

ORIGINAL ARTICLE

Epidemiology and Genetics

The neighbourhood natural environment is associated with asthma in children: A birth cohort study

João Cavaleiro Rufo^{1,2}  | Inês Paciência^{1,2}  | Elaine Hoffmann¹ | André Moreira^{1,2} | Henrique Barros^{1,2} | Ana Isabel Ribeiro^{1,2}

¹EPIUnit, Instituto de Saúde Pública da Universidade do Porto, Porto, Portugal

²Faculdade de Medicina da Universidade do Porto, Porto, Portugal

Correspondence

João Cavaleiro Rufo, Rua das Taipas 135, 4050-091 Porto, Portugal.
Email: jcruf@gmail.com

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Abstract

Background: A lower exposure to the natural environment has been hypothesized to adversely affect the human microbiome and its immunomodulatory capacity. However, the underlying effects of this hypothesis are still not understood. We aimed to evaluate the effect of early-life exposure to greenness and species richness on the development of allergic diseases and asthma in children.

Methods: A longitudinal study was conducted comprising 1050 children from a population-based birth cohort recruited in Portugal. Residential normalized difference vegetation index (NDVI) and species richness index (SRI) were assessed at baseline to estimate their association with allergic diseases and asthma at the ages of 4 and 7.

Results: Significant predisposing associations were observed between the exposure to species richness at baseline and the onset of asthma and wheezing at the age of 7. Children living in neighbourhoods surrounded by high levels of SRI were at a significantly higher risk developing allergic sensitization (OR [95% CI] = 2.00 [1.04:3.86] at age 4; 2.35 [1.20:4.63] at age 7). Living surrounded by greener environments was significantly associated with a lower prevalence of asthma and rhinitis at the age of 7 (0.41 [0.18:0.97] and 0.37 [0.15:0.93], respectively).

Conclusions: Living in close proximity to a greener environment at birth has a protective effect on the development of allergic diseases and asthma at the age of 7. Conversely, living in neighbourhoods with a high number of fauna species appears to be associated with a higher risk for allergy, asthma and wheezing.

KEYWORDS

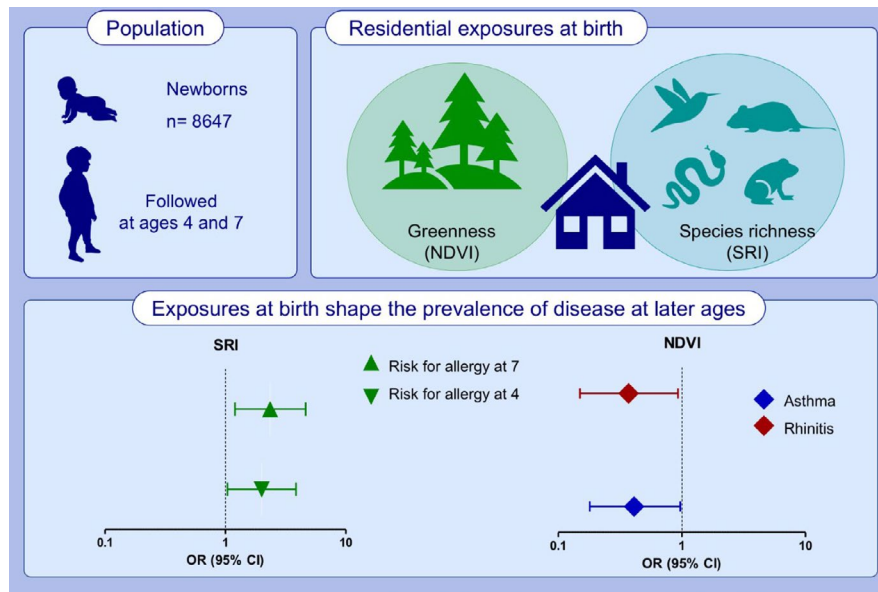
biodiversity, birth cohort, greenness, neighbourhood, species richness

1 | INTRODUCTION

The human microbiome plays an important role in the development of allergic diseases and asthma.¹ According to the "biodiversity hypothesis," a lower exposure to the natural environment and biodiversity may adversely affect the human microbiome and its immunomodulatory capacity.² This hypothesis has been demonstrated in

studies either showing lower microbiota diversity in the airways of children with asthma or finding a lower prevalence of atopic disease in children living the first years of their lives surrounded by a diverse microbiological environment.²⁻⁶

The human microbiome (particularly skin and nasal microbiota) may be influenced through the exposure to different microbiota found in fauna and flora species.^{7,8} Therefore, it is possible



GRAPHICAL ABSTRACT

Living in a greener neighbourhood at birth is associated with a lower prevalence of asthma and rhinitis at the age of seven. Living in a neighbourhood with high numbers of fauna species is associated with an increased risk for allergic diseases and asthma in children. The associations between the contact with nature and the development of asthma are stronger among children with a later onset of disease.

to hypothesize that early-life exposure to certain fauna and flora species may indirectly influence allergy and asthma onset in children. For instance, Hanksi et al were able to demonstrate that environmental microbiota, in the form of species richness of native flowering plants and land use types in the wider environment, were strongly associated with atopy.² However, estimating macroscopic biodiversity exposure is a very complex procedure, requiring the combination of taxonomic identification of species with their spatio-temporal distribution.⁹ Probably due to this complexity, most studies usually focus on estimating normalized difference vegetation index (NDVI) to search for associations between green spaces and asthma or allergic disease development,¹⁰ although resultant effects of exposure are somehow inconsistent between studies.^{11,12} An epidemiological study in Lithuania found that children exposed to greener areas (city parks and other high density of vegetation areas) were at a higher risk of developing asthma.¹⁰ Similar results were found in Spain with a 60% higher prevalence of children with asthma living close to parks.¹³ However, other studies, performed in considerably different environments (USA, Finland and Canada), showed a tendency for lower allergic diseases and asthma prevalence in greener areas.¹⁴⁻¹⁶

A possible reason for these contradicting results may derive from the fact that these studies only took into account availability/accessibility to green space, disregarding the richness of species in these areas. Some of the reported protective effects of green environments may actually be associated with the autonomic nervous system changes attributed to the contact with nature rather than a higher biodiversity, leading to stress reduction, lower heart rate and lower allostatic load in individuals living in those areas.^{12,17}

In other words, larger green areas do not necessarily hold higher number of species, and, in fact, even small green spaces can hold a significant amount of species richness depending on their habitat quality.¹⁸ Hence, species richness, the number of different species represented in a determined landscape,¹⁹ should be considered to understand how neighbouring greenness associates to allergic diseases and asthma development.

Therefore, this longitudinal study aims to evaluate the effect of early-life exposure to greenness and species richness in home surroundings on the development of allergic diseases and asthma in children.

2 | METHODS

2.1 | Participants and setting

Data were retrieved from the Generation XXI (G21) birth cohort, which assembles 8647 newborns delivered in 2005/6 in Porto Metropolitan Area.²⁰⁻²² Recruitment occurred at the five public maternity units where 95% of the births in the region occurred. The children were re-evaluated at 4 (2009/11) and 7 (2012/4) years of age. The study was approved by the Hospital S. João Ethics Committee, and a signed informed consent was obtained from the participants' legal guardians. All the phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. Having showed a good positional accuracy in a previous study,²³ participants were georeferenced according to their residential address using ArcGIS

Online World Geocoding Service and Google Earth, thereby allowing matching participants to places and the tracking of environmental exposures. The residential geolocation corresponded to the participant's address at birth.

For the present study, and since species richness data was only available for the city of Porto, a subset of all children living (upon birth) in the municipality of Porto were included in the present study, totalling 1050 children (546 females). Porto municipality is located in the northwest of Continental Portugal and has approximately 215 000 inhabitants, distributed across 41.7 km². Porto is limited by the Atlantic coast and extends along the Douro River estuary. It is an industrial and port city within the second largest metropolitan area of Portugal, with roughly 1.3 million inhabitants.

2.2 | Health outcomes

A self-reported symptoms and history of medical diagnosis questionnaire, based on the International Study on Allergy and Asthma Meeting (ISAAC) standardized questionnaire,²⁴ was completed by the participants' legal guardians at the ages of 4 and 7 cohort follow-up.

Relevant questions included: "Has your child ever been diagnosed with allergy by a physician?"; "Has your child experienced episodes of wheezing in the past 12 months, not associated with a cold or flu?"; "Has your child suffered from recurrent dry cough episodes in the past 12 months, not associated with a cold or flu?"; "Has your child ever been diagnosed with asthma by a physician?"; "Has your child suffered from recurrent sneezing episodes in the past 12 months, not associated with a cold or flu?"; "Has your child ever been diagnosed with rhinitis by a physician?"; and "Has your child ever been diagnosed with eczema by a physician?". A summary of the health outcomes at the ages of 4 and 7 is presented in Table 1. For the purpose of the study, individuals who reported allergy, rhinitis or eczema diagnosed by a physician were regarded as having allergic disease.

TABLE 1 Summary of the reported health outcomes for the 1050 participants

Characteristics	Age 4 follow-up		Age 7 follow-up	
	n	%	n	%
Allergy diagnosed by a physician	119	14.9	124	16.1
Wheezing in the last 12 mo	166	47.8	112	33.8
Dry cough in the last 12 mo	242	37.3	220	34.5
Asthma diagnosed by a physician	36	4.4	39	5.0
Rhinitis diagnosed by a physician	42	5.2	58	7.5
Eczema diagnosed by a physician	83	12.9	107	14.5

2.3 | Covariates

Covariates comprised sex, as well as other factors known to influence the onset of asthma in children, including maternal history of asthma, household crowding (characterized as <1.5 or ≥1.5 inhabitants per room²⁵) and maternal education (characterized as ≤9 years of education or >9 years of education). These variables were selected due to the influence of genetics and socioeconomic status on the prevalence of allergy and asthma.^{26,27} In addition, possible spatial confounders for NDVI and SRI included the distance to the nearest major road, motorway or highway and neighbourhood socioeconomic deprivation. These variables were included as confounders because residence in disadvantaged neighbourhoods was found to be associated with reduced access to green space,^{26,28} and residence near major roads constitutes an important predictor of asthma and allergy.²⁹ Neighbourhood deprivation was previously determined based on the weighted sum of the following standardized variables obtained from census data: % overcrowded households, % households with no bath/shower, % households without indoor flushing, % households occupied by nonowners, % women aged 65 or more, % individuals with low education, % individuals in low-income occupation and % unemployed individuals. The index was categorized into five quintiles (Q1-least deprived to Q5-most deprived).^{30,31}

A digital road network was obtained from the HERE[®] (formerly NAVTEQ Maps) dataset for the year 2012, provided by courtesy of the Environmental Systems Research Institute (ESRI). The network includes road class, allowing us to determine the distance from the child home to the nearest major road, motorway or highway, which are the road classes associated with higher traffic intensity. ArcMap 10.5.1 was used to compute the distance from the child home to the nearest major road, motorway or highway.

2.4 | Exposure: Normalized difference vegetation index

We also computed the mean NDVI within 100, 200 and 300 m from the child home. The NDVI is calculated based on land surface reflectance of visible red (VISR) and near-infrared (NIR) wavelengths. The underlying principle employed in the NDVI calculation is that chlorophyll in healthy vegetation absorbs radiation in the visible red region (630-690 nm) of the electromagnetic spectrum and reflects radiation in the near-infrared region (760-900 nm). It is a unit-less index that ranges from -1 to 1, with higher values corresponding to a higher density of healthy vegetation. For this study, we used images with 5% or less cloud coverage from Landsat 8 (spatial resolution: 30 m) during the spring/summer period (peak of vegetation) of 2005-2006. Average NDVI was calculated to represent the residential greenspace exposure during the period of the G21 baseline evaluation. We used ArcMap 10.5.1 to process satellite images and QGIS 3.8. to extract average NDVI within 100, 200 and 300 m from the child residence.



FIGURE 1 Distribution of participants' homes and SRI levels across Porto municipality.(for confidentiality reasons, the points representing home locations have been jittered)

2.5 | Exposure: Species richness index

The species richness index (SRI) for Porto was obtained from a species richness report previously compiled by Porto Municipality and the Research Centre in Biodiversity and Genetic Resources (CIBIO).^{32,33} A full description on how the species richness report was conducted is presented as (Appendix S1). Included species consisted in four vertebrate groups (amphibians, birds, reptiles and small mammals), totalling 89 different species.

Each participant was matched to a SRI value based on their residential location. To express the SRI (as the concentration of species in the habitats that surrounded the participants' home), the sum of the number of different species present in habitats located at a 100, 200 and 300 m radius from the participants' house at birth was multiplied by the total area of each habitat and, subsequently, divided by the buffer area.

2.6 | Statistical analysis

We computed absolute frequencies and percentages or medians and interquartile range for categorical and continuous variables, respectively.

Distribution of continuous variables was analysed for normality check. Since non-Gaussian distributions were observed, nonparametric tests were performed for inferential statistics. Mann-Whitney tests were performed to analyse differences between continuous variables, and chi-square test was used to compare proportions. Generalized linear mixed models were fitted to estimate adjusted association between species richness and greenness exposure and the studied outcomes at the ages of 4 and 7, considering the neighbourhood geographical location as a random effect. Since SRI and NDVI were significantly correlated ($\rho = 0.49$, $P < .001$), both variables were mutually included in the models in order to mitigate overlapping effects.

Models were adjusted for the following confounders: distance to the nearest major road, motorway or highway, sex, household crowding, maternal education, neighbourhood socioeconomic deprivation and maternal history of diagnosed asthma. Associations were

expressed as Odds Ratio (OR) and 95% Confidence Intervals (95% CI). In the models, for each unitary increase in SRI or NDVI, the risk for the dependent variables (health outcomes increase or decreased in accordance to the respective OR. Statistical analysis was performed using R software, package "lme4".³⁴

To ensure that the chosen distance thresholds did not affect the results, sensitivity analyses were performed to compare the results obtained using two other buffer sizes (200 and 300 m).

3 | RESULTS

Participants were evenly distributed across Porto municipality. The number of habitats in a 100 m radius from participants' homes ranged between 1 and 134, with a median of 13 habitats. Figure 1 shows the distribution of SRI and participants' homes across the municipality, with a median [25%-75%] index of 2.17 [0.75-4.64], while NDVI had a median [25%-75%] index of 0.12 [0.09-0.33]. Descriptive statistics for NDVI and SRI at 100, 200 and 300 m radius surrounding participants' homes are presented in Figure 2.

There were no significant associations between either NDVI or SRI and the prevalence of asthma or allergy at the age of 4. However, a significant predisposing association was identified between living in neighbourhoods with higher SRI within a 100 m radius and the prevalence of asthma at the age of 7 (OR [95% CI] = 3.58 [1.09:11.79], Figure 3). Interestingly, an opposing effect was observed for NDVI at 100 m, where living in neighbourhoods surrounded by more vegetation was significantly associated with a lower prevalence of asthma at the age of 7 (OR [95% CI] = 0.41 [0.18:0.97]). Both effects disappeared at the 200 and 300 m radius. These results are also observable when computing the empirical cumulative distribution of the participants in function of the respective NDVI and SRI (Figures S1 and S2).

To further understand the effects of SRI and NDVI at the 100m radius, the variables were categorized into terciles with the lower index values being treated as the reference tercile ([0-1.34] and [0.01-0.10], respectively) followed by the 2nd tercile ([1.34-3.79] and [0.10-0.14], respectively) and 3rd tercile ([3.79-23.6] and [0.14-0.33], respectively).

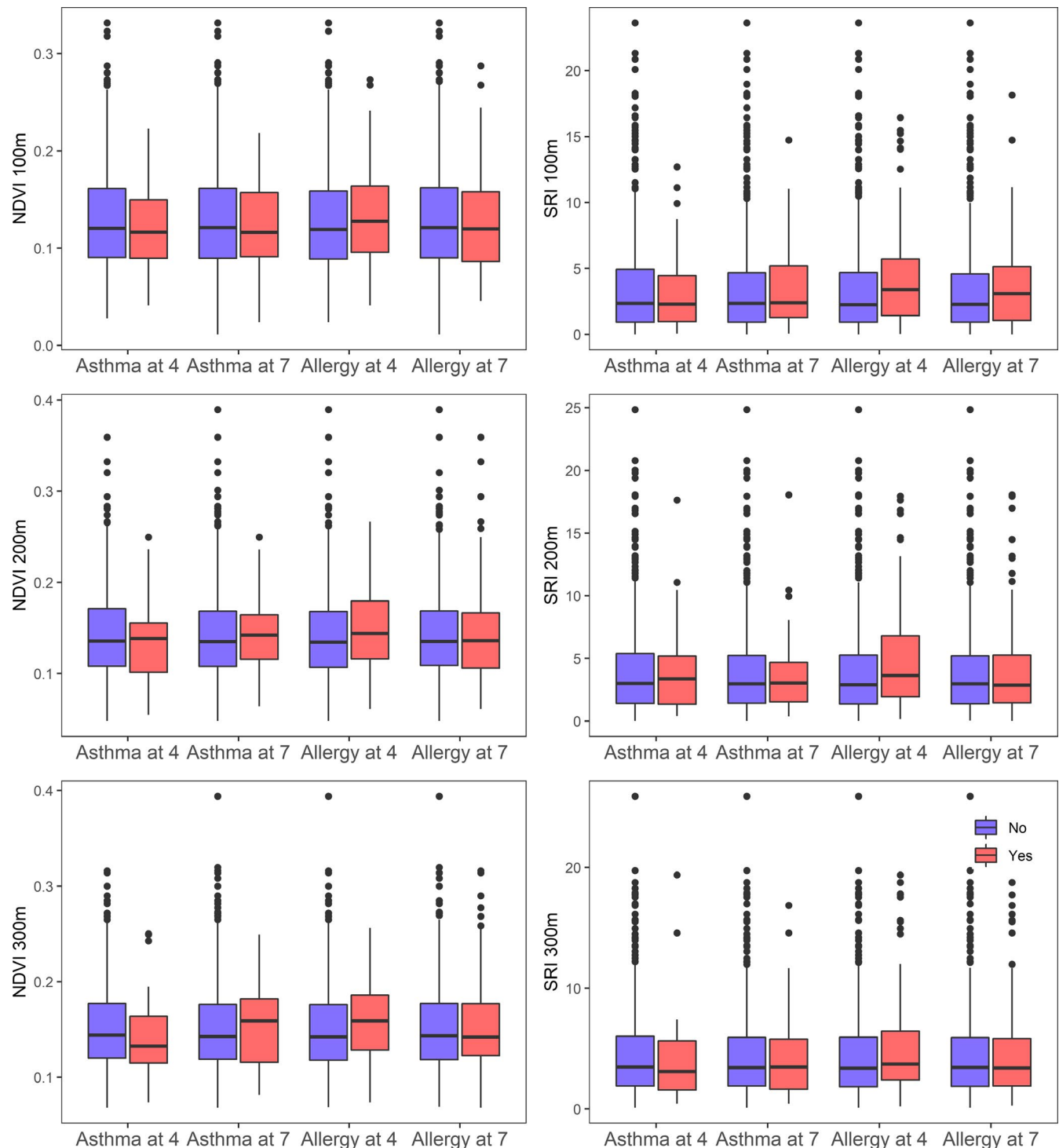


FIGURE 2 Distribution of early-life normalized difference vegetation index (NDVI) and species richness index, within 100, 200 and 300 m from home, grouped by the presence (yes/no) of allergy and asthma at age 4 and 7. Horizontal bars in bold correspond to median values. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles), while whiskers correspond to value range within 1.5 times the interquartile range. Dots correspond to outliers (outside the whiskers range)

As depicted in Figure 4, fitted models for categorized indexes showed a significantly higher risk of allergic sensitization in children who lived in neighbourhoods with the highest tercile of neighbouring species richness both at age 4 and 7 (OR [95% CI] = 2.00 [1.04:3.86] and 2.35 [1.20:4.63], respectively).

Moreover, although no significant SRI associations were identified for asthma, a tendency for increased prevalence of asthma at the age of 7 is discernible within the highest tier of SRI (OR [95% CI] = 2.28 [0.67:7.64]), supporting the previously observed association.

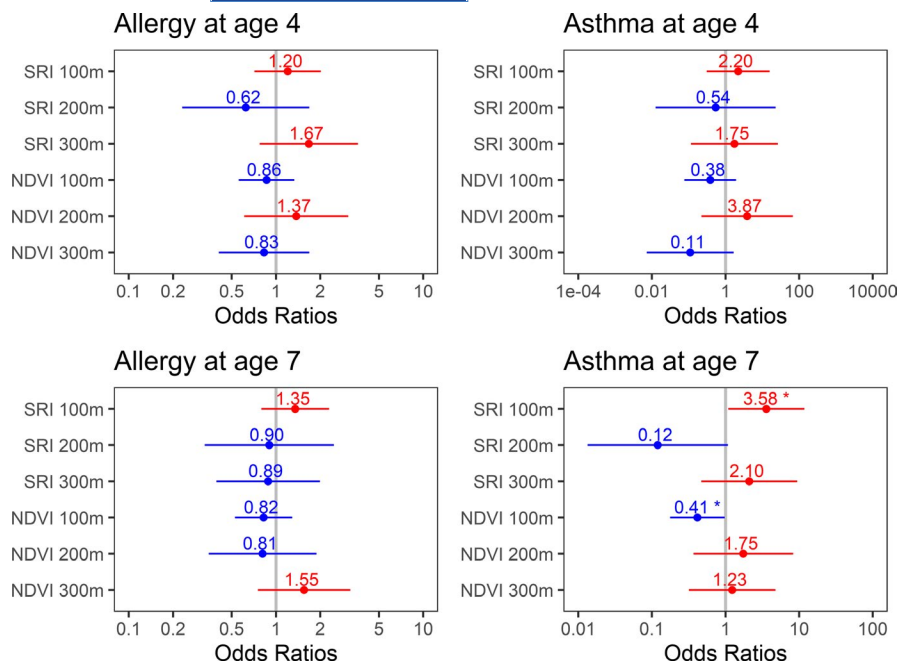


FIGURE 3 Associations between species richness index (SRI) and normalized difference vegetation index (NDVI), and the onset of allergy and asthma at age 4 and 7. Results presented as odds ratio (OR) and 95% confidence intervals (95% CI). Neighbourhood geographical location was considered as a random effect in the model. Models were adjusted for sex, neighbourhood deprivation, distance to major roads, maternal history of asthma, crowding and maternal education. Estimates in red represent risk factors (OR > 1), while estimates in blue represent protective effects (OR < 1)

Although nonsignificant, an opposing tendency was observed for NDVI, particularly at age 7, where the highest tercile was associated with a lower risk for both allergy and asthma (OR [95% CI] = 0.58 [0.31:1.09] and 0.37 [0.12:1.14]).

The association between SRI and asthma was similarly observed for wheezing at age 7, where children living in neighbourhoods within the highest tercile of SRI at a 100 m radius were at a significantly higher risk of wheezing (OR [95% CI] = 2.51 [1.08:5.88], Figure 5). Contrarily, living in a neighbourhood within the 2nd and 3rd terciles of NDVI in a 100 m radius showed a tendency for a lower prevalence of wheezing (OR [95% CI] = 0.48 [0.22:1.04] for the 2nd tercile; 0.49 [0.22:1.08] for the 3rd tercile). However, the most evident protective effect of NDVI was observed for the prevalence of rhinitis at the age of 7, where living in neighbourhoods within the highest tercile of NDVI at 100 m was significantly associated with a lower prevalence of this disease (OR [95% CI] = 0.37 [0.15:0.93]). No significant associations were observed for the remaining symptoms or allergic manifestations.

The associations between NDVI and SRI and the studied outcomes remained the same in a sensitivity analysis where the participants were divided according to whether they moved or remained in the same residence through the study (Table S1).

4 | DISCUSSION AND CONCLUSION

The study results showed significant predisposing associations between the exposure to species richness at baseline and the onset of physician's diagnosed asthma and wheezing symptoms at the age of 7. Moreover, children living in neighbourhoods surrounded by very high levels of species richness (SRI over 3.79) were at a significantly higher risk of having developed allergic sensitization at the age of 4, at an average of 2 times increased risk and, in a larger extent, at

the age of 7, at an average of approximately 2.4 times increased risk. On the other hand, living in neighbourhoods surrounded by greener environments was significantly associated with a lower prevalence of asthma at the age of 7, as well as slightly, but not significantly, associated with a lower prevalence of wheezing at that age. In addition, higher levels of neighbourhood greenness at birth (NDVI over 0.14) were significantly associated with a lower prevalence of rhinitis at the age of 7, at an average of 2.7 times lower risk. These results were observed for a radius of 100 m from participants' homes, and effects disappeared when considering 200 and 300 m radius, suggesting that relevant exposures occur mainly to vegetation and species in the immediate home proximities. Therefore, the biodiversity hypothesis, where being more exposed to a natural environment at birth promotes a lower risk for allergy and asthma, is both supported and contradicted by this study's results.

Recent evidence suggests that environmental biodiversity exposure may influence the human microbiome during growth, consequently having a major role in allergic sensitization and asthma development in children.^{35,36} It is hypothesized that early contact with multiple microbiological agents contributes to a preventive immunomodulation, inducing tolerance to allergens through both adaptive and innate immune mechanisms.³⁷

Based on the present results, and although only indirect associations are possible since no measurements of the participants' microbiome were performed, it is possible to hypothesize that being in contact with a greener environment at birth, independently of species richness, may induce a more natural development of the human microbiome, consequently resulting in the decreased prevalence of asthma and allergic rhinitis at the age of 7. These results are in accordance with those obtained by Donovan and co-workers in a study where they showed that children living in greener areas, as measured by the NDVI, were less likely to be asthmatic.³⁸ The protective effect of greenness on allergic rhinitis has also been reported in the GINI/

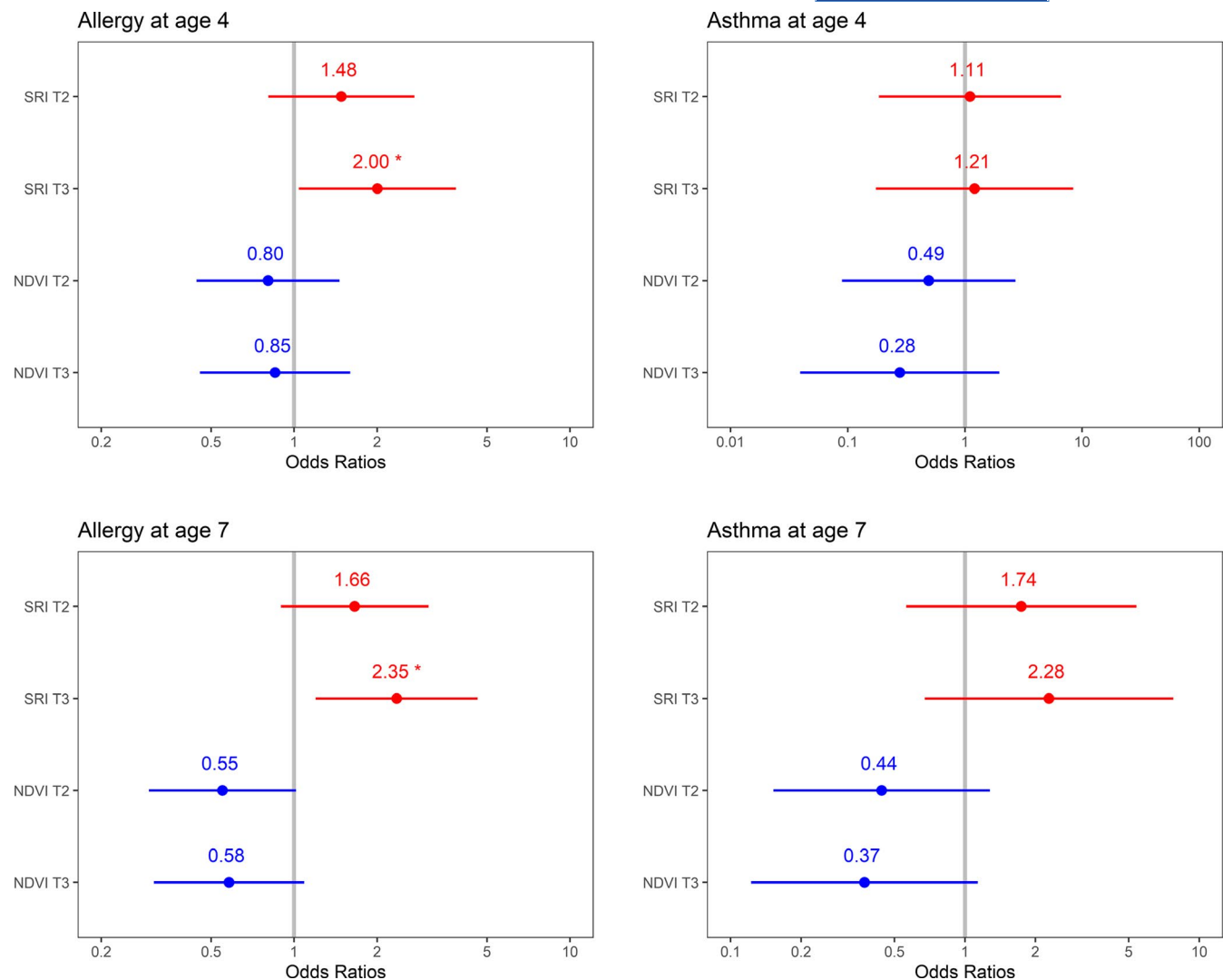


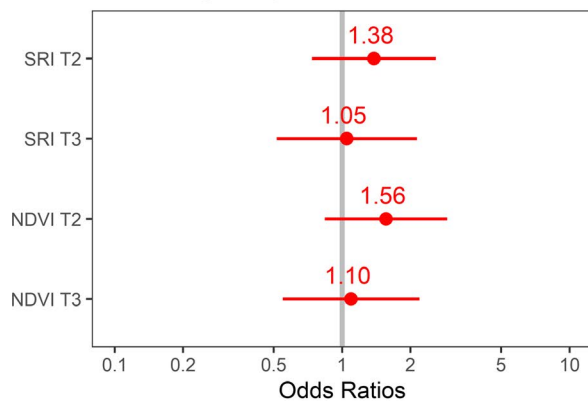
FIGURE 4 Associations between normalized difference vegetation index (NDVI) and species richness index (SRI) terciles within 100 m from home, and the onset of allergy and asthma at age 4 and 7. Results presented as odds ratio (OR) and 95% confidence intervals (95% CI). Neighbourhood geographical location was considered as a random effect in the model. Models were adjusted for sex, neighbourhood deprivation, distance to major roads, maternal history of asthma, crowding and maternal education. Estimates in red represent risk factors (OR > 1), while estimates in blue represent protective effects (OR < 1)

LISA North and PIAMA cohorts (OR [95% CI] = 0.61 [0.36, 1.01] and 0.67 [0.47:0.95], respectively, when compared to 0.37 [0.15:0.93] in the present study).³⁹⁻⁴¹ However, in these cases, a buffer of 500 m radius was used, and effects changed upon location, with the effects being contradictory in GINI/LISA South and BAMSE cohorts (OR [95% CI] = 1.69 [1.19, 2.41] and 1.42 [1.13, 1.79], respectively).^{39,40,42}

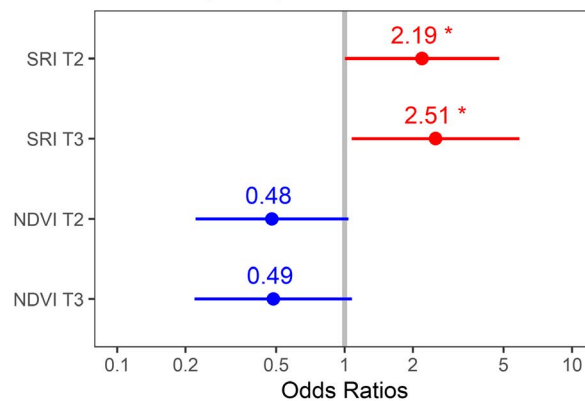
Nevertheless, the mechanisms behind this protective effect are not completely understood and have been attributed not only to the biodiversity associated with vegetation itself, but also to the natural barriers composed by arboreal vegetation that may protect neighbourhoods from environmental pollutants, or even to the stress-releasing effect of green landscapes on autonomic nervous system.^{12,38,43,44} Although the present study results do not allow the identification of the protective mechanisms associated with the NDVI, the species richness results certainly suggest that effects are not caused by the number of habitats and fauna diversity involved.

Although exposure to macrospecies might manifest different effects when compared to microspecies, one could expect that an increase in neighbouring macrospecies at birth would still promote microbiome diversity and indirectly be associated with a lower risk for allergy and asthma. Farm environments, generally characterized by higher species richness, have been associated with a lower prevalence of asthma in children.⁴⁵ Although microbiome diversity was not evaluated during the present study—and, thus, no conclusions can be made on the SRI-microbiome interaction—there was clearly no protective effect associated with living in neighbourhood with a larger number of species at birth. In fact, present study's results suggest that living proximate to more species at early-life increases the risk of asthma and symptoms of asthma at the age of 7, as well as the prevalence of allergic disease in those living in neighbourhoods with a very high number of species, both at the ages of 4 and 7. This results are in agreement with those observed in a study where high

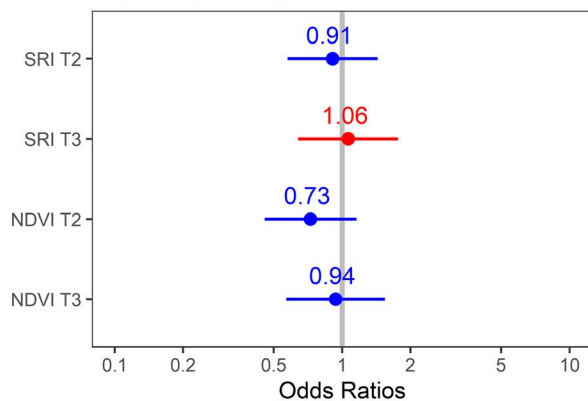
Wheezing at age 4



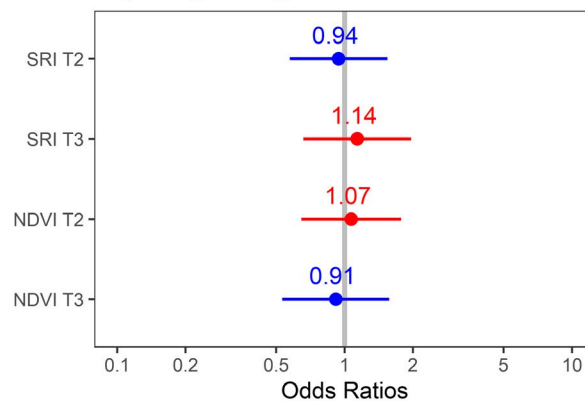
Wheezing at age 7



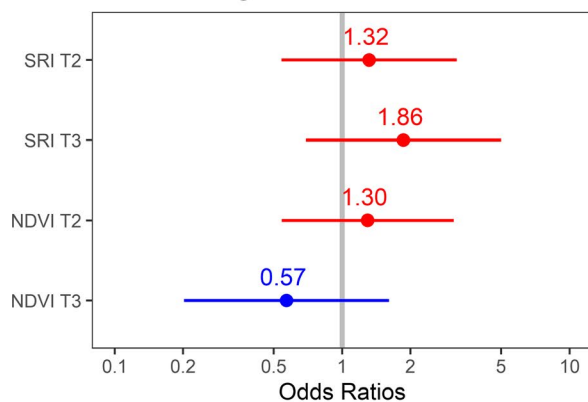
Dry cough at age 4



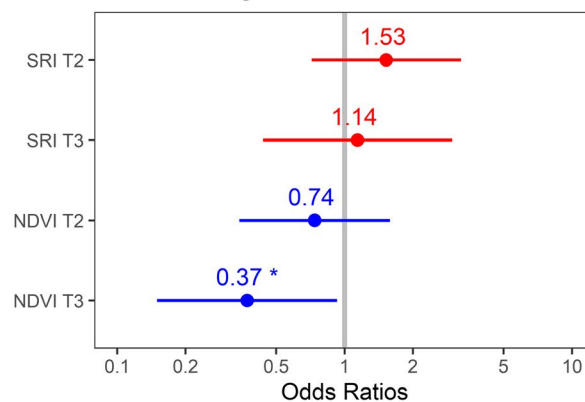
Dry cough at age 7



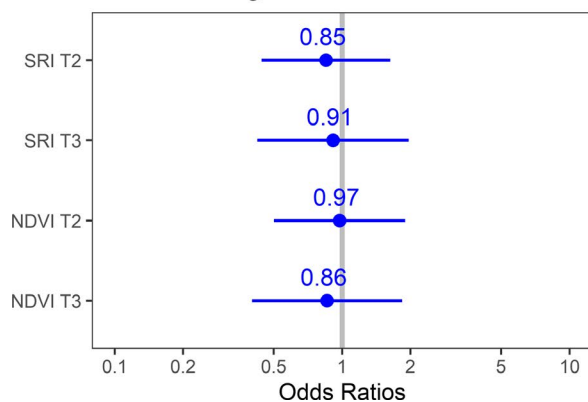
Rhinitis at age 4



Rhinitis at age 7



Eczema at age 4



Eczema at age 7

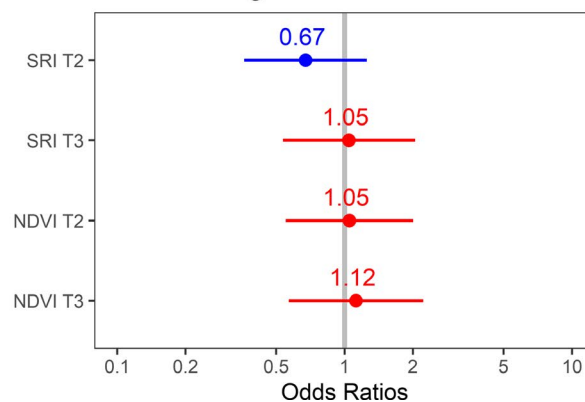


FIGURE 5 Associations between normalized difference vegetation index (NDVI) and species richness index (SRI) terciles within 100 m from home, and the onset of wheezing, dry cough, rhinitis and eczema at age 4 and 7. Results presented as odds ratio (OR) and 95% confidence intervals (95% CI). Neighbourhood geographical location was considered as a random effect in the model. Models were adjusted for sex, neighbourhood deprivation, distance to major roads, maternal history of asthma, crowding and maternal education. Estimates in red represent risk factors (OR > 1), while estimates in blue represent protective effects (OR < 1)

levels of species richness surrounding primary schools were shown to be associated with a tendency for a higher prevalence of allergic diseases.⁴⁶ Interestingly, the association between SRI and eczema is not so evident in the present study, what could be explained by the differences in criteria for classifying the diseases, as well as the effect of vegetation. It is plausible that the higher prevalence of allergic diseases would be associated with an increase in IgE levels in children growing in neighbourhoods with higher species richness. However, higher IgE levels do not necessarily mean allergic disease, and thus, it would be interesting to measure the direct impact of SRI and NDVI on children IgE levels. Unfortunately, and to this date, IgE levels have not been measured in the G21 cohort.

Another possible explanation for these results may be that having more neighbouring species will mean more neighbouring aeroallergens, more exacerbations and, consequently, more allergy and asthma symptoms being reported. However, this would also be true for aeroallergens originated from vegetation,⁴⁷ but the vegetation's effects observed in this study were actually protective. Moreover, and similarly to microbes, one could also hypothesize that exposure to a wider variety of antigens could educate the immune system, which was not observed in the present results in the form of species richness. The association between outdoor allergen exposure and the development of allergic sensitization is very complex, as it may be modelled by the interaction with other factors, such as air pollutants or climate aspects.^{48,49} In addition, and despite being a simple measure of biodiversity, it is important to notice that species richness does not account for species evenness, which refers to how equally in numbers different species are distributed in a given environment, and therefore, does not completely explain the exposure to species diversity and to the natural ecosystem. In fact, a decrease in environmental quality may actually lead to transient increases in species and, therefore, higher species richness.⁵⁰ Nevertheless, for the city of Porto, it is clear that living in neighbourhoods with high number of species during early-life is a risk factor for the onset of allergy and asthma.

Another variable that was not measured in the study cohort but may be influencing the results is the indoor exposure at birth. Young children are significantly more exposed to indoors than outdoors, and although outdoor exposure appears to play a role on immunomodulation, the impact of indoor biodiversity might be more determinant.^{51,52} Moreover, although the indoor environment is influenced by the outdoor air, there are several factors mainly originated from indoor sources that have been associated with allergy and asthma development, such as certain allergens, combustion and disinfectant by-products, household pets, among others.^{47,53-57} However, even concerning the influence of indoor environment, there is lack of clarity with exposures reducing or increasing risk

of allergy depending on what perspective and/or experimental approach is applied.⁵² Nevertheless, one cannot overlook that indoor biodiversity may be masking the effect of neighbouring species richness in the present study.

Interestingly, the observed effects were clearly stronger at the age of 7. Although the factors that influence the age of onset of asthma are not yet fully understood, Thomsen and co-workers showed, in a twin cohort study, that genetic variables only explain 34% of the variability in the age at onset for asthma, while environmental factors explain 66%.⁵⁸ Therefore, it is possible that asthma in children experiencing their first symptoms at later ages may have been predominantly induced by environmental aspects. Further research is needed to clarify these results.

Some study limitations should be disclosed. First, data on symptoms and diagnosis of allergic diseases and asthma were self-reported, which may introduce self-reporting bias. Nevertheless, ISAAC-based questionnaires are standardized and widely used throughout the clinical immunology community.²⁴ It is also important to consider that although these associations are estimated for NDVI and SRI exposure at birth, having changed the place of residence later in life may have had some influence on the results. However, the sensitivity analysis showed no differences in the drawn observations when considering only the population who maintained residence throughout the study. Another limitation may be associated with the possibility of different habitats being present at the participants' year of birth, since the species and habitat observation that supported the SRI estimation was conducted in a different year. Still, although changes in urban habitats may occur with time, these usually take several decades to significantly impact species richness.⁵⁹ The NDVI, however, does not capture richness of the vegetation as it is a biomass diversity index, thus limiting the assumptions when concerning exposure to flora biodiversity. Finally, having pets at birth may have acted as a confounder and, unfortunately, this information was not collected during baseline assessment. However, this information was collected in the follow-up evaluations and, when adjusting models for having a pet at the age of 4, no significant differences are observable in the models.

On the other hand, this is the first longitudinal study to investigate the impact of early-life exposure to neighbouring species richness in the development of allergic diseases and asthma using a combination of an uniquely developed SRI, estimated through four different groups of vertebrate fauna, and the NDVI. Although it may seem a roughly estimation on macrospecies diversity, the developed SRI is one of the few environmental biodiversity surrogates currently applied in a cohort study. This is also the first study to show the opposing effects of neighbouring species richness and greenness on the development of allergic diseases and asthma in children.

Despite no concrete conclusions could be drawn on the immunomodulatory effects of early-life biodiversity exposure, the present work suggests that living in close proximity to a greener environment at birth has a protective effect on the development of allergic diseases and asthma at the age of 7. Unlike the effects of vegetation, living in neighbourhoods with high numbers of fauna species in close proximity appears to be associated with a higher risk for allergy, asthma and asthma symptoms. To further understand the biodiversity hypothesis, the interactions and mechanisms between these environmental factors and the human microbiome should be explored.

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CONFLICT OF INTEREST

Authors declare to have no conflict of interests.

ORCID

João Cavaleiro Rufo  <https://orcid.org/0000-0003-1175-242X>
Inês Paciência  <https://orcid.org/0000-0001-5762-7814>

REFERENCES

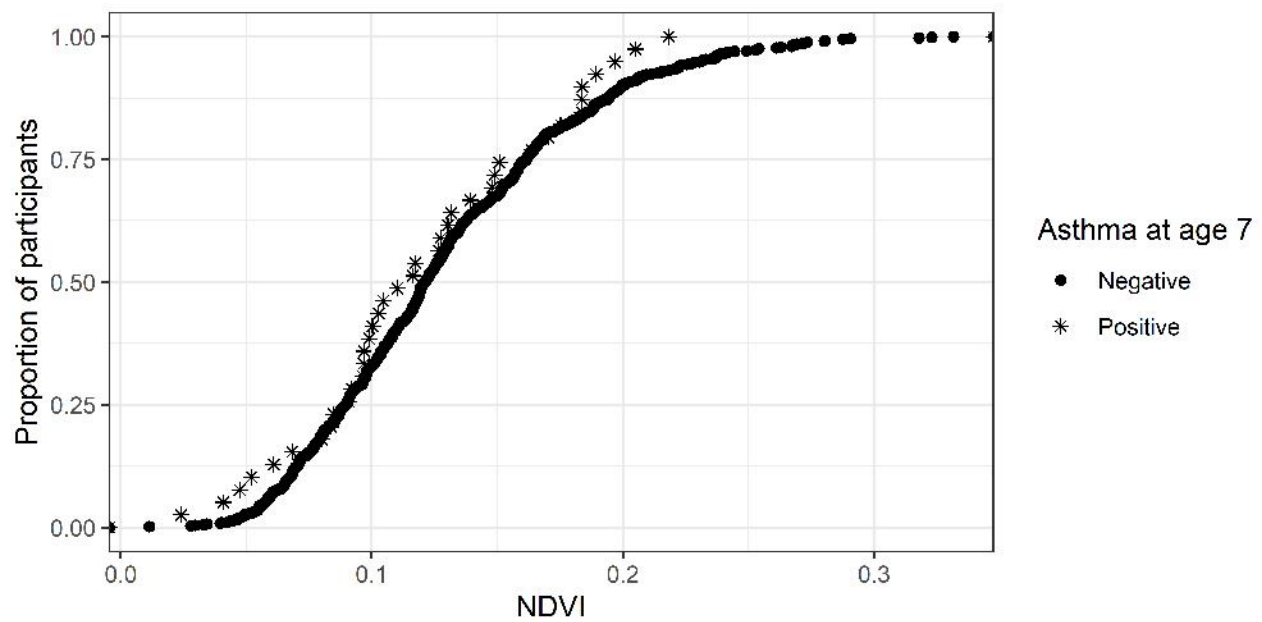
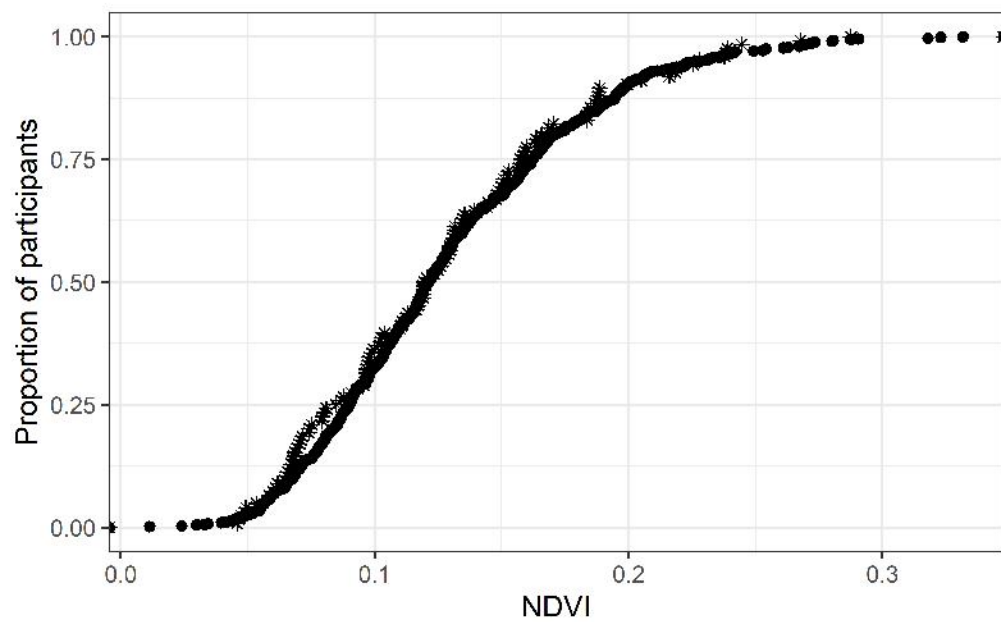
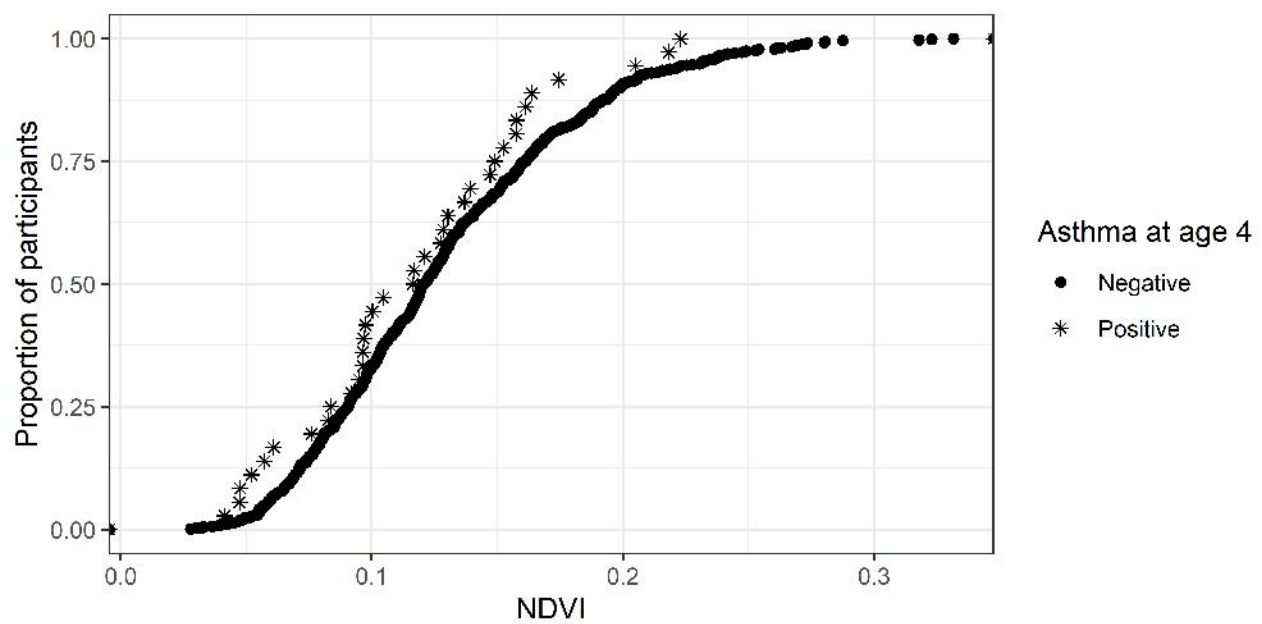
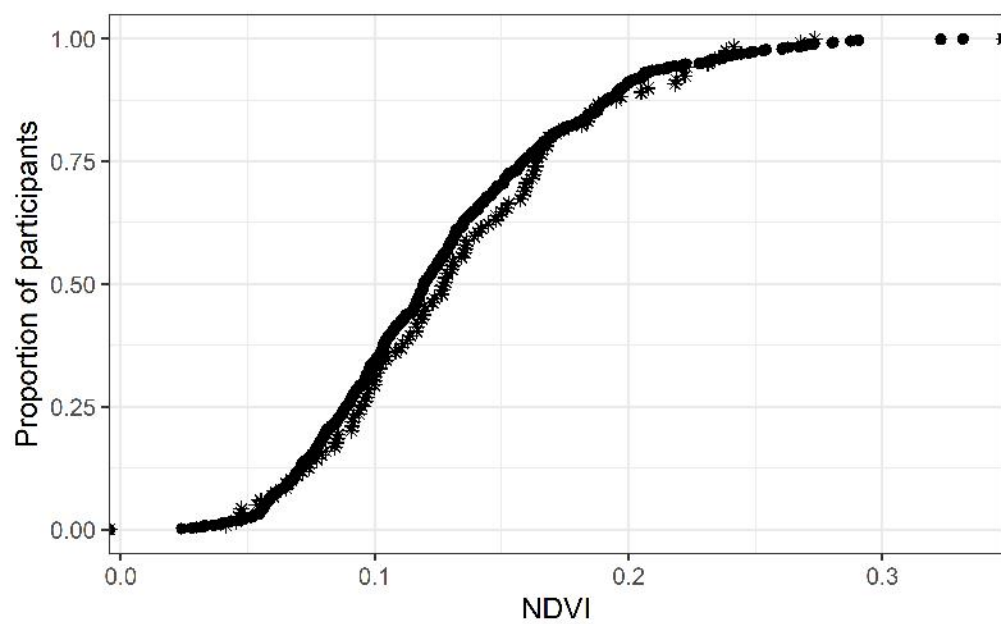
- Riiser A. The human microbiome, asthma, and allergy. *Allergy Asthma Clin Immunol*. 2015;11:35.
- Hanski I, von Hertzen L, Fyhrquist N, et al. Environmental biodiversity, human microbiota, and allergy are interrelated. *Proc Natl Acad Sci USA*. 2012;109(21):8334-8339.
- Chiu C-Y, Chan Y-L, Tsai Y-S, et al. Airway microbial diversity is inversely associated with mite-sensitized rhinitis and asthma in early childhood. *Sci Rep*. 2017;7(1):1820.
- Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS One*. 2010;5(1):e8578.
- Mathieu E, Escribano-Vazquez U, Descamps D, et al. Paradigms of lung microbiota functions in health and disease, particularly, in asthma. *Front Physiol*. 2018;9(1168):1-11.
- Cavaleiro Rufo J, Madureira J, Paciência I, et al. Indoor fungal diversity in primary schools may differently influence allergic sensitization and asthma in children. *Pediatr Allergy Immunol*. 2017;28(4):332-339.
- Flandroy L, Poutahidis T, Berg G, et al. The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems. *Sci Total Environ*. 2018;627:1018-1038.
- Prescott SL, Larcombe D-L, Logan AC, et al. The skin microbiome: impact of modern environments on skin ecology, barrier integrity, and systemic immune programming. *World Allergy Organ J*. 2017;10(1):29.
- Kissling WD, Ahumada JA, Bowser A, et al. Building essential biodiversity variables (EBVs) of species distribution and abundance at a global scale. *Biol Rev Camb Philos Soc*. 2018;93(1):600-625.
- Andrusaityte S, Grazuleviciene R, Kudzyte J, Bernotiene A, Dedele A, Nieuwenhuijsen MJ. Associations between neighbourhood greenness and asthma in preschool children in Kaunas, Lithuania: a case-control study. *BMJ Open*. 2016;6(4):1-9.
- Rufo JC, Paciência I, Ribeiro AI. Green environments and allergic diseases in children: a scoping review. *Curr Epidemiol Rep*. 2019;6(4):442-448.
- Paciência I, Rufo JC, Silva D, et al. School environment associates with lung function and autonomic nervous system activity in children: a cross-sectional study. *Sci Rep*. 2019;9(1):15156.
- Dadvand P, Villanueva CM, Font-Ribera L, et al. Risks and benefits of green spaces for children: a cross-sectional study of associations with sedentary behavior, obesity, asthma, and allergy. *Environ Health Perspect*. 2014;122(12):1329-1335.
- Chen E, Miller GE, Shalowitz MU, et al. Difficult family relationships, residential greenspace, and childhood asthma. *Pediatrics*. 2017;139(4):1-10.
- Sbihi H, Tamburic L, Koehoorn M, Brauer M. Greenness and incident childhood asthma: a 10-year follow-up in a population-based birth cohort. *Am J Respir Crit Care Med*. 2015;192(9):1131-1133.
- Ruokolainen L, Hertzen L, Fyhrquist N, et al. Green areas around homes reduce atopic sensitization in children. *Allergy*. 2015;70(2):195-202.
- Ribeiro AI, Tavares C, Guttentag A, Barros H. Association between neighbourhood green space and biological markers in school-aged children. Findings from the Generation XXI birth cohort. *Environ Int*. 2019;132:105070.
- Lepczyk CA, Aronson MFJ, Evans KL, Goddard MA, Lerman SB, MacIvor JS. Biodiversity in the city: fundamental questions for understanding the ecology of urban green spaces for biodiversity conservation. *Bioscience*. 2017;67(9):799-807.
- Colwell RK III. 1 Biodiversity: concepts, patterns, and measurement. In: Levin SA, ed. *The Princeton Guide to Ecology*. Princeton, NJ: Princeton University Press; 2009: 257-263.
- Sonnenschein-van der Voort AMM, Arends LR, de Jongste JC, et al. Preterm birth, infant weight gain, and childhood asthma risk: a meta-analysis of 147,000 European children. *J Allergy Clin Immunol*. 2014;133(5):1317-1329.
- Alves E, Correia S, Barros H, Azevedo A. Prevalence of self-reported cardiovascular risk factors in Portuguese women: a survey after delivery. *Int J Public Health*. 2012;57(5):837-847.
- Larsen PS, Kamper-Jorgensen M, Adamson A, et al. Pregnancy and birth cohort resources in Europe: a large opportunity for aetiological child health research. *Paediatr Perinat Epidemiol*. 2013;27(4):393-414.
- Ribeiro AI, Olhero A, Teixeira H, Magalhaes A, Pina MF. Tools for address georeferencing - limitations and opportunities every public health professional should be aware of. *PLoS One*. 2014;9(12):e114130.
- Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J*. 1995;8(3):483-491.
- Fraga S, Severo M, Ramos E, et al. Childhood socioeconomic conditions are associated with increased chronic low-grade inflammation over adolescence: findings from the EPITeen cohort study. *Arch Dis Child*. 2020;105(7):677-683.
- Kozyrskyj AL, Kendall GE, Jacoby P, Sly PD, Zubrick SR. Association between socioeconomic status and the development of asthma: analyses of income trajectories. *Am J Public Health*. 2010;100(3):540-546.
- Lim RH, Kobzik L, Dahl M. Risk for asthma in offspring of asthmatic mothers versus fathers: a meta-analysis. *PLoS One*. 2010;5(4):e10134.
- Hoffmann E, Barros H, Ribeiro AI. Socioeconomic inequalities in green space quality and accessibility-evidence from a southern European city. *Int J Environ Res Public Health*. 2017;14(8):916.

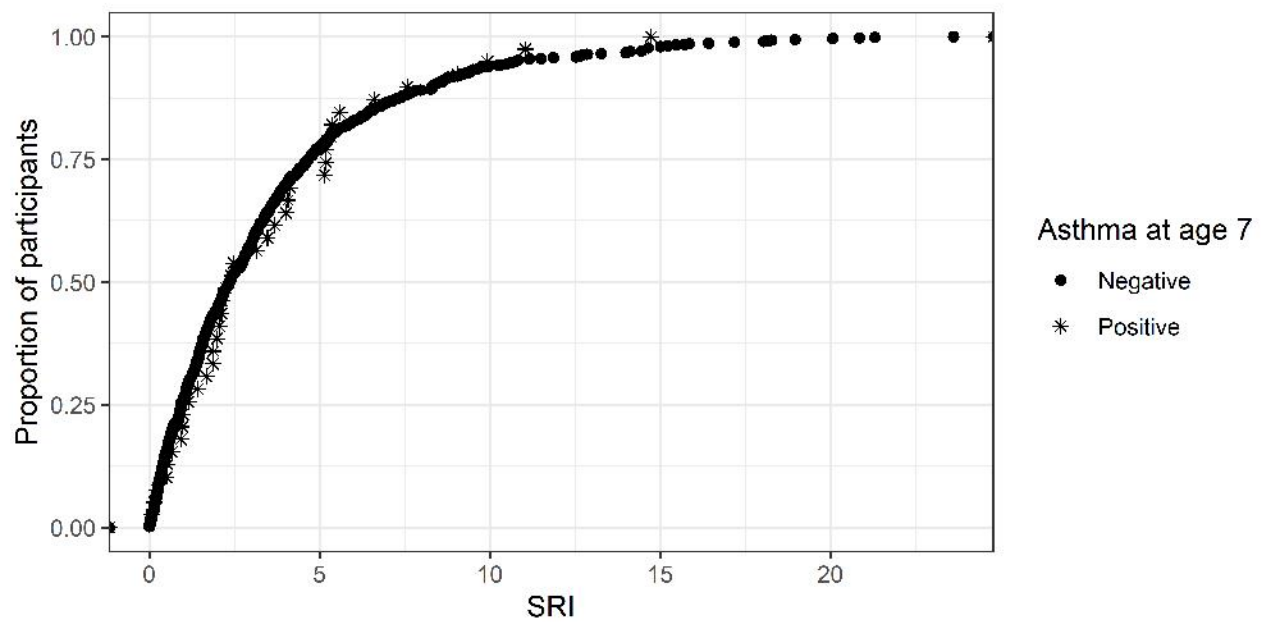
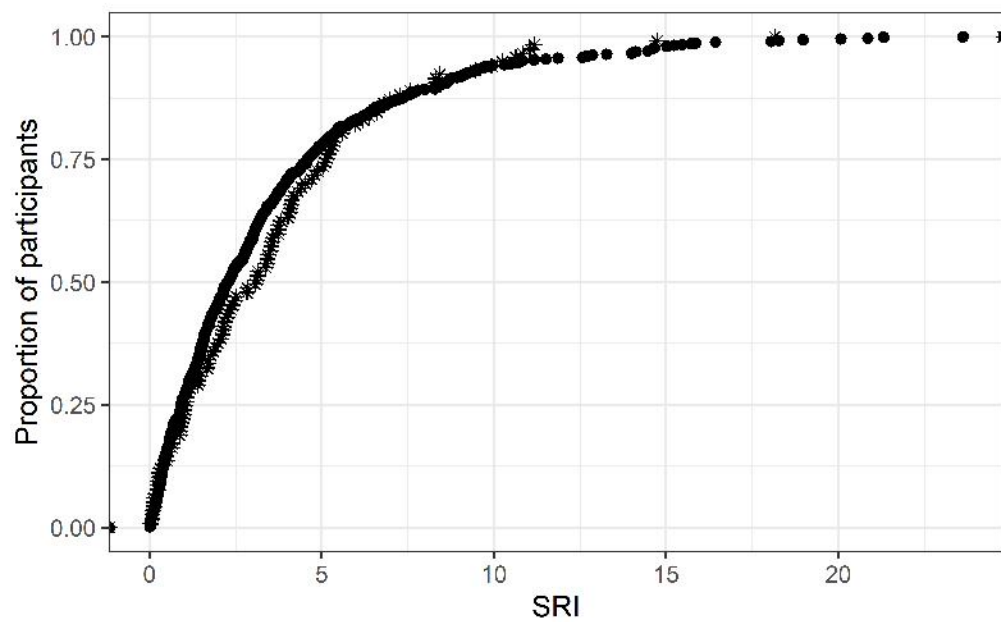
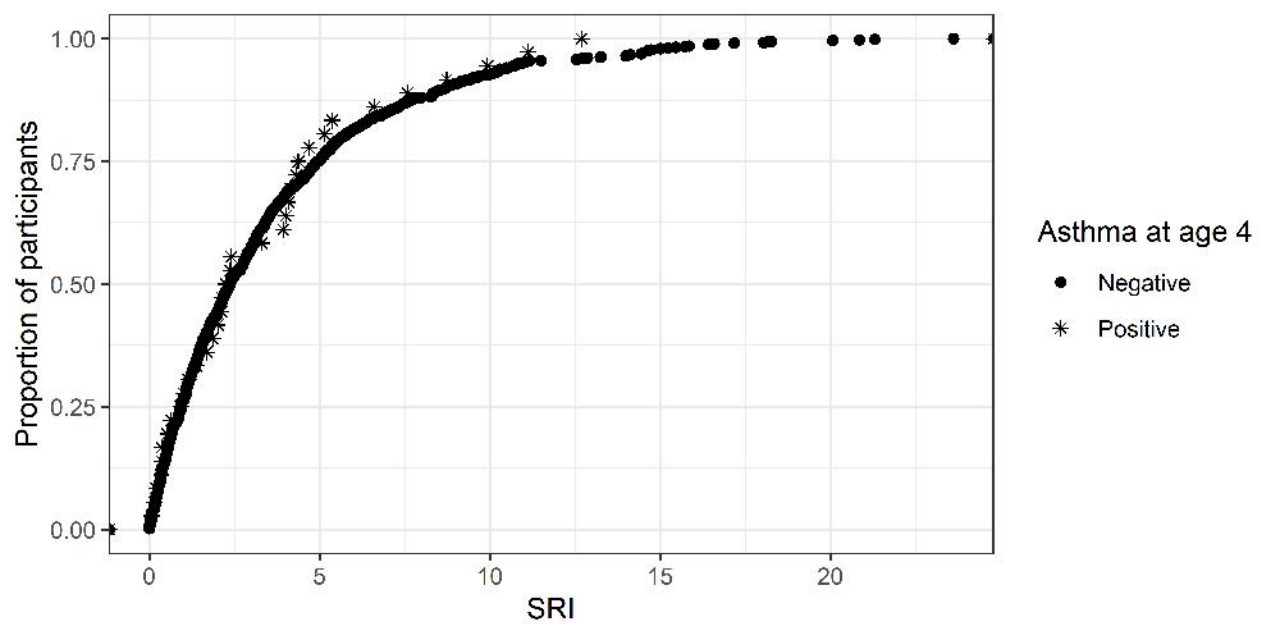
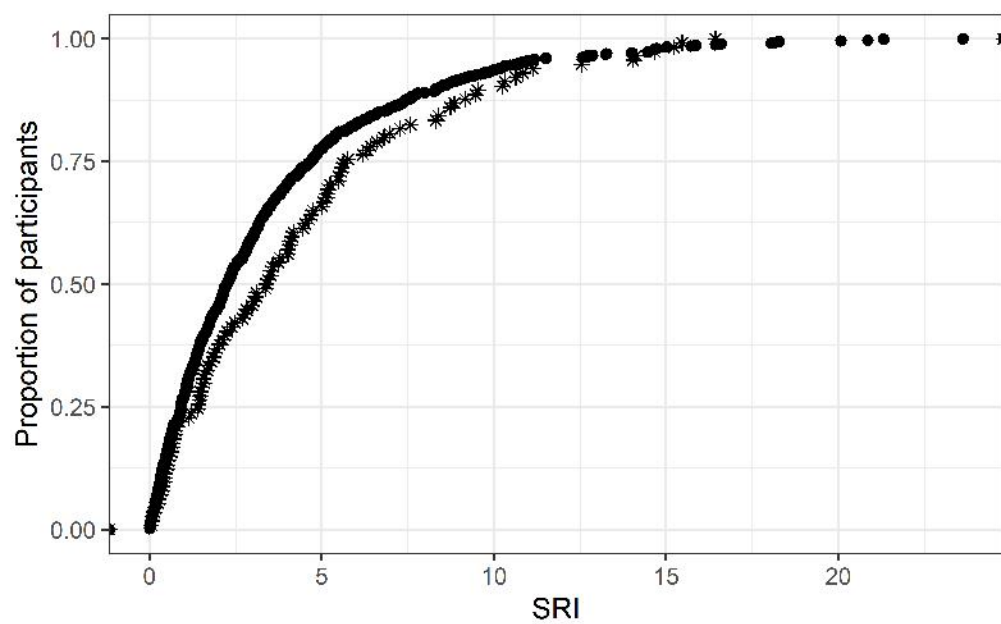
29. Li S, Batterman S, Wasilevich E, Elasaad H, Wahl R, Mukherjee B. Asthma exacerbation and proximity of residence to major roads: a population-based matched case-control study among the pediatric Medicaid population in Detroit, Michigan. *Environ Health*. 2011;10:34.
30. Ribeiro AI, Mayer A, Miranda A, Pina MF. The Portuguese version of the European deprivation index: an instrument to study health inequalities. *Acta Med Port*. 2017;30(1):17-25.
31. Guillaume E, Pornet C, De Jardin O, et al. Development of a cross-cultural deprivation index in five European countries. *J Epidemiol Commun Health*. 2016;70(5):493-499.
32. Gomes V, Ribeiro RM, Carretero MA. Effects of urban habitat fragmentation on common small mammals: species versus communities. *Biodivers Conserv*. 2011;20:3577-3590.
33. Guilherme F, Carretero MA, Farinha-Marques P. Vertebrates and invertebrates of European cities: selected non-avian fauna. In: Kelcey JG, ed. *Vertebrates and Invertebrates of European Cities*. New York, NY: Springer New York; 2015:1-667.
34. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*. 2015;67(1):48.
35. Haahtela T. What is needed for allergic children? *Pediatr Allergy Immunol*. 2014;25(1):21-24.
36. Haahtela T, Holgate S, Pawankar R, et al. The biodiversity hypothesis and allergic disease: world allergy organization position statement. *World Allergy Organ J*. 2013;6(1):1-18.
37. Rook GA. Regulation of the immune system by biodiversity from the natural environment: An ecosystem service essential to health. *Proc Natl Acad Sci USA*. 2013;110(46):18360-18367.
38. Donovan GH, Gatzolis D, Longley I, Douwes J. Vegetation diversity protects against childhood asthma: results from a large New Zealand birth cohort. *Nat Plants*. 2018;4(6):358-364.
39. Fuertes E, Markevych I, Bowatte G, et al. Residential greenness is differentially associated with childhood allergic rhinitis and aeroallergen sensitization in seven birth cohorts. *Allergy*. 2016;71(10):1461-1471.
40. Berg A, Kramer U, Link E, et al. Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years. *Clin Exp Allergy*. 2010;40(4):627-636.
41. Brunekreef B, Smit J, de Jongste J, et al. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol*. 2002;13(s15):55-60.
42. Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: presentation of a prospective longitudinal birth cohort study. *Pediatr Allergy Immunol*. 2002;13(s15):11-13.
43. Cilluffo G, Ferrante G, Fasola S, et al. Associations of greenness, greyness and air pollution exposure with children's health: a cross-sectional study in Southern Italy. *Environ Health*. 2018;17(1):86.
44. Ribeiro AI, Amaro J, Lisi C, Fraga S. Neighborhood socioeconomic deprivation and allostatic load: a scoping review. *Int J Environ Res Public Health*. 2018;15(6):1-15.
45. Ege MJ, Mayer M, Normand AC, et al. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med*. 2011;364(8):701-709.
46. Cavaleiro Rufo J, Ribeiro AI, Paciência IR, Delgado JLD, Moreira AMA. The influence of species richness in primary school surroundings on children lung function and allergic disease development. *Pediatr Allergy Immunol*. 2020;31(4):358-363.
47. Peden D, Reed CE. Environmental and occupational allergies. *J Allergy Clin Immunol*. 2010;125(2):S150-S160.
48. Cecchi L, D'Amato G, Annesi-Maesano I. External exposome and allergic respiratory and skin diseases. *J Allergy Clin Immunol*. 2018;141(3):846-857.
49. Custovic A. To what extent is allergen exposure a risk factor for the development of allergic disease? *Clin Exp Allergy*. 2015;45(1):54-62.
50. Hillebrand H, Blasius B, Borer ET, et al. Biodiversity change is uncoupled from species richness trends: consequences for conservation and monitoring. *J Appl Ecol*. 2018;55(1):169-184.
51. Dunn RR, Fierer N, Henley JB, Leff JW, Menninger HL. Home life: factors structuring the bacterial diversity found within and between homes. *PLoS One*. 2013;8(5):e64133.
52. Burbank AJ, Sood AK, Kesic MJ, Peden DB, Hernandez ML. Environmental determinants of allergy and asthma in early life. *J Allergy Clin Immunol*. 2017;140(1):1-12.
53. Barberan A, Dunn RR, Reich BJ, et al. The ecology of microscopic life in household dust. *Proc Royal Soc B Biol Sci*. 2015;282(1814):20151139.
54. Paciência I, Cavaleiro Rufo J, Silva D, et al. Exposure to indoor endocrine-disrupting chemicals and childhood asthma and obesity. *Allergy*. 2019;74(7):1277-1291.
55. Jie Y, Ismail NH, Jie X, Isa ZM. Do indoor environments influence asthma and asthma-related symptoms among adults in homes? A review of the literature. *J Formos Med Assoc*. 2011;110(9):555-563.
56. Bernstein JA, Alexis N, Bacchus H, et al. The health effects of nonindustrial indoor air pollution. *J Allergy Clin Immunol*. 2008;121(3):585-591.
57. Kanchongkittiphon W, Mendell MJ, Gaffin JM, Wang G, Phipatanakul W. Indoor environmental exposures and exacerbation of asthma: an update to the 2000 review by the Institute of Medicine. *Environ Health Perspect*. 2015;123(1):6-20.
58. Thomsen SF, Duffy DL, Kyvik KO, Backer V. Genetic influence on the age at onset of asthma: a twin study. *J Allergy Clin Immunol*. 2010;126(3):626-630.
59. Elmqvist T, Alfsen C, Colding J. Urban systems. In: Jørgensen SE, Fath BD, eds. *Encyclopedia of Ecology*. Oxford, UK: Academic Press; 2008: 3665-3672.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Study population					Population who maintained residence throughout the study		
Allergy at age 7							
		OR	95%		OR	95%	
SRI	Low	1.00	--	--	1.00	--	--
	Medium	1.66	0.90	3.07	1.59	0.74	3.43
	High	2.35	1.20	4.63	2.14	0.91	5.00
NDVI	Low	1.00	--	--	1.00	--	--
	Medium	0.55	0.30	1.02	0.42	0.19	0.90
	High	0.58	0.31	1.09	0.49	0.22	1.08
Asthma at age 7							
		OR	95%		OR	95%	
SRI	Low	1.00	--	--	1.00	--	--
	Medium	1.74	0.56	5.39	1.51	0.35	6.57
	High	2.28	0.67	7.74	2.03	0.41	9.97
NDVI	Low	1.00	--	--	1.00	--	--
	Medium	0.44	0.15	1.28	0.37	0.09	1.53
	High	0.37	0.12	1.14	0.49	0.12	2.06
Wheezing at age 7							
		OR	95%		OR	95%	
SRI	Low	1.00	--	--	1.00	--	--
	Medium	2.19	1.00	4.79	2.26	0.73	7.03
	High	2.51	1.08	5.88	3.50	1.02	11.97
NDVI	Low	1.00	--	--	1.00	--	--
	Medium	0.48	0.22	1.04	0.40	0.14	1.19
	High	0.49	0.22	1.08	0.45	0.15	1.35
Rhinitis at age 7							
		OR	95%		OR	95%	
SRI	Low	1.00	--	--	1.00	--	--
	Medium	1.53	0.72	3.25	1.65	0.63	4.34
	High	1.14	0.44	2.97	1.24	0.36	4.23
NDVI	Low	1.00	--	--	1.00	--	--
	Medium	0.74	0.35	1.58	0.39	0.15	1.03
	High	0.37	0.15	0.93	0.18	0.06	0.60

Supplementary table 1 – Comparison in the most relevant associations’ results between the study population and the subpopulation of those who did not change residence throughout the study.

Guide to species richness calculation for the city of Porto

The species richness index (SRI) for Porto was obtained from a species richness report previously compiled by Porto Municipality and the Research Centre in Biodiversity and Genetic Resources (CIBIO), according to the UrHBA methodology (1, 2). The original version of the report (in Portuguese) can be found in:

http://www.cm-porto.pt/assets/misc/img/PDM/ECD/23_PDMP_ECD_Estrut_Eco_Biod.pdf.

Urban habitats segmentation

The index first compiled information concerning the physiognomic characteristics of vegetation areas, such as being arboreal, shrub or herbaceous. According to these characteristics, habitats were divided into three different types of urban green areas: clearings, thickets and woods.

Clearings comprised green spaces predominantly composed by herbaceous flora, where the percentage of shrub or arboreal plants (phanerophyte cover) was inferior to 50% of the total green area. On the other hand, green spaces dominated by phanerophyte cover (over 50% of shrub and/or arboreal plants) were considered thickets, if highly populated by shrubs, or woods, if highly populated by trees. The area was calculated for each of these habitats and afterwards crossed with the urban fauna data.

Urban fauna

The fauna habiting the previously sectioned urban green spaces was studied according to four groups of vertebrates: birds, amphibians, reptiles and small mammals. A total of 89 species of vertebrates were accounted for the city of Porto (full list can be consulted in attachments 2.1 to 2.4 of the original report). These species included 69 birds, 7 amphibians, 7 reptiles and 6 small mammals.

Species richness calculation

A species richness potential value was calculated for each previously sectioned habitat according to its area and type (clearing, thicket or woods). A full description of this methodology is explained in attachment 2.5 of the original document.

Once the residential addresses of the participants were georeferenced using ArcGIS Online World Geocoding Service and Google Earth (shown good positional accuracy in a previous study (24)), each participant was matched to a SRI value. To express the concentration of species in the habitats that surrounded the participants' home, the sum of the number of different species present in a certain radius from the participants' house at birth multiplied by the total area, per habitat, was calculated, and subsequently divided by that area buffer, according to the equation:

$$SRI_{x=y} = \frac{1}{n} \sum_{y=1}^n N^{\circ} \text{ of species}(x, y) \times \text{Habitat area}(x, y) / B$$

The equation represents the SRI calculation formula, where “x” represents the participant and “y” a specific habitat in a determined radius from the participant's house, with “n” being the total habitat number for a given participant. The area buffer (B, in m²) is given by $\pi(\text{radius})^2$. As an example, if participant x = 1 has n = 8 habitats surrounding his/her home, the associated SRI will be equal to the sum of the number of species multiplied by the habitat area (in meters) for each of the habitats (y = 1, ..., 8), divided by the area buffer.

References

- 1 Gomes V, Ribeiro RM, Carretero MA. Effects of urban habitat fragmentation on common small mammals: species versus communities. *Biodiversity and Conservation*. 2011; **20**: 3577-90.
- 2 Guilherme F, Carretero MA, Farinha-Marques P. Vertebrates and Invertebrates of European Cities: Selected Non-Avian Fauna. In: Kelcey JG (ed.). *Vertebrates and Invertebrates of European Cities*. New York, NY: Springer New York 2015.