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Spatiotemporal ecological factors influencing the ecology of *Ixodes ricinus* ticks and the Lyme disease risk



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“No matter how much the wind howls, the mountain cannot bow to it.”

Walt Disney Company, Mulan

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1 Abstract

Ticks and tick-borne diseases represent a significant health problem for humans and their companion animals. Tick-borne pathogens cause morbidity and mortality in their vertebrate hosts (including humans). The incidence of Lyme borreliosis and other tick-borne diseases is increasing in Europe and North America. To predict the risk of tick-borne disease, it is critical to understand the ecological factors that determine the abundance of ticks and the density of infected ticks as this variable determines the risk of Lyme borreliosis to vertebrate hosts including humans. In Europe, the sheep tick (*Ixodes ricinus*) transmits a number of important diseases including Lyme borreliosis, which is caused by the bacterium *Borrelia burgdorferi* sensu lato (sl). In continental Europe, the nymphal stage of *I. ricinus* often has a bimodal phenology with a large spring peak and a smaller fall peak. There is consensus about the origin of the spring nymphal peak, but the origin of the fall nymphal peak remains unclear. Two alternative hypotheses predict different time lags (0 year versus 1 year) between the fall peak of the nymphal ticks and the blood meal of the previous stage (larval tick). The feeding success of the larval ticks depends on the density of their small mammal hosts, which in turn, depends on the seed production (masting) by deciduous trees. This PhD thesis aimed to determine the abiotic and biotic factors that influence the seasonal and inter-annual variation in the density of nymphs (DON) and density of infected nymphs (DIN) at a location in Switzerland where Lyme borreliosis is endemic.

Over a 15-year period (2004 to 2018), we monitored the monthly abundance of *I. ricinus* ticks (nymphs and adults) and their *B. burgdorferi* sl infection status at four different elevations on Chaumont Mountain in Neuchâtel, western Switzerland. We collected climate variables in the field and from nearby weather stations. We obtained data on beech tree seed production (masting) from the literature, as the abundance of *Ixodes* nymphs can increase dramatically two years after a masting event. We used generalized linear models and AIC-based model selection to identify the ecological factors that influence inter-annual variation in the DON, DIN and, the nymphal infection prevalence (NIP). We used generalized additive models and AIC-based model selection to model the complex bimodal seasonal phenology of *I. ricinus* nymphs and whether this approach yields additional insights into the factors that explain seasonal variation in tick abundance.

In chapter 1, we found that elevation, year, masting events two years prior, and relative humidity together explained 73.2% of the variation in our annual estimates of the DON.

According to the parameter estimates of our models, (i) the annual density of nymphs almost doubled over the 15-year study period, (ii) changing the beech tree seed production index from 1 to 5 increased the abundance of nymphs by 86.2% two years later, and (iii) increasing the field-collected relative humidity from 50.0% to 75.0% decreased the abundance of nymphs by 46.4% in the same year.

In chapter 2, we found that the inter-annual variation in the NIP decreased by 78% over the study period and was explained by the mean precipitation in the present year, and the duration that the DNA extraction was stored in the freezer prior to pathogen detection. The DIN decreased over the study period at all four elevation sites, but the decrease was only significant at the top elevation. Inter-annual variation in the DIN was best explained by elevation site, year, masting events two years prior, and the mean relative humidity in the present year.

In chapter 3, we found that the *I. ricinus* nymphs had a bimodal phenology at the three lower elevation sites, but a unimodal phenology at the top elevation site. We also found that the inter-annual variation in the densities of *I. ricinus* nymphs in the fall and spring were best explained by a 1-year versus a 2-year time lag with the beech tree masting index. The direct development hypothesis is the best explanation for the fall peak of *I. ricinus* nymphs at our study site. Our study provides new insight into the complex bimodal phenology of this important disease vector.

This PhD thesis demonstrated that masting events by deciduous trees is a critical driver of the seasonal and inter-annual abundance of *I. ricinus* ticks, and hence the risk of tick-borne disease. This work provides new information on the bimodal phenology of *I. ricinus* ticks, particularly that the direct development hypothesis is the best explanation for the spring and fall peaks of *I. ricinus* nymphs.

Keywords: Beech tree, *Borrelia burgdorferi*, climate, *Fagus sylvatica*, *Ixodes ricinus*, Lyme borreliosis, masting, phenology, tick-borne disease, tick population ecology

2 Résumé

Les tiques et les maladies transmises par les tiques représentent un problème de santé important pour l'homme et ses animaux de compagnie. Les agents pathogènes transmis par les tiques provoquent une morbidité et une mortalité chez leurs hôtes vertébrés (y compris les humains). L'incidence de la borréliose de Lyme et d'autres maladies transmises par les tiques est en augmentation en Europe et en Amérique du Nord. Afin de prédire le risque de maladies transmises par les tiques, il est essentiel de comprendre les facteurs écologiques qui déterminent l'abondance des tiques ainsi que la densité des tiques infectées, car cette variable détermine le risque de borréliose de Lyme pour les hôtes vertébrés, y compris les humains. En Europe, la tique du mouton (*Ixodes ricinus*) transmet un certain nombre de maladies importantes, dont la borréliose de Lyme, causée par la bactérie *Borrelia burgdorferi* sensu lato (sl). En Europe continentale, le stade nymphal de l'espèce de tiques *I. ricinus* a souvent une phénologie bimodale avec un pic printanier important et un pic automnal plus petit. Il existe un consensus sur l'origine du pic nymphal printanier, mais l'origine du pic nymphal d'automne reste incertaine. Deux hypothèses alternatives prédisent des décalages temporels différents (0 an versus 1 an) entre le pic automnal des tiques nymphales et le repas sanguin du stade précédent (tique larvaire). Le succès d'alimentation des larves de tiques dépend de la densité de leurs hôtes de petits mammifères, qui à son tour dépend de la production de graines (*masting*) par les arbres à feuilles caduques. Cette thèse de doctorat visait à déterminer les facteurs abiotiques et biotiques qui influencent la variation saisonnière et interannuelle du DON et du DIN dans un endroit en Suisse où la borréliose de Lyme est endémique.

Sur une période de 15 ans (2004 à 2018), nous avons suivi l'abondance mensuelle de l'espèce de tiques *I. ricinus* (nymphes et adultes) et leur statut infectieux de *B. burgdorferi* sl à quatre altitudes différentes sur la montagne de Chaumont à Neuchâtel en Suisse romande. Nous avons collecté des variables climatiques sur le terrain et à partir de stations météorologiques à proximité. Nous avons obtenu des données sur la production de graines de hêtres dans la littérature, car l'abondance des nymphes du genre *Ixodes* peut augmenter considérablement deux ans après un événement de *masting*. Nous avons utilisé des modèles linéaires généralisés et une sélection de modèles basée sur l'AIC pour identifier les facteurs écologiques qui influencent la variation interannuelle de la densité des nymphes (DON), la densité des nymphes infectées (DIN) et la prévalence de l'infection nymphale (NIP). Nous avons utilisé des modèles additifs généralisés et une sélection de modèles basée sur l'AIC pour modéliser la phénologie

saisonnière bimodale complexe des nymphes de l'espèce de tiques *I. ricinus* et investigué si cette approche fournit des informations supplémentaires sur les facteurs qui expliquent la variation saisonnière de l'abondance des tiques.

Dans le chapitre 1, nous avons constaté que l'altitude, l'année, les événements de *masting* deux ans auparavant et l'humidité relative expliquaient ensemble 73,2 % de la variation de nos estimations annuelles du DON. Selon les estimations des paramètres de nos modèles, (i) la densité annuelle de nymphes a presque doublé au cours de la période d'étude de 15 ans, (ii) le changement de l'indice de production de graines de hêtre de 1 à 5 a augmenté l'abondance des nymphes de 86,2% deux années plus tard, et (iii) l'augmentation de l'humidité relative recueillie sur le terrain de 50,0% à 75,0% a diminué l'abondance des nymphes de 46,4% au cours de la même année.

Dans le chapitre 2, nous avons constaté que la variation interannuelle du NIP a diminué de 78% au cours de la période d'étude et ceci s'explique par la moyenne des précipitations de l'année en cours, et la durée pendant laquelle l'extraction d'ADN a été stockée dans le congélateur avant la détection de l'agent pathogène. Le DIN a diminué au cours de la période d'étude aux quatre sites d'altitude différente, mais la diminution n'était significative qu'au site d'altitude supérieure. La variation interannuelle du DIN s'expliquait le mieux par le site d'altitude, l'année, les événements de *masting* deux ans auparavant et l'humidité relative moyenne de l'année en cours.

Dans le chapitre 3, nous avons constaté que les nymphes de l'espèce de tiques *I. ricinus* avaient une phénologie bimodale aux trois sites d'altitude inférieure, mais une phénologie unimodale au site d'altitude supérieure. Nous avons également constaté que la variation interannuelle des densités de nymphes de l'espèce de tiques *I. ricinus* à l'automne et au printemps s'expliquait le mieux par un décalage d'un an par rapport à un décalage de 2 ans avec l'indice de *masting* des hêtres. L'hypothèse du *direct development* est la meilleure explication du pic automnal des nymphes de l'espèce de tiques *I. ricinus* sur notre site d'étude. Notre étude fournit un nouvel aperçu de la phénologie bimodale complexe de cet important vecteur de maladie.

Cette thèse de doctorat a démontré que la production de graines par les arbres à feuilles caduques est un facteur critique de l'abondance saisonnière et interannuelle de l'espèce de tiques *I. ricinus*, et donc du risque de maladie transmise par les tiques. Ce travail fournit de nouvelles informations sur la phénologie bimodale de l'espèce de tiques *I. ricinus*, en particulier que l'hypothèse du *direct development* est la meilleure explication des pics printaniers et automnaux des nymphes de *I. ricinus*.

Mots-clés : Hêtres, *Borrelia burgdorferi*, climat, *Fagus sylvatica*, *Ixodes ricinus*, Maladie de Lyme, fructification des arbres, phénologie, maladies transmises par les tiques, écologie des populations de tique

3 General introduction

3.1 Tick vector

Ticks are hematophagous, obligate ectoparasites that belong to the class Arachnida, which also includes spiders, scorpions, and mites and the subclass Acari. About 900 different existing species are divided into three families, of which the *Argasidae* or soft-bodied ticks, and the *Ixodidae* or hard-bodied ticks, are the two most important families (>99% of the total species), the third family, *Nuttalliellidae*, contains only a single species [1]. Along with mosquitoes, ticks are of veterinary and medical importance because they act as vectors of diseases caused by viruses, bacteria, protozoa, and nematodes [2, 3]. The most important and representative tick-borne diseases, particularly in humans, companion animals, and livestock, are babesiosis, anaplasmosis, Lyme borreliosis, ehrlichiosis, theileriosis, cowdriosis, tick-borne relapsing fevers, and encephalitis [4]. Lyme borreliosis is recognized as the most commonly reported vector-borne disease in the northern hemisphere, accounting for hundreds of thousands of new cases each year [5]. It is caused by spirochete bacteria of the *Borrelia burgdorferi* sensu lato (sl) complex. The arthropod vectors of the spirochetes in the *B. burgdorferi* sl complex are hard ticks from the genus *Ixodes*. At least four different *Ixodes* ticks are known as main and competent vectors; the blacklegged tick or deer tick (*Ixodes scapularis*), the western blacklegged tick (*Ixodes pacificus*), the sheep tick or castor bean tick (*Ixodes ricinus*) and the taiga tick (*Ixodes persulcatus*). *Ixodes scapularis* and *I. ricinus* are found in North America on the east and west of the Rocky Mountains. *Ixodes ricinus* is found in western Europe, whereas *I. persulcatus* is found in Russia and Asia [6-8]. The three-host life cycle of these ticks involves four stages, eggs (inactive stage), larvae, nymphs, and adults, and blood meals from vertebrate hosts are needed for development to the next stage in larvae and nymphs, and for the production of eggs in the adult female stage. These tick species are considered competent vectors for *B. burgdorferi* sl because they are able to acquire the spirochetes during a blood meal taken from an infected vertebrate host, to maintain a spirochete population in the tick midgut across the life stages (e.g., transstadial transmission), and to transmit these pathogens to other hosts when feeding again [9]. *Ixodes* ticks feed on their hosts for only a short period of time and spend ~98% of their time in the environment (e.g., more than a year for *I. ricinus* ticks) [6, 10]. Approximately 3 days after feeding, larvae detach from the host and moult into nymphs, which takes at least 6 weeks and often much longer. Nymphs take their 5-day blood meal from a different host followed by detachment from the host and moulting into adult ticks. Mating

between males and females ticks usually occurs in the vegetation (e.g., off the host) but can continue throughout the feeding period (e.g., on the host) [11-13]. Counter to adult male ticks which do not blood feed, adult female ticks take a 7-day blood meal, which gives them enough resources to lay a batch of ~2000 eggs [10]; the female ticks die after laying their eggs. From a public health perspective, these four *Ixodes* tick species are important because they are what is called host generalists; they feed readily on a wide range of vertebrate hosts including mammals, birds, and reptiles, and at least 50 different vertebrate species have been described suitable reservoir hosts for the genospecies of the *B. burgdorferi* sl complex [14, 15]. In contrast, other species of *Ixodes* ticks are highly specialized on certain host species. For example, *I. frontalis*, *I. arboricola*, and *I. uriae* only feed on birds [6, 7, 16]. Similarly, *I. hexagonus* only feed on hedge hogs, while *I. dentatus* only feed on rabbits [6, 7]. As a result, these specialist tick species never bite humans and thus do not result in public health problems.

Ixodes ricinus is the central tick species discussed in the present work because it is the most common tick species and the principal vector of *B. burgdorferi* sl in Europe [17]. The life cycle length of *I. ricinus* ticks ranges from 2 to 6 years [18]. Larvae is the active stage that has the smallest body size (0.8 mm), followed by nymphs (1.2 – 1.5 mm), males (1.5 – 2.5 mm), and females (2.0 – 4.0 mm; [19]). The same individual tick takes its larval, nymphal, and adult female blood meal in different years. Each active stage searches for a vertebrate host in a behaviour often referred to as questing. Questing ticks climb up the vegetation and wait with its first pair of legs outstretched in the air to grab onto a passing host. During questing, *I. ricinus* ticks are highly susceptible to desiccation because loss of water takes place through their cuticle and spiracles by evaporation and respiration, respectively [20, 21]. After bouts of questing, *I. ricinus* ticks often return to the moist leaf litter layer for rehydration by taking up water from the atmosphere [18, 22]. As desiccation tolerance increases with stage [23], immature ticks usually quest for shorter periods and lower on the vegetation than mature ticks. Larvae and nymphs are generally observed between 0 and 30 cm and 30 to 60 cm above ground, respectively, whereas adults are mainly observed between 60 to 80 cm but can be found up to 140 cm above ground [22]. These differences in questing height may be one reason why the active stages quest for different hosts. Immature *I. ricinus* ticks prefer to feed on smaller hosts such as small mammals (e.g., rodents and insectivores), ground-dwelling birds, and lizards, but can feed on carnivores and ungulates [24]. In contrast, adult female ticks prefer to feed on larger hosts, and thus quest at higher height (Figure 1) [25]. Accordingly, a diverse host community is generally required for the successful completion of the life cycle of *I. ricinus* ticks.

Among blood-feeding arthropods, hard ticks are unique in the duration of attachment to the host. The long period of tick engorgement allows the vertebrate host to mount immune responses directed towards the tick. In response, ticks have evolved a wide diversity of salivary gland molecules that maintain blood flow, reduce detection, and evade immune system of the vertebrate host. During engorgement, ticks alternate between blood feeding and secreting their saliva into the host skin [26]. Tick saliva contains an extraordinary array of salivary proteins that have anti-hemostatic, anti-inflammatory, and immunomodulatory functions. The saliva containing these salivary proteins is injected into the host to prevent any disruption to the feeding process [27-29]. Tick saliva is produced by the salivary glands, which also ensure tick attachment to the host skin (e.g., production of cement) and homeostasis (e.g., concentration of nutrients from the blood meal by eliminating excess fluid), and absorption of moisture from the environment [30]. Salivary proteins not only ensure the success of the blood meal but they also facilitate transmission of tick-borne pathogens like *B. burgdorferi* sl. For example, *B. burgdorferi* ss uses its OspC proteins to bind to a tick salivary protein called Salp15, which allows the spirochetes to evade the host immune response and to establish infection in the vertebrate host [31]. Similarly, salivary gland extracts from *I. ricinus* caused *B. burgdorferi* sl spirochetes to grow faster into rodent skin, which facilitates earlier pathogen transmission to the vertebrate host [32]. Another study found that co-inoculation of tick salivary glands and *B. burgdorferi* sl into rodent skin enhances the success of the infection [33].

I. ricinus ticks are exposed to the seasonal changes in temperature, relative humidity, precipitation, and other climate-related abiotic variables during the off host periods. In general, temperature increases development rates and the life cycle is accelerated in a warmer climate, resulting in more abundant tick populations [34-36]. Questing for hosts is when ticks are particularly vulnerable to the drying power of the atmosphere and when they have to expend considerable energy to maintain their water balance [21]. During questing, ticks climb up the vegetation where they are exposed to lower relative humidity (e.g., < 80%), which results in desiccation and requires the ticks to return to the litter layer for rehydration [6, 22]. Ticks get all of their energetic resources from their blood meals; hence, the trips between questing sites and the litter layer exhaust their non-renewable energetic resources, which increases the probability of tick death [38]. When ticks encounter adverse microclimate conditions, they seek shelter in the litter layer, which has favourable conditions (e.g., high relative humidity and moderate temperatures) that enhance survival [21]. In addition to being a refuge for questing ticks, the layer-litter also provides a shelter for the developing or inactive stages. For this reason, typical habitats of *I. ricinus* ticks include deciduous forest, coniferous forest, or mixed forest

with reasonable rainfall, where the vegetation cover allows a certain relative humidity (>80%) at the litter layer [6].

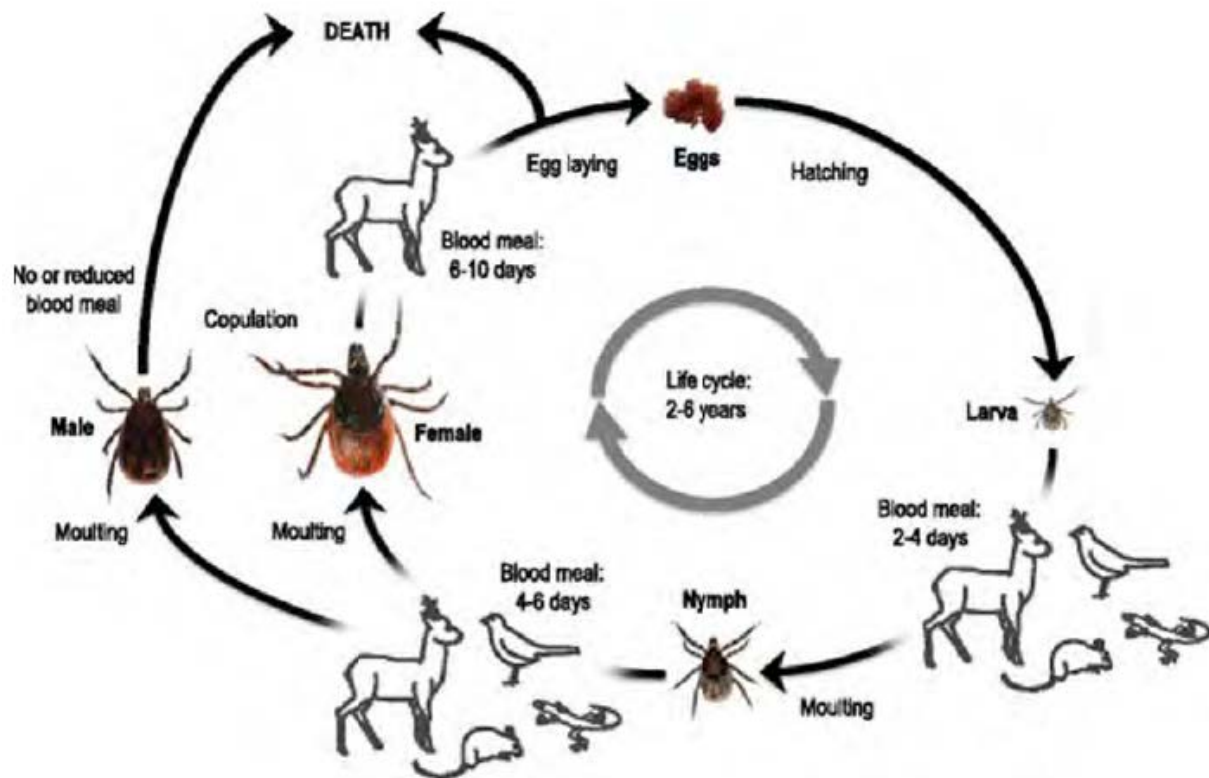


Figure 1. Life cycle of *I. ricinus* [25].

3.1.1 Tick distribution and abundance

Ixodes ricinus has a wide distribution across Europe, ranging from Ireland to the Ural Mountains in Russia, and from northern Sweden to North Africa [40]. In the past two decades, *I. ricinus* ticks were reported from new locations in Europe, being found at more northern latitudes and higher altitudes [41-46], while its abundance in endemic areas remains controversial [47-54]. The reasons for these changes are generally attributed to different ecological factors that act on the tick, the host, or their habitat. These ecological factors are often strongly interlinked; for example, climate influences the population ecology of the ticks, their hosts, and the plants on which the hosts feed. However, identifying the ecological factors that drive these changes in the distribution and abundance of *I. ricinus* is critical from a public health perspective. The risk of tick-borne disease differs among the three motile stages; nymphs and adults can both carry and transmit *B. burgdorferi* s.l. whereas the infection prevalence of *B. burgdorferi* s.l. in larvae is essentially zero [55-57]. In nature, the force of infection of *B. burgdorferi* s.l. depends on the density of infected nymphs because adult female ticks feed on

hosts that are incompetent for the spirochete. Nymphs and adult ticks both bite humans, the nymphs are more abundant than adults ticks as well as much smaller than the adult female ticks and therefore less likely to be noticed and removed by humans before they can transmit the infection [36]. Thus, the risk of human exposure to *B. burgdorferi* s.l. is determined by the DIN, which are responsible for the majority of Lyme disease cases [58-60]. The DIN describes the probability (per unit time) that an animal or a person will encounter an infected tick in that particular habitat [61]. The DIN is often estimated as the product of the DON and the NIP [62]. In summary, understanding the factors that determine the population ecology of *I. ricinus* is important for predicting the risk of exposure to tick-borne pathogens in a changing world. Plausible abiotic and biotic drivers of change which are relevant for the present work are discussed below.

3.1.1.1 Climate change

Global warming has resulted in dramatic changes in the distribution and abundance of medically important vectors, such as mosquitoes, ticks and biting flies [63, 64]. These range expansions are expected to increase the incidence of vector-borne diseases that are transmitted by these arthropod vectors with serious consequences for human health [65]. For example, *Ixodes* ticks have expanded their range towards northern latitudes, which has resulted in dramatic increases in the incidence of Lyme borreliosis and other tick-borne diseases in Canada and Scandinavia [41-46]. For example, the northern boundary for *I. ricinus* has expanded from 60°N to 66°N (e.g., > 600 kilometres) in northern Sweden over almost two decades [45]. Similarly, the number of counties in which *I. scapularis* has been documented increased by 44.7% between 1998 and 2016, particularly in northern states [35]. In Europe, endemic *I. ricinus* populations have shifted their distribution towards higher altitudes [66-72]. For example, in Switzerland, *I. ricinus* has been recorded at altitudes up to 1070 m above sea level (ASL) [68, 73], and in Czech Republic this species has shifted its altitudinal range limit from 700 m to 1100 m ASL in less than two decades [70]. These range expansions are directly related to the idea that global warming has made these environments more favourable for *Ixodes* ticks.

Given these extensive researches on increased range expansions towards northern latitudes, fewer studies have investigated whether climate change has influenced *Ixodes* tick abundance in areas where Lyme disease is endemic [47-54]. Evidence that climate change in increasing the abundance of ticks is difficult to assess as it requires long-term studies and seems to be species-, stage-, and location-specific. A 10-year study in the Netherlands found an increase in the abundance of *I. ricinus* larvae and adults [74]. In contrast, a 15-year study in

Switzerland found a significant decrease in the abundance of *I. ricinus* nymphs [48]. Another 8-year study in central New Jersey found no significant directional change in the abundance of *I. scapularis* nymphs [49]. Other long-term studies have found large inter-annual fluctuations in density, but none of them found that *Ixodes* tick density was increasing [47, 50, 51]. Increased tick abundance could have important consequences for the incidence of tick-borne diseases and public health. The risk of exposure to tick-borne diseases depends on the DIN, which is the product of the DON and the NIP [62]. All else being equal, an increase in tick abundance suggests that the risk of tick-borne disease could increase at a specific location. However and due to the rarity of long-term studies, we currently do not have a good understanding of how climate change will influence the abundance of *Ixodes* ticks in endemic areas [34, 64].

Many climate variables, such as temperature, precipitation, and relative humidity are expected to be impacted by global warming, all of which can influence the population ecology of ticks. Like all arthropods, *Ixodes* ticks are cold-blooded (ectothermic); the external temperature determines their internal temperature, and the life history traits of ticks are therefore highly sensitive to temperature and other abiotic factors. A better understanding of the relationships between climate variables and the critical life history traits (development, survival, reproduction) is necessary to understand how climate change will influence the distribution and abundance of *Ixodes* ticks [34, 64, 75]. In general, development rates increase with temperature [34, 76, 77], although high temperatures can also reduce tick abundance by increasing mortality rates through dehydration, by reducing moulting and oviposition success, or by inhibiting host seeking activity which reduces the probability that the tick will find a host before starvation [35, 36]. Very low temperatures can reduce tick questing activity, or slow down rates of development, which ultimately reduces the probability for a tick to survive to the next stage or reproduce [35, 36]. Nevertheless, temperature is not the only climate variable that influences the ecology of *Ixodes* ticks. A warmer climate increases water evaporation and allows the atmosphere to hold more moisture, which can change the relative humidity and precipitation patterns. *Ixodes* ticks require a high relative humidity to maintain a stable water balance for increasing their survival [21, 37], which is known to increase with relative humidity [6, 36, 79]. However, excessive precipitation is also bad because it can drown ticks, inhibit questing activity, and facilitate the development of entomopathogenic fungi [36, 39, 47, 80]. Each of these three adverse outcomes of excessive precipitation can thus directly reduce tick fitness or decrease the probability of encountering a host, both being detrimental to their survival. Climate variables can operate independently or interact with each other to drive the ecology of species. For example, the saturation deficit (SD) is a combination of temperature

and relative humidity and measures the drying power of the atmosphere. Hot and dry environments have high SD values ($> 24^{\circ}\text{C}$ and $< 45\%$ [81-83]), which decrease tick survival and abundance. Conversely, cool and humid environments have low SD values, which are favourable for tick survival and abundance [23, 67, 73, 83-86]. Hence, although an increase in temperature may be the main driver of the observed changes in distribution and abundance of *Ixodes* ticks, it is likely that other climatic factors are also involved. In summary, climate change will have an impact on the life history traits of *I. ricinus* ticks (survival, development, and reproduction), which will inevitably influence the risk of tick-borne diseases.

3.1.1.2 Vertebrate hosts

The abundance of suitable vertebrate hosts is a critical factor for tick population ecology because all motile stages (larvae, nymphs, and adult females) must take a blood meal from a vertebrate host to complete the life cycle [87, 88]. The presence of a significant number of large animals, such as deer, is required to feed adult female ticks which cannot feed on small animals [6]. Tick abundance is low in habitats where the density of deer or other ungulates is low, whereas tick abundance is high in habitats where the density of deer is moderate to high [36]. Although deer are important in feeding adult females ticks (i.e., they are reproduction hosts), they are incompetent for *B. burgdorferi* sl and do not transmit this pathogen to feeding ticks [14, 89, 90]. Consequently, with respect to *B. burgdorferi* it is important to make the distinction between reproduction hosts that produce more ticks and competent reservoir hosts that amplify the tick-borne pathogen.

Reservoir hosts are determined by their capacity to harbour *B. burgdorferi* sl and present long-term infection source for feeding tick vectors, which transmit the pathogens to other vertebrate hosts [14]. In Europe, at least 50 different vertebrate species have been described as competent reservoir hosts for the genospecies of the *B. burgdorferi* sl complex [14, 15]. However, vertebrate host species vary considerably in their reservoir competence [91], which in turn strongly influences the transmission dynamics of *B. burgdorferi* sl. A vertebrate host species with high reservoir competence is one upon which a great number of ticks successfully feed, and from which many become infected with *B. burgdorferi* sl. For example, white-footed mice successfully feed a large proportion of the *I. scapularis* larval ticks that encounter them [92] and transmit *B. burgdorferi* ss to 70 – 90% of the ticks [91, 93]. In contrast, opossums successfully feed only few *I. scapularis* larval ticks, making opossums a low reservoir competence host species [92]. Similarly, ungulates are considered as low reservoir competence host species because they successfully feed a large proportion of *Ixodes* female adult ticks but

are unable to transmit *B. burgdorferi* sl to *Ixodes* ticks [6, 89, 90]. The tick feeding success and infectivity of a particular vertebrate species for *Ixodes* ticks have been explored through host blood meal studies. These studies have tested field-collected *Ixodes* ticks for the presence of vertebrate host DNA [94-97]. Taken together, these results emphasize the fact that not all vertebrate host species are equally infected, nor equally infectious.

Beyond tick feeding success and host infectivity, host density and mean body burden of feeding ticks have to be considered and measured to capture how host community influences the transmission of *B. burgdorferi* sl [91, 98-100]. For example, rodent occurring in high densities and having large tick burden are considered as the most important vertebrate host species for feeding and infecting larval *I. ricinus* ticks [99]. These studies also explored how changing the community of vertebrate hosts (adding or subtracting species) influences the prevalence of infected nymphs. Thus, the main focus of these studies was on comparing host communities with different species of vertebrate hosts and their impact on the NIP under the assumption that host biomass and tick abundance were relatively constant. However, communities of vertebrate of hosts are not perfectly constant from year to year and the abundance of individual host species can vary dramatically.

Tick infection prevalence depends on a number of factors including (1) density of hosts, (2) mean number of larvae fed by those hosts, (3) percentage of hosts infected with *B. burgdorferi* sl, and (4) host-to-tick transmission for those hosts [58, 101]. In the North American Lyme disease system of *B. burgdorferi* ss and *I. scapularis*, rodents play an important role in determining the nymphal infection prevalence as they are abundant and heavily infested with ticks, inefficient in killing ticks while grooming, and are highly competent hosts for *B. burgdorferi* ss [58, 92, 102]. Thus, rodents increase the probability for *I. scapularis* larvae to become infected during the blood meal and to reach the nymphal stage [92]. Similarly in Europe, rodents are highly competent hosts for rodent-associated genospecies like *B. afzelii*, *B. bavariensis*, and *B. burgdorferi* ss [14, 103], are heavily infested with *I. ricinus* ticks [104-106], and can reach extremes densities after mast years [107-109]. Moreover, the survival of *I. ricinus* larvae feeding on rodents might be exceptionally high [110], confirming that vertebrate host community might have a great impact on infected nymph number and thus on Lyme disease risk.

Inter-annual variation in the abundance of vertebrate hosts can have important effects on year-to-year fluctuations in tick abundance and the risk of Lyme disease. Small mammals such as rodent and insectivores are important hosts because they feed large numbers of immature ticks (larva and nymphs) and because they are highly competent reservoir hosts for

some *B. burgdorferi* s.l. genospecies. Studies in North America and Europe have shown that small mammals, such as rodents, often exhibit dramatic inter-annual fluctuations in population size [107-109, 111, 112]. An important source of food for many rodent species is the mast or fruit of forest trees, and the annual production of this fruit can also vary dramatically among years [113-116]. Many vertebrate host species respond to masting occurring in the fall, including rodents, birds, carnivores, and omnivores [117]. Higher food supply results in increased overwinter survival, winter breeding, and higher spring reproductive success of small mammals [117, 118]. Consequently, rodent population is boosted the following year [47, 111, 118-121]. In turn, the increase in the rodent population size will enhance the feeding success of larvae, which will increase the abundance of nymphs in the following year [47, 120, 122, 123]. Thus, the local composition and abundance of vertebrate hosts greatly affects the numbers of ticks being fed, and by extension, tick infection prevalence.

3.1.1.3 Masting

Immature *Ixodes* ticks mostly feed on vertebrate hosts that are primary consumers of mast seeding. Mast years refer to years where beech and oak trees synchronously produce exceptionally large seed crops in autumn, with low production or total failure in the intervening years [124]. The synchronized seed production in masting tree species is driven by temperature and precipitation during the summers one or two years prior to a mast year [113-116]. Climate is influencing the frequency and the quantity of seed production of masting events [113, 116, 125, 126], which affects the population dynamics of animal species that use these seeds as a food resource, such as deer, small mammals and birds [111, 112, 117, 127]. For example, deer increase their home range and spend more time feeding in autumns of mast years compared with low seed production years [121, 128]. Similarly, seed fall in autumn is a critical food source for rodents, determining their survival and breeding through the winter [108, 118, 129]. In turn, fluctuations in the population size of deer and rodents can drive fluctuations in the abundance of *Ixodes* ticks because ticks feed on deer and rodents [47, 119, 120]. Ostfeld and colleagues in the eastern USA first discovered that the density of *I. scapularis* and the risk of Lyme disease depend on the abundance of rodents in the previous year and on the abundance of acorns two years previously [47, 120, 122, 123]. The chain of causality is as follows: mast seeding produces lots of food in the autumn of year y , which attracts deer carrying large number of female adult ticks in the autumn and increases the survival and reproduction of small mammals over the winter and these two phenomena increase the abundance of larvae and small mammals in year $y + 1$ [47, 107, 108, 111, 118-121, 128, 130, 131]. High densities of larvae

coincide with and feed on high densities of small mammals, which in turn increases the abundance of nymphs in year $y + 2$ [47, 120, 122, 123, 132, 133]. Most of the research demonstrating that masting increases the abundance of *Ixodes* nymphs with a 2-year time lag was done by the research group of Rick Ostfeld in New York State. In contrast, in Europe there is not much evidence that tree seed production is driving the population dynamics of *Ixodes* ticks [50]. However, if similar ecological relationships occur in areas endemic for Lyme disease in Europe, we predict that inter-annual fluctuations in tree seed production will influence tick abundance via their effects on the abundance of vertebrate hosts.

Masting intensity is already an important predictor for other tick-borne diseases in Europe. Tick-borne encephalitis virus (TBEV) is the causative agent of tick-borne encephalitis (TBE), a serious neurological infection affecting humans. Similarly to *B. burgdorferi* s.l., TBEV cycle between small mammals and *I. ricinus* ticks [134]. In Europe, there are 3,000 reported cases of TBE each year, with increasing number in the past decades [134]. TBE incidence has been recently shown to be determined by beech tree fructification index 2 years prior [135-137]. In the same manner as previously described, high fructification of beech trees increases the population density of rodents and the probability that larval *I. ricinus* ticks obtain a blood meal one year later. Consequently, higher densities of questing *I. ricinus* nymphs are responsible for the more frequent transmission of the TBE virus to humans two years later [50]. A recent study verify this causative chain in Austria, Germany, and Switzerland where high peaks in TBE cases were detected 2 years after a masting year [137]. Thus, similarly as is the natural cycle of TBE transmission, masting intensity could be used as important predictor for tick abundance and the incidence of Lyme disease.

3.1.2 Tick phenology

The seasonal activity pattern or phenology of *Ixodes* ticks within a year can be complex and varies considerably among geographic locations. In southern Europe where it is hot and dry, ticks are mostly active in the winter when abiotic conditions (temperature and humidity) are optimal for them to search for vertebrate hosts [85, 138]. In contrast, in the temperate regions of the northern hemisphere, ticks are active in the spring, summer, and fall [18]. Ticks search for hosts in a behaviour called questing. As mentioned previously, *I. ricinus* tick populations consist of discrete stages (e.g., larvae, nymphs, adults) that co-exist in the same year, but belong to different generations (i.e., the life cycle is slow); a larva that quests in year y will exist as a nymph in year $y+1$, and as an adult tick in year $y+2$ [6]. Thus, the abundance of a given tick stage in a given year often depends on ecological factors of one, two, or even three years prior.

In continental Europe, questing activity of *I. ricinus* is essentially non-existent in winter and early spring (November – February), with some exceptions depending on the region and weather conditions [18, 139]. Larvae start to quest in spring (May) and larval peak usually reaches its maximum in mid-summer (June – July), whereas nymphs and adult ticks start to quest earlier (February – March) with a peak of activity usually observed in spring and early summer (May – June), followed by a lower levels of activity in summer [18, 38, 140]. In many areas, a second peak of activity of nymphs and adults occurs in autumn (September – October), which is usually of a lower intensity than the spring peak (Table 1) [18]. In some parts of Europe, *I. ricinus* nymphs and adult ticks also exhibit unimodal phenology where questing activity peaks in late spring or early summer and ends in the fall (Table 1) [74, 83, 141-143]. Several theories have been proposed to explain these seasonal activity patterns of *I. ricinus* [140], but there is now general agreement that ticks avoid questing at unfavourable times of the year (winter and summer), using dormancy strategies to maximize their survival and development with respect to the environmental conditions. For example, *I. ricinus* ticks usually stop questing when the temperature exceeds 35°C or relative humidity is inferior to 80% [18, 37, 83]. Ticks also avoid direct sunshine, and they decrease their host-seeking activity as the saturation deficit increases [18, 37, 83]. However, the depletion of tick activity in mid-summer and winter cannot only be attributed to dormancy strategies to avoid unfavourable environmental conditions, but also to the removal of a substantial portion of fed ticks from the population (e.g., ticks that found a host by that time).

Table 1. Type of phenology for *I. ricinus* ticks in different countries in Europe. *Ixodes ricinus* ticks (nymphs and adults) either have a unimodal or bimodal phenology. The phenology is shown for different countries and regions in Europe. The studies that documented the phenology of these tick populations are also shown.

Phenology	Country	Region	Studies
Unimodal	UK	Powys	[141]
Unimodal	Sweden	Bogesund	[142]
Unimodal	Switzerland	Chaumont Mountain	[83, 143]
Unimodal	Netherlands		[74]
Bimodal	Ireland	County Wicklow	[144]
Bimodal	Sweden	Bogesund	[142]
Bimodal	Crimea		[145]
Bimodal	Switzerland	Chaumont Mountain, Salins	[48, 67, 73, 83, 84, 143]
Bimodal	UK	Exmoor National Park, Mynydd Mallaen, Dorset	[38]
Bimodal	Spain	Rioja region	[85]
Bimodal	Czech Republic	Prague	[51]
Bimodal	Finland	Seili, Jyväskylä	[146, 147]
Bimodal	Germany	Haselmuehl	[148]

Dormancy is by definition a state of reduced metabolic activity which enables organisms to survive adverse environmental conditions and conserve energy until conditions improve. With respect to *Ixodes* ticks, most authors subdivide dormancy into two distinct phenomena: quiescence, which refers to a behavioural state of inactivity that ticks adopt under adverse environmental conditions (e.g., low temperature), and diapause, which refers to a hormonally controlled state of inactivity triggered by environmental factors that are neither favourable nor unfavourable (e.g., day length). While quiescence lasts only until favourable conditions return, diapause has a fixed latency period that must be completed before development can resume [18]. Both forms of dormancy are not necessarily exclusive; in some years, unfavourable conditions may last longer than the length of diapause and thus ticks may enter a post-diapause quiescence and remain inactive until favourable conditions return [149]. *Ixodes ricinus* ticks show diapause, becoming inactive in autumn and throughout winter either through behavioural diapause of unfed ticks or developmental diapause of engorged ticks [18, 34]. Specifically, behavioural diapause is characterized by the suppression of host-seeking activity and/or host attachment by freshly moulted unfed nymphs and adults during summer in anticipation of unfavourable winter conditions, whereas developmental diapause involves cessation of development of recently laid eggs, or of engorged larvae and nymphs in autumn to enhance overwintering survival [150]. Accordingly, the spring and autumn activity peaks for each stage are thought to include different generational cohorts with respect to the two diapause strategies.

However, both forms of diapause cause delays, which also explains why the life cycle length ranges from 2 to 6 years for *I. ricinus* ticks [18]. While the nymphal and adult spring peaks seem to originate exclusively from larvae and nymphs that fed in the spring or early summer of the previous year (e.g., behavioural diapause), respectively, the origin of the nymphal and adult autumn peaks is less clear. Two alternative explanations for this bimodal phenology of questing *I. ricinus* nymphs are the developmental diapause hypothesis and the direct development hypothesis (Figure 2) [18, 38, 83, 143, 145, 151, 152]. The developmental diapause hypothesis (Figure 2) suggests that the timing of the larval blood meal splits the larval cohort into two groups of nymphs that are active in the spring and fall of the following year [18, 140]. Larvae that obtain their blood meal in spring or early summer, moult into unfed nymphs, enter behavioural diapause in fall, and quest the following spring. In contrast, larvae that obtain their blood meal in late summer, enter developmental diapause, complete their development the following summer, and quest that same fall [18]. The direct development hypothesis (Figure 2) suggests that the timing of the larval blood meal splits the larval cohort into two groups of nymphs that are active in the fall of that year and the spring of the following year [18, 140]. Larvae that obtain their blood meal in early summer, moult into unfed nymphs, and quest that same fall. In contrast, larvae that obtain their blood meal in late summer, moult into unfed nymphs, enter behavioural diapause, and quest the following spring [18]. In both hypotheses, there is a 1-year time lag between larval feeding and the spring nymphal peak. The critical distinction between these two hypotheses is the time lag between larval feeding and the fall nymphal peak, which is 1 year for the developmental diapause hypothesis and 0 years for the direct development hypothesis. To date, it is not clear which of these two explanations is more important for explaining the fall peak of *I. ricinus* nymphs. However, ticks that overwinter in developmental diapause are more vulnerable to cold temperatures and have shown lower survival rates than those that fed and moulted in the same year [153]. Thus, the autumn peak of nymphs is believed to be come from larvae that fed in the spring of the same year, but this assumption requires further investigation.

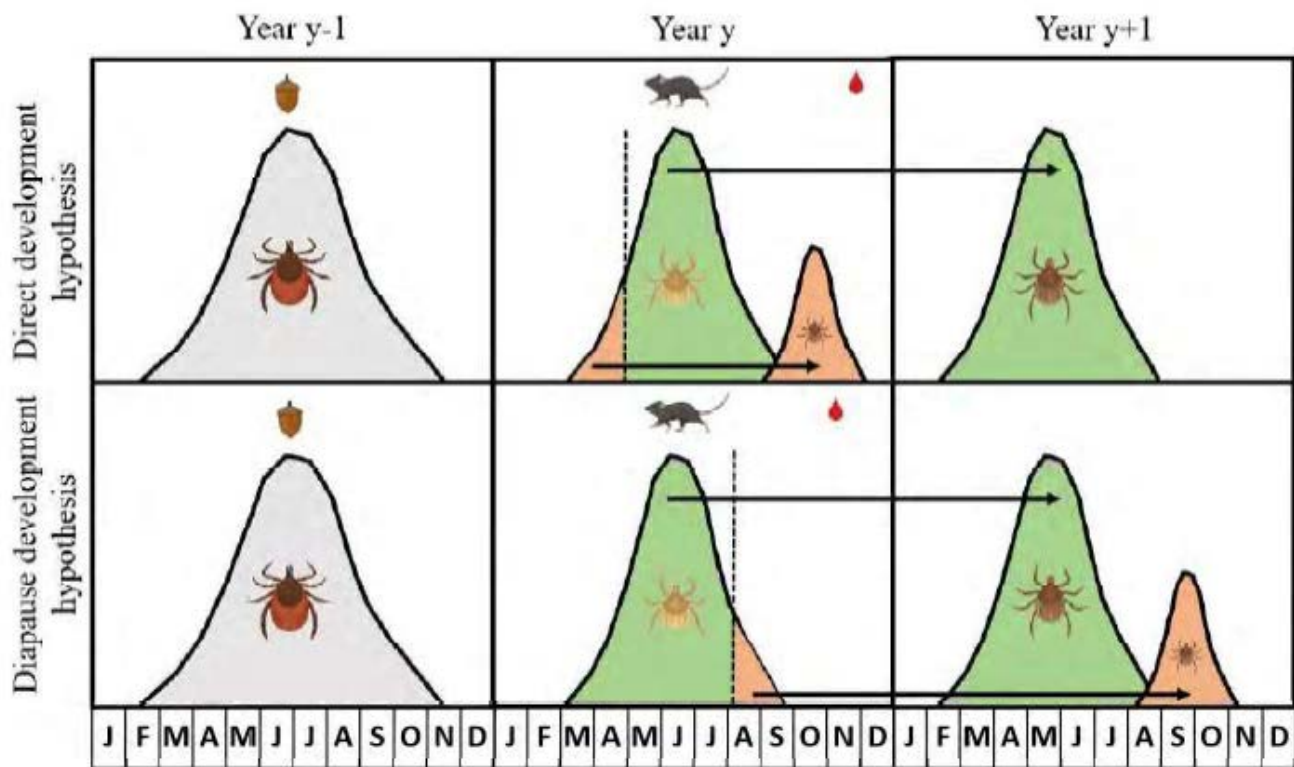


Figure 2. Two alternative (but not mutually exclusive) hypotheses for the fall peak are shown: the direct development hypothesis (top panel) and the developmental diapause hypothesis (bottom panel). The approximate 3-year life cycle of *I. ricinus* is shown by adult ticks (Year $y - 1$ in the left panel) that lay eggs, the larvae (Year y in the middle panel), and the nymphs (Year $y + 1$ in the right panel). The origin of the spring peak of nymphs (green peak in Year $y + 1$) is the same for both hypotheses and is as follows. Larvae that obtain their blood meal in the summer (green fraction in Year y), moult into unfed nymphs in the same year, enter behavioural diapause, overwinter, and quest as unfed nymphs the following spring (green peak in Year $y + 1$). The two hypotheses differ with respect to the origin of the fall nymphal peak (small orange peak). In the direct development hypothesis, larvae that obtain their blood meal early in the summer (orange fraction in Year y) moult into unfed nymphs in summer and quest as unfed nymphs that same fall (small orange peak in Year y). In the developmental diapause hypothesis, larvae that obtain their blood meal late in the summer (orange fraction in Year y), overwinter as engorged larvae, moult into unfed nymphs the following summer and quest as unfed nymphs the following fall (small orange peak in Year $y + 1$). The time lag between a masting event (Year $y - 1$) and the spring peak of nymphs (green peak in Year $y + 1$) is 2 years. In contrast, the time lag between a masting event and the fall peak of nymphs (small orange peak) is 1 year under the direct development hypothesis and 2 years under the developmental diapause hypothesis.

The onset, maintenance, and termination of both tick diapauses are thought to depend upon a combination of environmental factors (e.g., seasonal and/or annual). Factors of major importance include seasonal changes in day length and temperature, which might operate on the tick nervous and endocrine systems that are responsible to initiate or cease moulting processes [18]. Seasonal changes in day length (or photoperiod), particularly at high latitudes, are considered to be the primary mechanism regulating diapause. Ticks show diverse reactions to day length; long-day reaction in which activity (host seeking, engorgement, and post-engorgement development) occurs in response to long days and short-day reaction in which diapause occurs in response to short days [150]. Furthermore, the tick reaction to day length is determined by their critical threshold photoperiod. For example, *I. ricinus* ticks maintain normal activity at photoperiods longer than the critical threshold but enter diapause when photoperiods become shorter than the critical threshold [150]. This critical threshold photoperiod is believed to shape the diapause decision window in summer. Individuals feeding before this critical threshold undergo moulting before entering the behavioural diapause as unfed ticks until the following spring, while individuals feeding after this critical threshold enter developmental diapause as engorged ticks until development resumes in the following summer [18]. Thus, the perception of changes in day length appears to be the first step towards these physiological mechanisms that lead to diapause. However, several studies have shown that some individuals remain active throughout winter (i.e., despite short day lengths) when temperatures are sufficiently mild [139, 154]. In the same manner, the gradual increase of questing *I. ricinus* ticks in spring is regarded mainly as temperature-driven as the onset of nymphal and adult activity coincides with a weekly mean temperature of 7°C [34, 37, 83], and larval activity does not begin until temperature have reached a threshold of 10°C [34]. In summary, the onset, maintenance, and termination of diapause regarding unfavourable conditions depend on a combination of seasonal changes in day length and temperature.

3.2 Lyme disease

Lyme disease, also known as Lyme borreliosis, is the most common vector-borne disease in the temperate regions of the northern hemisphere. In Europe, there are an estimated 85,000 cases of Lyme disease each year while approximately 30,000 cases of Lyme disease are reported annually to the Center for Disease Control (CDC) in the United States [155, 156]. A recent report by the CDC found that tick-borne diseases in the USA more than doubled over a period of 13 years (>22,000 cases in 2004 to >48,000 cases in 2016) with Lyme disease accounting for 82% of all tick-borne disease reports [157]. In other part of the world such as Asia and

Northern Africa, the number of cases is lower with an estimated 3,450 and 10 cases of Lyme disease each year, respectively [5]. However, several factors suggest that the actual world annual incidence is an underestimate and might be up to tenfold higher than the final reported cases to the public health system. Persons diagnosed clinically for whom no testing were performed or the used of non-validated tests that have lower sensitivity and specificity are two of the many reasons that can explain this disparity [156, 158].

Originally mistaken for juvenile rheumatoid arthritis, Lyme disease was diagnosed in children and adult patients as a clinical condition for the first time in 1976 in Lyme, Connecticut, USA by physicians Allen Steere, Stephen Malawista, and David Snydman and others [159]. The epidemiology and clinical symptoms observed in this study led the authors to conclude that the disease was transmitted by an arthropod vector. This hypothesis was confirmed in 1981 by the Swiss-American scientist Willy Burgdorfer when he isolated the etiological agent of Lyme disease, spirochetes from the genus *Borrelia*, from the blacklegged tick (also called deer tick) *Ixodes scapularis* in the United States and the sheep tick *Ixodes ricinus* in Europe [160, 161]. The spirochete responsible for Lyme Disease was named *Borrelia burgdorferi* in honour of its discoverer [162].

In humans, the symptoms of Lyme disease can occur 3 to 30 days after the tick bite and they can vary widely depending on the stage of the infection [163]. Early signs include flu-like symptoms such as fever, headache, fatigue, muscle and joint pain, and swollen lymph nodes [164]. In up to 80% of Lyme disease cases, the patient has a bull's eye rash around the site of the tick bite, and this cutaneous infection is called an erythema migrans [10, 163]. After the dissemination of the spirochete from the skin to other organs, early Lyme disease clinical manifestations can evolve into complicated pathologies (e.g., late Lyme disease) involving the skin (acrodermatitis chronica atrophicans), nervous system (Lyme neuroborreliosis), heart (Lyme carditis), and joints (Lyme arthritis) [165]. Without the recommended treatment, the infection can progress to the chronic phase and symptoms might persist for years after the initial tick bite [10, 163, 165].

3.3 *Borrelia burgdorferi* sensu lato

Borrelia burgdorferi sensu lato (sl), the tick-borne agent of Lyme disease, is a complex of spirochete bacteria comprising at least 21 genospecies [166, 167]. At least seven different genospecies of the *B. burgdorferi* sl complex, namely *Borrelia afzelii*, *Borrelia bavariensis*, *Borrelia burgdorferi* sensu stricto (ss), *Borrelia garinii*, *Borrelia lusitaniae*, *Borrelia mayonii*, and *Borrelia spielmanii*, are known to be pathogenic to humans [168]. *Borrelia burgdorferi* ss

is by far the most important cause of human disease in North America, and this genospecies is also found in Eurasia [167-169]. *Borrelia mayonii* was discovered in 2016 at the Mayo Clinic in Minnesota and has not been found outside North America [170]. The other five *Borrelia* genospecies, *B. afzelii*, *B. bavariensis*, *B. garinii*, *B. lusitaniae*, and *B. spielmanii* occur in Eurasia and have not been found in North America [167-169]. The pathogenicity of other genospecies is still unclear, although *B. bissettii* and *B. valaisiana* have been detected in Lyme disease-infected patients but are not considered as important pathogens [10]. In Europe, the three most common Lyme disease pathogens are *B. afzelii*, *B. garinii*, and *B. burgdorferi* ss, each of which is associated with different types of Lyme disease in human. *B. afzelii* is mostly responsible for acrodermatitis, *B. garinii* mostly cause neuroborreliosis, and *B. burgdorferi* ss is mostly associated with arthritis [10, 169, 171].

Borrelia burgdorferi sl is maintained in nature in transmission cycles involving ticks and their vertebrate hosts. *Borrelia burgdorferi* sl can be acquired by larval ticks while feeding on an infected host. The pathogen is maintained via transstadial transmission from larvae to nymphs [17]. The spirochetes inhabit the midgut of the tick until the next blood meal, which establishes the beginning of their migration to the salivary glands. The spirochete dissemination from the midgut to the salivary glands takes place 36 to 48 hours after attachment [172], but earlier transmission can occur [173, 174]. The infection is transmitted to the host through the saliva deposited into the feeding lesion. Transstadial transmission also occur from nymphs to female adult ticks, which transmit subsequently the infection to the vertebrate host. Female ticks usually feed on incompetent hosts (e.g., deer) that cannot transmit the spirochetes [6, 89, 90]. As transovarial transmission of *B. burgdorferi* sl from infected mothers to their offspring is believed to be rare or non-existent [55-57], female ticks seem to have little if any role in the maintenance of *B. burgdorferi* sl in nature, except from producing larval ticks.

To persist and establish infection, *B. burgdorferi* sl pathogen have developed many strategies to adapt to the different environments that are present in the arthropod vector and the vertebrate hosts. The genome of *B. burgdorferi* sl contains genes that encode for a variety of outer surface proteins (Osps) and changes in the expression of these Osps allow the spirochetes to adapt to these markedly varied environments. For example, outer surface protein A (OspA) is expressed when the spirochetes are in the midgut of the tick and downregulated during the migration to the salivary glands and transmission to the vertebrate host [172, 175]. Spirochetes also use OspA to bind the *Ixodes* tick TROSPA receptor, which facilitates their persistence in the tick midgut [176]. In contrast, outer surface protein C (OspC) is upregulated during the migration of the spirochetes to the salivary glands, and during the establishment of infection

and dissemination in the vertebrate host [177, 178]. The temporal expression of these proteins is consistent with their function, OspA is necessary for the colonization of and persistence in the midgut of the tick vector, while OspC plays a critical role for infecting the vertebrate host [179-181].

During infection of the vertebrate host, *B. burgdorferi* sl is exposed to the host complement system, which plays a key role in the innate immunity of vertebrates. The complement system consists of more than 30 molecules that are located in the blood of the vertebrate host. The activation of this biochemical cascade results in the formation of the membrane attack complex (MAC), which allows the vertebrate host organism to clear a diversity of pathogens. The complement cascade is activated by three major pathways, namely the classical pathway, the lectin pathway, and the alternative pathway [182, 183]. All three pathways generate C3 and C5 convertases (enzymes) that convert the protein C5 to C5a and C5b fragments. During the activation of the complement system via the alternative pathway, a C3b protein is deposited on all local cells irrespective of whether the cell is host or foreign. In addition, complement regulatory factors, such as factor H and factor H-like protein 1 (FHL-1), bind to host cells and protect them against complement activation [184, 185]. The C5b fragments are then converted to the membrane attack complex (MAC) [182], and this macromolecule structure is responsible for binding and breaking through the cell walls or envelop of pathogens, which subsequently leads to their death (Figure 3) [183, 186-188].

To avoid being killed by the vertebrate host complement system, *B. burgdorferi* sl has developed resistance mechanisms that have been the focus of extensive research. Many *B. burgdorferi* sl genospecies are able to acquire host complement regulatory factors, such as factor H and factor H-like protein 1 (FHL-1). Both factors are regulators of complement activation that have a major role in the alternative pathway of the vertebrate complement cascade. *B. burgdorferi* sl harbours many different surface-exposed lipoproteins such as members of the Erp protein family (e.g., OspE) and the complement regulatory-acquiring surface proteins (CRASPs) that can bind the complement regulatory factors of the vertebrate host (e.g. factor H and FHL-1) [186, 189-198]. When host-derived complement regulatory factors (e.g., factor H and/or FHL-1) are bound to these spirochete membrane proteins (e.g., Erps and CRASPs), the former can inhibit complement activation by rapidly inactivating newly formed C3b at the spirochete surface. Subsequently, factor H and FHL-1 accelerate the decomposition of C3 convertase thereby preventing the formation of the MAC and lysis of the spirochetes [199]. Thus, the ability of the *B. burgdorferi* sl Erps and CRASPs to bind the host

complement regulatory factors allows the spirochetes to avoid or suppress complement activation and thereby evade this component of the host immune system.

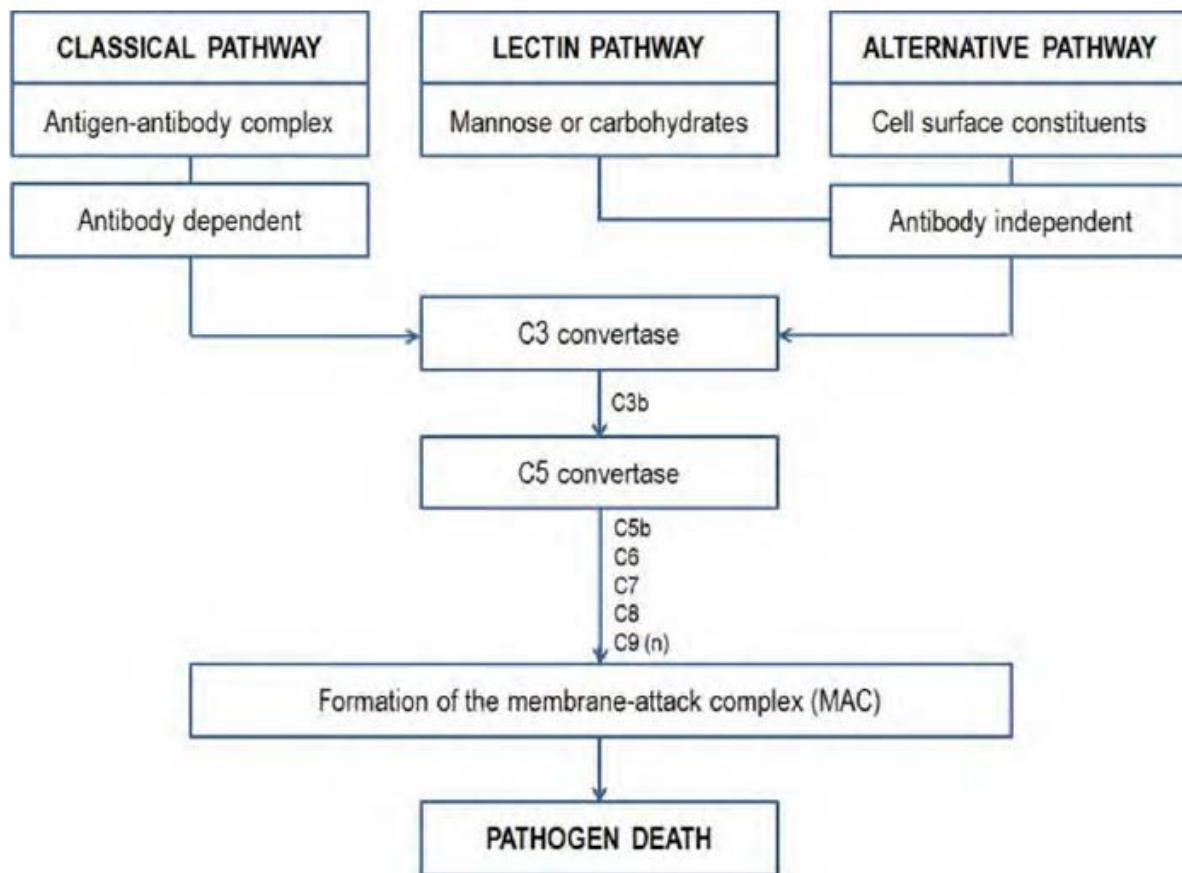


Figure 3. Overview of the three complement system activation pathways. The classical pathway is initiated by the binding of antibodies to antigens on a suitable target, such as a bacterial cell. The lectin pathway is initiated by the binding of the serum protein mannose-binding lectin (MBL) to mannose residues on glycoproteins or carbohydrates on the surface of pathogens. The alternative pathway is activated by the cell surface constituents of the pathogen that are foreign to the host. All pathways convert C3 to its active form, C3b, which participates in the formation of C5 convertase. With the formation of C5b, all pathways initiate the formation of the membrane attack complex (MAC), which eventually kills the pathogen. Reviewed from [182].

3.3.1 *Host-specificity*

In the 1990s, a great heterogeneity was observed among spirochetes circulating in Europe. Scientists knew at the time that these spirochetes present in ticks originate from vertebrate hosts and this incited them to reconsider the relationship existing between vertebrate hosts and the pathogens of Lyme disease. Subsequent field studies reported that majority of infections in the

skin or in ticks feeding on wild rodents were attributed to *B. afzelii*, whereas the majority of infections in questing ticks were attributed to *B. afzelii* and *B. garinii* [103, 200-202]. The absence of *B. garinii* in ticks feeding on rodents, despite its significant presence in local tick populations, suggested that rodent hosts are not competent reservoirs for this genospecies. Similarly, ticks collected from passerine birds were frequently infected with *B. garinii* despite the fact that *B. afzelii* was common in questing ticks [203-213]. The absence of *B. afzelii* in ticks feeding on passerine birds, despite its high prevalence in local tick populations, suggested that birds are not competent reservoir hosts for this genospecies. At the same time, individual ticks were reported to be infected with multiple genospecies of *B. burgdorferi* sl [214-217] but the analysis of mixed infections reported that *B. garinii* segregate from *B. afzelii* [218]. These findings emphasized the concept that *B. afzelii* and *B. garinii* evolve in different vertebrate host species. It was not until very much later that experimental infection studies reported host specificity. *B. garinii* was able to establish infection and achieve high host-to-tick transmission in avian reservoir hosts, whereas *B. afzelii* did not survive [219]. The fact that *B. afzelii* and *B. garinii* are sympatric (e.g., occur in the same local tick population) further supports the host-specificity hypothesis. In brief, *B. afzelii* cycles between ticks and rodents, and is negatively selected in ticks feeding on avian hosts, while *B. garinii* cycles between ticks and birds, but the negative selection in ticks feeding on rodent hosts remains to be determined.

The origin of the host-specificity of *B. afzelii* and *B. garinii* was attributed to the host complement system [187, 218, 220]. The complement-mediated lysis of a particular *B. burgdorferi* sl genospecies was shown to depend on its resistance and/or sensitivity to the blood serum complement of a given vertebrate host [220]. For example, *B. afzelii* was found to be sensitive to bird complement but resistant to rodent complement, which makes rodents appropriate hosts for this genospecies [220]. In contrast, *B. garinii* was sensitive to rodent complement but resistant to bird complement, which makes birds appropriate hosts for this genospecies [220]. *B. burgdorferi* ss had an intermediate resistance to both rodent and bird complements [220]. Another important finding of this study was that serum from ungulates (e.g., deer) lysed all *B. burgdorferi* sl genospecies, which correlated with their reported reservoir incompetence [89, 90].

Complement mediated lysis can occur in the vertebrate host but it can also occur in the midgut of the tick when that tick ingests blood that contains active components of the complement system [221]. Kurtenbach and colleagues suggested that genospecies-specific borreliacidal effects might amplify or clear the spirochete population depending on whether the host blood meal is matched or mismatched with the *B. burgdorferi* sl genospecies [187, 218].

For example, a study infested pheasants with nymphs infected with either *B. afzelii*, *B. burgdorferi* ss, *B. garinii*, or *B. valaisiana*, and tested the nymphs for the presence of spirochetes after they had fed to repletion [219]. Engorged nymphs tested positive for *B. burgdorferi* ss and *B. garinii* whereas *B. afzelii* was not detected in any nymphs. A later study showed that cultures of *B. afzelii*-infected nymphs that were allowed to feed on common blackbirds and great tits did not yield any viable *B. afzelii* spirochetes [222]. These two studies indicate that the rodent-associated *B. afzelii* was cleared from the midgut of nymphs that were fed on birds whereas the bird-associated *B. garinii* remained detectable. Thus, the genospecies-specific borreliacidal effects of the complement system are consistent with the associations between *B. burgdorferi* sl genospecies and their vertebrate hosts observed in the laboratory and the field and are summarized in Figure 4.

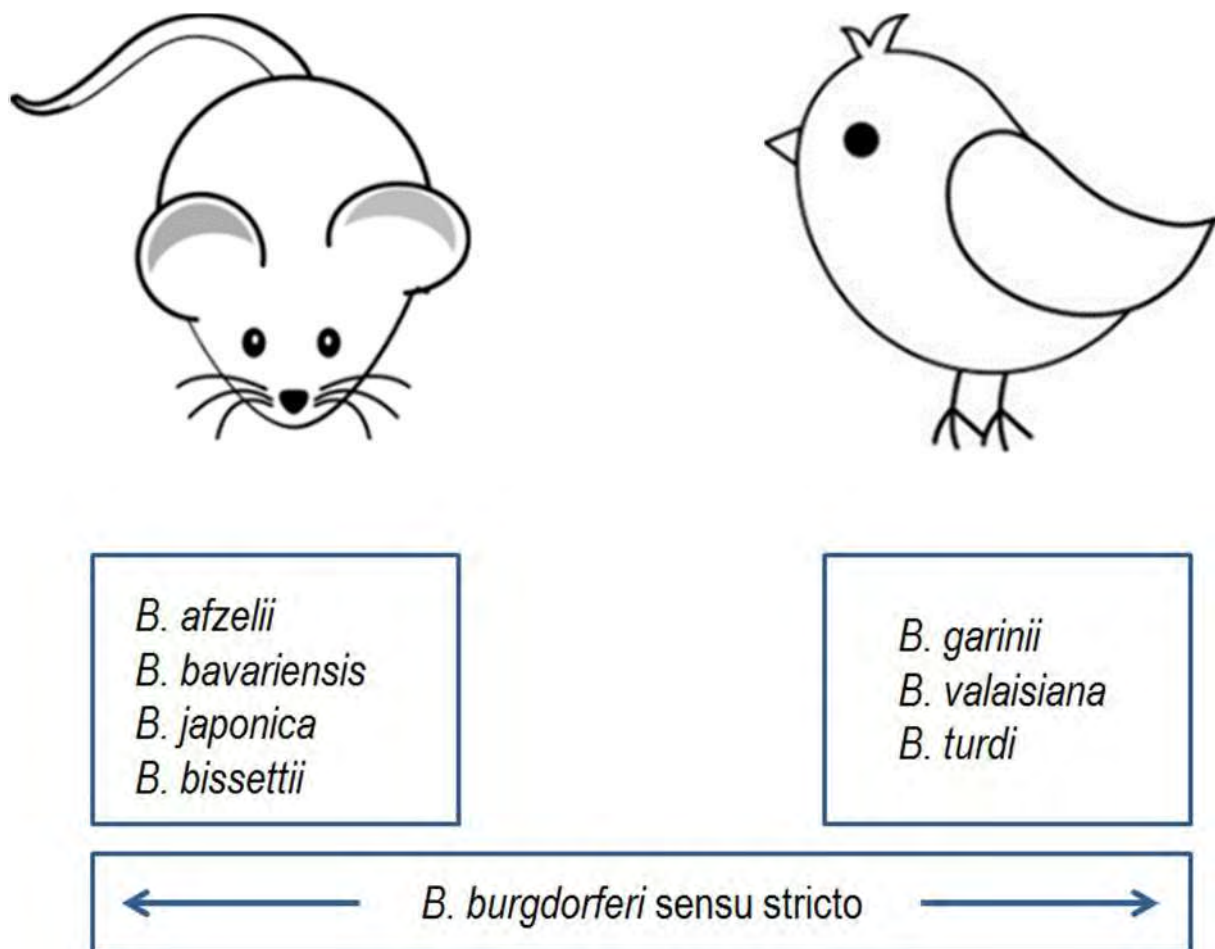


Figure 4. Host reservoir competence is shown for some members of the *B. burgdorferi* sl genospecies complex. The rodent-associated genospecies include *B. afzelii*, *B. bavariensis* (formerly referred to as *B. garinii* OspA serotype 4), *B. japonica* and *B. bissettii*. The bird-associated genospecies include *B. garinii*, *B. valaisiana*, and *B. turdi* [223-226]. *Borrelia*

burgdorferi ss genospecies can be found in both rodent and avian reservoir hosts. This picture was taken from [187].

3.4 Aims of the PhD thesis

The purpose of the present work was to better understand how ecological factors influence the abundance of *I. ricinus* ticks and hence the risk of Lyme disease. We used data from a 15-year study that monitored the monthly abundance of *I. ricinus* nymphs and adult ticks at four different elevations on Chaumont Mountain, in the canton of Neuchâtel, Switzerland. We collected climate variables that are known to be important for tick ecology (e.g., temperature, relative humidity, saturation deficit, precipitation, and snow height) and we obtained data on seed production by beech trees from the literature. Whenever the density of nymphs, the density of infected nymphs, and/or the nymphal infection prevalence are discussed, we referred to the density of *questing* (infected) nymphs and, by extension, the *questing* nymphal infection prevalence. However, we used the DON, DIN, and NIP for brevity throughout the present work.

In the first chapter, we studied whether the density of *I. ricinus* nymphs and adult ticks changed over time at the four different elevation sites, and whether any such changes in tick abundance were correlated with climate change. We expected tick abundance to be higher at the lower elevations compared to higher elevations. We expected tick abundance to increase over time at all elevations, and that this increase would be more pronounced at the higher elevations compared to the lower elevations. Finally, we expected that the intensity of seed production by beech trees would drive the DON two years later and the density of adult ticks (DOA) three years later.

In the second chapter, we tested whether the NIP and the DIN changed over time at the four different elevation sites. We had previously shown that the DON doubled over time at this study location and that seed production by beech trees had a strong and positive effect on the DON two years later [227]. Assuming that the NIP remains constant over time, we expected that the DIN would double over the duration of the study and that beech masting would be important for explaining inter-annual variation in the DIN with the expected 2-year time lag.

In the third chapter, we built on our previous modelling efforts of the same data [227, 228] to investigate three objectives. First, determine whether the developmental diapause hypothesis or the direct development hypothesis is better at explaining inter-annual variation in the fall peak of nymphs. Second determine whether seasonal climate means are better than annual climate means at predicting inter-annual variation in the density of nymphs, and which seasonal climate variables are important. Third, determine whether we can use generalized

additive models (GAMs) to model the complex bimodal seasonal phenology of *I. ricinus* nymphs and whether this approach yields additional insights into the factors that explain seasonal variation in tick abundance.

The fourth chapter contains the work that was my original PhD thesis project. This project used experimental infections to study the host-specificity of the rodent-adapted *B. afzelii* and the bird-adapted *B. garinii*. The purpose of the study was to create *Borrelia*-infected *I. ricinus* via needle-inoculation of laboratory mice (*Mus musculus*) and canary finches (*Serina canaria*) with different strains of *B. afzelii* and *B. garinii*, and great tits (*Parus major*) with different strains of *B. garinii*. All vertebrate hosts were subsequently infested with *I. ricinus* larvae, and the engorged larvae were allowed to moult into nymphs. With respect to *B. afzelii*, our predictions were that *B. afzelii* strains would establish infection in mice but not in birds and that only mice would transmit *B. afzelii* to immature *I. ricinus* ticks. Conversely, with respect to *B. garinii*, our predictions were that *B. garinii* strains would establish infection in birds but not in mice and that only birds would transmit *B. garinii* to immature *I. ricinus* ticks. However, we did not succeed in creating *Borrelia*-infected nymphs, which was essential for the completion of the project. Since reinvesting time and resources into a second attempt was not possible in the situation of our laboratory, we took the decision to abandon this project and developed a new PhD project (see Chapters 1, 2, and 3).

4 Chapter 1

Climate and tree seed production predict the abundance of the European Lyme disease vector over a 15-year period

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RESEARCH

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Climate and tree seed production predict the abundance of the European Lyme disease vector over a 15-year period

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Abstract

Background: To predict the risk of tick-borne disease, it is critical to understand the ecological factors that determine the abundance of ticks. In Europe, the sheep tick (*Ixodes ricinus*) transmits a number of important diseases including Lyme borreliosis. The aim of this long-term study was to determine the abiotic and biotic factors driving the annual abundance of *I. ricinus* at a location in Switzerland where Lyme borreliosis is endemic.

Methods: Over a 15-year period (2004 to 2018), we monitored the abundance of *I. ricinus* ticks on a monthly basis at three different elevations on Chaumont Mountain in Neuchâtel, Switzerland. We collected climate variables in the field and from nearby weather stations. We obtained data on beech tree seed production from the literature, as the abundance of *Ixodes* nymphs can increase dramatically two years after a masting event. We used AIC-based model selection to determine which ecological variables drive annual variation in tick density.

Results: We found that elevation site, year, seed production by beech trees two years prior, and mean annual relative humidity together explained 73.2% of the variation in our annual estimates of nymph density. According to the parameter estimates of our models, (i) the annual density of nymphs almost doubled over the 15-year study period, (ii) changing the beech tree seed production index from very poor mast (1) to full mast (5) increased the abundance of nymphs by 86.2% two years later, and (iii) increasing the field-collected mean annual relative humidity from 50.0 to 75.0% decreased the abundance of nymphs by 46.4% in the same year. Climate variables collected in the field were better predictors of tick abundance than those from nearby weather stations indicating the importance of the microhabitat.

Conclusions: From a public health perspective, the increase in nymph abundance is likely to have increased the risk of tick-borne disease in this region of Switzerland. Public health officials in Europe should be aware that seed production by deciduous trees is a critical driver of the abundance of *I. ricinus*, and hence the risk of tick-borne disease.

Keywords: Beech tree, Climate change, *Fagus sylvatica*, *Ixodes ricinus*, Lyme disease, Mast years, Tick population ecology, Tick-borne disease

Background

Climate change has resulted in dramatic changes in the distribution and abundance of medically important arthropod vectors, such as mosquitoes, ticks and biting flies [1, 2]. These range expansions are expected to increase the incidence of vector-borne diseases that are transmitted by these arthropod vectors with serious consequences for human health [3]. For example, the

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range expansion of *Ixodes* ticks into northern latitudes has resulted in a dramatic increase in the incidence of Lyme borreliosis and other tick-borne diseases in Canada and Scandinavia [4–9]. In contrast, whether climate change is affecting the abundance of *Ixodes* ticks in areas where they are endemic is not clear [10–17].

Climate change will influence the population ecology of *Ixodes* ticks via its effects on both abiotic factors (e.g. climate variables) and biotic factors (e.g. vegetation or host community). *Ixodes* ticks spend ~98% of their time off the host and have to cope with seasonal changes in temperature and precipitation. Their life history traits (development, survival and reproduction) are highly sensitive to climate variables that are changing due to global warming [18]. For example, tick development rates and survival increase with temperature and relative humidity, respectively [19–22]. However, we currently do not have a good understanding of how climate change will influence the abundance of *Ixodes* ticks in endemic areas [2, 20].

With respect to biotic factors, climate change is expected to drive changes in plant and animal communities that will feed back on the population ecology of *Ixodes* ticks. For example, climate influences seed production in beech and oak trees [23–26], which affects the population dynamics of animal species that use these seeds as a food resource, such as rodents and birds [27, 28]. In turn, fluctuations in rodent abundance can drive variation in the abundance of *Ixodes* ticks because ticks feed on rodents [10, 27, 29–31]. Previous work in North America has shown that the density of *Ixodes* ticks and the risk of Lyme disease depend on the abundance of rodents in the previous year and on the abundance of acorns two years previously [10, 31–33]. In contrast, in Europe there is no direct evidence demonstrating that tree seed production is driving the population dynamics of *Ixodes* ticks [34–36].

In Europe, the sheep tick (*Ixodes ricinus*) transmits a number of important diseases including Lyme borreliosis [37]. *Ixodes ricinus* is a generalist tick species that feeds on a variety of vertebrate hosts, including mammals, birds and reptiles [38]. *Ixodes* ticks have three active stages: larva, nymph and adult; each stage requires a single blood meal to develop to the next stage (female ticks take a blood meal to produce eggs). Blood meals often occur in different years, and the total life-cycle can range from 3 to 5 years. Thus, *Ixodes* tick population ecology is characterized by cohorts of larvae, nymphs and adults that were born in different years, but that search for hosts at the same time of year. In addition, *Ixodes* ticks have highly seasonal activity patterns; they search for hosts during the spring, summer, and fall and enter diapause during the winter. As most tick sampling methods only

capture questing ticks (i.e. ticks searching for a blood meal), within-year field studies inevitably capture the dramatic seasonal changes in the abundance of questing ticks.

The aim of this study was to better understand how climate and altitude influence the ecology of *I. ricinus* ticks. We used a long-term study to test whether the density of *I. ricinus* has increased along an altitudinal gradient in Switzerland, and whether any such increase in tick abundance was correlated with climate change. Specifically, we used data from a 15-year study that monitored the monthly abundance of *I. ricinus* nymphs and adult ticks at four different elevations on Chaumont Mountain, in the canton of Neuchâtel, Switzerland. We collected climate variables that are known to be important for tick ecology (e.g. temperature, relative humidity, saturation deficit and precipitation) and we obtained data on seed production by beech trees from the literature. We expected tick abundance to be higher at the lower elevations compared to the higher elevations. We predicted tick abundance to increase over time at all elevations, and that this increase would be more pronounced at the higher elevations compared to the lower elevations. Finally, we predicted that the intensity of seed production by beech trees would drive the abundance of nymphs two years later and the abundance of adult ticks three years later.

Methods

Study location

The study was conducted on the south-facing slope of Chaumont Mountain, which is part of the Jura mountains, and is located in the canton of Neuchâtel, in western Switzerland. The forest on Chaumont Mountain is mainly composed of European beech (*Fagus sylvatica*; 28.6%), Norway spruce (*Picea abies*; 28.5%), European silver fir (*Abies alba*; 20.4%), sycamore maple (*Acer pseudo-platanus*; 5.9%), European ash (*Fraxinus excelsior*; 3.7%), Scots pine (*Pinus sylvestris*; 2.3%), sessile oak (*Quercus petraea*; 2.3%), willows (*Salix* spp.; 2.1%), common whitebeam (*Sorbus aria*; 1.6%), and European hornbeam (*Carpinus betulus*; 1.0%). There is logging activity in the area, and there are also hiking trails and recreation areas for the public.

Four tick sampling sites, referred to as low, medium, high and top, were established at elevations of 620, 740, 900 and 1073 m above sea level (ASL), respectively. The four sites have been previously described [39, 40]. During the study, the canton of Neuchâtel approved the construction of a network of mountain bike trails and an adventure park, which were located at a distance of 25 m from the top site. The construction of the network of mountain bike trails occurred from 2006 to 2010, and

maintenance work on the trail is done every year. The construction of the adventure park started in 2011 and each year it is heavily used by the public from April to October. We believe that the construction of these recreation facilities reduced the quality of the forest habitat at the top elevation and compromised its integrity as a long-term field site.

Tick collection

Questing *I. ricinus* nymphal and adult ticks were collected on a monthly basis over a period of 15 years from January 2004 to December 2018 at each of the four elevations. The sampling protocol has been previously described [41]. Briefly, a 1-m² cotton flag was dragged across low vegetation over a distance of 100 m (low elevation) or 120 m (other elevations). The flag was inspected every 20 m and nymphs and adults were counted separately. The same person (Olivier Rais) conducted all of the 3427 drags (15 years × 12 months × 5 or 6 drags × 4 elevations = 4140 drags). Drags were not performed when there was snow on the ground (713 drags).

Field-collected climate data

Temperature (T; units are °C) and relative humidity (RH; units are %) were recorded at 60 cm above ground at one moment in time on the day of tick collection (usually between 10:00 am and 2:00 pm) at each sampling site using a thermohygrometer (Model 615, Testo SA, Lonay, Switzerland). Thus, for each combination of elevation and year, we had a total of 12 field-collected measurements of temperature and relative humidity. The saturation deficit (SD) is a measure of the drying power of the atmosphere (units are mm of mercury) and is calculated using temperature (T) and relative humidity (RH) as follows: $SD = (1 - RH/100) \times 4.9463 \times e^{0.0621T}$ [42, 43]. We confirmed that our field-collected climate data were accurate by comparing them to the Climap-net data (Additional file 1: Sections 1 and 2).

Climap-net data

In addition to the field-collected data, we obtained climate data from the Climap-net database of the Swiss meteorological center (MeteoSuisse). Two weather stations close to our four elevation sites are located in Neuchâtel at 485 m ASL and in Chaumont at 1136 m ASL. These weather stations sample at 200 cm above ground the temperature and relative humidity every hour, and the total precipitation each day. We used the daily mean temperature (average of the 24-hourly measurements), the daily mean relative humidity, and the daily total precipitation. Thus, for each year, we had a total of 365

Climap-net measurements of the daily mean temperature, the daily mean relative humidity, and the daily total precipitation. The saturation deficit was calculated as previously described. For each of the four elevation sites, we calculated site-specific climate variables by interpolating the data between the two weather stations (Additional file 1: Section 3).

Tree masting data

Mast refers to the fruit of forest trees, such as the acorns of oak trees and the beech nuts of beech trees. Data on masting (or seed production) were obtained for Neuchâtel from the MASTREE database for two important tree species: European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) [44]. These two species accounted for 57.1% of the trees at our study location. In this database, the mast intensity is classified into five classes: 1, 2, 3, 4, and 5, which refer to very poor mast, poor mast, moderate mast, good mast, and full mast, respectively.

Statistical methods

Statistical analyses were restricted to the three lower elevation sites (low, medium and high). The top elevation was excluded because tick abundance at this site declined dramatically following the construction of the network of mountain bike trails and the adventure park. In Additional file 1: Sections 4 and 5, the data are analyzed with all four elevation sites together.

Annual cumulative tick density

The cumulative nymph density (CND) is a measure of the total annual abundance of questing nymphs per 100 m² and was estimated by integrating the area under the curve (AUC) of the 12 monthly questing nymph densities for each year [43, 45]. We used this AUC approach because it is less likely to be biased by missing data compared to calculating a simple average for each year. We assume that the CND represents a small unknown fraction of the nymphs that were actually present in the area. No dragging was performed on days when there was snow on the ground (hereafter referred to as snow days). Over the study period (15 years × 12 months = 180 days), a total of 34 snow days occurred, and these were coded as missing data. In summary, tick abundance data from 2581 individual drags were collapsed into 45 estimates of CND (15 years × 3 elevations = 45 annual estimates of abundance). The same approach was used to calculate the cumulative adult tick density (CAD).

Annual mean climate variables

To investigate the relationship between climate and our annual estimates of tick abundance, we collapsed our

monthly or daily weather data into a single annual value. For the field-collected data, the annual means were calculated over the 12 monthly measurements (i.e. a single measurement for each month). For the Climap-net data, the annual means were calculated over 365 daily means (i.e. a total of $365 \text{ days} \times 24 \text{ measurements/day} = 8760$ hourly measurements). Thus, the Climap-net annual means were based on 730 times more data than the field-collected annual means. However, an important advantage of the field-collected data was that they were specific for each of the four elevation sites. In contrast, the Climap-net data came from two weather stations that were located at some distance from the four elevation sites. To facilitate comparison between the slopes of the climate variables, we standardized the climate variables to z-scores (mean of 0, standard deviation of 1).

Annual tree masting variables

The model by Ostfeld et al. [10] predicts a 2-year time lag between masting events and the CND and a 3-year time lag between masting events and the CAD. To validate the model by Ostfeld et al. [10], we compared five different time lags where the CND is predicted by mast events that occurred 0, 1, 2, 3 and 4 years into the past (Additional file 1: Section 9). This analysis confirmed that the 2-year time lag is an order of magnitude better than all of the other time lags considered. The 2-year time lag has a partial r^2 value of 26.3% and a P -value of 0.0002. In contrast, the partial r^2 values of the other four time lags (0, 1, 3 and 4 years in the past) are much lower (range: 0.0–5.1%) and none of the P -values are statistically significant. This preliminary analysis validates our decision to model the CND and CAD as a function of the tree mast scores (of European beech and Norway spruce) two years previously (year $y-2$) and three years previously (year $y-3$), respectively. For example, tree mast scores from the year 2001 predicted the CND in year 2003 (2 years later) and the CAD in year 2004 (three years later).

Analysis of the annual variation in nymph and adult tick abundance using linear models

Count data follow a Poisson distribution or a negative binomial distribution. However, our estimates of the CND (or CAD) are summary statistics (sums or integrals) that are based on the counts of ~60 drags (12 dates $\times$ 5 drags per date). According to the central limit theorem of statistics, summary statistics will follow a normal distribution even if the observations on which they are based are drawn from a non-normal distribution. We therefore assumed that the residuals of our CND values follow a normal distribution. The CND values were $\log_{10}$ -transformed to further improve their fit to the normal distribution. The $\log_{10}$ -transformed CND values ($n =$

45) were analyzed using linear models (LM) with normal errors. In section 11 of Additional file 1, we show that the analysis of the CND using generalized linear models with negative binomial errors (which are appropriate for over-dispersed count data) gives the same results.

The explanatory variables included the elevation site (3 levels: low, medium, high), the covariate year (rescaled as 1, 2, 3, ... 15), the covariate beech mast score (range: 1–5), the covariate spruce mast score (range: 1–5), mean annual temperature, mean annual RH, mean annual SD, and mean annual precipitation (all annual climate variables were transformed to z-scores). We used mean annual climate variables based on the field-collected data and on the Climap-net data to determine which climate variables were better for explaining variation in tick abundance. As time lags are known to be important in tick ecology, we modelled the CND as a function of the mean climate variable in the present year or the previous year. The same approach was used to model the CAD ($n = 45$).

Model selection approach

We used a model selection approach based on the Akaike information criterion (AIC) to find the most parsimonious model. Models were ranked according to their AIC values and the Akaike weights were calculated for each model. We used the Akaike weights to calculate the model-averaged parameter estimates and their 95% confidence intervals (CIs) (Additional file 1: Section 6). For the best model from the model selection table for the $\log_{10}$ -transformed CND (and CAD), we confirmed that the residuals met the assumptions of normality and equal variances (Additional file 1: Section 7). This result shows that we were justified in using linear models to analyze the CND and CAD.

We used R version 3.6.1 for all statistical analyses [46]. We used the *auc()* function (integrates the area under the curve) in the *flux* package to estimate the CND and the CAD for each year. We used the *lm()* function in the *base* package to model the $\log_{10}$ -transformed CND as a linear model of the explanatory variables. We used the *mod.sel()* function and the *model.av()* function in the *MuMIn* package. The *mod.sel()* function creates an automated model selection table using previously generated models (linear models with normal errors in our case), and the *model.av()* function calculates model-averaged parameter estimates and their standard errors. The raw data used for these statistical analyses can be found in Additional file 2: Table S1.

Results

Differences in climate between the elevation sites

The three elevation sites differed in climate. Across the 15 years of the study, the mean annual temperature was

warmest at the low elevation site (15.4 °C), intermediate at the medium elevation site (14.3 °C), and coldest at the high elevation site (13.2 °C) and these differences were significant (ANOVA: $F_{(2, 42)} = 9.431$, $P < 0.0001$). The mean annual relative humidity was lowest at the low elevation site (61.9%), intermediate at the medium elevation site (63.7%), and highest at the high elevation site (66.5%), but these differences were not significant (ANOVA: $F_{(2, 42)} = 1.948$, $P = 0.155$). The mean annual saturation deficit was highest at the low elevation site (6.3 mmHg), intermediate at the medium elevation site (5.5 mmHg), and lowest at the high elevation site (4.6 mmHg), and these differences were significant (ANOVA: $F_{(2, 42)} = 7.199$, $P = 0.002$). These analyses show that the three elevation sites differed with respect to temperature, humidity and saturation deficit.

Total number of ticks collected

Over the 15 years of the study, we collected a total of 39,255 *I. ricinus* ticks: 31,067 nymphs and 8188 adult ticks (4168 males and 4020 females) at the three lower elevation sites.

Variation in nymph abundance among elevation sites

We used a one-way ANOVA to compare the mean CND between the three elevations (i.e. this analysis ignores the other explanatory variables). The interpretation of the CND is the theoretical number of questing nymphs that would have been collected in an area of 100 m² over the course of the year if we had sampled the tick population each day (i.e. 365 sampling occasions per year). The mean CND was significantly different between the three elevation sites (ANOVA: $F_{(2, 42)} = 6661.4$, $P < 0.0001$). The mean CND (and 95% CI) for the low, medium, and high elevations were as follows: 21,532 (95% CI: 16,936–27,374), 17,793 (95% CI: 13,996–22,621), and 11,535 (95%

CI: 9073–14,665). The CND can be converted to a daily mean number of nymphs per 100 m² by dividing by 365 days. Thus, the mean number of nymphs collected in an area of 100 m² (and 95% CI) for the low, medium, and high elevations were as follows: 59.0 (95% CI: 46.4–75.0), 48.7 (95% CI: 38.3–62.0), and 31.6 (95% CI: 24.9–40.2). In summary, the mean nymph density was inversely related to the altitudinal gradient and it was highest at the low elevation site and lowest at the high elevation site.

Effect of the climate variables and mast years on variation in nymph abundance among years

For the log₁₀-transformed CND, the best six models had a combined support of 95.0% (Table 1). The best model had 76.0% of the support (Table 1), explained 73.2% of the variation in the log₁₀-transformed CND, and contained the explanatory variables of elevation site (*partial* $r^2 = 26.6\%$), year (*partial* $r^2 = 14.8\%$), beech mast score 2 years prior (*partial* $r^2 = 26.9\%$), and the field-collected relative humidity from the same year (i.e. no time lag; *partial* $r^2 = 7.6\%$). The five next-best models all contained the same explanatory variables of elevation site, year, and beech mast score, but differed with respect to the fourth explanatory variable, which included the site:year interaction, field-collected SD from the same year, field-collected temperature from the same year, field-collected RH from the previous year, and field-collected SD from the previous year (Table 1). The full model selection analysis with 52 models is presented in Additional file 1: Section 6.

Using the parameter estimates from the top models in Table 1, the effect sizes were calculated on the original scale of the CND with respect to the following baseline: the site was low elevation, the year was 2004, the beech tree mast index 2 years prior was set to 1, and the field-collected relative humidity from the same year was set to

Table 1 Model selection results for the linear models of the log₁₀-transformed cumulative nymphal density

Rank	Model structure	df	logLik	AIC	ΔAIC	Weight1	Weight2	r ²
1	log ₁₀ (CND) ~ S+Y+B+RH2	7	33.0	−48.7	0.0	76.0	76.0	73.2
2	log ₁₀ (CND) ~ S+Y+B+RH2+S:Y	9	33.6	−43.6	5.2	6.0	82.0	72.4
3	log ₁₀ (CND) ~ S+Y+B+SD2	7	30.4	−43.5	5.3	5.0	87.0	69.7
4	log ₁₀ (CND) ~ S+Y+B+T2	7	30.4	−43.4	5.3	5.0	92.0	69.6
5	log ₁₀ (CND) ~ S+Y+B+RH2 _{y-1}	7	29.4	−41.4	7.3	2.0	94.0	68.1
6	log ₁₀ (CND) ~ S+Y+B+SD2 _{y-1}	7	29.1	−40.8	7.9	1.0	95.0	67.7

Notes: Model selection results are shown for the linear models with normal errors of the log₁₀-transformed CND response variable. The explanatory variables were elevation site, year, beech masting 2 years prior, and the climate variables from the field and the Climap-net database. The models are ranked according to their Akaike Information Criterion (AIC). Of the 52 models in the set, only the 6 top models are shown for which the cumulative support (Weight 2) is 95%. Shown for each model are the model rank (Rank), model structure (see below for explanation of variable acronyms), model degrees of freedom (Df), log-likelihood (logLik), Akaike information criterion (AIC), difference in the AIC value from the top model (ΔAIC), model weight (Weight1), cumulative model weight (Weight2), and adjusted r-squared value (r²). Additional file 1: Section 6 shows the results from the full model selection. The acronyms for the explanatory variables are as follows: S, site; Y, year; B, beech mast score 2 years prior; S:Y, interaction between site and year; RH2, mean annual relative humidity from the field data in the same year; SD2, mean annual saturation deficit from the field data in the same year; T2, mean annual temperature from the field data in the same year; RH2_{y-1}, mean annual relative humidity from the field data in the previous year (y-1); SD2_{y-1}, mean annual saturation deficit from the field data in the previous year (y-1)

50.0%. For simplicity, we present the parameter estimates from the top model in Table 1 (Additional file 1: Section 8), but we note that these are qualitatively similar to the model-averaged parameter estimates for the 52 models in the full model selection analysis (Additional file 1: Section 6).

The $\log_{10}$ -transformed CND was significantly different between the three elevation sites (Figs. 1 and 2; Additional file 1: Section 8). The CND (on the original scale) at the low elevation was 11.4% higher than the medium elevation (*Medium-Low contrast in Y-intercept* = -0.053, *SE* = 0.045, *P* = 0.253). The CND (on the original scale) at the low elevation was 43.1% higher than the high elevation (*High-Low contrast in Y-intercept* = -0.245, *SE* =

0.005, *P* < 0.0001). In summary, the effect of elevation site in this multiple regression approach was the same as the results from the one-way ANOVA. The CND was inversely related to the altitudinal gradient; it was highest at the low elevation and lowest at the high elevation (Figs. 1 and 2; Additional file 1: Section 8).

The slope of the covariate year was positive (*slope* = 0.020 per year, *SE* = 0.005, *P* < 0.0001; Additional file 1: Section 8), indicating that the $\log_{10}$ -transformed CND was increasing over time at the three elevation sites. According to the estimate of the general slope for the three elevation sites on Chaumont Mountain, the CND (on the original scale) increased by 88.4% over the 15-year period of the study (2004–2018) (Fig. 3).

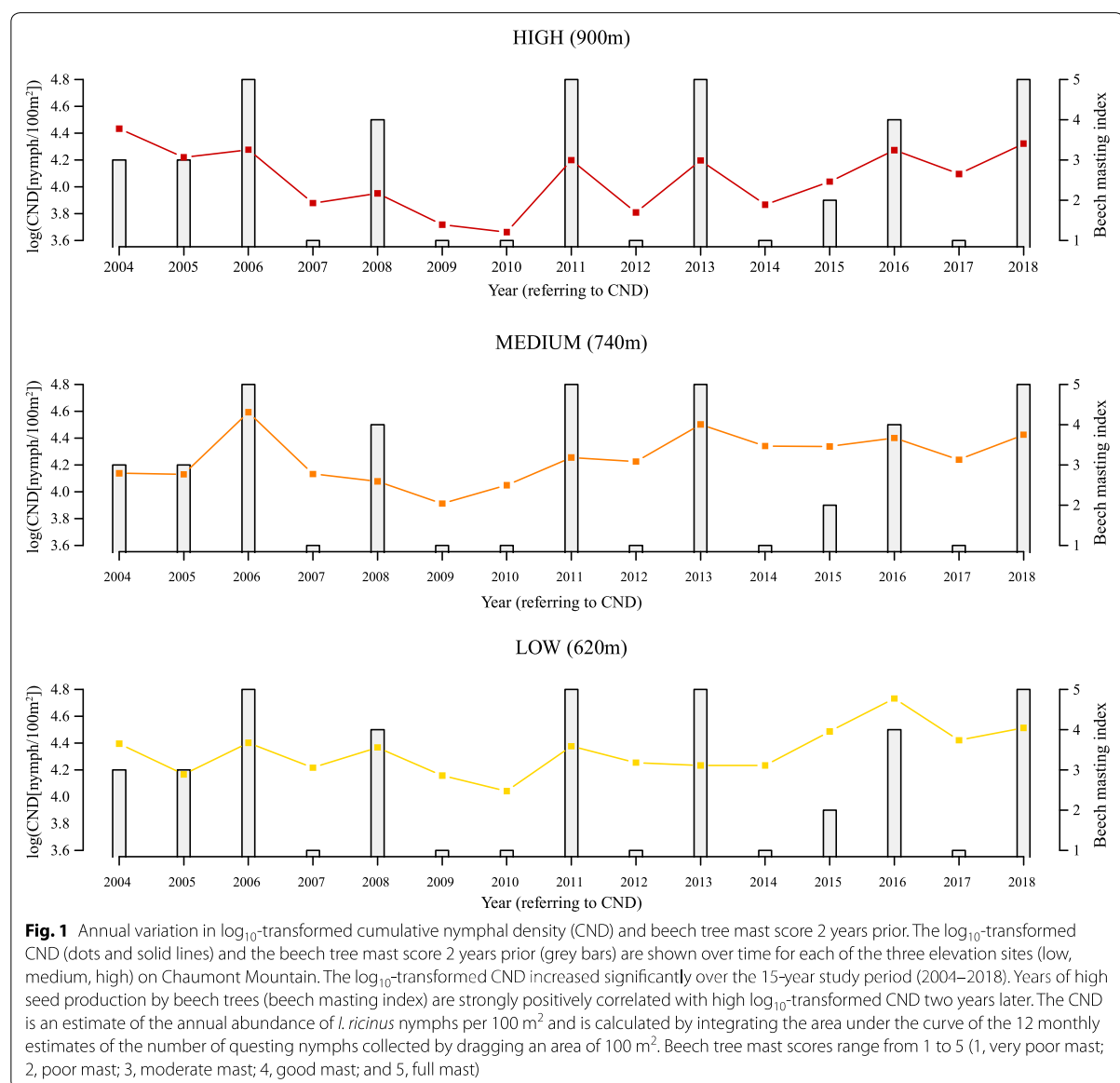


Fig. 1 Annual variation in $\log_{10}$ -transformed cumulative nymphal density (CND) and beech tree mast score 2 years prior. The $\log_{10}$ -transformed CND (dots and solid lines) and the beech tree mast score 2 years prior (grey bars) are shown over time for each of the three elevation sites (low, medium, high) on Chaumont Mountain. The $\log_{10}$ -transformed CND increased significantly over the 15-year study period (2004–2018). Years of high seed production by beech trees (beech masting index) are strongly positively correlated with high $\log_{10}$ -transformed CND two years later. The CND is an estimate of the annual abundance of *I. ricinus* nymphs per 100 m² and is calculated by integrating the area under the curve of the 12 monthly estimates of the number of questing nymphs collected by dragging an area of 100 m². Beech tree mast scores range from 1 to 5 (1, very poor mast; 2, poor mast; 3, moderate mast; 4, good mast; and 5, full mast)

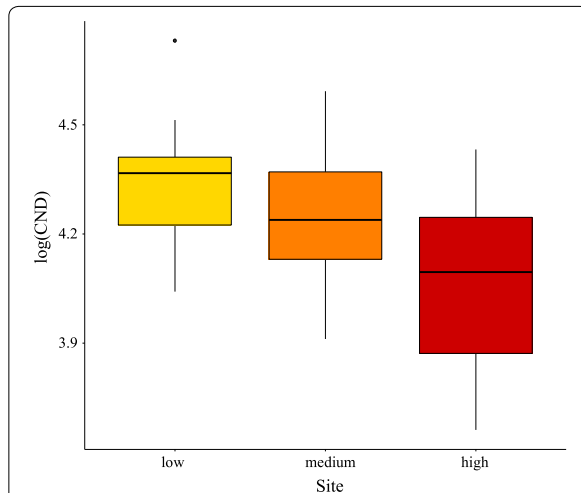


Fig. 2 Effect of elevation on the $\log_{10}$ -transformed cumulative nymphal density (CND). According to the model parameter estimates, the CND (on the original scale) at the low elevation was 11.4% higher than the medium elevation and 43.1% higher than the high elevation ($\text{partial } r^2 = 26.6\%$). The parameter estimates used to calculate the effect sizes were taken from the best model in Table 1, which had 76.0% of the support and explained 73.2% of the inter-annual variation in the $\log_{10}$ -transformed CND

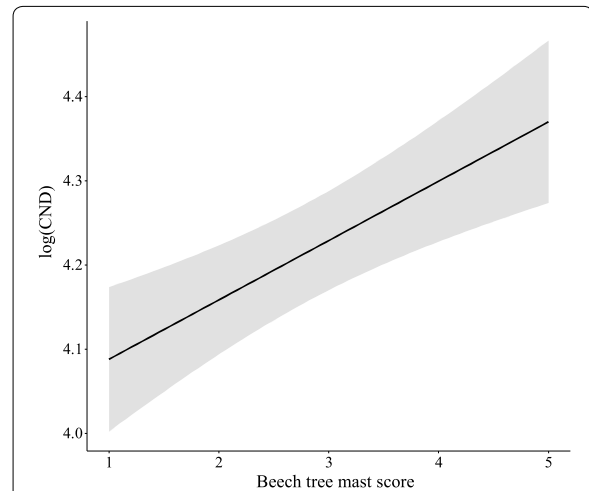


Fig. 4 Effect of beech mast score 2 years prior on the $\log_{10}$ -transformed cumulative nymphal density (CND). According to the model parameter estimates, increasing the beech mast score from 1 (poor mast) to 5 (full mast) increased the CND (on the original scale) by 86.2% ($\text{partial } r^2 = 26.9\%$). The parameter estimates used to calculate the effect sizes were taken from the best model in Table 1, which had 76.0% of the support and explained 73.2% of the inter-annual variation in the $\log_{10}$ -transformed CND

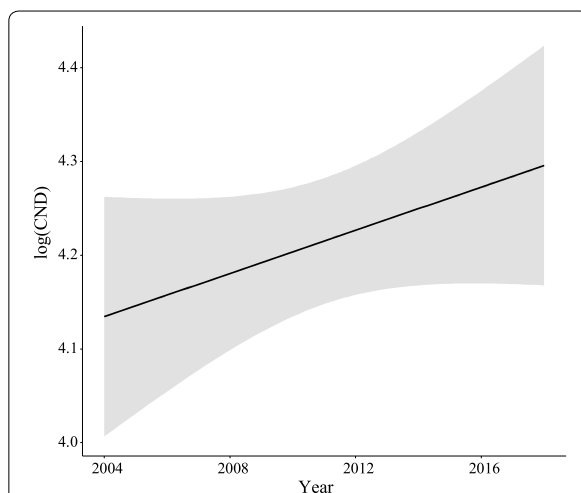
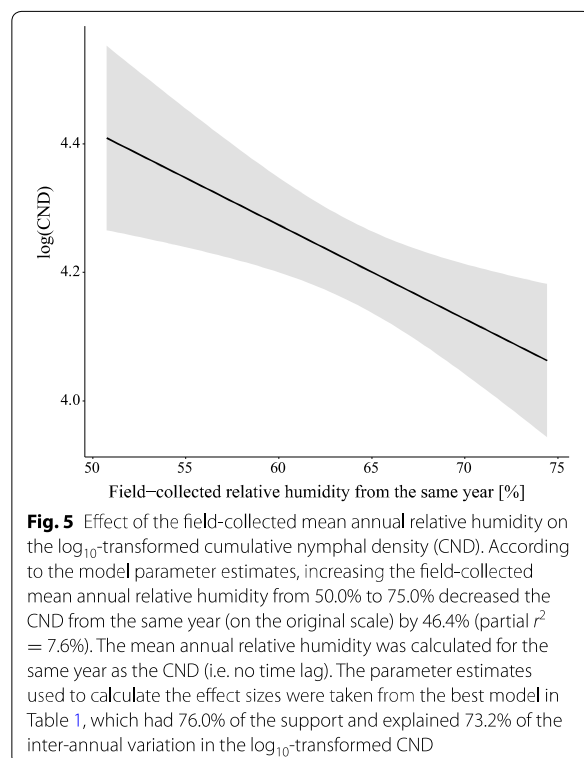


Fig. 3 Effect of year on the $\log_{10}$ -transformed cumulative nymphal density (CND). According to the model parameter estimates, the CND (on the original scale) increased by 88.4% over the 15-year study period ($\text{partial } r^2 = 14.8\%$). The parameter estimates used to calculate the effect sizes were taken from the best model in Table 1, which had 76.0% of the support and explained 73.2% of the inter-annual variation in the $\log_{10}$ -transformed CND

The beech mast score had a strong positive effect on the $\log_{10}$ -transformed CND two years later ($\text{slope} = 0.067$ per class, $SE = 0.011$, $P < 0.0001$; Fig. 1 and Additional

file 1: Section 8). According to the estimate of the general slope for the three elevation sites on Chaumont Mountain, increasing the beech mast score from 1 (poor mast) to 5 (full mast) increased the CND (on the original scale) by 86.2% (Fig. 4). Over the 15-year study period, beech trees produced 6 years of good or full mast scores (2004, 2006, 2009, 2011, 2014 and 2016), where high CND years were expected to occur two years later (2006, 2008, 2011, 2013, 2016 and 2018, respectively). At the low, medium, and high site, these 6 good mast years produced 3 (2016, 2018 and 2006), 4 (2006, 2013, 2018 and 2016), and 4 (2018, 2006, 2016 and 2011) of the six-highest CND values two years later (Fig. 1). For the adult ticks, the beech tree mast score had a strong positive effect on the $\log_{10}$ -transformed CAD three years later ($\text{slope} = 0.101$, $SE = 0.019$, $P < 0.001$, $\text{partial } r^2 = 21.0\%$; Additional file 1: Section 5).

The field-collected mean annual relative humidity at 60 cm above ground had a negative effect on the $\log_{10}$ -transformed CND in the same year ($\text{slope} = -0.074$ per standard deviation, $SE = 0.021$, $P = 0.001$; Additional file 1: Section 8). According to the estimate of the general slope for the three elevation sites on Chaumont Mountain, increasing the field-collected mean annual relative humidity from 50.0% to 75.0% decreased the CND from the same year (on the original scale) by 46.4% (Fig. 5).



The models with other climate variables had much less support (Table 1): field-collected mean annual SD from the same year (5%), field-collected mean annual temperature from the same year (5%), field-collected mean annual RH from the previous year (2%), and field-collected mean annual SD from the previous year (1%). We note here that field-collected mean annual RH (regardless of the time lag) always had a negative effect on nymph abundance, whereas field-collected mean annual temperature and field-collected mean annual SD always had a positive effect on tick abundance (Additional file 1: Section 6).

The analysis of the $\log_{10}$ -transformed CND for the four elevation sites gave very similar results (Additional file 1: Section 4). The only difference was that the best model contained an interaction between elevation and year. This interaction was driven by the dramatic decline in CND over time at the top site. The best model for the four-site analysis contained the effects of elevation site, year, beech mast score, and mean annual relative humidity in the same year (i.e. no time lag), which is identical to the three-site analysis. The parameter estimates were also very similar between the 3-site and 4-site analyses. We believe that the dramatic decline in CND at the top site was caused by

the construction of the recreation facilities during the study. For this reason, we presented the 3-site analysis of the CND in the main text. The analysis of the $\log_{10}$ -transformed cumulative adult density (CAD) for all four elevation sites is also presented in Additional file 1: Section 5.

In summary, the CND increased significantly over time and with tree seed production two years earlier while it decreased significantly with elevation and with the field-collected relative humidity in the same year.

Discussion

Climate change is driving the range expansion of arthropod vectors and the increased incidence of vector-borne diseases [3, 47]. In Europe and North America, there is currently much interest in determining which abiotic and biotic factors are influencing the abundance of *Ixodes* ticks and their associated pathogens. To address this question, we quantified the abundance of *I. ricinus* ticks over a period of 15 years in an area of Switzerland that is endemic for Lyme borreliosis. Variation in our annual estimates of cumulative nymph density was best explained by elevation site, year, abundance of beech nuts 2 years prior, and mean annual relative humidity in the same year. Nymph density increased by 88.4% (i.e. almost doubled) over the 15-year study period at the three elevation sites.

One of the most important results of this study is our demonstration of a strong positive association between seed production by beech trees and the density of nymphs two years later. The effect of beech seed production two years prior on the $\log_{10}$ -transformed CND was highly statistically significant ($P < 0.000001$). According to our parameter estimates, the effect size was large; changing the beech mast score two years prior from 1 to 5 increased the CND by 86.2%. Beech nut abundance 2-years prior explained at least 26.9% of the variation in our annual estimates of nymph abundance. By accounting for large inter-annual fluctuations in the CND, the inclusion of the beech mast index in our models revealed the effects of more subtle ecological factors, such as mean annual relative humidity and year. In keeping with the slow life-cycle of *Ixodes* ticks, the beech seed production increased the abundance of adult ticks three years later (Additional file 1: Section 5). The synchronized seed production in beech trees is driven by climate variables such as temperature and precipitation [23–26]. Some recent studies have suggested that climate change is increasing the frequency of masting events [23, 25, 48]. If true, this suggests that climate change could be increasing tick abundance in endemic areas *via* indirect effects on tree seed production; more frequent masting events would increase the long-term abundance of

reservoir hosts for ticks and tick-borne pathogens. Future long-term studies investigating whether climate change is affecting the abundance of *Ixodes* ticks and the incidence of tick-borne diseases should include the proper time-lagged estimates of tree seed production into their models.

The 2-year time lag between masting events and the abundance of nymphs was first discovered by Ostfeld and colleagues in the eastern USA where the blacklegged tick (*Ixodes scapularis*) is the vector of Lyme borreliosis [10, 31, 32]. The chain of causality is as follows. Mast seeding in year y increases the abundance of small mammals, deer, and larval ticks in year $y + 1$ [10, 27, 29, 31, 49, 50]. Higher densities of larval ticks coincide with and feed on higher densities of small mammals, which in turn increases the abundance of nymphs in year $y + 2$ [10, 31–33, 51–53]. In summary, there is good evidence in the eastern USA that mast events drive the abundance of *I. scapularis* nymphs with a 2-year time lag. In contrast, there are few studies in Europe that have demonstrated this phenomenon for *I. ricinus*. A 7-year study in Poland found the expected 2-year time lag between oak mast seeding and the incidence of Lyme borreliosis, but the causal link between tree seed production and nymphal abundance was not demonstrated [35]. A 10-year study in the Netherlands found that the mast scores of common oak trees and beech trees in year y had a negative effect on larvae, nymphs and adults in year $y + 1$ [34]. This result is expected because once ticks obtain a blood meal, they are no longer available to be sampled *via* dragging. An 18-year study in central Europe found a strong correlation between rodent densities in year y and tick-borne diseases in year $y + 1$, but did not demonstrate a direct link to ticks or tree seed production [36]. Thus, our study is the first demonstration that mast seeding events are strongly associated with the inter-annual abundance of *I. ricinus* ticks in Europe, which in turn influences the risk of tick-borne diseases [35].

An important contribution of our study is the demonstration that only the 2-year time lag was important for explaining variation in the CND (Additional file 1; Section 9). Masting with a 2-year time lag had a P -value of < 0.000001 . When the time lag between masting and the CND was changed to 0, 1, 3 or 4 years, the relationship was no longer statistically significant. Masting with a 2-year time lag had a partial r^2 value of 26.3%. In contrast, for the other time lags (0, 1, 3 and 4 years in the past), the partial r^2 values of masting ranged from 0.0 to 5.1%. This analysis clearly shows that variation in the CND at our study location was strongly associated with masting with a 2-year time lag and not with any other time lag.

Our long-term study is a rare demonstration that the abundance of *Ixodes* ticks has increased in a Lyme

disease-endemic area. We found that the abundance of nymphs at the three lower elevation sites increased by 88.4% from 2004 to 2018. A 10-year study in the Netherlands found an increase in the abundance of *I. ricinus* larvae and adults [34]. In contrast, a 15-year study at a site in Neuchâtel, Switzerland, that is very close to the location of the present study found a significant decrease in the abundance of *I. ricinus* nymphs [11]. Another 8-year study in central New Jersey, USA, found no significant directional change in the abundance of *I. scapularis* nymphs [12]. Other long-term studies have found large inter-annual fluctuations in density, but none of them found that *Ixodes* tick density was increasing [10, 13, 14]. Increased tick abundance could have important consequences for the incidence of tick-borne diseases and public health. All else being equal, our study suggests that the risk of tick-borne disease has doubled at this location in Switzerland.

Theoretical models have predicted that *Ixodes* ticks will increase their distribution and abundance under global warming [6, 54–56]. Numerous empirical studies have shown that *Ixodes* ticks and tick-borne diseases are emerging public health problems at the northern limit of their geographic distribution [8, 57–59]. In contrast, fewer studies have investigated whether global warming has influenced *Ixodes* tick abundance in areas where Lyme disease is endemic [10–17]. The present study did not find direct links between climate change and the observed doubling in tick abundance. Mean annual relative humidity in the same year was the climate variable that had the greatest impact on the CND, but there was no evidence that it had undergone directional change over the 15-year study period (Additional file 1: Section 10). Conversely, the mean annual temperature increased significantly over the 15-year duration of the study (i.e. evidence of directional climate change; Additional file 1: Section 10), and while temperature had a significant and positive effect on the CND (Additional file 1: Section 6), models that contained temperature had low support (5.7%; Additional file 1: Section 6). In summary, despite finding a significant increase in temperature and ticks over the 15-year study period, the evidence for a causal link between the two is weak.

An interesting result is our demonstration that the mean annual relative humidity had a negative effect on the abundance of nymphs in the same year. This result was unexpected, as numerous studies have shown that survival of immature *Ixodes* ticks increases with relative humidity [18, 19, 60]. However, this result is not without precedent and other studies in Europe have found a negative relationship between relative humidity and the abundance of *I. ricinus* nymphs [61–65]. One explanation

is that humid environments are favorable for the development of entomopathogenic fungi, which have been shown to cause high mortality in *Ixodes* ticks [66, 67]. A striking result was that the field-collected climate variables (measured at 60 cm above ground) were much more important for predicting tick abundance than climate variables from nearby weather stations (measured at 200 cm above ground). This result is even more remarkable when one considers that the annual means from the Climap-net data are based on 730 times more data (365 days $\times$ 24 hours = 8760 measurements per year) compared to the field-collected data (12 measurements per year). A recent long-term study at a nearby site in Neuchâtel [11] found the same result: field-collected climate variables were better than weather station climate variables at explaining inter-annual variation in tick abundance. The explanation is that the field-collected data measure the local conditions, whereas the weather stations are some distance away from the elevation sites. We suspect that microclimate factors like soil and vegetation are influencing the field-collected annual climate means to such an extent that they have only a weak resemblance to the weather station annual climate means (Additional file 1: Section 10). Our study suggests that researchers should make the effort to measure local climate variables rather than relying on data from nearby weather stations.

Ticks were found at all three elevations (620, 740 and 900 m ASL), but there were important differences in their abundance. Nymphal tick abundance was negatively correlated with elevation, which is in agreement with previous studies [39, 40]. A mechanistic explanation for this phenomenon is the relationship between temperature and tick development rates [18, 21]. At higher and colder elevations, eggs and larvae have much slower development rates, which ultimately reduces the number of larvae that reach the nymphal stage [20, 68, 69].

The nymphal tick abundance was lowest at the top site (1073 m ASL). At the top site, the CND decreased by 81.0% over the 15 years of the study, whereas at the other three elevation sites, the CND increased by 88.4% over the same time period (Additional file 1: Section 4). Thus, the population dynamics at the top site were very different from the low, medium, and high elevation sites. One explanation is the construction of recreation facilities at a distance of ~25 meters from our top site. These recreation facilities, which include a network of mountain bike trails (constructed from 2006 to 2010) and an adventure park with a zip line and outdoor laser games (constructed in 2011), have greatly increased the number of human visitors to the top of Chaumont Mountain. Thus, the destruction of the forest habitat and subsequent human disturbance may have caused the decline of the *I. ricinus*

tick populations over time at our top site. An interesting alternative explanation is that the monthly tick sampling over a period of 15 years decreased the CND at the top site. Field studies typically assume that dragging removes a small fraction of the available tick population, but this assumption may not be true in habitats where tick density is already low.

Conclusions

We found that the abundance of *I. ricinus* nymphs almost doubled over 15 years at our study location in Switzerland. From a public health perspective, this increase in nymph abundance is likely to have increased the risk of tick-borne disease in this region. Beech mast years at 2 and 3 years prior were strongly and positively associated with the abundance of nymphs and adult ticks, respectively. We found that relative humidity had a negative effect on nymph abundance, which was surprising because immature *Ixodes* ticks are known to be sensitive to desiccation. We found no direct association between climate change and the doubling of nymphal tick abundance. However, there could be indirect effects if climate change is increasing the frequency of masting events and thereby increasing the abundance of reservoir hosts and ticks. Public health officials should be aware that mast years are an important time-lagged predictor of tick abundance and the incidence of tick-borne diseases.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13071-020-04291-z>.

Additional file 1: Section 1. Validation of the field-collected temperature data. **Section 2.** Validation of the field-collected relative humidity data. **Section 3.** Interpolation of the Climap-net climate data. **Section 4.** Full statistical analysis of the cumulative nymph abundance (CND) for the four elevation sites. **Section 5.** Full statistical analysis of the cumulative adult abundance (CAD) for the four elevation sites. **Section 6.** Full model selection table, support of each individual explanatory variable, and model-averaged parameter estimates of the nymph abundance for the three lowest elevation sites. **Section 7.** Assumptions of the linear models for the best models from the AIC-based model selection approach of the nymph and adult abundance. **Section 8.** Parameter estimates of the top model in the model selection table of the nymph abundance for the three lowest elevation sites. **Section 9.** Analysis of different time lags between the cumulative nymph abundance and beech masting. **Section 10.** Climate change over the 15-year study period. **Section 11.** Analysis of CND using Generalized Linear Models with negative binomial errors.

Additional file 2: Table S1. Raw data used for all statistical analyses.

Abbreviations

AIC: Akaike information criterion; ASL: above sea level; CAD: cumulative adult density; CND: cumulative nymph density; LM: linear models; RH: relative humidity; SD: saturation deficit; SE: standard error.

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Authors' contributions

CB and MJV analyzed the data and prepared the manuscript. OR collected the ticks and the meteorological data in the field, and managed the data. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data for this study are stored in the Additional file 2. The climate data are available from the Climap-net database of the Federal Office for Meteorology and Climatology (<http://www.meteosuisse.admin.ch/home/service-et-publications/conseil-et-service/portail-de-donnees-dedie-aux-specialistes.html>). Accessed 4 Mar 2019). The tree seed production dataset is available in the Ecology–Ecological Society of America repository (<http://online.library.wiley.com/doi/10.1002/ecy.1785/supinfo>).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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5 Chapter 2

Masting by beech trees predicts the risk of Lyme disease

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