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Title: The influence of species richness in primary school surroundings on children lung function and allergic disease development

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Abstract

Background: Primary schools represent important environments for biodiversity exposure and thus may play a crucial role on early-life immunomodulation, protecting against allergic sensitization. The aim of this study was to understand how the exposure to different levels of species richness surrounding urban primary schools may influence the development of allergic diseases and asthma in children.

Methods: A species richness index (SRI), evaluating habitat diversity in terms of amphibians, birds, reptiles and small mammals, was estimated and attributed to 20 primary schools in the city of Porto, Portugal. The SRI was measured considering a 100 m straight-line buffer around the schools. Children who attended the participating schools were invited to participate in the study and performed spirometry with bronchodilation, skin-prick tests and had their parents fill a questionnaire concerning allergy and asthma symptoms, as well as demographic and socioeconomic data. Asthma was defined according to three distinct criteria.

Results: The study results showed significant and positive associations between the exposure to species richness in schools and the forced expiratory volume at the first second (FEV₁) and forced vital capacity (FVC) parameters both before and after bronchodilation, independently of the asthma and atopic status. Fully adjusted models revealed that a unitary increment in the SRI was associated with an average increase of approximately 2 and 3 mL in FEV₁ and FVC, respectively.

Conclusion: Species richness in school surroundings may impact lung function development in children. However, this increase in lung function was not associated with any clinically relevant protective effect on allergy and asthma development.

Keywords

Allergy; Asthma; Biodiversity; Lung function; Schoolchildren; Schools; Species richness

Introduction

Evidence provided by recent studies on determinants of allergic disease and asthma suggest a protective effect associated with early-life exposure to a more biodiverse environment (1-4). The presented hypothesis imply that exposure to a higher number of species has an impact on the human microbiome, which in its turn may play an important role in the development of allergic diseases and asthma (2, 5). Consequently, children living in urbanized areas are more prone to develop these conditions when compared to those living in rural environments. This has been demonstrated back in 2001 by Riedler and co-workers, who showed that children growing in contact with livestock had a superior immunomodulation and were thus more protected from developing allergic sensitization and asthma (6). Since then, a significant number of studies have supported these findings, as reviewed by von Mutius and Vercelli (7), and even in urban areas, children living in inner-city environments with more diverse microbiological species have been shown less likely to develop allergy and asthma symptoms (8).

However, children's homes may not be the only important setting for biodiversity exposure in urban areas. Apart from home, children spend most of their time at school, where they may contact with different species, either in classrooms or outdoor, during recess periods (4). In fact, Cavaleiro Rufo *et al.* showed, in 2017, that children exposed to a higher number of fungal species

in primary schools were less likely to develop allergic sensitization (9). One year later, Lai and co-workers reported that microbial diversity in inner-city schools was significantly higher than at children's homes (10). Therefore, evidence appears to support the importance of biodiversity exposure at schools for children living in urban environments. If this is the case, primary schools may not only have the important role of educating children, but may also play a crucial role on educating their immune systems, potentially protecting them against allergic sensitization development.

However, city schools are usually located in different urban environments and, therefore, species richness surrounding the schools may vary considerably according to neighbouring habitats. In fact, even small green spaces in highly urbanized areas can hold a significant amount of species richness depending on their habitat quality (11). Hence, the impact of the species richness in the surroundings of urban schools on children allergy and asthma prevalence should be further scrutinized.

The aim of this study was to understand how the exposure to different levels of species richness surrounding urban primary schools may influence the development of allergic diseases and asthma in children.

Methods

Study participants

The present study was conducted using the ARIA study database (9). Briefly, a total of 20 public primary schools located in the city of Porto, Portugal, were invited to participate in this cross-sectional survey, corresponding to a total of 71 assessed classrooms. The children's recruitment and clinical assessment were performed at the selected schools and occurred from January 2014 to March 2015.

All schools where the study was performed, along with the Ethical Committee of the University of Porto, approved the study protocol. Children attending the 71 participating classrooms were invited to enrol in the study, corresponding to 1602 invited children. After receiving the written consent from their legal guardians, 916 children were enrolled in the study (participation rate of 57%). Considering a confidence level of 95%, the estimated confidence interval for the sample

size was 2.42. A total of 58 children refused to perform the clinical tests having only the questionnaire data available, thus spirometry, skin prick testing and exhaled nitric oxide data were retrieved from 858 children. Among these individuals, 13 had poor quality lung function test data, and were thus excluded. The excluded children had no significant differences in gender, age, parental education and reported respiratory symptoms. Hence, 845 individuals were included in the present study (50.8% males).

Clinical assessment

All participating children had their lung function (spirometry with bronchodilation) and exhaled level of nitric oxide (FeNO) measured by a trained professional. Skin-prick-tests (SPT) were also performed to check allergic sensitization status. The participants' parents fulfilled a questionnaire based on the International Study of Asthma and Allergies in Childhood (ISAAC) where they reported the children's symptomatology history.

Lung function and airway reversibility were assessed according to the official ATS/ERS guidelines (12). Lung function data was retrieved before and 15 minutes after the administration of 400 µg of inhaled salbutamol.

Airway inflammation was assessed by measuring FeNO levels using the NObreath device (Bedfont Scientific Ltd. UK). The results were expressed as parts per billion (ppb) and stratified according to the official ATS guidelines for children (13).

Allergic sensitization was evaluated by SPT on the participants' forearm using a QuickTest™ applicator containing *Der p1*, mix of weeds (*Urtica dioica*, *Plantago lanceolata* and *Artemisia vulgaris*), mix of grasses (*Agrostis stolonifera*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Poa pratensis*, *Holcus lanatus*, *Phleum pratense*, *Secale cereal*), *Fel d1*, *Can f1* and *Alt a1*, negative control (extracts diluent), and a positive control (histamine at 10mg/mL), all belonging to the same batches (Hall Allergy, Netherlands). Results were read 20 minutes afterwards and allergic sensitization was defined by a positive SPT to at least one of the allergens. If children were on antihistamines or topical corticosteroids on the skin within the previous 7 days, SPTs would be postponed.

The following operational asthma definitions were adopted: Asthma by clinical criteria – at least a 12% increase in FEV₁ after bronchodilation and over 200mL and/or asthma diagnosed by a physician with reported symptoms of asthma (wheezing, dyspnoea or dry cough) occurring in the past 12 months; Asthma by functional criteria – at least a 12% increase in FEV₁ from baseline after bronchodilation and over 200mL; and Asthma diagnosed by a physician – having answered “yes” to the question “*Has your child ever been diagnosed with asthma by a physician?*”. Children were classified as having allergic rhinitis when their legal guardians answered positively to the question “Did the participant suffer from recurrent sneezing, rhinorrhea or nasal congestion in the last 12 months, while not having a cold or flu?”. Atopic dermatitis was defined in accordance with the United Kingdom Working Party’s Diagnostic Criteria for Atopic Dermatitis (14). Characterization of the participants is presented in Table 1.

Species richness index

The species richness index (SRI) for the Porto Metropolitan Area was obtained from a species richness report previously compiled by Porto Municipality and the Research Centre in Biodiversity and Genetic Resources (CIBIO) (15, 16). A full description on how the species richness report was conducted is presented as supplementary information. Briefly, the number, type and area of habitats in Porto were retrieved, followed by an estimation of the number of species living in those habitats. Included species consisted in four vertebrate groups (amphibians, birds, reptiles and small mammals), totalizing 89 different species.

Once the participating school addresses were georeferenced using ArcGIS Online World Geocoding Service and Google Earth, each participant was matched to a SRI value according to their respective school. This georeferencing methodology showed good positional accuracy in a previous study (17). To express the concentration of species in the habitats that surrounded the schools, the sum of the number of different species present in the habitats located within a 100 m radius multiplied by the total area of each habitat and subsequently divided by the buffer area in square meters (Equation 1).

Equation 1 – The SRI calculation formula, where “x” represents the school and “y” a specific habitat in the 100m radius from school $x=I$, with “n” being the total habitat number for school I . The 100m radius area buffer (B) corresponds to the total 100m area surrounding the schools ($B=\pi.100^2$). As an example, if school I has $n=8$ neighbouring habitats, 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 ($y = I, \dots, 8$), divided by the area buffer (B).

$$SRI_x = \frac{\sum_{y=1}^n N^o \text{ of species}_{(x,y)} \times \text{Habitat area}_{(x,y)}}{B}$$

Statistical analysis

Absolute frequencies and percentages or medians and interquartile-range were computed for categorical and continuous variables, respectively. Distribution of continuous variables were analysed for normality. Whenever non-Gaussian distributions were observed, non-parametric 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. Statistical significant results were reported when $p < 0.050$.

Mixed models were then performed with school and classroom specified as random-effect variables in order to estimate the influence of SRI exposure on the measured health outcomes. The following confounders were considered in the adjusted models: age (in years), sex, school, classroom and maternal education. Whenever stated, allergic sensitization status and diagnosis of asthma were also included as confounders.

Associations were expressed as odds ratio and 95% confidence intervals (95% CI) or standardized beta coefficients, β , and 95%CI for dichotomous and continuous variables, respectively. Statistical analysis was performed using R.

Results

Analysis revealed no protective effect associated with school neighbouring SRI on the participating children allergic disease and asthma prevalence (Figure 1). Moreover, in regards to atopic dermatitis, the prevalence was significantly higher in schools with higher SRI (mean SRI for present vs not present atopic dermatitis: 3.52 vs 2.87, $p=0.033$).

Mixed models corroborated the aforementioned results, as there was no significant association observed between school SRI and the risk of allergic diseases or asthma (Table 2), with the exception of atopic dermatitis, which showed a significantly higher risk associated with SRI (OR [95%IC] = 1.011 [1.000 : 1.023]; 1.009 [1.000 : 1.0195] after adjustment).

While observing Pearson's correlations between biomonitoring parameters and school SRI, significant positive correlations were observed for FVC and FEV₁ before bronchodilation, and for FVC after bronchodilation ($\rho=0.092$, 0.080 , and 0.094 , respectively). The median and interquartile range for each of these parameters, distributed through the participating schools, is available as supplementary material (Supplementary table 1). As the results suggest, it appears that there is an association between school SRI and the expiratory spirometry parameters, where the exposure to a higher SRI improves the lung function of children. To further explore these results, mixed models were fitted using school and classroom as random-effect variables (Figure 2) and, when adjusted for asthma diagnosis (clinical criteria) and allergic sensitization status, a significant association was still observed for FVC parameters (β [95%CI] = FVC pre: 10.8 [$1.1 : 20.7$]; FVC pos: 11.5 [$1.2 : 21.9$]). No significant association was observed for FEV₁ and FVC reversibility. Models were then adjusted for further confounders (age, sex, school, classroom and maternal education). After full adjustment, the positive association between SRI and the FCV parameters remained, although the tendency was no longer significant (β [95%CI] = FVC pre: 3.2 [$-5.2 : 11.7$]; FVC pos: 4.2 [$-3.8 : 12.2$]). A unitary increment in school SRI was associated with an average increase of approximately 2mL and 3mL in baseline FEV₁ and FVC, respectively.

Discussion

Children attending primary schools with higher species richness in the surroundings presented a tendency for improved lung function when compared to children in schools with a lower SRI. However, no protective effect was observed for species richness on allergic diseases or asthma. In fact, there is a higher prevalence of atopic dermatitis in schools with higher SRI. This is the first

study showing how species richness surrounding primary schools may influence lung function and allergic diseases in children.

The association between lung function and species richness observed in this study supports the results obtained by Liddicoat and co-workers in 2018, where they showed the protective effect of species richness on public hospital admissions for respiratory disease in the moderate majority socio-geographic cluster of the Australian population (18). Moreover, authors interestingly showed that the protective effect of major vegetation diversity on respiratory diseases was more significant than the overall species richness. This may actually be caused by the protective effect on urban pollution associated with some arboreous species, which may reduce the levels of particulate matter and noxious gases in those areas (19). Unfortunately, the quantification of species for the SRI used in the present study was provided as the cumulative value of species, thus it was not possible to consider only the impact of large vegetative species on the model.

Recently, Mills *et al.* (2019) demonstrated that habitat changes from urbanisation strongly associates with disease (20). Now, having in mind the present results and the SRI calculation method, we may assume that a school with a higher number of habitats has a higher chance of having a higher SRI, thus children studying in those schools would be less prone to develop allergic and respiratory diseases. Moreover, since the exposure to the environmental biodiversity appears to influence the human microbiome (21-23), and considering that airway microbial diversity has been associated with mite-sensitized rhinitis and asthma in early childhood (24-26), a lower prevalence of allergic diseases and asthma was expected to be observed on schools with higher species richness. However, this was not observed in the present study, with the prevalence of atopic dermatitis actually increasing in schools with higher SRI. Green environments and human microbiome interactions should be further studied in order to completely understand if green surroundings indeed affect the microbiome and, consequently, the immune regulation.

The increased atopic dermatitis prevalence observed in schools with higher SRI may be associated with the self-reported methodology used to classify participants, which also considered skin symptoms and/or exacerbations occurring in the previous 12 months (14). It is expected that, in a more diverse environment, there will also be a higher diversity of allergens, thus further inducing the occurrence of atopic dermatitis symptomatology, consequently leading to higher parental-reported prevalence in schools with higher SRI.

Lung function may also be affected by lung microbial composition that, as previously mentioned, can be determined by environmental exposure (27, 28). In this case, lung function was increased in participants attending higher SRI schools, thus supporting the protective effect of a more biodiverse environment on pulmonary health. However, it is very important to understand that the improved lung function found in children attending high SRI schools has no clinical implications for the outcomes screened in the present study. This is supported by absence of SRI effect on both FEV₁ and FVC reversibility. The increase in FEV₁ and FVC is also relatively low, with an average of 2 and 3 mL increase, respectively, for each unit of SRI. Nevertheless, these changes may provide a significant protective effect over time, supporting the concept of a better health and wellbeing associated with exposure to a natural environment (29).

The present study has some limitations, mainly based on the SRI calculation. Although considering habitat area measurements and different macroscopic taxa, the estimation does not account for microbial species richness. Children home environment, which was not considered in the present analysis, is known to have a major influence on their exposome and adopted lifestyle, possibly influencing the results. Moreover, participants' behaviour during recess periods and school boundaries structure in the proximity of their playground may also influence species exposure. Unfortunately, since it was not possible to collect children behavioural data, these eventual confounders were not considered in the analysis. In addition, the study follows a cross-sectional approach, which does not allow causal associations nor exposure-dose assumptions to be established. On the other hand, this is the first study to investigate the impact of exposure to school species richness in the development of allergic diseases and asthma. Moreover, asthma outcomes were established through three different criteria, and lung function was measured using spirometry with bronchodilation, which is the most validated method for this application (30).

In face of the observed results, it is possible to suggest that species richness in school surroundings may have an impact on lung function development in children. However, despite this increase in lung function, a higher species richness in schools was not associated with a protective effect for allergy and asthma, suggesting a need for better markers of the eventual role of exposure to species diversity. Ultimately, this work supports the concept that a more biodiverse urban environment increases lung function, which may be translated in an improved pulmonary health in the long-term. However, the lack of clinical significance of this improved lung function, as well as the significantly higher prevalence of atopic dermatitis in children attending schools with higher

species richness, does not allow us to support the hypothesis of a protective effect being associated with higher number of species in school surroundings. Longitudinal studies are needed for a causal relationship to be established.

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Table 1 – Characteristics of the included participants.

Characteristics	N (845)
Males (%)	50.8
Age (mean $\pm$ SD, years)	9 ($\pm$ 1)
Maternal education higher than 9 years (%)	43.4
Allergic sensitization (%)	35.4
FeNO (mean $\pm$ SD, ppb)	16.8 ($\pm$ 19.6)
Wheezing in the last 12 months (%)	25.2
Asthma by functional criteria [¶] (%)	6.7
Asthma diagnosed by a physician (%)	6.5
Asthma by clinical criteria [†] (%)	9.5
Allergic rhinitis (%)	64.4
Atopic dermatitis (%)	20.4

[¶] Corresponds to a 12% and 200mL increase in FEV₁ from baseline after bronchodilation, according to official guidelines (12). [†] Asthma diagnosed by a physician with associated respiratory symptoms, or having a positive bronchodilation.

Table 2 – Associations between the participants' clinical outcomes and the species richness index in respective schools.

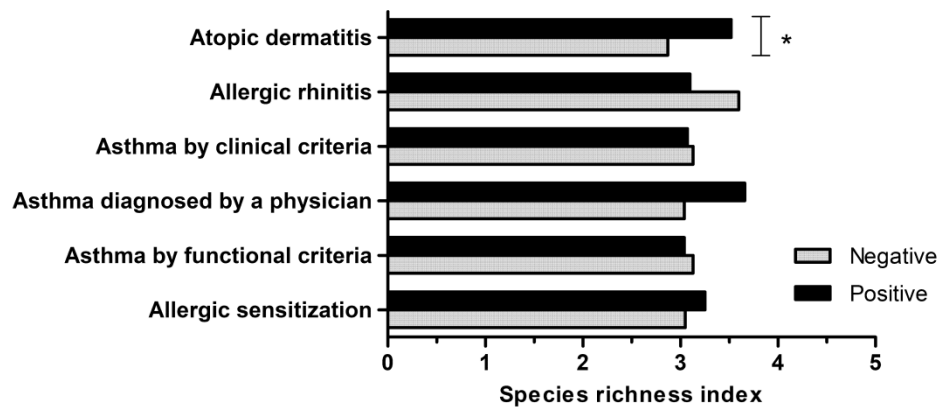
	Crude model	Adjusted model^a
	OR (95% CI)	OR (95% CI)
Allergic sensitization	1.004 (0.982 : 1.027)	1.005 (0.980 : 1.030)
Asthma by functional criteria	0.993 (0.994 : 1.005)	0.998 (0.992 : 1.005)
Asthma diagnosed by physician	1.004 (0.998 : 1.010)	1.004 (0.998 : 1.011)
Asthma by clinical criteria	1.000 (0.993 : 1.006)	1.000 (0.992 : 1.007)
Allergic rhinitis	0.990 (0.973 : 1.007)	0.991 (0.973 : 1.009)
Atopic dermatitis	1.011 (1.000 : 1.023)	1.009 (1.000 : 1.019)

^aAdjusted for age, sex and maternal education. School and classroom variables were specified as random-effects in the models. Values in bold represent statistically significant values.

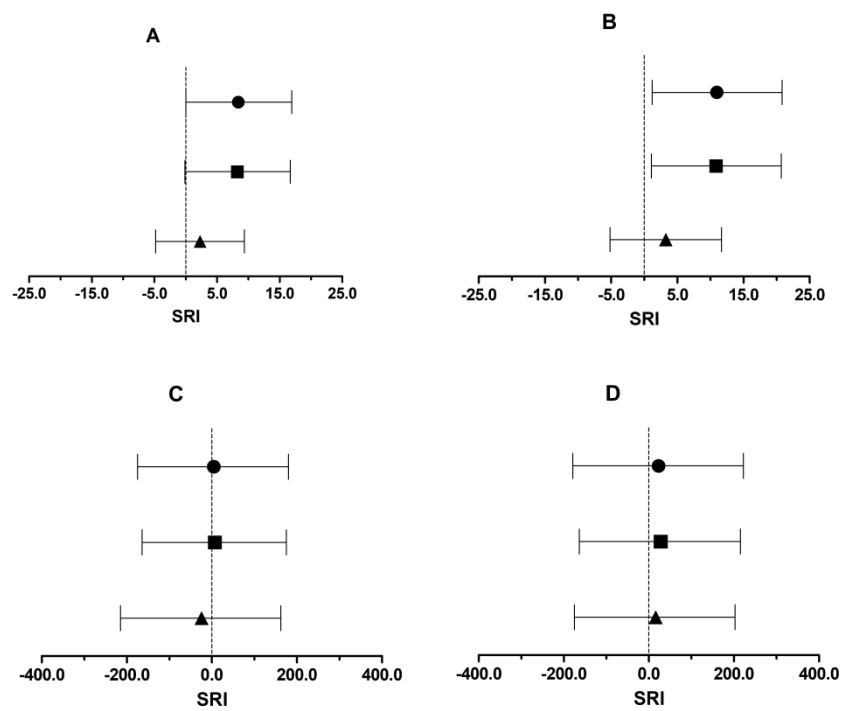
Figure legends

Figure 1 – School SRI and the prevalence of allergic diseases and asthma in participating children.

Figure 2 – Associations between the mean change (in millilitres) of lung function parameters and the species richness index (SRI). A: FEV₁ at baseline; B: FVC at baseline; C: FEV₁ reversibility; D: FVC reversibility; •Crude model; ■Model 1: Adjusted for asthma and atopy; ▲Model 2: Adjusted for asthma, atopy and socio-demographic confounders (sex, school, classroom, maternal education and age).



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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 participating school addresses were georeferenced using ArcGIS Online World Geocoding Service and Google Earth (shown good positional accuracy in a previous study (24)), each school was matched to a SRI value. To express the concentration of species in the habitats that surrounded the schools, the sum of the number of different species present in a 100 m radius around the school, per habitat, was calculated, and subsequently divided by the 100m area buffer, according to the equation:

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

The equation represents the SRI calculation formula, where “x” represents the school and “y” a specific habitat in the 100m surrounding radius, with “n” being the total habitat number for a given school. The 100m radius area buffer ($B = \pi \cdot 100^2$) is 31415.93m². As an example, if school $x = 1$ has $n = 8$ surrounding habitats, 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 ($y = 1, \dots, 8$), divided by the area buffer.

References

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