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Par Audrey Bourdin

Effet de la diversité des arbres sur la réduction des risques liés aux tiques pour la santé humaine

Sous la direction de : Hervé JACTEL

Soutenue le 9 Juin 2023

Membres du jury :

Mme LEBERT, Isabelle	Ingénieure de Recherches	INRAE, Clermont	Rapporteure
Mme VERHEYDEN, Hélène	Chargée de Recherches	INRAE, Toulouse	Rapporteure
M. PLANTARD, Olivier	Directeur de Recherches	INRAE, Nantes	Président
Mme VANWAMBEKE, Sophie	Professeure	UC Louvain, Belgique	Examinatrice
M. JACTEL, Hervé	Directeur de Recherches	INRAE, Bordeaux	Directeur

Titre : Effets de la diversité des arbres sur la réduction des risques liés aux tiques pour la santé humaine

Résumé : Les changements globaux, climatiques ou de surexploitation des habitats, menant à l'érosion de la biodiversité et des contacts plus étroits entre les populations humaines et la faune sauvage, concourent à augmenter le risque de zoonoses. Ces modifications concernent en particulier les maladies transmises par les tiques comme la borréliose de Lyme, causée par des bactéries du genre *Borrelia*. En effet, les densités de tiques et la fréquence des maladies qu'elles transmettent dépendraient en grande partie de la biodiversité des vertébrés hôtes. Des études américaines ont ainsi montré que la prévalence de borrélies chez les tiques *Ixodes scapularis* diminuait par effet de dilution quand augmentait la proportion de vertébrés hôtes incompétents. En Europe, de récentes études sur *Ixodes ricinus* suggèrent que la structure et la diversité de l'habitat peuvent aussi avoir des effets diluants ou amplificateurs sur les risques de maladies transmises par les tiques.

A l'aide d'une revue systématique de la littérature scientifique et d'expériences sur le terrain, nous avons cherché dans cette thèse à répondre aux trois questions suivantes : (i) Quel est le rôle de la forêt comme réservoir de tiques et comment leur densité dépend-elle de l'abondance et de la diversité de leurs hôtes vertébrés ; (ii) Quel est l'effet de la biodiversité des forêts sur les densités de tiques et leur taux d'infection par les bactéries; (iii) Quelles sont les composantes de la biodiversité des forêts qui exercent un effet direct ou indirect sur les densités de tiques et la prévalence des bactéries associées. Pour répondre à ces interrogations, nous avons réalisé une méta-analyse pour comparer les densités de tiques entre les habitats forestiers et les habitats ouverts (naturels ou anthropiques) suivie d'une méta-régression pour tester comment les abondances de tiques sont influencées par l'abondance de leurs hôtes vertébrés en forêt. Nous avons également testé l'effet de la diversité des arbres sur les densités de tiques et la proportion de tiques infectées au sein d'un dispositif expérimental manipulant la diversité des essences forestières. Enfin, nous avons échantillonné les tiques le long de gradients de diversité des essences forestières dans un réseau de sept sites en Europe pour évaluer l’effet combiné de la diversité des arbres et des plantes du sous-bois sur les tiques et les pathogènes associés.

Nos résultats montrent que les forêts, et plus particulièrement les forêts mixtes décidues-conifères, constituent les habitats avec les plus grandes densités de tiques. Nos expériences de terrain ont aussi révélé que la richesse spécifique en arbres induit une réduction de la prévalence des borrélies associées aux nymphes d’*Ixodes ricinus*. Enfin, la diversité des plantes de sous-bois et la proportion de plantes appétentes pour le chevreuil impactent à la fois la densité des tiques et les taux d'infection par plusieurs pathogènes.

Ces résultats suggèrent donc que la biodiversité des forêts, au niveau de la strate arborée et du sous-bois, peuvent affecter l'abondance des tiques et la prévalence des agents pathogènes de façon indirecte en modifiant la composition des communautés de vertébrés et en favorisant les processus de dilution par les hôtes incompétents. Des recherches supplémentaires seraient cependant nécessaires pour quantifier plus directement l’effet de la diversité des forêts sur la faune de vertébrés et leur charge en tiques vectrices de maladie. Elles permettront de confirmer que l’augmentation de la biodiversité forestière peut aider à réduire le risque de transmission des maladies associées aux tiques. Combinés aux effets connus de la diversité des essences forestières sur la réduction des maladies des arbres, nos résultats sur la santé humaine contribuent à établir les liens entre biodiversité et santé globale selon le concept One Health.

Mots clés : Biodiversité, Tiques, Santé humaine

Title : Effects of tree diversity on the reduction of risks to human health related to ticks

Abstract : Global change and habitat overexploitation leading to biodiversity erosion and closer contacts between human populations and wildlife, contribute to increase the risk of zoonosis. This is particularly the case for tick-borne diseases such as Lyme borreliosis, caused by bacteria of the genus *Borrelia*. Indeed, the densities of ticks and the frequency of the diseases they transmit would largely depend on the biodiversity of their vertebrate hosts. American studies showed that the prevalence of Borrelia in *Ixodes scapularis* ticks decreased by dilution effect when the proportion of incompetent vertebrate hosts increased. In Europe, recent studies on *Ixodes ricinus* suggest that habitat structure and diversity may also have diluting or amplifying effects on tick-borne disease risk.

Using a systematic review of the literature and field experiments, we aimed to answer the following three questions: (i) What is the role of forest habitat as a reservoir for ticks and how does their density depend on the abundance and diversity of their vertebrate hosts; (ii) What is the effect of forest biodiversity on tick densities and their infection rates; (iii) Which components of forest biodiversity have a direct or indirect effect on tick densities and the prevalence of associated bacteria.

To answer these questions, we conducted a meta-analysis to compare tick densities between forest and open habitats (natural or anthropogenic) followed by a meta-regression to test how tick abundance was influenced by the abundance of their vertebrate hosts in forests. We also tested the effect of tree diversity on tick densities and the proportion of infected ticks within an experimental plantation manipulating tree species diversity. Finally, we sampled ticks along gradients of forest species diversity in a network of seven sites in Europe to assess the combined effect of tree and understorey plant diversity on ticks and associated pathogens.

Our results showed that forests harbour on average more ticks than open habitats and that mixed deciduous - coniferous forests have the highest tick densities. Our field experiments revealed that tree species richness induces a reduction in the prevalence of *Borrelia* pathogens associated with Ixodes ricinus nymphs. Finally, understorey plant diversity and the proportion of plants palatable to roe deer impact both tick density and infection rates for several pathogens.

These results therefore suggest that forest biodiversity, at both tree and understorey tiers, may affect tick abundance and pathogen prevalence indirectly by altering vertebrate community composition and promoting dilution processes by incompetent hosts. However, further research is needed to quantify more directly the effect of forest diversity on vertebrate fauna and their load of disease-carrying ticks. This will confirm that increasing forest biodiversity can help reduce the risk of tick-associated disease transmission. Combined with the known effects of forest species diversity on reducing tree diseases, our results on human health contribute to establishing the links between biodiversity and global health according to the One Health concept.

Keywords : Biodiversity, Ticks, Human health

Unité de recherche

INRAE – Institut National de la Recherche Agronomique

UMR 1202 – Biodiversité, Gènes et Ecosystèmes

Site de Recherches Forêt Bois de Pierroton – Domaine de l’Hermitage

69, route d’Arcachon

33612 CESTAS Cedex-France

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TABLE DES MATIERES

1. Introduction

La littérature en écologie compte de nombreuses preuves du lien entre la biodiversité (voir Box 1 pour la définition) et le maintien de nombreux processus et les fonctions des écosystèmes dont certains impactent la survie et le bien-être de l'humain (Diaz et al., 2006 ; Loreau, 2010 ; Naeem et al., 2012 ; Norris, 2012 ; Gamfeldt et al., 2013 ; Loreau & de Mazancourt, 2013 ; Sandifer et al., 2015 ; Bennett et al., 2015 ; Wang et al., 2021). Issus des processus écosystémiques, les services écosystémiques sont les avantages en termes économiques, de bien-être et de santé que les personnes tirent directement ou indirectement des écosystèmes (Évaluation des écosystèmes pour le Millénaire, 2005 ; Fu et al., 2013). En raison de leur large distribution et utilisation par l'humain, les forêts sont généralement bien placées pour fournir la plupart des services écosystémiques énumérés par plusieurs rapports internationaux tels que l'Evaluation des écosystèmes pour le millénaire (Millennium Ecosystem Asessment 2005) ou le CICES (CICES 2013). Toutefois, les études empiriques qui établissent des relations entre la biodiversité forestière (voir Box 1 pour la définition) et les services écosystémiques font défaut pour de nombreux services importants comme la régulation des maladies humaines (Brockerhoff et al., 2017 ; Mori et al. 2017).

Box 1

Biodiversité: "La variabilité des organismes vivants de toute origine y compris, entre autres, les écosystèmes terrestres, marins et autres écosystèmes aquatiques et les complexes écologiques dont ils font partie. Cela comprend la diversité au sein des espèces et entre espèces ainsi que celle des écosystèmes" (Convention sur la Diversité Biologique).

Diversité forestière: Dans cette thèse, ce terme comprendra à la fois la diversité compositionnelle, fonctionnelle et structurelle végétale des forêts.

1.1 SANTE ET BIEN-ETRE DES HUMAINS: LE ROLE DE LA BIODIVERSITE

La biodiversité influence la santé humaine de bien des manières, qu'elles soient psychologiques, sociales ou biophysiques. D'après Marselle et al. (2021), elle permet (i) d'atténuer des atteintes néfastes à la santé (ii) restaurer et renforcer des aptitudes diminuées par les exigences de la vie quotidienne (Hartig, 2017) et joue un rôle dans (iii) la transmission de maladies ou la survenue d'allergies.

La biodiversité permet l’accès à des services d'approvisionnement essentiels pour l’humain, tels que les médicaments (i.e. les plantes médicinales représentent la principale source de médicaments à base de produits naturels pour une majorité de la population humaine (Romanelli et al., 2015)), une alimentation diversifiée et sécurisée face aux menaces du changement climatique (Bernstein, 2014) ainsi que l’eau potable (Dudley & Stolton, 2003). Elle diminue des facteurs de stress environnementaux via une bonne régulation de la pollution atmosphérique et sonore (Churkina et al., 2015, Grote et al., 2016) ou des chaleurs extrêmes (Lindley et al., 2019).

Des travaux en psychologie ont montré qu’une richesse réelle en espèces de plantes et d’oiseaux pouvait diminuer le stress et faciliter la réflexion (Fuller et al., 2007, Dallimer et al., 2012). Des impressions de détente et de bien-être ont été positivement associées à l'hétérogénéité du paysage d’espaces verts urbains (Meyer-Grandbastien et al., 2020), à la structure de la végétation et aux espèces végétales des jardins (Hoyle et al., 2017), mais aussi à la diversité réelle (Wood et al., 2018) et perçue (White et al., 2017). Björk et al. (2008) et de Jong et al. (2012) ont trouvé une association positive entre les environnements riches en espèces et une plus grande activité physique de la part des habitants.

Enfin, les habitats naturels peuvent présenter des risques pour la santé, que la biodiversité influence. Parmi ces risques, nous retrouvons l’exposition aux allergènes, composés organiques volatils et aux agents pathogènes causant des maladies humaines. D’après Prescott (2020), une diminution de l’exposition à la biodiversité augmente notre susceptibilité aux allergies. L'hypothèse de la biodiversité stipule que "le contact avec les environnements naturels enrichit le microbiome humain, favorise l'équilibre immunitaire et protège des allergies et des troubles inflammatoires" (Haahtela, 2019). Cette hypothèse est soutenue par plusieurs études (Hanski et al., 2012 ; Haahtela et al., 2013). Finalement, de plus en plus de preuves montrent des corrélations entre changement environnementaux globaux, perte de biodiversité et émergence ou augmentation de la prévalence de maladies infectieuses. Les perturbations/réorganisations des communautés écologiques induites par des changements de biodiversité affectent les dynamiques de transmission des agents pathogènes et impactent ainsi le risque zoonotique.

1.2 BIODIVERSITE ET ZOONOSES

Le lien entre biodiversité et maladies infectieuses semble très dépendant de l'identité du pathogène, de son cycle de vie et de son mode de transmission (Rohr et al., 2020). Par exemple, des agents pathogènes très spécialisés, sans stade de vie libre, d'hôtes intermédiaires ou de vecteurs (e.g. maladies sexuellement transmissibles chez l'humain) (voir Box 2 pour les définitions) sont peu susceptibles d'être impactés par des changements de biodiversité. En revanche, des pathogènes capables d'infecter les humains mais aussi d'autres vertébrés sont plus susceptibles de répondre à des changements de biodiversité dans la communauté de vertébrés. Cette interaction entre maladies et biodiversité, sera d'autant plus forte pour les systèmes parasites-hôtes dont les contacts mutuels sont limités. Par exemple, des vecteurs qui prennent un nombre de repas sanguins limité au cours de leur vie, seront plus sensibles aux modifications de diversité des hôtes et non-hôtes dans l'environnement que des parasites qui y répandent des milliards de stades infectieux (e.g. *Escherichia coli*, *Giarda sp.*). C'est notamment le cas des tiques et des moustiques impliqués dans la transmission de maladies vectorielles, dont la totalité représentent 17 % de la charge mondiale de toutes les maladies infectieuses selon l'Organisation Mondiale de la Santé.

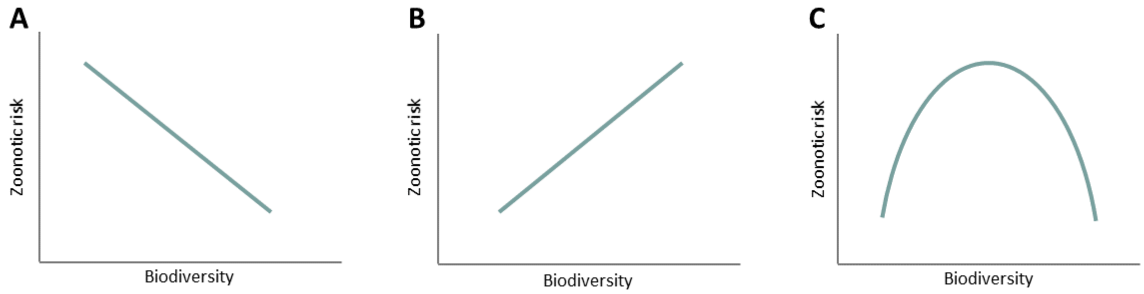


Fig. 1 : Relations schématiques et hypothétiques entre la biodiversité et le risque de maladie. A. Effet de dilution. B. Effet d'amplification. C. Dilution et amplification en fonction des circonstances.

D'après plusieurs auteurs, la relation entre biodiversité et maladies peut avoir plusieurs formes (e.g. linéaire, unimodale) et directions (Rohr et al., 2020 ; Keesing et al., 2021) (Fig.1). Des relations linéaires positives et négatives soutiennent respectivement les théories d'amplification et de dilution des maladies par la biodiversité des hôtes et non-hôtes. Une relation unimodale implique des phases de dilution et d'amplification selon différentes circonstances. D'après Keesing et al. (2021), les relations linéaires négatives sont le plus représentées dans la littérature, mais cela pourrait venir du fait que peu d'études ont pu être réalisées dans des cas de nulle (cas hypothétique) ou très faible biodiversité. Un environnement théorique avec une abondance nulle en hôte implique forcément l'absence du pathogène. Il est alors logique que l'apparition des premiers hôtes ait un effet amplificateur de la prévalence du pathogène.

Enfin, le sens de relation entre biodiversité et maladie semble diverger en fonction de l'échelle d'étude (Lafferty & Wood, 2013 ; Wood & Lafferty, 2013 ; Kilpatrick et al., 2017), la dilution semble prédominer à petite échelle mais l'amplification à plus large échelle. Johnson et al. (2015) pense que la relation biodiversité-maladie est plus forte à petite échelle car c'est à l'échelle locale que les interactions entre espèces tiennent place. Cohen et al. (2016) montrent que les facteurs susceptibles d'affecter la prévalence de maladies infectieuses comptent davantage aux échelles auxquelles ils varient le plus (i.e. la diversité des hôtes à échelle locale, et les facteurs climatiques à plus grande échelle).

Finalement, une meilleure compréhension du lien entre les maladies et la biodiversité permettrait un grand progrès dans le contrôle efficace des maladies infectieuses (Young et al., 2014). Pour cela, il est important de comprendre (i) quels pathogènes sont susceptibles d'être influencés par la biodiversité, (ii) le lien qui existe entre la biodiversité et le risque de maladie et (iii) lier correctement les changements de biodiversité avec chaque métrique du risque d'infection (e.g. densité de vecteurs, de vecteurs infectés, prévalence de pathogènes…) (Rohr et al., 2020).

Box 2

Zoonose: Une zoonose est une maladie ou une infection naturellement transmissible des animaux vertébrés à l'être humain (Organisation Mondiale de la Santé). Elle est dite vectorielle lorsqu'elle est transmise entre deux individus par un vecteur.

Vecteur: Un vecteur est un arthropode, souvent hématophage (dans cette thèse : la tique), qui transmet un agent pathogène : un virus, une bactérie ou un parasite (anses). Il acquière cet agent pathogène en se nourrissant sur un hôte puis le transmet à d'autres individus (Organisation Mondiale de la Santé).

Hôte: Dans cette thèse, nous emploierons le terme « hôte » pour désigner un vertébré que les tiques peuvent parasiter. Nous parlerons d'« hôte compétent » pour désigner un animal vertébré qui héberge et assure la survie prolongée d'un agent pathogène transmissible à l'homme par la tique. A l'inverse, nous parlerons d'«hôte incompétent» pour désigner un animal vertébré qui n'héberge pas (ou quasiment pas) un agent pathogène transmissible à l'humain par la tique.

Compétence: La compétence de l'hôte est sa capacité à transmettre efficacement l'infection à un autre hôte ou vecteur sensible (Gervasi et al., 2015).

1.3 TIQUES ET MALADIES A TIQUES

Les tiques sont des acariens hématophages stricts impliqués en tant que vecteurs dans le cycle de transmission de plusieurs maladies vectorielles humaines. L'espèce de tiques la plus répandue en Europe est *Ixodes ricinus* (L., 1758) représentant le principal vecteur des maladies transmises par les tiques (De la Fuente, 2008) (Fig. 2).

Fig. 2: Photo d'une nymphe *I. ricinus* prise à la loupe binoculaire. Face dorsale à gauche. Face ventrale à droite.

C'est une espèce qui a besoin de trois repas de sang pour compléter son cycle de vie et passer de la larve à la nymphe, de la nymphe à l'adulte et d'un troisième repas servant à la reproduction. *I. ricinus* est responsable de la transmission de plusieurs bactéries, virus et protozoaires pathogènes (Prinz et al. 2017 ; Rizzoli et al., 2014) dont le complexe bactérien *Borrelia burgdorferi* sensu lato, l'agent de la borréliose de Lyme transmis par les tiques (Fig. 3) qui peut causer divers troubles nerveux, articulaires ou encore cutanés chez l'humain sur le long terme (O' Connell, 2014). Mais d'autres pathologies émergent également, liées aux espèces *Anaplasma*, *Neoehrlichia*, *Rickettsia* et *Babesia* (Keesing et Ostfeld, 2021 ; Kilpatrick et Randolph, 2012), responsables d'Anaplasmoses, Neoehrlichioses, fièvres boutonneuses et Babésioses (Cosson, 2019).

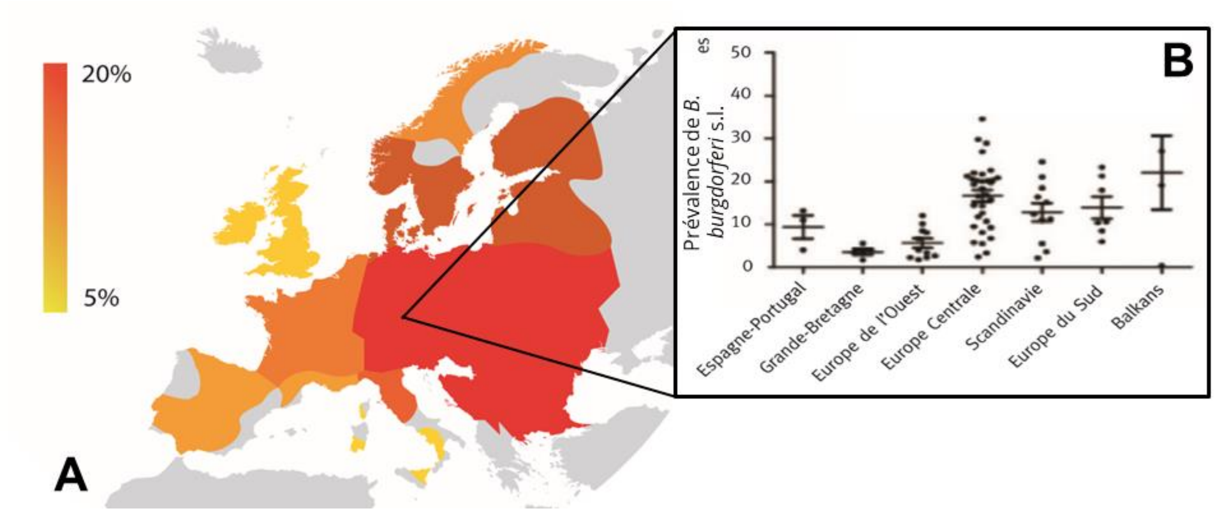


Fig. 3 a) Prévalence de *B. burgdorferi* sensu lato en Europe. Carte ©CiTIQUE **b)** Prévalence de *B. burgdorferi* sensu lato chez les nymphes *I. ricinus* dans des régions définies d'Europe (Strnad et al. (2017)).

Les tiques s'infectent pendant un repas de sang contaminé sur un hôte ou bien par transmission transovarienne (ou verticale). Cette dernière voie n'est possible que pour quelques agents pathogènes (Sprong et al., 2009 ; Burri et al., 2014) tels que certaines espèces de *Rickettsia* ou *Babesia* et constitue le principal moyen pour les larves d'être infectées avant un premier repas sanguin. Sachant que la probabilité pour une tique d'être infectée augmente avec le nombre de repas sanguins pris et que les nymphes sont plus abondantes dans l'environnement que les adultes, de nombreuses études considèrent la densité de nymphes infectées ou leur prévalence d'infection comme les meilleurs proxys du risque de contamination dans l'environnement (Diuk-Wasser et al., 2012; Pepin et al., 2012; Stafford et al., 1998).

I. ricinus est généraliste et peut infester une large gamme d'hôte. Les larves vivant plus près du sol, se nourrissent en majorité sur les micromammifères (e.g. souris, campagnols), alors que les nymphes et les adultes, chassant plus haut dans la végétation, sont plus souvent retrouvées sur les grands mammifères (e.g. ongulés sauvages) mais aussi en plus faible proportion sur les oiseaux et reptiles

(Hofmeester et al., 2016). Les espèces d’hôtes varient dans leur capacité de dilution ou d'amplification du risque en fonction de leur compétence, leur abondance et leur potentiel de transmission. Des études américaines sur les maladies transmises par *Ixodes scapularis* ont montré que la diversité des hôtes vertébrés pouvait diminuer la prévalence de borrélies chez les tiques par effet de dilution des hôtes compétents par les incompétents (Linske et al., 2018 ; Ostfeld et Keesing, 2012) (voir Box 2 pour les définitions). Dans les forêts européennes, ce résultat est rarement retrouvé sur *I. ricinus*. Esser et al. (2022) n'ont trouvé aucun effet de la richesse des hôtes vertébrés sur la prévalence des pathogènes associés à *I. ricinus* en forêts néerlandaises. Si l'effet global de la richesse en hôtes est faible ou nul, plusieurs auteurs s'accordent à dire que les ongulés sauvages (e.g. chevreuil, cerf) jouent un rôle important dans la dynamique des tiques et des pathogènes associés (Tagliapietra et al., 2011). Ces cervidés constituent des hôtes majeurs pour la reproduction des adultes d'*I. ricinus* (Vor et al., 2010), leur présence ou abondance pourrait amplifier les densités de larves et de nymphes (Vor et al., 2010 ; Gilbert et al., 2012; Hofmeester et al., 2017). En contrepartie, ils sont connus pour être des hôtes incompétents pour les borrélies, et pourraient diminuer le risque de maladie de Lyme dans l'environnement (Vour’ch et al., 2016 ; Rosef et al., 2009). Ils sont toutefois compétents pour de nombreux autres agents infectieux d'importance relative pour la santé publique et vétérinaire (e.g. *Anaplasma* spp. et *Rickettsia* spp.) (Hornok et al., 2018; Wijbur et al., 2022). Plusieurs espèces de petits mammifères (e.g. souris sylvestre, campagnol roussâtre) sont connues pour être des hôtes compétents pour les borrélies (e.g. *B. afzelii,* Hanincová et al., 2003) mais aussi pour d’autres pathogènes (e.g. *Anaplasma* spp*.*, Perez et al., 2017). Ils sont considérés comme des hôtes amplificateurs du risque de maladie de Lyme dans l’environnement (Ostfeld et al., 2006 ; Levi et al., 2016). Les oiseaux constituent également un réservoir compétent important dans la dynamique de la maladie de Lyme, en portant par exemple *B. garinii* ou *B. valaisianna* (Taragel'ová et al., 2008), mais aussi les lézards, compétents pour *B. lusitaniae* (Younsi et al., 2001).

Box 3

Risque: Le risque pour l'humain d'être infecté par un agent infectieux (bactérie, parasite ou virus) transmis par les tiques. Il est le résultat de la combinaison de trois composantes: l'aléa (cause du dommage), l'exposition (individus ou systèmes qui subiront les conséquences de ce dommage) et la vulnérabilité (capacité des systèmes ou des individus à résister au dommage) (IPCC). Dans le cas du risque de maladie humaine transmise par les tiques, l'aléa peut être considéré comme la densité de tiques (infectées ou non par des pathogènes transmissibles), l'exposition est constituée des personnes et des animaux potentiellement piqués par les tiques et la vulnérabilité correspond à la résistance d'être infecté par les agents pathogènes.

Dilution: Nous utilisons ici la définition globale de l'effet de dilution qui fait référence aux scénarios dans lesquels la biodiversité mène à une réduction de la prévalence de pathogènes associés aux tiques dans l'environnement (Ogden & Tsao, 2009). Cet effet se produit lorsque l'ajout d'une ou plusieurs espèces hôtes à une communauté rend un agent pathogène moins abondant et moins susceptible de persister dans l'environnement (Begon, 2008).

Amplification: Fait référence aux scenarios dans lesquels la biodiversité mène à une augmentation de la prévalence de pathogènes dans l'environnement (Ogden & Tsao, 2009).

1.4 LA DIVERSITE DES FORETS: UN ATOUT POUR REDUIRE LES MALADIES A TIQUES?

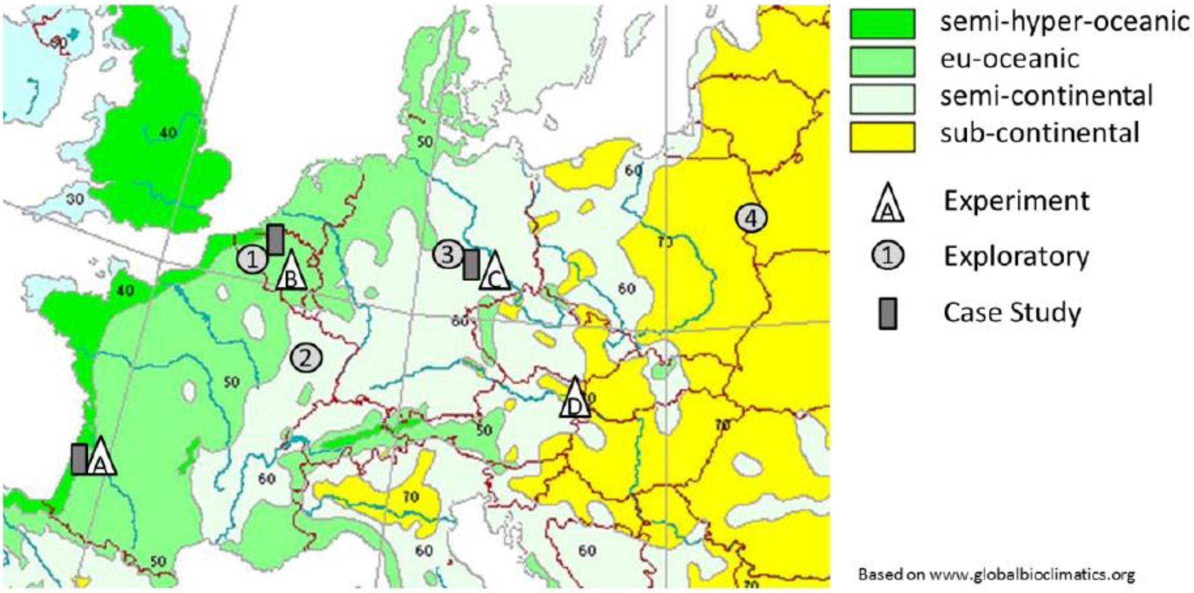
Il existe de nombreuses preuves que les forêts mixtes fournissent beaucoup de services écosystémiques tels qu'une productivité plus élevée que les forêts pures (Paquette et Messier 2011), avec une meilleure séquestration du carbone ou une meilleure capacité à se remettre des perturbations (Thompson et al., 2009), et moins de ravageurs (Jactel et al., 2017). Cependant, les liens plus directs avec le bien-être et la santé des humains sont moins connus (Marselle et al., 2021). En abritant un grand nombre d'hôtes et de vecteurs, la forêt peut présenter des dangers pour les humains en augmentant la probabilité de transmission des maladies par les animaux sauvages. Plusieurs études ont déjà établi un lien entre la déforestation et l'émergence ou le développement de zoonoses en raison de l'augmentation de l'interface entre les villes et les forêts, qui accroît les contacts entre les humains ou les animaux domestiques et les animaux sauvages (Olivero et al., 2017; Ellwanger et al., 2020). La fragmentation des forêts peut entraîner une réorganisation des communautés de vertébrés, dont les assemblages et désassemblages dans le temps et l'espace affectent directement les populations de vecteurs, la transmission et la prévalence de plusieurs agents pathogènes dans l'environnement (Diuk-Wasser et al., 2020). Plusieurs études montrent que les strates hautes et basses de la forêt peuvent impacter les populations de tiques et les pathogènes associés (Ehrmann et al., 2017, 2018). Cela directement ou indirectement via l'effet qu'elles ont sur les communautés d'hôtes vertébrés.

L'ombre, l'humidité et les fruits générés par la canopée ou le sous-bois peuvent fournir des sites d'alimentation, de reproduction ou de repos plus ou moins favorables aux hôtes vertébrés. En conditionnant le microclimat, arbres et plantes du sous-bois affectent la survie et l'activité de quête des tiques qui sont sensibles à la dessiccation (Ehrmann et al. 2017 ; McCoy et Boulanger, 2015, Boyard et al., 2007; Estrada-Peña, 2008).

Plusieurs études ont établi un lien de causalité entre la diversité des arbres et la diversité ou la composition du sous-bois (Corcket et al., 2020 ; Ampoorter et al., 2014 ; Gazol et Ibáñez, 2009). Il a également été démontré que la diversité et la composition des espèces d'arbres protégeaient le microclimat du sous-étage de variations intenses (De Frenne et al., 2019). Les forêts mixtes se caractérisent par un degré élevé de fermeture de la canopée, rendu possible par une plus grande complémentarité dans la forme des couronnes (théorie du « canopy packing » (Jucker et al. 2015 ; Williams et al. 2017)). Une canopée plus dense peut produire une meilleure qualité d'ombrage favorisant l'humidité dans le sous-étage, ce qui pourrait favoriser la survie des tiques. D'autres études ont démontré que la diversité des espèces forestières peut modifier l'abondance et le comportement alimentaire des vertébrés herbivores avec des effets souvent contrastés selon la taille de ces animaux, réduisant l'activité des petits (par exemple les souris et les campagnols) et augmentant celle des grands (par exemple les élans et les cerfs) (Jactel et al., 2017 ; Milligan et Koricheva, 2013). Cependant, très peu d'études ont tenté de mesurer les effets de la diversité des espèces forestières sur l'abondance des tiques et la prévalence des agents pathogènes transmis par les tiques (mais voir Ehrmann et al. 2017, 2018 ; Ruyts et al., 2016).

1.5 PROBLEMATIQUE DE LA THESE

Les écosystèmes forestiers constituent un réservoir important de biodiversité dans les paysages habités d'Europe centrale, et fournissent de nombreux services écosystémiques. Ils présentent plusieurs bénéfices pour la santé humaine, mais aussi des risques. L'importance de la biodiversité pour la santé est de plus en plus reconnue par les organisations politiques et les Nations Unies. La Convention sur la Diversité Biologique et l'Organisation Mondiale de la Santé collaborent pour promouvoir les avantages de la biodiversité, notamment en ce qui concerne la prévention des maladies infectieuses (Convention on Biological Diversity, 2016). Les maladies transmises par les tiques représentent la majorité des maladies humaines à transmission vectorielle en Europe avec *I. ricinus*, surtout présente dans les forêts, comme principal vecteur. L'hypothèse de dilution stipule que la diversité des hôtes des tiques dans l'environnement peut réduire le risque d'infection (Ostfeld et al. 2020) (voir Box 3 pour les définitions). En Europe, ce résultat reste peu trouvé sur *I. ricinus*, mais de récentes études suggèrent que la structure et la diversité de l'habitat peuvent elles-mêmes avoir des effets diluants ou amplificateurs sur les risques liés aux tiques. En agissant directement sur la survie et l'activité des tiques ou indirectement via les effets qu'elles ont sur les vertébrés, notre hypothèse principale est que l'abondance des tiques et la prévalence des pathogènes associés diminuent avec l'augmentation de la diversité des arbres. Dans cette thèse, nous nous sommes intéressés à comprendre quelles variables de risque liées aux tiques (densité de tiques, prévalences d'infection) répondaient à la diversité forestière, dans quel sens et selon quels mécanismes.



Based on www.globalbioclimatics.org

Fig.3 : Emplacement des sites forestiers plantés (Experiments) et semi-naturels (Exploratories) établis le long d’un gradient de diversité en arbres du projet BiodivERsA Dr. Forest.

En se concentrant sur la diversité compositionnelle, fonctionnelle et structurelle des arbres forestiers, et en la testant à travers l'Europe sur plusieurs sites le long d'un gradient climatique (Fig. 3), cette thèse a étudié les effets potentiellement positifs et négatifs de la biodiversité forestière sur les risques liés aux tiques pour la santé humaine, ainsi que les mécanismes sous-jacents. Tous les sites forestiers européens échantillonnés présentent l'avantage de disposer de gradients de biodiversité conçus pour minimiser les effets environnementaux confondants.

Fig. 4. Photos des sites forestiers du projet Dr Forest. A. MixLor (France). B**.** FORBIO (Belgique). C. TREEWEB (Belgique). D. Bialowieza (Pologne).

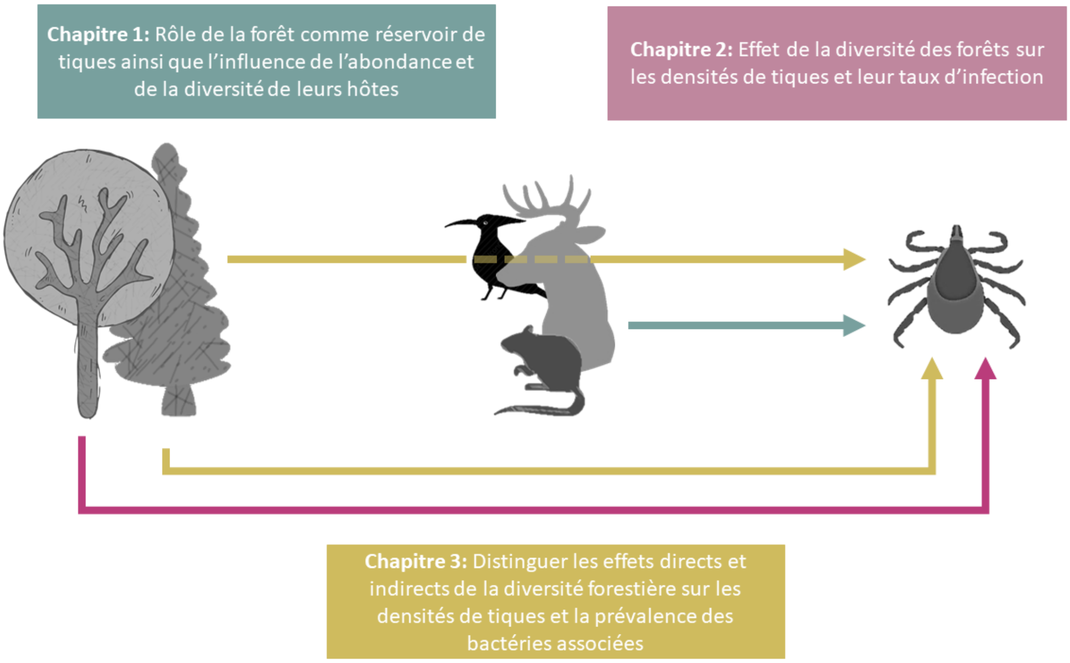


Fig. 5: Schéma récapitulatif des différents chapitres de la thèse.

Dans un premier chapitre de résultats, nous avons réalisé une méta-analyse de la littérature scientifique pour comparer les densités de tiques entre les habitats forestiers et les habitats ouverts, suivie d'une méta-régression pour tester comment les abondances de tiques sont influencées par l'abondance de leurs hôtes vertébrés en forêt. Nos principales hypothèses étaient :

(i) Les abondances de tiques sont plus grandes en forêt que dans les habitats non-forestiers dans le même paysage.

(ii) En forêt, l'abondance des tiques augmente avec l'abondance des hôtes vertébrés.

Dans un deuxième chapitre, nous avons testé l'effet de la diversité des arbres sur les densités de tiques et la proportion de tiques infectées au sein d'un dispositif expérimental manipulant la diversité des essences forestières. Nos principales hypothèses étaient :

(iii) L'abondance des tiques et la proportion de nymphes infectées sont plus faibles dans les parcelles mixtes que dans monocultures, en raison des caractéristiques du peuplement associées aux effets de " dilution de l'habitat ".

(iv) Les effets de la diversité des espèces d'arbres sur les tiques sont principalement médiés par les changements dans la densité et la composition du sous-bois.

Enfin, dans un dernier chapitre, nous avons évalué l'effet combiné de la diversité des arbres et des plantes du sous-bois, appétentes ou non pour le chevreuil, sur les tiques et les pathogènes associés. Cette dernière étude a été réalisée le long de sept sites forestiers européens, en France, Belgique et Pologne. Nos principales hypothèses étaient :

(v) L'abondance des tiques augmente mais la prévalence des bactéries transmises par les tiques diminue dans les forêts mixtes par rapport aux forêts pures.

(vi) Les différentes espèces de *Borrelia* répondent différemment aux variables environnementales en raison de leurs exigences en matière d'habitat liées à leur spécificité d'hôte vertébré.

(vii) La proportion de plantes appétentes pour le chevreuil a un effet positif sur les densités de tiques mais diminue les taux d'infection des nymphes.

Cette thèse fait partie du projet ERA-Net BiodivERsA Dr.Forest (dans le cadre de l'appel à propositions de recherche 2018-2019 BiodivERsA) (www.dr-forest.eu). Ce projet a pour objectif de quantifier les risques et bénéfices de la biodiversité des forêts sur la santé humaine, cela en combinant la conservation de la biodiversité et la gestion des écosystèmes, qui favorisent plusieurs co-bénéfices pour la santé. Particulièrement, les équipes étudient le lien entre la diversité des forêts sur la restauration psychologique (i.e. restauration de l'attention et la diminution du stress), la jouissance de loisirs (i.e. microclimat), l'abondance de produits (i.e. plantes médicinales, baies, champignons), la pollution de l'air (i.e. allergènes volatiles) et les vecteurs de maladies (e.g. tiques, chenilles urticantes).

2. Quel est le rôle de la forêt comme réservoir de tiques et comment leur densité dépend-elle de l'abondance et de la diversité de leurs hôtes vertébrés?

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ABSTRACT

With increasing deforestation, questions are being raised about the risk of zoonotic disease to humans. To better assess the role of forest in the emergence of tick-borne diseases, we conducted a meta-analysis of the scientific literature to compare the abundance or diversity of ticks between forest and open habitats (natural or anthropogenic) and a meta-regression to test how tick abundance is influenced by the abundance of their vertebrate hosts in forest habitats. We found that *Ixodes* ticks were on average more abundant and diverse in forests than in any other non-forested habitats, the difference being more pronounced with mixed deciduous- coniferous than with deciduous forests. At the forest scale, exophilic *Ixodes* tick abundance was positively influenced by the abundance of their ungulate hosts. Our results suggest that mixed forests represent the habitats with the highest level of tick hazard. However, more studies are needed to assess the risk of transmission of tick-borne diseases in forests, which also depends on the prevalence of pathogens and the exposure of people.

Keywords:

Ixodes, ungulates, zoonosis, urban, mixed forests

RESUME

Avec l'augmentation de la déforestation, des questions sont soulevées quant au risque zoonotique pour l'homme. Pour mieux évaluer le rôle de la forêt dans l'émergence des maladies transmises par les tiques, nous avons effectué une méta-analyse de la littérature scientifique pour comparer l'abondance ou la diversité des tiques entre la forêt et les habitats ouverts et une méta-régression afin de tester l'abondance des tiques est influencée par l'abondance de leurs hôtes vertébrés dans les habitats forestiers. Nous avons constaté que les tiques *Ixodes* étaient en moyenne plus abondantes et diversifiées dans les forêts que dans tout autre habitat non forestier, la différence étant plus prononcée dans les forêts mixtes de feuillus et de conifères que dans les forêts de feuillus. À l'échelle de la forêt, l'abondance des tiques exophiles *Ixodes* était positivement influencée par l'abondance de leurs hôtes ongulés. Nos résultats suggèrent que les forêts mixtes représentent les habitats présentant le plus haut niveau de risque lié aux tiques. Cependant, d'autres études sont nécessaires pour évaluer le risque de transmission des maladies à tiques dans les forêts, qui dépend également de la prévalence des agents pathogènes et de l'exposition des personnes.

2.1. INTRODUCTION

Tick-borne diseases (TBDs) are spreading worldwide (Madison-Antenucci et al., 2020) with increasing medical consequences for domestic animals and humans (Maggi & Krämer, 2019). Lyme disease, caused by bacteria of genospecies *Borrelia bugdorferi* s.l. group and carried by ticks of the genus *Ixodes,* represents the largest human disease burden of all vector-borne diseases in the European Union (Semenza & Suk, 2018). The ticks *Ixodes ricinus* (L., 1758) and *Ixodes scapularis* (Say, 1821) are the most widespread and well-studied tick species in Europe and the United States, and are prominent vectors of a variety of bacteria and viruses (Rizzoli et al., 2014; Prinz et al. 2017). Other tick genera are less common (i.e. *Dermacentor*, *Hyalomma*, *Rhipicephalus* or *Argas*) but can also transmit bacteria or protozoa (e.g. *Rickettsia* spp., *Anaplasma* spp., *Babesia* spp., *Ehrlicchia* spp.) to humans and vertebrates.

The risk of tick-borne diseases is generally assessed in a variety of ways (nymph density, infected nymph density, nymph infection prevalence, pathogen prevalence) (Mather et al., 1996; Stafford et al., 1998; Diuk-Wasser et al., 2012; Pepin et al., 2012, Vourc'h et al., 2016), but all appear to be influenced by several multi-scale variables that are principally related to abiotic conditions, vegetation and host vertebrates. Vegetation and microclimate can directly impact tick survival and activity as they are sensitive to cold and desiccation (Randolph et al., 2002; Estrada-Peña, 2008; Allan et al., 2010; McCoy and Boulanger, 2015; Ehrmann et al., 2017; Wongnak et al., 2022a,b), which denser vegetation cover can mitigate (Scheffers et al., 2014). Indirectly these local conditions influence the abundance and diversity of potential hosts for blood meals, which may have an impact on tick fitness and infection prevalence differently according to the tick stage (Ostfeld et al., 1995; LoGiudice et al., 2003). Small mammals, such as voles, mice or shrews are considered most common hosts of *I. ricinus* larvae, because of their abundance and the high probability of encounter close to the ground (Anderson et al., 1986). Several studies found a strong positive relationship between rodent abundance in a year and *I. ricinus* nymph density in the next year (Perez et al., 2016; Krawczyk et al., 2020). However, this relationship seems to vary with the rodent species or the sampling season. For example, Perez et al. (2016) found that bank vole abundance had a negative effect on *I. ricinus* nymph density in spring but a positive effect in fall. Nymphs and adults, being more generalist and questing higher up on the vegetation, are most frequently found on medium to large-sized mammals, such as ungulates, and in less proportion on lizards and passerines (Hofmeester et al., 2016). Cervids are known to constitute major hosts for *I. ricinus* and *I. scapularis* adult reproduction (Vor et al., 2010) and several studies showed a positive correlation between cervids abundance -or activity- and abundance of *I. scapularis* nymphs (Schulze et al., 2001; Rand et al., 2004 ; Werden et al., 2014) or *I. ricinus* nymphs (Vor et al., 2010 ; Vourc'h et al., 2016; Hofmeester et al., 2017b). Inclusion/exclusion experiments in forests confirmed the positive effect of cervid presence on the nymph abundance of both tick species (Daniels & Fish, 1995; Ginsberg et al., 2004; Rand et al., 2004; Gilbert et al., 2012). However, some authors question the link between deer density reduction and reduction of tick-related risk (Kugeler et al., 2016). More recently, a few studies focused on the impact of predators on tick vector populations, showing that their presence and activity may indirectly induce a reduction in tick-related risks (Levi et al., 2012; Takumi et al. 2019). In Europe, Hofmeester et al. (2017a) showed that the density of *I. ricinus* nymphs decreased with the red fox and stone marten activities in forests. In North America, Ostfeld et al., (2018) found a negative relationship between bobcat presence and *I. scapularis* nymph infection prevalence by three pathogens (i.e. *B. burgdorferi* s.s., *Anaplasma phagocytophilum* and *Babesia microti*). This phenomenon can be explained by the reduction of host density caused by predation activity (Roy & Holt, 2008), but also through change in the behavior of prey hosts, which can result in a decrease in their activity and therefore a reduced likelihood of catching ticks locally (Moll et al., 2020).

Compared to other types of habitats, forests seem to combine different factors that make them particularly suitable for the development of ticks, with abundant understory vegetation, a generally more humid microclimate and a richer biodiversity of vertebrates than in open areas. Studies on *I. ricinus* or *I. scapularis* have shown that wooded environments have higher tick densities than open habitats (L'Hostis et al., 1995; Estrada-Peña, 2001; Boyard et al., 2007; Boyard et al. 2011), due to their higher moisture content (Walker et al., 2001). However, this result appears to depend on forest composition. For example, Fryxell et al. (2015) found that coniferous forests had more ticks than grasslands, but this was not the case for deciduous forests. At the landscape scale, connectivity between favorable habitat patches determines their use by wild vertebrates that can benefit from complementary or supplementary resources, and thus shapes local tick-host interactions (Werden et al., 2014; Heylen et al., 2019). Perez et al. (2016) found a positive relationship between the abundance of rodents in open areas, the percentage of adjacent forest cover and the abundance of *I. ricinus* nymphs. Allan et al. (2003) showed that *I. scapularis* nymph density was inversely related to forest stand size, which determined the abundance of white footed mice. In fragmented agricultural landscapes, Boyard et al. (2008) hypothesized that the density and continued presence of *I. ricinus* in open pastures was mainly due to the immigration of rodents from nearby forest remnants. However, few studies have attempted to verify if forests are the preferred habitats of ticks, by systematically evaluating tick abundance or diversity in different habitat types within the same region or landscape (e.g. Mejlon & Jaenson, 1993; Schulz et al., 2014). This is important to better assess the zoonotic risks associated with increased human activities adjacent to or in forests (Ortiz et al., 2021; Gibb et al., 2020). With the expansion of land dedicated to agriculture, transportation infrastructure and urbanization, humans and their domestic animals have moved closer to forests and thus to the parasites and diseases of which they are reservoirs.

To better assess the role of forest in the global dynamics of tick-borne diseases, we undertook a meta-analysis of the scientific literature to compare the abundance or diversity of ticks between forested and open habitats (natural or anthropogenic) sampled with the same method in the same landscapes (or regions) and seasons. We proceeded in two steps. First, we quantitatively synthesized the literature testing the effect of habitat type by selecting published studies that used sampling of ticks in forested habitat and at least one other type of non-forested habitat, whether natural open, agricultural, or urban habitats. Second, we implemented a meta-regression to test the hypothesis that, in forested habitats, tick abundance is positively correlated with the abundance of their vertebrate hosts. In both steps, we considered different forest compositions and tick developmental stages.

According to a common definition (e.g. IPCC), risk is the result of the combination between three components, the hazard (cause of the damage), the exposure (how many individuals or systems will suffer the consequences of this damage) and the vulnerability (capacity of systems or individuals to resist the damage). In the case of the risk of tick-borne disease, the hazard can be seen as the population of ticks that are potential vectors of pathogens, the exposure is the people and animals potentially bitten by the ticks and the vulnerability is their resistance to the pathogens and/or their knowledge of the tick risk after an exposure and therefore the quality of the prevention measures adopted. Our main objective here was to contribute to a better assessment of the risk of tick-borne zoonosis in forest environments by focusing on its hazard component, seeking to better understand how and why forests harbor more or less ticks than other habitats within the same landscape. Detailed hypotheses are listed in Table 1.

2.2. MATERIAL AND METHODS

2.1 Data collection

2.1.1. Comparison of forest and non-forest habitats

We searched the published scientific literature reporting tick abundance or diversity in both forest and non-forest habitats. We defined the following equation that we applied in the Web of Science search engine:

Eq (0): ((Tick* OR Dermacentor OR Hyalomma OR Ixodes*OR Argas* OR Amblyomma) NEAR (abundance OR density OR richness* OR diversity OR Shannon)) AND (forest* OR woodland* OR tree*)

We limited the search to papers published in English, between 1982 and January 2023. We screened the title, abstract and material and methods section of returned articles and included them in the meta-analysis if they met the following criteria: Studies (1) were conducted in both forest and non-forest habitats the same season and in the same area (if they were not all spatially close, we split the analyses by comparing the subsets of sites that were spatially closest to each other); (2) focused on the abundance (or density) or species diversity of ticks as response variable; (3) reported the mean of the response variable, any measure of variability around mean (e.g. standard deviation, standard error or confidence interval) and the sample size used in the study, with at least two independent replicates per sampled habitat type. These data were retrieved from the text, tables or by digitizing figures with the WebPlotDigitizer software (version 4.5, Rohatgi, 2021). When data were not available, the corresponding authors were contacted and asked to share the missing information. In total, we contacted 57 authors and 12 sent us missing data.

We then completed our dataset by checking for relevant papers in the cited references of each article retained after the first initial search. A total of 453 articles were screened, of which 153 were eventually kept (PRISMA diagram #1, as appendix A1, Fig. S0A; appendix A3).

2.1.2. Effects of covariates in forest habitats

In a second step, we investigated how three types of variables could explain the abundance or species diversity of ticks in forest habitats. In accordance with our hypotheses, we focused on the effect of microclimate, understory vegetation and vertebrate host abundance. We therefore developed three new keyword equations and then followed the same approach to sort the articles:

Eq (1): Eq (0) AND (abiotic OR microclimate OR climate OR temperature OR humidity OR VPD)

Eq (2): Eq (0) AND (herbaceous* OR vegetation* OR understor* OR soil* OR litter OR vascular plant* OR grass*)

Eq (3): Eq (0) AND (host* OR mamm*OR mice OR lizard* OR rodent* OR mouse OR bird* OR squirrel* OR vertebrate* OR deer* OR vole*)

Too few articles on the effect of microclimate (N=3) and understorey vegetation (N=7) on ticks in forests meeting the selection criteria were found. We then focused on papers that studied the effect of vertebrate host abundance or diversity on ticks (Eq (3)). A total of 316 articles were screened, of which 166 were eventually retained (PRISMA diagram #2, as appendix A1, Fig. S0B; appendix A4).

2.2 Calculating effect sizes

For comparison of habitat types, we calculated Hedges' *d* effect size (Hedges & Olkin, 1985) as the standardized differences between tick abundance or diversity in forest habitat (as *control*) vs non-forest habitat (as *treatment*). A negative d value therefore indicated that ticks were more abundance or diverse in forest habitats as compared to non-forest habitats. For the effect of host abundance in forest habitat, we extracted the Pearson's coefficient of correlation with tick abundance or diversity and the sample size (*n*), that we used to calculate the Fisher's *z*-score and its variance as $1/(n-3)$. Positive values of Fisher's z mean that there was a positive correlation between ticks and host abundances or diversities. For the sake of convenience, we back-transformed *z* values to obtain correlation coefficients (*r*) in the *Results* section. We also extracted the slope coefficient (*b*) of the linear regression of tick abundance (or diversity, *y*) against host abundance (or diversity, *x*) from the equation $y = a + bx$. The slope value and its variance were used as effect size (Becker & Wu, 2007) to inform on the magnitude of the effect of host abundance. All effect sizes were calculated with the "metafor" package, using R version 4.2 (R Core Team, 2022).

2.3 Moderators

For each study case, we extracted the following moderators: *Tick stage* was characterized as larvae, nymphs or adults. *Tick genus* was considered with the exception of *Rhipicephalus* because it is exclusively associated with domestic host vertebrates (such as dogs or horses) and was not thus not relevant to compare tick abundance in natural habitats. *Forest composition* was categorized as deciduous (>80% broadleaves), coniferous (<20% broadleaves) or mixed. *Non-forest habitats* compared to forest were divided into two categories: open habitat (including grasslands, meadows, shrublands, croplands and pastures) and urban habitats (including urban parks and green areas). *Host communities* were grouped in four categories: birds, small mammals (including rodents and lagomorphs), ungulates (including cervids and wild boars) and carnivores (foxes, opossums).

In addition to moderators, each study case was attributed a single identifier (Case ID) and assigned to one original paper (Paper ID). A Paper ID corresponded to a single published paper retained in our analysis. Within each Paper, we considered as a Case ID any response variable (i.e. tick abundance and species diversity) measured for each pair comparing forest and non-forest habitats (for example in a given paper (i.e. Paper ID), the comparison of tick abundance in forest vs urban habitats and in forest vs grassland habitats generated two case IDs).

2.4 Statistical analyses

First, we estimated the grand mean effect size using the complete data set to assess whether there was an overall effect of habitat type (forest vs non-forest) and host abundance and host species diversity in forest, on tick abundance and tick species diversity. This effect was considered significant if the 95% confidence interval around the grand mean effect size did not include zero. We estimated consistency among studies by calculating between-study heterogeneity (τ^2) (i.e. how the studies were distributed around the grand mean effect size), and the standardized estimate of total heterogeneity (I^2) ranging from 0 to 1, indicating how much of the variation in effect sizes was due to the between-study variance (τ^2) (Koricheva et al., 2013; Nakagawa et al., 2017). The total heterogeneity around the grand mean effect size was accounted for by using moderators in a second step. To test moderators, we selected subsets of data for which there were enough observations (Case studies (k) $\geq$ 4 with at least more than two original papers (N) for each level of moderator) to enable testing their effects according to eight hypotheses (Table 1). To avoid confounding factors, moderators were tested using a hierarchical approach (Castagneyrol and Jactel 2012). Each moderator was added to the model only in the subset of the dataset when it was well distributed in terms of the number of case IDs among the different modalities.

Only cases dealing with *Tick abundance* response of the genus *Ixodes* could be analyzed because too few studies addressing tick diversity (N= 5) or other tick genera (N = 15) were present. Too few cases addressed endophilic *Ixodes* species (k=2/382 for the habitat type dataset, and k=16/258 for the dataset of host abundance) to test this criterion as moderator. For the habitat type analysis (see Material and Methods, 2.4.1), all models were tested with and without the two case studies dealing with endophilic ticks. Since the changes in the results were negligible, we decided to keep these two cases in our final models. In contrast, the test of moderators in the analysis of the effect of host abundance on tick abundance (see Material and Methods, 2.4.2) was only performed on the subset of data with exophilic *Ixodes* ticks (Table 1). Hypotheses (H) 2, 3 and 4 (Table.1) were thus tested for the response variable *Tick abundance* of the genus *Ixodes* and hypotheses (H) 6, 7, and 8 were tested only on case studies dealing with exophilic *Ixodes* abundance.

2.4.1 *Statistical analyses for the comparison of forest and non-forest habitats*

The effect of tick stage (H2) on tick abundance in different habitats was tested in an univariate model and because no significant differences in tick abundance between tick stages emerged for the different habitat types, all stages were pooled for further analyses.

Ixodes tick abundance study cases were not evenly distributed among moderators (Appendix A5, Table S1). For instance, there were no study cases comparing *Ixodes* tick abundance between coniferous forest and urban habitats. To avoid confounding the effects of habitat with the effect of forest composition, we therefore removed cases dealing with coniferous forests in final model testing H3 and H4 (Table.1).

2.4.2 Statistical analyses of moderators in forest habitats

Because the univariate model showed no significant effect of tick stage (H6) (Table 2) on the impact of host abundance on exophilic *Ixodes* ticks, all stages were pooled for further analyses. Too few studies addressing the effect of vertebrate host diversity (N=4), carnivores abundance (N=4) or bird abundance (N=2) in forest on *Ixodes* abundance were available (appendix A5, Table S2), so we focused on the effect of small mammals or ungulates abundance on exophilic *Ixodes* abundance in forest to test H7 and H8.

2.4.3 Models description and sensitivity analyses

For each model (Table 2) we used Case ID nested within Study ID as random factor to account for correlation among multiple case studies within the same primary study. The use of multiple comparisons to the same control was controlled by using a variance-covariance matrix among effect sizes. We built full models fitting the most complex model consistent with the experimental design including all moderators and random factors (Table 2). Finally, we applied model simplification by sequentially removing one-by-one, the less significant terms to allow a non-singular fit (Barr, 2013). We ran each model using the *rma.mv* function of the “metafor” package (Viechtbauer, 2010). Finally, we used complementary analyses (see details in appendix A2) to evaluate the sensitivity and robustness of our results to several sources of bias. Statistical analyses were performed using the R software version 4.2.0 (R Core Team, 2022).

Table 1: List of assumptions tested concerning the effect of habitat type, host abundance and species diversity of host on tick abundance and species diversity. For each hypothesis tested in the meta-analysis, the dataset used and key references are given. *k* = number of case studies, followed by the number of corresponding articles in parentheses (N).

		Hypotheses	**Key references**
Effect of habitat type on ticks			
Full dataset, *k*(N) = 382(62)			
	Overall response of ticks to habitat type	**H1:** Tick abundance and diversity are greater in forested habitats	Halos et al., 2010 Boyard et al., 2011
Subset *k*(N) = 288(46) *Ixodes* abundance			
	Tick stage	**H2:** The effect of habitat type on tick abundance depends on tick stage	Piedmonte et al., 2018
Subset *k*(N) = 201(31) Ixodes abundance in mixed and deciduous forests compared to open and urban habitats			
	Forest tree composition	**H3:** The difference in tick abundance between forest and compared land depends on the forest tree composition	Hauck et al., 2020
	Type of non-forest habitat compared to forest	**H4:** The difference in tick abundance between forest and compared land depends on the compared land type	Mejlon & Jaenson, 1993
Effect of host variables on ticks in forests			
Full dataset, k(N) = 258(34)			
	Overall response of ticks to host abundance and diversity	**H5:** Tick abundance and diversity increase with host abundance and diversity in forest	Krawczyk et al., 2020 Tagliapietra et al., 2011
Subset k(N) = 65(15) exophilic *Ixodes* abundance, small mammals and ungulates abundance in mixed and deciduous forest			
	Tick stage	**H6:** The effect of host abundance on tick abundance depends on tick stage	Keesing et al., 2013

Subset k(N)=84(21) exophilic *Ixodes* abundance, small mammals and ungulates abundance in forests			
	Forest tree composition	**H7:** The effect of host abundance on tick abundance depends on forest composition	Rizzoli et al., 2009
	Host community	**H8:** The effect of host abundance on tick abundance depends on host community	Ostfeld et al., 2018

2.3. RESULTS

A total of 382 case studies (*k*) from 62 original papers (N) were retained to test the effect of habitat type (forest *vs* non-forest) on tick abundance and 258 case studies from 34 original papers to test the effect of host abundance and species diversity on tick abundance and species diversity. Studies conducted in deciduous forests dominated (k=290, N=39), followed by mixed (k=118, N=24) and coniferous (k=79, N=20) forests. There was a strong disproportion in terms of tick genera studied: the genus *Ixodes* was the most frequent (k= 465, with (i) 7 exophilic species: *I. ricinus* (k=375), *I. scapularis* (k=34), *I. pacificus* (k=12) , *I. inopinatus* (k=4), *I. frontalis* (k=3), *I. persulcatus* (k=2), *I. affinis* (k=3), (ii) 3 endophilic species: *I. trianguliceps* (k=13), *I. angustus* (k=3), *I. ventalloi* (k=2) and (iii) some indeterminate species (k=14)) in contrast to other genera: *Amblyomma* (k=56), *Haemaphysalis* (k=29) and *Dermacentor* (k=15). *Ixodes* stages were fairly well distributed between adults (k=109), nymphs (k=223), and larvae (k=104). The "tick abundance" response was the most studied and accounted for 90% of cases; we therefore decided to exclude cases dealing with tick species diversity for testing our hypotheses (H2, H3, H4, H6, H7 and H8).

3.1 Effect of habitat type on *Ixodes* tick abundance

The grand mean effect size calculated with the full data set ($k = 382$) was significantly negative and equaled −0.41 ± CI [−0.64; −0.19] ($\tau^2 = 0.84$, $I^2 = 0.66$) (appendix A1, Fig. S1), indicating that ticks were on average more abundant and diverse in forests than in any other compared habitat type (H1, in Table 1). For the subset *Ixodes* tick abundance only ($k = 289$), the mean effect size was also significantly negative and equaled −0.51 ± CI [−0.73; −0.30] ($\tau^2 = 0.71$, $I^2 = 0.59$) (Fig. 1).

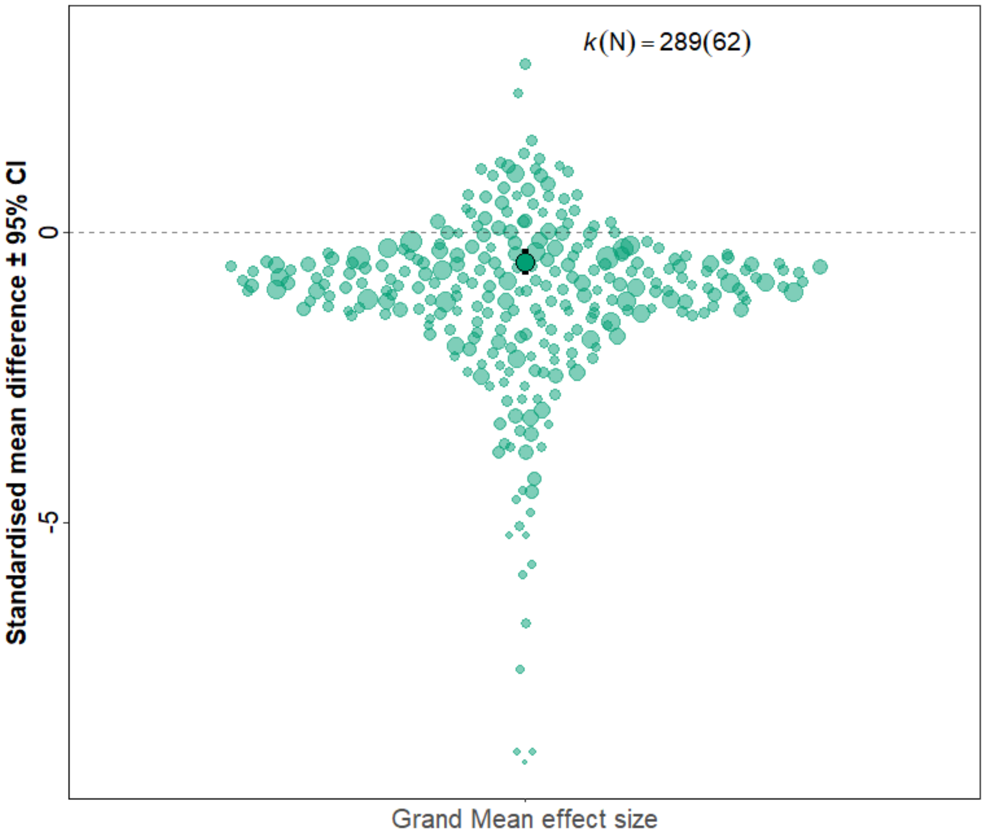

Fig. 1. Grand mean effect size (Hedges' d) on the effect of habitat type on *Ixodes* tick abundance. The green circled dot represents the grand mean effect size. The vertical thick line represents the 95% confidence interval. k(N) represents the number of cases (k) and the number of primary papers (N). The size of the dots corresponds to the accuracy of the study case (1/standard error).

We found a significant effect of forest composition on *Ixodes* tick abundance (H3, Table 1). The differences in *Ixodes* tick abundance between forest and non-forest habitats were greater when non-forested habitats were compared to mixed deciduous-coniferous than to deciduous forests. (Fig. 2A; there were not enough published papers comparing coniferous forests to non-forest habitats). The influence of habitat type on tick abundance did not differ between tick stages (H2, Table 1) or non-forest habitat types compared to forest (H4, Table 1, Fig. 2B).

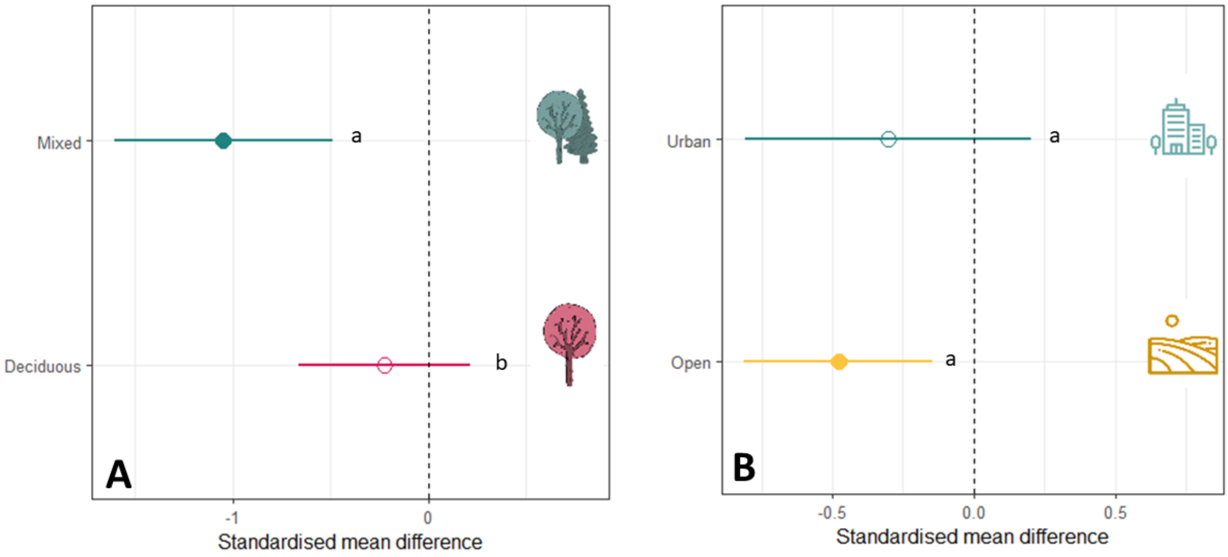

Fig. 2 A. Effect of the habitat type on *Ixodes* tick abundance as a function of tree composition of the forest compared to the other habitat. **B.** Effect of the habitat type on *Ixodes* tick abundance as a function of the type of non-forest land compared to forest. Circled dots represent the mean effect size for each moderator and thick lines represent the 95% confidence interval. Mixed forests *k*(N) = 30(10), deciduous forests *k*(N) = 130(18), urban habitats *k*(N) = 41(10) and open habitats *k*(N) = 160(24).

3.2 Effect of host abundance on tick abundance in forest

The mean correlation and slope values across all dataset were significantly positive ($r_{\text{grand mean}}$ = 0.31± CI [0.17; 0.47] and $b_{\text{grand mean}}$ = 0.18, $s^2 b_{\text{grand mean}}$ = 0.0008), indicating that tick abundance and diversity increased with host abundance and diversity in forest (H5, Table 1). A low variation in effect sizes was due to the between-study variance ($\tau^2 = 0.11$, $I^2 = 0.25$) and on average, host abundance and host species diversity accounted for 10% of the variability (r^2) of tick abundance. This effect depended on the host community (H8, Table 1), irrespective of tick stage (H6, Table 1) or forest composition (H7, Table 1). Tick abundance significantly increased with ungulates abundance ($r_{\text{ungulates mean}}$ = 0.27 ± CI [0.03; 0.5] and $b_{\text{ungulates mean}}$ = 0.73, $s^2 b_{\text{ungulates mean}}$ = 0.011) but not with small mammal abundance ($r_{\text{small mammals mean}}$ = -0.02± CI [-0.28; 0.24] and $b_{\text{small mammals mean}}$ = 0.07, $s^2 b_{\text{small mammals mean}}$ = 0.002) (Fig. 3).

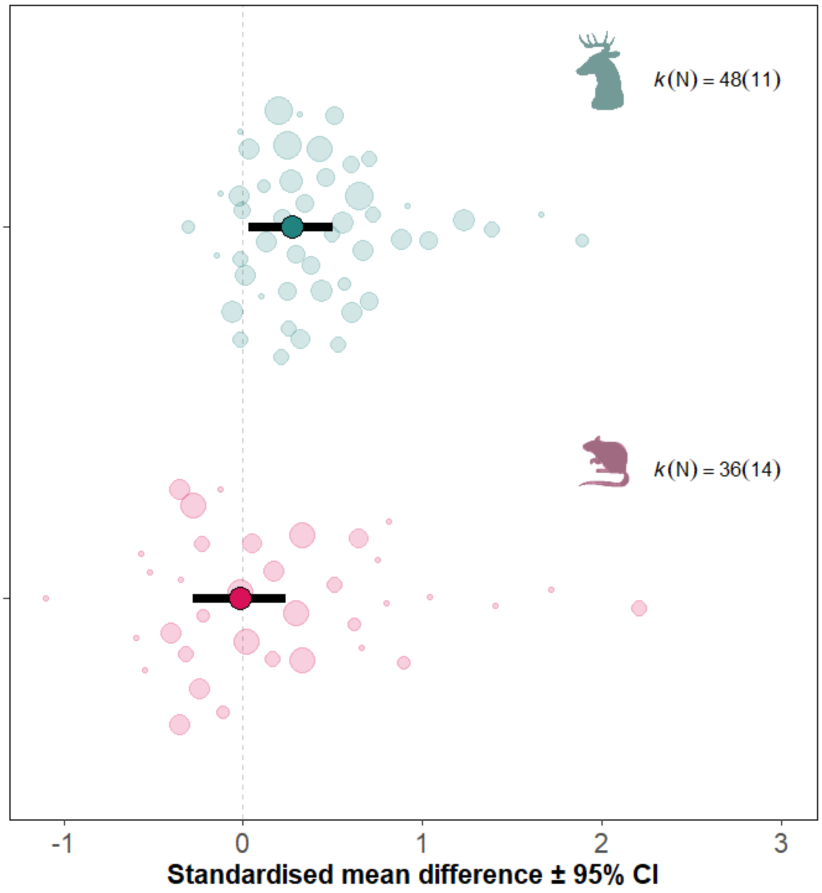

Fig. 3. Effect of the host abundance on exophilic *Ixodes* tick abundance as a function of the host type: ungulates in blue and small mammals in red. Circled dots represent the mean effect size for each moderator and thick lines represent the 95% confidence interval. k(N) represents the number of cases (k) and the number of primary papers (N). The size of the dots corresponds to the accuracy of the study case (1/se).

Table 2 Summary of model values for the different moderators tested. Given are the predictors, hypothesis tested, number of case studies (k), model heterogeneity (Q_M) and associated P value. Bold P values are significant.

Assumptions	Predictors	*k*	QM	*P*-value
H2	Tick stage	289	1.70	0.42
H3, H4	Forest tree composition + Non-forest land type	201	10.25	**0.006**
H6	Tick stage	75	0.01	0.99
H7, H8	Forest tree composition + Host community	84	8.16	**0.04**

3.3 Publication bias and sensitivity analyses

The funnel plots show symmetrical distributions of effect sizes (appendix A2, Fig. S8, S9) which suggests a lack of publication bias. The Rosenberg's safe numbers were 76948 for analysis of habitat type and 5037 for analysis of the impact of host abundance, both above the confidence thresholds of 1915 and 1300 (i.e. $5n+10$ with n corresponds to the number of study cases), which indicates that if there was a publication bias, it could be ignored (Rosenberg 2005). For both analyses, the QQ plot indicates that the data did not follow a Normal distribution, but no outlier was detected (appendix A2, Fig. S6, S7). All recalculated grand mean effect sizes and CIs (with leave-one-out and random case-study pick analysis) were comparable to the initial grand mean effect sizes (appendix A2, Fig. S2, S3, S4, S5). These analyses indicated that our findings were robust and unbiased.

2.4. DISCUSSION

Our results show that ticks were overall more abundant in forests than in any other type of habitat within the same landscape, regardless of tick stage. However, the composition of the forest influenced this pattern, the abundance of ticks being only significantly greater in mixed deciduous-coniferous forests compared to their nearby habitats. At forest scale, tick abundance was overall positively influenced by the abundance of their vertebrate host. This effect varied in magnitude according to host size, being stronger for large ungulates than for small mammals.

4.1 Limitations of the study

I. ricinus and *I. scapularis* are the two species over-represented in the published studies, probably because they are the main vectors of Lyme disease in humans. This consideration also leads the authors to choose the sampling period according to their specific activity, resulting in miss sampling of other tick genera with earlier or later activity in the season. Moreover, *Ixodes* species are known to be more suitable for the drag/flag technique (the most used), due to their questing behavior on the vegetation. Many studies were conducted in natural open environments and we noticed a gradual increase in tick ecology studies in urban environments, but far fewer studies were realized in cultivated lands (but see Boyard et al., 2007; Boyard et al., 2011). The explanation may be that cultivated fields are little or not visited by the public and would represent in this sense a lesser direct risk, via a lower exposure of the population. However, these fields can serve as bridges between forest patches in mosaic landscapes, and some studies showed that the (meta)population dynamics of potential hosts were dependent on landscape connectivity, with consequences for tick populations (Medlock et al., 2013). More tick studies should therefore be conducted at the landscape level sampling across all land use types to account for transmission issues.

Studies in coniferous forests were the least represented, probably because conifer forests are more abundant in northern, boreal countries while the Lyme disease is more prevalent in southern, warmer countries (Li et al., 2019). However, this geographic mismatch may change with global warming, making studies in conifer plantations that have long been favored by European private foresters more urgent. Too few studies have been published on vertebrate tick predators to analyze how habitat type influences top-down trophic interactions controlling the risk of tick-borne disease transmission. Nevertheless, studies on predators are growing, and tend to show net effects on tick abundance (Hofmeester et al., 2017), via direct regulation of host population or deterrence of their foraging behavior (Moll et al., 2020). Although data on the effect of microclimate on tick survival are numerous, they are mostly tested through temporal replications over seasons, and thus without simultaneously comparing the microclimate of different habitat types.

4.2 Forests: a preferential habitat for ticks

In agreement with our hypothesis, forests had higher tick abundances than any other compared habitat. This is consistent with results from studies conducted on ticks of the genus *Ixodes*, both in Europe and the United States (Estrada-Peña, 2001; Boyard et al., 2007; Halos et al., 2010; Boyard et al., 2011, Vacek et al., 2023). Authors suggested that this was mainly due to the more humid microclimate in forests than in open environments, which is more favorable to the survival of the ticks (Knülle and Devine, 1972; Tukahirwa, 1976; Davey et al., 1991; Gray, 1998). Daniel et al. (1977) actually found that ticks suffered from desiccation in open pastures, resulting in low survival rates.

Our meta-analysis showed that tick abundance was positively correlated with vertebrate host abundance in forests, but more significantly with the density of ungulates than rodents. This result suggests that forests are a more favorable habitat for ticks because they obviously have a better capacity to host these large animals than open, agricultural or urban environments. Deer abundance is known as a key driver of the abundance of tick nymphs (Barbour and Fish 1993; Rand et al., 2004). Recent studies suggest that the relationships between cervids and tick abundance are not always linear but asymptotic, depending on the environment and the tick stage (Van Buskirk & Ostfeld, 1995; Vourc'h et al., 2016). However, cervids are considered non-competent hosts for several tick-borne pathogens, therefore can participate in reducing the prevalence of pathogens in the environment (i.e. dilution effect) (Perkins, 2006). Rodents are known to be competent hosts for borrelia, but their influence on tick abundance appears to be highly species dependent (Perez et al., 2016). In our meta-analysis, small mammal species were grouped, a more fine-grained analysis of rodent species would be relevant to disentangle specific heterogeneous effects on tick abundances.

At the landscape scale, forests constitute key elements influencing tick host population dynamics by providing and connecting habitat patches or providing complementarity resources. Boyard et al. (2007) suggested that the presence and maintenance of *I. ricinus* populations in open areas was primarily due to high density of rodents living at the interface between forest patches and open lands. The expansion of forests in former agricultural areas, or the revegetation of urban environments, is therefore considered an important driver of tick-borne disease (Pfäffle et al., 2013; Wood & Lafferty, 2013; VanAcker et al., 2019).

4.3 Forest diversity and tick-related hazard

The biodiversity-diseases relationship is complex and seems to be dependent on the pathogen identity, the distribution of infectious stages and their mode of transmission (Keesing et al., 2010; Rohr et al., 2020). According to our results, differences in tick abundance between forest and non-forest habitats were greater when non-forested habitats were compared to mixed deciduous-coniferous than to deciduous forests. Several studies linked forest composition with tick abundance and many consider deciduous forests as the most prone to high tick densities (Guerra et al., 2002; Tack et al., 2012; Ferrell & Brinkerhoff, 2018 ; Ginsberg et al., 2020). In Czechia, Vacek et al. (2023) compared *I. ricinus* abundance between deciduous, coniferous and mixed forests and found the lowest nymph densities in mixed stands, but this result is no longer true when all tick stages were considered. Although our study does not directly compare relative tick abundance between different tree compositions, our results support the hypothesis that forest diversity is an important factor in explaining tick densities. Forest stand diversity can affect tick abundance and related pathogen prevalence by changing microclimate, understorey vegetation and potential host communities (McCoy and Boulanger, 2015; Ehrmann et al., 2017; Bourdin et al., 2022). The “canopy packing” hypothesis (Jucker et al., 2015; Williams et al., 2017) suggests that canopy cover is higher in mixed species forests due to complementarity between crown architectures of different tree species. This leads to stronger shade that allows the maintenance of humidity conditions more favorable to the survival of ticks. In addition, mixed deciduous-conifer forests can maintain a more constant microclimate with the presence of dense cover of needles throughout the year (Gillerot et al., 2022) which can protect ticks from temperature extremes over the seasons. Moreover, several studies indeed established a causal link between tree diversity and understorey diversity or composition (Gazol and Ibáñez, 2009; Ampoorter et al., 2014; Corcket et al., 2020) stabilizing understorey microclimate (De Frenne et al., 2019). Forest species diversity can also alter the abundance and foraging behavior of herbivorous vertebrates, with ungulates more abundant and active in mixed species forests (Milligan & Koricheva, 2013; Jactel et al., 2017).

4.4 Forest and tick-borne disease risk

Vanwambeke & Schimit (2021) show that the risk of tick-borne diseases is due to the combination of the three risk components (hazard, exposure and vulnerability) but not to each of them individually. Hazard is represented by the abundance of ticks and their pathogen load. Exposure corresponds to the spatial overlap between tick-infested areas and human visits, reflecting a number of potential bites. Vulnerability is associated with the capacity of humans to cope with hazard (“coping capacity”), and to protect themselves from potentially dangerous bites.

Although forests, as confirmed by our meta-analysis, are the habitats with the highest level of hazard (tick abundance), there is evidence that the incidence of the Lyme disease is not necessarily higher in forest habitats due to lower exposure. Vanwambeke & Schimit (2021) showed that forests were indeed the highest hazard environments, but ultimately all other environments combined accounted for the same number of tick bites. Piedmonte et al. (2018) found that some TBD were more prevalent in urban environments than in forests (i.e. *A. phagocytophilum* and *B. miyamotoi*). Garcia-Marti et al. (2017) explained that factors linked to human activity are more relevant to model tick bites than microclimate or vegetation. These results converge to underline the importance that exposure and individual protective behavior can have in the resulting risk associated with ticks. Several studies have shown that exposure to ticks is mainly peridomestic (Hinckley et al., 2016; Stafford et al., 2017; Mead et al., 2018) and that personal protective behaviors appear to be more neglected or not implemented in non-forested habitats (Due et al., 2013), leading to lower TBD transmission in forests. In addition, Larsen et al., (2014) found that the higher the incidence of Lyme disease in a country, the fewer people live at the interface between urban and wilderness habitat, which they translate into evidence of adaptation to risk. Other studies suggest that the prevalence of TBD tends to be lower in human populations that were aware of the hazard than in naïve populations, with a better conception of the risk in countries with high incidence of Lyme borreliosis (Herrington et al., 1997, Herrington, 2004). This means that, at least in the developed countries concerned by Lyme disease, it is possible to limit the risk associated with the tick hazard in forests, despite its greater amplitude, by reducing the vulnerability (through personal protective measures) and exposure (by changing the type of use) of humans who work or walk in them.

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2.6. SUPPLEMENTARY MATERIALS

Supplementary Material 1

Bourdin, A., Dokhelar, T., Bord, S., Van Halder, I., Stemmelen, A., Scherer-Lorenzen, M., Jactel, H. (n.d.) – **Forests harbor more ticks than other habitats: a meta-analysis**

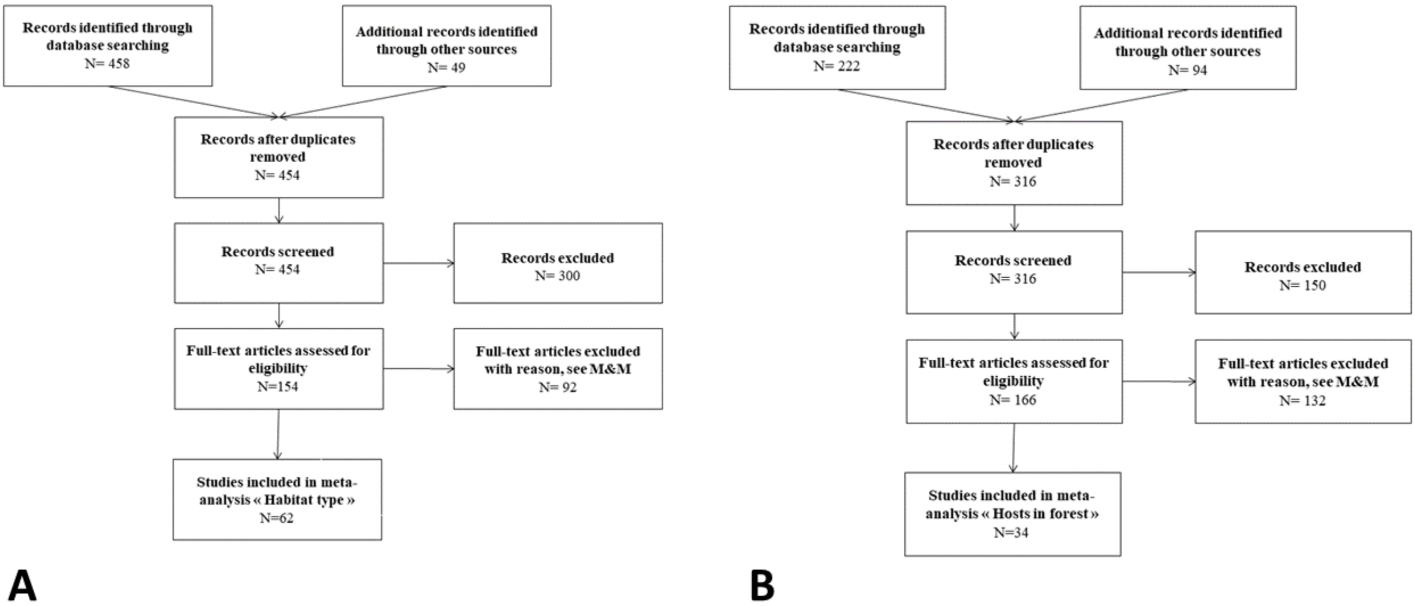

Fig. S0. **A.** Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA #1) workflow diagram for comparison of forest and non-forest habitats. **B.** PRSIMA #2 Effects of covariates in forest habitats.

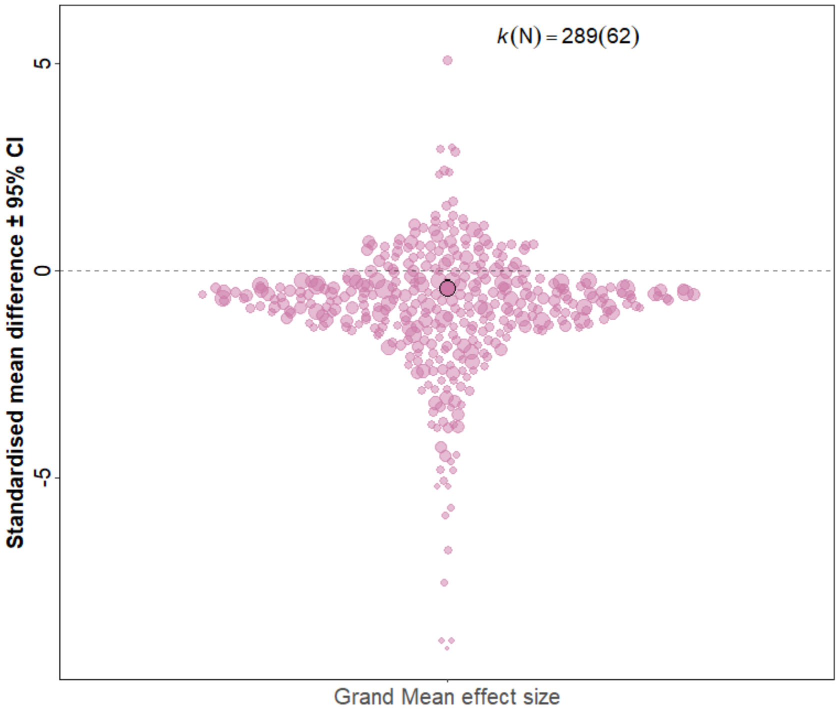

Fig. S1: Grand mean effect size (Hedges' d) on the effect habitat type on tick abundance and diversity. Circled dot represents the grand mean effect size. Vertical thick lines represent the 95% confidence interval. k(N) represents the number of cases (k) and the number of primary papers (N). The size of the dots corresponds to the accuracy of the study case (1/se).

Supplementary Material 2

Bourdin, A., Dokhelar, T., Bord, S., Van Halder, I., Stemmelen, A., Scherer-Lorenzen, M., Jactel, H. (n.d.) – **Forests harbor more ticks than other habitats: a meta-analysis**

Sensitivity analyses

First, we randomly selected one study case per studies 100 times, and re-ran the grand mean effects sizes analysis with this random dataset to check if the grand mean effect size values were included in the 95% CI interval calculated on the full dataset (Fig. S2, S3).

Second, we use the "leave-one-out method" to check if the grand mean effect size was particularly influenced by some studies. To that aim, we recalculated the results of our meta-analysis K-1 times, each times leaving out one study. We checked that model parameters (mean effect size and 95% CI) were still comparable to the grand mean effect size (Fig. S4, S5).

We addressed publication bias using funnel a plot and the Rosenthal's fail-safe number. Funnel plots effect size estimates (in the x-axis) against a measure of their precision (in the y-axis). It is expected that effect size for less precise studies should scatter more widely on the plot and lead to a funnel shape of the plot. If a bias is present, this will lead to an asymmetrical appearance of the funnel plot. We calculated the fail-safe number as described by Rosenberg (2005). It represents the number of non-significant, unpublished or missing studies that would be needed to make the overall meta-analysis turn non-significant.

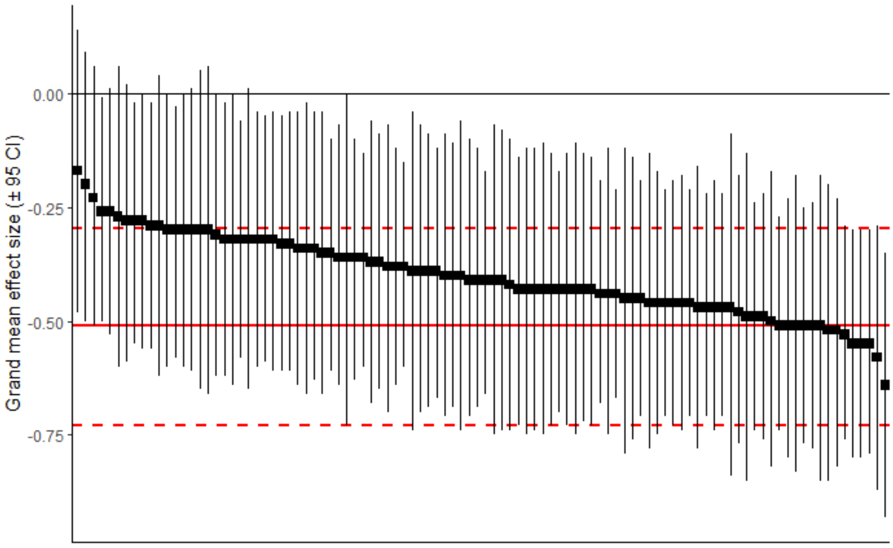

Fig. S2: Grand mean effect size ± 95% CI on the effect of habitat types on ticks for 100 subsets composed of one randomly selected case study per study. Solid and dashed red line represents the grand mean effect size ± 95% CI calculated on the whole dataset.

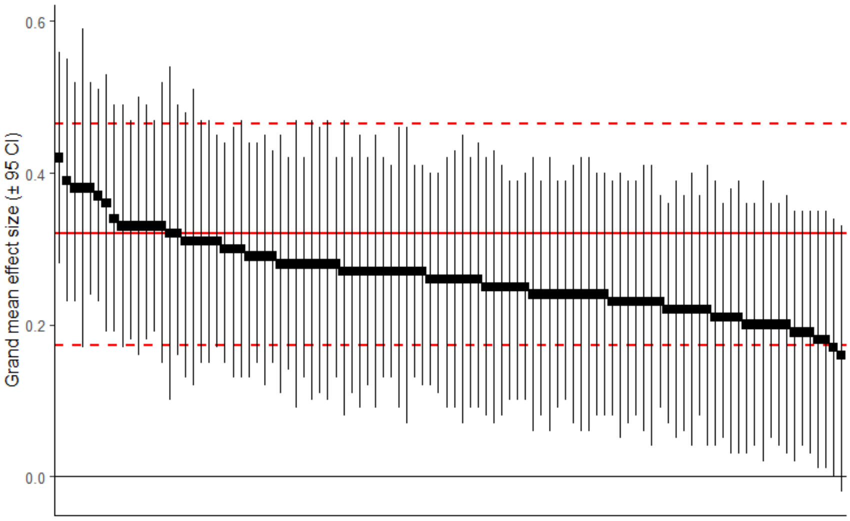

Fig. S3: Grand mean effect size ± 95% CI on the effect of host variables on ticks for 100 subsets composed of one randomly selected case study per study. Solid and dashed red line represents the grand mean effect size ± 95% CI calculated on the whole dataset.

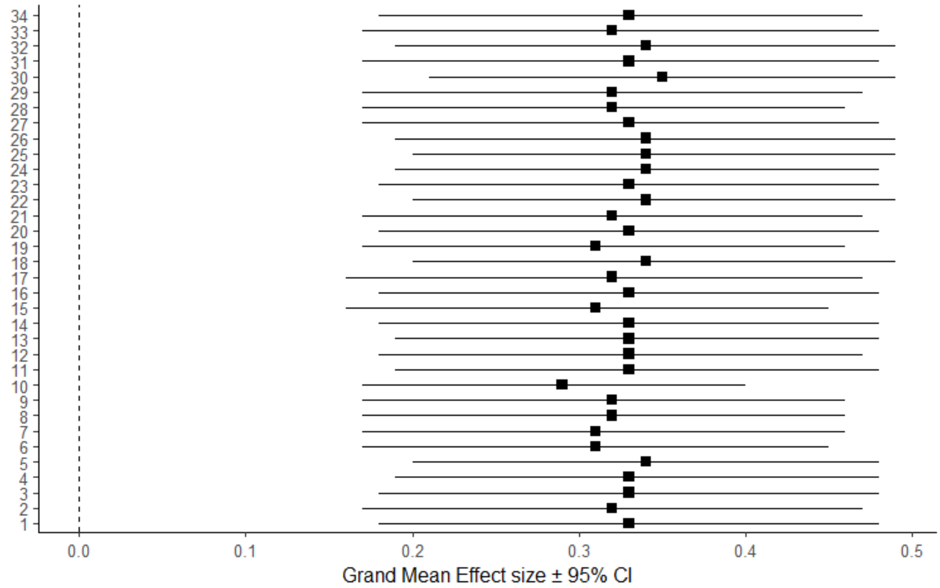

Fig. S4: Leave-one-out analysis on the effect of host abundance and diversity on ticks.

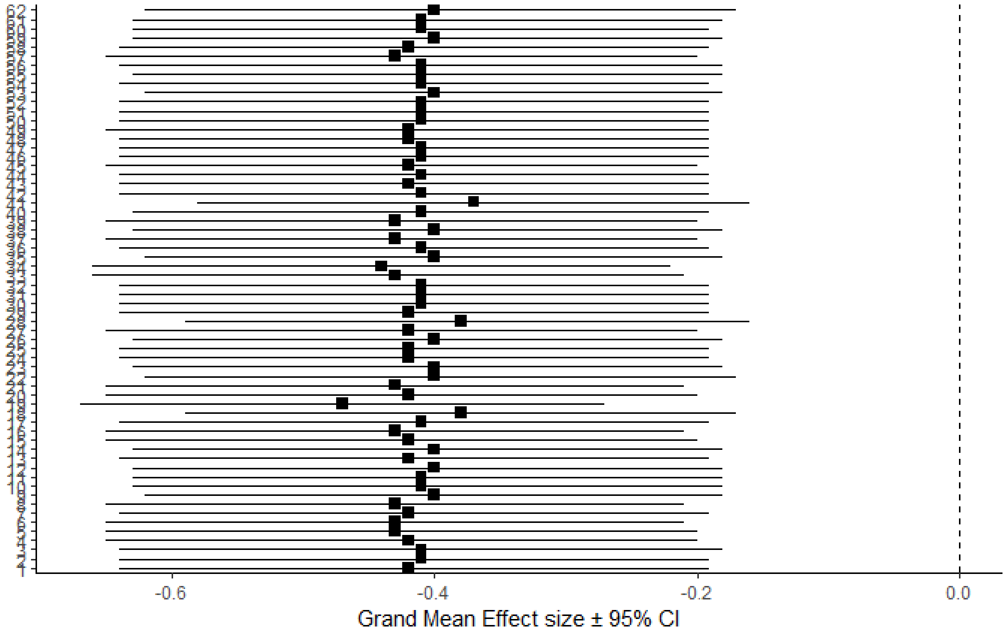

Fig. S5: Leave-one-out analysis on the effect of habitat type on ticks.

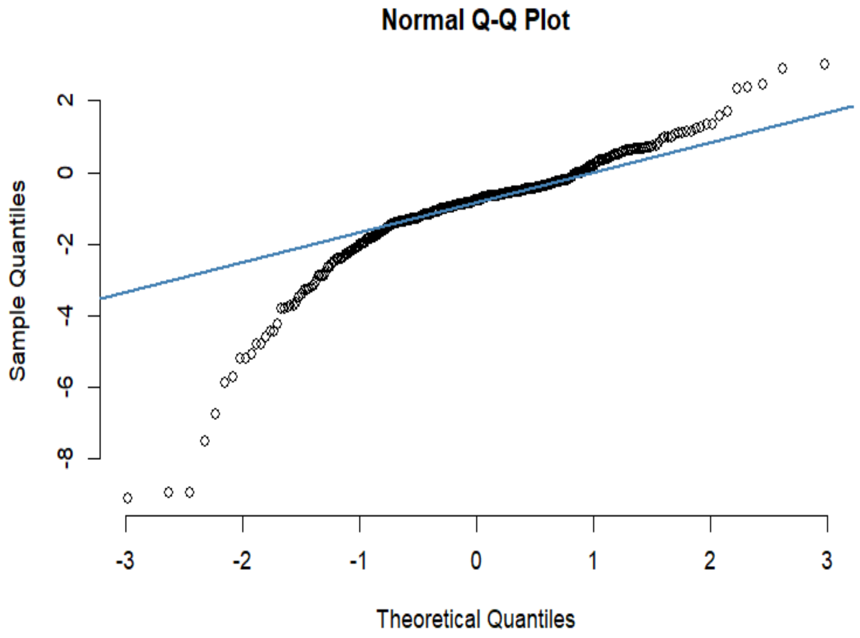

Fig. S6 : QQ-Plot of Hedge’s d effect size on the effect of habitat type on ticks.

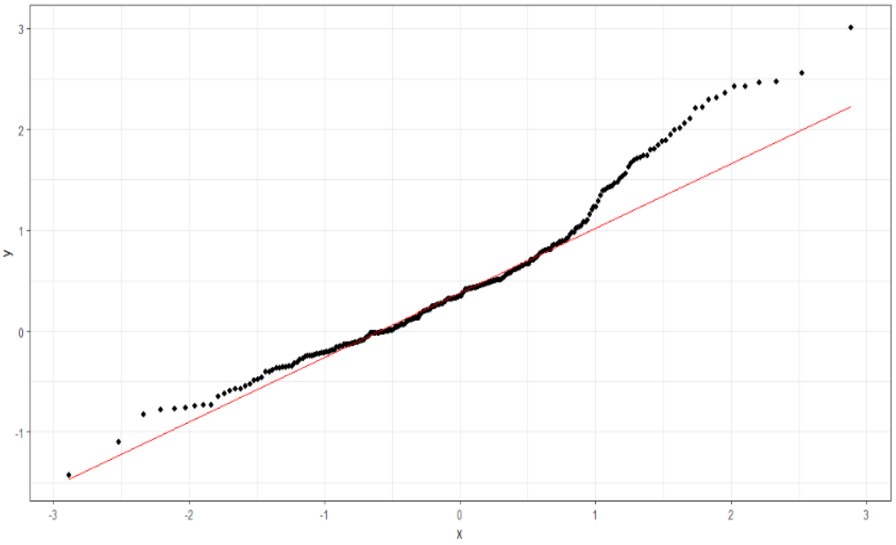

Fig. S7: QQ-Plot of Fisher-z effect size on the effect of hosts on ticks.

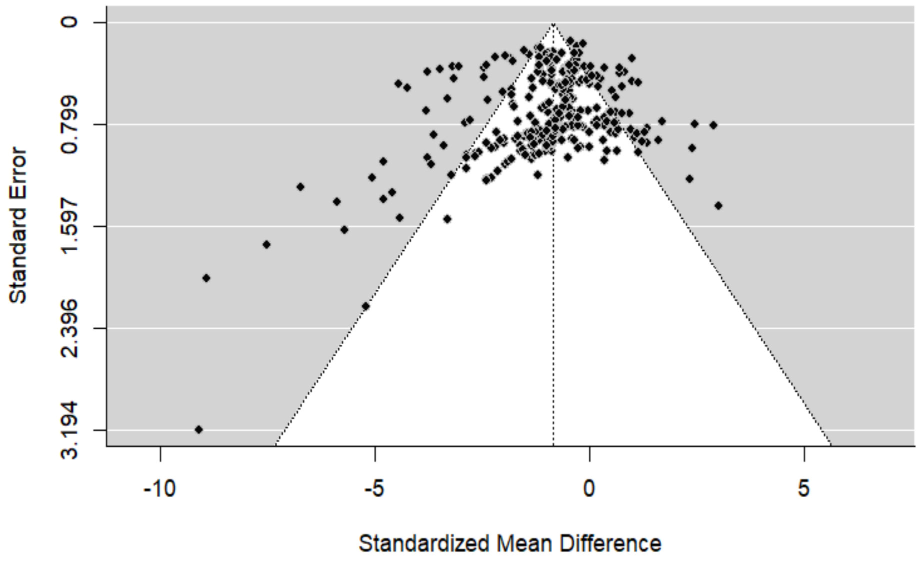

Fig. S8: Asymmetric funnel plot representing all 62 studies used in the habitat type meta-analysis.

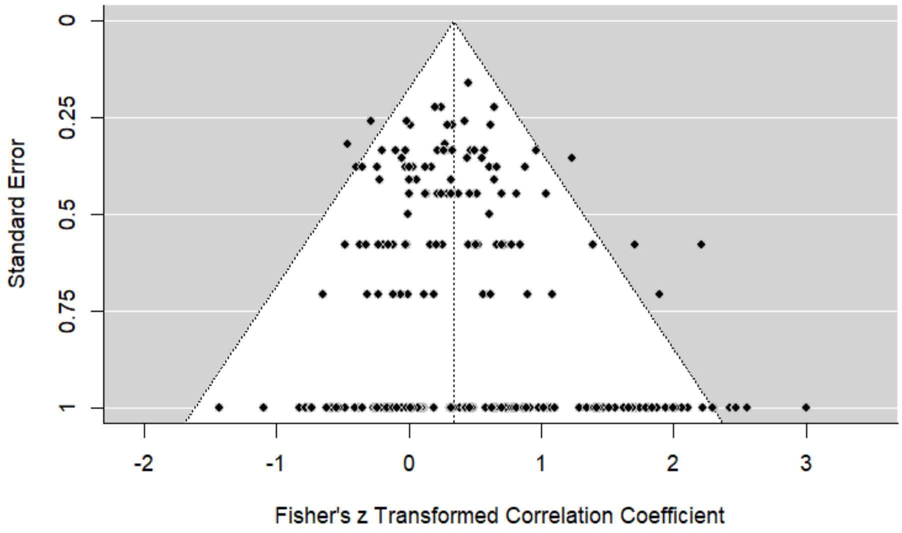

Fig. S9: Asymmetric funnel plot representing all 34 studies used in the effect of hosts abundance and diversity on ticks meta-analysis.

Supplementary Material 3

Bourdin, A., Dokhelar, T., Bord, S., Van Halder, I., Stemmelen, A., Scherer-Lorenzen, M., Jactel, H. (n.d.) – **Forests harbor more ticks than other habitats: a meta-analysis**

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Supplementary Material 4

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Supplementary Material 5

Bourdin, A., Dokhelar, T., Bord, S., Van Halder, I., Stemmelen, A., Scherer-Lorenzen, M., Jactel, H. (n.d.) – **Forests harbor more ticks than other habitats: a meta-analysis**

Table S1 Distribution of case studies among moderators for the meta-analysis testing the effect of habitat type on ticks.

Tick genus	**Tick stasis**	**Forest composition**	**Non-forest land**	**Total cases (*k*)**
Ixodes	adult	coniferous	open	7
		deciduous	open	33
			urban	8
		mixed	open	5
			urban	12
	larvae	coniferous	open	14
		deciduous	open	35
			urban	6
		mixed	open	3
			urban	5
	nymph	coniferous	open	52
		deciduous	open	75
			urban	10
		mixed	open	19
			urban	13

Table S2 Distribution of case studies among moderators for the meta-analysis testing the effect of host abundance on ticks in forest.

Tick genus	Tick stasis	Forest composition	Host community	Total cases (*k*)
Ixodes	Adult	Coniferous	Small Mammals	2
			Ungulates	1
		Deciduous	Birds	3
			Carnivorous	2
			Small Mammals	3
			Ungulates	2
		Mixed	Carnivorous	2
			Small Mammals	4
			Ungulates	2
	Larvae	Coniferous	Small Mammals	1
			Ungulates	1
		Deciduous	Birds	5
			Carnivorous	5
			Small Mammals	7
			Ungulates	9
		Mixed	Carnivorous	4
			Small Mammals	7
			Ungulates	5
	Nymph	Coniferous	Small Mammals	2
			Ungulates	2
		Deciduous	Birds	5
			Carnivorous	3
			Small Mammals	3
			Ungulates	7
		Mixed	Carnivorous	8
			Small Mammals	10
			Ungulates	8

3. Quel est l’effet de la biodiversité des forêts sur les densités de tiques et leur taux d’infection?

Bourdin, A., Bord, S., Durand, J., Galon, C., Moutailler, S., Scherer-Lorenzen, M., Jactel, H. (2022) – **Forest diversity reduces the prevalence of pathogens transmitted by the tick Ixodes ricinus**– *Frontiers in Ecology and Evolution* 10:891908

ABSTRACT

Tick-borne diseases represent the majority of vector-borne human diseases in Europe, with *Ixodes ricinus*, mostly present in forests, as the main vector. Studies show that vertebrate hosts diversification would decrease the prevalence of these pathogens. However, it is not well known whether habitat diversity can have similar impact on ticks and their infection rates. We measured the presence and abundance of different stages of *I. ricinus*, and the prevalence of associated pathogens in a large-scale forest experiment in which we manipulated tree diversity and moisture level. We showed that larval abundance was influenced by tree species identity, with larvae being more present in pine plots than in oak plots, while nymph abundance increased with canopy tree density. The proportion of *Borrelia bugdorferi* s.l.-infected nymphs decreased with increasing tree diversity. Our findings suggest that tree overstorey composition, structure and diversity, can affect tick abundance and pathogen prevalence. They support the idea that forest habitats may have “diluting” or “amplifying” effects on tick-borne diseases with direct relevance for human health.

Key words

Biodiversity, Forest ecosystems, Human health, Orphee, Ticks, Tick-borne disease

RESUME

Les maladies transmises par les tiques représentent la majorité des maladies humaines à transmission vectorielle en Europe, *Ixodes ricinus*, surtout présente dans les forêts, étant le principal vecteur. Des études montrent que la diversification des hôtes vertébrés diminuerait la prévalence de ces pathogènes. Cependant, on ne sait pas encore si la diversité des habitats peut avoir un impact similaire sur les tiques et leurs taux d'infection. Nous avons mesuré la présence et l'abondance des différents stades d'*I. ricinus*, ainsi que la prévalence des pathogènes associés dans une expérience forestière à grande échelle dans laquelle nous avons manipulé la diversité des arbres et le niveau d'humidité. Nous avons montré que l'abondance des larves était influencée par l'identité des espèces d'arbres, les larves étant plus présentes dans les parcelles de pins que dans les parcelles de chênes, tandis que l'abondance des nymphes augmentait avec la densité des arbres de la canopée. La proportion de nymphes infectées par *Borrelia bugdorferi* s.l. diminuait avec l'augmentation de la diversité des arbres. Nos résultats suggèrent que la composition, la structure et la diversité de la canopée des arbres peuvent affecter l'abondance des tiques et la prévalence des agents pathogènes. Ils soutiennent l'idée que les habitats forestiers peuvent avoir des effets de dilution ou d'amplification du risque de maladies à tiques, avec un intérêt direct pour la santé humaine.

3.1. INTRODUCTION

A growing body of observational and experimental evidence confirms that more diverse forests provide better support to the provision of ecosystem services and improve nature's contributions to people (Brockerhoff et al., 2017; Messier et al., 2021). Provisioning services such as biomass production (Zhang et al., 2012; Jactel et al., 2018), regulating services such as climate change mitigation through carbon sequestration (Harmon et al., 2013; Lange et al., 2015), pest and fungal disease control (Jactel et al., 2017, 2021), habitat provision for biodiversity (Ampoorter et al., 2020; Stemmelen et al., 2021) are on average often higher in mixed forests than in pure forests. However, it is important to note that forests and other ecosystems can also provide "disservices", i.e. risks for humans (Dunn 2010), such as those related to human health. Ecosystem disservices have been much less in the focus of science, although they have recently gained attention with the emergence of epidemics affecting human populations that are increasingly in contact with the wilderness due to deforestation (Jones et al., 2013; Smith et al., 2014; Everard et al., 2020). Even less known is the role of forest diversity in reducing the risk of zoonotic diseases, i.e. whether biodiversity in forests can mitigate ecosystem disservices. The dilution effect hypothesis (Halliday et al., 2020; Schmidt and Ostfeld, 2001) suggests that host species diversity would play a protective role in the spread of pathogens because some host species would not allow the multiplication of viruses or bacteria (low reservoir competence), constituting transmission dead ends, or because the complexity of food webs would allow top down regulation of disease hosts. This theory seems to apply well to the risk of tick-borne diseases (Diuk-Wasser et al., 2021). For example, LoGiudice et al., (2003) predicted a reduction in the prevalence of Lyme disease caused by bacteria of the genus *Borrelia*, with increasing host diversity. Hofmeester et al., (2017) reported that increasing the abundance of predatory mammals reduced the density of disease-carrying tick nymphs in prey rodents. American studies on *Ixodes scapularis*-borne disease showed that the diversity of vertebrate hosts could decrease *Borrelia* prevalence in ticks through the dilution effect of competent hosts by incompetent hosts (Linske et al., 2018; Ostfeld and Keesing, 2012). However, these arguments remain controversial, because the density of competent hosts may not always decrease sufficiently with increasing host diversity to reduce the density of infected nymphs, or because ticks can show a strong preference for competent hosts (Randolph and Dobson, 2012). For example, Ruyts et al. (2018) found no dilution effects in European forests for *Ixodes ricinus,* the most common tick species in western Europe (De la Fuente, 2008). In addition to this host-dilution hypothesis, Ehrmann et al. (2018) suggested that habitats can also be classified into “dilution” and “amplification” habitats that reduce or increase the prevalence of tick-borne disease vectors, respectively, at the local patch or even regional landscape scale. Such “dilution habitats” were characterised by a structurally and functionally diverse tree layer, diverse and abundant understorey vegetation, and increased levels of deadwood in European forests. However, we still lack investigations that are able to disentangle the role of tree diversity from that of other environmental factors in regulating tick abundance and the prevalence of tick-borne diseases. Experiments that manipulate tree diversity independently from other factors offer one promising way into this direction.

The main vector of tick-borne diseases in Europe (De la Fuente, 2008), *I.ricinus* (castor bean tick) is mainly present in forests and woodlands (Estrada-Peña, 2001). It is an obligate haematophagous that requires three blood meals to complete its life cycle and pass from larva to nymph, from nymph to immature adult and from immature adult to egg-laying female, respectively. These different stages depend on different habitat characteristics and microclimates (Ehrmann et al. 2017). *I. ricinus* is responsible for the transmission of several pathogenic bacteria, viruses and protozoa (Prinz et al. 2017; Rizzoli et al., 2014) including the bacterial complex *Borrelia bugdorferi* sensu lato, the tick-borne agent of Lyme borreliosis. But other pathologies are also emerging, related to *Anaplasma*, *Borrelia*, *Neoehrlichia*, *Rickettsia* and *Babesia* species (Keesing and Ostfeld, 2021; Kilpatrick and Randolph, 2012). Because there is no vaccine available against these growing diseases (Steere et al., 2004) with sometimes very serious health consequences, it is very important to search for methods to prevent the risk of transmission.

Ticks can be infected in three ways: (i) by feeding a blood meal on infected hosts (ii) by co-feeding, which is a direct transmission from a tick to another one feeding on the same host at the same time, and (iii) by transovarial (or vertical) transmission. This latter pathway is only possible for a few pathogens (Sprong et al., 2009; Burri et al., 2014) such as some *Rickettsia* or *Babesia* species for unfed *I. ricinus* larvae, and is the main way for larvae to become infected before a first blood meal. Knowing that the probability for one tick to be infected increases with the number of blood meals taken and that nymphs are more abundant in the environment than adults, many studies consider the density of infected nymphs as an indicator of the level of contamination risk in the environment (Diuk-Wasser et al., 2012; Pepin et al., 2012; Stafford et al., 1998; Mather et al., 1996). As a result, the presence and abundance of tick larvae remain less frequently monitored. Although larvae have a lower potential for transmission, their survival in the environment influences directly the abundance of future nymphs, infected or not, and indirectly the epidemiological risk associated (Ehrmann et al. 2017).

The risk of tick bites and disease transmission in forests is directly influenced by the density of infected ticks in the environment (Fischhoff et al., 2019), which in turn depends on interactions between biotic (e.g. host community) and abiotic (e.g. microclimate) conditions. Moreover, understorey vegetation plays a central role in the population dynamics of ticks. First of all, the structure of the understorey vegetation directly conditions the ability to reach the hosts for the questing ticks that are hanging to plant parts (McCoy and Boulanger, 2015). Then the understorey may provide more or less favorable feeding, breeding or resting sites for the vertebrate hosts that ensure the survival, infection and transport of ticks (Allan et al., 2010). Finally, the structure and composition of the understorey condition the microclimate that affects the survival and questing activity of ticks (Ehrmann et al. 2017; McCoy and Boulanger, 2015) and more particularly the humidity (Boyard et al., 2007; Estrada-Peña, 2008). Forest diversity may therefore indirectly influence tick abundance through its effect on understorey vegetation. Several studies have indeed established a causal link between tree diversity and understorey diversity or composition (Corcket et al., 2020; Ampoorter et al., 2014; Gazol and Ibáñez, 2009). Tree species diversity and composition have also been shown to mitigate understorey microclimate (De Frenne et al., 2019). Mixed forests are characterized by a high degree of canopy closure, made possible by a greater complementarity in crown shape, a process known as canopy packing (Jucker et al. 2015; Williams et al. 2017). A denser canopy then produces a shading effect that can increase the humidity in the understory, which favors the survival of ticks in case of drought. Other studies have demonstrated that forest species diversity can alter the abundance and foraging behavior of herbivorous vertebrates (i.e. potential tick hosts), often with contrasting effects depending on the size of these animals, reducing the browsing activity of small animals (e.g. mice and voles) and increasing that of large ones (e.g. moose and deer) (Jactel et al., 2017; Milligan and Koricheva, 2013). However, very few studies have attempted to measure the effects of forest species diversity on tick abundance and tick-borne pathogen prevalence (but see Ehrmann et al. 2017, 2018; Ruyts et al., 2016) and, to our knowledge, none has isolated the effect of tree species richness from other environmental drivers in an experimental setting.

In this study, we used a long-term experiment where both tree diversity and water supply are manipulated to test their interactive effects on *I. ricinus* larvae and nymphs abundance and on associated pathogens. We sampled ticks in forest plots ranging from one (monoculture) to three tree species mixtures, in both irrigated and non-irrigated conditions. More particularly, we tested the following hypotheses:

1) Tick abundance and proportion of infected tick nymphs are lower in mixed than in pure forest plots, due to stand characteristics associated with “habitat-dilution” effects.
2) Effects of tree species diversity on ticks are principally mediated by changes in understorey density and composition, being the main habitat for larvae and nymphs.
3) The negative effects of drought (in non-irrigated plots) on ticks are mitigated by forest diversity, due to higher crown space filling and shading of the understorey.

Although we were unable to sample vertebrate hosts, our goal was to quantify the direct and indirect (microclimate, understorey) drivers of the effects of overstorey tree diversity on the risk of disease transmission by ticks (Fig. 1).

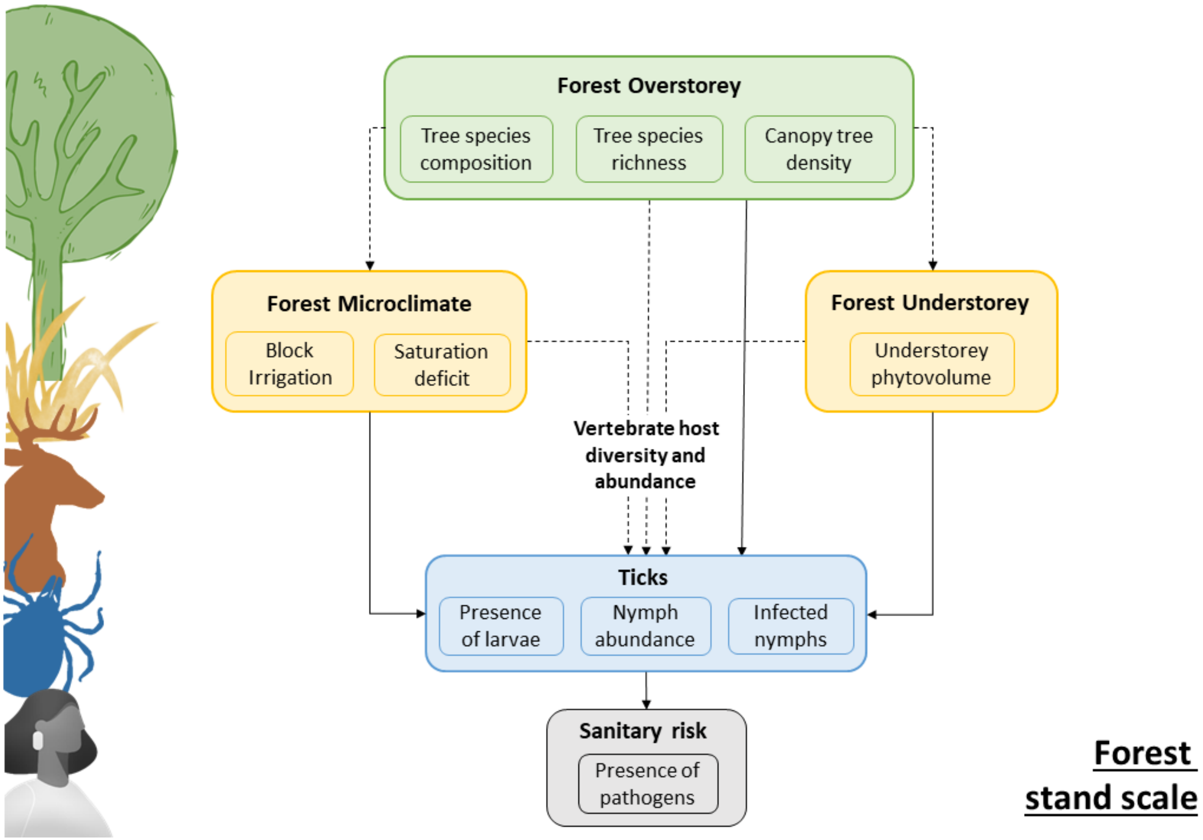

Fig. 1. Explanatory diagram showing our study hypotheses at the forest stand level. The biophysical and structural characteristics of the overstorey influence the ticks and associated pathogens through an influence on the within-stand microclimate, the understorey, and the host community in the environment. Diversifying host communities may result in a dilution of competent hosts in the environment and thus affects the prevalence of tick-borne diseases. Dashed arrows represent effects not tested in our study.

3.2. MATERIALS AND METHODS

2.1 Study site

The study was conducted in the tree diversity experiment ORPHEE, located in southwestern France, in the Landes de Gascogne forest. The region is characterised by a mild oceanic climate, with mean annual temperature of 12.5 °C and mean total precipitations of ca. 950 mm. The ORPHEE experiment, which belongs to the global network of tree diversity experiments TreeDivNet (Verheyen et al., 2016; Paquette et al., 2018), was established in 2008 on a 12 ha clearcut of maritime pine stands, on a sandy podzolic soil. It is composed of eight blocks of 32 randomly distributed plots corresponding to all the possible combinations of one to five tree species. Five native tree species were planted in monocultures (European birch: *Betula pendula*, ROTH; Pedunculate oak: *Quercus robur*, L.; Pyrenean oak: *Quercus pyrenaica*, WILLD.; Holm oak: *Quercus ilex*, L.; and Maritime pine: *Pinus pinaster*, AIT.) as well as all combinations with two, three, four and five tree species. Each plot is composed of 10 × 10 trees, planted 2 meters apart, resulting in 100 trees per plot, with a plot area of 400m^2. Tree species are planted according to a systematic alternate pattern (see details in the study by Castagneyrol et al., 2013; Damien et al., 2016). Four out of eight blocks are irrigated since 2015. Blocks are sprinkled at night with 42m^3 of water (i.e. 3mm precipitation, block area of 1.28 ha), daily from May to October, which significantly increases water availability as compared to non-irrigated blocks (Maxwell et al., 2020). The site has been fenced, which may have reduced the presence or passage of large mammal in the plots.

In our study, we focused on 14 different plots with the following four tree species: *Q. robur*, *Q. ilex*, *P. pinaster*, and *B. pendula*, planted in monocultures (n = 4), two-species mixtures (n=6), and three-species tree mixtures (n=4). All plot compositions were replicated eight times, four in irrigated blocks and four in non-irrigated blocks, for a total of 112 plots.

2.2 Tick sampling and identification

Free-living ticks, being larvae, nymphs or adults, were collected in June and July 2020 on days without rain during the peak of tick activity season, using the dragflag technique (Macleod, 1932; Vassallo et al., 2000). The total number of ticks collected along four parallel 20 meter long transects in each plot (80 m² sampled area) were denoted $N_{\{i,j,k\}}$ where $i \in [1;14]$ represents the plot composition, $j \in [1;8]$ represents the replication of plot composition and $k \in [1;4]$ reprensents the kth parallel long transect. To take into account the effect of different understorey diversity or composition on the sampling rate (Bord et al., 2014), *i.e.* the proportion of ticks collected by the drag in the questing tick population, each transect was passed four times, corresponding to two return trips, with a 1×1m white flannel cloth of dragged on the ground at a constant speed (walking pace). Ticks were counted after each successive sampling, stage determined and kept in a 70% ethanol tube at the end of each pass by transect. Abundance of adults, nymphs and larvae was confirmed after binocular examination in the laboratory.

Three plots could not be sampled due to the presence of dense gorse (*Ulex europaeus*) that would have torn the flannel. A total of 109 plots were therefore sampled, corresponding to 436 transects and 1744 sampling passes. Tick species were determined after binocular examination using the systematic key from Estrada-Peña et al. (2017). Too few adults were collected (n=35 including 29 *I. ricinus*). We therefore decided to focus only on *I. ricinus* nymphs and larvae.

2.3 Identification of tick-borne pathogens in *I. ricinus* nymphs

All *I. ricinus* collected nymphs (n=173) were isolated for pathogen identification. We used a microfluidic PCR technique that provides information on the presence of 35 different pathogens of the following genius: *Borrelia, Anaplasma, Rickettsia, Francisella, Bartonella, Babesia* and *Coxiella* potentially present in tick nymphs (developed by Michelet et al., 2014).

Tick nymphs were rinsed twice with distilled water. Prior to extraction, ticks were crushed and processed in a homogeniser at 5500 rpm for 20 s with 100 µl of elution buffer BE, 40 µl of lysis buffer MG and 10 µl of liquid proteinase K and six steel balls. DNA was extracted using a Nucleospin DNA insect kit (Macherey-Nagel, Germany), and DNA from each sample was stored at -20◦C in 50µl of rehydration solution until next step.

DNA pre-amplification reactions was performed with 2.5µl of PreAmp Master Mix according to the manufacturer's instructions (Fluidigm, CA, USA). Prior to experiments, primers targeting all pathogens were pooled in equal volumes (200 nM each). The experiment was performed with 1 µL of PreAmp

Master Mix, 1.25 µL of pooled primers mix, 1.5 µL distilled water, and 1.25 µL DNA for 5 µL final volume. The pre-amplifications were performed with one cycle at 95°C for two minutes, 14 cycles at 95°C for 15 s and 60°C for four minutes. Pre-amplified DNA was diluted 1:10 and stored at -20°C. Finally, a high-throughput real-time PCR was performed with the BioMark system (Fluidigm, USA) using the method explained in Michelet et al. (2014).

Pathogens detected with BioMark system were confirmed by conventional PCR or nested PCR on several samples presenting low Ct values. Primers targeting different genes or regions than those of the BioMark system were selected from the literature (listed Table S1) and PCR were performed with Phusion High fidelity kit (Thermo scientific, MA, USA). The positive samples after gel migration were sent to sequencing at Eurofins (Germany) and assembled using BioEdit software (Ibis Biosciences). An online BLAST (NCBI; National Center for Biotechnology Information) was used to identify the sequenced organisms.

2.4 Response variables

We analysed four response variables for *I. ricinus*: *larvae presence, nymph abundance* and *proportion of infected nymphs by Borrelia burgdorferi s.l.* and *proportion of infected nymphs by Rickettsia*. *Nymph abundance* indicator was the cumulative number of nymphs collected after the four successives samplings $\sum_{k=1}^{4} N_{\{i,j,k\}_l}$ where $i \in [1;14], j \in [1;8], k \in [1;4]$ and $l \in [1;4]$ corresponding to the plot composition, the repetition number, the parallel long transect and the successive samplings, respectively. *Larvae presence* variable is binomial: it is set to 1 when at least one larva was found in the plot, and 0 if none was found. As we performed individual PCR's, the proportion of nymphs infected by each pathogen was tested in the model as a vector including the number of pathogen-positive nymphs and pathogen-negative nymphs in the plot.

2.5 Explanatory variables

Table 1
Table representing the combination of the different tree compositions chosen for the analyses. Bp: *Betula pendula*, Qr: *Quercus robur*, Qi: *Quercus ilex*, Pp: *Pinus pinaster*.

Initial tree compositions		Combined tree compositions	
Monocultures	Bp	Birch	Bp
	Qr		BpQr
	Qi		BpQi
	Pp		BpQrQi
		Pine	Pp
2 species mixtures	BpQr		PpQr
	BpQi		PpQi
	BpPp		PpQrQi
	PpQr		
	PpQi	Pine and Birch	PpBp
			PpBpQr
			PpBpQi
3 species mixtures	BPQrQi	Oaks	
	BpQrPp		QiQr
	BpQiPp		Qr
	PpQrQi		Qi

We selected three tree layer variables. First, *canopy tree density,* corresponding to the percentage of canopy individuals in the plot (here pine and/or birch, since oak trees are much smaller in ORPHEE, still being part of the understorey layer; Damien et al. 2016).

Second, *tree composition* corresponds to different types of tree mixtures (a total of nine type of mixtures). In order to obtain sufficient statistical power to test the effect of plot composition on ticks variables, individual plot compositions were combined into four composition types: 1) Pure pine (including monocultures of pines and mixtures of pines and oaks, which are still small enough to be considered part of the understorey); 2) Pure birch (including monocultures of birches and mixtures of birches and oaks); 3) Mixtures of pine and birch (including mixtures of pines and birches, and mixtures of pines, birches and oaks); 4) Oaks (including monocultures of oaks and mixtures of only oaks) (Table 1).

Third, *tree species richness* corresponds to the number of different tree species in the plot (including the oak species). Further, we included understorey (*understorey phytovolume*) and microclimatic variables (presence/absence of *irrigation* and the *saturation deficit* of the plot) into the models.

To describe the understorey vegetation, we sampled one 1×1m quadrat in each transect passed for the ticks (i.e. four quadrats per plot). In each quadrat, we estimated the percentage of bare soil, litter and moss cover, the percentage cover of the main plant types found in ORPHEE (Corcket et al. 2020), i.e. grasses, heath, ferns and shrubs (mainly gorses), and the maximum height of each plant type. We considered not only plants directly rooted in the quadrat but also those whose aerial parts partially or completely covered the surface of the quadrat. The percentage cover of each type was transformed in eight categorical classes: O: 0%; A: 1-5%; B: 6-15%; C: 16-25%; D:26-50%, E:51-75%; F:75-99%; G: 100% (inspired by the Braun-Blanquet approach (Braun-Blanquet, 1964)). The maximal height of each plant type was measured on the highest individual in the quadrat using a measuring pole. Finally, we aggregated the data at the plot level by averaging across the four quadrats to calculate the *understorey phytovolume* (cm^3) according to equation (1):

$$Understorey\ phytovolume = \sum_{types} mean\ \left[\frac{cover\ types}{100}\right] \max height\ types \qquad \text{eqn (1)}$$

Finally, temperature (T) and relative atmospheric humidity (RH) were measured in each plot $\mathrm{N}_{\mathrm{i,j}}$ before each sampling (with Kestrel 3500 NV), in order to calculate the *Saturation Deficit* in millimeters of mercury, according to equation (2) (Vourc’h et al., 2016):

$$Saturation\ Deficit\ = \left(1 - \frac{RH}{100}\right) 4.9463 \exp(0.0621\, T) \qquad \text{eqn (2)}$$

Saturation Deficit, a proxy for tick desiccation potential, is considered a relevant predictor of nymph foraging activity (Gray, 2008).

2.6 Statistical analysis

According to our hypotheses we were interested in testing the effects of tree layer variables, understorey vegetation variables and microclimatic variables on tick response variables. A first sorting of the explanatory variables was done with univariate tests. Variables with a p value < 0.1 were selected as candidates for the full models.

Tick nymphs, larvae and associated pathogens were analysed separately. For *nymph abundance*, we built one negative-binomial generalized linear model (glmer.nb) suitable for overdispersed data (Breslow, 1984). We modelled the *presence of larvae* and *proportion of infected nymphs* with binomial generalized linear models (glm, binomial family).

Nymph abundance, *presence of larvae,* and *proportion of infected nymphs* were modelled as a function of *tree composition* or *canopy tree density*, *irrigation, tree species richness*, *understorey phytovolume* and their interactions, and *saturation deficit* as fixed effects. Spatial autocorrelation of plots in the block was accounted for by including *Block* as random factor.

All continuous predictor variables were scaled and centred prior to modelling to make their coefficients comparable (Schielzeth, 2010). We first built full models fitting the most complex model consistent with the experimental design including all fixed effect factors, interactions and random factors. Then we applied model simplification by sequentially removing one-by-one, the less significant terms to allow a non-singular fit (Barr et al. 2013). Once optimally simplified, we selected the best fitted model with the lowest AIC. Statistical analyses were performed using the R software version 4.0.5 (R Core Team 2021).

3.3. RESULTS

We collected three tick species in ORPHEE, namely *I. ricinus* (L., 1758), *Ixodes frontalis* (PANZER 1795), and *Dermacentor reticulatus* (FABRICIUS, 1794). However, *I. ricinus* was by far the most abundant with 1458/1535 larvae and 173/179 nymphs. We therefore focused our analyses on this species. The abundance of *I. ricinus* nymphs greatly varied between tree composition and the water supply of forest plots (Fig.2).

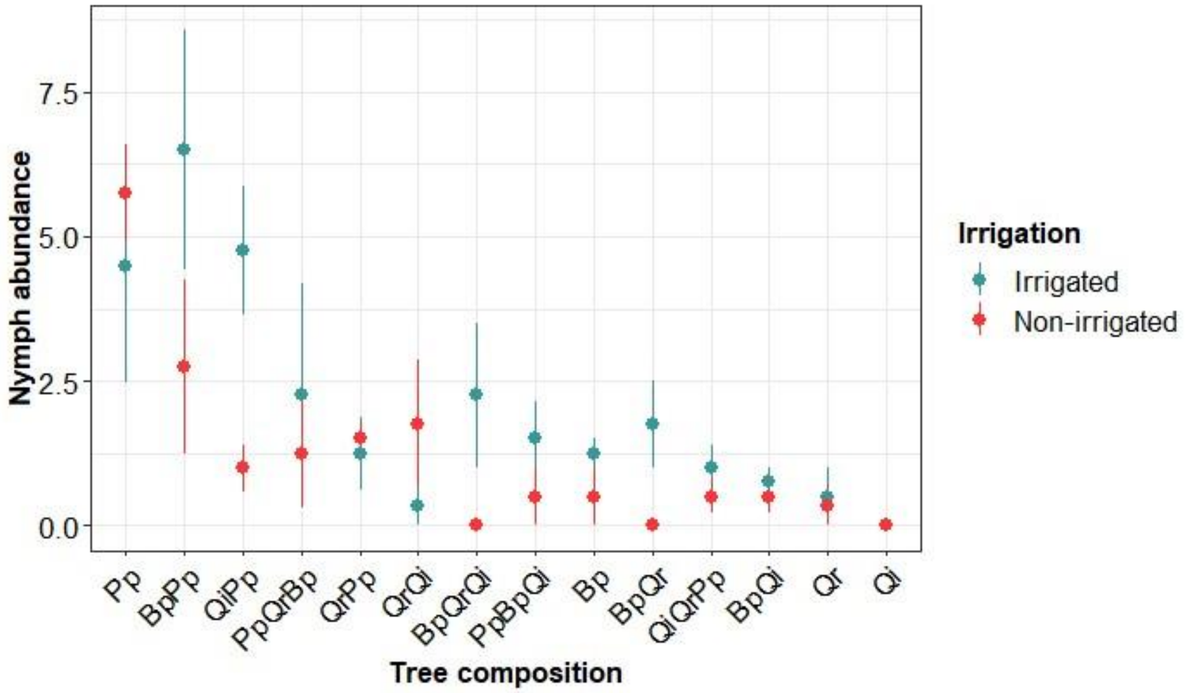

Fig. 2. *Ixodes ricinus* nymph abundance as a function of tree composition in all sampled plots, in irrigated plots (blue) and non-irrigated plots (red). Pp*: Pinus pinaster,* Bp*: Betula pendula,* Qr*: Quercus robur,* Qi*: Quercus ilex.* Dots and solid lines represents mean ± SE calculated on raw data.

3.1 Presence of *I. ricinus* larvae along the tree diversity gradient

Larvae were present in 56 out of the 109 sampled plots and their abundance was on average (± sd, n=1458) 13.38 ± 0.37 per plot and 26.03 ± 0.46 in the plots where they were present. However, because larval distribution was highly aggregated due to clustering in larval nests, we analysed only presence/absence data.

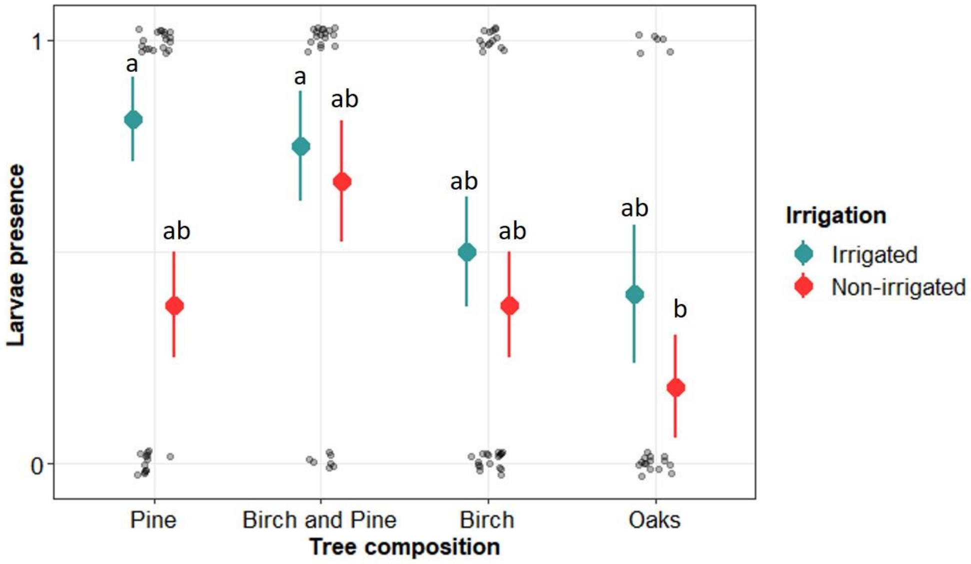

Fig. 3. Presence of *Ixodes ricinus* larva as a function of tree composition and irrigation in the plot. Graph made on raw data. Pine include Pine with or without oaks, Birch includes Birch with or without oaks, Birch and Pine include Birch with pine with or without oaks, and Oaks includes oaks only. Mean values with common letters were not significantly different (Tukey's post hoc test). Dots and solid lines represents mean ± SE calculated on raw data.

The probability of presence of the larvae significantly differed between tree compositions (Fig. 3). Presence of larvae was highest in pine plots (pure and mixed with birch), intermediate in birch plots and lowest in oak plots. We also found a significantly positive effect of irrigation, with higher probability of presence in irrigated plots, irrespective of plot composition (i.e. lack of significant interaction) (Table 2, Fig.3). The probability of larvae presence was significantly higher in irrigated pure and mixed pine plots than in non-irrigated oak plots (Fig.3).

3.2 Abundance of tick nymphs

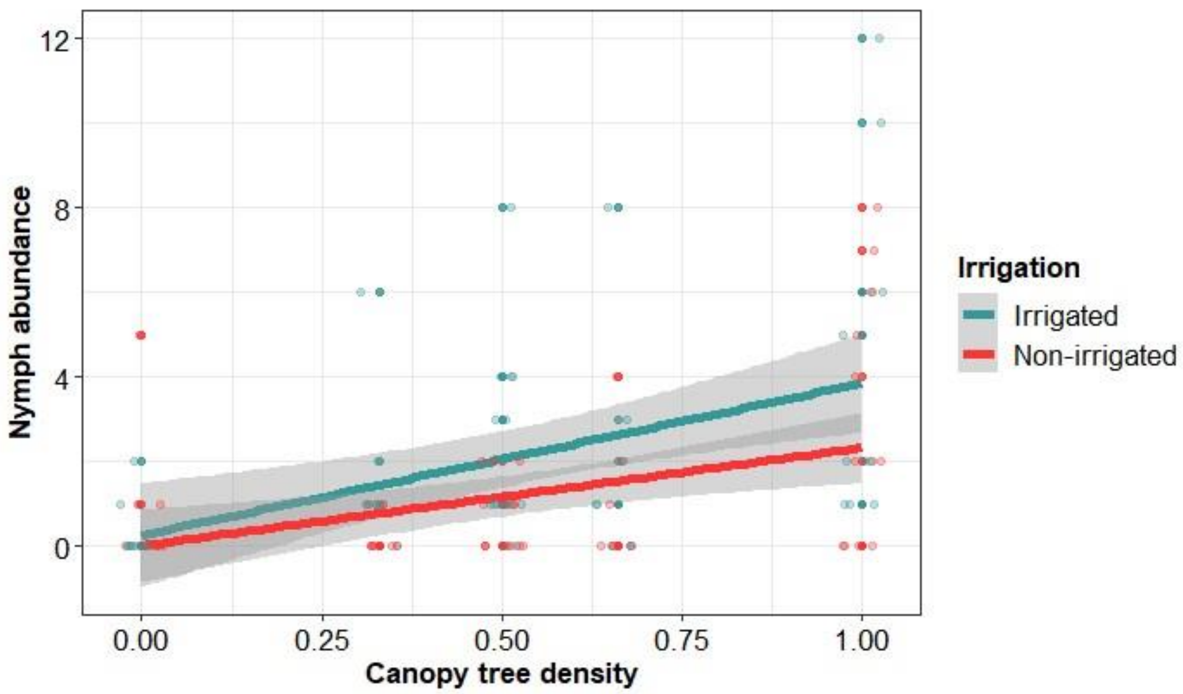

Fig. 4. *Ixodes ricinus* nymph abundance as a function of canopy tree density and irrigation in the plot. Canopy tree density is the ratio of the number of birch and/or pine trees to 100 total trees in the plot. Solid lines and grey shaded area correspond to the regression line and its 95% confidence interval, calculated on raw data.

We collect *I. ricinus* nymphs in 65 out of 109 plots. Nymph abundance was on average (± sd, *n* =173) 1.59 ± 0.02, with a median of 1 nymph per plot and 2.66 ± 0.02 in the plots with at least one nymph was present. According to the AIC of compared models, three explanatory variables were retained as significantly influencing the abundance of tick nymphs (Table 2). Nymph abundance increased with the density of canopy trees (Fig.4) and decreased with the phytovolume of understorey vegetation (Fig.5), being significantly higher in irrigated plots, irrespective of their composition (no interaction effect). We did not find any effect of the saturation deficit or tree species richness.

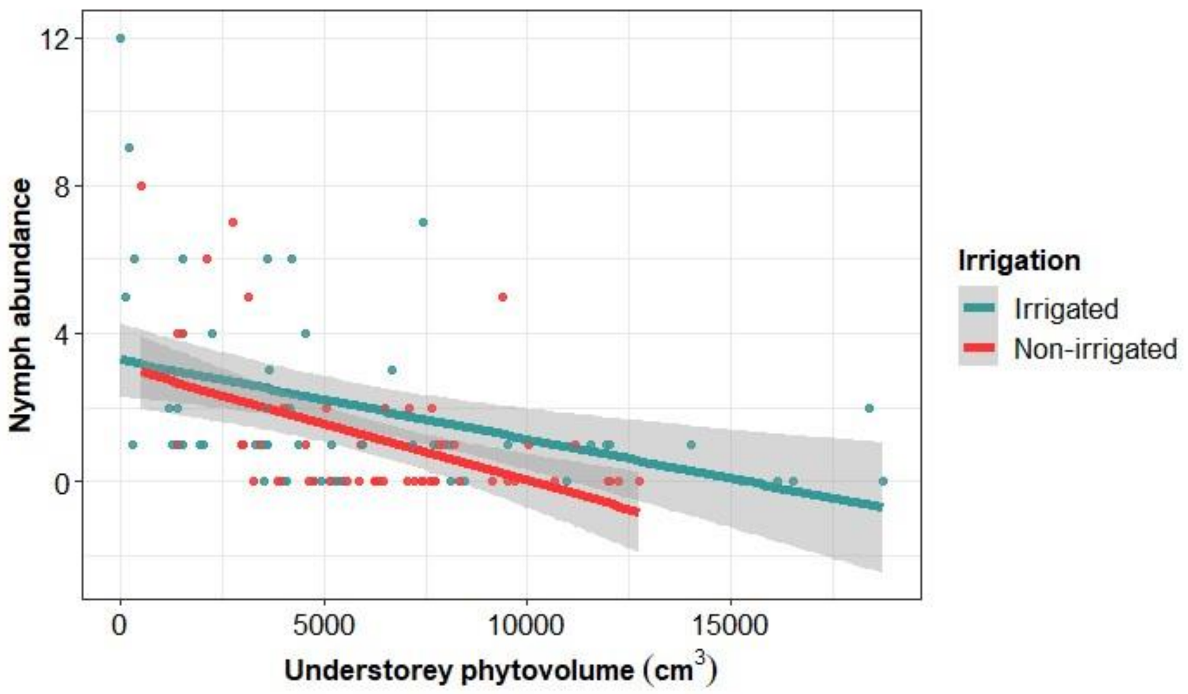

Fig. 5. *Ixodes ricinus* nymph abundance as a function of understorey phytovolume in irrigated (blue) and non-irrigated plots (red). Solid lines and grey shaded area correspond to the regression line and its 95% confidence interval, calculated on raw data.

3.3 Presence of infected nymphs

The control probe was positive for 172 of 173 collected nymphs, so one DNA extraction failed. Infected nymphs were found in 40 of the 65 plots with nymphs, 72 nymphs were infected with at least one pathogen and 6 nymphs presented a co-infection with several pathogens. Among the 72 infected nymphs, the majority of associated pathogens were from the *B. bugdorferi* s.l. complex (*n=35*), and *Rickettsia* spp. (*n=32*, with 69% of *Rickettsia helvetica* and 31% of *Rickettsia monacensis*), the other pathogens were not found frequently enough to test for a significant relationship with covariates.

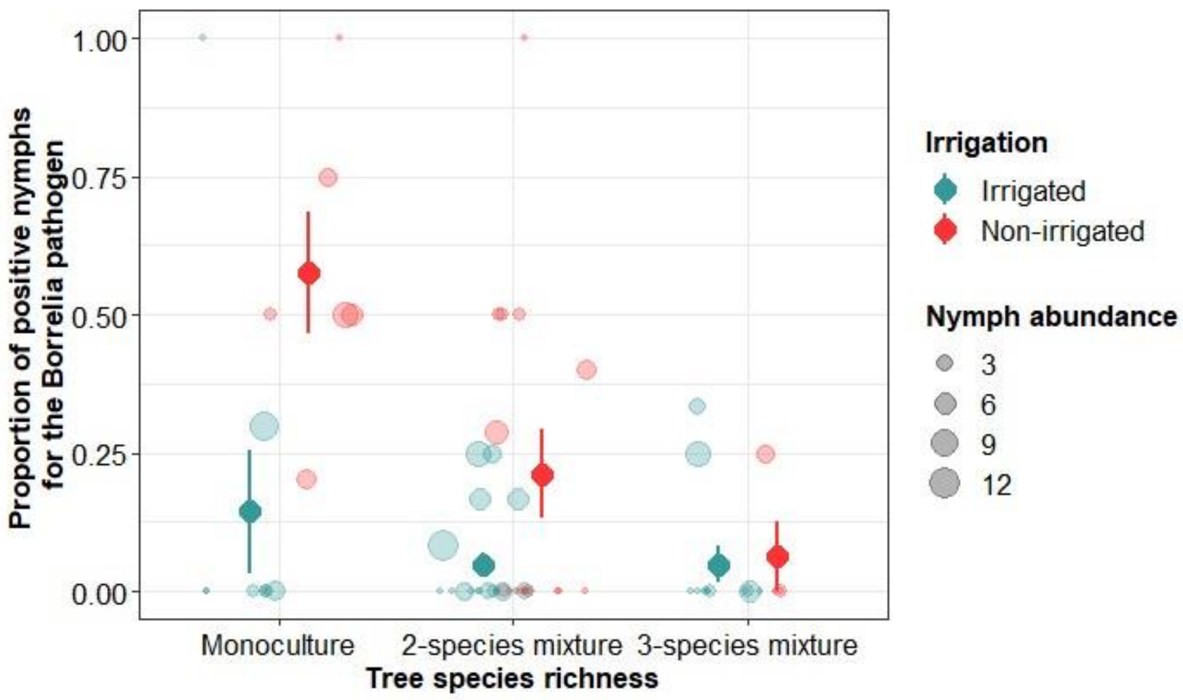

Fig. 6. Proportion of positive *Ixodes ricinus* nymphs for *Borrelia bugdorferi* s.l. pathogen as a function of tree species richness and irrigation. The size of the dots is proportional to the total number of nymphs on which the proportion of positive nymphs was calculated. Dots and solid lines represents mean ± SE calculated on raw data.

The *Borrelia* complex included five different species: 80% of *B. lusitaniae*, 11% *B. afzelii*, 3% *B. valaisiana*, 3% *B. garinii* and 3% *B. burgdorferi s.s.*. No *B. spielmanii, B. bissetti,* and no *B. miyamotoi* (relapsing fever *Borrelia*) were found. *B. lusitaniae*, which was the more frequently detected species, is known to be vectored by ticks with reptiles as hosts. Two other reptile-borne pathogens were found: 10 nymphs carried *Rickettsia monacensis* and one carried *Candidatus cryptoplasma*. The proportion of nymphs infected by *B. bugdorferi* s.l. was significantly influenced by the richness of tree species and also by irrigation, with no interaction between these factors (Table 2). The occurrence of nymphs infected by *B. bugdorferi* s.l. pathogen was lower in irrigated plots and decreased with increasing tree species richness, being highest in non-irrigated pure plots and lowest in irrigated three-species mixtures (Fig.6).

None of our variables were significantly related to the proportion of ticks infected with a *Rickettsia* pathogen (data not shown).

Table 2

Summary of results of final negative binomial GLM models for Ixodes ricinus nymph abundance, binomial GLMMs for presence of larva and binomial logit model for proportion of infected nymph by Borrelia and Rickettsia pathogens. χ2 and P-values are reported for each model, values in bold are those that were significant. Marginal (R^2m) and conditional (R^2c) regression coefficients, and AIC are given for the minimal model obtained by model simplification. The random effect corresponds to the "block" effect. The dispersion parameter is indicated for the model with over-dispersed data.

	Presence of larvae		Nymph Abundance		Nymph w *Borrelia* s.l.		Nymph w *Rickettsia* spp.	
Predictors	*df*	**χ^2-value** (*p*)	*df*	**χ^2-value** (*p*)	*df*	**χ^2-value** (*p*)	*df*	**χ^2-value** (*p*)
(Intercept)		(0.706)		(0.798)		**(0.001)**		**(0.03)**
Tree composition	3	**9.00** (0.03)						
Canopy tree density			1	**5.04** (0.02)				
Tree species richness	2	2.05 (0.36)			2	**6.72** (0.003)	2	2.24 (0.33)
Irrigation	1	**4.12** (0.04)	1	**4.13** (0.04)	1	**9.34** (0.02)	1	0.65 (0.41)
Understorey phytovolume			1	**10.16** (0.001)			1	0.98 (0.32)
Saturation deficit					1	2.30 (0.12)		
Random Effect								
σ^2	3.29		0.75		3.29		3.29	
τ_{00}	0.19 Block		0.00 Block		0.00 Block		0.00 Block	
ICC	0.05		0.00		0.00		0.00	
N	8 blocks		8 blocks		8 blocks		8 blocks	
Observations	109		109		65		65	
R^2m / R^2c	0.204 / 0.247		0.483 / 0.483		0.198 / 0.198		0.059 / 0.059	
AIC	148.2		348.7		88.5		105.5	
ɵ (Dispersion parameter)			2.11					

3.4. DISCUSSION

Using a dedicated experiment where both tree diversity and water supply were manipulated, our study demonstrated that the abundance and pathogen load of the most common tick species in Central

Europe, *Ixodes ricinus*, were significantly influenced by tree species composition, canopy density, tree species richness, volume of understorey vegetation, and by irrigation. The main trend was a decrease in presence of larvae, abundance of nymphs and proportion of nymphs carrying *Borrelia* pathogens in mixed forests where pine trees were substituted with broadleaved trees, irrespective of plot humidity (be it driven by the weather or controlled via irrigation).

4.1 Effects of forest diversity, composition and structure

Our results show that forest diversity and composition can influence tick populations across different stages and their level of infection by pathogens. The probability of presence of larvae was lowest in oak plots, intermediate in birch plots and greatest in pure and mixed pine plots, suggesting a strong tree species identity effect. The abundance of nymphs was significantly affected by canopy tree density, being greater in denser forest plots (e.g. 100% pines, 100% birches or 50% pine – 50% birch mixtures), suggesting a stronger effect of forest structure than tree identity. For a tick to be present in an environment, it must (i) have been deposited by a host for nymphal and adult stages or have been laid by a female for the larval stages. (ii) survived in a favorable environment. Overstorey trees can thus have an impact on young tick population by controling local microclimate (Renaud et al., 2011) and host presence. For example, dense, shaded forests as a result of canopy packing (Jucker et al. 2015; Williams et al. 2017) can be optimal habitats because they provide host animals with food resources while maintaining sufficient moisture for tick survival. A 19-year study in a mature oak-maple mixt forest showed a bottom-up, cascading effect of acorns yield on increasing mouse population levels, resulting in increased abundance of *Ixodes scapularis* (Say, 1821) nymph and associated Lyme disease risks (Ostfeld et al., 2018), and similar results were recently found with *I. ricinus* (Bregnard et al., 2020, 2021). High moisture is important for survival of tick larvae and nymphs (Knülle and Devine, 1972; Tukahirwa, 1976; Davey et al., 1991) and Gray (1998) explains that *I. ricinus* are usually found in environments with 80% atmospheric humidity. The questing activity of tick nymphs occurs higher in the understorey vegetation than that of larvae, so that they can attached to a passing animal, but this presents a greater risk of desiccation during this questing period (Schulze and Jordan., 2005). Denser forests with more trees and with a higher tree canopy packing could therefore limit this risk of desiccation and thus favoring the survival of tick nymphs. This explanation for the positive correlation between nymph abundance and proportion of canopy trees in our experimental plots is also consistent with our result of a positive effect of irrigation.

The presence of larvae and their questing activity are related to the microclimate closer to the ground (Yoder et al., 2006, Ehrmann et al., 2017). Leal et al., (2020) suggest that forest types where the most larvae are found differ between tick species. Indeed, several studies have found greater abundance of *I. ricinus* larvae in deciduous forests (Walker et al. 2001, Tack.et al., 2012, Ruyts et al., 2018). Due to the much slower growth of these species, the plots with only oaks in ORPHEE are currently characterized by small trees of about 2m height and thus an open canopy. Our results in deciduous plots are therefore to be distinguished from the scientific literature on mature deciduous forests. These conditions on ORPHEE were probably less favorable for the maintenance of suitable humidity in the understorey and for the visit of host animals. In contrast, pine plots characterized by dense and tall trees, may have favored larval survival by providing thick needle litter as a protective barrier against desiccation. Birch plots are in an intermediate position in terms of likelihood of larvae presence, probably because they are composed of high trees but with lower canopy closure and perhaps lower quality litter for ticks.

The proportion of nymphs vectoring *Borrelia* pathogens significantly decreased with increasing tree species richness, with the highest frequency in pure pine plots and the lowest in mixtures associating pine, birch and oaks. Our results thus support the finding by Ehrmann et al. (2018) that a reduction in the prevalence of tick-borne diseases is associated with greater forest diversity, confirming the beneficial role of tree species mixing for natural risk reduction (Jactel et al. 2017). Ruyts et al. (2016) did not find a tree species diversity effect on the prevalence of tick-borne pathogens but they found that the risk of Lyme borreliosis differed between different forest compositions. They showed that *B. afzelii* was more often found on ticks in pine plots while *B. garinii* and *B. burgdorferi* s.s. were more prevalent in oak plots. A negative effect of tree species richness on infected nymphs could be explained by an increase in ecological niches for host species, resulting in a change in the ratio of competent to incompetent reservoir hosts, with diluting effects on *Borrelia* (Ehrmann et al. 2018; Levi et al. 2016). Empirical multitaxonomic studies (e.g. Ampoorter et al. 2020) and meta-analyses (e.g. Stemmelen et al. 2021) have indeed shown greater faunal diversity in mixed forests than in pure forests. Additional research is now needed to inventory potential tick host animals in pure and mixed forests and better assess their level of competence for tick-borne pathogens.

Ruyts et al. (2016) suggested that the dilution effect would depend on the species of *B. burgdorferi* s.l.. In our study, 80% of *B. burgdorferi* s.l. were in fact *B. lusitaniae,* which appears to be carried and multiplied by lizards in several countries around the Mediterranean rim (Michelis et al., 2000; Younsi et al., 2001; Sarih et al., 2003; Tomassone et al., 2017). As ectothermic animals, lizards often prefer environments that allow them significant sun exposure for active thermoregulation (Smith and Ballinger, 2001). This may explain the negative effect of irrigation on the proportion of nymphs infected by *Borrelia burgdorferi* s.l. pathogens, either directly by increasing soil humidity or indirectly by increasing the density and thus shading of understorey vegetation (Corcket et al., 2020). We do not have yet direct data from the ORPHEE experiment but surveys in the Aquitaine region reported the presence of three lizard species in forests: the common wall lizard (*Podarcis muralis* (Laurenti, 1768)), the viviparous lizard (*Zootoca vivipara* (Lichtentein, 1823)) and the western green lizard (*Lacerta bilineata*, Daudin, 1802) (Berroneau M. 2015). All are known as *I. ricinus* potential hosts (Gwiazdowicz et al., 2020; Wu et al., 2019; Richter and Matuschka, 2006; Scali et al., 2001), and two of them (common wall and western green lizards) have already been involved in the transmission of *B. lusitaniae* in *I. ricinus* populations (Mendoza-Roldan et al., 2021; Richter and Matuschka, 2006). To date, no studies on the dilution effect of lizard hosts have been tested on *B. lusitaniae;* it would therefore be interesting to inventory them along a gradient of tree diversity and also to classify them according to their level of competence.

Finally, no variable explained the proportion of *I. ricinus* nymphs infected with *Rickettsia* spp. pathogens. The ecological drivers of distribution and prevalence of *Rickettsia* spp. remain poorly understood in the literature and even less the effect of forest composition or diversity.

4.2 Effects of understorey

Our study showed a distinct negative effect of the phytovolume of understorey vegetation on nymph abundance, independently from canopy diversity or composition. Several studies found opposite results with the abundance of nymphs positively correlated with high density of understorey vegetation, a condition considered favorable for visit by potential tick host animals (Tack et al., 2013, Ostfeld et al., 2018). However, other studies found a negative relationship between high density of understorey vegetation and nymph abundance, suggesting on the contrary that these environments would be less visited by hosts or would not be associated with good tick survival rates (Stein et al., 2008). Similarly, a denser understorey vegetation can also dilute tick infection prevalence (Ehrmann et al. 2018). A dense understorey can provide a suitable habitat for ticks but also for small mammals; this increases the likelihood of ticks finding a host, but at the same time decreases the likelihood that many ticks can make contact with the same host species. Thus, a lower average tick load and a lower co-feeding probability on hosts could result in dilution of *Borrelia* prevalence.

Bord et al., (2014) also highlighted that the dragflag technique, although very effective for sampling free-living ticks in forest, is less effective when the understorey is too dense, thus resulting in underestimation of tick abundance. In our study, the negative effect of understorey phytovolume on nymph abundance is however still present at low levels of understorey phytovolume. In addition, the phytovolume does not take into account plant species identity, an important factor for host attraction. In ORPHEE, the main understorey plant species were *Molinia caerulae, Ulex europaeus, Pteridium aquilinum* and some *Ericaceae* spp. (Corcket et al., 2020), which are not or only little palatable for many herbivorous hosts and whose density or height may limit the passage of large ones.

4.3 Medical relevance and practical perspectives

All *Rickettsia* and *Borrelia* pathogens detected in our study are transmissible to humans and of medical importance (Fournier and Raoult, 2009; Steere et al., 2004). *R. helvetica* and *R. monacensis* are belonging to the spotted fever group (Merhej and Raoult, 2011) and *B. afzelii*, *B. valaisiana*, *B. garinii* and *B. burgdorferi* s.s. are bacteria involved in the Lyme disease in humans (Cutler et al., 2017). Although the transmission of *B. lusitaniae* to humans has been demonstrated, the link with Lyme disease has yet to be investigated (Stanek and Reiter, 2011). Previous studies found *B. lusitaniae* associated with medical symptoms in humans after a tick bite (Collares-Pereira et al., 2004; Franca et al., 2005; Lopes de Carvalho et al., 2008). In our study, the proportion of infected nymphs did not correlate with nymph abundance, which confirms that it is important to distinguish tick abundance vs. tick-borne pathogen dynamics and to study pathogens at the species level, due to their host and home range specificity.

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3.6. SUPPLEMENTARY MATERIAL

Bourdin, A., Bord, S., Durand, J., Galon, C., Moutailler, S., Scherer-Lorenzen, M., Jactel, H. (2022) – **Forest diversity reduces the prevalence of pathogens transmitted by the tick Ixodes ricinus**– *Frontiers in Ecology and Evolution* 10:891908

Table S1: Primers used for the detection of *pathogen* species via PCR.

Pathogens	Primer name	Primer sequence (5'-3')	Tm (°C)	Fragment length (bp)	References
Borrelia s.l.	FlaB280F	GCAGTTCARTCAGGTAACGG	55	645	Loh et al. 2016
	FlaRL	GCAATCATAGCCATTGCAGATTGT			
	FlaB_737F	GCATCAACTGTRGTTGTAACATTAACAGG	59	407	
	FlaLL	ACATATTCAGATGCAGACAGAGGT			
Rickettsia spp.	Rsfg877	GGG GGC CTG CTC ACG GCG G	69	381	Regnery et al., 1991
	Rsfg1258	ATT GCA AAA AGT ACA GTG AAC	52		
Babesia spp., *Theileria* spp., *Hepatozoon* spp.	BTH 18S 1st F	GTGAAACTGCGAATGGCTCATTAC	58	1500	Masatani et al., 2017
	BTH 18S 1st R	AAGTGATAAGGTTCACAAAACTTCCC			
	BTH 18S 2nd F	GGCTCATTACAACAGTTATAGTTTATTTG			
	BTH 18S 2nd R	CGGTCCGAATAATTCACCGGAT			
Anaplasma spp., *Erlhichia* spp.	EHR1	GAACGAACGCTGGCGGCAAGC	60	ND/693	Rar et al. 2005
	EHR2	AGTA(T/C)CG(A/G)ACCAGATAGCCGC			
	EHR3	TGCATAGGAATCTACCTAGTAG	55	629/592	

4. Quelles sont les composantes de la biodiversité des forêts qui exercent un effet direct ou indirect sur les densités de tiques et la prévalence des bactéries associées?

Audrey Bourdin, Katarzyna Stojek, Bogdan Jaroszewicz, Damien Bonal, Helge Bruelheide, Clémence Galon, Loïc Gillerot, Yasmine Kadiri, Violette Maugis, Sara Moutailler, Bart Muys, Quentin Ponette, Michael Scherer-Lorenzen, Elena Valdes-Correcher, Kris Verheyen, Hervé Jactel (n.d.) –**Lower prevalence of Lyme disease pathogens in mixed deciduous – coniferous forests.** *In prep*

RESUME

Un nombre croissant d'études démontre les conséquences de la perte de biodiversité sur l'apparition de maladies à transmission vectorielle telles que la borréliose de Lyme. Comme les espèces hôtes diffèrent dans leur capacité à transmettre les borrélies aux tiques, plusieurs auteurs pensent qu'une diversité accrue en hôtes pourrait diminuer la prévalence de la maladie en augmentant la proportion d'hôtes de dilution, c'est-à-dire d'espèces hôtes qui transmettent les agents pathogènes moins efficacement. Cependant, ce résultat semble peu trouvé en Europe, et de récentes études ont montré que l'habitat lui-même, pouvait avoir des effets de dilution ou d'amplification du risque causé par les tiques. Nous avons échantillonné des tiques le long de gradients de diversité des essences forestières dans un réseau de sept sites en Europe pour évaluer l'effet combiné de la diversité des arbres et des plantes du sous-bois sur les tiques et les pathogènes associés. Nous avons trouvé que plusieurs caractéristiques de l'habitat impactaient les densités d'*Ixodes ricinus* et leurs taux d'infection. La proportion de nymphes *I. ricinus* infectées par une bactérie du genre *Borrelia* était moins importante dans les forêts mélangées que dans les forêts pures. Les prévalences de *Borrelia* et de *Rickettsia* augmentaient et diminuaient respectivement avec la richesse en plantes du sous-bois. Enfin, la densité de larves augmentait avec la proportion de plantes appétantes pour le chevreuil (*Capreolus capreolus*). Nos résultats suggèrent que la diversification de l'habitat et des ressources des hôtes vertébrés constituerait une base théorique utile pour orienter la gestion forestière.

4.1. INTRODUCTION

Biodiversity supports multiple forest ecosystem services (van der Plaas et al. 2016), including biomass production (Paquette and Messier 2011, Zhang et al. 2012; Jactel et al. 2018), carbon sequestration (Liu et al. 2018), pest regulation (Jactel et al. 2021), and habitat for species (Ampoorter et al. 2020). However, the more direct benefits to human well-being, such as reduced health risks, are less well known (Marselle et al., 2021). However, forests pose a number of hazards to humans, including diseases transmitted by wild animals. Several studies have already linked increased deforestation to the emergence or spread of zoonotic diseases due to the increased interface between cities and forests, which increases contact between humans or domestic animals and wild animals (Olivero et al., 2017; Ellwanger et al., 2020). In addition, forest fragmentation can lead to changes in vertebrate community composition, directly affecting disease vector animal populations, transmission and prevalence of several pathogens in the forest environment (Diuk-Wasser et al., 2020). It would therefore be interesting to know whether forest biodiversity also plays a role in regulating these zoonotic diseases in order to curb the increase of risks to human health.

The greatest health-hazard among all vector-borne diseases in the European Union is the Lyme disease. It is caused by *Borrelia burgdorferi* s.l. bacteria (Semenza & Suk, 2018) which are carried by ixodid ticks that mainly reside in the forest (Estrada-Peña, 2001; Bourdin et al. submitted). The composition of the vertebrate community, the understorey vegetation and the microclimate are well-known environmental drivers of tick population dynamics (Tack et al., 2012; Ehrmann et al., 2017), each of which is potentially influenced by forest diversity.

American studies on *Ixodes scapularis*-borne disease showed that the diversity of vertebrate hosts could decrease *Borrelia* prevalence in ticks through the dilution effect of competent hosts by incompetent ones (Linske et al., 2018; Ostfeld and Keesing, 2012). By contrast, Esser et al., (2022) found no effect of forest vertebrate host richness on the prevalence of pathogens associated with *Ixodes ricinus* in the Netherlands. In addition to rodents, which are prime hosts for the juvenile stages of *I. ricinus* and competent for *Borrelia*, ungulates might play an important role in the dynamics of ticks and tick-borne diseases (Tagliapietra et al., 2011). As cervids constitute major hosts for *I. ricinus* reproduction (Vor et al., 2010), their presence and their abundance could amplify larvae and nymph densities (Vor et al., 2010; Gilbert et al., 2012; Hofmeester et al., 2017). In addition, they are known to be incompetent hosts for *Borrelia*, and could thus decrease the risk of lyme-disease by diluting *Borrelia*-infected nymphs density. Vourc'h et al. (2016) indeed found fewer *I. ricinus* nymphs infected with *B. burgdorferi* s.l. in forests with high roe deer (*Capreolus capreolus* L.) densities than in those with medium densities. Rosef et al., (2009) found similar results in Norwegian mixed forests*,* where *I. ricinus* infection rates were significantly lower in sites with high density of roe deer and red deer (*Cervus elaphus L.*) compared to sites with low density.

Dry microclimates are known to reduce the survival of ticks as they are prone to desiccation (Gray, 2008). The microclimate in the forest is generally cooler and more humid, with less extreme variations, due to the protection provided by the canopy and the transpiration of the vegetation (De Frenne et al., 2019). This could favour higher tick abundances in forests (Gray, 2008). Mixed forests, through a greater complementarity in crown shape (Jucker et al. 2015), foster thicker canopies and therefore deeper shading which can promote tick survival by limiting dry conditions (Walker et al., 2001).

Tree species richness also influences the composition and diversity of understorey vegetation (Ampoorter et al., 2014; Corcket et al., 2020), the structure of which could directly impact tick accessibility to hosts and indirectly affect tick abundance through attraction of their vertebrate hosts (Allan et al., 2010). Ungulates are more abundant in mixed forests (Ampoorter et al. 2020), but the composition of mammal communities also seems to be influenced by tree diversity with mixed forests favouring large species (e.g. cervids) while reducing the activity of small ones (e.g. mice and voles) (Milligan and Koricheva, 2013; Jactel et al., 2017).

Few studies have attempted to specifically measure the effects of forest diversity on tick abundance and on prevalence of tick-borne pathogens. In their experiment conducted in five European countries, Ehrmann et al., (2018) found that the prevalence of *B. burgdorferi* s.l. in *I. ricinus* was amplified in denser forest stands but diluted in functionally more diverse ones (characterized by higher tree species richness, greater tree diameter heterogeneity, and greater seed size diversity for understory plants). Using a long-term experiment where tree species diversity is manipulated, Bourdin et al., (2022) also found a decrease in the proportion of *Borrelia burgdorferi* s.l.-infected nymphs with increasing tree species richness. In Belgium, Ruyts et al. (2016) did not find any influence of tree diversity on *I. ricinus* infection prevalence but they detected an effect of forest composition on *Borrelia* species proportion. *I. ricinus* nymphs infected by *B. afzelii* (carried by small mammals) were more frequent in coniferous stands while nymphs infected by *B. garinii* (carried by birds) were more abundant in deciduous stands.

These findings suggest that forest habitat diversity may have amplifying or diluting effects on tick-related disease risk. Yet, few studies have investigated these relationships along explicit gradients of diversity or composition of mixed forests. Even fewer have considered the ecology of different *Borrelia* species, even though their host range specificity may lead to divergent responses to biodiversity. To fill these gaps, we sampled ticks and associated pathogens in pure and mixed forests with different tree species compositions in a network of seven sites in Europe spanning a large biogeographical gradient. Our objective was to test the following hypotheses:

1. Tick abundance increases but prevalence of tick-borne bacteria decreases in mixed species forests as compared to pure ones
2. Different species of *Borrelia* bacteria respond differently to environmental variables due to their habitat requirements and vertebrate host specificity.
3. The proportion of plants that are palatable to roe deer has a positive effect on tick densities but results in lowest nymph infection rates because it is an incompetent host.

4.2. MATERIAL AND METHODS

2.1 Study sites

The study was conducted in 136 forest plots distributed over seven sites in three European countries (Belgium, France and Poland). Among these seven sites, four were young forest plantations that are part of the TreeDivNet network (Verheyen et al., 2016) and three were mature semi-natural forests, of which two were from the exploratory platform of the FundivEurope project (Baeten et al., 2013) and one from the TREEWEB network (De Groote et al., 2017). At each site, we focused on three-species mixtures and the three monocultures of their component species to enable comparing pure and mixed forests of different compositions (see Table 1). All plot compositions were replicated at least twice per site, except for mixed plots of the Bialowieza site (Poland) that were not replicated.

Table 1: Summary of the composition of the 3-species mixture plots with the mean proportion of each species expressed in basal area (in parentheses). *Q. robur* = *Quercus robur* (L., 1753), *Q. petraea* = *Quercus petraea* (Liebl., 1784), *Q. ilex* = *Quercus ilex* (L., 1753), *Q. rubra* = *Quercus rubra* (L., 1753), *B. pendula* = *Betula pendula* (Roth, 1788), *P. pinaster* = *Pinus pinaster* (Aiton, 1789), *F. sylvatica* = *Fagus sylvatica* (L., 1753), *C. betulus* = *Carpinus betulus* (L., 1753), *T. cordata* = *Tilia cordata* (Mill., 1768), *P. menziesii* = *Pseudotsuga menziesii* (Franco, 1950), *L. kaempferi*= *Larix kaempferi* (Carrière, 1856), *A. pseudoplatanus* = *Acer pseudoplatanus* (L., 1753), *L. eurolepsis* = *Larix eurolepsis* (Mill., 1754).

Site	Country	Species 1	Species 2	Species 3
Orphee	France	*B. pendula* (67%)	*Q. robur* (17%)	*Q. ilex* (11%)
Orphee	France	*B. pendula* (8%)	*Q. ilex* (2%)	*P. pinaster* (90%)
Orphee	France	*B. pendula* (9%)	*Q. robur* (2%)	*P. pinaster* (88%)
Orphee	France	*Q. robur* (1%)	*Q. ilex* (8%)	*P. pinaster* (91%)
Mixlor	France	*F. sylvatica* (32%)	*Q. petraea* (40%)	*C. betulus* (27%)
Mixlor	France	*C. betulus* (51%)	*Q. petraea* (23%)	*Q. robur* (27%)
Mixlor	France	*C. betulus* (27%)	*F. sylvatica* (32%)	*Q. petraea* (40%)
Mixlor	France	*C. betulus* (24%)	*F. sylvatica* (39%)	*Q. robur* (38%)
Zedelgem	Belgium	*Q. robur* (14%)	*B. pendula* (65%)	*T. cordata* (20%)
Zedelgem	Belgium	*Q. robur* (20%)	*T. cordata* (9%)	*P. sylvestris (*65%)
Zedelgem	Belgium	*F. sylvatica* (10%)	*B. pendula* (65%)	*T. cordata* (23%)
Zedelgem	Belgium	*F. sylvatica* (19%)	*Q. robur* (26%)	*P. sylvestris* (54%)
Zedelgem	Belgium	*F. sylvatica (12%)*	*B. pendula (45%)*	*P. sylvestris (42%)*
Hechtel-Eksel	Belgium	*P. sylvestris* (60%)	*B. pendula* (32%)	*P. menziesii* (7%)
Hechtel-Eksel	Belgium	*Q. petraea* (8%)	*P. menziesii* (56%)	*L. kaempferi* (36%)
Hechtel-Eksel	Belgium	*P. sylvestris* (72%)	*Q. petraea* (8%)	*L. kaempferi* (20%)
Hechtel-Eksel	Belgium	*Q. petraea* (9%)	*B. pendula* (74%)	*P. menziesii* (16%)
Hechtel-Eksel	Belgium	*P. sylvestris* (56%)	*B. pendula* (26%)	*L. kaempferi* (18%)
Gedinne	Belgium	*F. sylvatica* (7%)	*P. menziesii* (85%)	*Q. petraea* (7%)
Gedinne	Belgium	*F. sylvatica* (4%)	*L. eurolepsis* (95%)	*A. pseudoplatanus* (1%)
Gedinne	Belgium	*P. menziesii* (73%)	*A. pseudoplatanus* (3%)	*Q. petraea* (11%)
Gedinne	Belgium	*F. sylvatica* (5%)	*P. menziesii* (20%)	*L. eurolepsis* (75%)
Gedinne	Belgium	*L. eurolepsis (91%)*	*A. pseudoplatanus (1%)*	*Q. petraea* (7%)
Treeweb	Belgium	*F. sylvatica (26%)*	*Q. robur (23%)*	*Q. rubra (49%)*
Bialowieza	Poland	*B. pendula* (32%)	*C. betulus* (37%)	*P. sylvestris* (22%)
Bialowieza	Poland	*B. pendula* (30%)	*P. sylvestris* (14%)	*Q. robur* (27%)
Bialowieza	Poland	*B. pendula* (50%)	*C. betulus* (39%)	*Q. robur* (11%)
Bialowieza	Poland	*P. sylvestris* (34%)	*C. betulus* (41%)	*Q. robur* (24%)

2.2 Tick sampling and identification

Free-living ticks were collected in June 2020 in the Orphee site (SW France) and between 25th May and 2nd August 2021 in all other sites, using a 1m×1m white flannel cloth dragged on the ground at a constant speed (Macleod, 1932; Vassallo et al., 2000). The total number of ticks collected along four parallel 10 meter long transects in each plot (40 m² sampled area) was denoted $N_{\{i,j,k\}}$ where $i \in [1;4]$ represents the plot composition, $j \in [1;2]$ represents the replication of plot composition and $k \in [1;4]$ represents the kth parallel long transect. Each transect was sampled four times (i.e. two return trips), which accounts for the effect of understorey heterogeneity on the sampling rate (Bord et al., 2014). All samplings took place on days without rain during the local peak of tick activity. Larvae, nymphs and adults were counted after each successive pass and summed up at the plot level ($\sum_{k=1}^{4} \quad N_{i,j}$). Nymphs and adults were kept in a 70% ethanol tube at the end of each pass in a transect and the larvae were released immediately after each pass (it was not possible to screen for pathogens associated with the larvae). Temperature (T) and relative atmospheric humidity (RH) were measured in each plot $N_{i,j}$ before each sampling (with a Kestrel 3500 NV thermometer), in order to calculate the *Saturation Deficit* (important predictor of nymphs questing activity) in millimeters of mercury, according to equation (1) (Gray, 2008; Vourc'h et al., 2016):

$$Saturation\ Deficit\ = \left(1 - \frac{RH}{100}\right) 4.9463 exp(0.0621\, T) \qquad \text{eqn (1)}$$

All nymph and adult species were identified after binocular examination in the laboratory and using the systematic key from Estrada-Peña et al. (2017) by a single operator (AB).

2.3 Identification of tick-borne pathogens in *Ixodes ricinus* nymphs

All 1,105 *I. ricinus* nymphs collected were individually analyzed for the identification of associated pathogens. Four DNA extractions failed so the results are based on 1,101 nymphs. We used a microfluidic real-time PCR technique to detect the presence of 35 different pathogen strains of the following genus: *Borrelia, Anaplasma, Rickettsia, Francisella, Bartonella, Babesia* and *Coxiella* (developed by Michelet et al., 2014).

Tick nymphs were rinsed twice with distilled water. Prior to extraction, ticks were crushed and processed in a homogeniser at 5500 rpm for 20 s with 100 µL of elution buffer BE, 40 µL of lysis buffer MG and 10 µL of liquid proteinase K and six steel balls. DNA was extracted using a Nucleospin DNA insect kit (Macherey-Nagel, Germany), and DNA from each sample was stored at -20◦C in 50µl of rehydration solution until next step.

DNA pre-amplification reactions were performed with 2.5µL of PreAmp Master Mix according to the manufacturer's instructions (Standard Biotools, CA, USA). Prior to experiments, primers targeting all pathogens were pooled in equal volumes (200 nM each). The experiment was performed with 1 µL of PreAmp Master Mix, 1.25 µL of pooled primers mix, 1.5 µL distilled water, and 1.25 µL DNA for 5 µL final volume. The pre-amplifications were performed with one cycle at 95°C for two minutes, 14 cycles at 95°C for 15 s and 60°C for four minutes. Pre-amplified DNA was diluted 1:10 and stored at -20°C. Finally, a high-throughput real-time PCR was performed with the BioMark system (Standard Biotools, USA) using the method explained in Michelet et al. (2014).

Pathogens detected with BioMark system were confirmed by conventional PCR or nested PCR on several samples presenting low Ct values. Primers targeting different genes or regions than those of the BioMark system were selected from the literature (listed Table S1) and PCR were performed with Phusion High fidelity kit (Thermo scientific, MA, USA). The positive samples after gel migration were sent to sequencing at Eurofins (Germany) and assembled using BioEdit software (Ibis Biosciences). An online BLAST (NCBI; National Center for Biotechnology Information) was used to identify the sequenced organisms.

2.4 Response variables

We analyzed five response variables for *I. ricinus*. The densities of larvae (*DOL*)*,* nymphs (*DON*) and adults (*DOA*) were estimated as the cumulative number of larvae, nymphs and adults respectively collected in the four 10m long transects. NIP_{Bor} was the proportion of infected nymphs by *Borrelia* spp. (including all detected borrelia genospecies and of relative importance to human health: *B. afzelii*, *B. burgdorferi* s.s., *B. garinii*, *B. valaisiana*, *B. lusitaniae*, *B. miyamotoi* and *B. spielmanii*). NIP_{Rick} was the proportion of ticks infected with *Rickettsia spp.* All data were analyzed at the plot level (40m^2 sampled).

2.5 Explanatory variables

We used two tree layer variables. *Tree species richness* was the number of different tree species in the plot, i.e. one or three. *Tree composition* took four values: 1) Pure coniferous, 2) Pure deciduous, 4) 3–species mixtures composed of three deciduous species; 4) 3-species mixtures composed of coniferous and deciduous species (there were no three-species mixtures composed of only conifer species). With this functional group approach, each site had replicates of compositional types of pure and mixed forests. We used three understorey vegetation variables: *Total understorey cover*, *Total understorey plant richness* and the *Proportion of roe deer* (*Capreolus capreolus* L.) *palatable plants*. European roe deer (*Capreolus capreolus*) is very common all over Europe and constitutes a key host species for ticks (Walker et al. 2001; Rizzoli et al. 2007, Zeman and Pecha 2008). It is known as an incompetent host for *B. burgdorferi* s.l. pathogens but competent for *Anaplasma* spp. (Hornok et al., 2018) and *Rickettsia* spp. (Stefanidesova et al., 2008). All vascular plant species less than 1.3 m tall were recorded in three 5 m × 5 m subplots (including one that is crossed by tick sampling transects), integrated into each sampled plot, to estimate understorey species richness and cover (Ampoorter et al. 2014). Each plant species was then considered palatable or not for roe deer according to the references listed in Supplementary Material SM1. The *saturation deficit* has been included as a microclimatic variable.

2.7 Statistical analysis

2.7.1 Drivers of tick and tick borne disease prevalence

We investigated the effect of forest diversity (pure vs mixed plots) and composition on tick and tick borne disease abundance or prevalence while taking into account the understorey vegetation and local microclimate.

DOL, *DON*, *DOA*, NIP_{Bor} and NIP_{Rick} were analysed separately. We first sorted out the explanatory variables with univariate tests (see univariate test results in SM2 Table S3). Variables with a p value ≤ 0.1 were selected as candidates for the full models. For *DON* and *DOA*, we built negative-binomial generalized linear models (glmer.nb) suitable for overdispersed data (Breslow, 1984). We modelled *DOL* with a zero-inflated negative binomial model (zinb GLM). We modelled NIP_{Bor} and NIP_{Rick} with binomial generalized linear models (glm, binomial family). NIP_{Bor} and NIP_{Rick} were modelled as vectors including the number of pathogen-positive nymphs and pathogen-negative nymphs at the plot level. All fixed effects used in final models are detailed in Table 2. All continuous predictor variables were scaled and centered prior to modeling to make their coefficients comparable (Schielzeth, 2010) and we included the *Site* as a random factor for each model.

We first built full models fitting the most complex model consistent with the experimental design including the random factor and all candidates fixed effects. We applied model simplification by sequentially removing the less significant terms one-by-one to allow a non-singular fit (Barr et al. 2013). Finally, models with the lowest AIC were selected.

We did not have enough replicates to perform proper structural equation modeling but we eventually tested the effect of tree composition and tree species richness on the understory variables selected in the final models with χ^2 tests to help with the interpretation of detected patterns.

Statistical analyses were performed using the R software version 4.2.0 (R Core Team 2022).

2.7.2 Net Biodiversity Effect

To further analyze the effect of tree species mixing on ticks and tick-borne diseases, we calculated the net biodiversity effect as the difference between mean observed values of tick abundance or TBD prevalence in the three-species plots and their expected values based on their mean values in the three monocultures of component tree species and the relative proportion of tree species in the mixed plots (Loreau & Hector 2001).

More specifically we calculated the net biodiversity effect (*NBE*) as the rate of the differences between observed and expected tick mean densities (*DOL*, *DON* or *DOA*) or proportion of infected nymphs (NIP_{Bor} or NIP_{Rick}) of the component species in the mixture:

$$NBE = (\underline{X}_{\mathrm{mx}} - \sum p_i \underline{X}_{\mathrm{mo}\text{-}i}) / \sum p_i \underline{X}_{\mathrm{mo}\text{-}i} \qquad \text{eqn(2)}$$

Where $\underline{X}_{\mathrm{mx}}$ = mean tick density or NIP in mixture; $\underline{X}_{\mathrm{mo}}$= mean tick density or NIP in monoculture plot; p_{i} is the relative proportion of the tree species *i* in the mixture (% basal area of each tree species in the mixture, Table 1). Then we averaged all NBEs across sites and considered the effect significant if the 95% confidence interval around the mean *NBE* did not include zero. Negative values of NBE meant that tick abundance was lower than expected in mixed forests. We could not calculate the selection and complementarity components of NBE because we could not relate tick abundance to specific tree species in the mixtures. NBE was calculated for *DOL*, *DON*, *DOA*, NIP_{Bor} and NIP_{Rick}. NBE could not be calculated for the Bialowieza site because the mixed plots were not replicated and when $\sum \underline{X}_{\mathrm{mo}i} = 0$ (i.e. when all pure plot values of species *i* in the mixture were equal to 0).

4.3. RESULTS

Tick larvae were present in 38 out of the 136 sampled plots and their density was on average (± sd) 82.5 ± 0.9 in the plots where they were present. A total of 1,106 nymphs, including 1,105 *I. ricinus* and a total of 103 adults including 96 *I. ricinus* were collected. We found *I. ricinus* nymphs in 125 out of the 136 sampled plots and their density was on average 10.05 ± 0.1 in the plots where they were present. *Dermacentor reticulatus* (Fabricius, 1794) and *Ixodes trianguliceps* (Birula, 1895) were also found but in numbers too low to be included in the analyses. *I. ricinus* adults were present in 44 plots and their abundance was on average 1.91 ± 1.2 in plots where they were present. *DON* was positively correlated with *DOL*.

Among the 1,101 *I. ricinus* nymphs analyzed, 308 carried at least one pathogen and 30 presented co-infection. The majority of the pathogens were part of *Borrelia* spp. (*n*=176) and by *Rickettsia* spp. (*n*=163) (SM2, Table S1). Among the total number of nymphs infected with a *Borrelia* spp., 38% were infected by *B. afzelii*, 25% by *B. garinii,* 14% by *B. valaisiana*, 10% by *B. miyamotoi*, 6% by *B. lusitaniae,* 5% by *B. burgdorferi* s.s., and 2% by *B. spielmanii*. Among the nymphs infected with *Rickettsia* spp., 95% corresponded to *R. helvetica*, 3% to *R. monacencis* and 2% to undetermined *Rickettsia* species.

3.1 Effect of tree diversity and composition on *I. ricinus*

None of the response variables (*DOL, DON, DOA,* NIP_{Bor} and NIP_{Rick}) were significantly influenced by tree species richness (one vs three) in the generalized linear models. There was no effect of tree composition on tick densities (*DOL, DON* and *DOA*) and NIP_{Rick} but we found a significant effect of tree composition on NIP_{Bor}. NIP_{Bor} were significantly lower in mixed deciduous-coniferous plots than in the other forest compositions (Fig. 1).

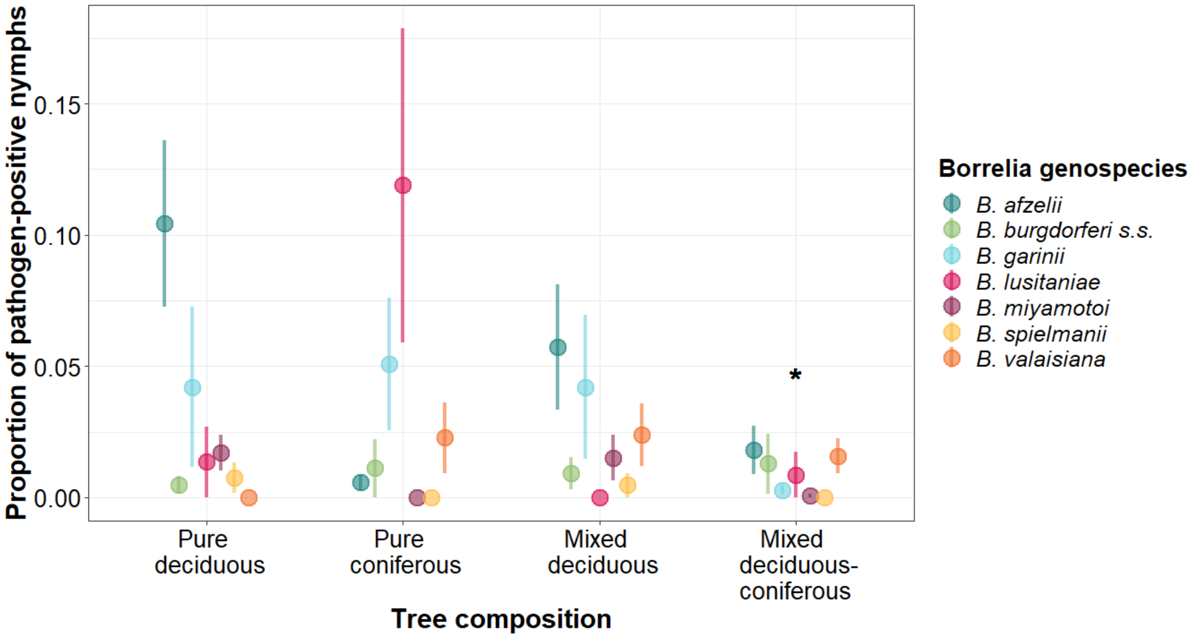

Fig. 1. Proportion of *I. ricinus* nymphs positive to different species of *Borrelia* spp. (all genospecies composing NIP_{Bor}) as a function of tree composition. Dots and solid lines represent mean ± SD.

The net biodiversity effects calculated on *DOL, DON, DOA and* NIP_{Rick} were not significantly different from zero (see SM3, Fig S1, Fig 2b). In contrast, the mean NBE calculated on NIP_{Bor} was significantly negative and equaled −0.39 ± CI [−0.75; −0.03], indicating a significant reduction in the proportion of nymphs infected by *Borrelia* spp. pathogens in mixed plots compared to the expected value from the monocultures of their component species (Fig. 2a).

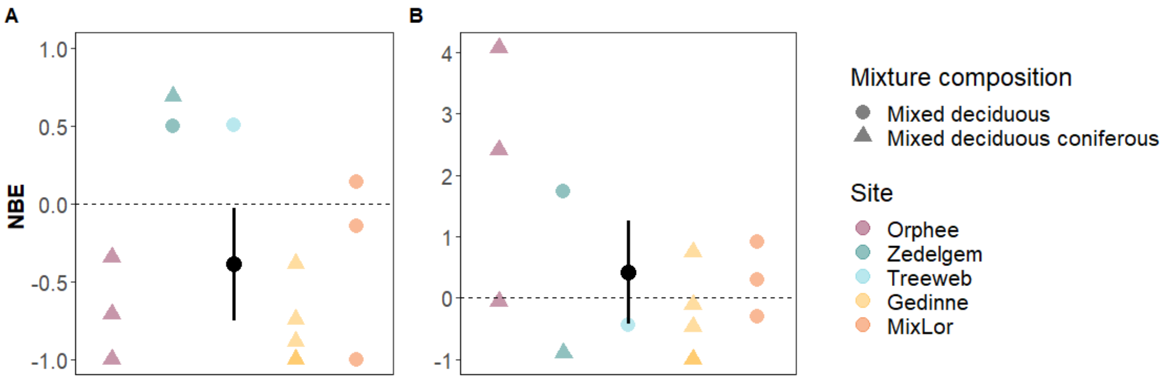

Fig. 2.a : Net tree biodiversity effect on proportion of nymphs infected by *Borrelia* spp.. **b)** Net tree biodiversity effect on proportion of nymphs infected by *Rickettsia* spp. Black dot and associated solid line represent mean ± CI, the colors correspond to the sites and the shape of the dots indicates the tree composition of the mixture.

3.2 Effect of the composition and richness of understorey plants on *I. ricinus*

None of the understory variables were significantly influenced by tree composition or richness in our analyses.

DOL increased with the proportion of roe deer palatable plants in the understorey (Fig. 3a) but were not influenced by other understorey variables. *DOA*, and NIP_{Bor} increased whereas NIP_{Rick} decreased with increasing species richness of understorey plants (Fig. 3b, c, d). We found no effect of understorey variables on *DON*.

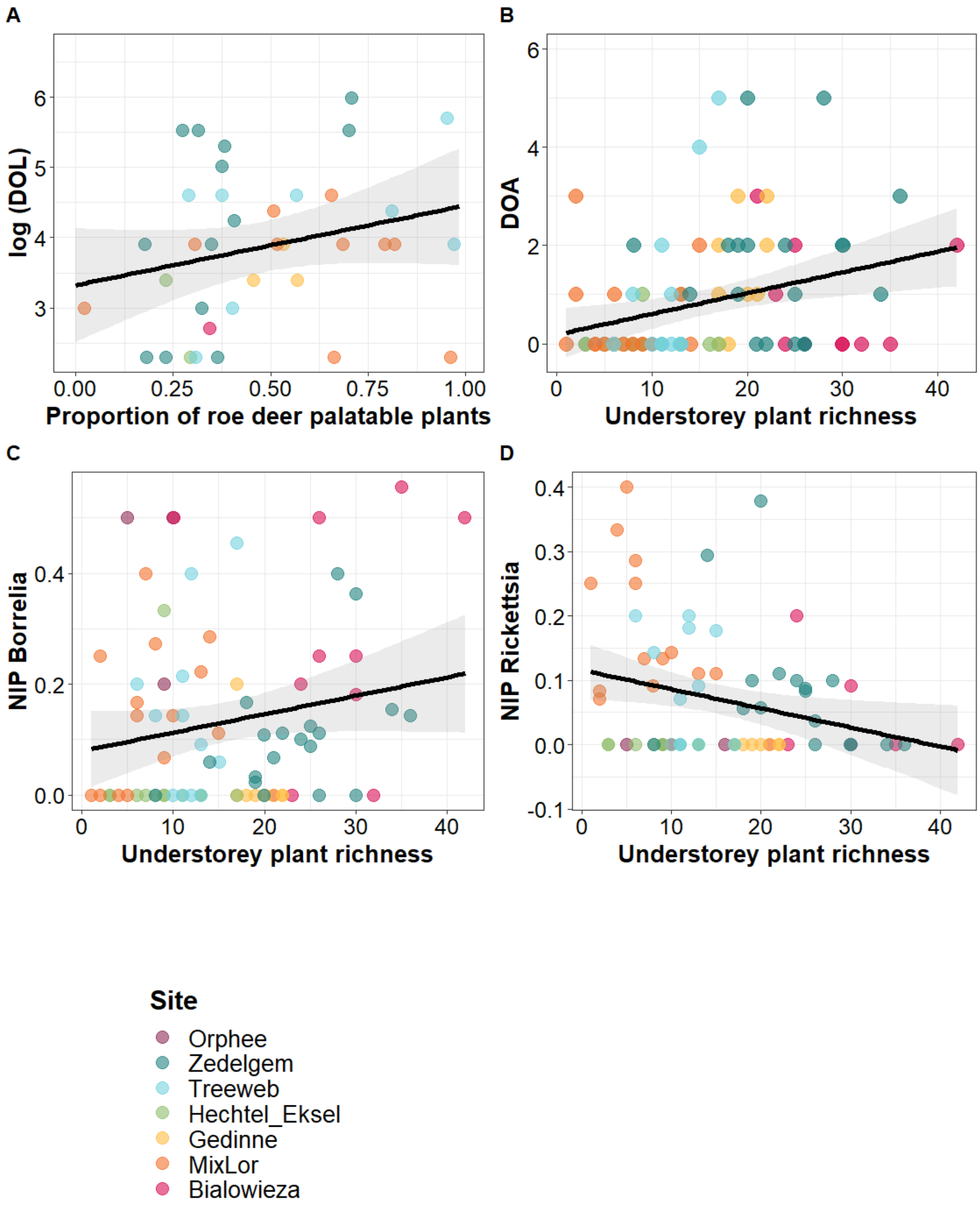

Fig. 3.a: Proportion of *I. ricinus* larvae as a function of the cover proportion of roe deer palatable plants in the understorey vegetation. **b)** Density of *I. ricinus* adults as a function of understorey plant species richness; **c)** Proportion of nymphs infected by *Borrelia* spp. (*NIP*$_{Bor}$) as a function of understorey plant species richness; **d)** Proportion of nymphs infected by *Rickettsia* spp. (*NIP*$_{Rick}$) as a function of understorey plant species richness

Table 2: Summary of results of final models performed on the previously selected variables by univariate tests (p value < 0.1). Negative binomial GLM models for *I. ricinus* nymphs and adult densities, zero-inflated negative binomial GLM for larval density and binomial logit model for proportion of infected nymph by *Borrelia* and *Rickettsia* pathogens. χ2 and p-values are reported for each model, values in bold are those that were significant. The sign of the estimate is indicated in parentheses following the p-value.

		DOL	DON	DOA	NIP$_{Bor}$	NIP$_{Rick}$
Predictors	df	χ2-value (p)	χ2-value (p)	χ2-value (p)	χ2-value (p)	χ2-value (p)
Tree composition	3				15.95 (**0.007**)**	
Understorey cover	1		-0.19 (0.10)			
Understorey richness	1			7.5 **(6.2e-3)**** (+)	6.1 **(0.01)*** (+)	9.34 **(0.002)**** (-)
Proportion of roe deer palatable plants	1	5.72 **(0.02)*** (+)				2.62 (0.11)
Tree composition × *Borrelia species*	18				34.99 **(0.009)****	
DOL	1		0.19 **(0.03*)** (+)			
DON	1			1.64 (0.19)		
DOA	1	0.03 (0.8)				

4.4. DISCUSSION

Our study demonstrated that the abundance and pathogen load of *I. ricinus* were significantly but not consistently influenced by tree species composition, tree species richness, species richness of understorey plants and the proportion of palatable understorey plants for roe deer over the geographical gradient studied here (Fig. 4).

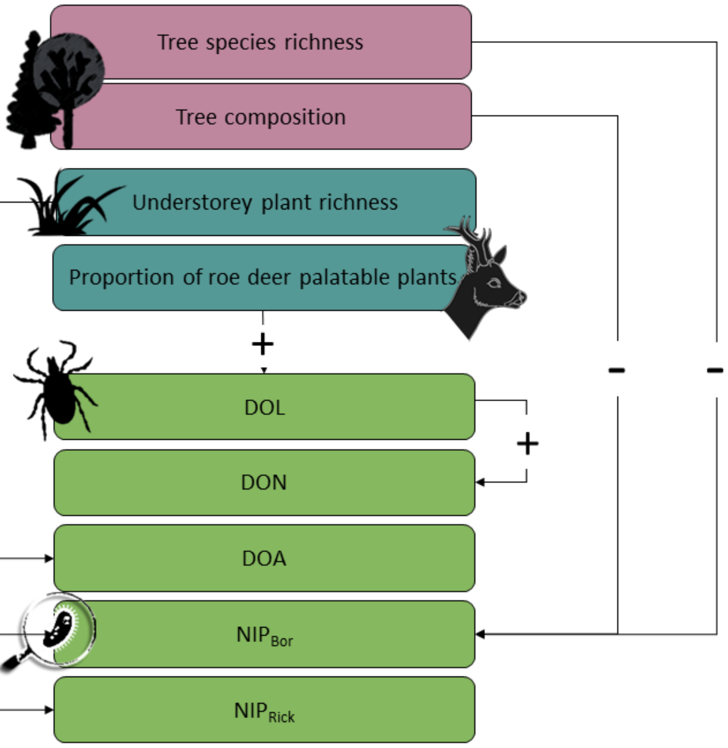

Fig. 4. Explanatory diagram showing our study results at the forest stand level. Tree species richness and mixed deciduous-coniferous composition had a negative effect on the proportion of nymphs infected by *Borrelia* bacteria (NIP_{Bor}). Understorey plant richness had a positive influence on the density of *Ixodes ricinus* adults (*DOA*) and NIP_{Bor} but a negative effect on *Rickettsia* prevalence (NIP_{Rick}). Proportion of roe deer palatable plants had a positive effect on the density of *I. ricinus* larvae (*DOL*). Density of nymphs (*DON*) increased with *DOL*. Black arrows represent significant effects with their signs.

4.1 Tree composition and diversity effects on ticks and tick-associated pathogens

We found that forest diversity or composition had no influence on tick densities but on the prevalence of pathogens associated with tick nymphs. More specifically, we found that the proportion of nymphs infected by *Borrelia* spp., responsible for the Lyme disease, was lower in mixed deciduous-coniferous stands than in other forest compositions. We also demonstrated a net biodiversity effect on *Borrelia* spp., with a significantly lower prevalence in mixed forests than the expected value calculated from the monocultures of their component species. These findings are consistent with the results of recent studies showing lower prevalence of tick-borne diseases in mixed species forests (Ehrmann et al., 2018; Bourdin et al., 2022), which confirms the beneficial role of tree species mixing in reducing natural risks (Jactel et al. 2017). The negative effect of tree species mixtures on the proportion of infected nymphs could be explained by an increase in ecological niches for vertebrate host species, resulting in a change in the ratio of competent to incompetent reservoir hosts, ultimately leading to diluting effects on *Borrelia* (Ehrmann et al. 2018; Levi et al. 2016). Empirical multitaxonomic studies (e.g. Ampoorter et al. 2020) and meta-analyses (e.g. Stemmelen et al. 2021) showed greater faunal diversity in mixed than in pure forests. However, a dilution effect of *I. ricinus*-associated pathogens via higher host richness remains debated as it is not yet confirmed in Europe (Esser et al., 2022). In particular, it remains to be demonstrated whether habitat richness induces dilution of *I. ricinus*-associated bacteria by increasing overall host richness, or by altering the ratio of competent to incompetent hosts for *Borrelia*. For example, Vehviläinen and Koricheva (2006) showed that vole damage was lower in mixed than in pure forests but found the opposite pattern for moose, which could be explained by different foraging behaviour and diet breadth between small and large mammals.

The proportion of nymphs infected with *Rickettsia* was not influenced by tree composition or richness. We already found no effect of forest diversity on the prevalence of *Rickettsia* in a tree diversity experiment (Bourdin et al. 2022) and to our knowledge no other study tested the effects of forest variables on the prevalence of *Rickettsia* associated to *I. ricinus*.

Our results thus indicate that tree diversity does not directly affect tick densities but rather the prevalence of associated *Borrelia* pathogens*,* suggesting that the effects of trees on tick-related risk are mediated by changes in vertebrate hosts diversity or composition.

4.2 Understorey richness and attractiveness for roe deer

In our study, the abundance of *I. ricinus* adults and the proportion of nymphs infected by *B. burgdorferi* s.l. increased with the species richness of understorey plants whereas the prevalence of *Rickettsia* decreased. In addition to providing access to their hosts, the understorey vegetation can also directly impact tick populations by modulating and buffering the microclimate (De Frenne et al., 2019), as mild and wet conditions are necessary for the survival and questing activity of ticks (Ehrmann et al. 2017; McCoy and Boulanger, 2015). Understorey can also indirectly affect tick populations by providing more or less favourable feeding, breeding or resting sites for the vertebrate hosts that ensure the survival, infection and transport of ticks (Allan et al., 2010). For example, Ehrmann et al. (2017) found that *I. ricinus* adult abundance responded to the richness of plant seeds according to a hump-shaped relationship. Because they promoted the prevalence of *Borrelia* spp., plots with high understorey richness might have represented prime locations for *borrelia*-amplifying hosts like rodents. However, Erhmann et al. (2018) found the opposite, with a dilution of *Borrelia* associated with *I. ricinus* in forests associated with richer understory vegetation.

Bacteria from the genus *Rickettsia* are much less studied than *Borrelia*. *Rickettsia* species can be carried by birds (Spitalska et al. 2011; Hornok et al., 2014), rodents (Schex et al., 2011), deer (Stefanidesova et al. 2008) and authors indicated possible trans-ovarian transmission in *I. ricinus* (Sprong et al., 2009). Wijbur et al. (2022) proposed that deer may act sporadically as competent hosts for the transmission of *Rickettsia* to ticks. The negative effect of understorey richness that we found on prevalence of *Rickettsia* suggests again a dilution of pathogen load by higher host richness but more studies are needed to understand the underlying mechanisms. In our study, *I. ricinus* larval density increased with the proportion of plants palatable to roe deer, but not with the total understorey cover or richness. The effect of food resources on tick-host relationships was already found with small mammals and the amount of tree fruits (Ostfeld et al., 2001). Although it can be parasitized by all stages of *I. ricinus*, roe deer is a major host for *I. ricinus* adult reproduction in Europe (Vor et al., 2010). As a result, its presence and abundance have already been positively related to the densities of *I. ricinus* larvae and nymphs in forests (Gilbert et al., 2012; Hofmeester et al., 2017). According to Charnov (1976), a vertebrate herbivore stays longer in stands with its preferred plants. Stands with higher understorey cover of palatable plants may represent areas where deer spend more time, return more often, and thus have more opportunity to deposit adult females that will ultimately lay eggs and produce more larvae.

Our results therefore suggest that understorey vegetation has a more direct influence on ticks (densities) than trees but that it could also have an indirect impact on associated pathogens by attracting vertebrate hosts.

4.3 Conclusions and future research

Our results showed that forest diversity, at both tree and understorey levels, influences ticks and associated pathogens through several direct and indirect mechanisms such as effects on vertebrate hosts, particularly herbivores. The majority of studies examining the effects of tree diversity on herbivores have been conducted on insects (Jactel et al. 2017, 2021) while more research is needed to examine the influence of forest diversity, in terms of tree species composition or richness, on mammalian herbivores (but see Milligan and Koricheva, 2013) to better understand the ecology of tick-borne diseases.

Nevertheless, our results provide new empirical evidence that the risk of becoming infected with tick-borne diseases is lower in more diverse forest stands, particularly mixed conifer – broadleaved forests. It would be interesting to complement these results by studying human populations and their pets that frequent forests to see if their behaviour is influenced by the species composition of trees and understory plants, altering their exposure to Lyme disease risk.

If confirmed by additional research, the process of dilution of tick-borne pathogen load through diversification of vertebrate host habitat and resources would provide a useful theoretical foundation for shifting forest management toward greater plant species diversity. The diversification of forests to better regulate the health risk associated with ticks while preserving biodiversity would then represent a new example of a Nature-based Solution.

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4.6. SUPPLEMENTARY MATERIALS

Supplementary Material 1

Lower prevalence of Lyme disease pathogens in mixed deciduous – coniferous forests - Audrey Bourdin, Katarzyna Stojek, Bogdan Jaroszewicz, Damien Bonal, Helge Bruelheide, Clémence Galon, Loïc Gillerot, Yasmine Kadiri, Violette Maugis, Sara Moutailler, Bart Muys, Quentin Ponette, Michael Scherer-Lorenzen, Elena Valdes-Correcher, Kris Verheyen, Hervé Jactel – *in prep*.

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Supplementary Material 2

Lower prevalence of Lyme disease pathogens in mixed deciduous – coniferous forests - Audrey Bourdin, Katarzyna Stojek, Bogdan Jaroszewicz, Damien Bonal, Helge Bruelheide, Clémence Galon, Loïc Gillerot, Yasmine Kadiri, Violette Maugis, Sara Moutailler, Bart Muys, Quentin Ponette, Michael Scherer-Lorenzen, Elena Valdes-Correcher, Kris Verheyen, Hervé Jactel – *in prep*.

Table S1 : Complete list of pathogens associated with *I. ricinus* nymphs detected.

Pathogen	**Total number of infected nymphs**
B. burgdorferi s.s.	8
B. garinii	38
B. afzelii	67
B. valaisiana	31
B. lusitaniae	12
B. spielmanii	3
B. miyamotoi	19
Anaplasma phagocytophilum	54
Ehrlichia spp	20
Neoehrlichia mikurensis	20
Rickettsia spp.	163
Apicomplexa	16
Babesia spp.	15

Table S2 : Primers used for the detection of pathogen species via PCR.

Pathogens	**Primer name**	**Primer sequence (5'-3')**	**Tm (°C)**	**Fragment length (bp)**	**References**
Borrelia s.l.	FlaB280F	GCAGTTCARTCAGGTAACGG	55	645	Loh et al. 2016
	FlaRL	GCAATCATAGCCATTGCAGATTGT			
	FlaB_737F	GCATCAACTGTRGTTGTAACATTAACAGG	59	407	
	FlaLL	ACATATTCAGATGCAGACAGAGGT			
Rickettsia spp.	Rsfg877	GGG GGC CTG CTC ACG GCG G	69	381	Regnery et al., 1991
	Rsfg1258	ATT GCA AAA AGT ACA GTG AAC	52		
Babesia spp., *Theileria* spp., *Hepatozoon* spp.	BTH 18S 1st F	GTGAAACTGCGAATGGCTCATTAC	58	1500	Masatani et al., 2017
	BTH 18S 1st R	AAGTGATAAGGTTCACAAAACTTCCC			
	BTH 18S 2nd F	GGCTCATTACAACAGTTATAGTTTATTTG			
	BTH 18S 2nd R	CGGTCCGAATAATTCACCGGAT			
Anaplasma spp., *Erlhichia* spp.	EHR1	GAACGAACGCTGGCGGCAAGC	60	ND/693	Rar et al. 2005
	EHR2	AGTA(T/C)CG(A/G)ACCAGATAGCCGC			
	EHR3	TGCATAGGAATCTACCTAGTAG	55	629/592	

Table S3 : Table presenting all the *p*-values of the univariate tests for the selection of variables.

	DOL	**DON**	**DOA**	**NIP_{Bor}**	**NIP_{Rick}**
Tree species richness	0,7	0,73	0,25	0,37	0,1
Tree composition	0,14	0,42	0,3	0,3	0,22
Understorey cover	0,38	**0,01***	0,13	0,3	0,62
Understorey richness	0,5	0,5	0,08	**0,01***	**0,0002*****
Proportion of roe deer palatable plants	0,1	0,2	0,9	0,7	**0,007****
Saturation Deficit	0,25	0,7	0,6	0,81	0,4
Tree composition × Borrelia species				**0.009***	
DOL		**0,0003 *****			
DON			0,2		
DOA	**0,01***				

Supplementary Material 3

Lower prevalence of Lyme disease pathogens in mixed deciduous – coniferous forests - Audrey Bourdin, Katarzyna Stojek, Bogdan Jaroszewicz, Damien Bonal, Helge Bruelheide, Clémence Galon, Loïc Gillerot, Yasmine Kadiri, Violette Maugis, Sara Moutailler, Bart Muys, Quentin Ponette, Michael Scherer-Lorenzen, Elena Valdes-Correcher, Kris Verheyen, Hervé Jactel – *in prep*.

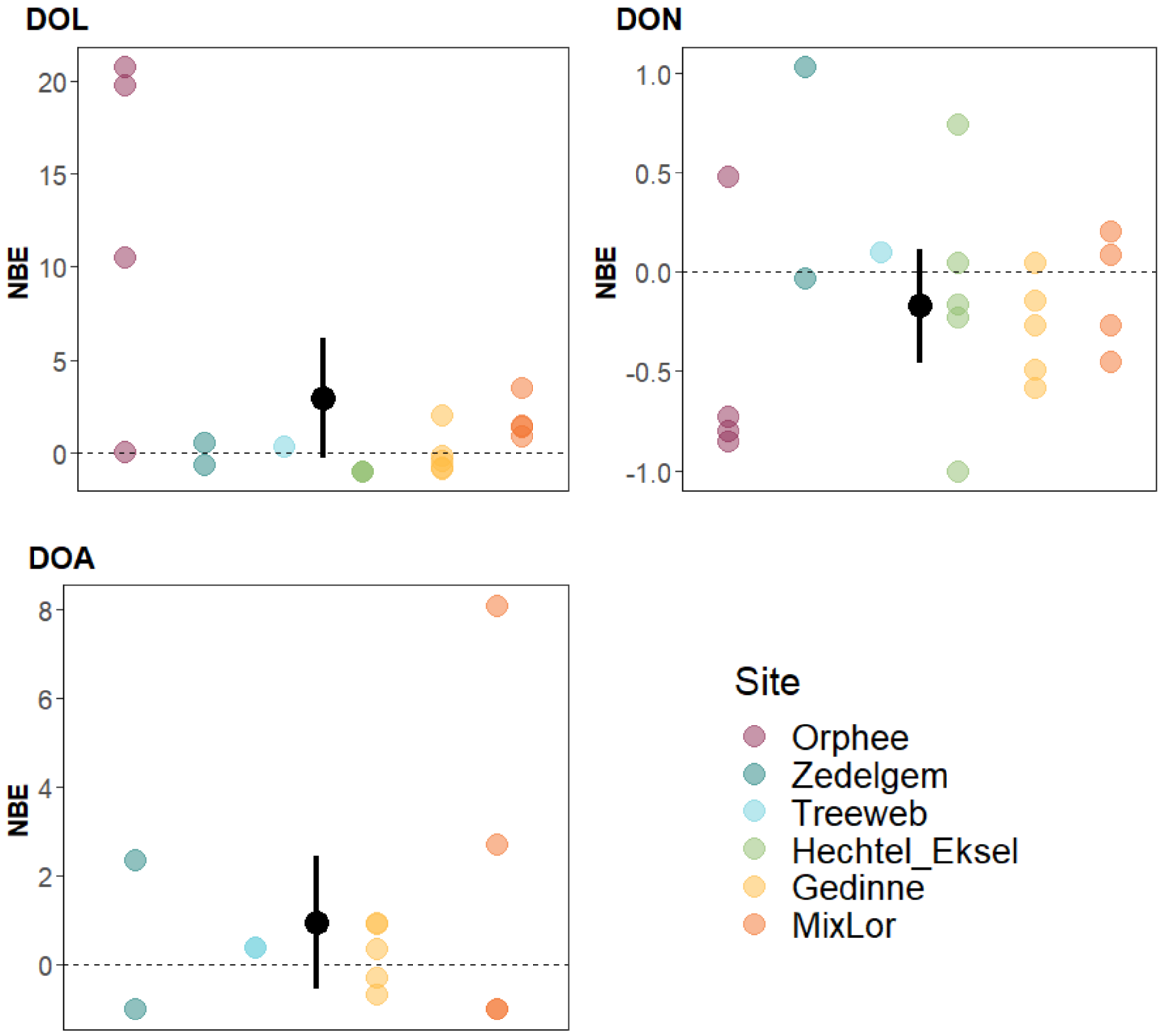

Fig. S1 Net Biodiversity Effect (NBE) calculated with DOL, DON and DOA

5. Discussion générale

Dans le contexte actuel de changements globaux et de changement d’utilisation des terres, les activités de déforestation et/ou de reboisement ont été intimement liées à des modifications de l'incidence et de la distribution géographique des tiques, des hôtes, des agents pathogènes et des maladies humaines impliquées (Ginsberg et al. 2020, VanAcker et al., 2019). Ces perturbations affectent la diversité et la composition des communautés d'hôtes et de non-hôtes et leur dynamique d’assemblage au fil du temps (Rhor et al., 2020). Les assemblages substitutifs ou additifs d'hôtes et de non-hôtes affectent les populations de vecteurs, la prévalence et la transmission des agents pathogènes, et peuvent entraîner une modification du risque (dilution, amplification, effet neutre) (Halliday et al., 2017). Bien que l'hypothèse de l'effet de dilution appliquée aux maladies transmises par les tiques se concentre principalement sur la diversité des hôtes, les trois chapitres de cette thèse s’insèrent dans l’étude de l’effet diluant ou amplificateur potentiel de l’habitat de ces hôtes sur la prévalence des pathogènes associés aux tiques, soutenu par de récentes études européennes (Erhmann et al., 2017, 2018). En Europe, les tiques constituent une menace importante pour la santé humaine dans les forêts et il est important aujourd’hui d'approfondir les recherches sur l'influence de la gestion des forêts et des paysages sur les tiques et pathogènes associés afin d’établir des plans de gestions optimaux minimisant les risques pour la population.

Par le biais de synthèse de la littérature et d’expériences empiriques le long de sites forestiers spécifiquement conçus pour étudier les effets de la biodiversité sur le fonctionnement des écosystèmes, nous avons mis en évidence que la forêt représentait un puit d’abondance de tiques au sein du paysage, et que la composition, la structure et la diversité des différents étages forestiers pouvaient affecter l'abondance des tiques et la prévalence des pathogènes associés.

5.1 : LA FORET: UNE COMBINAISON DE FACTEURS FAVORABLES AUX TIQUES

Les résultats de notre premier chapitre montrent que les forêts (en climat tempéré) représentent l'habitat abritant les plus grandes densités de tiques du genre *Ixodes* au sein d'un paysage hétérogène. La qualité d'ombrage et l'humidité permises par la canopée des arbres et la structure du sous-bois, ainsi que la fréquentation de nombreux hôtes favorisent la survie et la multiplication des tiques en les protégeant de la dessiccation à laquelle elles sont sensibles (Gray, 2008) et en fournissant des repas de sang. À cet égard, certains chercheurs pensent que l'expansion des forêts dans d'anciennes zones agricoles, ou la revégétalisation des environnements urbains, ont été et continuent d'être des moteurs importants des maladies transmises par les tiques (Pfäffle et al., 2013 ; Wood & Lafferty, 2013; VanAcker et al., 2019). Un habitat où l'abondance des tiques est généralement élevée, peut abriter un nombre considérable de tiques infectées même avec une prévalence modérée ou faible de pathogènes associés. La forêt, si elle est visitée, peut ainsi représenter un habitat à risque élevé de morsures potentiellement dangereuses pour les promeneurs. Cela peut également avoir un impact à l'échelle du paysage et des études montrent que les forêts jouent un rôle dans le maintien et la présence de tiques dans les habitats adjacents via la dynamique des communautés d'hôtes vertébrés dont les niches écologiques croisent différents biomes (Boyard et al., 2008). Les lisières entourant les parcelles de forêt sont connues pour leur abondance accrue d'hôtes vertébrés en raison des conditions idéales de reproduction ou d'alimentation (Zohdy et al., 2019). Cela crée de nouvelles opportunités pour ces organismes d'agir comme hôtes relais, en route pour mobiliser les agents pathogènes présents dans les habitats fragmentés vers les animaux domestiques et les humains occupant les habitats environnants (Gibb et al., 2020). Estrada-Peña (2003) montre par exemple que des habitats aux conditions abiotiques peu favorables (e.g. prairies) portent d'autant plus de tiques lorsque ceux-ci présentent une forte connectivité avec des parcelles à haute densité de tiques (i.e. zones boisées). Cela signifie que les densités de tiques au sein de la forêt conditionnent également l'infestation à plus large échelle dans le paysage. Nous savons que le risque réel d'être mordu par une tique est en fait la combinaison de trois composantes: l'aléa (la densité de tiques ou de tique infectées dans l’environnement), l’exposition (le degré de fréquentation de cette zone par l’humain) et la vulnérabilité (les comportements de protections individuelles). Certains auteurs pensent ainsi que les bordures de forêt constituent les habitats au plus grand risque car elles combinent de relativement grandes abondances de tiques (Vacek et al., 2023), globalement une plus forte exposition globale de la population humaine (habitations à l’orée de bois, sentiers de promenade plus accessibles…) qui y adopte moins de comportements de protection (vêtements couvrants, répulsifs) (Due et al., 2013).

Finalement, les risques au sein de la forêt et à sa périphérie semblent être tous deux directement liés aux abondances de tiques qu’elle héberge. Il est important maintenant de comprendre si des variables forestières impactent les densités de tiques, lesquelles et comment, afin d’établir au mieux des plans de gestion des forêts minimisant les risques relatifs aux tiques pour la population humaine.

5.2 BIODIVERSITE DES FORETS: DILUTION OU AMPLIFICATION DU RISQUE ?

En plus de montrer que la forêt représentait l’habitat avec les plus grandes abondances de tiques comparée aux milieux ouverts et urbains d’un même paysage, notre chapitre 1 a également mis en évidence que les différences d’abondance de tiques (entre forêt vs non-forêt) pouvaient être plus ou moins grandes selon la composition en arbre de la forêt comparée (Chapitre 1). Les différences d’abondances de tiques entre la forêt et l’habitat non-forestier, étaient plus grandes lorsque la forêt était composée de mélanges décidus et conifères, plutôt qu’uniquement décidus. Bien que ce résultat ne compare pas directement les abondances de tiques entre ces deux compositions forestières, il constitue un premier indice révélateur d’un potentiel effet de la composition forestière sur les tiques. Les expériences de terrain des chapitres 2 et 3, ont ensuite permis de mettre en lumière différentes relations entre la diversité forestière et *I. ricinus* et ses taux d’infection. Les larves étaient impactées par une composition spécifique en arbres ou en sous-bois, alors que l’abondance des nymphes et des adultes était plutôt expliquée par la richesse en arbre et la structure (i.e. densité en arbres) de la parcelle (Fig. 1). Ces divergences peuvent résulter des différences biologiques et l'écologiques inhérentes aux différents stades de la tique *I. ricinus*.

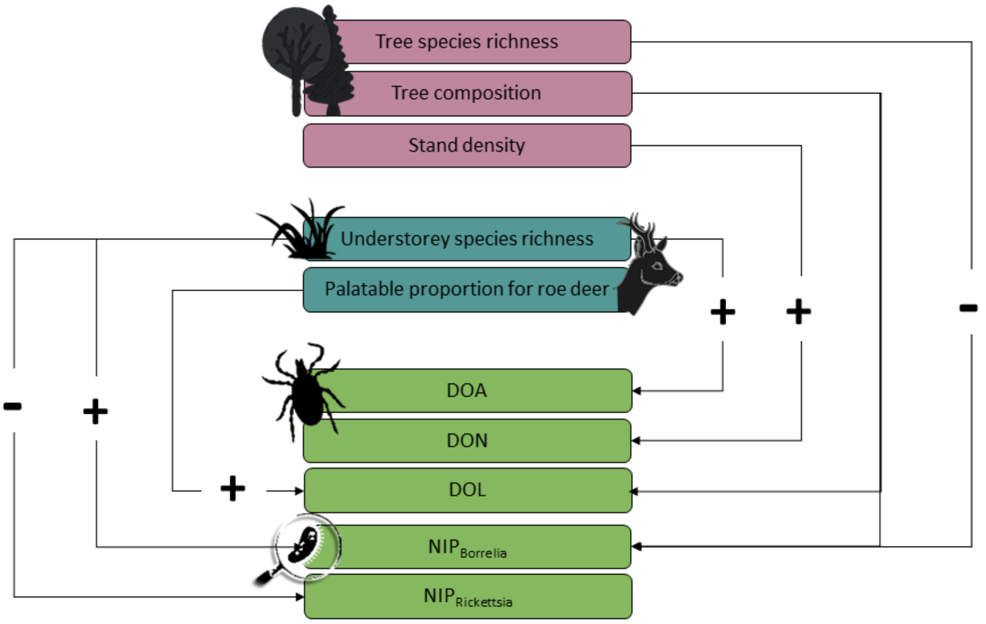

Fig. 1: Schéma explicatif montrant les résultats de la thèse au niveau du peuplement forestier. La diversité des arbres a un effet négatif sur la prévalence de *B. burgdorferi* s.l. qui est la plus faible dans les parcelles mixtes décidues-conifères. Les densités de larves étaient plus importantes dans les parcelles de conifères et les abondances de nymphes étaient favorisées par la densité en arbre de la parcelle. La richesse en plantes de sous-bois a un impact positif sur la densité d'adultes et la prévalence de *B. burgdorferi* s.l. associées aux nymphes. La proportion de plantes appétentes pour le chevreuil a un impact positif sur la densité des larves. DON augmente avec DOL. Les flèches noires représentent les effets significatifs avec leurs signes.

5.2.1 Effets diluants de la diversité forestière

Nos résultats suggèrent que la richesse et la composition en arbres mais aussi la richesse du sous-bois peuvent avoir des effets diluants sur le risque associé aux tiques pour la santé humaine en forêt (Fig. 4). Particulièrement, nous avons trouvé des prévalences plus faibles de *B. burgdorferi* s.l. associées aux nymphes de *I. ricinus* dans les forêts mélangées que dans les forêts pures. Ce résultat était surtout marqué pour les compositions feuillus-conifères (et moins pour les mélanges de feuillus), ce qui semble révéler l'importance non négligeable de l'identité des espèces dans l'effet du mélange. Nous avons également mis en évidence une diminution de la prévalence de *Rickettsia* spp (i.e. bactéries responsables de diverses fièvres boutonneuses, Fournier & Raoult, (2009); Merhej & Raoult, (2011)) avec l'augmentation de la richesse en plantes du sous-bois.

La dilution de la prévalence de pathogènes se met en place lorsqu'un ratio entre le nombre de tiques s'alimentant sur des hôtes incompétents et celles se nourrissant sur des hôtes compétents augmente. Cette augmentation du ratio peut intervenir lorsque les assemblages des communautés d'hôtes favorisent les hôtes incompétents (Wood & Lafferty, 2013 ; LoGiudice, *et al. 2003*) et/ou défavorisent les plus compétents via par exemple des processus de compétition, ou de prédation entre espèces, ou liés aux niches écologiques dépendantes des caractéristiques végétales ou microclimatiques de l'habitat. Bien que l'étude de la diversité de l'habitat sur les risques liés aux tiques soit encore balbutiante, Ehrmann et al. (2018), ont auparavant trouvé une dilution des borrélies dans les forêts plus diversifiées en terme d'espèces d'arbres et de sous-bois.

Si l'hypothèse de l'effet de dilution (Halliday et al., 2020 ; Schmidt et Ostfeld, 2001) suggère que la richesse en espèces hôtes jouerait un rôle de réduction dans la propagation des agents pathogènes parce que certaines espèces ne permettraient pas la multiplication des virus ou des bactéries (réservoir de faible compétence), ce résultat reste mal démontré en Europe (Esser et al., 2022). Cela peut s'expliquer par le fait que la densité d'hôtes compétents ne diminue pas toujours suffisamment avec l'augmentation de la richesse globale de la communauté de vertébrés pour réduire la densité des nymphes infectées. De plus, les tiques peuvent présenter une forte préférence pour les hôtes compétents (Randolph et Dobson, 2012). Des études empiriques et des méta-analyses montrent de plus grandes diversités faunistiques dans les forêts mixtes que dans les forêts pures (e.g. Ampoorter et al., 2020, Stemmelen et al., 2021), mais si une richesse globale des hôtes n'est pas systématiquement associée à une dilution des pathogènes associés aux tiques, cela signifie que la diversité forestière agit probablement en induisant une richesse " orientée " en faveur des hôtes incompétents et/ou en défaveur des hôtes compétents pour les borrélies. Vehviläinen and Koricheva (2006) ont par exemple montré que la diversité des forêts impactait positivement l'activité de certains cervidés (e.g. élans) et diminuait l'activité de petits mammifères (e.g. campagnols). Dans l'étude de Cook-Patton et al. (2014), les parcelles mixtes étaient deux fois plus susceptibles d'être visitées et broutées par des cerfs de Virginie que les monocultures. Une augmentation de l'activité des ongulés sauvages (incompétents pour les borrélies) et la diminution de l'activité ou fréquentation de petits mammifères (compétents pour les borrélies) dans les parcelles mixtes pourrait expliquer nos résultats.

Concernant les *Rickettsies*, peu de connaissances sont encore solides concernant le degré relatif de compétence ou de transmission à *I. ricinus* de différentes espèces hôtes. Nous savons que certaines Rickettsies peuvent être portées par des oiseaux (Spitalska et al. 2011), des rongeurs (Schex et al., 2011) et en moindre proportion par des ongulés sauvages (Wijbur et al., 2022). D'après nos résultats, la dilution des Rickettsies a lieu à l'échelle du sous-bois et non des arbres contrairement aux borrélies. Cela suggère que l'écologie des *Rickettsia* serait plus intimement liée à des hôtes sensibles aux changements de biodiversité de la végétation du sous-bois (e.g. petits mammifères, Melo et al., 2013) plutôt que de la strate arborée (contrairement aux borrélies).

5.2.2 Effet amplificateur de la diversité forestière

D'après nos études, si des caractéristiques de la diversité forestière participaient à diluer le risque local lié aux tiques, certaines au contraire le favorisaient. Les parcelles de conifères et la proportion de plantes appétentes pour le chevreuil favorisaient les densités de larves, les densités de nymphes étaient favorisées par la densité en arbres de la parcelle et la richesse en plantes du sous-bois était positivement corrélée à de plus grandes abondances de tiques adultes et de proportion de nymphes infectées par *Borrelia* spp..

Un microclimat favorable et une forte abondance d'hôtes peuvent être, localement, des variables amplificatrices des densités de tiques (Gray, 2008 ; Wongnak et al., 2022), et le ratio hôtes/non-hôtes présent détermine théoriquement la prévalence des pathogènes associés (Rohr et al., 2020).

Nous avons proposé que la qualité d'ombrage et l'épaisseur de la litière d'aiguilles dans les parcelles de pin ont pu favoriser la survie des larves près du sol (Chapitre 2). Les parcelles à forte couverture de plantes appétentes peuvent représenter des zones où les chevreuils passent plus de temps (Charnov, 1976) avec donc plus de chances de déposer des femelles adultes qui finiront par pondre des œufs et produire des larves (Chapitre 3). L'abondance des nymphes, en quête plus haut dans la végétation pourrait être plutôt expliquée par un microclimat favorable à mi-hauteur entre canopée et sous-bois, d'où l'effet amplificateur de la densité en arbres qui influence la qualité d'ombrage (Williams et al. 2017). La densité des adultes, moins sensibles à la dessiccation, semblait être plus expliquée par des variables liées aux hôtes (richesse du sous-bois) qu'au microclimat. Enfin, parce qu'elles favorisaient la prévalence de *Borrelia* spp. associée aux nymphes, les parcelles à haute richesse floristique dans le sous-bois représentaient possiblement des lieux de choix pour les hôtes amplificateurs de *borrélies* comme certains rongeurs (Ostfeld et al., 2006) et/ou favorisaient peu les hôtes diluants. Cependant, les cervidés théoriquement « diluants » des *borrélies*, sont des hôtes minoritaires pour l'alimentation des larves lorsqu'il y a abondance d'hôtes « préférés » (e.g. rongeurs) (Hofmeester et al., 2016). Par conséquent, une dilution des taux d'infestation des nymphes en borrélies devrait plutôt intervenir à la suite d'une perte en hôtes compétents dans la communauté, plutôt qu'à l'ajout seul d'hôtes incompétents. Mais d'autres études sont nécessaires pour comprendre quels hôtes peuvent être qualifiés d'amplificateurs de *borrélies* ou d'autres pathogènes associés à *I. ricinus*.

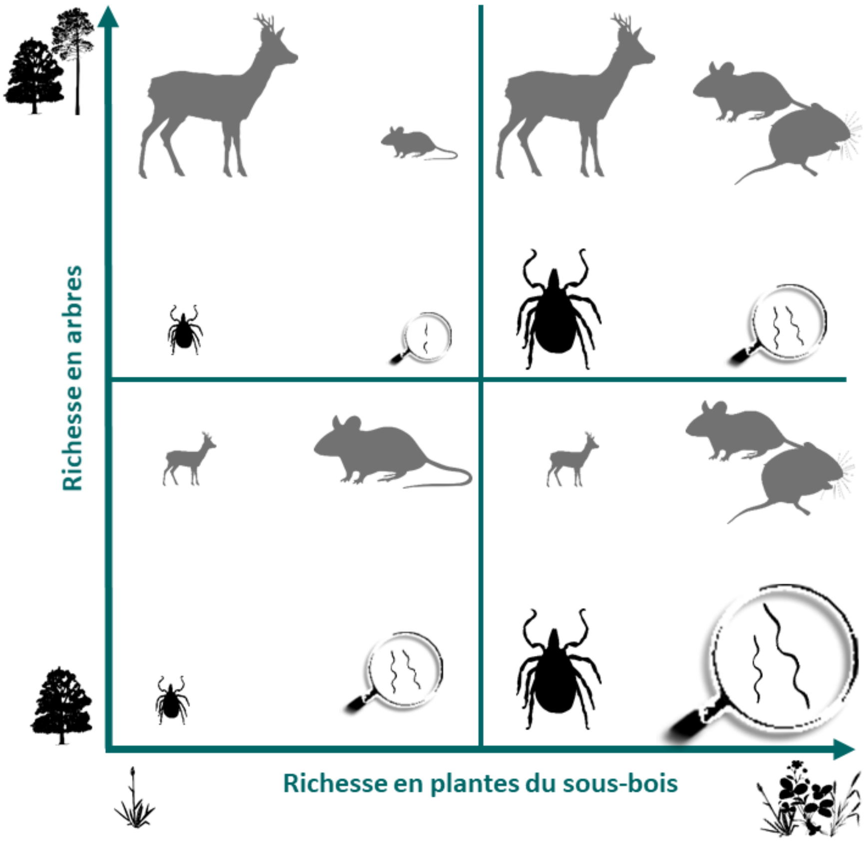

Fig. 2. Proposition de relations entre la richesse en arbre et la richesse en plantes du sous-bois, les hôtes compétents et incompétents, les densités de tiques *I. ricinus* et les taux d'infection pour *Borrelia* associés. En noir, nos résultats obtenus le long de gradient de diversité en arbres et en plantes du sous-bois (Chapitres 2 et 3) : 🕷 : densités de tiques *I. ricinus* ; 🔍 : prévalence de *Borrelia* associées aux nymphes *I. ricinus*. En gris, nos hypothèses pour expliquer nos résultats : 🦌 : proportion de tiques nourries sur un hôte incompétent (i.e. cervidé) pour *Borrelia* ; 🐁 : proportion de tiques nourries sur un hôte compétent (i.e. rongeurs) pour *Borrelia*. Lorsque les richesses en arbre et en sous-bois sont faibles l'activité des cervidés est faible mais l'activité des rongeurs est importante (Milligan & Koricheva, 2013 ; Cook-Patton et al., 2014), ce qui induirait de plus grandes proportions de tiques nourries sur les rongeurs et une faible dilution des borrélies associées aux tiques. Lorsque la richesse spécifique en arbre est importante, l'activité des cervidés augmente se traduisant par un plus grand effet de dilution et donc la diminution de la prévalence des borrélies (Chapitres 2 et 3). Une augmentation de la richesse du sous-bois impliquait de plus fortes prévalences de borrélies associées aux nymphes (Chapitre 3), ce qui pourrait s'expliquer par une plus grande activité et ou richesse d'hôtes compétents (i.e. rongeurs). Les parcelles à faible richesse en arbre et à forte richesse en plantes du sous-bois représenteraient les environnements les plus à risque car défavoriseraient l'activité des cervidés, favoriseraient la richesse et l'abondance des rongeurs mais aussi les densités de tiques (Chapitre 3).

5.3 PERSPECTIVES ET FUTURES RECHERCHES

Les résultats de cette thèse ont contribué aux connaissances actuelles concernant l'écologie de plusieurs pathogènes associés aux tiques dans un contexte de forêts européennes. Néanmoins, plusieurs questions doivent encore être abordées dans le cadre de recherches ultérieures.

5.3.1 Effets de la diversité forestière sur les tiques : distinguer la part des hôtes, de la végétation et du microclimat

La principale limite a été de ne pas pouvoir clairement distinguer les mécanismes directs et indirects sous-jacents des effets de la diversité forestière sur les variables du risque (Fig. 2). Le lien que nous avons établi entre les plantes appétentes pour le chevreuil et les tiques représente une première observation d'un effet indirect de l'habitat sur les vecteurs via des hôtes spécifiques. Cette caractérisation de variables environnementales ciblant certaines espèces hôtes doit être approfondie et complétée par un suivi plus direct de ces vertébrés le long d'un gradient de diversité forestière. Effectivement, beaucoup d'études ont testé l'impact des mammifères sur la diversité des forêts (e.g. Stutz et al., 2014), mais à notre connaissance, beaucoup moins l'inverse (mais voir Milligan & Koricheva, 2013). Plusieurs études ont par contre analysé cela sur les oiseaux (e.g. Charbonnier et al., 2016).

Tout d'abord, il serait intéressant de travailler sur un gradient de richesse spécifique en arbres plus étendu, qui nous permettrait de vérifier la forme linéaire de la relation entre diversité de l'habitat forestier et le risque causé par les tiques. En plus d'échantillonnages de tiques avec la méthode du drap (MacLeod, 1932), un suivi multi-taxonomique des hôtes le long de ce gradient serait intéressant à mettre en place, avec en priorité, le suivi des chevreuils et des rongeurs.

Bien que les techniques de Capture-Marquage-Recapture ou bien d'indice kilométriques semblent être les plus fiables pour la détermination des effectifs de cervidés (Morellet, 2008), les résultats qu'ils confèrent sont plutôt à large échelle. Ainsi, la mise en place de nombreux pièges photographiques/vidéo pourrait être plus pertinente pour comparer des abondances et temps de fréquentation plus localisées entre parcelles forestières plus ou moins diversifiées. Une capture des rongeurs pourrait aussi se réaliser à l'échelle locale (de la parcelle) à l'aide de pièges (e.g. pièges avec boîtes-dortoirs), complétée par la détermination de leur charge en tiques (particulièrement les larves) ainsi que leur teneur en pathogènes via des prélèvements sanguins (Chastagner et al., 2016). Avec un suivi à long terme du microclimat et une caractérisation de la végétation du sous-bois, cette expérience pourrait nous permettre de caractériser des habitats plus ou moins favorables aux différents types d'hôtes pour révéler les mécanismes sous-jacents de l'effet de la diversité forestière sur les tiques libres et les prévalences en pathogènes associées en distinguant l'effet médié par les hôtes, le microclimat ou la végétation du sous-bois.

5.3.2 Lier des données de couverture et de diversité forestière avec les piqûres signalées sur les humains

De plus, il serait intéressant de coupler ces résultats avec un suivi de l'exposition de la population humaine au cœur de forêts plus ou moins diversifiées afin de quantifier le réel risque posé par ces environnements. Par exemple, Garcia-Marti et al. (2017) montraient que les facteurs liés à l'activité humaine étaient plus pertinents que le microclimat ou la végétation pour modéliser les piqûres de tiques. Plus tard, Vanwambeke & Schimit (2021) trouvaient que les forêts représentaient bel et bien des habitats à haut risque, mais que la combinaison de tous les autres habitats comparés comptait le même nombre total de piqûres de tiques. Des outils numériques nationaux de science participative (i.e. applications smartphones) sont d'ores et déjà mis en place pour permettre aux citoyens de déclarer les piqûres de tiques dont ils ont été victimes mais aussi de les faire parvenir aux laboratoires impliqués, pour l'analyse des pathogènes associés (e.g. programme CiTIQUE en France, www.citique.fr). Il pourrait être très intéressant de croiser les données collectées (de piqûres et de prévalences de pathogènes) géolocalisées avec des données de diversité forestière (e.g. cartes IGN, CORINE land cover, voire télédétection à partir d'images satellites). En établissant ainsi une cartographie des taux de piqûres et taux d'infection en forêt et idéalement les mettre en lien avec leur composition ou diversité en arbres, cette étude permettrait de lier directement les proxys du risque trouvés dans notre thèse, avec les réels taux de piqûres sur la population.

5.3.3 Recommandations pour la gestion des forêts

Nos résultats confirment le risque généralement plus élevé d'être infecté par des maladies transmises par les tiques dans les forêts non diversifiées, ombragées, humides et denses pendant la période d'activité des tiques. Les forêts mixtes feuillus-conifères abritaient les plus faibles prévalences de borrélies, ce qui représente un critère important à prendre en compte dans la conception de nouvelles plantations mélangées. En plus de cela, il serait important de conseiller d'entretenir des sentiers « propres » pour l'accueil du public en ramassant la litière où se situent les pontes. Egalement, il reste indispensable de sensibiliser les promeneurs au risque représenté par les forêts et favoriser l'adoption de protections individuelles telles que les vêtements couvrants ou répulsifs qui s'avèrent être très efficaces pour réduire le risque de morsures d'après d'autres études (Due et al., 2013).

5.4 CONCLUSIONS

Finalement, les résultats de cette thèse montrent que les habitats forestiers peuvent avoir des caractéristiques " diluantes " ou " amplificatrices " du risque lié aux tiques. Ces concepts semblent prometteurs pour comprendre les processus épidémiologiques au sein de la forêt et traduire en action la grande quantité de recherches déjà effectuées pour, in-fine, parvenir à réduire le risque sanitaire causé par les tiques. La complexité de l'écologie des maladies à tiques, due notamment au caractère généraliste des vecteurs, la multitude d'hôtes potentiels aux compétences diverses et à la diversité des pathogènes, mène à préférer des approches interdisciplinaires pour leur gestion (One Health approach, voir Dantas-Torres et al. (2012)), nécessitant une communication accrue entre différents domaines tels que l'écologie, la zoologie, l'épidémiologie, la biologie moléculaire, les sciences médicales et vétérinaires, les études climatologiques et sociales et la foresterie. Globalement, nos résultats suggèrent que les "habitats de dilution" sont caractérisés par une grande diversité compositionnelle de la strate arborée, avec probablement des effets sur la composition et l'abondance des espèces hôtes. S’ils sont étayés par des recherches supplémentaires, cette perspective ouvrirait des possibilités de contrôle actif des tiques réduisant leur survie et/ou diminuant les taux d’infection par une gestion forestière adaptée.

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