Effect of the presence of apple trees on the behaviour of growing rabbits raised outdoors

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A **THESIS**
submitted for a Master's Degree
Predictive and integrative animal biology (PRIAM)

Effect of the presence of apple trees on the behaviour of growing rabbits raised outdoors

by

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- 2024 -

Table of contents

List of figures

List of appendices

List of abbreviations:

ANSES: National Agency for Food, Environmental and Occupational Safety

AO: Apple orchard

CIWF: Compassion in World Farming

EFSA: European Food Safety Authority

FAWC: Farm Animal Welfare Committee

GL: Grassland

IFPO: French Institute of Public Opinion

INRAE: National Research Institute for Agriculture, food and Environment

ITAVI: Technical Institute of Poultry, rabbit and fish Sector

LAPOESIE: Rabbit, Apple tree and Interspecific Ecosystem Services

SFECA: French society for Study of Animal Welfare

VHD: Viral Haemorrhagic Disease

Preamble

The study conducted during this internship is based on data collected during a trial carried out in 2022 (LAPOESIE project 2020-2022). The author of this manuscript did not participate at the experimental trials. Her participation lies in the creation of protocols to process collected data, in order to answer a scientific question. The author is also behind the analyses of these data. The results obtained during this internship were presented by the author at the congress of the French Society for the Study of Animal Behaviour in May 2024 (Appendix 9)

Abstract

In France, 90% of rabbits are raised in buildings in wire cages. These living conditions impact their welfare and therefore are criticized by citizens. One of the alternative systems studied aimed to develop an agroecological system breeding young rabbits outdoor in an apple orchard. We were interested in the effect of trees on the rabbits' behaviours with the hypothesis that trees could enrich the environment and secure rabbits. We therefore compared the behaviour of rabbits raised in mobile pens of 18m^2 in an apple orchard or in a grassland. We have implemented a spatial dispersion analysis, created a behavioural repertoire and compared their occurrences and the rabbits' time budgets, in both modalities. Our analyses showed that the presence of trees tend to increase space occupancy by rabbits. Behavioural analyses demonstrated the expression of a large behaviour's repertoire (N = 44 observable behaviours) in both modalities. However, some behaviours are differentially expressed in the two modalities. Indeed, we observe higher number of occurrences of resting and exploration behaviours in apple orchard. We also observed higher number of fleeing behaviours and a higher time budget of expression of alert behaviours in grassland compared to apple orchard. In future studies, quantification of the levels of expression of behaviours could allow us to refine our understanding of the effect of trees. So far, we can already affirm that presence of trees allows enrichment (diversity of expressed species-specific behaviours) and security (lower expression of vigilance) of rabbit's environment and thus contribute to rabbit's welfare.

Keywords

Rabbits, Welfare, Apple trees**,** Breeding**,** Agroecology

Figure 1: Diagram illustrating the 5 degrees of freedom defined by the FAWC

Introduction

According to the technical institute of the poultry, rabbit and fish sectors (ITAVI, 2021) global rabbit meat production is declining (-6.5% per year since 2014). Nevertheless, ITAVI designates France as the second European producer. The France Agrimer report (2024) lists more than 20 million slaughtered rabbits in France, with fewer than 800 professional rabbit breeders in 2022. In 90% of cases, they raise animals above ground, in wire cages, with a maximum animal density of 21*29.7 cm^2 per rabbit of 2.8 kg, in long narrow buildings, without enrichment, often without access to natural light and fed exclusively with pellets diets. Other systems, such as farming on the ground or with access to the outdoor access exist. Outdoor access is mandatory for farms respecting the French organic farming charter but represents only fifty farms in France. This low representation is mainly explained by a lack of technical-economic references on this type of exploitation (Gidenne *et al.,*2022). However, recent studies have shown that outdoor breeding would allow an acceptable growth and survival rate under good production conditions (Fetiveau *et al*., 2021 Savietto *et al.*, 2024).

Cage systems are strongly challenged today particularly because of their negative impacts on animal welfare. This consideration of animal welfare in livestock farming systems is reflected in the opinion of citizens. Indeed, a survey conducted in 2024 by 30 Million d'amis and the French Institute of Public Opinion, suggests that 83% of French people would declare themselves in favour of the prohibition of intensive breeding. This consideration is also reflected in the European Citizens' Initiative « For a new age without cages » launched in 2020 by the Compassion In World Farming. This initiative garnered over 1.3 million signatures and was therefore presented to the European Commission. It committed, at the end of 2023, to propose a new law for the breeding of some species including rabbits (ICE, 2021). Although postponed by the European Union in December 2023, this initiative continues with a complaint to the EU Court of Justice by the citizens' committee of the initiative in March 2024 (CIWF, 2024).

However, to improve the welfare of farmed animals, it is necessary to understand this notion and be able to assess it. The National Agency for Food, Environmental and Occupational Safety defines, in its annual report of 2018, the welfare of an animal as: “The positive mental and physical state related to the satisfaction of its physiological and behavioural needs, as well as its expectations. This condition varies according to the perception of the situation by the animal”. This definition considers the subjective point of view of the animal. It is based on freedoms concept, established in 2009 by the Farm Animal Welfare Committee. It requires the breeder to bring freedoms animals from hunger and thirst, discomfort, pain, injury, and disease, to express normal behaviour and from fear and distress (Figure 1). In term of physiological needs, according to Joshi and Herdt (2006) intensive livestock farming induces specific pathologies, called production pathologies. Boucher and Nouaille (2002) also showed that rabbits claustration limits, in other things, the proper functioning of the proximal colon and therefore constrains the regulation of intestinal transit. They also demonstrated that this loose of function prevents the removal of urinary calcium, which can cause fatal calcifications and kidney failure. Finally, Masthoff and Hoy (2019) have proved that cage systems induce pododermatitis.

Moreover, ANSES asserts that behavioural changes are first indicators of individuals' ill-being and are also markers of individuals' perception. Therefore, a behavioural approach is essential for the assessment of animal welfare. A behaviour is defined as an observable expression triggered by an internal or external event. Studies report that cage housing reduces the number of behaviours that rabbits can express, such as leaping, jumping, running, standing up and grazing (Lehmann, 1987; Masthoff and Hoy, 2019; Coda *et al*., 2020). We can thus say that intensive rabbit farming does not respect animal welfare, as stated in the 2020 report of the European Food Safety Authority.

Knowing that domestic rabbit is the same species as wild rabbit (*Oryctolagus cuniculus*), it is relevant to think that the behaviour of farm rabbits should be similar to that of their wild counterparts. They are mainly found in ecotones, transition zones between bush and grassland, rich in grasses and covered with shrubs and trees (Lombardi *et al*., 2007). Indeed, in the light of its prey status (Monclús *et al*., 2005), the rabbit favours the topology of its habit to the resource presence on the latter (Villafuerte and Moreno, 1997). This status also explains that activity outside their burrow occurs mostly at sunrise and sunset (Mykytowycz and Rowley 1958). Rabbits are phytophagous and graze (Southern 1940). They live in hierarchical groups and their activity depends on their social status, gender and age (Mykytowycz and Rowley 1958, Rödel *et al*., 2015). Studies by Fetiveau *et al*. [a] (2023) qualitatively observed that farm rabbit seemed to positively anticipated a going out when outside time is restricted. In addition, the behaviours observed in wild rabbits are found well in rabbits raised in enclosure in grasslands. Indeed, they are more active when they have access to the outside with a larger behavioural repertoire. Grazing behaviour is predominant. Finally, their rest time outside increases when the environment has refuge zones.

Researchers of the National Research Institute for Agriculture, Food and the Environment set up LAPOESIE (Rabbits, Apple trees and Interspecific Ecosystem Services) project (Savietto *et al.,* 2024). It is a total redesign of rabbit breeding systems because, for the first time in France, rabbits were raised in the context of agroforestry. Therefore, it also presents a redesign of the management of apple orchards. This project aimed to serve as proof of concept. Previous studies on this project have shown satisfactory zootechnical results on the growth of rabbits outdoors as well as on the interspecific services provided by the combination of animal breeding and tree cultivation.

The present study aims to continue the analysis of this new system by focusing on the animal welfare challenge. Our goal was to assess the welfare of rabbits raised outdoor under apple orchard (AO) or in grassland (GL) conditions and evaluate the effects of the presence of trees on rabbits' behaviours. Our initial hypothesis was that trees could be a source of enrichment and security and therefore of well-being for rabbits. To evaluate this, we conducted spatial distribution, quantification of behaviours' occurrences and time budget analyses in both modalities. We expected to observe the use of a larger pen area and an attraction for trees in the apple orchard. We also expect a decrease of behaviours related to environmental insecurity in this modality.

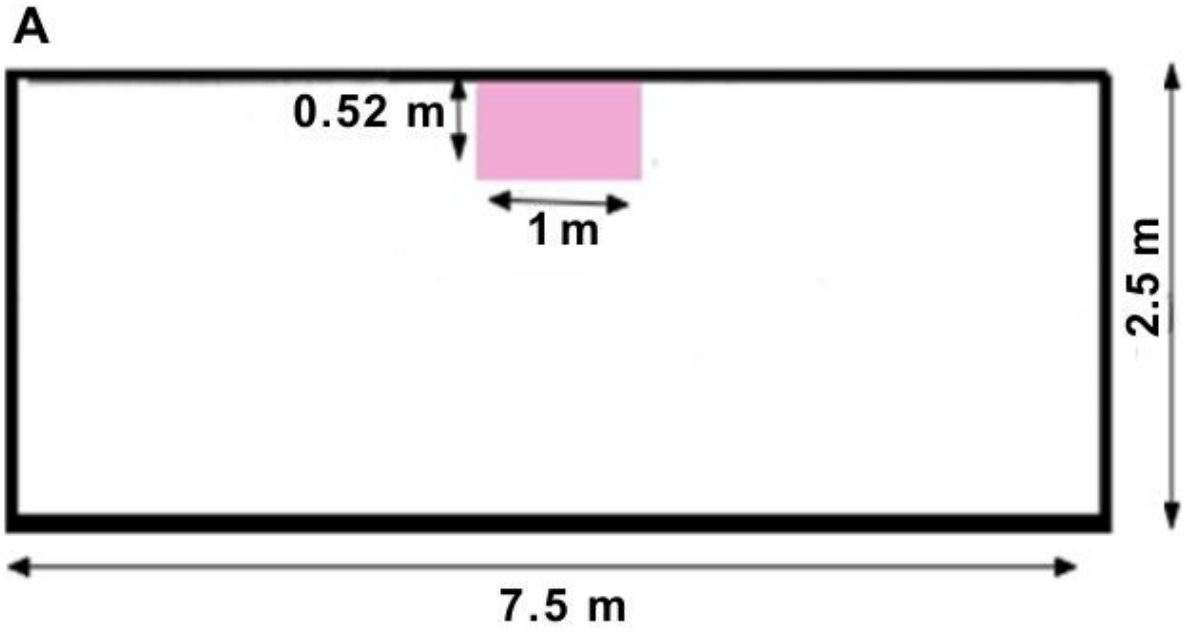

Figure 2: Scheme of pens (A) and picture of hut (B) use during the experimentation

Materials and methods

0- Ethical note

Animals were handled in accordance with the recommendations of the European Union (2010) and French legislation on the protection of animals used for scientific purposes (EU Directive 2010/63/EU, Official Journal of the French Republic (Decree No. 2013-118)). All the protocols were approved by the Ethics Committee n° 115 of the French Ministry of National Education, Higher Education and Research (authorization number APAFIS #35391-2021091717004334 v6). The experimental farm was approved by the French Ministry of Agriculture (approval number A263131402).

1- Animals and housing conditions

32 rabbits (Fauves of burgundy X New Zealand X Lop) were inseminated by semen of PS119 males (Hypharm) and gave birth to 330 young rabbits (½ PS119 × ⅛ French Giant Lop × ¼ INRA1777 × ⅛ Fauves of Burgundy) (Appendix 1), on September 2nd 2022. These crosses were intended to obtain colourful rabbits, with a thick undercoat and having production characteristics. Rabbits offspring were born in experimental cages and allocated to a mother (biological or nurse) in balanced group. They were vaccinated on September 30th for the Viral haemorrhagic disease (FILAVAC® VHD K C+V) and myxomatosis (FILAVAC MYX). Their weaning was done at 45 days of age and their vaccination reminder at 76 days of age. 144 of these rabbits were selected for the experiment and divided into 24 groups. The composition of the latter was established in order to maintain an intra-group morphological diversity but an inter-group homogeneity. Rabbits from the same litter were most often separated (17 lots/24).

During the experiment, that started at 45 days of age, rabbits were housed in mobile pens consisting of 8 wire panels forming a space of 7.5*2.5 = 18.75 m^2 (Figure 2.A). Each pen had a water pipette attached to a wire panel. A hut of 100*52 *92 cm (Width*Depth*Height) (Figure 2.B). Pens were moved every week, in both conditions (Appendix 2), in order to offer access to grass all throughout the experience. Pellets were offered in the huts, ad libitum.

2- Experimental design

The 144 rabbits were housed in two distinct modalities: 72 under apple orchard and 72 in cultivated grassland. In both conditions, rabbits were raised in groups of 6, with the same composition as defined during their selection to the experiment, within mobile-pens (12 pens per conditions). The rabbits were raised from October 17th, 2022, at 45 days of age to the 21st of November (81 days of age).

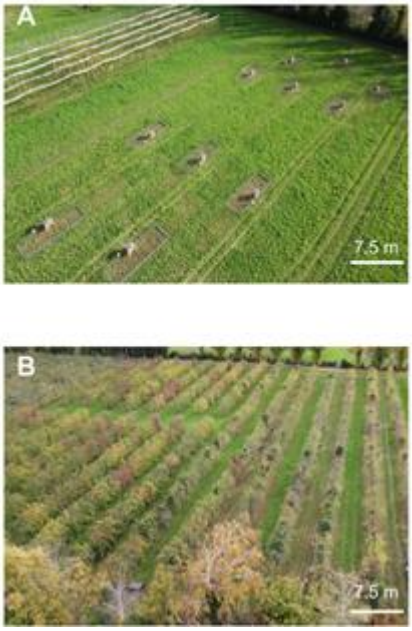

Figure 3: Picture of the apple orchard (A) and the cultivated grassland (B) of the Gotheron experimental unit

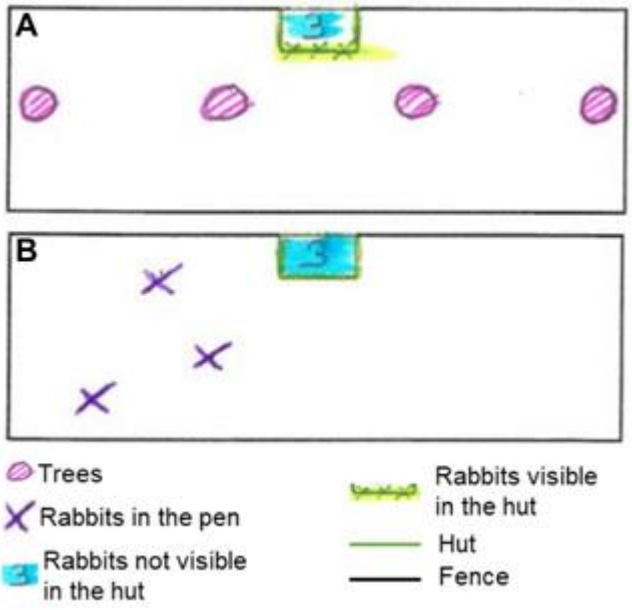

Figure 4: Example of schemes of the field records of spatial distributions within a pen in apple orchards (A) and a pen in grassland (B)

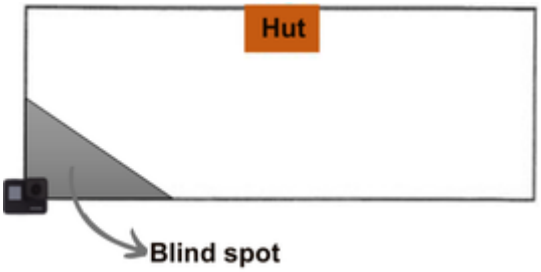

Figure 5: Scheme of camera position in pens

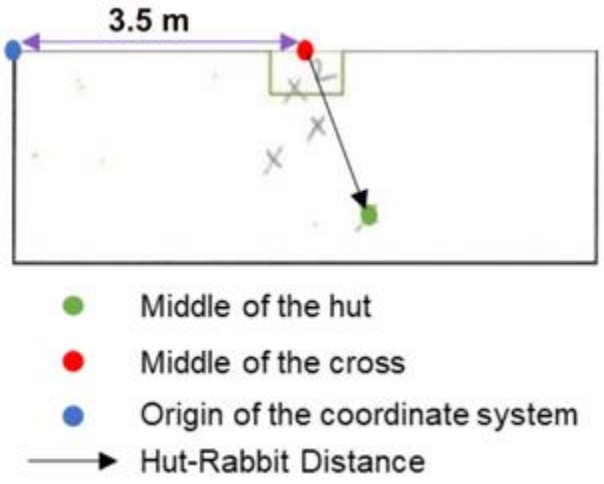

Figure 6: Scheme of the spatial coordinate measurement protocol used on FIJI

a. Apple orchard characteristics

Apple orchard (Figure 3.A) were cultivated in 2005, on the site of INRAE Gotheron (Simon *et al*., 2018). Tree lines were 5m apart and each tree was 2m apart within a line. Each pen of this modality was thus composed of 4 trees.The apple trees were CG 10 Yellow Delicious Smoothee. The trees were irrigated with micro-diffusers placed 60cm from the ground. The tree trunks were protected by plastic sleeves of 50cm high, held by strings. Apple orchard soil was covered with various plant species.

b. Grassland Characteristics

The cultivated grassland (Figure 3.B) was also located at INRAE Gotheron, about 350m from the orchards. It consisted of two types of grass (*Lolium* and *Dactylis*).

3- Data collection

a. Spatial distribution

Field surveys of rabbits' positions in pens were represented by crosses on paper schemes (Figure 4). These locations were collected in each pen, of each modality, in the morning or in evening, by three observers. A total of 25 observations were made between October 19th, 2022, and November 18th, 2022, between 47 and 77 days of age.

b. Video recording

We had 8 cameras GoPro (GoPro Hero 7). 4 pens in AO (P1, P5, P8 and P12) and in 4 pens in GL (P13, P16, P23 and P24) were chosen. As rabbits were not marked this choice was based on the high colour diversity of rabbits in the pen to maximize individual identification. A parallel study on the human-animal relationship was conducted on these rabbits (Fetiveau *et al*., 2024). The pens were therefore also selected to have 2 groups of individuals linked to man and 2 groups of individuals not linked, by modality. Video recordings were made on November 20th, 2022, 6 days after the last transfer of pens, at 79 days of age for rabbits. One observation consisted in a two hours video recording (except for the pen P8, with only around 50 minutes of recording because of a premature camera stop). Cameras were placed on the top of the fence, in an angle, in order to observe the majority of the pen and the entrance of the hut (Figure 5). The recording was carried out between 3:45 pm and 6pm, the most active diurnal period of rabbits (Fetiveau *et al*., 2021)

4- Analyses

a. Spatial distribution

Knowing the real dimension of a pens, a scale of 43.33 pixels/metres was applied to each image on FIJI software (ImageJ 1.54f) using Set scale function. The distance between the middle of the hut (x=3.5m and y= 0m) and the middle of a cross was called "Hut-Rabbit distance" (Figure 6). Theses distances was measured using Fiji software Straight Line and Set measurement functions.

Category	Behaviour	Description
Locomotion	To walk	The rabbit moves slowly by moving its anterior one after the other and then bringing back its posteriors after a short moment
Locomotion	To leap	The rabbit advances at moderate speed by propelling itself slightly with its posteriors
Locomotion	To run	The rabbit advances at high speed by making several successive bounds
Locomotion	To flee	All the rabbits run and enter the hut
Locomotion	To cavort	The rabbit makes one or more disorderly or stationary jumps by kicking or shaking his head
Locomotion	To enter	The rabbit enters into the hut
Locomotion	To go out	The rabbit goes out of the hut
Locomotion	To go up	The rabbit goes up into the hut using the ramp
Locomotion	To go down	The rabbit goes down into the hut using the ramp
Locomotion	To jump	The rabbit jumps over an obstacle
Feeding	To eat an apple	The rabbit chews an apple
Feeding	To eat a leaf	The rabbit grabs or chews a leaf
Feeding	To graze	The rabbit pulls out blades of grass and chews. Mandible movements can be seen indicating chewing
Feeding	To drink	The head of the rabbit is under the water pipette, the head slightly tilted. It is possible to observe swallowing
Feeding	To forage	The rabbit moves slowly, nose to ground, looking for food
Grooming	To lick oneself	The rabbit licks its body. Repetitive head movements are observed
Grooming	To snort	The rabbit shakes its head or all its body
Grooming	To chew oneself	The rabbit chews slightly a part of its body
Grooming	To scratch oneself	The rabbit scratches the front of his body with one of its posterior
Grooming	To rub oneself	The rabbits rub its head with its anterior
Resting	To stretch oneself/ To yawn	The rabbit stretches its muscles by lengthening the body, anterior and/or posteriors. The rabbit can also yawn
Resting	To make a flop	The rabbit flips abruptly into the extended position
Resting	To rest	The rabbit is in a ball, lying or extended position, in a specific area
Exploration	To observe	The rabbit is motionless, its eyes focused on something, its ears erected, well opened and directed slightly forward. The head is high
Exploration	To rise up	The rabbit grows, buttocks on the ground, or slightly raised on the hind legs. The forelegs do not touch the ground
Exploration	To lean on	The rabbit is rises up and leans on a support (hut, fence or tree)
Exploration	Out of sight in the hut	We know the rabbit is in the hut but we don't see it
Exploration	Invisible	The rabbit is out of sight, in a camera blind spot or hidden by an element of its environment
Exploration	To smell	The rabbit smells an element of its environment
Exploration	To scrape	The rabbit quickly scratches an element of its environment with its anterior
Exploration	To mark	The rabbit rubs his chin against an element of its environment
Social	To escape	The rabbit runs to escape a congener
Social	To pursue	The rabbit pursues a congener
Social	Allogrooming	The rabbit grooms a congener
Social	Side by side	The rabbit rests/ is present near to a congener when they might walk away
Social	Nose to nose	The rabbit has its nose in front of that of a congener and feels it
Social	On the top	The rabbit jumps over a congener, one or several time
Social	Overlapping	A rabbit overlaps another with the will to reproduce or establish the hierarchy
Social	To attack	A rabbit jumps abruptly on another. Teeth may be visible
Building	To gnaw	The rabbit gnaws an element of its environment with its incisors
Building	To dig	The rabbit digs with its anterior to form or enlarge a hole
Building	To move	The rabbit takes an element of its environment with its teeth to move it. It can also push the earth with its head
Excretion	To urinate	The rabbit urinates
Excretion	To defecate	The rabbit defecates

Figure 7: Behavioural repertoire associating the 8 categories and descriptions to the 44 behaviours observable in the 2 modalities

We also collected the coordinates of each cross. A new software named LOC Tracker 20240206, created by Téo COCHOU, was used to analyse the spatial distribution data. This software generates automatically heatmaps of the coordinates measured via FIJI. Fineness of these maps was defined of 60 tiles per 20 tiles to respect the ratio of 1/3 of the enclosure. Each tile is coloured in a red gradient. The darker the tile colour, the higher the rabbit density at this point in the pen.

Only rabbits outside of the hut are represented in these maps. Considering the potential variability between observers on the position of the rabbits, no statistical analyses were performed on spatial distributions but heatmaps were used as exploratory illustrations.

b. Behavioural repertoire

The entirety of the films collected during the experiment was observed, without distinction of individuals and using Ad libitum sampling method (Martin and Bateson, 2007). A name and a definition were assigned to each behaviour observed, allowing the construction of a comprehensive behavioural repertoire (Figure 7) composed of 44 behaviours, gathered in 8 categories and was used to develop ethograms for behavioural quantifications.

c. Number of occurrences

The number of occurrences of each behaviour of the behavioural repertoire were counted. These were obtained thanks to the viewing of the video recordings and the Behaviour sampling method (Martin and Bateson, 2007). Around one hour of recording was analyse in each pen. As the rabbits in this study are not marked, each individual was followed as soon as it was visible in the pen, and the observation stopped as soon as it was no longer visible in his hut. Descriptive analyses were conducted on the number of occurrences data via the R software (R version 4.4.0.). The rare behaviours (number of occurrences < 20) and the behaviours specific to a modality (interaction with trees) have been excluded from our statistical analysis, as well as occurrences of moments when individuals were not visible (Invisible and out of sight). To limit the number of behaviours omitted, we grouped together behaviour of Grooming category. To allow comparisons, data were transformed in rate (number of occurrences per hour). Statistical analysis of the rate of occurrences therefore focused on 24 behaviours: To walk, To leap, To run, To flee, To cavort, To enter, To go out, To jump, To graze, To drink, To forage, Grooming (=∑Behaviours of the grooming category), To stretch, To rest, To Observe, To Rise up, To smell, To scrap, To mark, To escape, To pursue, Side-by-side, Nose-to-nose, To gnaw. Data were again transformed, on percentage of occurrences per hour.

Behaviours	Description
Stopped	The rabbit does not move. It stays near to a specific element
To move	The rabbit walks in a particular direction, moving distinctly its anterior one after the other, then bringing its posteriors to its anterior or jumps in a particular direction, simultaneously advancing its anterior and propelling itself with its posteriors with a rocking movement of the body
To run	The rabbit moves in a particular direction, making several jumps that cannot be counted, pushing itself firmly with its posteriors. Its body is in suspense above the ground between each leap. There is no movement of bascule of the body
To drink	The rabbit is stopped with its head tilted under the water pipette. Swallowing can be observed
Feed intake	The rabbit grazes, ingests food from its environment (excluding pellets) and mastic. It can be stopped or move
To stretch/ To yawn	The rabbit is stopped and stretches its muscles by lengthening its body, anterior and/or posteriors. The rabbit can also yawn
To groom	The rabbit is stopped and groom itself by licking, biting its body or scratching itself
To snort	The rabbit is stopped and shakes its head or entire body
To smell	The rabbit is stopped and smell an element of its environment
To lean on	The rabbit is stopped and raised up. It put its anterior on an element of its environment
To scrape/ To dig	The rabbit is stopped and scrapes the ground with its anterior. It can also scrape deep to enlarge an existing hole or form a new one
To mark	The rabbit is stopped and rubs its chin on an element of its environment
Allogrooming	The rabbit is stopped and licks or nibbles a congener
Proximity	The rabbit is stopped near to a congener, during more than 5 second with a distance equivalent to the size of three rabbit width maximum
Side-by-side	The rabbit is stooped in contact with a congener during more than 5 seconds, when it might walk away
Four-legged	The rabbit is stopped. Its four paws are on the ground, its belly does not touch the ground
Sitting	The rabbit is stopped. Its butt is on the ground, its anterior are on the ground, between its posteriors
Upright	The rabbit is stopped. It grows by leaning on its posteriors. Its body is perpendicular on the ground. Its buttocks are on the ground or slightly raised. its anterior do not touch the ground
Lying	The rabbit is stopped. Its four paws are on the ground and its belly touches the ground. Its body is in a lump or ball. Its anterior and/or posteriors may be stretched in the prolongation of its body
Submission	The rabbit is stopped, near to a congener. It is pressed to the ground, its four legs under its body, its belly against the ground, its back sponsors the ground or with the buttocks slightly towards the sky. Its ears are on its back, its head between its shoulders and its chin on the ground
Ears Posture	The rabbit is stopped and inactive with a specific ears' posture
Head Posture	The rabbit is stopped and inactive with a specific head 's posture
Invisible	The rabbit's behaviour is not clearly identifiable because the rabbit is in a camera blind spot, hidden by a congener or by an element of the environment, or in the up stair of the hut
Inactive	The rabbit does not express any locomotion, feeding, grooming, resting or exploration behaviours
To gnaw	The rabbit is stopped and gnaws an element of its environment with its incisors

Figure 8: Ethogram use in Boris software

Category	Computation
Activity	100- (Inactive + Invisible)
Relaxation	Lying_ball + Lying_side + Groom
Social	Move_congener + Proximity + Side_by_side
Exploration	Smell_fence + Smell_ground + Smell_hut + Smell_tree + Smell_indeterminated + Lean_on_fence + Gnaw_hut + Gnaw_tree
Warning	Run_hut + Ears_raised
Feeding	Feed_intake + Drink
Locomotion	Move_fence + Move_hut + Move_tree + Move_nothing

Figure 9: Table of categories created for time budgets' statistical analyses

Given our counting data, having a high over-dispersion, the rate of occurrences were modelled by a Quasi-Poisson law, by considering the pens as repetitions. Generalised linear models were used, such as:

$$Log(\mu_i) = \beta 0 + \beta 1 * Modality$$

Where μ_i represents the mean of the Quasi-Poisson distribution for the observation of the pen i, β0 is the intercept of the model and β1 is the coefficient associated with the explanatory variable «Modality». Estimated marginal means of each occurrence rates were computed and ANOVA on them were performed.

d. Time budgets

Time budgets of the activity of the rabbits visible in the pen were scored with the intent to control for the animal identity. Since not all rabbits had individual pattern to recognize them, a method was developed to allow the scoring individual time budget without over sampling the data. More precisely, the videos of each pen were sampled in 8 observation windows (excepted for P8 with 6), a window corresponding to 2 min of observation and each window was spaced eight minutes apart (Appendix 3). This analysis was conducted on the first hour of videos of each pen recorded the first 50 minutes for P8). For each of these windows, the same monitoring method as the quantification of occurrence was used (see above): one individual was monitored as long as it was in the focus of the camera. So, for one individual, a windowed time budget lasted maximum two minutes, less if the animal went out of focus.

An ethogram of 25 behaviours (Figure 8) associated to 17 modifiers (Appendix 4) was considered. This ethogram was implemented on BORIS software (BORIS v.8.22.16). Before the encoding phase, reliability tests (Kappa coefficient), include in BORIS functions, were conducted on samples of 3 minutes of video, for each pen. These tests were accepted when kappa coefficients were greater than or equal to 0.65 (Appendix 5). After Boris coding, time budgets were exported and transformed to obtain percentages of time of expression of each behaviour.

According to categories defined in the behavioural repertoire, we grouped some behaviours. We thus created 7 categories containing only independent behaviours with average percentage of expression time greater than 5% (Figure 9). In view of our integer discrete data, presenting an over-dispersion of values in 0, the time budgets were modelled by a negative binomial law such as:

$$Log(\mu_{ij}) = \beta 0 + \beta 1 * Modality * Y_{ij} + \mu_i, \text{ with: } \mu i \sim N(0, \sigma_\mu 2) \text{ and } Y_{ij} \sim NB(\mu_{ij}, \theta)$$

Where, μ_{ij} represents the random effect of pen i during observation j, β0 is the global intercept, β1 is the coefficient associated with the fixed effect «Modality» and Y_{ij} is the percentage of time spent expressing a behaviour Y in pen i during observation j. The estimated marginal means were calculated for each category and pairs tests, adjusted by a Bonferroni correction were performed on these means.

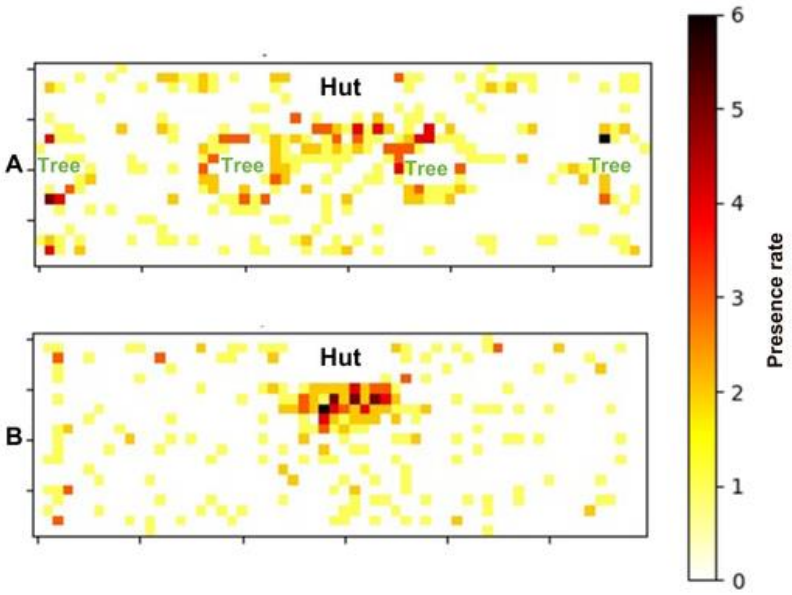

Figure 10: Heatmaps of the cumulative spatial distribution and rabbits' presence rate on 13 observations in apple orchard (A) and 12 observations in grassland (B). Each pen is cut in 1200 tiles. Each tile is coloured in a red gradient. The darker the tile colour, the higher the rabbit density at this point in the pen.

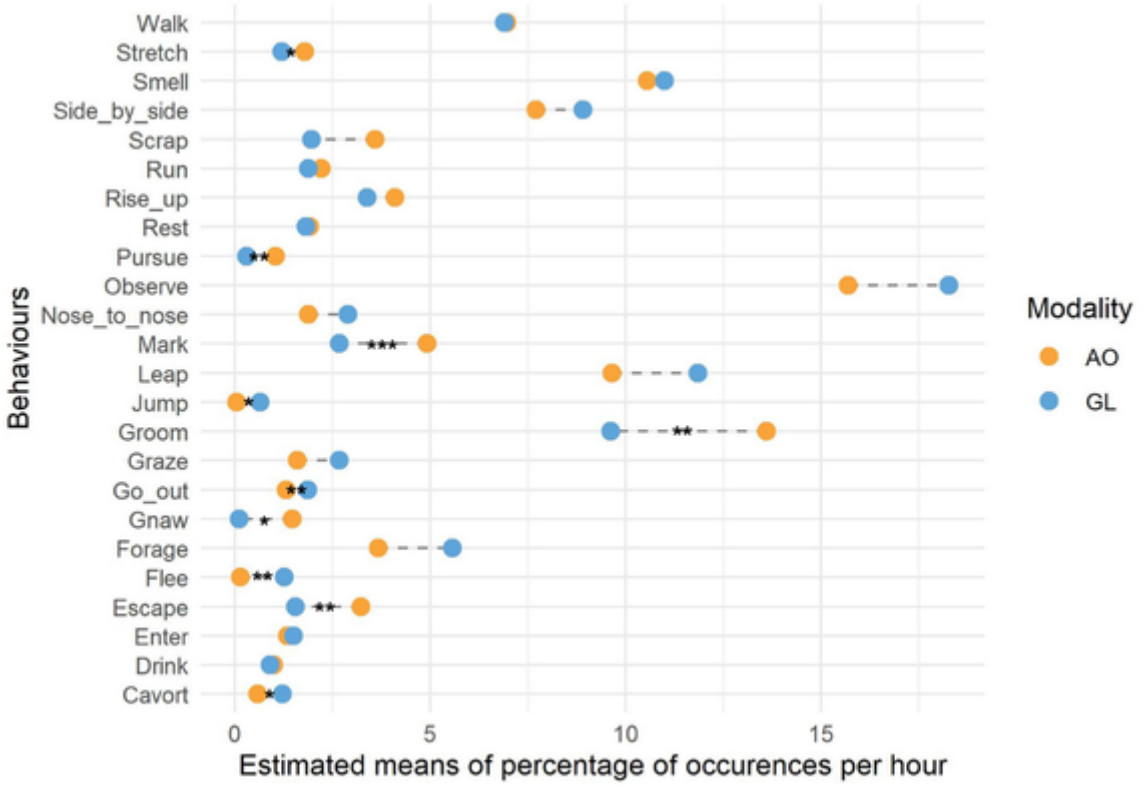

Figure 11: Cleveland chart comparing the estimated means of percentages of occurrences per hour, of 24 behaviours observed on 48 rabbits homogeneously distributed in 8 pens (4 in AO and 4 in GL) with significance of ANOVA test (P-val: *<0.5, **<0.001, ***<0)

Results

1- Spatial distribution

Heatmaps showing the distribution and density of rabbits in pens are presented in Figure 10. For both modalities, rabbits seem to locate more often in front the hut. In addition, and contrary to GL condition, in AO condition, the presence rate of rabbits seem to be mainly marked around trees and along the fence (Figure 10.A). On the other hand, in GL condition, some rabbits are present along the fence but mainly in corners (Figure10.B). In the two conditions, rabbits do not seem to spread homogeneously in the enclosure area.

2- Number of occurrences

Descriptive analyses of the counts of the behavioural repertoire (Appendix 6) highlight the rarity of certain behaviours (less than 20 cumulative occurrences on all observations) such as: Up, Down, Flop, Lean-on, On the top, Overlap, Attack, Move, Dig, Urinate, and Defecate. Some behaviours are also specific to a modality such as: Eating an apple or a leaf and resting near a tree.

Statistics show that, on the one hand some behaviours were more observed GL than in AO condition: “To jump” ($df=1$, $\chi^2=6.296$, $P\text{-val}\leq 0.05$), “Going-out” ($df=1$, $\chi^2=9.150$, $P\text{-val}\leq 0.01$), “To Flee” ($df=1$, $\chi^2=6.669$, $P\text{-val}\leq 0.01$) and “To cavort” ($df=1$, $\chi^2=4.722$, $P\text{-val}\leq 0.05$). On the other hand, other behaviours were more observed in AO than GL condition: “To Stretch” ($df=1$, $\chi^2=4.894$, $P\text{-val}\leq 0.05$), “To groom” ($df=1$, $\chi^2=9.992$, $P\text{-val}\leq 0.01$) “To Mark” ($df=1$, $\chi^2=25.282$, $P\text{-val}\leq 0.001$) , “To Gnaw” ($df=1$, $\chi^2=6.594$, $P\text{-val}\leq 0.05$), “To pursue” ($df=1$, $\chi^2=8.163$, $P\text{-val}\leq 0.01$) and “To Escape” ($df=1$, $\chi^2=7.417$, $P\text{-val}\leq 0.01$, , see appendix 7 for statistical table).

The estimated means of the occurrence percentages of the 24 analysed behaviours were represented on a Cleveland chart (Figure 11). On this chart, estimated means computed in AO are represented in orange, and those in GL in blue.

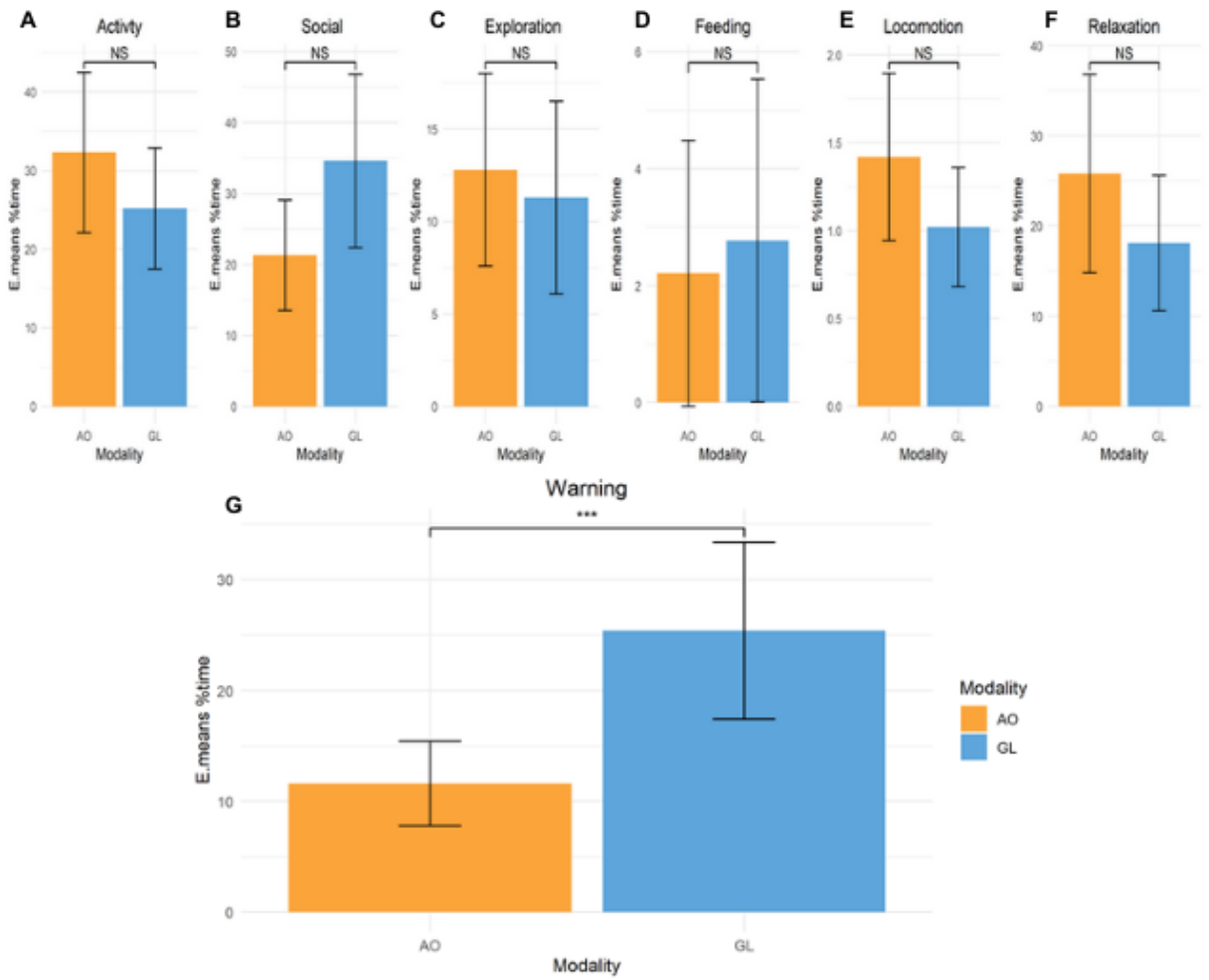

Figure 12: Bar plots comparing the estimated means of percentages of time, of Activity (A), Social (B), Exploration (C), Feeding (D), Locomotion (E), Relaxation (F) and Warning (G) behaviours observed on 48 rabbits homogeneously distributed in 8 pens (4 in AO and 4 in GL) with significance of Pairs test (P-val: NS> 0.05, ***<0.001)

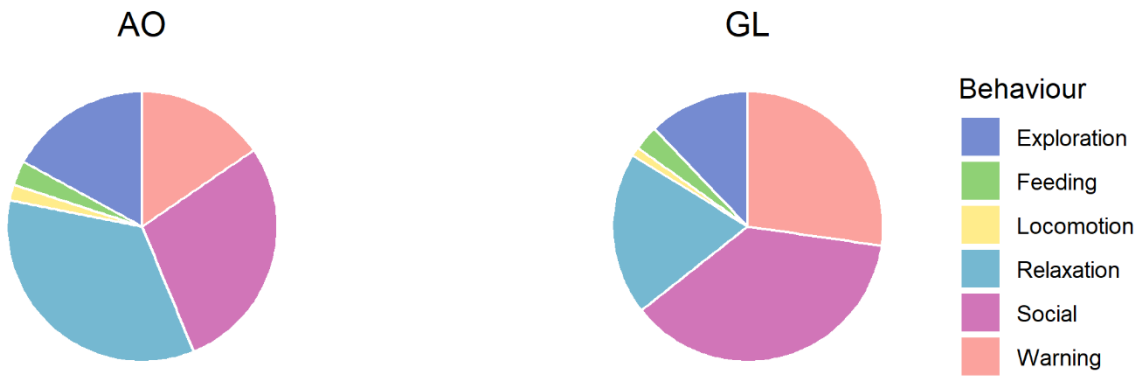

Figure 13: Pie charts comparing time budget of rabbits in the 2 modalities

3- Time budgets

The estimated marginal means are not different for Activity, Social, Exploration, Feeding, Locomotion and Relaxation (Figure 12.A, B, C, D, E and F, df=1, r.ratio < 1.43, p-val > 0.76). However, statistical analyses show differences in Warning with a higher value in GL (Figure 12.G, r.ratio = 0.458, P≤ $7e^{-4}$, see appendix 8 for statistical table).

Even if the majority of the results were not significant, pie chart illustrate time budget of rabbits in both modalities (Figure 13).

Discussion

1- Better use of space in apple orchard

The qualitative visualisation of spatial distribution of rabbits within pens (Figure 10) of the AO modality suggest an emancipation of the dependence of rabbits from the hut, in favour of trees and fence. In addition, individuals of this modality appear to occupy more different areas of the pen than individuals in grassland.

Study of Fetiveau *et al* [b]. (2023) indicating that the presence of hiding places on the enclosure favours the presence of rabbits on the latter. Moreover, use and exploration of a territory is correlated with the presence of refuge in wild rabbits (Villafuerte and Moreno, 1997). We can therefore provide that tress are perceived as safe zone for individuals of AO and that their presence spread throughout the pen allows rabbits to more use the land at their disposal.

However, it is necessary to consider that the spatial distribution data at our disposal have not allowed us to carry out statistical analyses. Our qualitative analyses gives us access only to hypotheses that must be validated in future studies. Finally, our analysis only considers the dispersion of rabbits observed outside the enclosure. It could be interesting to compare also the number of rabbits remaining in the hut in both modalities in order to reinforce the hypothesis of valorisation of use of the pen in the presence of trees.

2- A large behavioural repertoire

The comprehensive repertoire established in this study. Among these behaviours, some cannot be observed in cages, such as behaviours related to locomotion, nutrition, social interactions, construction and some exploration behaviours. The grazing behaviour was observed in both modalities of our study. Moreover, rabbits in apple orchard have been observed eating leaves, branches and apples. Both modalities also reveal warning behaviour and social interactions (Figure 7).

As demonstrated in previous studies (Fetiveau *et al*., 2021; Gohier *et al*., 2023), rabbits inherently have complex behavioural expression. Lehmann (1987), also testifies observed in rabbits in cages, early expressions of behaviours such as “To leap”, “To jump” or “To run” that could not be fully realised. Give outdoor access enables rabbits to returning to their natural nutrition behaviour. It is particularly true in AO where, as researchers already demonstrated (Chapman and Reiss 1999, Savietto *et al*., 2023), environment fits with rabbits’ natural mixed diet. Our study therefore reinforce the idea that livestock farming in outdoor pens therefore helps to resolve the frustration caused by the lack of satisfaction of behavioural needs

Valence of social interactions cannot be confirmed in the context of our study. These behaviours, as well as hypervigilance, although intrinsic to rabbits, can therefore be a sign of animal stress. Langbein et *al.* (2004) showed that a moderately challenging environment can, in the long run, contribute to animal welfare. However, it could be interesting to complete our studies in order to certify that our livestock systems do not induce overexpression of behaviours that can be considered negative in terms of stress induced in animals, but also in terms of livestock management.

3- Different modes of behavioural expression

We observed a decrease in the number of fleeing behaviours and an increase of exploration behaviours in rabbits in AO compared to rabbits in GL. We also saw more social interactions (high-speed chase) in the AO. On the other hand, in GL, the number of occurrences of behaviour related to a high reactivity ("To Cavort "and "To Jump") is higher (Figure 11).

Alert behaviours are linked to the natural prey status of rabbits. Their diminution in AO reinforces our hypothesis that the presence of trees brings a sense of security to rabbits. We know that wild rabbits live in habitat divided into a common safe zone (the burrow) and a vital zone, shared by a group and composed of various refuges (Burt, 1943). This vital area, including the use of shelters, is subject to group hierarchy (Cowan and Bell, 1986). If we consider that, in our experiment, trees represent safe zones within the vital space. We can thus assume that they allow rabbits a better use of the space offered. In addition, trees could serve as enrichment with which rabbits could interact. They increase the number of supports to mark or gnaw in AO compared to the grassland modality. If trees are perceived as sub-territories, rabbits of AO, aged 72 days at the time of recording the videos, could also begin to express behaviours of territoriality. However, this interpretation can be criticised since the young rabbits have been weaned and raised together.

Finally, behaviours related to a high reactivity cannot be attributed to a positive valence more than negative. Positive anticipation having been demonstrated in previous studies (Fetiveau *et al*. 2021), we can suggest that the rabbits, forced to limit their use of the outdoor place, due to a lack of safety, were all the more satisfied when outings were possible. But this over-satisfaction can also imply frustration when rabbits of this modality were obliged to limit the use of an attractive environment. To confirmed that, it could be interesting to design a behavioural experiment whit rabbits, alternatively raised in AO and GL.

4- Hypervigilance in grassland

First, the estimated means calculated on our different models allowed us to highlight that, in both modalities, the smallest time budget was allocated to locomotion. Pie chart also suggest that rabbits in AO spend most of their time relaxing while rabbits in grassland spend most of their time expressing social behaviours (Figure 13). In addition, our time budget analyses on behaviour categories allowed us to highlight the significant overexpression of alert behaviours in rabbits raised in GL (Figure 12.G). Unfortunately, comparisons of the other categories did not show significant differences between the two modalities.

Mykytowycz and Rowley (1958) demonstrated that locomotion behaviours are less expressed in wild rabbits. It is therefore consistent to find a low expression time in this category. This result can also be explained by the fact that the behaviours considered in this category only concern the movements from point A to point B and does not consider the movements that occur when expressing other behaviours (for example during pursuit races or during exploration). Hypervigilance observe in GL confirms results obtained during the analysis of the number of occurrences of behaviours (Figure 11). We can therefore confirm that the presence of trees in the environment brings safety to rabbits.

Nevertheless, it is important to take into account that the categories analysed are composed of different behaviours. Therefore, there could be significant differences in terms of specific behaviour expression time. This hypothesis is based on the repeated observation of rabbits in both modalities. Indeed, we observed that some behaviours were expressed only in one modality. This is, for example, the case of «Lying_side» behaviour which has been observed exclusively by rabbits in AO. The behaviours in the categories can also represent different activities. Behaviours in the social category, for example, can be passive or active interactions. For example, proximity of rabbits, which seems to be more frequent in GL rabbits when viewing videos, can be perceived by an attempt at protection by the group effect. On the other hand, pursuit races in AO do not have the same interpretation in terms of social behaviour. In other words, it is not because the categories of behaviour are not significantly different in terms of expression time that the behaviours expressed by rabbits are the same.

Finally, we take into account that the models used to analyse these categories do not take into account the repetition of observations within each pen (represented by the different observation windows), or the various independent factors identified during the programming of the project on the BORIS software. We can therefore assume that a precision factor used in our models would allow a better adjustment of the latter.

Conclusion

This study allowed us to enrich the scientific knowledge on the behaviour of rabbits raised in an alternative system combining livestock and agroforestry. We were able to show that there is indeed an effect of the presence of trees on the behaviour of rabbits. The latter serve as safe zone. Therefore, they bring safety to rabbits and allow them: (i) Better use of their living environment, (ii) A decrease in alert behaviours. Trees also serve as enrichment and allow for increased recovery of natural behaviours such as gnawing and marking. Future studies on quantitative analyses of spatial distributions and on specifics behaviours, could allow to precise our understanding of effects of trees on rabbit behaviours. In conclusion, this study allowed us to demonstrate the benefit of apple trees to improve the welfare of growing rabbits raised outdoor.

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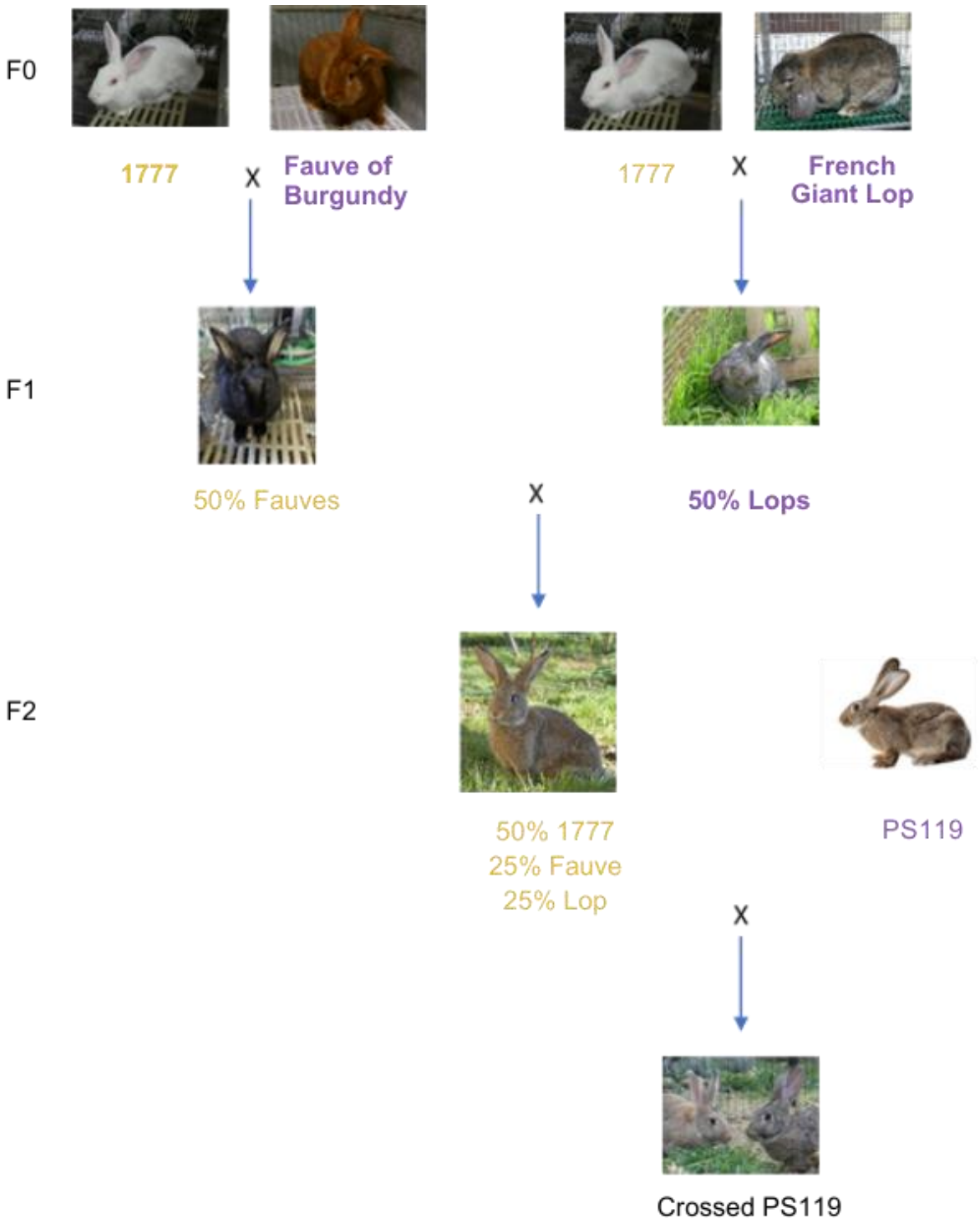
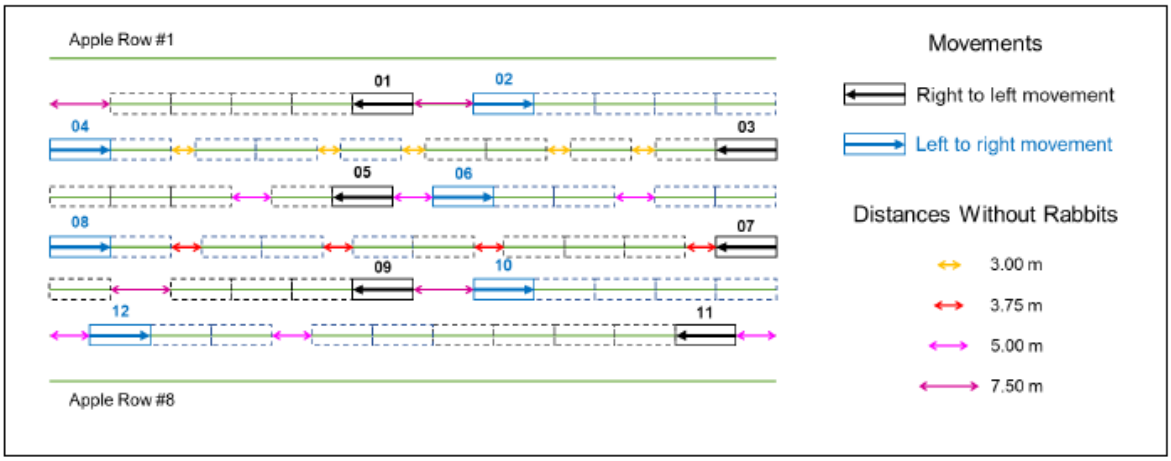
Appendices



Appendix 1: Phylogeny of rabbits used in the experiment with the female line in yellow and the male line in purple



Appendix 2: Mobile-pen locations in the apple orchard (same layout in grassland). Numbers represent the initial location of each mobile-pen. Arrows indicate the direction of the mobile-pens transfer and the dashed areas (within each apple row; green lines) indicated their future location (Savietto et al., 2024, under review)

Appendix 3: Diagram of video sampling protocol (black line = camera setup time, green line = coding time, red line = latency time, dotted line = video time)

Behaviours	Associated modifier	Description
Stopped Run Smell Lean-on Mark Gnaw	Tree	The rabbit interacts, moves towards or is next to a tree
	Hut	The rabbit interacts, moves towards or is next the hut
	Fence	The rabbit interacts, moves towards or is next to the fence
Run Smell	Congener	The rabbit interacts or moves towards a congener
Smell	Ground	The rabbit smells the ground
Mark	The graze	The rabbit marks the graze
Stopped	Nothing	The rabbit is stopped at a random location in the parks
Move Smell Lean-on Mark Gnaw	Indeterminate	The rabbit interacts or moves towards something indeterminate
Ears Posture	Raised	Rabbit's ears are raised in a specific direction
	Asymmetric	One of the rabbit's ear is released along its body and the other is raised
	Relaxed	Rabbit's ears are released along its body
Head Posture	Up	Rabbit's head is well raised above its shoulders
	Right	Rabbit's head is straight in the continuity of its body
	On the floor	Rabbit's head is laid on the ground
Lying	Side	The rabbit is lying on the side and pads are visible
	Ball	The rabbit is lying, its belly on the ground and its four legs under his body
Ears Posture Head Posture	Invisible	We can't determine the rabbit's posture

Appendix 4: Table of the modifiers use with Boris ethogram

	Kappa on AO pens			Kappa on GL pens		
	Min	Mean	Max	Min	Mean	Max
Kappa	0.71	0.81	0.90	0.65	0.74	0.92

Appendix 5: Table of minimums (min), means and maximum (max) of kappa's value obtained by comparing 3 minutes of video in each parc in both modality

	Numbers of occurrences/ Hour in AO			Numbers of occurrences/ Hour in GL		
Behaviours	Min	Mean	Max	Min	Mean	Max
To walk	72.88	85.71	98.89	62.71	86.65	140.86
To leap	103.30	117.80	144.60	103.40	149.60	187.30
To run	19.01	27.29	33.88	20.34	22.67	24.58
To flee	0	0.48	5.08	5.93	15.89	22.89
To cavort	4.13	6.96	8.47	7.63	15.47	22.89
To enter	14.05	16.32	19.83	11.86	19.28	26.27
To go-out	14.05	15.90	18.18	15.25	23.73	32.20
To jump	0	0.62	2.48	4.24	7.84	11.86
To graze	10.74	19.02	27.97	23.73	33.05	44.92
To drink	0	11.83	19.83	7.63	11.65	18.64
To forage	34.71	45.36	66.12	37.29	69.92	109.32
To stretch	17.80	21.93	26.45	9.32	14.83	19.49
To rest	18.65	23.73	29.75	18.64	22.88	31.36
To observe	143.20	194.70	247.10	155.10	230.30	302.50
To rise-up	29.75	48.72	73.73	36.44	42.37	55.08
To smell	103.40	130.70	162.20	105.10	136.40	161.00
To scrape	24.58	44.96	66.94	3.39	25.43	35.59
To mark	54.24	60.38	65.29	27.12	32.63	39.83
To escape	28.89	39.26	51.24	8.47	20.76	41.83
To pursue	8.26	12.45	16.95	0.85	3.81	5.93
Side-by-side	75.21	94.33	112.40	83.90	112.92	146.62
Nose-to-nose	15.25	23.55	40.50	16.95	36.44	62.71
To gnaw	1.65	18.62	37.78	0.85	1.48	1.69
To groom	106.10	169.80	204.10	104.20	118.90	137.30

Appendix 6: Table of brut data of number of occurrences per hour of 24 behaviours

Behaviours	Estimated marginal means in apple orchard (% of occurrences/h)	Estimated marginal means in grassland (% of occurrences/h)	Df	χ^2	P
To walk	6.96 ± 8.62	6.89 ± 8.58	1	$3e^{-3}$	0.95
To leap	9.65 ± 7.89	11.85 ± 8.74	1	3.475	0.06
To run	2.22 ± 2.53	1.88 ± 3.32	1	0.985	0.32
To flee	0.14 ± 0.11	1.27 ± 0.34	1	6.669	0.01
To cavort	0.58 ± 0.17	1.23 ± 0.24	1	4.722	0.03
To enter	1.35 ± 0.16	1.51 ± 0.17	1	0.498	0.48
To go out	1.31 ± 0.12	1.86 ± 0.14	1	9.150	$2e^{-3}$
To jump	0.05 ± 0.05	0.66 ± 0.18	1	6.296	0.01
To graze	1.60 ± 0.33	2.67 ± 0.43	1	3.770	0.06
To drink	1 ± 0.27	0.91 ± 0.26	1	$6e^{-2}$	0.81
To forage	3.66 ± 0.77	5.57 ± 0.96	1	2.364	0.12
To groom	13.60 ± 0.96	9.62 ± 0.81	1	9.992	$1e^{-3}$
To stretch	1.78 ± 0.20	1.21 ± 0.17	1	4.893	0.0 3
To rest	1.91 ± 0.11	1.82 ± 0.11	1	0.389	0.53
To scrape	3.58 ± 0.67	1.97 ± 0.5	1	3.631	0.06
To observe	15.7 ± 1.12	18.3 ± 1.21	1	2.428	0.12
To rise up	4.10 ± 0.8	3.39 ± 0.73	1	0.427	0.51
To smell	10.6 ± 0.67	11 ± 0.68	1	0.208	0.65
To mark	4.92 ± 0.35	2.68 ± 0.26	1	25.282	$5e^{-7}$
To escape	3.22 ± 0.5	1.55 ± 0.34	1	7.417	$6e^{-3}$
To pursue	1.05 ± 0.22	0.30 ± 0.12	1	8.163	$4e^{-3}$
Side-by-side	7.75 ± 0.56	8.91 ± 0.61	1	2.131	0.15
Nose-to-nose	1.88 ± 0.43	2.89 ± 0.54	1	2.110	0.15
To gnaw	1.47 ± 0.40	0.12 ± 0.11	1	6.594	0.01

Appendix 7: Table of estimated marginal means, in percentage of behaviours' occurrences per hour, considered, in both modalities and ANOVA test results (df= degrees of freedom, χ^2= value of the computed chi square, P= p-value)

Categories	Estimated marginal means in apple orchard (% of time)	Estimated marginal means in grassland (% of time)	Ratio	P
Activity	32.3 ± 5.21	25.2 ± 3.93	1.28 ± 0.29	0.27
Relaxation	25.8 ± 5.62	18.1 ± 3.83	1.42 ± 0.43	0.25
Social	21.3 ± 3.98	34.6 ± 6.23	0.617 ± 0.16	0.06
Exploration	12.8 ± 2.66	11.3 ± 2.66	1.14 ± 0.33	0.65
Warning	11.6 ± 1.94	25.4 ± 4.07	0.46 ± 0.11	$7e^{-4}$
Feeding	2.21 ± 1.16	2.77 ± 1.41	0.80 ± 0.58	0.76
Locomotion	1.42 ± 0.243	1.02 ± 0.173	1.39 ± 0.34	0.17

Appendix 8: Table of estimated marginal means, in percentage of time, in both modalities and Pairs test results (Ratio = Estimated marginal mean in AO / Estimated marginal mean in GL, P= p-value)

GenPhySE, Université de Toulouse, INRAE, ENVT- UERI de Gotheron, INRAE- Institut Agro Dijon / CESAER (UMR 1041)

Effect of the presence of apple trees on the behaviour of growing rabbits bred outdoors

Morgane GENIN, Avelyne S. VILLAIN, Davi SAVIETTO, Téo COCHOU, Manon FETIVEAU, Stéphanie DRUSCH, Floriane DERBEZ, Valérie FILLON

Why?

- In France about 90% of farm rabbits are housed indoors in wired cages with **no enrichment or** access to natural light. They are also exclusively fed with pellets [1].
- Impact rabbits ability to express behaviours specific to their species [2]
- **Impair their welfare** [3] and [4].
- Designing an innovative Agroecological system [5] combining rabbits and apple orchards.

Could apple trees enrich their living environment and secure rabbits?

How?

Apple Orchard

Grass Land

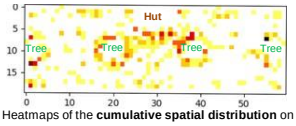
- Spatial distribution: Observation of 72 rabbits per modality
- Behaviours quantification: Observation of ~1h of video in 8 parks
- Time budget: Observation of 2 min of video each 8 min during ~1h in 8 parks

- 24 mobile pens (12 per modality)
- 1 pen: rigid panels (~ 18.75 m²)
- 144 young crossbreed rabbits: 6 per pen, both sexes

Rabbits' behaviours…

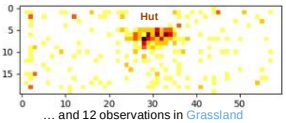
Spatial Distribution

… in Apple Orchard



Heatmaps of the **cumulative spatial distribution** on 13 observations in Apple orchard…

… in Grass Land

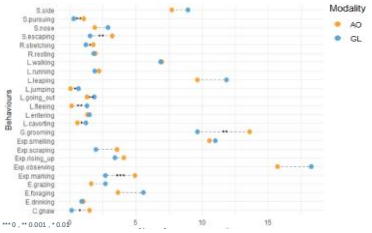


… and 12 observations in Grassland

Ethogram

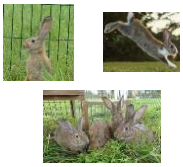
Behaviours significantly more expressed:

Grooming
Pursuing/escaping a congener
Marking
Gnawing



Cleveland chart comparing the mean number of 24 **behaviour occurrences** per hour in Apple Orchard and 4 in Grass Land

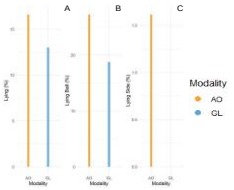
Behaviours significantly more expressed:



Fleeing
Jumping
Cavorting
Frequently going in and out

Time Budget

Rabbit **lying** on the side
Only observed when rabbits **feel safe**



Bar plots comparing the mean percentage of time rabbits lying (A) in ball (B) or on the side (C) in Apple Orchard and 4 in Grass Land (analysis still on going for other behaviours).

Rabbits lying on the side **not observed**

Take home message

- **Outdoor** breeding **restore natural** rabbits' **behaviours**
- Presence of trees **increases the occupation of space** by rabbits
- Rabbits have **affinity for trees**
- Trees promote **exploration** and amplify **deep resting behaviours**
- Trees seem to **decrease alert behaviours**

Apple trees provide a spatial enrichment that confers protection and improve the welfare of rabbits raised outdoors

References & Acknowledgements

[1] France Agrimer, 2024
[2] Delanoue *et al.*, 2018
[3] ANSES, 2018
[4] EFSA Panel on Animal Health and Welfare, 2020
[5] Savietto *et al.*, 2023

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We would like to thank all the rabbits we work with and Le graal, ethoschR and white rabbit for the rehoming of these.

PDF view here

53rd conference of the French Society for the Study of Animal Behaviour (SFECA), 22 to 24 May 20234, Albi - INU Champollion

Appendix 9: Poster SFECA

Title: Effect of the presence of apple trees on the behaviour of growing rabbits raised outdoors

Keywords: Rabbits, Welfare, Apple trees**,** Breeding**,** Agroecology

Abstract: In France, 90% of rabbits are raised in buildings in wire cages. These living conditions impact their welfare and therefore are criticized by citizens. One of the alternative systems studied aimed to develop an agroecological system breeding young rabbits outdoor in an apple orchard. We were interested in the effect of trees on the rabbits' behaviours with the hypothesis that trees could enrich the environment and secure rabbits. We therefore compared the behaviour of rabbits raised in mobile pens of 18m^2 in an apple orchard or in a grassland. We have implemented a spatial dispersion analysis, created a behavioural repertoire and compared their occurrences and the rabbits' time budgets, in both modalities. Our analyses showed that the presence of trees tend to increase space occupancy by rabbits. Behavioural analyses demonstrated the expression of a large behaviour's repertoire (N = 44 observable behaviours) in both modalities. However, some behaviours are differentially expressed in the two modalities. Indeed, we observe higher number of occurrences of resting and exploration behaviours in apple orchard. We also observed higher number of fleeing behaviours and a higher time budget of expression of alert behaviours in grassland compared to Apple Orchard. In future studies, quantification of the levels of expression of behaviours could allow us to refine our understanding of the effect of trees. So far, we can already affirm that presence of trees allows enrichment (diversity of expressed species-specific behaviours) and security (lower expression of vigilance) of rabbit's environment and thus contribute to rabbit's welfare.