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Does childhood exposure to biodiverse greenspace reduce the risk of developing asthma?

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HIGHLIGHTS

- Living one's early years in relatively biodiverse environments does not unequivocally reduce the risk of developing asthma.
- High levels of greenspace cover are linked to increased risk of developing asthma, but may reduce the risk of severe asthma.
- Density of urban greenspace is associated with a decreased risk of asthma.

GRAPHICAL ABSTRACT

ARTICLE INFO

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ABSTRACT

The prevalence of inflammatory diseases is increasing in populations throughout the industrialized world. An increasing proportion of human populations grow up and live in urban areas, probably with reduced exposure to biodiversity, including diverse soil biotas. Decreased exposure to microorganisms from natural environments, in particular in early childhood, has been hypothesized to hamper development of the human immune system and lead to increasing risks of inflammatory diseases, such as asthma.

We investigated 40,249 Danish individuals born 1995–2015. Percentage greenspace was assessed in a 2 km buffer around home addresses of individuals. The Danish Biodiversity Map, charting occurrence density of red-listed animals, plants and macrofungi, was used as a proxy for multi-taxon biodiversity.

For asthma defined broadly, we found no evidence of decreasing risk of developing asthma with higher levels of biodiversity, while greenspace exposure was associated with higher risk of asthma. In contrast, exposure to total and biodiverse greenspace was associated with reduced risk of developing severe asthma. Exposure to farmland, which in Denmark is heavily industrialized cropland, also showed association with elevated risk of developing asthma, even at relatively low agricultural landcover. In the subset of children growing up in highly urbanized settings, we found high exposures to urban greenspace to be associated with reduced risk of developing asthma.

Our results lend limited support to the hypothesis that childhood exposure to biodiverse environments reduces the risk of acquiring inflammatory diseases later in life. However, access to urban greenspace, such as parks, which typically harbour low levels of biodiversity, seems to reduce asthma risk, potentially through exposure to common soil

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microbiota. Our results suggest that effects of biodiversity exposure on human health is set by a balance between ecosystem services and disservices and that biodiversity conservation is best motivated with other arguments than reduction of risks from inflammatory diseases.

1. Introduction

Inflammatory diseases, such as asthma have become more prevalent in some, but not all countries (Eder et al., 2006; Sevelsted et al., 2021). There is evidence that the increase in prevalence is a global phenomenon tied to urbanization trends (Pearce et al., 2007). While the underlying causes of the increase in inflammatory diseases remain contested, it is often argued that changes in the ecological interactions between humans and other species play a role. Modern urban lifestyles, for example, have been shown to be associated with changes in the composition, diversity and structure of the environmental microbiota, to which humans are exposed during their life (Barberán et al., 2015; McCall et al., 2020). These changes undoubtedly have multiple causes, but key drivers include increased consumption of processed food, increased use of antibiotics and changes in water treatment and domestic sanitation (Scudellari, 2017; Sonnenburg and Sonnenburg, 2019).

In recent decades, the degradation of natural environments, and its consequent effect on microbial environments, has been proposed as an additional driver of inflammatory diseases. Haahtela (2019), building on Rook et al. (2003), proposed the hypothesis that the reduced microbial biomass and diversity of some kinds of cities, and hence reduced exposure to a diversity of microorganisms, deprives humans of microbial exposures that stimulated and upheld the human immune system function throughout the evolution of our species (Haahtela, 2019; Rook et al., 2003). In its simplest form, this "hygiene hypothesis" (sensu Rook) posits that access to greenspace – i.e. areas not farmed and not built-up – is the crucial factor, with no additional effect of microbial or macroscopic biodiversity. In that case, contact with ubiquitous soil microorganisms should be sufficient to stimulate the development of the human immune system in children. Meanwhile, the related "biodiversity hypothesis" posits that the human species has evolved in interaction with stimuli from wild nature and, hence, has become reliant on the environmental microbiota for optimal immune system development. In that case, exposure to high-biodiversity habitats early in life should outperform exposure to mere greenspace in terms of risk prevention. Finally, more recent work has suggested that the effects of "hygiene" or "biodiversity" might be contingent not only on the presence of microbial biomass or biodiversity, but also on human interactions therewith. For example, recent studies of Amish and Hutterite communities found that, although both communities continue to farm, it is only in the latter community where daily interactions with farm animals - and their microbes - remain common enough that the beneficial effects of microbial exposure are manifest (Stein et al., 2016).

A related consideration with regard to the value of exposures to microbes relates to the broader consideration of the value of nature to human societies. If biodiversity near people offers benefits to human health, such biodiversity effects have the potential to serve as additional justification, not just for the maintenance of biodiversity in general, but instead for the maintenance of biodiversity where "we" live (Mace et al., 2012; Pearson, 2016). On the other hand, it is also true that species that are part of biodiversity can kill humans. In any particular locale, biodiversity's net contribution to people is set by a balance between ecosystem services and disservices, with the latter including tetanus, zoonoses and other diseases acquired from nature (Blanco et al., 2019; Dunn, 2010; Lyytimäki et al., 2008), as well as threats to livestock presented by parasites and large predators (Nyhus, 2016; Svenning et al., 2016). However, it remains little investigated whether a beneficial effect of microbes in general or of particular microbes on human health depends on diverse communities comprising habitat-specific, range-restricted or otherwise unique species or if a large biomass of relatively widespread microbial lineages might suffice (Marselle et al., 2021).

Conservation ethics and legislation almost exclusively focuses on biodiversity of animals (particularly birds and mammals), plants and, to a lesser extent, macrofungi. Motivating conservation of this "macrobial" biodiversity with potential benefits of associated microorganisms is only justified if there are cascading health consequences from the effects of plant and animal diversity on microbial diversity and its net benefits to humans.

Using spatial analyses, a number of studies have sought to explore the association between early-life exposure to natural areas and inflammatory diseases, reporting protective effects of plant diversity and extent of natural greenspace on asthma and atopy in Finland (Hanski et al., 2012; Ruokolainen et al., 2015), New Zealand (Donovan et al., 2018) and Australia (Liddicoat et al., 2018). Such studies build on earlier work that noted strong immunological differences among individuals living in the Finnish and Russian parts of Karelia. Individuals on the Russian side of the border are more exposed to greenspace, outdoor work and biodiversity and suffer less from immunological conditions than do individuals living on the Finnish side (Ruokolainen et al., 2020). However, other studies have failed to find benefits effects of exposure to natural environments (Andrusaityte et al., 2016; Lovasi et al., 2013) or to species richness (Cavaleiro Rufo et al., 2020). Here, we take advantage of Danish nationwide heath registers to consider the links between lifetime biodiversity exposure and asthma across the Danish population. In Denmark, all citizens have easy and free access to one unique healthcare system, which for more than four decades has kept record of all diagnoses related to hospital contacts and for more than two decades all medical treatments in the entire population. This enables unselected regional and national studies of occurrence of diseases over a lifespan.

Studies of asthma are typically constrained by small sizes of study populations, but may use strict definitions of asthma diagnoses. Here, we use two alternative types of data on asthma incidence in large register databases. The first metric – the use of asthma medicines – is broad and includes both severe and less severe cases. The second metric – hospital diagnosis of asthma – is a subset of the former, mainly comprising individuals with severe asthma. However, some individuals with severe asthma are not in contact with hospitals.

Our main hypothesis was that exposure of children to a wide array of microorganisms early in life will decrease the risk of later developing asthma. It has been shown that variation in richness and community composition of soil fungal and bacterial communities follow patterns similar to those of animals and plants, at least qualitatively, along major natural and anthropogenic environmental gradients in the Danish landscape (Brunbjerg et al., 2018; Frøslev et al., 2019; Frøslev et al., 2022), as well as elsewhere (Baruch et al., 2021). Further, many plant and animal species are known to have species-specific microbial associates (Emmett et al., 2017; Harrison and Griffin, 2020; Ma et al., 2022). We therefore used high-resolution data on the "macrobial" biodiversity (plants, animals and macrofungi) in Denmark as a proxy for microbial diversity. More specifically, we aimed at teasing apart an effect of greenspace alone, which would likely entail exposure to the subset of the microbiota common in parks and other urban greenspace (e.g. Reese et al., 2016), from an effect of biodiverse greenspace, which would entail exposure to more diverse (alpha diversity) or more variable (spatial or temporal beta diversity) microbial communities and hence increased probability of exposure to rarer microorganisms with particular effects (Lehtimäki et al., 2017).

For Denmark specifically, we anticipated that greenspace land-cover and biodiversity would both be negatively related to urban/suburban land-cover and to farmland cover, the two latter themselves negatively correlated. Some recent field and lab studies have shown contact with farmyard dust to reduce the risk of developing asthma later in life (Stein et al., 2016). Nonetheless, given the strong negative correlation between

farmland and greenspace land-cover in Denmark, we hypothesized higher risk of asthma associated with growing up in rural settings due to lack of non-farm greenspace. The majority of the Danish population live in urban and suburban settings, while natural ecosystems have low cover generally situated far from urban centres. Thus, we also anticipated that urban greenspace cover might have a health effect on urban dwellers independently of any effect of total greenspace on the entire population. Using Danish biodiversity data and nationwide health register data, we aimed at testing the following non-exclusive hypotheses:

(1) early-life exposure to greenspace land-cover would reduce risk of asthma,
(2) early-life exposure to farmland cover would increase risk of asthma (in part, because the negative correlation between greenspace and farmland cover),
(3) early-life exposure to biodiverse greenspace land-cover would reduce risk of asthma, and more so than greenspace alone, and
(4) early-life exposure to urban greenspace would reduce risk of asthma for individuals growing up in urban and suburban settings, independently of the previous hypotheses.

2. Materials and data sources

2.1. Study area

Denmark is a small, relatively homogeneous country with a population of 5.9 million people and a total area of about 43,000 km^2. The Danish health care system is highly developed and publicly funded, i.e. free to the citizens. Human-dominated environments prevail throughout the country. For example, farmland constitutes 74 % of the land area and urban/infrastructure constitutes another 12 % (Statistics Denmark, 2018), which are both above the European average (European Environment Agency, 2019). Less than 4 % percent of the land area is regarded as natural.

We used the National Square Grid as a geographic standard to combine data on disease history of individuals with spatial land-cover and biodiversity data. This allowed data analysis to be independent of changes through time to administrative units. It also had the advantage of standardizing, coastlines notwithstanding, the shape and size of grid cells. We used the smallest grid cell extent, i.e. 100 m grid cell. The coordinate system used by the National Square Grid is based on the UTM projection zone 32 and datum ETRS89 (EPSG code 25832) (Statistics Denmark, 2019).

2.2. Biodiversity data

For our present purposes, Denmark has the advantage as a study site of being relatively well-studied with regard to biodiversity. This is possible thanks to the rather modest biodiversity of Denmark (e.g. c 1500 native plant species as compared to c 10,000 in. for example, similarly-sized Costa Rica), but also because of a long history of natural history research in the country. In characterizing Danish biodiversity, we took advantage of the Danish Biodiversity Map (Ejrnæs et al., 2014) and the so-called *bioscore*. The Danish Biodiversity Map includes occurrence data for 1735 species reviewed in the Danish Red List (Moeslund et al., 2019, IUCN categories from near-threatened to regionally extinct), including plants, macrofungi, vertebrates and a subset of well-studied insect taxa. The resulting data consists of expert-validated geolocated point occurrences (for sedentary species) and habitat polygons (for mobile species) from the last twenty years (in total, 62,883 points and polygons; Ejrnæs et al., 2018). In Denmark, red-listed species constitute about a quarter of the total biota and the local density of red-listed species is highly indicative of total alpha diversity and, in particular, of the occurrence of range-restricted and habitat-specific species across all major taxa (Brunbjerg et al., 2019; Ejrnæs et al., 2014). The *bioscore* is the sum (re-scaled to 1–20) of red-listed species' incidences and occurrence of a number of validated proxy variables (e.g. terrain ruggedness, organic soils, proximity to the coast, structurally complex forest cover, low Nitrogen deposition) indicative

of rare habitat conditions increasing the probability of un-recorded occurrences of red-listed species. In essence, the bioscore is predominantly determined by species occurrences, but "bumped up" where validated GIS variables indicate high biodiversity.

2.3. Land-cover data

We retrieved land-cover information from BaseMap (Levin et al., 2012), which is a GIS map of land use and land cover in Denmark, detailed to 35 major classes spanning anthropogenic landscapes such as buildings, roads, urban greenspace, farmland, production forest and protected natural areas. BaseMap has predecessors and updated successors, but we chose the version closest in time to the childhood years of our study individuals.

For the present analysis, land-cover categories were merged to a level matching our aims, i.e. bog, heathland, dune, salt marsh, meadow, grassland and forest areas were combined into one "natural habitat" variable. Total greenspace included natural and semi-natural areas, plantation forests as well as urban parks and leisure areas. Urban greenspace was defined as areas of land allocated to permanently vegetated and water surface in an urban setting, typically sports facilities, cemeteries and parks. Although urban greenspace covered as little as 0.7 % of Denmark's total area, most of which was sports facilities with highly modified swards, we hypothesized that these areas may provide exposure to soil biota, in particular where natural habitats are absent.

Extensive, intensive and undefined agriculture within the 2 km circle of each individual were combined into one category, farmland. The land-cover type constitutes 59 % of Denmark's total area (Levin et al., 2012), with intensive agriculture making up 54 % of the total.

Natural habitat heterogeneity at the landscape level was assessed as the number of different natural habitat types within the 2 km buffer (range 0 to 8), with the requirement of area > 1 ha of a given land use type for it to be counted.

2.4. Data from Danish health registers

To determine asthma outcome in the study population, we used asthma diagnoses in the Danish National Patient Register and filled prescriptions on asthma medication from the Danish National Prescription Registry.

Since 1968, Danish citizens have been assigned a unique identification number used in all contacts within the healthcare system, educational system etc., i.e. the Danish Civil Registration System, CRS (Pedersen, 2011). This allows for linkage between healthcare registers, residence address and the National Square Grid.

Initially launched in 1977, the Danish National Patient Register holds information on inpatients admitted to somatic treatment. Since 1995, the register expanded to cover psychiatric inpatients and outpatients from emergency departments and specialty clinics (Schmidt et al., 2015).

Since 1995, all individual-level medicine purchases at all Danish pharmacies have been recorded in the Danish National Prescription Registry (Wallach Kildemoes et al., 2011).

2.5. Definition of study population

We restricted our study population to inhabitants of six Danish municipalities (out of 98 in total), selected from the proportion urban area and the Natural Capital Index (NCI) for Denmark (Skov et al., 2017) (Fig. 1). The NCI scores Denmark's municipalities on a 0–100 scale based on land-cover types weighted by their value as habitat for nationally red-listed species. The average score across all municipalities is 24. Individuals living the first 2 years of their lives in one of six municipalities between January 1, 1995 and December 31, 2015 were selected, in total 40,249 individuals (Table 1). The time interval was chosen from two criteria: 1) recording of medication began by 1995; 2) diagnosis data and medication data were
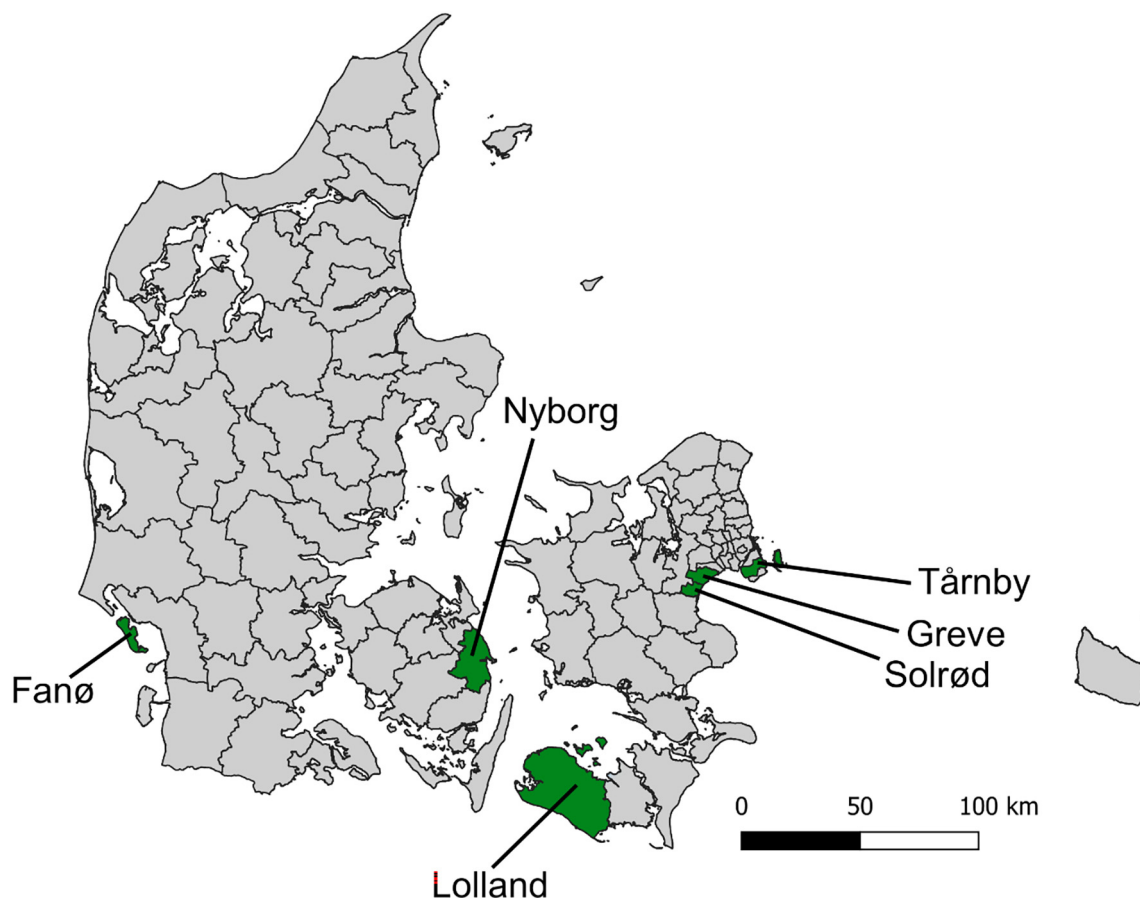
Fig. 1. Denmark with the six municipalities selected (out of 98) highlighted in green. Fanø and Tårnby have high Natural Capital Index values, the rest have low values. Tårnby, Greve and Solrød are urban, while Lolland, Nyborg and Fanø are rural.

available until 2018, hence using 2015 as end year allowed for asthma take at least 3 years to manifest itself.

Data on residence at birth were obtained for all individuals from the Danish Civil Registration System. Regarding residential history of individuals, the CRS allows for extrapolation of residence at birth, but also later residences. We used timestamps assigned to each residential address to calculate a time-weighted exposure to greenspace and biodiversity throughout the two first years of life, taking change of address into account.

An urban subpopulation was delimited across our total study population. It was based on >50 % urban land use within a 2 km radius buffer.

2.6. Definition of the outcome asthma

Individuals with asthma were identified based on hospital contacts for asthma ICD-10 diagnoses or filled prescriptions on asthma medication from 1995 to 2018, using a validated algorithm (Henriksen et al., 2015). Individuals were considered asthmatic if they had ≥1 hospital contact with ICD-10 codes J45-J46. Similarly, we combined several ATC codes related to asthma medication to search for cases of asthma. If a patient had >1 filled prescription of one of the listed medications within 12 months, they were considered a case. Individuals that had been prescribed montelukast (ATC-code R03DC03) and had a diagnosis of allergic rhinitis (J30) and no asthma diagnosis, were considered non-asthmatic.

Additionally, we used the subset of individuals based on hospital contact. We included inpatient and outpatient contacts, which means that patients were not necessarily hospitalized. Nonetheless, we consider this subset to likely represent more severe cases on average. We informally consider this additional analysis some sort of sensitivity analysis of the primary results.

Table 1

Key land-cover and biodiversity properties of the study populations from six selected municipalities. Land-use and bioscore variables were calculated using circular 2 km buffers surrounding residences inhabited by study individuals during their first two years of life. Greyspace refers to the cover of buildings, roads, city centres and other highly urbanized land-use types (Levin et al., 2012). Farmland refers to all agricultural areas, incl. extensive agriculture. Natural habitats include bogs, marshes, heathland, dunes, grasslands and forests. The bioscore was calculated from the Danish Biodiversity Map (Ejrnæs et al., 2014). The total population and the Natural Capital Index score refers to the municipality as such, not the study population.

Municipality name	Total population 2015 (N)	Study population size (N)	Greyspace cover (ha, mean SD)	Farmland cover (ha, mean SD)	Natural habitats cover (ha, mean SD)	Bioscore (mean and SD)	Natural Capital Index score
Fanø	3263	505	279 (73)	117 (32)	436 (110)	5.98 (1.22)	80
Tårnby	42,573	8821	946 (220)	48 (63)	120 (174)	2.26 (2.82)	72
Lolland	43,024	8075	298 (202)	708 (303)	72 (73)	0.84 (1.08)	14
Nyborg	31,573	6550	342 (197)	560 (348)	144 (51)	0.74 (0.49)	12
Greve	48,835	11,314	671 (184)	216 (239)	72 (51)	0.91 (0.47)	12
Solrød	21,552	4984	464 (134)	403 (316)	112 (67)	1.19 (0.78)	13

Register data did not allow us to distinguish between allergic and non-allergic asthma, precluding analyses of the link between biodiversity exposure and allergic asthma vs. non-allergic asthma.

2.7. Potentially confounding factors

Parents' educational and income level was obtained from the Danish Civil Registration System. The "highest educational level" and the mean income of both parents from five years prior to the birth of the study subjects was assigned to each household. The educational data were transformed to reflect the International Standard Classification of Education 2011 (ISCED 2011) and grouped into three groups, with ISCED levels 0–2 considered low education, 3–5 considered medium education and 6–8 considered high education. The income variable was grouped into three equal-sized groups, henceforth "tertiles," with low, medium, and high-income tiers.

To determine familial disposition to asthma, parents of the study subjects were identified and their asthma disease history between 1977 and 2018 was gathered from the Danish National Patient Register. Parents were considered to have asthma if the requirement ≥1 hospital admission was met. Prescription data was not used to determine asthma status in the parents as this information was only available from 1995 onwards.

2.8. Environmental exposures to biodiversity and natural land cover using GIS

The geolocation of the address served as the centre of a buffer used to gather spatial data on environmental exposures from the surrounding area. We chose a 2 km circular buffer (area c. 1256 ha) around the homes of each study individual. Landlocked buffers contained c. 1256 grid cells at the 100×100 m resolution, corresponding to 1256 ha or 12.56 km^2.

Each address in the Danish Civil Registration System was attributed to a single grid cell in the National Square Grid. Linkage to residence data and extraction of environmental metrics was performed by Statistics Denmark in compliance with the Data Protection Act. Next, the spatial data was linked to each individual using their residence as a key and weighted exposures were calculated.

Biodiversity exposure was assessed as the mean bioscore per grid cell within the 2 km buffer, what we call the individual bioscore. The individual bioscore is reflective of an individual's exposure to biodiversity during early childhood. The study population was split into quintiles with regard to biodiversity exposure (Fig. S1). To assess the effects on asthma risk of growing up in highly biodiverse environments, the 95th percentile, or upper 5 %, was determined to be the cut-off through assessment of the mean bioscore in Fanø municipality, the Danish municipality having the highest NCI and commonly known as biodiversity hotspot.

2.9. Statistical modelling

Baseline differences between individuals exposed to high and low levels of biodiversity in their environment were evaluated, pertaining to asthma prevalence, socio-economic metrics and familial disposition to asthma, using χ^2-tests conducted for categorical variables and Kruskal-Wallis tests for continuous variables. A two-sided p-value <0.05 was considered statistically significant. Spearman's rank correlation was used to assess the relationships between biodiversity and land-cover data.

Associations between asthma and exposure to nature, i.e. biodiversity and particular landcover types, were examined using univariate and multivariate Cox proportional hazards regression adjusting for age, sex, parental income and education, and familial disposition to asthma.

We considered the exposure in two ways. First, we considered the greenspace to which individuals were exposed. For all types of exposure (farmland cover, total greenspace cover, urban greenspace cover, bioscore, landscape diversity), the study population was divided into quintiles in order to quantify the asthma risk in classes of increasing exposure. An additional model was made for each type of exposure, focusing on extremes, i.e. the individuals in the 95th percentile of exposure compared to individuals in the lower quintile (20th percentile and below).

Second, we compared individuals as a function of their early childhood exposure to biodiversity, measured as the individual bioscore. Individuals were grouped in two parallel ways, i.e. quintiles as above, and in three classes (low, medium and high categories) of individual bioscore (Fig. S1). The highest possible personal bioscore is 20, which would mean an individual lived their entire early childhood in the highest biodiversity conditions. In practice, the observed maximum individual bioscore was 11 and all five quintiles were overwhelmingly dominated by study individuals with very modest individual bioscore values. Reflecting this reality, we defined three uneven classes, with high exposure set to a bioscore level equal to or higher than the level of Fanø municipality, i.e. mean bioscore ≥5.41 ($n = 1972$). The medium bioscore tier consisted of individuals exposed to a mean bioscore of 0.31 to 5.40 ($n = 29,568$), whereas individual in the low bioscore tier were exposed to bioscore levels below 0.31 ($n = 7878$).

For farmland coverage, the study population was divided in quintiles. However, as for the individual bioscore, the distribution was skewed towards low exposure. Thus, as an alternative, we divided study individuals into three groups based on farmland exposure, i.e. urban (<200 ha farmland), semi-rural (200–800 ha farmland) and truly rural (>800 ha farmland). Rural status was set to >63.7 % agricultural landcover in the 2 km buffer area.

Initial data management was done in SAS 9.4 and further data management and analysis was done using R (version 4.0.2) and the packages 'survival' ver. 3.2–11 (Therneau, 2021) and 'survminer' ver. 0.4–9 (Kassambara et al., 2021). From the Cox regression, hazard ratios (HR) were estimated and reported with 95 % confidence intervals (CI).

The study was approved by the Danish Data Protection Agency and data were analysed on a secure server at Statistics Denmark, to secure the anonymity of study individuals. Ethical approval is not required for registry-based research in Denmark.

3. Results

3.1. Population characteristics

The study population consisted of 40,249 individuals born between 1995 and 2015 (Table 2). For the window of time we studied, the reference dataset categorized individuals as either male or female. Given this system, the two sexes were almost equally represented (51.5 % males, 48.5 % females). However, we recognize that this binary model of categorizing sex fails to capture health outcomes for non-binary, intersex and other gender diverse subpopulations. Based on records of prescribed asthma medications and hospital admissions, 6192 (15.4 %) individuals suffered from asthma. When examining parental hospital admissions for asthma, we found that 3675 individuals (9.1 %) had a family history of asthma.

The average individual bioscore of the study population was 1.26 ± 1.67 for exposure during the first two years of life. The low level reflects that most residential areas have low or very low levels of "macrobiotic" biodiversity. For contrast, individuals spending their early years in the island municipality of Fanø had a mean bioscore of 5.98 ± 1.22 (Table 1). In short, the biodiversity, to which the average modern Dane is exposed, is a tiny proportion of the biodiversity, to which preindustrial and, particularly, prehistoric Danes would have been exposed. In addition, because the biodiversity of Denmark is modest compared to much of the world, particularly the tropical world, it is likely that bioscores of modern Denmark are among the lowest globally, although this awaits empirical confirmation.

Mean individual bioscore in quantiles varied from 0.15 ± 0.10 in Q1 to 7.46 ± 1.31 in the "high exposure" quantile (Table 2). The distribution of sexes did not vary across the quantiles. High income and long education of parents were associated with upper bioscore quantiles. That is to say, more affluent, educated Danes had more access to high biodiversity environments. For example, 26.8 % of the individuals in the lower quintile (Q1) had parents with high-income, while the number was 41.5 % in the upper quintile (Q5) and 43.2 % in the "high exposure" quantile for biodiversity.

Table 2

Distribution of sex, parental asthma cases, parental income, parental education levels and bioscore among quantiles of biodiversity defined based on the bioscore variable extracted from the biodiversity map of Denmark. For the extreme quantile, exact values for parental education are replaced by rough values in order to comply with data protection rules. Statistically significant results are in bold. Q1 refers to the 1st quantile, Q2 to the 2nd quantile, Q3 to the 3rd quantile, Q4 to the 4th quantile. Q5 represents the lower bracket of the 5th quintile while EX refers to extreme values of bioscore >5.40, deemed synonymous with high biological diversity in the surroundings. ISCED stands for International Standard Classification of Education and SD is short for standard deviation. *P*-values come from χ^2-tests for categorical variables and Kruskal-Wallis tests for the Bioscore.

|	Total (N = 40,249)	Q1 (N = 7878)	Q2 (N = 7885)	Q3 (N = 7884)	Q4 (N = 7885)	Q5 (N = 5914)	EX (N = 1972)	P-value
Sex								
Male	20,727 (51.5 %)	4072 (51.7 %)	4104 (52.0 %)	4087 (51.8 %)	4025 (51.0 %)	2997 (50.7 %)	1002 (50.8 %)	0.54
Female	19,522 (48.5 %)	3806 (48.3 %)	3781 (48.0 %)	3797 (48.2 %)	3860 (49.0 %)	2917 (49.3 %)	970 (49.2 %)	
Parental asthma								
No	36,574 (90.9 %)	7119 (90.4 %)	7122 (90.3 %)	7213 (91.5 %)	7156 (90.8 %)	5417 (91.6 %)	1797 (91.1 %)	**0.03**
Yes	3675 (9.1 %)	759 (9.6 %)	763 (9.7 %)	671 (8.5 %)	729 (9.2 %)	497 (8.4 %)	175 (8.9 %)	
Parental income tertiles								
Low	12,659 (31.5 %)	2670 (33.9 %)	2551 (32.4 %)	2817 (35.7 %)	2294 (29.1 %)	1422 (24.0 %)	363 (18.4 %)	**<0.001**
Medium	12,659 (31.5 %)	2650 (33.6 %)	2642 (33.5 %)	2430 (30.8 %)	2446 (31.0 %)	1706 (28.8 %)	661 (33.5 %)	
High	12,659 (31.5 %)	2114 (26.8 %)	2288 (29.0 %)	2158 (27.4 %)	2728 (34.6 %)	2455 (41.5 %)	852 (43.2 %)	
Parental education (ISCED)								
0–2	4840 (12.0 %)	907 (11.5 %)	929 (11.8 %)	1104 (14.0 %)	863 (10.9 %)	605 (10.2 %)	n < 200	**<0.001**
3–5	30,537 (75.9 %)	6373 (80.9 %)	6142 (77.9 %)	5844 (74.1 %)	5883 (74.6 %)	4262 (72.1 %)	n < 1600	
6–8	4740 (11.8 %)	589 (7.5 %)	796 (10.1 %)	877 (11.1 %)	1124 (14.3 %)	1031 (17.4 %)	n < 300	
Bioscore								
Mean (SD)	1.26 (1.67)	0.15 (0.10)	0.47 (0.09)	0.81 (0.11)	1.21 (0.14)	2.43 (1.04)	7.46 (1.31)	**<0.001**
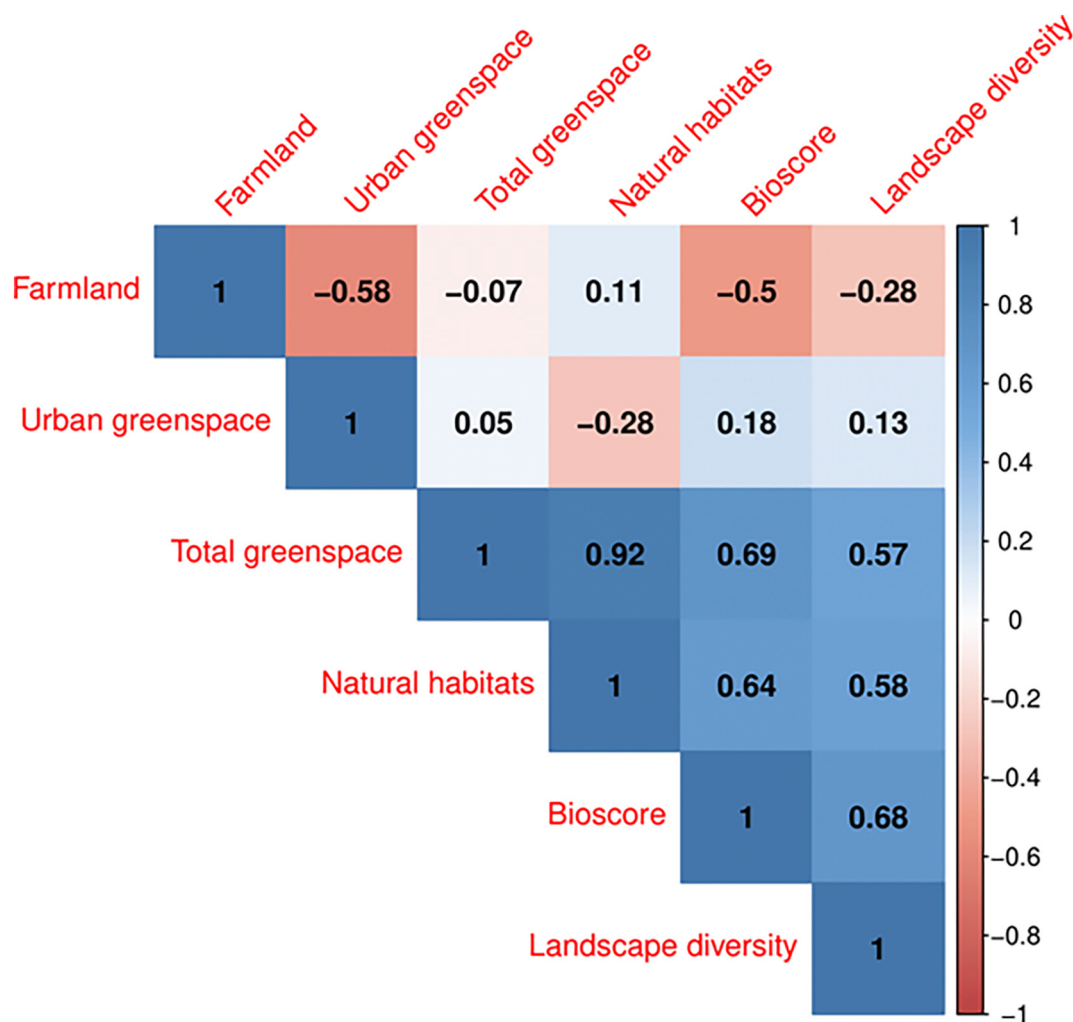
Fig. 2. Spearman's rank correlation coefficients for relationships between biological land use variables (N = 40,249). Blue colours represent positive correlations while red colours represent negative correlations. Weak correlations, *r* < 0.3, are lighter while stronger relationships are more colourful. The *p*-value was found to be P < 0.001 in all relationships.

3.2. Associations between biodiversity and land-use types

Individual bioscores were positively correlated to the proportional area of natural areas (and hence to total greenspace) and to landscape heterogeneity, while being negatively correlated to the proportional area of farmland and showing little relationship with urban greenspace (Fig. 2). The proportional area of natural habitat was weakly positively correlated with that of farmland, probably because both tend to occur away from urban areas. The proportional area of farmland was negatively correlated to most other metrics. For the dataset as a whole, urban greenspace appeared to be weakly positively correlated with individual bioscore ($r = 0.18$, $P < 0.001$).

3.3. Biodiversity and asthma

Contrary to predictions of the biodiversity hypothesis, we found no association between quintiles of the bioscore and risk of asthma using the broad asthma definition (Table S1). When analysing low, medium, and high levels of the bioscore, we also found no indications of any risk-reducing associations pertaining to growing up in biodiverse environments (Table 3). However, looking at more severe asthma cases, we found support for the hypothesis, i.e. that the risk of developing asthma was reduced by exposure to biodiverse greenspace.

3.4. Total greenspace and asthma

Aligned with the above results, we found that increased exposure to greenspace land-cover – which included natural and semi-natural areas, plantation forests and urban parks – was associated with slightly increased risk of asthma (Table S2). When assessing the highest exposure levels to greenspace by comparing individuals with high exposure (i.e. >347 ha total greenspace, corresponding to >28 % greenspace coverage), medium exposure (i.e. 62–347 ha = 5–28 % greenspace coverage) and low exposure (i.e. 0–62 ha or 0–5 % greenspace coverage), we also found an increased risk of asthma at medium and high levels of greenspace exposure as compared to low exposure (Table 4). In contrast, for the more severe asthma cases, total greenspace was associated with reduced asthma risk (Table 4).

3.5. Urban greenspace and asthma

In the urban subpopulation, the highest level of urban greenspace cover seemed to be associated with lower risk of asthma (Table S3 and 5). Exposure to high levels of urban greenspace in the surroundings (the 90th percentile of individuals and above; equivalent of >62 ha greenspace) was associated with decreased risk of asthma as compared to low exposure, i.e. <24 ha urban greenspace (Table 5). In contrast, for the more severe asthma cases, urban greenspace showed no effect on asthma risk (Table 5).

3.6. Farmland and asthma

Independent of asthma definition, higher farmland coverage was associated with increased risk of asthma (Table S4 and 6). Using a coarse farmland categorization, individuals living in semi-rural areas (200–800 ha farmland) had higher asthma risk than either urban (<200 ha farmland) and truly rural (>800 ha farmland)individuals. For the severe asthma cases, both semi-rural and truly rural settings were associated with increased risk (Table 6).

4. Discussion

We calculated personal bioscores for individuals, reflecting the levels of biodiversity of plants, animals and macrofungi those individuals could have been exposed to during the early years of their lives. We did so in the context of a country, Denmark, in which we found asthma rates to be very high (~15 %) in the focal generation of children, both relative to historic rates in Denmark (Henriksen et al., 2015) and relative to other countries (Asher et al., 2021). Using personal bioscores as proxies for exposure to biodiversity, we found no evidence that increased exposure to biodiverse natural environments during early childhood led to reduced risks of developing asthma among >40,000 individuals in the study population. For most levels of biodiversity exposure realized in Danish residential areas, little variation in asthma risk was detectable. However, we surprisingly found some evidence (although not statistically significant) that spending one's early years in the most biodiverse settings found in Denmark was associated with an *increased* risk of developing asthma in the our broad diagnosis definition. Likewise, higher exposure to greenspace, which is dominated by natural and semi-natural habitats outside urban areas, was associated with increased risk of asthma. When considering the subset of more severe asthma cases, the opposite patterns emerged, i.e. exposure to total greenspace and to biodiverse greenspace was associated with reduced risk of developing severe asthma. These apparently contradictory results suggest that the net effect of biodiversity exposure is the result of a balance between positive and negative effects (von Döhren and Haase, 2015), including higher asthma risk from exposure to antigens, such as pollen, and enhanced stimulation of the immune system from exposure to soil biota.

Viewed across our entire study population, we did not find unequivocal support for either the "hygiene hypothesis" (sensu Rook et al., 2003) or the "biodiversity hypothesis" (Haahtela, 2019). Both hypotheses revolve around two key items, namely soil and plant associated microorganisms and human exposure to such microbes. As no data directly addressing richness or composition of soil microbiotas at any given point in space were available for our study area, we used multi-taxon multicellular biodiversity as a proxy. This choice was underpinned by previous demonstrations that variation in richness and community composition of soil fungal and bacterial communities is largely congruent with "macrobial" communities along major natural and anthropogenic environmental gradients in the Danish landscape (e.g. Brunbjerg et al., 2018). Thus, we find it justified to use the Biodiversity Map of Denmark as a proxy for the microbial diversity of sites and the probability of occurrence of microbes with particular effects,

Table 3

Cox univariate and multivariate regression models fitted to asthma data using biodiversity as the main exposure. Asthma cases are grouped into categories 1) Medication and hospital diagnosis asthma and 2) Hospital diagnosis asthma. Family history of asthma, income, education, age and sex were included in the multivariate regression. Statistically significant results are in bold.

	Bioscore	Univariate hazard ratio (95 % CI)	P-value	Multivariate hazard ratio (95 % CI)	n	P-value
Medication and hospital diagnosis asthma						
Low	0.0–0.3	1.00 (ref)	-	1.00 (ref)	7878	-
Medium	0.3–5.4	1.00 (0.94–1.06)	0.92	1.01 (0.95–1.08)	29,568	0.66
High	5.4–10.6	1.09 (0.96–1.23)	0.17	1.13 (0.99–1.28)	1972	0.07
Hospital diagnosis asthma						
Low	0.0–0.3	1.00 (ref)	-	1.00 (ref)	7878	-
Medium	0.3–5.4	0.95 (0.85–1.06)	0.33	0.97 (0.87–1.09)	29,568	0.62
High	5.4–10.6	0.64 (0.49–0.83)	**<0.001**	0.72 (0.55–0.94)	1972	**0.01**

Table 4

Cox univariate and multivariate regression models fitted to asthma data using total greenspace land cover as the main exposure. Asthma cases are grouped into categories 1) Medication and hospital diagnosis asthma and 2) Hospital diagnosis asthma. Family history of asthma, income, education, age and sex were included in the multivariate regression. Statistically significant results are in bold.

	Total greenspace cover (ha)	Univariate hazard ratio (95 % CI)	P-value	Multivariate hazard ratio (95 % CI)	n	P-value
Medication and hospital diagnosis asthma						
Low	0–62	1.00 (ref)	-	1.00 (ref)	8258	-
Medium	62–347	1.06 (0.99–1.12)	0.09	1.07 (1.00–1.14)	29,986	0.05
High	347–838	1.13 (1.00–1.28)	**0.04**	1.17 (1.03–1.32)	2005	**0.02**
Hospital diagnosis asthma						
Low	0–62	1.00 (ref)	-	1.00 (ref)	8258	-
Medium	62–347	0.99 (0.89–1.10)	0.86	1.02 (0.91–1.13)	29,986	0.77
High	347–838	0.65 (0.50–0.84)	**<0.001**	0.71 (0.55–0.93)	2005	**0.01**

Table 5

Cox univariate and multivariate regression models fitted to asthma data using urban greenspace land cover as the main exposure. Asthma cases are grouped into categories 1) Medication and hospital diagnosis asthma and 2) Hospital diagnosis asthma. Family history of asthma, income, education, age and sex were included in the multivariate regression. Statistically significant results are in bold.

	Urban greenspace cover (ha)	Univariate hazard ratio (95 % CI)	P-value	Multivariate hazard ratio (95 % CI)	n	P-value
Medication and hospital diagnosis asthma						
Low	12–26	1.00 (ref)	-	1.00 (ref)	3475	-
Medium	26–62	0.96 (0.88–1.06)	0.46	0.94 (0.85–1.04)	11,932	0.25
High	62–99	0.82 (0.70–0.97)	**0.02**	0.80 (0.68–0.95)	1594	**0.01**
Hospital diagnosis asthma						
Low	12–26	1.00 (ref)	-	1.00 (ref)	3475	-
Medium	26–62	1.12 (0.92–1.36)	0.24	1.04 (0.85–1.27)	11,932	0.74
High	62–99	1.05 (0.78–1.42)	0.76	0.92 (0.67–1.27)	1594	0.62

which is specifically related to the "biodiversity hypothesis". The link between biomass of common soil microbes and conservation value is less straightforward, so our evidence does not necessarily contradict to the "hygiene hypothesis".

Our results assume that the physical proximity between a residential address and (biodiverse) greenspace predicts exposure. There are several ways in which that assumption could be violated. First, both the hygiene hypothesis and the biodiversity hypothesis are predicated on the presence of microbial biodiversity or microbial biomass near where people live, but also the interaction of such microbes with the human immune system. Such interaction is conditional on individuals actually spending sufficient time outdoors in the surroundings of their home address or elsewhere (e.g. day-care, second home). It may be that the average Dane spends insufficient time outdoors to benefit from a high individual bioscore, or that the lack of time spend outdoors decouples the biodiversity near to individuals and that which their immune systems experience. In rural areas, microbial diversity does enter homes more so than it does in urban areas (Barberán et al., 2015; Parajuli et al., 2018), but this drift of organisms, some being dead, may be insufficient to trigger immunological benefits. Such a possibility is suggested by studies of Amish and Hutterite peoples in the United States. Both groups live in traditional agricultural settings, but Hutterite carry out a form of agriculture that leads to much more contact with soils,

plants and domestic animals. At the same time, rates of Hutterite asthma are very low and dust from Hutterite homes has been shown to be anti-asthmatic in mouse models, while the same is not true of the Amish (Stein et al., 2016). A related possibility is that the benefits of microbial biodiversity and biomass are dominated in modern lives by the effects of the highest exposure to such microbes in daily lives, such as with furry pets (Dunn et al., 2013; Kates et al., 2020; Tun et al., 2017) or access to private or day-care gardens (Parajuli et al., 2020; Roslund et al., 2020). If direct immunological exposure is more important than outdoor environments, human behaviour and lifestyles may decouple biodiversity/health benefits for many individuals and interventions may need to focus on combined actions of making sure greenspace is present and then, also, making sure individuals, particularly children, get outdoors. However, with a study population of >40,000 individuals, we find it likely that potentially confounding factors, e.g. getting outdoors, were evenly distributed over fractions of individual bioscore.

Domestic risk factors for developing asthma other than those related to microbiota, e.g. exposure to tobacco smoke, which we did not have information on, could potentially have confounded our results. However, prevalence of smoking in the Danish population is linked to socioeconomic factors such as length of education (Eek et al., 2010), for which we did control. Moreover, residences on addresses in greenspace-dense areas are likely

Table 6

Cox univariate and multivariate regression models fitted to asthma data using farmland cover as the main exposure. Asthma cases are grouped into categories 1) Medication and hospital diagnosis asthma and 2) Hospital diagnosis asthma. Family history of asthma, income, education, age and sex were included in the multivariate regression. Statistically significant results are in bold.

	Farmland cover (ha)	Univariate hazard ratio (95 % CI)	P-value	Multivariate hazard ratio (95 % CI)	n	P-value
Medication and hospital diagnosis asthma						
Low	0–200	1.00 (ref)	-	1.00 (ref)	20,146	-
Medium	200–800	1.11 (1.04–1.17)	**<0.001**	1.10 (1.03–1.16)	12,291	**0.002**
High	>800	1.04 (0.98–1.11)	0.220	1.02 (0.95–1.10)	7812	0.526
Hospital diagnosis asthma						
Low	0–200	1.00 (ref)	-	1.00 (ref)	20,146	-
Medium	200–800	1.48 (1.34–1.63)	**<0.001**	1.42 (1.28–1.57)	12,291	**<0.001**
High	>800	1.56 (1.40–1.75)	**<0.001**	1.45 (1.29–1.63)	7812	**<0.001**

to be above the average price level for real estate in Denmark, and thus generally being inhabited by parents at higher educational and income levels, which would suggest reduced risk of their children developing asthma, if there were a strong risk-reducing effect associated with biodiverse greenspace.

It is of note that individual bioscore was correlated with other aspects of individual lifestyle. Particularly, bioscores were higher for more affluent families. A quarter of the individuals in the lower quintile (Q1) with regard to personal bioscore had high-income parents, while the number was 41.5 % in the upper quintile (Q5) and 43.2 % in the "high exposure" quantile for biodiversity. Compared to many countries, Denmark is widely known for its low level of income inequality (Atkinson and Søgaard, 2016). Nonetheless, Denmark mirrors many other regions in the world in that more affluent people have more access to biodiversity, a phenomenon termed the "luxury effect" (Leong et al., 2018). The bioscore-economics effect that we have seen here suggests the broader value of the use of individual bioscore. While previous studies have considered the relationship between wealth and biodiversity, they have typically done so in the context of the biodiversity present where an individual is living at a particular moment. Here, we extend this work to show an effect on the cumulated exposures of individuals.

Potential mechanisms for increased asthma risk with higher greenspace cover include exposure to allergenic particles, such as plant pollen and fungal spores (DellaValle et al., 2012; Lovasi et al., 2013). Allergen exposure is thought to cause increased sensitization. For allergic individuals, avoidance is considered a way to avoid triggering inflammations and worsening symptoms (Baxi and Phipatanakul, 2010). It cannot be ruled out that allergenic effects of "macrobial" biodiversity, such as exposure to airborne pollen, affect individuals even when they stay inside, while benefits, in effect, may "stop at the front door." Such a balance between opposed effects of biodiversity on asthma risk probably is behind our apparently contradictory results.

Denmark is among the most intensively farmed countries of the world and farming practices are highly industrialized. Thus, there is generally a strongly negative association between biodiversity and farmland cover. It may on that background seem at odds with the previous results that we found individuals spending their early years in farmland settings to have increased risk of developing asthma in our broad definition. Recent studies have found that upbringing in more traditional rural settings to be associated with lower asthma risk (Stein et al., 2016), and homes at farms to have more diverse microbiotas (Kirjavainen et al., 2019; Timm et al., 2016). It may be that semi-rural life mostly lived indoors is insufficient to capture potential beneficial effects of rural microbial diversity, but nonetheless bears the effects of a higher abundance of allergens, which may explain also other studies finding an elevated risk of asthma in rural settings compared to urban (e.g. Valet et al., 2011).

Our analysis of the subpopulation living in urban and suburban settings suggested that growing up with higher cover of urban greenspace led to reduced risk of developing asthma. This finding lends support to the hygiene hypothesis, but not the biodiversity hypothesis, as - for this subset - there was a negative correlation between urban greenspace and individual bioscore. Urban greenspace in Denmark tends to consist of well-tended lawns and shrubbery with low levels of "macrobial" biodiversity. Nevertheless, even a turfgrass monoculture may promote human contact with ubiquitous soil microbes. Moreover, exposure to urban greenspace may result in indoor microbiotas with greater asthma preventing properties (Kirjavainen et al., 2019). It is, however, not possible to separate this effect from other potential explanations, such as access to urban greenspace promoting physical exercise during leisure time (McMorris et al., 2015; Pearson et al., 2014), which could lower the risk of childhood obesity, a known risk factor for asthma (Pearson et al., 2014). Similarly, high urban greenspace landcover could potentially be associated with lower exposure to air pollutants, such nitrogen dioxide, ozone and particulate matter, which are thought to increase exacerbations of asthma, but also increase the sensitization of the airways, leading to inflammation and asthma (Guarnieri and

Balmes, 2014). Nevertheless, this part of our results is perhaps the most relevant to planning of cityscapes in a world inhabited by an ever more urbanized population, as they suggest that easy access to urban greenspace (Methorst et al., 2021) is important to certain aspects of human health, although not necessarily to all (Aerts et al., 2020).

Stepping back to put our study in context, there is clear evidence from other studies in some contexts of the beneficial effects of exposure to farm animals and their microbes on immune health (Stein et al., 2016), of links between backyard biodiversity, skin biodiversity and asthma in Finland (Ruokolainen et al., 2015), and, in some regions, of immune benefits of exposure to dogs and their microbes (Tun et al., 2017). Our results stand out as distinct from these previous studies. However, although these studies are superficially similar, they differ in many respects. The studies of Hutterites and Amish include a case, the Hutterites, in which daily interactions with microbes - or their proxies and vectors, farm animals - proved to be key to the beneficial effects of microbes. While agricultural landcover is very high in Denmark, Danish agriculture is highly industrialized and hence more similar to that of the Amish than that of the Hutterites. Superficially, our study is most similar to the studies done in Finland. Finnish and Danish nature are quite similar in terms of biotas. However, whereas the Danish landscape is dominated by intensive agriculture, that of Finland is dominated by forest, albeit intensively used plantation forest. Even plantation forest with low "microbial" biodiversity may effectively promote direct interaction between humans and environmental microbiotas more than arable land. More importantly, as we look at the globe, the data points represented by our study and other studies of the hygiene and biodiversity hypothesis cover just a tiny portion of the variability that exists within and among countries in terms of biodiversity and microbial exposure.

In the later decades, biodiversity conservation has increasingly been advocated from a utilitarian and anthropocentric point of view, i.e. motivated by "ecosystem services." Under this instrumental framing, a positive effect of biodiverse ecosystems on human somatic and mental health would provide support for setting aside areas for nature at the expense of areas for housing and production of commodities (Mace et al., 2012; Pearce et al., 2007). However, as demonstrated here and elsewhere, biodiverse greenspace may come - in addition to ecosystem services - with disservices (von Döhren and Haase, 2015), e.g. higher asthma risk from exposure to antigens. Our results expose the limits of the utilitarian framework, but obviously not biodiversity conservation as such (Batavia and Nelson, 2017). While there is compelling evidence for positive effects of urban greenspaces on various aspects of mental and somatic health and general well-being (e.g. Bratman et al., 2019; Engemann et al., 2018; Engemann et al., 2020; Pearson et al., 2014), our results suggest that biodiversity conservation is better motivated with other arguments than prevention of asthma. Perhaps, exposure to microbial diversity is unimportant to asthma risk. Or, microbial diversity indeed has an effect on asthma risk, but the link between microbial diversity and the diversity of animals, plants and fungi, which is the obvious scope of biodiversity conservation, is weak. Either way, our findings suggest caution before the prevention of inflammatory diseases, such as asthma, are used as an argument for biodiversity conservation.

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CRediT authorship contribution statement

Martin Holm Winnicki: Conceptualization, Data curation, Formal analysis, Writing – review & editing. **Robert R. Dunn:** Conceptualization, Writing – review & editing. **Matilde Winther-Jensen:** Data curation, Formal analysis, Project administration, Writing – review & editing. **Tine Jess:** Conceptualization, Funding acquisition, Writing – review & editing. **Kristine Højgaard Allin:** Conceptualization, Funding

acquisition, Methodology, Supervision, Writing – review & editing. **Hans Henrik Bruun:** Conceptualization, Methodology, Supervision, Writing – original draft.

Data availability

The data that have been used are confidential.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157853.

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