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Effects of pesticides and vineyard management on the breeding success of the Eurasian wryneck (*Jynx torquilla*)

Master thesis

by

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Abstract

The Eurasian wryneck's population has experienced a decline due to agricultural intensification, particularly the reduction of available nest cavities and the increase in pesticide use. However, recent efforts in Switzerland have shown a positive trend in the wryneck population through measures such as implementing management methods in vineyards that enhance biodiversity. This study aims to investigate the effects of copper, the most widely used pesticide in our study area, and the impact of different vineyard management practices on the wryneck's breeding success. Ant samples, feathers and feces were collected in vineyards along the littoral of the lakes of Neuchâtel and Bienne and copper was quantified. The proportion of eco-networks were calculated around each nest box occupied from 2017 to 2022. To see the impact of the proportion of eco-networks, the position of the nest boxes and the amount of copper in samples on the breeding success, LMMs and GLMMs were applied. Our analysis unexpectedly found higher copper levels in ants collected from eco-network plots compared to ants from conventionally managed plots. In addition, we found a positive correlation between the amount of copper in ants and in feces. The proportion of eco-networks seems to impact positively the breeding success, without being statistically significant. Moreover, the position of the nest boxes does not seem to impact the breeding success. In conclusion, copper might be accumulating in chicks through ant's bioaccumulation, without impacting the breeding success of the wryneck. Lastly, eco-networks measures seem to be working for helping the wryneck in vineyards, regardless of the position of the nest box.

1. Introduction

The Eurasian wryneck (*Jynx torquilla*) is a peculiar bird species from the *Picidae* family. Unlike the other woodpecker species, it is unable to excavate its nesting cavities and must use old woodpecker nests or natural cavities from old trees (Freitag, 1998; Maumary *et al.*, 2007). It has a highly specialized diet, as it solely feeds on ants (Freitag, 1998; Maumary *et al.*, 2007). The wryneck is also the only woodpecker species that migrates to sub-Saharan Africa (Maumary *et al.*, 2007; Wink *et al.*, 2011).

Until the end of the 19th century, the Eurasian wryneck was breeding throughout Europe (Keller *et al.*, 2020). In Switzerland, between 1972-76 and 1993-96, a decline of 22% in the breeding range was observed (Maumary *et al.*, 2007). In Neuchâtel, the survey for the Neuchâtel breeding bird atlas of 1997 to 2002 only accounted for one to eight couples left (Mulhauser *et al.*, 2007). However, since 2010, the wryneck population seems to follow a positive trend in Switzerland (Knaus *et al.*, 2018).

The major identified cause for the wryneck population decline in Switzerland and Europe has been the intensification of agriculture, which decreases the number of available nest cavities and the abundance of ants (Knaus *et al.*, 2018; Keller *et al.*, 2020). Two major parts of agricultural intensification play a role: the use of pesticides and the removal of high-stem orchards (Knaus *et al.*, 2018; Fenestraz *et al.*, 2022).

In Switzerland, high-stem orchards number has declined by 80% from 1950 to 1991 (Blickenstorfer *et al.*, 1996), strongly impacting wryneck populations. Since then, even though fruit production has increased by 30% between 2000 and 2016, the increase has been mostly driven by intensive fruit plantations, despite high-stem orchards being promoted through compensation payments for farmers, (Knaus *et al.*, 2018).

As for pesticides, their negative impacts on human health and the environment have already raised concern in the 60s, and have been confirmed since then (Carson, 1962; Boatman *et al.*, 2004). Some pesticides' effects can be direct and others indirect. For example, insecticides have indirect negative effects on the breeding success of insectivore birds, in diminishing the number of insects available (Boatman *et al.*, 2004; Stanton *et al.*, 2018). Pesticides can also have direct lethal and sublethal effects. A lethal effect is when the individual dies of pesticide exposure, whereas a sublethal effect is when the individual suffers from effects of a dose that does not kill instantly (Tosi *et al.*, 2022). For example, as the wryneck feeds itself and its chicks with ants captured inside the vineyard plots, it may suffer from both indirect (i.e., reduction in the availability of ants) and direct (i.e., exposure to and ingestion of potentially toxic substances) effects of pesticides (Stanton *et al.*, 2018; Poisson *et al.*, 2021).

Fortunately, measures can be taken to mitigate the problems caused by agricultural intensification.

First, different management methods exist and are used in vineyards, some providing better conditions for the installation and survival of the wryneck. One noticeable management measure in Switzerland is the standards for SPB, which stands for *Surface de Promotion de la Biodiversité*. On the European level, those types of measures are called AES, for Agri-Environmental Scheme. In Switzerland, these are management measures to promote biodiversity that farmers must implement to 3.5% of their agricultural area, and 7% to their specialized cultures (i.e., vineyards or orchards) and for which they may receive compensation payments under specific conditions (art. 14 al.1 OPD). Farmers can even receive additional

compensation payments for organizing their SPB lands in networks which are called “eco-networks”. These measures consist of, for example, the use of manuring and foliar herbicides solely under the vines or the alternate mowing every other row (Graf *et al.*, 2016), the latter having already proven to promote the wryneck in vineyards (Weisshaupt *et al.*, 2011; Knaus *et al.*, 2018).

Measures such as the installation of semi-natural structural elements (dry stone wall, hedges, groves, etc.) and the provision of artificial nesting cavities such as nest boxes are not required for farmers to receive payments in the vineyards. However, they are highly encouraged because they have been shown to be effective measures to promote biodiversity (Graf *et al.*, 2016; Fenestraz *et al.*, 2022).

In this study, we focused on two different aspects.

First, we investigated the potential indirect effects of pesticides on the wryneck’s breeding success. We focused on copper-based fungicides (hereafter copper), since it is the pesticide (fungicide) that is most widely used in the vineyards of Neuchâtel and Bern in both organic and conventional vineyards. Indeed, the use of synthetic insecticides is prohibited in vineyards in the Canton of Neuchâtel. Copper has been used since 1880 to fight mainly against mildew (*Plasmopara viticola*), and up to 50 kg a.s./ha between 1920 and 1950 has been used in Switzerland (Räz *et al.*, 1987). As of today, it is the only powerful fungicide allowed in organic viticulture that has a broad-spectrum effect (Andrivon *et al.*, 2018). The acute and long-term risks of copper exposure were assessed as high for mammals as for birds for application rates up to 5 kg a.s./ha (EFSA *et al.*, 2018). However, only few studies looked at the sublethal effects of copper on birds. Most of them were done on poultry, meaning that the lethal effects on the birds were not considered and studies focused on the benefits of copper for growth, or as an antibacterial agent (Kamel *et al.*, 1971; Sharif *et al.*, 2021). However, it has been shown that adding 250 mg/kg of copper in the diet of chicks can already erode their gizzard (Poupoulis & Jensen, 1976).

Secondly, we aimed at understanding the effects of different management methods in vineyards, such as eco-networks, on the breeding success of this bird species. The positive effects of these measures on the wryneck have been proved, by increasing the number of ants available, or by allowing it to extend its territory (Weisshaupt *et al.*, 2011; Fenestraz *et al.*, 2022). However, their effects on the breeding success have never been investigated.

To this end, this study was done in collaboration with the SORBUS organization, which stands for *Sauvegarde des Oiseaux Rares et de la Biodiversité Utile à leur Survie*, who installed a wide network of nest boxes starting in 2005 in vineyards along the littoral of Neuchâtel and in 2008 in Bern (Fenestraz *et al.*, 2022; Pauli, 2022).

We collected ants in plots conventionally managed and in eco-network plots to analyze their copper amount, and to see if this amount was different in ants collected in plots managed differently. We then analyzed data collected by SORBUS on the breeding success of the wryneck between 2017 and 2022 to see if the amount of copper in samples and if the proportion of eco-network around nest boxes or the location of nest boxes (within or outside the vineyard) had an impact.

We expect the ants collected in eco-network plots to contain less copper compared to ants collected in conventionally managed plots, since eco-networks management practices are restrictive in pesticides’ applications and amounts applied. Additionally, the proportion of eco-network around the nest boxes is expected to have a positive effect on the breeding success of the wryneck, since eco-network plots are managed with practices that are supposed to help this

species. Lastly, nest boxes positioned inside are expected to have a lower breeding success, due to disturbances created by direct exposure to pesticides or work done in the vineyards.

2. Material and methods

2.1. Study sites

The study area included vineyards extending from Gorgier in the Canton of Neuchâtel to Twann in the Canton of Bern, Switzerland, with a total of 15 municipalities in the area. They were installed by SORBUS from 2005 on in vineyards along the littoral of Neuchâtel and by Hans Rudolf Pauli from 2008 on in Bern (Fenestraz *et al.*, 2022; Pauli, 2022; Fig.1b).

Some nest boxes were located within the vineyard plots, mostly on the vineyard's poles, on the wall of vine huts or telephone poles. Those will be referred to as “in”. Others were placed on the border or a few meters away from it, mostly on trees, and those nest boxes will be referred to as “out”.

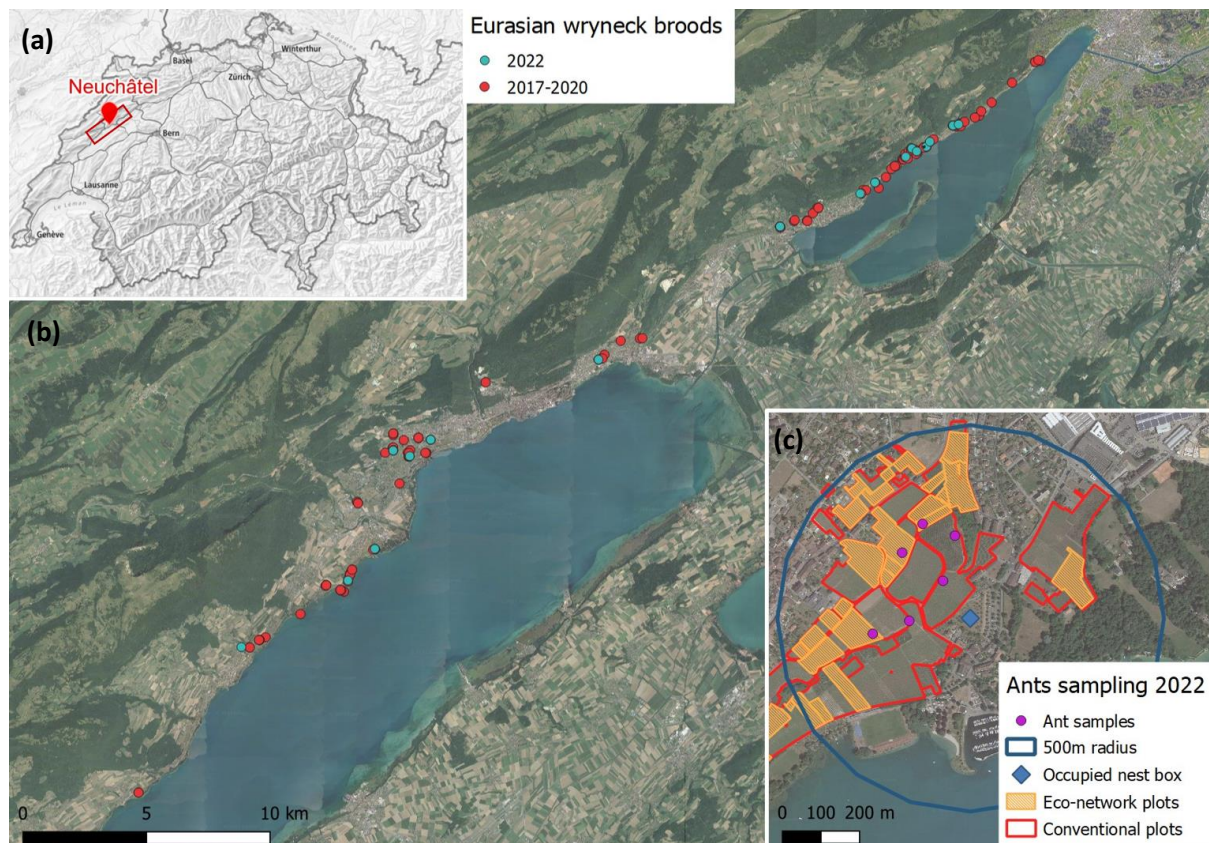


Figure 1 : Study area. (a) Map of Switzerland with a red box highlighting the study area and a red pin indicating the city of Neuchâtel. Map created using swisstopo geodata. (b) Map of the study area. Each red dot represents a brood that was documented by SORBUS between 2017 and 2020. Each blue dot represents a nest box for which we collected the feathers, feces and ants in 2022. (c) Example of the ant sampling around a nest box.

Maps from (b) and (c) were created using QGIS v. 3.28.5 and map data ©2023 Google.

2.2. Data on breeding success

Each year, all next boxes deployed by SORBUS in Neuchâtel and Hans Rudolf Pauli in Bern are controlled by a team of volunteers. When a next box is occupied by a wryneck, additional

controls are done to collect information on the breeding success (see Fenestraz *et al.*, 2022 and Pauli, 2022 for more information). This information is gathered in a database maintained by SORBUS. For this study, we only used data from the years 2017-2022, as it corresponds to the current eco-network period (contracts with wine growers are renewed every eight years). Note that no data were collected in 2021 due to logistic reasons, and data from 2022 were collected for the purpose of this study (see section 2.3).

2.3. Data collection for 2022 samples analyses

Field work was conducted between June and July 2022, which corresponds to the breeding season of *J. torquilla* (Wink *et al.*, 2011; Fenestraz *et al.*, 2022). Each nest box was visited to check if chicks were still present in the nest before collecting samples.

Feces and feather samples

We collected the remaining feces and feathers inside nest boxes after the chicks had fledged to avoid any disturbance. Feces were stored in a tube filled with silicate beads to remove any humidity. Feathers were put in an envelope to avoid any excessive electrostatic forces. Both were stored at -20 °C until further analysis.

Ant samples

Ant samples were collected in a 500-meter radius around the nest boxes. For each nest box, we took three samples in vineyard plots that were conventionally managed and three others in vineyard plots that were part of an eco-network (Fig. 1c). We used the QField application from the QGIS program (v. 3.28.5-Firenze; QGIS.org, 2023) to know where to collect the samples on the field. The Eurasian wryneck forages food by pecking the ground in search of ant nests (Freitag, 1998). Therefore, the ground was observed to find any sign of ant nest, and a big kitchen spoon was used to harvest the nest in round boxes that were 9.5 cm wide and 8 or 4.5 cm deep. This had to be done quite quickly, since ants, when they are attacked, can hide their brood out of reach in their nest within a minute (Freitag, 1998). Coordinates were then written down. Finally, samples were stored at -80 °C until further preparation.

2.4. Samples preparation

Feces and feather samples

Feces were dried in a heat chamber at 50 °C for 48 hours and stored at -20 °C until further analysis. Feathers required no further preparation and remained stored at -20 °C until further analysis.

Ant samples

Ants needed to be cleaned of any dirt (e.g. leftovers of the nest). Each sample was sorted using two sieves, 2 mm and 0.6 mm. Then the remaining soil was searched manually using forceps. Ants and brood were put in a 10 ml tube and stored again at -80 °C. Samples were then freeze-dried for 48 hours using a Telstar LyoQuest freeze-drier (Hernandez *et al.*, 2010).

2.5. Copper analyses

The same method was used to quantify the amount of copper in all the samples.

First, samples were crushed using liquid nitrogen to be homogeneous. Then, it was mixed with 2.5 mL of 65% nitric acid and 2.5 mL of oxygenated water in a Teflon reactor. They were

mineralized at a high temperature and under pressure in a Titan microwave preparation system. This step gave us a liquid that can be stocked at 4 °C. For further analysis, the sample had to be diluted to get a concentration of 0 to 2 ppm. The last step before quantification was the addition of yttrium, an element that does not occur in nature, for calibration.

Finally, the copper amount was quantified using an Optima 7300 DV, which is an ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) device.

2.6. Statistical analysis

Weather data and data for eco-network proportions

Raster weather data were retrieved from MétéoSuisse, which is the Swiss federal office of meteorology and climatology. From these raster data, we extracted the daily mean temperature and the daily amount of precipitation for each nest box location and calculated the mean for both variables from March to July (R-package *raster*; Hijmans & Van Etten, 2023).

Shapefiles data to calculate the proportions of eco-networks were provided by the Cantons of Neuchâtel and Bern.

Amount of copper in ants, feces and feather samples

We built a linear mixed model (LMM) to test the effect of the plots' management method (samples collected in an eco-network plot or in a conventionally managed plot) on the amount of copper in ants. We added the site as a random intercept to account for the fact that six ant samples have been collected for each nest boxes (R-package *lme4*; Bates *et al.*, 2015). A Rosner's outlier test was performed on the amount of copper in ants, and it highlighted that the two highest values were outliers. Those were therefore removed from the data set.

To test the effect of eco-network in a 500 m radius around the nest boxes on the amount of copper in feces and feather samples, we calculated the proportion of vineyard plots that were part of an eco-network within a 500 m radius around the nest boxes. In addition, to test if the amount of copper found in ants could predict the amount of copper in feces and feathers, we calculated the mean amount of copper in the ants' samples. Moreover, we also tested the effects of the nest box position (within or outside the vineyard plots). We built two linear mixed models (LMMs), with the proportion of eco-network (% eco-network), the position of the nest boxes (within or outside) and the mean amount of copper in ants as fixed effects. We added the site as a random intercept.

We also tested if the copper amounts in feces and feathers could predict the proportion of mortality (% dead chicks) and the number of fledglings. To this end, we built generalized linear mixed models (GLMMs) with the amount of copper in feces or in feathers, the position of the nest boxes (within or without) and the proportion of eco-network in a 500 m radius as fixed effects. The site was added as a random intercept (R-package *lme4*; Bates *et al.*, 2015). The proportion of mortality was modelled with a binomial error distribution, and the number of fledglings with a Poisson error distribution. Since the chicks' mortality was calculated as a proportion, we included the brood size as weight in the models. Lastly, when modeling the proportion of dead chicks in response to the amount of copper in feathers, we had to remove the position of nest boxes as a fixed effect, since there was a problem of convergence.

All continuous predictors were scaled to make a direct comparison of effect sizes possible.

Breeding success from 2017 to 2022

We estimated the breeding success with four response variables that are commonly used for this purpose (Poisson *et al.*, 2021; Senzaki *et al.*, 2020) : the brood size (number of eggs laid), the hatching success (proportion of eggs that hatched), the number of fledglings (number of chicks that fledge the nest) and the mortality rate (here measured as a binary response with 1 when at least one chick was found dead and 0 otherwise). To this end, we built generalized linear mixed models (GLMMs) with the proportion of eco-network vineyard plots (% eco-network) and the position of the nest boxes (within or outside) as fixed effects and the municipality of the nest box location and the date and year of laying as random intercepts (R-package *lme4*; Bates *et al.*, 2015). The wryneck can make two consecutive broods. We therefore added the brood number as a covariate in the models. We chose the date of 1st of June as cut-off for the separation of the first and second brood, following Mulhauser & Zimmerman (2014). We included an interaction term between the brood number and % eco-network and nest box position. Moreover, as meteorological conditions are known to strongly influence the breeding success of birds, including wrynecks (Geiser *et al.*, 2008), we added the mean temperature and the mean amount of precipitation as covariates in the models. We selected the best error distributions based on the models' fit and AICc values. Brood size and fledging success were modelled with a Gaussian error distribution, while hatching success and mortality rate with a binomial error distribution. Since hatching success was calculated as a proportion, we included the brood size as weight in the models.

All continuous predictors were scaled to make a direct comparison possible.

All model's diagnostics were checked using the DHARMA R-package (Hartig, 2022).

All analyses were done using R Statistical Software (v.4.2.2; R core team, 2022).

3. Results

Overall, 17 nest boxes were occupied during 2022. We collected a total of 84 ant samples, 14 feather samples and 15 feces samples. For the breeding success analysis, we had a total of 147 broods from 2017 to 2020 and 2022.

3.1. Copper amount in ants

Copper was quantified in a total of 81 samples. Our analysis indicated that ant samples that were collected from an eco-network plot contained a higher amount of copper than samples collected from conventionally managed plots (estimate = 24.78, SE = 11.02, p-value = <0.05, Table 1; Fig. 2).



Figure 2 : Copper amount in ants according to the plots' management. The red dots represent the raw data, the two black dots the predicted means and the black lines represent the 95% confidence interval. Ants collected in eco-network plots contain higher amount of copper than ants collected in conventional plots (estimate = 24.78, SE = 11.02, p -value = <0.05).

3.2. Copper amount in feathers and in feces

The models suggest that there is less copper in feces when there is a higher proportion of eco-network around the nest boxes and when the nest boxes are positioned out of the vineyard plots. They also indicate a positive correlation between the amount of copper in ants and the amount of copper in feces. None of these effects were significant, but we reported them as trends (Fig. 3; Table 1).

Copper amount in feces according to the mean amount of copper in ants

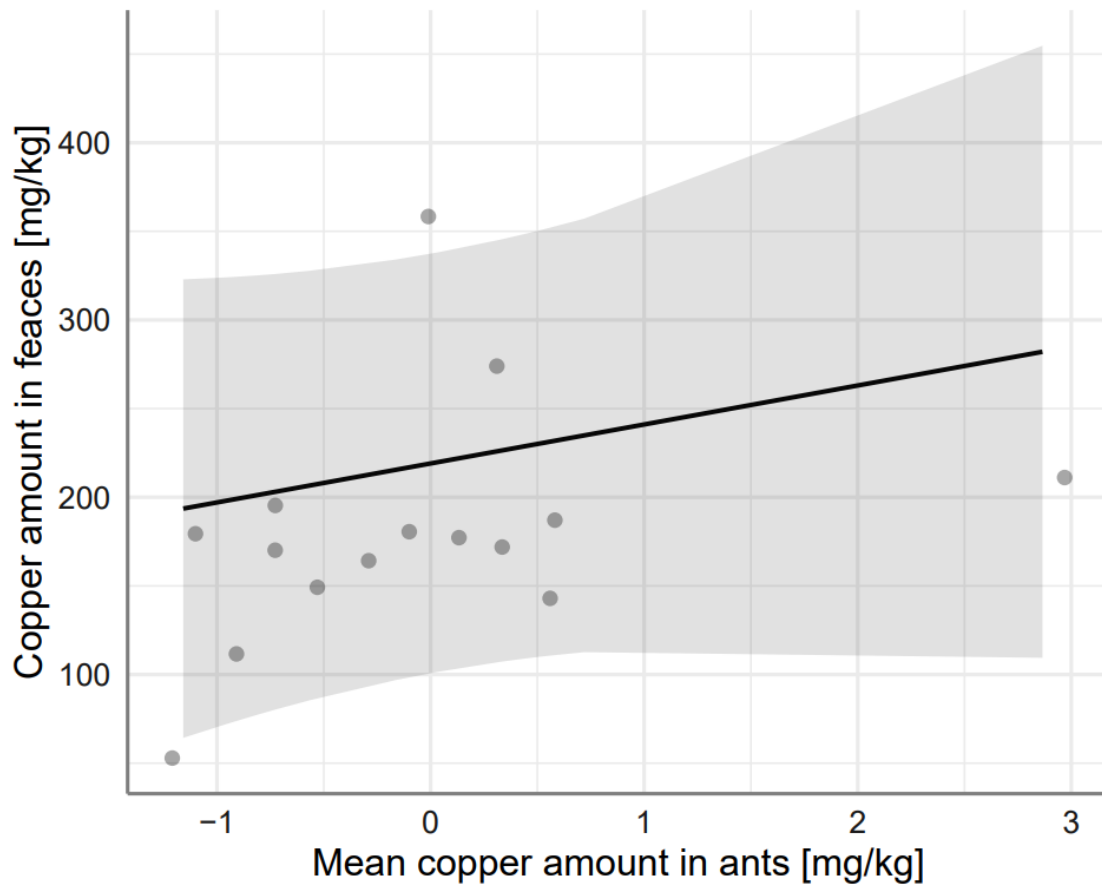


Figure 3 : Copper amount in feces according to the mean amount of copper in ants. Higher amounts of copper in feces seem to be linked to higher amounts of copper in ants (note that this relationship is not significant). The black line and the grey area represent model prediction and 95% confidence intervals. The grey points are the raw data. The mean amount of copper in ants has been scaled.

The models indicate no effect of copper in feces and in feathers on the breeding success, except for the proportion of dead chicks which seems to be negatively influenced by the amount of copper in feathers (estimate = -19.68, SE = 7.69, p-value <0.05, Table 1).

Table 1 : Parameters estimates of the copper models. R^2_m and R^2_c represent respectively the marginal R^2 and the conditional R^2 .

Response	Predictors	Estimates	SE	p-value
Copper in ants	Intercept	79.803	8.341	<0.001***
Error distribution = normal	Eco-network or conventional	-24.778	11.02	<0.05*
$R^2_m = 0.058$	[conventional]			
$R^2_c = 0.187$				
Copper in feces	Intercept	219.06	52.30	<0.005**
Error distribution = normal	Proportion of eco-network	-43.19	61.65	0.498
$R^2_m = 0.121$	Within or outside [outside]	-96.57	121.30	0.443
$R^2_c = 0.159$	Mean copper in ants	21.99	19.55	0.285
Proportion of dead chicks	Intercept	-1.64	1.67	0.325
Error distribution = binomial	Copper in feces	-0.48	0.49	0.324
$R^2_m = 0.036$	Proportion of eco-network	-0.59	1.67	0.726
$R^2_c = 0.541$	Within or outside [outside]	-1.11	3.48	0.749
Number of fledglings	Intercept	2.04	0.31	<0.001***
Error distribution = normal	Copper in feces	-0.001	0.10	0.913
$R^2_m = 0.004$	Proportion of eco-network	-0.06	0.35	0.856
$R^2_c = \text{NA}$	Within or outside [outside]	-0.14	0.70	0.843
Copper in feathers	Intercept	48.90	309.90	0.885
Error distribution = normal	Proportion of eco-network	67.33	340.16	0.856
$R^2_m = 0.155$	Within or outside [outside]	-0.05	175.78	1.000
$R^2_c = \text{NA}$	Mean copper in ants	21.19	44.45	0.666
Proportion of dead chicks	Intercept	-18.78	7.42	<0.05*
Error distribution = binomial	Copper in feathers	-19.68	7.69	<0.05*
$R^2_m = 0.991$	Proportion of eco-network	2.94	2.56	0.250
$R^2_c = \text{NA}$				
Number of fledglings	Intercept	1.98	0.39	<0.001***
Error distribution = normal	Copper in feathers	-0.07	0.15	0.634
$R^2_m = 0.049$	Proportion of eco-network	0.02	0.40	0.969
$R^2_c = \text{NA}$	Within or outside [outside]	-0.12	0.85	0.891

3.3. Breeding success from 2017 to 2022

Proportion of eco-network

The models suggest that a higher proportion of eco-network leads to a larger brood size and a larger number of fledglings. The mortality rate seems to be lower when there is a higher proportion of eco-network around the nest boxes. However, none of these effects were statistically significant. Lastly, the model indicates no effect of the proportion of eco-networks on the hatching success (Table 2).

Position of nest boxes

Overall, the models seem to indicate that positioning the nest boxes out of the vineyards plots is better for the breeding success. However, none of the effects were statistically significant (Table 2).

The model indicated an interaction between the number of the brood and the position of the nest box. In fact, when the nest boxes were outside the vineyard, the number of fledglings was significantly lower only for the first brood (estimate = -1.70, SE = 0.85, p-value < 0.05; fig 4). The brood size also showed this tendency, but the effect was not significant (Table 2).

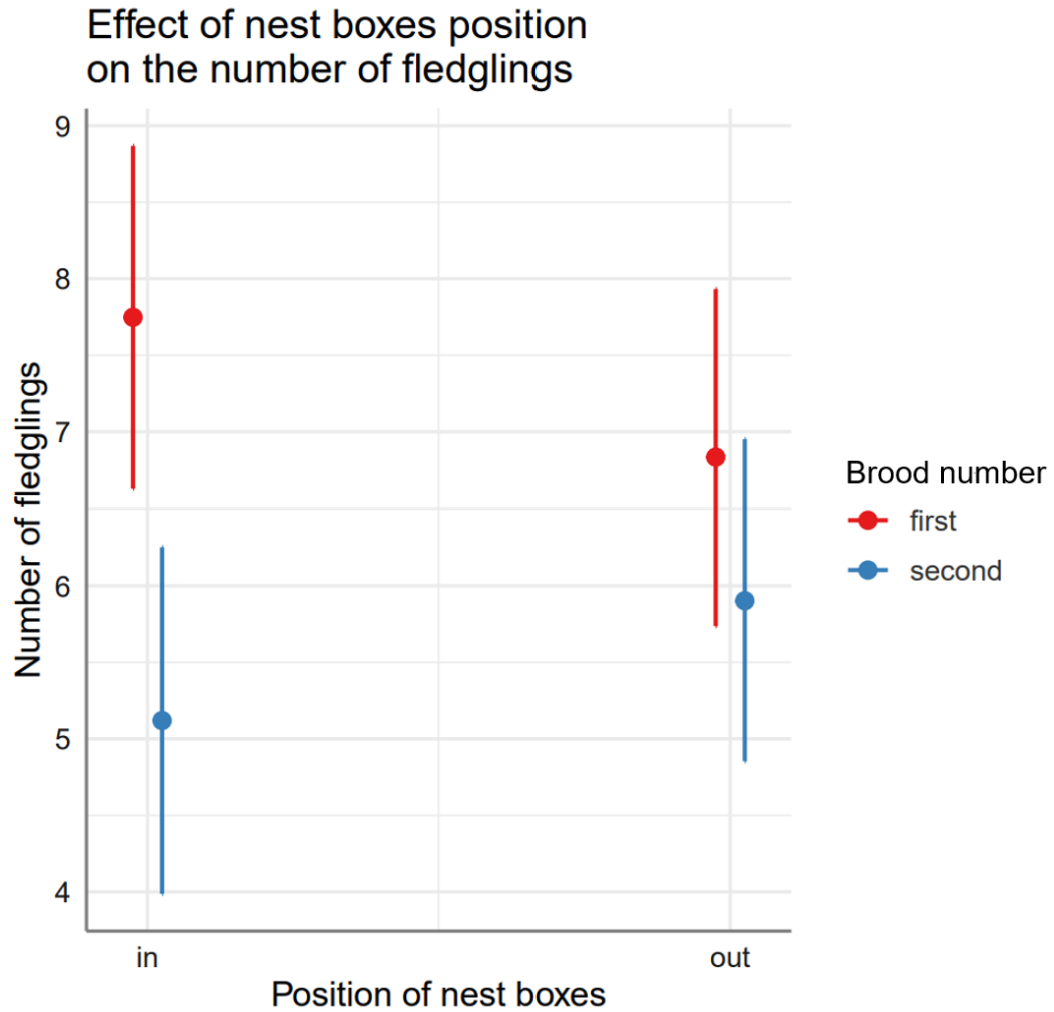


Figure 4 : Effect of nest boxes position on the number of fledglings. The data represented is the number of fledglings, the dots represent the predicted means and the lines the 95% confidence, according to their nest box position and if they were a first (in red) or a second (in blue) brood. As the model indicated an interaction between those two fixed effects, the effect of the position of the nest boxes therefore depends on the number of the brood. For the first brood, the number of fledglings was lower if the nest box was positioned out of the vineyard plots (estimate = -1.70, SE = 0.85, p -value < 0.05).

Weather effects

The mean precipitation had two different effects on the breeding success. First, the mean amount of precipitation had a negative effect on the brood size (estimate = -0.53, SE = 0.24, p -value = <0.05; Table 2). Surprisingly, a higher amount of precipitation led to a better hatching success (estimate = 0.92, SE = 0.34, p -value <0.01) and reduced the mortality rate (estimate = -1.42, SE = 0.34, p -value <0.001; Table 2).

Effect of the first brood

The models showed that, the brood size (estimate = 2.06, SE = 0.52, p -value <0.001) and the number of fledglings (estimate = 2.63, SE = 0.58, p -value <0.001) were significantly higher for the first brood (Table 2).

Table 2 : Parameters estimates of the breeding success models. R^2_m and R^2_c represent respectively the marginal R^2 and the conditional R^2 . The interactions are represented by the symbol “*”.

Response	Predictors	Estimates	SE	p-value
Brood size	Intercept	6.51	0.37	<0.001***
Error distribution = normal	Proportion of eco-network	0.41	0.25	0.109
$R^2_m = 0.228$	Within or outside [outside]	0.55	0.53	0.301
$R^2_c = NA$	Brood number [first]	2.06	0.52	<0.001***
	Mean temperature	-0.39	0.23	0.101
	Mean precipitations	-0.53	0.24	<0.05*
	Proportion of eco-network * first brood	-0.01	0.39	0.982
	Outside * first brood	-1.30	0.77	0.095
Hatching success	Intercept	1.95	0.52	<0.001***
Error distribution = binomial	Proportion of eco-network	0.07	0.34	0.826
$R^2_m = 0.094$	Within or outside [outside]	0.78	0.70	0.261
$R^2_c = 0.482$	Brood number [first]	0.86	0.64	0.183
	Mean temperature	0.16	0.32	0.622
	Mean precipitations	0.92	0.34	<0.01**
	Proportion of eco-network * first brood	-0.33	0.50	0.507
	Outside * first brood	-0.44	0.94	0.639
Number of fledglings	Intercept	5.10	0.58	<0.001***
Error distribution = normal	Proportion of eco-network	0.32	0.29	0.276
$R^2_m = 0.373$	Within or outside [outside]	0.78	0.62	0.208
$R^2_c = NA$	Brood number [first]	2.63	0.58	<0.001**
	Mean temperature	-0.38	0.29	0.192
	Mean precipitations	-0.30	0.38	0.443
	Proportion of eco-network * first brood	-0.10	0.43	0.812
	Outside * first brood	-1.70	0.85	<0.05*
Mortality rate	Intercept	0.44	0.63	0.486
Error distribution = binomial	Proportion of eco-network	-0.34	0.41	0.405
$R^2_m = 0.188$	Within or outside [outside]	-0.72	0.82	0.378
$R^2_c = 0.400$	Brood number [first]	-0.39	0.73	0.595
	Mean temperature	-0.69	0.36	0.055
	Mean precipitations	-1.42	0.43	0.001**
	Proportion of eco-network * first brood	0.18	0.56	0.744
	Outside * first brood	-0.003	1.08	0.998

4. Discussion

4.1. Higher amount of copper in ants from eco-network plots

Our results indicate that there is a higher amount of copper in ants collected from eco-network plots than from conventionally managed plots, which is unexpected. Indeed, we predicted the contrary, since eco-network plots are managed with methods that promote biodiversity by using lower amount of pesticide (Graf *et al.*, 2016). A few hypotheses could explain these results.

First, as farmers who manage their vineyards following the eco-network management are restricted to pesticides that are organic or biotechnical (Graf *et al.*, 2016), they might need to use more copper-based fungicides, whereas farmers who follow a conventional management can use other types of fungicides such as synthetic fungicides that are not copper-based. This might explain why we found more copper in ants collected from eco-network plots than ants from conventionally managed plots.

Second, the copper used as fungicide in vineyards accumulates in the soil, especially in the top layers (Pietrzak & McPhail, 2004), meaning that the amount of copper present in the plots from

which we sampled the ants does not depend on the current management methods, but more on the historical management of those vineyards (Wightwick *et al.*, 2008). Ants collected from plots that were historically managed using more copper, or from older vineyards, might be accumulating copper from the soil pollution, as insects have been shown to bioaccumulate copper in their environment (Augusto *et al.*, 2022). The results we got might be due to that exposition to copper from historical use and might not reflect the exposition of ants to copper used by farmers following eco-network management methods.

However, this hypothesis is based on ants accumulating copper from the soil. As Augusto *et al.* (2022) focused on aquatic insects that create a flux of copper from streams to land, their results might not be applicable to ants, which might not be accumulating copper or other chemicals in the same way. In addition, no studies have been done on the sublethal effect of copper on ants. In fact, studies have focused on the acute effect of copper exposure, and mainly on nematodes, collembola, enchytraeids and earthworms (Karimi *et al.*, 2021). This means that copper might affect ants in an unknown way, instead of being accumulated in them from historically contaminated vineyard soils. In this case, this hypothesis would not explain why we found more copper in ants from eco-network plots than from conventional plots.

Overall, the proportion of the variance explained by the model is relatively low (conditional $R^2 = 0.187$), meaning that these results should be taken with precaution. In addition, the two highest values were removed because they were detected as outliers and the vineyard might have been sprayed by a pesticide that day. But without removing those outliers, the effect was not statistically significant.

To go further, we would need more information on the effect of the weather (i.e., precipitations) on copper accumulation in soils. Indeed, it has been proven that, when there is a higher amount of copper in the soil, there is more copper in vineyard's run-off waters as well (Banas *et al.*, 2010). Yet, we have no information if the presence of copper in run-off waters means that there is lower amount of it in vineyard soils. We would as well need additional information on the vineyard management, for example more details on the types of fungicides that are used, and the amount applied to the vineyards, either in eco-network or in conventional vineyard management. Moreover, as discussed above, there is a lack of information on the bioaccumulation of copper in ants, especially in vineyards, as well as on the impact of the historical accumulation of copper in soils on ants. To try and consider this, we could include the amount of copper accumulated in vineyard soils, or the age of the vineyards in the analysis. Lastly, our sample size was limited, with only 17 nest boxes occupied in 2022. To go further, we would need to extend our study on a few years.

4.2. Copper amount in feces and feathers

First, our results suggest that there is less copper in feces when there is a higher proportion of eco-network around the nest boxes and when the nest boxes are positioned out of the vineyard plots. This seems to indicate that nest boxes that are outside the vineyards might be less exposed to copper than nest boxes inside of them. This is not surprising since nest boxes positioned out of the vineyards are usually placed on trees and not directly sprayed by the copper-based fungicides.

Second, results also show a positive correlation between the amount of copper in ants and the amount of copper in feces. This could indicate that copper might be accumulating in the wryneck chicks through the ants (Augusto *et al.*, 2022). However, as discussed above, we have

limited information on the mechanisms of bioaccumulation of copper in ants. This means that this correlation might not be due to the bioaccumulation of copper in ants, but to other factors such as the direct exposure of ants to copper when it is sprayed in the vineyard for example (Leonzio *et al.*, 2009).

Nevertheless, the breeding success does not look impacted since none of our results are significant. This is in fact surprising, because the amount of copper we found in feces and in feathers is high when compared to results found in other studies, such as the ones from Dauwe *et al.* (2004). In their study, they have analyzed the copper concentration in feces and feathers of great tits (*Parus major*) along a pollution gradient. When comparing the concentrations of copper in the birds from their most polluted site to our results, we have found copper concentrations almost three times higher in feces and thirty times higher in feathers. As feces have been found to be a good monitor of the amount of non-essential metal such as copper in the environment of passerine birds, our results would suggest that our study sites present a high level of copper pollution (Dauwe *et al.*, 2004). This would lead us to think that the breeding success should be negatively impacted.

However, our results might be not statistically significant because of the small sample size ($n = 15$). Another hypothesis would be that the variables used to estimate the breeding success in our study are too coarse. Moreover, the chicks' survival might also be impacted in later life-stages, such as the post-fledgling, which would not reflect in our estimation of the breeding success (Naef-Daenzer & Gruebler, 2016).

To go further, our study might have benefited from more detailed breeding success variables such as the mass and wing length of chicks (Naef-Daenzer & Gruebler, 2016). Lastly, other well-known pesticides might be implicated in having negative sub-lethal effects and could have been included, such as glyphosate or DDT (Dichlorodiphenyltrichloroethane). Indeed, Patinha *et al.* (2018) found residues of DDT and its metabolites in the soils of old vineyards in Portugal. It would not be surprising to find similar residues in Swiss soils. We could investigate their presence in Swiss vineyard soils, their effects on the breeding success of the wryneck and the possible interactions with copper.

Lastly, the proportion of dead chicks seems to be negatively influenced by the amount of copper in feathers. This would indicate that more copper in feathers reduces the proportion of dead chicks, which is surprising, although it has been proven that copper in the diet of chicks improves the body weight gain and the feed efficiency (Poupoulis & Jensen, 1976; Hamdi *et al.*, 2018). Indeed, Hamdi *et al.*, (2018) showed that broilers given a supplementation of copper at a dose of 150 mg/kg during the first 35 days gained more body weight than with a lower supplemental copper dose (15 mg/kg), whereas higher doses (300 mg/kg) reduced bodyweight gains. However, in our study, we cannot directly link the amount of copper in feathers to the proportion of dead chicks, because copper might come from an exogenous source, such as the direct exposure of chicks to the copper sprayed in vineyards (Leonzio *et al.*, 2009). Moreover, this result should be taken with caution, since the sample size was very small ($n = 9$), and that the residuals were problematic.

4.3. Effect of the proportion of eco-network on the breeding success

Overall, even if our results were not statistically significant, a higher proportion of eco-network had a positive impact on the breeding success. These results are not surprising and confirm that implementing biodiversity enhancing measures such as eco-networks are working and are

efficient conservation measures (Weisshaupt *et al.*, 2011; Fenestraz *et al.*, 2022). For example, the alternated mowing provides the best foraging conditions for the wryneck, which consists of approximately 50% of bare soil (Coudrain *et al.*, 2010; Weisshaupt *et al.*, 2011). The vegetation provides habitats for ants, and the wryneck can easily forage on the bare soil, where rows are mowed.

As eco-networks management methods consist of different types of measures, it would be interesting to go further in details by considering the different types of measures applied in each plot. For example, we could have calculated the proportion of plots where the mowing is alternated every other row, since it plays an important role for the wryneck's foraging (Weisshaupt *et al.*, 2011). Other types of management are practiced in vineyards as well, such as biodynamic farming (Bosco *et al.*, 2022). This type of management includes other practices, such as ploughing. It has been shown by Bosco *et al.* (2022) that biodynamic farming does not provide an ideal habitat for invertebrates in vineyards, possibly impacting the ant's abundance and therefore the breeding success of the wryneck. Moreover, conventional farming in vineyards is not homogeneous either. Farmers do not need to be labeled to practice organic farming or to implement practices that have a positive impact on the wryneck's breeding success, which blur the lines between eco-network plots and conventionally managed plots.

To go further, we would therefore need more information and details on the different practices used on plots to understand how they impact the ants and the wryneck's breeding success. In addition, as said above, more detailed data on what type of pesticides and the amounts applied would also be interesting and relevant to implement more efficient practices as well.

The fact that the results are not significant might be explained by the lack of precision in the shapefile data provided by the cantons, but by the lack of details on the management methods as well. Additionally, our models might have benefited from more differences in the spectrum of the proportion of eco-networks. Indeed, most of our nest boxes were surrounded by at least 40% of eco-networks. Extending our study area to other cantons, such as the Canton of Valais, where farmers are less prone to create eco-networks, might be interesting (Bosco *et al.*, 2022).

4.4. Effect of the position of the nest boxes on the breeding success

Interestingly, the position of the nest boxes does not seem to impact the breeding success. This means that the disturbances from the vineyard management does not impact the breeding success of broods in nest boxes placed inside the vineyards.

Two models also indicated that placing the nest boxes out of the vineyard had a negative impact on the breeding success of the first brood. This might be because of a higher predation rate outside the vineyard. As nest boxes are placed usually on trees when they are not inside the vineyard, this might allow predators that live in hedges or groves, such as dormouse, to predate on these nest boxes (Miller, 2002; Adamík & Král, 2008). In fact, the European edible Dormouse (*Glis glis*) terminates its hibernation at the end of May on average, which would coincide with the first brood of the Eurasian wryneck (Adamík & Král, 2008).

4.5. Management recommendations and implications

Our results seem to indicate that the wryneck's breeding success is not impacted by the work done in the vineyards when nest boxes are placed in them. In addition, placing the nest boxes

outside the vineyards seems to have a negative impact on the breeding success of the wryneck, certainly due to higher predation rate.

This leads us to advise placing nest boxes both inside and outside the vineyards to maximize the number of artificial cavities provided for the wryneck. However, we would suggest installing additional guards to mitigate predation on nest boxes placed on trees outside the vineyards. The most optimal one has been found to be the cone baffle installed on the structure that supports the nest box or a hole extender, as described in Bailey & Bonter (2017).

Lastly, as mentioned above, installing nest boxes is not required in an SPB, meaning that plots from eco-networks might not contain any nest boxes. We would advise, when organizing eco-networks, to have the installation of nest boxes in vineyards as a criterion to join the network, since it has been shown to be an effective conservation measure to extend the wryneck's territories (Coudrain *et al.*, 2010).

4.6. Study limitations and outlook

We encountered many difficulties that created limitations to our study.

First, the number of wryneck's broods was low in 2022. This reflected in our study in the small sample sizes for the copper analysis in feces and feathers. We think it might be due to extreme weather events, with several days of heatwaves and storms with heavy rainfall during the breeding period.

Our sample size and the precision of our analysis could be increased by sampling feathers and feces directly on the chicks. However, this sampling technique would be more invasive. Otherwise, we could extend the duration of the sampling period to multiple breeding seasons, but this would be more time-consuming.

Additionally, we sampled feces and feathers that were in the nests after fledglings left, meaning that we cannot directly link the amount of copper in our samples with the ants we collected, because feathers and feces might have been from previous broods.

Moreover, we had difficulties getting access to data concerning vineyard management, which caused a lack of details in our analysis concerning the management methods. As discussed above, using only "eco-networks" as one category is simplifying the multitude of practices used by farmers on vineyards, which was convenient for us, but not as representative of the reality as it should be.

Lastly, it would have been interesting to investigate glyphosate, a herbicide still frequently used under the vines, and which has been shown to have deleterious effects on organisms, even in eco-network plots. However, quantifying glyphosate in ants was not possible, for technical reasons.

5. Conclusion

This study investigated the effects of copper, the main pesticide used in our study area, and the influence of vineyard management practices on the breeding success of the Eurasian wryneck. Our analysis revealed higher copper levels in ants collected from eco-network plots compared to ants from conventionally managed plots. Additionally, a positive correlation was observed between the amount of copper found in ants and the amount detected in feces. These findings suggest that copper might be accumulating in wryneck chicks through the ants. Moreover, we

found that the proportion of eco-networks appeared to positively influence breeding success, although this effect was not statistically significant. Lastly, the position of the nest boxes did not seem to have a significant impact on the breeding success.

These findings were an interesting first step for understanding the complex interactions between copper-based fungicides, vineyards management, and the breeding success of the Eurasian wryneck. They underscore the importance of implementing biodiversity enhancing practices in agricultural landscapes to support the conservation efforts for the wryneck and other vulnerable farmland bird species. It led us to suggest placing nest boxes inside and outside of vineyards, with additional guards against predation for the latter, as well as establishing the installation of nest boxes as a criterion for eco-networks in vineyards.

Future research could delve further into the mechanisms of copper accumulation in vineyard soils and in ants, as well as of other pesticides such as glyphosate or DDT, and explore the diversity of vineyards management practices and their impact on the breeding success of the wryneck.

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