadows of our study area by considering data collected only from natural behaviors (food stores and DNA barcoding in stomach contents). This defined the effective diet in the meadows of our study area.

Third, we investigated the variability in vole dietary preferences by comparing the grassland composition through botanical, roots, and DNA data (available niche) with the plants eaten/collected by voles (realized trophic niche).

These three points complete the first part of the study to describe the realized niche of voles in spring and winter and document plant selection. For the second part, the variation in preferences was studied through triple food choice tests conducted using Campascope throughout the season, between outbreak and non-outbreak areas, and with different availabilities of preferred food. Triple food choice tests were based on the results of the niche description.

Data use and concepts are summarized in Figure 1.

Study area and agricultural plots choice

The study was conducted in the natural habitat of the fossorial form of water voles, specifically in natural meadows managed by farmers. The plots were selected based on a gradient of productivity in order to encompass all prairie types (Gaillot et al., 2020) (Appendix S1). Productivity was estimated for each field plot within the study area by combining the administrative register of parcels and plant productivity from satellite imagery (HR-VPP products from Copernicus Land Monitoring Service by the European Environmental Agency and VITO, average value for years 2020, 2021, and 2022). At the local scale (i.e., across several equivalent field plots), selection was based on technical considerations: road access, farmers' knowledge and agreement, size of field plot (i.e., field plots smaller than 1.5 ha were avoided), and the surrounding habitat of the plot (i.e., open field), aligning with the random process inherent to stratified sampling. Vole density values on the studied field plots are available in Appendix S2.

In the first part of the study, we used 22 plots to compare the realized niche with the available niche in winter and spring. All the plots used for this part of the study were located in an outbreak area above 900 m high and within the high-density phase of the cycle. Using outbreak areas and the high-density phase ensured the diet was studied in an environment favorable to vole outbreaks. Samplings of realized and available niches were systematically paired on plots to avoid confusion factors. Sampling types (roots or DNA) were of different nature in winter and spring. We also sampled botanical species on 25 plots in outbreak areas ($n = 15$) and in a control area ($n = 10$). The control area is defined as under 550 m and over 1100 m high,

where no outbreaks are documented. For the second part of the study, we selected 15 plots, 9 from outbreak areas and 6 from the control area. The vole densities on plots were estimated by remote sensing from UAV photography of the plots. The plots in non-outbreak areas were de facto all in low densities. We also considered a gradient of dandelion density on all selected plots. The dandelion index was calculated using the year of the study by remote sensing. We set up the study in four different seasons (Table 1).

Data collection

Voles' fundamental niche

Plants' palatability

We developed an original design to propose food to voles inside their burrows. This device, the Campascope (Lisse & Pinot, 2024), consists of a box buried in a gallery and equipped with a camera to film voles' behavior. We offered a fresh small piece (~20 g) of each plant species ($n = 51$) found in meadows (based on a list of botanical data and representing the available niche) and observed whether the voles consumed it or carried it away. We also tested plants that water voles may not find in meadows but are consumed by other vole species (spruce, silver fir; Jacob & Tkadlec, 2010) or were formerly used as livestock fodder in the study area (common alder, European ash). The appetite test was performed in five grasslands.

Diet from previous trapping studies

To expand our knowledge on the fundamental trophic niche, we completed the list of eaten plants with opportunistic data collected from observations reported in the field by professionals (farmers and scientists) on cultivated plants. For each of the data, we confirmed that a vole was the eater of a plant through systematic trapping. The traps were placed in the dead-end gallery leading to the eaten plant (at a couple of centimeters away from the plant). Data were collected in Massif Central mountain gardens and orchards from 2017 to 2023. Not all identified plants were systematically represented in natural grasslands.

Voles' available and realized niches

Available plants in the studied area (summer samplings)

To assess the available niche, plant species were inventoried in natural grasslands of the voles' repartition area. Three main types of meadows (Gaillot et al., 2020) were sampled: mountain hay meadow with *Umbelliferae*

TABLE 1 Sampling design for field-based feeding choice tests.

Season	Plots (total = 15)		Feeding choice sequences (total = 136)	
	Outbreaks (total = 9)	Control (total = 6)	Outbreaks (total = 103)	Control (total = 33)
Winter	4	1	13	7
Fall	5	2	36	6
Spring	2	1	24	11
Summer	4	1	30	9

Note: The table summarizes the distribution of feeding choice sequences conducted in natural habitat across 15 plots and four seasons, in outbreak and control areas. Control areas were defined as sites below 550 m and above 1100 m elevation, where no outbreaks have been documented. Plots were sampled in multiple seasons.

on healthy to fresh and fertile soil, mountain hay meadow with *Rumex crispus* on healthy to fresh and very fertile soil, and mountain grazed meadow with *Lolium perenne* and *Taraxacum officinale* on healthy to fresh and fertile to very fertile soil. The plots were natural meadows used for hay production in the outbreaks area. Twenty-five plots were sampled, 15 in the outbreaks area (where voles' samplings were realized) and 10 outside this area. Botanical determinations were made in summer when most species were in flower. For each plot, 9 random subplots of 0.33 m² were described along a 100-m transect in the middle of the grassland plot. Braun-Blanquet (1964) cover indices were noted for each plant. The average proportion of each species was calculated over all plots.

Available and realized niche during the vegetation growth season (spring)

DNA analysis of stomach contents was the main tool used to investigate diet during the growing season. Collecting fecal samples is also an effective method of investigating the diet of rodents (e.g., Neby et al., 2024) and is more respectful of animal welfare. However, these voles are strictly subterranean and have subterranean latrines. Their droppings deteriorate very quickly when exposed to trampling and dampness of the burrows. Therefore, collecting fecal samples directly from burrows was not realistic. In our study area, fossorial water voles were classified as pests at the time of the study, so it was illegal to release them once caught. This made it impossible to collect fresh feces by placing a vole in a box for a few minutes without killing it afterwards. Therefore, we concluded that stomach sampling was the best option, as it permitted us to work with less degraded material.

DNA metabarcoding analysis was conducted in spring. In each of 22 plots, seven adult voles were trapped using Topcat lethal traps and dissected to extract their stomach contents. Fifteen traps were set on each plot on 5 colonies. Concurrently, 10 soil samples were collected with 8-cm diameter augers to a depth of 5–8 cm. Soil samples were

made to assess vegetation diversity through eDNA, especially for plants that were not/few visible during botanical sampling but could represent substantial resources due to their big roots. Samples from the same plots were pooled into a single sample (1 sample for soil and 1 sample for stomach content by plot). They were prepared by scraping stomach contents or by removing soil particles from plants, dried in accordance with Argaly laboratory protocols (see dedicated protocol in Appendix S3), conditioned in 20-g sterile tea bags with a calcium chloride additive and sent to the laboratory (Argaly, Saint-Hélène-du-Lac, –73800 France). All field and laboratory work were conducted under extreme hygienic conditions (individual protection equipment and fire-cleaning of utensils) to avoid DNA contamination. DNA extraction and metabarcoding were done at the Argaly laboratory according to procedures detailed in Appendix S3. DNA identification and the associated number of sequences were obtained for each batch of stomach and soil samples. We first cleaned data provided by Argaly by eliminating matches with species and genus that were not local plants. Plant families were pooled to match identification between soil samples and stomach contents. To avoid interpretation bias when analyzing DNA sequences quantitatively, caused by preferentially amplified sequences (Pompanon, 2012; Taberlet et al., 2012), we compared the number of occurrences observed in the soils and in the stomachs.

Available and realized niche during the vegetation non-growing season (winter)

From 2017 to 2019, we monitored *A. amphibius* populations in the same 22 field plots several times each year (with at least one trapping in spring and one in winter). During excavation for trap placement, we discovered 15 food stores across 8 different plots. For each food store, the contents were collected, cleaned, and the plant species identified. We also recorded the dates of findings to analyze the seasonality of food store discovery. For 6 stores of roots, each piece was weighed, measured, and dried. Additionally, we supplemented our field

observations with information from the literature (Airoldi, 1976; Kopp, 1998).

In winter 2018, the 22 plots were also sampled for root biomass. In each plot, roots were sampled with 10 cores along two intersecting transects, with 3 m between each sampling point. The soil samples containing roots were collected using an 8-cm diameter root auger to a depth of 12 cm. The samples were then sieved using different mesh to separate roots by diameter and to remove soil. Root species were identified by visual inspection (*T. officinale*, *Trifolium*, *Ranunculus*, *Dactylis glomerata*, bulbs, and thin roots or other roots that could not be determined to species/genus) and weighed. These samples allowed for the estimation of the available niche during winter.

Variability of feeding preferences

Campascope triple-choice test

To measure the representativity of the food preferences pattern, the variability of these preferences in relation to environmental factors of voles' natural habitat was tested. Behavioral tests were conducted on wild voles in natural grasslands in outbreak and control areas (Table 1). These tests of feeding choices were carried out using the Campascope (Lisse & Pinot, 2024) with three plants of interest. The plants were chosen from each major family group available in the field, based on species previously mentioned in the literature or identified as significant during the realized trophic niche analysis. Specifically, preference tests were conducted on a triptych of dandelion (*T. officinale*, noted as the preferred species), white clover (*Trifolium repens*, present in all grassland and also selected), and cocksfoot (*D. glomerata*, present in all grasslands and apparently the most selected gramineous species). We tested variability of food preferences between these three plants under different environmental conditions. Field plots were chosen on a gradient of vole density and with variable plant composition. Four seasonal sessions were chosen, in March (late winter), May (spring), June/July (summer), and September/October (fall). Due to snow cover and frozen soils, devices could not be set up during full winter. The densities of voles and dandelions were determined using remote sensing by analyzing the amount of bare soil and the number of dandelion flower heads in drone-captured photos (see Buronfosse et al., 2025 for full method description). A total of 128 Campascopes were set up, with at least 25 per season and 5 per plot. Fresh plants were collected directly from the field plot or its border. The three species, *T. repens*, *T. officinale*, and *D. glomerata*, were each calibrated to be approximately the same size and mass (~15 g) and randomly positioned together in the box.

When plants were all consumed, fresh plants were placed in the Campascope. After the first choice, plants were left for a maximum of 1 h and 30 m (i.e., the standard length of a vole's activity period during the day; Airoldi, 1979), before resetting the choice with fresh plants. The age class of the vole (juvenile, subadult, or adult) was estimated from each video based on its size (Saucy, 1988). For each plant species, the rate of removal or consumption was recorded, as well as the order in which removal or consumption occurred.

All applicable institutional and national guidelines for the care and use of animals were followed. This study was conducted in accordance with the guidelines declared in the Declaration of Helsinki and received approval from the relevant authorities (number 37713, project authorization number 2238) and from the ethics committee of the veterinary school of Lyon.

Statistical analysis

Comparison between plant availability (available niche) in meadows and vole samples

Vole preferences were assessed by comparing the realized trophic niche with the available niche in paired samples and with plants of the studied area. The spring selection coefficient was estimated by comparing plant occurrences in stomach samples with plant frequencies in meadows (botanic: Braun-Blanquet and barcoding: occurrence of sample). The winter selection coefficient was similarly estimated by comparing the proportion of roots in food stores with the proportion of roots in meadows. These analyses were conducted at the family level. Shannon diversity indices (Shannon, 1948) were calculated for each type of field data collected (botanical samples, stomach contents DNA, soil DNA, roots in food stores, and roots in soil). These indexes were used to evaluate the broadness of the two different niches comparatively to one another. They provided a general pattern and were used to rank plants in order of importance for the vole diet.

Variability of feeding preference

A generalized linear mixed model (Poisson) was employed to estimate the variation in average choice rank for each of three plants tested. This model enabled us to determine whether significant differences existed among the three plants. The influence of external variables on the ranking was tested with sub-models for each plant. The following variables were tested: season (factorial variable; spring, $n = 42$; summer, $n = 35$, fall,

$n = 39$, winter, $n = 20$), area type (outbreak area between 550 and 1100 m, control outside), apparent vole density (factorial variable, class of density of earth mounds: low, middle, high), dandelion density (average number of flowers per 0.58 ha recorded in the field plot on May 15), and individual age class (three classes: juveniles, subadults, and adults). A random effect of plot was included. Each variable was tested independently and results were compared with the null model using corrected Akaike information criterion (AIC_c) comparison. Models with a delta $AIC_c < 2$ were assumed equivalent models and the best model was selected as the one with the fewest parameters considered (Burnham & Anderson, 2002).

RESULTS

Fundamental trophic niche

Water voles were found to consume at least 79 different plant species belonging to 30 different plant families. These species are listed in Table 2, specifying the method of recording and their presence in the meadows within our study area. The water vole did not exhibit neophobic behavior, as it consumed leaves of forest trees. It also consumed plants with toxic components (e.g., *Narcissus*, *Rumex*, and *Ranunculus*) as well as those with urticant properties (*Urtica*). Voles were able to eat a large variety of herbaceous plants, but also roots of creepers (two species) and trees (six species). Voles also ate plants formerly used for feed livestock (common alder, European ash), but ignored resinous trees (spruce, silver fir). Voles were able to eat all the available plants in grasslands (for a complete description of the grassland plant community, see “Plant availability: grasslands composition and diversity” in the “Food preference” section and Figure 2a).

The fossorial form of water vole showed a very large fundamental trophic niche with no specific specialization for the flora of meadows.

Realized trophic niche

DNA in stomachs

DNA barcoding analyses of stomach contents identified 11 families of plants representing at least 20 different species. The Shannon index of diversity for the “all” dataset was 4.15, indicating a large and equilibrate diet *in natura*, at least in summer. During this period, 71% of stomachs contained *Poaceae*, 71% *Asteraceae* (mainly *T. officinale*), 39% *Polygonaceae* (mainly *Rumex*), 32%

Fabaceae, 23% *Plantaginaceae*, 16% *Brassicaceae* and 13% *Caryophyllaceae* (Figure 2b), highlighting the dominant role of *Poaceae* and dandelion in the diet, but also the significant contribution of other plant families. The Shannon index of diversity per vole was approximately 0.84 ± 0.71 (mean $\pm$ SD). In comparison with the Shannon index of the “all” dataset, it suggests that vole diet is variable between individuals.

Food stores

Over the course of 184 capture sessions and almost 1000 colonies sampled, 15 different food stores were discovered, exclusively in fall and winter, during the non-growing period (Figure 3a).

Food stores were composed exclusively of roots cut into pieces and of low diameters. Among the 1596 fragments measured from 6 different food stores, a bimodal distribution was observed with short fragments (4.4 ± 1.5 cm; 8.4 mm of average diameter) and long fragments (7.5 ± 2.6 cm; 8.2 mm of average diameter). The pieces of roots were well preserved, as voles isolated them from one another by surrounding each fragment with soil. The tops of dandelions were present in silos. The mass of the food stores (excluding soil) was between 37 and 1380 g, with a mean of 545 g.

The food stores contained plant material from four families representing five species (Figure 3b). The Shannon index of diversity was 0.366, indicating a low level of diversity. This suggests a highly constrained realized trophic niche for food stores. Indeed, 94.5% of the food stores were composed of dandelions roots, revealing a strong dependence on dandelions in winter in our study area.

Food preferences

Plant availability: Grasslands composition and diversity

At the community scale, we observed that voles inhabit highly diverse meadows. A total of 66 plant species were identified before mowing. Figure 2a shows the average plant composition of the 22 sampled grasslands located in outbreak areas (measured before outbreak to avoid vole disturbance). The average percent cover was calculated for each species (mean of percent cover across 22 grasslands) and allowed better description of the habitat of voles: grasslands were dominated by grasses, which accounted for approximately 40% of the cover and included 19 species. The most prevalent *Poaceae* was *L.*

TABLE 2 Plant species eaten by the fossorial form of water vole (*Arvicola amphibius*).

Species	Habitat	Braun-Blanquet	Damages	Food stores	Stomach	Campascope	Bibliography
<i>Achillea millefolium</i>	G	1				x	
<i>Agropyrum repens</i>	G	...	x	x			
<i>Allium ampeloprasum</i>	M	...	x				
<i>Alnus glutinosa</i>		...				x	
<i>Alopecurus pratensis</i>	G	1				x	
<i>Anethum graveolens</i>	M	...	x				
<i>Anthriscus sylvestris</i>	G	0				x	
<i>Avena sterilis</i>	G	...			x		
<i>Bellis perennis</i>	G	...				x	
<i>Beta vulgaris</i> subsp. <i>Vulgaris</i>	M	...	x				x
<i>Borago officinalis</i>	G	...	x				
<i>Bromus hordeaceus</i>	G	1				x	
<i>Capsella Bursa-pastoris</i>	G	0			x	x	x
<i>Castanea sativa</i>	O	...	x				
<i>Cerastium arvense</i>	G	1				x	
<i>Cichorium intybus</i>	M	...	x	x	x		
<i>Cirsium arvense</i>	G	0	x			x	
<i>Convolvulus arvensis</i>	G	...					x
<i>Crocus albiflorus</i>	G	...					x
<i>Cucurbita pepo</i>	M	...	x				
<i>Cydonia oblonga</i>	O	...	x				
<i>Cynosurus cristatus</i>	G	0				x	
<i>Dactylis glomerata</i>	G	6		x		x	x
<i>Daucus carota</i>	G	0			x	x	x
<i>Daucus carota</i> subsp. <i>Sativus</i>	M	...	x		x	x	x
<i>Euphorbia cyparissias</i>	G	...				x	
<i>Festuca arundinacea</i>	G	2				x	
<i>Festuca rubra</i>	G	1			x	x	
<i>Fraxinus excelsior</i>	O	...					
<i>Galium verum</i>	G	0				x	
<i>Heracleum sphondylium</i>	G	2				x	x
<i>Holcus lanatus</i>	G	5				x	
<i>Humulus lupulus</i>	C	...					x
<i>Knautia arvensis</i>	G	1				x	
<i>Lactuca sativa</i>	M	...	x				
<i>Lathyrus pratensis</i>	G	0				x	
<i>Leontodon hispidus</i>	G	...		x			
<i>Leucanthemum vulgare</i>	G	2				x	
<i>Lolium perenne</i>	G	9				x	x
<i>Lotus corniculata</i>	G	0				x	
<i>Malus domestica</i>	O	...	x				
<i>Medicago Sativa</i>	C	0				x	x

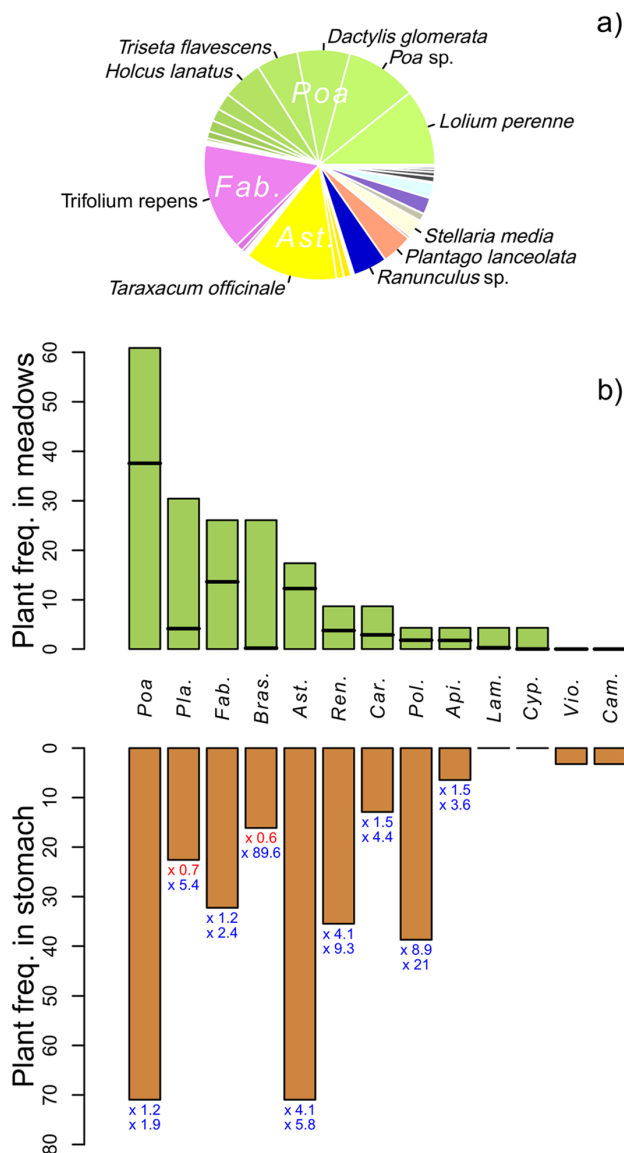
(Continues)

TABLE 2 (Continued)

Species	Habitat	Braun-Blanquet	Damages	Food stores	Stomach	Campascope	Bibliography
<i>Myosotis arvensis</i>	G	0				x	
<i>Narcissus pseudonarcissus</i>	G	0	x				
<i>Petroselinum crispum</i>	M	...	x				
<i>Phleum pratense</i>	G	0				x	x
<i>Plantago lanceolata</i>	G	3			x	x	
<i>Plantago major</i>	G	0			x	x	
<i>Poa pratensis</i>	G	8				x	
<i>Poa trivialis</i>	G	8			x	x	
<i>Potentilla erecta</i>	G	0		x		x	
<i>Pyrus communis</i>	O	...	x				
<i>Ranunculus acris</i>	G	4				x	
<i>Ranunculus bulbosus</i>	G	0	x	x		x	
<i>Ranunculus repens</i>	G	0		x		x	
<i>Rhodiola rosea</i>	M	...	x				
<i>Rumex acetosa</i>	G	2		x		x	x
<i>Rumex crispus</i>	G	1				x	
<i>Sanguisorba minor</i>	G	0					x
<i>Scorzoneroideis autumnalis</i>	G	0				x	
<i>Silene vulgaris</i>	G	0			x		
<i>Solanum tuberosum</i>	M	...	x				x
<i>Spinacia oleracea</i>	M	...	x				
<i>Stachys officinalis</i>	G	1				x	
<i>Stellaria media</i>	G	2				x	
<i>Taraxacum officinale</i>	G	#	x	x	x	x	x
<i>Tragopodon pratensis</i>	G	0			x		
<i>Trifolium dubium</i>	G	1				x	
<i>Trifolium pratense</i>	G	1			x	x	x
<i>Trifolium repens</i>	G	#	x	x	x	x	x
<i>Trisetum flavescens</i>	G	5			x	x	
<i>Tulipa</i> sp.	M	...	x				
<i>Urtica dioica</i>	M	...	x				
<i>Veronica arvensis</i>	G	0				x	
<i>Vicia faba</i>	M	2	x			x	
<i>Vicia sativa</i>	G	...				x	
<i>Vicia sepium</i>	G	...				x	
<i>Viola tricolor</i>	G	0				x	
<i>Vitis vinifera</i>	O	...	x				
<i>Zea mays</i>	G	...				x	

Note: Habitat indicates the main habitat of each species (G, grasslands; M, market garden; C, croplands; O, orchards). Braun-Blanquet indicates the abundance–dominance index observed in our study area (in percentage composition; # if >0% but <1%). In the next five columns, an “x” indicates that the species was recorded in the vole diet using the corresponding method. For the “Bibliography” method, publications referred to the fossorial form in Western Europe.

perenne with 8.5% cover, followed by *D. glomerata* (5.9%) and *Trisetia flavescens* (4.61%). Fabaceae were also strongly represented, accounting for 13.6%. *T. repens* was the most abundant species, followed closely by *T. officinale* (10.3%). Other notable species included *Plantago lanceolata* and several *Ranunculus*. The Shannon index for plant diversity of grasslands was 4.13.



Soil samples for DNA analysis were collected after mowing and revealed the presence of 11 families of plants representing at least 22 different species. The Shannon index of diversity for the soil samples was 3.81, which is the same order of magnitude as the Shannon index obtained from botanical samplings. The dominant family identified was *Poaceae*, present in 61% of the samples. Other prominent families included *Plantaginaceae*, *Fabaceae*, and *Brassicaceae* (found in 30%, 26%, and 26% of the samples, respectively). The *Asteraceae* family was present in 17% of the samples. Those results can be compared to the botanical results in Figure 3b, though correspondence may be challenging due to potential inaccuracies in the recognition of DNA sequences. The relative contribution of plant families to the overall plant community was globally consistent between methods except for two families: *Plantaginaceae* and *Brassicaceae*, which were over-represented in DNA soil samples compared to botanical samples.

In the soil, root biomass was dominated by root hairs from graminoids or other plants that could not be visually identified in winter (Figure 3b). They accounted for 61.1% of the total dry mass. The remaining biomass was composed of roots from *D. glomerata* (20.8%), *T. officinale* (9.4%), *Ranunculus* (6.1%), and *T. repens* (2.6%). The Shannon diversity index for root biomass was 1.61. This low value should be interpreted with caution because of the great difficulty in identifying small root species.

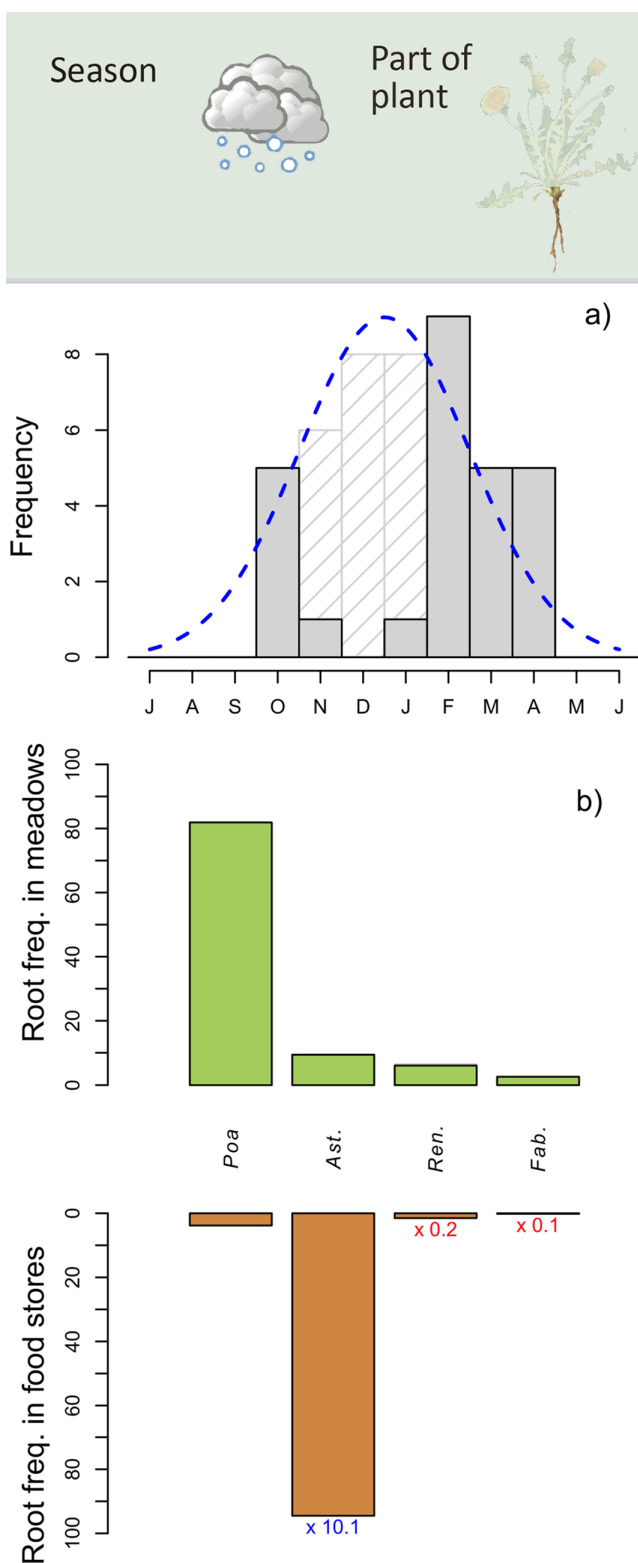
Preferences observed in DNA analyses

The Shannon diversity index for vole stomach samples was approximately 4.15, higher than that observed for soil DNA samples, but similar to the index obtained on botanical

FIGURE 2 Water vole diet depends on plant availability in grasslands. Icons at the top of the figure indicate the sampling season and the plant part concerned. Illustration credit: A. Pinot. (a) Grasslands composition in late spring estimated using Braun-Blanquet method. (b) Plant distribution in summer estimated using DNA detected in soil (green) and in vole stomach contents sampled at the same time (brown). Horizontal bars in the green part of the graph represent occurrence of the family in the botanical sampling (Braun-Blanquet). Below the brown bar plot, two coefficients are reported: the upper one compares DNA occurrence in soil (green bars) and in stomach contents (brown bars), and the lower one compares Braun-Blanquet occurrence (horizontal bars) and DNA occurrence in stomach (brown bars). Api., Apiaceae; Ast., Asteraceae; Bras., Brassicaceae; Cam., Campanulaceae; Car., Caryophyllaceae; Cyp., Cyperaceae; Fab., Fabaceae; Lam., Lamiaceae; Pla., Plantaginaceae; Pol., Polygonaceae; Ren., Ranunculaceae; Vio., Violaceae.

samples. However, the diversity in vole stomach contents was mainly due to diversity between individuals rather than diversity within the same bolus. Indeed, the average Shannon index of diversity for an individual was approximately 0.84. Despite this, it is unlikely that individuals were

specialized as the average species richness per stomach was 5.22 (SD: 3.52). The most selected family seemed to be *Polygonaceae* (mainly *Rumex acetosa*) with an 8-fold increase compared to soil DNA and a 21-fold increase compared to botanical samples, then *Ranunculaceae* (4.1–9.3-fold increase, probably *Ranunculus bulbosus*) and *Asteraceae* (4.1–5.8-fold increase, *T. officinale*). Some other families also seemed to be common in stomachs, despite being relatively rare in meadows: the *Caryophyllaceae* (1.5–4.4-fold, mainly *Silene vulgaris*) and the *Apiaceae* (1.5–3.6-fold, mainly *Daucus carota*). Voles also appeared to select a *Brassicaceae* (*Capsella bursa-pastoris*) that was not detected in botanical samples. While the voles' stomach contents consisted mainly of *Poaceae*, it was unclear whether selection had occurred, as the family was highly dominant in grasslands. *Fabaceae* (mainly *Trifolium*) and *Plantaginaceae* (*Plantago*) were probably in the same case. None of the dominant plants were avoided. Because comparisons between botanical and environmental DNA samples showed that *Plantaginaceae* and *Brassicaceae* were overrepresented in DNA soil samples compared to botanical samples, quantitative results from DNA stomach samples should then be taken with caution. With this caution, a pattern of selection in summer is found for *Rumex*, *Ranunculus*, and *Taraxacum*. The common feature of the apparently selected species is a taproot.



Preferences observed in food stores

Food stores were composed of 94.5% of dandelion roots (*T. officinale*), even though this species accounted for only 9.4% of the available root biomass in grassland samples (Figure 4b). The remaining 5.5% comprised little bulbs, which were absent from the soil samples, indicating that voles are likely to find and select them, and of *Trifolium* and *Ranunculus*, which were also found in the soil. When comparing the proportions of plant families between food stores and soil samples (Figure 3b), all families were markedly

FIGURE 3 Root storage by water vole. Icons at the top of the figure indicate the sampling season and the plant part concerned. Illustration credit: A. Pinot. (a) Seasonality of vole food stores discovery. Gray bars represent food stores reported in bibliography and recorded in the field by month (from July to June); light bars represent the expected but undetected food stores due to limited fieldwork (snow and frozen soil). The blue dotted line represents the expected seasonal dynamics of food stores. (b) Root species available in grasslands during winter (green) and found in voles' food stores (brown). Frequencies are shown for four plants families: Ast., Asteraceae; Fab., Fabaceae; Poa, Poaceae; Ren., Ranunculaceae. Below the brown bar plot, the comparison between root occurrence in grasslands and in food stores is reported.

underrepresented, except for *Asteraceae* (10-fold increase), indicating strong selection of *T. officinale* by water voles.

Variability of food preferences

In the field, we recorded voles' food choices on video throughout several seasons in an entire year. After visioning records, 140 sequences of vole choice, involving at least 70 animals, divided into 4 batches of 20–42 videos (1 batch per season) were available for statistical treatment (Table 3). Choice ranks for *T. officinale*, *T. repens*, and *D. glomerata* were 1.42 ± 0.106 , 1.77 ± 0.122 , and 2.6 ± 0.159 , respectively (mean $\pm$ SE from the Poisson model; Figure 4). Voles showed a high preference for *T. officinale*, which was taken in 95% of cases and chosen first in 60% of cases. *T. repens* was taken in 85% of cases and chosen first in 32% of cases, while *D. glomerata* was taken in 75% of cases and chosen first only in 8% of cases.

The best-fitting model in *T. officinale* and *D. glomerata* models were the null model, which only estimated the

average choice rank for each plant (as shown above or in Figure 4 for estimated coefficients and standard errors). Thus, none of the tested factors (seasons, elevation, average grassland productivity, apparent vole density, dandelion density, and individual age class) significantly influenced the explained variable (i.e., choice rank of each tested plant) according to the AIC_c selection model procedure (Table 3).

Nevertheless, for the *T. repens* selection model, the best model selected was the one with dandelion densities in the model. Examining the coefficient of the model, we only detected a trend according to dandelion density in plots. This trend was significant only at 7% ($p = 0.07$).

DISCUSSION

Multi-methods approach

Investigating diet during growth and non-growth seasons

In this study, DNA analysis provided valuable insights into the trophic niche, consistent with the results of feeding tests on voles using the Campascope device. However, the DNA metabarcoding of stomach contents exhibited several biases that could alter the quantitative interpretation of the data (Neby et al., 2021), especially in estimating the realized trophic niche. Indeed, DNA sequencing lacked precision for some taxa and the DNA database used for DNA comparison might have influenced species determination (Pompanon et al., 2012). To compensate for this bias, the precision of the data had to be degraded to the taxonomic level of family. Although this allowed a consistent pattern to be drawn, it was unsatisfactory for families such as *Fabaceae*, which are thought to be important for voles and encompass several known species. Additionally, stomach content analyses may be biased by over-representation of the last meal, particularly in species such as voles, where food intake is limited by stomach size (Zynel & Wunder, 2002). To compensate for some of the last-meal effect, the number of occurrences in stomachs was used rather than the total number of sequences.

Complementarily, assessing the winter diet is challenging but crucial for understanding rodent–plant interactions (Soininen & Neby, 2023). Analysis of food stores provided valuable data, although such data remain opportunistic and partial. Indeed, only 15 food stores were discovered in 184 trapping sessions. Although trapping was conducted throughout the year, we only found food stores from middle fall to early spring. We also observed a peak in October and another in February to

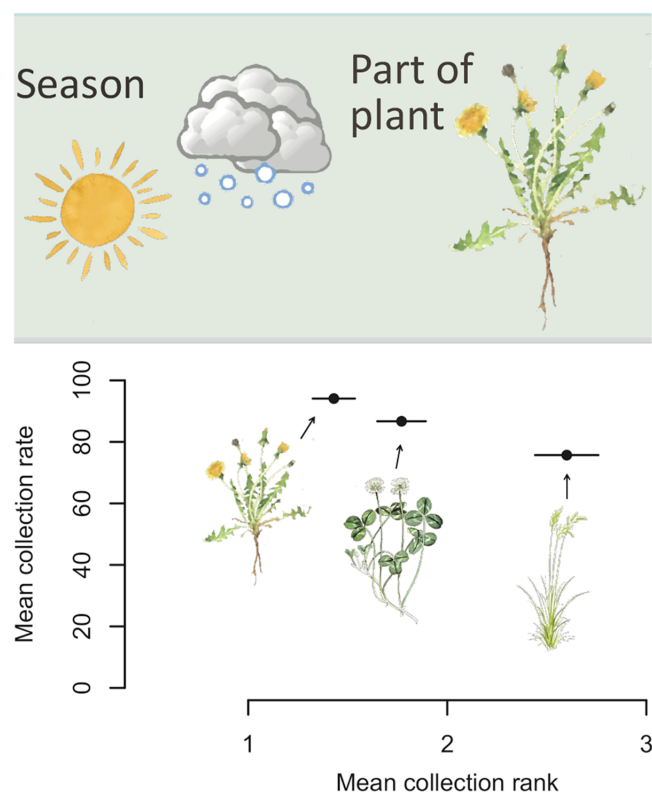


FIGURE 4 Feeding preferences of the water vole. Mean percentage of plants eaten or carried by voles as a function of mean choice rank for three plant species (left to right: *Taraxacum officinale*, *Trifolium repens*, and *Dactylis glomerata*). These three plant species were systematically offered simultaneously during behavioral tests. Tests were conducted along environmental gradients and across seasons. Icons at the top of the figure indicate the sampling season and the plant part concerned. Illustration credit: A. Pinot.

TABLE 3 Water voles' feeding choice variability.

Formula	df	AIC _c	ΔAIC _c	Estimated coefficient	SE
Dandelion_order ~ 1 + (1 Field_plot)	2	323.62	0.00		
Dandelion_order ~ Area + (1 Field_plot)	3	325.65	2.03		
Dandelion_order ~ Season + (1 Field_plot)	5	329.99	6.37		
Dandelion_order ~ Stage + (1 Field_plot)	5	325.70	2.07		
Dandelion_order ~ Vole_D + (1 Field_plot)	3	325.48	1.86		
Dandelion_order ~ Dand._D + (1 Field_plot)	3	325.45	1.82		
Clover_order ~ 1 + (1 Field_plot)	2	322.28	1.01		
Clover_order ~ Area + (1 Field_plot)	3	323.82	2.54		
Clover_order ~ Season + (1 Field_plot)	5	327.59	6.31		
Clover_order ~ Stage + (1 Field_plot)	5	325.38	4.10		
Clover_order ~ Vole_D + (1 Field_plot)	3	324.38	3.10		
Clover_order ~ Dandelion_D + (1 Field_plot)	3	321.28	0.00	4.17E-03	2.31E-03
Cockfoot_order ~ 1 + (1 Field_plot)	2	314.69	0.00		
Cockfoot_order ~ Area + (1 Field_plot)	3	315.69	1.00	1.58E-01	1.47E-01
Cockfoot_order ~ Season + (1 Field_plot)	5	319.74	5.05		
Cockfoot_order ~ Stage + (1 Field_plot)	5	321.17	6.48		
Cockfoot_order ~ Vole_D + (1 Field_plot)	3	316.81	2.12		
Cockfoot_order ~ Dand._D + (1 Field_plot)	3	316.51	1.82	−1.19E-03	2.16E-03

Note: Corrected Akaike information criterion (AIC_c) for three sets of models explaining plant choice ranks in voles' behavioral tests. Parameters tested included Season (spring, summer, fall, and winter), a vole age class (Juvenile or Adult), a vole density index (Vole_D), a dandelion density index (Dand._D), and outbreak versus control area (Area; reference = outbreak). Control areas are defined as sites below 550 m and above 1100 m elevation, where no outbreaks have been documented. Selected models appear in boldface: lowest AIC first and lowest number of parameters when ΔAIC < 2.

April. However, it is possible that the number of food stores in November, December, and January was significantly underestimated due to the reduced number of trapping sessions during these months associated with frozen ground and snow cover. Nevertheless, the seasonal pattern is consistent with other studies made on this vole species (Airolidi, 1978; Potapov et al., 2004) despite the relatively small sample size.

These approaches are therefore complementary, revealing seasonal variations that could not otherwise be described. This is important because accurate predictions of feeding behavior in winter would not have been possible based on the diet during the vegetation growth period, and vice versa.

Investigating variability of food preferences

To assess feeding preferences in the wild, we compared eaten/storage plants to available plants in field. This is a contemporary method (e.g., Lopes et al., 2020; Scopin et al., 2020; Soininen et al., 2013). In addition, we used the Campascope device, which provided accurate behavioral data and allowed the testing of potential sources of variation on wild animals. Although this method

provided high-quality data, it did not allow for the simultaneous testing of many plants and requires significant time for fieldwork. To optimize our fieldwork, we used previous literature and DNA analysis of stomach and food store contents to select the three most important species of the three most important families of plants. This method was partial since only three plants were tested, but it permitted the investigation of the variation in food preferences.

Ultimately, the use of several complementary methods made it possible to outline general patterns in the diet of the common vole, emphasizing what is variable and what is not.

Trophic niche of water vole: Consistency of general pattern

This study identified a general pattern for the diet of water voles in the outbreak area. First, water voles have a very broad fundamental niche. Their feeding behavior also displays a strong seasonal pattern, with food stores being used during the winter season and likely only direct intake of food during the growing season. Furthermore, voles consistently prefer dandelions

regardless of environmental conditions or individual status. The only exception was when dandelions were overabundant on the plot.

Seasonality of trophic niche depending on plant growth season

As a generalist species, water voles seem to be well adapted to all grasslands during the growing season, but less so in winter. During the growing season, grasslands contain abundant high-quality plants. Indeed, as the grasslands in our study area are devoted to livestock farming, most farmers tend to maintain high productivity in their plots. It provides voles with abundant food and certainly results in a broad realized niche. During the growing season, voles are probably more dependent on grassland productivity than on specific plant composition. Despite a large realized niche, water voles overselected plants with taproots during this season. Their diet also contained *Poaceae*, which are widespread in the studied grasslands. Additionally, voles consume clovers (*Fabaceae*). The preference for protein-rich species, such as clovers, vetches, or alfalfa, has been assessed in previous studies on other vole species (Bergeron & Jodoin, 1987; Cole & Batzli, 1978; Curtis et al., 2002; DeJaco & Batzli, 2013; Marquis & Batzli, 1989). These preferences are consistent with previous research on the diet of fossorial water voles in Switzerland (Kopp, 1988; Saucy, 1988; Saucy et al., 1999). However, as clovers are abundant, they do not appear to be over-selected. Saucy et al. (1999) noted that clover may contain a cyanogenic component that makes it toxic and limits its intake. Interestingly, results on feeding preference variability showed that clovers (*T. repens*) tend to be preferred when dandelions (*T. officinale*) are highly abundant, suggesting that nitrogen is not the main limiting factor in winter.

In the winter season, the realized trophic niche seems to be much narrower than that observed during the growing season, and voles appear to be constrained by their reliance on dandelions for food stores. Almost all grasslands are grazed by livestock in the late fall, leaving only lawn-height vegetation for the winter. As a result, the amount of food available to herbivores decreases drastically in winter, making rodents highly dependent on food storage behavior. Such storage behavior is well documented in several rodent species and has been selected in evolutionary processes to survive the winter season (e.g., Soulairac & Lapetite, 1946 review on rats; Airolidi, 1978; Potapov et al., 2004, on water voles). In our study, the storage behavior of water voles was only observed in winter, suggesting their dependence on these food storages to survive the

winter season. Food storage of water voles seems to be mainly dependent on only one species, the dandelion (*T. officinale*), which is consistent with their food preferences observed during the growing season. However, we cannot assert that voles did not consume other plants that they did not store. Airolidi (1978) reported more diverse food stores, indicating that the food storage behavior of the water vole in our outbreak area is specific to dandelion, but it might be more diverse in other areas. The low proportions of *Rumex* and *Ranunculus* in the food storages may be due to the presence of toxic components, oxalic acid and protoanemonin, respectively, as well as due to their lower densities in our meadows.

The preference for dandelion could therefore be due to the fact that, as far as we know, it does not contain any toxic compounds. Its near exclusivity in food storage is probably due to its abundance in fertile soil.

Strong feeding preferences for dandelion independent of outbreak context and seasonality

If dandelions (*T. officinale*) seem to be the main choice in winter, it should be noted that they are also the preferred choice in other seasons. Behavioral tests show that they are favored over clover (*T. repens*) and cocksfoot (*D. glomerata*) throughout the year. This order of choices is also independent of the outbreak/non-outbreak areas. This result suggests an innate preference of voles that may provide a significant advantage in seasonal environments. Previous studies have also found a feeding preference for dandelion (*T. officinale*) in the montane vole (DeJaco & Batzli, 2013; Marquis & Batzli, 1989), but in third place after *Medicago sativa* and *Trifolium pratense*, and in the prairie vole (*Microtus ochrogaster*; Curtis et al., 2002). Dandelion has also been noted as a strongly selected species by the water vole in Switzerland (Airolidi, 1976; Kopp, 1988).

The fossorial water vole is therefore probably a facultative specialist, as defined by Shipley et al. (2009). While its realized niche is broad during the growing season, it becomes narrower in winter (Figure 5). During this period, voles appear to forage on roots, a difficult resource requiring morphological adaptation for burrowing. However, the water vole is clearly not a specialist, as it shows a broad realized niche in summer and does not specialize in difficult chemical plants: it is able to eat *Ranunculus* or *Rumex*, which also have large roots, but does not show strong selection for them. Furthermore, it is obvious that water voles are able to live in low-productivity areas (Fichet-Calvet et al., 2010), where dandelions are expected to be scarce.

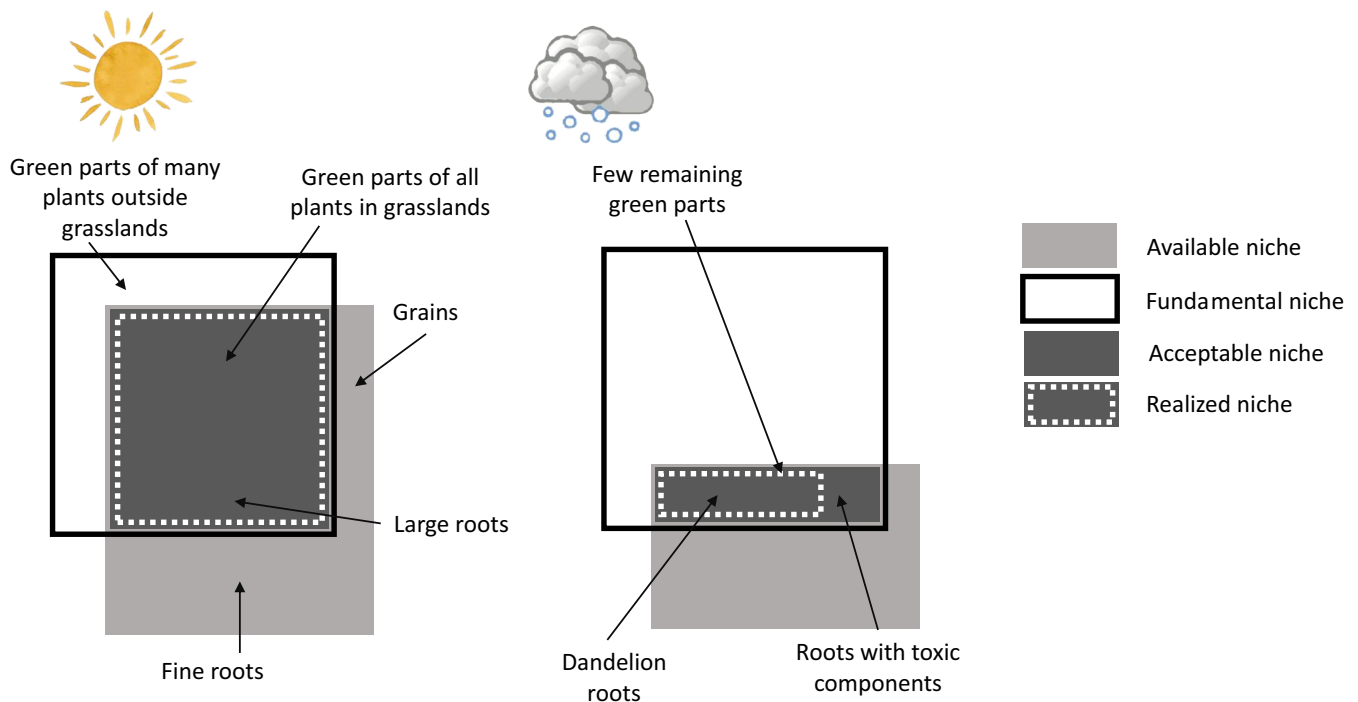


FIGURE 5 Conceptual diagram of water vole niches across seasons. The realized niche of the water vole appears to be highly constrained during winter by resource availability. This results in a strong specialization on dandelion in our study area during that season; however, water voles showed a preference for dandelion throughout the year. Illustration credit: A. Pinot.

Insights for plant hypothesis in explaining vole population regulation

From the plant hypothesis framework, two of the three main points have been investigated in the study. (1) Plants show strong seasonality in their growth period, involving shift in voles' realized trophic niche during the year. (2) Voles have a strong preference for dandelions and are dependent on them, at least during the winter. The third point was not investigated, but seems also to be promising. (3) After rodents have damaged their preferred food sources, a recovery period is needed to restore its quality or quantity. Indeed, in our outbreaks study area, dandelions can be very abundant in grasslands, with more than 10 plants/m², representing a potential resource of hundreds of grams per square meter. Voles therefore collect the entire plant, suggesting that they reduce the number of dandelions in grasslands. This was also recorded in Buronfosse et al. (2025). In line with the maximum number of dandelion apexes found in a food store (233) and the local density of dandelions on the same plot (27 individuals/m² in uncolonized areas), the storage behavior of voles impacts the local density of dandelions: a food store may contain all the dandelions from an area of 8.5 m² for a vole territory size of approximately 50 m² (Airolidi, 1978; Lisse et al., 2022), but several generations of voles of several years might be required for

resource depletion. At high densities (e.g., 600 adults/ha), all available territories are occupied by voles, enabling widespread depletion. Dandelions can live for several years and do not reproduce in their first year. It is unlikely that the dandelion root mass will recover immediately after depletion.

Thus, given the current state of knowledge, the plant hypothesis for explaining water vole cycle dynamics in the mid-mountains should be seriously considered. To further follow this path, it could be useful to investigate (1) whether voles affect the population of dandelions during outbreaks at field plot scales, (2) whether dandelion deficiencies affect the reproduction, survival, or dispersion of voles at population scales, and (3) if (1) is observed, whether the dandelion population regrows after being heavily foraged and with what duration of delay.

AUTHOR CONTRIBUTIONS

All authors of the manuscript have seen and approved the article, all authors have contributed substantially to the work, and all co-authors have been included. The study comprised four complementary parts (A—root stores, B—stomach contents, C—food preference trial, and D—consumed species). H  l  ne Lisse led the conception and fieldwork (C and D), led the analysis (A, B, C, and D), and led the writing and revision.

Cédric Jacquet participated in the conception of part B, led the fieldwork for part B, and initiated the analyses for part B. He participated in the revision. Gaëlle Sobczyk-Moran contributed to the conception (A) and fieldwork (led A, participated B), and initiated the analysis (A). Ambre Fafournoux participated in the fieldwork (C). Etienne Ramadier led the conception (A, B) and participated in the fieldwork (A, B). Virginie Lattard contributed to the management (C). Marion Buronfosse and Virginie Lattard provided editorial advice. Adrien Pinot managed the overall program, led the conception (A, B, C, and D), participated in the fieldwork (A, B, C, and D), led the analyses (A, B, C, and D), and participated in the writing and revision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Lisse & Pinot, 2026) are available from Zenodo: <https://doi.org/10.5281/zenodo.18597968>.

ETHICS STATEMENT

This study follows the guidelines of the Declaration of Helsinki, and was approved by the APAFiS (number 37713, project authorization number 2238). The study was carried out according to an experimental protocol in accordance with international guidelines and with the approval of the Ethics Committee of the Veterinary School of Lyon (authorization number 2017110209446108).

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SUPPORTING INFORMATION

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

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