# Human pathogen co-occurrence in *Ixodes ricinus* ticks: effects of landscape topography, climatic factors and microbiota interactions

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## Abstract

The factors shaping microbial communities within organisms are still poorly understood. Besides ecological factors and host characteristics, direct interactions among microbes may shape the occurrence of microbes and the structure of communities. In the past it has been difficult to disentangle if patterns of microbial co-occurrence are due to facilitation or competition effects, or shaped by shared ecological preferences (i.e., environmental filtering). Here we use a joint species distribution model to characterize the bacterial microbiota composition of an important human disease vector, the sheep tick *Ixodes ricinus*, along ecological gradients in the Swiss Alps, and to test for facilitation or competition effects among human pathogens and tick endosymbionts. We identify a number of ecological variables that significantly predicted the diversity of tick microbial community and the occurrence of specific tick endosymbionts and human pathogens. However, ecological associations were generally microbe-specific rather than universal. We also found evidence for significant microbe interactions, in particular widespread facilitation among pathogens, which promotes pathogen co-infection within ticks, as well as competition between the tick endosymbiont *Spiroplasma* and a number of human pathogens. These findings highlight that direct interactions among microbes can affect the vector competence of ticks and thereby tick-borne disease dynamics.

**Keywords**: disease ecology, tick-borne pathogens, species distribution modelling, community composition, Borrelia burgdorferi, Lyme disease, facilitation, microbiome, endosymbionts

## Introduction

Microbial communities within organisms consist of symbionts, commensals, mutualists and pathogens that co-occur simultaneously and potentially influence each other [1–3]. These microbial communities may be shaped by a range of factors and processes, including the environment, host and microbe genetics and the occurrence and abundance of other microbial species. For example, certain microbial species might tolerate only certain biotic or abiotic conditions, which makes it more likely that species with similar requirements co-occur within a host ('environmental filtering' [4]). Similarly, the host's immune system can influence colonization success of microbes [5], with cross-immunity preventing the colonization of different microbes with similar antigenic properties [6]. Finally, direct interactions among microbes might affect colonization, or replication success after colonization, through competition or facilitation processes. Competition may occur when different microbes use the same, limited resources within a host [7], whereas facilitation may occur directly through the production of public goods [8] or indirectly through the modification of the host's physiology [9] or immune defense [10].

Ticks are important vectors for a range of zoonotic pathogens, including *Borrelia* spp., the causative agents of Lyme borreliosis [11] and *Rickettsia* sp., the causative agents of, among others, spotted fever and typhus [12]. Recent studies have found that pathogen co-infection is common in ticks [13–16]. Yet, the causes of pathogen co-occurrence remain poorly understood. Gaining insights into the factors that shape microbial communities in ticks in general, and the co-occurrence of human pathogens in particular, is of relevance from a fundamental ecological perspective, but also for public health, and it might inform tick-borne disease control programs.

Ticks have complex lifecycles, and require two or three bloodmeals to reach sexual maturity. They thus come into contact with several vertebrate hosts species throughout their life [17]. The composition of the host community might thus be a major determinant of the microbial community in ticks as different hosts might harbor different microbial (including pathogen) species [18, 19].

Host communities in turn are likely influenced by a range of biotic and abiotic factors such as vegetation structure, food abundance, elevation, temperature or rainfall. In addition, these biotic and abiotic factors might influence tick microbial communities directly, through effects on tick physiology or activity patterns [20].

In addition, there is some evidence that facilitation and competition processes shape the microbial community of ticks [21, 22]. For example, pathogenic *Rickettsia* species prevent co-infection with other *Rickettsia* species in *Dermacentor variabilis* ticks [23], whereas the presence of *Francisella* sp. endosymbionts increases the colonization success of pathogenic *Francisella novicida* in *D. andersoni* ticks [24]. Facilitation has also been suggested to promote co-infection with different *Borrelia afzelii* strains in *Ixodes ricinus* ticks [15]. Most strikingly, dysbiosed *I. scapularis* ticks (i.e., ticks with low microbiotal diversity) have a defective peritrophic matrix which decreases the colonization success of *B. burgdorferi* s.s., suggesting that the pathogen requires the presence of an intact microbiota to be able to infect ticks [25]. Thus, the microbial community may have a crucial impact on vector competence of ticks and thereby on disease dynamics.

Previous studies on the bacterial community of *I. ricinus* ticks have found approximately 100 OTUs (operational taxonomic units; [26]) per tick [27]. Most of them are environmental and free-living bacteria but also several endosymbionts and human pathogens were found [28, 29]. Differences in the bacterial community structure of ticks across habitats [30], geographical sites [28] and tick life stages and sexes [28, 31] have been documented. Yet, the processes that shape these patterns remain poorly understood [32]. In particular, whereas previous studies have investigated the potential interactions among microbes in ticks, they have not accounted for potential confounding variables such as shared ecological requirements, and are thus limited in their ability to differentiate between accidental co-occurrences due to shared environmental niches, and co-occurrence caused by direct interactions (such as facilitation or competition) among microbes. To address this gap, we use a

joint species distribution modelling (JSDM) approach to disentangle environmental effects on tick microbiota composition from direct microbial interactions [33, 34].

*I. ricinus* is the most common tick species in Europe and its distribution and abundance are strongly influenced by environmental conditions, in particular temperature and humidity [35, 36]. *I. ricinus* harbours a range of human pathogens, including several genospecies of *Borrelia* [37], *Rickettsia* sp. [38], *Anaplasma* sp. [39] and *Candidatus* Neoehrlichia [40]. Spatial variation in pathogen occurrence and prevalence is often pronounced [41]. For some pathogens, such as *B. afzelii*, previous studies have identified a range of ecological factors such as elevation and its associated abiotic factors [42, 43] or vegetation type and its associated effects on host communities [44], which contribute to this spatial variation. For other pathogens, however, the ecological drivers of distribution and prevalence remain poorly understood and little is known about the role of other microbes in influencing pathogen occurrence in *I. ricinus* ticks.

In our study, we exploited the environmental heterogeneity along replicated elevational gradients in the Swiss Alps to quantify the relative importance of environmental factors, tick characteristics and direct microbial interactions in influencing the structure of bacterial communities in *I. ricinus* ticks in general, and the occurrence of human pathogens in particular, using a combination of 16S sequencing and JSDM [45, 46]. Specifically, we ask (i) how do abiotic factors and tick-related variables affect tick microbiota composition, (ii) which abiotic and tick-related variables predict pathogen occurrence, and (iii) whether there are patterns of non-random microbial co-occurrence that cannot be explained by environmental similarities and are thus likely caused by direct facilitation or competition among microbes.

## Materials and methods

*Tick sampling*

Questing *Ixodes ricinus* ticks were collected at three locations in the Swiss Alps (Kanton Graubünden). At each location, one site at low (630 - 732 m above sea level), one at medium (1 094 – 1 138 masl) and one at high (1 454 – 1 673 masl) elevation were identified (Figure 1, Table 1, N = 9 sampling sites). At each site, questing ticks were sampled thrice, once in June, once in July, and once in August 2014 by dragging a white blanket (1 m x 1 m) over the ground vegetation as described previously [47]. Ticks were collected from the blanket and stored in 95% ethanol. Tick species and life stage were verified using a stereomicroscope.

*Environmental variables*

For each sampling site, we compiled information on elevation, slope and aspect using DHM25 and land use data from swissTLM3D (both from Federal Office of Topography swisstopo) and information on temperature and precipitation provided by Landscape Dynamics (Swiss Federal Research Institute for Water, Snow and Landscape Research WSL and Federal Office of Meteorology and Climatology MeteoSwiss, [48]). Information on *I. ricinus* abundance and the abundance of a key tick host, the bank vole (*Myodes glareolus*), as well as the ratio of bank vole to other rodents at our sampling sites were obtained from [43]. Details on and justification for variables is provided in the Supplementary Material.

*Tick DNA isolation and quantification of neutral genetic diversity*

We randomly selected *I. ricinus* ticks from each sampling site for analysis (Table 1). Variation in the number of ticks per site was due to variation in tick abundance [47].To avoid environmental

contamination, we performed DNA isolation and amplifications in a laminar flow cabinet. Each tick was washed thrice with sterile water before sterilizing it with 3% hydrogen peroxide. Ticks were then cut in half with a sterilized blade to facilitate DNA isolation. DNA was extracted using DNeasy Blood & Tissue kit (Qiagen; Hilden, Germany).

In order to quantify individual and population-level genetic diversity, we genotyped ticks at 11 microsatellite markers in two multiplexed amplifications (see Supplementary Material for details). Not all markers were successfully amplified in all samples, but none of the samples contained more than two failed markers. We used package 'poppr' [49] in R 3.4.1 [50] to test for linkage disequilibrium and deviation from Hardy-Weinberg equilibrium. Individual observed heterozygosity was determined for each tick as a proportion of heterozygous markers to all successfully amplified markers. Expected population level heterozygosity was determined with 'poppr'.

*Tick microbiota sequencing*

We quantified tick bacterial community composition by sequencing the hypervariable V3-V4 region of the 16S gene. Negative controls (N=5) were processed alongside the tick samples. Sequencing libraries were prepared following the Earth Microbiome 16S Illumina Amplicon protocol, using the primers 515FB and 806RB[51] (see Supplementary Material for details). Samples and negative controls were randomized across two plates. The libraries were sequenced on Illumina MiSeq at the Functional Genomic Center Zurich with a target length of 250 bp following the manufacturer's protocol. The obtained sequence data were analyzed following the *mothur* pipeline with MiSeq standard operation procedures [52].

Sequences have been deposited to the Sequence Read Archive under BioProject PRJNA50687. The complete metadata of the samples and their matching sequence accession numbers have been submitted to FigShare (doi: 10.6084/m9.figshare.7380767).

There has been a debate whether ticks have a stable microbiota [53], mirroring the wider debate on how common resident microbiota is in arthropod hosts [54]. Thus, in addition to analyses of overall microbiota diversity and composition, a special focus of our analysis was on tick endosymbionts and tick-borne human pathogens (Table 2), which are obligate residents.

*Tick microbiota diversity*

Only samples with > 500 amplicons and OTUs which were present in at least two samples were included in the analyses. We removed the most common OTU, the intra-mitochondrial endosymbiont *Candidatus* Midichloria [55], as it was present in all samples and compromised 19.3 % of all amplicons. After exclusion of Midichloria, we rarified the samples to lowest sample size to account for variation in amplicon numbers.

Bacterial alpha diversity (inverse Simpson index; [56]) and beta diversity (Bray-Curtis dissimilarity index, which takes shared species proportions into account when calculating dissimilarities; [57]) was calculated with the R package ‘vegan’ [58]. We ran linear mixed models with the package ‘lme4’ [59] to test for associations between bacterial alpha diversity and elevation, tick life stage, sex and sampling month, with full interactions. Site was included as a random effect in the model. We performed both permutational ANOVA with dissimilarity matrices and analysis of multivariate homogeneity of group dispersion using the package ‘vegan’ to test for association between measures of beta diversity and elevation, tick life stage, sex, site and sampling month.

For each model, non-significant factors were removed from the model starting with the least significant term. In addition, we used a model selection approach based on Akaike’s Information

Criterion [60] and model fit with conditional $R^2$ [61] in the package 'piecewiseSEM' [62] to test which combination of factors best describes variation in tick bacterial diversity.

*Joint species distribution modelling of microbiota composition*

We used a framework for JSDM called Hierarchical Modelling of Species Communities (HMSC, [45]) to test how environmental variables correlate with human pathogen and tick endosymbiont occurrence and whether there are non-random residual associations among different OTUs and/or oligotypes, implying direct facilitation or competition effects among microbes.

This approach combines information on environmental covariates, bacterial species traits, spatiotemporal context and sampling design to explain the presence or absence of OTUs (Figure S3). The associations among OTUs are modelled with the latent part of the framework, using the residual variance after accounting for the effects of the environment. Correlations among OTUs in this analysis thus reflect (dis)associations which cannot be explained by shared responses to the environment.

We compiled occurrence matrices for OTUs for each individual tick as a response variable with the same sample exclusion criteria as for the diversity analyses. An OTU was determined to be present in a tick if >5 non-rarefied amplicons were identified in a sample. For each sampling unit, i.e. a row in our response variable matrix, we included information on the identity of the sampling unit (tick ID), its location, sampling site and month, describing the study design.

To reach a better resolution within specific OTUs, we analyzed known human pathogens, tick endosymbionts and their close relatives within the 100 most common OTUs with oligotyping pipeline [63]. Oligotyping uses all the sequences, which form an OTU, and performs Shannon Entropy Analysis to regroup sequences based on within-OTU variation. This results in higher-

resolution grouping than OTUs as the different oligotypes might differ only by a single nucleotide [63]. We used the standard operation procedures of the oligotyping pipeline software [64].

Including a large number of explanatory variables in statistical models is inherently challenging. To reduce the number of variables, while maintaining their information value, we used two variable sets in the model: a) a set of full-effect explanatory variables, and b) explanatory variables under variable selection [46]. The full-effect variable set included an intercept, two individual-tick level variables (tick sex or life stage and individual heterozygosity) and two site-level variables (tick abundance and elevation of the sampling site). Additionally, we included information whether a specific OTU is an endosymbiont and/or a pathogen as traits [65]. This allowed us to test if endosymbionts and/or pathogen respond differentially to environmental conditions than other OTUs. The set of explanatory variables under variable selection included additional information on the environmental conditions of the sites (namely the number of days above 7 C° during the year, monthly precipitation, mean monthly temperature, forest coverage, slope, aspect, bank vole abundance, the proportion of voles to other rodents and expected tick heterozygosity) (Table S1). We considered all parameter estimates, including associations among bacterial OTUs, having strong statistical support if the 90% central credible interval of the parameter did not overlap with zero [following 73]. See Supplementary Material for additional model details.

Although JSDM is a powerful approach to model microbial community structure, it has a number of limitations. First, it assumes that interactions among microbes are similar across environments [but see 66]. This is not necessarily the case as both abiotic and biotic factors may shape microbial interactions [68]. Second, the model assumes that the explanatory variables affect the microbial community composition (or rather, the presence or absence of individual OTU), but not vice versa. However, this is a valid assumption for most environmental (e.g. elevation and temperature) and tick-related variables (e.g. tick sex, life stage) included in our models. Thirdly, covariation among variables poses a problem to any modelling approach. Our model is built on two distinct variable

sets to account for such covariation: the full variable set includes elevation, whereas the variables with the strongest covariation (i.e., temperature and precipitation) are included in the variable selection set.

## Results

### Ixodes ricinus *microbiota composition*

We 16S sequenced the bacterial community of 92 *Ixodes ricinus* ticks which resulted in 13 214 477 amplicons. No amplification was observed in the negative controls. After contig assembly and quality control 1 656 287 sequences were retained. There was a median of 1 562 quality-controlled amplicons per sample, with an interquartile range of 6 319. After exclusion of *Ca*. Midichloria, 82 samples with more than 340 amplicons per sample and a Good's coverage estimator ≥0.95 were included in the subsequent analyses. In total, 5 181 bacterial OTUs were identified. The median number of OTUs per rarified sample was 89 OTUs, with a 95% confidence interval of 78.3 - 98.5 OTUs.

Six OTUs were present in at least 90% of the samples: *Ca*. Midichloria (Otu0001), *Sphingomonas* (Otu0002, 0006 and 0007*)*, *Pseudomonas* (Otu0011) and *Delftia* (Otu0012). Together, they represented 50.2 % of all amplicons. We used oligotyping to further divide OTU0031 '*Rickettsia*' into two oligotypes labelled as '*R. helvetica*' and '*R. monacensis*', and OTU0086 '*Borrelia*' into four oligotypes labelled as '*B. afzelii*', '*B. valaisiana*' and '*B. garinii*' and '*B. miyamotoi*'. After excluding rare OTUs, we used 635 OTUs and oligotypes in subsequent analyses, including 14 endosymbionts and / or human pathogens (Table 2).

### Ixodes ricinus *microbiota diversity*

We observed a significant association between tick microbiota alpha diversity and elevation with lower bacterial diversity observed at higher elevations ($F_{1,78}$=3.98, p = 0.05, conditional $R^2$ = 0.07, Figure 2). No other environmental variables were significantly associated with tick microbiota alpha diversity (Table S2).

The analysis of tick microbiota beta diversity revealed a significant interaction effect between tick life stages/sex and elevation, indicating that differences in tick microbiota beta diversity across tick life stages/sex are shaped by elevation (pseudo-$F_1$ = 1.59, p = 0.01, $R^2$ = 0.03, Figure S1;Table S3). For other variables no significant association with microbial beta diversity was observed (Table S3).There was a significantly larger group dispersion at lower elevation sites ($F_{8,73}$ = 19.25, p < 0.001, Figure S2) suggesting that among-individual variation in bacterial community composition is higher at lower elevation. For other variables, no significant heterogeneity in bacterial community composition was observed (Table S4).

*Tick microbiota community modelling*

*Tick microbiota variance partitioning*

Variance partitioning revealed that most of the variation in tick microbiota composition was explained by tick ID: for the hundred most common OTUs, tick ID accounted for 83.5% of the variation explained by the JSDM model. Fixed effects accounted for 9.3% and other random effects (location, site and month) explained 7.2% (Figure 3). This suggests that there is extensive among-individual variation which cannot be accounted for by environmental variation. Notably, the situation differed for endosymbionts or pathogens: while tick ID was still the most important variable, fixed effects explained 21.9% and random effects explained 11.2% of the total variation explained by the model, when averaged over all pathogens and endosymbionts (Figure 3).

*Tick-specific and environmental factors associated with OTU occurrence*

The occurrence of some OTUs was strongly predicted by specific explanatory variables (Table 3). Slope, for example, was a strong predictor of the occurrence of a number of endosymbionts and pathogens with a higher probability of *R. helvetica*, *Rickettsia sp*., *Anaplasma* and *B. afzelii* and a lower probability of *Ca.* Neoehrlichia occurrence on steeper slopes. Females were less likely to harbour the endosymbionts *Spiroplasma*, *Lariskella* and *Rickettsia* spp. (Table 3) and ticks at higher elevations had higher probability to harbour *R. helvetica* and *R. monacensis*, but were less probable to harbour *B. garinii* (Table 3). Ticks from sites facing northwards had a higher probability of harbouring *Spiroplasma* and *B. afzelii*, but a lower probability to harbor *Anaplasma* (Table 3). Higher tick density was associated with a higher probability of *Rickettsiella* and *Ca.* Neoehrlichia occurrence (Table 3), while more extensive forest coverage was associated with a higher probability of *R. helvetica* and *B. afzelii* occurrence (Table 3). Associations between tick life stage, temperature, the number of days > 7 C°, precipitation or relative vole abundance and the occurrence of specific OTUs were not strongly statistically supported.

The effect sizes of strongly statistically supported associations varied substantially (Figure S5a-i). For example, threefold increase in vole abundance corresponded to one percentage point decrease of *R. monacensis* prevalence (Figure S5b), whereas a threefold increase in tick abundance corresponded to a twofold increase in *Neoehrlichia* prevalence from 20% to 40% (Figure S5e).

*Microbial facilitation or competition effects within ticks*

Numerous bacterial OTUs were either significantly more or less likely to co-occur within a tick than expected by chance after accounting for shared environmental preferences (Figure 4; Table S5). The occurrence of the tick endosymbiont *Spiroplasma* was negatively associated with the occurrence of the endosymbiont *Lariskella* and several tick-borne human pathogens, namely *Anaplasma* sp., *Ca*. Neoehrlichia and *B. miyamotoi* (Figure 4). Associations among pathogens, if

they occurred, were all positive (Figure 4), suggesting that ticks are more likely to be co-infected with several human pathogens simultaneously than expected by chance or based on shared environmental preferences. *Borrelia* oligotypes showed positive co-occurrence patterns among each other, except for *B. miyamotoi*, which was not associated with other *Borrelia sp.*, but negatively with *Spiroplasma* and positively with *Lariskella*. As our approach allowed us to control for shared environmental preferences, the significant negative or positive associations among endosymbionts and pathogens can be considered as indications of competition and facilitation.

## Discussion

Using a Joint Species Distribution Modelling approach, our study provides evidence that a range of tick endosymbionts and human pathogens are more or less likely to co-occur within *I. ricinus* ticks than expected by chance or due to shared environmental preferences. Although the co-occurrence of microbes within ticks has been documented before, both in *I. ricinus* [16, 69] and other tick species [70, 71], previous studies did not control for shared environmental preferences (i.e., environmental filtering) and were therefore unable to disentangle shared responses to the environment from direct microbe-microbe interactions. Our study reveals that when accounting for shared environmental preferences, associations among tick endosymbionts and human pathogens are mostly positive, suggesting that facilitation processes significantly contribute to endosymbiont-pathogen co-occurrence as well as pathogen co-infections within ticks. Such facilitation processes will affect the structure and dynamics of microbial communities [9, 71]. At the same time, the resulting co-occurrence of pathogens within ticks has implications for the severity, diagnosis, treatment and control of tick-borne diseases.

Although associations among microbes were mostly positive, there were negative associations between the tick endosymbiont *Spiroplasma* sp. and several human pathogens, which is indicative

of competition. Similar protective effects have been previously described in *Drosophila sp.*, where *Spiroplasma* sp. is associated with a decreased probability of nematode and parasitoids infections [72, 73]. Such competition effects among tick endosymbionts and human pathogens might open up new and exciting avenues for the control of tick-borne pathogens in areas with high disease incidence.

Among the significant microbe-microbe interactions, the strong positive associations among the Lyme disease-causing *Borrelia* genospecies (*B. afzelii*, *B. garinii* and *B. valaisiana*) were particularly striking and indicative of facilitation (see also [15]). The co-occurrence of these *Borrelia* genospecies within ticks is surprising because *B. garinii* and *B. valaisiana* are bird specialists [74, 75], whereas *B. afzelii* is a rodent specialist [76]. It suggests that *I. ricinus* ticks typically feed on multiple, phylogenetically diverse host species during their life cycle and do not show pathogen-mediated host specialization as has been suggested previously [77, 78].

In addition to testing for facilitation and competition among microbes, we used a JSDM framework to quantify the role of tick-specific and environmental variables in explaining spatial variation in tick microbiota composition. Even though environmental variation across our sampling sites was large (spanning an elevational gradient from 630 – 1 580 masl) and a large number of ecological and tick-related variables was considered in our models, overall they accounted for only 9.3% of the variation in bacterial microbiota composition. This finding highlights that unexplained variation in microbiota composition across individual ticks is substantial, which is in line with the idea that at least a part of the bacteria present in ticks may be non-resident [53]. Nevertheless, this can also result from missing relevant environmental variables in our data. However, this conclusion is further supported by the finding that the explanatory power of tick-related and environmental variables was higher for tick endosymbionts and human pathogens, which are obligate resident.

Despite the large among-tick variation in microbiota composition, we found significant associations between specific environmental variables and OTUs. Particularly pronounced was the association

between tick microbiota diversity and elevation. Across three independent elevational gradients we found that alpha diversity was consistently higher at lower elevations. Although a decrease in plant and animal diversity with increasing elevation is a widely documented pattern in ecology, it has been suggested that the biogeographical patterns exhibited by bacteria may be fundamentally different from those of plants and animals [79]. Our findings do not support this hypothesis but are in line with the idea that microbiota diversity decreases with increasing distance from the host's environmental optimum, such as at a host's range edge or during torpor [80]. Similarly, we observed changes in microbial beta diversity with elevation, particularly in female ticks, and a significantly larger group dispersion in microbial beta diversity at lower elevations. Together with the decrease in tick microbiota alpha diversity with increasing elevation, it highlights that suboptimal environmental conditions permit the occurrence of only a subset of bacterial OTUs in ticks, leading to more similar, depleted microbiota at such sites. The consequences of low microbial diversity for individual ticks and tick populations are currently unknown. Yet, given the lack of an association between microbial alpha or beta diversity and the occurrence of human pathogens it is unlikely to affect human disease risk.

We identified a range of tick-specific and environmental variables that significantly predicted the occurrence of various tick endosymbionts and human pathogens. However, these associations were typically OTU specific rather than universal, with the same variable being both positively and negatively associated with the probability of occurrence of different OTUs. For example, *B. garinii* was *less* likely to occur at higher elevations whereas *R. helvetica* and *R. monascensis* were *more* likely to occur at higher elevations. Generally, the environmental factors shaping *Rickettsia* spp. distribution are poorly understood, as is their range of host species [44, 81]. Yet, it has previously been found that spotted fever incidence in humans, caused by *R. ricketsii*, is highest in suboptimal tick habitats [82]. This is in line with our findings and suggests that *Rickettsia* spp. are more likely to colonize ticks living under suboptimal conditions. The finding that *B. garinii* is less likely to

occur at higher elevations is in line with previous observations [42] and may be explained by elevational clines in the diversity and/or abundance of birds, the natural hosts of *B. garinii* [74]. In contrast, the occurrence of the mammal specialist *B. afzelii* was not associated with elevation, potentially because elevational clines in mammal diversity and/or abundance are less pronounced [83]. Indeed we did not observe an association between elevation and bank vole abundance in our study (ANOVA: $F_{1,8}$=0.357, p = 0.57, $R^2$ = 0.05).

Interestingly, temperature and precipitation, which vary strongly across elevational gradients (average temperature and precipitation: high elevation sites: 11.8 C° and 17.8 mm per month; in low sites: 16.5 C° and 12.1 mm per month), were not significant predictors of the occurrence of endosymbionts or pathogens. This may be partly due to the temperature and precipitation measures included in our models not fully capturing microclimatic variation across sites and along elevational clines. Indeed, slope and aspect, which are important determinants of the topography, and thus microclimate [84], were significant predictors of pathogen and endosymbiont occurrence. The probability of pathogen and endosymbiont occurrence was higher on steeper slopes. Furthermore, the probability of occurrence was higher on north-facing slopes for *B. afzelii* and *Spiroplasma* on north-facing slopes and and higher on south-facing slopes for *Rickettsiella* and *Anaplasma* sp. (see also [85]). Microclimatic conditions may affect microbial occurrence directly, or indirectly via affecting tick behavior or host community composition [86, 87]. Furthermore, topography can affect population connectivity and dispersal in metapopulation networks [88].

Host community composition and density are crucial for pathogen and endosymbiont occurrence and dispersal [41]. Previous work has found that tick abundance is a strong predictor of *Borrelia* spp. prevalence, potentially because larger tick populations facilitate co-feeding transmission [89]. No association between *Borrelia* spp occurrence and tick abundance was observed in our study. However, both *Ca.* Neoehrlichia and *Rickettsiella* were more common at sites where ticks were

more abundant, suggesting that co-feeding transmission may also play a role in the life cycle of these microbes.

Finally, differences in host competence can lead to dilution effects and thus affect the prevalence of tick-borne pathogens [90]. Whereas for some tick-borne pathogens the vertebrate hosts are known or suspected (e.g. small mammals for *B. afzelii* [76] and *Ca*. Neoehrlichia [91], birds for *B. garinii* and *B. valaisiana* [75], both for *Anaplasma* [92] and *R. helvetica* [93]), for others the host species range is less well understood (e.g. *B. miyamotoi* [94]). The bank vole is a common tick host at our study sites and their abundance was a significant positive predictor of *B. valaisiana* and negative predictor of *R. monacensis* occurrence. Interestingly, bank voles are known hosts for neither of these microbes [95]. Most likely, the association is thus indirect, explained by a biotic or abiotic variable that correlates with bank vole abundance. For example, bank voles avoid open habitats and prefer woodlands and heterogeneous habitats [96, 97]. Overall, no evidence was found that the proportion of bank voles to other rodents affects the prevalence of tick-borne pathogens.

In conclusion, our results show how we can jointly model tick-associated bacterial communities, and taking into account of the environmental effects, tease apart patterns in the data indicating facilitation and competition among microbes. We identified a number of ecological variables that predict the occurrence of specific tick endosymbionts and human pathogens with strong statistical support, but these effects were generally microbe-specific rather than universal. This highlights that environmental change can have different, even opposite effects on the probability of occurrence of different pathogens, and thus human disease risk. Furthermore, our modelling approach reveals significant among-microbe interactions, suggesting in particular widespread facilitation among pathogens, which promotes pathogen co-infection within ticks, as well as competition between *Spiroplasma* and a number of human pathogens. The latter opens up new and exciting avenues for the control and management of tick-borne diseases in regions with high human disease incidence.

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## References

1. Sofonea MT, Alizon S, Michalakis Y. From within-host interactions to epidemiological competition: A general model for multiple infections. *Philos Trans R Soc B Biol Sci* 2015; **370**: 20140303.

2. Petney TN, Andrews RH. Multiparasite communities in animals and humans: frequency , structure and pathogenic significance. *Int J Parasitol* 1998; **28**: 377–393.

3. Rigaud T, Perrot-Minnot M-J, Brown MJF. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proc R Soc B Biol Sci* 2010; **277**: 3693–3702.

4. Dallas T, Presley SJ. Relative importance of host environment, transmission potential and host phylogeny to the structure of parasite metacommunities. *Oikos* 2014; **123**: 866–874.

5. Hawley DM, Altizer SM. Disease ecology meets ecological immunology: understanding the links

between organismal immunity and infection dynamics in natural populations. *Funct Ecol* 2011; **25**: 48–60.

6. Durand J, Jacquet M, Paillard L, Rais O, Gern L, Voordouw MJ. Cross-immunity and community structure of a multiple-strain pathogen in the tick vector. *Appl Environ Microbiol* 2015; **22**.

7. Lello J, Boag B, Fenton A, Stevenson IR, Hudson PJ. Competition and mutualism among the gut helminths of a mammalian host. *Nature* 2004; **428**: 20–24.

8. West SA, Buckling A. Cooperation, virulence and siderophore production in bacterial parasites. *Proc R Soc B Biol Sci* 2003; **270**: 37–44.

9. Abraham NM, Liu L, Jutras BL, Yadav AK, Narasimhan S, Gopalakrishnan V, et al. Pathogen-mediated manipulation of arthropod microbiota to promote infection. *Proc Natl Acad Sci* 2017; **114**: E781–E790.

10. Rodríguez M, Terrazas LI, Márquez R, Bojalil R. Susceptibility to Trypanosoma cruzi is modified by a previous non-related infection. *Parasite Immunol* 1999; **21**: 177–185.

11. Lane JR., Piesman K, Burgdoifer W. Lyme Borreliosis: Relation of its causative agent to its vectors and hosts in North America and Europe. *Annu Rev Entomol* 1991; **36**: 587–609.

12. Raoult D. Rickettsioses as paradigms of new or emerging infectious diseases. *Clin Microbiol Rev* 1997; **10**: 694–719.

13. Diuk-Wasser MA, Vannier E, Krause PJ. Coinfection by Ixodes Tick-Borne Pathogens: Ecological, Epidemiological, and Clinical Consequences. *Trends Parasitol* 2016; **32**: 30–42.

14. Moutailler S, Valiente Moro C, Vaumourin E, Michelet L, Tran FH, Devillers E, et al. Co-infection of ticks: the rule rather than the exception. *PLoS Negl Trop Dis* 2016; **10**: e0004539.

15. Andersson M, Scherman K, Råberg L. Multiple-strain infections of Borrelia afzelii: a role for within-host interactions in the maintenance of antigenic diversity? *Am Nat* 2013; **181**: 545–54.

16. Michelet L, Delannoy S, Devillers E, Umhang G, Aspan A, Juremalm M, et al. High-throughput

screening of tick-borne pathogens in Europe. *Front Cell Infect Microbiol* 2014; **4**: 103.

17. Gern L. Life cycle of Borrelia burgdorferi sensu lato and transmission to humans. In: Lipsker D, Jaulhac B (eds). *Lyme Borreliosis*. 2009. Karger, Basel, Switzerland., pp 18–30.

18. Randolph SE, Miklisová D, Lysy J, Rogers DJ, Labuda M. Incidence from coincidence: Patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. *Parasitology* 1999; **118**: 177–186.

19. MacDonald AJ, Hyon DW, Iii JBB, Connor KEO, Swei A, Briggs CJ. Lyme disease risk in southern California : abiotic and environmental drivers of Ixodes pacificus (Acari: Ixodidae) density and infection prevalence with Borrelia burgdorferi. *Parasit Vectors* 2017; 7.

20. van Treuren W, Ponnusamy L, Brinkerhoff RJ, Gonzalez A, Parobek CM, Juliano JJ, et al. Variation in the microbiota of Ixodes ticks with regard to geography, species, and sex. *Appl Environ Microbiol* 2015; **81**: 6200–6209.

21. Haine ER. Symbiont-mediated protection. *Proc R Soc B Biol Sci* 2008; **275**: 353–361.

22. Bonnet SI, Binetruy F, Hernández-Jarguín AM, Duron O. The tick microbiome: Why non-pathogenic microorganisms matter in tick biology and pathogen transmission. *Front Cell Infect Microbiol* 2017; **7**: 236.

23. Macaluso KR, Sonenshine DE, Ceraul SM, Azad AF. Rickettsial Infection in *Dermacentor variabilis* (Acari: Ixodidae) Inhibits Transovarial Transmission of a Second *Rickettsia*. *J Med Entomol* 2002; **39**: 809–813.

24. Gall CA, Reif KE, Scoles GA, Mason KL, Mousel M, Noh SM, et al. The bacterial microbiome of Dermacentor andersoni ticks influences pathogen susceptibility. *ISME J* 2016; **10**: 1846–1855.

25. Narasimhan S, Rajeevan N, Liu L, Zhao YO, Heisig J, Pan J, et al. Gut microbiota of the tick vector Ixodes scapularis modulate colonization of the Lyme disease spirochete. *Cell Host Microbe* 2014; **15**: 58–71.

26. Stackebrandt E, Goebel BM. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA

sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 1994; **44**: 846–849.

27. Greay TL, Gofton AW, Paparini A, Ryan UM, Oskam CL, Irwin PJ. Recent insights into the tick microbiome gained through next-generation sequencing. *Parasites and Vectors* 2018; **11**: 12.

28. Carpi G, Cagnacci F, Wittekindt NE, Zhao F, Qi J, Tomsho LP, et al. Metagenomic profile of the bacterial communities associated with Ixodes ricinus ticks. *PLoS One* 2011; **6**: e25604.

29. Gofton A, Oskam C, Lo N, Beninati T, Wei H, McCarl V, et al. Inhibition of the endosymbiont 'Candidatus Midichloria mitochondrii' during 16S rRNA gene profiling reveals potential pathogens in Ixodes ticks from Australia. *Parasit Vectors* 2015; **8**: 345.

30. Estrada-Peña A, Cabezas-Cruz A, Pollet T, Vayssier-Taussat M, Cosson J-F. High Throughput Sequencing and Network Analysis Disentangle the Microbial Communities of Ticks and Hosts Within and Between Ecosystems. *Front Cell Infect Microbiol* 2018; **8**: 236.

31. Vayssier-Taussat M, Moutailler S, Michelet L, Devillers E, Bonnet S, Cheval J, et al. Next generation sequencing uncovers unexpected bacterial pathogens in ticks in western Europe. *PLoS One* 2013; **8**: e81439.

32. Norman RA, Worton AJ, Gilbert L. Past and future perspectives on mathematical models of tick-borne pathogens. *Parasitology* 2016; **143**: 850–859.

33. Ovaskainen O, Abrego N, Halme P, Dunson D. Using latent variable models to identify large networks of species-to-species associations at different spatial scales. *Methods Ecol Evol* 2015; **7**: 549–555.

34. Warton DI, Blanchet FG, O'Hara RB, Ovaskainen O, Taskinen S, Walker SC, et al. So many variables: joint modeling in community ecology. *Trends Ecol Evol* 2015; **30**: 766–779.

35. Gatewood AG, Liebman K a, Vourc'h G, Bunikis J, Hamer S a, Cortinas R, et al. Climate and tick seasonality are predictors of Borrelia burgdorferi genotype distribution. *Appl Environ Microbiol* 2009; **75**: 2476–83.

36. Cortinas MR, Guerra MA, Jones CJ, Kitron U. Detection, characterization, and prediction of tick-borne disease foci. *Int J Med Microbiol* 2002; **291**: 11–20.

37. Mannelli A, Bertolotti L, Gern L, Gray J. Ecology of Borrelia burgdorferi sensu lato in Europe: transmission dynamics in multi-host systems, influence of molecular processes and effects of climate change. *FEMS Microbiol Rev* 2012; **36**: 837–61.

38. Venclikova K, Rudolf I, Mendel J, Betasova L, Hubalek Z. Rickettsiae in questing Ixodes ricinus ticks in the Czech Republic. *Ticks Tick Borne Dis* 2014; **5**: 135–138.

39. Jahfari S, Herremans T, Platonov AE, Kuiper H, Karan LS, Vasilieva O, et al. High seroprevalence of Borrelia miyamotoi antibodies in forestry workers and individuals suspected of human granulocytic anaplasmosis in the Netherlands. *New Microbes New Infect* 2014; **2**: 144–149.

40. Kawahara M, Rikihisa Y, Isogai E, Takahashi M, Misumi H, Suto C, et al. Ultrastructure and phylogenetic analysis of 'Candidatus Neoehrlichia mikurensis' in the family Anaplasmataceae, isolated from wild rats and found in Ixodes ovatus ticks. *Int J Syst Evol Microbiol* 2004; **54**: 1837–1843.

41. Ruyts SC, Tack W, Ampoorter E, Coipan EC, Matthysen E, Heylen D, et al. Year-to-year variation in the density of Ixodes ricinus ticks and the prevalence of the rodent-associated human pathogens Borrelia afzelii and B. miyamotoi in different forest types. *Ticks Tick Borne Dis* 2017; **9**: 141–145.

42. Jouda F, Perret J, Gern L. Ixodes ricinus density, and distribution and prevalence of Borrelia burgdorferi sensu lato infection along an altitudinal gradient. *J Med Entomol* 2004; **41**: 162–169.

43. Cornetti L, Hilfiker D, Lemoine M, Tschirren B. Small-scale spatial variation in infection risk shapes the evolution of a *Borrelia* resistance gene in wild rodents. *Mol Ecol* 2018; 3515–3524.

44. Halos L, Bord S, Cotté V, Gasqui P, Abrial D, Barnouin J, et al. Ecological factors characterizing the prevalence of bacterial tick-borne pathogens in ixodes ricinus ticks in pastures and woodlands. *Appl Environ Microbiol* 2010; **76**: 4413–4420.

45. Ovaskainen O, Gleb Tikhonov, Norberg A, Blanchet FG, Duan L, Dunson D, et al. How to make

more out of community data? A conceptual framework and its implementation as models and software. *Ecol Lett* 2017; **20**: 561–576.

46. Ovaskainen O, Tikhonov G, Dunson D, Grøtan V, Engen S, Sæther B-E, et al. How are species interactions structured in species-rich communities? A new method for analysing time-series data. *Proc R Soc B Biol Sci* 2017; **284**: 20170768.

47. Lemoine M, Cornetti L, Tschirren B. Does Borrelia burgdorferi sensu lato facilitate the colonisation of marginal habitats by Ixodes ricinus? A correlative study in the Swiss Alps. *bioRxiv* 2018.

48. Thornton PE, Running SW, White MA. Generating surfaces of daily meteorological variables over large regions of complex terrain. *J Hydrol* 1997; **190**: 214–251.

49. Kamvar ZN, Tabima JF, Grünwald NJ. *Poppr*: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2014; **2**: e281.

50. Team RC. R: A language and environment for statistical computing. 2013. R Foundation for Statistical Computing, Vienna, Austria.

51. Carey H V, Walters WA, Knight R. Seasonal restructuring of the ground squirrel gut microbiota over the annual hibernation cycle. *Am J Physiol Regul Integr Comp Physiol* 2013; **304**: R33-42.

52. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* 2013; **79**: 5112–20.

53. Ross BD, Hayes B, Radey MC, Lee X, Josek T, Bjork J, et al. Ixodes scapularis does not harbor a stable midgut microbiome. *bioRxiv* 2017; 198267.

54. Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP, Fierer N. Caterpillars lack a resident gut microbiome. *Proc Natl Acad Sci* 2017; **114**: 9641–9646.

55. Lo N, Beninati T, Sassera D, Bouman EAP, Santagati S, Gern L, et al. Widespread distribution and high prevalence of an alpha-proteobacterial symbiont in the tick Ixodes ricinus. *Environ Microbiol* 2006; **8**: 1280–1287.

56. Simpson EH. Measurement of diversity. *Nature* . 1949. , **163**: 688

57. Bray JR, Curtis JT. An ordination of the upland forest community of Southern Wisconsin. *Ecol Monogr* 1957; **27**: 325–349.

58. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al. vegan: Community Ecology Package. 2013.

59. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* 2015; **67**.

60. Akaike H. A new look at the statistical model identification. *IEEE Trans Automat Contr* 1974; **19**: 716–723.

61. Nakagawa S, Schielzeth H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol Evol* 2013; **4**: 133–142.

62. Lefcheck JS. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods Ecol Evol* 2016; **7**: 573–579.

63. Eren AM, Borisy GG, Huse SM, Mark Welch JL. Oligotyping analysis of the human oral microbiome. *Proc Natl Acad Sci* 2014; **111**: E2875–E2884.

64. Eren AM, Maignien L, Sul WJ, Murphy LG, Grim SL, Morrison HG, et al. Oligotyping: Differentiating between closely related microbial taxa using 16S rRNA gene data. *Methods Ecol Evol* 2013; **4**: 1111–1119.

65. Abrego N, Norberg A, Ovaskainen O. Measuring and predicting the influence of traits on the assembly processes of wood-inhabiting fungi. *J Ecol* 2016; **(accepted)**.

66. Aivelo T, Norberg A. Parasite-microbiota interactions potentially affect intestinal communities in wild mammals. *J Anim Ecol* 2017; **87**: 438–447.

67. Tikhonov G, Abrego N, Dunson D, Ovaskainen O. Using joint species distribution models for evaluating how species-to-species associations depend on the environmental context. *Methods Ecol*

*Evol* 2017; **8**: 443–452.

68. Elliot SL, Blanford S, Thomas MB. Host-pathogen interactions in a varying environment: temperature, behavioural fever and fitness. *Proc R Soc B Biol Sci* 2002; **269**: 1599–607.

69. Lommano E, Bertaiola L, Dupasquier C, Gern L. Infections and coinfections of questing Ixodes ricinus ticks by emerging zoonotic pathogens in Western Switzerland. *Appl Environ Microbiol* 2012; **78**: 4606–4612.

70. Laaksonen M, Klemola T, Feuth E, Sormunen JJ, Puisto A, Mäkelä S, et al. Tick-borne pathogens in Finland: comparison of Ixodes ricinus and I. persulcatus in sympatric and parapatric areas. *Parasit Vectors* 2018; **11**: 556.

71. Gurfield N, Grewal S, Cua LS, Torres PJ, Kelley ST. Endosymbiont interference and microbial diversity of the Pacific coast tick, Dermacentor occidentalis, in San Diego County, California. *PeerJ* 2017; **5**: e3202.

72. Xie J, Vilchez I, Mateos M. Spiroplasma bacteria enhance survival of Drosophila hydei attacked by the parasitic wasp Leptopilina heterotoma. *PLoS One* 2010; **5**: e12149.

73. Jaenike J, Unckless R, Cockburn SN, Boelio LM, Perlman SJ. Adaptation via symbiosis: recent spread of a Drosophila defensive symbiont. *Science (80- )* 2013; **329**: 212–215.

74. Comstedt P, Jakobsson T, Bergström S. Global ecology and epidemiology of Borrelia garinii spirochetes. *Infect Ecol Epidemiol* 2011; **1**: 9545.

75. Hanincova K, Taragelova V, Koci J, Schafer SM, Hails R, Ullmann AJ, et al. Songbirds in Slovakia. *Appl Environ Microbiol* 2003; **69**: 2825–2830.

76. Hanincova K, Schäfer SM, Etti S, Sewell HS, Taragelová V, Ziak D, et al. Association of Borrelia afzelii with rodents in Europe. *Parasitology* 2003; **126**: 11–20.

77. McCoy KD, Chapuis E, Tirard C, Boulinier T, Michalakis Y, Le Bohec C, et al. Recurrent evolution of host-specialized races in a globally distributed parasite. *Proc R Soc B Biol Sci* 2005; **272**: 2389–2395.

78. McCoy KD, Léger E, Dietrich M. Host specialization in ticks and transmission of tick-borne diseases: a review. *Front Cell Infect Microbiol* 2013; **3**: 57.

79. Fierer N, Mccain CM, Meir P, Zimmermann M, Rapp JM, Silman MR, et al. Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology* 2011; **92**: 797–804.

80. Geiger A, Ponton F, Simo G. Adult blood-feeding tsetse flies, trypanosomes, microbiota and the fluctuating environment in sub-Saharan Africa. *ISME J* 2015; **9**: 1496–1507.

81. Eremeeva ME, Dasch GA. Challenges Posed by Tick-Borne Rickettsiae: Eco-Epidemiology and Public Health Implications. *Front Public Heal* 2015; **3**: 1–17.

82. Atkinson SF, Sarkar S, Aviña A, Schuermann JA, Williamson P. Modelling spatial concordance between Rocky Mountain spotted fever disease incidence and habitat probability of its vector Dermacentor variabilis (American dog tick). *Geospat Health* 2013; **7**: 91–100.

83. McCain CM. Elevational gradients in diversity of small mammals. *Ecology* 2005; **86**: 366–372.

84. Bennie J, Huntley B, Wiltshire A, Hill MO, Baxter R, Wood CEHM, et al. Slope, aspect and climate□: Spatially explicit and implicit models of topographic microclimate in chalk grassland. *Ecol Modell* 2008; **6**: 47–59.

85. Stuen S, Granquist EG, Silaghi C. Anaplasma phagocytophilum—a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol* 2013; **3**: 31.

86. Lawson CR, Bennie J, Hodgson JA, Thomas CD, Wilson RJ. Topographic microclimates drive microhabitat associations at the range margin of a butterfly. *Ecography (Cop)* 2014; **37**: 732–740.

87. Swei A, Meentemeyer R, Briggs CJ. Influence of abiotic and environmental factors on the density and infection prevalence of Ixodes pacificus (Acari: Ixodidae ) with Borrelia burgdorferi. *J Med Entomol* 2011; **48**: 20–28.

88. Swei A, Kwan JY. Tick microbiome and pathogen acquisition altered by host blood meal. *ISME J* 2017; **11**: 813–816.

89. Jouda F, Perret J-L, Gern L. Density of questing Ixodes ricinus nymphs and adults infected by Borrelia burgdorferi sensu lato in Switzerland: spatiotemporal pattern at a regional scale. *Vector-borne Zoonotic Dis* 2004; **4**: 23–32.

90. Keesing F, Holt RD, Ostfeld RS. Effects of species diversity on disease risk. *Ecol Lett* 2006; **9**: 485–98.

91. Jahfari S, Fonville M, Hengeveld P, Reusken C, Scholte E, Takken W, et al. Prevalence of Neoehrlichia mikurensis in ticks and rodents from North-west Europe. *Parasit Vectors* 2012; **5**: 74.

92. Keesing F, Hersh MH, Tibbetts M, McHenry DJ, Duerr S, Brunner J, et al. Reservoir competence of vertebrate hosts for Anaplasma phagocytophilum. *Emerg Infect Dis* 2012; **18**: 2013–2016.

93. Sprong H, Wielinga PR, Fonville M, Reusken C, Brandenburg AH, Borgsteede F, et al. Ixodes ricinus ticks are reservoir hosts for Rickettsia helvetica and potentially carry flea-borne Rickettsia species. *Parasites and Vectors* 2009; **2**: 41.

94. Wagemakers A, Staarink PJ, Sprong H, Hovius JWR. Borrelia miyamotoi: A widespread tick-borne relapsing fever spirochete. *Trends Parasitol* 2015; **31**: 260–269.

95. Burri C, Schumann O, Schumann C, Gern L. Are Apodemus spp. mice and Myodes glareolus reservoirs for Borrelia miyamotoi, Candidatus Neoehrlichia mikurensis, Rickettsia helvetica, R. monacensis and Anaplasma phagocytophilum? *Ticks Tick Borne Dis* 2014; **5**: 245–251.

96. Tattersall AFH, Macdonald DW, Hart BJ, Johnson P, Manley W, Feber R. Is habitat linearity important for small mammal communities on farmland? *J Appl Ecol* 2002; **39**: 643–652.

97. Ecke F, Löfgren O, Sörlin D. Population dynamics of small mammals in relation to forest age and structural habitat factors in northern Sweden. *J Appl Ecol* 2002; **39**: 781–792.

98. Cornetti L, Lemoine M, Hilfiker D, Morger J, Reeh K, Tschirren B. Higher genetic diversity on mountain tops: The role of historical and contemporary processes in shaping genetic variation in the bank vole. *Biol J Linn Soc* 2016; **118**: 233–244.

Table 1: Tick sampling sites in the Swiss Alps.

| Location | Site | Coordinates | | Elevation | Sequenced *Ixodes ricinus* ticks | | |
|---|---|---|---|---|---|---|---|
| | | North | East | (masl) | nymphs | males | females |
| 1 | Sagogn | 46.783 | 9.233 | 693 | 0 | 9 | 15 |
| | Flims | 46.827 | 9.280 | 1138 | 3 | 5 | 3 |
| | Ruschein | 46.795 | 9.169 | 1454 | 0 | 1 | 1 |
| 2 | Rodels | 46.760 | 9.425 | 630 | 2 | 5 | 4 |
| | Tomils | 46.772 | 9.453 | 1144 | 3 | 6 | 4 |
| | Feldis | 46.789 | 9.453 | 1673 | 1 | 1 | 0 |
| 3 | Passug | 46.840 | 9.538 | 732 | 0 | 5 | 6 |
| | Castiel | 46.826 | 9.569 | 1094 | 0 | 3 | 3 |
| | Praden | 46.817 | 9.589 | 1582 | 1 | 0 | 1 |

Table 2: Common tick endosymbionts and/or putative human pathogens observed in I. ricinus ticks. See Supplementary Materials for information on OTU assignment.

| OTU | Label | Human pathogen / tick endosymbiont | Occurrence (% of analyzed ticks) |
|---|---|---|---|
| Otu0001 | *Midichloria* | endosymbiont | 100 |
| Otu0003 | *Spiroplasma* | endosymbiont | 41 |
| Otu0005 | *Rickettsiella* | endosymbiont | 63 |
| Otu0021 | *Lariskella* | endosymbiont | 49 |
| Otu0031 | *Rickettsia helvetica* | both | 16 |
| | *R. monacensis* | both | 6 |
| Otu0067 | *Rickettsia sp.* | both | 25 |
| Otu0076 | *Anaplasma* | both | 33 |
| Otu0086 | *Candidatus* Neoehrlichia | both | 22 |
| Otu0088 | *Borrelia afzelii* | pathogen | 9 |
| | *B. miyamotoi* | pathogen | 10 |
| | *B. garinii* | pathogen | 6 |
| | *B. valaisiana* | pathogen | 2 |

Table 3. Associations between tick-specific and environmental variables and the occurrence of endosymbionts and human pathogens in *I. ricinus* ticks. A positive sign indicates that higher variable values are associated with a higher probability of OTU occurrence. A higher aspect value means that a site is facing northwards. Only associations with strong statistical support (based on the 90% central credible interval) are presented.

| Full variable set | | | | | | | Variable selection set | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tick sex (Female) | Tick life stage (Nymph) | Tick abundance | Tick heterozygosity | Elevation | Tick population expected heterozygosity | | Number of days > 7C° | Precipitation | Mean temperature | Forest cover | Slope | Aspect | Vole abundance | Vole/ other rodents ratio |
| Otu0003 | *Spiroplasma* | − | | | | | | | | | | | | + | | |
| Otu0005 | *Rickettsiella* | − | | + | | | | | | | | | | − | | |
| Otu0022 | *Lariskella* | − | | | − | | | | | | | | | | | |
| Otu0031 | *Rickettsia helvetica* | | | | | + | | | − | | | + | + | | | |
| | *R. monacensis* | | | | | + | | − | | | | | | | − | |
| Otu0067 | *Rickettsia sp.* | − | | | | | | | | | | | + | | | |
| Otu0076 | *Anaplasma* | | | | | | | | | | | | + | − | | |
| Otu0086 | *Ca.* Neoehrlichia | | | + | | | | | | | | | − | | | |
| Otu0088 | *Borrelia afzelii* | | | | | | | | | | | + | + | + | | |
| | *B. miyamotoi* | | | | | | | | | | | | | | | |
| | *B. garinii* | | | | | − | | | | | | | | | | |
| | *B. valaisiana* | | | | | | | + | | | | | | | + | |

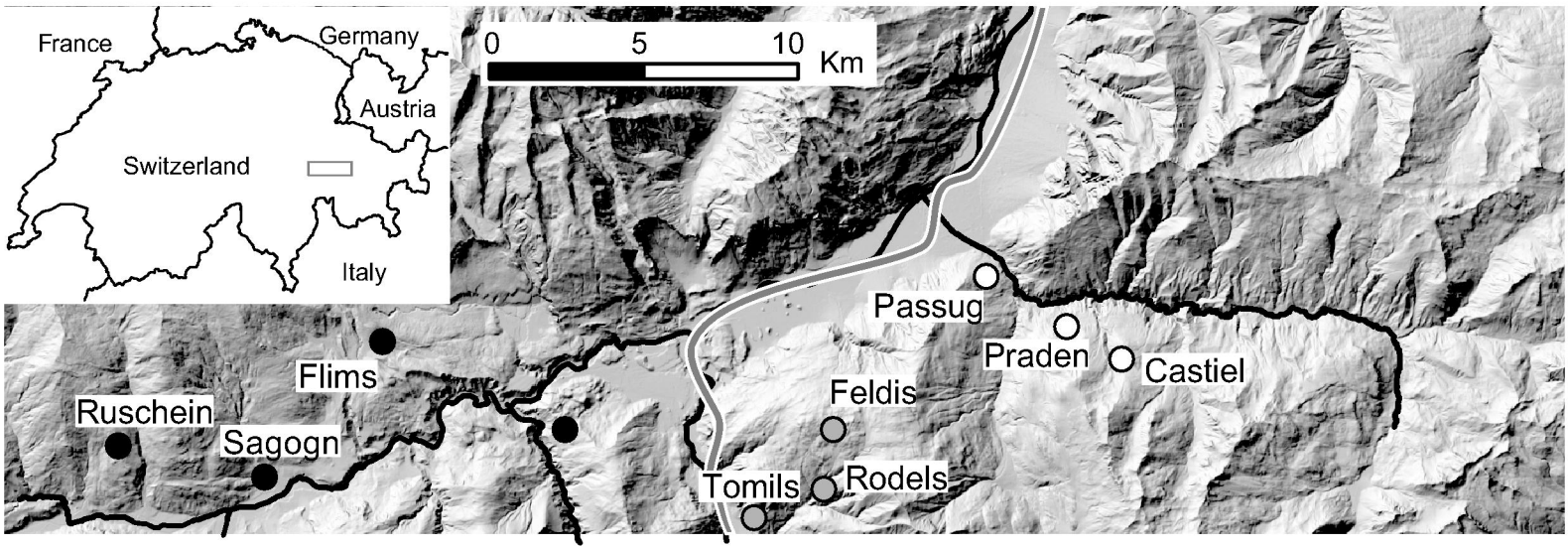
## Figure legends

Figure 1: Location of tick sampling sites in the Swiss Alps. Different colours of circles (i.e., black, grey, white) represent the different locations. Rivers and motorway are shown in black. Modified from [98].

Figure 2: Association between bacterial alpha diversity (inverse Simpson index) and elevation at three locations in the Swiss Alps. Bacterial alpha diversity was consistently lower at higher elevations. Model predictions for each location are plotted as lines.

Figure 3: Tick microbial community variance partitioning for different fixed and random effects. The first three columns represent tick endosymbionts, the next three columns are OTUs which are both tick endosymbionts and human pathogens and the subsequent six columns represent human pathogens. The other columns represent the 88 most common OTUs found in *I. ricinus*, ordered by amplicon frequency. Month, sampling site, location and tick ID were included in the model as random effects, whereas fixed effects were divided into environmental (elevation, temperature, precipitation, forest coverage, slope, aspect, vole abundance and vole to other rodents ratio) and tick-related variables (life stage or sex, individual heterozygosity, abundance, expected population heterozygosity). See raw data in Figshare for information on OTU labels.

Figure 4: Facilitation or competition among endosymbionts and pathogens within ticks. Relationships after accounting for shared environmental preferences are shown. Red lines represent positive associations (i.e., facilitation) and blue lines negative associations (i.e., competition). Only associations with strong statistical support (i.e., based on the 90% central credible interval) are presented. Darker colors indicate stronger associations.


France
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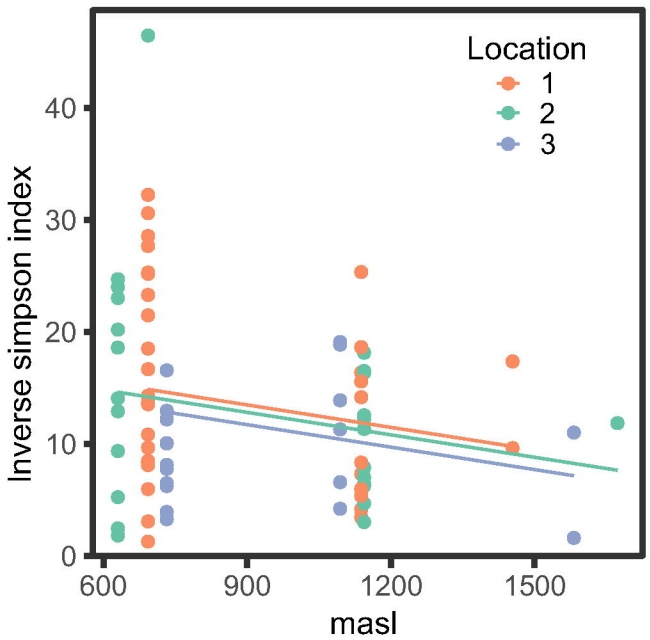
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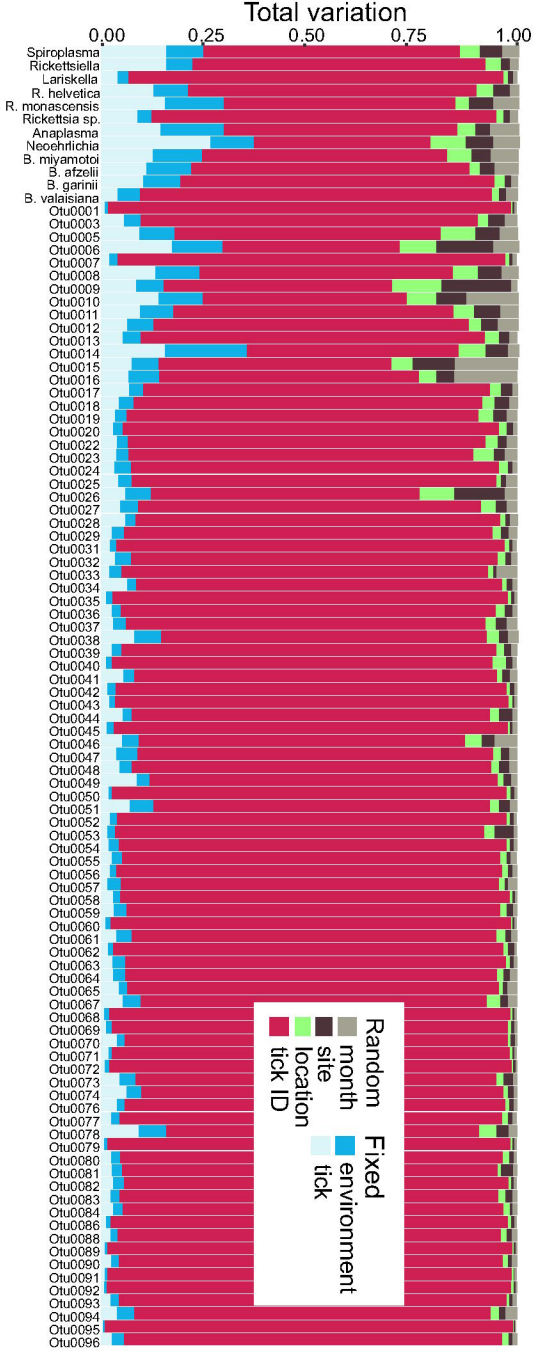
Total variation
0.00
0.25
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1.00
Spiroplasma
Rickettsiella
Lariskella
R. helvetica
R. monascensis
Rickettsia sp.
Anaplasma
Neoehrlichia
B. miyamotoi
B. afzelii
B. garinii
B. valaisiana
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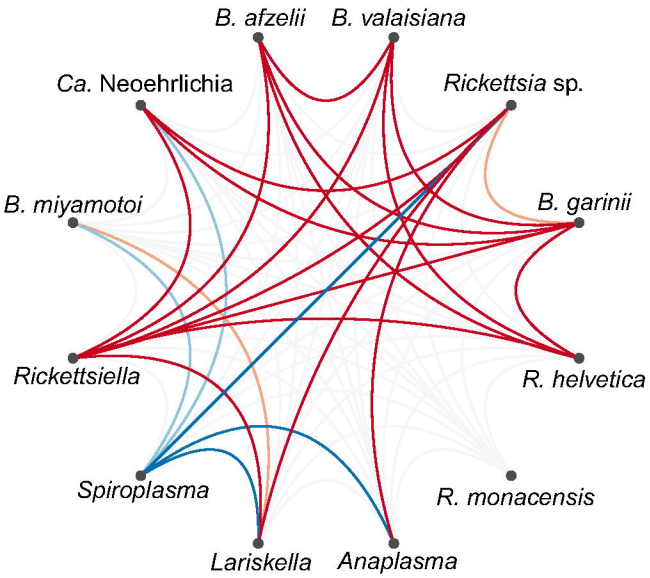
Random
month
site
location
tick ID
Fixed
environment
tick


B. afzelii
B. valaisiana
Ca. Neoehrlichia
Rickettsia sp.
B. miyamotoi
B. garinii
Rickettsiella
R. helvetica
Spiroplasma
R. monacensis
Lariskella
Anaplasma