The Association of Urban Greenspace Characteristics with Tick Densities and *Borrelia burgdorferi* prevalence in Scotland

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List of Abbreviations

ABM	Agent-based Model
AF	Adult Females
AM	Adult Males
B. burgdorferi	Borrelia burgdorferi
CI	Confidence Interval
df	degrees of freedom
DIN	Density of Infected Nymphs
DNA	Deoxyribonucleic Acid
DON	Density of Nymphs
EDS	Ecosystem Disservices
ELISA	Enzyme-Linked Immunosorbent Assay
ES	Ecosystem Services
ESRI	Environmental Systems Research Institute
EU	European Union
GI	Green Infrastructure
GLMM	General Linear Mixed Model
I. ricinus	Ixodes ricinus
LB	Lyme borreliosis
LCM	Land Cover Map
LEcoS	Landscape Ecology Statistics
MEASURE	Maximising Ecosystem Services in Urban Environments
N	Nymphs
NBN	National Biodiversity Network
NERC	Natural Environment Research Council
NH4OH	Ammonium hydroxide
NIP	Nymph Infection Prevalence
PCR	Polymerase Chain Reaction
QGIS	Quantum Geographic Information System
RCP	Relative Concentration Pathway
s.l	sensu lato

SL	Single Large
SLOSS	Single Large Or Several Small
SS	Several Small
Std.	Standard
UK	United Kingdom
UKCEH	United Kingdom Centre for Ecology and Hydrology
USA	United States of America
VIF	Variance Inflation Factor
X2	Chi Squared

Abstract

Ticks are an ecosystem disservice in urban greenspaces, with the potential to transmit diseases. The characteristics of an urban greenspace can impact the hazard of ticks and tick-borne pathogens both within a greenspace and in the surrounding area. This research aimed to understand how the configuration, connectivity, area, and land cover of urban greenspaces can influence the population densities of ticks and the associated hazard of *Borrelia* burgdorferi, the agent of Lyme disease. Tick densities were estimated at 34 sites across Scotland in 2022 and 2023, and tick samples were analysed to detect the prevalence of *B. burgdorferi* pathogens. The area and connectivity of each greenspace was calculated, as well as the proportions of four land cover types within a 1 km buffer around each greenspace. An agent-based model was used to explore how the configurations of single large vs several small greenspaces may influence the risk of tick bites and *Borrelia* infections. Increased connectivity of urban greenspaces was significantly correlated with increased density of nymphs (DON) and the density of infected nymphs (DIN) within greenspaces. Increased greenspace area was associated with increased DIN, but not DON. Land cover was found to have varying effects on DON and DIN; Increased woodland cover was associated with increased DIN but decreased DON. The proportion of built-up area was negatively associated with the DIN. Increased areas of improved grassland were associated with increased DIN, while the proportion of semi-natural grassland had the opposite effect. Modelling outputs suggested that while the risk of tick bites may be significantly higher in a ‘several small’ greenspace configuration, the risk of *Borrelia* infections is significantly higher in a ‘single large’ greenspace. These results highlight the need for urban planners to recognise these potential disservices when designing greenspaces, and the importance of educating the public about tick awareness.

Chapter 1 Introduction

1.1 Ecosystem Services and Disservices of Urban Green Infrastructure

1.1.1 Ecosystem Services

The majority of the world's population now live in urban areas, the figure has risen from just 30% of people in 1950 to 55% in 2018 (United Nations Department of Economic and Social Affairs: Population Division, 2018), and urbanisation is still on the rise. This puts pressure on towns and cities to expand and create new urban housing developments, often making use of brownfield and greenfield sites which are important hotspots for nature (Kattwinkel, Biedermann, and Kleyer, 2011). Urban greenspaces can provide many ecosystem services (ES), including: water and air purification (Yang et al., 2015), biodiversity conservation (Strohbach and Haase, 2012), reduction of urban heat island effects (Lin et al., 2020), carbon capture and storage (Strohbach and Haase, 2012), as well as aesthetic, cultural and social values for users (Langemeyer et al., 2015). There is now increasing pressure on urban planners to implement more green infrastructure (GI) into new areas of development, thanks to new urban planning policy implemented to increase the provision of ecosystem services (Pamukcu-Albers et al., 2021). This is often achieved by the creation or conservation of public or semi-private urban greenspaces including parks, sports fields, road verges, wetland and waterways, cemeteries, and vegetated external areas to public buildings (Boulton, Dedekorkut-Howes, and Byrne, 2018). However, greenspaces need to be sited and planned appropriately, so that they suit the needs of people and can continue to be maintained long term. Green infrastructure needs to be good quality, provide facilities and services, be easily accessible to meet the needs of diverse populations (Wolch, Byrne, and Newell, 2014), while also preserving ecosystem functions and services.

1.1.2 Ecosystem Disservices

While the benefits of urban green infrastructure from ecosystem services have been well researched, research focusing on ES often overlooks any negative impacts that urban greenspaces may have on human wellbeing (Dunn, 2010). Ecosystem disservices have been defined as the functions generated by an ecosystem's processes and attributes that can result in a negative impact on the wellbeing of humans (Shackleton et al., 2016). Some authors have suggested that the introduction of this concept may promote a black and white approach where studies focus specifically on either ecosystem services or ecosystem disservices, when in reality most ecosystems contribute to both (Saunders and Luck, 2016). On the other hand, targeting ecosystem disservices reduction may be more effective than focusing on ecosystem service increase, because negative impacts are more likely to influence peoples actions than the benefits provided by ecosystem services (Blanco et al., 2019). This idea has been termed the "EDS-biased behaviour" hypothesis and is supported by a number of studies (Blanco et al., 2019; Shapiro and Báldi, 2014). Therefore, studying EDS is important to try and reduce them without compromising ecosystem resilience (Lyytimäki, 2015), this can ultimately help to achieve more balanced sustainability policies (Schaubroeck, 2017; Shackleton et al., 2016) and encourage nature-friendly societies. EDS of urban greenspaces include certain sounds, smells and behaviour of plants and animals which can cause inconvenience, fear, and irritation to people. Large blue (water bodies) or green (vegetated) areas can make transportation through the greenspace more difficult, and people may feel unsafe travelling through woodland, particularly at night in urban areas (Petersen et al., 2007). In addition, soil and water can be sources of infection in some areas, and some animal species can be vectors for diseases such as avian flu, rabies, and Lyme disease (Petersen et al., 2007), which this dissertation will focus on specifically.

1.2 Urban Green Infrastructure Planning

GI in the urban context refers to the network of natural and semi-natural areas, such as greenspaces, creating ecosystem services which contribute to both the health of ecosystems and humans (Naumann et al., 2011). GI strategies, such as the EU biodiversity strategy, are often created to help design and maintain green spaces with the maximum ES (European Commission, 2011). To maintain biodiversity in an urban setting, GI must provide suitable habitat area and functional networks of habitats for wildlife movement (Bolliger and Silbernagel, 2020). This is challenging because habitat fragmentation occurs in areas of urban development. Habitat fragmentation is defined as the transformation of a large habitat into numerous smaller patches that are isolated by a matrix of habitats unlike the original (Wilcove, 1986). This creates a mosaic of smaller greenspaces surrounded by a matrix of urbanised built-up areas. Smaller greenspaces may be unable to support certain species, and these patches are sometimes disconnected, making wildlife movement between patches difficult (Braaker et al., 2017; Nielsen et al., 2014).

Several key factors must be considered in green infrastructure creation in order to maximise ecosystem services and minimise ecosystem disservices. SLOSS stands for ‘Single Large Or Several Small’ (Fahrig, 2020), referring to landscape structure. It is an important consideration for GI planning, determining whether it is more beneficial to create a single large greenspace, reducing habitat fragmentation, or multiple smaller greenspaces to provide a network of habitats. Whether or not single large or several small approaches are more beneficial to biodiversity is still debated in literature (Fahrig et al., 2019; Fletcher et al., 2018). GI planning to increase connectivity between habitat patches can help to reduce the negative impacts of habitat fragmentation, allowing wildlife to move between and utilise different habitat patches.

To understand how habitat fragmentation and connectivity can affect the ES and EDS provided by urban GI, research must incorporate a number of these factors together to understand how they interact. This can be difficult to achieve at a large scale through empirical studies alone, due to the substantial amounts of time and resource required. Increasingly, simulation models are being implemented alongside empirical studies to support real-world decision making (Grimm and Railsback, 2012). These models have the power to incorporate heterogeneity of landscapes and the behaviours of species within the landscapes, and explore how they change under different environmental management strategies (Zellner, 2008). This can help to explain the trends in empirical data.

1.2.1 Connectivity

Connectivity can be focused on in two different aspects. The functional concept of connectivity considers the behaviour of a specific organism when moving through fragmented landscapes (Tischendorf and Fahrig, 2000). However, it is the structural connectivity which underpins the physical contact of one patch to another, determining the ability of many species to transition between patches to forage and hunt (Forman, 1995). The connectivity of a patch depends on how well a landscape facilitates or impedes movement (Taylor et al., 1993), which involves consideration of the matrix habitat between patches, as well as the distance between the patches. The matrix quality may determine whether fragmentation is more likely to have a positive or negative effect on a species (Fahrig, 2017). For example, a matrix of dense road networks may be overall negative because of roadkill accidents, making it highly resistant to the movement of species. On the contrary, a matrix of mostly gardens or agricultural land may be less negative, with less resistance, allowing animals to move through these areas and travel between habitat patches.

In addition to the impacts on biodiversity, connectivity can also affect the provision of other ecosystem services, such as flood mitigation and water quality which rely on the control of water and nutrient flow through connected adjacent riparian ecosystems (Barbier et al., 2011). Furthermore, connected GI can be more accessible for humans as well as wildlife (Wolch, Byrne, and Newell, 2014), and so can provide ecosystem services to a greater population of people.

1.2.2 Habitat Fragmentation

In addition to the negative impacts of patch isolation, habitat fragmentation creates more edge habitat (perimeter-area ratio increases as the patch area decreases), which may have a different structure to both adjacent habitats. Whether or not the creation of edge habitat is beneficial can depend on number of factors such as the location of the habitat. For example, tropical forests contain many species which require large patches of forest for survival (Fletcher et al., 2018; Phalan, 2018). These forest interior specialist species may be strongly negatively affected by edge effects (Fahrig, 2017). Edge effects can also be positive, however, since edges can be more productive and contain more diverse flora than the habitat interior, creating new foraging opportunities and predator refuge (Henden et al., 2011; Moore, Van Niel, and Harvey, 2011). Whether or not habitat fragmentation has a positive or negative overall effect on the ecosystem service of biodiversity is still debated (Fahrig et al., 2019; Fletcher et al., 2018), making GI planning more challenging.

Fragmentation has also been linked to a reduction in carbon storage capacities of urban greenspace, because if the area and cohesion of vegetated areas is reduced then plants and trees may not grow as effectively (Liu et al., 2017). Fragmentation of vegetated areas, especially surrounding riparian habitat, may also reduce infiltration, therefore reducing the ES of flood management (Li et al., 2022).

1.2.3 The SLOSS Debate

The SLOSS debate in an urban context is often referred to as the land sparing (single large) versus land sharing (several small) model (Soga et al., 2014) (Figure 1). Land sparing aims to create more dense, highly urbanised areas which take up as little space as possible, leaving larger contiguous patches of habitat for wildlife. On the other hand, land sharing suggests creating less dense urban areas, which might cover more area overall, but can allow for many small areas of greenspace within the urban matrix.

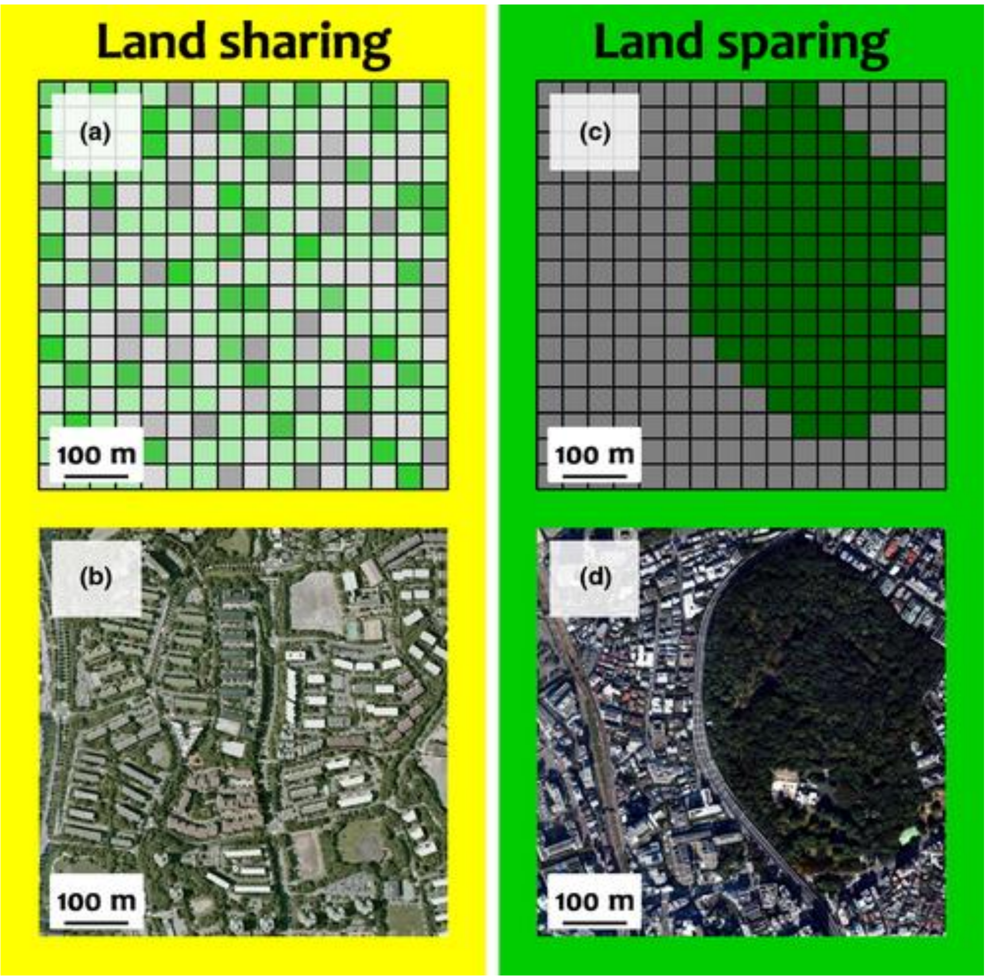

Figure 1: Land sharing vs land sparing green infrastructure in an urban setting (Soga et al., 2014). Panels a and b demonstrate land sharing configuration and panels c and d demonstrate land sparing.

There is not currently a definitive answer for whether land sharing or land sparing is a better approach for urban greenspace configuration. This depends on how well wildlife species can cope in fragmented habitats, and how connected the habitat fragments are.

The creation of GI with land sparing configurations leaves a larger area of interior habitat, and less edge habitat. This can benefit species which are negatively impacted by edge effects. These ecotone habitats may be closer to high foot and road traffic in urban areas and have reduced shelter from tree cover. Larger patches of woodland and vegetated areas may also be more effective for carbon capture (Liu et al., 2017), and infiltration to reduce flood risks (Li et al., 2022). On the other hand, it is suggested that groups of smaller habitat patches are likely to include greater environmental heterogeneity (e.g. soil structure) than a single large area (Fahrig, 2020; Quinn and Harrison, 1988; Simberloff and Abele, 1982), therefore land sharing may benefit a broader range of wildlife species overall. Furthermore, edge effects can be positive for some species, so increased edge habitat may not always mean a reduction in biodiversity (Fahrig et al., 2019).

Since land sparing GI is surrounded by a denser urban matrix, and greater distances between greenspaces, the connectivity is often poor. If some animals are unable to pass through these highly resistant matrix habitats, they may become isolated and extirpated from patches over time (Galán-Acedo et al., 2019; Morante-Filho et al., 2015; Pardini et al., 2010). Land sharing has a higher number of habitat patches and wildlife corridors, and may also have a less resistant matrix, so connectivity is greater (Asensio et al., 2009), and species can move from one patch to another to take advantage of the different habitat types available. Improved connectivity from land sharing GI approaches can also benefit local residents, who might otherwise have to travel long distances to access a single large greenspace in town (Wolch, Byrne, and Newell, 2014).

1.2.4 Agent-based Modelling Tools to Aid Urban Planning

An agent-based model (ABM) is a type of simulation model. In ecology they often include two elements: a physical landscape (made up of immobile cells), and agents within the landscape (Levy, Martens, and Heijden, 2016). The agents can be made autonomous by setting specific rules based on their characteristics and interactions with other aspects of the model (Zellner, 2008). By assigning such behaviours to organisms within a model, it is possible to create reasonable, natural scenarios to expand people's understanding (Zellner, 2008) and aid decision making by urban planners (Grimm and Railsback, 2012). In addition, it is often not possible to perform empirical studies of different environmental management strategies in urban settings at the large scale which is required (Van Buskirk and Ostfeld, 1995), which is where an ABM may become a particularly useful tool to facilitate the exploration of these management scenarios for urban planners.

There are many other modelling techniques available to researchers, such as matrix modelling and negative binomials. These models provide exploration of potential outcomes following different scenarios. These scenarios can model outcomes for many events, such as climate change or new infrastructure development. However, these models are often limited by their broad assumptions, which do not consider key population dynamics (Halsey and Miller, 2018). Many models are also restricted to the use of homogenous landscapes, which are not representative for urban planners who usually deal with highly heterogenous urban landscapes in reality. ABMs have the ability to include both biotic and abiotic factors to include more population dynamics (Halsey and Miller, 2018), and can more easily incorporate heterogenous landscapes which are more in-tune with real world situations (Levy, Martens, and Heijden, 2016). ABMs can become very complex, however, if too many parameters are included. They are also particularly sensitive to the initial conditions when the model starts, which can then cause disproportions in the outputs. (Levy 2016).

ABMs can be useful alongside empirical studies to further understand and explain data trends. Models are often also used to extrapolate these trends and predict future scenarios. This can be difficult because ABM outcomes vary depending on decisions made early on in the model run, however an ABM can still provide insight into the importance of different parameters (Zellner, 2008).

1.3 Ticks and Tick-borne Disease

1.3.1 Ticks

Ticks are ectoparasites, feeding on bloodmeals from vertebrates, including humans. It is estimated that there are almost 900 species of tick across the world (Barker and Murrell, 2004; Nava, Guglielmone, and Mangold, 2009) In the UK there are 20 endemic species (Cull et al., 2018). These 20 species consist of three soft tick (family *Argasidae*) species, and 17 hard tick (family *Ixodidae*) species (Cull et al., 2018). The species which most frequently bites humans in the UK, and across much of Europe, is *Ixodes ricinus* (commonly known as the sheep tick or castor bean tick) because they quest for hosts on vegetation rather than in nests or burrows unlike other nidicolous species. It is recognised that *I. ricinus* ticks are widespread across the UK (Figure 2), and their recorded distribution in Great Britain has expanded (Scharlemann et al., 2008). However, this could be because recording has improved over time (Cull et al., 2018). Furthermore, there are still limited data in some locations, and the actual distribution of ticks is likely to be wider than currently recognised, particularly where the data is from a voluntary reporting schemes (like Figure 2) which may be biased to key focal tourist sites (Public Health England, 2020).

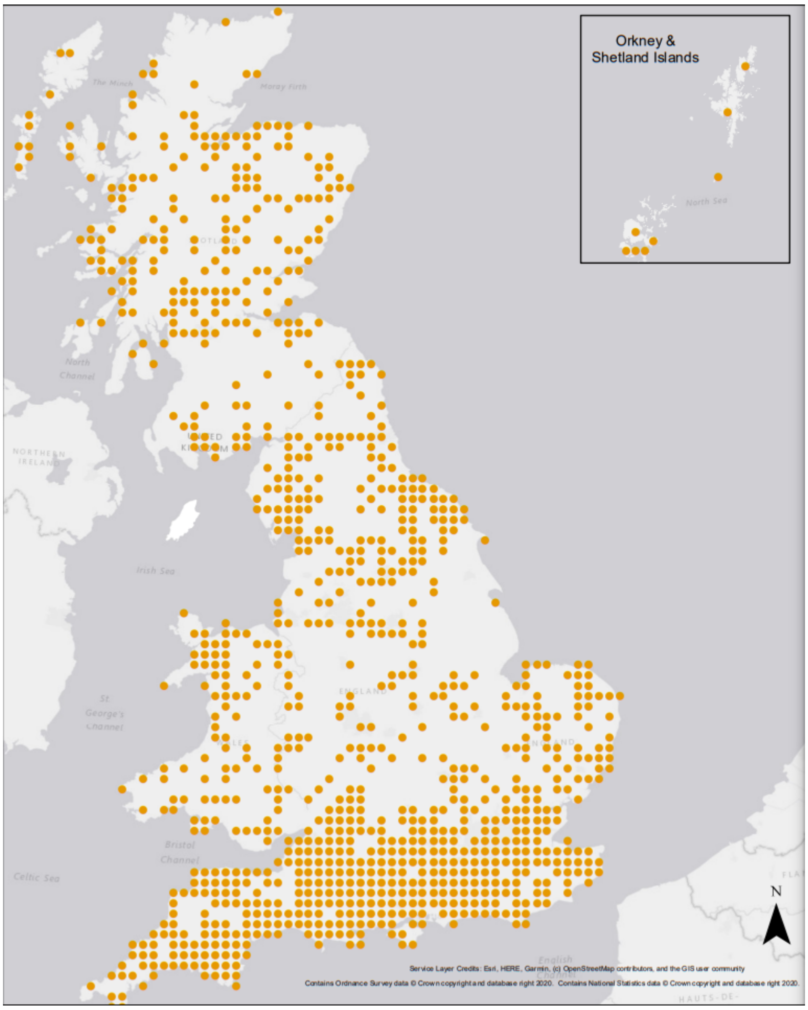

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Figure 2: Distribution of Ixodes ricinus across Great Britain in 10 km by 10 km grids. An absence of a point does not necessarily mean the tick is absent, but it has not yet been recorded here (Public Health England, 2020).

Ixodes ricinus has a four stage life cycle; eggs, larvae, nymphs and adults (Figure 3) during each of which the tick takes a blood meal to provide the resources needed to moult or reproduce (Cull et al., 2018). Ixodes *ricinus* ticks are able to feed on a wide range of vertebrate hosts although the relative importance of different hosts varies between life stages, with smaller species such as rodents being more important for feeding larvae and nymphs and larger species such as deer feeding more adult females. In the UK, common wildlife *I. ricinus* hosts include bank voles (*Myodes glareolus)*, wood mice (*Apodemus sylvaticus*), grey squirrels (*Sciurus carolinensis*), woodland specialist bird species (such as pheasants, blackbirds, thrushes, and robins), hedgehogs (*Erinaceus europaeus*), badgers (*Meles meles*), foxes (*Vulpes vulpes*), and deer. Many of these species can be found in urban greenspaces in cities across the UK (Hansford et al., 2017). Grey squirrels are thought to be especially important hosts for tick nymphs (Craine, Randolph, and Nuttall, 1995), and are often present in large numbers in urban greenspaces. As well as these wildlife species, sheep, cattle, cats, and dogs can also host *I. ricinus* ticks, and pet dogs may be particularly important for helping sustain populations of adult ticks in urban greenspaces (Cull et al., 2018).

While any *I. ricinus* life stage can feed on humans, most tick bites on humans are from nymphs as they are far more abundant than adults (Robertson, Gray, and Stewart, 2000). Ticks are most frequently encountered in woodland habitats as these habitats not only harbour high densities of vertebrate hosts but also provide appropriate climatic conditions for ticks to survive. Indeed, off-host ticks require at least 80% humidity to survive (Medlock et al., 2013) and can be easily desiccated in hot, dry areas. Many urban greenspaces can provide this favourable habitat for tick survival.

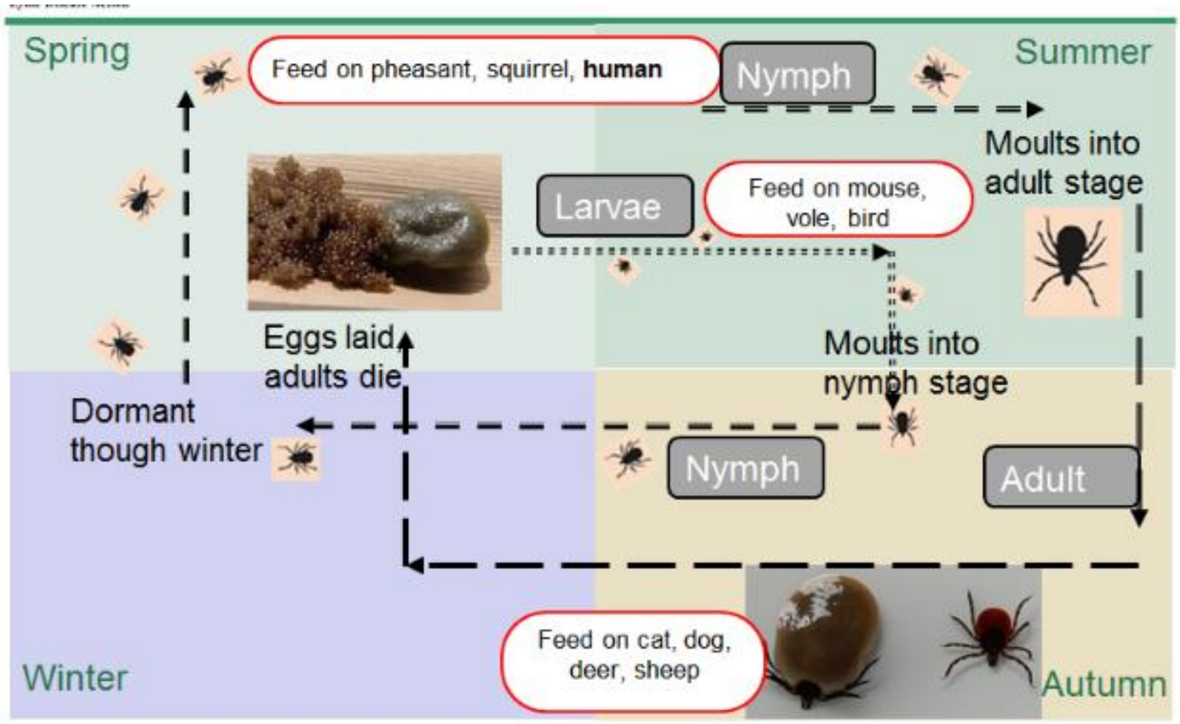

Figure 3: The life cycle of an Ixodes ricinus tick (Lyme Disease Action, 2022). Only adult females will take a blood meal, adult males only reproduce and then die.

1.3.2 Deer as Key Tick Hosts

Deer can feed all active life stages of *I. ricinus* and are important hosts for adult female ticks (Gilbert et al., 2012; Kilpatrick, Labonte, and Stafford, 2014a), especially as there are very few other large wild mammal species present in the UK. Deer provide female ticks with the bloodmeal required for successful reproduction, and therefore are considered key to sustaining a tick population. Deer have large home ranges (Zolnik et al., 2015) so they are capable of transporting ticks between greenspaces, a journey which ticks would be unable to make themselves.

Most deer species are predominantly found in woodland habitat, but many species in Europe and North America are expanding into other habitat types and patches of peri-urban and urban woodlands (Duarte et al., 2015). Urban woodland patches, gardens, and cemeteries provide good grazing opportunities for deer (Duarte et al., 2015; Kilpatrick, Labonte, and Stafford, 2014a). However, some deer species are becoming more habituated in urban areas (Geist, 2011), which can have negative impacts on both the deer and local residents. As well as transporting ticks through urban areas, deer grazing may cause damages to gardens and woodland, and deer moving close to roads may lead to an increase in road collisions (Hesse, 2010).

In the UK there are six species of deer, including two native species, red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*). The fallow deer (*Dama* dama) is considered naturalised as it was present in the last inter-glacial period and then reintroduced by the Normans (Ward, 2005). The other three species are non-native but have all been present in the UK for at least 90 years. They include sika deer (*Cervus nippon*), Reeves's Muntjac (*Muntiacus reevesi*), and the Chinese water deer (*Hydropotes inermis*) (Dolman et al., 2010). The range of all six species expanded from 1972 to 2007, despite challenges with fragmentation of woodland habitat (Dolman et al., 2010). This is due to many factors, such as more controlled hunting, reforested areas, warmer winters, more winter grazing from ornamental plants and autumn-sown cereal crops (Fuller and Gill, 2001).

Roe deer have the greatest range expansion rate of all species in the UK (Ward, 2005), and they are being seen more frequently in peri-urban and urban greenspace areas (Dandy et al., 2009). Roe deer home range varies depending on the sex, season, and the land cover (Lovari, Serrao, and Mori, 2017). One study of radio tracked roe deer found that during the colder seasons the median habitat range size was 14.95 ha for males, and 28.53 ha for females. This changed to 18.24 ha for males, and 19.92 ha for females in the warmer months (Lovari, Serrao, and Mori, 2017). Other studies have suggested roe deer home ranges could be as large as 100ha (Li et al., 2012). All the deer tracked in Lovari, Serrao, and Mori's study (2017) had at least one patch of woodland in their home range, but many also moved into other habitat types. It is suggested that ecotone habitats (at the edges of woodland) provides good grazing land close to the shelter of woodland for the roe deer, with vegetation similar to woodland glades which they have grazed in historically (Lovari, Serrao, and Mori, 2017).

Deer often carry large burdens of ticks, which may have an impact on the densities of ticks present in the urban greenspaces that deer move through. Tick burdens vary greatly, but one study of 142 culled roe deer found an average of 65 ticks per deer (range 0 to 270) (Vor et al., 2010). Another study of 367 culled roe deer found an average of 43 ticks per deer (range 1-269) (Vázquez et al., 2011), and a third study of 80 culled roe deer found 65 ticks (including all life stages) per deer on average (range 10-582) (Kiffner et al., 2010). All these studies were conducted in Europe where roe deer populations are expanding in many places.

1.3.3 Ecosystem Disservices of Ticks

The presence of ticks can put people off spending time in a greenspace, because of the risk of getting tick bites. However, arguably the greater disservice of ticks is that they are key vector for the transmission of pathogens causing many diseases, and wildlife present in urban greenspaces can act as a reservoir of tick-borne pathogens (Pfäffle et al., 2013). This is becoming a key issue in urban greenspaces across Europe and North America as ticks are responsible for the transmission of pathogens causing many diseases (Rizzoli et al., 2014), including tick-borne encephalitis, anaplasmosis, babesiosis, and Lyme borreliosis.

Lyme borreliosis (LB) is commonly referred to as Lyme disease and is caused by various genospecies of the bacteria complex *Borrelia burgdorferi* sensu lato (s.l) (Robertson, Gray, and Stewart, 2000). There are an increasing number of studies in Europe and North America focusing on the densities of ticks, and densities of ticks infected with *B. burgdorferi* in urban greenspaces. It is important to understand the potential impact of these disservices in urban environments, as more urban greenspaces, used by members of the public, are created (Pamukcu-Albers et al., 2021). A recent meta-analysis looked based on European studies found that, the estimated density of *I. ricinus* nymphs (DON) in urban green space was 12.2 (range; 0 – 159.5) nymphs per 100 m^2 (Hansford et al., 2022). The mean *B. burgdorferi* prevalence for nymphs was 14.2% (range; 0.5%–86.7%), and the density of infected nymphs (DIN) was 1.7 (range; 0–5.6) per 100 m^2 in urban green space (Hansford et al., 2022). In the UK, DON has been found to be between 1.6 – 26.1 per 100m^2 (Greenfield, 2011; Hansford et al., 2017; Hansford et al., 2023a; Hansford et al., 2021; Nelson et al., 2015) within urban greenspaces but varies greatly depending on the location.

High DON and DIN in urban greenspace greatly increase the risk of human tick bites and thus the acquisition of LB. Lyme borreliosis is the most common tick-borne disease across temperate climates in the northern hemisphere (Radolf et al., 2012). Human incidence estimates vary across different studies, but one study in the UK conducted between 2000 and 2018 estimated that the average annual incidence was 5.18 cases per 100,000 people, increasing from 2.55 to 9.33 from 2012 to 2018 (Brellier et al., 2022). The early recognition and diagnosis of LB is essential, as the condition can progress into a multi-system disease which can affect the musculoskeletal, nervous, and cardiovascular systems if it goes untreated (Steere, Coburn, and Glickstein, 2004). Initial symptoms may include redness and swelling around the bite, and sometimes fever-like symptoms. Many physicians also look for the trademark "bull's eye rash" lesions (Figure 4), however studies have found that the rash only manifests in this way in 17% of cases (Mavin, Watson, and Evans, 2015). This leads to delayed or missed diagnoses for many patients (Stonehouse, Studdiford, and Henry, 2010). Serology tests can be carried out to screen for LB, using an enzyme-linked immunosorbent assay (ELISA) or an indirect fluorescent antibody test (Roos, 2014). Antibiotics are prescribed to treat cases of LB, the course may vary depending on patient’s symptoms, and in serious cases they can be administered intravenously. Some symptoms such as fatigue and muscle ache, however, can continue even after treatment (NHS, 2021).

Upon feeding on people, ticks can transmit the pathogens they carry. Tick larvae are very rarely infected with *B. burgdorferi* upon hatching, however they can contract the pathogens from an infected host following their first bloodmeal, and then moult to become an infected nymph the following year (Matuschka et al., 1998; Richter et al., 2012). Infected nymphs and adults may bite humans and other hosts, infecting them with the pathogen. People are most often infected by nymphs, as they are more abundant yet less noticeable than adults, and they quest during spring and early summer, which coincides with popular periods for human outdoor activity (Levi et al., 2015).

Figure 4: Bull's eye rash which is sometimes seen surrounding the tick bite site following Lyme borreliosis infection (NHS, 2021).

1.3.4 Importance of Hosts in *B. burgdorferi* Ecology

As discussed, *I. ricinus* have many different host species which they can feed on. However, not all these hosts are competent reservoir hosts for *B. burgdorferi* s.l.. Competent reservoir hosts can become infected with *B. burgdorferi* s.l. and can subsequently infect feeding ticks, creating an enzootic cycle (Goethert and Telford, 2003; LoGiudice et al., 2003). In the UK, many small mammals are competent reservoir hosts, such as voles, mice (Kurtenbach et al., 1998), and squirrels (Craine, Randolph, and Nuttall, 1995). Passerine birds and foxes (Gern et al., 1998) may also be competent hosts. Deer are not reservoir competent, so they do not contribute to the transmission of *B. burgdorferi* s.l. (Goethert and Telford, 2003; LoGiudice et al., 2003). They are still key reproductive hosts and are known to drive tick populations. It has been suggested that high densities of deer can reduce pathogen prevalence (Ostfeld and Keesing, 2000), because if they are the most abundant host then more larvae and nymphs may feed on them instead of reservoir competent smaller mammals (dilution effect). However, more recently research suggests that this reduction in pathogen prevalence is cancelled out by the increase in tick densities where deer are present (Gandy et al., 2022) and deer have an overall positive effect on the density of infected ticks (tick density x pathogen prevalence). Species that are well adapted to urban settings often consist of highly reservoir competent rodents (e.g., mice and grey squirrels) and deer (e.g., roe deer) (Allan, Keesing, and Ostfeld, 2003). Thus, high concentrations of reservoir competent hosts and reproductive hosts may increase the density of ticks infected with *B. burgdorferi* in an urban greenspace (LoGiudice et al., 2008).

1.4 Research Context

1.4.1 The MEASURE Project

The MEASURE project (Maximising Ecosystem Services in Urban Environments) is a four-year project running from 2021 to 2025, funded by the Natural Environment Research Council (NERC). The project involves multidisciplinary research teams from three different universities across the UK: the University of Manchester, the University of Glasgow, and the University of Salford.

MEASURE aims to create models and tools to assist with urban planning for the maximisation of ecosystem services, by developing a better understanding of how biodiversity is associated with green infrastructure. This will help to bridge the current knowledge gap of how different characteristics of urban greenspaces can affect the ecological relationships and biodiversity of the ecosystems that exist here. The research involves studying how landscape characteristics can impact the provision of ecosystem services and disservices within urban greenspaces. The project focuses not only on maximising the ecosystem services, but also mitigating potential ecosystem disservices. Services and disservices have not yet been integrated into a single operational framework (Blanco et al., 2019) so this is a key area to focus on. The ecosystem services captured by this project include carbon capture and storage, urban cooling, and flooding prevention. The disservices focus on greenhouse gas emissions and pathogen (including tick-borne pathogen) hazard. The research includes the sampling and modelling of many different habitats in diverse urban settings, from both a small-scale and a UK-wide approach to achieve the project objectives. The project team includes people from many different research areas, bringing together different aspects of the One Health agenda. One health recognises that animals, humans and the environment are all closely linked, and so sectors need to collaborate to create better public health outcomes (World Health Organisation, 2017).

Empirical work is carried out to understand how physical characteristics and connectivity of greenspaces on an urban to rural gradient are associated with ecosystem services and disservices provided by green infrastructure. The project also involves the use of this empirical data to develop statistical and ABM which can assist with urban planning tools. These tools can be used to test urban planning scenarios and predict how different attributes may promote or reduce ecosystem services and disservices provided by urban green infrastructure.

1.4.2 The MEASURE Project’s Link to My Research

My research involves the use of data collected by the MEASURE project team, which I have been given permission to analyse as secondary data. These data were collected through field surveys carried out in spring and summer of 2022 and 2023. I was involved with the data collection process during the 2023 survey season. My study aims to understand how connectivity, area, and land cover of urban greenspaces can influence tick densities and the associated hazard of *B. burgdorferi* infection. This links closely with one of MEASURE’s objectives, which is focused on determining how attributes of greenspaces in urban, peri-urban, and rural settings are associated with ecosystem services of vertebrate diversity and disservices of ticks and tick-borne disease. My study also uses the ABM developed by the MEASURE project team at the University of Manchester. The outputs of the model are used to assess how the density and distribution of deer, ticks, and infected ticks are affected by changing greenspace characteristics.

1.5 Aims and Objectives

This project aims to understand how the configuration, connectivity, area, and land cover of urban greenspace could influence the population densities of ticks and the associated hazard of *B. burgdorferi* s.l. infection. To achieve this aim, my research has three objectives:

1. Investigate how the area and connectivity of an urban greenspace affects the density of *Ixodes ricinus* ticks and *Borrelia burgdorferi* (s.l) prevalence across two cities in Scotland.
2. Explore how different proportions of woodland, grassland, and non-vegetated areas within an urban greenspace influence the density of ticks and the density of *B. burgdorferi* s.l.-infected ticks.
3. Test the use of an ABM to explore how the configurations of single large vs several small greenspaces may influence the presence of ticks and the associated hazard of *B. burgdorferi* s.l.infection.

Chapter 2 Literature Review

2.1 Introduction

This chapter will provide a critical analysis of existing literature relating to how the characteristics of an urban greenspace can affect the relative density of ticks, the density of ticks infected with *Borrelia burgdorferi* (s.l), and the prevalence of tick host species. It then discusses the use of ABM as a tool to explore the interactions between ticks, their hosts, and their environment. The literature review includes any gaps or limitations of previous studies, therefore highlighting the relevance of this study within the field. Understanding how different urban greenspace characteristics together can affect the abundance of ticks, and therefore the risk to people of being bitten by an infected tick is important research to aid urban planning. Urban greenspaces provide many ecosystem services, and their creation and maintenance is becoming more important with ongoing urban population growth. It is necessary to be able to create green infrastructure which can maximise these services, while keeping disservices to a minimum. This ensures that people can continue to enjoy and appreciate nature safely in an urban setting, while also ensuring that wildlife is still able to exist alongside human populations.

Studies often refer to the density of nymphs (DON), calculated as the number of nymphs collected per unit area, to study distributions in urban greenspaces. The DON is particularly important because this is the life stage which bites humans most frequently (Robertson, Gray, and Stewart, 2000). If nymphs are collected and sent for *B. burgdorferi* s.l. testing, then the nymph infection prevalence (NIP) can be calculated, which is the percentage of nymphs which were infected with *B. burgdorferi* s.l.. Studies often multiply NIP and DON to calculate the density of infected nymphs (DIN), providing an estimation of the likelihood of humans encountering an infected tick (McClure and Diuk-Wasser, 2018).

2.2 The Relationship Between Urban Greenspace Connectivity and Densities of Ticks

Only a small number of studies have focussed on how habitat connectivity in urban greenspaces relates to ecosystem disservices, such as tick-borne diseases. The consensus in existing research is that increasing habitat connectivity also leads to an increase in the relative density of ticks (Estrada-Peña, 2002; Hansford et al., 2023a; Heylen et al., 2019; VanAcker et al., 2019).

Estrada-Peña's study (2002) was one of the first studies to link habitat fragmentation and isolation to the population densities of *I. ricinus* ticks. This study was carried out over 572 sites of at least $1km^2$ in a large area in northern Spain (230km x 60km), consisting of suitable tick habitats surrounded by unsuitable habitat (Estrada-Peña, 2002). These sites were not focussed on urban greenspaces specifically. The authors found the highest densities of ticks in sites with the highest connectivity values, and even when sites had suitable habitats ticks were not always present due to their isolation from the main connection web (Estrada-Peña, 2002). The main mammalian tick hosts identified in the area were cattle and sheep, with only a few deer, yet within urban greenspace studies deer are key hosts for tick movement (Rizzoli et al., 2014). Deer may have different behaviour, habitat preferences to cattle and sheep so this could affect the results.

On the other hand, a study of 30 sites across southern Connecticut (USA) in 2005 found a significant negative relationship between tick density, tick infection prevalence and habitat connectivity, suggesting that increasing connectivity may decrease DON and DIN (Brownstein et al., 2005). However, they used minimum distance between patch edges as a metric for connectivity rather than more appropriate methods, and they only tested a small sample of ticks for *B. burgdorferi* (20) from each site. On the contrary, the study also looked at how connectivity was related to reported human incidence of Lyme disease and they found that increasing connectivity was associated with higher Lyme disease incidence (Brownstein et al., 2005) which is similar to what later studies suggested (Heylen et al., 2019; VanAcker et al., 2019). The authors acknowledge that the relationship between DIN and Lyme disease incidence may be weak because DON and DIN cannot account for human behaviour. Human incidences may be higher or lower depending on the season, the location, how well used the site is, or how educated visitors are on tick bites. Even if the hazard is high (i.e. high DIN), if there are very few visitors to the area then the risk is low. Collecting this risk information requires additional project time and resource to gather data on human behaviour. DON and DIN are still useful for understanding the environmental hazard of infected tick bites because the risk per person is strongly correlated with the DIN (Ribeiro et al., 2023). In addition, using DIN is more valuable than just infection prevalence data alone (Kilpatrick et al., 2017).

Surveys completed between 2014 and 2016 in Antwerp, Belgium found the same trend (Heylen et al., 2019) as found in Spain in 2002. The Antwerp study was the first of its kind, analysing how characteristics and connectivity affect tick populations within urban greenspaces. Heylen et al. (2019) sampled 22 greenspaces which included mostly open and some woodland areas along an urban to rural gradient; the study area was much smaller than in Estrada-Peña's study in 2002. Connectivity was calculated in relation to the cost distance to the nearest greenspace or to the closest deer population according to a database of roe deer observations. This is a step further than Estrada-Peña's (2002) study where hosts were not considered in the connectivity metric. As ticks cannot travel far themselves, incorporating host movements into tick distribution studies is important (Diuk-Wasser, VanAcker, and Fernandez, 2020). Heylen et al., (2019) measured the DON and DIN, finding that increased connectivity of urban greenspaces correlated with increased both DON and DIN values (Heylen et al., 2019). There were limitations in the analysis of the data in this study though, as the urban sites within the city ring road were completely isolated and had no ticks or deer (which are a key host), so no comparisons could be made between them. This also means that values for DON and DIN could only be correlated for a few sites where ticks were present.

Further evidence of the importance of connectivity was demonstrated by Van Acker et al. (2019), with similar methods to Heylen et al. (2019), surveying 24 forest sites across New York City in 2017 and screening at least 50 ticks from each site where possible for *B. burgdorferi* (VanAcker et al., 2019). They found that DON and DIN increased as connectivity of urban greenspaces increased. They concluded that, like the Spanish study, connectivity had a much greater positive effect on DON and DIN than physical factors such as the type and size of habitat patches (Estrada-Peña, 2002; VanAcker et al., 2019). The study used a different metric for connectivity than the studies in Antwerp and northern Spain which both used least cost methods to determine the easiest movement pathway for hosts. VanAcker et al. (2019) calculated the number of connections to each park within 4.8 km (up to 5 connections). Then, for Staten Island where tick densities were particularly high, VanAcker et al. (2019) used circuit-based theory to find the lowest resistance routes for deer movement. This connectivity metric is a relatively new concept which is more accurate than the simpler least cost models (McRae et al., 2008), however it is also more complex which could be why it was only used for one specific area in VanAcker's study.

A positive relationship between tick density and connectivity was also found in a study by Hansford et al. (2023). This was the first study in England which focused on tick densities in urban greenspaces relating to connectivity, and it encompassed 72 sites across three different cities (Bristol, Bath, Southampton). However, in contrast to the studies in Belgium and Spain, Hansford et al. (2023) found no significant relationship between connectivity and DIN (Hansford et al., 2023a) and there was no explanation as to why this may be. There were large variations in the infection prevalence though depending on the city (Bristol had no infected ticks at the time of the survey), which demonstrates the importance of incorporating multiple cities, as they can vary significantly. Hansford et al., (2023) used distance to the closest woodland patch and greenspace area within a buffer as a metric for connectivity. However, this method is fairly simplistic compared to least cost and circuit-based theory metrics because it cannot include any host factors or incorporate matrix habitats (Hansford et al., 2023a). Connectivity was calculated for patches which were within a 1km buffer, which is smaller than the buffer size used by VanAcker et al. (2019) (4.8 km), but this is still appropriate for modelling host movement as the roe deer has a smaller home range size than the white-tailed deer present in North America (DeNicola, 2017; Lovari, Serrao, and Mori, 2017).

2.3 The Relationship Between Urban Greenspace Area and Densities of Ticks

Researching area or patch size often comes hand in hand with connectivity, as habitat fragmentation causes reduced patch sizes and often reduces connectivity too. There are only a few previous studies which have focused on how fragmentation affects the relative density of ticks and *B. burgdorferi* prevalence in urban greenspaces (Allan, Keesing, and Ostfeld, 2003; Brownstein et al., 2005; Li et al., 2012; Millins et al., 2018; Simon et al., 2014; Tran and Waller, 2013)

Most studies concluded that increasing fragmentation leads to an increase in DON and DIN (Allan, Keesing, and Ostfeld, 2003; Brownstein et al., 2005; Simon et al., 2014; Tran and Waller, 2013). Allan, Keesing and Ostfeld (2003) carried out a study of 14 woodland greenspaces in south-eastern New York. In this area of the United States the white-footed mouse (*Peromyscus leucopus*) is a key tick host, and the species is a highly competent reservoir for *B. burgdorferi* s.l. (Allan, Keesing, and Ostfeld, 2003; Simon et al., 2014). The sites in this study were between 0.7 – 7.6 hectares, and they were all at least 1.6k m apart to ensure each mouse population was isolated. Sampling at each site was extensive (blanket dragging for 400m) but only 20 ticks were tested for *B. burgdorferi* s.l. per site. Allan, Keesing and Ostfeld (2003) found that both the DON and DIN were higher in the fragments smaller than 1.2ha (0.1 nymphs/m^2) compared to fragments over 1.2ha (0.03 nymphs/m^2). The five smallest patches contained around seven times as many infected nymphs as the other larger patches (0.07 nymphs/m^2 and 0.01 nymphs/m^2 respectively) (Allan, Keesing, and Ostfeld, 2003). It is possible that when habitat areas are small within an urban matrix, the density of mice increases, because other less generalist species are outcompeted and therefore there is a higher density of hosts for questing ticks. Allan, Keesing and Ostfeld (2003) found higher densities of adult ticks in larger patches however, and it is suggested that these patches may be more favourable to large mammals such as deer which they suggest should be further explored.

Brownstein et al. (2005) quantified the level of fragmentation using mean patch size as well as patch isolation to measure connectivity. This study was not at site level like the New York study though, patch size was measured as the average forested area within buffers (250 m, 500 m, 750 m, 1 km and 5 km). Brownstein et al. (2005) found a significant negative relationship between the mean patch size and NIP, but the opposite trend for mean patch size and Lyme disease incidence (Brownstein et al., 2005). They suggest this could be because there is less forest area for people to spend time in, and they may not travel to visit small, fragmented patches (Brownstein et al., 2005). In contrast to Allan, Keesing and Ostfeld (2003), they suggest that small, fragmented patches may benefit deer due to positive edge effects.

Li et al. (2012) used a cellular automata approach, which modelled ticks and tick hosts in different habitats. The study concluded that DON was greater in larger patches, however the results for DIN were conflicting. Reducing woodland patch size lead to an increase in DIN when woodland is adjacent to non-vegetated areas (Li et al., 2012). By contrast, they found DIN decreased with patch size when woodland was adjacent to grassland (Li et al., 2012). Li et al. suggest this could be because deer are more likely to venture into neighbouring grassland than neighbouring non-vegetated areas, so neighbouring grassland may become a sink for ticks. If the ticks attach in the woodland patch, but drop off in neighbouring grassland where there is no canopy cover they may become desiccated, therefore reducing the population in the woodland patch (Li et al., 2012). Using a cellular automata approach was beneficial, as it provided a degree of flexibility to change parameters which may take a long time or be limited by resources in empirical studies (Li et al., 2012). However, it is highlighted that field data and host behaviour should be used to inform models to ensure greater accuracy (Diuk-Wasser, VanAcker, and Fernandez, 2020; Kilpatrick et al., 2017).

Another study which focused on Lyme disease incidence concludes that fragmented habitats may put people at greater risk of Lyme disease (Tran and Waller, 2013) which contrasts Brownstein et al. (2005) findings. Tran and Waller (2013) included Lyme disease data from 13 US states overlayed onto land cover data layers to analyse the effects of fragmentation on Lyme disease incidence. The study is more recent than that by Brownstein et al. (2005), and covers a bigger area, but they did not account for the effects of connectivity like Brownstein et al. (2005) did. Tran and Waller highlight the importance of studies focusing more on the interactions of fragmentation (i.e. connectivity) rather than patch area alone (Tran and Waller, 2013), and if connectivity is not controlled or measured it may skew the results.

A later study (2014) in Quebec found higher densities of ticks in smaller habitat patches (Simon et al., 2014). White-footed mice were a key host in this area, like the New York study, and they were trapped in order to collect feeding ticks in addition to questing ticks on the vegetation across 34 sites. This is a more in-depth sampling method than the methods in Allan, Keesing and Ostfeld (2003) and the number of sites is greater, but they did not explore how DIN was related to patch size. As hypothesized in the New York study, there was a high *B. burgdorferi* prevalence in the trapped mice, and higher densities of mice were found in smaller fragments (Simon et al., 2014).

One study of 18 sites in western Scotland found no significant relationship between the size of habitat patches and the infection prevalence of nymphs (Millins et al., 2018). Six of these survey areas were on mainland Scotland, and 12 were island sites (0.03 km^2 to 1.2 km^2), but all were mostly deciduous woodland with some coniferous areas (Millins et al., 2018). Both mainland and island sites had populations of fallow deer (*Dama dama*), smaller woodland mammals and birds. The mean infection prevalence of nymphs on the mainland was 2.5%, compared to just 0.9% on the islands (Millins et al., 2018). However, when the island sites with no ticks were removed the infection prevalence was more comparable (2.4% for islands and 2.7% for mainland) (Millins et al., 2018). The study was only carried out in rural areas, which may explain a variation in the trends seen in studies of urban and peri-urban greenspaces. Furthermore, the islands were surrounded by water which is a highly resistant and high cost matrix for many host species, and there is no ecotone which can be important for hosts (Barbour and Fish, 1993; Brownstein et al., 2005; Diuk-Wasser, VanAcker, and Fernandez, 2020; Lovari, Serrao, and Mori, 2017). The sampling was extensive, 200 ticks were collected from each site where possible and deer dung surveys were included. Using indirect deer estimates from dung can provide good estimates of deer abundance over several months (Fernanda et al., 2001), and this is a good way to gain an understanding of how deer populations are linked to tick densities. Very few studies focusing on connectivity or area of urban greenspaces have included host surveys alongside tick surveying, but it is recognised as important (Diuk-Wasser, VanAcker, and Fernandez, 2020; Hansford et al., 2023a; Kilpatrick, Labonte, and Stafford, 2014a) and so needs to be explored more by future studies.

2.4 The Relationship Between Urban Greenspace Land Cover and Densities of Ticks

While fragmentation and patch isolation are both important factors to understand the movement of ticks, their hosts, and the prevalence of *B. burgdorferi* across urban greenspaces, habitat is a key factor to determine whether tick populations can be sustained. Without suitable habitat available, the connectivity or size of a patch is negligible, as there may be fewer tick hosts and ticks may be unable to survive (Ogden, Mechai, and Margos, 2013). Furthermore, many studies of urban greenspaces only focus on one habitat type (usually woodland). However, the urban environment is characterised by a mosaic of heterogeneous habitats (Dautel and Kahl, 1999) which tick hosts can utilise and ticks are able to survive in.

One study in Hungary surveyed 30 sites including cemeteries, woodlands, and parks (Hornok et al., 2014). Cemeteries were characterised by their wild uncut grass areas, parks by sparse trees, bushes, and areas of cut grass, and forest by dense woodland canopy. Ticks were found in all but one site which had cut grass and trimmed vegetation (Hornok et al., 2014), and the density of ticks was low in cut grass where the humidity was low. They concluded that woodland had the highest nymph infection prevalence, but unmanaged grassland in cemeteries had the highest tick densities (Hornok et al., 2014). This highlights the importance of specifying between managed and unmanaged grassland in habitat studies. The woodland in this study, however, was characterised by sparse, low, vegetation which may have affected the results.

Hansford et al. (2017) surveyed 25 sites in Salisbury, England in 2013 and 2014. They were classified into grassland, hedge, park, woodland, and woodland edge (ecotone) habitat. Ticks were found in every category, but there were higher densities within woodland edge than woodland (Hansford et al., 2017). Infected ticks were found in grassland, hedge, woodland, and woodland edge. They did not specifically categorise managed and unmanaged grassland, but they highlight that one site with unmanaged long grass at the edges had high densities of ticks, which is similar to what Hornok et al. (2014) suggested. Hansford et al. (2017) also highlighted that future studies should perhaps distinguish between types of grassland. They found no significant difference in infection prevalence across different habitats (Hansford et al., 2017), unlike the Poland study, but Hansford et al. (2017) highlight that results can vary between cities, and note the importance of studies that encompass multiple cities (Hansford et al., 2017).

Van Acker et al. (2019) considered habitat in their connectivity study, including proportions of tree cover, impervious surfaces, water, grass/shrubs, and soil within a 100 m buffer around each park as well as the canopy area within each park (VanAcker et al., 2019). Increasing tick densities were found with increasing tree canopy within the park and park buffer (VanAcker et al., 2019). Hansford et al. (2017) also found high densities in woodland, but the highest densities were in woodland edge, which VanAcker et al. (2019) did not explore. Increasing proportions of water and soil had a significant negative correlation with tick densities (VanAcker et al., 2019). This was the first study to include soil cover specifically, and to analyse the effect of different land covers within and surrounding each greenspace, but they did not discuss the impacts of the land cover on infection prevalence.

Matthews-Martin et al. (2020) surveyed two urban parks and a peri-urban park in the city of Lyon, France in 2019. They categorised habitats into forest, forest pathways, forest edge, meadow, or pathway in open area. Tick densities were 32 times higher in closed canopy than open areas, supporting the study by VanAcker et al. (2019), and 16 times higher in the woodland than the woodland edge (Mathews-Martin et al., 2020), in contrast to the observations of Hansford et al. (2017). They found 100 times more ticks in the peri-urban site and suggested this may be because the proportion of woodland was much greater here (Mathews-Martin et al., 2020). They did highlight that connectivity for large mammals like deer could have impacted the results between the sites as they did not control for this, and that deer movement/surveying should also be included in further studies (Mathews-Martin et al., 2020).

Another study by Hansford et al. (2023) focused on the effects of habitat as well as connectivity on tick densities and *B. burgdorferi* s.l. prevalence, using broad habitat categories: open (long or short grass), under canopy (scattered trees), woodland, and woodland edge (ecotone). Hansford et al. (2023) found very few ticks in open managed habitats (cut grass), supporting the observations of Hornok et al. (2014), and there were no infected ticks in open areas (Hansford et al., 2023a). The 2023 study also found a difference in seasonality, with significantly more ticks in the woodland in summer than in autumn (Hansford et al., 2023a). Ticks can become easily desiccated in hot, dry weather (Berger et al., 2014), therefore remaining closer to woodland in summer helps to increase survival and their abundance in woodland. In Autumn, due to the cooler and wetter weather ticks may be able to survive in more open areas, decreasing the difference in abundance between woodland and open ground. This highlights the importance of sampling across different seasons or stating the specific season of sampling, as the season may affect the reliability of the data. Overall, the DON was greater in the woodland than in the woodland edge, which contrasts with the results of Hansford et al. *(*2017). Hansford et al. (2023) found that NIP was highest in woodland edge, at 4.2%

compared to 2.8% in woodlands (Hansford et al., 2023a). Deer behaviour or presence was not explored in this study, but the authors recognised this may affect the results.

2.5 The Use of Agent-based Modelling for Tick Populations and Tick-borne Diseases

Agent-based models are an important tool which can improve the understanding and explanations for the trends found in empirical research to aid urban planning. Models can be created to include many different parameters for species and landscapes over a large scale, which may not be possible to study in cases with limited resources for empirical data collection. The use of ABMs to focus on how the interactions of ticks, landscape characteristics, and tick hosts can affect tick densities and pathogen prevalence is a relatively recent development in literature, therefore there are a limited number of existing studies.

Wang, Grant, and Teel (2012) carried out a study which focused on the interactions of *Amblyomma Americanum* ticks, their hosts, and a heterogenous landscape in Texas, USA. This was one of the first studies to model the spatial-temporal dynamics of ticks and include both seasonality and a heterogeneous landscape (Wang, Grant, and Teel, 2012). Adding seasonality allowed Wang, Grant, and Teel (2012) to add and remove hosts according to their population changes through the year, and they removed hosts which reached the end of their life expectancy. The hosts had many different attributes, such as habitat preferences, home range sizes, and maximum number of feeding ticks, however the hosts did not represent individual species. 47 potential host species were identified in the study area which was modelled around the city of Houston, and hosts were categorised into small, medium or large (Wang, Grant, and Teel, 2012). Ticks were not represented as individual agents, but all life stages (including eggs) were included, and they could be affected by environmental conditions (temperature and humidity). The use of these additional population dynamics by Wang, Grant, and Teel (2012) contribute to the accuracy of the ABM, as these are all factors at play in the real world. The study included nine different habitat classifications based on the Houston landscape, which included urban greenspace in the form of a greenbelt surrounding the built-up urban areas. Ticks populated the new greenbelt, and tick densities in surrounding woodlands and the urban park increased (although this varied yearly depending on host populations and climate) (Wang, Grant, and Teel, 2012). This is one of the only studies using an ABM to explore how increasing connectivity through the addition of greenspace can increase tick densities in urban areas. It is highlighted that studies like this are needed to explore how tick populations can be controlled, because this data can be challenging to gather from empirical work alone (Wang, Grant, and Teel, 2012).

A later study created an ABM of a heterogeneous landscape to assess the Lyme disease risk in Scotland (Li et al., 2016), using DIN values as an indicator of Lyme disease risk. This study included environmental factors like the study of Wang, Grant, and Teel (2012), but focused specifically on the effects of temperature and elevation (which can affect temperature and habitat type) on tick ecology and host seasonality. The study also used three host categories based on transmission hosts, livestock, and reproduction hosts (Li et al., 2016) rather than small, medium and large hosts (Wang, Grant, and Teel, 2012). These categories are more appropriate for the Scotland study because the ABM is concentrating on pathogen transmission which is dependent on these different host categories. In Li et al. (2016), the modelled larvae preferred transmission hosts (rodents), adults preferred reproductive hosts (deer), and nymphs were generalists. Four habitat categories were used: woodland, heathland (both favoured by deer but occasionally used by livestock), grassland (favoured by livestock and occasionally used by deer), and non-vegetated areas (only used as a transport route by hosts) (Li et al., 2016). There were no specific urban habitat categories. The NIP values generated by the model were similar to field data, predicting that higher global temperatures may increase the risk of *Borrelia* infections due to extended questing periods, and the risk may expand to higher altitudes. The findings suggested that the data used to design the ABM was accurate, but there was a lack of field data on DIN for comparison (Li et al., 2016).

Halsey and Miller (2018) designed an ABM of *Ixodes scapularis* ticks interacting with wildlife hosts. The model included specific host species population dynamics and seasonality for white-footed mice and white-tailed deer (key hosts in North America), and key tick behavioural factors such as questing and feeding period (Halsey and Miller, 2018). This information was based on ecological data from many empirical studies, and focusing on specific tick and host species allows for the creation of species specific parameters, which may be more accurate than methods in previous studies (Li et al., 2016; Wang, Grant, and Teel, 2012). However, the complexity of adding these parameters meant that limiting assumptions had to be made in other areas. For example, humidity and elevation were not included as environmental variables, and larvae and nymphs were limited to feeding on the mice while adults were limited to deer (Halsey and Miller, 2018) even though this has been proven not to be exclusive (Bosler et al., 1984). Furthermore, the study area was based on one hectare of homogenous forest (Halsey and Miller, 2018), when landscapes are highly heterogeneous in reality. Halsey and Miller (2018) did state that the use of a larger heterogenous habitat would require the inclusion of more than two host species to be realistic. The model was run with hourly timesteps, which is more accurate than the weekly timesteps in previous studies (Li et al., 2016; Wang, Grant, and Teel, 2012), but it has been argued that 400 timesteps per 24 hours would be more realistic for wildlife movement (Wang, Grant, and Teel, 2012). Using 400 timesteps per day would take a long time to run the model though, and Halsey and Miller (2018) ran the model up to 10 years so 400 timesteps daily may not be achievable. The study found that it took up to 10 years for a reduction in adult tick populations (due to deer management) to reduce larvae and nymph populations by around 30% (Halsey and Miller, 2018), highlighting the need to run ABMs for a number of years to see the trends form.

Another study by Li et al. (2019) also focused on two groups of tick hosts, rodents, and deer, in an ABM of how climate change may affect Lyme disease risk in Europe. The study did not focus on specific species dynamics like Halsey and Miller (2018) though, and generalising species simplifies the model (Li et al., 2019). The ABM was further simplified by using monthly timesteps, however this allowed the model to run up to 2050 to run all relative concentration pathway (RCP) values for climate change scenarios (Li et al., 2019). The landscape in Li et al. (2019) was heterogenous but included fewer habitat categories than previous studies using heterogenous landscapes (Li et al., 2016; Wang, Grant, and Teel, 2012). The model focused on woodland, shrubs, and grassland, with no element of urbanised/ non-vegetated areas (Li et al., 2019). Like Li et al. (2016), DIN values were used to indicate Lyme disease risk, and the output values suggested that rising temperatures due to climate change may not always amplify Lyme disease risk (Li et al., 2019). However, Li et al. (2019) do highlight that future studies should focus on how fragmentation and connectivity may be linked to disease risk, as this may be more influential than temperature change.

A more recent study created an ABM to study specifically how interactions of *Ixodes scapularis* ticks and their hosts can increase *B. burgdorferi* s.l. prevalence (Tardy et al., 2022). The study only modelled a single host species, but added them as individual agents with their own personal behaviours, in addition to the general ecological behaviours of the species (Tardy et al., 2022). Previous models included assumptions that all individuals of a host species behave the same way (Halsey and Miller, 2018; Li et al., 2012), but in reality, this is not the case. Tardy et al. (2022) found that individual hosts which had particularly high tick burdens were more likely to be present in fragmented landscapes, suggesting like Li et al. (2019) that future studies should focus on the effects of habitat fragmentation on tick densities and pathogen prevalence. The model was carried out over 10 years (Tardy et al., 2022) which is similar to Halsey and Miller (2018), but Tardy et al. (2022) used 24 hour timesteps instead of hourly timesteps (which would be more accurate). The study also didn’t include any climate input parameters (Tardy et al., 2022), while previous studies included at least temperature (Halsey and Miller, 2018; Li et al., 2016; Li et al., 2019; Wang, Grant, and Teel, 2012) because this can impact host and tick behaviour.

While these studies demonstrate that ABMs can be useful for ecological modelling, there are often concerns about the need for model validation before using them for policy implementation and infrastructure planning. In many cases, validation is difficult due to a lack of empirical data which can be compared to model outputs (Filatova et al., 2013). This may be particularly difficult where empirical data is currently limited, as is the case for studying ecosystem disservices of ticks and tick-borne diseases in urban greenspaces, which is a relatively new area of research. It has been argued that in open system environmental models, there will always be some external parameters and hidden variables unaccounted for (and there often are in empirical studies too) (Heppenstall et al., 2021). It has been suggested that if a model were to match empirical findings exactly, this would be more questionable (Polhill et al., 2017).

2.6 Summary

Overall, there are a limited number of studies focussing on how the characteristics of urban greenspaces can affect the movement and distribution of ticks and tick-borne diseases. The studies using ABMs to model tick population distributions all highlighted the importance of deer and large reproduction hosts on tick populations, however there is a gap in the data for empirical studies relating to these reproductive hosts. Ticks cannot travel long distances on their own, so their movement and dispersion is dependent on hosts, and particularly large mammalian hosts with a large home range size. Deer are known to be a key tick host, particularly for adult ticks (Rizzoli et al., 2014), and it is suggested that they frequently occupy urban greenspaces, therefore it is key to consider their movements and distributions in studies (Diuk-Wasser, VanAcker, and Fernandez, 2020). Many of the empirical studies discussed did not include any host data (Brownstein et al., 2005; Estrada-Peña, 2002; Hansford et al., 2017; Hansford et al., 2023a; Hornok et al., 2014; Kowalec et al., 2017; Mathews-Martin et al., 2020), and only two included data from public observation records (Heylen et al., 2019; VanAcker et al., 2019). Public deer observation data is important and can be useful, however it may be biased depending on how many people visit the site and how many people take recordings, and this can produce contrasting results (VanAcker et al., 2019).

Much of the current literature recognises the importance of habitat structure in sustaining tick hosts and ticks within urban greenspaces (Hansford et al., 2023a; Heylen et al., 2019; Li et al., 2012; VanAcker et al., 2019), but many studies include very broad habitat categories which doesn’t provide a particularly in-depth explanation for tick distributions. For example, it is well reported that woodland is the favourable habitat for ticks (Allan, Keesing, and Ostfeld, 2003; Brownstein et al., 2005; Hansford et al., 2023a; Medlock et al., 2013). However, there were a small number of studies which distinguished tick densities in long, unmanaged grassland from densities in short, managed grassland. They found high densities of ticks in unmanaged grassland areas too (Hansford et al., 2017; Hornok et al., 2014), which the other studies did not pick up. In addition, most of the ABM studies include the use of a heterogeneous landscape with different habitat categories to form a more realistic landscape layer, but very few include urban habitat patches (Li et al., 2016; Li et al., 2019; Wang, Grant, and Teel, 2012) so there is a gap focussing on ABMs to model tick populations in urban landscapes.

Another limitation of the current literature is that it empirical studies focus mostly on urban greenspaces within a single city, yet the densities of ticks within different cities can be highly variable. Hansford et al. (2023) was the only study which included multiple cities, and despite them all being in the south of England the tick densities and infection prevalence varied greatly (Hansford et al., 2023a). They therefore suggested that future studies should try and include more cities where possible, to improve the reliability of the data.

The literature suggests that connectivity, patch size, and habitat are all important characteristics which are associated with the DON and NIP in urban greenspaces, and studies highlight the need for future ABMs to focus more on the impact of habitat fragmentation and connectivity on tick populations (Li et al., 2019; Tardy et al., 2022). All of these characteristics are closely linked (Diuk-Wasser, VanAcker, and Fernandez, 2020; Estrada-Peña, 2002; Hansford et al., 2023a; Heylen et al., 2019; Mathews-Martin et al., 2020; VanAcker et al., 2019), and ticks may not thrive when any one of the optimal conditions are met if there is another lacking. Some field studies have suggested that while patch size is important, connectivity may be more important for deer (Estrada-Peña, 2002; Tran and Waller, 2013; VanAcker et al., 2019). It has also been suggested that the habitat within and surrounding a greenspace may affect whether fragmentation is beneficial or detrimental to tick populations (Li et al., 2012).

Chapter 3 Methodology

3.1 Field Surveying

3.1.1 Study Sites

The empirical data used in this study was collected from field surveys of Glasgow and Edinburgh in Scotland during spring and summer of 2022 and 2023. Glasgow has the highest population of all cities in Scotland and the third largest population of all cities in the UK (Statista, 2021). In 2021 the city of Glasgow's population was around 635,130 (National Records of Scotland, 2022). The city is also home to more than 3,500 hectares of greenspace (Glasgow City Council, 2019). Edinburgh has a smaller population (526,470 in 2021 (National Records of Scotland, 2021)), but it is still the sixth highest population of all cities in the UK (Statista, 2021). The city of Edinburgh has 1,600 hectares of urban greenspace (City of Edinburgh Council, 2019).

A range of urban, suburban, and rural greenspaces were selected in each city and the surrounding urban periphery. The sites surveyed were a range of sizes, from small parks and urban woodland patches to large rural woodlands. An area was selected within each site to survey which was representative of the larger site area. Urban sites were selected within the city ring roads, suburban and rural sites were all more than 2 km from the city centre but remained within 10 km. Where motorways or waterways were present sites were surveyed along either side. In Glasgow there were 18 sites (Table 1, Figure 5) surveyed in total (6 urban, 6 suburban, and 6 rural). and in Edinburgh there were 16 sites (Table 2, Figure 6) in total (5 urban, 6 suburban, and 5 rural). Three sites were added in Glasgow during the second year of surveys; however, while it was aimed to survey each site twice, the additional sites could only be surveyed once in 2023.

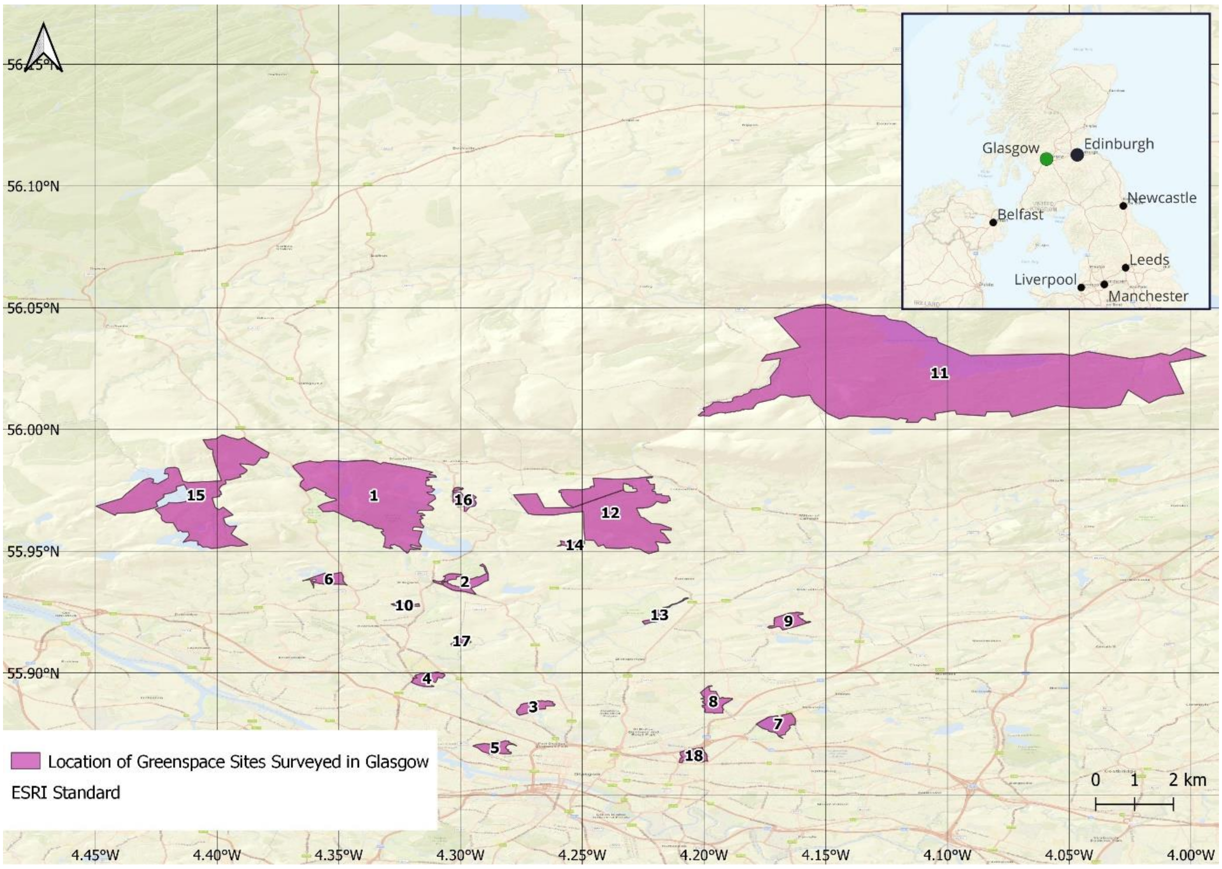

Figure 5: A map of the 18 field study sites in Glasgow city and the periphery.

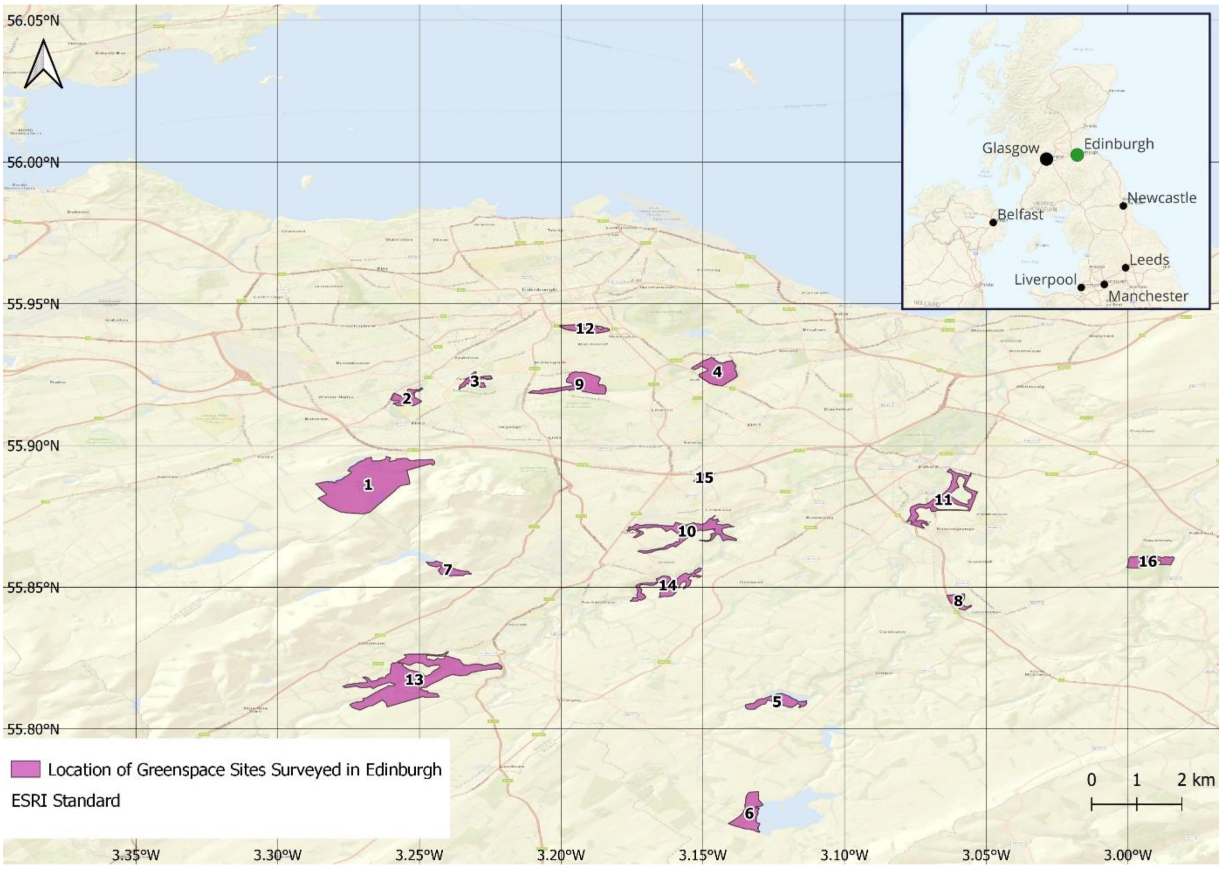

Figure 6: A map of the 16 field study sites in Edinburgh city and the periphery.

Table 1: Glasgow site numbers and corresponding site names, location, and area. Highlighted sites were only surveyed once in 2023.

Glasgow Greenspace Sites	Site Number	Site Grid Reference	Site Area
Mugdock Country Park	1	NS 53870 78107	2.11
Dougalston	2	NS 56623 73666	0.17
Ruchill Park	3	NS 57873 68241	0.21
Dawsholm Park	4	NS 55294 69495	0.33
Kelvingrove Park	5	NS 57218 66345	0.37
Mains Plantation Bearsden	6	NS 53058 74201	0.37
Hogganfield Park	7	NS 64601 67188	0.61
Robroyston Park	8	NS 62952 68391	0.53
Lenzie Moss	9	NS 64780 71877	0.46
Kilmadinny Loch	10	NS 54832 73020	0.13
Carron Valley Campsies	11	NS 68747 82386	0.91
Lennoxtown	12	NS 60492 76935	0.31
Cadder Church	13	NS 57916 69247	0.04
Blairskaith Linn	14	NS 59361 75633	0.17
Queens View	15	NS 49734 77427	0.30
Loch Ardling	16	NS 56583 77575	0.05
Temple Hill Wood	17	NS 56503 71278	0.07
Alexandra Park	18	NS 62205 65813	0.37

Table 2: Edinburgh site numbers and corresponding site names, location, and area.

Edinburgh Greenspace Sites	Site Number	Site Grid Reference	Site Area (Km2)
Bonaly Country Park	1	NT 20855 66249	0.34
Collinton	2	NT 21525 69320	0.20
Craiglockheart	3	NT 23066 70425	0.16
Craigmiller	4	NT 28534 71091	0.66
Edgelaw	5	NT 29736 58063	0.21
Gladhouse	6	NT 28909 53770	0.37
Glencourse	7	NT 22222 63437	0.17
Goreglen	8	NT 33631 61848	0.07
Hermitage	9	NT 25330 70373	0.52
Loanhead	10	NT 28235 64843	0.14
Lord Ancrum Wood	11	NT 33435 65844	0.07
Meadows	12	NT 25639 72666	0.23
Penicuik	13	NT 21828 59039	0.31
Roslin Glen Country Park	14	NT 27425 62513	0.07
Straiton	15	NT 28246 66760	0.07
Vogrie Country Park	16	NT 38032 63488	0.22

3.1.2 Tick Collection

Tick surveys were carried out following the blanket dragging method (Milne, 1943) using a 1m^2 woollen piece of fabric. A 10 m long transect was randomly selected, and the blanket was dragged slowly. After each drag, the blanket was overturned to collect ticks with forceps. Only the adults and nymphs were counted and recorded before being placed into 70% ethanol in Eppendorf tubes. Presence of larvae was noted but not counted.

Fifteen transects were carried out at each study site/visit. The drags were spread out across the site so that a representative sample was collected. Temperature and humidity measures were taken upon arrival and prior to leaving each site to ensure that conditions were suitable for tick questing activity. Sampling was postponed if the temperature was below 13˚C to ensure the conditions were appropriate for tick questing behaviour. Surveys were not carried out in wet weather. At least 50 nymphs were collected from each site where possible to get a representative sample for testing infection prevalence, but this was not always possible due to a lack of ticks and/or time restraints at some sites. Any extra ticks collected from clothing were added to the samples and noted as extras on the recording sheet. The DON was then calculated from these abundances for each survey:

DON (per 100m^2) = (Number of nymphs collected / 150 (the area of survey transects in m^2)) * 100.

3.1.3 Host Observations

While carrying out blanket drags, the surveyor made a note of any deer signs observed in the transect. This involved counting the number of deer dung piles and noting any deer tracks. Any observations of deer grazing or moving through the site were also noted on the recording sheet, including deer seen by recorders or accounts provided by walkers in the area. This data was used to generate a deer present (1) or deer absent (0) value for each site. Any sign of deer on site resulted in a 'deer present' value at site level. It was assumed that a false positive deer observation from a walker was unlikely, and so these records contributed to a 'deer present' result. The nature of this data did now allow for more precise calculations such as deer density to be calculated, because there was no way of counting individual deer.

Data points were also downloaded from the NBN Atlas (NBN Trust, 2023) for verified deer sightings in Scotland within the last 5 years (2018 onwards). The data was exported for sightings of all six species present in the UK (*Cervus elaphus*, *Capreolus capreolus, Dama* dama, *Cervus nippo*n, *Muntiacus reevesi*, *Hydropotes inermis*)[1]. All the observation points were added to QGIS so that they could be overlayed with the field survey data. It was assumed false positive observations were unlikely in this dataset. If deer signs were observed either in field surveys or NBN observations, the site would receive a deer present value. Where there were no deer signs noted in surveys, and no observation point(s) in the NBN dataset, the site received a deer absent value.

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[1] See Appendix 1 for the full list of citations and records collated for deer observation datasets on the NBN Atlas website.

3.2 Pathogen Screening

All nymphs and adult ticks collected from field surveys were morphologically identified and tested for *B. burgdorferi* s.l.

DNA extraction was performed for all ticks using ammonium hydroxide (NH_4OH) (Guy and Stanek, 1991). Briefly, each tick was added to an Eppendorf with NH_4OH (100 µl for nymphs and 500 µl for adults), and then added to a tissue lyser for one minute at 30Hz. Samples were heated at 100˚C for 20 minutes, and then for a further 15 minutes with the lids open. The samples were stored at -20˚C until polymerase chain reaction (PCR) tests were carried out.

PCR was carried out following methods for real-time PCR detection of *B. burgdorferi* s.l. (Courtney et al., 2004). Briefly, 2x MyTaq mix (Meridian BioSciences), 10 ρmol μl^{-1} primers, and 3.2 ρmol μl^{-1} probe were thawed on ice before making the PCR mix. Each mix consisted of 12.5µl of 2xMyTaq mix, 1 µl of each primer (Bb23SF and Bb23Sr) and the probe (Taqman), and 7.5 µl of water. This mixture was added to PCR tubes in aliquots of 23 µl along with 2 µl of DNA extract, ensuring there was one negative control with two positives. Thermal cycling was carried out using Opticon Realtime machine using 95˚C for five minutes, then 39 cycles of 95˚C for 15 seconds and 57˚C for one minute. The plate was then read after each 57˚C step. Positive and negative controls were included in each run.

NIP was calculated as the proportion of ticks collected at a site which tested positive for *B. burgdorferi* s.l.. DIN could then be calculated using the following equation:

Density of infected nymphs: Nymph infection prevalence for a site * Density of nymphs at the site

3.3 Calculating Greenspace Area

To calculate the area of each site, the OpenStreetMap ESRI Standard and ESRI Satellite layers (available under the Open Database Licence) were added as base maps onto QGIS version 3.28.11 (Firenze). Then the perimeter of each site was traced using the base maps. For sites such as urban parks, country parks, and nature reserves, the ESRI standard map was used to follow the boundaries. For patches of woodland with no clear site boundary, the ESRI satellite layer was used in combination with the ESRI standard to trace around the boundaries of the woodland patch as the woodland edge was more clearly visible from satellite view. Once all survey sites were digitalised, the $Area function was used to calculate the area of each feature in metres squared, which was converted to hectares for analysis.

3.4 Determining Land Cover

To understand how the density of ticks in urban greenspace is associated with land cover the proportions of different land cover types were calculated for each site. The land cover proportions were calculated within a 1 km buffer around the centroid of each site to understand why roe deer may be present at certain sites depending on the surrounding land cover. Roe deer have home ranges of between 0.14-1 km^2 (Li et al., 2012; Lovari, Serrao, and Mori, 2017) which can expand beyond the boundaries of the smaller survey patches, so a buffer of 1 km was selected to capture the usual range of roe deer around a central point.

The 10 m classified pixel Scotland Land Cover Map (LCM) from the UK Centre for Ecology and Hydrology (UKCEH) 2021 (Marston et al., 2022) was downloaded and added as a raster layer in QGIS. The LCM is created by combining multiple classification scenes into a single mosaic of land cover for the UK (Marston et al., 2022).

After adding the layer onto QGIS it was classified according to the 21 UKCEH land cover classes (Appendix 2). The UKCEH classes are similar to the Biodiversity Action Plan classifications, but not identical, as UKCEH is modified specially for use with satellite remote sensing (Marston et al., 2022).

The centroid tool was used to create a central point for each greenspace site polygon, so and a 1 km buffer was generated around the centroid using the Buffer tool. Using the Landscape Ecology Statistics (LEcoS) plugin and the buffer layer as a vector overlay, the land cover proportions of specific land cover classes of interest (classes 1,2,4,5,6,7,8,20,21) covered by each buffer were calculated. The proportions were exported to a comma delimited .csv file. In Microsoft Excel these proportions were grouped into generalised land cover aggregates (Table 3), similar to the UKCEH aggregate classes, which would be most appropriate for analysis in this study. These land covers were selected as they would be the most common habitats encountered by and utilised by deer in and around Glasgow and Edinburgh.

Table 3: UKCEH land cover aggregate classes defined for data analysis.

Generalised Aggregate Land Cover	UKCEH Land Cover Classes
Woodland	1,2
Improved Grassland	4
Semi-natural Grassland	5,6,7,8
Built-up Areas	20,21

3.5 Connectivity Metric

Least cost paths were generated as a measure of connectivity for each greenspace site surveyed. The calculation of least cost paths required the creation of a cost raster from assigned cost values derived for each UKCEH land cover class (Table 4). The cost layer depicts the 'cost' for deer to move through each land cover type, depending on their habitat preferences. It is then assumed that deer would choose the movement path with the least cost to them. The cost values were modified from previous studies which used expert-based ranking systems for forest species such as deer to measure landscape resistance (Balčiauskas et al., 2020; Girardet, Conruyt-Rogeon, and Foltête, 2015; Gurrutxaga, Rubio, and Saura, 2011; VanAcker et al., 2019).

Table 4: Cost values assigned to each UKCEH land cover class to create cost raster layer.

Land Cover Class	Cost Value
Deciduous Woodland	1
Coniferous Woodland	1
Arable	60
Improved Grassland	40
Neutral Grassland	30
Calcareous Grassland	30
Acid Grassland	30
Fen	30
Heather	30
Heather Grassland	30
Bog	30
Inland Rock	40
Saltwater	1000
Freshwater	1000
Supralittoral Rock	100

Supralittoral Sediment	100
Littoral Rock	100
Littoral Sediment	100
Saltmarsh	300
Urban	1000
Suburban	300

To create the cost layer as a raster, the land cover raster was first vectorised. This allowed the use of the field calculator in the attribute table to assign each land cover polygon a cost value depending on the land cover classification according to Table 4. The layer was then rasterised back to a 10 m pixel resolution, burning in the new cost attribute field rather than land cover class (Figures 7 and 8).

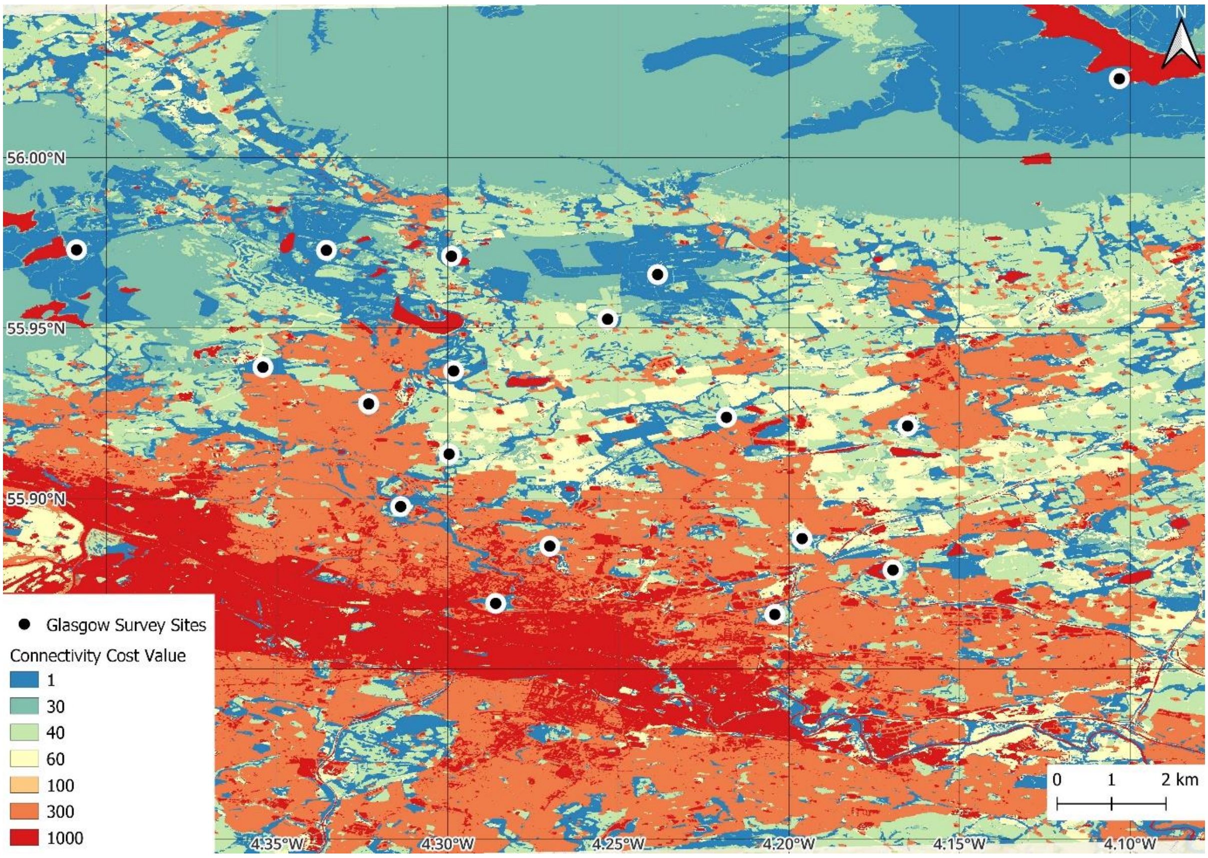

Figure 7: Connectivity raster map layer for Glasgow.

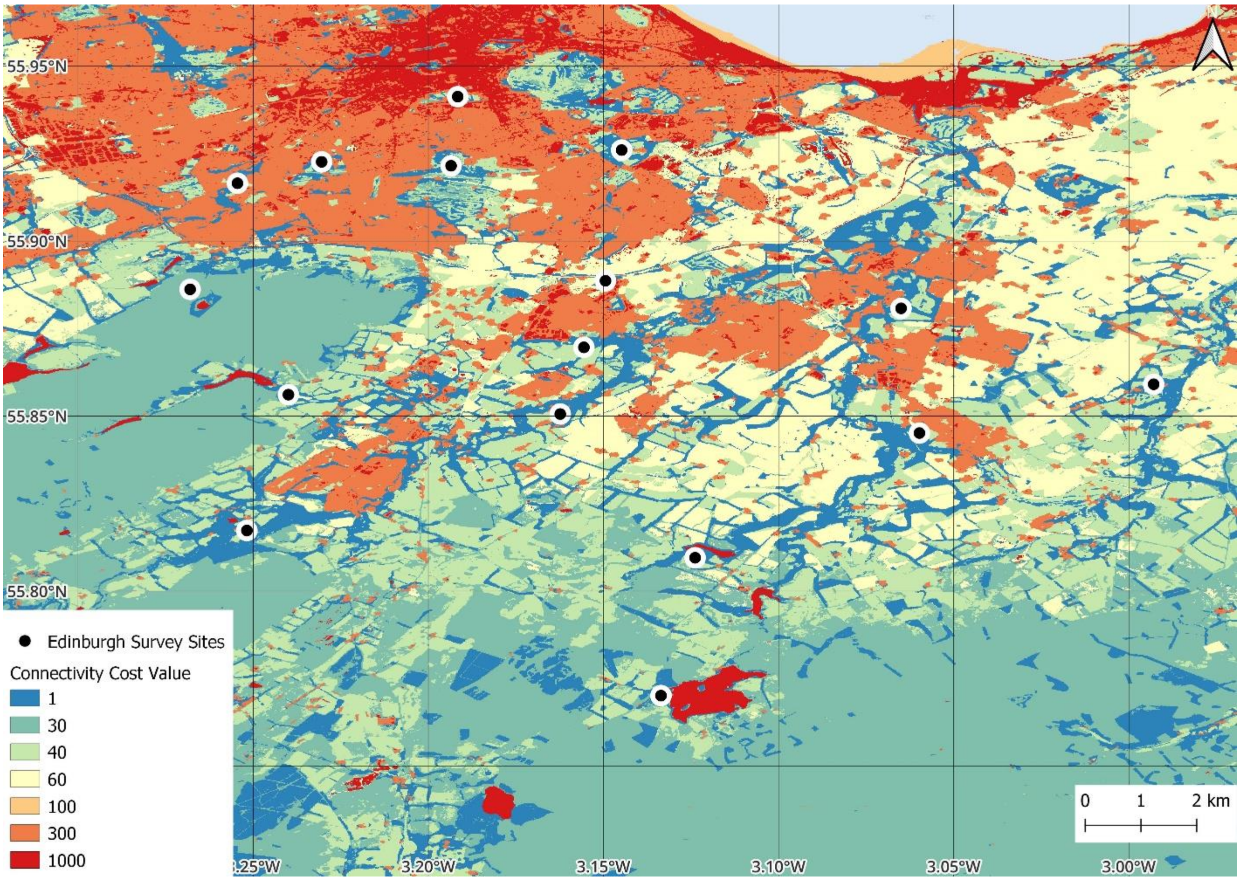

Figure 8: Connectivity raster map layer for Edinburgh.

After creating the cost layer, a source and a sink had to be defined to specify where deer would be moving to and from. Since there was no accessible, accurate dataset of deer observations in the cities of Glasgow and Edinburgh, as roe deer are known to have at least one woodland patch within their home range, all woodland patches of over 10 ha were assumed to be potential sources (Heylen et al., 2019; Lovari, Serrao, and Mori, 2017). All woodland features in the land cover layer with areas over 10 ha were selected and exported to a new layer. The difference tool was then used to remove woodland patches which were situated within or overlapping the greenspace sites so that the least cost path did not stay within the site. As the least cost tool only works between point attributes, the extract vertices tool was used to add vertex points for each site and woodland patch. The Least-Cost Path was then calculated between the closest vertices of each site and the nearest woodland patch. Using the vertices was more accurate than the centroid points for each site as this can generate inaccuracies in the path length for large patches where the centroid is far from the edge.

3.6 Agent-based Modelling

3.6.1 Agent-based Model Design

In this study, an ABM was used to create scenarios to understand how tick densities and *B. burgdorferi* s.l. infection prevalence may be associated with different forms of greenspace creation (e.g., as either a single large greenspace, or as several small greenspaces. The model was developed in Java, using Geomason, by members of the MEASURE project team based at the University of Manchester (Watkinson and Huck, 2023). Within the ABM there was a 10 m^2 grid-based land cover layer covering a 250 km^2 area of Glasgow and its periphery. There are four mobile agents, based on roe deer in their adult (1+ years) and juvenile (under 1 year) form, *I. ricinus* ticks (as eggs, larvae, nymphs, and adults), and humans. Each of the agents can interact intraspecifically and interspecifically. To reduce computational complexity, other tick hosts are not modelled but are represented as probability distributions describing the likelihood a questing tick will attach to another host species. Temperature data are also included, as they can affect the behaviour of the agents. The ABM runs over a seven-year period (approximately 2 tick life cycles), beginning in January, with a minutely timestep, generating outputs at daily intervals.

At the start of the model, ticks are distributed based on the findings of the field survey data in 2022 (Table 5), and 90% of ticks were in diapause (an inactive development phase over winter months). Once out of diapause (when the temperature reaches over 7°C) ticks were either questing, feeding, or in a development phase into the next life stage, which is determined by boolean state variables (see Appendix 3 for decision process flow chart). A boolean variable also determines whether a tick is infected with *Borrelia* or not, and consequently whether it can infect a human. While questing, ticks are aware of hosts (deer or human) within a 5m radius and attach to the closest host. If a tick attaches to a roe deer, they move with the deer and drop off after 3-10 days. If a tick attaches to a human, it is removed from the simulation. If no deer or humans are present, ticks may randomly attach to an alternative host. When attached to an alternative host, ticks remain stationary and cannot carry out other behaviours. After 3-10 days ticks are moved to a different woodland or grassland patch. When attached to deer, adult male ticks can mate with females on the same host, before being removed from the simulation. Females feed for seven days after mating, and then drop off to lay eggs. After laying eggs, females are also removed from the model.

Table 5: ABM Tick Parameter Values

Tick Parameters	**Value**	**Source**
Tick sex	0.5	Li et al., 2014
Adult tick mortality	1.98E-06	Li et al., 2019
Nymph tick mortality	2.98E-06	Li et al., 2019
Larvae tick mortality	2.98E-06	Li et al., 2019
Egg to larvae development	0.00001, 0.002, 0.019, 8.4	Hancock, Brackley, and Palmer, 2011
Larvae to nymph development	0.00003, 0.00073, 0.007, 7.4	Hancock, Brackley, and Palmer, 2011
Nymph to adult development	0.000008, 0.0019, 0.016, 8.7	Hancock, Brackley, and Palmer, 2011
Adult egg laying	0.0001, 0.01, -0.062, 8.7	Hancock, Brackley, and Palmer, 2011
Transmission of Lyme Disease to humans after tick bite	0.267	Maiwald et al., 1998
Larvae transmission host finding (non-deer)	0.000000881	Li et al., 2019
Larvae reproduction host finding (deer)	0.000000722	Li et al., 2019
Nymph transmission host finding (non-deer)	0.000000881	Li et al., 2019
Nymph reproduction host finding (deer)	0.000000722	Li et al., 2019
Adult transmission host finding (non-deer)	0.000001696	Li et al., 2019
Probability of adult female becoming oviparous	0.5	Own/Li et al. 2014

Maximum time a larvae can quest for before dying (days)	56	Hancock, Brackley, and Palmer, 2011
Maximum time a nymph can quest for before dying (days)	84	Hancock, Brackley, and Palmer, 2011
Maximum time an adult can quest for before dying (days)	112	Hancock, Brackley, and Palmer, 2011
Time attached to host as a larva (days)	4	Steinbrink et al., 2022
Time attached to host as a nymph (days)	5	Steinbrink et al., 2022
Time attached to host as an oviparous female (days)	10	Steinbrink et al., 2022
Infection prevalence of Lyme Disease in ticks at model initialisation	0.5	Randolph and Craine, 1995
Life stage ratios used to determine number of ticks per life stage at model initialisation	2000:200:10:2	Randolph, 1998
Probability of going into diapause over winter	0.9	Li et al., 2019

The deer behaviours modelled in the ABM were based on Topping et al. (2003), and their initial distributions in the model were also based on 2022 field data (Table 6). Roe deer can move freely through the model, they begin by selecting a home range (based on woodland cover) using model stochasticity and can then roam throughout this range (see Appendix 4 for decision process flow chart). They begin initially in winter herds, moving collectively and then dispersing over summer months. Foraging behaviour also changes seasonally through Summer and Winter, which is stored in the model as lists, and will forage for a maximum period each day. The herd size is dependent on tolerance, which is determined by the amount of woodland cover. Rutting behaviour is also exhibited by the males. Deer move preferentially through woodland and seek shelter in woodland patches when human agents are nearby. Deer can also feed, ruminate, rut, mate, and raise young depending on their sex and the season, which is based on Boolean state variables. Females remain with their young for one year- while they remain juvenile, and adult deer can live up to 7 years (the chance of deer death increases with age and is dependent on their rank). Juvenile fawns exhibit similar behaviour to adults, but there are fewer fawns in the model. They use their location to feed or follow their mother and will inherit the foraging patterns of their mother.

Table 6: ABM Deer Parameter Values

Deer Parameters	**Value**	**Source**
Number of deer at model initialisation	57	Dandy et al., 2009
Probability of deer having fawn at model initialisation	0.1	Own
Deer sex	0.5	Strandgaard, 1972
Minimum home range radius	1500	Jepsen and Topping, 2004
Maximum home range radius	3000	Jepsen and Topping, 2004
Female deer fertile window	Day 195 to 227	Jepsen and Topping, 2004
Probability female becomes fertile	0.65	Own
Gestation period of roe deer	300	Jepsen and Topping, 2004
Number of forage cells required within home range	4000	Jepsen and Topping, 2004
Minimum percentage of woodland required within home range	15	Jepsen and Topping, 2004

Maximum time spent eating (hours) - summer/winter	5.5/7.5	Wallach, Shanas, and Inbar, 2010
Tick capacity	800	Li et al., 2019
Preferred group size	17.1 * woodland ^0.5	Jepsen and Topping, 2004
Foraging habitats	Arable, grassland	
Cover habitats	Woodland, arable	
Female Deer Background Mortality Rates (Per Minute)		
> 10 years	0.00001984	Jepsen and Topping, 2004
7-10 years	0.000004712	Jepsen and Topping, 2004
2-7 years	4.96E-07	Jepsen and Topping, 2004
1-2 years	0.000003968	Jepsen and Topping, 2004
Male Deer Background Mortality Rates (Per Minute)		
> 10 years	0.00001984	Jepsen and Topping, 2004
7-10 years	3.22E-06	Jepsen and Topping, 2004
2-7 years	2.48E-06	Jepsen and Topping, 2004
1-2 years	4.64E-06	Jepsen and Topping, 2004

Initially, 1744 humans are loaded into the model. This figure was based on urban greenspace visitation data in Scotland (NatureScot, 2018) (Table 7). Humans were limited to movement into and around urban greenspace areas in the ABM. Human agents decided on each day whether to visit a greenspace, where to visit, how long for, which route to take, and what time (see Appendix 5 for decision process flow chart). Humans can choose whether to walk through or around a greenspace, and whether to follow a designated path, or walk randomly. The decisions were largely dependent on the day of the week and temperature.

Table 7: ABM Human Parameter Values

Human Parameters	**Value**	**Source**
Probaility person will walk around a greenspace, if not walk through	0.47	NatureScot, 2018
Human Visit Probabilities for Each Site		
Hogganfield	0.125906228	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Kelvingrove	0.430385672	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Robyroyston	0.018985633	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Ruchill	0.026270339	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Dawsholm	0.050692718	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Lenzie Moss	0.045315828	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Kilmardinny Loch	0.045445684	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr

Dougalston	0.025358595	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Mains Plantation Bearsden	0.001407696	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Blairskaith Linn	0.013048376	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Mugdock	0.177391489	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Lennoxtown	0.010387484	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Carron Valley Campsies	0.000232342	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Cadder Church	0.00411191	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Queens View	0.025060006	Survey data, Google, TripAdvisor, Facebook, Instagram, Flickr
Off-path Walking Probability In Greenspaces		
Hogganfield	0.239316239	Survey data
Kelvingrove	0.238095238	Survey data
Robyroyston	0.304347826	Survey data
Ruchill	0.380952381	Survey data
Dawsholm	0.388349515	Survey data
Lenzie Moss	0.355371901	Survey data
Kilmardinny Loch	0.158536585	Survey data
Dougalston	0.342105263	Survey data
Mains Plantation Bearsden	0.2	Survey data
Blairskaith Linn	0.357142857	Survey data
Mugdock	0.372093023	Survey data
Lennoxtown	0.27	NatureScot, 2018
Carron Valley Campsies	0.27	NatureScot, 2018
Cadder Church	0.444444444	Survey data
Queens View	0.275	Survey data

3.6.2 Developing Scenarios

The agent-based model scenarios were created in QGIS version 3.28.11 (Firenze); one several large (SL) scenario and one several small (SS) were mapped. A new polygon vector layer was created for each scenario so that new greenspace(s) could be drawn. A point polygon layer was created to add four entrances/ exits for each theoretical greenspace site. The area where the virtual greenspaces were sited consisted mostly of housing estates, which were replaced with a new theoretical greenspace of around 30Ha for the SL scenario. To develop the SS scenario, smaller polygons were drawn around the same geographical area, ensuring that the total area of the smaller polygons still approximately summed up to 30Ha, to control for the effects of habitat loss on tick and host populations.

The UKCEH land cover map then had to be updated to account for the new greenspace(s) created in each scenario. To do this the difference tool was used, selecting the land cover vector layer as the input layer and the new greenspace polygon layer as the overlay. This removed the area of current land cover polygons which overlap with new greenspace area. Then, on the layer with the new greenspace polygon, all features were selected and copied to clipboard. The features were pasted onto the land cover difference layer and the land cover was specified with the value 1 (for broadleaf woodland). All greenspaces in the scenarios were specified as woodland to control for the effects of habitat type and proportion on tick and host populations, and the surrounding land cover was predominantly urban in both scenarios. The rasterise tool was used to burn in the new land cover types, and then to burn in the cost values for each. This produced a raster land cover and raster cost layer for each greenspace scenario to enter into the ABM. The connectivity was calculated for the theoretical greenspaces using the methods described above (section 3.5) so that this could be analysed with the outputs to compare to the empirical data findings.

3.6.3 Running Scenarios

To run the ABM, the land cover and cost rasters were loaded into the model. These layers determine how deer move preferentially through different areas in the model (Figures 9 and 10). The cost layer is also used to determine human movement through greenspaces; however, the cost layer was inversed for human movement in line with human vs deer behaviour. This assumes that it is a lower cost (easier and preferential) for humans to move through a built-up area or paved path than through grassland or woodland, however it does not prevent humans from using woodland. Deer on the other hand, are less likely to utilise built-up area, and move primarily through forests. Climate data were also loaded in as this can affect the behaviour of the agents through the simulation.

The greenspace scenario polygons were added to determine the greenspace boundaries for visitors, along with the point layer of entrances and exits. The configuration of these points determined how humans move through the greenspace when visiting, and whether they enter and leave via the same point. 961 humans were loaded into the model initially, based on data from the Scotland People and Nature survey of greenspace usage (NatureScot, 2018), which found on average 96,904 people in Glasgow visit a greenspace at least once a week. Normal distribution statistics determined the likelihood of humans visiting each greenspace in this study based on this figure. 46,537 ticks were loaded into the model initially, with distributions based on the 2022 Spring and Summer field survey data. 18 deer were loaded into the model, based on medium estimates of deer density per km^2 in a Forest Research report in peri-urban Scotland (Dandy et al., 2009). The SS scenario was run 36 times, and the SL was run 25 times (as many runs as possible within time constraints) to generate repeat results which could be averaged. The model outputted estimates for DON, DIN, deer density, number of tick bites and infected tick bites of humans at each greenspace, which could then be statistically analysed.

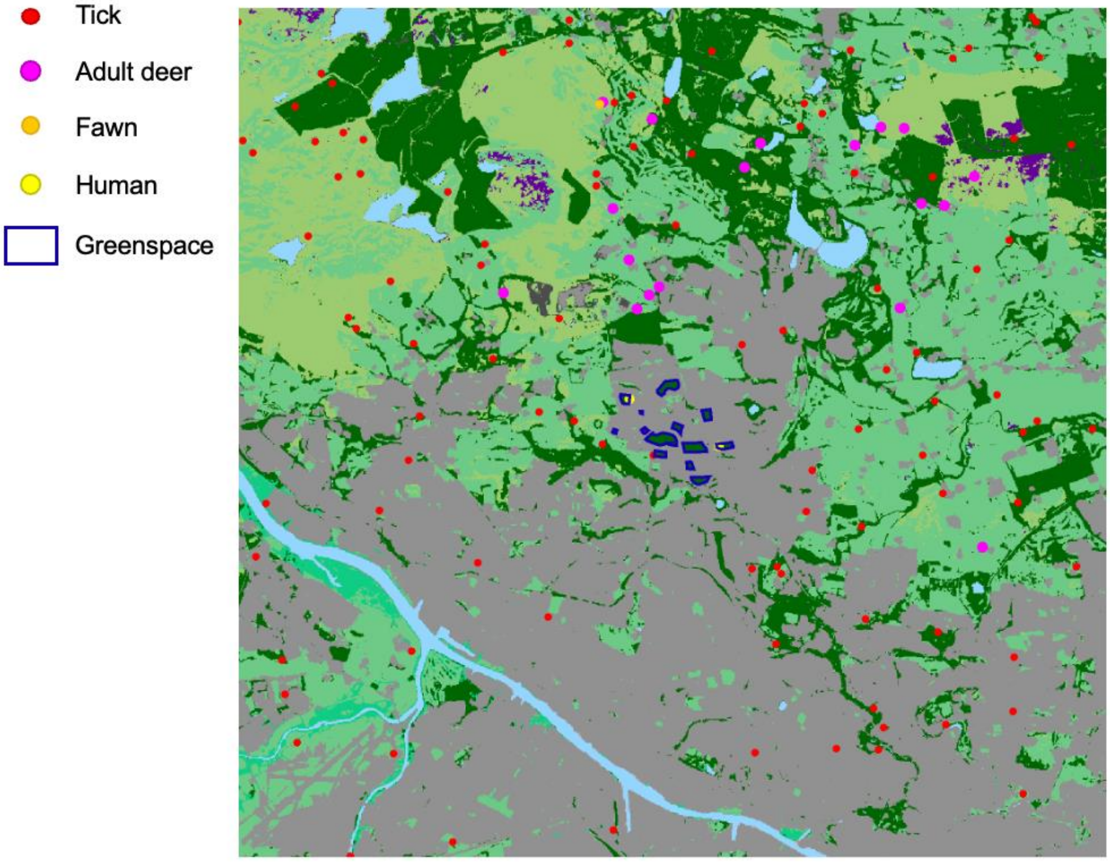

Figure 9: A screenshot from a model run with 'several small' configuration, showing the different agents on a land cover map.

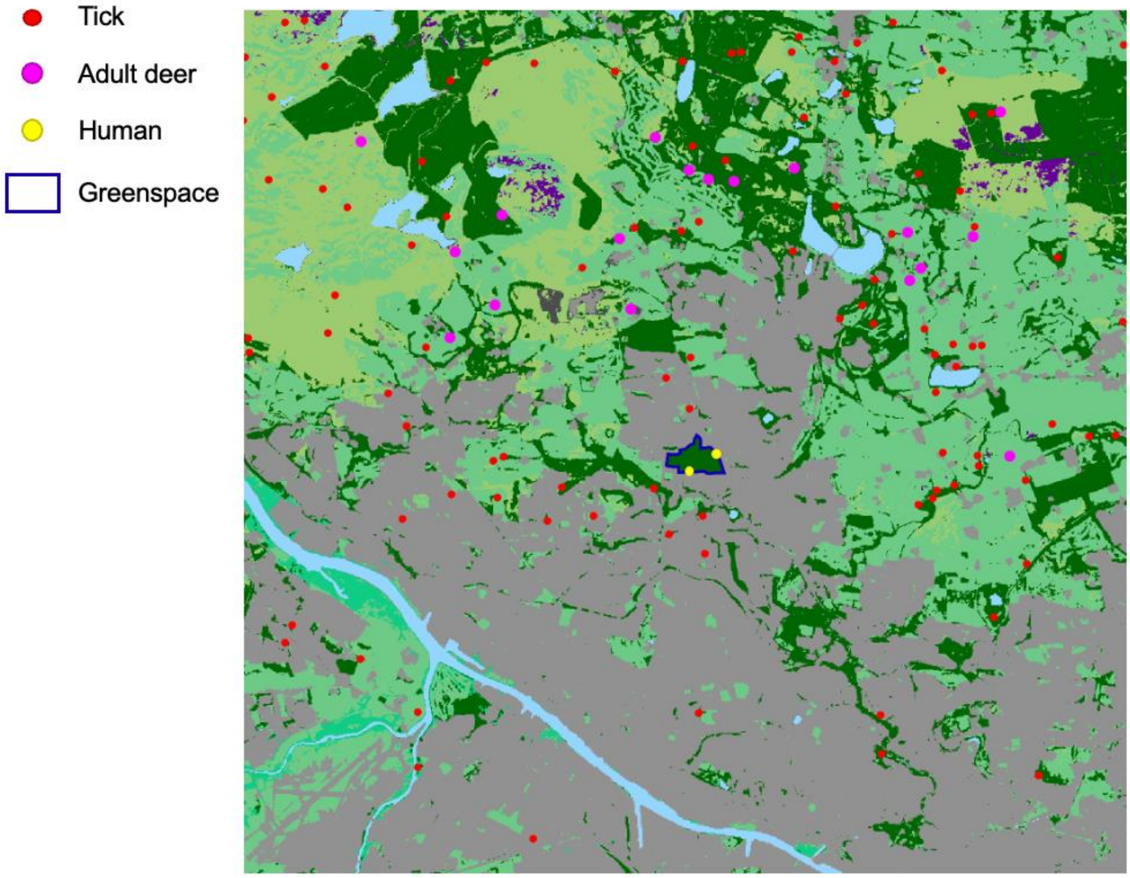

Figure 10: A screenshot from a model run with 'single large' configuration, showing the different agents on a land cover map.

3.7 Statistical Analysis

All statistical analysis was carried out using R (version 4.3.1) and R studio software (R Core Team, 2023). First, the continuous data were visualised using histogram plots to check the distributions. GLMMs (General Linear Mixed Models) were used to investigate whether there were significant relationships between these continuous variables (Table 5). These models were selected because there were multiple independent variables and sources of random variability in the dataset. They can also handle data which is not normally distributed. Fitted vs residual plots, dispersion plots and VIF (Variance Inflation Factor) values were generated to check that the data met the GLMM assumptions of linearity, homoscedasticity, overdispersion and multicollinearity for each model. 95% confidence intervals (CI) were used, with a P value of ≤0.05 as significant.

GLMM 1 was used to test for significant correlations between DON (per 100m^2) and path cost, site area, and proportions of woodland, improved grassland, semi-natural grassland, and built-up area within 1 km of a site. All fixed effects were scaled before running GLMM1, as the scales varied greatly between effects. The data were not normally distributed, and was heavily right skewed, so negative binomial distribution was used to account for the overdispersion of the data. Month, Year and City were added as random effects in the model, to account for differences in tick presence with seasonal variation and in different locations. None of the VIF values were over 3 in this GLMM, so none of the variables needed to be excluded (Zuur et al., 2009).

The second GLMM was used to test for significant correlations between DIN (per 1 km^2) and path cost, site area, and proportions of woodland, improved grassland, semi-natural grassland, and built-up area (Table 5). Sites where no ticks or a single tick was collected during surveying were excluded from DIN analysis, and a zero-inflation model was run to account for the large number of sites where no infected ticks were found. Negative binomial was used because the data was not normally distributed and was heavily right skewed. The original values per 100m^2 had to be multiplied out of their decimal form to run the model, hence why they were transformed to values per 1km^2 instead. Values for DON could instead be rounded as they were greater than 0. For GLMM 2, the built-up area proportion VIF value was 3.2, suggesting slight collinearity, this was noted but the fixed effect was left in the model. Year and City were added as random effects to account for differences in DIN with seasonal variation and in different locations. Month could not be included as a random effect due to identifiability issues in the smaller dataset.

GLMM 3 and GLMM 4 were used to analyse the ABM outputs of tick bite data, to assess whether the path cost or site area for the SS scenarios affected the number of tick bites and number of *Borrelia burgdorferi s.l.* infected bites in a greenspace. This could then be compared with the results of the field survey data. The land cover and city were already controlled for in the design of the scenarios. Temperature was added as a random effect since the model was run over entire years, where temperature can vary greatly in comparison to the three summer months of field surveying. The VIF values were all low, however the data was right skewed so negative binomial GLMMs were used.

For the field data, Chi-squared were used to test for significant differences between DON or DIN in different cities (Glasgow vs Edinburgh) or across different years (2022 vs 2023). For the ABM data, Wilcox Rank tests were used to test for significant differences between the number of tick bites and *B. burgdorferi s.l.* infected bites in the SS scenario, compared to the SL scenario, as Wilcox allows for data with lower expected frequencies. Chi-squared tests were also performed with deer presence data Average DON and DIN were calculated for sites where deer signs were present (sites with a deer presence value of 1), and for sites where deer signs/ observations were absent (value of 0). Chi-squared tests were then performed to assess whether there were significant differences in DON and DIN between sites where deer were present compared to sites where deer were absent.

Chapter 4 Results

4.1 Tick Surveys

4.1.1 Summary

A total of 946 *I. ricinus* ticks were collected from 28 of 34 (82.4%) greenspaces surveyed in Glasgow and Edinburgh, including 861 nymphs (N), 34 adult females (AF), and 51 adult males (AM)[2]. These figures varied between different survey years, with 178 ticks (153 N, 14 AF, 11 AM) in 2022 compared to 672 ticks (615 N, 20 AF, 37 AM) in 2023, excluding ticks found in the additional three sites in 2023 (Temple Hill Wood, Alexandra Park, Loch Ardling). The figures also varied between cities. 753 ticks were collected in Glasgow (683 N, 28 AF, 42 AM), excluding the ticks found in Alexandra Park and Loch Ardling which were added in 2023, to even out the number of urban, suburban, and rural sites between Glasgow and Edinburgh. Ticks were collected in 16 of the 18

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[2] Appendices 3 and 4 show the field survey data collected at each site.

(88.9%) sites surveyed in Glasgow (15 of 16 sites excluding Alexandra Park and Loch Ardling). 100 ticks were found in Edinburgh (88 N, 6 AF, 6 AM), in 12 of the 16 sites surveyed (75%). These figures exclude the ticks found in Alexandra Park and Loch Ardling, which were added to Glasgow in 2023, so that the number of urban, suburban, and rural sites in Glasgow and Edinburgh are even. *B. burgdorferi s.l.*-infected ticks were found in 15 of the 18 (83.3%) survey sites in Glasgow (14 of 16 sites excluding Alexandra Park and Loch Ardling), but only 1 of 16 (6.3%) sites surveyed in Edinburgh (Gladhouse). In total, 44 ticks (43 nymphs) tested positive for *B. burgdorferi s.l.*. 40 nymphs (90.9%) were collected in Glasgow, and 38 (86.4%) were collected during the 2023 surveys. Evidence of deer presence was found in 25 of 34 sites (73.5%), according to field survey and NBN data (Figures 11 and 12).

The mean DON across both cities was 8.83 (95% CI: 7.51 - 10.30) per 100 m^2, and the maximum DON for a greenspace was 62 per 100 m^2. The densities varied between sites, in Glasgow the density was generally higher in rural sites, and in the sites located in north-west Glasgow (Figure 11). In Edinburgh densities were highest in suburban sites, followed by rural sites, and in the sites to the south-west of the city. The mean NIP was 2.81% (95% CI 2.28 - 3.32), and the mean DIN was 0.36 (95% CI 0.3 - 0.42) per 100 m^2. The figures varied across sites in Glasgow, rural sites generally had a higher DIN, but there was no specific trend across different areas of the city (Figure 13). For the single suburban site in Edinburgh where infected ticks were found, DIN was 0.63 per 100m^2. The maximum DIN value was 3.03 per 100m^2, from Dougalston in Glasgow (suburban), surveyed in 2023.

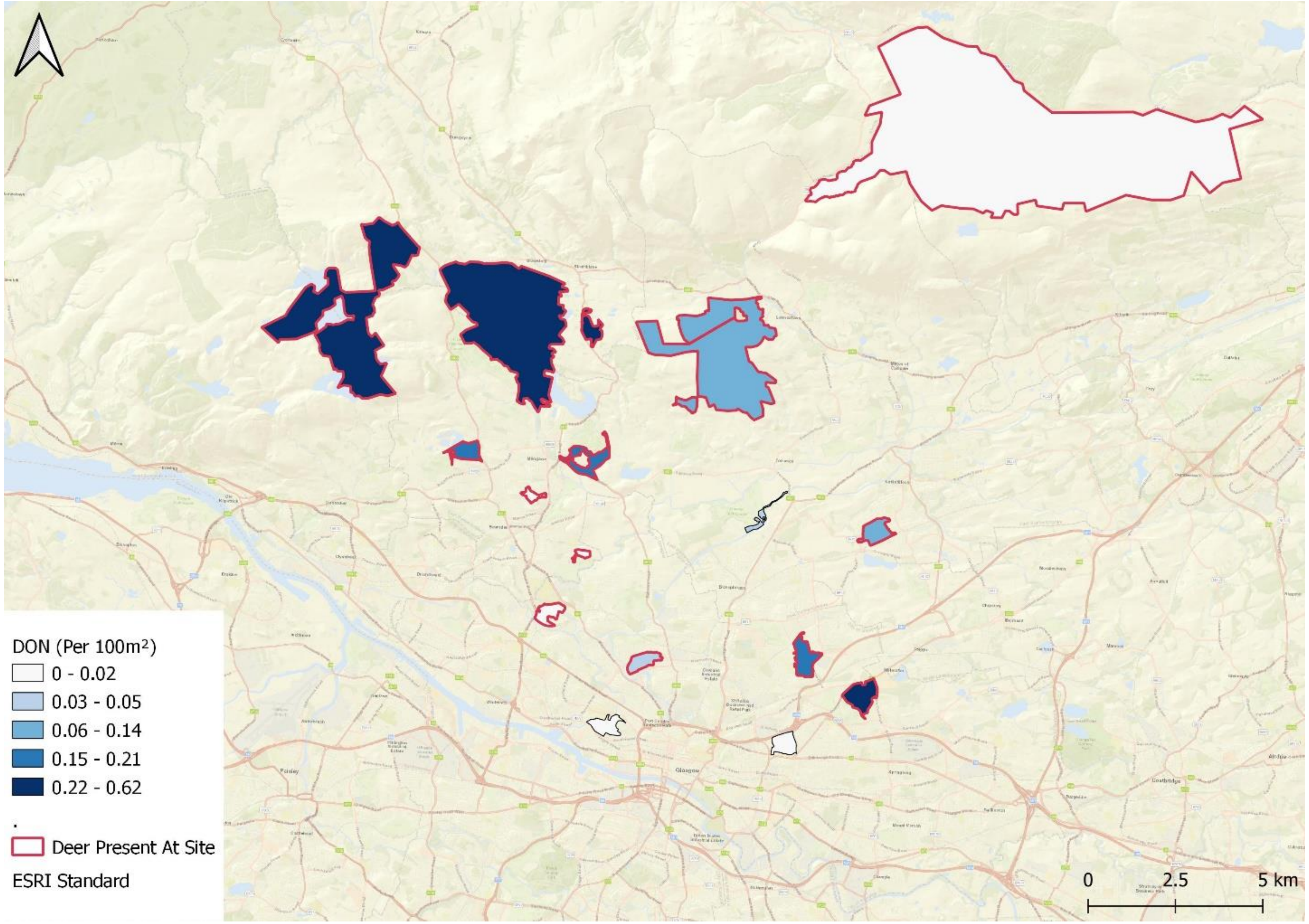


Figure 11: Density of nymphs per 100m^2 and deer presence within Glasgow survey sites.

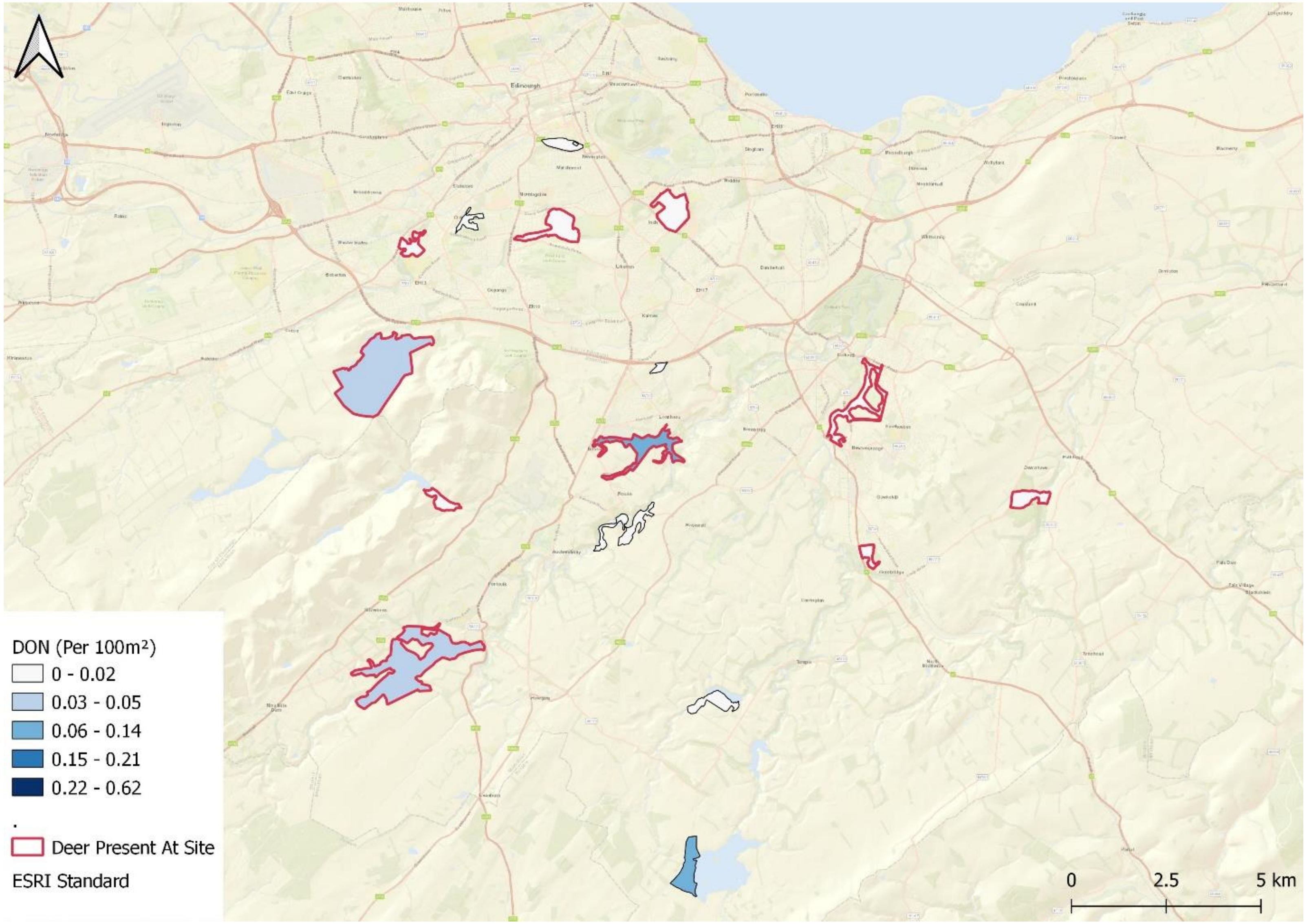


Figure 12: Density of nymphs per 100m^2 and deer presence within Edinburgh survey sites.

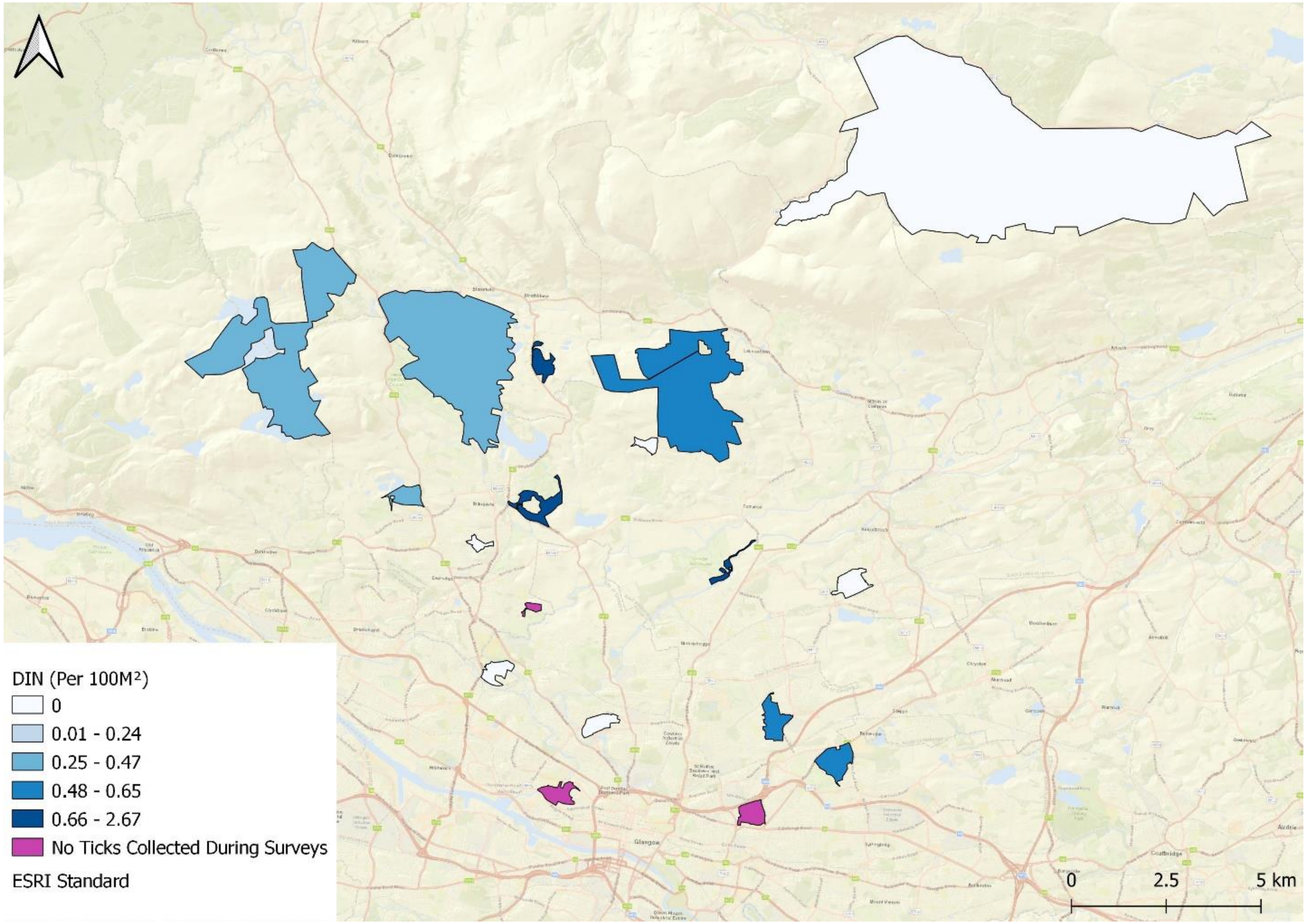


Figure 13: Density of infected nymphs per 100m² within Glasgow survey sites.

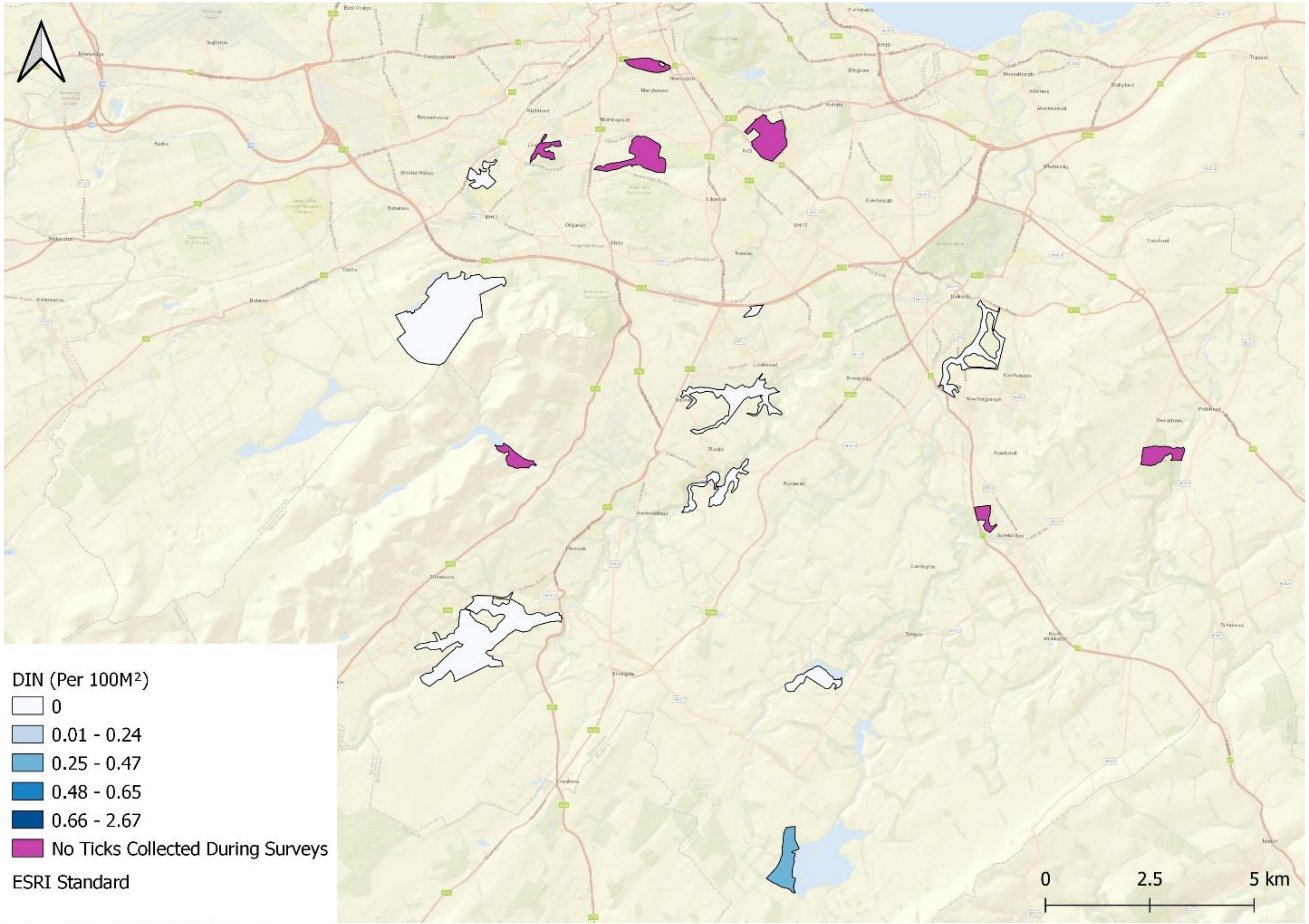


Figure 14: Density of infected nymphs per 100m^2 within Edinburgh survey sites.

4.1.2 Tick Presence

A Chi-squared test confirmed that DON was significantly higher in 2023 than 2022 ($X^2 = 71.438$, df = 17, $p < 0.001$) in both cities (Figure 15). DON was also significantly greater in Glasgow than Edinburgh ($X^2 = 137.74$, df = 17, $p < 0.001$), with the mean DON for Glasgow at 15.62 (95% CI 13.17, 18.38) per 100 m^2 compared to 1.83 (95% CI 1.46, 2.27) per 100 m^2 (Figure 15) for Edinburgh.

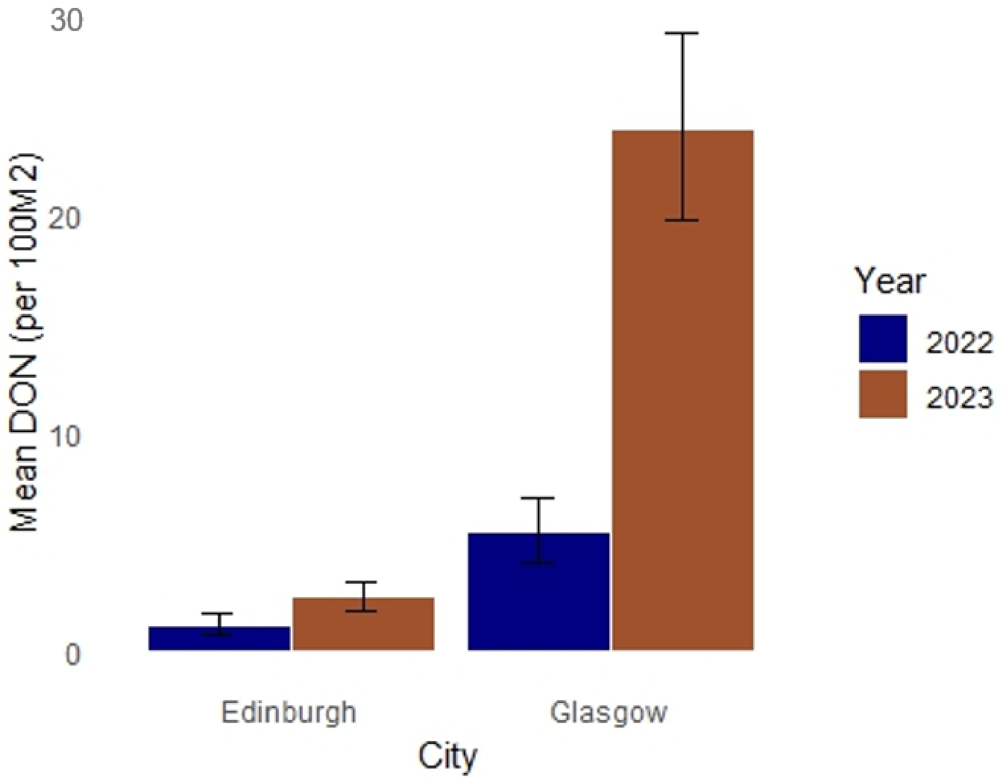


Figure 15: Mean density of nymphs per 100m^2 across the different cities and years, with error bars representing 95% confidence intervals.

Chi-squared tests confirmed similar trends for DON and DIN. The mean DIN differed significantly between 2022 and 2023 ($X^2 = 184.4$, df = 13, $p < 0.001$), and was again higher in 2023 (0.52 per 100 m^2, 95% CI 0.43, 0.61), compared to just 0.12 (95% CI 0.10, 0.16) per 100 m^2 in 2022 (Figure 16). As in GLMM 1, DIN was significantly higher in Glasgow than Edinburgh ($X^2 = 138.12$, df = 13, $p < 0.001$).

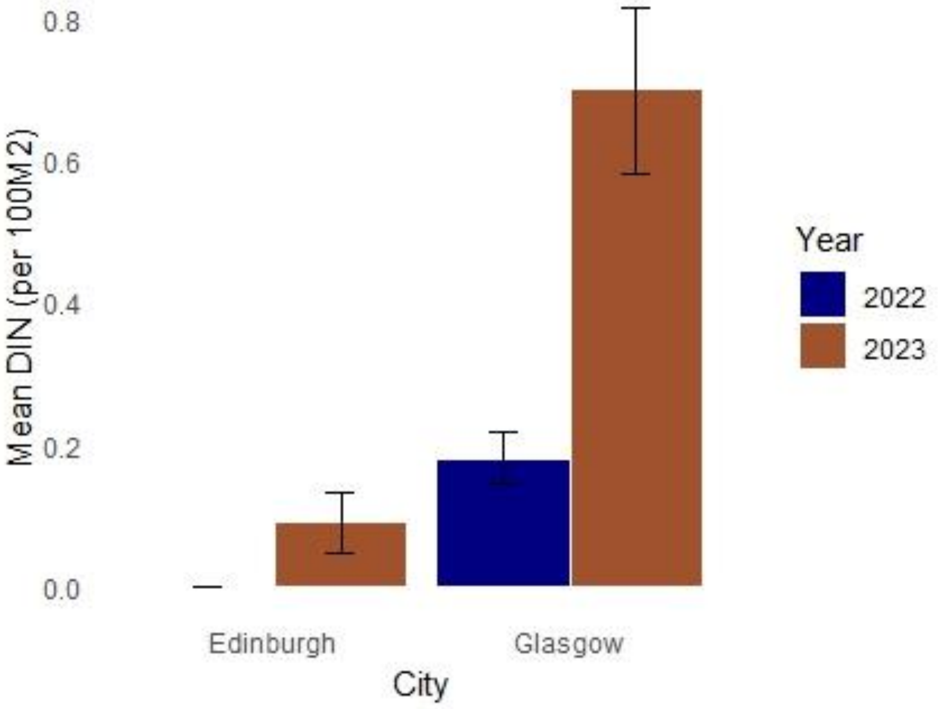


Figure 16: Mean density of infected nymphs per 100m^2 across the different cities and years, with error bars representing 95% confidence intervals.

4.1.3 Deer Presence and Tick Abundance

Evidence of deer presence was also found to be significantly different (based on Chi-squared Test) between cities ($X^2 = 146.3$, df = 1, $p < 0.001$). Deer signs were recorded by surveyors or from NBN data at least once in 62.5% of sites in Edinburgh and 83.3% of sites in Glasgow.

Deer presence was found to be correlated with both DON and DIN. DON was found to be greater in a greenspace when deer signs were recorded present than when deer signs were absent ($X^2 = 86.253$, df = 17, $p < 0.001$) (Figure 17). DIN values showed the same trend, and DIN was higher in a greenspace when deer were present at a site according to the Wilcox test ($X^2 = 174.74$, df = 13, $p < 0.001$) (Figure 18).

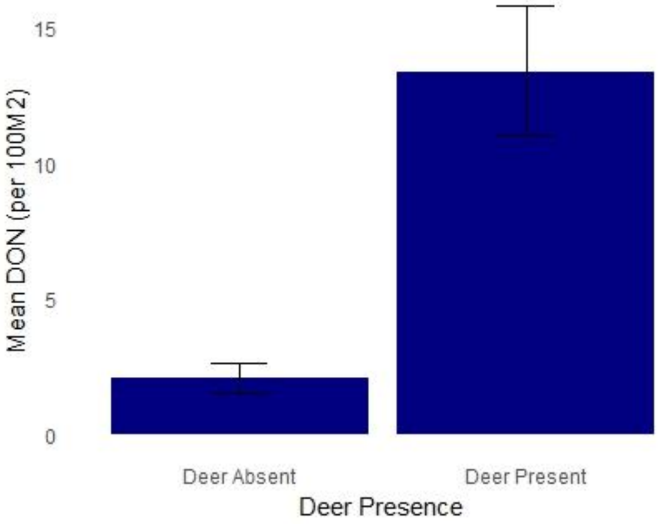


Figure 17: Mean density of nymphs per 100m^2 when deer are present vs absent in an urban greenspace, with error bars representing 95% confidence intervals.

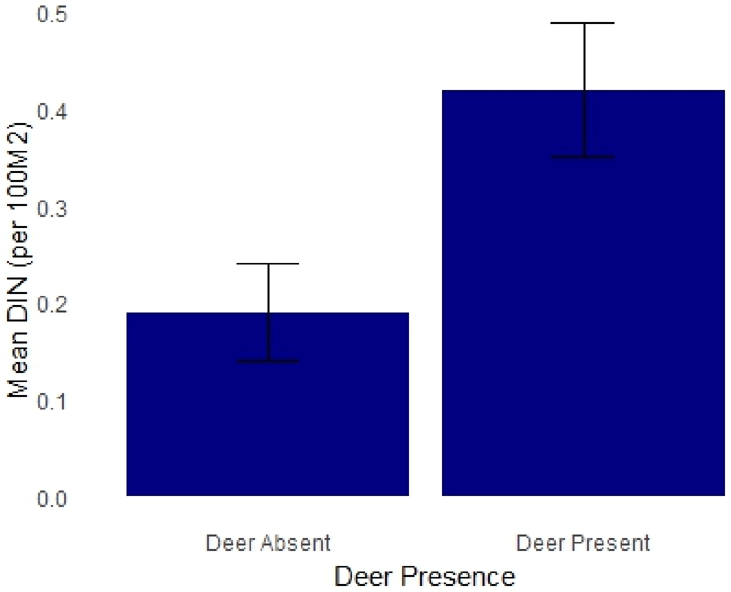


Figure 18: Mean density of infected nymphs per 100m^2 when deer are present vs absent in an urban greenspace, with error bars representing 95% confidence intervals.

4.1.4 Greenspace Characteristics and Tick Abundance

4.1.4.1 Density of Nymphs

The results from GLMM 1 suggest that the connectivity between a greenspace to the nearest woodland of ≥10 ha (the inverse of path cost) is the characteristic which most significantly affects the DON within an urban greenspace (Table 8). Path cost was significantly negatively correlated with DON (Figure 19).

While there was a slight increase in the predicted DON as greenspace area increases, the results of the GLMM revealed no significant correlation between the area of a greenspace and the DON within the greenspace (Table 8). The correlation value between observations and predictions was 0.463 per m2, and the root mean square error was 21.41 suggesting moderate positive correlation. The month was the largest random effect in GLMM 1 (Table 9), but other random effects did not show strong significance.

The land cover proportions within and surrounding (1km buffer area) urban greenspaces was found to have varying effects on the DON within greenspaces (Table 8). The proportion of woodland cover was significantly positively correlated with the DON (Figure 20). The proportion of built-up area was negatively correlated with DON; however, this correlation was not quite significant (P = 0.08) (Figure 20). The proportion of grassland habitats had a negligible effect on DON.

Table 8: GLMM 1 outputs for correlations between density of nymphs per 100m^2 and path cost, site area, and proportions of woodland, improved grassland, semi-natural grassland, and built-up area within 1km of a site.

	Estimate	**Std. Error**	**Z value**	**P value**
Intercept	0.981	0.561	1.749	0.080
Path Cost	-0.509	0.191	-2.669	0.008
Site Area	0.024	0.108	0.224	0.822
Woodland Cover Proportion within 1km	0.378	0.145	2.526	0.012
Improved Grassland Proportion within 1km	-0.008	0.149	-0.057	0.955
Semi-natural Grassland Proportion within 1km	-0.293	0.184	-1.591	0.112
Built-up Area Cover Proportion within 1km	-0.364	0.213	-1.710	0.087

Table 9: GLMM 1 table of random effect standard deviations.

Effect	**Standard Deviation**
City	0.270
Month	1.025
Year	0.000

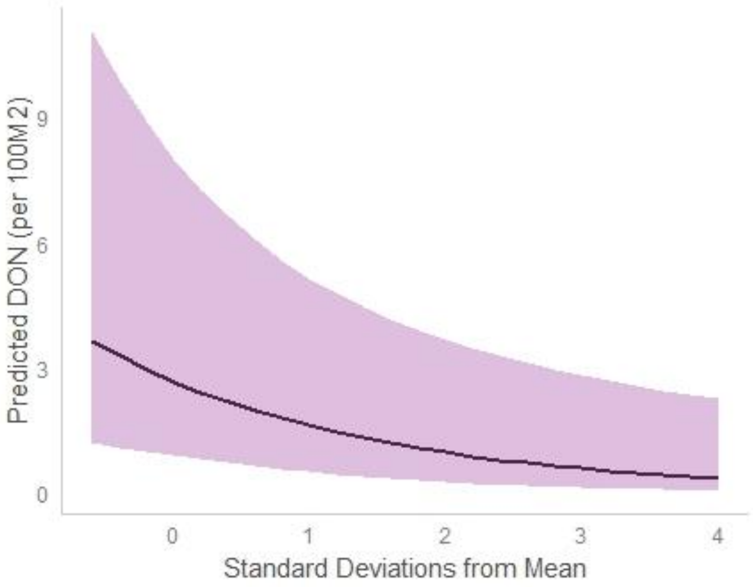
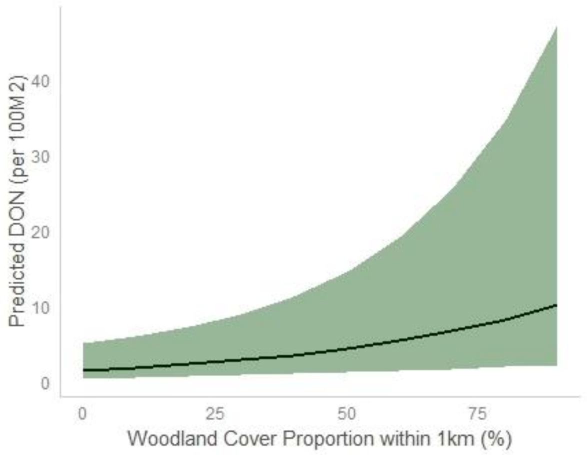


Figure 19: Predicted density of nymphs per 100m^2 in urban greenspaces in relation to standard deviations from mean path cost, with 95% confidence interval (shaded).



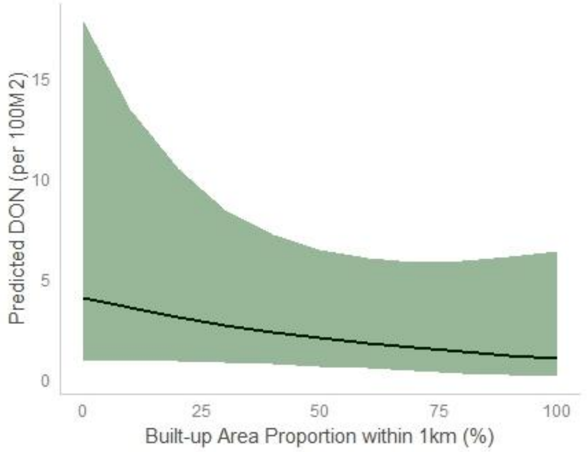


Figure 20: Predicted density of nymphs per $100m^2$ in urban greenspaces in relation to the proportion of different land covers within a 1km buffer, with 95% confidence interval (shaded).

4.1.4.2 Density of *B. burgdorferi*-Infected Nymphs

The results from GLMM 2 suggest that increasing connectivity between a greenspace to the nearest woodland of ≥10ha is associated with a significant increase in DIN (Table 10, Figure 21), similar to the relationship between DON and path cost (Figure 19).

Site area was found to correlate positively with DIN (Figure 22), which was also suggested for DON. This correlation is small but highly significant for DIN (Table 10).

The proportions of different land cover types within and surrounding urban greenspaces was also revealed to be significantly correlated with DIN (Table 10, Figure 23). Unlike DON, DIN is significantly negatively correlated with woodland cover proportion. The proportion of semi-natural grassland and built-up area within 1km also had a significant negative correlation with DIN in a greenspace. The opposite relationship was found for improved grassland proportion, however, as there was a significant positive correlation between the proportion of improved grassland and DIN. The correlation value between observations and predictions is 0.441, and the root mean square error was 19.736 per m2. These values suggests that despite accounting for zero-inflation, this model does not fit as well as GLMM 1. However, the random effects of year and city were moderately significant (Table 11).

Table 10: GLMM 2 outputs for correlations between density of infected nymphs per 1km^2 and path cost, site area, and proportions of woodland, improved grassland, semi-natural grassland, and built-up area within 1km of a site as log odds ratios.

	Estimate	**Std. Error**	**Z value**	**P value**
Intercept	7.085	0.275	25.748	p<0.001
Path Cost	-0.000	0.000	-7.933	p<0.001
Site Area	0.004	0.001	6.268	p<0.001
Woodland Cover Proportion within 1km	-0.010	0.004	-2.455	0.01409
Improved Grassland Proportion within 1km	0.022	0.005	4.118	p<0.001
Semi-natural Grassland Proportion within 1km	-0.032	0.008	-4.031	p<0.001
Built-up Area Cover Proportion within 1km	-0.014	0.004	-3.673	p<0.001

Table 11: GLMM 1 table of random effect standard deviations.

Effect	**Standard Deviation**
City	0.892
Year	0.777

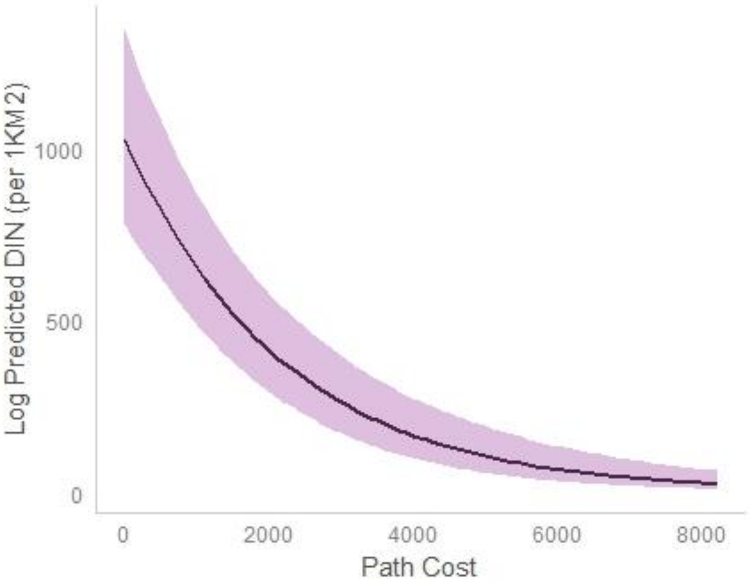


Figure 21: Log predicted density of infected nymphs per 1km^2 in urban greenspaces in relation to standard deviations from mean cost path (path cost has been scaled), with 95% confidence interval (shaded).

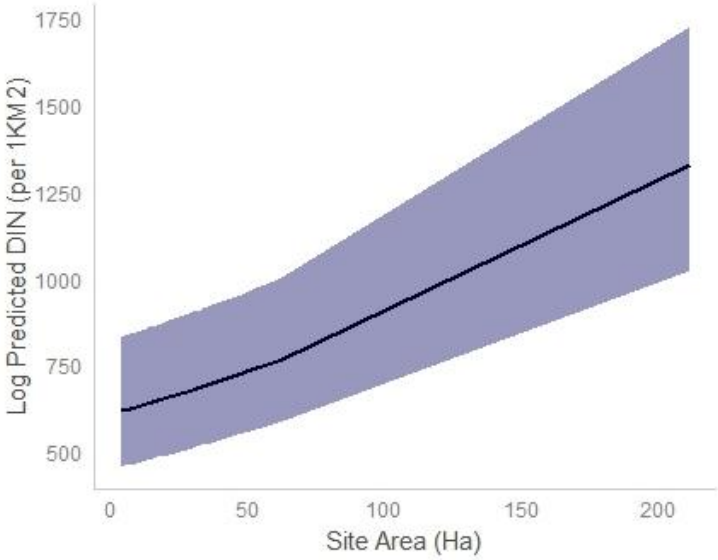


Figure 22: Log predicted density of infected nymphs per 1km^2 in urban greenspaces in relation to the greenspace area, with 95% confidence interval (shaded).

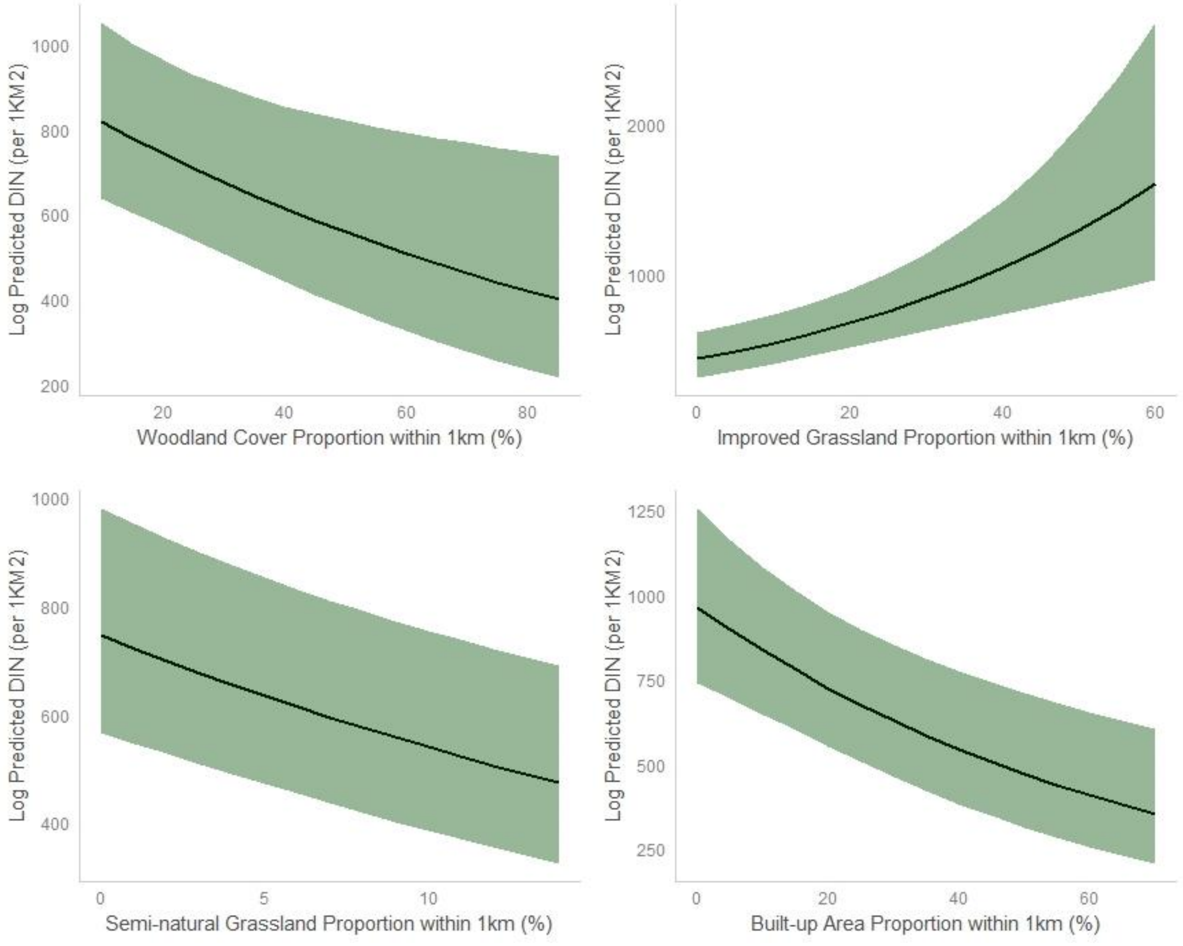


Figure 23: Log predicted density of infected nymphs per 1km^2 in urban greenspaces in relation to the proportion of different land covers within a 1km buffer, with 95% confidence interval (shaded).

4.2 Agent-based Modelling

4.2.1 Connectivity and Site Area

4.2.1.1 Number of Tick Bites

In contrast to DON and DIN, the results from GLMM 3 suggest that as connectivity increases, the number of tick bites within a greenspace decreases (Table 12, Figure 24). However, tick bites were only observed when the connectivity decreased below a certain threshold, and the number of tick bites rapidly increased beyond this.

Increasing the area of urban greenspaces was found to significantly decrease the number of tick bites (Table 12, Figure 25), also different to the findings for DON and DIN. A drop off was also observed in the graph, and no tick bites were observed in greenspaces greater than 4ha.

Table 12: GLMM 3 outputs for correlations between number of tick bites and the path cost or site area.

	Estimate	**Std. Error**	**Z value**	**P value**
Intercept	-8.987	0.827	-10.862	p<0.001
Path Cost	0.000	0.000	13.897	p<0.001
Site Area	-1.343	0.170	-8.001	p<0.001

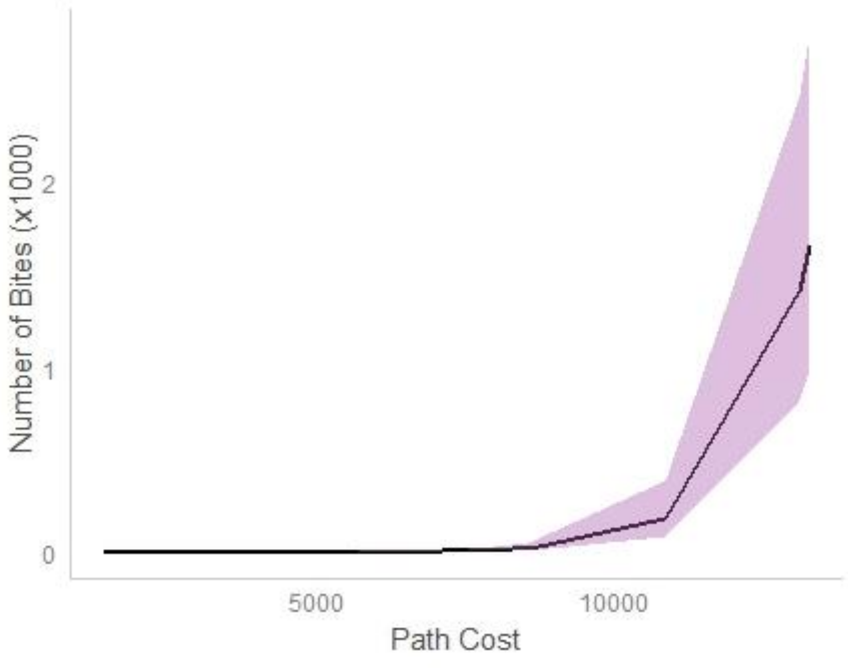


Figure 24: Predicted number of tick bites (X1000) in urban greenspaces in relation to cost path, with 95% confidence interval (shaded).

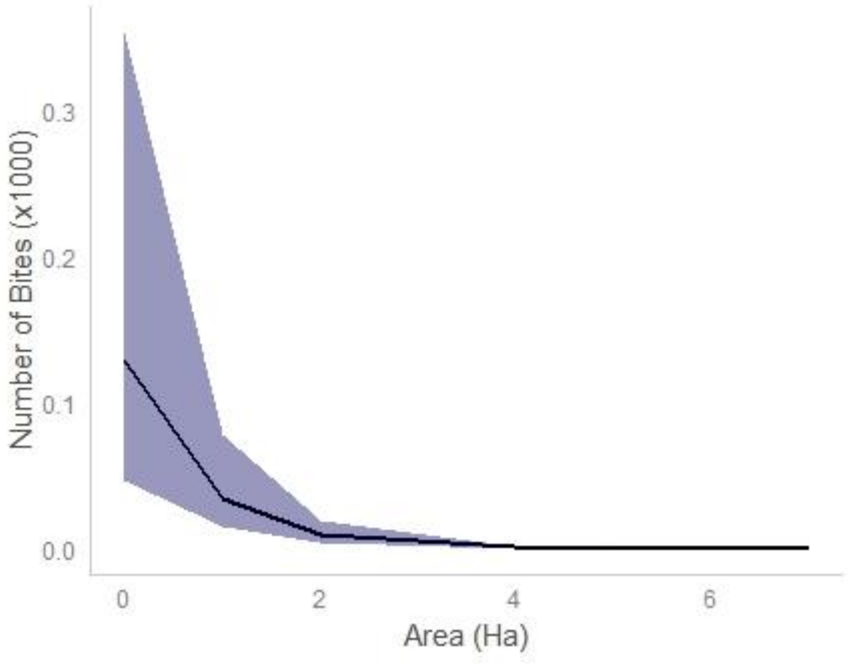


Figure 25: Predicted number of tick bites (X1000) in urban greenspaces in relation to site area, with 95% confidence interval (shaded).

4.2.1.2 Number of *B. burgdorferi*-Infected Bites

The results from GLMM 4 show a similar association between greenspace connectivity and the number of *B. burgdorferi*-infected tick bites as the total number of tick bites (Table 13), suggesting that an increase in connectivity leads to a decrease in the number of bites by *B. burgorferi sl.* Infected ticks. The graph also showed a similar trend, with slightly different thresholds (Figure 26).

The number of infected bites was found to decrease as site area increased, with infections dropping off beyond 4ha, similar to the finding for total number of bites. However, for *B. burgdorferi* infections this wasn't significant (Table 13).

Table 13: GLMM 4 outputs for correlations between number of Borrelia infected tick bites and the path cost or site area.

	Estimate	**Std. Error**	**Z value**	**P value**
Intercept	-17.800	2.301	-7.738	p<0.001
Path Cost	0.001	0.000	8.685	p<0.001
Site Area	-0.487	0.311	-1.565	0.118

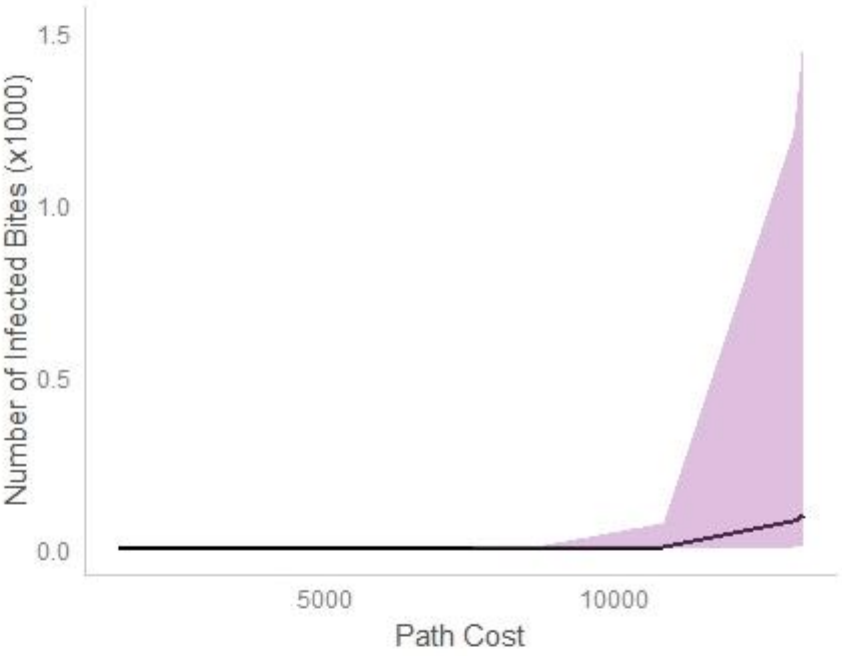


Figure 26: Predicted number of B. burgdorferi-infected tick bites (X1000) in urban greenspaces in relation to cost path, with 95% confidence interval (shaded).

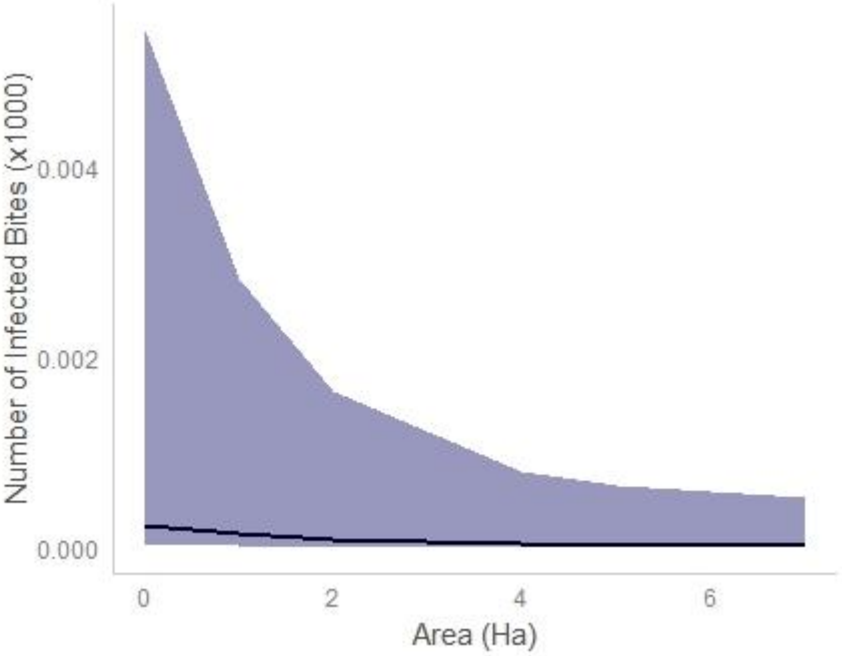


Figure 27: Predicted number of B. burgdorferi-infected tick bites (X1000) in urban greenspaces in relation to site area, with 95% confidence interval (shaded).

4.2.2 Single Large or Several Small

Single large and several small greenspace configurations showed varying effects on the model outputs. The mean number of tick bites was found to be significantly greater in the several small configurations than the single large greenspace (W = 3798414, p-value < 2.2e-16) (Figure 28). In contrast, the mean number of *B. burgdorferi*-infected tick bites was significantly greater in the single large greenspace than the several small greenspaces (W = 4078242, p-value = 5.165e-10) (Figure 29), suggesting that infection prevalence is higher within a single large greenspace.

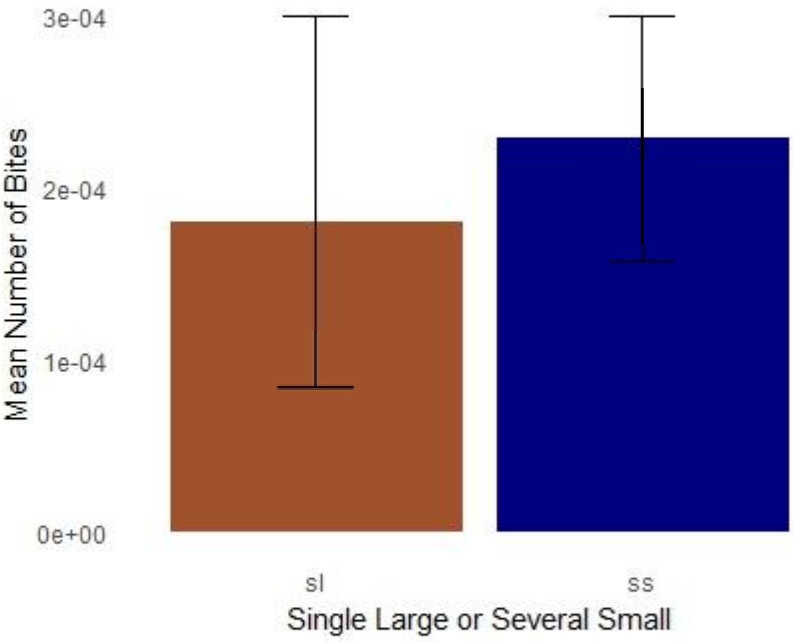


Figure 28: Mean number of tick bites in a greenspace according to single large or several small configuration, with 95% confidence intervals.

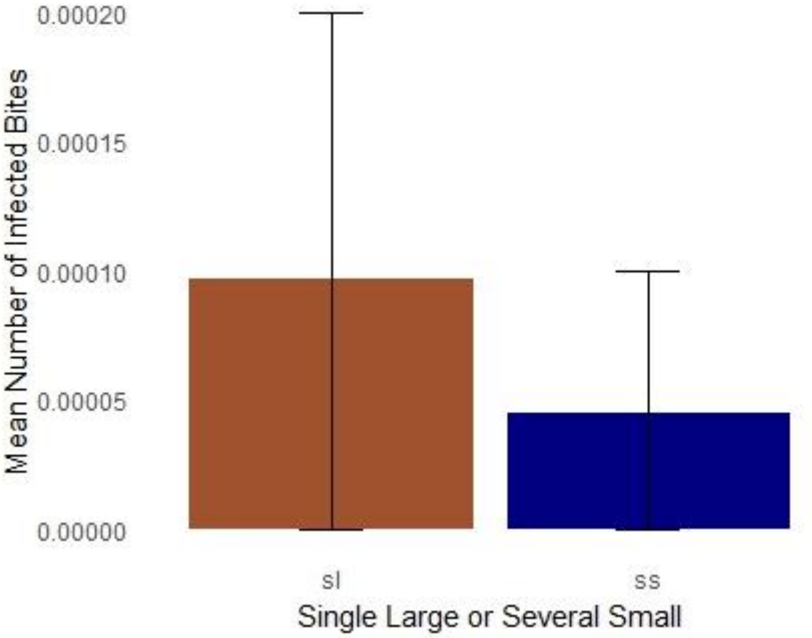


Figure 29: Mean number of B. burgdorferi-infected tick bites in a greenspace according to single large or several small configuration, with 95% confidence intervals.

Chapter 5 Discussion

5.1 Introduction

Understanding how the characteristics of urban greenspaces can contribute to an increase in biodiversity while minimising the associated EDS is an important consideration for urban GI planning (Dunn, 2010). Literature suggests that connectivity, patch size, and habitat are key characteristics associated with the density of ticks and infection prevalence in urban greenspaces, however there are currently few studies linking these factors to tick densities and disease prevalence in an urban context, especially within the UK. This research was conducted to understand how the configuration, connectivity, area, and land cover of urban greenspace could influence the population densities of ticks and the associated hazard of *B. burgdorferi* s.l. infection. To investigate this, urban greenspaces across Glasgow and Edinburgh were surveyed for tick and deer presence. The connectivity, area, and land cover proportions were calculated for each site, and analysed to explore the correlation between these urban greenspace characteristics and the densities of ticks and *B. burgdorferi* s.l. infected ticks within the greenspace. The influence of greenspace configuration, connectivity and area was also explored using an ABM. The number of bites, and number of infections were compared between two scenarios of future greenspace in Glasgow were compared (land sparing vs land sharing) and their relationship between configuration, connectivity and area compared.

5.2 Field Survey Data

5.2.1 Tick Presence

Overall, 946 *I. ricinus* ticks were collected through surveys during Spring and Summer 2022 and 2023, across Glasgow and Edinburgh. The analysis focuses on nymphs specifically, as they are responsible for the majority of human tick bites. Nymphal ticks are small in size, so are less likely to be noticed and removed, and are more abundant in the environment (Robertson, Gray, and Stewart, 2000). The high abundance of nymphal ticks is demonstrated by the fact that 91% (861/946) of ticks collected in this study were nymphs. Other studies across Europe have also found high proportions of nymphs collected in blanket dragging surveys (64.5% and 88.3%) (Hansford et al., 2022; Hansford et al., 2023b).

The mean DON was 8.83 (95% CI 7.40 - 10.26) per 100m^2, which is slightly lower than the average across urban green spaces in Europe for *I. ricinus* nymphs, which is estimated to be around 12.2 (range; 0 – 159.5) per 100m^2 (Hansford et al., 2022). DON can vary depending on the type and characteristics of greenspace sites included within different studies, as some studies may only select sites where tick populations are known to exist, whereas this study included sites where no ticks were found. Even the range of DON values from studies within urban greenspaces in the UK vary greatly from 1.6 – 27.1 per 100m^2 (Greenfield, 2011; Hansford et al., 2017; Hansford et al., 2023a; Hansford et al., 2023b; Hansford et al., 2021; Nelson et al., 2015). While there is currently no data for DON in urban greenspaces in Scotland specifically, a study in rural woodland in Scotland previously found a mean DON value of 36.5 per 100m^2 (Millins et al., 2016). This is much greater than the values calculated in this study for urban greenspaces, but rural habitat generally includes large contiguous patches of habitat, ideal for ticks and their hosts. The mean infection prevalence of nymphs infected with *B. burgdorferi* in this study was 2.81% (95% CI 2.29 - 3.32), with a mean DIN value of 0.36 (95% CI 0.3 - 0.41) per 100m^2. This is also lower than the averages for urban greenspaces in Europe, which are 14.2% (range; 0.5%–86.7%) and 1.7 (range; 0–5.6) per 100m^2 respectively (Hansford et al., 2022). DIN also varies greatly between other studies; one study of urban greenspaces in Southampton and Bath found 2.73 infected nymphs per 100 m^2, yet another study of Southampton, Bath, and Bristol found DIN was only 0.03 per 100m^2. There are no existing figures for DIN in urban greenspaces within Scottish cities currently, however studies within rural Scotland by Millins et al. (2016) and Millins et al. (2018) found NIP values of 1.7% and 2.5% respectively. Despite the lower-than-average DON and DIN values in this study, there is still a risk of *B. burgdorferi* s.l. transmission to humans. Human risk is a combination of factors, including the hazard (DON and DIN), but also exposure factors (e.g., how long visitors spend in a greenspace and whether they stick to a defined, open path where they are less exposed). The risk is also determined by vulnerability factors such as tick awareness (e.g., whether people regularly check for ticks, or wear long clothing to protect them from bites). This means that the risk may be similar in a greenspace with a low DIN and lots of visitors to a greenspace with few visitors and a high DIN. The risk can also be increased if tick awareness is low, as people may not take precautions to prevent tick bites and infections. In Scotland, 60% of the population visited a greenspace at least once a week during 2019, rising to 77% during the COVID-19 pandemic (Public Health Scotland, 2022). If tick awareness is low, this population may be exposed regularly, and therefore at higher risk of tick bites and *B. burgdorferi* s.l. infections, even if the hazard (DIN) is low.

In this study, there were significantly more ticks and infected ticks collected in 2023 compared to 2022, which highlights the importance of gathering data over multiple years. Heylen et al. (2019) also found that tick densities varied significantly between survey years, however other studies have found the variation across years to be insignificant (Hansford et al., 2017; Millins et al., 2018). This difference could depend on the extent of variation in environmental & climatic factors and host populations between years. There were also significantly more ticks and *B. burgdorferi* s.l. infected nymphs in Glasgow compared to Edinburgh. Other studies which included multiple cities in the UK found conflicting results on whether DON and infection prevalence differs between different cities (Hansford et al., 2023a; Hansford et al., 2023b).

The method of tick surveying used in this study is well used and accepted, however there are limitations to the data collected. At each site, the total blanket dragging area was 150m^2. Other studies have sampled greater areas of each site (Allan, Keesing, and Ostfeld, 2003; Brownstein et al., 2005; James et al., 2014; Mathews-Martin et al., 2020; Rosà et al., 2018) which is more accurate when calculating DON values. However, this requires more time and labour, which was infeasible within the wider project this research was conducted in, which carried out tick sampling at many sites. Though some studies surveyed over larger areas, others have carried out sampling of only 100m^2 (Ehrmann et al., 2018; Hansford et al., 2017; Hansford et al., 2023b), suggesting that 150m^2 is still a reasonable area to cover. The number of ticks and nymphs collected for *B. burgdorferi* s.l. testing was also lower than is suggested (a minimum of 75 ticks (Daniels et al., 1996) or 55 nymphs (Zolnik et al., 2015)) for an accurate representation of *B. burgdorferi* prevalence. However, time constraints in this project also meant it was difficult to collect 50 nymphs from some sites, particularly where ticks were less abundant, and for some highly urban sites there are very few ticks, so it was not possible to collect higher numbers. Since this is the first study of urban greenspaces in Scotland there are no literature values for comparison, but these results are the first to demonstrate the importance of education on tick awareness and Lyme disease in and around Glasgow.

5.2.2 Deer Presence

This study is the first to explore the relationship between deer, tick densities and disease prevalence in an urban setting using host survey methods, rather than secondary data on deer observations. These associations have been recognised in previous studies, but are often not well quantified (Rand et al., 2003). The results show that DON is correlated with deer presence; DON is significantly higher in sites where deer are present. The importance of deer in the maintenance of tick populations is well reported (Rizzoli et al., 2014), as they provide vital bloodmeals for adult female ticks before they lay eggs (Gilbert et al., 2012). Deer are particularly important in the UK, where there are few other large wildlife species, and roe deer are particularly well adapted to living within urban areas, due to their abundance, behavioural plasticity, and small size (Ciach and Fröhlich, 2019). They can move between greenspaces whereas smaller hosts would not disperse as far. Roe deer are a common species in the UK, and they are known to have high tick burdens (Kiffner et al., 2010; Vázquez et al., 2011; Vor et al., 2010). In this study, it was found that there were a greater number of sites where deer signs were present in Glasgow compared to Edinburgh, which could be related to the higher tick densities in Glasgow sites. Glasgow has a higher-than-average canopy cover (18%) (Glasgow City Council, 2024) and is a short distance from the Loch Lomond and the Trossachs National Park in the highlands, which may explain the high deer populations present. Many other studies have suggested that areas with more deer also generally have higher densities of ticks (Gilbert et al., 2012; Kilpatrick, Labonte, and Stafford, 2014b; Ruiz-Fons and Gilbert, 2010). A study in rural Scotland in 2007 and 2008 also found an increased nymph abundance where deer were present (James et al., 2014). These studies imply that understanding and studying deer movements and ecology around urban areas is important for understanding the hazard of tick presence, demonstrating that they are important drivers of tick densities in these settings.

DIN also demonstrated the same relationship with deer presence as DON, suggesting that deer presence can also increase the prevalence of *B. burgdorferi*, which has been reported in previous studies (James et al., 2014). Even though deer are not competent *B. burgdorferi* s.l. hosts, they may still contribute to an increase in DIN because of the higher tick densities associated with deer presence, cancelling out any pathogen dilution effects (Gandy et al., 2022). However, other studies have found conflicting views on the effect of deer presence on DIN. Millins et al. (2016) and James et al. (2013) also carried out studies in rural areas of Scotland and found no significant association between the two (James et al., 2013; Millins et al., 2016). None of these studies were in urban areas though where host densities and movement may be different to rural areas.

The quantification of the relationship between tick densities and deer prevalence beyond absence/ presence can be difficult. It is difficult to specify whether deer signs or sightings are related to the same individual, unless many are physically seen at one time. NBN data may also be biased to where there are more people visiting to observe deer; hence presence vs absence was selected in my study. False positives were unlikely as all field surveyors were given guidance in identifying deer tracks and dung, and only verified NBN data was included. False negatives may be more likely if deer densities are low at a site, or if a site is large and deer can spread out more, so deer were marked as present if there were deer signs in at least one field survey, and/or if deer observation points were present on the NBN dataset. Furthermore, deer dung is difficult to see in areas where the vegetation was tall and dense so some may be missed. Deer dung surveys are still a useful method for providing data on deer presence at a site (Fernanda et al., 2001) in the months prior to surveys- this is emphasised by the fact that all sites which did not have deer signs noted in surveys also had no observation points from the NBN data.

5.2.3 Density of Nymphs

5.2.3.1 Connectivity of Urban Greenspaces

The results from this study found that increasing connectivity between urban greenspaces significantly increases DON within the greenspace. Increasing path cost is the inverse of increasing connectivity. The majority of previous studies which have looked at how connectivity affects DON in urban greenspaces have also found this correlation (Estrada-Peña, 2002; Hansford et al., 2023a; Heylen et al., 2019; VanAcker et al., 2019). More connected areas are better suited for tick host movement, especially larger hosts such as roe deer which can move over large distances through their home ranges (Zolnik et al., 2015). Smaller mammalian and avian hosts may still be present in isolated greenspaces, however without larger hosts, such as deer, to sustain adult tick populations, the tick population cannot be sustained in these areas (Heylen et al., 2019). Estrada Pena et a.l (2002) and VanAcker et al. (2019) also suggested that connectivity has a greater effect on DON than environmental factors such as land cover. My data also suggest that connectivity had a greater effect on DON than land cover proportions. Even if a patch has optimal habitat available for deer (and favourable tick habitat), if the patch is too isolated and the path cost is too high deer will not likely travel to the area, hence ticks will only be transported there by other smaller hosts such as birds. Estrada Pena et al. (2002) also suggested that the removal of individual highly connected patches with high tick and host densities can greatly reduce the densities of ticks in surrounding areas. These results are important to note, as many GI projects are working towards better connected habitat areas for improving biodiversity, but the possible introduction of greater disservices such as tick populations needs to be considered too, with the right balance to minimise them while retaining ecosystem resilience (Lyytimäki, 2015) and encouraging environmentally sustainable planning policies (Schaubroeck, 2017).

Brownstein et al. (2005) found conflicting results, suggesting that increasing connectivity reduced DON. However, it was not specified that the greenspaces in this study were in urban areas, so it is possible there is a variation when studying only rural greenspace. They also used minimum distance to patch edge as a connectivity metric, which is less accurate than other methods (Hansford et al., 2023a). My study used least cost methods which is recognised as an appropriate, accurate method for creating connectivity metrics (Estrada-Peña, 2002; Heylen et al., 2019). This is more accurate than closest distance to woodland metrics used by Brownstein et al. (2005) and Hansford et al. (2023), as connectivity for host movements can depend on the matrix habitat characteristics between two patches, which isn't captured by distance alone.

While path cost is a more accurate measure of connectivity than closest distance to woodland, there are still limitations to this metric. My study assumed that all woodlands over 10 ha could be a deer population source, a metric also used by Heylen et al. (2019), and so path cost was calculated between the survey sites and any woodland of at least 10 ha. In reality this may not be the case, as 10 ha is not a definitive area of woodland that roe deer would use or travel through. Furthermore, the path cost was calculated from each site, however if there were sites where there was less than 10 ha of woodland present, deer may not travel there regardless of the connectivity value. One alternative to this would be to look at connectivity on a town/ city level rather than at site level.

5.2.3.2 Urban Greenspace Site Area

No significant difference in DON as patch area increases (fragmentation decreases) was found. This lack of difference suggests that connectivity and other environmental factors, such as land cover proportions, may be more important to consider for urban planning to mitigate ecosystem disservices, and that the creation of large greenspaces may not necessarily put people at greater risk of tick bites, as this may depend on other factors in the greenspace, such as connectivity and land cover Though in this study no relationship between patch area and DON was found, other studies have found that fragmentation does significantly affect DON. For example, Li et al. (2012) found that DON decreased with increasing fragmentation and suggested that this was related to deer presence. The authors state that due to their size, deer prefer larger habitat patches, and as a key driver of tick population, this preference could explain the observed relationship between patch size and tick density (Li et al., 2012). Allan, Keesing, and Ostfeld (2003) found increasing fragmentation increases nymph density, but that the opposite was true for adult ticks. Similarly, Simon et al. (2014) also found greater DON in fragmented patches where white-footed mice (*Peromyscus leucopus*) were also the key hosts in their study area. White-footed mice were a prominent host for nymphs and larvae in their study area due to their small size and being a generalist species, which means they only require small habitat patches and can outcompete other hosts (Allan, Keesing, and Ostfeld, 2003). On the other hand, their results suggest that in larger patches where deer are present, more adult ticks are present because adult ticks often feed on larger hosts, which supports the work of Li et al. (2012). The studies demonstrate that fragmentation can affect different hosts in different ways and may explain why there was not a significant relationship in this study. It is likely that both small and large hosts were present in some areas surveyed, but smaller hosts were not surveyed so further work would be needed to understand whether small mammals are affected by these metrics. Brownstein et al. (2005) suggested fragmented patches may benefit deer due to positive edge effects, and therefore increase tick density. This demonstrates that edge effects can complicate the relationship between fragmentation and tick host populations. Brownstein et al. (2005) did not study at the patch level though, as area was calculated as the average forest area within a buffer, and the area was also not specifically urban. Edge effects may be more positive for deer in rural patches of woodland, as the greenspace may border grassland, which is favourable grazing habitat, rather than built-up area. In urban areas, however, there is often a smaller ecotone area (Hansford et al 2023) backing onto built up areas, which may be less beneficial to deer and explain these differences in results.

While the area of the sites in this study could be calculated quite accurately through simple methods using satellite data on GIS, where greenspaces were very large or well-connected in the urban periphery the site perimeter was difficult to define. There were also small discrepancies in the boundaries between the ESRI standard and ESRI satellite layer creating challenges in calculating the greenspace areas. Given more time, I could have accounted for this by taking several coordinates from the perimeter while surveying, to match up to the satellite data on QGIS. Furthermore, due to time constraints and access difficulties when sampling, it was difficult to carry out blanket drags in all areas of large study sites. Therefore, while it is assumed that the tick samples collected are representative of tick densities across the site, this may not be entirely accurate. If possible, it would be beneficial to spend more time at each site, carrying out a greater number of drags across all areas of the site to gather a more representative sample. The correlation value and root mean square value suggested a moderate positive correlation; however, they suggest there may be other random effects that were not included here, and there is possibly variability due to different microhabitats and other climate variability such as temperature. This could not be further explored due to time constraints.

5.2.3.3 Land Cover of Urban Greenspaces

The results demonstrate that DON was significantly positively correlated with the proportion of woodland, and negatively correlated with the proportion of built-up areas and grassland proportions, however these negative relationships were not significant. VanAcker et al. (2019) report a similar relationship between the proportion of areas with closed canopy and DON. As in this study, VanAcker et al. (2019) used buffers to capture the land cover proportions within and surrounding urban greenspaces to more accurately understand how land cover relates to deer (as key tick hosts). Using a land cover layer with a 1 m resolution, they found that increasing soil and water proportions in and surrounding each park significantly reduced DON. While this study did not focus on soil and water specifically, a reduction in DON was observed where the proportion of built-up area was greater (even though this finding was not quite significant; p=0.08). Since *I. ricinus* ticks cannot survive for long periods in unvegetated areas due to risk of desiccation (Medlock et al., 2013), built-up areas, areas of bare ground and water are largely non-vegetated areas, so there may be a negative correlation between these habitats and tick densities. Several studies also found that tick densities were higher within closed canopy woodland compared to open ground (Matthews-Martin et al., 2020; Hansford et al., 2023). Although neither study explored the different types of habitats within "open areas", the finding that tick densities are highest where there is woodland is in line with the findings of this study. Woodland is an ideal sheltered habitat for ticks, protecting them from wind and sun which can increase desiccation risk (Berger et al., 2014). Additionally, woodlands can provide shelter and food for many tick hosts, including roe deer (Estrada-Peña, 2002), which are a key host in Scotland and woodland specialists (Lovari, Serrao, and Mori, 2017). These findings suggest that woodland areas within urban greenspaces need to be carefully planned and managed, and it is important to also consider other woodland patches within the surrounding area, not just the patches within the greenspace. Where there are woodland patches used for recreation, it is important that people are tick aware, and stick to paths where vegetation is likely lower and less dense, and ticks are less likely to quest.

Though many studies support the dominance of woodland as a suitable tick habitat, Hornok et al. (2014) found contrasting results; they found that areas of uncut, unmanaged grassland in cemeteries support a higher tick density than woodlands. The authors did highlight however that the woodland in their study was very dense and had sparse low ground vegetation. *Ixodes ricinus* ticks can become desiccated where vegetation is sparse as the humidity is often low (Medlock et al., 2013), which could explain why fewer ticks were found in woodlands. This suggests that mowing grass in urban greenspaces could reduce tick densities. As ground vegetation within woodland was not studied, it is not possible to establish a link between ground vegetation and DON in this study.

In this study, a buffer of 1km radius was selected as this most accurately reflects the home range size of roe deer (Li et al., 2012; Lovari, Serrao, and Mori, 2017), which are the most common deer species in urban greenspaces in Scotland. Other studies have used similar buffers of 800m – 1km around a central point to account for the maximum home ranges of roe deer (Coulon et al., 2006; Hansford et al., 2023a). Although these buffer sizes are created to accurately represent deer home range, they assume that the central point of each site is the central point of the deer home range, which is not necessarily the case. It is important to recognise this when interpreting the results, as it may appear that deer are using sites with certain land cover proportions when they are in fact also using the land covers outside of the buffer.

5.2.4 Density of Infected Nymphs

5.2.4.1 Connectivity of Urban Greenspaces

Like DON, increasing connectivity was found to increase DIN significantly, which was also found by previous studies (Heylen et al., 2019; VanAcker et al., 2019). This is likely due to the fact that DIN is a product of DIN and the prevalence of *B. burgdorferi* s.l. Unlike DON, the results of this study suggest that connectivity has a much smaller association with DIN than other greenspace characteristics, which has also been reported by others (Brownstein et al., 2005; Hansford et al., 2023a). Brownstein et al. (2005) found that DIN decreased as connectivity increased and suggested that if infected ticks and transmission hosts exist within an isolated area and no new uninfected tick populations or hosts enter, then prevalence will increase over time. However, the authors found that that Lyme disease cases in humans increased with increasing connectivity. As discussed above (5.2.3.1), the reason for the potential mixed findings could be due to the less accurate methods used by Brownstein et al. (2005) to calculate connectivity. In their study, Hansford et al. (2023) calculated connectivity as the closest distance to woodland, and their results, like Brownstein et al. (2005), found no significant relationship between connectivity and the DIN figures. Again, this could be affected by host presence, as deer are non-competent *B. burgdorferi* hosts, therefore if deer are the primary host in a greenspace DIN could be lower, compared to greenspaces where there are more reservoir competent hosts.

DIN is also more difficult to study than DON because the values are often quite low in urban greenspaces and variation can be small compared to DON, and so there is a less of a contrast in results for analysis to see key trends according to different characteristics than it is with DON. In this study, low values of DIN created challenges with the GLMM. As there were fewer samples for sites with infected ticks to add to the GLMM, compared to the number of sites and samples in the DON GLMM the data was zero inflated particularly for Edinburgh DIN data. The correlation value for this zero-inflated model suggested a moderate positive correlation, however the root mean square error was very high, indicating the model had a poor fit and limited ability to accurately predict the DIN in a greenspace. This could be related to the skewness of the data, or due to effects of other factors which were not explored. This could not be further explored due to time constraints. To more effectively analyse the relationship between DIN and urban greenspace characteristics, it may be beneficial for future studies to focus on more sites than were surveyed in this study, so that there is more data.

5.2.4.2 Urban Greenspace Site Area

In this study, results showed that increasing fragmentation significantly reduced DIN and though the relationship between DON and area was not significant, the correlation was also positive. As discussed above (5.2.3.2), there are conflicting views on the effect of patch size on deer, but if larger habitat patches are more favourable to deer (as suggested by Allan, Keesing, and Ostfeld (2003)) then there may be fewer deer in more fragmented patches, hence a lower density of ticks and lower density of infected ticks. If this is the case, then the risk of exposure to Lyme disease may be greater in larger, less fragmented greenspaces. It is therefore important to consider greenspace size when planning urban areas. Li et al. (2012) also found a lower Lyme disease hazard in woodlands with increasing fragmentation, when woodland patches were adjacent to grassland. They suggest that, as deer are key reproductive hosts and may venture into grassland for grazing, deer have the potential to introduce ticks into grassland areas. Ticks introduced into grassland areas may become desiccated or be unable to find another host, causing the tick population to decline and DIN to decrease. However, where woodland was adjacent to non-vegetated areas DIN was observed to increase in woodland with increasing fragmentation (Li et al., 2012). This may be due to deer being more likely to stay in woodland if the adjacent habitat is non-vegetated, concentrating ticks in areas where they are more likely to survive and find a suitable host, increasing the density of infected ticks. Though studies suggest a link between DIN and patch size, one study in Scotland suggested that there was no significant relationship between fragmentation and infection prevalence (Millins et al., 2018). However, the sites studied were islands and the *B. burgdorferi* s.l. pathogens were mostly bird-associated genospecies (*B. garinii* and *B. valaisiana*). Patch size is thought to affect large mammalian hosts more than smaller mammals and birds (Allan, Keesing, and Ostfeld, 2003) as their home ranges are larger, which may explain why no significant relationship was found by Millins et al.. Contrasting results were found in Allan, Keesing, and Ostfeld (2003); Their study focused on sites where reservoir competent white-footed mice were the prominent tick host and suggested that the density of mice, which are transmission hosts, is higher in smaller patches. This may differ from my study because the hosts in my sites were not always limited to small mammals and birds, as deer were sometimes present. This suggests that consideration of the different host species within urban greenspaces may be important to understand how smaller, fragmented greenspaces may affect *B. burgdorferi* s.l. hazard or not, because host densities may vary depending on the greenspace area. Brownstein et al. (2005) found that increasing fragmentation increased Lyme disease incidence, which also contrasts what my results suggest, but Brownstein et al. (2005) suggest this could be linked to human behaviour rather than the prevalence of the pathogen, as humans may be less likely to travel for recreation in small woodland patches. Since my study focuses on DIN, I can only comment on the potential exposure risks, which may explain the differences. Furthermore, the actual incidence may be underreported, because there could be missed/ misdiagnosed cases (Stonehouse, Studdiford, and Henry, 2010) which are missed out from incidence data.

5.2.4.3 Land Cover of Urban Greenspaces

This study suggests that DIN decreases as the proportion of woodland cover increases. Such a relationship has not previously been reported, this could suggest that there is another factor which wasn’t controlled for which may be driving the relationship. Indeed, ground vegetation type, height and density, which was not studied in this case, could be affecting these results. Therefore, future studies should include these environmental variables that may affect DON and DIN. The proportion of built-up area was significantly negatively correlated with DIN, suggesting that infection prevalence and the risk of infected tick bites is low in parks close to city centres, where the proportion of surrounding built up area is high.

Though this study suggests increasing the proportion of woodland around a site decreases DIN, other studies report mixed findings. Hornok et al. (2014) found that NIP was higher in woodlands compared to other habitat types, whereas Hansford et al. (2017) reported no significant difference in NIP between woodland and grassland habitats. This failure to find a significant difference may be attributed to a lack of differentiation in grassland type. This study found that increasing both the proportion of woodland and semi-natural grassland reduced DIN, but that the proportion of semi-natural grassland had the greatest negative effect on DIN. It was also found that increasing the proportion of improved grassland increased DIN significantly. This contrasts results from previous studies and could also be related to vegetation type and density which were not explored in this study. Both Hornok et al. (2014) and Hansford et al. (2017) used NIP rather than DIN to explore the relationship between landcover and infection prevalence, which could contribute to the difference in results as NIP is a measure of hazard, but not exposure risk. A later study did find differences between habitat types, however they found infected ticks in woodland and woodland edge habitat, and no infected ticks in open areas (Hansford et al., 2023a). Their findings suggest that greater proportions of woodlands would increase infection prevalence which contrasts the findings of my study. Again, using open areas is less specific than my study which distinguished between different grassland categories and built-up areas which could all otherwise be categorised together as open areas. Hansford et al. (2023) also focused specifically on greenspaces within the urbanised city centre, excluding peri-urban and rural sites within the city periphery which my data included.

5.3 Agent-based Modelling

5.3.1 Connectivity of Urban Greenspaces

There are limited studies which use ABMs to study the effects of habitat connectivity and greenspace size on tick populations and tick-borne disease, but it is recognised that modelling these characteristics may be key to understand and predict the disease risk to urban populations (Li et al., 2019). The results from this study found that increasing greenspace connectivity decreased the number of tick bites within a greenspace, as well as the number of *B. burgdorferi* s.l. infected tick bites. This disagrees with most of the findings of previous studies (Estrada-Peña, 2002; Hansford et al., 2023a; Heylen et al., 2019; VanAcker et al., 2019; Wang, Grant, and Teel, 2012), however most of these studies focussed on the densities of ticks rather than tick bite data, and only one other study has previously used an ABM to study how connectivity affects tick densities specifically (Wang, Grant, and Teel, 2012); Their study used a tick and tick host layer and created a greenbelt around the city of Houston (USA) to assess the effect on tick densities in the greenbelt and surrounding greenspaces. It was demonstrated that the addition of the greenbelt (i.e. an increase in connectivity), increased the density of ticks within neighbouring greenspaces. The authors did suggest, however, that this was dependent on host populations and climate, for which the parameters were likely very different for the Texas ABM compared to the model of Glasgow used in this study. In this study, the ABM focused on one host species, as it has been suggested that using ecological data and generating species specific data can improve model accuracy (Halsey and Miller 2018). Wang, Grant, and Teel's study (2012) did not focus on specific host species in their model, which may explain the difference in results.

One empirical study in Connecticut, USA found results which partially agree with the results of this study (Brownstein et al., 2005). They measured the closest distance to woodland from greenspace patches and found that the tick density and infection prevalence decreased as connectivity increased, suggesting that the number of tick bites and infected tick bites may also decrease with increasing connectivity. They suggest that this is likely because deer populations are higher in suburban areas (often characterised by reduced connectivity), because there is good grazing year-round from ornamental vegetation, and there is no hunting/ predation of deer in these urban areas. Brownstein et al. (2005) did find conflicting results for Lyme disease incidence though, which suggested that the opposite was true. They note that this could be linked more to human behavioural factors but did not explore these factors specifically.

Though there are possible explanations for the results obtained from the ABM, the lack of agreement with previous empirical studies suggest ABMs should be used with caution and may not always be suited to replace empirical studies if resources are available. In addition, this model using tick bite data could not be validated within the time frame available, and validation can be difficult for tick bite data, as people may not report a bite if medical treatment was not required. While area and habitat were controlled in my scenarios, it was difficult to control the connectivity for each new scenario, and connectivity may impact the deer presence and tick population in these theoretical greenspaces. However, the ABM used in this study could be useful as a pilot, as data outputs have not previously been statistically analysed from this model version. Models in future could specifically focus on seasons when ticks are usually more abundant, and scenarios could be developed in areas where tick populations would be expected, based on surrounding land cover and host behaviours. Direct comparisons could not be made between the empirical findings and the ABM outputs in this study, because the model was not run with the same greenspace sites as the sites surveyed, and bite data was analysed rather than tick density data, because the figures for DON and DIN were not being correctly outputted for the modelled greenspaces. Given more time, it would have been possible to further develop the model to generate DON and DIN outputs, and to process additional model runs for the surveyed greenspaces to assess the power of using ABMs in place of empirical studies. Furthermore, the number of ticks and bites output in the model were low overall in the ABM, while this is partially expected due to the low likelihood of humans interacting with ticks in an urban environment, it could have been exacerbated by the model being run over the whole year, and therefore includes winter figures where temperatures were low, bringing averages down. Analysing the model outputs from Spring and Summer months alone may have resolved this challenge. Having access to more data on people's use of greenspaces would also make the modelling more accurate.

5.3.2 Urban Greenspace Site Area

In contrast to connectivity, the outputs from the ABM used in this study agree with the findings for previous studies focused on site area (Allan, Keesing, and Ostfeld, 2003; Brownstein *et al.,* 2005; Simon *et al.*, 2014; Tran and Waller, 2013). The ABM findings suggest that increasing site area (i.e. reducing fragmentation) reduces tick bites, as well as the number of *Borrelia* infected bites. The effect of greenspace area on tick populations has only been studied using an ABM once prior to this study (Tardy *et al.* 2022). The authors found that tick burdens on hosts were higher in smaller greenspaces, suggesting the number of tick bites may be higher in smaller areas (Tardy *et al.*, 2022), which agrees with the findings of this study. Furthermore, in smaller greenspaces, human visitors may be more concentrated, and therefore a tick is more likely to find a host. However, it should be noted that Tardy et al. (2022) focussed specifically on the relationship between site area and tick host burdens on deer, compared to my study which focused on human bites. Humans are more likely to notice and remove ticks than other hosts, so tick burdens would not be appropriate for human studies such as this one.

The results of the ABM are also supported by the results of empirical studies. For example, Allan, Keesing, and Ostfeld's study of white-footed mice in New York (2003) found that the five smallest study patches contained around seven times as many infected nymphs as other larger patches. A study of white-footed mice by Simon et al. (2013) also reported higher tick densities in smaller greenspace sites. The authors suggest that when tick hosts are limited to smaller areas, the density of small mammalian transmission hosts for *B. burgdorferi* s.l. is higher. This means ticks have a higher chance of finding hosts to feed on, and a higher proportion of hosts are infected. In addition to host composition, Li et al. (2012) suggest that adjacent habitat type could impact the effect of greenspace area on tick and tick host populations. Their study found that while DON increased with increasing patch size, DIN showed varying results depending on the surrounding land cover (Li *et al.*, 2012). Their study found that increasing woodland patch size significantly decreased DIN when adjacent to un-vegetated land. While the scenarios in my study were predominantly surrounded by urban (non-vegetated) land, this factor was not controlled specifically and could have impacted the results. There are only two previous studies which focussed on the risk to humans of Lyme disease infection. Tran and Waller (2013) used Lyme disease incidence data from 13 US states to assess how the area of a greenspace is associated with *B. burgdorferi* s.l. infection, and they also found that increasing site area reduces the risk of people contracting Lyme disease. Another study also studied Lyme disease incidence but found that the incidence was lower in smaller greenspaces (Brownstein et al., 2005), contrasting the results of Tran and Waller (2013) and the results of this study. Brownstein et al. (2005) suggest that people may be less likely to visit small fragmented rural woodland patches, so the number of cases in these small patches is small. The ABM used in my study did not incorporate this specific human behaviour, but Brownstein et al. (2005) studied rural and suburban areas, rather than urban cities, where this may not be the case if choice of greenspace is more limited. Future studies could develop an ABM with the ability to specify the likelihood of humans visiting a greenspace depending on the size and cover of different habitats.

5.3.3 Single Large or Several Small Urban Greenspace Configuration

'Land sparing' (single large - SL) and 'land sharing' (several small - SS) are two key concepts to consider for the management of urban spaces (Perfecto and Vandermeer, 2008; Phalan, 2018). Land sparing involves maximising the density of built-up areas within urban areas to free up as much land as possible for a single large area of urban greenspace, whereas land sharing involves urban communities and urban greenspaces being interspersed in the same part of the city or town, with the creation of many small greenspace patches. It is important to understand whether the creation of GI in the form of single large urban greenspaces, or several small urban greenspaces, may increase the risk of exposure to ticks, and hazard of *B. burgdorferi* s.l. infections so that this can be reduced where possible. The resources required to perform empirical studies on different urban greenspace development configurations are often consequent (Van Buskirk and Ostfeld, 1995), therefore the use of an ABM was beneficial as theoretical scenarios could be simulated.

The ABM used in this study suggests that the risk of tick bites may be higher in a SS greenspace configuration as there were more bites recorded in this configuration. However, the risk of Lyme disease infection was higher in a SL configuration, as there were more infections recorded in the singular large scenario compared to the several small scenario, which may be a more important consideration for greenspace creation than the number of tick bites alone. Single large versus several small habitat configurations are still debated in literature (Fahrig et al., 2019; Fletcher et al., 2018), however very few studies have used an ABM to explore how fragmentation and isolation may affect ticks and tick-borne disease in urban settings. This is the first study to consider how a single large or several small urban greenspaces could affect tick-borne disease risk, and the use of ABMs to explore singular large versus several small should be encouraged, as they can be used to simulate situations which are not possible to create in the field. Though they have not considered the several small versus singular large debate, previous studies have demonstrated the importance of using ABMs to explore how habitat fragmentation may affect ticks and their hosts. Tardy et al. (2022) found that individual hosts which had particularly high tick burdens were more likely to be present in fragmented landscapes, suggesting that, as demonstrated in this study, the risk of tick bites may be higher in a SS configuration. Their study, however, suggests that the effects of different greenspace configurations may depend on the species, as some species may favour SS and other SL (Soga *et al.*, 2014), and on how well connected the small greenspaces are. For example, Jokimäki et al. (2020) found that passerine birds can be found in higher densities in urban areas with a land sparing configuration (SL) (2020), and since passerine birds are tick hosts (Gern *et al.*, 1998) and can transmit the human pathogenic *B. garinii*, this could affect Lyme disease hazard. This wasn't included in this study, as a balance between model complexity and computational performance was necessary, however this is something that could be explored by future ABM studies.

While the outputs of the ABM model used in this study suggest that ABMs could be a useful tool for town planners to predict the impact of future GI development on tick populations and the risk of tick-borne diseases, there are limitations to the SLOSS scenarios created, which may have affected the results. As the landscape generated was theoretical, new greenspaces replaced existing sub-urban housing estates, which is not likely to happen in reality. Whilst the findings are still useful for predicting the effects of creating SL or SS on tick densities and *B. burgdorferi* s.l. prevalence, it would be more appropriate for town planners and policy development to add greenspaces in Glasgow's proposed greenspace development areas. However, specific planning maps for greenspace creation in Glasgow where the impact of SL versus SS could be tested could not be found. The scenarios were also only tested over a short period of time (6 years). In the future, it would be beneficial to test scenarios over a longer period of time because it allows the tick and host populations to develop over time and the results are then less likely to be impacted by the initial set-up numbers. It would also be beneficial to create a dataset with more scenarios with different surrounding landcovers, but due to time constraints this wasn't possible for my project. It is also important to consider that the ABM agents' behaviours were developed from empirical data collected as part of the MEaSURE project and a previously developed ABM about roe deer behaviour (Topping et al 2003). This is noted as important in literature (Spear et al., 2010).

5.3.4 The Limitations of Agent-based Modelling

Agent-based models often focus on either a detailed, realistic landscape/ environment, or detailed ecology and physiology of the organisms they are modelling (Topping et al., 2003) due to constraints on time and model complexity. This model was designed to represent a heterogeneous urban greenspace landscape, as well as the behaviour of three very different organisms (ticks, humans, and deer) within the model, so a number of real-life complexities regarding microclimates and habitat structures have to be simplified for computational modelling capacity. It has been suggested that simplification of a model can produce quite different results to a more complex version with the same data (Stephens et al., 2002). It is therefore important to recognise these simplifications when using an ABM for informing policy and planning.

This is particularly relevant in the case of modelling tick-borne diseases. Unlike many other vector-borne diseases, where transmission is largely affected by temperature, tick-borne diseases are impacted by many other factors too, including host populations and habitats (Li et al., 2019). This is another reason why it was important for deer behaviour was incorporated into the ABM used in this study, as well as temperature. It is, however, particularly difficult to model deer behaviour accurately in an urban environment, because the effect that this has on the provision of ecosystem services and disservices (such as ticks and tick-borne disease) has only been recently studied. Furthermore, roe deer have the ability to exhibit behavioural plasticity in changing environments (Jepsen and Topping, 2004). This makes it more challenging to model their social and spatial behaviours in urban areas accurately (Jepsen and Topping, 2004).

The modelling of human behaviour is also challenging to model accurately without the introduction of broad assumptions about human behaviour, as fine-grained decision making cannot be assigned to each human agent within the model. To use these models in a land planning scenario, it may be necessary to incorporate additional models of social psychology and behavioural economics to accurately represent human decision making (Schulze et al., 2017). It is also suggested that models should be tested under different socioeconomic scenarios, specific to different community areas (Li et al., 2019). The inclusion of human behaviour in ABMs also makes it more difficult to validate the model, because it is difficult to gather empirical, real-world data that are specific to and relevant for the study (Filatova et al., 2013). The collection of more specific human behaviour data relating to urban greenspace use is being collected as a part of the MEASURE project to improve the ABM, however time constraints meant that these refinements could not be made before using the model in this study.

5.4 Future Studies

Future studies should further explore how connectivity, site area and land cover can affect tick densities. Studies which include host surveys for other species such as passerine birds, small mammals and squirrels would be beneficial to further explain the trends in tick populations. The data could be analysed with the specific *B. burgdorferi* s.l. genospecies to allow for stronger links to be made between the importance of different host species in the prevalence of *B. burgdorferi* s.l in urban greenspaces and how they are each specifically impacted by different greenspace characteristics. Future studies could also explore the differences in behaviours of host species in urban settings, compared to rural settings, and how this may relate to DON and DIN in urban areas. Measures of human activity and behaviour could be combined with field observations of DIN and DON to explore the exposure risk to ticks and the hazard of tick bites, which can be used by urban planners to create spaces where ticks are less likely to be present in high densities. The risk to people can also be explored, so that that tick awareness raising can be targeted in specific areas of higher risk. Incorporating data on human's usage of urban greenspaces could also help understand tick-borne disease risks in urban settings. The composite risk metric could also be compared against field observations of DIN and DON to provide further insight on the appropriateness of DIN as an estimate of *B. burgdorferi* s.l. transmission risk and how DIN/DON is correlated with risk based on visitor behaviour data. It will also be important to further investigate the applicability of ABMs for studying Lyme disease transmission so that the accuracy of these models can be improved and validated, which may make local governments more open to using models to inform urban planning decisions.

Chapter 6 Conclusion

The majority of the world's population are living in urban areas, and the figures are continuing to rise (United Nations Department of Economic and Social Affairs: Population Division, 2018). As urbanisation rises, there is an increasing pressure for governments to put more plans in place to help mitigate against the impact of climate change in urban areas (Pamukcu-Albers et al., 2021). As a part of these plans, there is an increase in the development of green infrastructure and urban greenspaces in new urban developments (Pamukcu-Albers et al., 2021). Urban greenspaces can provide many ecosystem services which can benefit the local communities, mitigate climate change, and improve biodiversity (Strohbach and Haase, 2012). However, the provision of urban greenspaces also brings ecosystem disservices, such as concerns for safety, certain sounds and smells, and vector borne diseases (Wolch, Byrne, and Newell, 2014). It is therefore important for urban planners to understand how the creation of urban greenspaces in different locations, configurations, and with different characteristics may be associated with different ecosystem services and disservices (Dunn, 2010).

In this research, the risk of vector-borne transmission, specifically tick-borne diseases, and the associated risk of Lyme disease transmission was explored. The research aimed to understand how the configuration, connectivity, area, and land cover of urban greenspace influences DON and the associated hazard of *B. burgdorferi* s.l. infection. Field surveys revealed the presence of both tick populations and *B. burgdorferi* s.l. within and surrounding urban greenspaces in Scotland (Glasgow and Edinburgh), which until now, had not been studied. DON and DIN in greenspaces were demonstrated to be correlated with deer presence, a key host of ticks in Scotland. The correlation with deer presence highlights the importance of future studies to include tick host surveys, as they can be drivers of tick densities in greenspaces. The results also suggest that increasing connectivity and woodland cover within and surrounding urban greenspaces increases the DON significantly. However, the greenspace area and proportions of improved grassland, semi-natural grassland and built-up area within and surrounding the greenspace did not significantly affect DON. DIN was also found to increase significantly with increasing connectivity, as well as increasing site area and the proportion of improved grassland. Increasing woodland cover, semi-natural grassland, and built-up area proportions within and surrounding a greenspace significantly reduced DIN. These results are important to note for future green infrastructure projects, as the addition of new greenspaces may improve the connectivity of habitat networks for tick hosts and may therefore lead to higher densities of ticks and infected ticks. The expansion of existing greenspace, or creation of new large greenspace areas may also increase the *B. burgdorferi* s.l. infection hazard. It was also demonstrated that the creation and management of habitats *within* urban greenspaces will also require careful planning and consideration, as different land cover types may affect the density of nymphs and density of infected nymphs in different ways. An increase in woodland cover and decrease in built-up area cover could significantly increase DON in urban environments. Reducing the proportion of built-up areas, or increasing grassland habitat, could also increase NIP. If DIN is higher within greenspaces, then visitors may be at greater risk of contracting Lyme disease. Future studies should try to identify areas where visitor numbers and infection prevalence is high, to further explore public health risk, and to develop current measures of Lyme disease risk to consider both frequency of occurrence as well as hazard (DIN). It will also be vital to educate visitors to urban greenspace to improve tick awareness and lower the risk of Lyme disease. Outputs from the agent-based model used in my study suggest that while the risk of tick bites may be higher in a ‘several small’ greenspace configuration, the risk of contracting Lyme disease may be higher in a single large greenspace. This is important to consider, because even though tick bites are seen as unpleasant, tick-borne diseases, such as Lyme disease, can have major negative impacts on people’s health in the short and long term. The figures generated from model outputs were not dissimilar to the findings from empirical work, however more studies are needed in future to test the use of ABMs as tools for urban planners to plan GI creation, as current literature is limited, and model validation is difficult. Model results always need to be interpreted with caution, as there are inevitable assumptions which models are sensitive to, that cannot be removed due to computational complexity.

This research provides important insight that local governments and urban planners can use to create urban greenspace in a way that can benefit people without introducing unintended consequences such as Lyme disease transmission. This means that greenspace can be designed with all the ecosystem services of climate mitigation, mental health, physical health benefits – without risking negative health impacts from the ecosystem disservices of tick and tick-borne disease.

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Appendix 1- Citations for NBN deer sighting data:

- Argyll Biological Records Centre (2023). Argyll Biological Records Dataset. Occurrence dataset on the NBN Atlas
- Bat Conservation Trust (2023) National Bat Monitoring Programme. The BCT/MTUK Bats & Roadside Mammals Survey. Occurrence dataset on the NBN Atlas
- Biological Records Centre (2023). Mammal records from Britain from the Atlas of Mammals (1993), with some subsequent records. Occurrence dataset on the NBN Atlas
- David Dodds Associates Ltd - Species Records, TWIC (2020)
- East Ayrshire Countryside Ranger Service (2023). East Ayrshire Species Database. Occurrence dataset on the NBN Atlas
- East Lothian Council - Woodland Plants Survey 2011 (public), TWIC (2020)
- Fife Nature Records Centre (2023). Fife Nature Records Centre combined dataset. Occurrence dataset on the NBN Atlas
- Glasgow Museums BRC (2023). Glasgow Museums BRC: Mammals. Occurrence dataset on the NBN Atlas
- Glen Finglas Species Records, The Woodland Trust (2020)
- Highland Biological Recording Group (2023). Vertebrates (not Badger) Dataset. Occurrence dataset on the NBN Atlas
- Hsing, P.-Y., Hill, R. A., Smith, G. C., Bradley, S., Green, S. E., Kent, V. T., Mason, S. S., Rees, J., Whittingham, M. J., Cokill, J., MammalWeb citizen scientists & Stephens, P. A. (2022). Large-scale mammal monitoring: The potential of a citizen science camera-trapping project in the United Kingdom. Ecological Solutions and Evidence, 3, e12180. https://doi.org/10.1002/2688-8319.12180
- iSpot (2023). iSpot British Isles observations. Occurrence dataset on the NBN Atlas
- Lothian Wildlife Information Centre (2023). General Records (1900-1989). Occurrence dataset on the NBN Atlas
- Mammal Society 12/12/2023 Mammal Mapper App Sighting Records
- Natalie Harmsworth's Records (2010-2019), TWIC (2019)
- National Bat Monitoring Programme (accessed: 12/12/2023) Sunset/Sunrise Survey, Bat Conservation Trust.
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- National Trust for Scotland (2023). National Trust for Scotland Species Records. Occurrence dataset on the NBN Atlas
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- People's Trust for Endangered Species (2023). Living with Mammals survey. Occurrence dataset on the NBN Atlas
- Recording Invasive Species Counts (accessed: 12/12/2023) RISC Non-Native Species Records for Muntjac.
- Records provided by Argyll Biological Records Centre, accessed through NBN Atlas website.
- Records provided by Bat Conservation Trust, accessed through NBN Atlas website.
- Records provided by Biological Records Centre, accessed through NBN Atlas website.
- Records provided by British Trust for Ornithology, accessed through NBN Atlas website.
- Records provided by BTO, accessed through NBN Atlas website.
- Records provided by BTO, accessed through NBN Atlas website.

- Records provided by Central Scotland Green Network Trust, accessed through NBN Atlas website.
- Records provided by East Ayrshire Countryside Ranger Service, accessed through NBN Atlas website.
- Records provided by Fife Nature Records Centre, accessed through NBN Atlas website.
- Records provided by Glasgow Museums Biological Records Centre, accessed through NBN Atlas website.
- Records provided by Highland Biological Recording Group, accessed through NBN Atlas website.
- Records provided by Invasive non-native species records from SEWeb, accessed through NBN Atlas website.
- Records provided by iSpot, accessed through NBN Atlas website.
- Records provided by Mammal Society, accessed through NBN Atlas website.
- Records provided by MammalWeb, accessed through NBN Atlas website.
- Records provided by National Trust for Scotland, accessed through NBN Atlas website.
- Records provided by North Ayrshire Countryside Ranger Service, accessed through NBN Atlas website.
- Records provided by Northeast Scotland Biological Records Centre, accessed through NBN Atlas website.
- Records provided by Northeast Scotland Terrestrial Mammals 1900-2017 (excluding squirrels, wild cats and marine mammals), accessed through NBN Atlas website.
- Records provided by People's Trust for Endangered Species, accessed through NBN Atlas website.
- Records provided by Royal Society for the Protection of Birds, accessed through NBN Atlas website.
- Records provided by Scotland's Environment Web, accessed through NBN Atlas website.
- Records provided by Scottish Wildlife Trust, accessed through NBN Atlas website.
- Records provided by South Lanarkshire peatland records 2013, accessed through NBN Atlas website.
- Records provided by The British Association for Shooting and Conservation, accessed through NBN Atlas website.
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- Scottish Wildlife Trust (2023). Casual records for Scottish Wildlife Trust reserves - Verified data. Occurrence dataset on the NBN Atlas
- Scottish Wildlife Trust (2023). Commissioned surveys and staff surveys and reports for Scottish Wildlife Trust reserves - Verified data. Occurrence dataset on the NBN Atlas
- Scottish Wildlife Trust (2023). Survey and monitoring records for Scottish Wildlife Trust reserves from reserve convenors and Trust volunteers - Verified data. Occurrence dataset on the NBN Atlas
- The British Association for Shooting and Conservation (2023). UK casual records from members of BASC - 1980 onwards. Occurrence dataset on the NBN Atlas
- The Road Lab (2023). UK Roadkill Records. Occurrence dataset on the NBN Atlas
- The Royal Society for the Protection of Birds (2023). RSPB Lochwinnoch incidental species records. Occurrence dataset on the NBN Atlas

- The Wildlife Information Centre (2023). City of Edinburgh Natural Heritage Service - Historical Records. Occurrence dataset on the NBN Atlas
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- The Wildlife Information Centre (2023). City of Edinburgh Natural Heritage Service - Natural Heritage Service Data. Occurrence dataset on the NBN Atlas
- The Wildlife Information Centre (2023). City of Edinburgh Natural Heritage Service - Ranger Ad-hoc records and sightings. Occurrence dataset on the NBN Atlas
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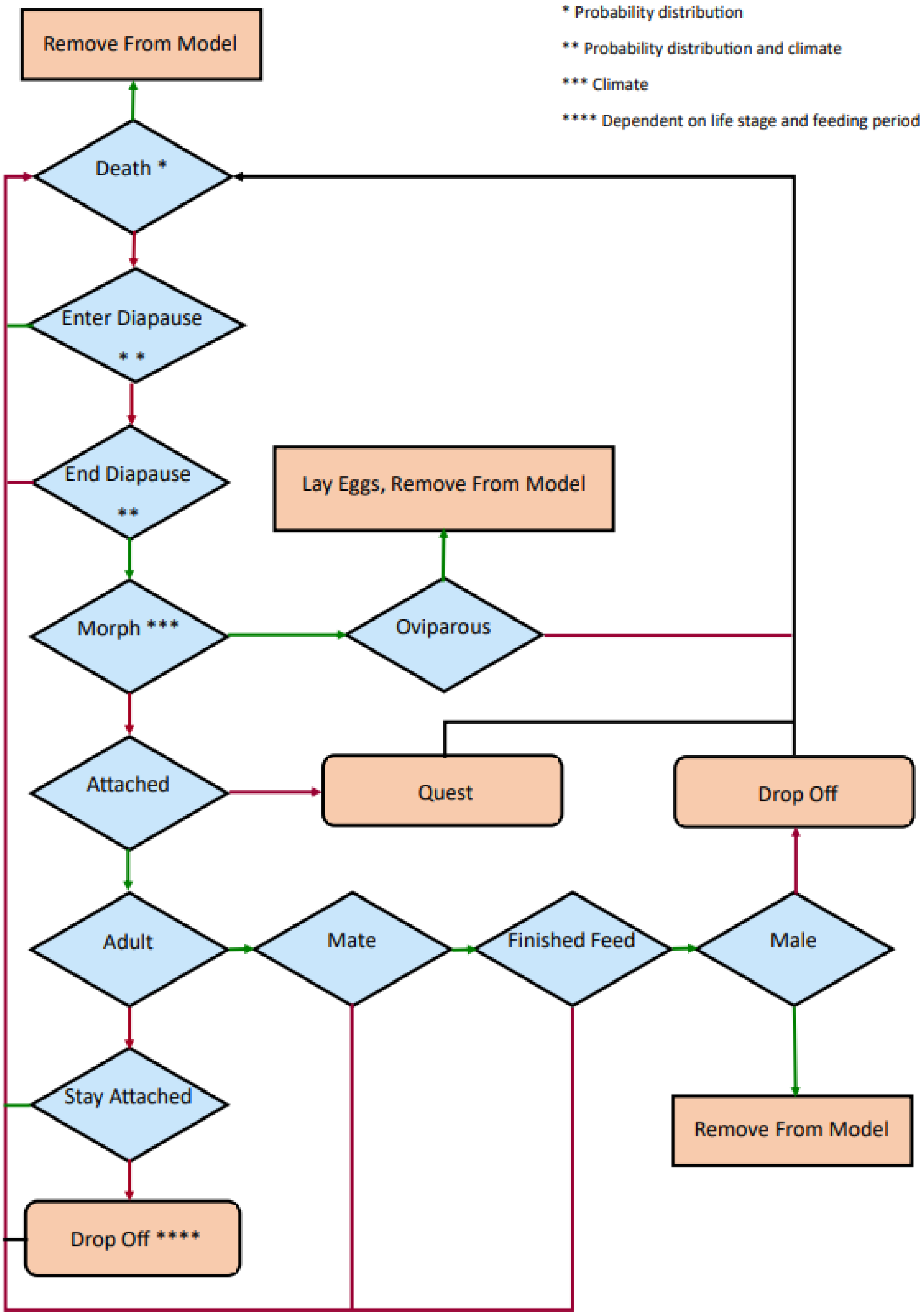
- The Wildlife Information Centre (2023). Lothian Wildlife Information Centre Secret Garden Survey. Occurrence dataset on the NBN Atlas
- The Wildlife Information Centre (2023). Lothian Wildlife Information Centre surveys (non-invertebrate - general). Occurrence dataset on the NBN Atlas
- The Wildlife Information Centre (2023). LWIC - Local Patch Project. Occurrence dataset on the NBN Atlas
- The Wildlife Information Centre (2023). R. Manning's Records. Occurrence dataset on the NBN Atlas
- The Wildlife Information Centre (2023). The Lothian Mammal Survey. Occurrence dataset on the NBN Atlas
- The Wildlife Information Centre (2023). TWIC Biodiversity Field Trip Data (1995-present). Occurrence dataset on the NBN Atlas
- The Wildlife Information Centre or BRISC (depends on originator in dataset name)
- The Wildlife Information Centre or BRISC (depends on originator in dataset name)
- TWIC General Records (2015 - present), TWIC (2020)
- TWIC Site Surveys (2010 - present) (2020)
- West Lothian Council (2023). Local Biodiversity Site Surveys. Occurrence dataset on the NBN Atlas

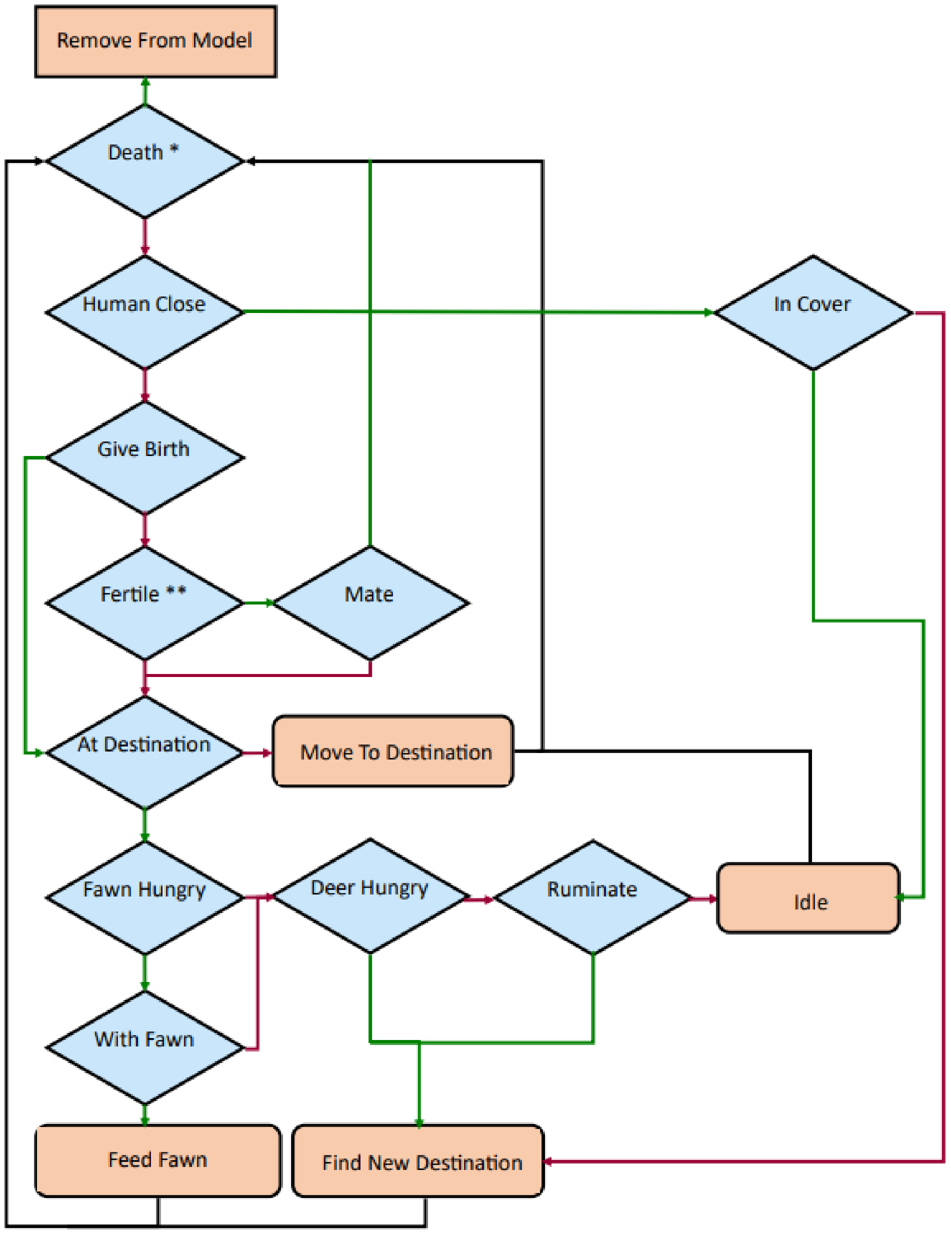
Appendix 2 - UKCEH land cover classes

Class	Land Cover
1	Deciduous Woodland
2	Coniferous Woodland
3	Arable
4	Improved Grassland
5	Neutral Grassland
6	Calcareous Grassland
7	Acid Grassland
8	Fen
9	Heather
10	Heather Grassland
11	Bog
12	Inland Rock
13	Saltwater
14	Freshwater
15	Supralittoral Rock
16	Supralittoral Sediment
17	Littoral Rock
18	Littoral Sediment
19	Saltmarsh
20	Urban
21	Suburban

Appendix 3 – Agent-based Model Decision Flow Chart for Ticks



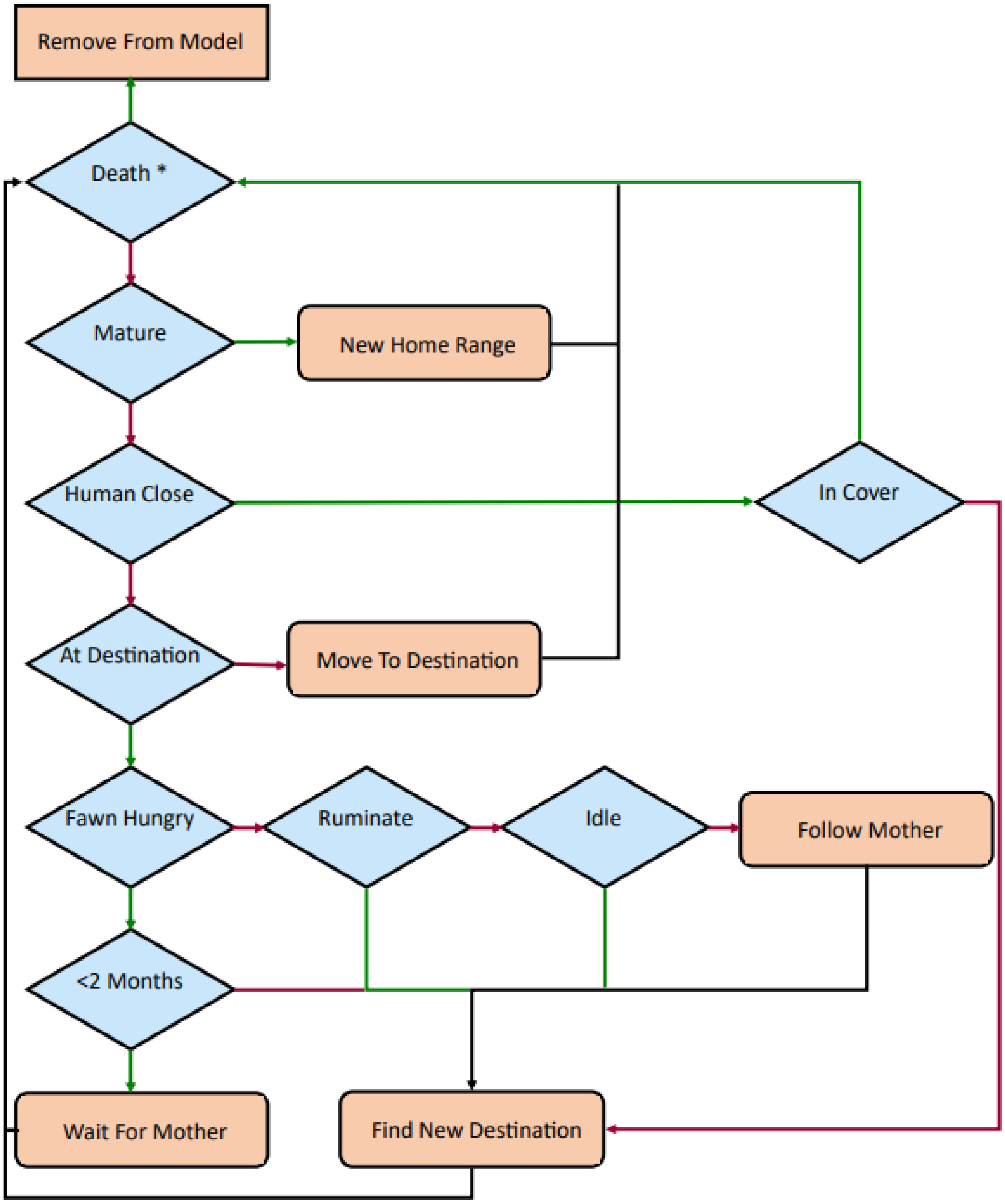
Appendix 4 – Agent-based Model Decision Flow Chart for Female Deer



* Probability distribution

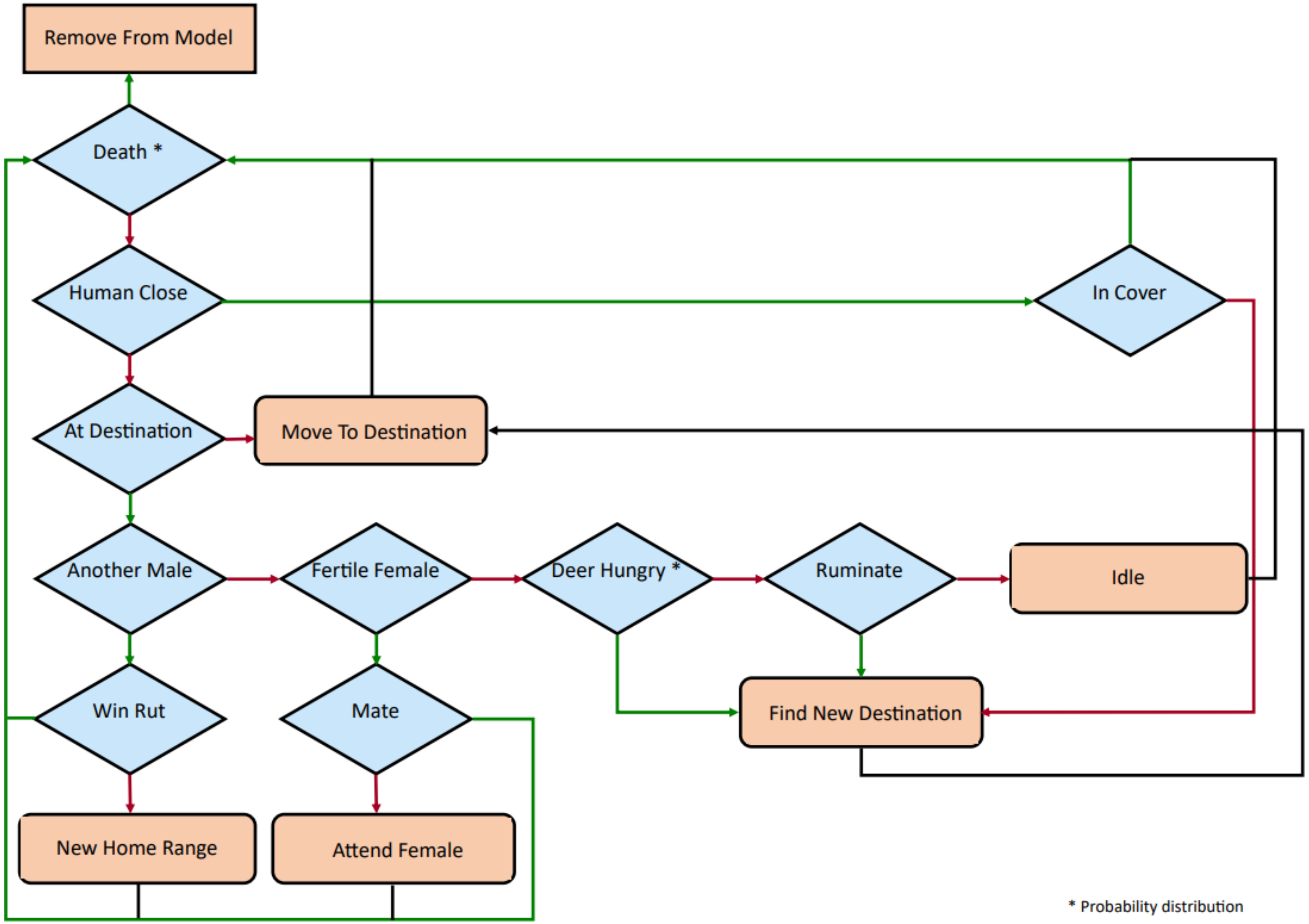
** If not pregnant or with fawn, dependant on time of year.

Appendix 5 – Agent-based Model Decision Flow Chart for Juvenile Deer



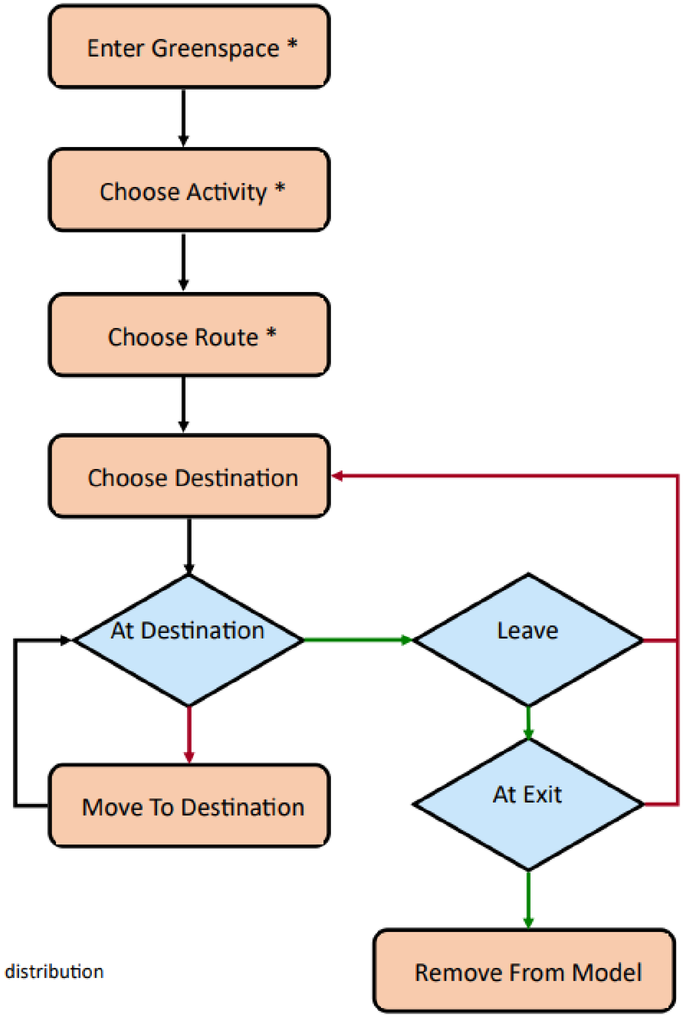
* Probability distribution

Appendix 6 – Agent-based Model Decision Flow Chart for Male Deer



* Probability distribution

Appendix 7 – Agent-based Model Decision Flow Chart for Humans



* Probability distribution

Appendix 8 – Raw Tick Survey Data from Glasgow Sites

Site	Urban /Suburban/ Rural	Year	Larvae Present?	Deer signs present at site?	Squirrel signs present at site?	Other hosts at site?	Nymphs	Adult Female	Adult Male	Deer Piles	Deer Hoof
Alexandra Park	Urban	2023	NA	0	1	0	0	0	0	0	0
Bearsden	Suburban	2022	1	1	0	0	11	2	0	0	1
Bearsden	Suburban	2023	NA	1	0	0	44	0	2	1	7
Blairskaith Linn	Rural	2022	0	1	0	cows, sheep	2	0	1	0	0
Blairskaith Linn	Rural	2023	NA	1	0	hares	32	1	1	0	4
Cadder Church	Suburban	2022	1	0	0	0	5	0	1	0	0
Cadder Church	Suburban	2023	NA	0	0	foxes	11	0	1	0	0
Carron Valley Campsies	Rural	2022	0	0	1	0	0	0	0	0	0
Carron Valley Campsies	Rural	2023	NA	1	0	foxes	7	0	1	5	2
Dawsholm	Urban	2022	1	1	1	0	2	0	0	1	0
Dawsholm	Urban	2023	0	1	0	0	4	1	3	0	0
Dougalston	Suburban	2022	1	1	0	0	28	0	0	0	0
Dougalston	Suburban	2023	NA	1	0	voles	31	0	1	0	4
Hogganfield	Urban	2022	0	1	0	0	2	1	0	2	0
Hogganfield	Urban	2023	NA	1	0	voles	63	1	5	3	8
Kelvingrove	Urban	2022	0	0	1	0	0	0	0	0	0
Kelvingrove	Urban	2023	NA	0	1	foxes	0	0	0	0	0
Kilmadinny Loch	Suburban	2022	0	1	1	foxes	0	0	0	0	0
Kilmadinny Loch	Suburban	2023	NA	1	0	0	3	0	0	7	6
Lennoxtown	Rural	2022	0	1	0	0	6	0	0	2	0
Lennoxtown	Rural	2023	NA	1	0	foxes	35	0	1	3	3
Lenzie Moss	Suburban	2022	0	1	0	0	6	0	0	0	0

Lenzie Moss	Suburban	2023	NA	1	0	foxes	19	3	3	9	0
Loch Ardling	Rural	2023	NA	1	0	0	80	0	3	1	0
Mugdock	Rural	2022	0	1	0	0	17	0	1	0	2
Mugdock	Rural	2023	NA	1	0	0	104	2	2	2	2
Queens View	Rural	2022	1	1	0	foxes	40	4	4	4	1
Queens View	Rural	2023	NA	1	0	foxes	147	6	9	10	2
Robroyson Park	Urban	2022	0	0	0	foxes	4	0	0	0	0
Robroyson Park	Urban	2023	NA	1	0	foxes, voles, badgers	51	5	4	3	3
Ruchhill	Urban	2022	0	1	0	0	1	1	1	3	0
Ruchhill	Urban	2023	0	1	0	0	15	0	1	6	0
Temple Hill Wood	Suburban	2023	NA	1	0	voles	3	0	0	0	2

Appendix 9 – Raw Tick Survey Data from Edinburgh Sites

Site	Urban /Suburban / Rural	Year	Larvae Present ?	Deer signs at site?	squirrel signs at site?	Other hosts on site?	Nymphs	Adult Female	Adult Male	Deer Piles	Deer Hoof
Bonaly Country Park	Suburban	2022	0	1	0	deer, foxes, badger	3	0	0	2	0
Bonaly Country Park	Suburban	2023	1	1	0	Voles	6	1	0	1	0
Collinton	Urban	2022	0	1	0	deer, foxes	0	1	0	0	0
Collinton	Urban	2023	0	0	0	NA	3	0	0	0	1
Craiglockheart	Urban	2023	0	0	0	NA	0	0	0	0	0
Craiglockheart	Urban	2022	0	0	0	Foxes	1	0	0	0	0
Craigmiller	Urban	2022	0	1	1	rabbit, foxes	1	0	0	0	0
Craigmiller	Urban	2023	0	0	1	Foxes, Bats, Badgers	0	0	0	0	0
Edgelaw	Rural	2022	0	0	1	NA	1	0	0	0	0
Edgelaw	Rural	2023	0	0	0	NA	2	0	0	0	0
Gladhouse	Suburban	2022	1	0	0	cows	5	2	2	0	0
Gladhouse	Suburban	2023	1	0	0	NA	12	0	2	0	0
Glencourse	Rural	2022	0	1	1	NA	0	0	0	0	0
Glencourse	Rural	2023	0	0	1	NA	0	0	0	0	0
Goreglen	Rural	2022	0	1	1	rabbit, badgers	0	0	0	5	1
Goreglen	Rural	2023	0	1	0	Badgers, Rabbits	0	0	1	0	0
Hermitage	Urban	2022	0	1	1	Rabbit	0	0	0	0	0
Hermitage	Urban	2023	0	0	0	NA	0	0	0	0	0
Loanhead	Suburban	2022	1	1	1	mice, voles, foxes	10	1	1	2	0
Loanhead	Suburban	2023	1	0	1	NA	16	0	0	0	0
Lord Ancrum Wood	Suburban	2022	1	1	1	hare	0	0	0	0	0
Lord Ancrum Wood	Suburban	2023	1	0	1	NA	6	0	0	0	0
Meadows	Urban	2022	0	0	1	foxes, rabbits	0	0	0	0	0
Meadows	Urban	2023	0	0	0	NA	0	0	0	0	0

Penicuik	Rural	2022	0	1	1	badger, otter, mice, voles, hedgehogs	6	1	0	0	0
Penicuik	Rural	2023	0	0	0	NA	2	0	0	7	1
Roslin Glen	Suburban	2022	1	0	0	NA	2	0	0	0	0
Straiton	Suburban	2022	0	0	0	possible badgers	0	0	0	0	0
Straiton	Suburban	2023	1	0	0	NA	5	0	0	0	0
Vogrie Country Park	Rural	2022	0	1	1	horses	0	0	0	1	0
Vogrie Country Park	Rural	2023	0	1	0	Rabbits	1	0	0	0	0