Host weight, seasonality and anthropogenic factors contribute to parasite community differences between urban and rural foxes

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HIGHLIGHTS

- Comprehensive helminth community assessment including environment, host- and parasite traits
- Reduced weight in wildlife infested with many different helminth species
- More helminth multi-infections (higher species richness) in winter and spring
- Urbanisation affects the parasite community: urban vs. rural helminth species.
- Urban species show unexpected traits: more trophic transmission in the city.

GRAPHICAL ABSTRACT
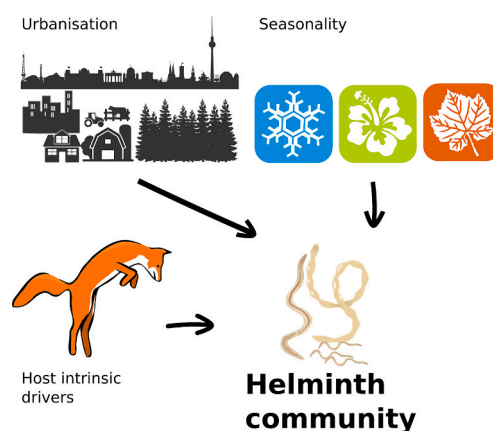
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ABSTRACT

Pathogens often occur at different prevalence along environmental gradients. This is of particular importance for gradients of anthropogenic impact such as rural-urban transitions presenting a changing interface between humans and wildlife. The assembly of parasite communities is affected by both the external environmental conditions and individual host characteristics. Hosts with low body weight (smaller individuals or animals with poor body condition) might be more susceptible to infection. Furthermore, parasites' mode of transmission might

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affect their occurrence: rural environments with better availability of intermediate hosts might favour trophic transmission, while urban environments, typically with dense definitive host populations, might favour direct transmission. We here study helminth communities (141 intestinal samples) within the red fox (*Vulpes vulpes*), a synanthropic host, using DNA metabarcoding of multiple marker genes. We analysed the effect of urbanisation, seasonality and host-intrinsic (weight, sex) variables on helminth communities. Helminth species richness increased in foxes with lower body weight and in winter and spring. Season and urbanisation, however, had strong effects on the community composition, i.e., on the identity of the detected species. Surprisingly, transmission in two-host life cycles (trophic transmission) was more pronounced in urban Berlin than in rural Brandenburg. This disagrees with the prevailing hypothesis that trophically transmitted helminths are less prevalent in urban areas than in rural areas. Generally, co-infestations with multiple helminths and high infection intensity are associated with lighter (younger, smaller or low body condition) animals. Both host-intrinsic traits and environmental drivers together shape parasite community composition and turnover along urban-rural gradients.

1. Introduction

Human land use alters the composition of ecological communities, i. e., the identity and abundance of species (Dale, 2018; Kampichler et al., 2012). For communities of parasites this has implications for potential spill-overs to humans, pets, and livestock (Huang et al., 2021; Schwensow et al., 2022). Understanding the relationship between land use change and parasite community change is therefore paramount for disease management. Community turnover means a change in species composition along spatial gradients or across time (Dunson and Travis, 1991; Legendre and Caceres, 2013). This process can be accompanied by changes in species richness meaning that a higher or lower overall number of species identities can be observed along the gradient. Alternatively, species can be replaced by other species, whereby species richness may stay the same. Understanding the reasons for community composition and turnover requires extensive consideration of factors at different levels of organisation: environmental differences and habitat heterogeneity and the species' traits (Clark et al., 2018; Lewthwaite et al., 2017; Morin, 2009; Travis, 1996).

Urbanisation is an extreme form of land use change, in which human land use intensifies and human population density increases (French et al., 2022); this has effects on wildlife due to habitat alterations and fragmentation, shifts in food sources, changes in animal behaviour, and increased contact with humans (Bonnington et al., 2014; Petersen et al., 2021; Wang et al., 2017). Such effects can cascade down to the parasitic communities within their wildlife hosts (Alfieri and Anderson, 2019; Calegaro-Marques and Amato, 2014; Schwensow et al., 2022). Urbanised areas therefore provide important study systems to disentangle host factors from environmental ones in parasite communities.

When analysing host-associated communities, such as those of helminths, it is important to keep in mind that they live in two "nested environments": the internal environment of their host and the external environment (Nieberding et al., 2005; Poulin, 2011). Helminths are parasitic worms with various, in some cases complex, but well-known transmission routes and pose a significant public health and economic concern. Parasites with direct life cycles exploit only one host throughout their development and reproduction and are usually transmitted via a faecal-oral route (Lucius et al., 2018). Parasites with a more complex life cycle involving multiple hosts are usually transmitted trophically, meaning that a host is infected by ingesting the parasite most commonly with its prey (Poulin, 2011). Additionally, helminths show a large diversity in host-specificity, from a narrow host range (i.e. infecting only one or few species) to a broader range of hosts from different taxonomic levels (Schmid-Hempel, 2011). Such differences in host range can occur at different life cycle stages, but the host range is usually more limited in definitive hosts than in the intermediate hosts (Benesh et al., 2014, 2021). This diversity of life cycles makes helminth communities an interesting system to study the differential impact of the host and environment on pathogen community composition and turnover.

Seasonality, i. e. predictable and cyclic variations or changes that occur within a year in natural phenomena, or biological processes, can have additional impacts on the composition of biological communities (Tonkin et al., 2017), including those of parasites (de Angeli Dutra et al., 2023). For parasites, the effects of seasonality can be expected to be mediated via altered survival probability of external life cycle stages or via their hosts (Arcenillas-Hernández et al., 2022; Shearer and Ezenwa, 2020).

In addition to anthropogenic factors and seasonality, a multitude of host-intrinsic traits can be expected to affect pathogen communities. Hosts partially control or resist infections depending on sex or life-history stage (e.g. age or reproductive status). Pathogen control can further depend on resource availability and come with behavioural adaptations (Korte et al., 2005). In this context, high parasite loads can lead to a higher susceptibility to infections and a vicious circle of further diminishing body condition (Beldomenico et al., 2008; Beldomenico and Begon, 2010). This would predict higher parasite species richness in animals with impaired body condition and lower weight. Such host-intrinsic effects are often a result of phenotypic adjustment or evolutionary adaptation of hosts to the environment (Turner et al., 2021). An opposing hypothesis could be derived from theoretical (Hechinger, 2013) and empirical (e.g., Horn et al., 2023) work on "carrying capacity". Under this hypothesis larger host can support the space or metabolic demands of more parasites and higher parasite prevalence would thus be predicted in heavier or larger animals. In both cases, host and intraspecific variation of host traits, thus play a key role in the assembly of pathogen communities.

We choose the urban dwelling red fox (*Vulpes vulpes*; hereafter 'fox') in the metropolitan area of Berlin, Germany, as a suitable study species, as it is a common and generalist species thriving along the urban-rural environmental gradient (Adkins and Stott, 1998; Soulsbury et al., 2010). Foxes in our study area show a distinct genetic population structure differentiating them into rural and urban populations (Kimmig et al., 2020). Although not proven for the study area, fox population density is often considered to be higher in urban context, potentially affecting pathogen transmission dynamics (Gras et al., 2018). Urban foxes often rely on anthropogenic food provisioning, whereas foxes in the adjoint rural Brandenburg have a larger dietary niche than conspecifics in urban Berlin (Scholz et al., 2020), which may in turn affect parasite community richness within the fox host. Foxes can contain a multitude of parasites of zoonotic potential, such as the fox-tapeworm *Echinococcus multilocularis* (Deplazes et al., 2004; Fischer et al., 2005; Gloor, 2002; Hofer et al., 2000), which put them in the focus of parasite prevalence studies, e.g., (Davidson et al., 2006). However, comprehensive studies on the whole community of parasites in a certain area and systematically accounting for external (environment, seasonality) as well as internal factors (host and parasite traits) are missing up to date.

We here analysed and contrasted the effects of environmental factors represented by (1) administrative areas, and (2) seasonality, with those of host-intrinsic factors, represented by (3) body weight, and sex, and (4) parasitic traits (4a - transmission type; 4b - host range; 4c - life cycle; 4d - zoonotic potential). We analysed how these impact parasitic helminth

species richness, and community compositional turnover in intestinal samples of foxes from the urban Berlin and rural Brandenburg (North-East Germany) administrative areas using DNA metabarcoding. We expect (1) higher parasite species richness in rural than in urbanised areas due to higher exposure via intermediate hosts and (2) increased helminth species richness in winter potentially pointing to decreased disease resistance of hosts. We also (3) expected higher helminth species richness and higher helminth prevalence in hosts with a low body weight. We (4) expected that trophically transmitted helminths with intermediate hosts should be more affected by the external environment than directly transmitted parasites due to the combined effects of the environment and intermediate hosts. Furthermore, (5) parasites with direct transmission should be more prevalent in urban areas than parasites with complex life cycles due to the reduced availability of intermediate hosts in urban environments compared to the rural surroundings.

2. Methods

2.1. Study area

The study was conducted in the federal states Berlin and Brandenburg in the north-east of Germany (Fig. 1). Berlin has an area of ~892 km^2 and an overall human population density of ~4126 inhabitants/km^2. The urban structure is heterogeneous, from highly urbanised areas with high-rise buildings, multi-lane roads and areas with a high proportion of sealed surfaces to districts dominated by lakes, forests and green spaces. About 2500 city parks, 160 km^2 of forest and several lakes characterise the city nature. In total, nearly 60 % of the city surface is sealed with housing and traffic infrastructure. Berlin is enclosed by the federal state of Brandenburg, which has an area of ~29,479 km^2 and a human population density of ~87 inhabitants/km^2. Brandenburg is dominated by agricultural land (49 % of surface area), small forests (37

%), and relatively few cities and villages (7 %). Since several forests, lakes and green spaces stretch into the city and the periphery of the city is mainly characterised by single-family housing communities with gardens, there is a suburban transition from the rural areas of Brandenburg to the highly urbanised city centre of Berlin (www.statistik-berlin-brandenburg.de, 2020).

2.2. Fox carcass sampling

In cooperation with the Berlin-Brandenburg State Laboratory (LLBB; https://www.landeslabor.berlin-brandenburg.de), we collected 141 intestinal samples from dead adult foxes from Berlin and Brandenburg (from now on referred to as "intestinal samples"). The complete intestine was taken out of the carcasses, and the small intestine was separated from the large intestine (colon, rectum). The large intestine was not opened and the contents were pressed out of it into plastic sample vessels. These large intestine contents constitute the material for all further analyses. Samples originated from foxes that were either involved in road accidents, hunted or died of natural causes between the summer of 2016 and the spring of 2018. No animal was harmed to provide samples for this study. For all individuals, location, sex, carcass weight and the decomposition stage of the carcass (slightly autolytic or fresh) were recorded. All intestinal samples were stored at −80 °C until further analysis. The final set comprised 141 intestinal samples of adult foxes. A total of 91 samples originated from males and 50 from females. The majority of animals were sampled in winter (Dec-Feb; Berlin (B): $n = 56$, Brandenburg (BB): $n = 18$), with fewer samples available in spring (March–May; B: 9, BB: 23) and combined summer and autumn seasons (Jun-Nov; B: 33, BB: 2).
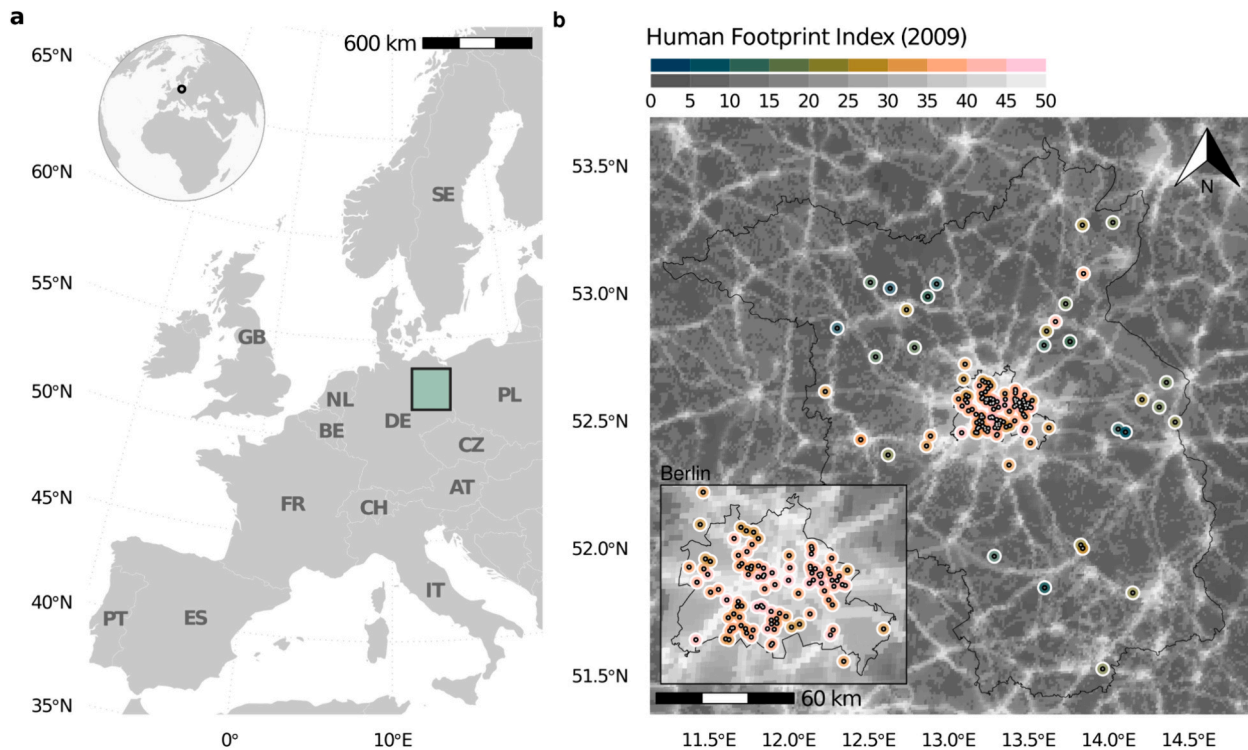

Fig. 1. Study area and geographical distribution of samples. a) The study area is located in North-Eastern Germany and comprises the city of Berlin and the surrounding rural area of Brandenburg, located in the area highlighted by the green square. b) Circles represent sample locations (*n* = 141). The map is coloured according to the human footprint index (grey shades; Venter et al., 2016), and the sampling locations are coloured according to the same index averaged in a 1 km radius surrounding them (green-purple gradient). Berlin, highlighted in the inner frame, is located in the centre of the area, enclosed by Brandenburg.

2.3. Laboratory analyses

2.3.1. DNA extraction and metabarcoding procedure

Whole DNA was extracted using the NucleoSpin© Soil kit (Macherey-Nagel GmbH & KG, Düren, Germany) following the instructions of the manufacturer. Kits developed for soil samples have been found to be suitable for extraction of DNA from intestinal samples for amplicon sequencing (Sinha, 2017). The concentration of the DNA (in ng/µl) and the quality in regard to the ratio of absorption at 260/280 nm and 260/230 nm was measured with a NanoDrop 2000c (Thermo Scientific, Walham, USA). DNA extracts with high quantity and quality, were diluted to a concentration of 30 ng/µL for further analysis.

Samples were randomised in their order and amplified in parallel with 10 non-template negative controls using a microfluidics PCR on the Fluidigm Access Array 48 × 48 system (Fluidigm, San Francisco, California, USA). Microfluidic PCR allows amplifying multiple fragments (amplicons) for different marker genes with universal primer pairs targeting a wide diversity of eukaryotes (suppl. Table 1). Library preparation is integrated into the amplification procedure and was performed according to the protocol Access Array Barcode Library for Illumina Sequencers (single direction indexing) as described by the manufacturer (Fluidigm, San Francisco, California, USA). The amplicon mixes were quantified using Qubit fluorometric quantification dsDNA High Sensitivity Kit (Thermo Fisher Scientific, Walham, USA) and pooled in equimolar concentration. The final library was purified using Agencourt AMPure XP Reagent beads (Beckman Coulter Life Sciences, Krefeld, Germany). Quality and integrity of the library were checked using the Agilent 2200 TapeStation with D1000 ScreenTapes (Agilent Technologies, Santa Clara, California, USA). Sequences were generated at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv) in two runs on the Illumina MiSeq platform (Illumina, San Diego, California, USA) using v2 chemistry with 500 cycles. All sequencing raw data can be accessed through the BioProject PRJNA386767 in the NCBI Short Read Archive (SRA).

2.3.2. Sequencing data analysis

All following analyses were performed in R version 4.2.2 (R core team 2022). Sequencing reads were processed using the package MultiAmplicon (version 0.1.2; Heitlinger, 2019). In brief, sequencing reads were trimmed to 250 bp, allowing a maximum error of two nucleotides and zero ambiguous nucleotides. They were sorted according to different amplicons based on the sequence of the primer pairs, which was removed for further analysis. Forward and reverse sequencing reads were de-replicated and concatenated; chimaeras (self-primed PCR artefacts) were removed using the R package dada2 (Callahan et al., 2016). Sequences were trimmed, allowing only read pairs with zero mismatches in both forward and reverse reads. Samples for which sequencing read numbers clustered with negative controls (providing low numbers of non-helminth sequence artefacts) were removed and Amplified Sequence Variants (ASVs) were summarised across samples. ASVs are probabilistically credible variants of sequences (Callahan et al., 2016).

Taxonomy was automatically assigned based on the single best hit or at the lowest common taxonomic level in case multiple best hits based on BLAST+ (standalone version 2.11.0+) searches against sequence in the NCBI nt database (downloaded May 13th 2023) unbiased for suspected target sequences. Blast results were validated and refined manually for all helminth species (suppl. Table 2). Annotations at the genus level were scrutinised for fox parasites distinguishing parasitic helminths from worms taken up as part of the diet. Where possible, species identities were assigned by confronting BLAST resluts with previous reports of the parasites in foxes. We used this refined automatic taxonomic annotation (suppl. Table 2) to identify fox-infecting helminth species or species groups (see also suppl. Table 3; for references). Assigned ASVs were compiled by sample ID and added up by genus annotation to form a single abundance matrix for analysis in the package

phyloseq v1.28.0 (Holmens and McMurdie, 2013). Ferreira et al. (2023) demonstrated that the multi-amplicon sequencing approach processed with the listed methods results in robust assessments robustly correlated with those from quantitative PCR.

2.4. Statistical analyses

2.4.1. Explanatory variables and descriptors

In addition to the administrative areas of urban Berlin and rural Brandenburg, we used a set of different geographical raster layers related to red fox habitat use. We obtained data on tree cover and impervious surface from the Copernicus online database (https://land.copernicus.eu; available within our studies data repository, see "data and code availability"). The original rasters included data at the European scale at 20 m resolution from the year 2015, representing the percentage of a cell covered by trees or impervious surface, respectively. A geographic representation of human modifications to the environment was obtained in the form of the "Human Footprint Index" from the Centre for International Earth Science Information Network (CIESIN) (Venter et al., 2016). This raster contains a synthetic index representing human modification to the landscape, that ranges from 0 (no human effect) to 50 (landscape completely modified by human activity, e.g., city centre), at 1 km resolution. For these three environmental layers, we extracted the average values of each environmental variable within a 1 km radius (3.14 km^2 area) of each individual's sampling location. We selected this radius to represent the habitat used by individuals and the uncertainty introduced by potential dispersal (Gras et al., 2018). All analysed individuals were adults and thus primarily residents, and the buffer area for environmental variables covers their home range of ca. 200 ha (Drygala and Zoller, 2013).

We first tested for the association of continuous and categorical predictors as well as for collinearity between the continuous predictors. We included only those with low correlation (Pearson's $|r| < 0.7$) in the same models (suppl. Fig. 1). We modelled the administrative area of origin (urban Berlin vs rural Brandenburg) and associated environmental gradients (tree cover, impervious surface percentage, and human footprint index) separately.

The data set included only one sample for warmer summer months (thus June, July and August in our study region). Therefore we pooled these samples with the autumn data into one summer/autumn season. An overview of predictors used in all following statistical analyses is given in Table 1.

2.4.2. Analyses of helminth species richness

We estimated species richness of helminths within each fox using

Table 1

Predictor variables used in the statistical analyses throughout this study.

Variable level	Name	Range
Environmental (external)	Administrative area of origin	Categorical: urban Berlin, rural Brandenburg
	Human footprint index[a]	Cont. [5–46] in 1 km radius around sampling site
	Tree cover[a]	Cont. [0.08–84.34 %] in 1 km radius
	Impervious surface[a]	Cont. [0–83.37 %] in 1 km radius
Seasonality	Season	Categorical: Spring, summer/autumn, winter
Host intrinsic	Sex	Categorical: female, male
	Weight	Cont. [2.4–8.4 kg]
Helminth traits	Transmission type	Categorical: trophic, other (non-trophic)
	Host range	Categorical: moderate (specific to the taxonomic family of the host), wide (not specific to taxonomic family)
	Life cycle	Categorical: One, two or three hosts
	Zoonotic potential	Categorical: yes, no

[a] These variables were analysed in separate models, as alternative to the categorical predictor 'Administrative area of origin'. See suppl. Fig. 1.

"Hill numbers" with levels q = 0 (Chao, 1984; Jost, 2006) in the R package 'iNEXT' 3.0.0 (Hsieh et al., 2016). We rarefied sequencing reads to assess the saturation of sequencing depths for the detection of helminth taxa. We used generalised linear models (GLMs) with the predictors listed in Table 1 to assess their effects on species richness (the same predictors were investigated throughout analyses described below).

2.4.3. Factors associated with parasite community composition and turnover

For all composition analyses, we used the helminth data from 131 foxes with at least one positive detection. We analysed community turnover using Jaccard distance to avoidestimation of abundance. Abundance is potentially affected by biases, asthe number of sequencing reads obtained from parasites of different sizes contain different amounts of DNA (Luo et al., 2023). To highlight predictor variables and helminth species contributing to community dissimilarity, we applied a non-metric multidimensional scaling (NMDS) ordination, using the R package 'vegan' v2.6–4 and the function "envfit" to fit covariates to the ordination surface. We performed a PERMANOVA analysis (Anderson, 2017; Anderson and Walsh, 2013) using the function "adonis2" to discern significant variables driving community differences, i.e. using the Jaccard dissimilarity index as response.

We further analysed the effects of the predictor variables (Table 1) on community composition using hierarchical joint species distribution models (jSDMs) (Ovaskainen et al., 2017; Pollock et al., 2014), implemented in the R package 'Hmsc' v3.0–13 (Tikhonov et al., 2020). With this model we further analysed the community responses at two additional levels: the species-specific response and the trait response. We used presence/absence data and a 'probit' distribution, while accounting for spatial autocorrelation of the samples in the random structure of the model (Tikhonov et al., 2020). Prevalence is defined as the number of hosts infected by a particular parasite devided by the number of sampled host (Bush et al., 1997). This is equivalent with the probabilty that a newly sampled host would be infected. The probabilty estimated in jSDMs can thus be interpreted as "inferred prevalence" for a specified configuration of predictor variables. We use the term prevalence for jSDM results to highlight the conceptual concordance of prevalence and probability estimates. We restricted the helminth data in jSDM analyses to those 9 genera with at least 10 % "basic" prevalence (within all samples, irrespective of predictor variables) to ensure model convergence. Sample location (latitude and longitude) was included as a random effect to control for potential spatial autocorrelation in the data based on distances between sampling locations and to provide a measure of clustering in space.

We ran the jSDMs using non-informative priors (default option) in the 'Hmsc' package, and performed 210,000 Markov chain Monte Carlo (MCMC) iterations with three chains. We removed first 10,000 iterations as burn-in, and we used a thinning rate of 10, obtaining 20,000 posterior samples per chain (60,000 posterior samples in total). We evaluated model convergence visually by plotting the chains and using Gelman-Rubin diagnostics (Gelman and Rubin, 1992). We assessed the explanatory power of the model by computing the area under the curve (AUC) value for the occurrence of each helminth and averaged it across all helminths. Additionally, we applied variance partitioning to calculate the relative importance of the variables.

3. Results

3.1. Helminth detection

We obtained 6,115,166 high quality sequencing reads for 21 universal amplicons (COI, 18S and 28S rRNA; suppl. Table 1). In 141 red fox samples, we identified 762 different amplified sequence variants (ASVs) for nematodes and platyhelminthes. We assigned these ASVs to 75 genera by automated taxonomic annotation, out of which 16 were fox-

infecting helminths detected by 403,843 sequencing reads. The other 59 genera of nematodes and platyhelminthes were considered part of the diet or accidentally ingested (e.g. plant parasitic and soil-dwelling nematodes).

One hundred and thirty one of the 141 red foxes (93 %) were infested with at least one parasitic helminth. Table 2 gives an overview of the prevalence of all recorded helminths in the rural and urban administrative areas. Overall, 9 out of 16 genera had prevalence higher than 10 %, while seven genera showed relatively low prevalences of under 5 %.

Sequencing depth (with a mean of 40,116 reads across samples) was not impacted by any of the technical or biological characteristics associated with the sample (suppl. Fig. 2). Rarefaction analysis showed that sampling bias (e.g., low DNA recovery for individual samples leading to insufficient sequencing depth) did not impact the detection of helminths in our samples, as even with the minimum number of 3320 sequence reads the rarefaction reaches a plateau for helminths (Fig. 2a). We observed a maximum richness of eight helminth species in one animal, and five animals were infected with seven species.

3.2. Effects of urbanisation, seasonality and host-intrinsic factors on helminth species richness

The weight of the fox carcass was overall the strongest predictor for helminth species richness, as heavier foxes had less species rich communities (Table 3, Fig. 2b). We also detected a seasonality effect, as foxes found in winter and spring seasons showed significantly elevated helminth species richness over those sampled in summer and autumn. Also male foxes carried a slightly but significantly higher species richness of helminths. We tested for potential bias of these species richness estimates by carcass autolysis, in which the availability of DNA for helminth detection would be impacted by autolysis of a deteriorating sample. The sample classes with higher helminth species richness (sampled in winter and lighter carcasses) were associated with more autolytic decomposition stages, making such bias very unlikely (suppl. Fig. 3 a-b). We note, however, that slightly autolytic carcasses tended to be lighter (suppl. Fig. 3 c). No sample bias towards carcasses at different decomposition stages was detected between urban and rural foxes.

We did not detect significant differences in helminth species richness between administrative areas of rural Brandenburg and urban Berlin when analysed either as binary variables (administrative areas) or using continuous environmental predictors for the urbanisation gradient, namely the proportions of impervious surface or the tree cover and human footprint around the sampling site (Table 3).

Table 2
Prevalence of helminths found in this study, sorted by total helminth prevalence in descending order. Echinococcus multilocularis was not detected.

| Genus	% Prevalence Berlin (n = 98)	%Prevalence Brandenburg (n = 43)	% Prevalence Total (n = 141)	95% confidence interval in %
Angiostrongylus	80.61	37.21	67.38	±7.74
Eucoleus	54.08	69.77	58.87	±8.12
Uncinaria	46.94	60.47	51.06	±8.25
Crenosoma	32.65	20.93	29.08	±7.5
Mesocestoides	21.43	46.51	29.08	±7.5
Alaria	7.14	37.21	16.31	±6.1
Toxocara	16.33	11.63	14.89	±5.88
Clonorchis	8.16	25.58	13.48	±5.64
Strongyloides	4.08	25.58	10.64	±5.09
Ancylostoma	3.06	6.98	4.26	±3.33
Pearsonema	4.08	4.65	4.26	±3.33
Toxascaris	0.00	9.30	2.84	±2.74
Taenia	3.06	0.00	2.13	±2.38
Brachylaima	2.04	0.00	1.42	±1.95
Opisthorchis	1.02	2.33	1.42	±1.95
Aelurostrongylus	0.00	2.33	0.71	±1.39
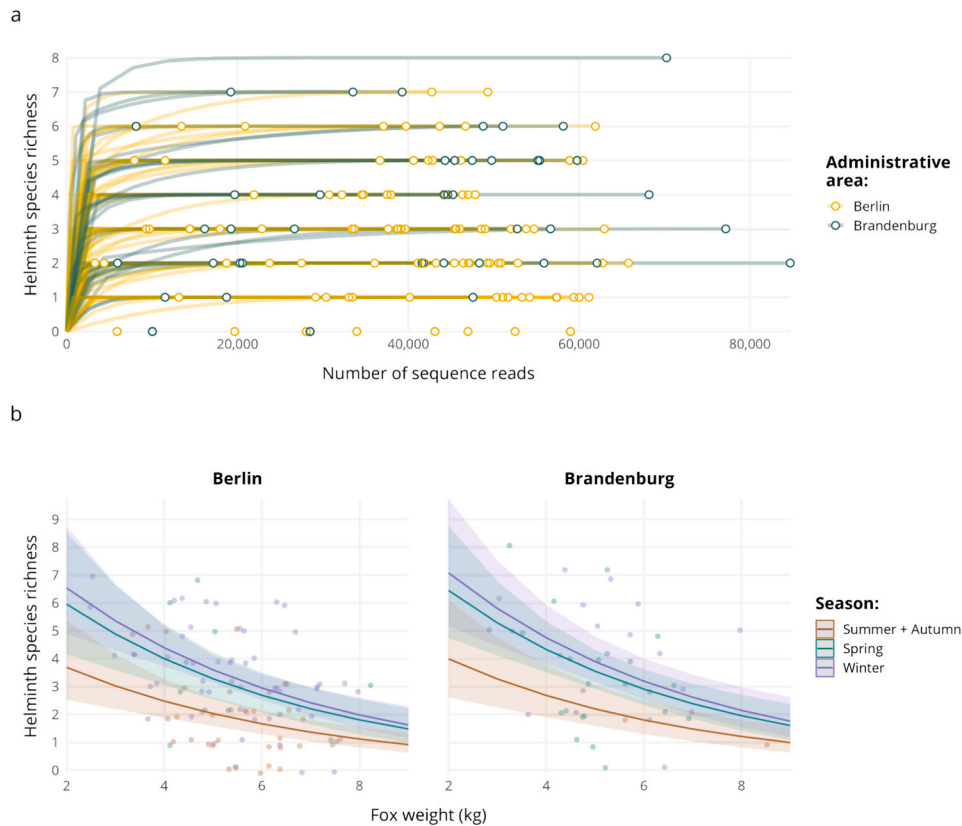
Fig. 2. Association between helminth species richness, body weight and seasonality a) Rarefaction analysis shows that sequencing depth (sampling) for helminths is saturated in our dataset. b) Helminth species richness (Hill number q = 0) was assessed for the effects of host-intrinsic and environmental predictors. Predictions from a Poisson generalised linear model (lines, with respective 95 % confidence intervals; full model details in Table 3) show that with increasing body weight and in summer the helminth species richness decreases in foxes.

Table 3

Linear models explaining helminth species richness by host and environment. Coefficients of the poisson model are given with 95 % confidence intervals in brackets.

	Dependent variable			
	Estimator			
	(1)	(2)	(3)	(4)
Area Brandenburg	0.080 (0.117)			
Imperv 1000 m		−0.001 (0.002)		
Tree cover 1000 m			0.002 (0.003)	
Human fpl 1000 m				−0.001 (0.005)
Weight kg	−0.199*** (0.042)	−0.200*** (0.042)	−0.201*** (0.042)	−0.202*** (0.042)
Sex male	0.173 (0.111)	0.177 (0.111)	0.188* (0.110)	0.182 (0.111)
Season spring	0.479*** (0.177)	0.501*** (0.168)	0.537*** (0.159)	0.520*** (0.168)
Seasons winter	0.572*** (0.143)	0.569*** (0.145)	0.580*** (0.142)	0.584*** (0.142)
Constant	1.591*** (0.245)	1.664*** (0.267)	1.535*** (0.262)	1.648*** (0.325)
Observations	141	141	141	141
Log Likelihood	−260.135	−260.195	−260.099	−260.342
Akaike Inf. Crit.	532.271	532.390	532.198	532.684

* $p < 0.1$.

*** $p < 0.01$.

3.3. Helminth community turnover between urban and rural areas

We observed strong compositional differences (community turnover) between urban Berlin and rural Brandenburg, as helminth communities showed clear differences according to the administrative area the samples originated from (Fig. 4; Table 4).

Together, all assessed predictors explained 16.7 % of the variance in the helminth community (PERMANOVA). The administrative area where the fox carcass was found was a significant predictor of the helminth community, explaining 4.7 % of the variance (Table 4) and pointing to community turnover between urban Berlin and rural Brandenburg. Seasonality affected the composition significantly at a similar effect size of 4.1 %. Fox weight explained a lower proportion of the variance in helminth community composition at 2.7 %, but this effect was still significant. Similarly, the sex of the fox had a small (1.7 % of variance) but significant effect on the helminth community. Continuous environmental gradients did not explain more of the variance in composition than the categorical administrative area difference.

3.4. Influence of predictors on parasite community composition and trait distribution

The jSDM analysis based on a fully converged model with an average AUC value 0.87 showed that host-intrinsic factors (weight and sex) had substantial effects on the helminth community composition, explaining 27 % and 11 % of the variance, as a mean across helminth genera, respectively. The effects of administrative area (28 %) and seasonality (27 %) also had a similarly large effect (Fig. 5).

Both host intrinsic factors and the natural environment impacted helminth occurrence. For example, >50 % of the explained variance for
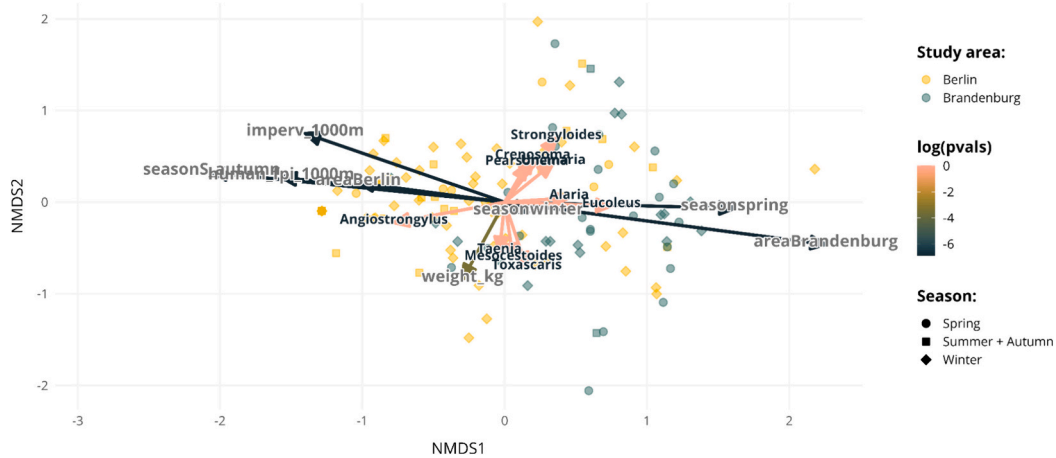
Fig. 4. Community composition and its drivers. Non-metric multidimensional scaling (nMDS) "biplot" showing placement of fox samples ("scores") and the contribution of variables ("loadings"). Sampling units (red fox samples) are displayed by circles, triangles or squares to indicate different sampling seasons; the colour of circles (yellow = urban Berlin, blue = rural Brandenburg) represents the administrative area of red fox sampling origin. The closeness of samples indicates similarity in their helminth community. Only variables and contributing taxa with a significant effect in a permutation test are presented. Line lengths along ordination axes indicate the effect sizes, colour indicates significance levels (*P*-values, log transformed "log(pvals)", with darker/ black colours indicating more significant findings). Eight of sixteen species of helminths correlate significantly with community composition. The origin from different administrative areas, together with seasonality, are drivers for turnover, visible as a separation of samples along nMDS axis 1.

Table 4
PERMANOVA results for helminth species turnover.

	Df	Sum of Squares	R2	F	Pr(>F)
Area	1	1.625	0.047	7.116	0.001***
Weight	1	0.932	0.027	4.078	0.001***
Sex	1	0.587	0.017	2.571	0.015*
Season	2	1.418	0.041	3.104	0.001***
Residual	125	28.55	0.832		
Total	130	34.311	1		

*The displayed statistics are "marginal contributions" for focal factors within the "background" of the remaining model.
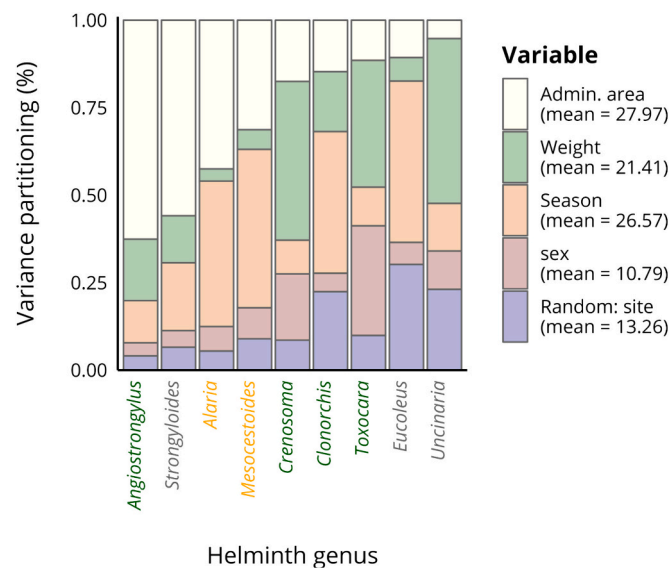

Fig. 5. Variance partitioning plot showing the amount of variance explained by each variable group in the jSDM model. Helminth taxa (> 10 % prevalence) have been ordered by decreasing values of variance explained by the administrative area (urban Berlin vs. rural Brandenburg). Helminth names are colour-coded to represent one-host transmission -grey-, or multi-host (trophic transmission): two-host -green-, three-host -orange-.

Crenosoma presence was related to host weight and sex, while only ~10 % was explained by the combined variables administrative area and seasonality. On the other hand, *Angiostrongylus* presence was explained to ~50 % by the variables of administrative areas and seasonality and only to ~20 % by host intrinsic factors of weight and sex. These results indicate specific effects on helminth species to arise either more from the outside environment or the host, not both (or one variable from each category): either weight and sex have a large influence (*Crenosoma*, *Toxocara* and *Uncinaria*) or administrative area and seasonality (*Angiostrongylus*, *Strongyloides*, *Alaria*, *Mesocestoides*, *Clonorchis* and *Eucoleus*).

Reduced carcass weight was associated with higher prevalence of *Uncinaria*, *Toxocara*, *Crenosoma* and *Angiostrongylus* (the jSDM estimates are probabilites of infection, equivalent ot a proportion of infected individuals in a sample and can therefore be understood as inferrential prevalence estimates; compare also with basic prevalence estimates in Table 2). The spring season was associated with higher prevalence of *Clonorchis* and *Alaria*, the winter season with *Mesocestoides*, *Eucoleus* and *Alaria* (Fig. 6). No helminth was found in increased prevalence in summer. *Agiostrongylus* and *Crenosoma* were significantly associated with the more urbanised environment in the administrative area of Berlin. At the same time, *Alaria*, *Eucoleus,* and *Strongyloides* were found to have increased prevalence in the rural administrative area of Brandenburg. Parasites that depend on two hosts were negatively related to the rural Brandenburg area (Fig. 7).

Using environmental gradients (human footprint index, tree cover and impervious surface; Table 1) instead of categorical administrative area did not result in overall better models (suppl. Figs. 4, 5 and 6). For at least one taxon, however, we found more nuanced effects for the detailed gradients: *Angiostrongylus* (associated with urban Berlin as administrative area) showed a positive association with a high human footprint, but, surprisingly, increased tree cover also had a positive effect (suppl. Fig. 5).

4. Discussion

We examined how anthropogenic, seasonal, host- and helminth-intrinsic factors affect helminth communities of red foxes along a rural-urban gradient. We found no effect of urbanisation on helminth species richness, but an evident turnover in community composition associated with urbanisation. The hypothesis that helminths with
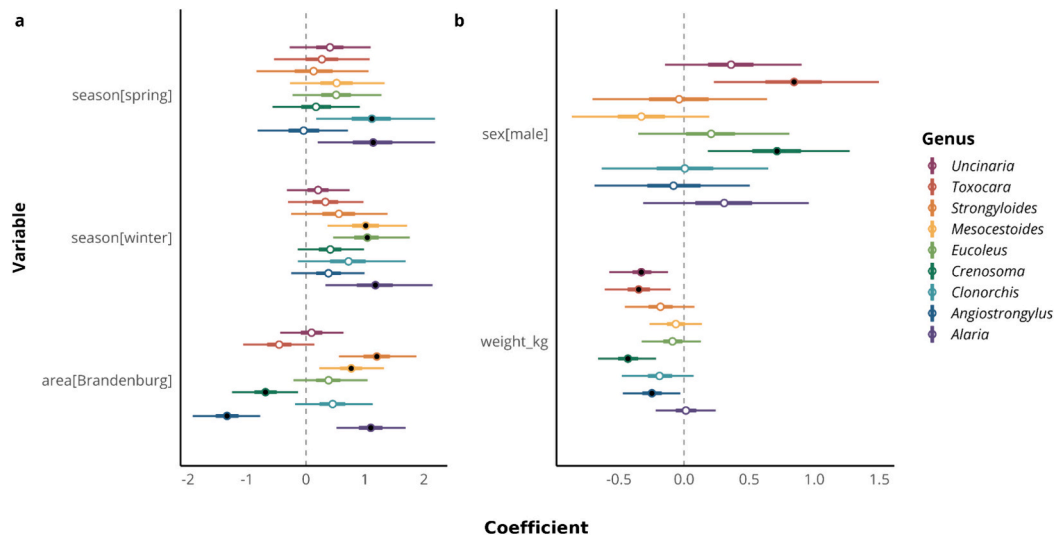
Fig. 6. Effect of host-intrinsic, seasonal and environmental factors on the occurrence of helminths. For effects of helminth traits see Fig. 7. X-Axis: Coefficients of the explanatory variables in the jSDM for the different helminth taxa. Explanatory variables are presented in two panels: a) Season and administrative area; b) Host intrinsic variables sex and weight. Lines indicate the 95 % posterior probability of the estimate in the model; for significant estimates, these lines do not overlap zero and points are filled in black. All effects are displayed contrasted against estimates for samples from female foxes in Berlin in the summer season.
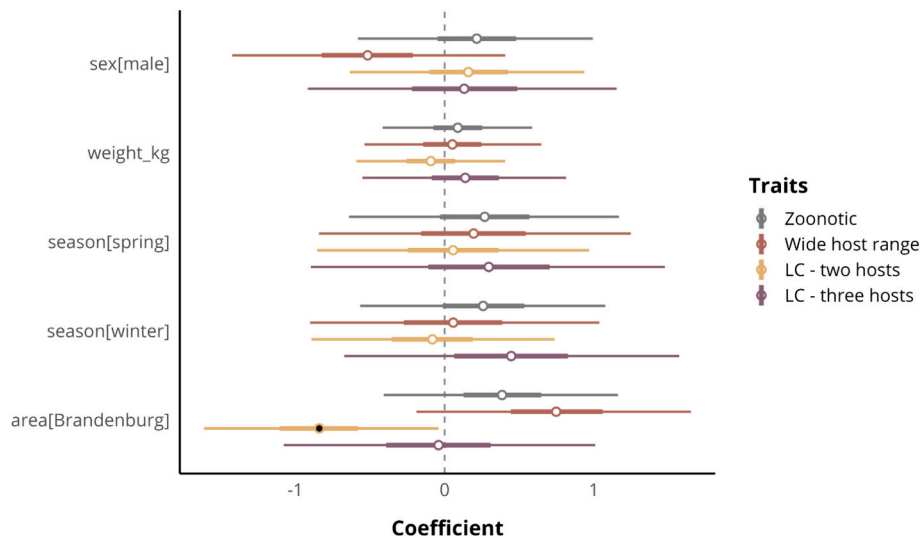
Fig. 7. Effect of helminth traits on the occurrence of helminths according to environmental and host-intrinsic variables. Coefficients of the explanatory variables in the jSDM for the different helminth taxa are summarised for traits of the helminth species. Lines indicate the 95 % posterior probability of the estimate in the model; for significant estimates, these lines do not overlap zero, and points are filled in black. LC: life cycle. A two-host life cycle is associated with significantly reduced occurrence in the rural area of Brandenburg.

complex life cycles should be more affected by the external environment while those with direct life cycles should be more affected by their host was not supported. Surprisingly, we found trophic transmission in two-host life cycles to occur more in the urban than in rural areas. For our study area, this refutes the hypothesis that rural areas would support more trophic transmission, which was proposed to explain patterns of helminths in foxes in Geneva (Reperant et al., 2007). This is also contrasting to the pattern of higher urban prevalence of helminths with complex life cycle found in a meta-analysis of helminth prevalence across multiple host species (Werner and Nunn, 2020).

4.1. Urbanisation and other environmental effects

Definitions of "urbanisation" are discordant across the literature (French et al., 2022), and hence no commonly agreed-on environmental

variables capture the phenomenon. Here, we tested the readily available measures of tree cover and impervious surface, as well as a human footprint index, capturing multiple sources of environmental change associated with human presence and activity. It is noticeable that some parasites occur preferentially in one of the two areas and thus the prevalence differs clearly between urban and rural areas (e.g. *Alaria* and *Strongyloides*). Intriguingly, throughout our analyses, the administrative areas of rural Brandenburg and urban Berlin provided similar explanatory value compared to these more sophisticated environmental gradients. This can have multiple reasons: first, the binary classification could act as a proxy for multiple variables that are difficult to measure in the field but that could capture the needs of helminths for their transmission. For example, the availability or density of intermediate hosts, such as arthropods, might be different in rural and urban areas (Chatelain et al., 2023; Planillo et al., 2021; Van Nuland and Whitlow, 2014).

Secondly, the spatial scale (approx. 3 km^2) we used to extract environmental variables around fox carcasses might not reflect a suitable environmental context, as some individuals might have been dispersing when they succumbed to e.g. traffic accidents and were sampled. Thirdly, each helminth species has different intermediate hosts, and this would require separate, detailed hypotheses for each taxon. It might be hard to capture these hypotheses within one general pattern for the entire helminth community when contrasted against general environmental gradients. As a final reason for the prevailing effect of administrative areas, these might reflect population differentiation in urban vs. rural foxes through behaviour, which cannot be captured by environmental variables (Kimmig et al., 2020). Generally, similar observations have been termed "urban filters" and can lead to abrupt changes in response to a gradient, which in turn might be better captured by a binary variable (Aronson et al., 2016). Therefore, the binary variable of the administrative area might be a sufficient predictor in our model, although the environmental gradients might still refine results for specific taxa. In this regard, we found clear occurrence patterns for helminth taxa in our analysis, which are consistent across the two sets of variables. *Strongyloides*, for example, was positively associated with the rural Brandenburg area, and lower values of human footprint. *Angiostrongylus* was associated with urban Berlin in the binary administrative area approach, which was then refined by the environmental gradient analysis. Interestingly, the latter showed a consistent positive effect of human footprint but also a positive association with tree cover. *Angiostrongylus* is prevalent in areas with high human disturbance and with trees, which are associated with resources - food or resting sites - for the fox. This might point to a taxon-specific generally positive effect of urban green spaces on host and pathogen prevalence (cf. also Gecchele et al., 2020; Gras et al., 2018).

Seasonality had a large impact on helminth species richness, as in winter and spring more diverse communities were observed than in summer and autumn. An explanation for this could be seasonal patterns in climatic conditions, e.g. rainfall or temperature, affecting parasite survival and transmission. Such processes are well described generally (Shearer and Ezenwa, 2020) and specifically for fox parasites (Arcenillas-Hernández et al., 2022). We could expect such effects to primarily act on species with infective stages accumulating in the outside environment. Our results, however, showed that the prevalence of helminth species is affected by seasonality in general and not for helminth species with particular traits. This might indicate a general negative influence of adverse climatic conditions in winter and spring on the host, as they are associated with demanding life history stages of the foxes (Iossa et al., 2008) and reduced food availability at the same time, which could lead to a weakened physiological state (Halvorsrud, 2014). Stress responses and allocation of energy to vital processes such as movement and reproduction (allostatic load and allocation trade-offs) (Korte et al., 2005) would then affect infections with helminth parasites, making them more prevalent during the "hard times" of the year. Because of these reasons, it is conceptually difficult to discern body weight (or even body condition; see below) effects on helminth richness from potentially intertwined effects of seasonality. A future avenue of research would be analysing changes in host susceptibility and effects on single helminth species' transmission mechanisms along seasons.

4.2. Host-intrinsic effects

Lighter carcasses showed increased helminth species richness. The sampled fox carcasses' weight is only a proxy of the weight of the living animal, as it was also reduced by decomposition prior to sampling. We remark that the association of more autolytic carcasses with both reduced weight and winter and spring seasons might reduce our ability to differentiate the effects of seasonality from those of body weight. While body weight is not necessarily a direct reflection of body condition and health (Barnett et al., 2015), weight may be impacted by helminthic infections or vice versa; helminths could infect primarily weak,

susceptible individuals. In a recent meta-analysis, such negative associations between body weight and helminth loads have been shown robustly across study systems, albeit with lower support in carnivores (Shanebeck et al., 2022). Reduced body weight—if it reflected diminished body condition—and diverse coinfections might be both a cause and a consequence in a "vicious circle" (Beldomenico et al., 2008). The opposing hypothesis of carrying capacity limiting infections with parasites (Hechinger, 2013) and larger animals thus carrying more species, was clearly not supported in our data.

To differentiate the effects of seasonality and host weight on helminth species richness, a finer control of decomposition status is required in future studies. Additionally, structural measurement of foxes' body size and variations in their age should be taken into account to differentiate age and life history effects on weight from health effects on body condition (Stevenson and Woods Jr., 2006). For the present work, we could not fully disentangle the effects of weight and seasonality and could not link them directly to body condition. However, we were able to differentiate those effects from the effects of urbanisation.

Interestingly, *E. multilocularis* and *Taenia* spp. are less prevalent in the city of Geneva than in surrounding areas. As they also detected fewer rodent intermediate hosts in stomach contents, Reperant et al. (2007) concluded that trophic transmission in complex life cycles might be less likely in urban areas. Our study, in contrast, found a higher prevalence of trophically transmitted parasites with two hosts, namely *A. vasorum and C. vulpis*, in the city of Berlin; *E. multilocularis* was not present. Such disparate results across studies can be reconciled: only the overarching conclusions seem contradictory, while the basic results (species identities) are not comparable. The obligatory intermediate hosts of "Berlin's city helminths" *A. vasorum* and *C. vulpis* are a large variety of invertebrates (Suppl. Table 3), definitely present in urban areas. The clustering of resources in urban areas (Scholz et al., 2020) might then result in dense populations, leading to elevated predation and trophic transmission. Consequently, higher prevalences and occurrences of even trophically transmitted helminths can be observed in the urban area.

5. Conclusion

In conclusion, we found turnover of the helminth community between foxes from urban and rural areas, while species richness remained stable. The individual species' identities underlying this turnover are specific to our study area and different from those previously reported in other urban-rural comparisons in central Europe (Reperant et al., 2007; Azian et al. 2008; Gecchele et al., 2020). Our findings are incompatible with a breakdown of trophic transmission cycles in Berlin, especially for parasites with invertebrate intermediate hosts. We found environmental effects on helminth community composition and host-intrinsic factors impacting both composition and species richness. Overall, pathogen communities along urban/rural gradients should be studied considering seasonality and both host and parasite traits to prevent zoonoses.

Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2024.173355.

CRediT authorship contribution statement

Carolin Scholz: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation. **Víctor Hugo Jarquín-Díaz:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Aimara Planillo:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Viktoriia Radchuk:** Writing – review & editing, Supervision, Conceptualization. **Cédric Scherer:** Writing – original draft, Visualization, Validation. **Christoph Schulze:** Writing – review & editing, Resources, Data curation. **Sylvia Ortmann:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Stephanie Kramer-Schadt:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources,

Data availability

Raw sequencing data is available under BioProject PRJNA386767 in the NCBI Short Read Archive (SRA). All code and metadata for the analysis described here is available at https://github.com/VictorHJD/AA_Fox/ (non-static version under development) and archived at Zenodo (static version; https://doi.org/10.5281/zenodo.11198275).

Code availability

All code for the analysis described here is available at https://github.com/VictorHJD/AA_Fox/ (non-static; version under development) and archived at Zenodo (static version; https://doi.org/10.5281/zenodo.11198275).

Benefits generated

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

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