Title: **Relationships between landscape structure and the prevalence of two tick-borne infectious agents, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato, in small mammal communities**

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Landscape structure and prevalence of two tick-borne infections in small mammals

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Abstract

Context

By modifying ecosystems, land cover changes influence the emergence and the incidence of vector-borne diseases.

Objective

We aimed to identify the relationships between the prevalence of two tick-borne infectious agents, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l., in small mammal communities and the landscape structure.

Methods

Small mammals were sampled in 24 sites along a gradient of woodland fragmentation and hedgerow network density, and screened for infectious agents with rt-PCR techniques. Functional variables of wooded habitats connectivity based on graph theory and least cost path distances for the two dominant species, Bank voles (*Myodes glareolus*) and Wood mouse (*Apodemus sylvaticus*), as well as structural variables (composition and configuration) of the surrounding landscape at various scales (50-500 m) were computed for each site.

Results

The *A. phagocytophilum* prevalence increased with wooded habitats cover (50-500 m), which is explained by host population size, and increased also slightly with Bank vole abundance, which has a higher reservoir competence than Wood mouse. The *B. burgdorferi* s.l. prevalence only locally increased with wooded ecotones (50-100 m). Wooded habitats connectivity measures did not improve models built with simple land cover variables. A more marked spatial pattern was observed for the prevalence of *A. phagocytophilum* than *B. burgdorferi* s.l..

Conclusions

This study highlights the interest of considering together the life traits of infectious agents (e.g. host specificity) and the host species community ecology to better understand the influence of the landscape structure on the spatial distribution of vector-borne infectious agents.

Keywords

Tick-borne diseases; Small mammal community; Landscape connectivity; Graph theory; Least cost paths

Introduction

Landscape changes are suspected to cause infectious disease emergence or reemergence worldwide (Jones et al. 2008). Several authors depicted the complexity of the interactions between the landscape, hosts, vectors and humans, which drive the emergence of these diseases (reviewed in Lambin et al. 2010). The spread of infectious agent in ecosystems relies partly on their life history traits shaped by their evolutionary histories (e.g. transmission modalities). Thus, because species within communities exhibiting various levels of reservoir or vector competence, it relies on hosts and vectors diversity and abundance (Keesing et al. 2009).

Landscape heterogeneity (composition and configuration) and habitat connectivity, by filtering and regulating richness, abundance and dispersal of species in hosts and vectors communities, may influence the transmission rate and the diffusion of infectious agents, which ultimately determine their prevalence and distribution (Suzán et al. 2015). For instance, an increase in the proportion of non-reservoir host species may induce an increased number of missed transmissions from reservoir hosts, or an increased number of wasted bites by vectors for vector-borne diseases. This may reduce the number of transmission events which may have a so called “dilution effect” on prevalence (Ostfeld and Keesing 2000; Clay et al. 2009). Conversely, an increased number of competent host species may increase the number of transmission events or efficient bites by vectors (Roche et al. 2013). When less competent species are more prone to local extinction, this non-random species loss in host communities seems to be a driver of the increased direct and vector-borne infectious disease transmission (Ostfeld and LoGiudice 2003; Johnson et al. 2013; Roche et al. 2013).

Habitat connectivity partly shapes the host community structure. Large-bodied species, with small clutch/litter size and long gestation/incubation time are supposed to be more sensitive to habitat fragmentation because they experience higher local extinction risk. Conversely, small-bodied species, with large clutch/litter size and short incubation/gestation time can persist in fragmented habitat landscapes. These species need less habitat surface and benefit from a competition and predation release in these landscapes that are less favorable to predators and specialist species (Nupp and Swihart 1998, 2000; Gehring and Swihart 2003; Ferrante et al. 2017). As it happens, the latter kind of species also seem to be better reservoirs for infectious agents (Gottdenker et al. 2012; Huang et al. 2013; Ostfeld et al. 2014). Thus, in fragmented habitat landscapes, one could expect an amplification of infectious diseases supported by a higher density of highly competent hosts (Allan et al. 2003; Rubio et al. 2014). However, habitat connectivity can also indirectly influence the risk of local extinction of infectious agents by shaping hosts – and vectors – population size, as demonstrated on Hantavirus in Bank voles (Guivier et al. 2011). Thus, more studies explicitly assessing the relationships between land cover composition and configuration, landscape habitat connectivity, the communities of hosts, vectors, and infectious agents, and their interactions are needed for a better assessment of the effects of land use changes on infectious disease risk.

Ticks are obligate hematophagous arachnids vectors of infectious agents responsible of diseases of medical and veterinary importance (Jongejan and Uilenberg 2005). To complete their life cycle, hard ticks need one blood meal on a vertebrate host to molt from larva to nymph, and a second to molt from nymph to adult. Adult females need a third one to produce eggs fertilized by adult males. Tick-borne infectious agents can be acquired by ticks or transmitted to hosts at each blood meal. Such systems are interesting to study the influence of land cover and habitat connectivity on the distribution of infectious agents. As non-flying arthropods, tick displacements *per se* are reduced and their dispersal occurs while feeding on their hosts. Tick-borne infectious agents disperse either by the dissemination of ticks getting infected by feeding on infected hosts, or by the dispersal of already infected ticks while attached on hosts at the subsequent blood meal. Thus, their dispersal distance depends on the home range and competence of tick hosts (Kurtenbach et al. 2002b).

Small mammals are important reservoir hosts of many tick-borne infectious agent taxa: protozoan (e.g. *Babesia* sp., Hersh et al. 2012), bacteria (e.g. *Borrelia* sp., Gern et al. 1998; *Anaplasma* sp., Stuen et al. 2013) and viruses (e.g. tick-borne encephalitis virus, Mansfield et al. 2009). Small mammal communities composition is known to vary according to landscape features like habitat patch size, shape and isolation (Nupp and Swihart 2000; Michel et al. 2006) and small mammals displacements to be constrained by the landscape matrix (Szacki et al. 1993).

In this study, we searched for two infectious agents, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato (s.l.). The *A. phagocytophilum* bacteria ecotype associated to small mammals are apparently transmitted mostly by *Ixodes trianguliceps* and maybe other endophilic (i.e. burrow dwelling) tick species (Bown et al. 2009; Blaňarová et al. 2014; Jahfari et al. 2014). Infection in small mammals is short-lived, for about a couple of months post-infection (Bown et al. 2003). No transovarial transmission (i.e. from engorged females to their offspring) of these bacteria is known in *Ixodes* ticks (Stuen et al. 2013).

In Europe, several genospecies of the *B. burgdorferi* s.l. complex can be hosted by small mammals: *B. afzelli*, *B. bavariensis* (formerly *garinii* OspA serotype 4 strain), *B. bissetti*, *B. burgdorferi* sensu stricto (s.s.), and *B. spielmani* (Kurtenbach et al. 2002a, 2006; Margos et al. 2009; Coipan and Sprong 2016). However, host ranges of some of these genospecies include also larger mammals like hedgehogs (*Erinaceus europaeus*) and squirrels (*Sciurus vulgaris*) (Skuballa et al. 2012; Pisanu et al. 2014). The *B. burgdorferi* s.l. bacteria can be transmitted by several tick species, but the exophilic (i.e. questing for host on the vegetation) tick species *I. ricinus* is assumed to be its main vector in Europe (Rizzoli et al. 2011). Small mammals are major hosts for *I. ricinus* larvae and only occasional hosts for nymphs, but they also host endophilic tick species like *I. trianguliceps* and *I. acuminatus* at all life stages (Boyard et al. 2008; Bown et al. 2008; Hofmeester et al. 2016; Perez et al. 2017). Therefore, the maintenance of the zoonotic cycle of these bacteria may partly rely on other tick species (Hubbard et al. 1998; Heylen et al. 2013; Szekeres et al. 2015). Infections in small mammals are lifelong (Gern et al. 1994; Humair et al. 1999). Transovarial transmission is assumed to be null or rare (Rollend et al. 2013).

Here, we investigated at a local scale how landscape composition, configuration and connectivity directly, or *via* relationships with host species abundances, can explain the spatial variation of *A. phagocytophilum* and *B. burgdorferi* s.l. prevalence in small mammals. We specifically tested the three following hypothesis on the prevalence drivers:

(**H1**) Host population size: prevalence would be higher in the more connected wooded habitats patches, larger patches and/or patches with more surrounding wooded habitats, which ones support larger populations.

(**H2**) Overall small mammal community competence: the landscape would indirectly influence prevalence by acting on the relative abundances of species with different reservoir competence, modifying the overall community competence (leading to dilution or amplification).

(**H3**) Infectious agents specificity: a stronger relationship of prevalence with the landscape variables would be observed for *A. phagocytophilum*, for which the small mammal ecotype is specific, than for *B. burgdorferi* s.l., which show a wider range of host and of vector species.

Materials and Methods

Study area

The study took place in the ‘Zone Atelier Armorique’ (Brittany, France), a 132 km² Long-Term Ecological Research (LTER) area labeled by the CNRS (‘Centre National de la Recherche Scientifique’) and belonging to the International LTER network. The ‘Zone Atelier Armorique’ includes different landscapes with various land use, from deciduous mixed forest, to livestock-crop mixed farming landscapes, to cereal-oriented farming open landscapes. Compared to the livestock-crop mixed farming landscapes, the open landscapes have more crops (mainly maize and cereals) and fewer grasslands, larger patches (mean 2.3 ha compared to 1.3 ha), fewer and smaller woodlots, and a looser hedgerow network (Michel et al. 2007).

Sampling strategy

Wooded habitats support higher abundances of small mammals, better abiotic conditions for ticks (i.e. moisture) and are as such supposed to be key habitats for small mammals-ticks interactions (Boyard et al. 2008). For these reasons, 24 sampling sites were selected in wooded habitat patches in various contexts of land use and habitat connectivity (the exact locations are available in Perez et al. 2016). The 24 sampling sites at least 500 m apart from each other to ensure their spatial independence, were distributed as follows: 6 in the forest core; 6 in forest edge landscapes; 6 in the mixed farming landscapes; and 6 in the open farming landscapes. In each of the two agricultural landscape types, 3 sites were selected along hedgerows (6 sites overall) and 3 along woodlots edges (6 sites overall). At forest edges and in agricultural landscapes, the sampling sites (n = 18) were selected at the ecotone between wooded habitats and grasslands, as meadows are expected to be more favorable to ticks than more disturbed cultivated plots. Because small mammals start breeding in spring and populations peak generally in autumn, we sampled in May-June and in October in 2012 and 2013. These periods also correspond to the main and the secondary abundance peak of *I. ricinus* nymphs, respectively, and conversely for *I. trianguliceps* adult females, while the nymphs of this latter species are active mainly through summer and until October (Randolph 1975a).

Small mammal sampling and ethic statement

Small mammals were trapped using French model (INRA) live traps with wooden dormitory boxes. These traps can catch small mammals from 4 to 40 grams, including shrews and small rodents. Trap lines (100 m long) were constituted of 34 traps (3 m one apart from each other) baited with a mix of commercial seeds for pet rodents (sunflower, wheat, etc.), dry cat food, and a piece of apple for water supply. Traps were checked at the morning after 24 and 48 hours. Animals were brought to the field lab to be identified at the species level, euthanized by pentobarbital injection, weighted, sexed and dissected.

Traps were designed to limit as much as possible the stress or injury of the animals. The bait was designed to obtain an optimal catch rate across small mammal species and a good survival of individuals. The targeted animals were not protected species and thus no special authorization was needed, according to the French law in force. All individuals were euthanized by authorized experimenters according to current French law and to the European guidelines on the use of animals in science (Close et al. 1997).

Molecular detection of infectious agents

DNA was extracted from small mammal ear and spleen samples with Macherey-Nagel NucleoSpin Tissue kits (Chastagner et al. 2016). The screening for *A. phagocytophilum* was performed on DNA extracts from small mammal spleens by real-time PCR targeting the *msp2* genes according to the protocol of Courtney et al. (2004). The screening for *B. burgdorferi* s.l. was performed on DNA extracts from small mammal ears by real-time PCR in SybrGreen according to the protocol of Halos et al. (2010). The *B. burgdorferi* s.l. prevalence was low and, for technical reasons, all geno-species (genetically discriminated *Borrelia* species) could not be identified (data not shown). Thus, all the *B. burgdorferi* s.l. genospecies were combined hereafter. Although all geno-species which can infect small mammals do not share the same host range, we assumed that this simplification did not bias our results, because *B. afzelii* is known to be predominant in the studied species (Kurtenbach et al. 2002a; Marsot et al. 2013). In the present study, as temporal variation of prevalence has been studied elsewhere (Perez et al. 2017), we summed the data from all the sampling sessions for each site to consider only the spatial variations hereafter.

Landscape data

The used landscape data were: a land cover shape file of the year 2012 kindly provided by the 'LETG-COSTEL-Rennes' lab *via* the 'Zone Atelier Armorique'; a road network shape file provided by the 'Institut National de l'Information Géographique et Forestière' (IGN); and a 5 m-resolution raster land cover file of the 2010 year from Gil Tena et al. (2014) for woodland and hedgerows. The land covers were characterized as "woodland", "hedgerows" (abandoned lands and hedgerows extracted from the 5 m-resolution raster file), "grassland", "crops", "roads" (ranked according to the 'road importance index' of the 'IGN'), "urban areas" and "water areas". All files were aggregated into a single 5 m-resolution raster file that was used to create resistance maps and to compute metrics. This work was performed with ArcGIS 10.3.1 for Desktop, Esri ®.

Landscape functional connectivity

The dispersal of the two studied infectious agents is known to be mostly realized by larval ticks, which acquire them by feeding on infected adult small mammal hosts and falling off in the latter's home ranges. The dispersal of infectious agents by young small mammal hosts, which have not yet been highly exposed to ticks and thus are unlikely yet infected, can be considered as negligible (Randolph 1975b; Sinski et al. 2006; Kallio et al. 2014; Perez et al. 2017). Furthermore, despite *I. ricinus* nymphs can disperse bacteria on longer distances attached on other hosts with larger home ranges, small mammals are unlikely to be infected by *I. ricinus* adult females because they rarely host them. For these reasons, we selected for the functional connectivity analysis the spatial scale corresponding to the home range of adult small mammals.

Land cover cost files were built only for the two dominant rodent species with contrasted habitat preference: Wood mice and Bank voles (Boyard et al. 2008). Because these species move preferentially along linear structures or across the shortest path between suitable habitat patches (Zhang and Usher 1991), the functional distance between two habitat patches was set using least cost path distance. For "woodland", "grassland" and "crops", the resistance costs were assigned like the inverse of the relative species abundance from the literature (Ouin et al. 2000; Tattersall et al. 2002; Boyard et al. 2008; Vourc'h et al. 2008). A maximum resistance value of 100 was assigned arbitrary to "water areas" and "buildings". Roads are poor habitats for small mammals because they impede their displacements (Mader 1984; Rico et al. 2007). They also cause a traffic-related mortality (Ruiz-

Capillas et al. 2015). Eskilkdsen (2010) reported that finding bank voles were 15 times less likely on the opposite side of a barrier (forest path or road) than on the same side. Thus, a gradient of resistance indices from 15 (countryside and forest tracks) to 75 (expressways) was arbitrary assigned to “roads” according to the ‘road importance index’. Resistance coefficients for each land cover are summarized in **Table 1**.

The median distance travelled by an individual was modelled using negative exponential functions based on data from the same landscapes, Papillon et al (Papillon et al. 2002), and from a forest landscape of the Berkshire, United-Kingdom (Kikkawa 1964). In consistency with the lower mobility of the Bank vole compared to the Wood mouse, these distances were 100 m and 25 m, respectively (Zhang and Usher 1991). The nodes were defined as patches with an area of at least 0.025 ha, that corresponds to the minimum home range size of the studied species (Kikkawa 1964).

Graphs were created for each least cost path networks (i.e. for each species) and for different Euclidian distances (25 m, 50 m, 100 m, and 250 m), which represent an ‘isolation by distance’ only model. Nodes were adjacent woodland habitats patches (“woodland” and “hedgerows”). Connectivity measures were computed at the patch level for each graph. The measure was the difference in Probability of Connectivity (‘dPC’), which is based on a negative exponential function (Saura and Pascual-Hortal 2007). Because of the high number of considered nodes (3 819), dPC values were very small and thus were multiplied by 10^6. As this connectivity index is dependent on the patch surface itself (intra-patch connectivity; range: 0.025 – 212 ha), this variable was also used as a reference connectivity measure (i.e. no inter-patch displacement/dispersal). Because habitat patch areas and dPC values were over-dispersed, those variables were log-transformed hereafter. Least cost paths, graphs, and connectivity measures were computed using Graphab 1.2.3 (Foltête et al. 2012). An example of resulting graphs is shown in **Figure 1**.

Landscape composition and configuration

To evaluate the relationship between each landscape variable and the prevalence of infectious agents or small mammal species abundance at various spatial scale, they were computed in circular zones of different size around trap-line centres with Chloe2012 software (Boussard and Baudry 2014). The radius distances corresponded to twice the median Euclidian distances used for the ‘isolation by distance only’ model described above (i.e. 50 m, 100 m, 200 m, and 500 m). Because eighteen sites had similar local configuration (wooded habitat-grassland ecotones) and the first 50 m zones around each 100 m trap-line centre were not informative, we added this distance. The landscape composition variable ‘Wooded’ was computed as the proportion of “woodland”/“hedgerows” pixels. This variable is representative of the amount of permanent – wooded – habitat in the surrounding landscape (wooded habitats hereafter). the landscape configuration variable ‘Ecotones’ was computed as the proportion of ‘wooded habitat’ and “grassland”/“crops”/“roads” pixel couples. Such ecotones are known to be favorable for most small mammal species (Meunier et al. 1999; Ouin et al. 2000; Boyard et al. 2008). For an equal amount of permanent habitat, this latter variable is also an index of landscape fragmentation. A summary of all the landscape variables is shown in **Table 2**.

Data analyses

To determine whether prevalence in Wood mouse and Bank vole should be modelled separately, we first tested, for both infectious agents, the correlation between prevalence and the correlation between numbers of infected individuals of each of the two species. Because of the non-normal distribution of prevalence data (Shapiro-Wilk test: $p < 0.05$), we used Spearman's correlation tests.

To assess indirect effects of landscape structure on prevalence *via* the abundances of small mammal species, the relationship between these latter and landscape variables was tested. First, to determine whether Wood mouse and Bank vole abundances could be modelled separately, their correlation was tested through Pearson's correlation test. Then, to choose the most appropriate error distribution of species abundances, AICc values of the null models fitted with a normal error distribution and with a Poisson error distribution were compared. The normal error distribution had a better support (lower AICc-value) for Wood mouse abundance (AICc-normal = 180.2, AICc-Poisson = 232.0) and for Bank vole abundance (AICc-normal = 141.6, AICc-Poisson = 154.9). Species abundances were thus modelled using Linear Models (LMs). The set of explanatory landscape variables is summarized in **Table 2.** The variables were first selected in single explanatory variable models ($p < 0.1$). Then, multiple explanatory variable LMs were built with the selected variables. To avoid collinearity problems, we excluded models with correlated variables ($|r| > 0.5$). The models were then ranked based on the Akaike Information Criterion corrected for finite sample size (AICc; Hurvich and Tsai 1989), and the significance of the variables in the best models (ΔAICc < 2) was evaluated with type II ANOVAs ($p < 0.05$).

To assess whether tick-borne infectious agents were related to landscapes variables or species abundances, prevalence in small mammals of each infectious agent per site was modelled using binomial Generalized Linear Models (GLMs) with the same explanatory landscape variables as above, the Wood mouse abundance (N.As), Bank vole abundance (N.Mg), and the Bank vole/Wood mouse abundances ratio (ratio.Mg:As). Abundances were expressed as the total number of captured individuals per site. The variables were first selected in single explanatory variable models ($p < 0.1$). Then, multiple explanatory variable GLMs were built with the selected variables. The same AICc-based model ranking and variable significance evaluation as described above was performed on each variable set ($p < 0.1$). A selection and a variables significance evaluation was finally performed on multiple explanatory GLMs with the selected landscape and species abundances variables ($p < 0.05$).

All statistical analyses were performed using R software (R Development Core Team 2014). The R-package ‘car’ was used for ANOVAs, ‘MuMIn’ for model selections, and ‘psych’ for pairwise correlation tests between explanatory variables. The absence of autocorrelation in the dependent variables was checked using the ‘correlog’ function of the ‘ncf’ R-package.

Results

A total of 612 small mammals belonging to five species were caught during the two sampling years (see **Table 3** for detailed results; the whole data set is available in supplementary material). The Wood mouse (*Apodemus sylvaticus*) and the Bank vole (*Myodes glareolus*) were largely dominant (i.e. 74.2% and 24.3% of all animals caught, respectively) and found in all the 24 sites. We captured three other species at seven sites: the Field vole (*Microtus agrestis*; 3 sites, 4 individuals captured), the Common pine vole (*Microtus subterraneus*; 2 sites, 2 individuals captured) and the Crowned shrew (*Sorex coronatus*; 3 sites, 3 individuals captured). Four species (all but the Common pine vole) occurred only in one forest core site.

The Wood mouse abundance and the Bank vole abundance were not correlated ($p = 0.281$, $rho = 0.229$) and thus have been analysed separately hereafter. We found that the best model for the Wood mouse abundance included the proportion of ecotones in 500 m-radius zones ($R^2 = 0.290$, $p = 0.003$) with a positive relationship. The Bank vole abundance was not significantly related to any landscape variable ($p > 0.05$).

Results for A. phagocytophilum

The analyses of *A. phagocytophilum* prevalence were based on the PCR results for 452 wood mice, 147 bank voles, 4 field voles, 2 common pine voles, and 3 crowned shrews. Twenty wood mice, twenty bank voles and two crowned shrews were positive (**Table 3**). The number of positive wood mice and bank voles per site were significantly correlated ($p = 0.038$, $rho = 0.425$). However, the prevalence were not ($p = 0.177$, $rho = 0.291$). The results of the selection procedure of the GLMs of *A. phagocytophilum* prevalence as a function of the landscape variables and the abundances of the two dominant species are detailed in **Table 4**.

The most supported GLM of *A. phagocytophilum* prevalence in all small mammals included the proportion of wooded habitats in 50 m-radius zones, which displayed a significant positive relationship (**Figure 2a**). This model also included the Bank vole abundance, which also displayed a significant positive relationship with the prevalence (**Figure 2b**).

For wood mice separately, the most supported GLM of *A. phagocytophilum* prevalence included the proportion of wooded habitats in 50 m-radius zones, which displayed a significant positive relationship (**Figure 2c**). This model also included the Bank vole abundance, which also displayed a positively relationship, but not significantly (p = 0.073; **Figure 2d**). The ratio between the Bank vole abundance and the Wood mouse abundance was significantly positively related to the prevalence in wood mice in the univariate model (not shown).

Finally, the most supported GLM of *A. phagocytophilum* prevalence in bank voles included the proportion of wooded habitats in 50 m-radius zones, the proportion of ecotones in 200 m-radius zones, and the bank vole abundance. Those three variables displayed significant positive relationships with the prevalence (**Figure 2e**, **2f**, and **2g** respectively).

Results for B. burgdorferi *s.l.*

The analyses of *B. burgdorferi* s.l. prevalence were based on the PCR results for 450 wood mice, 147 bank voles, 4 field voles, 2 common pine voles, and 3 crowned shrews. Fifteen wood mice, ten bank voles and one common pine vole were positive (**Table 3**). The numbers of positive wood mice and bank voles per site was not significantly correlated neither was the prevalence (p > 0.05). The results of the selection procedure of the GLMs of *B. burgdorferi* s.l. prevalence as a function of the landscape and small mammal species abundances variables are detailed in **Table 5**.

For all small mammals, the most supported GLM of *B. burgdorferi* s.l. prevalence included the proportion of ecotones in 50 m-radius zones, which displayed a significant positive relationship (**Figure 3a**). No other variable was significant.

When considering *B. burgdorferi* s.l. prevalence in each species separately, no landscape variable displayed a significant relationship for wood mice. For bank voles, the most supported GLM of this prevalence included the proportion of ecotones in 100 m-radius zones (**Figure 3b**), which displayed a positive relationship, while the proportion of wooded habitats in 50 and 100 m-radius zones also displayed a significant relationship, but negatively. No species abundances variable was significantly related to any of the prevalence.

Discussion

The small mammal community at the woodland/hedgerows-grassland ecotones or in forest sites was dominated by two rodent species, the Wood mouse and the Bank vole. These two species responded differently to the landscape structure, and so are interesting to compare at this landscape scale. The Wood mouse abundance was significantly correlated to the proportion of ecotones at the largest scale considered (500 m), which is consistent with the propensity of this generalist species to use complementary various habitats (Tew and Macdonald 1994; Ouin et al. 2000). Conversely, no landscape variable was related to the Bank vole abundance. According to several studies (Szacki 1987;

Paillat and Butet 1996; Michel et al. 2007; van Apeldoorn et al. 2012), one could have expected a relationship between the woodland habitats connectivity and the abundance of this species, counterintuitively with an increased density in isolated patches, but not detected even with complex habitat connectivity measures.

Prevalence of Anaplasma phagocytophilum

The numbers of positive individuals as well as the prevalence rate in wood mice and bank voles were correlated, suggesting a common infection factor and/or an increased exposition of one species in the presence of the other. We observed a higher prevalence in bank voles and a positive, despite weak, relationship of the abundance of bank voles with the prevalence, in all small mammals and by species separately. Although not related to landscape variables, these results support an effect of relative host species abundances on prevalence (**H2**): Bank vole acts likely as an amplification host species for the transmission of these bacteria, while Wood mouse acts, comparatively, rather as a dilution host species (Rosso et al. 2017; Perez et al. 2017).

Several studies demonstrated that the prevalence of directly transmitted infectious agents decreased with host population size or isolation as a consequence of a higher local extinction risk (Begon et al. 2003; Guivier et al. 2011). Here, *A. phagocytophilum* prevalence, for all small mammals and by species separately, was positively related to the proportion of wooded habitats in the surrounding landscape. The more wooded landscapes can probably support larger and more connected populations of bank voles resulting in a lower local extinction probability in a given habitat patch (Paillat and Butet 1996). Consequently, it is expected a lower local extinction probability of their parasites (including tick species specialized on small mammals, like *I. trianguliceps* and *I. acuminatus*), and subsequently/or of the infectious agents they could transmit, like *A. phagocytophilum* (**H1**).

The relationship between the proportion of ecotones and prevalence was negative in single explanatory variable models but positive in multiple explanatory variables models. Actually, a low proportion of ecotones can either correspond to fragmented landscapes with few wooded habitats patches and to landscapes with a high proportion of wooded habitats where edges are scarce. This variable may thus not be sufficient *per se* to compare highly contrasted landscapes.

Prevalence of Borrelia burgdorferi *sensu lato*

The *B. burgdorferi* s.l. prevalence in small mammals, particularly bank voles, was positively related to the proportion of ecotones within 100 m. These results suggest an enhanced transmission of these bacteria at the interface between wooded and open (i.e. crops and grassland) habitats and therefore in fragmented landscapes where such ecotones are frequent. A possible explanation of increased *B. burgdorferi* s.l. prevalence in such landscapes is an increased density of *I. ricinus* nymphs resulting of an increased small mammal host density amplifying the transmission cycle (Hoch et al. 2010; Agoulon et al. 2012; Cayol et al. 2018). However, in a previous study, no relationship between the abundance of *I. ricinus* nymphs and *B. burgdorferi* s.l. prevalence was observed (Perez et al. 2017).

The absence of spatial pattern in *B. burgdorferi* s.l. prevalence in wood mice could be explained the mobility of this species and its ability to use crops and grassland. Several studies showed that wood mice, which are more prone to host *Ixodes* sp. larvae than bank voles, despite displaying lower bacterial load, may yield more nymphs infected by those bacteria than the latter species (Humair et al. 1993, 1999). It is thus possible that wood mice act as medium distance dispersers (at least up to 500 m) of infected *Ixodes* sp. larvae that become infective nymphs, as previously suggested in other studies (Boyard et al. 2008; Gassner et al. 2008). These results support a role of the specificity of infectious agents in their distribution patterns (**H3**).

For both infectious agents, the measures of wooded habitats connectivity did not improve the models compared to simple composition and configuration variables. In our study area, variation in the connectivity of these habitats was maybe not contrasted enough to be capture (Michel et al. 2007). Alternatively, the connectivity measures may not be at the appropriate scale for small mammal species, which are highly influenced by habitats quality and heterogeneity within each land cover (Michel et al. 2007), which should be accounted for in higher resolution models and/or habitat quality based landscape connectivity models (Mortelliti et al. 2010). It would also be interesting to account for other potential factors affecting small mammal communities and the prevalence of their tick-borne infectious agents, like predation pressure (Hofmeester et al. 2017).

Finally, species which were rare in the sampled habitats (i.e. Crowned shrew, Field vole and Common pine vole) can also host *A. phagocytophilum* and/or *B. burgdorferi* s.l. (Bown et al. 2008, 2009, 2011). Further studies including habitats where these species can be more abundant (e.g. fallow lands, fields' margins, clear cuts) and focusing on more hosts species, especially for *B. burgdorferi* s.l., would allow a better overall view of the studied infectious agents distribution and their spread in the landscape.

Conclusion

The increase in *A. phagocytophilum* prevalence can be substantially explained by an increasing proportion of wooded habitats in the surrounding landscape, at least up to 500 m, validating the host population size hypothesis (**H1**). Despite a weak positive relationship between bank vole abundance and *A. phagocytophilum* prevalence, no indirect effect of the landscape structure on this prevalence has been detected, questioning the overall small mammal community competence hypothesis (**H2**). Indeed, only the abundance of wood mice was significantly linked to our landscape variables, but at larger scales than the landscape variables related to the prevalence of the studied tick-borne infectious agents. The *B. burgdorferi* s.l. prevalence could not be explained by any landscape variables, excepted by the presence of ecotones at small scale (< 100 m). Thus, the prevalence of the two studied tick-borne infectious agents displayed contrasted spatial patterns, a difference which likely results of the wider ranges of hosts and vectors of *B. burgdorferi* s.l., as predicted by the infectious agent specificity hypothesis (**H3**). As a whole, our results demonstrate the interest of integrating complementary approaches such as landscape ecology and community ecology to the study of tick-borne diseases ecology to better understand their spatial distribution in the landscape in a risk prevention objective.

Competing interests

The authors declare no competing interests

Authors' contributions

GP, SB, AA, GV, OP and AB designed the study. GP, SB, AA, YR, OP and AB participated to the small mammal field sampling. AC performed most of the DNA extractions and the molecular analyses. YR managed the GIS data. GP performed all data analyses and drafted the manuscript. All authors read, commented and approved the manuscript.

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Tables and figures captions

Table 1: Resistance coefficients attributed to each land cover class for the Wood mouse and the Bank vole.

Land cover	Wood mouse friction coefficients	Bank vole friction coefficients
Forest, woodlots, copses, hedegerows and fallow lands	1	1
Grassland (meadows and grassy strips)	4	15
Crops	5	15
Unasphalted roads	15	15
Small roads	30	30
Medium roads	45	45
Major roads	60	60
Expressways	75	75
Buildings, dependencies, water bodies and streams (ponds and rivers)	100	100

Table 2: Landscape variables used in the study

Variable	Scale (m)[a]	Unit	Mean	Range (min; max)
Ratio of wooded habitats				
Wooded.50m	50	Ratio of pixels	0.511	0.053; 0.986
Wooded.100m	100	Ratio of pixels	0.487	0.039; 0.988
Wooded.200m	200	Ratio of pixels	0.453	0.042; 0.990
Wooded.500m	500	Ratio of pixels	0.420	0.034; 0.970
Ratio of ecotones				
Ecotones.50m	50	Ratio of pixel couples	0.048	0.021; 0.090
Ecotones.100m	100	Ratio of pixel couples	0.042	0.017; 0.075
Ecotones.200m	200	Ratio of pixel couples	0.034	0.014; 0.057
Ecotones.500m	500	Ratio of pixel couples	0.027	0.012; 0.047
Connectivity measures				
log(dPC.graph.Euclidian.25m)	25	none	6.17	-2.00; 12.73
log(dPC.graph.Euclidian.50m)	50	none	6.26	-2.08; 12.73
log(dPC.graph.Euclidian.100m)	100	none	6.35	-2.12; 12.71
log(dPC.graph.Euclidian.250m)	250	none	6.62	-1.82; 12.72
log(dPC.graph.least.cost.As.100m)[b]	100	none	6.52	-2.01; 12.79
log(dPC.graph.least.cost.Mg.25m)[c]	25	none	5.99	-3.20; 12.76
log(Area)	Patch	m²	11.15	6.48; 14.57

[a] Buffer zone radius (ratios) or median movement distance (connectivity).

[b] As: with the friction coefficients set for *Apodemus sylvaticus*.

[c] Mg: with the friction coefficients set for *Myodes glareolus*.

Table 3: Results of of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. PCR detection per sampling site over the two-year sampling (2012-2013).

Site	Landscape context[a]	Tested and positive individuals for *A. phagocytophilum* (*Aph*) and *B. burgdorferi* s.l. (*Bb* sl)[b]																	
		Asyl			Mgla			Magr			Msub			Scor			**Total**		
		Nbr[c]	*Aph*	*Bb* sl	Nbr[c]	*Aph*	*Bb* sl	Nbr[c]	*Aph*	*Bb* sl	Nbr[c]	*Aph*	*Bb* sl	Nbr[c]	*Aph*	*Bb* sl	**Nbr[c]**	***Aph***	***Bb* sl**
1	FC	11	*1*	*0*	2	*0*	*0*										**13**	***1***	***0***
2	FC	9	*0*	*0*	10	*1*	*0*										**19**	***1***	***0***
3	FC	9	*0*	*0*	2	*1*	*0*										**11**	***1***	***0***
4	FC	19	*2*	*1*	11	*9*	*0*	2	*0*	*0*				1	*1*	*0*	**33**	***12***	***1***
5	FC	19	*2*	*1*	10	*2*	*1*	1	*0*	*0*							**30**	***4***	***2***
6	FC	19[d]	*1*	*1*	1	*0*	*0*										**20**	***1***	***1***
7	FE	18	*2*	*0*	13	*1*	*0*	1	*0*	*0*							**32**	***3***	***0***
8	FE	32	*2*	*2*	3	*1*	*0*										**35**	***3***	***2***
9	FE	20	*0*	*0*	11	*1*	*1*										**31**	***1***	***1***
10	FE	18	*1*	*1*	1	*0*	*0*				1	*0*	*0*				**20**	***1***	***1***
11	FE	2	*0*	*0*	1[d]												**3**	***0***	***0***
12	FE	8[d]	*0*	*0*	2	*0*	*0*										**10**	***0***	***0***
13	ME	21	*2*	*0*	6[d]	*0*	*0*										**27**	***2***	***0***
14	ME	32	*3*	*2*	12	*4*	*1*										**44**	***7***	***3***
15	ME	24	*0*	*0*	7	*0*	*1*										**31**	***0***	***1***
16	MH	29	*2*	*1*	13	*0*	*2*										**42**	***2***	***3***
17	MH	21	*0*	*0*	6	*0*	*1*										**27**	***0***	***1***
18	MH	40[e]	*0*	*1*	4	*0*	*0*				1	*0*	*1*				**45**	***0***	***2***
19	OE	32	*0*	*1*	4	*0*	*0*										**36**	***0***	***1***
20	OE	9	*0*	*0*	5	*0*	*0*										**14**	***0***	***0***
21	OE	17	*0*	*2*	4	*0*	*1*										**21**	***0***	***3***
22	OH	16	*0*	*1*	6	*0*	*0*							1	*0*	*0*	**23**	***0***	***1***
23	OH	23	*0*	*1*	8	*0*	*2*										**31**	***0***	***3***
24	OH	6	*2*	*0*	7	*0*	*0*							1	*1*	*0*	**14**	***3***	***0***
Total	**24**	**454**	***20***	***15***	**149**	***20***	***10***	**4**	***0***	***0***	**2**	***0***	***1***	**3**	***2***	***0***	**612**	***42***	***26***
% of total		**74.2**	***3.3***	***2.5***	**24.3**	***3.3***	***1.6***	**0.65**	***0***	***0***	**0.33**	***0***	***0.16***	**0.49**	***0.3***	***0***	**100**	***6.91***	***4.29***
Prevalence (%)			***4.42***	***3.33***		***13.6***	***6.80***		***0***	***0***		***0***	***50***		***66.7***	***0***		***6.91***	***4.29***

[a] FC:forest core; FE: forest edge; ME: mixed farming at woodlot edge; MH: mixed farming along hedgerows; OE: open farming at woodlot edge; and OH: open farming along hedgerows.

[b] Asyl: *Apodemus sylvaticus*; Mgla: *Myodes glareolus*; Magr: *Microtus agrestis*; Msub: *M. subterraneus*; and Scor: *Sorex coronatus*.

[c] Total number of individuals over the two-year sampling.

[d] Including one individual not tested.

[e] Including two individuals not tested for *B. burgdorferi* s.l. only.

Table 4: The most supported binomial GLMs of *Anaplasma phagocytophilum* prevalence as a function of the landscape and species abundances variables according to the AICc-based selection procedure.

All small mammals

Model set	Rank	Variables and their significativity	AICc	ΔAICc	R^2
Null model			106.8		
Landscape	1	**Wooded.50m*****	86.4		0.331
Community	1	**N.Mg*****	97.5		0.170
	2	N.Mg° + Ratio.Mg:As	98.9	1.37	0.188
Community and landscape	1	**Wooded.50m*** + N.Mg****	79.0		0.478

Wood mice (*Apodemus sylvaticus*)

Model set	Rank	Variables and their significativity	AICc	ΔAICc	R^2
Null model			61.4		
Landscape	1	**Wooded.50m***	59.0		0.144
	2	Ecotones.100m°	60.3	1.89	0.105
	3	Wooded.100m°	60.3	1.90	0.104
	4	log(Area)°	60.6	2.17	0.096
	5	Wooded.200m°	60.8	2.37	0.090
Community	1	**Ratio.Mg:As***	59.1		0.141
	2	N.Mg°	60.5	1.40	0.099
Community and landscape	1	**Wooded.50m*** + N.Mg°	58.4		0.241
	2	Wooded.50m° + Ratio.Mg:As°	58.5	0.06	0.239
	5	log(Area) + Ratio.Mg:As*	59.5	1.07	0.227
	6	Wooded.100m + Ratio.Mg:As°	59.6	1.20	0.205
	7	log(Area)° + N.Mg°	59.8	1.36	0.200
	8	Wooded.100m° + N.Mg°	59.8	1.37	0.200
	9	Ecotones.100m° + N.Mg°	59.8	1.40	0.199
	10	Wooded.200m + Ratio.Mg:As°	60.1	1.68	0.190
	13	Wooded.200m° + N.Mg°	60.3	1.92	0.183

Bank voles (*Myodes glareolus*)

Model set	Rank	Variables and their significativity	AICc	ΔAICc	R^2
Null model			73.0		
Landscape	1	**Ecotones.200m* + Wooded.50m*****	52.1		0.477
	2	Ecotones.200m° + **Wooded.100m*****	52.9	0.87	0.461
	3	**Ecotones.200m* + Wooded.200m*****	52.9	0.89	0.461
	4	**Wooded.100m*****	54.0	1.94	0.393
Community	1	**N.Mg***	71.3		0.074
Community and landscape	1	**Ecotones.200m** + Wooded.50m*** + N.Mg***	48.9		0.588
	2	**Ecotones.100m* + Wooded.50m*** + N.Mg***	50.7	1.78	0.555

Only models with ΔAICc < 2 are shown. Because the connectivity variables are very strongly correlated to wooded habitat patch size and give similar results, only this latter variable is considered here. Significant codes are: "***" when $p \leq 0.001$; "**" when $0.001 < p \leq 0.01$; "*" when $0.01 < p \leq 0.05$; "°" when $0.05 < p \leq 0.1$; otherwise, $0.1 < p$. Significant variables at $p < 0.05$ are in bold.

Table 5: The most supported binomial GLMs of *Borrelia burgdorferi* s.l. prevalence as a function of the landscape and species abundances variables according to the AICc-based selection procedure.

All small mammals

Model set	Rank	Variables and their significativity	AICc	ΔAICc	R²
Null model			56.7		
Landscape	1	**Ecotones.50m***	55.2	0.00	0.201
	2	Ecotones.100m°	55.4	0.25	0.188
Community	None				

Wood mice (*Apodemus sylvaticus*)

Model set	Rank	Variables and their significativity	AICc	ΔAICc	R²
Null model			43.0		
Landscape	None				
Community	None				

Bank voles (*Myodes glareolus*)

Model set	Rank	Variables and their significativity	AICc	ΔAICc	R²
Null model			35.2		
Landscape	1	**Ecotones.100m***	33.3	0.00	0.252
	2	**Wooded.50m***	33.7	0.40	0.229
	3	Ecotones.50m°	34.0	0.67	0.213
	4	**Wooded.100m***	34.1	0.78	0.206
	5	Ecotones.100m + Wooded.50m	34.3	1.01	0.347
	6	log(Area)°	34.3	1.02	0.193
	7	Wooded.200m°	34.5	1.18	0.183
	8	Ecotones.100m + Wooded.100m	34.5	1.22	0.311
	9	Ecotones.100m + log(Area)	34.7	1.39	0.324
	10	Wooded.500m°	34.7	1.44	0.168
	11	Ecotones.50m + log(Area)	34.9	1.61	0.311
	12	Ecotones.50m + Wooded.50m	35.0	1.66	0.309
	13	Ecotones.100m + Wooded.200m	35.0	1.66	0.309
	14	Ecotones.100m + Wooded.500m	35.2	1.89	0.295
Community	None				

Only models with ΔAICc < 2 are shown. Because the connectivity variables are very strongly correlated to wooded habitat patch size and give similar results, only this latter variable was considered here. Significant codes are: "***" when $p \leq 0.001$; "**" when $0.001 < p \leq 0.01$; "*" when $0.01 < p \leq 0.05$; "°" when $0.05 < p \leq$; otherwise, $0.1 < p$. Significant variables at $p < 0.05$ are in bold.

Figure 1: Example of the habitats landscape connectivity analyses.

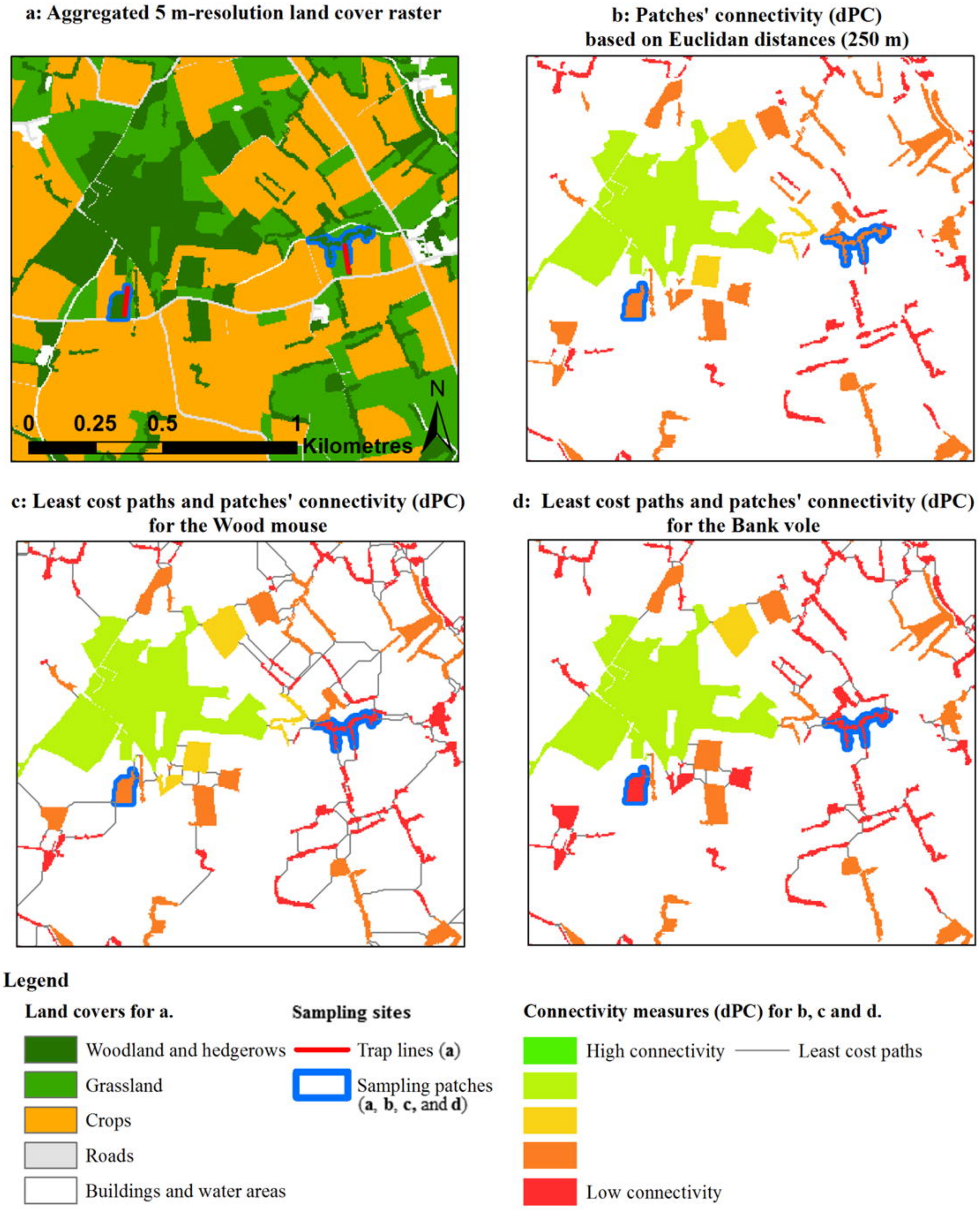
Final aggregated 5 m-resolution land cover raster file (**a**) and connectivity indices (dPC, for 'difference in Probability of Connectivity') computed on different graphs (**b**, **c**, and **d**). The sampling patch on the right (surrounded in blue) appears moderately connected according to Euclidian distances (**b**), while it is weakly connected when weighted by least cost paths (**c** and **d**) because it is surrounded by a river at north and roads otherwise (see **a**). The sampling patch on the left is moderately connected for the Wood mouse (**c**) while it is weakly connected for the Bank vole (**d**) because it is separated to other wooded habitats patches by large grassland or crops patches resulting in fewer connections in the graph built for this latter species. See **Materials and Methods** for more details.

Figure 2: The *Anaplasma phagocytophilum* prevalence of as a function of the landscape variables and species abundances variables in the most supported binomial Generalized Linear Models.

The *A. phagocytophilum* prevalence, expressed as the frequency of infected individuals per site, in all small mammals (**a** and **b**), in wood mice (**c** and **d**), and in bank voles (**e**, **f**, and **g**). Fitted binomial regression curves, according to the multiple explanatory variables GLMs, are shown with 95% confidence intervals (light grey). Prevalence is shown as a function of the proportion of wooded habitats in 50 m-radius zones (**a**, **c** and **e**), as a function of the Bank vole abundance (**b**, **d**, and **g**), and as a function of the proportion of ecotones in 200 m-radius zones (**f**).

Figure 3: The of *Borrelia burgdorferi* sensu lato prevalence as a function of the landscape variables in the most supported binomial Generalized Linear Models.

The *B. burgdorferi* s.l. prevalence, expressed as the frequency of infected individuals per site, in all small mammals (**a**), and in bank voles (**b**). Fitted binomial regression curves, according to single explanatory variable GLMs, are shown with 95% confidence intervals (light grey). Prevalence is shown as a function of the proportion of ecotones in 50 m-radius zones (**a**) and in 100 m-radius zones (**b**).



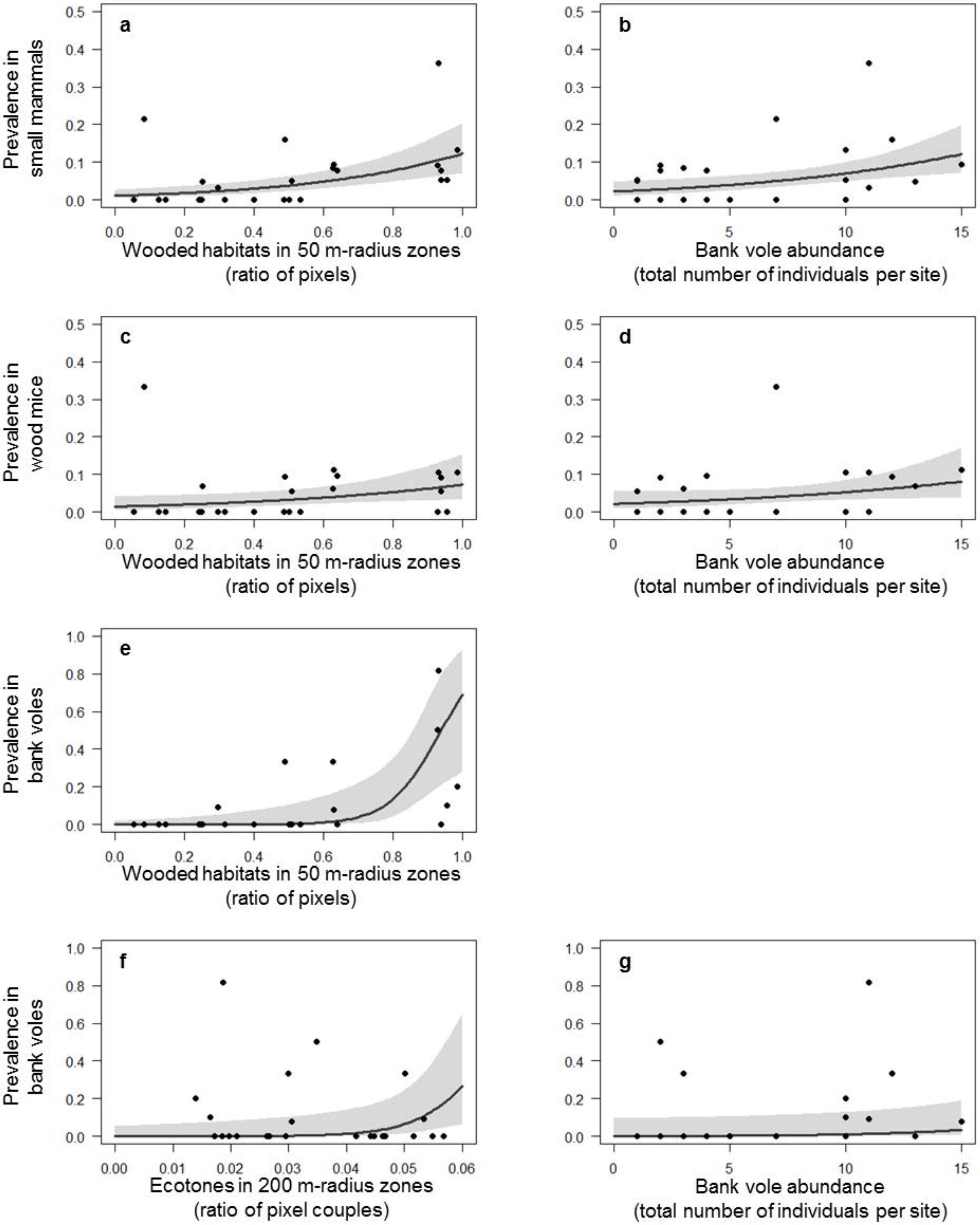
a: Aggregated 5 m-resolution land cover raster

b: Patches' connectivity (dPC) based on Euclidan distances (250 m)

c: Least cost paths and patches' connectivity (dPC) for the Wood mouse

d: Least cost paths and patches' connectivity (dPC) for the Bank vole

Figure 1



a
b
c
d
e
f
g
Prevalence in small mammals
Prevalence in wood mice
Prevalence in bank voles
Prevalence in bank voles
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.8
1.0
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0
5
10
15
Wooded habitats in 50 m-radius zones (ratio of pixels)
Bank vole abundance (total number of individuals per site)
Ecotones in 200 m-radius zones (ratio of pixel couples)

Figure 2

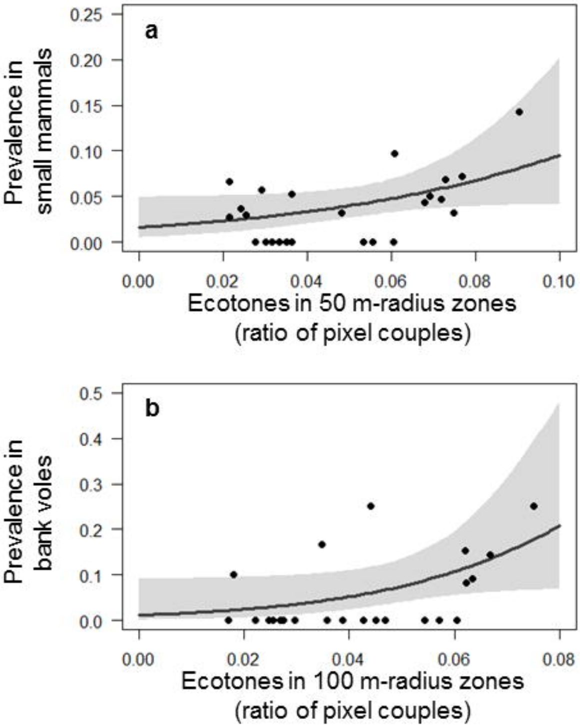

a
Prevalence in small mammals
0.25
0.20
0.15
0.10
0.05
0.00
0.00
0.02
0.04
0.06
0.08
0.10
Ecotones in 50 m-radius zones (ratio of pixel couples)
b
Prevalence in bank voles
0.5
0.4
0.3
0.2
0.1
0.0
0.00
0.02
0.04
0.06
0.08
Ecotones in 100 m-radius zones (ratio of pixel couples)

Figure 3