UNIVERSITÉ DU QUÉBEC À MONTRÉAL

CONSÉQUENCES ÉCOLOGIQUES DU MICROBIOME ET DU COMPORTEMENT CHEZ DEUX RONGEURS SYMPATRIQUES, LA SOURIS SYLVESTRE (PEROMYSCUS MANICULATUS) ET LE CAMPAGNOL À DOS ROUX (MYODES GAPPERI)

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JOËL W. JAMESON

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RÉSUMÉ

La communauté de bactéries qui occupe le système digestif d'un hôte joue un rôle important pour la santé et le métabolisme de l'hôte. Une vague récente d'études démontre une relation entre ce microbiome et de nombreux traits liés à l'hôte tels que l'âge, le sexe, la morphométrie, le génotype et l'habitat. Contrairement à la nouveauté de l'étude du microbiome, les chercheurs étudient depuis longtemps le comportement animal et les facteurs à l'origine de ce comportement. Des études récentes sur des souris et des rats en laboratoire révèlent une capacité du microbiome à moduler les comportements d'exploration, de sociabilité et d'anxiété de l'hôte. En milieu naturel, les individus d'une population peuvent différer les uns des autres en fonction de ces comportements, mais l'existence d'un lien entre le comportement et le microbiome intestinal n'a toujours pas été testée en milieu naturel. Étant donné les nombreux autres facteurs écologiques à diverses échelles pouvant aussi interagir avec l'hôte et son microbiome, seule une étude en milieu naturel permettrait une compréhension des conséquences écologiques et évolutives de la relation entre le microbiome et le comportement.

Dans cette thèse, je me penche sur la relation entre le microbiome intestinal et les comportements d'exploration, d'agressivité, d'anxiété et de sociabilité chez une métapopulation de souris sylvestres (*Peromyscus maniculatus*) et de campagnols à dos roux (*Myodes gapperi*). J'évalue premièrement la relation entre le microbiome de l'hôte et de multiples facteurs écologiques, dont le comportement, au niveau de l'hôte et du paysage. Je démontre que les associations entre les traits relatifs à l'hôte et son microbiome dépendent de l'échelle spatiale et de l'espèce. Je détecte un lien chez les deux espèces d'hôtes entre l'agressivité et le microbiome (composition taxonomique du microbiome bactérien) à un niveau intrapopulationnel. En revanche, entre les populations, le microbiome est plutôt lié au génotype des souris, et à la diversité et à l'isolement de la population et à l'habitat des campagnols. J'évalue ensuite l'effet direct du microbiome sur le comportement des souris et des campagnols en milieu naturel et ses conséquences pour les traits d'histoire de vie de l'hôte. A la suite d'une modification du microbiome par un antibiotique, j'observe une influence du microbiome sur le comportement d'exploration et une réduction du domaine vital des souris, ainsi qu'une réduction importante de la survie des campagnols. Finalement, pour mieux comprendre l'effet que peut avoir la dispersion de bactéries d'un hôte à

l'autre, j'examine l'effet de transferts potentiels entre deux hôtes de même espèce et d'espèces différentes. Je démontre un lien entre la composition du microbiome et l'importance des liens sociaux entre les individus d'une même espèce pour les campagnols, mais ce lien reste à être confirmé pour les souris. Je démontre aussi que la composition du microbiome de deux individus d'espèces différentes peut être liée à la probabilité qu'ils se rencontrent.

Cette thèse offre un aperçu écologique du lien entre le microbiome intestinal et le comportement et contribue à élucider ses conséquences écologiques et évolutives.

CHAPITRE I

1.1 Le microbiome, nouvelle frontière en écologie

Il n'y a pas si longtemps, la présence d'une bactérie dans un échantillon se confirmait seulement en la cultivant sur la surface d'un milieu gélosé. L'arrivée récente des techniques de séquençage à haut débit a permis de découvrir l'existence d'une diversité impressionnante de micro-organismes occupant tout être vivant. L'ensemble des micro-organismes occupant un hôte est désigné son microbiome, et la composante bactérienne du microbiome est la plus étudiée. Ces bactéries peuvent être pathogènes, symbiotiques ou commensales (Hooper et Gordon, 2001) et ces dernières peuvent apporter à l'hôte plusieurs bénéfices adaptatifs. Par exemple, certains organismes sont les hôtes de bactéries qui produisent des structures physiques et des composés odorants importants à la sélection sexuelle et à la communication de leur hôte (Hooper & Gordon, 2001 ; Archie & Theis, 2011 ; Ezenwa et al., 2012).

Le nombre d'études sur le microbiome augmente à un rythme exponentiel et la plupart de ces études ciblent le microbiome gastro-intestinal, en grande partie en raison du rôle de plus en plus évident qu'il joue dans la santé humaine. On trouve les plus grandes densité et diversité de bactéries de l'hôte dans le système gastro-intestinal (Donaldson et al., 2016). Ce microbiome est actif, entre autres, dans la protection immunitaire, l'absorption de nutriments (Hooper et Gordon, 2001), la régulation de sérotonine (Ridaura et Belkaid, 2015) et la production d'acide gras à chaîne courte qui peuvent affecter la motilité et l'homéostasie intestinale et le système nerveux sympathique (Nicholson *et al.*, 2012). Le microbiome pourrait même influencer des traits morphologiques de l'hôte comme la taille et la masse, en partie par son influence sur les hormones et les fonctions métaboliques (Dominianni *et al.*, 2015 ; Goodrich *et al.*,

2014). Plusieurs pathologies ou maladies du système gastro-intestinal chez les humains sont aussi liées à une dysbiose (une déstabilisation de la communauté bactérienne) du microbiome comme la maladie de Crohn (Joossens *et al.*, 2011), les maladies inflammatoires intestinales (Shim, 2013) et le syndrome du côlon irritable (Kennedy *et al.*, 2014). Étant donné le lien entre le microbiome et les multiples traits phénotypiques de l'hôte, on peut supposer que celui-ci devrait avoir de fortes conséquences écologiques et évolutives, mais ces conséquences demeurent très peu étudiées.

1.2 Le microbiome et les concepts de l'écologie des communautés

Pour tenter de comprendre les facteurs qui influencent la composition taxonomique du microbiome, les écologistes font des parallèles entre les réseaux de microbiomes et une métacommunauté. Tout comme une métacommunauté représente un réseau de communautés connectées par la dispersion de ses habitants et où les espèces peuvent potentiellement interagir (Wilson, 1992), le microbiome d'une population hôte représenterait une métacommunauté. Ainsi, les hôtes représenteraient des îles ou des parcelles d'habitats entre lesquels la dispersion de bactéries d'un hôte à l'autre est limitée (Costello et al., 2012). En traitant le microbiome d'un hôte comme faisant partie d'une métacommunauté, il est possible d'utiliser les théories de métacommunautés et de dynamiques des populations développées au cours des 100 derniers ans. Le triage des espèces et les effets neutres et de masses semblent les paradigmes de ces théories qui semblent le mieux expliquer la dynamique du microbiome gastro-intestinal.

1.2.1 Le triage des espèces microbiennes par l'hôte

Le triage des espèces décrit un système où chaque parcelle d'habitat représente une niche différente. Chaque espèce est spécialisée dans la colonisation d'une niche particulière. L'espèce la plus spécialisée dans l'utilisation d'une ressource exclura les autres espèces compétitionnant pour cette ressource (Leibold & Chase, 2018). Tout comme l'habitat d'une aire de forêt peut trier les espèces qui s'y trouvent, l'environnement dans le système gastro-intestinal peut aussi mener à la sélection des espèces qui y habitent. La diète peut influencer de manière importante les ressources alimentaires disponibles pour les bactéries dans le système gastro-intestinal et ainsi mener à une sélection de bactéries adaptées à la diète de l'hôte. Un changement de diète chez l'humain passant d'une diète à base de produits végétaux à une diète plutôt carnivore mène à une réduction de la proportion des bactéries appartenant au phylum *Firmicutes*, et à une augmentation de celle de bactéries résistantes à la bile et provenant des phylums *Proteobacteria* et *Bacteroidetes* (David et al., 2014). De tels changements de diète peuvent aussi mener à des changements d'expression de gènes et de spécialisation fonctionnelles (David et al., 2014) ainsi qu'à un changement potentiel dans la richesse des gènes du microbiome (Cotillard *et al.*, 2013). De plus, la consommation de fibres chez l'humain est liée à une abondance plus élevée d'*Actibobacteria* et de *Clostridia* (Dominianni *et al.*, 2015). Les changements de diètes peuvent ainsi être à l'origine des variations dans la composition du microbiome avec le temps et l'âge, et même entre les espèces différentes. Par exemple, le microbiome des souris et des humains varie selon le moment de la journée en raison de l'horaire d'alimentation qui est contrôlé par le cycle circadien (Thaiss et al. 2014), et une analyse de 33 mammifères a révélé une différence entre les espèces carnivores/omnivores et herbivores dans la composition génétique et fonctionnelle de leurs microbiomes (Muegge *et al.*, 2011).

Le système immunitaire de l'hôte exerce aussi une force sélective importante sur son microbiome intestinal et cela peut se faire de deux façons. Premièrement, le système immunitaire empêche les bactéries d'atteindre l'épithélium par un processus

nommé ‘stratification’. La stratification se fait à l’aide d’une barrière de couches de mucus, par la sécrétion d’Immunoglobine A (IgA), et par la sécrétion de peptides et de protéines antimicrobiennes (Hooper et al., 2012). IgA est un anticorps qui se lie aux bactéries dans la muqueuse près de la lumière de l’intestin. Quand une bactérie réussit à pénétrer l’épithélium, elle est souvent phagocytée par un macrophage (Kelsall, 2008), mais elle peut aussi être détectée par des cellules dendritiques qui induisent les cellules B à produire des IgA (Macpherson et Uhr, 2004). Les peptides antibactériens sont produits lorsque des bactéries à la surface de l’épithélium activent des récepteurs de type Toll à la surface de cellules épithéliales. Ceci active, par la suite, la protéine d’intervention primaire de différenciation de myéloïde (MyD88) menant à la production des cytokines pro-inflammatoires et de peptides antibactériens et à la détoxification des lipopolysaccharides produits par les bactéries (Burns *et al.*, 2017 ; Karmarkar et Rock, 2013). Si certaines bactéries sont détruites et empêchées de coloniser l’intestin, d’autres peuvent éviter une attaque par le système immunitaire. Par exemple, le polysaccharide A (PSA) de la bactérie *Bacteroides fragilis* incite les lymphocytes T-régulateurs du système immunitaire de l’hôte à bloquer la réaction pro-inflammatoire normalement induite par un recrutement du lymphocyte T auxiliaire T_H17. Le PSA permet donc à *B. fragilis* de coloniser l’hôte (Hooper et al., 2012 ; Round et al., 2011). Le deuxième mécanisme utilisé par le système immunitaire est un cloisonnement des bactéries commensales à des endroits qui limiteraient le contact entre ces bactéries bénéfiques et le système immunitaire. Ceci se fait spécialement à l’aide de la structure physique du système gastro-intestinal (Hooper et al., 2012).

L’effet de l’immunité sur le microbiome intestinal pourrait être à l’origine des liens entre la composition du microbiome et le génotype de l’hôte. Par exemple, Dimitriu et al. (2013) ont comparé le microbiome intestinal de quatre groupes de souris dont trois avaient des déficiences immunitaires génétiques. Le premier groupe montrait une déficience dans la protéine-tyrosine-phosphatase (CD45) nécessaire à l’activation des cellules T et B. Ces cellules baissent le seuil de signalisation des récepteurs à

antigène. La CD45 régule aussi le déclenchement de la signalisation de récepteurs dans les cellules mast et dans les récepteurs de type Toll (Altin et Sloan, 1997 ; Saunders et Johnson, 2010). Le deuxième groupe montrait une déficience dans les cellules B et T, et le troisième une déficience dans la CD45 et les cellules B et T. Le microbiome différait entre les génotypes de souris et celui des souris normales se distinguait le plus des trois autres. Burns et al. (2017) ont observé des différences de microbiome entre les poissons-zèbres de type wild-type (WT) et des poissons déficients du gène qui encode MyD88. Ces exemples montrent que la manipulation d'un,ou de quelques gènes peut affecter le microbiome de l'hôte, par l'entremise de son effet sur le système immunitaire. Même lorsque le système immunitaire n'a pas été ciblé, des différences de microbiome ont été trouvées pour des lignées différentes de souris WT (Loh *et al.*, 2008) et deux lignées de poules sélectionnées pour leur poids faible ou élevé (Zhao *et al.*, 2013). Dans un contexte écologique, un lien entre la distance génétique et les différences dans la composition du microbiome a été observé pour le microbiome buccal chez la grenouille *Amietia hymenopus* (Griffiths *et al.*, 2018), le microbiome intestinal chez l'humain (Spor *et al.*, 2011b), deux écotypes de guppy *Poecilia reticulata* (Sullam *et al.*, 2015), et chez la souris *Mus musculus* (Suzuki *et al.*, 2019). Malgré ceci, comparé à d'autres facteurs reliés à l'hôte, comme la diète, le génotype a un effet relativement faible sur la composition du microbiome de l'hôte.

À l'échelle évolutive, la distance phylogénique entre les hôtes semble aussi corrélée aux différences de composition du microbiome. En étudiant les microbiomes d'humains et de 59 autres espèces de mammifères, Ley et al. (2008) ont observé de plus fortes différences interspécifiques qu'intraspécifiques. Chez 17 espèces de chauves-souris des néotropiques, les différences de composition du microbiome de la muqueuse intestinale étaient associées au niveau taxonomique de la famille de l'hôte (Ingala *et al.*, 2018). Il est aussi intéressant de noter que, dans cette étude, la composition du microbiome fécal était plus associée aux différences de diète entre les hôtes qu'aux différences phylogéniques indiquant un effet du lieu d'échantillonnage

sur l'importance relative des facteurs liés à la composition du microbiome. Youngblut et al. (2019) ont comparé le microbiome intestinal de 128 espèces représentant huit classes (*Mammalia, Aves, Reptilia, Amphibia, and Actinopterygii*). Ils ont trouvé que la distance phylogénique expliquait 15% de la variance dans la composition du microbiome et, à l'intérieur des mammifères, la présence de certains taxons bactériens dépendait du groupe phylogénique de l'hôte. L'histoire phylogénique de l'hôte joue donc un rôle important dans la composition de son microbiome, potentiellement par l'entremise de différences dans le système immunitaire et la physiologie de l'hôte.

1.2.2 La dispersion des bactéries

Deux autres mécanismes écologiques qui semblent être en jeu dans la constitution du microbiome sont les effets neutres et les effets de masse. Dans le modèle neutre, la colonisation et la disparition d'espèces à l'échelle locale sont contrôlées par des processus stochastiques (Hubbell, 2001 ; Leibold et Chase, 2018). Ce ne sont pas toutes les espèces qui peuvent se rendre à tous les sites, mais une fois rendues, elles ont toutes la même capacité à coloniser le site tant qu'il n'est pas occupé (Leibold & Chase, 2018). Fukami et al. (2007) ont introduit des lignées différentes de la bactérie *Pseudomonas fluorescens* dans un milieu de culture en changeant la séquence d'introduction de chaque lignée à chaque relancement de l'expérience. Ils ont observé que l'ordre d'immigration/colonisation déterminait l'espèce dominante et, par conséquent, les espèces colonisatrices provenant de la diversification. Ce phénomène est souvent nommé "effet du fondateur" puisque c'est le premier arrivé qui réussit à dominer (Dawson et Lerner, 1966 ; Mayr, 1942). L'effet fondateur semble surtout important tôt dans la vie de l'hôte. À la naissance, le microbiome de la mère est la première source de bactéries qui colonise sa progéniture (Spor *et al.*, 2011b). L'effet de ce premier inoculum provenant de la mère sur le microbiome de l'hôte, souvent

nommé « effet maternel », a déjà été détecté jusqu'à la quatrième génération (Spor *et al.*, 2011b).

L'effet de masse décrit un système d'habitats/parcelles hétérogènes dans lequel la variation entre les espèces quant à leur capacité à utiliser un habitat favorise la co-existence régionale et l'immigration maintient la coexistence locale (Leibold & Chase, 2018 ; Mouquet et al., 2006). Une espèce peut être un compétiteur dominant dans une parcelle et un compétiteur inférieur dans un autre. La dynamique de l'effet de masse est souvent nommée dynamique 'source-puits' (Levin, 1974), puisque la coexistence locale d'un compétiteur inférieur avec un compétiteur supérieur est possible grâce à la dispersion de propagules d'une espèce d'une parcelle où elle est un compétiteur supérieur (source) jusqu'à une parcelle où elle est un compétiteur inférieur (puits). Ce paradigme se distingue parce qu'il permet à plus d'une espèce d'occuper la même parcelle (Leibold & Chase, 2018), et donc ce paradigme pourrait très bien expliquer la communauté de milliers d'espèces de bactéries dont est composé le microbiome. Les bactéries immigrantes pourraient provenir de la surface d'éléments biotiques et abiotiques dans l'environnement de l'hôte avec lesquels ils sont en contact, ou par le contact avec d'autres individus de la même espèce ou d'une espèce différente. La capacité des bactéries exogènes à coloniser et à modifier le microbiome intestinal a été démontrée par plusieurs études au cours desquelles des transferts expérimentaux du microbiome ont été effectués d'un hôte à un autre (Ellekilde *et al.*, 2014 ; Kassam *et al.*, 2013 ; Manichanh *et al.*, 2010). De plus, en transférant un prélèvement de microbiome de souris avec certains phénotypes à des souris axéniques (souris sans microbiome), il est même possible de transférer le phénotype associé à ce microbiome. Ceci a été démontré pour l'obésité (Ridaura *et al.*, 2013 ; Turnbaugh *et al.*, 2007), le syndrome métabolique (Vijay-Kumar *et al.*, 2010) et la maladie du foie gras (Henao-mejia *et al.*, 2012). Mais, tout comme la persistance d'une espèce dans une parcelle requiert une immigration continue de

l'espèce, l'effet des transferts expérimentaux de microbiome demeure généralement temporaire (Ellekilde *et al.*, 2014 ; Manichanh *et al.*, 2010).

L'importance de la dispersion sur le microbiome est d'autant plus apparente quand on étudie l'effet des réseaux sociaux sur le microbiome où la similarité du microbiome entre les individus dépend de leur fréquence de contact. Moeller et al. (2016) ont comparé les interactions sociales interindividuelles et le microbiome intestinal provenant de fèces d'une population de chimpanzés en Gombe, Tanzanie pendant huit ans. Les saisons pendant lesquelles le niveau de sociabilité des individus était plus élevé étaient corrélées avec une augmentation de la similarité entre les microbiomes des individus et une augmentation de la diversité alpha (richesse d'espèce bactérienne), et ce indépendamment de la diète. Ils ont aussi trouvé que les similarités entre les chimpanzés consanguins provenaient principalement de leurs contacts sociaux. Tung et al. (2015) ont analysé des données de contacts sociaux entre 48 babouins du Kenya pour lesquels ils ont aussi fait l'analyse de séquences métagénomiques du microbiome intestinal. Presque 19% de la variation du microbiome était liée au groupe social des babouins tandis que le sexe, l'âge et la profondeur du séquençage expliquaient moins de quatre pour cent de cette variation. L'effet de l'identité sociale persistait fortement même en contrôlant l'effet de la consanguinité. Un résultat similaire a été rapporté par Diakiw (2017) qui a étudié l'effet du groupe social sur le microbiome intestinal de lémuriens à ventre roux. Cette espèce forme des groupes sociaux relativement stables à l'âge adulte avec des territoires fixes. Dans cette étude, le niveau de consanguinité comptait pour seulement 2.4% de la variation microbienne, tandis que 25% de la variation était attribuable au groupe social de l'individu. La dispersion et la fréquence de transmission des bactéries semblent donc influencer la composition du microbiome intestinal à l'échelle locale. À l'échelle régionale, certains facteurs pourraient influencer la dispersion des bactéries en jouant sur les différences de microbiome entre les populations d'hôtes.

1.2.3 Le microbiome et la biogéographie insulaire

En macro-écologie, les facteurs déterminant la diversité des espèces occupant une île ont longtemps préoccupé les écologistes. MacArthur & Wilson (1963, 1967) furent parmi les premiers à développer un modèle offrant un moyen de prédire concrètement la richesse des espèces sur les îles. Leur modèle d'équilibre de la biogéographie insulaire dit que la richesse d'espèce est une fonction des taux d'immigration et d'extinction des espèces et que ces deux facteurs dépendent respectivement de l'isolement et de la superficie de l'île. Depuis, plusieurs études portant sur plusieurs systèmes à des échelles différentes ont trouvé un certain degré d'appui pour un effet positif de la superficie d'un territoire isolé, et un effet négatif de son degré d'isolement, sur la richesse des espèces qui s'y trouvent (Whittaker et Fernández-Palacios, 2007). Cependant, la relation entre l'isolement et la superficie sur la richesse d'espèces semble varier selon l'échelle géographique et le nombre d'habitats sur le terrain. De plus, la superficie du terrain peut, elle aussi, affecter le taux d'immigration en affectant la probabilité que le terrain soit colonisé quand la dispersion des propagules est aléatoire, et en affectant son attractivité aux immigrants potentiels (par son nombre d'habitats). L'isolement pourrait aussi affecter le taux d'extinction d'une espèce. Par exemple, en ayant recours à un effet de masse, une population sur une île avec une croissance négative pourrait être supplémentée par de nouveaux immigrants, un phénomène que Brown & Kodric-Brown (1977) nommèrent « effet de sauvetage ». Un flux régulier d'immigrants permettrait aussi un faible niveau de consanguinité dans la population (Charlesworth, 2003) ce qui lui permettrait de persister plus longtemps et d'accumuler une diversité microbienne plus importante. Finalement, l'ordre de colonisation des espèces ainsi que la diversification des espèces à l'échelle évolutive peuvent aussi influencer la richesse d'espèces (Fukami *et al.*, 2007). Plus une île est isolée, plus sa richesse en espèces proviendra de la diversification plutôt que de l'immigration (Whittaker et Fernández-Palacios, 2007). Si le microbiome agit comme une

communauté d'espèces occupant des parcelles d'habitats plus ou moins isolés, les hôtes, les mêmes forces à l'origine de la richesse en espèces sur les îles pourraient influencer la richesse du microbiome. Par exemple, en comparant le microbiome de populations d'hôtes occupant un réseau de sites insulaires, la richesse du microbiome devrait diminuer avec le niveau d'isolement du site occupé par l'hôte. De même, la richesse du microbiome devrait augmenter avec la superficie du site occupé par l'hôte étant donné l'effet positif de la superficie du site sur sa richesse d'habitats et son taux d'immigration. Bien que l'effet de la diversification sur la richesse en espèces soit quasi négligeable à une échelle temporelle macro-écologique, ce facteur influence probablement plus fortement la richesse du microbiome intestinal étant donné les taux de reproduction et de spéciation élevés des bactéries (Costello *et al.*, 2012).

Les caractéristiques démographiques d'une population pourraient aussi influencer la composition du microbiome des membres des populations avoisinantes. Par exemple, une forte densité dans une population pourrait non seulement limiter les mouvements des hôtes à l'intérieur de la population et rendre la distribution microbienne dans la population plus hétérogène, mais aussi favoriser une émigration plus importante de ses habitants vers les populations avoisinantes (Saether et al., 1999). Ceci devrait homogénéiser la diversité microbienne entre les populations proches.

1.3 Le microbiome et le comportement de l'hôte

1.3.1 Effets du microbiome intestinal sur le comportement de l'hôte

Les premières démonstrations de l'effet du microbiome sur le comportement de l'hôte ont été faites en comparant les comportements de souris axéniques et de souris à organismes pathogènes spécifiques (EOPS). Les souris axéniques sont des souris élevées sans microbiome dans un environnement stérile tandis que les EOPS ont un microbiome normal. Heijtz et al., (2011) ont observé que les souris axéniques se

déplaçaient plus loin et passaient plus de temps dans le centre d'une arène nouvelle que les EOPS. Elles passaient aussi plus de temps que les EOPS dans les bras ouverts d'un labyrinthe en croix surélevé (Heijtz *et al.*, 2011). Heijtz et al. (2011) ont également observé que les différences comportementales chez les axéniques disparaissaient lorsque le microbiome d'EOPS était transféré aux jeunes souris axéniques, mais pas aux adultes indiquant que l'effet du microbiome sur le développement comportemental/cérébral pourrait avoir lieu majoritairement pendant une période critique au début de la vie de l'hôte. Neufeld et al. (2011) ont effectué une étude similaire et n'ont pas pu confirmer l'effet de l'état axénique sur l'exploration des souris, mais, comme dans l'étude menée par Heijtz et al., (2011), les axéniques passaient plus de temps que les EOPS dans les bras ouverts du labyrinthe en croix surélevé. Les souris axéniques éprouvaient aussi des niveaux plus élevés de corticostérone (hormone de stress) dans le plasma et les auteurs ont mesuré des différences d'expression de certaines sous-unités des récepteurs NMDA (N-méthyl-D-aspartate) dans l'amygdale, et du facteur neurotrophique dérivé du cerveau (BDNF) et d'un récepteur de sérotonine dans l'hippocampe (Neufeld *et al.*, 2011). Contrairement aux études antérieures, Nishino et al. (2013) ont trouvé que l'inoculation du microbiome de souris axéniques par un microbiome EOPS diminuait leur comportement d'anxiété mesuré par le temps passé dans la périphérie de l'opennfield et leur tendance à enterrer des billes. Ces changements étaient accompagnés de changements dans les niveaux de neurotransmetteurs catécholaminergiques (ex. dopamine, sérotonine) et/ou de leurs métabolites dans le cerveau. Crumeyrolle-Arias et al. (2014) ont également observé un comportement plus anxieux dans le test openfield chez des rats axéniques que des EOPS, ainsi que des différences dans les niveaux de neurotransmetteurs catécholaminergiques basals et en réponse à un stress. Sudo et al. (2004) ont aussi montré que la réaction de l'axe hypothalamique-pituitaire-surrénal (HPS ; mesuré par les niveaux d'hormone adrénocorticotrope et de corticostérone dans le plasma) à un stress est plus élevée chez les souris axéniques que chez les EOPS. Ils ont mesuré une réduction des niveaux de neurotransmetteurs qui régulent l'expression du BDNF et une

réduction de BDNF dans le cortex et l'hippocampe des axéniques. De plus, l'expression élevée du HPS chez les axéniques a été amortie en transplantant le microbiome de souris EOPS aux axéniques, mais seulement lorsque celles-ci étaient jeunes. Finalement, l'axénie semble aussi modifier le comportement social. Dans un test de dyade, les rats axéniques explorent moins leur conspécifique (i.e. moins sociaux) que les EOPS (Crumeyrolle-Arias *et al.*, 2014) et les souris axéniques passent à la fois moins de temps dans la chambre contenant une autre souris et moins de temps à investiguer une souris étrangère que les souris avec un microbiome normal (Desbonnet *et al.*, 2014). L'état axénique est donc associé à un niveau anormal d'anxiété basal et en réponse à un stress, et à des troubles de comportement social. Ces différences sont aussi accompagnées de différences au niveau de la neurochimie de l'animal. L'effet du microbiome sur le comportement semble aussi varier selon l'étude, possiblement en fonction de l'espèce et de la lignée de l'hôte (González-Arancibia *et al.*, 2019).

L'effet du microbiome sur le comportement de l'hôte a aussi été démontré en modifiant le microbiome grâce à un traitement antibiotique ou probiotique. Bercik et al. (2011) ont administré un traitement antibiotique (neomycine, bacitracine) et un antifongique (pimiricine) à des souris. Les souris traitées montraient une anxiété réduite, illustrée par une faible latence pour descendre d'une plateforme surélevée et par un temps dans le compartiment éclairé d'un test d'obscurité/lumière. Les souris traitées ont aussi fait preuve d'une capacité d'exploration élevée indiquée par une fréquence plus élevée d'entrées dans chaque zone du test d'obscurité/lumière. Ces différences de comportement étaient accompagnées de niveaux de BDNF plus élevés dans l'hippocampe, mais plus faibles dans l'amygdale. En mesurant les niveaux de sérotonine, de dopamine et de noradrénaline dans l'intestin, ainsi qu'en sevrant le nerf vague (vagotomie), ils ont pu démontrer que les changements de comportement et de BDNF ne dépendaient pas des neurotransmetteurs intestinaux ni du système nerveux autonome. L'administration de l'antibiotique pénicilline, pendant les périodes pré- et

postnatales, réduit les comportements d'anxiété (visites des bras ouverts dans le LCS) et de sociabilité (sélection de la chambre avec un conspécifique), et augmente le niveau d'agressivité (réponses offensives à une attaque par un conspécifique) chez la souris (Leclercq *et al.*, 2017). Ce traitement n'a pas affecté l'inflammation (expression de cytokines et de jonctions serrées) au niveau de l'intestin, mais a affecté l'intégrité de la barrière hématoencéphalique et a augmenté l'inflammation (expression de cytokines) dans le cortex frontal (Leclercq *et al.*, 2017). L'administration du probiotique *Lactobacillus rhamnosus* a atténué ces effets (Leclercq *et al.*, 2017). L'administration du probiotique *Bifidobacterium longum* (*Actinobacteria*) dans un modèle de souris pour l'anxiété a rétabli les comportements d'anxiété et l'expression de BDNF dans l'hippocampe de celles-ci à des niveaux normaux, indépendamment des voies immunitaires et du nerf vague (Bercik *et al.*, 2010). De même, l'administration du probiotique *Bacteroides fragilis* à un modèle de souris pour l'autisme a permis de rétablir les comportements d'anxiété et de sociabilité chez celui-ci à un niveau normal (Hsiao *et al.*, 2013). *B. fragilis a* corrigé l'hyperperméabilité de l'intestin en normalisant les niveaux d'une cytokine capable de moduler certaines composantes des jonctions serrées dans l'intestin (Hsiao *et al.*, 2013). Par conséquent, *B. fragilis* a normalisé les niveaux de certains métabolites neurotoxiques dans le sang capables d'influencer le comportement de l'hôte (Hsiao *et al.*, 2013). Ces études démontrent clairement la capacité du microbiome, et même d'une seule bactérie, à influencer le comportement de l'hôte et indiquent que les mécanismes par lesquels ceci est accompli sont potentiellement nombreux et pourraient dépendre de l'hôte et de la composition de son microbiome.

Quoiqu'une discussion sur les mécanismes potentiels à l'origine de l'effet du microbiome sur le comportement dépasse le cadre de cette thèse, la façon dont le microbiome est capable d'influencer le comportement est une question qui revient souvent. Pour satisfaire à la curiosité du lecteur, une telle discussion est méritée. Il existe plusieurs mécanismes potentiels par lesquels une diaphonie entre le microbiome

intestinal et le cerveau pourrait se faire. Le nerf vague offre la voie la plus directe entre ces deux. Celui-ci établit des connexions directes jusqu'au cortex préfrontal (CPF) et il a été démontré que l'amygdale (Hoban *et al.*, 2016) du cerveau possède des connexions synaptiques avec des cellules entéroendocriniennes nommées cellules « neuropod » (Kaelberer *et al.*, 2018). Le deuxième mécanisme consiste en une translocation de produits bactériens de l'intestin vers le cerveau. Certaines bactéries produisent des métabolites capables d'affecter les niveaux de myéline et l'expression de gènes importants dans la régulation de myéline dans le CPF ainsi que dans les comportements de sociabilité et de dépression (Gacias *et al.*, 2016). Une fois qu'ils franchissent l'épithélium intestinal, ces métabolites circulent dans le sang et pénètrent la barrière hématoencéphalique. Les lipopolysaccharides (LPS) provenant de la membrane des bactéries peuvent aussi franchir l'épithélium intestinal et entrer dans le sang et un niveau élevé de LPS dans le sang est associé à des symptômes de dépression chez les rats (Ait-Belgnaoui *et al.*, 2012). Le microbiome est aussi impliqué dans la régulation de la sérotonine et de ses métabolites chez l'hôte et le bon fonctionnement de régions du cerveau impliquées dans le contrôle de l'anxiété et du comportement social, processus qui dépendent aussi du système sérotoninergique (Crumeyrolle-Arias *et al.*, 2014 ; Heijtz *et al.*, 2011 ; O'Mahony *et al.*, 2015). La sérotonine peut même protéger contre la dégénérescence neuronale et est importante dans le développement de l'intestin. Certaines bactéries telles qu'*Escherichia coli, Morganella morganii,* et *Klebsiella pneumoniae* et certains probiotiques peuvent même produire la sérotonine. Le tryptophane provenant de la nourriture est majoritairement métabolisé par la voie kynurenine. Celle-ci implique la production de métabolites neuroprotecteurs et neurotoxiques, dans le cerveau, capables d'influencer le développement du SNC. Certaines bactéries peuvent influencer les niveaux de tryptophane dans le système gastro-intestinal en le produisant ou en l'utilisant, et peuvent même s'en servir pour produire de la sérotonine (O'Mahony *et al.*, 2015). D'autres neurotransmetteurs produits par les bactéries du système gastro-intestinal incluent l'acétylcholine, l'histamine, l'acide γ-aminobutyrique (GABA), la noradrénaline, et la dopamine

(González-Arancibia *et al.*, 2019). Finalement, la capacité des produits microbiens à passer dans le sang et, par la suite, dans le cerveau dépend de l'intégrité de la barrière intestinale. Celle-ci peut être affectée par plusieurs facteurs. Par exemple, un des symptômes physiologiques causés par l'axe HPA en réponse à un stress est une augmentation de la perméabilité de l'intestin (Ait-Belgnaoui *et al.*, 2012). L'administration, à des rats, du probiotique *Lactobacillus farciminus* bloque l'hyperperméabilité de l'intestin, alors que l'administration d'un cocktail antibiotique composé de néomycine et d'ampicilline la réduit. Ainsi elles empêchent la translocation des LPS dans le sang (Ait-Belgnaoui *et al.*, 2012). Le probiotique *Bacteroides fragilis* corrige aussi l'hyperperméabilité de l'intestin chez un modèle de souris pour l'autisme en normalisant les niveaux d'une cytokine qui agit sur les jonctions serrées (Hsiao *et al.*, 2013).

1.3.2 L'effet du comportement sur le microbiome intestinal

Toutes les études décrites ci-haut ont été faites en laboratoire, mais l'effet direct du microbiome sur le comportement en milieu naturel n'a toujours pas été testé. Chez plusieurs espèces en milieu naturel on retrouve des différences entre les individus d'une population par rapport à leurs comportements d'exploration, de témérité, d'anxiété, d'agressivité, et de sociabilité (Réale *et al.*, 2007 ; Wolf et Weissing, 2012). En plus d'être associés à des traits métaboliques ou à la fréquence cardiaque, ces comportements sont souvent corrélés entre eux pour former un 'syndrome comportemental' qui varie selon un continuum d'explorateurs lents et rapides (Réale *et al.*, 2007, 2010). Ce sont les mêmes traits comportementaux qui sont influencés par le microbiome intestinal en laboratoire. Cependant, en laboratoire, le milieu est homogène et très contrôlé et donc pas du tout représentatif de l'hétérogénéité environnementale en milieu naturel. Par l'entremise de la dispersion des bactéries et de la diète, les différences de comportement entre les individus pourraient contribuer aux

différences dans la composition de leurs microbiomes. Les individus plus rapides ayant une exploration et une témérité plus élevées se déplaceraient ou disperseraient plus loin et donc entreraient en contact avec une plus grande diversité d'habitats et d'aliments. Ces comportements devraient donc modifier la composition et augmenter la diversité bactérienne du microbiome de ces individus. Les individus plus agressifs devraient aussi démontrer plus d'approches offensives envers les conspécifiques ou autres intrus ou prédateurs. Ceci devrait augmenter leurs fréquences de contacts avec ceux-ci et, par conséquent, augmenter la diversité taxonomique de leur microbiome. Tout comme l'agressivité, le comportement social devrait affecter le microbiome. Une corrélation entre les similarités dans la composition du microbiome entre les individus et l'importance de leurs liens sociaux (fréquence de contacts) a déjà été démontrée pour les chimpanzés (Moeller et al., 2016), les babouins (Tung et al., 2015), les poneys semi-sauvages (Antwis, Lea, Unwin, & Shultz, 2018) et les lémuriens à ventre rouge (Raulo *et al.*, 2018). De plus, les individus plus sociaux devraient être plus fréquemment en contact avec une plus grande diversité de conspécifiques et ainsi avoir un microbiome plus diversifié.

En milieu naturel, les différences comportementales interindividuelles peuvent avoir des répercussions sur la valeur adaptative de l'individu (Cote *et al.*, 2008 ; Smith et Blumstein, 2008). L'impact du microbiome sur ces traits, ainsi que l'effet potentiel de ces comportements sur le microbiome de l'hôte en retour, pourrait avoir des conséquences écologiques importantes et aller jusqu'à influencer le taux de survie de l'hôte, sa reproduction et ses contacts sociaux, son domaine vital et sa dispersion, et par conséquent la structure des populations (Boyer et al., 2010 ; Cote et al., 2010). Cependant, toutes les études à présent examinant l'effet du microbiome sur le comportement ont été faites en laboratoire et donc ne permettent pas une extrapolation à la situation en milieu naturel. Il est donc impossible d'inférer l'importance écologique et évolutive que pourrait avoir le lien entre le microbiome et le comportement de l'hôte.

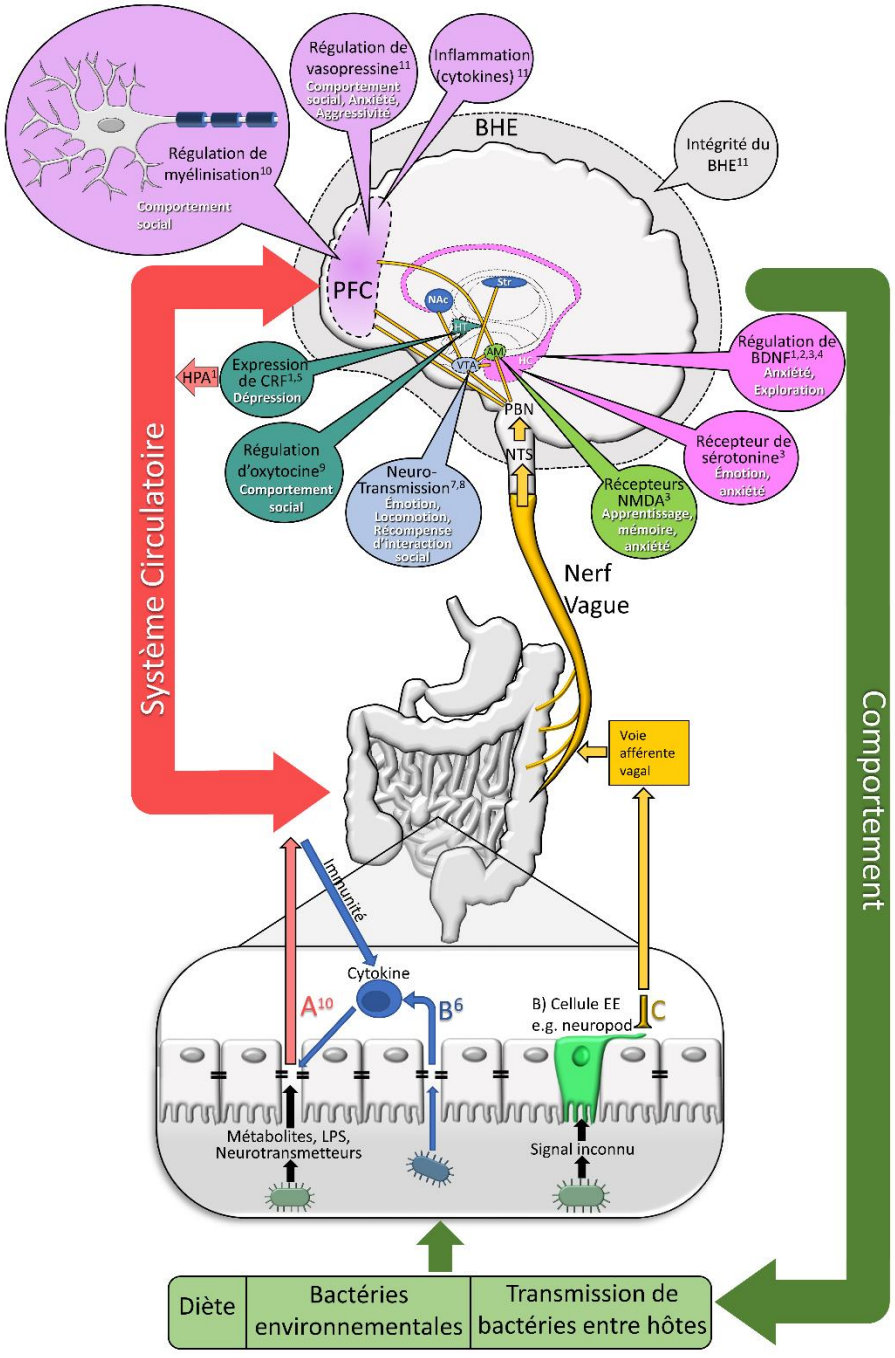

Figure 1.1 Schéma décrivant la rétroaction entre le microbiome et le comportement. Le microbiome intestinal semble pouvoir influencer le comportement par trois mécanismes : A) par les produits microbiens qui atteignent et affectent le cerveau ; B)

par la régulation de jonctions serrées (ex. en agissant sur des cytokines capables de réguler les jonctions serrées; et C) par une signalisation, qui se ferrait directement par le nerf vagal, mais le mode de signalisation n'est toujours pas connu. Acronymes : BHE= barrière hématoencéphalique, NTS= noyau du faisceau solitaire, PBN= noyau parabrachial, AM=amygdale, HT=hypothalamus, HC=hippocampe, BDNF=facteur neurotrophique dérivé du cerveau, VTA= aire tegmentale ventral, Str=striatum, NAc= noyau accumbens, LPS=lipopolysaccharides, CRF= facteur de libération de corticotrophine, HPA= Axe hypothalamique pituitaire surrénal, EE=entéroendocrénien. Références:1(Sudo *et al.*, 2004), 2(Bercik *et al.*, 2010), 3(Neufeld *et al.*, 2011), 4(Bercik *et al.*, 2011), 5(Ait-Belgnaoui *et al.*, 2012), 6(Hsiao *et al.*, 2013), 7(Nishino *et al.*, 2013), 8(Crumeyrolle-Arias *et al.*, 2014), 9(Desbonnet *et al.*, 2015), 10(Gacias *et al.*, 2016), 11(Leclercq *et al.*, 2017).

1.4 Objectifs et structure de la thèse

Dans cette thèse, j'analyse comment la composition du microbiome est liée à différents facteurs individuels, démographiques et environnementaux chez deux métapopulations insulaires et sympatriques de souris sylvestres (*P. maniculatus*) et de campagnol à dos roux (*M. gapperi*). La métapopulation est un système idéal pour étudier les relations entre ces facteurs et le microbiome puisqu'elle représente un ensemble de populations dynamiques liées à des degrés plus ou moins élevés par la dispersion (Hanski, 1998) et permet une étude de la relation hôte-microbiome à différents niveaux hiérarchiques.

Ce doctorat est divisé en trois chapitres qui ont pour but de mieux comprendre comment le lien entre le microbiome et le comportement de l'hôte se situe dans un contexte écologique :

1. Les facteurs individuels, démographiques et environnementaux reliés au microbiome intestinal ; ce chapitre a pour but de découvrir les caractéristiques à

l'échelle de l'hôte et du paysage liées au microbiome intestinal et de déterminer si, parmi ces facteurs, un lien entre le microbiome et le comportement existe, et de faire le point sur l'importance relative de ce lien.

2. Les effets proximaux du microbiome sur le comportement de l'hôte ; ce chapitre a pour but de tester premièrement si le microbiome est capable d'influencer directement les comportements d'exploration, d'anxiété et d'agressivité en milieu naturel et deuxièmement si cet effet a des conséquences sur les traits d'histoire de vie de l'hôte, plus précisément, son domaine vital et sa survie.
3. Les liens entre le microbiome et la dynamique sociale ; jusqu'à présent, toutes les études démontrant un lien entre la fréquence des contacts entre individus sur les similarités de leurs microbiomes ont été faites sur les humains, les primates non humains, et quelques autres espèces très sociales. Il est donc important de mieux comprendre ces associations chez d'autres espèces avec des structures sociales différentes. Ce chapitre aura donc pour objectif d'examiner l'importance des contacts entre individus sur la composition du microbiome chez les rongeurs *P. maniculatus* et de *M. gapperi*. Dans un premier temps, j'examine ces effets pour chaque espèce séparément. Dans un deuxième temps, vu l'importance potentielle des interactions interspécifiques sur la dynamique des populations et des communautés, j'examine l'association entre les contacts potentiels entre hôtes d'espèces différentes et la composition de leurs microbiomes.

1.5 Espèces modèles et populations d'étude

La souris sylvestre et le campagnol à dos roux sont parmi les espèces de rongeurs les plus communes en Amérique du Nord (Harper et Austad, 2001 ; Wood *et al.*, 2010). Les deux espèces sont aussi communes dans les forêts mixtes, mais la souris sylvestre a plus de succès que le campagnol à dos roux dans l'occupation des habitats plus

ouverts (Martell, 1983). Les deux espèces sont opportunistes dans leur alimentation et ont des diètes similaires. Elles se nourrissent de baies, de graines et d'arthropodes (Harper et Austad, 2001), mais le campagnol à dos roux est aussi reconnu pour sa consommation de champignons (Sullivan *et al.*, 2017). Leur système d'accouplement est aussi fondé sur la promiscuité (Dewsbury, 1981 ; Kawata, 1985). Les domaines vitaux des mâles des deux espèces sont plus grands que ceux des femelles et chevauchent beaucoup ceux des autres mâles et des femelles tandis que les domaines vitaux des femelles se chevauchent beaucoup moins (Mihok, 1981 ; Wood *et al.*, 2010).

Malgré leurs ressemblances, il semble exister quelques différences comportementales et physiologiques entre ces deux espèces. Le réseau des domaines vitaux des campagnols à dos roux ressemble à celui des souris sylvestres, mais les femelles semblent être plus territoriales et faire preuve de plus d'agressivité envers les mâles (McGuire, 1997 ; Mihok, 1981) tandis que, chez la souris sylvestre, les femelles démontrent très peu d'agressivité envers les mâles (Dewsbury, 1981). Les deux espèces semblent aussi faire preuve de réponses hormonales différentes face au stress causé par la capture (Harper et Austad, 2001) et à une densité de population élevée (Harper et Austad, 2004). Ces espèces semblent donc montrer des réponses physiologiques différentes face au stress, ce qui indique que l'association entre le comportement et le microbiome pourrait être différente pour chacune.

Les populations de souris sylvestres et de campagnols à dos roux étudiées dans ce doctorat occupent un réseau de sites insulaires et riverains sur la rivière Winnipeg dans le sud-ouest de l'Ontario près du village de Minaki. La dispersion des rongeurs entre les sites est présumée être limitée et varier selon le niveau d'isolement du site, faisant de ce système une métapopulation. L'échantillonnage pour le chapitre 1 de ce doctorat a été fait à 11 sites insulaires et 4 sites riverains. Les chapitres 2 et 3 se concentrent sur les dynamiques à l'intérieur d'une de ces populations insulaires.

CHAPITRE II

GUT MICROBIOME LINKED TO AGGRESSIVITY, GENOTYPE, AND LANDSCAPE ATTRIBUTES IN TWO RODENTS

Joël W. Jameson, Denis Réale, Tristan Juette, Josh Miller, Dany Garant, Louis Bernatchez, Steven Kembel

2.1 Abstract

The gut microbiome (GM) influences the development of host exploration and anxiety in laboratory studies, but the association between the GM and these behaviours have not been verified in wild animals. We tested whether the GM, derived from 16S rRNA gene sequencing, correlates with exploration, anxiety and aggressiveness in sympatric wild populations of deer mice (*Peromyscus maniculatus*) and red-backed voles (*Myodes gapperi*) sampled across a network of inland islands. To place behaviour-microbiome associations in a broader ecological context, we measured 11 host and landscape characteristics and assessed associations at within- and among-population scales. Mouse and vole GMs were distinctly different, with core microbiomes sharing only seven species. GM beta diversity correlated with aggressiveness in male mice and in voles. Mouse GM beta diversity depended mostly on population-level genetic distance and date, while associations with vole GM beta diversity were much weaker, and strongest for population density and connectivity among sites. GM alpha diversity was not correlated with any traits in mice but correlated with landscape characteristics in voles. Our results show the variety of individual, populational, phylogenetic, and landscape features explaining the diversity of host microbial communities.

2.2 Introduction

Coevolution between gut microbiome (GM) and host have led to strong associations between the GM and numerous host attributes including diet (David *et al.*, 2014 ; Ingala *et al.*, 2018 ; Ley *et al.*, 2008 ; Muegge *et al.*, 2011 ; Thaiss *et al.*, 2014), intrinsic host traits including host behaviour (Bercik *et al.*, 2010 ; De Palma *et al.*, 2015 ; Desbonnet *et al.*, 2014 ; Gacias *et al.*, 2016 ; Heijtz *et al.*, 2011 ; Hsiao *et al.*, 2013 ; Neufeld *et al.*, 2011), phylogeny and genotype (Griffiths *et al.*, 2018 ; Ingala *et al.*, 2018 ; Phillips *et al.*, 2012 ; Spor *et al.*, 2011a ; Sullam *et al.*, 2015 ; Suzuki *et al.*, 2019 ; Youngblut *et al.*, 2019 ; Zhao *et al.*, 2013), and spatial and environmental factors like habitat (Miller *et al.*, 2018). However, the strength and nature of these associations vary across spatial, environmental, and phylogenetic scales, and are poorly understood for wild animal populations (Amato, 2013). Studying these associations in wild animals is necessary for understanding their underlying co-evolutionary processes.

Diet is among the factors with the greatest influence on wild animal GMs (Ingala *et al.*, 2018 ; Ley *et al.*, 2008 ; Muegge *et al.*, 2011). It depends on resource availability dictated by habitat. Shared diet and environment are considered key drivers in the effect of geographic distance on the GM (Yatsunenko *et al.*, 2012). However, transfer of bacteria through social contacts or genetic similarity could also drive biogeographic patterns of GM composition. At broad taxonomic levels, compositional differences exist in the microbiome congruent with host phylogeny (Ingala *et al.*, 2018 ; Phillips *et al.*, 2012 ; Youngblut *et al.*, 2019) and, within various species, common garden experiments reveal an association between host genetic distance and differences in GM structure (Spor *et al.*, 2011a ; Sullam *et al.*, 2015 ; Suzuki *et al.*, 2019 ; Zhao *et al.*, 2013). Additional studied traits include age, sex, and morphology. Generally, juveniles show lower GM alpha diversity and greater interindividual variation in GM composition than adults, and compositional differences between juveniles and adults reflect changes in diet (Kohl *et al.*, 2019 ; Pafco *et al.*, 2019 ; Yatsunenko *et al.*, 2012). Differences due to sex are more subtle than those for age and may even

depend on age (Jaggar *et al.*, 2020 ; Pafco *et al.*, 2019 ; Schmidt *et al.*, 2019). Finally, microbiomes play key roles in nutrient assimilation and metabolism (Thaiss *et al.*, 2014), with GM diversity and composition associating with body mass index (BMI) and growth in some species (Le Chatelier *et al.*, 2013 ; Suzuki *et al.*, 2019 ; Turnbaugh *et al.*, 2007 ; Uren Webster *et al.*, 2018).

Laboratory studies show that the GM is important in determining host behaviour. Germ-free (GF) mice are less anxious (Heijtz *et al.*, 2011 ; Neufeld *et al.*, 2011), move more (Neufeld *et al.*, 2011) and display greater social avoidance (Desbonnet *et al.*, 2014) compared to mice harbouring a conventional GM, and these behaviours can be reversed by conventionalization of the GM of GF mice [26, 28]. Additionally, laboratory mice require a GM to express normal behavioural responses to stressors [29, 30]. While research on the GM-behaviour association has focused on the GM's effect on behaviour, this association is potentially bidirectional. In the wild, individuals from a given population often vary in their exploration, anxiety, aggressive and social behaviour (Réale *et al.*, 2007 ; Wolf et Weissing, 2012). These differences may persist across time and different contexts and are maintained by selection (Réale *et al.*, 2007 ; Wolf et Weissing, 2012). Often, these behaviours, and physiological traits like metabolism and heart rate, are correlated with individuals varying along a continuum of fast to slow explorers (Réale *et al.*, 2010). Faster explorers disperse more (Le Galliard *et al.*, 2012), which could impact the variation of habitats, conspecifics, and foods they encounter, ultimately impacting the GM.

We tested whether the GM correlates with exploration, anxiety, and aggressiveness in a metapopulation of sympatric wild deer mice (*Peromyscus maniculatus*) and red-backed voles (*Myodes gapperi*) inhabiting a network of inland islands. We also measured heart rate under stress, a physiological trait known to correlate with exploration and proactivity (Koolhaas *et al.*, 1999 ; Montiglio *et al.*, 2012). To place behaviour-microbiome associations in a broader ecological context, we measured five other host-level factors: sex, age, morphology, host-wise genetic distance, and habitat complexity; and four population-level factors: population density, habitat composition, site insularity and connectivity. When possible, we assessed associations at within- and among-population spatial scales. We hypothesized that faster, bolder, more aggressive

individuals have more diverse microbiomes. Table 2.1 summarizes our hypotheses on how GM alpha diversity correlates with each measured trait.

In addition to potential influences of host genotype, metacommunity theory predicts that the GM's composition depends on the rate of bacterial dispersal between the host and surrounding bacterial sources (Leibold *et al.*, 2004 ; Miller *et al.*, 2018). We hypothesized that similarity in microbiome composition among individuals (beta diversity) increases with genetic, phenotypic and environmental similarity and that more closely connected sites harbour hosts with similar microbiome compositions. Finally, differences in the GM of different species and strains can underlie differences in behaviour and neurochemistry (Bercik *et al.*, 2011 ; Gacias *et al.*, 2016 ; O'Mahony *et al.*, 2015). We therefore hypothesized that the patterns we observed would differ between mice and voles.

2.3 Methods

We conducted this study near Minaki (49°59′11″N 94°40′12″W), in the Winnipeg river basin, Ontario, Canada. We captured mice and voles on 11 islands and four mainland sites during three consecutive nights (weather permitting) at each site between 1 July–1 September 2015 (Fig. 1). Sampling locations were 350.1–4776.6 m apart and island sites were 72.9–1778.7 m from the nearest mainland shore. Islands varied in size from 7.2–316.4 ha. To minimize temporal autocorrelation and control for seasonal effects, we visited sites in random order, alternating between island and mainland sites.

2.3.1 Captures

We captured animals with Longworth and BIOEcoSS (BIOEcoSS Ltd.) live traps. Capture grids comprised 60 traps in pairs at 30 stations (5 × 6) distanced 10 m apart. Before deployment, we sterilized traps with Virkon™. We baited traps with peanut butter and carrot and included cotton for thermoregulation. Traps were active from 19:00–06:00. Each evening, we replaced traps that captured an animal the previous night with clean traps. From captured animals, we collected a tissue sample from ears with an ear punch for genetic analyses and used different ear-punch combinations as identification markers. We stored tissue samples in 1.5 ml Eppendorfs with 95% ethanol at -20 °C. We also recorded each individual's sex, age, and reproductive condition.

2.3.2 Genotype

Genetic distance among mice was quantified using single-nucleotide polymorphisms (SNP), while we quantified genetic distance among voles with microsatellites. The decision to use different methods for each species stemmed from an opportunistic availability of SNP data for mice, but not for voles, and SNPs provide greater accuracy in resolving genotypic differences than microsatellites (Morin *et al.*, 2004). We obtained data on genotypic differences for deer mice in our study from Miller et al. (submitted; see also supplementary methods). Samples were sequenced by double digest restriction-site associated DNA (RAD) sequencing (Peterson *et al.*, 2012). Reads were aligned against the genome assembly for *Peromyscus maniculatus* (GCA_000500345.1) to identify SNPs. Samples with too many missing SNP data were removed and the resulting SNP list was further filtered. We conducted principal component analysis (PCA) on the allele frequencies from the genotype matrix. Scores of the first two PCA axes (genotype-PC1, genotype-PC2) were used as genetic distance variables in our analyses for mice.

We conducted microsatellite analyses for voles (details in supplementary methods). We extracted DNA and PCR-amplified 12 microsatellites identified for bank voles (*Myodes glareolus*) (Rikalainen *et al.*, 2008). The amplified product was normalized and sequenced on an AB 3130xl (Applied Biosystems). We removed null alleles and genotyping errors from further analyses and tested the remaining eight loci for Hardy-Weinberg equilibrium (Rousset, 2008). We calculated a

matrix of pairwise distances among individuals averaged across all loci (R package polysat [Clark *et al.*, 2019]) and ran a PCA of the distance matrix. The scores of the first two PCA axes (genotype-PC1, genotype-PC2) were used as genetic distance variables in our analyses.

2.3.3 Microbiome

We collected two to three fecal pellets from each individuals' trap and placed them in a 2 ml Eppendorf with RNAlater (Ambion, Austin, TX). We kept samples on icepacks while we processed animals and afterwards, stored them at -20 °C. Upon returning from the field, we transferred samples to -80 °C. We extracted DNA from samples with the QIAamp Fast DNA Stool Mini Kit (QIAGEN), randomly attributing samples to sequencing kit. We manually homogenized ~10 mg of fecal material in extraction kit ASL buffer with a pipet tip. In the final extraction step, DNA was eluted into 100 µl of buffer ATE. PCR amplification of the 16S rRNA gene V5-V6 region (799F-1115R; [Kembel *et al.*, 2014]), and subsequent clean-up and normalization followed DeBellis et al. (DeBellis *et al.*, 2019). We sequenced DNA on an Illumina MiSeq platform (2x250bp V3 Illumina Reagents).

Following quality control, filtering, and chimera removal, we identified reads to amplicon sequence variants (ASV) with DADA2 V1.10 (Callahan *et al.*, 2016). We used default settings specifying forward and reverse read lengths of 210 and 200 respectively during read trimming. We used DADA2's RDP Naive Bayesian Classifier to assign taxonomy to ASV according to the SILVA database (V128). We rarefied samples to 4000 reads/sample. Because rarefaction only removed negative controls, we performed a non-metric multidimensional scaling ordination with Bray-Curtis distances (R package Vegan [Oksanen *et al.*, 2019]) to detect and remove three obvious outliers.

2.3.4 Behavioural and Physiological Response to Stress

Upon capturing individuals, we measured their aggressiveness towards a predator (human) by holding the animal at the base of the tail and having it rest on the back of the hand. Aggression was scored on a 4-point scale from very docile (immobile) to very aggressive (bites, struggles alot). Aggression has been scored similarly in various mammals including mice (Dingemanse et Réale, 2005 ; Geburt *et al.*, 2015 ; Moons *et al.*, 2004) and our measure of aggressiveness is heritable in mice from this study system (Miller et al. submitted).

We measured exploration and anxiety behaviours with an open-field test (Montiglio *et al.*, 2010 ; Wolfer *et al.*, 2004). We placed the animal alone in a 40 × 60 × 50 cm (w x l x h) arena and video-recorded its movements for three minutes. We extracted behaviours using Ethovision (V.9.0, Noldus Information Technology, Wageningen, The Netherlands). In some videos, tracking began two seconds late, so we measured exploration as the average speed during the test. We also defined a central square in the arena representing one quarter of the arena area (600 cm^2). We measured time spent in this zone as an indicator of the animal's propensity to take risks, an anxiety-related behaviour (Foster et Neufeld, 2013). Mean speed and time in the center remain consistent across years in mice and voles (Brehm *et al.*, 2019). We also counted the frequency of jumps and entries into the central zone.

Finally, we measured the animal's heart rate when handled as a physiological response to a stressful situation. Heart rate is shown to correlate with exploration speed and proactivity (Koolhaas *et al.*, 1999 ; Montiglio *et al.*, 2012) and with behavioural and steroidal responses to stress (Hollocks *et al.*, 2014 ; Kralj-fišer *et al.*, 2010). To measure heart rate, we placed the microphone of a voice recorder (Olympus S10) against the animals' chest and recorded for one minute. We used Avisoft-SASLab Pro (Berlin, Germany) to count the amplitude peaks in a sample of ~100 beats. We measured heart rate as the average number of beats/second, the maximum time between beats or inter-beat-interval and its variance. Heart rate is an indicator of sympathetic, and inter-beat-interval variance of parasympathetic nervous system reactivity (Koolhaas *et al.*, 1999).

2.3.5 Morphometry

We aged all animals to one of three classes (juvenile, subadult, adult) based on pelage colour (Naughton, 2012 ; Osgood, 1909) and sexed them based on urogenital distance. We weighed animals with a 60 g capacity (± 0.30%) Pesola ® Micro-Line Spring Scale. We used a Sony ® DSC-HX300 camera to photograph the hindfeet and tail with a ruler placed along each. We measured tail and hindfoot length from photos with ImageJ software V1.50i (Schneider *et al.*, 2012) (for more information see [Juette *et al.*, 2020]).

2.3.6 Landscape characteristics

We measured site-level habitat characteristics by establishing nine 10 × 10 m quadrats within each capture grid. We characterized the tree stratum by measuring canopy cover with a densiometer and by tallying each species of tree with a diameter at breast height (dbh)>10 cm in each quadrat. Within a 5 × 5 m square centered in each quadrat, we characterized the shrub stratum by estimating the percent cover of each plant species for plants >1 m tall with a dbh <10 cm. We characterized the herbaceous stratum by estimating the percent cover of remaining plant species including deadwood, litter, and rocks within five 1 × 1 m subquadrats in a cross configuration centered in the quadrat, and averaged results for each quadrat. For each stratum, we removed species with a total across-site relative abundance of <5%. We combined the remaining species/categories into a single dataframe. We also quantified habitat structure at each trap. Three to four researchers independently estimated the percent cover of deadwood within a 2 m radius around each trap and we averaged these. We also measured canopy cover at each trap.

To estimate the probability of dispersal between sites, we calculated an index of connectivity $S_i = \sum_{i \neq j} p_j A_i A_j e^{-\alpha d_{ij}}$, for each site *i* following Hanski et al. (Hanski *et al.*, 2017). Here, j is the source patch, pj is the incidence of occupancy of the source patch, Ai and Aj are the areas of the target and source patches respectively, α is a constant that models the behaviour of the dispersal kernel (Hanski, 1994), and dij is the minimal distance between i and j patches. We calculated island area and distance between sites with QGIS Girona 3.2.0 (QGIS Development Team, 2018). We

specified a pj of 1 for each source patch j because mice and voles occupied all sites. We used a model constant of α=1 which is suitable for our system (Juette *et al.*, 2020).

2.3.7 Analyses

We compared the GM of mice and voles by identifying their 50% core microbiome (R package microbiome [Lahti et Shetty, 2020]) and the taxa they shared. We used differential expression analysis (R package DESEQ2; model design: ~ site + species; transformation: addition of the pseudocount "1" to the microbiome community data) to identify taxa with the greatest difference in relative abundance between mice and voles (Love *et al.*, 2014). Including site first in the model removed site-level variation.

We tested predictions relating to environmental factors by running separate analyses on each species. We ran a PCA on each set of explanatory variables to obtain one or two variables (PCA scores) that explained most of the variance in each set (i.e. behaviour, morphology, heart rate, trap-level habitat structure, site-level habitat; Fig. S1). We retained the first two PCA axes for behaviour, which represented exploration speed (PC1) and aggressiveness (PC2) (Fig. S1a and d). For cardiac parameters, we retained the first axis (Fig. S1b and e), which largely represented heart rate. For morphology, we retained the first axis, which represented body size (Fig. S1c and f). For trap-level habitat structure, we retained the first axis, which represented habitat openness with lower canopy cover areas having less woody debris (Fig. S1g and h). Finally, for site-level habitat, we retained the first PCA axis, which represented a gradient going from open coniferous to dense-mixedwood forest (Fig. S1i). The variance explained by each axis is provided in Fig. S1.

We Hellinger-transformed (R package Vegan [Oksanen *et al.*, 2019]) the ASV matrices and ran principal coordinate analyses (PCoA) on Bray-Curtis distances (R package phyloseq [McMurdie et Holmes, 2013]). Using a scree test (D'agostino et Russell, 2014), we selected and retain the first three PCoA axes for further analyses. We tested our hypotheses on GM beta diversity by running separate mixed models (R package lme4 [Bates *et al.*, 2015]) on each PCoA axis score. The full model tested included, as fixed effects, the retained PCA axis scores for exploration,

aggressiveness, heart rate, genetic distance, habitat structure, forest type, age, sex, landscape type, site connectivity, habitat diversity, and population density, as well as each variables' interaction with age and each variables' interaction with sex. Date was a fixed effect accounting for seasonal variation and site was a random effect. To assess whether effects mostly occurred at the within- or at the among-population level, we tested the effect of the random effect (site), and compared marginal and conditional R-squared values (R^2m and R^2c) of the final models with the models where each variable was removed. R^2m represents the variance explained by the fixed effects, and R^2c the variance explained by the fixed and random effects (Nakagawa et Schielzeth, 2013). If removal of a fixed factor from the model reduced the R^2m but not R^2c, this indicated that the variance explained by the removed variable was shared with the random factor. To test predictions on microbiome alpha diversity, we ran a linear model with the same independent variables and with Shannon's diversity as the dependent variable. We did not include genetic distance in this analysis as alpha diversity is not a measure of dissimilarity. We used a linear model because our predictors accounted for all the site-level variance in alpha diversity. We selected the most parsimonious models with AIC-based backward stepwise selection (R function step for mixed models and stepAIC for linear models). We validated all final models regarding model assumptions and transformed dependent variables when necessary. For significant interactions, we compared least square means of each predictor combination (R package emmeans [Lenth *et al.*, 2020]). We used function envfit (R package Vegan) to identify microbial families correlated with each PCoA score used to test our hypotheses.

We used differential expression analysis (DESEQ2) to identify specific ASVs associated with aggressiveness. We ran separate models for each species and used the fixed effects model designs from the previous mixed models in which aggressiveness was significant. We included site as a fixed effect in the models.

2.4 Results

We captured 224 mice of which our analyses included 59 females (seven subadults, 52 adults), and 62 males (13 subadults, 49 adults) from nine island and four mainland sites. We captured 421 voles of which our analyses included 53 females (12 subadults, 41 adults), and 78 males (25 subadults, 53 adults) from nine island and three mainland sites. When testing hypotheses on GM alpha and beta diversity associations with host and landscape attributes, each model was selected from a full model that included all measured traits so that each model tested all hypotheses at once.

2.4.1 GM composition of mice and voles

Mouse and vole GMs mainly contained bacteria from the phyla Bacteroidetes and Firmicutes, with relatively fewer Proteobacteria, Spirochaetae and Tenericutes (Fig. 1). Mouse and vole 50% core microbiomes comprised 70 and 74 ASVs respectively and shared only seven of these. Regarding all ASVs, mice had higher GM diversity than voles (Shannon's: estimate=-0.3, SE=0.04, df=279, t=-7.9, $p<0.001$) and mouse and vole GMs differed in the proportions of 2 473 ASVs (DESEQ2 test; adjusted p-values < 0.05; Fig. 2). Most differentially abundant ASVs were of the phylum Bacteroidetes (Fig. 2). Within the phylum Spirochaetae, a single ASV, genus *Treponema_2*, was significantly more abundant in voles. Finally, within the phylum Firmicutes, different ASVs of the families Lactobacillaceae, Lachnospiraceae and Ruminococcaceae were also differentially abundant in each rodent species (Fig. 2).

2.4.2 Factors associated with the mouse GM

PCo1 largely represented the ratio between the phyla Bacteroidetes and Firmicutes. Specifically, PCo1 most correlated positively with the proportion of the family Bacteroidales_S24-7 (Bacteroidetes) and negatively with the proportions of Lachnospiraceae and Ruminococcaceae (Firmicutes; Fig. 3). PCo2 most correlated negatively with the proportions of Bacteroidaceae, Rikenellaceae and Porphyromonadaceae (Bacteroidetes). Finally, PCo3 correlated positively with the proportions of Prevotellaceae (Bacteroidetes) and Spirochaetaceae (Spirochaetae; Fig. 3).

At the within-site level, PCo1 was positively associated with body size and negatively associated with the interaction between aggressiveness and sex with more aggressive males harbouring lower PCo1 values (Table 2.2, Fig. 3). Across sites, PCo2 was positively associated with genotype-PC1 and date (Table 2.2, Fig. 3), and PCo3 was negatively associated with habitat with mice from dense, mixedwood forests harbouring lower PCo3 values. PCo3 was also positively associated with date (Table 2.2, Fig. 3). Microbiome composition was not associated with exploration, age, heart rate, habitat structure, landscape type, site connectivity or population density. Genotype-PC1 was correlated with landscape type, site connectivity, and forest type (Fig.S2) so we reran the analysis without genotype-PC1 to see how these traits were confounded with genotype. This new analysis gave a much poorer model (genotype-PC1 excluded: R^2m = 0.248, AICc = -314.3; genotype-PC1 included: R^2m = 0.749, AICc = -341.5) that lacked these landscape traits (Table S2.6). Shannon's diversity was not correlated with any measured traits in mice.

2.4.3 Factors associated with the vole GM

In voles, PCo1 was most positively correlated with the proportions of Spirochaetacea and Clostridiales_vadinBB60 (Firmicutes; Fig. 3). PCo2 was most positively correlated with Ruminococcaceae, Lachnospiraceae and most negatively correlated with Bacteroidales_S24-7 (Fig. 3). Finally, PCo3 was most positively correlated with Clostridiales_vadinBB60 (Fig. 3).

In voles, PCo1 was positively associated with population density and negatively associated with date and age, with the age effect occurring at the within-site level (Table 2.3, Fig. 3). PCo2 was positively associated with site connectivity and subadult heart rate, and negatively associated with aggressiveness. The aggressiveness effect occurred at the within-site level and all others, at the among-site level (Table 2.3, Fig. 3). Associations between host and landscape traits and PCo3 were weaker than those with PCo1 and PCo2. PCo3 was associated with habitat, with voles from dense mixedwood forests harbouring higher PCo3 values (Table 2.3, Fig. 3). PCo3 was also negatively associated with female body size, population density, and heart rate. The heart rate effect and the interaction with sex and body size occurred at within- and among-site levels and all others, at the among-site level (Table 2.3, Fig. 3). No associations with GM composition were found for

exploration, genotype, habitat structure, or landscape type. However, the association with site connectivity and habitat may indicate an association with landscape type as these three factors were correlated (Fig.S3). Contrary to mice, vole GM Shannon's diversity correlated with landscape traits. Voles from islands and more connected and enclosed sites with more woody debris had higher diversity (Table 2.4).

2.4.4 GM taxa linked to aggressiveness

In both species, aggressive and docile individuals differed with respect to 16 ASVs (DESEQ2 test; adjusted p-values < 0.05). These ASVs were different in each host species but largely belonged to similar families (Bacteroidales_S24-7, Lachnospiraceae, Clostridiales_vadinBB60; Table 2.5).

2.5 Discussion

Consistent with our hypotheses, various host, habitat and landscape attributes were associated with GM composition in mice and voles, including aggressiveness. We also found striking differences in these associations between host species.

2.5.1 Behaviour and the GM

Aggressiveness explained a small proportion of within-site variance in mouse and vole GM compositions. While behaviour may influence a host's contact with microbial sources which can impact the GM, contact with predators (which is akin to our assay) is unlikely to permanently alter a prey's GM. Since mice and voles in our study shared few ASVs, taxa obtained by mice and voles from their predators are unlikely to be adapted and competitive within the GMs of their prey. Metacommunity theory postulates that less competitive species require continued transmission to sustain their populations (Leibold *et al.*, 2004). It is, therefore, likely that aggressiveness covaries with another host trait that selects on the GM, or components of behaviour and the GM are under similar genetic control. Aggressiveness was associated with similar GM compositional gradients in mice and voles, and with a handful of different but taxonomically related ASVs. Such

associations could indicate codivergence and potentially coevolution (O'Brien *et al.*, 2019). This merits further investigation by comparing divergence times of host and behaviour-associated ASVs. Given the potentially bidirectional relationship between behaviour and the microbiome, these ASVs could play a role in modulating the aggressive phenotype and represent targets for investigating mechanisms underlying the GM-behaviour relationship.

Alternatively, by extending the interpretation of aggressiveness to encompass social aggression, transmission of bacteria via social contacts could explain our results if aggressive encounters are more likely among aggressive individuals and non-aggressive contacts among docile individuals. This would also explain the sex-dependent association with aggressiveness in mice but not voles. Both species exhibit aggressiveness during the reproductive period (Mihok, 1981 ; Wood *et al.*, 2010). Male-male aggression facilitates access to females and females are aggressive towards females and heterospecifics to protect nests (Dewsbury, 1981 ; Dracup *et al.*, 2016 ; Mihok, 1981). However, female mice are rarely aggressive towards males (Dewsbury, 1981), while female voles show greater interindividual variation in aggressiveness towards males (McGuire, 1997). Thus, bacterial transmission via aggressive contacts between males and females is likely less frequent in mice than in voles. Finally, although we found no association between exploration and GM structure, such an association may simply be weak or occur across a different gradient of GM variation not captured by our analyses.

2.5.2 Within-population effects on GM composition

In addition to aggressiveness, body size of mice and age, heart rate, and female body size of voles explained within-site variance in GM composition. Larger mice and voles had relatively more Bacteroidales_S24-7 mirroring studies showing an association between BMI/weight and a Bacteroidetes/Firmicutes ratio (Ley *et al.*, 2006 ; Turnbaugh *et al.*, 2007). Some authors report more Bacteroidetes with obesity (Gao *et al.*, 2018), while others report more Firmicutes (Ley *et al.*, 2006). Here, larger mice had relatively fewer Lachnospiraceae and Ruminococcaceae, both of

which break down carbohydrates and Lachnospiraceae impair lipid and glucose metabolism (Vacca *et al.*, 2020), which could influence digestion and consequently body size. Both groups are associated with social deficiencies and depression in rodents (De Palma *et al.*, 2015 ; Golubeva *et al.*, 2017 ; Reichelt *et al.*, 2018). Additionally, mice fed a diet deficient in Omega-3 fatty acids had GMs with relatively more Lachnospiraceae and fewer Bacteroidales_S24-7, and exhibited depressive and social deficiency behaviours (Robertson *et al.*, 2017). This is consistent with behaviour and body size associating with both these groups in mice, indicating that diet could play an important role in regulating the GM, body size, and behaviour in this species. In voles, larger females had relatively more Peptococcaceae and Ruminococcaceae and fewer Clostridiales (vadin BB60). In humans, females have more Ruminococcaceae (Gao *et al.*, 2018) than males and, in mice, pregnancy changes GM proportions of Clostridia, Ruminococcaceae and Bacteroides (Wallace *et al.*, 2018). The interaction between sex and body size may have resulted from larger voles being pregnant, though it is unclear why patterns of size and sex differ between species.

Age and heart rate associated with GM composition in voles but not mice. Associations between age and the GM are well established (Yatsunenko *et al.*, 2012). Since our analyses only included adults and subadults, the lack of an age effect in mice could indicate a more rapid stabilization of the mouse GM. This could result from differences between mice and voles in their rates of physiological or immunological development, though information on these differences is lacking. If development of gut function is slower in voles, young may depend more on the GM for metabolic performance while the gut matures to achieve greater functional independence, as proposed for some primates (Pafco *et al.*, 2019). Alternatively, a greater proportion of the GM in mice may be regulated via genetics/immunity leading to innate GM stability, but our genomic analyses offer insufficient resolution to assess this. Similarly, GM association with heart rate in voles and not mice may stem from species differences in their physiological reactions to stress (Harper et Austad, 2004). Additionally, cardiovascular disease is associated with the GM in humans (Bu et Wang, 2018), and voles are more sensitive to GM perturbations than mice (Jameson *et al.*, 2020), potentially indicating stronger regulation of host physiology or health by the vole GM.

2.5.3 Among-site effects on GM composition

Most host traits associated with GM composition at the among-site level, of which the most important was genetic distance (Genotype-PC1). Translocation experiments have shown a weak to no effect of genotype on the GM of wild deer mice from the same population (Schmidt *et al.*, 2019), but GMs of offspring from wild house mice from different populations raised in the laboratory retain population signatures (Suzuki *et al.*, 2019). Our results agree with these findings and suggest a retention of stronger genetic signatures at larger spatial scales. While genetic distance of voles varied with site, it was not associated with GM structure. The different association with genotype between mice and voles could stem from SNPs providing greater accuracy in resolving genotypic differences than microsatellites (Morin *et al.*, 2004). Although genotype in mice was also correlated with landscape type, forest type, site connectivity, and population density, it was a better predictor of GM composition. Nevertheless, we cannot discount the potentially weaker contributions to the mouse GM by these other influences. Indeed, forest type best explained a gradient of mouse GM dominated by Prevotellaceae. In humans, Prevotella are associated with nutrient breakdown and diet (Fielding *et al.*, 2019 ; Precup et Vodnar, 2019) so habitat may impact the GM through diet.

Contrary to mice, population density and connectivity best predicted site-level microbiome variation in voles, but still accounted for relatively little variance in GM composition. Population density and connectivity can influence contacts among individuals (Borremans *et al.*, 2017) and potentially impact GM composition through bacterial dispersal. Alternatively, site connectivity was also correlated with landscape type and forest type, so closely connected sites may harbour similar dietary resources leading to diet-induced similarities in GM composition. In voles, the axis of GM variation associated with site connectivity was also associated with subadult heart rate. More connected sites harboured denser mixed forest and lower population density (see Fig. S3). These conditions may provide subadults with optimal refuge from predators and competitors and increased dispersal capacity, ultimately affecting their physiological state (Harper et Austad, 2004) and GM.

2.5.4 Effects on GM Diversity

Vole GM diversity correlated mostly with site-level attributes. Island biogeography and metacommunity theory predict falling species diversity with isolation (Leibold *et al.*, 2004 ; MacArthur et Wilson, 1967b). This is partly reflected in our results with diversity increasing with site connectedness, but surprisingly, voles had greater GM diversity on island than mainland sites. Juette et al. (Juette *et al.*, 2020) showed that, in our study area, island mouse and vole populations were denser and skewed towards one of the two species, potentially favouring low interspecific and high intraspecific competition. This could broaden niche widths of island occupants or populations (Bolnick *et al.*, 2010) leading to greater diversity of host habitats, diets and thus GMs. If environmental conditions and resources are less predictable on islands, greater GM diversity may offer a selective advantage to island occupants. Vole GM diversity was also greater in areas with reduced deadwood and canopy cover. Reduced canopy and debris can favour greater plant diversity and increase dietary and thus GM diversity. Finally, GM diversity did not associate with behaviour. This and our differential abundance analysis results are consistent with host behaviour associating with few bacterial taxa. Phylogenetic relationships between bacterial taxa associated with behaviour from different host species may help reveal evolutionary patterns in animal behaviour.

The GM has been shown to directly impact host behaviour in laboratory settings, yet this association is not well studied in the wild where multiple intrinsic and extrinsic factors could drown out associations with behaviour. Our multi-scale, multi-factorial field study confirms an association between GM composition and aggressiveness of mice and voles. Additionally, we show how associations with host traits and GM vary with species and spatial scale and that associations with behaviour are relatively weak and likely occur at within-population scales. Future studies should aim to understand how these associations are influenced by diet, season, and host phylogeny.

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Tableau 2.1 Predicted relationships and their rationale between GM alpha diversity and 11 environmental and host factors.

	Variable	α diversity	Explanation	Reference
Intrinsic factors	Sex	+	Males have larger home ranges facilitating contact with a greater diversity of conspecifics, habitats and foods.	[36, 37]
	Age	+	Adults have a more diverse diet and associate with more conspecifics and environmental elements.	[19, 22–24]
	Body size	?	Obesity is linked to greater diversity but greater diversity in the wild may afford a better body condition and growth.	[27, 38]
	Exploration rate	+	Fast explorers should disperse further and associate with a greater diversity of conspecifics, habitats and foods.	[30]
	Boldness (Anxiety-related behaviour)	+	Bolder individuals engage in riskier activity, leading them to associate with a greater diversity of conspecifics, habitats and foods.	[30]
	Aggressiveness	+	Aggressive individuals are more likely to engage in active defense against intruders and predators, which may increase the likelihood of ingesting bacteria from a predator or opponent.	
	Heart rate	+	Heart rate reveals differences along the slow-fast continuum with high heart rate corresponding to aggressive and fast explorers.	[34, 35]
Extrinsic factors	Habitat	+	More complex habitats harbour greater diversity of bacteria and food resources.	[29]
	Landscape type (island/ mainland)	+	More isolated sites should harbour lower species diversity.	[39]
	Site connectivity	+	Closely connected sites facilitate the transfer of bacteria between hosts and environments from each site.	[40, 41]
	Population density	-	A greater density restricts movement and home ranges of individuals, restricting contact with different bacterial sources.	[37]

Tableau 2.2 Results from mixed models testing for a relationship between the gut microbiome of mice (P. maniculatus), expressed as the scores of the first three axes of a principal coordinates analysis on Bray-Curtis distances, and various host traits including behaviour. Marginal and conditional R-squared values are provided for each best model and for models where a variable (the corresponding variable in the table) was removed from the model. P-values are corrected for false discovery rate (Padj) and significant p-values are in bold.

Variable	Estimate	SE	df	t	Padj	LCL	UCL	R^2m	R^2c
PCo1 ~ Aggressivity + Sex + Body Size + Aggressivity:Sex								0.130	0.156
(Intercept)	0.002	0.019	39.72	0.11	0.915	-0.036	0.039		
Aggressiveness	0.013	0.018	114.53	0.73	0.585	-0.022	0.048	0.067	0.108
Sex(M)	0.006	0.025	114.13	0.23	0.859	-0.045	0.055	0.084	0.113
Body Size	0.034	0.013	110.03	2.63	**0.041**	0.009	0.060	0.080	0.107
Aggressivity : Sex(M)	-0.065	0.025	115.62	-2.57	**0.041**	-0.114	-0.016	0.084	0.114
Site (random effect)	*Variance (SD) = 0.00057 (0.0239)*			*Residual Variance (SD) = 0.01853 (0.1361)*					
PCo2 ~ Sex + Age + Genotype-PC1 + Genotype-PC2 + Date + Sex:Genotype-PC2								0.749	0.885
(Intercept)	0.023	0.017	26.60	1.31	0.293	-0.027	0.021		
Sex(M)	-0.004	0.008	104.56	-0.50	0.748	-0.019	0.011	0.738	0.881
Age(S)	-0.026	0.013	109.93	-1.98	0.085	0.000	0.051	0.749	0.880
Genotype-PC1	0.084	0.012	17.44	7.10	**0.000**	0.061	0.107	0.237	0.891
Genotype-PC2	0.002	0.011	33.55	0.22	0.859	-0.018	0.022	0.737	0.881
Date	0.045	0.012	10.29	3.67	**0.020**	0.023	0.068	0.538	0.863
Sex(M) : Genotype-PC2	-0.019	0.008	104.78	-2.46	0.050	-0.034	-0.004	0.737	0.880
Site (random effect)	*Variance (SD) = 0.00187 (0.0433)*			*Residual Variance (SD) = 0.00159 (0.0399)*					
log10(PCo3+0.5) ~ Aggressivity + Sex + Age + Genotype-PC2 + Body Size + Habitat + Population Density + Date + Sex:Genotype-PC2 + Aggressivity:Age								0.587	0.855
(Intercept)	-0.315	0.021	21.81	-15.14	0.000	-0.352	-0.279		
Aggressivity	0.007	0.008	103.38	0.91	0.506	-0.007	0.022	0.564	0.849
Sex(M)	-0.003	0.008	102.32	-0.39	0.806	-0.018	0.012	0.589	0.851
Age(S)	0.012	0.014	104.49	0.82	0.547	-0.015	0.041	0.559	0.853
Genotype-PC2	0.030	0.013	85.02	2.32	0.060	0.006	0.054	0.581	0.848
Body Size	0.010	0.005	103.81	2.22	0.061	0.001	0.019	0.578	0.850
Forest Type	-0.096	0.024	11.66	-3.99	**0.011**	-0.138	-0.054	0.305	0.879
Population Density	0.066	0.027	12.27	2.48	0.061	0.020	0.112	0.481	0.861
Date	0.080	0.016	12.17	4.89	**0.003**	0.052	0.109	0.129	0.872
Sex(M) : Genotype-PC2	-0.018	0.008	102.90	-2.32	0.060	-0.032	-0.003	0.588	0.849
Aggressivity : Age(S)	-0.019	0.009	104.10	-2.15	0.062	-0.037	-0.003	0.565	0.852
Site (random effect)	*Variance (SD) = 0.00298 (0.0546)*			*Residual Variance (SD) = 0.00162 (0.0402)*					

Tableau 2.3 Results from mixed models testing for a relationship between the gut microbiome of voles (M. gapperi), expressed as the scores of the first three axes of a principal coordinates analysis on Bray-Curtis distances, and various host traits including behaviour. Marginal and conditional R-squared values are provided for each best model and for models where a variable (the corresponding variable in the table) was removed from the model. P-values are corrected for false discovery rate (Padj) and significant p-values are in bold.

Variable	Estimate	SE	df	t	Padj	LCL	UCL	R^2m	R^2c
PCo1 ~ Age + Population Density + Date + (1|Site)								0.374	0.675
(Intercept)	0.040	0.029	16.60	1.40	0.228	-0.013	0.094		
Age(S)	-0.048	0.019	122.71	-2.55	**0.041**	-0.085	-0.010	0.361	0.648
Population Density	0.100	0.028	10.22	3.57	**0.028**	0.048	0.151	0.100	0.694
Date	-0.071	0.027	10.79	-2.62	**0.048**	-0.123	-0.021	0.232	0.660
Site (random effect)	*Variance (SD) = 0.00715 (0.0845)*				*Residual Variance (SD) = 0.00771 (0.0878)*				
PCo2 ~ Aggressivity + Age + Heart Rate + Landscape Type + Connectivity + Age:Heart Rate + (1|Site)								0.210	0.316
(Intercept)	-0.003	0.032	21.38	-0.10	0.930	-0.063	0.056		
Aggressivity	-0.021	0.009	121.84	-2.34	**0.048**	-0.038	-0.003	0.183	0.282
Age(S)	0.062	0.023	123.19	2.75	**0.030**	0.020	0.107	0.150	0.304
Heart Rate	0.046	0.023	122.87	2.02	0.068	0.004	0.093	0.176	0.319
Landscape Type (Mainland)	-0.222	0.092	11.22	-2.43	0.059	-0.391	-0.054	0.131	0.308
Connectivity	0.116	0.040	11.37	2.87	**0.045**	0.042	0.190	0.095	0.310
Age(S) : Heart Rate	-0.061	0.025	122.91	-2.45	**0.045**	-0.114	-0.015	0.176	0.318
Site (random effect)	*Variance (SD) = 0.00152 (0.0390)*				*Residual Variance (SD) = 0.00983 (0.0991)*				
PCo3 ~ Sex + Genotype-PC1 + Heart Rate + Body Size + Forest Type + Population Density + Sex:Genotype-PC1 + Sex:Body Size + (1|Site)								0.251	0.288
(Intercept)	-0.006	0.014	28.12	-0.42	0.743	-0.032	0.020		
Sex(M)	0.018	0.017	118.59	1.07	0.349	-0.017	0.049	0.177	0.233
Genotype-PC1	-0.013	0.012	99.44	-1.05	0.349	-0.039	0.009	0.229	0.263
Heart Rate	-0.023	0.008	121.96	-2.68	**0.031**	-0.040	-0.007	0.212	0.262
Body Size	-0.035	0.012	122.00	-3.01	**0.023**	-0.060	-0.014	0.186	0.261
Forest Type	0.029	0.011	8.84	2.72	**0.048**	0.010	0.047	0.187	0.293
Population Density	-0.032	0.011	7.48	-2.99	**0.046**	-0.051	-0.013	0.185	0.311
Genotype-PC1 : Sex(M)	0.034	0.017	117.61	2.07	0.068	0.004	0.068	0.226	0.273
Body Size : Sex(M)	0.052	0.017	121.99	2.99	**0.023**	0.020	0.087	0.197	0.255
Site (random effect)	*Variance (SD) = 0.00044 (0.0209)*				*Residual Variance (SD) = 0.00844 (0.0919)*				

Tableau 2.4 Results from linear regression models testing for a relationship between the gut microbiome Shannon's diversity of mice and voles and various host traits including behaviour. Adjusted R-squared values are provided for the best model and for models where a variable (the corresponding variable in the table) was removed from the best model. P-values are corrected for false discovery rate (Padj) and significant p-values are in bold.

Species	Variable	Estimate	SE	df	t	Padj	LCL	UCL	Adj.R2
Mice	**log10(Shannon's Diversity) ~ Sex + Habitat Structure + Landscape Type + Connectivity + Habitat Structure:Sex**								0.054
	(Intercept)	0.678	0.004	10,110	180.92	0.000	0.670	0.685	
	Sex(M)	-0.009	0.004	10,110	-2.17	0.062	-0.017	-0.001	0.014
	Habitat Structure	0.001	0.003	10,110	0.31	0.844	-0.005	0.007	0.042
	Landscape Type (Mainland)	0.020	0.011	10,110	1.90	0.097	-0.001	0.041	0.033
	Connectivity	-0.010	0.004	10,110	-2.20	0.061	-0.019	-0.001	0.023
	Habitat Structure : Sex(M)	-0.006	0.004	10,110	-1.54	0.193	-0.015	0.002	0.043
Vole	**(Shannon's Diversity)3 ~ Exploration + Aggressiveness + Sex + Age + Heart Rate + Body Size + Habitat Structure + Landscape Type + Connectivity + Exploration:Sex + Aggressiveness:Sex + Aggressiveness:Age + Habitat Structure:Age**								0.145
	(Intercept)	######	5.063	13,117	20.102	0.000	91.754	#####	
	Exploration	2.188	3.031	13,117	0.722	0.535	-3.815	8.191	0.134
	Aggressiveness	10.743	4.827	13,117	2.225	0.053	1.182	20.303	0.129
	Sex(M)	-0.323	3.655	13,117	-0.088	0.930	-7.561	6.914	0.115
	Age(A)	-1.087	4.496	13,117	-0.242	0.860	-9.991	7.816	0.127
	Heart Rate	-4.417	1.853	13,117	-2.384	**0.046**	-8.087	-0.748	0.111
	Body Size	3.140	2.107	13,117	1.490	0.189	-1.032	7.312	0.136
	Habitat Structure	9.381	3.004	13,117	3.123	**0.023**	3.432	15.329	0.086
	Landscape Type (Mainland)	-32.471	11.855	13,117	-2.739	**0.030**	-55.948	-8.994	0.098
	Connectivity	16.759	5.222	13,117	3.209	**0.023**	6.416	27.101	0.077
	Exploration : Sex(M)	-6.243	3.820	13,117	-1.634	0.149	-13.808	1.322	0.133
	Aggressiveness : Sex(M)	-8.102	4.021	13,117	-2.015	0.068	-16.066	-0.139	0.123
	Aggressiveness : Age(S)	-5.723	4.033		-1.419	0.207	-13.711	2.264	0.137
	Habitat Structure : Age(S)	-7.502	3.680	13,117	-2.039	0.068	-14.789	-0.215	0.122

Tableau 2.5 We used DESEQ2 to identify microbial taxa that most differentiated the microbiomes of aggressive and docile male mice (Peromyscus maniculatus) and aggressive and docile voles (Myodes gapperi). ASVs are sorted by lfc (log-fold change).

	ASV	baseMean	lfc	lfcSE	stat	Padj	Phylum	Family	Genus
P. maniculatus	SV312	7.09	3.33	0.91	3.64	0.017	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV51	15.91	3.11	0.87	3.57	0.017	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV671	6.36	3.03	0.90	3.39	0.018	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV31	56.14	2.90	0.94	3.09	0.033	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV316	8.95	2.65	0.77	3.45	0.017	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV276	13.55	2.55	0.83	3.07	0.033	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV520	6.00	2.59	0.77	3.36	0.018	Bacteroidetes	NA	NA
	SV248	13.41	-3.74	1.03	-3.62	0.017	Bacteroidetes	Bacteroidales_BS11_gut_group	NA
	SV506	12.32	-2.87	0.94	-3.05	0.033	Firmicutes	Clostridiales_vadinBB60_group	NA
	SV49	17.95	-2.95	1.02	-2.90	0.045	Proteobacteria	Helicobacteraceae	Helicobacter
	SV13	62.00	-2.58	0.89	-2.89	0.045	Firmicutes	Lachnospiraceae	NK4A136
	SV304	9.27	-3.17	1.05	-3.02	0.033	Firmicutes	Lachnospiraceae	NA
	SV511	8.05	-3.24	0.98	-3.30	0.020	Firmicutes	Lachnospiraceae	NA
	SV321	8.18	-3.47	0.99	-3.51	0.017	Firmicutes	Lachnospiraceae	NK4A136
	SV327	15.14	-3.74	1.15	-3.26	0.021	Firmicutes	Lachnospiraceae	NA
	SV64	19.09	-2.73	0.78	-3.50	0.017	Firmicutes	Ruminococcaceae	NA
M. gapperi	SV245	3.43	2.18	0.66	3.31	0.035	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV61	6.80	2.32	0.69	3.36	0.033	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV379	8.63	2.36	0.74	3.18	0.046	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV534	2.85	2.42	0.65	3.70	0.014	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV400	8.18	2.47	0.73	3.37	0.033	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV105	7.43	2.62	0.68	3.85	0.012	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV1004	5.95	2.82	0.68	4.14	0.004	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV55	44.48	2.87	0.89	3.23	0.042	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV140	6.93	3.60	0.67	5.36	0.000	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV6	66.00	2.93	0.77	3.81	0.012	Firmicutes	Lachnospiraceae	NK4A136
	SV123	13.15	-2.28	0.69	-3.30	0.035	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV735	3.68	-2.24	0.66	-3.38	0.033	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV71	23.98	-2.02	0.59	-3.43	0.033	Bacteroidetes	Bacteroidales_S24-7_group	NA
	SV186	6.53	-2.53	0.68	-3.71	0.014	Firmicutes	Clostridiales_vadinBB60_group	NA
	SV20	39.75	-3.79	0.91	-4.15	0.004	Firmicutes	Lachnospiraceae	NK4A136
	SV63	37.53	-3.95	0.77	-5.10	0.000	Tenericutes	Mycoplasmataceae	Mycoplasma

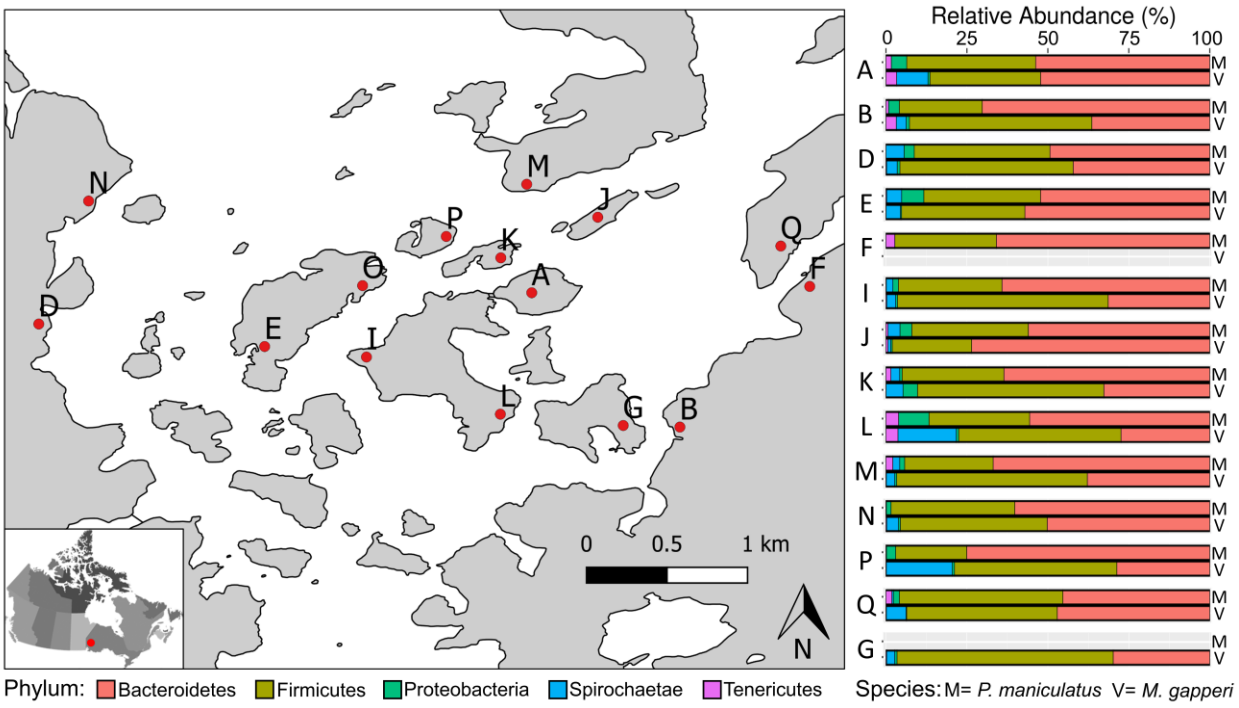

Figure 2.1 Left: Map of the study area in Western Ontario with specific sample sites indicated by red dots and site names indicated by letters. Right: Composition of the microbiome at each sample site for each species represented by the average relative abundance of each major phylum. The sum of the phyla was rescaled to equal 100.

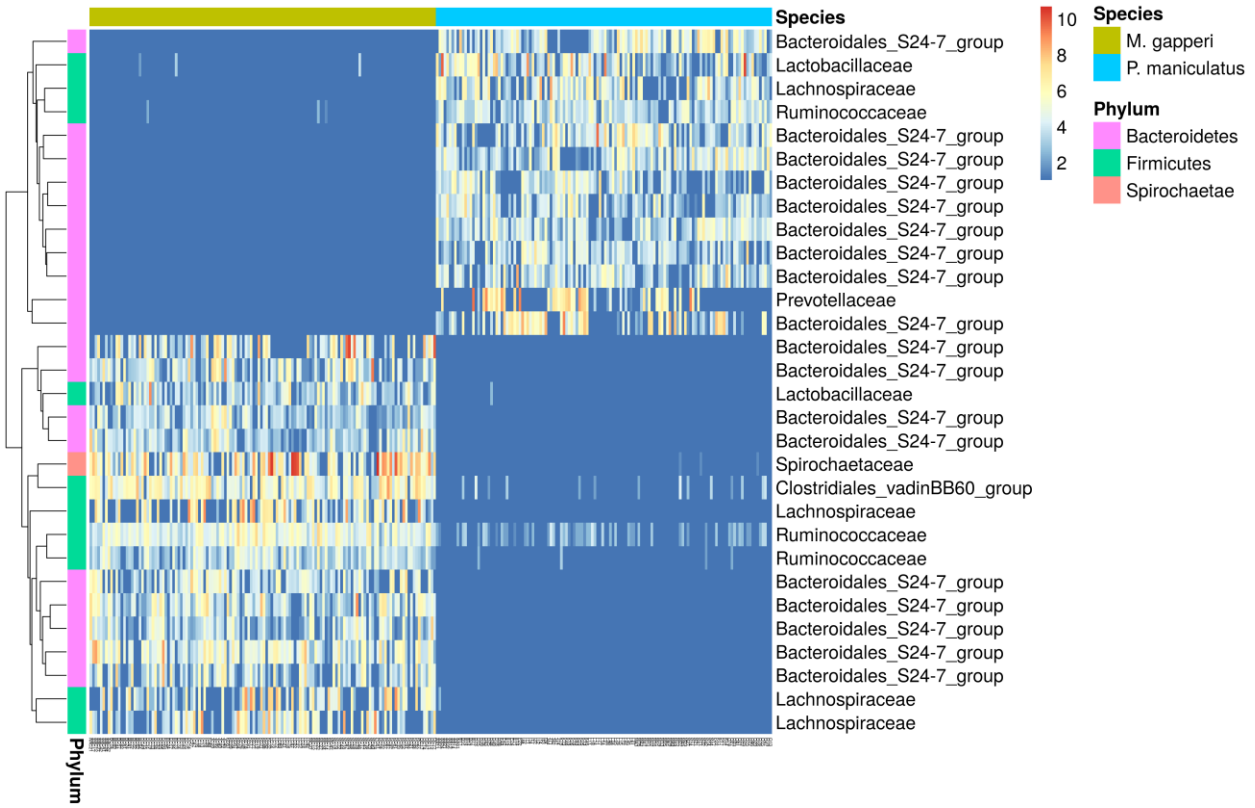

Figure 2.2 We used DESEQ2 to identify microbial taxa that most differentiated the microbiomes of mice (P. maniculatus) and voles (M. gapperi). The log2 transformed normalized counts of the 30 most significant ASVs (identified at the family level) are represented in the above heatmap by a colour gradient (blue are least abundant and red are most abundant).

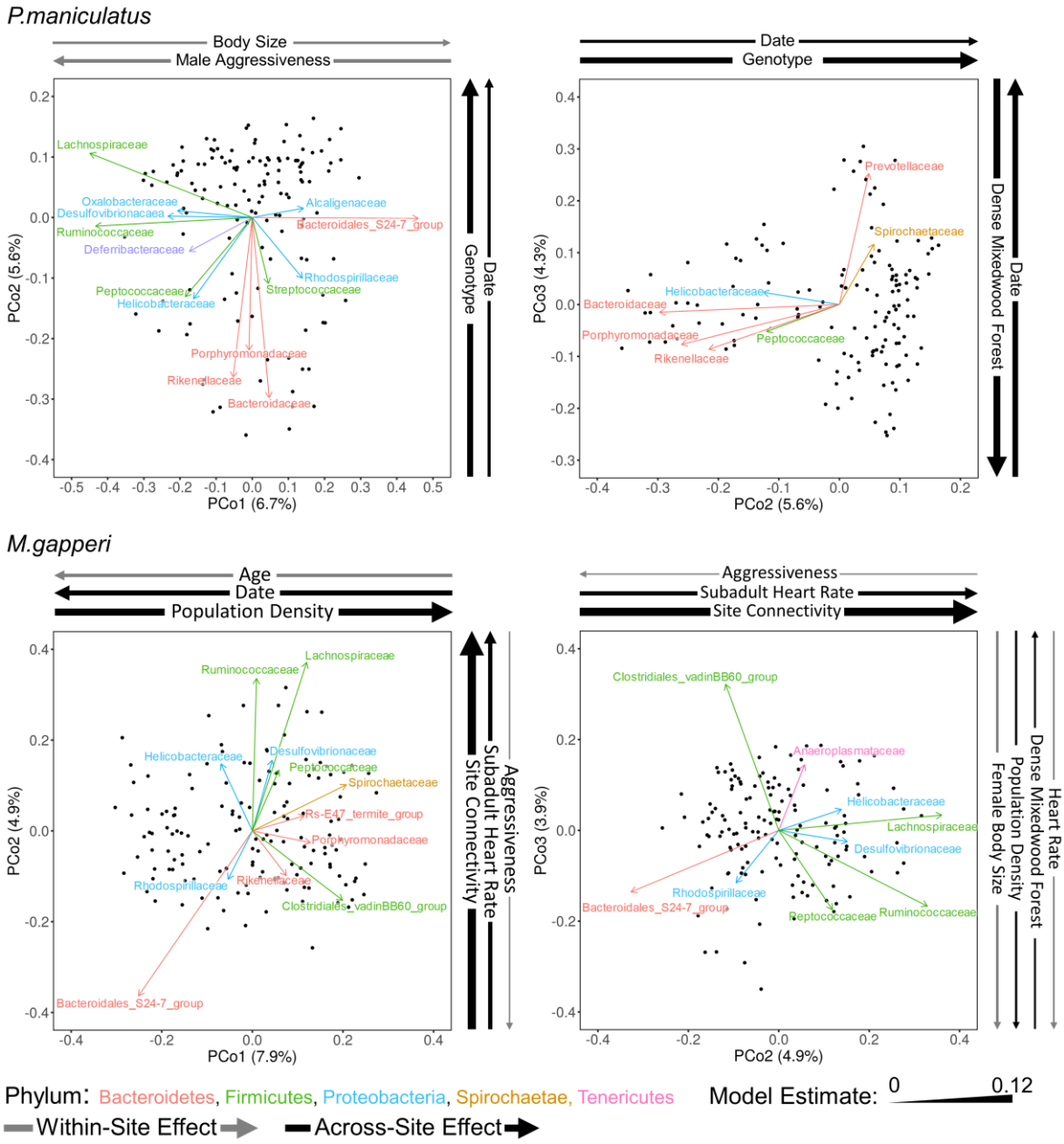

Figure 2.3 Ordination plots of the first three axes (left: 1 vs 2, right: 2 vs 3) of separate principal coordinates analyses on Bray-Curtis distances of the gut microbiome compositions of mice (P. maniculatus) and voles (M. gapperi). Microbial families significantly correlated with the ordination axes were identified by an envfit analysis and plotted as vectors whose direction and length represent the strength of the correlation with each axis. Their colour indicates their phylum. Host traits, and vegetation and landscape features, correlated with each axis (described in Tables

2.1 and 2.2) are indicated along the outside the axes with arrows whose direction represents the sign of the correlation, whose width represents the strength of the model estimate and whose colour represents the predominant spatial scale of the effect.

2.7 Supplementary information

Tableau S2.1 Summary of landscape characteristics estimated for each island and mainland site in the Minaki area, north-western Ontario, Canada

Site	Type	Area *(ha)*	Distance nearest island *(m)*	Distance nearest mainland shore *(m)*	S_i *(α=1)*	Kernel *(α=1)*
A	Island	15.59	23.95	1058.40	8786.41	7.89
E - O	Island	59.99	132.18	853.46	31657.14	8.71
G	Island	29.98	95.88	148.06	17095.05	6.22
I - L	Island	70.53	23.95	501.83	38550.78	9.80
J	Island	7.19	226.73	1263.35	4174.26	6.42
K	Island	7.72	120.23	1573.15	4429.10	7.82
M	Island	316.43	13.93	763.36	93183.75	6.79
P	Island	8.24	120.23	1778.74	4453.81	7.04
Q	Island	60.06	495.85	72.87	34683.81	4.79
F	Mainland	NA	363.11	NA	138140.47	3.33
B	Mainland	NA	239.55	NA	171161.99	4.42
N	Mainland	NA	301.78	NA	153034.84	3.58
D	Mainland	NA	747.50	NA	170328.25	3.63

Tableau S2.2 AICc values of full and final selected models testing for a relationship between the gut microbiome of mice (P. maniculatus) and various host traits including behaviour. Separate linear mixed models were run with the scores of the first three axes of a principal coordinates analysis on Bray-Curtis distances of mouse GMs (PCo1, PCo2 and PCo3) as dependent variables and host identity as a random effect. Linear regression was used to test for an association between the same explanatory factors and GM Shannon's diversity. All final models were selected by AIC-based backwards stepwise selection (R packages step [linear mixed models] and stepAIC [linear regression models]).

Dependent Variable	Full and Final Models	AICc
PCo1	PCo1 ~ Exploration + Aggressiveness + Sex + Age + Genotype-PC1 + Genotype-PC2 + Heart Rate + Body Size + Habitat Structure + Landscape Type + Site Connectivity + Forest Type + Habitat Diversity + Population Density + Date + Exploration:Sex + Aggressiveness:Sex + Age:Sex + Genotype-PC1:Sex + Genotype-PC2:Sex + Heart Rate:Sex + Body Size:Sex + Habitat Structure:Sex + Exploration:Age + Aggressiveness:Age + Genotype-PC1:Age + Genotype-PC2:Age + Heart Rate:Age + Body Size:Age + Habitat Structure:Age + (1|site)	99.7
	PCo1 ~ Aggressiveness + Sex + Body Size + Aggressiveness:Sex + (1|site)	-94.6
PCo2	PCo2 ~ Exploration + Aggressiveness + Sex + Age + Genotype-PC1 + Genotype-PC2 + Heart Rate + Body Size + Habitat Structure + Landscape Type + Site Connectivity + Forest Type + Habitat Diversity + Population Density + Date + Exploration:Sex + Aggressiveness:Sex + Age:Sex + Genotype-PC1:Sex + Genotype-PC2:Sex + Heart Rate:Sex + Body Size:Sex + Habitat Structure:Sex + Exploration:Age + Aggressiveness:Age + Genotype-PC1:Age + Genotype-PC2:Age + Heart Rate:Age + Body Size:Age + Habitat Structure:Age + (1|site)	-118.1
	PCo2 ~ Sex + Age + Genotype-PC1 + Genotype-PC2 + Date + Genotype-PC2:Sex + (1|site)	-341.5
PCo3	log10(PCo3+0.5) ~ Exploration + Aggressiveness + Sex + Age + Genotype-PC1 + Genotype-PC2 + Heart Rate + Body Size + Habitat Structure + Landscape Type + Site Connectivity + Forest Type + Habitat Diversity + Population Density + Date + Exploration:Sex + Aggressiveness:Sex + Age:Sex + Genotype-PC1:Sex + Genotype-PC2:Sex + Heart Rate:Sex + Body Size:Sex + Habitat Structure:Sex + Exploration:Age + Aggressiveness:Age + Genotype-PC1:Age + Genotype-PC2:Age + Heart Rate:Age + Body Size:Age + Habitat Structure:Age + (1|site)	-118.6
	log10(PCo3+0.5) ~ Aggressiveness + Sex + Age + Genotype-PC2 + Body Size + Forest Type + Population Density + Date + Genotype-PC2:Sex + Aggressiveness:Age + (1|site)	-298.9
Shannon's Diversity	log10(Shannon's Diversity) ~ Exploration + Aggressiveness + Sex + Age + Heart Rate + Body Size + Habitat Structure + Landscape Type + Site Connectivity + Forest Type + Habitat Diversity + Population Density + Date + Exploration:Sex + Aggressiveness:Sex + Age:Sex + Heart Rate:Sex + Body Size:Sex + Habitat Structure:Sex + Exploration:Age + Aggressiveness:Age + + Heart Rate:Age + Body Size:Age + Habitat Structure:Age	-523.6
	log10(Shannon's Diversity) ~ Sex + Habitat Structure + Landscape Type + Connectivity + Habitat Structure:Sex	-567.3

Tableau S2.3 AICc values of full and final selected models testing for a relationship between the gut microbiome of vole (M. gapperi) and various host traits including behaviour. Separate linear mixed models were run with the scores of the first three axes of a principal coordinates analysis on Bray-Curtis distances of mouse GMs (PCo1, PCo2 and PCo3) as dependent variables and host identity as a random effect. Linear regression was used to test for an association between the same explanatory factors and GM Shannon's diversity. All final models were selected by AIC-based backwards stepwise selection (R packages step [linear mixed models] and stepAIC [linear regression models]).

Dependent Variable	Full and Final Models	AICc
PCo1	PCo1 ~ Exploration + Aggressiveness + Sex + Age + Genotype-PC1 + Genotype-PC2 + Heart Rate + Body Size + Habitat Structure + Landscape Type + Site Connectivity + Forest Type + Habitat Diversity + Population Density + Date + Exploration:Sex + Aggressiveness:Sex + Age:Sex + Genotype-PC1:Sex + Genotype-PC2:Sex + Heart Rate:Sex + Body Size:Sex + Habitat Structure:Sex + Exploration:Age + Aggressiveness:Age + Genotype-PC1:Age + Genotype-PC2:Age + Heart Rate:Age + Body Size:Age + Habitat Structure:Age + (1|site)	11.3
	PCo1 ~ Age + Population Density + Date + (1|site)	-204.6
PCo2	PCo2 ~ Exploration + Aggressiveness + Sex + Age + Genotype-PC1 + Genotype-PC2 + Heart Rate + Body Size + Habitat Structure + Landscape Type + Site Connectivity + Forest Type + Habitat Diversity + Population Density + Date + Exploration:Sex + Aggressiveness:Sex + Age:Sex + Genotype-PC1:Sex + Genotype-PC2:Sex + Heart Rate:Sex + Body Size:Sex + Habitat Structure:Sex + Exploration:Age + Aggressiveness:Age + Genotype-PC1:Age + Genotype-PC2:Age + Heart Rate:Age + Body Size:Age + Habitat Structure:Age + (1|site)	29.2
	PCo2 ~ Aggressiveness + Age + Heart Rate + Landscape Type + Site Connectivity + Heart Rate:Age + (1|site)	-166.5
PCo3	PCo3 ~ Exploration + Aggressiveness + Sex + Age + Genotype-PC1 + Genotype-PC2 + Heart Rate + Body Size + Habitat Structure + Landscape Type + Site Connectivity + Forest Type + Habitat Diversity + Population Density + Date + Exploration:Sex + Aggressiveness:Sex + Age:Sex + Genotype-PC1:Sex + Genotype-PC2:Sex + Heart Rate:Sex + Body Size:Sex + Habitat Structure:Sex + Exploration:Age + Aggressiveness:Age + Genotype-PC1:Age + Genotype-PC2:Age + Heart Rate:Age + Body Size:Age + Habitat Structure:Age + (1|site)	10.0
	PCo3 ~ Sex + Genotype-PC1 + Heart Rate + Body Size + Forest Type + Population Density + Genotype-PC1:Sex + Body Size:Sex + (1|site)	-169.1
Shannon's Diversity	(Shannon's Diversity)^3 ~ Exploration + Aggressiveness + Sex + Age + Heart Rate + Body Size + Habitat Structure + Landscape Type + Site Connectivity + Forest Type + Habitat Diversity + Population Density + Date + Exploration:Sex + Aggressiveness:Sex + Age:Sex + Heart Rate:Sex + Body Size:Sex + Habitat Structure:Sex + Exploration:Age + Aggressiveness:Age + Heart Rate:Age + Body Size:Age + Habitat Structure:Age + (1|site)	1198.4
	(Shannon's Diversity)^3 ~ Exploration + Aggressiveness + Sex + Age + Heart Rate + Body Size + Habitat Structure + Landscape Type + Connectivity + Exploration:Sex + Aggressiveness:Sex + Aggressiveness:Age + Habitat Structure:Age	1169.7

Tableau S2.4 Final models testing for a relationship between gut microbiome beta diversity and host and landscape traits in mice (P. maniculatus) and voles (M. gapperi) were compared to models that included only the random variable (site), to test the effect of the random variable in the models. Results are output by the rand() function in the R package lmerTest. Dependent variables in each model were scores of the first three axes of a principal coordinates analysis on Bray-Curtis distances for each species.

Species	Final model	npar	logLik	AIC	LRT	Df	P
P. maniculatus	PCo1 ~ Aggressiveness + Sex + Body Size + Aggressiveness:Sex + (1|site)	6	54.47	-96.93	0.62	1	0.430
	PCo2 ~ Sex + Age + Genotype-PC1 + Genotype-PC2 + Date + Genotype-PC2:Sex + (1|site)	8	160.82	-305.63	39.46	1	<0.001
	log10(PCo3+0.5) ~ Aggressiveness + Sex + Age + Genotype-PC2 + Body Size + Forest Type + Population Density + Date + Genotype-PC2:Sex + Aggressiveness:Age + (1|site)	12	135.95	-247.89	56.44	1	<0.001
M. gapperi	PCo1 ~ Age + Population Density + Date + (1|site)	5	81.05	-152.10	55.22	1	<0.001
	PCo2 ~ Aggressiveness + Age + Heart Rate + Landscape Type + Site Connectivity + Heart Rate:Age + (1|site)	8	89.93	-163.86	6.14	1	0.013
	PCo3 ~ Sex + Genotype-PC1 + Heart Rate + Body Size + Forest Type + Population Density + Genotype-PC1:Sex + Body Size:Sex + (1|site)	10	96.28	-172.55	0.72	1	0.395

Tableau S2.5 Results from mixed models testing for a relationship between genotype and geographic location (scaled utm coordinates) of mice (P. maniculatus) and voles (M. gapperi). Genotype is given as the scores of the first two axes of allelic frequencies from SNP (SNP.PC1, SNP.PC2) and microsatellite (MSAT.PC1, MSAT.PC2) data for mice and microsatellite data for voles. Marginal and conditional R-squareds are provided for the whole models and for models where a single variable from the whole model was removed. Significant p-values are bolded.

Species	Variable	Estimate	Std.Error	df	t	p	R^2m	R^2c
P. maniculatus	**SNP.PC1 ~ x + y + (1|site)**						0.256	0.969
	(Intercept)	-0.006	0.016	10.16	-0.35	0.731		
	x	0.034	0.014	14.74	2.44	**0.028**	0.032	0.962
	y	0.006	0.011	19.04	0.57	0.579	0.210	0.964
	SNP.PC2 ~ x + y + (1|site)						0.568	0.927
	(Intercept)	-0.001	0.009	7.89	-0.15	0.888		
	x	-0.033	0.009	10.29	-3.61	**0.005**	0.012	0.917
	y	-0.026	0.008	13.05	-3.26	**0.006**	0.177	0.932
	MSAT.PC1 ~ x + y + (1|site)						0.174	0.783
	(Intercept)	0.027	0.035	10.14	0.77	0.459		
	x	0.055	0.035	12.61	1.59	0.138	0.026	0.773
	y	0.035	0.031	14.69	1.13	0.278	0.099	0.754
	MSAT.PC2 ~ x + y + (1|site)						0.146	0.473
	(Intercept)	-0.008	0.023	9.88	-0.32	0.753		
	x	-0.045	0.024	10.94	-1.89	0.086	0.019	0.463
	y	-0.021	0.022	11.24	-0.97	0.355	0.112	0.447
M. gapperi	**MSAT.PC1 ~ x + y + (1|site)**						0.203	0.371
	(Intercept)	-0.004	0.018	9.97	-0.24	0.818		
	x	0.043	0.019	10.12	2.30	**0.044**	0.084	0.355
	y	-0.031	0.018	10.98	-1.72	0.113	0.137	0.351
	MSAT.PC2 ~ x + y + (1|site)						0.089	0.243
	(Intercept)	0.019	0.017	9.29	1.16	0.277		
	x	0.038	0.018	9.45	2.14	0.060	0.004	0.229
	y	-0.001	0.017	10.38	-0.06	0.956	0.089	0.225

Tableau S2.6 Results from a mixed model testing for a relationship between the gut microbiome of mice (P. maniculatus), expressed as the score of the second axis of a principal coordinates analysis on Bray-Curtis distances, and various host traits including behaviour. Genotype-PC1 was removed from the analyses to assess whether its effect may be confounded with landscape variables (landscape type, forest type, site connectivity) with which it is correlated. The resulting model described below is much poorer (R2m = 0.248, AICc = -314.3) than the model including Genotype-PC1 (R2m = 0.749, AICc = -341.5). Marginal and conditional R-squared values are provided for the selected model and for models where a variable (the corresponding variable in the table) was removed from that model. P-values are corrected for false discovery rate and significant p-values are bolded.

Variable	**Estimate**	**SE**	**df**	**t**	**P**	**LCL**	**UCL**	**R^2m**	**R^2c**
PCo2 ~ Sex + Age + Genotype-PC2 + Habitat Structure + Date + Sex:Genotype-PC2 + Age:Habitat Structure								0.248	0.898
(Intercept)	0.010	0.031	16.37	0.32	0.811	-0.050	0.070		
Sex(M)	-0.003	0.008	102.66	-0.39	0.811	-0.018	0.012	0.249	0.895
Age(S)	-0.009	0.014	103.64	-0.63	0.695	-0.037	0.019	0.247	0.892
Genotype-PC2	-0.005	0.013	107.99	-0.36	0.811	-0.031	0.021	0.240	0.894
Habitat Structure	-0.037	0.014	102.43	-2.72	**0.045**	-0.064	-0.011	0.237	0.891
Date	0.063	0.025	17.43	2.54	0.057	0.016	0.112	0.030	0.884
Sex(M) : Genotype-PC2	-0.019	0.008	103.15	-2.49	**0.049**	-0.034	-0.004	0.247	0.894
Age(S) : Habitat Structure	0.036	0.014	102.29	2.50	**0.049**	0.008	0.063	0.242	0.892
Site (random effect)	*Variance (SD) = 0.01017 (0.1009)*				*Residual Variance (SD) = 0.00159 (0.0399)*				

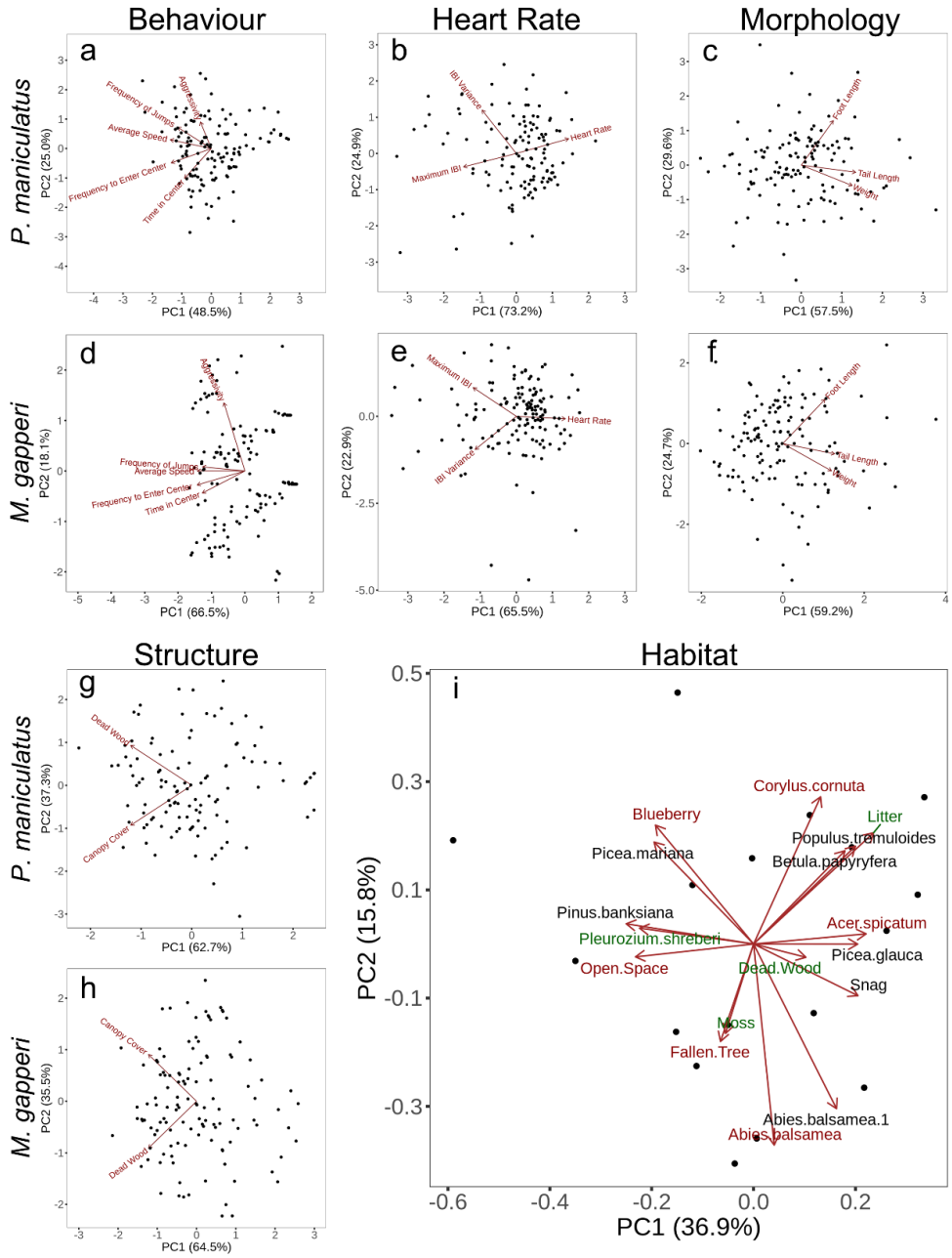

Figure S2.1 Principal coordinates analyses of variables measured to describe behaviour, heart rate, morphology, structure of the habitat around the trap in which an animal was captured, and the habitat at each site. Scores from the first one or two axes accounting for most of the variance in the data were included in mixed models examining the correlations among host traits and the gut microbiome

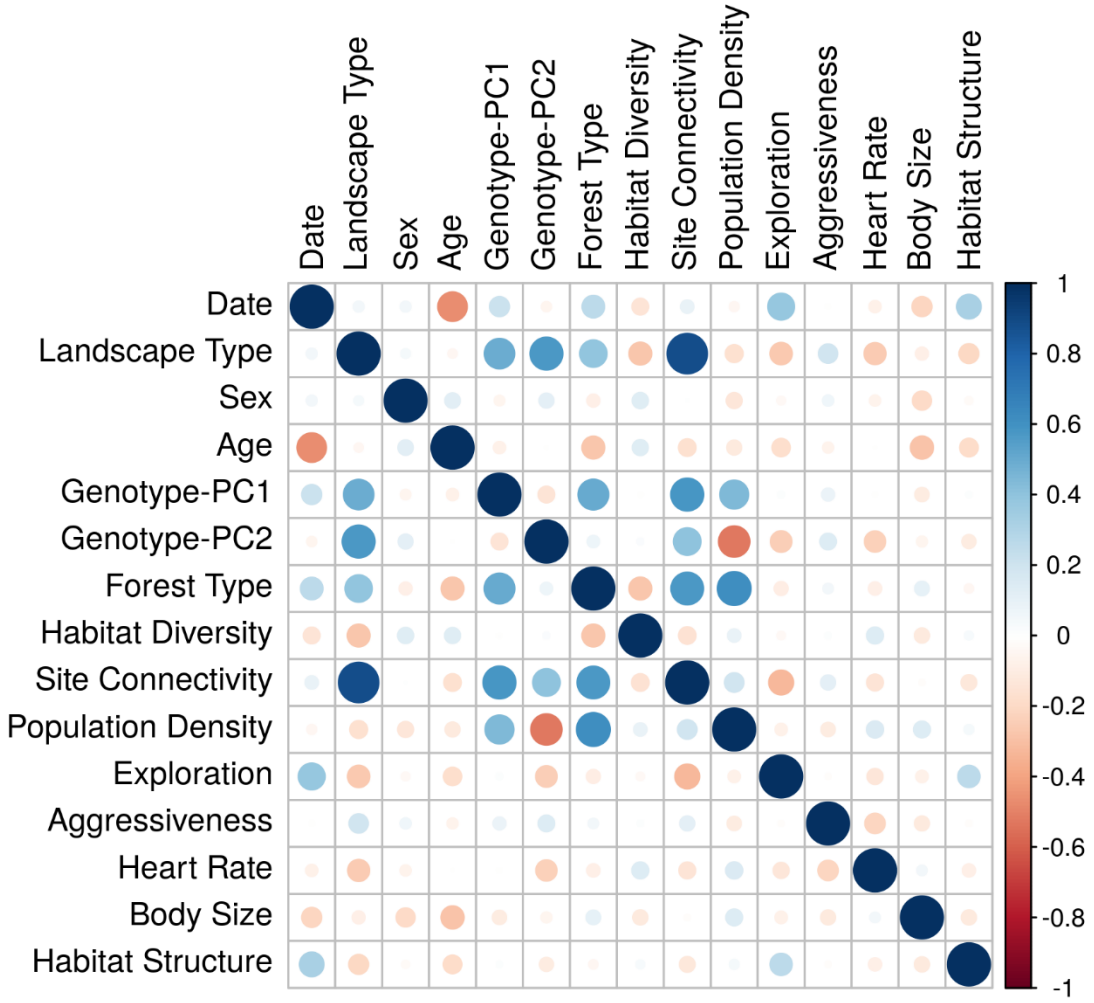

Figure S2.2 Pearson correlation matrix (index values represented by colour) of independent variables used in analyses testing for a relationship between the GM of mice and host- and site-level factors.

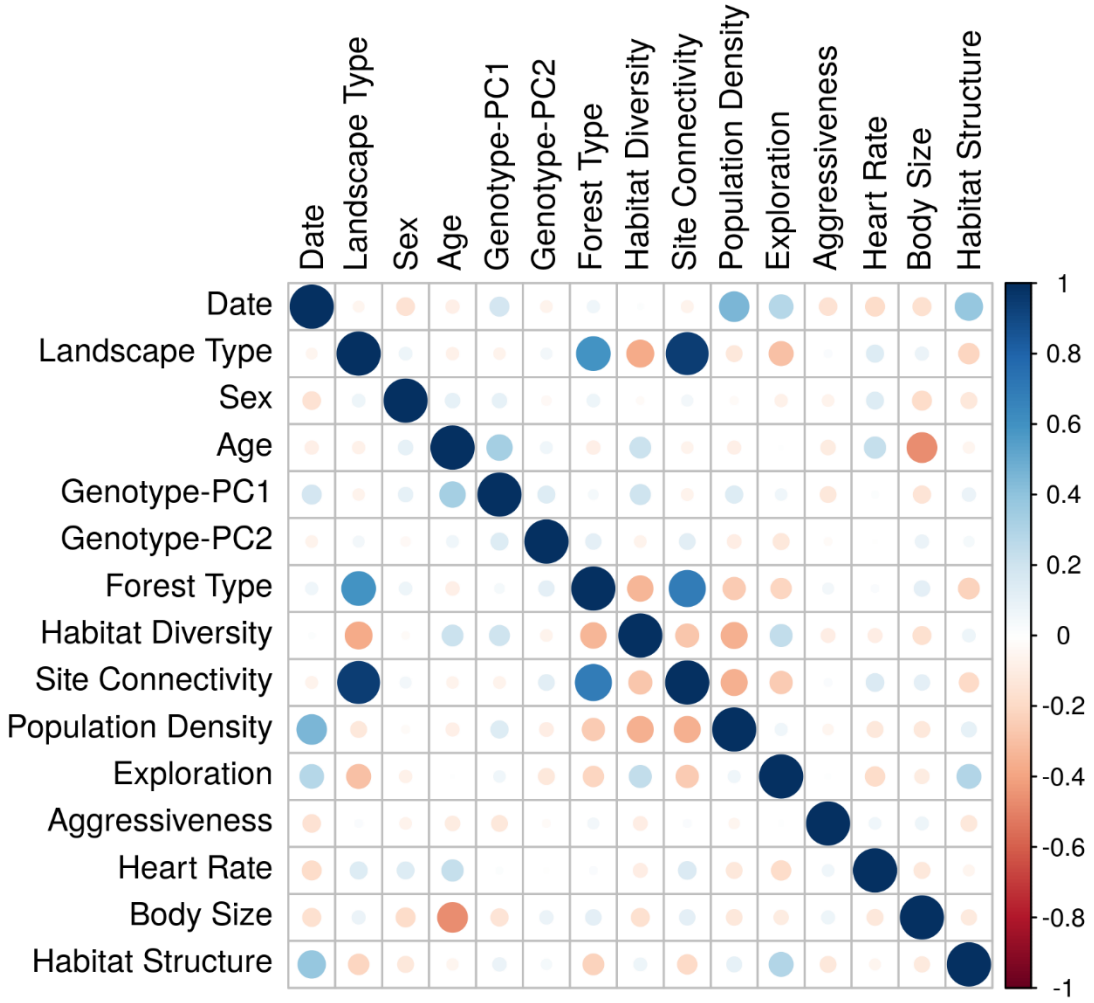

Figure S2.3 Pearson correlation matrix (index values represented by colour) of independent variables used in analyses testing for a relationship between the GM of voles and host- and site-level factors.

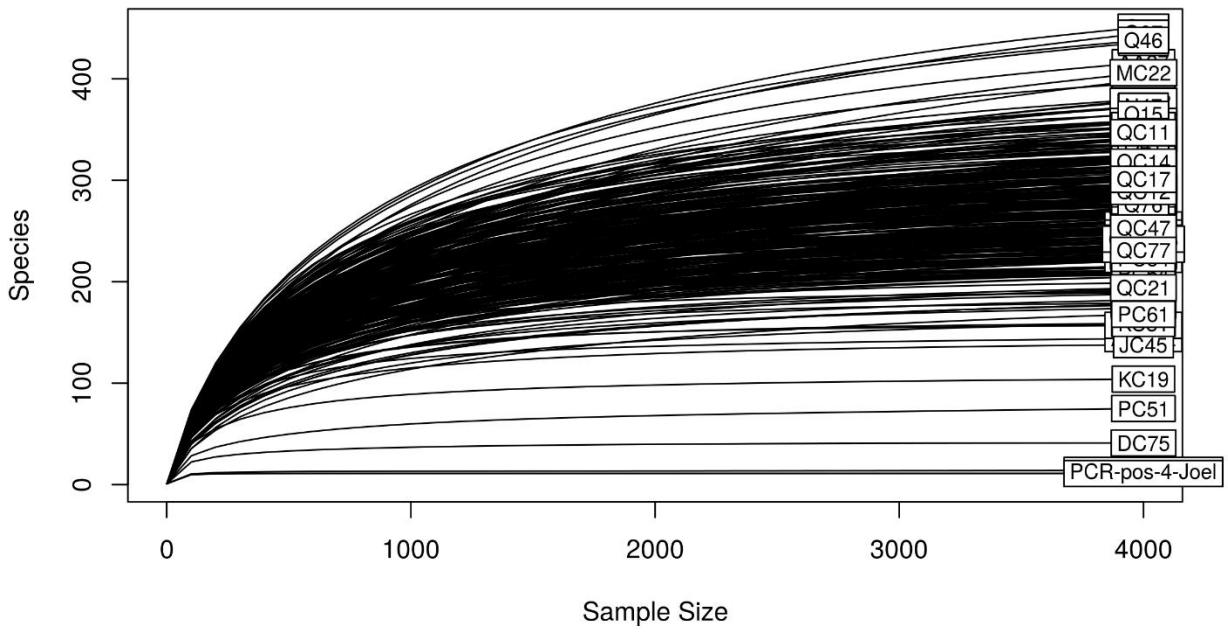

Figure S2.4 Rarefaction curves for all samples collected depicting the number of amplicon sequence variants (ASV) per number of sequences in each sample.

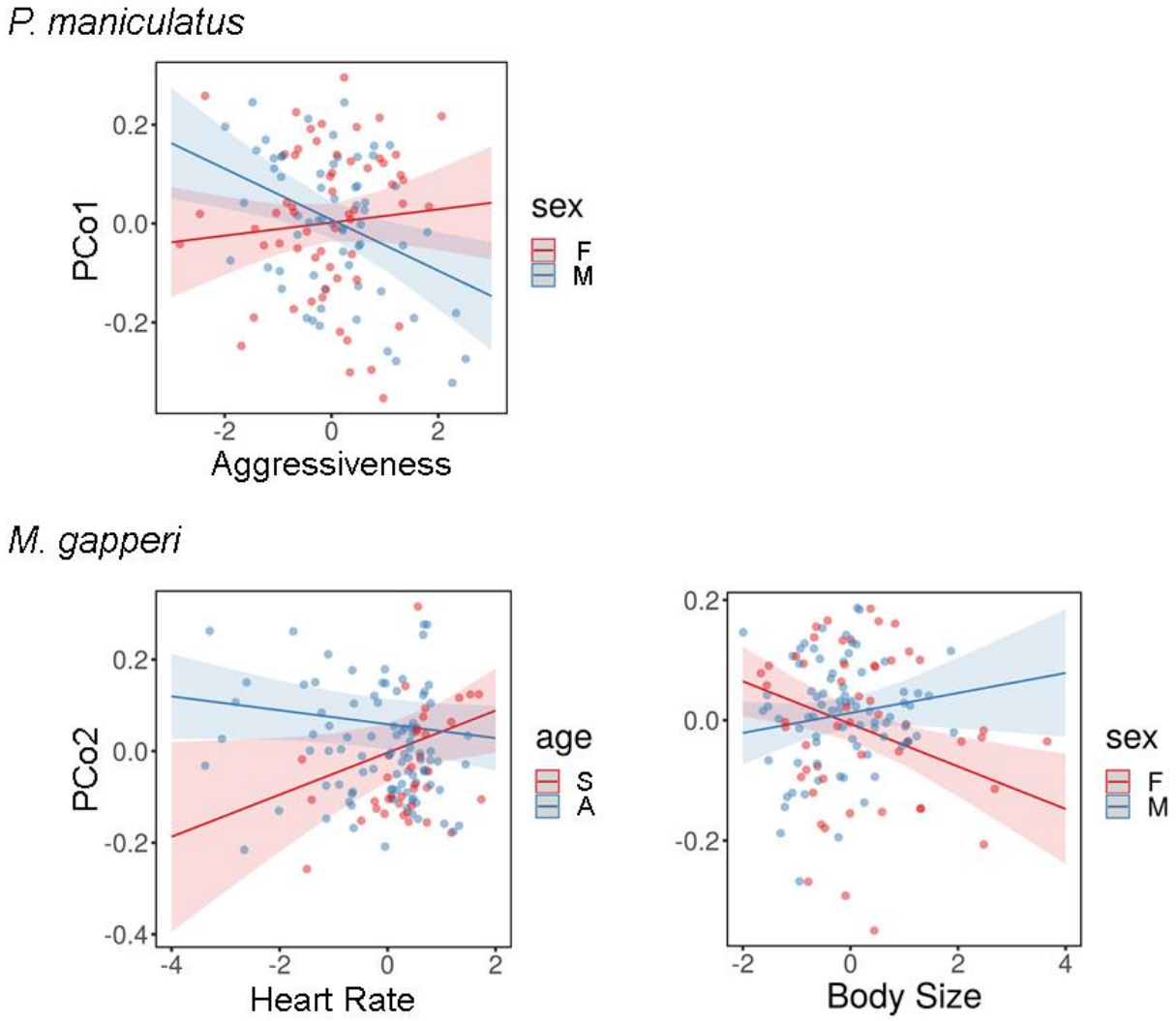

Figure S2.4 Graphical representation of significant interactions in mixed models testing for correlations among host traits and gut microbiome composition. In mice, PCo1 (the scores of the first axis of a principal coordinates analysis on Bray-Curtis distances of mouse GMs) was significantly associated with the interaction between aggressiveness and age (S: subadult or A: adult). In voles, PCo2 (the scores of the second axis of a principal coordinates analysis on Bray-Curtis distances of vole GMs) was significantly associated with the interaction between heart rate and age, and between body size and sex. Best fit lines (± SE) represent predicted correlations by the models and points represent the raw data.

2.7.1 Supplementary methods: Microsatellites

Microsatellite analyses were conducted for mice (*P. maniculatus*) and voles (*M. gapperi*). We performed DNA extraction from ear punches using the DNeasy Blood and Tissue Kit (QIAGEN). We followed all steps outlined by the kit except for the initial lysis step where we first added 180 ul of ATL buffer and 20 ul of proteinase K to the sample. We then cut the sample into smaller pieces if possible and vortexed for a few seconds. We then incubated the sample at 56C and left to shake at maximum speed overnight.

We PCR-amplified 13 microsatellites (Bw4-260, BwC-28, PmBw-385, PmBw-397, PmBw-447, Bw4-245, Bw4-112, Bw3-15, Bw4-28, PmBw-346, PmBw-390, PmBw-410, Bw4-SREL) from mouse DNA identified by Mullen et al. (2006) and Weber et al. (2010). For voles, we amplified 12 microsatellites (Cg1G12, Cg2C5, Cg5G6, Cg12E6, Cg8A5, Cg10H1, Cg13G2, Cg12A7, Cg12B9, Cg17A7, Cg1F11, Cg17E9) identified by Rikalainen et al. (2008) for bank voles (*Myodes glareolus*). PCR solutions consisted of H2O, buffer Taq (10 x), MgCl2 (25 mM), dNTPs (1 Mm), forward primer (10 uM), reverse primer (10 uM), Taq (1 unit/ul), extracted DNA (5ng/ul). PCR reagent quantities varied by microsatellite and were based on tests to determine optimal quantities for best results. The PCR cycle for voles consisted of an initial denaturation at 94C for 2 minutes followed by 30 cycles of 94C for 45sec, 50–60C (depending on the microsatellite) for 45sec, and 72C for 60sec and a final elongation at 72C for 5min. Most of the mouse microsatellites were amplified using an initial denaturation at 94C for 3 minutes followed by 30 cycles of 94C for 45sec, 55–62C (depending on the microsatellite) for 10sec, and 72C for 60sec and a final elongation at 72C for 5min. We placed 10ul of PCR mix in a SimpliAmp Thermal cycler (Applied Biosystems). The amplified product was normalized with 0,1 µl Liz and 8,9 µl Formamide Hi-Di (Applied Biosystems) and sequenced on a AB 3130xl (Applied Biosystems) sequencer running Genemapper version 4.0 (Applied Biosystems).

Null alleles and genotyping errors were identified with MICRO-CHECKER v2.2.3 using a 95% confidence interval (van Oosterhout et al. 2004). Null alleles were identified for loci Cg8A5, Cg12A7, Cg17A7, Cg17E9 in voles, and BwC-28 in mice so we removed these from further

analyses. We also tested for Hardy-Weinberg equilibrium in the remaining loci with GENEPOP v.4.3 (Rousset, 2008). We calculated a matrix of pairwise distances among individuals averaged across all loci (function meandistance.matrix, R package polysat) and ran a principal component analysis (PCA) of the distance matrix. The scores of the first two PCA axes were used as independent genotype variables in our analyses.

2.7.2 Supplementary methods: Single nucleotide polymorphisms (SNPs)

We obtained data on genotypic differences for deer mice captured in our study from (Miller *et al.*, s. d.) and analytical methods are described in detail therein. Briefly, samples were sequenced by double digest restriction-site associated DNA (RAD) sequencing (Peterson *et al.*, 2012) at the Institut de Biologie Intégrative et des Systèmes (IBIS) at Université Laval (Québec, Canada) following (Mascher *et al.*, 2013). Genomic DNA was first extracted from mouse tissue with a salt-extraction protocol adapted from (Aljanabi et Martinez, 1997). DNA from each sample was normalized to 20 ng/µl in 10 µl (200ng total) using PicoGreen (Fluoroskan Ascent FL, Thermo Labsystems). Samples were digested at 37 °C for two hours with NEB4 digest buffer and PstI and MspI restriction enzymes. Two adaptors (one unique to each sample and the second common) were added to each sample and samples were ligated at 22 °C for 2 hours with a ligation master mix and T4 ligase. Samples were pooled and purified with QIAquick PCR purification kits. Samples were PCR-amplified and sequenced on Ion Torrent Proton P1v2 chips.

The resulting reads were aligned against the genome assembly for Peromyscus maniculatus (GCA_000500345.1) with Burrows-Wheeler Aligner version 0.7.13 (Li et Durbin, 2010) and samtools view version 1.3 (Li *et al.*, 2009). STACKS version 1.44 (Catchen *et al.*, 2013) and custom scripts (https://github.com/enormandeau/stacks_workflow) were used to identify loci and SNPs and extract population-level statistics. Samples with too many missing SNP data were removed and new population-level statistics were extracted for the remaining samples. The resulting list of SNPs was further filtered such that each locus had a minimum read depth of 10, was present in at least 70% of individuals, and had less than 60% heterozygosity in all populations,

had a minimal global minor allele frequency (MAF) of 0.01 or had a minimal MAF of 0.05 in at least one population, had a minimum and maximum Fis value of -0.3 and 0.3, respectively, and was in a locus with a maximum of 10 SNPs. We summarized genetic structure by conducting principal component analysis (PCA) on the allele frequencies from the matrix of genotypes with lea package version 1.2.0 (Frichot et François, 2015). The scores of the first two PCA axes were used as independent genotype variables in our analyses for mice.

SNP and microsatellite data showed a strong site-level genetic structure in mice and microsatellite data showed a much weaker site-level structure in voles. Geographic location was a much weaker predictor of genotype than site. Genotype in neither of the species correlated with within-site geographic location suggesting poor within-site structure (Table S2.3).

2.7.3 Supplementary methods: Sequence data quality control methods

Following quality control, filtering, and removal of chimeras, we identified reads to amplicon sequence variants (ASVs) with package DADA2 version 1.10 (Callahan *et al.*, 2016). We used default settings for all DADA2 analysis parameters specifying a forward read length of 210 and a reverse read length of 200 during read trimming. When merging forward and reverse reads, we specified a minimum overlap length of 10 and a maximum number of mismatches allowed in the overlap region of 10. We removed chimeras using the "consensus" method. This resulted in an average (±SD) of 19,034 ± 10,319 sequences per sample (minimum=8 sequences, maximum=79,643 sequences). PCR negative controls (n=5) had an average (± SD) of 393 ± 378 reads, and PCR positive controls (n=4) averaged 39,948 ± 32,719 reads. We used the RDP Naïve Bayesian Classifier algorithm in DADA2 to assign taxonomy to ASVs according to the SILVA database (V128). We removed ASVs with <100 sequences and rarefied samples to 4,000 reads per sample, which was sufficient for rarefaction curves to reach a plateau and eliminated 11 samples from our dataset (Fig.S2.4).

CHAPITRE III

GUT MICROBIOME MODULATES BEHAVIOUR AND LIFE HISTORY IN TWO WILD RODENTS

Joël W. Jameson, Denis Réale, Steven Kembel

3.1 Abstract

BACKGROUND: Laboratory studies demonstrate that the gut microbiome can regulate host anxiety and exploratory behaviour. Besides its implications for human health, this could have ecological and evolutionary implications for wild populations, a hitherto untested hypothesis. We tested whether the microbiome can directly modulate host behaviour and affect aspects of life history in wild mice (*Peromyscus maniculatus*) and voles (*Myodes gapperi*). We compared the microbiome composition, exploration and anxiety behaviours, and home range of mice and voles before and after chronic antibiotic treatment and measured survival during treatment.

RESULTS: Treated animals had lower microbial diversity and higher relative abundance of Proteobacteria. In mice, antibiotics countered a decrease in exploration that was possibly induced by the gavage manipulation. While home range correlated with microbiome composition, antibiotics did not impact home range or survival. In voles, the antibiotic lowered survival such that we could not test its effect on behaviour.

CONCLUSION: The gut microbiome can directly impact behaviour and survival in the wild, but its effect is species-specific. It may help drive behavioural adaptation in the face of changing environmental factors and have a role in shaping population structure.

KEY WORDS

gut microbiome, behaviour, exploration, anxiety, home range, survival, wild

3.2 Background

Modulation of host behaviour by the gut microbiome is well established from studies on laboratory rodents, relying on comparisons between germ free (GF) animals, antibiotic- or probiotic-treated animals, and animals with a conventional gut microbiome (Bercik *et al.*, 2011 ; Desbonnet *et al.*, 2014 ; Heijtz *et al.*, 2011 ; Neufeld *et al.*, 2011). Gut microbiome-mediated behavioural changes in these studies also correlate with neurochemical changes in the brain (Ait-Belgnaoui *et al.*, 2012 ; De Palma *et al.*, 2015 ; Desbonnet *et al.*, 2014 ; Neufeld *et al.*, 2011). Despite the gut microbiome's potential to influence behaviour, this influence has only been studied in controlled laboratory experiments on genetically homogeneous groups of animals and in the context of studying human health and disease. However, the impact of the gut microbiome on behavioural traits like anxiety and exploration could have important ecological and evolutionary consequences in wild populations.

In laboratory studies, GF mice show reduced anxiety-like behaviour (Heijtz *et al.*, 2011 ; Neufeld *et al.*, 2011), increased locomotion (Heijtz *et al.*, 2011), increased social avoidance and reduced social cognition (avoidance of newly encountered conspecifics) (Desbonnet *et al.*, 2014) compared with specific pathogen free (SPF) mice. These differences are accompanied by increased levels of plasma stress hormones and gene-level changes in the hippocampus and amygdala (Neufeld *et al.*,

2011). In one study comparing GF and SPF mice, the microbiome was required for induction of an anxiety behaviour resulting from an early-life stressor (maternal separation) despite both groups presenting similar activity of the hypothalamic pituitary adrenal (HPA) axis (De Palma *et al.*, 2015). The use of antibiotics has also elucidated important interactions between host behaviour and the microbiome in lab mice. Mice given non-absorbable antimicrobials have shown reduced anxiety and elevated exploration accompanied by elevated brain-derived neurotrophic factor (BDNF) in the hippocampus and reduced BDNF levels in the amygdala (Bercik *et al.*, 2011). In a mouse model of anxiety, oral antibiotics suppressed the HPA stress response and promoted expression of proinflammatory cytokines in the hypothalamus (Ait-Belgnaoui *et al.*, 2012). Finally, in a study by Gacias et al. (Gacias *et al.*, 2016), offspring of C57BL/6J mice treated with antibiotics that lowered *Lactobacillus* and increased *Clostridium* abundances in the gut, exhibited reduced exploration and increased thigmotaxis in the open field (Tochitani *et al.*, 2016). However, the same antibiotic treatment given to BALB/c mice for seven days increased exploratory behaviour, despite causing similar changes to the microbiome (Bercik *et al.*, 2011), suggesting that the effect of the microbiome on behaviour can vary with species or even species strain.

In the wild, variation in anxiety and exploration-related behaviours can affect dispersal, reproduction, survival and fitness, with potential consequences for population structure (Ballew *et al.*, 2017 ; Smith et Blumstein, 2008 ; Spiegel *et al.*, 2017). Although these traits are often studied in the context of clinical behavioural disorders, variation in these traits also exists among individuals in wild populations (Réale *et al.*, 2007) where they are often correlated, forming a behavioural syndrome (Réale *et al.*, 2010). For example, animals with lower anxiety are generally more aggressive, less sociable, faster explorers (Aplin *et al.*, 2013 ; Jolles *et al.*, 2015 ; Kurvers *et al.*, 2010 ; Réale *et al.*, 2010), a pattern that is also observed when comparing GF mice to SPF mice (Desbonnet *et al.*, 2014 ; Heijtz *et al.*, 2011 ; Neufeld *et al.*, 2011). Potential drivers of individual behavioural variation in the wild include genotype, resource competition, sexual selection, predation, and parasite infection (Barber et Dingemanse, 2010 ; Bell et Sih, 2007 ; Cote *et al.*, 2008 ; Réale *et al.*, 2007 ; Schuett *et al.*, 2010). The gut microbiome may also play a key role in determining individual-level behavioural phenotypes in the wild. Gut microbiome structure is correlated with level of social interaction in wild chimpanzees (Moeller *et al.*, 2016) and social

structure is also correlated with gut microbiome variation in wild baboons and lemurs (Diakiw, 2017 ; Tung *et al.*, 2015). While this indicates a link between microbiome and social behaviour in the wild, we are not aware of any study that has empirically tested if the microbiome can modulate behaviour in wild animals. Given that the same behaviours under microbial control in the laboratory are also under selection in wild populations (Smith et Blumstein, 2008), the microbiome may also play a role in this latter context, though the extent of this role under natural ecological conditions remains to be determined.

We tested the hypothesis that the microbiome can directly modulate host exploration and anxiety-related behaviour in wild animals. We also aimed to determine whether a microbiome-induced change in host behaviour could influence aspects of host life history such as home range and survival. Since behavioural response to microbial changes can vary with host species, we conducted this study on two rodent species, the deer mouse (*Peromyscus maniculatus*) and the red-backed vole (*Myodes gapperi*). Both species are among the most common rodent species in North America (Harper et Austad, 2001). They often live in sympatry with overlapping home ranges (Harper et Austad, 2001). Both have a similar social structure and reproductive strategy (Harper et Austad, 2001). Both live less than one year in the wild (Blair, 1948 ; Innes et Millar, 1994) and share similar diets comprised of berries, seeds, and arthropods (Harper et Austad, 2001), with voles having a greater affinity for mushrooms (Sullivan *et al.*, 2017). Despite these similarities, fecal glucocorticoid levels correlate with population size and time confined in a trap in mice but not voles suggesting these species respond differently to stress, which may also manifest in their behaviour-microbiome associations (Harper et Austad, 2001). Studying wild rodents, especially mice, allowed us to make more direct comparisons with results from laboratory studies. To address the goals of this study, we compared the microbiome, exploration and anxiety-related behaviours, and home range of wild mice and voles before and after chronic antibiotic treatment and measured their survival during the treatment period.

3.3 Results

We live-trapped mice and voles approximately every two days over a period of 41 days from 14 July – 23 August 2016. During a pre-treatment period (first 17 days) animals were captured but received no treatment. During the treatment period (day 18 to 41), animals were randomly assigned to either a group given saline, or a group given an antibiotic cocktail. For ethical reasons, these groups comprised only adult males. We captured 19 male and 16 female deer mice, and 47 male and 28 female red-backed voles. Of the adult male mice, we gave a treatment of antibiotics to 10 and saline to eight. Of the adult male voles, 18 received antibiotics, and another 18 received saline. Every day an animal was captured during the treatment period, it received its respective treatment (saline or antibiotic) which we administered by oral gavage. On average (± SD), mice received 5.7 ± 2.0 treatments and were treated every 2.6 ± 0.5 days while voles received 3.9 ± 2.2 treatments and were treated every 2.3 ± 0.7 days.

3.3.1 Antibiotic treatment reduces microbiome diversity

We characterized the microbiome of mice and voles by sequencing the bacterial 16S barcode gene of two to three fecal samples per animal collected from traps throughout the study. We used DADA2 to identify amplicon sequence variants (ASV), our fundamental unit of ecological analysis (Callahan *et al.*, 2016), and assigned taxonomy using the SILVA database (V128). We calculated Shannon's and inverse Simpson's diversity of each sample and Bray-Curtis dissimilarity among the samples (Oksanen *et al.*, 2019). We then used principal coordinate analyses (PCoA) to plot pairwise dissimilarities in two dimensions and calculated the weighted average scores for taxa to identify those most associated with each PCoA axis (Oksanen *et al.*, 2019). Finally, we used DESEQ2 (package DESEQ2 in R) differential abundance analysis to identify specific ASVs impacted by the treatment (Love *et al.*, 2014). The antibiotic treatment significantly changed the gut microbiome composition of both species (Fig. 1, Tables S3.1, S3.2). In mice, variance along the first axis (PC1) was attributable to the antibiotic treatment, while variance along PC2 represented an unknown source, independent of the treatment (Figs. 2,3, Tables S3.3 and S3.4). In voles, the antibiotic treatment effect acted on both PC1 and PC2 (Figs. 2,3, Tables S3.3 and S3.4). Alpha diversity of both mice and voles decreased only in the antibiotic

group from the pre-treatment to the treatment period, and the extent of this effect on Shannon's diversity depended on the number of days since their last treatment (Fig. 3, Tables S3.3 and S3.4). However, while the antibiotic effect on inverse Simpson's diversity in mice also varied with the number of days since the last treatment, it did not in voles (Table S3.1). PC1 increased from the pre-treatment to the treatment period only in the antibiotic-treated mice and voles, with this effect also varying as a function of the number of days since their last treatment (Fig. 3, Tables S3.3 and S3.4). Only saline-treated mice showed an increase along the PC2 axis from the pre-treatment to treatment period indicating that PC2 represented variation independent of the antibiotic treatment. In voles, variation along PC2 corresponded with the antibiotic treatment, increasing only in antibiotic-treated voles during the treatment period and once again, varying as a function of the number of days since the animal was last captured (Fig. 3, Tables S3.3 and S3.4).

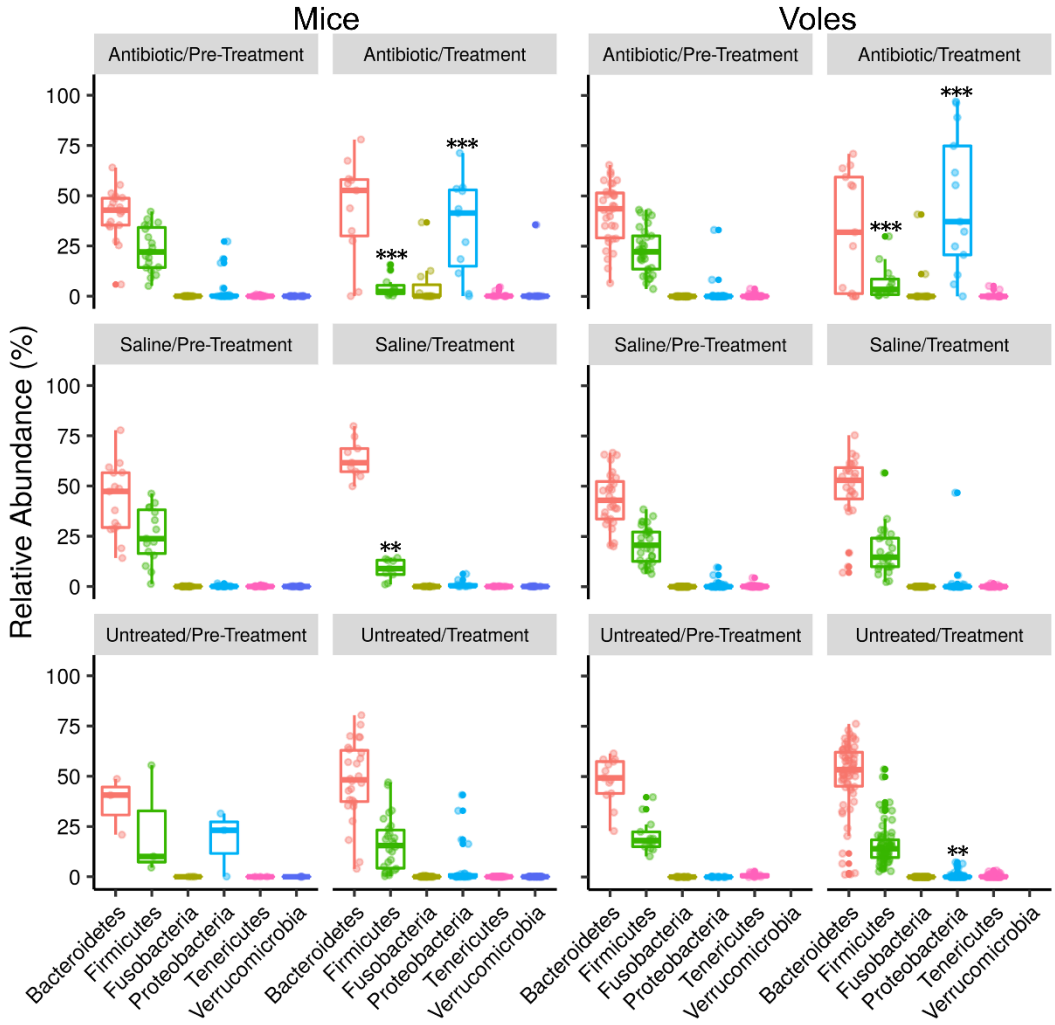

Figure 3.1 Effect of an antibiotic treatment on the relative abundance of major phyla in the gut microbiome of deer mice and red-backed voles that received antibiotic, saline, or no treatment before and during the treatment period. We ran zero-inflated negative binomial generalized linear models with relative abundance as the dependent variable and phylum, treatment, period, and their interactions as independent variables (see Tables S3.1 and S3.2). Untreated animals are included as an additional baseline and are mostly female. ***P<0.001, **P<0.01.

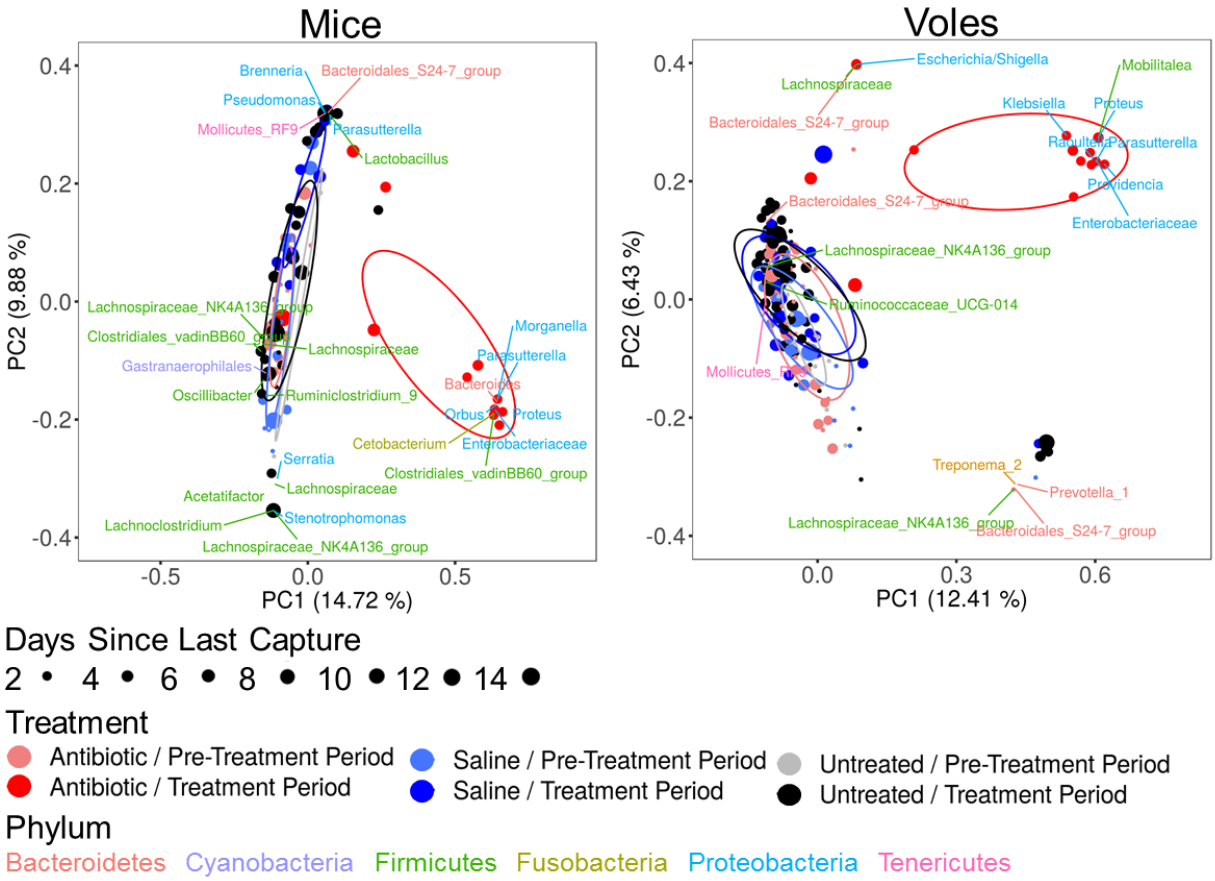

Figure 3.2 Principal coordinate analysis (PCoA) of Bray-Curtis distances among all samples collected from deer mice and red-backed voles. Treatment groups (Antibiotic, Saline, Untreated) before and during the treatment period are indicated by color and 95% confidence ellipses. Untreated animals are included as an additional baseline and are mostly female. Point size indicates the number of days since the last capture which, for the treatment period antibiotic group, represents the number of days since the last treatment. Phyla (color-coded) with the greatest weighted average scores in PCoA space are also plotted. Each point represents an individual sample.

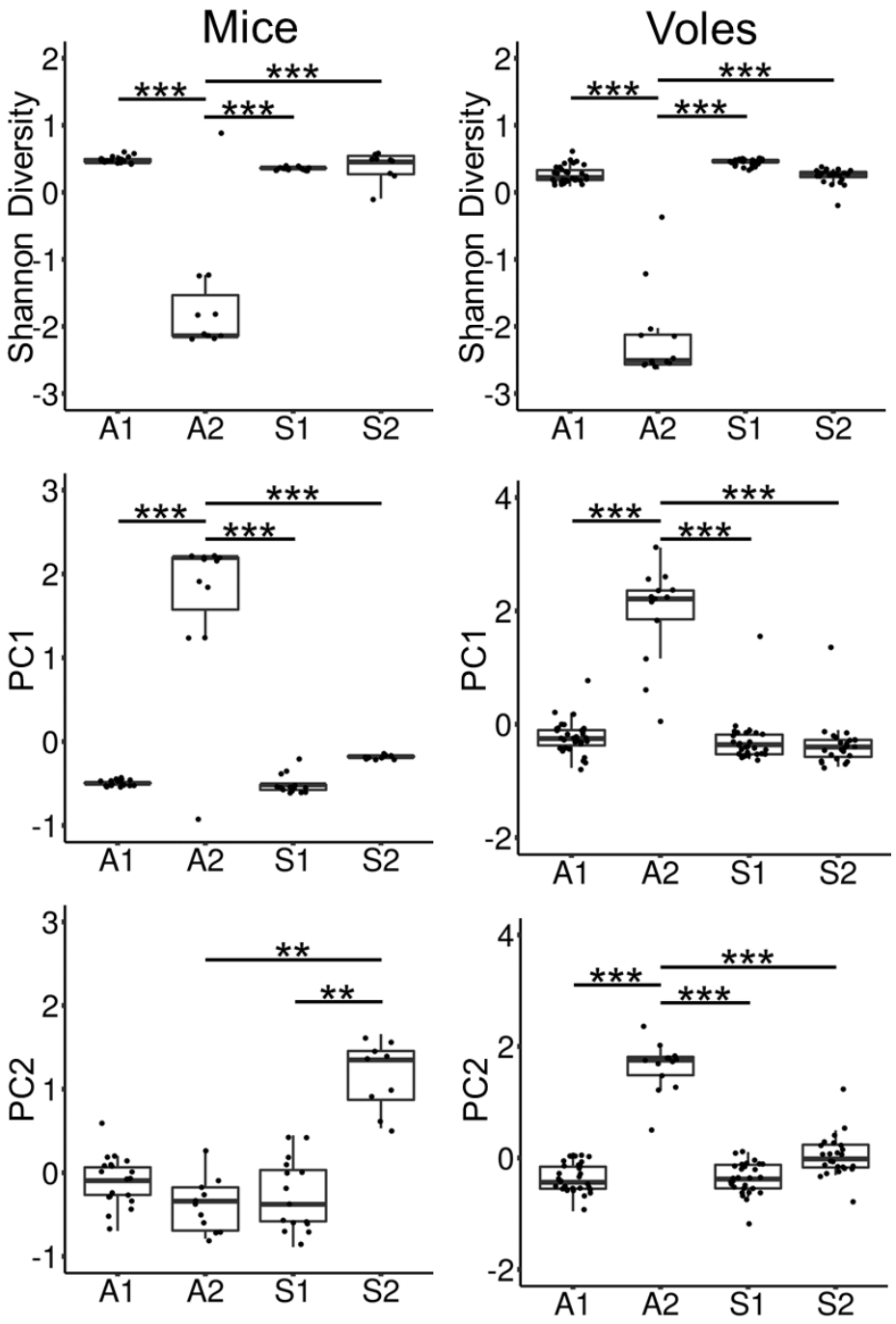

Figure 3.3 Shannon diversity and the scores of the first two axes from a PCoA of Bray-Curtis distances of deer mice and red-backed voles. Mixed models for each species (see Tables S3.3 and S3.4) were derived from full models that included treatment, period, days since last capture and

their interactions as fixed effects, with ID as a random effect. Between-group significance is assessed by pairwise comparisons of least squares means. A1=Antibiotic Pre-treatment, A2=Antibiotic Treatment, S1=Saline Pre-treatment, S2=Saline Treatment. ***P<0.001, **P<0.01.

In mice, weighted average scores of microbial taxa on ordination axes show that antibiotic-treated animals were mainly associated with an increase in relative abundance of taxa from the phylum Proteobacteria, with the greatest weight attributed to the genera *Morganella*, *Parasutterella*, *Orbus*, *Proteus,* and unidentified members of the family Enterobacteriaceae (Fig. 2). This was met with a relative decrease in the phylum Firmicutes. Antibiotic-treated animals also clustered with *Cetobacterium* of the phylum Fusobacteria, and an unknown Clostridiales taxon of the phylum Firmicutes. The increase in saline group PC2 was largely due to a relative decrease in Firmicutes, and a relative increase in Bacteroidetes, of which the group Bacteroidales S24-7 had the greatest weight. This change was also associated with higher relative levels of the genera *Brenneria*, *Pseudomonas* and *Parasutterella* of the phylum Proteobacteria, and of the class Mollicutes of the phylum Tenericutes (Fig. 2). Unlike mice, the microbiome composition of voles did not change independently of the antibiotic treatment. The treatment resulted in an increase in the relative abundance of Proteobacteria, with the greatest weight attributed to the genera *Klebsiella*, *Parasutterella*, *Raoultella*, *Providencia*, *Proteus* of the family Enterobacteriaceae, and the genus *Mobilitalea* of the phylum Firmicutes (Fig. 2). The above results were largely supported by a differential abundance analysis (Figs. S2,S3). Mice treated with the antibiotic showed a drastic loss in taxa of the phyla Bacteroidetes and Firmicutes, with an increase in relative abundance of *Klebsiella sp*., *Parasutterella*, *Morganella morganii*, other Enterobacteriaceae sp. (Proteobacteria), *Fusobacterium ulcerans* (Fusobacteria), *Bacteroides sp*. and Bacteroidales (Bacteroidetes), and *Erysipelatoclostridium ramosum* (Firmicutes). The change in composition of the saline group from the pre-treatment to treatment period comprised a decrease in Firmicutes which were mostly *Ruminoclostridium* (Clostridiales), and an increase in Bacteroidetes, mainly of ASVs assigned to Bacteroidales S24-7 (Fig. S2). For voles, taxa differentially enriched due to the

treatment were mainly Proteobacteria and included *Escherichia*/*Shigella sp*., *Klebsiella sp*., *M. morganii*, *Raoultella sp*., *Proteus sp*., other unknown Enterobacteriaceae and *Enterobacter*, and *Parasutterella*. The treatment also caused an increase in an unknown *Clostridium* taxon, *Enterococcus sp*., *Erysipelotrichaceae*, and *Lactobacillus sp*. (Firmicutes; Fig. S3).

3.3.2 Antibiotic Treatment Opposes Change in Exploration

Following the high vole mortality during the season (see next section), we restricted our analyses on the effects of antibiotics on behaviour to deer mice. We measured exploration (i.e. total distance travelled in the open field) and anxiety (i.e. the latency to enter the center of the open field; 29, 30) behaviour of each individual once just before they received their first treatment, and once again at the end of the treatment period. This limit of two tests per individual prevented animals from habituating to the test. To account for non-independence of behavioural data associated to replicated tests on each individual, we ran four mixed models for each behaviour, including individual ID as a random effect (distance travelled and latency to enter the center; Table S3.5). The first model had, as independent variables, treatment group, period, PC1, and their interactions. The second model was identical to the first but included the number of previous captures of each individual. The last two models were identical to the first two but included PC2 instead of PC1. The model that best explained distance travelled included PC2 but did not include the number of previous captures (model 4; Fig.4, Table S3.5, S3.6). In this model, the interaction between treatment and period was significant (estimate = -2.75 ± 0.51, $t_{6.3} = -5.36$, $P = 0.023$, Table S3.6). Mice from both the antibiotic and saline groups showed a decrease in exploration after the treatment period (estimate = -0.94 ± 0.24, $t_{6.0} = -4.01$, $P = 0.036$, Table S3.6), but this decrease was estimated to be almost three times greater in the saline compared to the antibiotic group (Fig. 4).

Three models were equivalent at explaining latency to enter the center of the open-field: the two models with PC1 and the model with PC2 and number of previous captures (Table S3.5). To limit the number of models run, when two models containing the same microbiome axis (e.g. PC1) are

equivalent (ΔAICc < 2), we only present results from the model that included number of previous captures (Table S3.6). We observed a change in association from positive to negative between microbiome composition and the latency to enter the center of the open field in the saline-treated group (Fig.4, Table S3.6). This change was significant for PC1 (estimate = -3.56 ± 1.09, $t_{17.8}$ = -3.26, P = 0.036), but not for PC2 (estimate = -1.90 ± 0.69, $t_{12.4}$ = -2.77, P = 0.057; Table S3.6). Furthermore, this shift in slope was not observed in the antibiotic-treated group. Additionally, contrary to distance travelled, latency to enter the center increased with the number of previous captures in both treatment groups in the model for PC2 (estimate = 0.99 ± 0.33, $t_{16.3}$ = 2.98; P = 0.023, Table S3.6), but not for PC1 (estimate = 0.75 ± 0.35, $t_{15.0}$ = 2.13; P = 0.109, Table S3.6).

3.3.3 Home Range Correlates with Microbiome Composition

We measured the 95% kernel home range of each mouse during the pre- and post-treatment periods. We ran the same series of four mixed models for home range, as for the open field behaviours (Table S3.5), but weighted each model by the number of times individuals were detected to correct for potential variation in the accuracy of the home range estimations. The model that best explained home range contained PC2 and included the number of previous captures (Fig.4, Table S3.5, S3.6). Home range size decreased in both the saline and antibiotic groups (estimate = -1.78 ± 0.39, $t_{20.8}$ = -4.60; P = 0.005, Table S3.6). Home range was also correlated with microbiome composition (PC2; estimate = -0.81 ± 0.22, $t_{9.1}$ = -3.73; P = 0.036, Table S3.6) and increased with the number of previous captures (estimate = 0.70 ± 0.22, $t_{18.3}$ = 3.12; P = 0.036, Table S3.6). However, we found no significant difference in change in home range between the two treatment groups.

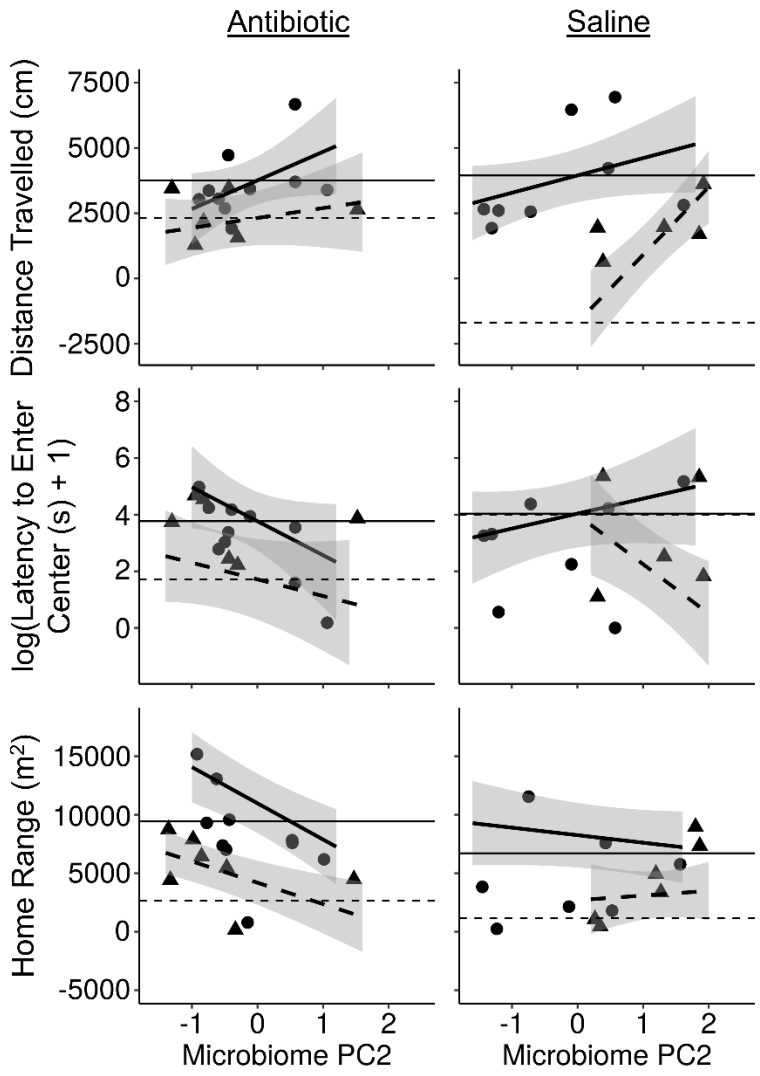

Figure 3.4 Effect of the antibiotic-mediated change in gut microbiome on open-field behaviours and home-range size in deer mice. We used mixed models to test for an effect of gut microbiome composition given by the scores of the first two axes of a PCoA of Bray-Curtis distances on behaviours measured in an open field and on the 95% kernel home range. We ran separate models for each behaviour and each microbiome composition axis (PC1 and PC2). Presented here are the results for the best models from model comparisons described in Table S3.5. Circles and solid lines represent the pre-treatment period, triangles and dashed lines represent the treatment period. See Table S3.6 for detailed model results.

3.3.4 Antibiotic treatment reduces vole survival

We compared survival during the treatment period of antibiotic-treated, saline-treated, and untreated animals with cox proportional hazards analysis (Therneau, 2015). Five voles (1 Antibiotic, 4 Untreated) were found dead in the trap when traps were collected, and 10 voles (5 Antibiotic, 1 Saline, 4 Untreated) died during the manipulation or before release. No mice died in captivity. The average (± SD) proportion of days animals were detected between the first and last detection during the treatment period (i.e. days detected / days the animal was known to be present in the capture grid) was 81.5 ± 20.7% for mice, and 84.6 ± 19.0% for voles. Capture rate of mice and voles during this period did not differ between antibiotic and saline groups (see Table S3.7 and Fig. S4). We found no differential change in weight between antibiotic- and saline-treated mice or voles but mice increased in weight (t=2.7, P =0.02) and voles maintained a stable weight (t=-2.0, P =0.07) throughout the study (Fig. S5, Table S3.8). For mice the antibiotic treatment did not change the hazard ratio when compared to the saline group (z=-0.44, P=0.66) or the untreated group (z=0.20, P=0.84, Fig. 5). Conversely, for voles, the antibiotic group showed a reduced hazard ratio compared to the untreated group (z=-3.1, P=0.002) and a non-significantly reduced hazard ratio compared to the saline group (z=-1.8, P=0.08, Fig. 5).

We compared the microbiomes of antibiotic-treated voles that died to those of antibiotic-treated voles that survived during the treatment period with a DESEQ2 differential abundance analysis. Voles that died had enriched Enterobacteriaceae including *Escherichia/Shigella* and *Raoultella*, and one Clostridiaceae ASV, *Clostridium sensu stricto*, and lower proportions of ASVs mainly classified to Bacteroidales S24-7, Lachnospiraceae, and Ruminococcaceae (Fig. S6).

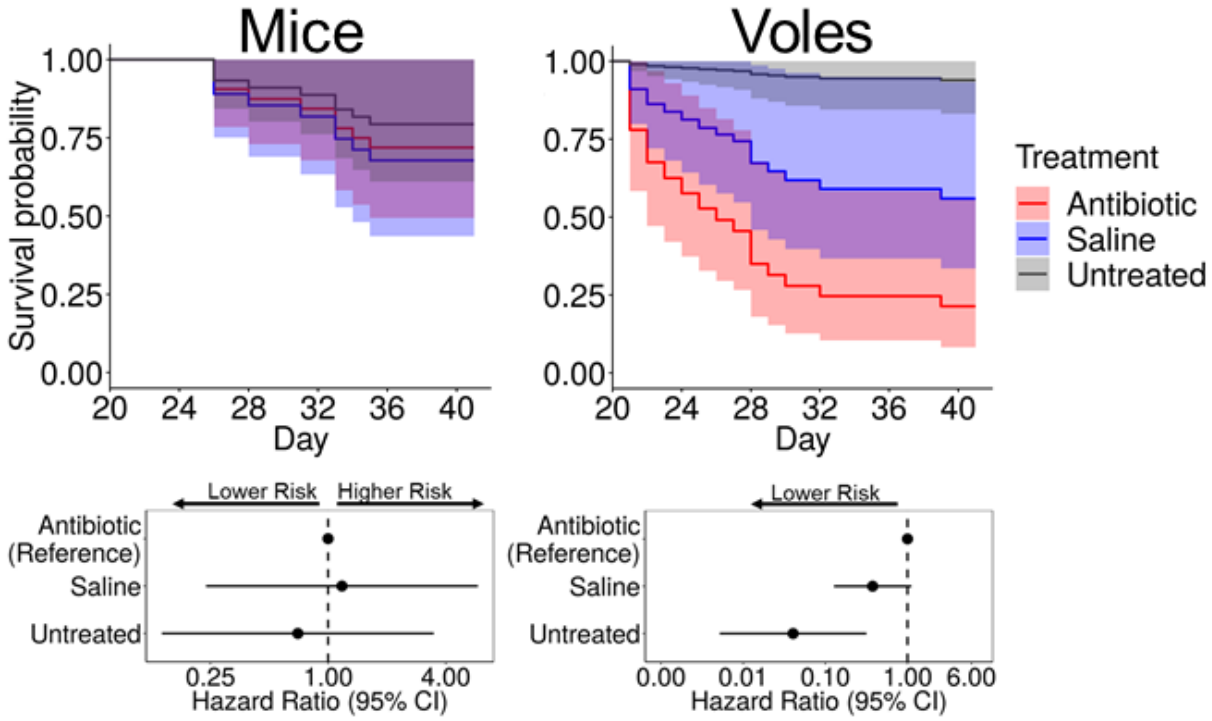

Figure 3.5 Comparison of survival of three treatment groups (Antibiotic, Saline, Untreated) during the treatment period using a Cox proportional hazards ratio analysis for deer mice and red-backed voles. Untreated animals are included as an additional baseline and are mostly female. Hazard ratios below 1.00 represent a lower likelihood of death, while values above 1.00 indicate a higher likelihood of death.

3.4 Discussion

Experimental studies have not yet addressed the potential impact of microbiome-mediated behavioural changes on the ecology and evolution of wild animals. Several ecological factors can influence the gut microbiome and behaviour of wild animals (Amato, 2013) and potentially overwhelm the effect of the microbiome on behaviour. Therefore, the goals of this study were to first, determine whether modifying the gut microbiome of wild mice and voles changes exploration

and anxiety-related behaviours, and second, whether these changes have repercussions on the life history of the animals as measured by home-range size and survival.

3.4.1 Oral Gavage and Antibiotic Treatment Alter the Gut Microbiome

The antibiotic treatment reduced the diversity of the gut microbiome of both mice and voles. The gut microbiota of antibiotic-treated mice decreased in relative abundance of Firmicutes and increased in relative abundance of Proteobacteria, namely *Klebsiella*, *Parasutterella*, *Morganella*, and other unidentified Enterobacteriaceae. These same taxa also characterized the microbiome of antibiotic-treated voles, with the addition of enriched *Escherichia/Shigella*. These changes in the microbiome are in line with those from other studies that have used an identical or similar antibiotic cocktail on laboratory mice showing enrichment of Proteobacteria including *Morganella* and *Proteus* and other unidentified Enterobacteriaceae (Gacias *et al.*, 2016 ; Rodrigues *et al.*, 2017). Interestingly, in mice, we also observed changes in microbiome composition (PC2) of the saline group independent of those (PC1) in the antibiotic group, namely an increase in the relative abundance Bacteroidetes, including Bacteroidales S24-7, and a decrease in Firmicutes, specifically Clostridiales. Differential abundance of these taxa was also identified by Gacias et al. (Gacias *et al.*, 2016) in NOD mice given distilled water by oral gavage though they observed an increase in Clostridiales. These changes in microbiota, which they attributed to the oral gavage manipulation, led to increased microbial gut metabolites that prevented myelin expression in the medial prefrontal cortex, inducing a depressive-like behaviour in the host. In our study, stress induced by the gavage manipulation may have driven the microbiome and behavioural (see below) changes in the saline group via a similar mechanism.

3.4.2 Antibiotic Treatment Opposed Change in Exploration

Exploration decreased in both antibiotic and saline-treated mice. This decrease in exploration in both treatment groups may have stemmed from stress induced by chronic gavage. In the study by Gacias et al. (Gacias *et al.*, 2016), administration of antibiotics prevented onset of the depressive-

like phenotype induced by chronic gavage (Gacias *et al.*, 2016). In our study, anxiety (i.e. latency to enter the center) and exploration (i.e. distance travelled) were correlated and, while we did not measure depression, anxiety and/or depression could have manifested as a decrease in exploration (Carter *et al.*, 2013). This decrease was significantly less pronounced in antibiotic-treated mice (Fig. 4), suggesting a role for the microbiome in expression of exploratory behaviour and supporting our hypothesis that the microbiome directly modulates exploration-related behaviour in the wild. The ability of the microbiome to modulate exploration might help promote cohesion among animals that live in dense populations and new migrants entering a population. Transfer of bacteria among animals that live in groups promotes homogenization of microbiota which could favor homogenization of behaviours to promote positive interspecific interactions/cooperation. Additionally, the strong relationship between diet and the microbiome (David *et al.*, 2014) means that seasonal changes in diet in wild animals could lead to microbiome-induced seasonal changes in behaviours that could potentially improve foraging success.

Since the best model for the change in exploration was that which contained PC2, the effect on behaviour by the antibiotic treatment was likely not driven by abundant bacteria in antibiotic-treated mice (Enterobacteriaceae), but instead by the absence of specific taxa or by preventing the compositional change along PC2 seen in saline-treated mice. The decrease in exploration with period was met with a decrease in the relative abundance of Ruminoclostridium, Lachnospiraceae, Lachnoclostridium, and Clostridiales (phylum Firmicutes) and an increase in the proportions of Bacteroidales S24-7 (phylum Bacteroidetes), one *Lactobacillus*. Higher proportions of Bacteroidales S24-7 relative to Lachnospiraceae have been linked to higher anxiety and social aversion in a mouse model for autism (Golubeva *et al.*, 2017). However, relative proportions of these taxa were reversed in mice fed a diet deficient in Omega-3 fatty acids which consequently developed depressive and social deficiency behaviours (Robertson *et al.*, 2017). While several members of the Lachnospiraceae and Bacteroidales S24-7 occupied the microbiomes of mice and voles in our study, many ASVs from each group showed opposite changes in relative abundance with the changes in behaviour. This is indicative of ASVs within each family having different functional profiles and, given that their effects may vary by host species, the ability to describe

and identify these ASVs at more refined taxonomic levels will be invaluable to understanding their role in modulating behaviour.

Unlike distance travelled in the open field, the mean latency to enter the center of the open field did not change between pre-treatment and treatment periods. However, the slope of the correlation between behaviour and microbiome composition (PC1 and PC2) in the saline group was reversed after the treatment period, but the antibiotic group showed no such change in slope. Since pre-treatment correlations between latency and microbiome composition differed between the antibiotic and saline groups, only a modest interpretation of results for this behaviour is justified. A change in slope is more likely to arise from variation in the change in microbiome composition rather than from an inversion in the behavioural phenotype, especially since relative behavioural differences are thought to remain consistent between individuals (Réale *et al.*, 2007). Bold mice may encounter many bacterial sources and thus harbour a greater diversity of transient bacteria, while shy mice may encounter fewer bacterial sources and harbour a more robust, simplified community. A small change in spatial behaviour could therefore have a large impact on the microbiome of bold mice, but a much smaller impact on that of shy mice. This idea that individual differences in microbiome composition plasticity associate with the behavioural phenotype of the host requires further study.

3.4.3 Antibiotic Treatment Did Not Affect Home Range Size

Home range size generally decreased during the treatment period, but this change was not modulated by the antibiotic treatment indicating that home range in deer mice may not be under microbiome control. It is not surprising that our results for home range and exploration differed since these two variables were not correlated and, while this seems counter-intuitive, associations between these variables can vary with species. For example, open field exploration does not predict home range size in bank voles and starlings (Minderman *et al.*, 2010 ; Schirmer *et al.*, 2019) but it seems to in red squirrels and chipmunks (Boon *et al.*, 2019 ; Montiglio *et al.*, 2012). Given that

our manipulation of the animals negatively impacted behaviours in the open field, we expected it to also drive a reduction in home range. However, home range increased with the number of times an individual was captured, likely because home range is measured, in part, from capture events. This suggests that the manipulation likely did not cause the reduction in home range which may have, instead, resulted from seasonal behavioural patterns not driven by the microbiome. For example, an increase in population size could drive population-wide reductions in home range and such density-dependent effects may overpower any behavioural effect of the microbiome.

We also found a significant correlation between PC2 and home range suggesting that different mechanisms may drive the change in home range observed between periods and the association between home range and the microbiome. For example, while seasonal changes in home range may stem from density-dependent or other seasonal processes, home range may influence the microbiome by influencing the environmental and dietary sources encountered by the host. Proportions of Lachnospiraceae, Ruminococcaceae decreased along PC2 and those of Bacteroidales S24-7 increased. While many members of these groups are associated with depressive and anxious phenotypes (Golubeva *et al.*, 2017 ; Inserra *et al.*, 2019), Lachnospiraceae and Ruminococcacea hydrolyze starch (Vacca *et al.*, 2020), and diet can also shape the Bacteroidetes community (Goodrich *et al.*, 2014).

3.4.4 Antibiotic treatment reduces vole survival

Survival of mice was unaffected by the antibiotic treatment and chronic gavage. In contrast, chronic gavage and the antibiotic treatment seemed to reduce vole survival in an additive manner with saline- and antibiotic-treated voles having respectively one third (nonsignificant) and two-thirds lower survival probabilities compared to untreated animals at the end of the study. Given that most of the voles that died during or just after the manipulation were antibiotic-treated and that the manipulation did not kill any mice, death is the likely cause of the disappearance in voles. Finding the exact causes of death in voles is not straightforward. However, our results show that

death was related to the treatment method and further to the change in the microbiome caused by the antibiotics.

Although variation in exploration-related behaviour should influence individual predation risk and thus, survival (Smith et Blumstein, 2008 ; Wolf et Weissing, 2012), our continued presence on the study site may have deterred potential predators thereby removing the influence of predators on survival. This would most likely have affected voles since mice are mostly active during the night when we were absent, while voles are active during the day and night (Gilbert *et al.*, 1986). It is therefore surprising that the treatment lowered the survival of voles and not that of mice. This difference may indicate potential differences in host-microbiome interactions between the two species. Enrichment of *Escherichia* and *Shigella* in voles but not in mice may have contributed to the observed differential survival. While most species of *Escherichia* and *Shigella* are non-pathogenic, some are. These taxa, along with other Enterobacteriaceae can promote intestinal inflammation and cause bacteremia and sepsis in the host (Menezes-Garcia *et al.*, 2020 ; Zeng *et al.*, 2017). Indeed, when compared to the three voles that survived the study, the voles that died had enriched Enterobacteriaceae including *Escherichia/Shigella* and *Raoultella*, and one Clostridiaceae ASV, *Clostridium sensu stricto*, and lower proportions of ASVs mainly classified as Bacteroidales S24-7, Lachnospiraceae, and Ruminococcaceae. Additionally, unlike mice, the number of days between antibiotic treatments did not affect Simpson's diversity of the vole microbiome, indicating a lower capacity of the vole microbiome to recover its initial composition. The vole microbiome may therefore be less resilient to change and succumb more easily to pathogens. Finally, high population density can suppres host immunity and fitness (Svensson *et al.*, 2001). Voles had a relatively high population density while the density of mice was low and ten times lower than in the previous year indicating a recent crash in the population. Density-dependent effects may therefore have contributed to the lower sensitivity of mice and to the higher sensitivity of voles to both the gavage and the change in microbiome.

3.5 Conclusions

Antibiotics offer a practical and efficient way of manipulating the microbiome to understand its function. While widely used in laboratory studies, to our knowledge our study is the first to apply this method to wild populations. This study provides the first confirmation that the microbiome can directly modulate behaviour and survival in a population of wild animals. Administration of antibiotics reduced gut microbiome diversity of deer mice, favoring members of the Enterobacteriaceae. This change countered a likely stress-related decrease in exploration, without impacting home range or short-term survival. A component of the microbiome unrelated to the treatment was also correlated with home range in mice. The effect of the microbiome on behaviour may help drive behavioural adaptation in the face of changing environmental factors such as population structure and resource availability. Conversely, it may weaken populations if it affects ecologically relevant traits or survival. Antibiotics reduced gut microbiome diversity of red-backed voles, favoring once again the Enterobacteriaceae, with strong effects on vole survival, preventing us from testing the effect of the microbiome on vole behaviour. Our results suggest that host microbiome interactions depend on species and that different bacterial taxa may modulate or relate to different behaviours.

3.6 Methods

We conducted this study on Harbour Island in the Winnipeg river basin, Ontario (50°2.580'N, 94°40,436'W). The site consists of mixed boreal forest dominated by balsam fir (*Abies balsama*), paper birch (*Betula papyryfera*), white spruce (*Picea glauca*), bigtooth aspen (*Populus gradidentata*) and trembling aspen (*Populus tremuloides*).

We captured mice and voles with Longworth and BIOEcoSS (BIOEcoSS Ltd.) live traps, over a period of 41 days from 14 July – 23 August 2016. We placed 192 traps at 96 stations (8 x 12) distanced 10 m apart. The capture grid (9600 m^2) was large enough to estimate individual home range sizes (Thompson *et al.*, 2009 ; Vanderwel *et al.*, 2010 ; Wolff, 1985 ; Wood *et al.*, 2010). We conducted captures approximately every two days, weather permitting, opening the traps in the evening and checking and closing them at 6:00 a.m. Before each capture session, we sterilized

traps with 70% ethanol and baited them with exactly 4 g of carrot and 1 ml of peanut butter. After processing, we returned animals to the precise location they were captured. During a pre-treatment period (first 17 days) animals were captured but received no treatment. During the treatment period (day 18 to 41), the adult males were randomly assigned to either a group given saline, or a group given an antibiotic cocktail of Metronidazole (75 mg/kg), Ampicillin (100 mg/kg), Vancomycin (50 mg/kg), and Neomycin (50 mg/kg). This cocktail has been successfully used to deplete the gut microbiome and induce a GF phenotype in lab mice (Reikvam *et al.*, 2011). We also included the antifungal Pimaricin (0.19 mg/kg) to stave off fungal infections (Bercik *et al.*, 2011 ; Bravo *et al.*, 2012 ; Foster et Neufeld, 2013). The duration and start/end dates of each period depended on each animal's capture history.

We collected fecal samples from traps and collected two to three samples per animal throughout the study. Tools were systematically sterilized with 70% ethanol and flame just before sampling. Samples were placed in 99% ethanol, frozen at -20°C until the end of the study, then moved to a -80°C freezer. For each sample, we bead-beat 10 mg of fecal material in extraction kit ASL buffer (beat 1 min, rest 3 min, beat 1 min). We extracted DNA with the QIAGEN QIamp DNA Stool Minikit and randomly attributed samples to sequencing kit. The V4-V5 regions (~410 bp) of the 16S rRNA gene of extracted DNA were amplified with 515f/926r primers (Walters *et al.*, 2015) and sequenced on an Illumina MiSeq at the Centre for Comparative Genomics and Evolutionary Bioinformatics (CGEB), Dalhousie University. We used the package DADA2 in R (R Core Team, 2018) to identify amplicon sequence variants (ASV) which were used as the fundamental unit of ecological analysis. It discriminates single nucleotide sequence variation by first modelling sequence error rate and correcting sequences for this error (Callahan *et al.*, 2016). We used the SILVA database (V128) for taxonomic assignment using the RDP Naive Bayesian Classifier algorithm in DADA2. We removed ASVs with <100 sequences and rarefied (package Vegan in R) ASVs to 4500 sequences per sample (Fig. S1).

and determine

To assess the impact of the antibiotics on the gut microbiome of each species, we first tested their effect on the relative abundance of major phyla in the gut microbiomes of mice and voles with

zero-inflated negative binomial generalized linear models (package pscl in R) with relative abundance as the dependent variable and phylum, treatment (antibiotic, saline, or untreated), period (pre-treatment period or treatment period), and their interactions as independent variables. We ran separate models for each species. We then compared each treatment-period contrast within each phylum with post-hoc pairwise comparisons of least squares means. We calculated Shannon's and inverse Simpson's diversities (alpha diversity; package Vegan in R) of each sample. We then Hellinger-transformed the ASV relative abundances and calculated the Bray-Curtis dissimilarity among the samples (Oksanen *et al.*, 2019). We used principal coordinate analysis to obtain pairwise dissimilarities in two dimensions. We ran separate mixed models on alpha diversity and on the scores for each of the first two PCoA axes (PC1, PC2) for each species using package nlme in R. Estimated degrees of freedom and p-values for all mixed models were obtained through Satterthwaite's degrees of freedom method with package lmerTest in R. We started with the fully parameterized model which included treatment, period, days since last capture and their interactions as fixed effects, and ID as a random effect. We performed model selection by sequentially removing a term and using the log-likelihood ratio to test the simplified model against the full model. Between-group significance was assessed by pairwise comparisons of least squares means. We validated all final models with respect to model assumptions. To ascertain the impact of the antibiotic at the ASV level, we calculated the weighted average scores for taxa to identify those most associated with each PCoA axis (Oksanen *et al.*, 2019). We overlaid taxa with the greatest weight onto PCoA plots. Finally, we used DESEQ2 (package DESEQ2 in R) differential expression analysis to identify microbial taxa that showed the greatest treatment-induced change in abundance and their log2 fold change (Love *et al.*, 2014). For each pairwise contrast in this analysis, the 30 most significant taxa were compiled, and we generated a heatmap of their log transformed normalized counts (see supplementary material). Factors included in the DESEQ2 model design were treatment, period, and their interaction.

We measured anxiety and exploration behaviour with an open-field test just before, and at the end of the treatment period (Montiglio *et al.*, 2010 ; Wolfer *et al.*, 2004). The arena was cleaned with 70% ethanol before each trial. We could not measure post-treatment behaviour of animals that were not recaptured at the end of the study. Animals were individually placed in the same corner

of an empty arena (46 cm^3) and video-recorded from above for five minutes. We tracked their movement in the arena and extracted behavioural variables using Ethovision software (V.9.0, Noldus Information Technology, Wageningen, The Netherlands). We measured exploration as the total distance travelled in the open field. We also defined a central square in the arena (23 cm^2) which is perceived by the animal as a riskier zone, and measured levels of anxiety as the latency to enter this zone.

To measure home range, when first captured, we outfitted each animal with a passive subcutaneous integrated transponder (PIT) tag. This allowed us to rapidly identify recaptured animals, and to follow their movement within the capture grid. We deployed 16 RFID antennae at equal distances throughout the capture grid from July 25 (Day 12) to August 23 (Day 41). The RFID system is described in the supplementary information section. When an animal passed through the antenna ring, a data logger recorded its identity and the time of detection. To measure home range, we counted multiple consecutive detections for an individual at the same RFID station and on the same day as a single detection. We calculated the 95% kernel home range for each individuals' pre-treatment and treatment period with the package adehabitatHR in R (Calenge, 2006).

Given the high mortality in voles throughout the season we restricted analyses of behaviour and home-range size to deer mice. We ran four mixed models (package lme4 in R) for each behaviour (distance travelled and latency to enter the center) and home range (Table S3.5). The first model had, as independent variables, treatment group, period, PC1, and their interactions. The second model was identical to the first but included the number of previous captures of each individual. The last two models were identical to the first two but included PC2 instead of PC1. We performed model selection by AICc, and likelihood ratio tests when the difference in AICc values was below two. In models for home range, we included number of detections as a weighting factor to correct for the potential effect of the number of detections on the accuracy of the home range estimation. All models had the identity of the mouse as a random factor.

We measured survival during the treatment period (days 18-41) using Cox proportional hazards analysis with the package survival in R (Therneau, 2015). Our data better suited this modelling approach, as opposed to capture-mark-recapture models due to the high detection rate of both mice

and voles (see results). For this analysis, we had enough data to include untreated animals. Most untreated mice were females, so we included all adult-untreated mice. There were enough untreated adult male voles that we excluded female and subadult male voles from the analysis. We pooled the capture and RFID detection data to obtain the first and last detections of each animal. We specified five days as the threshold after which an animal was considered either dead or no longer in the study site. We were confident in this assumption given the high detection rate of our animals. We excluded all animals that died while captive. We ran separate models for each species and included treatment, number of treatments, and their interaction as fixed effect in the models. To understand why some antibiotic-treated voles survived while most did not, we compared the microbiomes of antibiotic-treated voles that died to those of antibiotic-treated voles that survived during the treatment period with a DESEQ2 differential abundance analysis. We did not include animals that died in captivity and we assumed voles to have died if we stopped capturing them nine days or more before the last day of the study.

Finally, to assess whether the antibiotic treatment negatively impacted the health of the host, we tested its effect on capture rate during the treatment period and on the weight of the animals before and after the treatment period. For capture rate, we ran a linear regression analysis with treatment group, species, and their interaction as independent variables. For weight, we ran separate linear mixed models for each species that included period, treatment group, and their interaction as independent variables, with ID as a random factor.

3.7 Declarations

3.7.1 Ethics approval and consent to participate

Animal care and experimental procedures were performed in accordance with protocols approved by the Comité Institutionnel de Protection des Animaux (CIPA #917).

3.7.2 Consent for publication

Not applicable.

3.7.3 Availability of data and material

The 16S rRNA gene sequence data are available in the NCBI repository, BioProject ID PRJNA629807 (https://www.ncbi.nlm.nih.gov/sra/PRJNA629807). The datasets and R scripts supporting the conclusions of this article are included within the article (and its additional file[s]).

3.7.4 Competing interests

The authors of this article declare no competing interests.

3.7.5 Funding

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3.7.6 Authors' contributions

JWJ designed the study, collected and analysed the data and wrote the article, as well as provided a small proportion of funding for the study. DR and SWK provided most of the funding for the study and provided guidance in all aspects of the study including design, statistical analyses and writing the article.

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3.8 Supplementary material

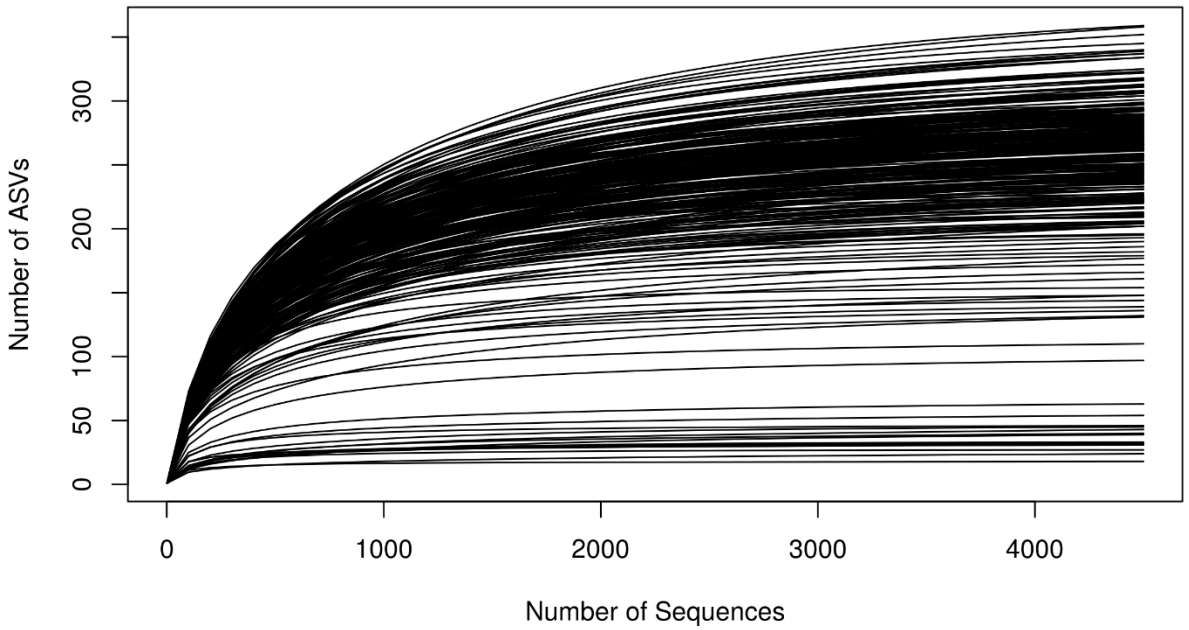

Figure S3.1 Rarefaction curves for all samples collected depicting the number of amplicon sequence variants (ASV) per number of sequences in each sample.

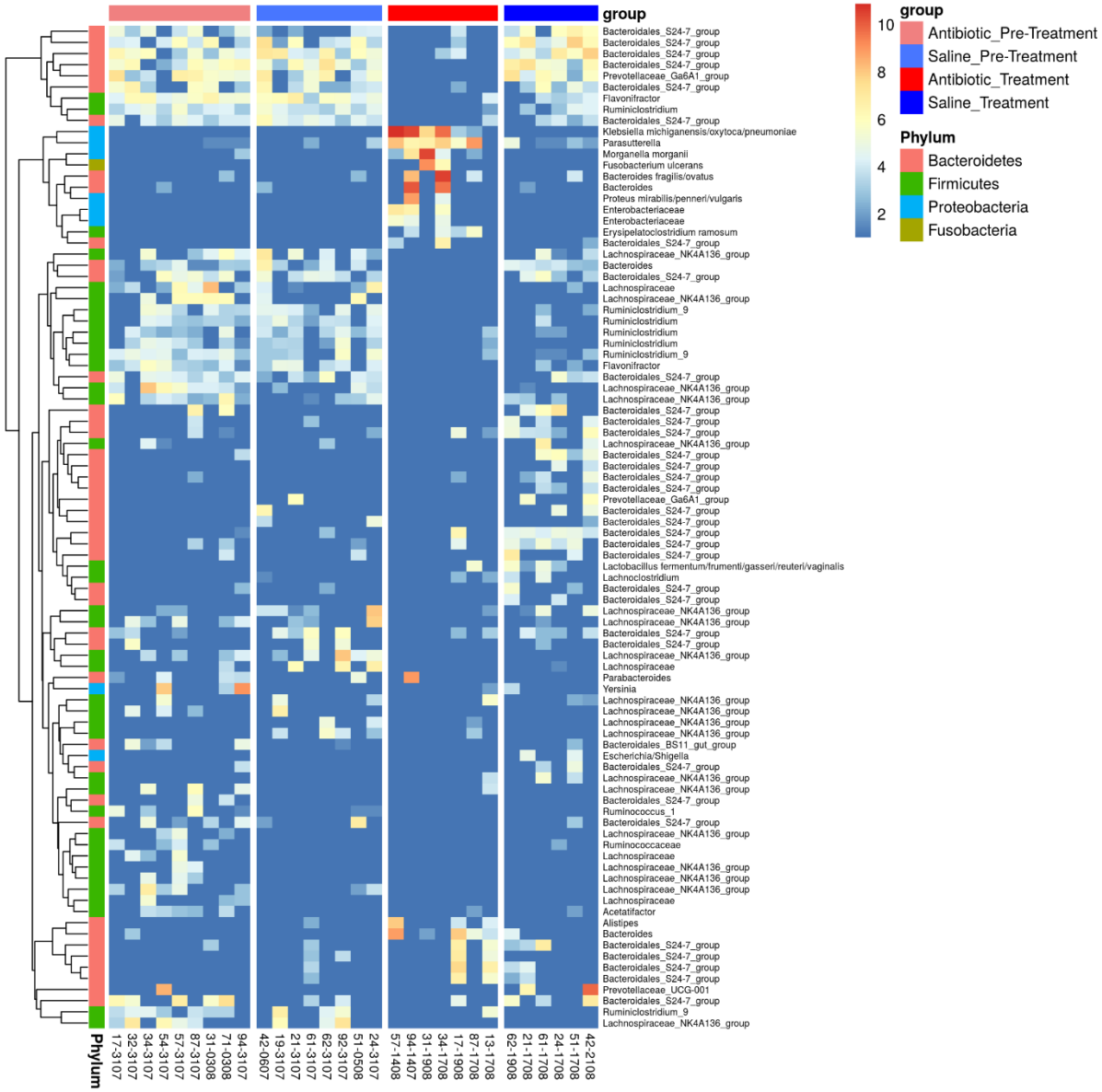

Figure S3.2 We used DESEQ2 differential expression analysis to identify microbial taxa that showed the greatest change in abundance among treatments and periods in deer mice (*Peromyscus maniculatis*). For each pairwise contrast in this analysis, the 30 most significant taxa (adjusted P-values < 0.05) were compiled, and we generated the above heatmap with their log transformed normalized counts.

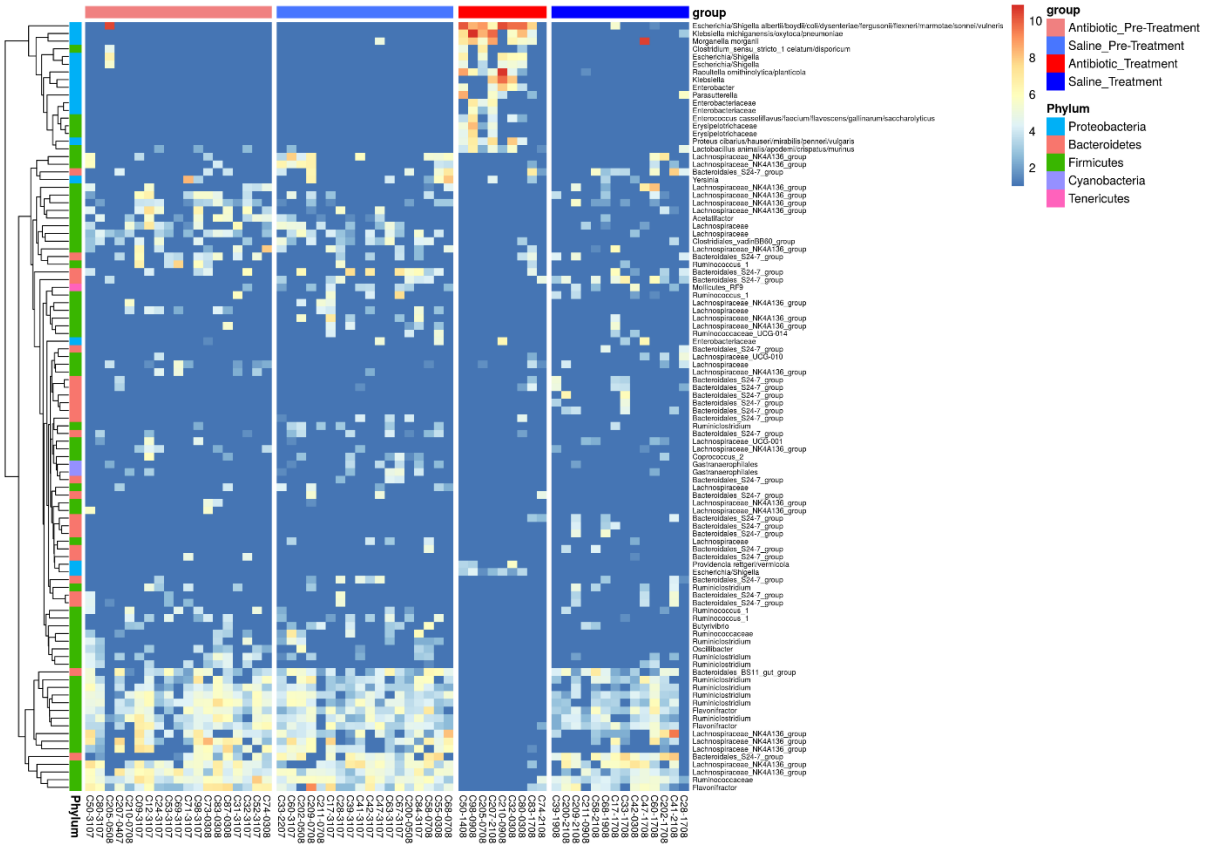

Figure S3.3 We used DESEQ2 differential expression analysis to identify microbial taxa that showed the greatest change in abundance among treatments and periods in red-backed voles (*Myodes gapperi*). For each pairwise contrast in this analysis, the 30 most significant taxa (adjusted P-values < 0.05) were compiled, and we generated the above heatmap with their log transformed normalized counts.

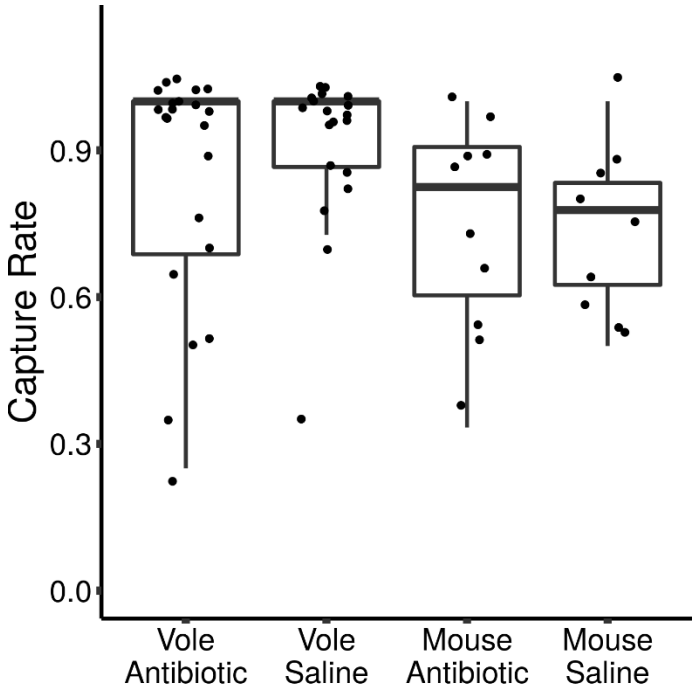

Figure S3.4 Capture rates during the treatment period of each species (deer mouse or red-backed vole) and by treatment group (antibiotic or saline). Capture rates were compared by regression analysis and did not differ statistically (see Table S3.7).

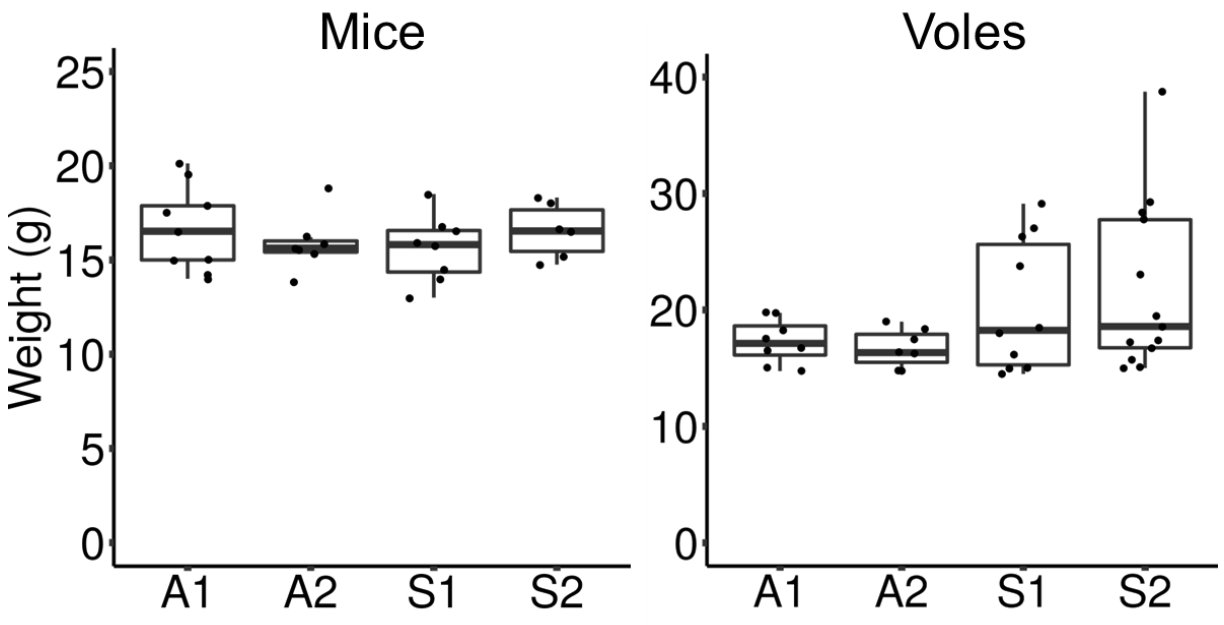

Figure S3.5 Weights of mice and voles given saline (S) or antibiotic (A) during the pre-treatment (1) and treatment (2) periods. There was no effect of the antibiotic on weight. Mice increased in weight from the pre-treatment to treatment period. The weight of voles remained stable throughout the experiment (see Table S3.8).

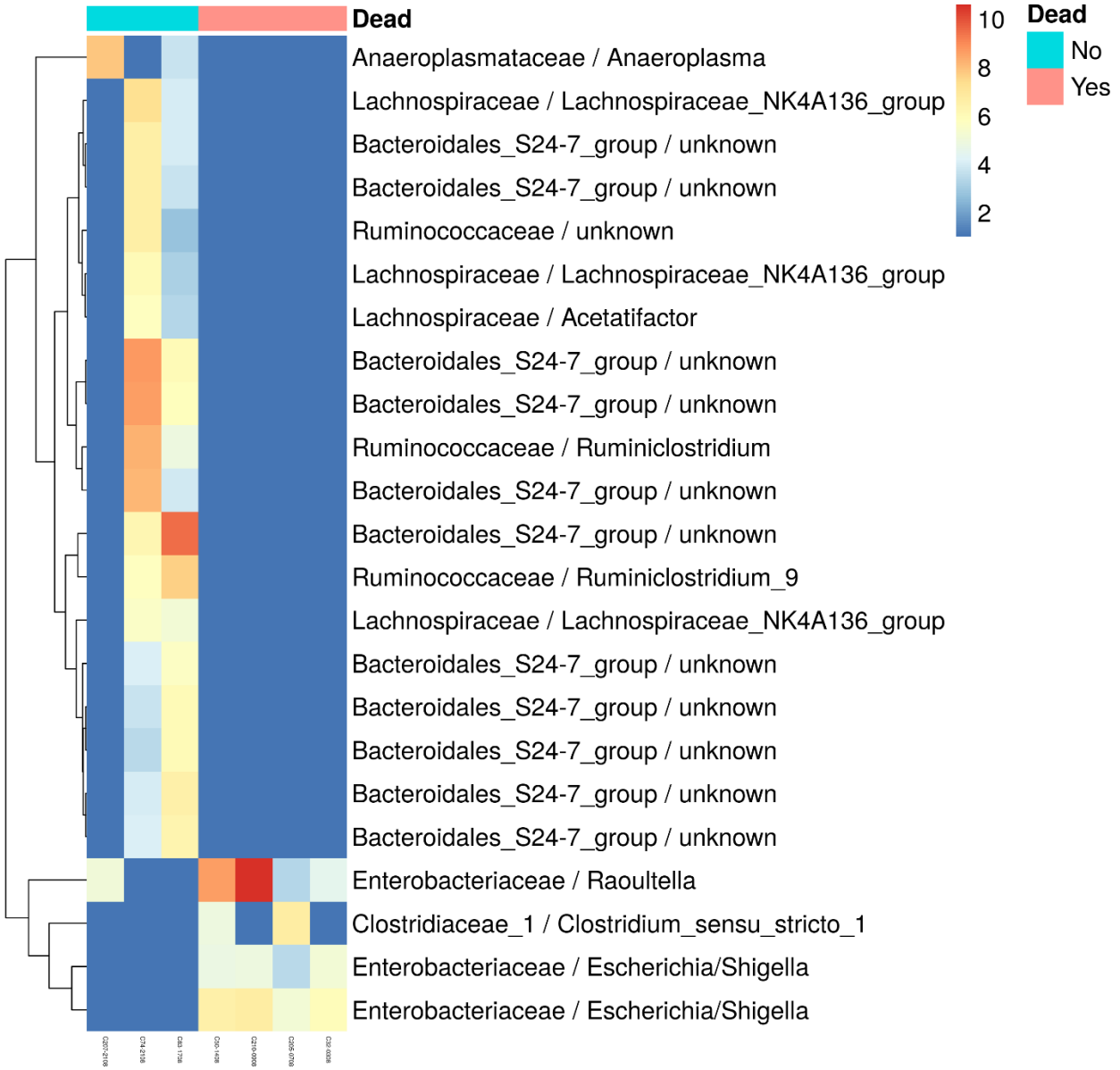

Figure S3.6 We used DESEQ2 differential expression analysis to identify microbial taxa differentially abundant in the gut microbiomes of antibiotic-treated voles that survived compared to antibiotic-treated voles that did not survive during the treatment period. We assumed voles to have died if they stopped being captured >= nine days before the last day of the study. The above heatmap depicts the log transformed normalized counts of differentially abundant ASVs (adjusted P-values < 0.05) classified to family / genus.

Tableau S3.1 Effect of the antibiotic treatment on the relative abundance of major phyla in the gut microbiome of deer mice (Peromyscus maniculatus) and red-backed boles (Myodes gapperi). Results are from zero-inflated negative binomial generalized linear model with relative abundance as the dependent variable and phylum, treatment (antibiotic, saline, or untreated), period (pre-treatment period or treatment period), and their interactions as independent variables. Separate models were run for each host species.

	Mice			Voles		
Effects	DF	Chisq	P	DF	Chisq	P
phylum	5	88.39	**<0.001**	4	57.19	**<0.001**
treatment	2	0.17	0.920	2	0.74	0.690
period	1	0.37	0.544	1	0.04	0.842
phylum : treatment	10	6.67	0.756	8	14.62	0.067
phylum : period	5	47.18	**<0.001**	4	30.13	**<0.001**
treatment : period	2	0.43	0.805	2	0.11	0.944
phylum : treatment : period	10	13.92	0.177	8	11.39	0.181

Tableau S3.2 Results from post-hoc pairwise comparisons of least squares means derived from zero-inflated negative binomial generalized linear models testing for an effect of the antibiotic treatment on the relative abundance of each major phylum in the gut microbiome of deer mice (Peromyscus maniculatus) and red-backed boles (Myodes gapperi) (see Table S3.1).

		Mice					Voles				
	contrast	Estimate	SE	DF	z ratio	P	Estimate	SE	DF	z ratio	P
Bacteroidetes	A1 vs A2	-2.32	11.29	Inf	-0.21	0.897	8.33	8.43	Inf	0.99	0.466
	S1 vs S2	-20.58	15.11	Inf	-1.36	0.433	-4.91	6.77	Inf	-0.73	0.589
	U1 vs U2	-10.96	14.85	Inf	-0.74	0.531	-2.24	7.78	Inf	-0.29	0.773
Firmicutes	A1 vs A2	18.81	3.67	Inf	5.12	**<0.001**	15.79	2.59	Inf	6.09	**<0.001**
	S1 vs S2	16.62	4.71	Inf	3.53	**0.002**	3.16	2.92	Inf	1.08	0.452
	U1 vs U2	6.95	9.03	Inf	0.77	0.531	4.46	3.33	Inf	1.34	0.345
Fusobacteria	A1 vs A2	-5.64	2.92	Inf	-1.93	0.200	-4.00	3.02	Inf	-1.32	0.345
	S1 vs S2	0.00	-	Inf	-	-	0.00	-	Inf	-	-
	U1 vs U2	-0.04	0.04	Inf	-0.99	0.507	0.00	-	Inf	-	-
Proteobacteria	A1 vs A2	-30.15	7.71	Inf	-3.91	**0.001**	-45.37	8.08	Inf	-5.62	**<0.001**
	S1 vs S2	-1.09	0.64	Inf	-1.71	0.260	-1.61	1.16	Inf	-1.39	0.345
	U1 vs U2	13.62	11.11	Inf	1.23	0.472	-0.56	0.17	Inf	-3.26	**0.005**
Tenericutes	A1 vs A2	-0.57	0.59	Inf	-0.96	0.507	-0.34	0.51	Inf	-0.68	0.589
	S1 vs S2	0.07	0.07	Inf	0.99	0.507	0.08	0.17	Inf	0.49	0.673
	U1 vs U2	0.00	0.00	Inf	0.01	0.991	0.47	0.30	Inf	1.58	0.345
Verrucomicrobia	A1 vs A2	-3.27	3.75	Inf	-0.87	0.522	-	-	-	-	-
	S1 vs S2	0.00	-	Inf	-	-	-	-	-	-	-
	U1 vs U2	0.00	-	Inf	-	-	-	-	-	-	-

Tableau S3.3 Effect of the antibiotic treatment on gut microbiome Shannon diversity, inverse Simpson diversity and composition (scores from the first two axes of a PCoA of Bray-Curtis distances) of deer mice (Peromyscus maniculatus) and red-backed boles (Myodes gapperi). Results are from a mixed-effect model with individual ID as a random effect.

		Mice					Voles				
	Effects	Value	SE	DF	t	P	Value	SE	DF	t	P
Shannon Diversity	(Intercept)	0.51	0.10	30	5.01	<0.001	0.32	0.09	56	3.52	0.001
	treatment (saline)	-0.14	0.14	16	-1.01	0.327	0.13	0.13	35	1.04	0.304
	period (treatment)	-2.48	0.16	30	-15.50	**<0.001**	-2.79	0.17	56	-16.30	**<0.001**
	days since the last capture	0.06	0.13	30	0.44	0.663	0.17	0.12	56	1.42	0.162
	treatment (saline): period (treatment)	2.62	0.24	30	10.90	**<0.001**	2.64	0.22	56	11.99	**<0.001**
	treatment (saline): days since the last capture	-0.05	0.15	30	-0.35	0.727	-0.18	0.14	56	-1.30	0.200
	period (treatment): days since the last capture	0.78	0.17	30	4.59	**<0.001**	0.97	0.26	56	3.76	**<0.001**
	treatment (saline): period (treatment): days since the last capture	-1.04	0.25	30	-4.11	**<0.001**	-1.06	0.28	56	-3.73	**<0.001**
Inverse Simpson	(Intercept)	0.39	0.18	30	2.13	0.042	0.16	0.15	60	1.10	0.278
	treatment (saline)	-0.13	0.26	16	-0.49	0.632	0.36	0.21	35	1.68	0.102
	period (treatment)	-2.01	0.25	30	-7.97	**<0.001**	-1.73	0.24	60	-7.30	**<0.001**
	days since the last capture	-0.06	0.20	30	-0.32	0.751	-	-	-	-	-
	treatment (saline): period (treatment)	2.43	0.38	30	6.40	**<0.001**	1.32	0.30	60	4.34	**<0.001**
	treatment (saline): days since the last capture	0.17	0.24	30	0.71	0.482	-	-	-	-	-
	period (treatment): days since the last capture	0.64	0.28	30	2.26	0.032	-	-	-	-	-
	treatment (saline): period (treatment): days since the last capture	-1.57	0.42	30	-3.73	**<0.001**	-	-	-	-	-
PC1	(Intercept)	-0.49	0.08	30	-6.27	0.000	-0.26	0.14	56	-1.92	0.060
	treatment (saline)	-0.01	0.11	16	-0.09	0.932	0.00	0.19	35	-0.01	0.996
	period (treatment)	2.52	0.12	30	20.36	**<0.001**	2.46	0.19	56	13.12	**<0.001**
	days since the last capture	0.01	0.10	30	0.09	0.928	-0.17	0.12	56	-1.46	0.151
	treatment (saline): period (treatment)	-2.20	0.19	30	-11.88	**<0.001**	-2.59	0.23	56	-11.17	**<0.001**

PC2	treatment (saline): days since the last capture	0.07	0.12	30	0.63	0.535	0.16	0.14	56	1.15	0.254
	period (treatment): days since the last capture	-0.87	0.13	30	-6.60	**<0.001**	-1.01	0.25	56	-4.00	**<0.001**
	treatment (saline): period (treatment): days since the last capture	0.80	0.20	30	4.08	**<0.001**	1.07	0.28	56	3.81	**<0.001**
	(Intercept)	-0.01	0.22	33	-0.06	0.955	-0.35	0.16	56	-2.19	0.033
	treatment (saline)	-0.25	0.33	16	-0.76	0.456	-0.06	0.22	35	-0.28	0.782
	period (treatment)	-0.54	0.31	33	-1.77	0.085	2.21	0.27	56	8.29	**<0.001**
	days since the last capture	0.24	0.11	33	2.08	**0.046**	0.01	0.18	56	0.08	0.935
	treatment (saline): period (treatment)	1.79	0.44	33	4.09	**<0.001**	-1.80	0.33	56	-5.38	**<0.001**
	treatment (saline): days since the last capture	-	-	-	-	-	-0.02	0.20	56	-0.08	0.933
	period (treatment): days since the last capture	-	-	-	-	-	-0.65	0.37	56	-1.72	**0.091**
	treatment (saline): period (treatment): days since the last capture	-	-	-	-	-	0.90	0.42	56	2.17	**0.035**

Tableau S3.4 Pairwise comparisons testing the interaction between the treatment (antibiotic or saline) and period (pre-treatment or treatment) on gut microbiome Shannon diversity, inverse Simpson diversity, and composition (scores from the first two axes of a PCoA of Bray-Curtis distances) of deer mice (*Peromyscus maniculatus*). A: antibiotic, S: saline, 1: pre-treatment period, 2: treatment period.

		Mice					Voles				
	contrast	Estimate	SE	DF	t	P	Estimate	SE	DF	t	P
Shannon Diversity	**A1 vs A2**	**2.48**	**0.16**	**30**	**15.50**	**<0.001**	**2.80**	**0.17**	**56**	**16.30**	**<0.001**
	A1 vs S1	0.14	0.14	16	1.01	0.745	-0.13	0.13	35	-1.04	0.725
	A1 vs S2	0.01	0.18	16	0.07	1.000	0.02	0.14	35	0.16	0.999
	A2 vs S1	**-2.34**	**0.16**	**16**	**-14.70**	**<0.001**	**-2.93**	**0.17**	**35**	**-16.97**	**<0.001**
	A2 vs S2	**-2.47**	**0.19**	**16**	**-12.78**	**<0.001**	**-2.77**	**0.18**	**35**	**-15.15**	**<0.001**
	S1 vs S2	-0.13	0.18	30	-0.74	0.882	0.16	0.14	56	1.14	0.666
Inverse Simpson	**A1 vs A2**	0.13	0.26	16	0.49	0.961	-0.36	0.21	35	-1.68	0.349
	A1 vs S1	2.01	0.25	30	7.97	**<0.001**	1.73	0.24	60	7.30	**<0.001**
	A1 vs S2	-0.29	0.32	16	-0.91	0.801	0.05	0.23	35	0.24	0.995
	A2 vs S1	1.88	0.29	16	6.43	**<0.001**	2.09	0.27	35	7.73	**<0.001**
	A2 vs S2	-0.42	0.28	30	-1.49	0.459	0.41	0.19	60	2.18	0.141
	S1 vs S2	-2.30	0.35	16	-6.61	**<0.001**	-1.68	0.28	35	-5.99	**<0.001**
PC1	A1 vs S1	0.01	0.11	16	0.09	1.000	0.00	0.19	35	0.01	1.000
	A1 vs A2	**-2.52**	**0.12**	**30**	**-20.36**	**<0.001**	**-2.46**	**0.19**	**56**	**-13.12**	**<0.001**
	A1 vs S2	-0.31	0.14	16	-2.22	0.159	0.13	0.21	35	0.64	0.920
	S1 vs A2	**-2.53**	**0.12**	**16**	**-20.56**	**<0.001**	**-2.46**	**0.23**	**35**	**-10.54**	**<0.001**
	S1 vs S2	-0.32	0.14	30	-2.30	0.120	0.13	0.14	56	0.95	0.779
	A2 vs S2	**2.21**	**0.15**	**16**	**14.81**	**<0.001**	**2.59**	**0.24**	**35**	**10.65**	**<0.001**
PC2	A1 vs S1	0.25	0.33	16	0.77	0.869	0.06	0.23	35	0.28	0.992
	A1 vs A2	0.54	0.31	33	1.77	0.304	-2.21	0.27	56	-8.29	**<0.001**
	A1 vs S2	-1.00	0.38	16	-2.64	0.076	-0.35	0.25	35	-1.42	0.497
	S1 vs A2	0.29	0.37	16	0.80	0.854	-2.27	0.29	35	-7.84	**<0.001**
	S1 vs S2	**-1.25**	**0.33**	**33**	**-3.80**	**0.003**	**-0.41**	**0.20**	**56**	**-2.03**	**0.191**
	A2 vs S2	**-1.54**	**0.40**	**16**	**-3.83**	**0.007**	**1.86**	**0.31**	**35**	**6.10**	**<0.001**

Tableau S3.5 Mixed models testing for an effect of the antibiotic-mediated change in gut microbiome on open-field behaviours and home-range size in deer mice (*Peromyscus maniculatus*). Gut microbiome composition is represented by the scores (PC1 and PC2) of the first two axes from a PCoA of Bray-Curtis distances. We ran separate models for each behaviour and each microbiome composition axis (PC1 and PC2). In models for home range, we included number of detections as a weighting factor. Models were compared by AICc (values presented under each behaviour heading). Final selected models have bolded AICc values. If two models containing the same microbiome axis (PC1 or PC2) were equivalent (ΔAICc < 2), we selected the model that included the number of previous captures.

Models Compared	Distance Travelled	Latency to Enter Center	Home Range
treatment + period + PC1 + number of previous captures + treatment:period + treatment:PC1 + period:PC1 + treatment:period:PC1 + (1|ID)	102.83	**96.98**	83.73
treatment + period + PC1 + treatment:period + treatment:PC1 + period:PC1 + treatment:period:PC1 + (1|ID)	100.75	97.77	82.93
treatment + period + PC2 + number of previous captures + treatment:period + treatment:PC2 + period:PC2 + treatment:period:PC2 + (1|ID)	91.25	**96.47**	**71.80**
treatment + period + PC2 + treatment:period + treatment:PC2 + period:PC2 + treatment:period:PC2 + (1|ID)	**88.33**	102.04	72.98

Tableau S3.6 Effect of the antibiotic-mediated change in gut microbiome on open-field behaviours and home-range size in deer mice (*Peromyscus maniculatus*). Results from mixed models testing for an effect of the gut microbiome composition given by the scores of the first two axes from a PCoA of Bray-Curtis distances on behaviours measured in an open field and on the 95% kernel home range. We ran separate models for each behaviour and each microbiome composition axis (PC1 and PC2). In models for home range, we included number of detections as a weighting factor. We present here the results for the best models from model comparisons described in Table S3.5. P-values are adjusted for false-discovery rate with a Benjamini-Hochberg correction. Significant (≤0.05) p-values are bolded.

	Effects	Estimate	SE	DF	t	P
Distance Travelled	(Intercept)	0.41	0.31	16.0	1.35	0.195
	treatment (saline)	0.12	0.46	16.1	0.27	0.816
	period (treatment)	-0.94	0.24	6.0	-4.01	**0.036**
	PC2	0.72	0.40	19.2	1.78	0.156
	treatment (saline):period (treatment)	-2.75	0.51	6.3	-5.36	**0.023**
	treatment (saline):PC2	-0.28	0.47	18.1	-0.60	0.641
	period (treatment):PC2	-0.47	0.35	9.8	-1.33	0.278
	treatment (saline):period (treatment):PC2	1.73	0.58	10.2	2.97	0.053
Latency to Enter Center	(Intercept)	-0.11	0.49	19.5	-0.21	0.834
	treatment (saline)	1.04	0.61	19.1	1.70	0.163
	period (treatment)	-0.97	0.73	18.6	-1.32	0.272
	PC1	-0.87	0.59	18.2	-1.47	0.236
	number of previous captures	0.75	0.35	15.0	2.13	0.109
	treatment (saline):period (treatment)	-0.41	0.74	10.9	-0.56	0.653
	treatment (saline):PC1	2.14	0.84	19.9	2.56	0.058
	period (treatment):PC1	1.25	0.72	17.4	1.73	0.163
	treatment (saline):period (treatment):PC1	-3.56	1.09	17.8	-3.26	**0.036**
Latency to Enter Center	(Intercept)	0.38	0.35	16.3	1.10	0.289
	treatment (saline)	0.18	0.45	15.9	0.39	0.750
	period (treatment)	-1.35	0.57	19.9	-2.37	0.080
	PC2	-0.79	0.43	20.0	-1.83	0.150
	number of previous captures	0.99	0.33	16.3	2.98	**0.039**
	treatment (saline):period (treatment)	1.33	0.65	7.4	2.05	0.150
	treatment (saline):PC2	1.14	0.51	19.7	2.21	0.097
	period (treatment):PC2	0.40	0.43	10.4	0.95	0.454
	treatment (saline):period (treatment):PC2	-1.90	0.69	12.4	-2.77	0.057
Ho	(Intercept)	0.89	0.25	16.1	3.53	0.003

treatment (saline)	-0.71	0.34	15.6	-2.09	0.109
period (treatment)	-1.78	0.39	20.8	-4.60	**0.005**
PC2	-0.81	0.22	9.1	-3.73	**0.036**
number of previous captures	0.70	0.22	18.3	3.12	**0.036**
treatment (saline):period (treatment)	0.32	0.46	7.9	0.69	0.605
treatment (saline):PC2	0.64	0.27	8.2	2.42	0.097
period (treatment):PC2	0.33	0.22	7.2	1.53	0.238
treatment (saline):period (treatment):PC2	-0.06	0.40	8.5	-0.15	0.884

Tableau S3.7 Comparison of capture rates during the treatment period of each species (deer mouse or red-backed vole) and by treatment group (antibiotic or saline). Capture rates were compared by linear regression and did not differ statistically.

Effects	Estimate	SE	DF	t	P
(Intercept)	0.84	0.04	3, 56	18.99	<0.001
treatment (saline)	0.07	0.07	3, 56	1.04	0.305
species (mouse)	-0.08	0.08	3, 56	-1.03	0.309
treatment (saline) : species (mouse)	-0.09	0.12	3, 56	-0.78	0.437

Tableau S3.8 Results from mixed models testing for an effect of the antibiotic treatment on the weights of mice and voles. Dependent variables in the models were the period (pre-treatment or treatment), the type of treatment (antibiotic or saline), and their interaction. We ran separate models for each species. Significant P-values are bolded.

	Mice					Voles				
Effects	Estimate	SE	DF	t	P	Estimate	SE	DF	t	P
(Intercept)	17.09	1.68	23	10.20	**<0.001**	16.30	0.66	16	24.53	**<0.001**
period (treatment)	-0.79	0.58	11	-1.36	0.202	0.72	0.27	10	2.65	0.024
treatment (saline)	3.63	2.24	23	1.62	0.118	-0.70	0.99	16	-0.71	0.490
period (treatment) : treatment (saline)	1.34	0.70	11	1.90	0.083	-0.17	0.39	10	-0.44	0.672

3.8.1 Description of RFID dataloggers

Dataloggers consisted of a ring-shaped antenna placed onto a wooden arena (20 x 20 x 5 cm). The arena contained closed compartments filled with peanut butter. The compartments were perforated so animals could smell the bait but not eat it. We regularly replaced the bait in these compartments. The floor of the arena was a plastic grid elevated 1-2 cm above the ground to prevent accumulation of feces within the arena and prevent unwanted cross-contamination among animals.

CHAPITRE IV

MOUSE AND VOLE SOCIAL NETWORKS ARE LINKED TO THEIR GUT MICROBIOME COMPOSITION AND DIVERSITY

Joël W. Jameson, Steven Kembel, Denis Réale

4.1 Abstract

Metacommunity theory predicts that strongly connected individuals will harbour similar gut microbiomes (GMs) and affiliating with more individuals should increase GM diversity. Additionally, cross-species bacterial transmission may play a role in how interspecific interactions affect host community dynamics. We tracked sympatric mice (Peromyscus maniculatus) and voles (Myodes gapperi) and constructed social networks for each species and both species together. We tested whether: 1) social proximity correlated with GM similarity between individuals within and across species; 2) A host's number of conspecific or heterospecific neighbours correlated with GM diversity. We could not differentiate associations between GM composition and mouse social proximity or habitat. In voles, social proximity explained part of the GM composition. GM composition associated with interspecific social proximity, and mouse GM diversity correlated with number of vole neighbours. Contributions of host-host bacterial transmission to the GM partly follow metacommunity theory but depend on host species.

4.2 Introduction

There is growing interest in how social interactions among animals relate to their microbiome. The gut microbiome (GM) is important for digestion (Neish, 2009), lipid storage (Bäckhed et al., 2004), immune activation (Karmarkar and Rock, 2013), and development of the central nervous system (Desbonnet et al., 2014; Heijtz et al., 2011; Hoban et al., 2016; Neufeld et al., 2011). These effects have downstream consequences for social behaviour (Desbonnet et al., 2014; Gacias et al., 2016). Dysbiosis of the GM is associated with autism spectrum disorder (Mohamadkhani, 2018), depression, anxiety and social impairment (Amato, 2013; De Palma et al., 2015; Golubeva et al., 2017) as well as intestinal pathologies including Crohn's disease (Joossens et al., 2011) and irritable bowl syndrome (Kennedy et al., 2014). Given the microbiome's association with host health and social behaviour, understanding how GM composition relates to interactions among hosts can help us better understand population dynamics.

Considering the host as a habitat patch inhabited by a community of species, the microbiome, allows the use of metacommunity theory to explain at least part of how host social interactions affect GM composition (Amato, 2013; Miller et al., 2018). Following metacommunity theory (Leibold et al., 2004; MacArthur and Wilson, 1967), if microbes disperse between hosts, then similar microbial communities should inhabit closely connected hosts, and more isolated hosts should show lower diversity. Abundant evidence demonstrates the capacity for host-host bacterial transmission to shape the GM. For example, in humans, the mother transfers perinatal GM via the uterus, vagina and milk (Amato, 2013). In adulthood, individuals that live together share more similar GMs than non-housemates (Song et al., 2013) with greater similarity and increased bacterial diversity in the GMs of close married couples (Dill-McFarland et al., 2019). Additionally, for people that live alone, higher levels of social interactions increase GM diversity (Dill-McFarland et al., 2019). Finally, mice sharing the same cage develop similar GM compositions, either through direct contacts or indirectly via the environment (Alexander et al., 2006; Antonopoulos et al., 2009; Hildebrand et al., 2013).

Besides studies on humans and laboratory animals, social network studies of wild animals provide further evidence for a strong role of host-host interactions in shaping the GM composition and diversity. During periods of elevated social aggregation, and regardless of diet, degree of social contacts among chimpanzees (Pan troglodytes) explained 7.6% of variation in GM richness and 4.8% of variation in GM composition (Moeller et al., 2016). Tung et al. (Tung et al., 2015) found that 19% of the GM of baboons (Papio cynocephalus) was attributable to social group. Antwis et al. (Antwis et al., 2018) identified within- and across-band variation in the GM composition of semi-feral ponies (Equus ferus caballus) consistent with social proximity, with band explaining 14% of GM composition. In red-bellied lemurs (Eulemur rubriventer), social group explained up to 28% of GM variance, once controlling for time, age and sex but not diet (Raulo et al., 2018). Interestingly, Raulo et al. observed a negative correlation between individual sociality (i.e. propensity of social grooming) and GM diversity, which they claim counters metacommunity theory. However, metacommunity theory predicts greater diversity at intermediate levels of dispersal (Kunin, 1998; Mouquet et al., 2006; Venail et al., 2010) meaning GM diversity may be lower in highly social species. Current evidence for an association between social interactions and host GM composition and diversity, thus, seems consistent with metacommunity theory. This evidence, however, comes from highly social species including humans and other primates, and domestic or laboratory animals (Alexander et al., 2006; Antwis et al., 2018; Hildebrand et al., 2013; Moeller et al., 2016; Raulo et al., 2018; Tung et al., 2015). We must extend our knowledge to other social architectures to fully understand the role of dispersal in shaping the GM.

Past studies have also focused on a single species at a time in varied geographic locations, but bacterial transmission may occur across co-occurring species. Adult humans share a more similar skin microbiome with their dogs than with other dogs, and dog owner's skin microbiomes resemble each other more than those of people without dogs (Song et al., 2013). Additionally, experimental GM transfers can humanize the GM of mice (Turnbaugh et al., 2009; Wrzosek et al., 2018), indicating that cross-species microbial dispersal could shape the GM of wild animals. Cross-species microbial transfer may occur directly through predator-prey relationships (Moeller et al., 2017), or through competition and agonistic interactions, or indirectly through shared habitats. Agonistic interactions have been observed between several small mammal species in neutral field

tests (Banks et al., 1979; Falkenberg and Clarke, 1998; Grant, 1972; Rowley and Christian, 1976; Rychlik and Zwolak, 2006). In the wild, agonistic interactions among species are suggested to facilitate coexistence (Rychlik and Zwolak, 2006). We suspect that competitive interactions between species or their sympatric use of resources can facilitate the transmission of GM members from one species to another, which could have strong eco-evolutionary consequences. For instance, transfer of beneficial or pathogenic bacteria could change the competition game between two species. It is also important to understand the extent of the role that some individuals might play in interspecies transfer, as it may create fitness differences among individuals within each species. However, the extent to which such transmission occurs in the wild has not been tested.

Here we examine the role of social proximity in two sympatric and ecologically similar species of rodent, the deer mouse (Peromyscus maniculatus) and the red-backed vole (Myodes gapperi). Both species are common and widespread in North America, have similar habitat preferences, population dynamics, and reproductive strategies (Harper and Austad, 2001). Both species mate promiscuously, and females are territorial while males are not (Mihok, 1981). Consequently, their home ranges overlap considerably, enabling within- and cross-species bacterial dispersal that could affect the GM. Additionally, neither species forms strong social groups (Kawata, 1985; Millar and Derrickson, 1992), though deer mice show some plasticity in social gregariousness (Millar and Derrickson, 1992; Savidge, 1974), so testing associations between GM and social proximity in this system should afford a better understanding of the extent to which metacommunity theory can predict GM composition. Specifically, we hypothesize that 1) similarity in GM composition between individuals will correlate with their likelihood of association, at the within- and across-species levels; and 2) an individual's GM diversity will increase with its number of conspecific or heterospecific neighbours.

4.3 Methods

This study took place from 14 July—2 August 2016 (20 days) on Harbour Island in the Winnipeg River basin, Ontario (50°2.580'N, 94°40,436'W). We captured mice and voles with Longworth and BIOEcoSS (BIOEcoSS Ltd.) live traps. We placed 192 traps at 96 stations (8 × 12) distanced 10 m apart providing a capture grid (9600 m2) large enough to estimate individual home ranges (Thompson et al., 2009; Vanderwel et al., 2010; Wolff, 1985; Wood et al., 2010). Weather permitting, we conducted captures every second day. We opened the traps in the evening and checked and closed them at 06:00. Before each trap deployment, we cleaned and sterilized them with 70% ethanol and baited them with 4 g of carrot and 1 ml of peanut butter. For each capture, we recorded age as one of three classes (juvenile, subadult, adult) based on pelage colour (Naughton, 2012; Osgood, 1909) and sex based on urogenital distance. After processing, we returned animals to their precise trapping location.

When first captured, we outfitted each animal with a subcutaneous, passive integrated transponder (PIT) tag. This allowed us to rapidly identify recaptured animals, and to collect movement data by deploying 16 RFID data loggers at equal distances throughout the capture grid starting on Day 12 (July 25). Data loggers consisted of a ring-shaped antenna placed onto a wooden arena (20 × 20 × 5 cm). The arena contained closed compartments filled with peanut butter which we regularly renewed. The compartments were perforated so animals could smell the bait but not eat it. The floor of the arena was a plastic grid elevated 1–2 cm above the ground to prevent accumulation of feces within the arena and prevent unwanted cross-contamination among animals. When an animal passed through the antenna ring, the data logger recorded its identity and time of detection. We counted multiple consecutive detections for an individual at the same RFID station and on the same day as one detection.

We obtained a dual-species social network based on the compiled capture and PIT tag data from both species. Since traps and data loggers do not sample equally, we randomly selected a single detection from each individual on capture days but used all detections on non-capture days. This allowed us to maximize the data used to estimate social networks while balancing survey effort. We also only retained individuals with at least five days of data. We estimated the social network using a novel approach described in (Wanelik and Farine, 2019) wherein we use logistic models

to model sex- and species-specific space use. Using the data from both species, we first built a distance matrix based on each individuals' centroid and, for each pair of individuals, we estimated their probability of occurring at each of 100 distance slices between their centroids. We then took the parallel minima and maxima of those probabilities and, finally, the ratio of the sum of the parallel minima and the sum of the parallel maxima gives a measure of the encounter probability between each pair of individuals. The matrix of encounter probabilities (hereafter "social matrix") can then be graphically represented as a social network with edge weights corresponding to the encounter probability. We then subsampled the social matrix to obtain a separate social matrix for each species. We assessed whether the dual (i.e. mouse and vole) and single-species social networks were non-randomly structured using a Monte Carlo approach by generating 1000 random networks and comparing the coefficients of variance of observed social matrices to those of random social matrices. We transformed the social matrices into social distance matrices by taking the log of the inverse of the social matrices. We did this because some subsequent analyses required input matrices to be in this format and it facilitated interpretation of results since microbiome composition was summarized as a dissimilarity matrix. After transformation, the log(social matrix) and the distance matrix were perfectly negatively correlated (R2=1, see supplementary information).

To account for potential confounding effects of shared habitat on the GM, we collected habitat data at each trapping station and included information on habitat composition in our analyses testing our first hypothesis on GM composition, and on habitat composition and diversity when testing our second hypothesis on GM diversity. At each station, within a 5 m-radius circle centered on the trap, we characterized the overstory by measuring the abundance of all tree species with a diameter at breast height (dbh)>10 cm and the percent total canopy cover. We characterized the understory by measuring, within a 2 m-radius circle centered on the trap, the percent cover of all other plant species. We did not identify mosses and lichens to species. We also noted the percent cover of deadwood, tree stumps, litter, and rock. Finally, we measured soil moisture and pH one meter from the trap in each cardinal direction. We simplified the data by removing overstory species with an abundance <5% of the total overstory data and by removing understory species with an abundance <1% of the understory data. We Hellinger-transformed the habitat community

data and summarized it with a principal component analysis (PCA) (Borcard et al., 2011). The first three PCA components cumulatively accounted for 43% of the variance in habitat data so we used their scores (Habitat-PC1, Habitat-PC2, Habitat-PC3) as habitat composition variables in subsequent analyses. Finally, we obtained a single value for each score per individuals by averaging the habitat scores over each individual's detection events. We quantified habitat diversity using Simpson's index.

We collected samples from feces left in the trap by the captured individual. We collected two to three fecal samples per animal throughout the study. We amplified and sequenced the V4-V5 regions (~410 bp) of the 16S rRNA gene. We identified bacterial sequences to amplicon sequence variants (ASV) with DADA2 (Callahan et al., 2016) and performed taxonomic assignment with SILVA database (V128). Details on sample storage, DNA extraction, amplification and sequencing are described in (Jameson et al., 2020). Sequence data quality control procedures are described in Appendix S1 in Supporting Information.

As a general test of our first hypothesis, we used a Procrustes analysis (least-squares orthogonal mapping; function protest, package Vegan; Oksanen et al., 2019; Peres-Neto and Jackson, 2001) to test for structural similarities between the microbiome and social distance matrices. We also ran the same analysis testing microbiome dissimilarity (Bray-Curtis) against habitat dissimilarity (Euclidean distance). This analysis generates a measure of fit (m122), which is the sum of squared deviations between two matrix configurations, from which it derives a correlation index (t). It then uses a permutation test to calculate the probability (p-value) of observing our m122 (Oksanen et al., 2019; Peres-Neto and Jackson, 2001). We ran these two tests for each species' social distance matrix and for the dual-species social distance matrix. We also ran this test on the subset of interspecific pairs from the dual-species social distance matrix which allowed us to focus the analysis on between-species interactions.

We then used a constrained redundancy analysis (RDA ; function rda, package Vegan; Borcard et al., 2011) as a more thorough test of our first hypothesis. This analysis allowed us to identify gradients of microbiome composition associated with social structure and to identify components of social structure correlated with the microbiome and not confounded with shared habitat. To

extract gradients of variation in social distance that we could use in the RDA, we ran principal coordinate analysis (PCoA; function pcoa, package ape; Paradis et al., 2020) on social distance and used the scores of the first three axes (Social-PCo1, Social-PCo2, Social-PCo3) in the RDA analyses. These axes cumulatively represented 47%, 43%, and 16% of the variance in the mouse, vole and dual-species networks respectively. We ran separate RDA analyses for each species and for the dual-species network. We included the Hellinger-transformed microbiome community as the dependent variable, and the social and habitat scores as independent variables. We also included sex in the analysis for voles, but not for mice as the mouse data for this analysis contained a single female. Additionally, for the dual species model, we first removed the variance associated with species and sex by conditioning for these variables in a partial RDA. We used forward stepwise model selection and set the selection procedure to stop when the adjusted R2 reached that from the full model. We used an ANOVA-like permutation test to assess the significance of the models and of each independent variable in the models. To understand which social and habitat variables were confounded, we ran the above analyses without the habitat variables, and once more conditioning on the habitat variables.

To address the effect of an individuals' position in the network relative to conspecifics and heterospecifics (i.e. number of social neighbours), we obtained three measures of degree centrality for each species. We used degree centrality since it is equivalent to other network parameters in predicting information flow but more suited to potentially incomplete datasets due to its simplicity and lower sensitivity to error (Drewe and Perkins, 2015). For each individual of both species, we first measured the degree centrality of an individual in its conspecific network, and its degree centrality taken from the dual-species network. For the third measure, we obtained that individual's degree centrality relative to the heterospecific network.

We then measured Shannon's, Simpson's, and richness indices of microbiome diversity (function diversity, package vegan). Simpson's diversity is more sensitive to abundant species, richness to rare species, and Shannon's diversity to rare and abundant species equally (Morris et al., 2014). We tested our hypothesis on GM diversity with all three indices as this allowed us to understand which component of diversity was affected by social transmission. We then ran linear models

(package stats) with microbiome diversity as the dependent variable and degree centrality, Habitat-PC1, Habitat-PC2, Habitat-PC3, and Simpson's habitat diversity as independent variables. When necessary, we log-transformed microbiome diversity to meet normality assumptions. We ran separate models for each measure of microbiome diversity, each measure of degree centrality, and for each species. Each of these full models was reduced using backwards stepwise model selection by AIC. Finally, we corrected p-values for false-discovery-rate with a Benjamini-Hochberg correction.

4.4 Results

We collected enough data to build a social network for 27 mice (20 males, 7 females) and 48 voles (26 males, 21 females, 1 unknown sex) and, of these, we obtained viable microbiome samples from 18 mice (17 males, 1 female) and 34 voles (24 males, 10 females). The social network constructed from both species, as well as those of each species, were more structured than random (Fig. 1, see Appendix S2 and Appendix S3). We tested for an effect of species and sex on degree centrality (linear model with species and sex as fixed effects) to assess how an over-representation of male mice might affect the interpretation of our results. Mice had a lower degree centrality than voles and this was driven by a lower degree centrality in female compared to male mice (species [vole] × sex [male]: $t=-3.43$, $P<0.001$, $R2adj=0.21$; see Appendix S4).

We used the scores of the first three axes of a PCA (see Appendix S5 and Appendix S6) on the habitat community data measured at each trapping location as habitat variables when testing our hypotheses about social proximity and microbiome structure and diversity. These axes accounted for 24.1%, 11.2%, and 7.4% of the variance in habitat data. Axis 1 (Habitat-PC1) represented a gradient going from open, coniferous forest with low fruiting shrubs (e.g. blueberry; Vaccinium myrtilloides) to mixed forest with coarse woody debris. Axis 2 (Habitat-PC2) represented a gradient going from coniferous forest with low understory comprised of mosses, starflower (Trientalis borealis) and bunchberry (Cornus canadensis), to poplar-dominated deciduous forest

with an understory of beaked hazel (Corylus cornuta) and red osier dogwood (Cornus stolonifera). Finally, axis 3 (Habitat-PC3) represented a gradient going from poplar-dominated deciduous forest with soils relatively high in moisture and pH, to mixed forest with low-moisture, low-pH, litter-covered soil.

The GM community dissimilarity of mice and voles were correlated with social network (Procrustes analyses; mice: m122=0.32, t=0.84, P<0.001; voles : m122=0. 47, t=0.74, P=0.01) and habitat dissimilarity (mice: m122=0.48, t=0.64, P<0.001; voles: m122=0.63, t=0.63, P=0.02). For the dual-species network, GM community dissimilarity correlated with the social network (m122=0.68, t=0.56, P<0.001), but not with habitat dissimilarity (m122=0.86, t=0.37, P=0.15). Similarly, for mouse-vole pairs, GM dissimilarity remained correlated with social distance (m122=0.70, t=0.55, P=0.03), but not with habitat dissimilarity (m122=0.84, t=0.40, P=0.22).

When analysing the effect of social distance on GM composition accounting for habitat by RDA, Mouse GM composition was positively correlated with Habitat-PC2 (F=2.05, P=0.001) and Habitat-PC3 (F=1.33, P=0.016; Fig. 2). The adjusted cumulative R-squared (R2adjcum) for these variables was 0.077. When we repeated the modelling procedure with habitat removed, GM correlated with Social-PCo1 (F=1.85, P=0.001) and Social-PCo2 (F=1.43, P=0.003) and both explanatory variables had a R2adjcum of 0.072 (Fig. 3). Finally, when we repeated the above procedure while conditioning on habitat, GM was no longer correlated with the social network.

Vole GM composition correlated with Habitat-PC2 (F=1.48, P=0.007; Fig. 4), Social-PCo1 (F=1.52, P=0.008) and sex (F=1.35, P=0.03) and these variables had a R2adjcum of 0.04. When we removed habitat from the full model, GM was correlated with Social=PCo1(F=1.48, P=0.005), Social-PCo2 (F=1.40, P=0.02), and sex (F=1.34, P=0.04; R2adjcum=0.04; Fig. 5). When we conditioned on habitat and sex, GM composition remained correlated with Social-PCo1(F=1.66, P=0.003; R2adjcum=0.06).

When we performed a combined analysis including mice and voles, conditioning on sex and species, GM composition was only correlated with Habitat-PC3 (F=2.43, P=0.005, R2=0.03). GM

composition did not correlate with social proximity when we excluded habitat from the analyses since no variables were retained in the model during the model selection procedure.

We tested if mice GM diversity increased with increasing degree centrality in the mouse social network. We only found an association between GM Shannon's diversity and Habitat-PC2 and between GM richness and both Habitat-PC1 and Habitat-PC2 (Table 4.1). When we regressed mouse GM diversity on habitat and degree centrality obtained from the dual-species network, GM Shannon's diversity and GM richness both correlated with Habitat-PC1 and Habitat-PC2, but GM Shannon's diversity also correlated with degree centrality (Table 4.1, see Appendix S7). Finally, when we tested for a relationship between GM diversity and degree centrality of each mouse with respect to the vole network, GM Shannon's diversity and richness correlated with Habitat-PC1 and degree centrality (Table 4.1). Additionally, using degree centrality with respect to the vole network gave models that explained four times as much of the mouse GM Shannon's diversity and twice as much of mouse GM richness as including degree centrality with respect to the mouse network (Table 4.1). Simpsons' diversity of the mouse GM did not correlate with habitat or any measure of degree centrality (Table 4.1). In contrast to mice, the only association with vole GM diversity we found was between GM Simpson's diversity and sex, with males harbouring higher diversity than females (Table 4.1, see Appendix S8).

4.5 Discussion

We hypothesized that similarity in GM composition between hosts, within and across host species, would correlate with their social distance. We found that, in mice, social position and habitat were related, and that habitat was a slightly better predictor of GM composition. In contrast, in voles, social position best explained part of their GM composition and a second component of vole GM varied with both habitat and social position but was best explained by habitat. Finally, we found some evidence for interspecific interactions driving GM composition. Our second hypothesis was

that GM diversity would vary with the number of neighbours in the social network, within and cross-species. This was only supported for the number of vole neighbours on the GM of mice.

The confounding effects of social position and habitat in mice made it impossible to confirm a role for social distance in shaping the mouse GM composition. This is likely a result of the method (Wanelik and Farine, 2019) used to generate the social network. We used spatial occupation data to estimate social connections and habitat is also spatially structured, rendering both factors related and difficult to dissociate. While observations of true contacts may provide a more accurate estimate of the social network, these may be rare (especially for less social individuals) and difficult to obtain. Our method allowed estimation of both strong and weak social connections. Additionally, our hypotheses were based on a host-as-patch paradigm which does not preclude indirect transmission between hosts via the environment and thus probability of transmission should still depend on host isolation. Separating social distance and habitat effects on GM in mice will require sampling in areas where habitat is homogeneous.

Given that our measure of social distance among individuals depended on their movement, the effect of social distance we observed may result from individual differences in spatial behaviours such as exploration. GM composition in wild deer mice has been shown to directly influence host exploration in an open field but does not seem to affect home range size (Jameson et al., 2020). We found that Bacteroidales-S24-7 (phylum Bacteroidetes) and Lachnospiraceae (phylum Firmicutes) strongly associated with position in the social network in both mice and voles (see Fig. 3 and Fig. 5), and these taxa have been shown to associate with open-field exploration in wild deer mice (Jameson et al., 2020). Fast explorers should have larger home ranges and a greater probability of encountering more conspecifics and heterospecifics and experience a greater diversity of habitats. These features should impact their GM. Specific GM members or communities could also directly influence host social behaviour by promoting host cohesive behaviour and rendering their hosts more likely to associate with each other thus facilitating their transmission among the hosts. Some members of Bacteroidales-S24-7 are also associated with social behaviour, with their abundance linked to social and cognitive impairment and depression in a mouse model of autism (Golubeva et al., 2017). That this bacterial group was strongly

associated with social distance in both mice and voles, suggests it could be associated with social behaviour in these species and that it may be transmissible between hosts.

Transmission of bacteria between hosts could also occur through indirect transmission routes. For example, members of the Enterobacteriaceae (phylum Proteobacteria), Escherischia/Shigella in mice and Yersinia in mice and voles, were associated with variation in social position and are transmitted via the fecal-oral route. However, these bacteria are most commonly dispersed by water (Percival and Williams, 2014) and, indeed, we found that this group associated most with humid habitats. These habitats may have facilitated transmission of these taxa among individuals. This could explain the confounded habitat and social effects we observed on the GM. Behaviours such as scent marking may also facilitate transmission of these and other bacterial species. Scent marking was proposed as a driver of socially transmitted E. coli in Verreaux's sifakas (Propithecus verreauxi) (Springer et al., 2016). In rodents, urine and feces-derived chemical cues are used to communicate identity and dominance, mark territories, navigate, select and attract mates, and influence social behaviour and reproductive physiology of conspecifics (Johnston, 2003; Ma et al., 1999). Contact with these bacterial sources offers an indirect mechanism for the social transmission of bacteria. Additionally, mice and voles are coprophagic (Cranford and Johnson, 1989), which could also drive bacterial transmission in these species, yet the extent to which they consume the feces of other individuals or species is unclear.

Finally, when we considered the interactions between both species on GM composition, Procrustes analysis supported our hypothesis that individuals of different species with more frequent interactions share greater similarity in their GM composition. While we did not observe this with the RDA, Procrustes targeted mouse-vole interactions which made it more suited to testing our hypothesis. Additionally, while we attempted to correct for species differences in the RDA, transmission between species may be confounded with species differences in GM. This is because taxa transmitted between species may be common in one species but rare in the other and thus be removed when correcting for species differences. Nevertheless, our results are consistent with bacterial dispersal within and between species following metacommunity theory, though the effect of potential interactions between mice and voles on their GM is likely weak.

We predicted that the number of potential encounters (i.e. degree centrality) would promote transmission of bacteria from different communities and result in greater GM diversity. This was not the case for either species when we examined degree centrality measured within each species' conspecific network. However, degree centrality of mice with respect to voles, and to a lesser degree, that of mice within the dual-species network, were strong predictors of mouse GM diversity. Though the lower density of mice may have limited our ability to detect a correlation between intraspecific degree centrality and GM diversity, these results provide further evidence for an influence of bacterial transmission across different host species on the GM of these animals. As far as we know, this has not yet been shown in wild species. GM diversity measures associated with degree centrality were sensitive to rare taxa suggesting that cross-species transfers mainly involved rare taxa. As such, bacteria obtained by mice from voles are more likely to be transient or may not share a commensal relationship with their new host.

We observed a robust association between GM composition and social proximity in voles. In mice, social proximity was correlated with both habitat and GM composition. Our measures of habitat may not account for some components of diet (e.g. invertebrates), and diet could be a stronger driver of spatial variation in GM than host-host transmission (David et al., 2014; Ley et al., 2006; Muegge et al., 2011). While some studies have found no evidence of deer mice forming long-lasting social groups with kin (Dewsbury, 1990), other studies suggest they can form family groups (Savidge, 1974) and demonstrate female-biased philopatry (Teferi, 1993). Thus, another factor that may be confounded with social distance is genotype and bacteria may also be transferred from parent to offspring and among kin in the natal nest. However, Schmidt et al. (2019) observed a weak effect of genotype on within-population GM composition of deer mice, and our hypotheses focus on host-host dispersal as a driver of GM composition which does not preclude dispersal among kin. We also found fundamental differences in the network structure of mice and voles based on sex with female mice being less central than male mice and found no such difference in voles. This is consistent with greater territoriality and lower home-range overlap in females compared to males in both species (Mihok, 1981). This difference in network structures suggests that associations between GM and frequency of encounters in mice may differ between sexes. Expression of aggressive behaviours differs between male and female deer mice but such

differences are less evident in red-backed voles (Dewsbury, 1981; Dracup et al., 2016; McGuire, 1997; Mihok, 1981). These behavioural differences could lead to sex and species-specific differences in bacterial transmission dynamics. Finally, we cannot discount the possibility that the presence of the bait may have facilitated microbial dispersal among individuals by possibly driving animals to congregate around the bait or possibly leading to territorial behaviour around the bait.

Our results demonstrate, besides the mounting evidence from highly social species such as non-human primates (Moeller et al., 2016; Raulo et al., 2018; Tung et al., 2015) that the GM composition and diversity of less social species follow a metacommunity theory framework. Of exceptional interest is the finding that the GM of a species is likely influenced by the GM of surrounding heterospecifics. The GM may therefore play a role in regulating host communities, either through dispersal of beneficial or pathogenic bacteria. Despite this, probability of contact among hosts explained a relatively small proportion of the GM in mice and voles. While metacommunity theory offers a convenient starting point to understand how host behaviourally-driven microbial dispersal shapes the GM, it currently does not account for the host's capacity to directly influence its GM and the GM's capacity to influence host health and behaviour. It also does not account for the resident host microbiome's ability to mitigate immigration of allochthonous strains. Integrating these host-GM feedbacks in current metacommunity theory should greatly improve its ability to explain GM composition within and across host species.

4.6 Acknowledgements

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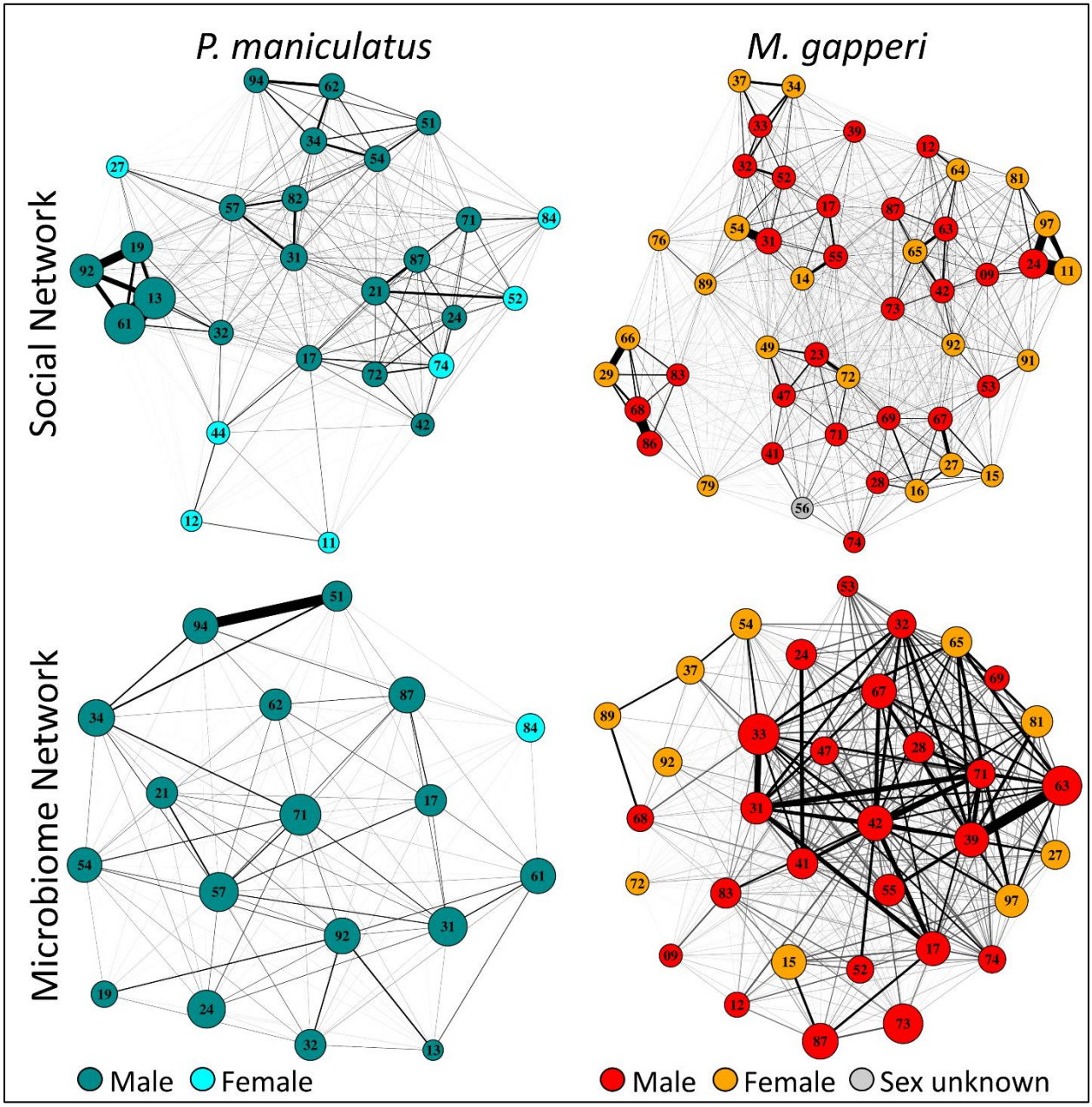

Figure 4.1 Social network (above) and microbiome distance network (below) of P. maniculatus and M. gapperi. Vertex size is proportional to degree centrality in social networks and GM Shannon's diversity in microbiome networks. Edge width is proportional to the strength of association in social networks and to compositional similarity in GM networks. To improve visualization of the networks, edge colour is made proportional to the strength of the association.

Edge weights were divided into 10% quantile categories and each category was assigned a colour following a gradient from white (weakest association) to black (strongest association). Numbers identify individuals and vertex colours identify species and sex.

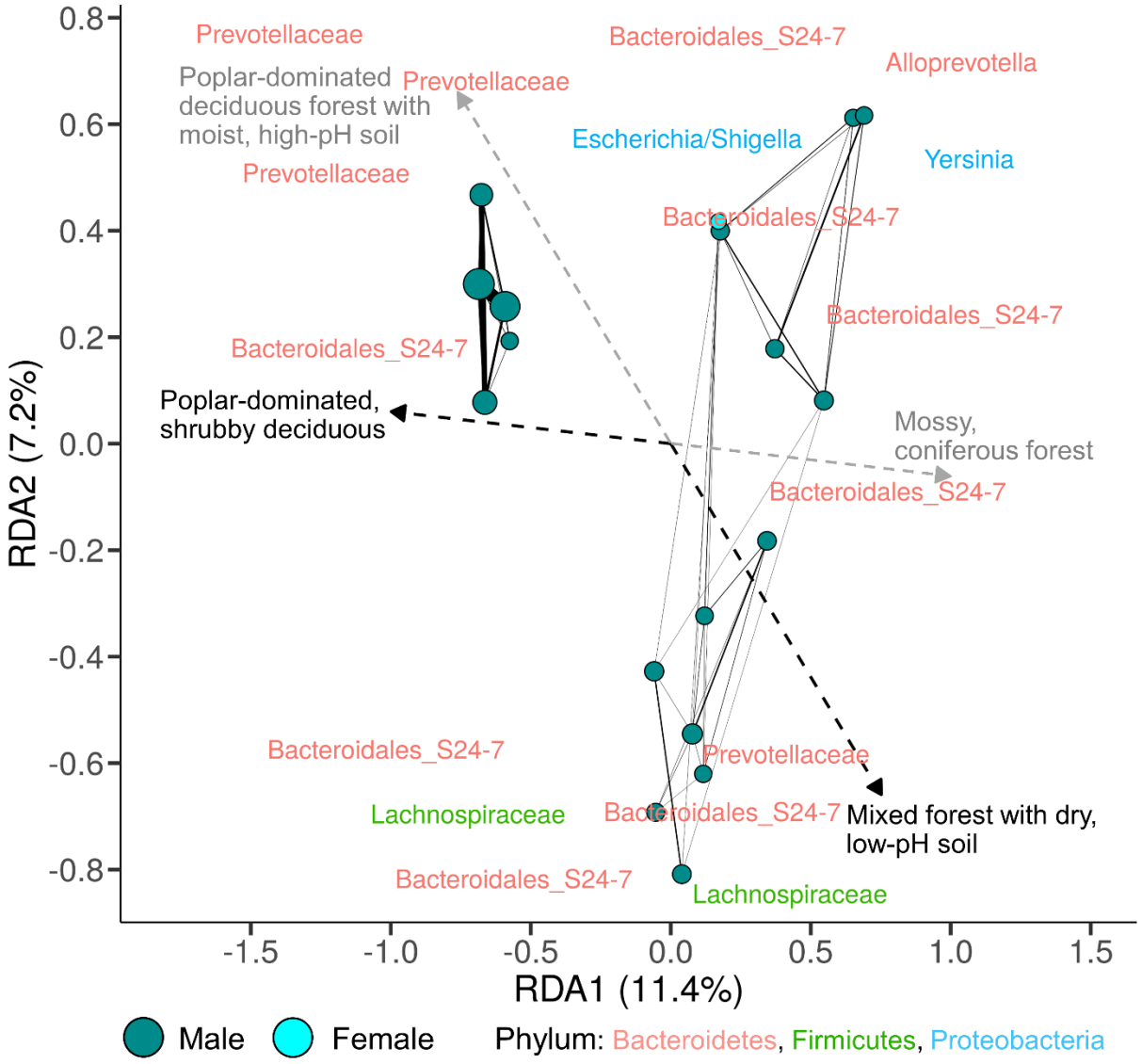

Figure 4.2 Biplot of RDA of the association between GM composition and habitat in P. maniculatus. The model was selected from a full model that included social network and habitat as explanatory factors. Variables correlated with GM composition and the direction and strength of the correlations are indicated by arrows. Habitat variables were scores of a PCA of the habitat data and are represented by descriptions of vegetation structure along these PCA axes. Social variables were scores of a PCoA on social distance. Individuals with the strongest social

associations (> third quartile of social distances) are connected by lines, line width is proportional to the strength of association, and point size is proportional to degree centrality in the mouse social network. The 10 most strongly loaded bacterial ASVs along each axis (identified to the finest-level taxonomic annotation for that ASV) are plotted (duplicate ASVs were removed).

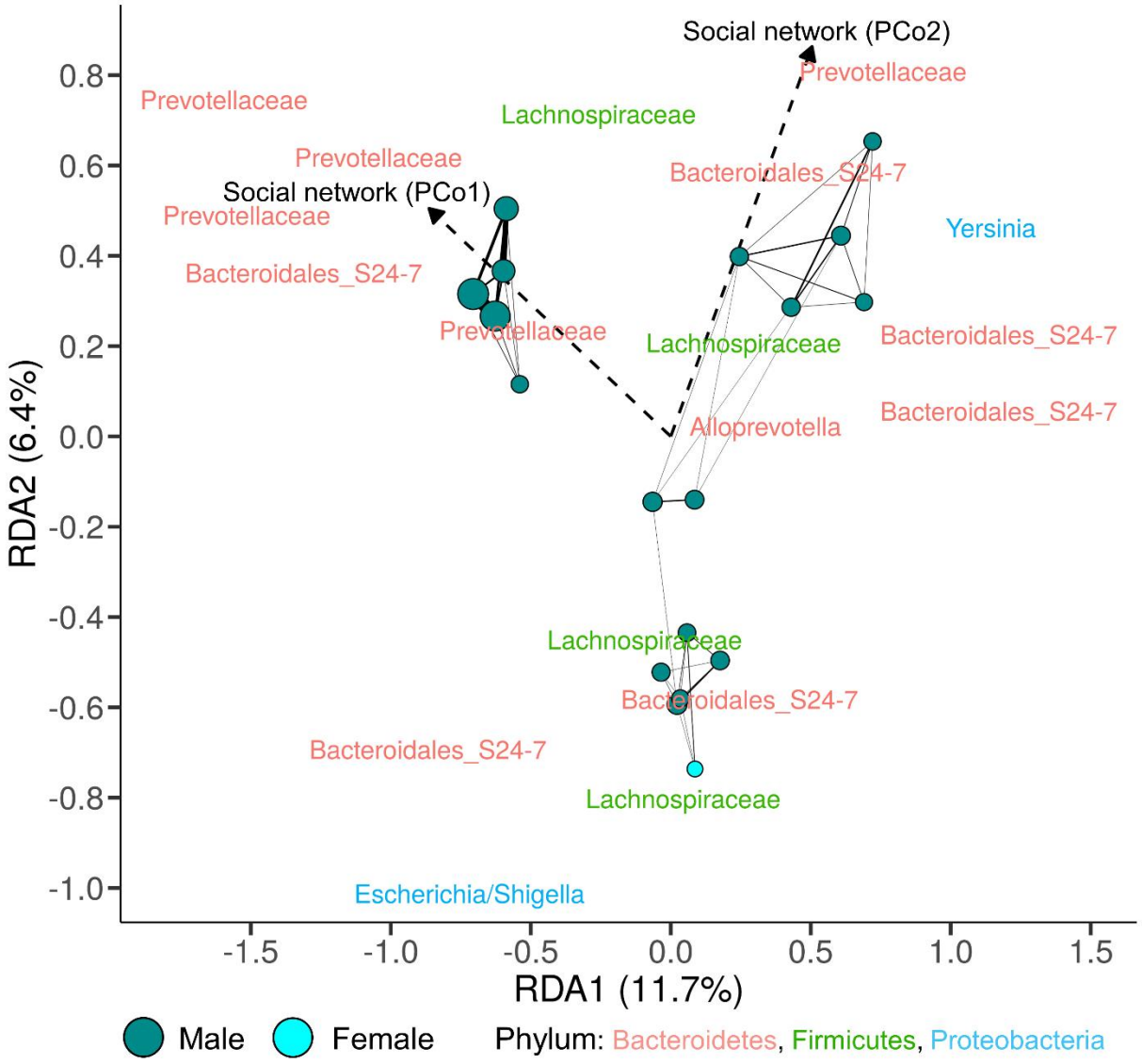

Figure 4.3 Biplot of RDA of the association between GM composition and social proximity in P. maniculatus. The model was selected from a full model that included only social network variables as explanatory factors. See Fig. 2 for a description of the plot.

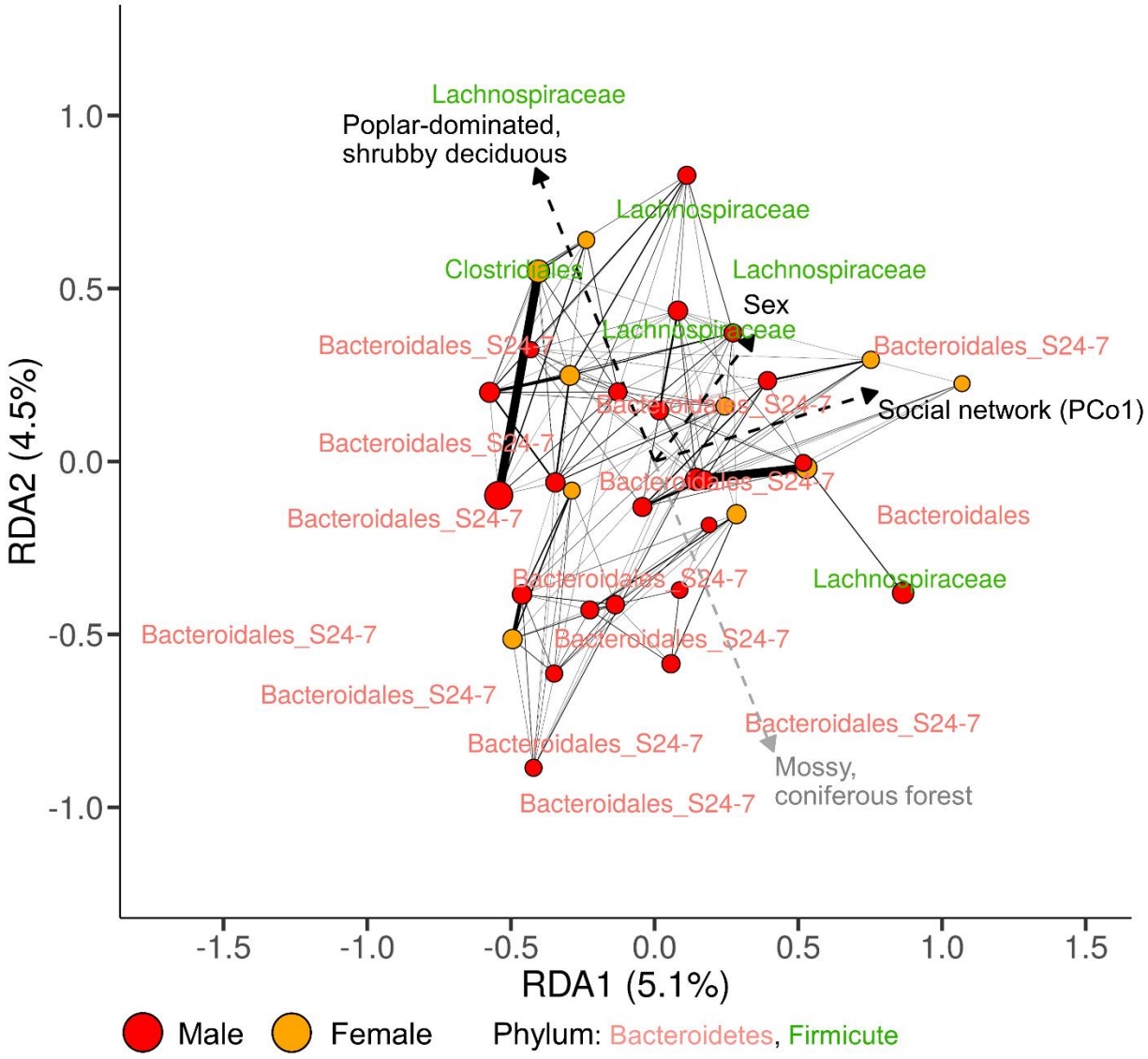

Figure 4.4 Biplot of RDA of the association between GM composition and social network, habitat and sex in M. gapperi. The model was selected from a full model that included social network, habitat, and sex as explanatory factors. Variables correlated with GM composition and the direction and strength of the correlations are indicated by arrows. Habitat variables were scores of a PCA of the habitat data and are represented by descriptions of variation along the PCA axes. Social variables were scores of a PCoA on social distance. Individuals with the strongest social

associations (> third quartile of social distances) are connected by lines, line width is proportional to the strength of association, and point size is proportional to degree centrality in the vole social network. The 10 most strongly loaded bacterial ASVs along each axis (identified to the finest-level taxonomic annotation for that ASV) are plotted (duplicate ASVs were removed).

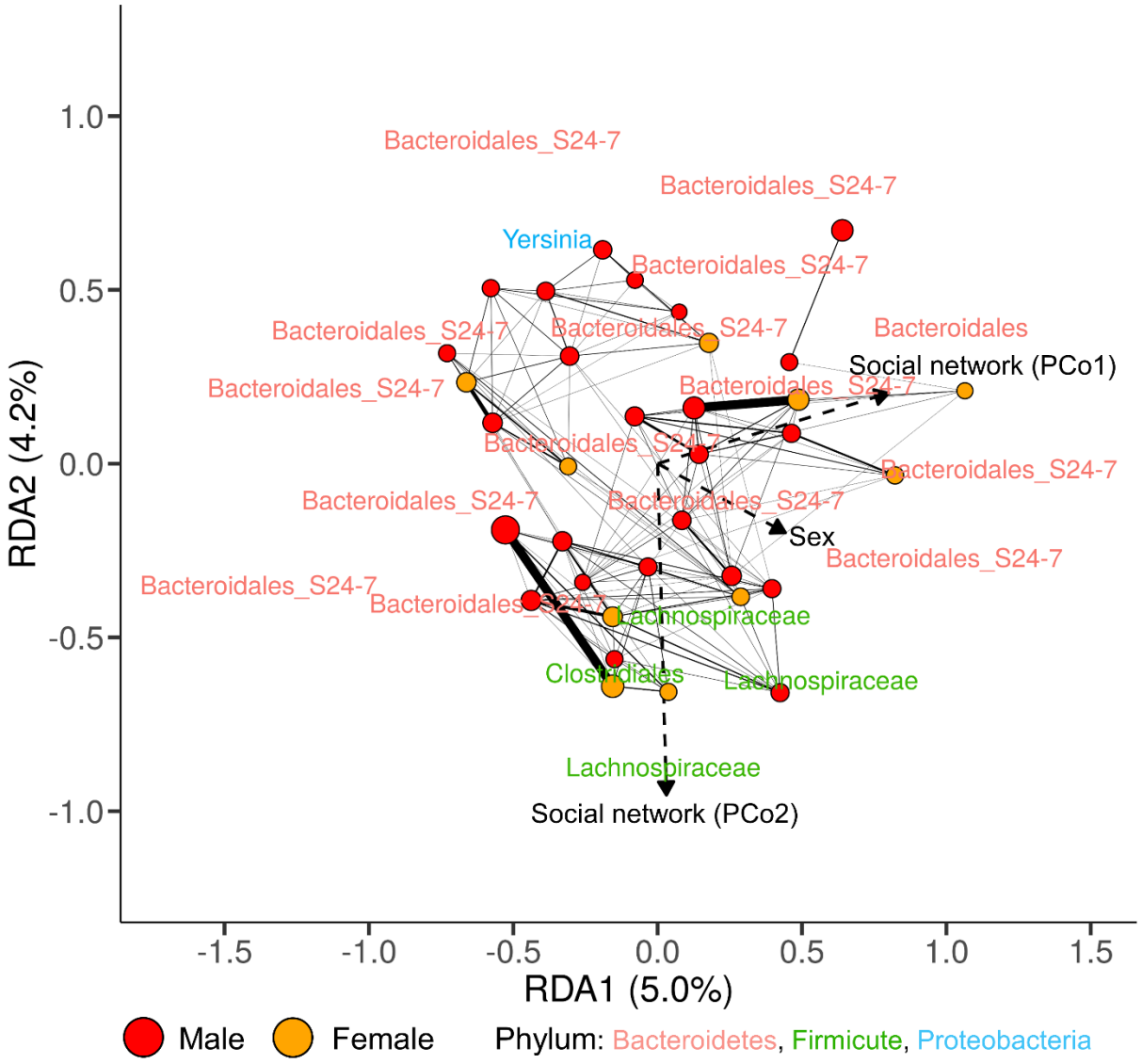

Figure 4.5 Biplot of RDA of the association between microbiome composition and social proximity and sex in M. gapperi. The model was selected from a full model that included social network and sex as explanatory factors. See Fig. 4 for a description of the plot.

Tableau 4.1 Separate linear regression analyses were conducted to test for a relationship between three measures of GM diversity (response; Shannon's, Simpson, and Richness) and host social degree centrality (predictor) in mice and voles. To compare the effects of within- and cross-species interactions on GM diversity for each species and for each measure of diversity, we conducted separate analyses including one of three measures of degree centrality: degree centrality of each individual with respect to its own species' social network, degree centrality with respect to the dual-species network, and degree centrality of each individual with respect to the other species' network. We accounted for habitat composition and diversity in analyses. In each analysis, a model was derived using stepwise model selection (comparing AIC values) on the following full model: diversity ~ degree centrality + Habitat-PC1 + Habitat-PC2 + Habitat-PC3 + Habitat Diversity. The best model for each measure of diversity is presented here (see Table S4.1 for results of the final models from analyses with each measure of degree centrality).

	GM Diversity	Model and Significant Variables	Estimate	SE	df	t	padj
P. maniculatus	Shannon	**~ Degree Centrality (vole network) + Habitat-PC1 + Habitat-PC3 + Habitat Diversity [R^2_{adj} = 0.54]**					
		(Intercept)	0.718	0.003	4, 12	236.11	0.000
		Degree Centrality(vole network)	0.018	0.005	4, 12	3.75	**0.009**
		Habitat(PC1)	0.013	0.004	4, 12	2.80	**0.034**
		Habitat(PC3)	-0.014	0.007	4, 12	-2.05	0.079
		simpson	-0.010	0.004	4, 12	-2.29	0.061
	Simpson	**~ Degree Centrality (vole network) + Habitat Diversity [R^2_{adj} = 0.18]**					
		(Intercept)	0.981	0.002	2, 14	605.25	0.000
		Degree Centrality(vole network)	0.004	0.002	2, 14	2.06	0.077
		Habitat Diversity	-0.003	0.002	2, 14	-1.73	0.123
	Richness	**~ Degree Centrality (vole network) + Habitat-PC1 + Habitat-PC3 + Habitat Diversity [R^2_{adj} = 0.45]**					
		(Intercept)	254.778	8.007	4, 13	31.82	0.000
		Degree Centrality(vole network)	43.876	12.830	4, 13	3.42	**0.014**
		Habitat(PC1)	31.511	12.134	4, 13	2.60	**0.041**
		Habitat(PC3)	-35.340	17.550	4, 13	-2.01	0.079
		simpson	-25.788	11.984	4, 13	-2.15	0.069
M. gapperi	Shannon	**~ Sex + Habitat-PC1 + Habitat-PC2 + Habitat-PC3 + Habitat Diversity + Sex:Habitat-PC1 + Sex:Habitat-PC2 + Sex:Habitat Diversity [R^2_{adj} = 0.21]**					
		(Intercept)	4.828	0.036	8, 24	134.23	0.000
		Sex	-0.160	0.072	8, 24	-2.23	0.128
		Habitat(PC1)	0.027	0.040	8, 24	0.67	0.570
		Habitat(PC2)	0.038	0.069	8, 24	0.55	0.624
		Habitat(PC3)	-0.065	0.035	8, 24	-1.83	0.153
		Habitat Diversity	-0.013	0.060	8, 24	-0.22	0.828
		Sex:Habitat(PC1)	0.120	0.096	8, 24	**1.26**	0.306
		Sex:Habitat(PC2)	-0.243	0.128	8, 24	-1.90	0.153

	Sex:Habitat Diversity	-0.222	0.147	8, 24	-1.51	0.236
Simpson	**~ Sex + Habitat-PC3 [R^2_{adj} = 0.34]**					
	(Intercept)	0.985	0.001	2, 30	931.11	0.000
	Sex	-0.008	0.002	2, 30	-3.74	**0.003**
	Habitat(PC3)	-0.002	0.001	2, 30	-1.82	0.153
Richness	**~ Sex + Habitat-PC2 + Habitat-PC3 + Sex:Habitat-PC2 + Sex:Habitat-PC3 [R^2_{adj} = 0.05]**					
	(Intercept)	271.643	7.362	5, 27	36.90	0.000
	Sex	-12.657	14.274	5, 27	-0.89	0.460
	Habitat(PC2)	9.300	8.019	5, 27	1.16	0.330
	Habitat(PC3)	-12.501	6.994	5, 27	-1.79	0.153
	Sex:Habitat(PC2)	-18.741	13.546	5, 27	-1.38	0.267
	Sex:Habitat(PC3)	31.989	17.540	5, 27	1.82	0.153

4.7 Supplemental material

4.7.1 Sequence data quality control methods

Following quality control, filtering, and removal of chimeras, we identified reads to amplicon sequence variants (ASVs) with package DADA2 version 1.10 [51]. We used default settings for all DADA2 analysis parameters specifying a forward read length of 250 and a reverse read length of 210 during read trimming. When merging forward and reverse reads, we specified a minimum overlap length of 10 and a maximum number of mismatches allowed in the overlap region of 10. We removed chimeras using the "consensus" method. This resulted in an average (±SD) of 18,878 ± 5,051 sequences per sample (minimum=4,775 sequences, maximum=39,164 sequences). We used the RDP Naïve Bayesian Classifier algorithm in DADA2 to assign taxonomy to ASVs according to the SILVA database (V128). We removed ASVs with <100 sequences and rarefied samples to 4 500 reads per sample, which was sufficient for rarefaction curves to reach a plateau without eliminating any samples for our dataset.

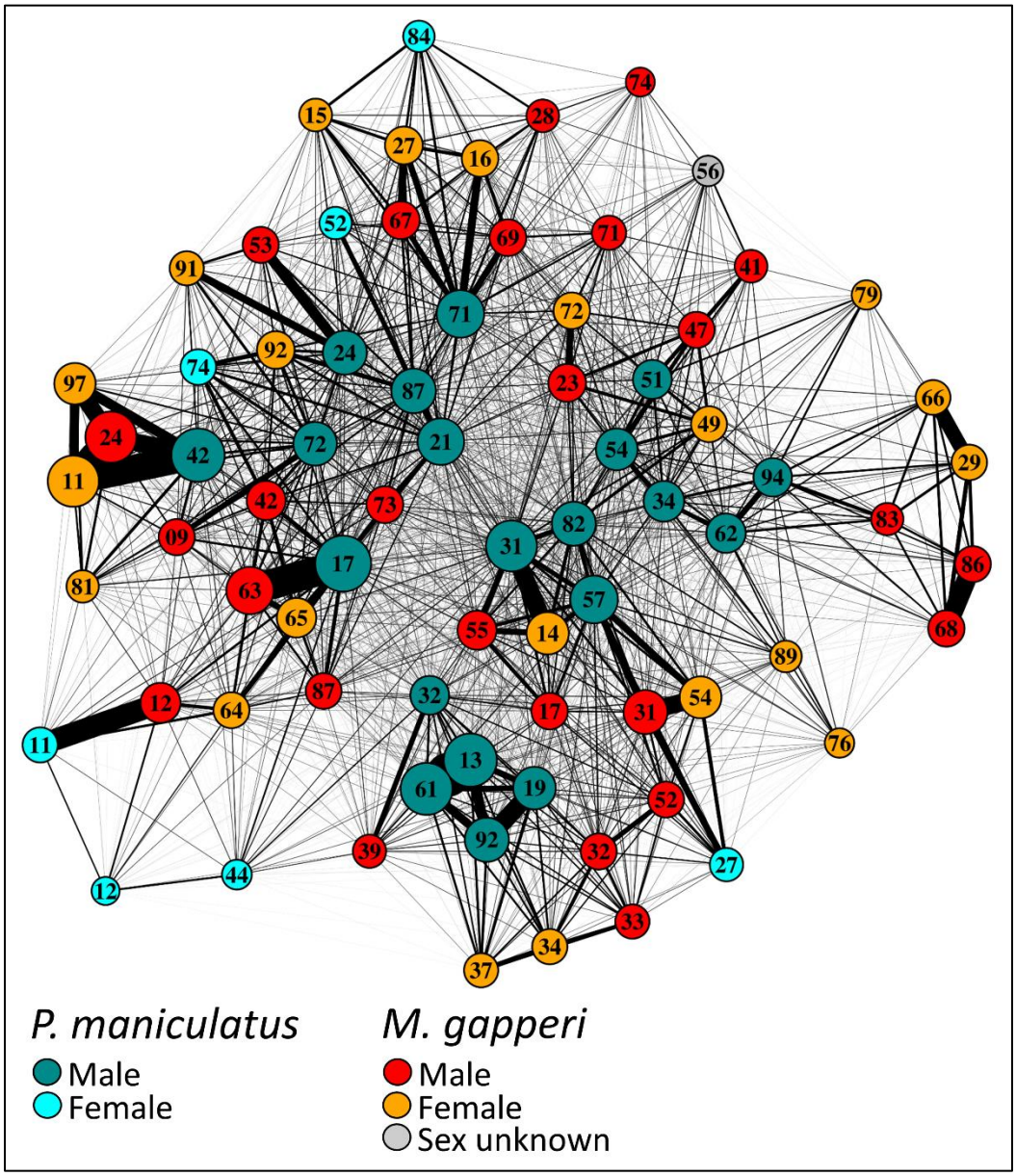

Figure S4.1 Combined social networks of mice and voles. Vertex size is proportional to degree centrality in social networks. Edge width is proportional to the strength of association. Numbers identify individuals.

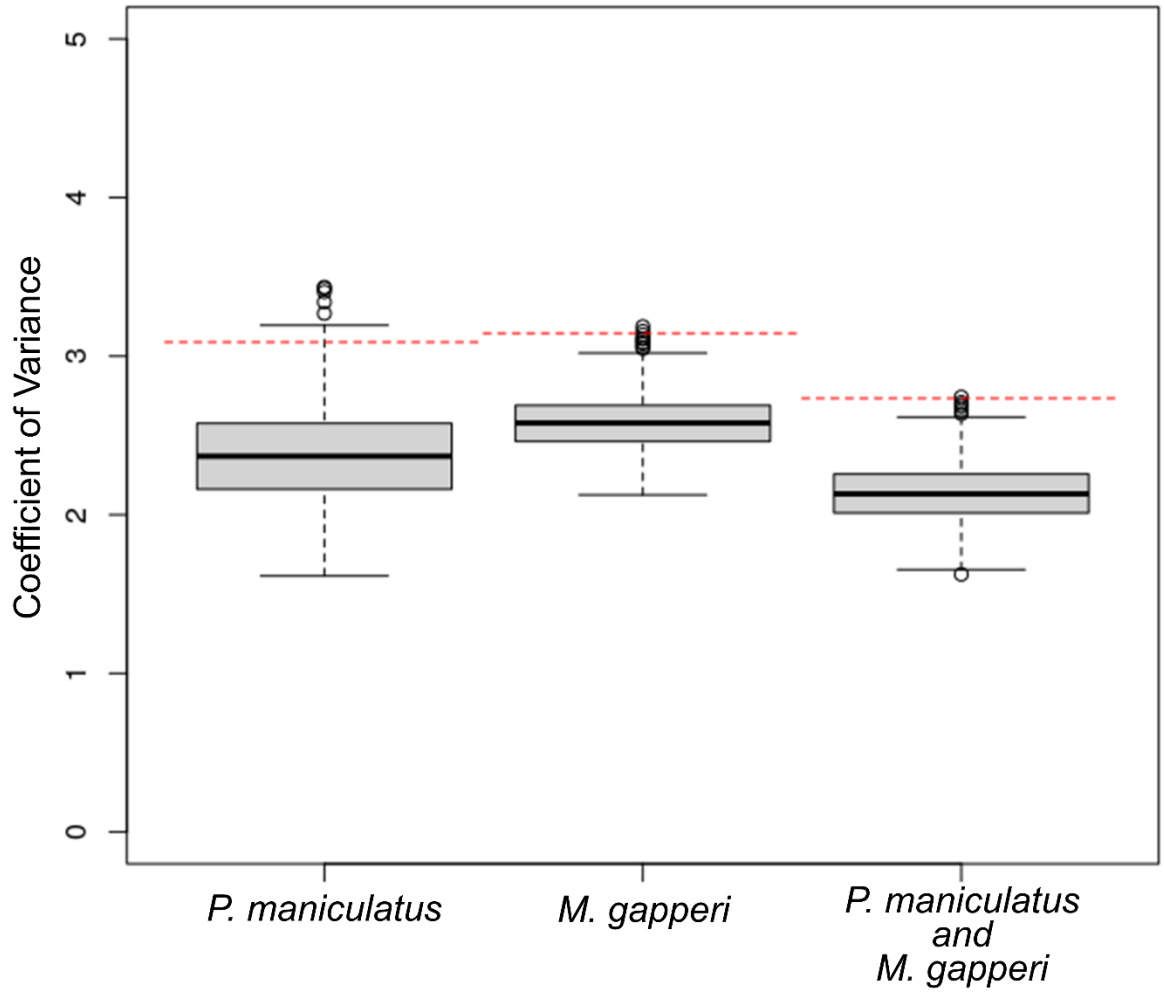

Figure S4.2 Boxplots of results from permutation tests to determine if social networks constructed for P. maniculatus, M. gapperi, and both species together, are more structured than random. Boxplots are derived from the coefficient of variance (y axis) for 1000 random permutations of the social network. The dotted red lines represent the coefficients of variance of the observed social networks (CVobs).

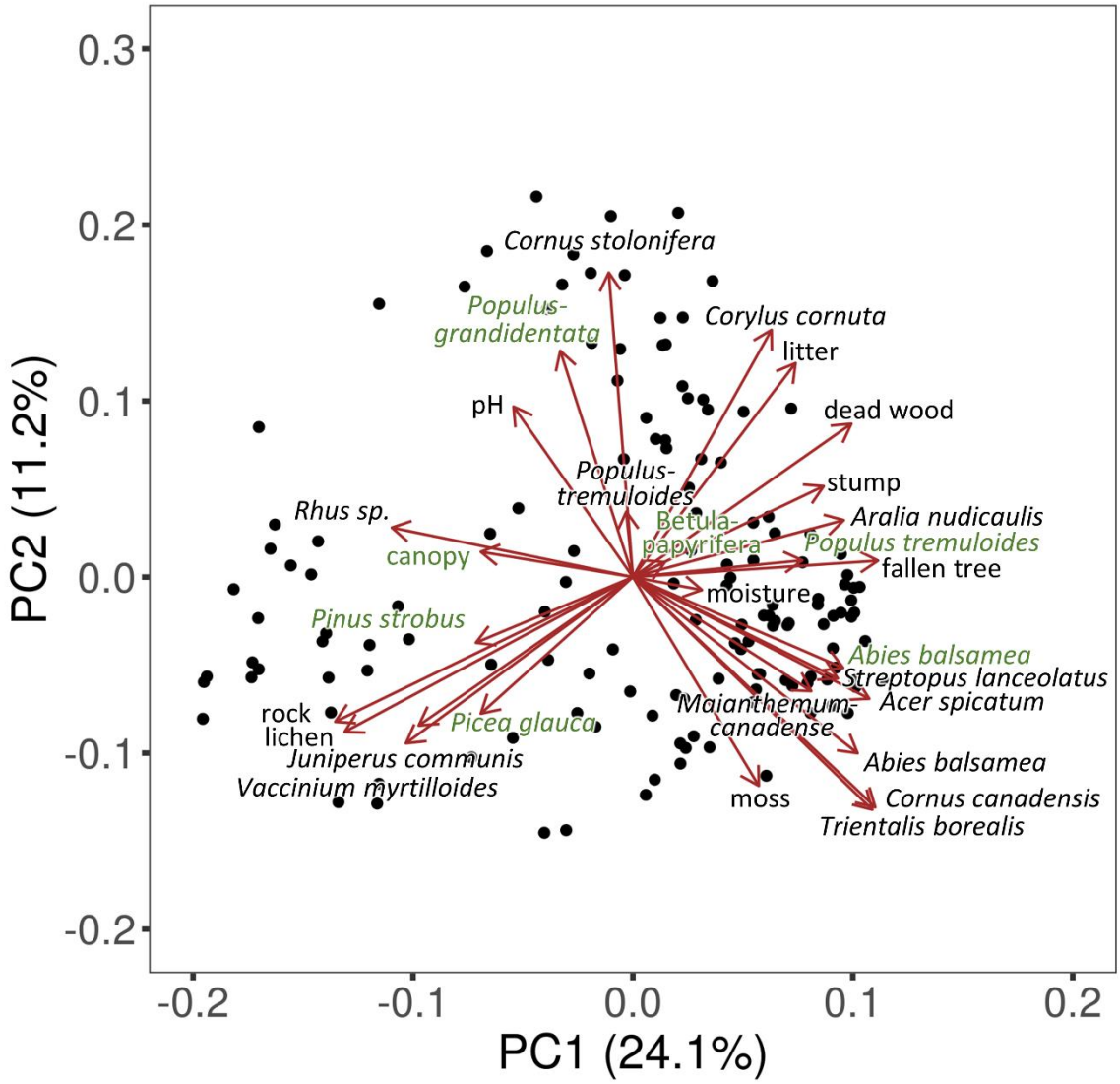

Figure S4.3 Biplot of axes 1 and 2 of a principal components analysis of habitat community data collected at each trapping location (points in the figure). Overstory species are indicated in green.

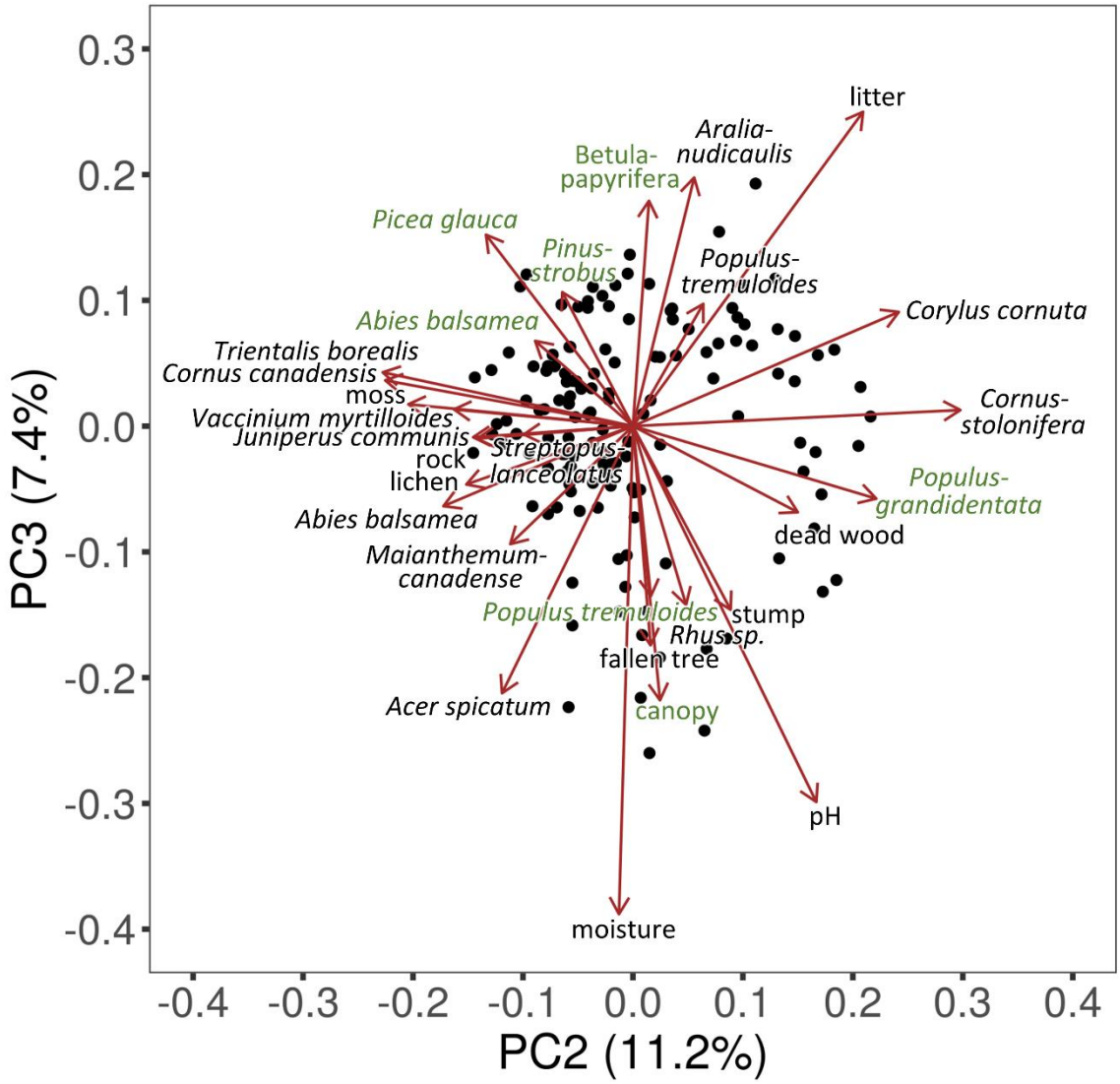

Figure S4.4 Biplot of axes 2 and 3 of a principal components analysis of habitat community data collected at each trapping location (points in the figure). Overstory species are indicated in green.

Tableau S4.1 Separate linear regression analyses were conducted to test for a relationship between three measures of GM diversity (response; Shannon's, Simpson, and Richness) and host social degree centrality (predictor) in mice. To compare the effects of within- and cross-species interactions on GM diversity for each species and for each measure of diversity, we conducted separate analyses including one of three measures of degree centrality: degree centrality of each individual with respect to its own species' social network, degree centrality with respect to the dual-species network, and degree centrality of each individual with respect to the other species' network. We accounted for habitat composition and diversity in analyses. In each analysis, a model was derived using stepwise model selection (comparing AIC values) on the following full model: diversity ~ degree centrality + Habitat-PC1 + Habitat-PC2 + Habitat-PC3 + Habitat Diversity.

GM Diversity	Model and Variables	Estimate	SE	df	t	padj	LCI	UCI
Shannon	**~ Habitat-PC1 + Habitat-PC2 + Habitat-PC3 [R^2_{adj} = 0.17]**							
	(Intercept)	0.718	0.004	3, 13	175.54	0.000	0.709	0.727
	Habitat(PC1)	0.020	0.009	3, 13	2.26	0.061	0.001	0.039
	Habitat(PC2)	0.028	0.011	3, 13	2.47	**0.046**	0.004	0.053
	Habitat(PC3)	0.010	0.007	3, 13	1.43	0.186	-0.005	0.026
	~ Degree Centrality(mouse/vole network) + Habitat-PC1 + Habitat-PC2 [R^2_{adj} = 0.38]							
	(Intercept)	0.718	0.004	3, 13	202.42	0.000	0.710	0.726
	Degree Centrality(mouse/vole network)	0.011	0.004	3, 13	2.65	**0.040**	0.002	0.020
	Habitat(PC1)	0.022	0.008	3, 13	2.91	**0.029**	0.006	0.039
	Habitat(PC2)	0.018	0.007	3, 13	2.48	**0.046**	0.002	0.034
	~ Degree Centrality(vole network) + Habitat-PC1 + Habitat-PC3 + Habitat Diversity [R^2_{adj} = 0.54]							
	(Intercept)	0.718	0.003	4, 12	236.11	0.000	0.711	0.725
	Degree Centrality(vole network)	0.018	0.005	4, 12	3.75	**0.009**	0.008	0.029
	Habitat(PC1)	0.013	0.004	4, 12	2.80	**0.034**	0.003	0.022
	Habitat(PC3)	-0.014	0.007	4, 12	-2.05	0.079	-0.028	0.001
	simpson	-0.010	0.004	4, 12	-2.29	0.061	-0.020	-0.001
Simpson	**No suitable model with Degree Centrality(mouse network)**							
	(Intercept)	0.981	0.002	0,16	548.30	0.000	0.977	0.985
	~ Degree Centrality(mouse/vole network) + Habitat-PC1 + Habitat Diversity [R^2_{adj} = 0.09]							
	(Intercept)	0.981	0.002	3, 13	575.42	0.000	0.977	0.985
	Degree Centrality(mouse/vole network)	0.003	0.002	3, 13	1.72	0.123	-0.001	0.008
	Habitat(PC1)	0.003	0.002	3, 13	1.32	0.211	-0.002	0.008
	simpson	-0.003	0.002	3, 13	-1.32	0.211	-0.008	0.002
	~ Degree Centrality(vole network) + Habitat Diversity [R^2_{adj} = 0.18]							
	(Intercept)	0.981	0.002	2, 14	605.25	0.000	0.978	0.985
	Degree Centrality(vole network)	0.004	0.002	2, 14	2.06	0.077	0.000	0.007
	Habitat Diversity	-0.003	0.002	2, 14	-1.73	0.123	-0.007	0.001
Richness	**~ Habitat-PC1 + Habitat-PC2 + Habitat-PC3 [R^2_{adj} = 0.25]**							
	(Intercept)	254.778	9.612	3, 14	26.51	0.000	234.162	275.393
	Habitat(PC1)	55.201	21.555	3, 14	2.56	**0.041**	8.970	101.432
	Habitat(PC2)	80.723	27.795	3, 14	2.90	**0.029**	21.108	140.338
	Habitat(PC3)	26.559	16.805	3, 14	1.58	0.150	-9.484	62.602
	~ Degree Centrality(mouse/vole network) + Habitat-PC1 + Habitat-PC2 [R^2_{adj} = 0.34]							
	(Intercept)	254.778	9.043	3, 14	28.17	0.000	235.382	274.173
	Degree Centrality(mouse/vole network)	22.890	10.629	3, 14	2.15	0.069	0.094	45.687
	Habitat(PC1)	58.741	20.396	3, 14	2.88	**0.029**	14.997	102.486
	Habitat(PC2)	53.689	19.510	3, 14	2.75	**0.034**	11.844	95.534
	~ Degree Centrality(vole network) + Habitat-PC1 + Habitat-PC3 + Habitat Diversity [R^2_{adj} = 0.45]							
	(Intercept)	254.778	8.007	4, 13	31.82	0.000	237.480	272.076
	Degree Centrality(vole network)	43.876	12.830	4, 13	3.42	**0.014**	16.157	71.594
	Habitat(PC1)	31.511	12.134	4, 13	2.60	**0.041**	5.297	57.725
	Habitat(PC3)	-35.340	17.550	4, 13	-2.01	0.079	-73.255	2.576
	simpson	-25.788	11.984	4, 13	-2.15	0.069	-51.677	0.102

Tableau S4.2 Separate linear regression analyses were conducted to test for a relationship between three measures of GM diversity (response; Shannon's, Simpson, and Richness) and host social degree centrality (predictor) in voles. To compare the effects of within- and cross-species interactions on GM diversity for each species and for each measure of diversity, we conducted separate analyses including one of three measures of degree centrality: degree centrality of each individual with respect to its own species' social network, degree centrality with respect to the dual-species network, and degree centrality of each individual with respect to the other species' network. We accounted for habitat composition and diversity in analyses. In each analysis, a model was derived using stepwise model selection (comparing AIC values) on the following full model: diversity ~ degree centrality + Habitat-PC1 + Habitat-PC2 + Habitat-PC3 + Habitat Diversity. In voles, final models did not contain degree centrality and therefore, the same model was obtained within each measure of GM diversity regardless of the measure of degree centrality used.

GM Diversity	Model and Variables	Estimate	SE	df	t	padj	LCI	UCI
Shannon	**~ Sex + Habitat-PC1 + Habitat-PC2 + Habitat-PC3 + Habitat Diversity + Sex:Habitat-PC1 + Sex:Habitat-PC2 + Sex:Habitat Diversity [R^2_{adj} = 0.21]**							
	(Intercept)	4.828	0.036	8, 24	134.23	0.000	4.753	4.902
	Sex	-0.160	0.072	8, 24	-2.23	0.128	-0.308	-0.012
	Habitat(PC1)	0.027	0.040	8, 24	0.67	0.570	-0.056	0.110
	Habitat(PC2)	0.038	0.069	8, 24	0.55	0.624	-0.104	0.179
	Habitat(PC3)	-0.065	0.035	8, 24	-1.83	0.153	-0.137	0.008
	Habitat Diversity	-0.013	0.060	8, 24	-0.22	0.828	-0.138	0.111
	Sex:Habitat(PC1)	0.120	0.096	8, 24	1.26	0.306	-0.077	0.317
	Sex:Habitat(PC2)	-0.243	0.128	8, 24	-1.90	0.153	-0.508	0.021
	Sex:Habitat Diversity	-0.222	0.147	8, 24	-1.51	0.236	-0.525	0.082
Simpson	**~ Sex + Habitat-PC3 [R^2_{adj} = 0.34]**							
	(Intercept)	0.985	0.001	2, 30	931.11	0.000	0.983	0.987
	Sex	-0.008	0.002	2, 30	-3.74	**0.003**	-0.012	-0.003
	Habitat(PC3)	-0.002	0.001	2, 30	-1.82	0.153	-0.004	0.000
Richness	**~ Sex + Habitat-PC2 + Habitat-PC3 + Sex:Habitat-PC2 + Sex:Habitat-PC3 [R^2_{adj} = 0.05]**							
	(Intercept)	271.643	7.362	5, 27	36.90	0.000	256.537	286.749
	Sex	-12.657	14.274	5, 27	-0.89	0.460	-41.945	16.630
	Habitat(PC2)	9.300	8.019	5, 27	1.16	0.330	-7.153	25.754
	Habitat(PC3)	-12.501	6.994	5, 27	-1.79	0.153	-26.851	1.849
	Sex:Habitat(PC2)	-18.741	13.546	5, 27	-1.38	0.267	-46.534	9.053
	Sex:Habitat(PC3)	31.989	17.540	5, 27	1.82	0.153	-4.001	67.979

Tableau S4.3 Results from a linear regression model testing for an effect of species (Mouse and Vole) and sex (Male and Female) on degree centrality of individuals in their respective species' social network.

Variable	Estimate	SE	t	p	LCI	UCI
(Intercept)	-1.45	0.15	-9.41	<0.001	-1.75	-1.14
species(V)	0.65	0.18	3.67	<0.001	0.30	1.01
Sex(M)	0.82	0.18	4.56	<0.001	0.46	1.17
species(V):Sex(M)	-0.74	0.22	-3.44	0.001	-1.17	-0.31

CHAPITRE V

CONCLUSIONS

5.1 Contributions et conclusions

Le premier objectif de cette thèse était de caractériser la relation entre la composition du microbiome intestinal et les nombreux facteurs écologiques ayant la capacité d'influencer cette composition en milieu naturel, et ce aux échelles intra- et inter-populationnelles. De plus, étant donné la relation abondamment étudiée uniquement en laboratoire entre le microbiome et les comportements d'exploration, d'anxiété et d'agressivité de l'hôte, il était aussi important de déterminer si cette relation pouvait se détecter chez les animaux en milieu naturel, malgré de nombreuses autres influences intrinsèques et extrinsèques à l'hôte. En étudiant le microbiome de métapopulations de souris sylvestres (*Peromyscus maniculatus*) et de campagnols à dos roux (*Myodes gapperi*), j'ai démontré que les associations entre les traits relatifs à l'hôte et son microbiome dépendent de l'échelle spatiale et de l'espèce. L'agressivité est associée au microbiome des souris mâles et des campagnols au niveau intra-site et cette association est relativement faible. Je n'ai pas détecté de relation entre le microbiome et le comportement d'exploration. Au niveau inter-site, le microbiome des souris est plus fortement lié aux différences génétiques entre les populations et avec l'habitat. Cependant, à cette même échelle spatiale, le microbiome des campagnols est plus fortement lié à la densité de la population et à l'isolement de la population. Outre les études qui existent déjà sur le microbiome et les contacts sociaux, ces résultats confirment pour la première fois, un lien entre le microbiome et le comportement en milieu naturel et confèrent une crédibilité à son importance évolutive potentielle.

L'inclusion de variables à plusieurs échelles spatiales demeure point fort du chapitre II. Cependant, certaines variables explicatives ont principalement représenté les différences microbiennes à une échelle seulement, ce qui limite cette approche. Par exemple, je n'ai pas observé de lien entre les

différences génétiques et la composition du microbiome de l'hôte au niveau intra-populationnel. Ceci ne veut pas dire que cette relation n'existe pas. Il se peut tout simplement que les méthodes utilisées (SNP et microsatellites) n'aient pas permis de différencier suffisamment les individus d'une même population au niveau génétique. Une approche qui pourrait permettre de véritablement détecter l'importance de la relation entre la distance génétique et le microbiome aux niveaux inter- et intra-populationnel serait une étude de jardin commun. En transférant un échantillon d'individus de plusieurs populations d'hôtes sauvages à des microcosmes munis d'environnements identiques, il serait possible d'identifier la part de la variance microbienne liée aux différences génétiques inter- et intra-populationnels. Si l'on répète cette expérience en manipulant l'habitat des microcosmes (par exemple, où chaque microcosme contient un de trois habitats différents), on peut d'autant plus différencier les effets génétiques des effets de l'habitat ; ces effets n'ont pas pu être véritablement démêlés dans le chapitre II. On pourrait même envisager de traiter les sujets avec un antibiotique avant l'expérience pour minimiser l'influence de l'effet maternel sur le microbiome.

Une autre limite du chapitre II réside dans le fait qu'elle reste corrélationnelle. Bien que le chapitre III eût pour but de tester l'effet direct du microbiome sur le comportement, l'agressivité n'a pas pu figurer parmi les comportements testés du fait d'une faible variance de ce comportement chez la population testée. Une étude visant à tester l'effet du microbiome sur l'agressivité pourrait ressembler au suivant : premièrement, mesurer le niveau d'agressivité des individus d'une population ; deuxièmement, conserver des prélèvements de fèces des individus les plus agressifs et dociles ; troisièmement, traiter les plus agressifs et dociles avec un traitement antibiotique visant la flore intestinale ; finalement, soumettre les individus agressifs à une transplantation fécale de fèces provenues des individus dociles, et les dociles avec les fèces des agressifs. Une inversion du comportement chez les animaux traités indiquerait un effet direct certain du microbiome sur le comportement.

Le deuxième objectif de cette thèse a été d'évaluer s'il y a un effet direct du microbiome sur le comportement des souris et des campagnols en milieu naturel et si cet effet engendre des conséquences pour l'histoire de vie de l'hôte (influence sur son domaine vital ou sur sa survie).

J'ai trouvé qu'une réduction de diversité et une perturbation de la composition du microbiome de souris en milieu naturel ont entraîné une baisse de comportement d'exploration moins importante que celle observée chez des témoins. Ce résultat concorde avec les études en laboratoire démontrant que le microbiome est nécessaire à l'expression de certains comportements (De Palma *et al.*, 2015 ; Heijtz *et al.*, 2011). Le changement du microbiome n'a cependant pas affecté le domaine vital ni la survie des souris, mais a causé une réduction importante de la survie des campagnols.

La manipulation du microbiome par traitement antibiotique oral offre un moyen de manipuler le microbiome rapidement et de manière efficace pour répondre à une panoplie d'hypothèses concernant les effets directs du microbiome sur l'hôte. Cependant, une des critiques de ce type de traitement est qu'il est impossible de savoir si l'effet observé du traitement résulte d'un changement du microbiome ou d'une toxicité induite par le médicament. Une solution qui est parfois exploitée pour démêler ces deux effets est d'inclure, en plus du groupe traité et du groupe témoin, un troisième groupe recevant l'antibiotique par voie sous-cutanée. En administrant un antibiotique non absorbable par voie sous-cutanée, l'effet de l'antibiotique sur le microbiome intestinal est minimisé, mais son effet systémique est maximisé. De cette façon, le chercheur peut isoler l'effet systémique indépendant du microbiome. Les futures études en milieu naturel faisant appel aux antibiotiques pour manipuler le microbiome intestinal devraient employer cette manipulation additionnelle.

Finalement, le troisième objectif de cette thèse consista à mieux comprendre le rôle que peut avoir la dynamique des communautés dans le modelage du microbiome des hôtes au sein d'une population. Plus spécifiquement, je me suis penché sur l'effet que peut avoir la dispersion de bactéries d'un hôte à l'autre. Dans un premier temps, j'ai examiné l'effet de transferts potentiels entre deux hôtes de même espèce. Dans un deuxième temps, j'ai examiné l'effet de transferts potentiels entre deux hôtes d'espèces différentes. Cette dernière facette, à ma connaissance, n'a jamais été abordée ni testée dans les études à présent sur le microbiome en milieu naturel et représente un élément nouveau et innovateur de cette thèse. J'ai pu confirmer que, chez les souris, la composition du microbiome est liée à l'importance des contacts sociaux entre celles-ci, mais cet

effet n'a pas pu être séparé d'un effet parallèle et légèrement plus important de l'habitat. Chez les campagnols, j'ai confirmé un lien entre la composition du microbiome et l'importance des liens sociaux entre les individus. Plus fascinant encore, c'est que la composition du microbiome de deux individus d'espèces différentes est liée à la probabilité qu'ils se rencontrent. De plus, la diversité du microbiome d'une souris est fortement liée au nombre de campagnols avec lesquels elle est potentiellement en contact. Ces résultats indiqueraient un rôle pour le transfert de bactéries entre espèces à influencer la composition du microbiome, et que l'importance de cette influence pourrait dépendre de l'espèce de l'hôte.

Une des raisons pour laquelle je n'ai pas pu différencier les effets sociaux des effets de l'habitat sur le microbiome des souris est que j'ai utilisé la dispersion spatiale des individus pour inférer la probabilité de rencontre entre ceux-ci. Par contre, la sélection d'habitats peut aussi fortement influencer cette distribution spatiale. Il serait donc plus prudent de quantifier les vraies interactions entre les individus. Ceci pourrait se faire en plaçant des caméras aux stations de détection PIT tag pour enregistrer les interactions entre les individus et la nature de ces interactions (ex. agressifs ou dociles). L'attraction des animaux à l'odeur d'appâts émise aux stations de détection et leur habituation possible à ces stations pourraient influencer les interactions entre les individus. Même s'ils n'avaient pas accès à l'appât et si les animaux visitant une station vivaient probablement à proximité de ces stations, le biais induit par l'appât aux stations pourrait être amorti en déplaçant les stations de détection régulièrement à des endroits aléatoires. Une méthode alternative serait d'enregistrer les contacts entre individus à l'aide de colliers de proximité ou d'avoir un plus grand nombre de stations de détection PIT tag (équipés de caméras), mais sans les munir d'attractif.

5.2 Perspectives

Cette thèse nous illumine sur la relation entre l'hôte et son microbiome en milieu naturel, particulièrement en ce qui concerne le comportement de l'hôte. Cependant, elle soulève de nouveaux questionnements.

Premièrement, les résultats de cette thèse soulignent l'importance d'étudier les relations entre l'hôte et son microbiome chez plusieurs espèces. Dans cette thèse, j'ai seulement étudié ces relations chez deux espèces et donc une analyse quantitative de l'effet de l'espèce n'était pas possible. J'ai monté des différences de composition de microbiome entre espèces hôtes, comme l'ont démontré d'autres études (Ingala *et al.*, 2018 ; Ley *et al.*, 2008 ; Youngblut *et al.*, 2019). J'ai également observé des différences fondamentales dans les mécanismes qui façonnent le microbiome entre deux rongeurs sympatriques. Si ces différences proviennent d'une sélection naturelle, la relation entre le microbiome et l'hôte pourrait nous illuminer sur l'évolution de certains comportements. La différence entre les souris et les campagnols a été d'autant plus évidente par la diminution marquée de la survie des campagnols après une suppression de leur microbiome. Par conséquent, je n'ai pas pu évaluer l'effet direct du microbiome sur le comportement chez les campagnols. Plusieurs raisons pourraient être au cœur de cette survie réduite. Par exemple, étant donné son lien au rythme cardiaque tel qu'observé dans le chapitre II, on pourrait se demander si le microbiome joue un rôle plus important dans le maintien de l'homéostasie physiologique chez les campagnols que chez les souris. Les campagnols dépendent-ils plus de leur microbiome pour la digestion que les souris ? Puisque la densité de la population des campagnols était plus élevée que celle des souris, cette densité élevée a-t-elle rendu les campagnols plus susceptibles que les souris aux effets néfastes d'une perte du microbiome ? Cette dernière question est intéressante puisqu'elle suggère que la densité de la population de l'hôte pourrait influencer les effets que d'autres facteurs peuvent avoir sur le microbiome. En effet, les effets différents des contacts sociaux sur le microbiome entre les espèces auraient pu être causées par des différences de densité de population. Il sera donc important à l'avenir de tenir compte des différences de densité de population lorsque les chercheurs comparent la relation entre l'hôte et son microbiome entre plusieurs espèces d'hôtes.

Deuxièmement, la dispersion potentielle de bactéries entre les souris et les campagnols comme le démontre cette thèse indique un effet des communautés d'hôtes sur les communautés de bactéries de leur microbiome. Les études à présent faisant appel aux théories des métacommunautés pour expliquer la composition du microbiome se sont concentrées sur une seule espèce. Par contre, si l'hôte représente une parcelle d'habitat, on pourrait imaginer que les hôtes d'une même espèce

contiennent des habitats optimaux à la colonisation par une communauté spécifique de bactéries. La théorie des métacommunautés nous apprend que la dispersion peut permettre à des espèces occupant une parcelle optimal d'occuper et de persister dans une parcelle non optimale (Leibold et Chase, 2018 ; Levin, 1974 ; Mouquet *et al.*, 2006). Donc, tout macro-organisme occupant un milieu peut servir de parcelle aux bactéries dans ce milieu. Ainsi, la présence d'une espèce d'hôte pourrait influencer la composition du microbiome d'une autre espèce. Les futures études sur le microbiome devraient donc prendre en compte l'effet de la communauté d'hôtes sur le microbiome et chercher à identifier les conséquences de cet effet sur le comportement et la valeur adaptative de l'hôte.

Troisièmement, les résultats de cette thèse indiquent que le lien entre le microbiome et le comportement est relativement faible. D'ailleurs, même en tenant compte de nombreux facteurs au niveau de l'hôte et du paysage, ces facteurs ont expliqué très peu de la variation dans la composition du microbiome des souris et des campagnols. Même si certains gradients de variation dans cette composition pouvaient être presque entièrement expliqués par des processus à l'échelle des sites, je n'ai pas réussi à identifier tous les facteurs potentiellement responsables de cette variation. Un facteur reconnu pour son effet très fort sur le microbiome c'est la diète (Cotillard *et al.*, 2013 ; David *et al.*, 2014 ; Dominianni *et al.*, 2015). Malgré mes efforts pour incorporer ce facteur dans le chapitre II, des contraintes techniques m'en ont empêché. La diète pourrait expliquer une grande partie de la variation du microbiome non expliquée par cette thèse. De plus, l'effet de la diète revient souvent dans les discussions de cette thèse et aurait pu possiblement permettre d'expliquer certains effets observés. Une partie de l'effet du microbiome sur le comportement pourrait provenir de l'effet de la diète sur le microbiome. Il serait intéressant de voir si la disponibilité saisonnière des ressources alimentaires mène à des changements de microbiome et si ces changements de microbiome mènent à des changements de comportements plus adaptés aux nouvelles conditions. L'effet du microbiome et son effet sur le comportement pourraient ainsi jouer un rôle dans la plasticité adaptative de l'hôte.

Quatrièmement, outre les effets à court terme du microbiome sur le comportement, tels qu'observés dans le chapitre III, l'effet à long terme du microbiome sur le comportement de l'hôte

est probablement plus important que celui à court terme. Les effets du microbiome sur le développement neurochimique de l'hôte ont principalement lieu à un jeune âge (Heijtz *et al.*, 2011 ; Sudo *et al.*, 2004), mais cette thèse aborde seulement la relation entre le comportement et le microbiome chez les subadultes et adultes dans le chapitre II, et seulement chez les adultes dans les chapitres III et IV. En raison des contraintes imposées par l'étude des animaux en milieu naturel, j'ai rarement capturé des juvéniles. Lorsque je les ai capturés, il était impossible de les marquer pour les identifier plus tard. En milieu naturel, après le sevrage, les souris et campagnols juvéniles dispersent du nid natal. Les conditions dans lesquelles ils sont capturés ne reflètent donc pas nécessairement celles du site natal où leur comportement s'est développé. Il est donc envisageable que la composition du microbiome reflète plus le comportement de l'hôte juste avant qu'il disperse du nid. D'un autre côté, le microbiome devrait se stabiliser au cours de la vie de l'hôte et le comportement de l'hôte pourrait influencer ses choix d'environnements et donc renforcer le lien entre le microbiome et le comportement à travers une rétroaction. Pour savoir laquelle des situations a lieu, il serait important de suivre le changement du microbiome et du comportement au cours de la vie de l'hôte.

Finalement, tout comme il existe une biogéographie microbienne à de grandes échelles spatiales, il en existe une au sein de l'hôte (Donaldson *et al.*, 2016 ; Ingala *et al.*, 2018). Des études démontrent des différences dans la composition du microbiome à différents endroits dans l'intestin (Donaldson *et al.*, 2016 ; Ingala *et al.*, 2018) et même que les relations entre cette composition et les facteurs qui la façonnent varient selon le lieu d'échantillonnage (Ingala *et al.*, 2018). Par exemple, chez les chauves-souris, le microbiome fécal retient une influence plus prononcée de la diète tandis que le microbiome de la muqueuse de l'intestin est plus fortement lié à la phylogénie de l'hôte (Ingala *et al.*, 2018). Étant donné que cette thèse porte sur la relation entre le microbiome fécal et l'hôte, il se peut que les conclusions ne soient pas généralisables à tous les microbiomes occupant l'intestin des souris et des campagnols. Quoique le lien observé entre le comportement et le microbiome soit relativement faible, le comportement pourrait être plus fortement lié à un microbiome échantillonné plus près de la paroi intestinale, puisque les bactéries les plus près de l'épithélium intestinal influencent probablement le plus l'hôte. Il serait aussi intéressant de voir si la communauté de bactéries dans le microbiome fécale est plutôt liée à l'effet du comportement de

l'hôte sur son microbiome et si le microbiome de la lumière intestinale est plutôt lié à l'effet direct du microbiome de l'hôte sur son comportement. Ceci représente une avenue de recherche intrigante offrant la possibilité de séparer les effets du microbiome sur le comportement et du comportement sur le microbiome dans les études en milieux naturels.

5.3 Conclusion générale

Cette thèse offre un aperçu écologique de la relation entre le microbiome intestinal et le comportement de l'hôte. Elle démontre que cette relation persiste en milieu naturel et qu'elle a le potentiel d'avoir d'importantes conséquences écologiques au niveau de l'hôte, des populations, et peut-être même des communautés.

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