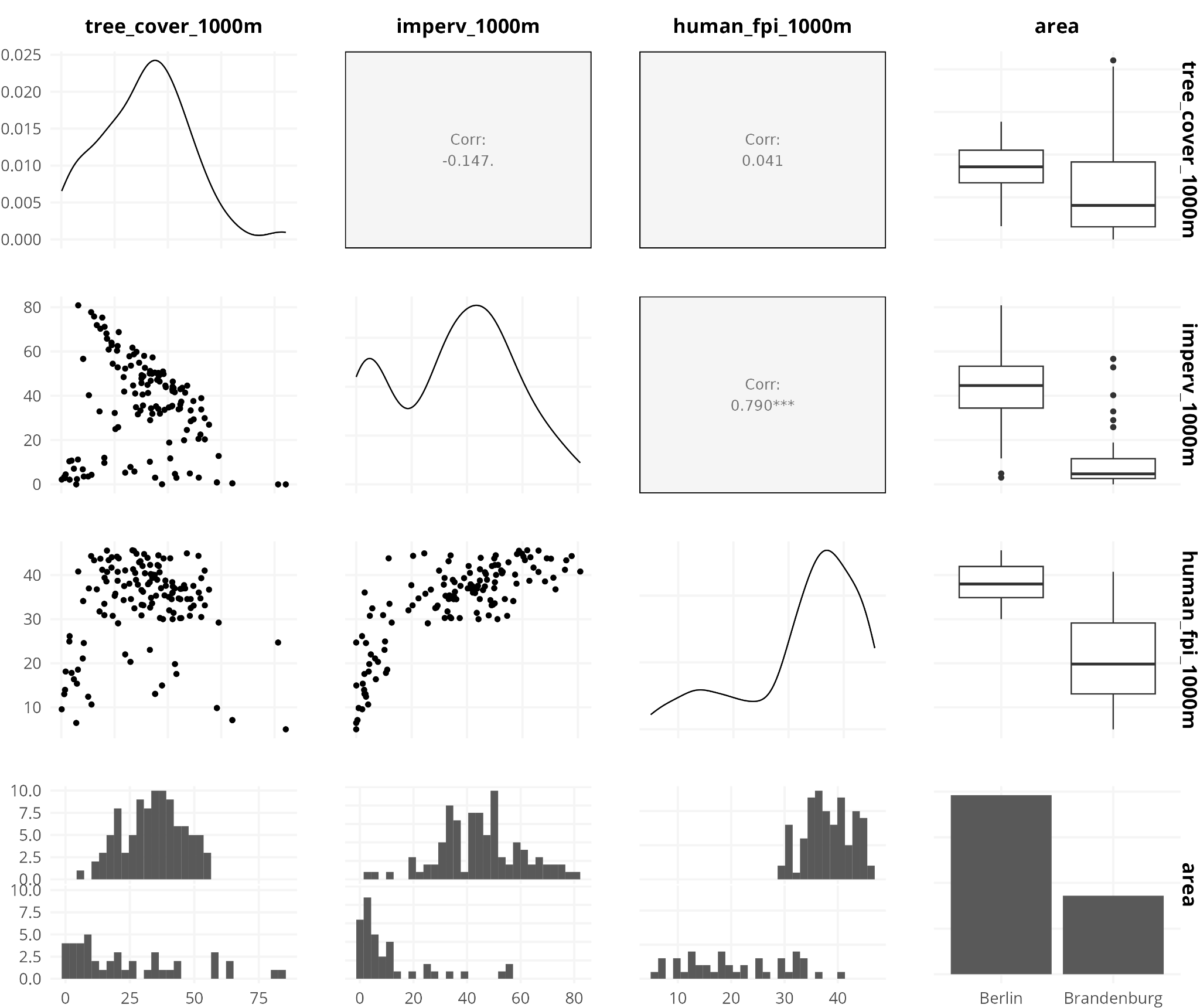
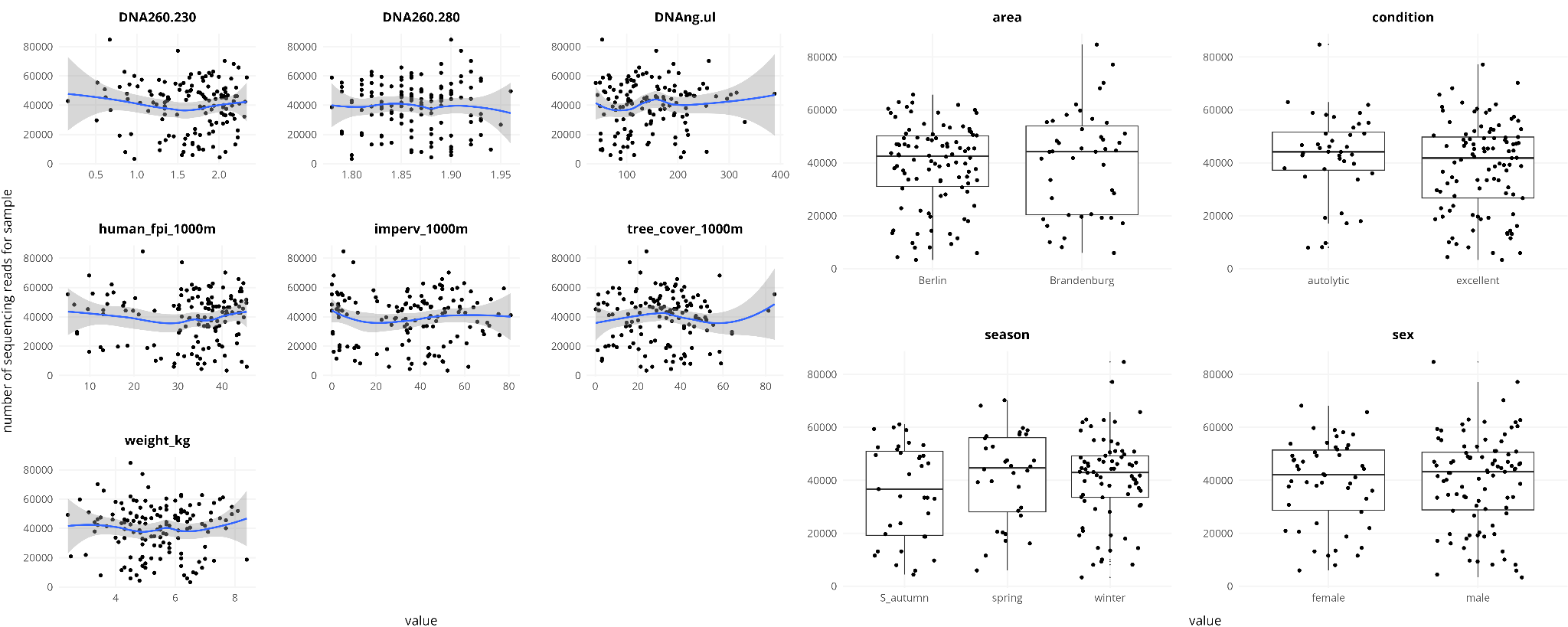
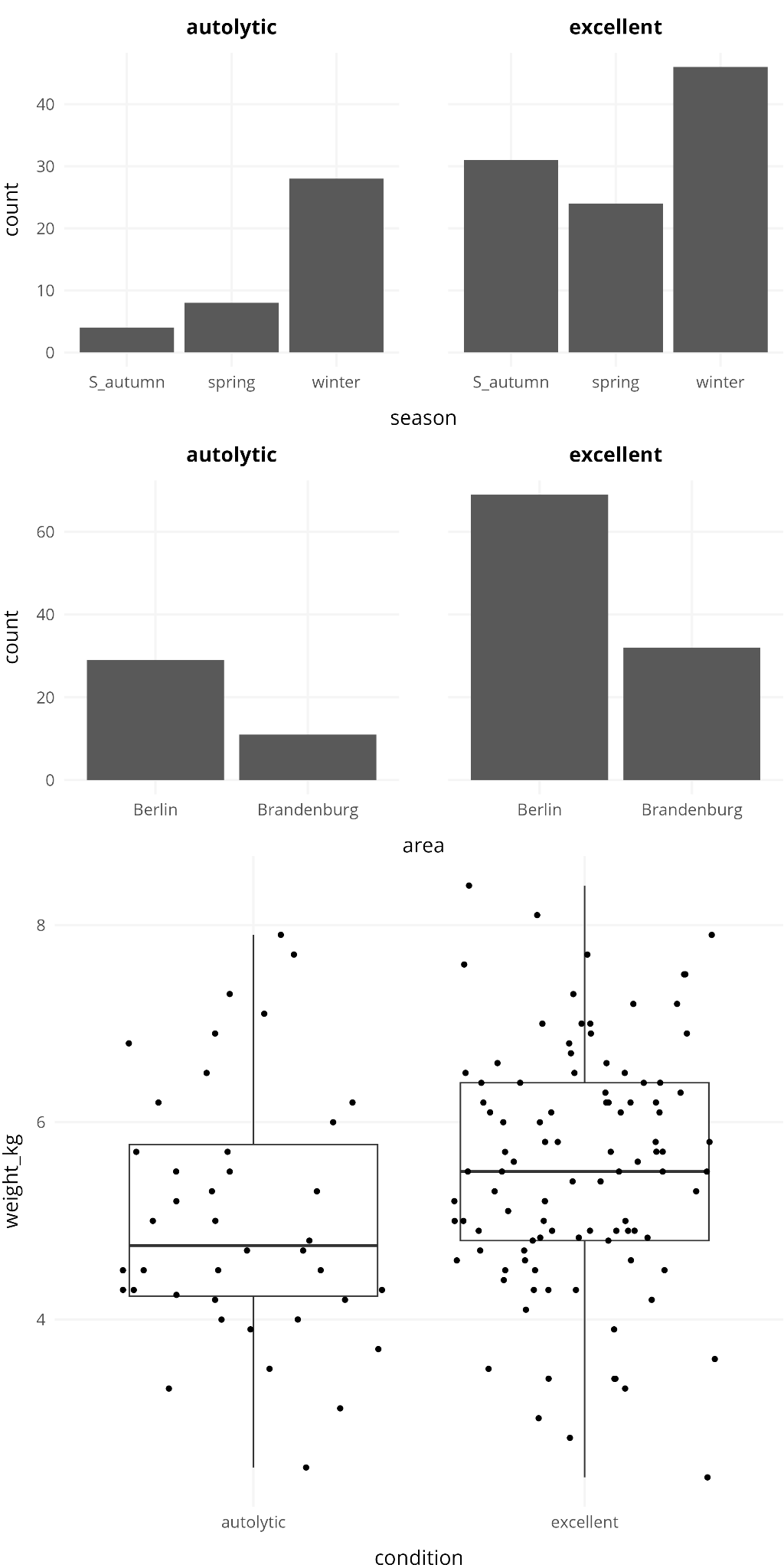
**Suppl. figures** (suppl tables 1 and 2 are available as files only due to their size).

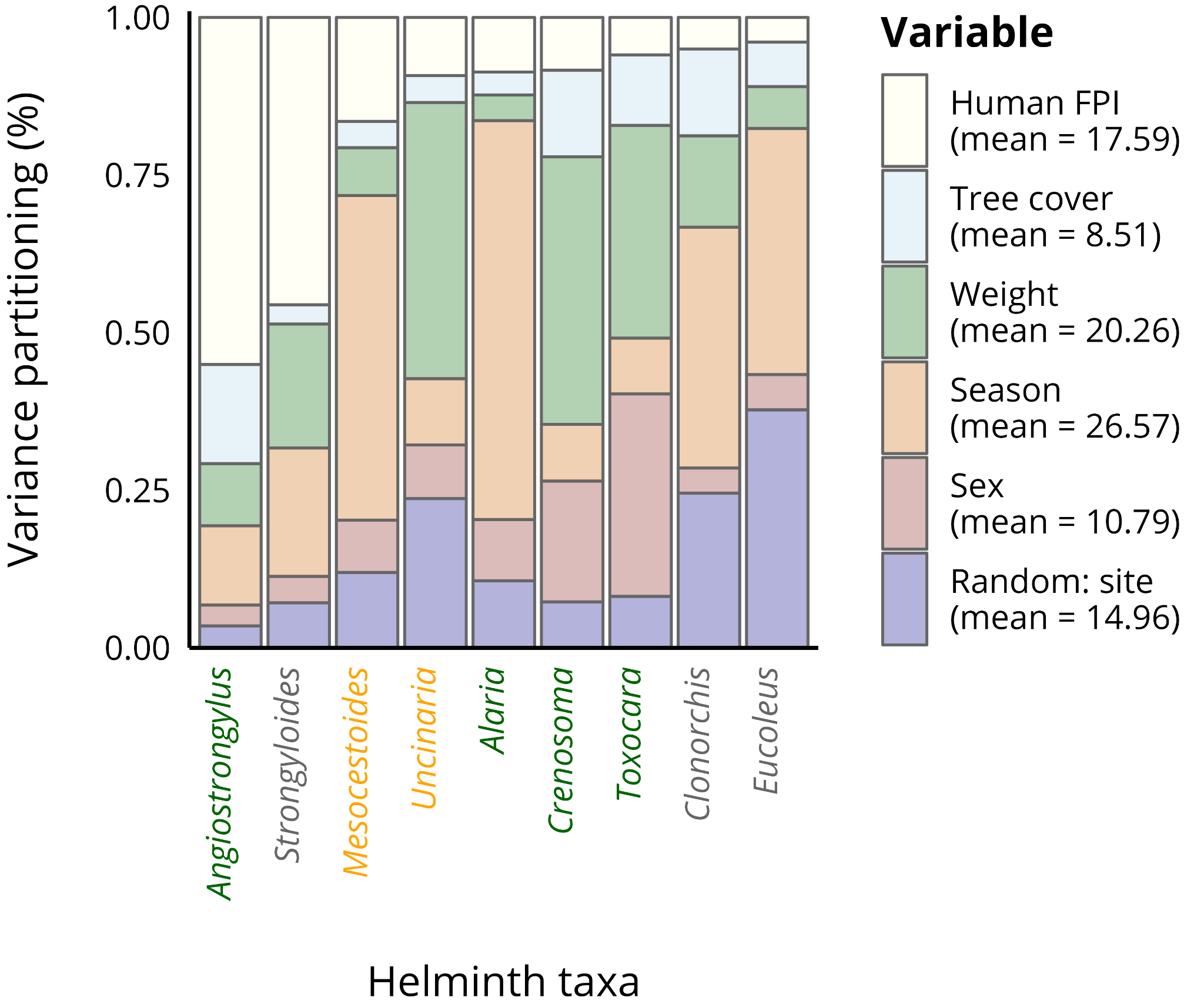


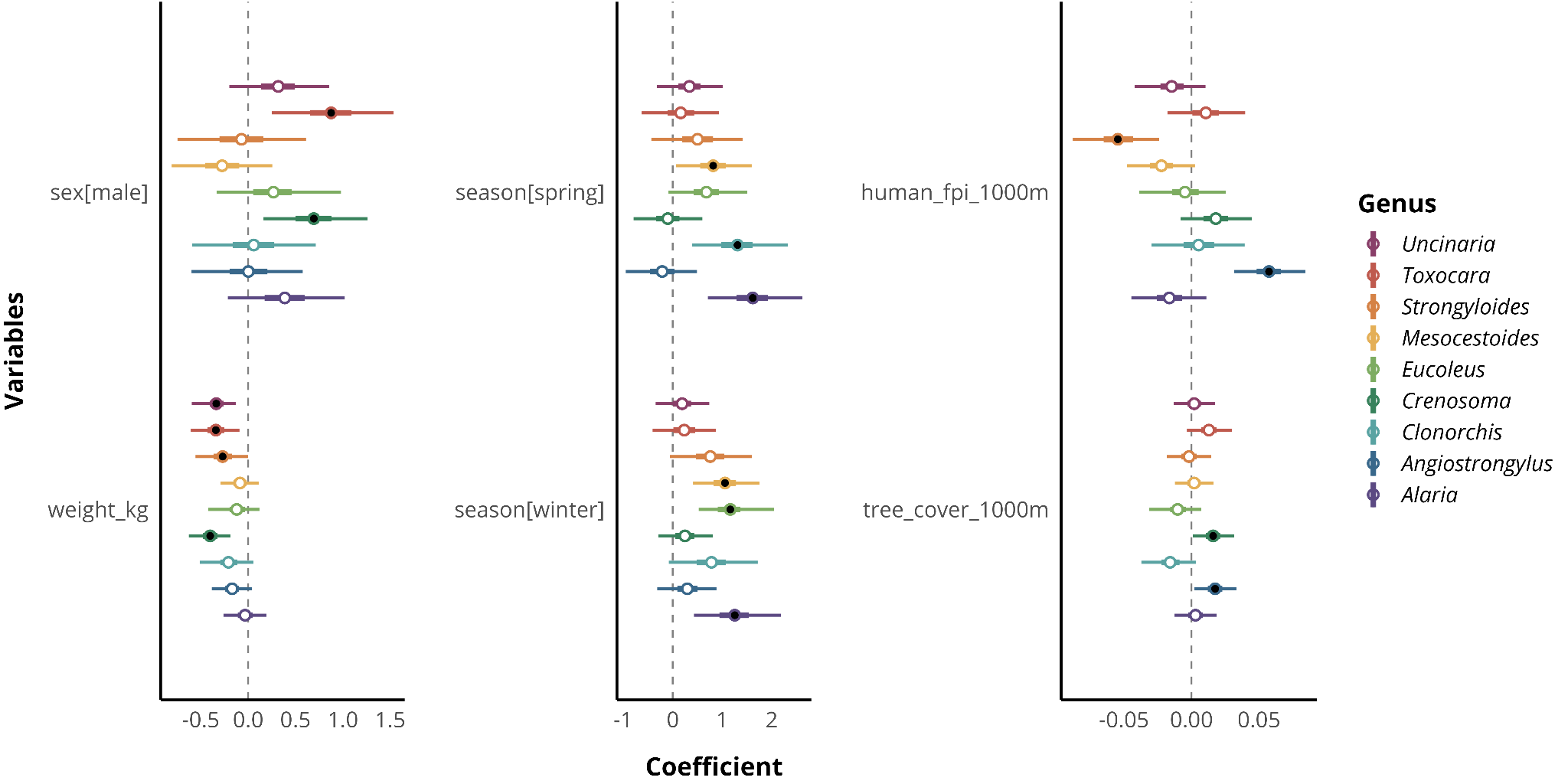
Suppl. figure 1 - Correlation between continuous variables for urbanisation and association with administrative area. Impervious surface and human footprint index are strongly correlated. All three continuous variables are strongly associated with differentiation between the rural and urban administrative area.



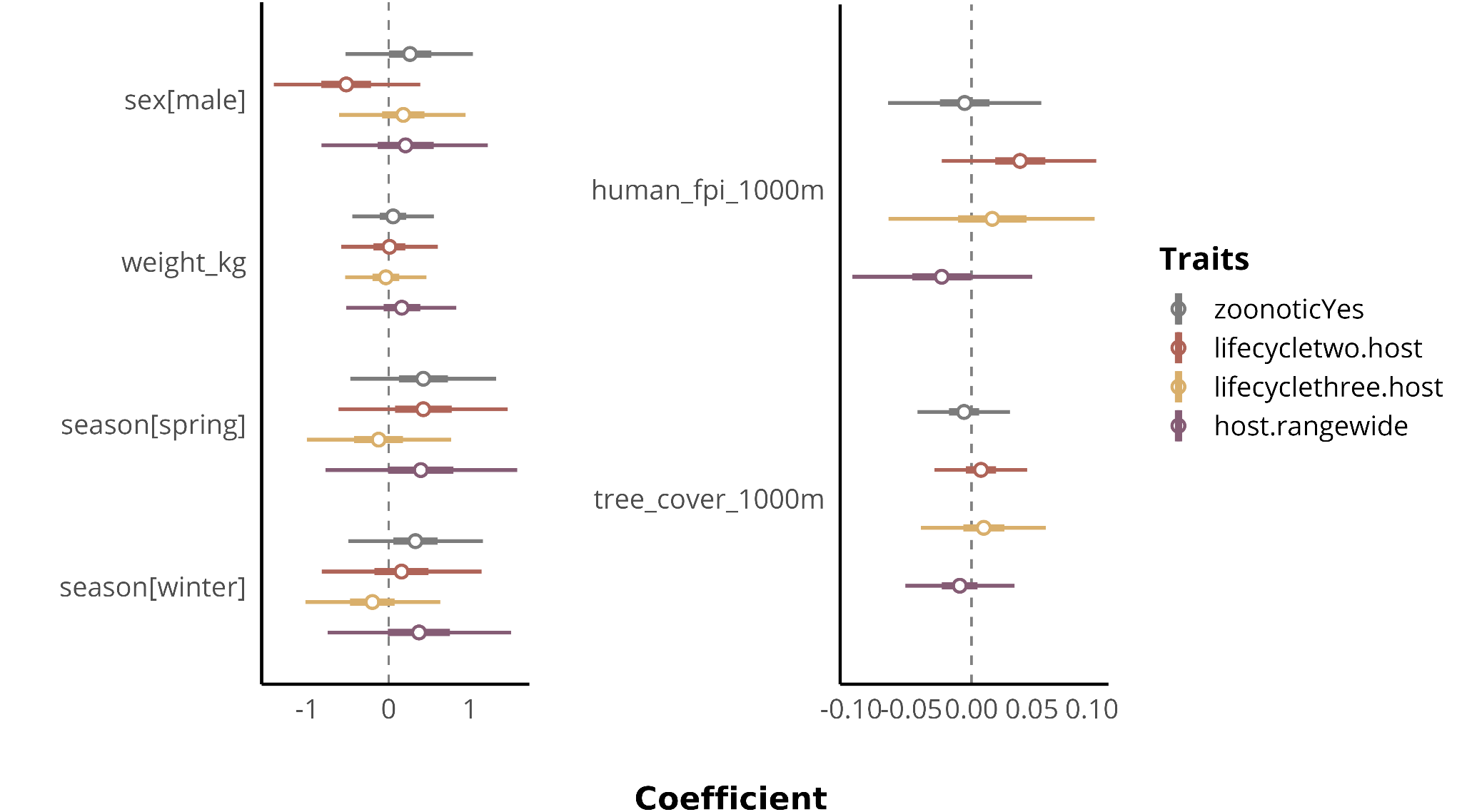
Suppl. figure 2 - Correlation or association of sampling depth (the number of sequencing reads recovered) with technical or biological variables. Sequencing depth is not associated or correlated with any of the technical (DNA quality and quantity) or biological (as reported in the manuscript) variables.

Suppl. figure 3 - Association of season, weight and administrative area with sample decomposition status. Slightly autolytic carcasses were sampled at higher proportions in winter and were lighter than excellently preserved carcasses. No difference in decomposition status was detected between administrative areas.

Suppl. figure 4 - The continuous environmental variables “human footprint index” and “tree cover” explain variance in helminth composition similarly to differences in administrative areas (see Figure 5).



Suppl. figure 5 - Host-intrinsic, seasonal and environmental factors affect the occurrence of helminths. Coefficients of the explanatory variables in the jSDM for the different helminth taxa are given. Environmental explanatory variables add some information i.e. *Crenosoma* and *Angiostrongylus* are associated with higher tree cover and *Angiostrongylus* and *Strongyloides* with higher and lower human footprint index, respectively. Compare this with figure 6, in which general effects of administrative areas are presented.

Suppl. figure 6 - Helminth traits are not significantly associated with any variables in this model. This does not provide increased resolution over an analysis of administrative area effects (compared with Figure 7).

Suppl. Table 3 - Prevalent helminth species detected in this study in their specific epidemiological context

|  |  |
| --- | --- |
| *Angiostrongylus vasorum* (67.4% prevalence). | Infection of definitive hosts (foxes or dogs) occurs by ingesting third larval stages while feeding on infected intermediate hosts (slugs or snails) or paratenic hosts (amphibians, birds and rodents) [(Mozzer and Lima 2015)](https://www.zotero.org/google-docs/?mi28j8). This relatively complex life cycle would predict strong effects of the natural environment and increased parasite occurrence in more biodiverse areas. However, we found the opposite: this trophically transmitted parasite was more abundant and prevalent in the city. The species is indeed the main driver for the overall effect of more pronounced trophic transmission of helminths with two-host life cycles in the city than in the rural area. Given observations of increasing prevalence and distribution of the species in recent times [(Gillis-Germitsch et al. 2020; Fuehrer et al. 2021)](https://www.zotero.org/google-docs/?Ecq6f4) and the high genetic diversity of urban populations [(Tayyrov et al. 2021)](https://www.zotero.org/google-docs/?AhWijK), we can conclude that *A. vasorum* is an urbanophile helminth with growing populations in an urbanised world. |
| *Eucoleus aerophilus* (58.9% prevalence) | Has a direct, faecal-oral life cycle and infection with *E. aerophilus* occurs through ingesting food or water contaminated with eggs [(Anderson 1999)](https://www.zotero.org/google-docs/?OPGl26). The larvae hatch in the intestines and enter the lungs via the bloodstream. The lung hairworm causes disease symptoms, such as bronchitis with coughing in severe infestation with accompanying bacterial infections [(Spratt 2002)](https://www.zotero.org/google-docs/?iivSC9). Indeed, poor body condition was not associated with this parasite. The parasite, however, showed the most substantial effects of seasonality and was significantly more prevalent in winter than in summer or spring. This is difficult to explain, as eggs need higher temperatures than usual observed in this season to develop in vitro [(Perrucci, Di Cesare, and Fichi 2014)](https://www.zotero.org/google-docs/?2ctC2h). Seasonality effects on *E. aerophilus* are one of findings contradicting the hypothesis that directly transmitted parasites should rather be impacted by the host-intrinsic traits and be less impacted by the environment. |
| *Uncinaria stenocephala* (51.1% prevalence) | Has a direct lifecycle with a faecal-oral transmission route. Among all taxa, it had the strongest association with a reduced weight of the foxes. This pattern fits with the hypothesis that directly transmitted parasites might be more impacted by host-intrinsic variables and is also supported by previous pathology reports [(Bowman et al. 2010)](https://www.zotero.org/google-docs/?K0NdnL). We found *U. stenocephala* not related to urbanisation, particularly not more prevalent in the urbanised areas, as reported previously from Geneva [(Reperant et al. 2007)](https://www.zotero.org/google-docs/?EWS4DQ). *U. stenocephala*, similar to *E. aerophilus,* also showed strong spatial effects (“random site” in Figure 6), meaning that infection might occur at geographical hotspots. The ecological or immunological drivers associated with increased occurrence of this potentially zoonotic hookworm in animals with low body weight are worth further investigation. |
| *Alaria alata* (16.3% prevalence) | Was strongly associated with rural Brandenburg and an increased prevalence was observed in winter and spring. This parasite is potentially zoonotic and has a complex life cycle with three hosts and additional paratenic hosts from a broad taxonomic spectrum [(Korpysa-Dzirba et al. 2021; Z, G, and G 2018)](https://www.zotero.org/google-docs/?O6798o). This life cycle might be better supported by intermediate and paratenic host availability in rural Brandenburg. A predisposition for the winter month might again point to increased susceptibility of foxes in winter and is more challenging to reconcile with the low availability of especially invertebrates as intermediate hosts in this season. |
| *Mesocestoides* spp. (29.1% prevalence) and *Strongyloides* spp. (10.6% prevalence) | Constitute groups of two or multiple species, respectively. Both groups showed increased prevalence in rural Brandenburg, pose the risk of zoonosis, but have strongly contrasting life cycles: *Mesocestoides* spp. is transmitted trophically in a complex cycle involving two or three hosts [(Fuentes, Galán-Puchades, and Malone 2003)](https://www.zotero.org/google-docs/?GI0Ixi), while *Strongyloides* sp. can complete development and reproduction in a single host or in the form of female pathogenesis in the outside environment [(A. F. White, Whiley, and E. Ross 2019)](https://www.zotero.org/google-docs/?JDW8DG). This shows that similar patterns of association with environmental drivers can be observed for parasites with very different traits. |
| *Crenosoma vulpis* (29.1% prevalence) and *Toxocara canis* (14.9% prevalence) | Were the parasites most strongly associated with host-intrinsic factors, i.e. with the foxes’ body weight but also (quite exceptionally among all taxa) with male sex. Associations of these species with environmental variables were less pronounced. This is despite the fact that both species are either obligatorily (*C. vulpis*) or facultatively (*T. canis*) transmitted trophically via intermediate or paratenic hosts, and have been reported to benefit from moist environmental conditions [(Arcenillas-Hernández et al. 2022)](https://www.zotero.org/google-docs/?vrxmiB). The species cause different diseases (*C.­ vulpis*: eosinophilic bronchitis, *T. canis*: toxocariasis with multiple different symptoms and sites of infections). The differences in the disease caused by these makes it remarkable that they are similarly associated with reduced weight and male sex. *C. vulpis* has been reported to cause coughing in dogs [(McGarry et al. 1995)](https://www.zotero.org/google-docs/?oZSrXT) and it seems possible that foxes suffer more pathology from these species than previously known, or that individuals in a physiologically weakened state caused by other factors (unmeasured here) are more susceptible. This is noteworthy as *T. canis* is an important zoonotic pathogen and *C. vulpis* is more prevalent in the city, despite having a life cycle with trophic transmission via an invertebrate intermediate host. |
| *Clonorchis* (13.5% prevalence) | represents a group of opisthorchiid flukes for which limited to no sequence data of related species is available for taxonomic annotation. The particular taxon we recorded is likely *Metorchis bilis* and we also detected *Ophistoris felinus* (1.4% prevalence) as a second low prevalence taxon in the group. These taxa have been reported before from our sampling area and are associated with the availability of surface water [(Schuster et al. 1999)](https://www.zotero.org/google-docs/?2Yviv8). We, however, did not detect any effect of environmental or host-intrinsic variables on these taxa. |
| *Trichinella spiralis* and *Echinococcus multilocularis*  NOT DETECTED | Although common for red foxes in other studies, these species were not detected in any sample. While we cannot exclude a false negative, we know that many of our primer pairs should bind the 18S and amplify fragments of the gene in both parasites (primer sequences match those of the taxa in databases). Infestation with *E. mulitlocularis* varies greatly from region to region. For example, *E. multilocularis* was found in ~20% of red fox samples tested in Belgium (Losson et al. 2003) and ~30% of foxes in the Slovak Republic (Miterpáková et al. 2009). In its core epidemic area (Switzerland) *E. multilocularis* is even more prevalent, commonly found in ~50% of foxes (Fischer et al. 2005; Hofer et al. 2000; Deplazes et al. 2004), but it is not found in the UK (Richards, Harris, and Lewis 1995; Gecchele, Pedersen, and Bell 2020). In foxes from a region overlapping with our study area in rural Brandenburg, a prevalence of ~13% has been recorded (Herzig et al. 2021), while only a single *E. multilocularis* infection in 2303 foxes from Berlin was found since 2013 (unpublished C. Schulze). These results were obtained with a specialised, highly sensitive technique for the detection of *E. multilocularis* (Tackmann, Mattis, and Conraths 2006), while our sequencing assay is not specifically validated for these parasites. Because of the high variation in occurrence of the zoonotic *E. multilocularis* and our, in principle, sensitive but not specifically validated molecular detection, our findings should be interpreted with caution regarding the prevalence of the parasite in the northeast of Germany. Similarly, for *T. spiralis* or any other parasite, we empathise that failure of detection in our assay does not constitute evidence for absence. |