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Meteorological and climatic variables predict the phenology of *Ixodes ricinus* nymphs activity in France, accounting for habitat heterogeneity

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Abstract

Ixodes ricinus ticks (Acari: Ixodidae) are the most important vector for Lyme borreliosis in Europe. As environmental and climate changes might affect the distribution and activity of tick populations, this study aimed to determine the effects of environmental factors, i.e., meteorological, bioclimatic, and habitat characteristics on questing activity of *I. ricinus* nymphs across diverse climatic types in France over an 8-year period. Questing activity was observed using a repeated removal sampling design with a cloth-dragging technique in 11 sampling sites from 7 tick observatories from 2014 to 2021 at approximately 1-month intervals, involving 631 sampling campaigns. Three phenological patterns were observed, potentially following a climatic gradient. The mixed-effects negative binomial regression revealed that observed nymph counts were driven by different interval-average meteorological variables, including one-month average temperature, previous-season average temperature, and half-year average minimum relative humidity. The interaction effects indicated that the phenology in colder climates peaked differently from that of warmer climates. Also, the land cover characteristics that support the highest baseline abundance were moderate forest fragmentation with transition borders with agricultural areas. Finally, our model could potentially be used to predict seasonal human-tick exposure risks in France that could contribute to mitigating Lyme borreliosis risks.

1. Introduction

In the light of ongoing anthropogenic climate change, redistributions of vector populations and vector-borne diseases have become increasingly concerned as an alarming public health threat¹. The effects of climate change on the population of *Ixodes ricinus* ticks (Acari: Ixodidae), the most important arthropod vector species in Europe, have become the topic of interest in this region². The life cycles and key ecological processes of ixodid ticks, such as mortality, development rate, and questing behaviour, are sensitive to weather conditions^{3,4}. Therefore, variations in meteorological conditions contribute to the regulation of the dynamics of tick abundance, phenology of questing activity, and eventually, human-tick exposure risks throughout the year. However, the direct impacts of climate change on tick population dynamics related to abiotic factors, such as temperature and relative humidity, are not always straightforward. Rising temperatures could promote the probability of questing activity and interstadial developmental rate; however, ticks may also face a higher mortality risk at the same time⁴. Meanwhile, relative humidity influences the water regulation of ticks, affecting their questing behaviour and survival⁵⁻⁷. Furthermore, climate change could also pose indirect effects on tick populations through biotic factors, such as habitats⁴ and host populations (both density and behaviours)^{2,8}. As a result, climate change has been expected to alter the distribution of tick populations, the activity of questing ticks, and human-tick exposure both geographically (poleward and upslope expansions)^{9,10} and seasonally (altered phenology)¹¹.

I. ricinus ticks have wide geographical and climatic ranges spanning all over Europe and some regions in northern Africa and western Asia¹². Long-term observation of the questing activity of *I. ricinus* nymphs, for both phenology and abundance, has been an important indicator for the exposure risks to ticks and tick-borne diseases in these regions, particularly Lyme borreliosis¹³⁻¹⁶. *I. ricinus* ticks have been observed to exhibit different phenology (a seasonal variation) of questing activity across their wide range of geo-climatic distributions, such as unimodal or bimodal patterns¹⁷, which determines the time of year when questing ticks are most or least likely to be encountered. While the abundance (a population size) of ticks in each area reflects the suitability/carrying capacity of the habitats for the populations, both land cover and climatic conditions¹⁸. Furthermore, when the suitability of the habitat is altered, long-term observations can reveal directional changes in questing activity over several years (inter-annual variation)¹⁵. To evaluate the effects of climate change on the distribution of tick-borne diseases, it is critical to understand the impacts of environmental factors, both abiotic and biotic, on all levels of tick activity variations over a long period of time across various geographical/climatic ranges.

However, most of the previous studies on the environmental effects on *I. ricinus* nymph activities in Europe did not capture both the temporal (seasonal and inter-annual) and spatial variations. Some studies focused on short-term observations at multiple sites, allowing for the comparison of abundance estimates but not seasonal and inter-annual variations¹⁹⁻²². While the other studies were long-term observations at a few sites, capturing seasonal and inter-annual variations and thus unable to compare with multiple sites¹³⁻¹⁶. Furthermore, the most widely used method for monitoring nymph activity, cloth-dragging sampling, has a low capture rate²³. Therefore, this method produces a high level of uncertainty in abundance estimates across different environmental conditions and field investigators²⁴, which makes comparing results from different observatories difficult.

France is a hotspot for *I. ricinus* and Lyme borreliosis²⁵ and has a wide range of geographical and climatic characteristics²⁶. An in-depth understanding of the characteristics of questing activity of *I. ricinus* across various geo-climatic areas is still needed to help control Lyme borreliosis in this area. The present study sought to investigate the activity of *I. ricinus* nymphs, both phenology and abundance, across a wide range of climatic region types in France over a long-term observation period. The sampling protocol suggested by a previous study was employed to reduce the uncertainty from the cloth-dragging method²⁴. Also, this study aimed to assess the impacts of environmental factors, such as meteorological, bioclimatic, and habitat characteristics on the variations of *I. ricinus* nymph questing activity, which could help better understand the effects of climate change on *I. ricinus* populations.

2. Material And Methods

2.1. Sampling sites

Longitudinal observation campaigns for *I. ricinus* nymph activity were carried out at 11 sampling sites in forest areas from 7 different tick observatories across France. Tick observatories are located at the following French municipal areas, where the coordinates of the centre of each municipal area and the climatic types²⁶ are also provided as: 1) La Tour de Salvagny (45°48'50.6"N 4°42'53.2"E; Mixed climates); 2) Saint-Genès-Champagnelle (45°43'23.8"N 3°01'08.0"E; Mountain climate); 3) Etiolles (48°37'59.9"N 2°28'00.1"E; Degraded oceanic climate); 4) Carquefou (47°17'58.5"N 1°29'26.0"W; Oceanic climate); 5) Gardouch (43°23'25.7"N 1°41'02.1"E; South-West Basin climate); 6) Velaine-en-Haye (48°42'13.4"N 6°01'16.1"E; Semi-continental climate); 7) Les Bordes

(47°48'47.3"N 2°24'01.3"E; Degraded-oceanic climate) (Figure 1). The observation campaigns were carried out from April/June 2014 to May/June 2021 in most observatories, except for Les Bordes, which began in April 2018.

Each tick observatory corresponds to one sampling site except La Tour de Salvagny, Gardouch, and Les Bordes (Table S1). In La Tour de Salvagny, we had to withdraw the observations at the original site (La Tour de Salvagny A) in September 2016 because the site became no longer accessible. In April 2017, we continued our observations at a nearby site, approximately 2 km apart (La Tour de Salvagny B). In Gardouch, the activity of questing nymphs was observed both inside and outside the enclosed area of an experimental station on roe deer (*Capreolus capreolus*), referred to as Gardouch Inside and Gardouch Outside, respectively. The estimated population density of roe deer in Gardouch Inside (mean 50 individuals per 100 ha) was higher than Gardouch Outside (estimated by experts as less than 20 individuals per 100 ha). Furthermore, three sampling sites in Les Bordes, approximately 1.2 km apart, were referred to as Les Bordes A, B, and C, respectively. Due to their geographical proximity, the meteorological/climatic factors of different sampling sites from the same observatories were considered identical in further analysis.

Field observation campaigns were planned and carried out by local investigators who had been trained on the sampling protocol. The locations of forests, sampling sites, and passages were chosen where their biotopes are known to be suitable for *I. ricinus* tick populations around each observatory at the time the field observation campaigns started²⁷. Besides, the observations were never carried out during the daytime when the weather was highly unfavourable to questing ticks, *e.g.*, heavy rain, snow, or snow cover.

2.2. Sampling protocol for questing *Ixodes ricinus* nymphs

Activity of questing *I. ricinus* nymphs was observed by a cloth-dragging sampling technique²⁸. Within a 1-km radius, a 1 m x 1 m white cloth was dragged over 10 observation units of 10 m short-grass vegetative forest floors, called transects. For each transect, a repeated removal sampling design was used²⁴. The cloth-dragging sampling process was successively repeated three times per sampling. Nymphs found the white cloth were removed and collected in a vial for morphological identification²⁹ by the same acarologists at the corresponding laboratories. As a result, the questing nymph activity of each sampling site was monitored as a total number of confirmed *I. ricinus* nymphs collected from three repeated sampling on 10 transects, equivalent to a surface area of 100 m². This measure was considered as a proxy for tick abundance on the day of sampling. The same transects were repeatedly sampled throughout the study period at approximately 1-month intervals.

2.3. Environmental data

We tested 28 environmental variables to explain the observed *I. ricinus* nymph activity (Table 1). These variables could be categorized as: 1) Daytime duration and meteorological variables (time-dependent, 9 variables); 2) Land cover, topography, and bioclimatic variables (time-independent, 19 variables).

Table 1
Environmental variables (meteorological, land cover, topography, and bioclimatic variables) used to explain the abundance of *I. ricinus* ticks in regression analysis.

Type	Group	Variable	Description
Meteorological	Temperature	$T_M^{0:1}$	One-month average temperature; average temperature from 30 preceding days to the sampling day (°C)
		$T_M^{3:6}$	Previous season average temperature; average mean temperature of preceding 3 to 6 months (°C)
		$T_M^{0:6}$	Half-year average mean temperature; between 6 preceding months to the sampling day (°C)
		$T_M^{0:12}$	Average annual mean temperature; between 12 preceding months to the sampling day (°C)
	Relative humidity	$U_N^{0:1}$	One-month minimum relative humidity; average value from 30 preceding days to the sampling day (%)
		$U_N^{3:6}$	Previous season minimum relative humidity of preceding 3 to 6 months (%)
		$U_N^{0:6}$	Half-year average minimum relative humidity; between 6 preceding months to the sampling day (%)
		$U_N^{0:12}$	Average annual minimum relative humidity; between 12 preceding months to the sampling day (%)
	Daytime	<i>daytime</i>	Daytime duration (h)
	Land cover, topography, and bioclimate ^a	Topography	$mean_{elv}$
sd_{elv}			Standard deviation of elevation (m)
p_{flat}			Proportion of flat area; slope $\leq 2.5\%$
p_{north}			Proportion of non-flat area facing north; aspect $< 45^\circ$ or $\geq 315^\circ$
p_{east}			Proportion of non-flat area facing east; $45^\circ \leq$ aspect $< 135^\circ$
p_{west}			Proportion of non-flat area facing west; $135^\circ \leq$ aspect $< 225^\circ$
p_{south}			Proportion of non-flat area facing south; $225^\circ \leq$ aspect $< 315^\circ$
<i>Catchment</i>			Catchment area
Bioclimate		$BIO1_{temp}$	Annual mean temperature (°C)
		$BIO2_{diur}$	Mean diurnal range (°C)
		$BIO5_{maxTemp}$	Maximum temperature of the hottest month (°C)
		$BIO12_{prec}$	Annual precipitation (mm)
Land cover		H_{CLC1}	Shannon's diversity index for level-1 CLC types
		H_{CLC2}	Shannon's diversity index for level-2 CLC types
		H_{Forest}	Shannon's diversity index for forest types
		p_{Forest}	Percentage of forest-covering area (%)
		n_{Forest}	Number of forest patches
		ED_{Forest}	Forest edge density (m/km ²)
Soil		pH_{soil}	Soil pH

^a Land cover, topography, and bioclimatic variables were transformed by the principal component analysis as coordinates of PCA dimensions before being used in the regression analysis.

2.3.1. Daytime duration and meteorological variables

Daytime duration (*daytime*) from January 2013 to June 2021 at each sampling site was obtained from the corresponding latitude using *geosphere* package³⁰. Hourly meteorological data (2-m temperature and relative humidity) were recorded locally at each forest. Subsequently, daily mean, minimum, and

maximum values of temperature (T_M , T_N and T_X ; in °C) and relative humidity (U_M , U_N and U_X ; in %) were derived from these hourly records.

Missing values found on these local daily-level variables were imputed by the *random forest* algorithm in *mice* package³¹. External daily meteorological data, *i.e.*, daily average temperature and relative humidity, derived from neighbouring weather stations (Météo-France or INRAE), as well as month and year information, were used as auxiliary variables (Table S2). As a result, the imputation process creates a total of 500 iterated values for each variable. The median values of 500 imputations were used to replace the missing values.

The imputed daily meteorological data were subsequently used to calculate the averaged values in different lagged time intervals for further analysis, called interval-average variables¹⁴. The interval-average variables were generated to reduce the uncertainty that might arise during the imputation process and to capture the cumulative effects of the meteorological variables, which were mean temperature T_M and minimum relative humidity U_N . The mean temperature was selected to represent the effects of overall temperature on the tick population, whereas the minimum relative humidity was chosen to represent the effects of desiccation stress. The interval-average variables were defined as the average values of a meteorological variable $M \in \{T_M, U_N\}$ during a period between t_1 to t_2 month(s) before the sampling, denoted as $M^{t_1:t_2}$, where one month consists of 28 days. For instance, $U_N^{0:6}$ represents an average of the minimum daily relative humidity between 0 to 6 months (0 to 168 days) before the day of sampling.

Here, we hypothesized that interval-average meteorological conditions influence the dynamics of observed nymph activity at different time lags in different manners. Short-term lags may have an impact on immediate responses, such as the probability of questing. At the same time, long-term lags may influence the dynamics of nymph abundance, which is associated with development and survival rates. Therefore, we explored the impact of each meteorological variable at following time lags on the observed nymphs activity in subsequent regression analysis: 1) One-month average condition, $M^{0:1}$; 2) Previous-season average condition, $M^{3:6}$; 3) Half-year average condition, $M^{0:6}$; 4) Annual average condition, $M^{0:12}$.

In addition to the interval-average variables, monthly and seasonal average values of mean temperature and minimum relative humidity during the observation period were also calculated to describe the characteristics of meteorological conditions of each sampling site.

2.3.2. Land cover, topography, and bioclimatic variables

We obtained land cover, topography, and bioclimatic data from a 1-km radius buffer area around the center of each sampling site to capture habitat characteristics across all 10 transects. All the variables were handled and obtained by using QGIS version 3.8.0³². The digital elevation model (DEM) data derived from the Shuttle Radar Topography Mission (SRTM) database³³ was used to describe the topographic features of sampling sites, which included the mean ($mean_{elv}$) and standard deviation (sd_{elv}) of the elevation (in m above sea level), the proportion of flat area (p_{flat} ; defined by the slope $\leq 2.5\%$ ³⁴), the proportion of area facing north (p_{north}), east (p_{east}), west (p_{west}), and south (p_{south}), and the catchment area ($catchment$) as a proxy variable for moisture. Bioclimatic variables for each site (historical average conditions during 1970 to 2000) were derived from the WorldClim database³⁵, including the annual mean temperature ($BIO1_{Temp}$; in °C), the mean diurnal range ($BIO2_{Diur}$; in °C), the maximum temperature of the warmest month ($BIO5_{maxTemp}$; in °C), and the annual precipitation ($BIO12_{Prec}$; in mm). The land cover features of each sampling site were described using the CORINE Land Cover (CLC) 2018³⁶, while the characteristics of forests were explained by the BD forêt version 2 data³⁷. The forest fragmentation was characterized by the percentage of forest-covering area (p_{Forest}), the forest edge density (ED_{Forest} ; in m/km²), and the number of forest patches (n_{Forest}). While the diversities of the land cover types (level-1 and level-2 CLC) and the forest types were calculated by using the Shannon's diversity index³⁸ (H) as $H = -\sum_{i=1}^S p_i \ln p_i$ where S is the total number of land cover/forest types and p_i is the proportion of land cover/forest type i within the 1-km radius buffer area. The Shannon's diversity index for level-1 CLC, level-2 CLC, and forest types were denoted as H_{CLC1} , H_{CLC2} , and H_{Forest} respectively. Finally, the soil pH data (pH_{soil}) was retrieved from the European Soil Data Centre (ESDC) database³⁹.

2.4. Statistical analysis

All the statistical analyses were carried out using the programming language R version 3.6.0⁴⁰. The phenological pattern of nymph activity of each site was assessed using normalized monthly median nymph counts, where 0 and 1 indicated minimum and maximum values, respectively. Subsequently, the phenological patterns were descriptively classified using the following criteria: 1) The season which the peaks of activity arrive; 2) Evidence of reduced activity during winter (November to January); 3) The number of activity waves in a year, whether the pattern is unimodal or bimodal. Further, the directional changes in the maximum nymph counts were tested using a Spearman's rank correlation coefficient, a p -value < 0.05 was considered significant.

The effects of environmental variables (meteorological, land cover, topographical and bioclimatic variables) on the abundance of *I. ricinus* nymphs, were evaluated by a multivariate mixed-effects negative binomial regression, using the *glmmTMB* package⁴¹. Recalled that the negative binomial distribution is a generalized form of the Poisson distribution with an additional shape parameter θ that allows the variance to be independent to the mean μ . The number of *I. ricinus* nymphs per 100 m² was considered the response variable, and the sampling site served as the random effects for intercepts as displayed in (1):

$$\begin{array}{l} N_i \sim \text{NegBinom}(\mu_i, \theta) \quad (1) \\ \ln \mu_i = \alpha_0 + \alpha_i + X_i \beta \end{array}$$

Where N_i denoted nymph counts per 100 m² of site i , μ_i and θ represented mean of site i and the shape parameter of the negative binomial distribution, respectively. α_0 was an intercept of the regression model, while α_i was the random intercept of site i . Finally, X_i and β denoted a covariate vector of site i and a vector of regression coefficients for fixed effects, respectively.

The multicollinearity among all environmental variables was evaluated using Pearson's correlation coefficient r . Highly correlated variables, with $|r| \geq 0.6$, were not included in the same model. The principal component analysis (PCA) was used to reduce the dimensions of multiple highly correlated Land cover, topography, and bioclimatic variables, using *FactoMineR*⁴² and *factoextra*⁴³ packages. To reduce the number of variables considered in the regression analysis, the coordinates of individual sampling sites on the PCA dimensions were subsequently explored as explanatory variables. Non-linearity and interaction effects of the fixed effects were also investigated.

Multivariable models were explored by both forward and backward selection of significant variables decreasing the Akaike information criterion (AIC). Variations of the data explained by fixed effects were monitored by a pseudo- R^2 statistic using the *MuMIn* package⁴⁴. Residuals of the models were evaluated using the *DHARMA* package^{45,46}. The performance of the best-fitted model was assessed by a resampling method. Briefly, 50% of the original dataset was randomly sampled to fit with the best model for 500 iterations, referred to as resampling models. The distributions of regression coefficients across 500 resampling models were compared against those of the model with the original dataset, called the complete model. Additionally, the coverage probability (CP) of each explanatory variable, defined as the percentage of resampling models that produced regression coefficients that were significantly different from zero ($p < 0.05$), were also calculated. The CP index is a measure of how robust each variable is to explain observed data. Finally, the effects of each environmental variable were predicted using the *effects* package⁴⁷.

3. Results

3.1. Characteristics of sampling sites

During 2014 to 2021, the activity of questing *I. ricinus* nymphs was observed from 11 sample sites at 7 tick observatories in France. The characteristics of each sampling site, *i.e.*, meteorological, land cover, topography, and bioclimatic features, appeared to be highly correlated (Figure S1). According to the meteorological conditions (Figures 2, S2, S3A, and S3B), the characteristics of tick observatories can be classified as: 1) cold average temperature with the average winter temperature $< 2.5^\circ\text{C}$ (Saint-Genès-Champanelle and Velaine-en-Haye); 2) warm average temperature with average winter temperature $\sim 7^\circ\text{C}$ and high summer humidity with average minimum relative humidity in summer $> 60\%$ (Gardouch and Carquefou); 3) intermediate average temperature with average winter temperature between 4 to 6°C and low summer humidity with average minimum relative humidity in summer $< 55\%$ (La Tour de Salvagny, and Etiolles).

The land cover, topography, and bioclimatic characteristics of each sampling site were described in Tables S3 to S5, respectively. The first two dimensions of the principal component analysis (PCA) explained 70.3% of the variation in the land cover, topography, and bioclimatic variables (Figures 3A and S4). The first dimension differentiated each sampling site predominantly based on forest and land cover characteristics, in which the first 4 contributions were the number of forest patches n_{Forest} , percentage of forest-covering area p_{Forest} , Shannon's diversity index for forest types H_{Forest} , and Shannon's diversity index for level-2 CORINE Land Cover (CLC) types H_{CLC2} , respectively (Figures 3A and S4). The Shannon's diversity index for forest types H_{Forest} was found to be negatively correlated with the Shannon's diversity index for the CLC types, H_{CLC1} and H_{CLC2} , (Figure S1). The sampling sites in La Tour de Salvagny A, La Tour de Salvagny B, Gardouch Inside and External were relatively high fragmented forests, less percentage of forest-covering areas, more diverse in the CORINE Land Cover types, and less diverse in the forest types. While the sampling sites in Saint-Genès-Champanelle, Les Bordes and Etiolles were covered by a high percentage of forest areas with less forest fragmentation and high diversity in forest types.

The second PCA dimension of was driven by topographic and bioclimatic features, such as mean $mean_{eIV}$ and standard deviation sd_{eIV} of elevation, proportion of non-flat area facing north p_{north} , annual precipitation $BIO12_{prec}$ and annual mean temperature $BIO1_{Temp}$ (Figures 3A and S4). The sampling site in Saint-Genès-Champanelle was located at a high uneven elevation above the sea level (mountainous) with higher annual precipitation and lower annual temperature compared to all other sampling sites. While sampling sites of Les Bordes and Etiolles were located at flat areas with the lowest annual precipitation. The coordinates for each sampling site on the first and second dimensions on the PCA plot (Figure 3B) were used to describe land cover, topography, and bioclimatic characteristics in subsequent regression analysis, denoted as *Dim1* and *Dim2*, respectively. In addition, soil in the forest of Les Bordes appeared to be acidic, with an estimated pH of 4 (Table S3).

3.2. Activity of *Ixodes ricinus* nymphs

During the observation period, 631 campaigns were carried out, yielding a total of 22,912 *I. ricinus* nymphs (Table S1). The baseline level of nymph counts was highest in Gardouch Inside, La Tour de Salvagny B, and Carquefou, while it was the lowest in Saint-Genès-Champanelle, La Tour de Salvagny A, and Les Bordes A to C (Figures 4 and S3C). The normalized nymph counts revealed three phenological patterns of questing nymph activity observed among the 11 sampling sites (Figures 5 and S3D): 1) Pattern 1, a unimodal pattern with a relatively long peak activity beginning in late spring, reaching its maximum in summer and fading out in autumn, with no or very low activity in winter (Saint-Genès-Champanelle, Velaine-en-Haye, and La Tour de Salvagny B); 2) Pattern 2: a bimodal pattern with an earlier nymph activity reaching its peak in spring (the first peak), fading out in summer, reappearing in autumn (the second peak), and fading out again in winter (Les Bordes A to C, La Tour de Salvagny A, and Etiolles); 3) Pattern C: a unimodal pattern with moderate nymph activity throughout autumn and winter, reaching its peak earlier in spring, and fading out in summer (Carquefou, Gardouch Inside, and Gardouch Outside). Interestingly, questing nymphs have also been observed during winter in most sites. In addition, a decreasing trend in annual maximum nymph counts was observed more evidently in Gardouch Outside (Spearman's rank correlation coefficient = -0.8982; p -value < 0.05), while no apparent directional change was observed in other sites (Figures 4 and S5).

3.3. Environmental impacts on nymphs activity

Several mixed-effects negative binomial regression models were evaluated to explain impacts of environmental factors on observed *I. ricinus* nymph counts. The best-fitted model was selected by the AIC value, while other model characteristics were compared by dispersion parameter, variance of the random

intercepts, and pseudo-R² statistics (Table 2). Fixed effects in Model 5, predominantly meteorological variables, explained 65.1% of the total variance of nymph counts.

Table 2

The Akaike information criterion (AIC), dispersion parameter of the negative binomial regression, and the variance of the intercepts when sampling site

Model	Description	AIC
0	Null	5447
1	$(T_M^{0:1})^3 + (T_M^{0:1})^2 + T_M^{0:1}$	5272
2	$(T_M^{0:1})^3 + (T_M^{0:1})^2 + T_M^{0:1} * BIO1_{temp}$	5256
3	$(T_M^{0:1})^3 + (T_M^{0:1})^2 + T_M^{0:1} * BIO1_{temp} + (T_M^{3:6})^3 + (T_M^{3:6})^2 + T_M^{3:6}$	4858
4	$(T_M^{0:1})^3 + (T_M^{0:1})^2 + T_M^{0:1} * BIO1_{temp} + (T_M^{3:6})^3 + (T_M^{3:6})^2 + T_M^{3:6} + (U_N^{0:6})^3 + (U_N^{0:6})^2 + U_N^{0:6}$	4823
5	$(T_M^{0:1})^3 + (T_M^{0:1})^2 + T_M^{0:1} * BIO1_{temp} + (T_M^{3:6})^3 + (T_M^{3:6})^2 + T_M^{3:6} + (U_N^{0:6})^3 + (U_N^{0:6})^2 + U_N^{0:6} + (Dim1)^3 + (Dim1)^2 + Dim1$	4811

Asterisks indicated the interaction terms between 2 variables.

Model diagnostics showed no indication of lack of fit (Kolmogorov–Smirnov test: p -value = 0.2067), under- or over-dispersion in the residuals (p -value = 0.08), or residual outliers (p -value = 1) (Figure S6). The strength of the best-fitted model was assessed by resampling half of the data to fit with this model. The regression coefficients yielded from 500 resampling models were similar to those of the complete model with mostly high coverage probability (Figure S7 and Table S6).

The best-fitted model (Model 5) suggested that phenology of *I. ricinus* nymphs non-linearly responded to meteorological factors, such as one-month average temperature $T_M^{0:1}$, previous season temperature $T_M^{3:6}$, and half-year average minimum relative humidity $U_N^{0:6}$. The optimal condition for one-month average temperature $T_M^{0:1}$ on nymph counts varied depending on the annual average temperature BIO_{Temp} (Figure 6A). Nymph counts were expected to be higher when the previous season was cold (Figure 6B) and when the humidity was high during the past 6 months (Figure 6C). It should be noted that because the daytime duration was highly correlated with several meteorological variables, such as one-month average temperature, previous season temperature, and one-month average minimum relative humidity (Figure S1), it was excluded from the best-fitted model.

The Dimension-1 PCA coordinates Dim1, representing the forest and land cover characteristics of different sampling sites, markedly reduced the variance of random intercepts from 1.0004 in Model 4 to 0.4224 in Model 5 (Table 2). Thus, the forest and land cover characteristics in Model 5 gave a significant explanation of the baseline nymph count levels. The landscape that supports more baseline nymph counts was suggested to have a moderate percentage of forest-covering areas $\{p\}_{Forest}$, number of forest patches $\{n\}_{Forest}$, and Shannon's diversity index for level-1 ($\{H\}_{CLC1}$) and level-2 CORINE Land Cover types ($\{H\}_{CLC2}$) (Figure 6D).

Finally, the prediction from the best-fitted model replicated well the phenological patterns of observed *I. ricinus* nymphs (Figures 5 and S8), particularly sampling sites with more observations, *i.e.*, Saint-Genès-Champanelle, Velaine-en-Haye, Etiolles, Carquefou, Gardouch Inside, and Gardouch Outside. However, the autumn peaks of the phenological Pattern 2 (Figure 5) and the interannual variations of the nymph counts (Figure S8) remain imperfectly predicted by the model using meteorological variables.

4. Discussion

The variations in tick activity across space and time can be classified into 4 levels: 1) Baseline abundance variation; 2) Sub-daily variation; 3) Seasonal variation; 4) Inter-annual variation. The baseline abundance reflects the suitability/carrying capacity of the habitats for the populations, both land cover and climatic conditions¹⁸. The sub-daily variation arises from the immediate responses of questing ticks to the environment, such as temperature, relative humidity, or photoperiod⁴⁸, reflecting the instantaneous probability of questing. While seasonal and inter-annual variation reflects the long-term cumulative results of the questing behaviour as well as reproduction, development, and mortality rates on the tick population³. However, previous studies on the effects of the environment on *I. ricinus* nymph activities in Europe did not capture all the aforementioned levels of variation. They largely focused on either a short-term period for multiple sites^{19–22} or a long-term period for a few areas^{13–16}. The present study attempted to explore the baseline abundance, seasonal, and inter-annual variations of *I. ricinus* nymph activity by expanding the observation across a wide range of geographical/climatic areas over a long period, involving a total of 11 sampling sites from 7 tick observatories in France over 8 years. With the repeated removal sampling design, we controlled the uncertainty on the proxy measure of tick abundance (nymph counts per 100 m²) that arises from the variability of sampling rate across different sites and times^{24,49}.

Furthermore, all transects were covered with low vegetations, which were shown to maximize sampling rates compared to other types of vegetative floors²⁴, resulting in higher accuracy in estimating the proxy of tick abundance. To avoid the uncertainty caused by the variations of the environment within a sampling site, we repeated tick sampling from the same transects throughout the observation period. Furthermore, we strived to minimize the interference of tick

populations and their environment during the long-term observation period by using a one-month sampling interval. As all of the transects were open areas, ticks were free to relocate in and out of the transects, either directly or through hosts, and compensate for the monthly removals of tick populations. Nevertheless, the sub-daily variations in questing behaviour or immediate response to meteorological conditions among nymphs from different regions were not captured by this study design.

We observed the phenological patterns of nymph activity tended to follow the characteristics of meteorological conditions: 1) Pattern 1, a unimodal pattern with a broad summer peak and a winter pause in colder climates (Saint-Genès-Champanelle, and Velaine-en-Haye); 2) Pattern 2, a bimodal pattern with spring and autumn peaks in intermediate climates with dry summer (Les Bordes A to C, and Etiolles); 3) Pattern 3, a unimodal pattern with an early spring peak without a winter pause in warm and humid climates (Carquefou and Gardouch). However, despite the phenological patterns 1 and 3 being clearly distinct, we found that the existence of the autumn peaks in pattern 2 was ambiguous for the following reasons: 1) Most sites with phenological pattern 2 had a low baseline abundance level. One additional nymph count due to variability of the sampling could lead to a significant false signal for the autumn activity; 2) Compared to other patterns, tick activity at most sites with phenological pattern 2 was observed for a shorter period (a smaller sample size). A longer observation period is required to confirm the presence of bimodal patterns at these sites. Furthermore, we observed different phenological patterns at La Tour de Salvagny A and B, despite these two sites being approximately 2-km apart. The heterogeneity of the phenological patterns could have arisen from the following reasons: 1) The phenological pattern 2 observed from a short-term period at La Tour de Salvagny A was ambiguous, as previously discussed; 2) Different landscapes and host densities influenced tick population dynamics and apparent tick activities differently.

The overall phenology patterns observed in our study followed a climatic gradient previously reported on *I. ricinus* adults across northern Africa to eastern Europe, with a unimodal pattern with a summer peak and a winter pause in freezing climates (Karelia, Russia), a bimodal pattern with spring and autumn peaks in intermediate climates (European Russia's temperate zone), and a unimodal pattern with a late winter peak in warm climates (Algeria)¹⁷. Also, a similar phenology trend observed in one mountain region in Switzerland was reported to follow an elevation gradient where the spring-autumn bimodal phenology shifted to a late-spring unimodal pattern at a higher elevation, presumably due to a temperature gradient¹⁶. Therefore, it is conceivable to hypothesize that a gradually warming climate will result in the following phenological changes to *I. ricinus*: 1) At colder climates, the activity peaks could have shifted towards late-winter or early-spring; 2) At warmer and drier climates, the habitats would no longer support the physiological needs of *I. ricinus*. However, a spring-autumn bimodal phenology was also observed in a freezing climate in Finland⁵⁰, where the population genetics structure was completely different from that of *I. ricinus* in western Europe⁵¹. This suggested that *I. ricinus* from different climatic regions could have adapted/evolved to questing for a host at different temperature thresholds⁵² and responded differently to climate change. Furthermore, our findings indicated that describing phenological patterns solely based on their shape, such as unimodal or bimodal, was insufficient. Rather, the timing of the phenology peak and the winter traits should also be addressed. As a consequence, in order to better understand the relationship between climatic gradient and phenological patterns, we recommend longitudinal studies conducted over large geographic areas to report the phenological pattern of each sampling location separately. Moreover, determining the age of nymphs through fat content analysis has been demonstrated to guide formulating hypotheses about the mechanisms for the population dynamics of each phenological pattern¹⁶.

As the computation for cumulated nymph counts using areas under the density curve¹⁵ requires strictly regular sampling intervals throughout the study period, we used annual maximum nymph counts as a proxy to explore inter-annual variations in questing nymph activity. Directional changes in the annual maximum nymph counts were observed only in Gardouch Outside but not Gardouch Inside, despite being located in the same area. We hypothesized that it could have occurred as a result of 1) A possible decrease in roe deer population outside of the experimental station, while the number of roe deer was always maintained vastly high inside the experimental station; or 2) The periods of heatwaves that impacted this area in summer 2019 and summer 2020 affected the tick survival, while the high host density in Gardouch Inside compensated the surge in tick mortality. Furthermore, a recent study suggested that the inter-annual fluctuation of nymph abundance could be explained by the level of tree seed production, and thus the abundance level of small mammal hosts, in previous years⁵³. However, retrospective data on seed production were not available for all of our sampling sites during the study period, which precluded us from exploring this hypothesis in this study.

The regression analysis suggested that high nymph counts were non-linearly associated with different interval-average variables, including lower previous season temperature $\{T\}_{(M)}^{(3:6)}$, higher half-year average minimum relative humidity $\{U\}_{(N)}^{(0:6)}$, and a range of optimal one-month average temperature $\{T\}_{(M)}^{(0:1)}$. As the number of nymphs collected in each sampling was a product of total population size of nymphs at the time of sampling (true abundance), the proportion of nymphs questing for a host, and the sampling rates, we hypothesized that the meteorological conditions influenced each of these unobserved parameters at different time lags. The population size is determined by the cumulative effect of past meteorological conditions, either through mortality rate or larva-to-nymph developmental rate. While the proportion of questing nymphs tended to respond to short-term fluctuation of the weather conditions⁵⁴. A longitudinal observation on one site in Germany found similar effects of interval-average variables that explain the phenological pattern and inter-annual variation of *I. ricinus* activity¹⁴, which were current temperature $\{T\}_{(M)}^{(0:0)}$, four-to-six previous months average temperature $\{T\}_{(M)}^{(4:6)}$, and one-month average relative humidity $\{U\}_{(M)}^{(0:1)}$. Furthermore, the best-fitted model also showed a significant interaction between the one-month average temperature and a bioclimatic variable (average annual temperature $\{BIO\}_{(Temp)}$). This result indicated that although *I. ricinus* from colder climates start questing at lower temperature thresholds⁵², their activity arrives at its peak at higher temperatures (in summer; temperature ~ 14 to 16°C). While tick activity in warmer climates peaks at lower temperatures (in spring; temperature ~ 11 to 14°C). Presumably, the population size of active nymphs in cold climates is unlikely to reach its peak during a colder spring due to developmental or behavioural diapause. In addition, photoperiod has also been proposed to regulate both behavioural and developmental diapause of ixodid ticks⁵⁵; therefore, the exclusion of daytime duration from the best-fitted model, which was highly correlated with several meteorological variables, does not rule out the effects of photoperiod on tick activity dynamics.

The regression model also suggested that the baseline abundance level was associated with land cover characteristics. Sampling sites with a transition between moderately fragmented forest and non-forest areas, such as grassland or agricultural areas, support higher baseline nymph abundance (La Tour de

Salvagny B, Gardouch Inside, Gardouch Outside, and Carquefou; $\{p\}_{\text{Forest}} \sim 17$ to 52%). While landscapes with a high proportion of forest covers ($\{p\}_{\text{Forest}} > 90\%$) tended to have intermediate (Velaine-en-Haye and Etiolles) or low (Les Bordes A to C, and Saint-Genès-Champanelle) baseline abundance level. These findings are consistent with previous studies^{56–58}, indicating that forest fragmentation created transitional forest areas known as ecotones, which attract several mammal hosts²¹ and lead to a higher tick abundance. In addition, it has been suggested that forest fragmentation increases not only tick abundance but also the prevalence of pathogen-infected ticks⁶¹ and the risks of human-tick exposure⁶², which may lead to a higher incidence of tick-borne diseases. We also observed markedly low baseline *I. ricinus* abundance in the highly fragmented forest with a low proportion of forest-covering area (La Tour de Salvagny A; $\{p\}_{\text{Forest}} = 6.5\%$), possibly due to the lack of large foraging hosts in such a landscape⁶³. Furthermore, we found a remarkably low baseline abundance in all three sampling sites in Les Bordes, which could not be fully explained biologically by our available environmental factors. This could be explained by the unsuitable conditions of Les Bordes forest, where superimposed layers of clay and sand lead to a temporary perched water table in winter, and the low soil water storage capacity leads to arid soil conditions in the upper parts of the soil profile during summer months with low rainfall. Other additional factors could also contribute to explain the low tick abundance at Les Bordes site such as host abundance, competition, or predation of a forest with such high biodiversity.

Desirable model diagnostic results and repeated resampling models using half of the original data produced similar estimate values over 500 iterations, indicating that all explanatory variables in the best-fitted model are robust. Model predictions also successfully predicted baseline abundance, phenology patterns, and inter-annual variations of *I. ricinus* nymph activity. However, the prediction from the one-month average model still imperfectly captured the autumn peak in the phenology Pattern 2, where the sampling sites with this pattern had both fewer observations and lower abundance. This has brought us to the following inconclusive explanations: 1) The model failed to replicate the apparent autumn activity peak more clearly; 2) The apparent autumn peak was observed at random, and one additional nymph collected could generate a false signal when the sample size and baseline abundance were both small. As a result, it is envisioned that a more extended observation period and more sampling sites encompassing all phenological patterns should be implemented. Finally, although the regression analysis has been primarily used to find robust associations of environmental factors and help formulate ecological hypotheses on tick activity, it is considered a phenomenological model that does not fully explain the mechanisms of the observed phenomena³. In light of growing concerns about the impact of climate change on tick-borne diseases, particularly Lyme borreliosis, the mechanistic modelling framework for *I. ricinus* population dynamics is still required to investigate these ecological hypotheses.

5. Conclusions

The study design in this study allowed us to longitudinally monitor the questing *I. ricinus* nymph activity, which is comparable across multiple tick observatories. Two distinct phenological patterns were identified in colder climates (a unimodal pattern with a summer peak and complete winter pause) and warmer climates (a unimodal pattern with an early-spring peak without winter pause). While a longer observation period is still required to confirm the presence of a spring-autumn bimodal phenological pattern in intermediate climates. Furthermore, the regression analysis revealed that phenological patterns and inter-annual variations in questing activity were associated with meteorological variables at different lag times. Our results also supported the notion that moderate forest fragmentation promotes higher nymph abundance. Our findings can guide formulating ecological hypotheses about the effects of climate change on the distribution, abundance, and phenology of this tick species, which can then be assessed using a mechanistic population dynamics model. Finally, the phenological patterns of *I. ricinus* activity predicted by our model could potentially be employed to develop tick-borne disease prevention tools, such as developing a seasonally dynamic human-tick exposure risk map, which could help to reduce Lyme borreliosis cases in France.

Declarations

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Authors' contributions

Conceptualization and design of field observations: SB, KCM; Acquisition of data: PW, MJ, SB, AA, LB, AC, JFC, ND, TH, FH, NK, IL, AMd, AMr, SM, OP, TP, VP, MRM, MVT, HV, NC, GV, KCM; Statistical analysis and interpretation of data: PW, MJ, SB, KCM; Drafting the manuscript: PW, MJ, SB, GV, KCM; All authors revised and approved the final manuscript.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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Figures



Figure 1
The distribution of tick observatories according to the climatic region types of the continental France²⁶: 1) Etiolles (degraded oceanic); 2) Velaine-en-Haye (semi-continental); 3) Les Bordes (degraded oceanic); 4) Carquefou (oceanic); 5) La Tour de Salvagny (mixed); 6) Saint-Genès-Champanelle (mountain); 7) Gardouch (south-west basin).

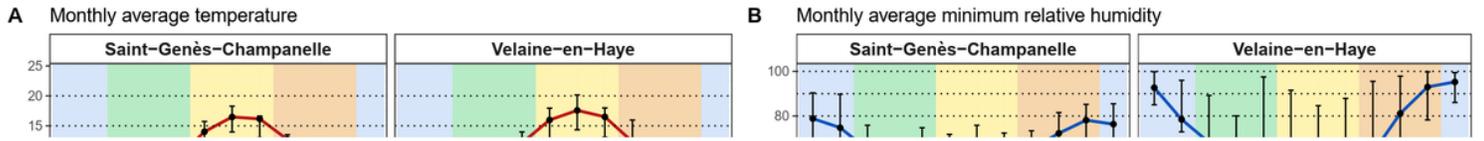


Figure 2

Monthly average meteorological conditions of 7 tick observatories: A) Average temperature ; B) Minimum relative humidity . The average values were calculated using the imputed data from January 2013 to June 2021, with the exception of Les Bordes, which was calculated from January 2017 to June 2021. The points represented the median of the monthly average values over the observed period, while the error bars represent the maximum and minimum monthly average values. Background colours indicated meteorological seasons of the northern hemisphere: Spring, 1st March to 31st May (green); Summer, 1st June to 31st August (yellow); Autumn, 1st September to 30th November (brown); Winter, 1st December to 28th February (blue).

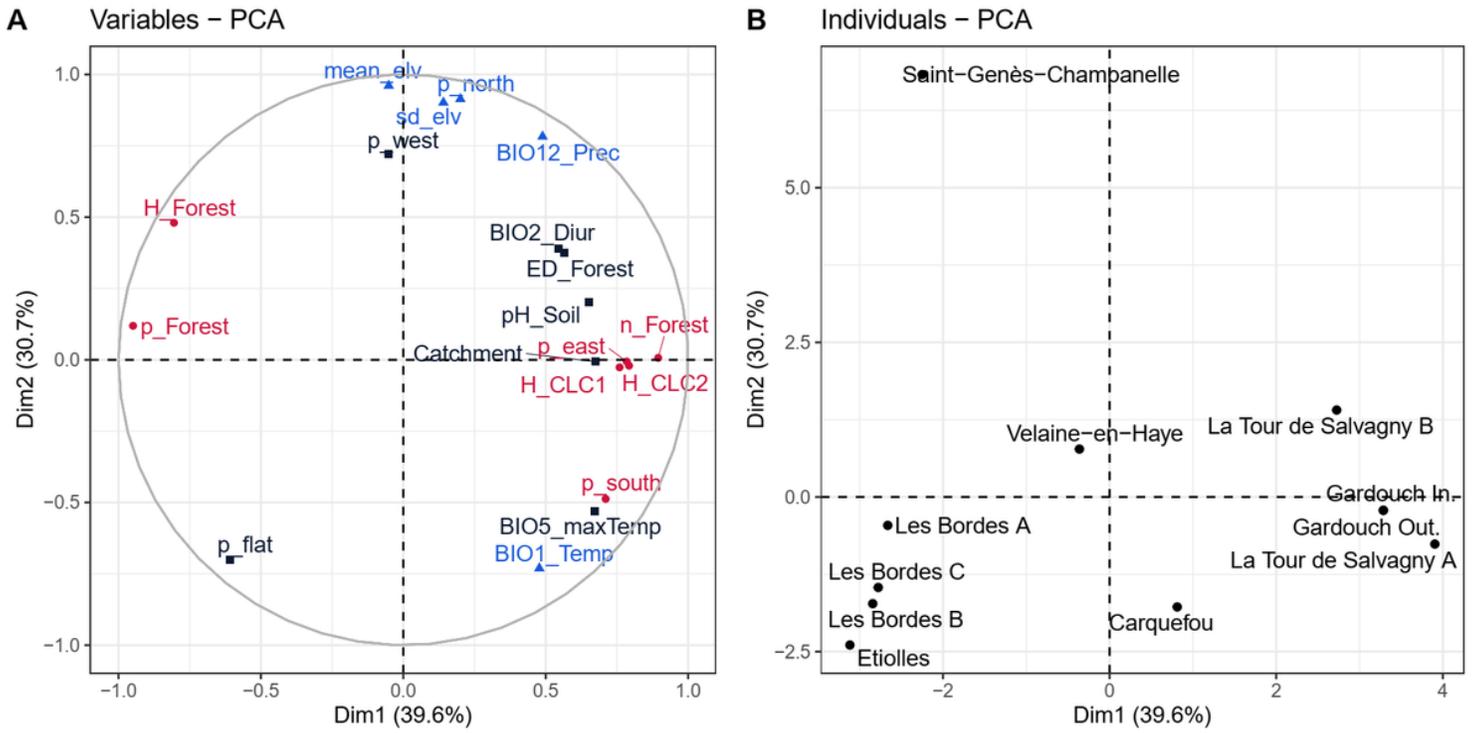


Figure 3
 Principal component analysis (PCA) for land cover, topography, and bioclimate characteristics of all sampling sites: A) PCA plot for land cover, topographical, and bioclimatic variables. Colours indicate whether the variable contributes more to Dimension 1 (red), Dimension 2 (blue), or other higher dimensions (black), using the cumulative contribution of ~60%; B) PCA plot for individual sampling sites. Coordinates of the individual PCA plot were subsequently used in the regression analysis.

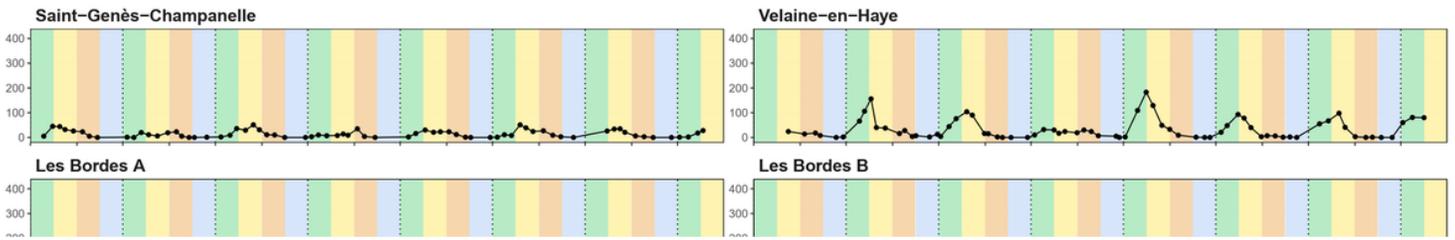


Figure 4

Number of *Ixodes ricinus* nymphs per 100 m² obtained from 11 sampling sites. Background colours indicate meteorological seasons of the northern hemisphere: Spring, 1st March to 31st May (green); Summer, 1st June to 31st August (yellow); Autumn, 1st September to 30th November (brown); Winter, 1st December to 28th February (blue). Vertical dashed lines indicate 1-year interval.

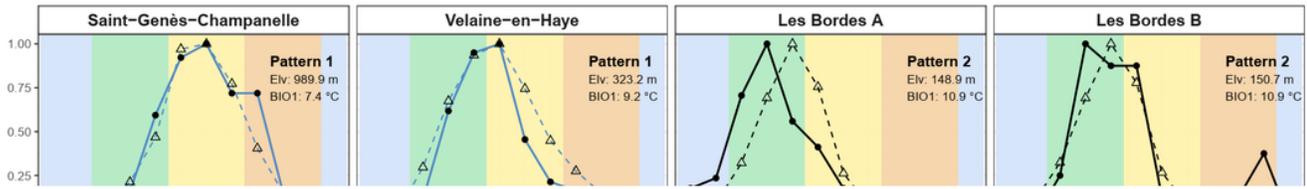


Figure 5

Observed and predicted normalized median nymph counts of each sampling site by month; the values of 0 and 1 indicate minimum and maximum normalized median nymph count, respectively. Line types distinguish between the observation (solid) and prediction from the best-fitted model (dashed). Line colours indicate different phenology patterns of the observed data: Pattern 1, a unimodal pattern with a summer peak and a winter pause (blue); Pattern 2, a bimodal pattern with a greater activity peak in spring and a smaller peak in autumn (black); Pattern 3, a unimodal pattern with a spring peak without winter pause (red). Text annotations demonstrate mean elevation above the sea level (m) and average annual temperature (°C). Numbers at the bottom represent the number of observations of each month throughout the study period. Background colours indicate meteorological seasons of the northern hemisphere: Spring, 1st March to 31st May (green); Summer, 1st June to 31st August (yellow); Autumn, 1st September to 30th November (brown); Winter, 1st December to 28th February (blue).

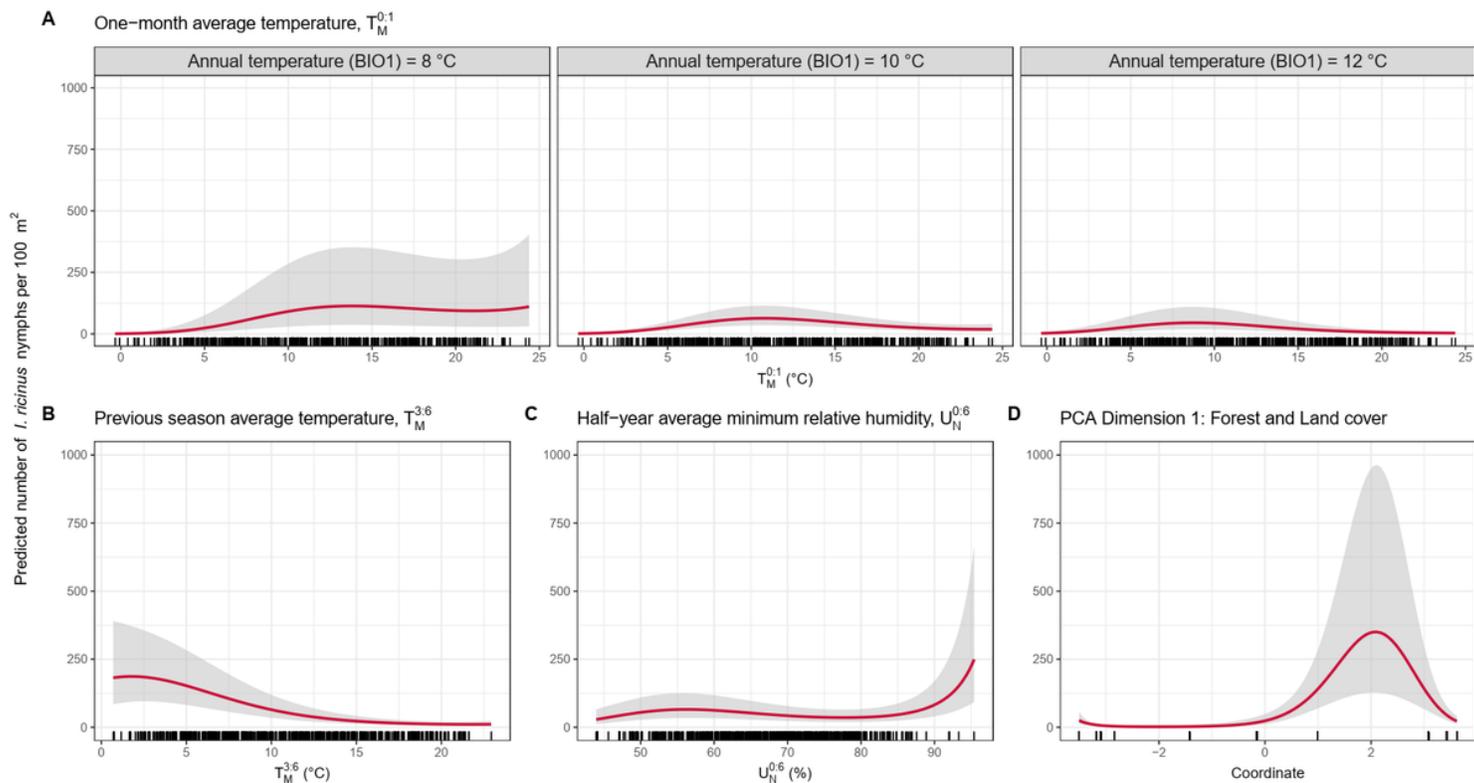


Figure 6

Predicted effects of environmental factors on the number of *I. ricinus* nymphs per 100 m² given the average values of all other variables: A) One-month average temperature, an averaged mean temperature between previous 30 days and sampling day ($T_M^{0.1}$) with an interaction effect with annual temperature ($T_M^{0.1}$); B) Previous season average temperature, an averaged mean temperature between 3 to 6 months prior to the sampling day ($T_M^{3.6}$); C) Half-year average minimum relative humidity, an averaged minimum relative humidity between previous 6 months and the sampling day ($U_N^{0.6}$); D) Coordinates on Dimension 1 of the principal component analysis ($Coordinate$), representing forest and land cover characteristics. Gray shaded areas indicate a 95% confidence interval. Rugs display the distribution of observed values.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryClimaticActivity211222.docx](#)