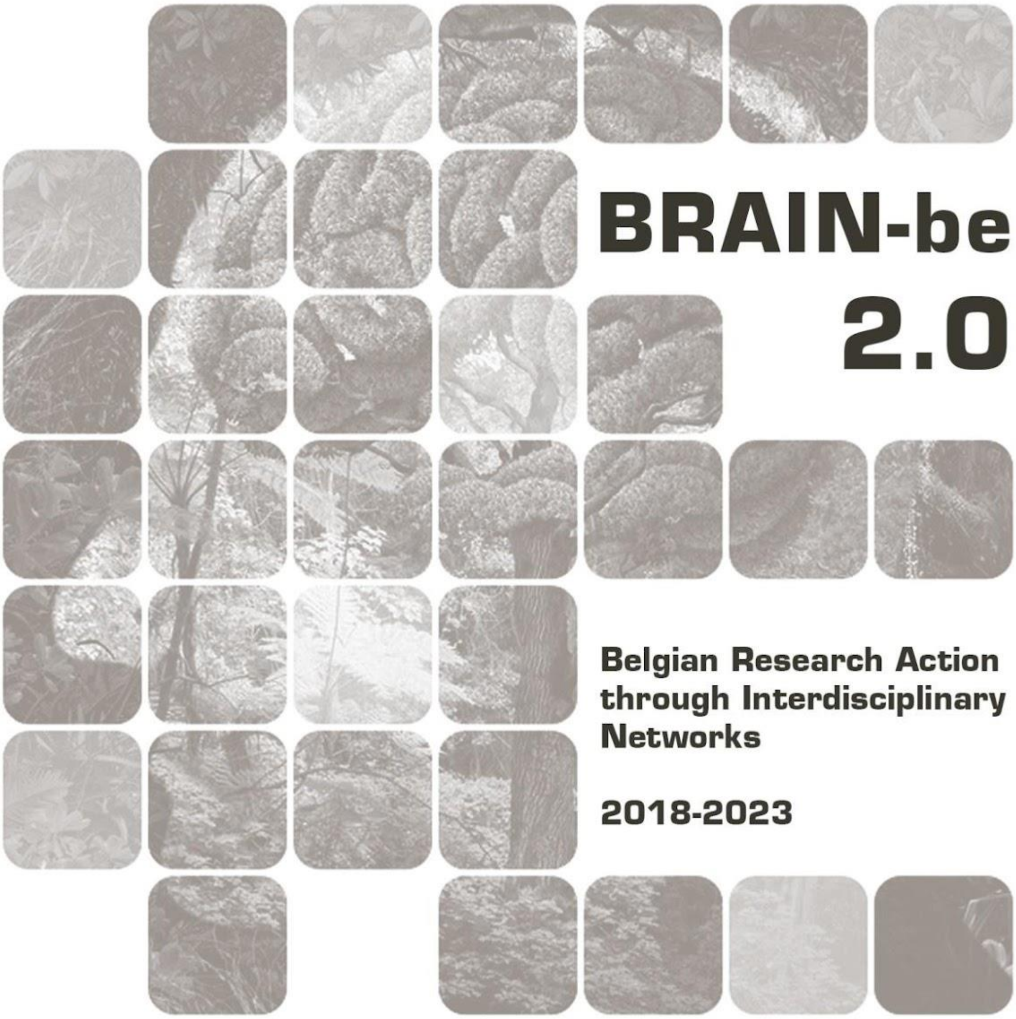

B@SEBALL

Biodiversity at School Environments Benefits for ALL

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Pillar 3: Federal societal challenges

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Belgian Research Action through Interdisciplinary Networks

2018-2023

NETWORK PROJECT

B@SEBALL

Biodiversity at School Environments Benefits for ALL

Contract - B2/191/P3/B@SEBALL FINAL REPORT

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ABSTRACT

B@SEBALL investigated the health contribution of biodiversity at school environments, and how this knowledge can be relevant to reducing health inequality among children. The B@SEBALL study was conducted as planned, and all planned deliverables were achieved as outlined in this document. B@SEBALL evidence supports the importance of a green school environment for mental well-being and healthy immune system development. B@SEBALL evidence supports the importance of diverse natural elements and plant-associated bacteria on the playgrounds for less reported rhinitis symptoms in children and a healthier immune development of school children reflected in less reported allergic symptoms. B@SEBALL evidence underlines the importance of tackling health inequality due to unequal access to a green environment: the results of B@SEBALL show that self-reported well-being of children is higher in greener school playgrounds. Additionally, this positive association is even greater for children with lower socio-economic status (SES). Funds and other guidance support may be targeted toward greening of schools for which socio-economic indicators are low (irrespective of landscape type). Schools can contribute to children's physical and mental well-being by increase of the level of biodiversity: different natural elements at the school playground. Also awareness raising activities may be promoted among local communities and schools.

INTRODUCTION

B@SEBALL aimed to contribute to more equal health opportunities for children, by investigating the health contribution of biodiversity at school environments, and how this was distributed among children with different socio-economic and cultural backgrounds. Reducing health inequality is an important challenge of primary health care. According to the biodiversity hypothesis (Hanski et al. 2012), microbial contact of people with biodiversity is important for human health, especially in childhood. Access to environments with biodiversity, such as urban green spaces and nature sites, are not evenly distributed among children.

Chronic health conditions associated with urban lifestyle are on the rise (Dye 2008). Especially mental health appears to be lower in urban environments (Pelgrims et al 2021). One of the main current challenges in this field of research is unravelling the importance of the specific quality of nature (Frumkin et al. 2017) and biodiversity (Aerts et al. 2018). Enhanced immune functioning emerges as one promising candidate for a central pathway between nature and health (Kuo 2015). The relation between the diversity of the environmental microbiomes and the human microbiome of the people exposed to this environment is currently underexplored, particularly in children.

STATE OF THE ART AND OBJECTIVES

Key research foci in the field of the research project

B@SEBALL focused on the following research foci:

1) How biodiversity in the school environment affects children's health and mental well-being and can be linked to human microbiome diversity

2) How this knowledge is relevant to school management and design

3) How this knowledge is relevant to reducing health inequality among children

Main findings of past research (<2022)

Residential green space has been linked to lower rates of mortality (Bauwelinck et al 2021). Many studies report positive effects of nature on mental health, stress reduction and attention restoration (e.g. Alcock et al. 2014, Thompson et al. 2012, Mitchell and Popham 2008, Van den Berg et al. 2014, Hartig et al. 2003, Ulrich et al. 1991). Convincing evidence points towards the contribution of exposure to nature during childhood in improving cognitive development and mental health, varying from reducing children's emotional and behavioral difficulties (Vanaken and Danckaerts 2018) to lowering the risk of developing mental health disorders later in life (Engemann et al. 2019). Residential green areas and their potential health benefits have thus received increasing attention, also in the context of environmental health inequalities, where an unequal social distribution of these resources may contribute to health inequalities. Vulnerable population groups lack both individual green space (such as gardens and green street segments) and community resources (green public place, such as parks, playgrounds and green schools) (Schüle et al. 2019). This inequality is exacerbated by the findings from the leisure sciences that certain social groups may be less likely to visit green spaces or to be less likely to use them for active recreation (Cole et al. 2019, Hunt et al. 2016). This observation heightens the importance of green school environments. The existing literature on the health benefits of green school environments is limited, but growing (van den Bogerd et al. 2020a, b; van Dijk-Wesselius et al. 2018, 2020; van den Berg et al. 2017; Maas et al. 2013) There are sufficient indications to warrant further research on this topic (Browning et al. 2019). There are however few studies comprehensively investigating biodiversity and its influence on health and mental wellbeing (Aerts et al. 2018). Furthermore studies are not conclusive: while some studies found a positive relation between plant species richness and well-being/reflection (Fuller et al. 2007, Carrus, et al. 2015) others found a negative association (Dallimer et al. 2012). Recently, a link is emerging between environmental microbial biodiversity, the microbiome of children and positive effects on immunological and other health outcomes (Gisler et al. 2021, Roslund et al. 2020). Especially during childhood, exposure to specific microorganisms and/or a diverse environmental microbiomes has been associated with a lower risk for developing inflammatory conditions, such as allergies and asthma (Kirjavainen et al. 2019, Fyhrquist et al. 2014, Ege et al. 2009), although the exact link is yet to be elucidated.

Existing gaps of past research

A recent study indicates that nearby green space can determine the diversity of environment microbiota (Dockx et al 2021), but further research can provide more detail in this relation, as well as their contribution to health benefits. Despite mounting evidence that people with a diverse microbiome or who interact with green spaces enjoy better health, studies have yet to directly examine how biodiverse urban green spaces might modify the human microbiome and reduce chronic disease. Another challenge is to enhance access to nature for all, which is unevenly distributed among social groups with different socio-economic and cultural backgrounds. We will also investigate how child behaviour and parent attitude with respect to playing in nature are of influence. In this project, the environmental microbiome, defined as the microbes in soil, on plants, and associated with air dust, will be targeted, as well as its social distribution and link with the microbiome of children. Limited environmental, social, and behavioural data on study participants continue to be an important limitation in the interpretation and generalizability of many published results (Soga and Gaston. 2020). In our study, all these data will be collected in a sample of schools from a study design that as much as possible controls for possible confounding variables. This study will also address the adverse effects of green space, looking into the occurrence of allergic disease caused by aeroallergens (Aerts et al 2018), which is often overlooked, and which can also be influenced by the surrounding green space. The study design entails a comparison of schools with low naturalness playground (control) versus schools with high naturalness playground (case). Each case-control will be matched to have similar location, outdoor air pollution levels and socio-economic status. Although this is a cross-sectional study (within the timeframe of the project only a single survey can be undertaken), the design can be easily repeated so that it becomes a longitudinal study. When combined with interventions this can further improve the understanding of possible mechanisms behind the associations.

New research contributions

The B@SEBALL project was conducted in the school environment with different playground types, because children spend a significant amount of time in this environment. By collecting and integrating a wide range of data, including environmental biodiversity and microbiome data, child microbiome and a detailed health and socio-economic status questionnaire, including self-reported allergies and well-being indicators, B@SEBALL contributed to knowledge linking biodiversity and health, and to knowledge about social distribution effects.

B@SEBALL contributed to the capacity of Sciensano and other involved research institutes in building capacity in this promising field of research. B@SEBALL also contributed to tackling the prominent problem of health inequalities among children, due to unequal health access to natural surroundings.

B@SEBALL particularly focused on the socially vulnerable group of young children, who are more susceptible to unequal opportunities and risks for their health. In order to enhance chances for fair treatment in relation to the benefits of contact with green spaces, B@SEBALL investigated how resulting health inequalities for these groups of the population can be partly compensated by green playgrounds at primary schools. B@SEBALL particularly investigated the health potential of microbial contact with biodiversity of green spaces for children's health. Recently Finlay et al. (2021) have even suggested that Covid-19 protection measures may have long-term impacts on human microbiome, which can complicate matters.

Discussion on what is expected in terms of policy maker recommendations

Based on the research outcomes of B@SEBALL, stakeholder groups, families and policy makers have additional practice relevant knowledge and arguments for enhancing a more equal distribution of children's access to nature for their health. Knowledge on the health benefits of environmental biodiversity within different school playgrounds can contribute to optimal school playground design and environmental contact recommendations for children.

METHODOLOGY

WP1 Integrated assessment

Task 1.1 Methodological implementation sampling design

Sampling design

A stratified matched case-control design was employed to compare schools with low versus high greenness playgrounds (Rosenbaum 2010). The study paired schools with similar characteristics in location, outdoor air pollution levels, and socio-economic status. Socio-economic status and outdoor air quality are considered potential confounders for effects of biodiversity on health (Aerts, Honnay, and Van Nieuwenhuyse 2018; Aerts et al. 2020). The sample was divided into two strata based on the naturalness of the landscape within a 2000 m buffer around each school:

1. Low naturalness landscape: Schools in urbanized areas with high impervious surface levels and low combined forest and grassland cover.
2. High naturalness landscape: Schools in rural areas with low impervious surface levels and high combined forest and grassland cover.

The following criteria were used to select eligible schools:

1. Minimum enrollment of 120 children
2. Ordinary primary schools (excluding specialized schools for children with special needs)
3. Available geo-information (school parcel perimeter)
4. Fit into one of four categories based on: low vs. high greenness at school level crossed with low vs. high naturalness at landscape level

These criteria reduced the initial pool of 5582 schools to 600 eligible schools. A stratified matched case-control sample was drawn from this set of 600 eligible schools. The matching and sampling procedure consisted of the following steps:

1. Within each landscape context, matching was based on Mahalanobis distance between potential cases and controls.
2. Case-control pairs exceeding the median Mahalanobis distance were discarded.
3. The optimal full matching algorithm (Hansen and Olsen Klopfer 2006) was used to select 1:1 matched case-control pairs.
4. The local pivotal method (Grafström, Lundström, and Schelin 2011; Grafström and Lundström 2013) was employed to draw a probabilistic sample from the case-control pairs.

This sampling procedure was used to draw a sample of a sample size larger than was needed for the study in order to be able to replace school(-pairs) in case of unwillingness to participate in the study. The sampling order was used to contact and recruit schools. Adherence to the sample order ensured that the final sample was approximately balanced and well-spread regarding mean location and socio-economic status compared to the 600 eligible schools in the sampling frame.

The study protocol was approved by the Committee for Medical Ethics UZA-UA of the University of Antwerp prior to recruitment (Belgian registration number B3002020000242). Informed consent was obtained and documented.

The participant-level inclusion criteria of the study required that participants were attending fifth grade of primary school (in Belgium, education is compulsory between the ages 6 and 18; the primary school consists of a period of six years) and that parents agreed to provide detailed background information and that their children were allowed to complete questionnaires.

A total of 167 schools were contacted in 2021 out of which 40 expressed interest to participate and 37 participated (Figure 1.1); these 37 schools had 929 children in the fifth grade out of which 527 children (57%) gave consent to participate in at least one of the tests (Figure 1.2).

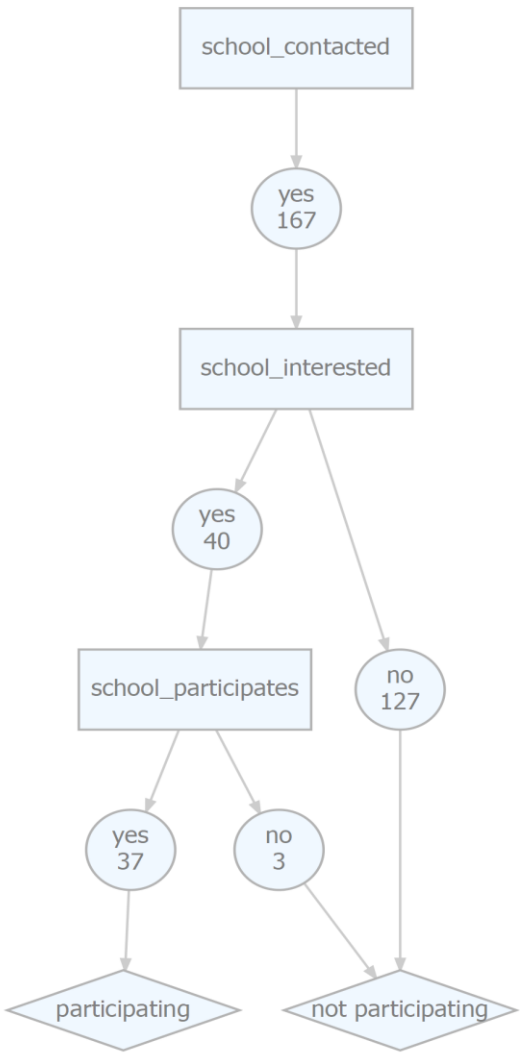


Figure 1.1 Number of contacted and participating schools.

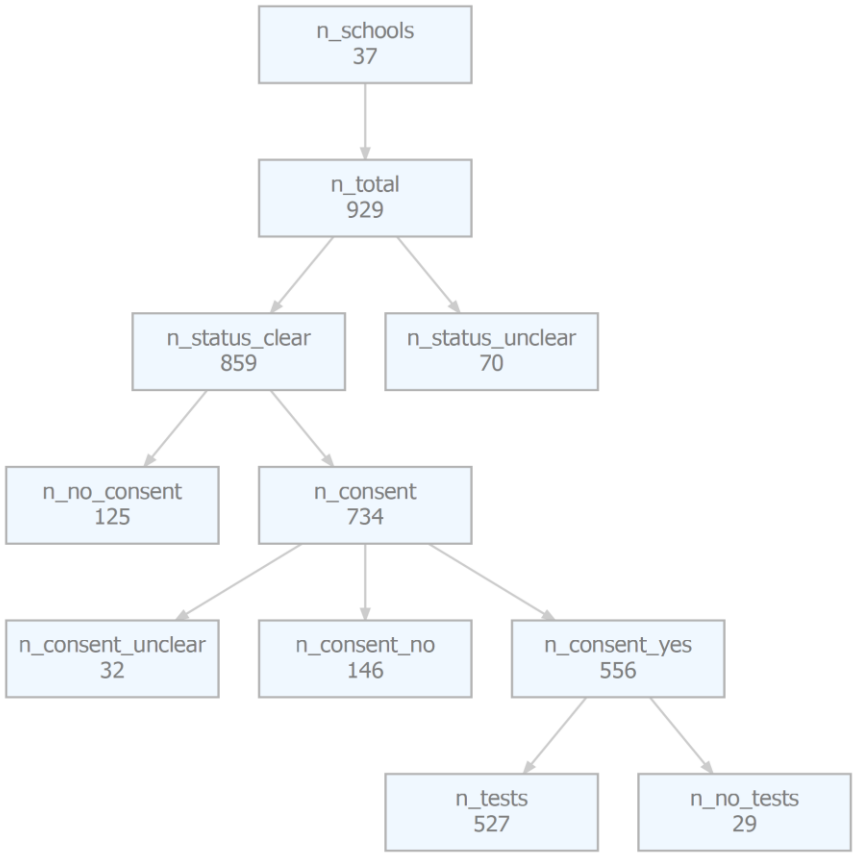


Figure 1.2 Breakdown of the number of children of the fifth grade in the 37 recruited schools.

We originally planned to recruit 72 schools, 18 in each combination of landscape type and school context with an assumed number of 20 children per school. The realised number of recruited schools and participating children can found in Table 1.1.

		School context		
	Landscape type	Low greenness	High greenness	Paired
Planned	High naturalness	18 (360)	18 (360)	18
	Low naturalness	18 (360)	18 (360)	18
Realised	High naturalness	13 (154)	9 (122)	6
	Low naturalness	9 (164)	6 (89)	4

Table 1.1 Planned versus realised number of recruited schools and children (the number of children is given between brackets).

Power analysis

The original design and sample sizes were determined using a power analysis. We based the power analysis on the outcome prevalence of allergy or asthma. The prevalence of allergy is a binary scale variable (child is sensitized to an allergen or not) for which the relative margin of error will likely be higher for a given sample size compared to the other measurement types. We used the procedure outlined in to obtain estimates of statistical power when testing for a significant difference between low and high biodiverse schools in the prevalence of allergies (Bolker 2008):

- Simulate many data sets assuming that the alternative hypothesis is true, that is, the effect of interest is not zero (i.e. prevalence of allergy differs between low and high biodiverse schools).
- Using each simulated data set, perform a statistical test of the null hypothesis that the effect size is zero (i.e. prevalence of allergy does not differ between both types of schools).
- Calculate the proportion of simulated data sets in which the null hypothesis was rejected. This proportion is the power estimate.
- The effect of different sample sizes (number of schools and number of children per school) was explored by repeating steps 1–3 across a range of realistic scenarios.
- We simulated data from logistic multilevel models with fixed effects for landscape and school context and random effects for school and school cluster.

The following assumptions were made for the power calculations:

- Prior knowledge suggests allergy effects start to be more pronounced starting from age 6. We fix the age at 10-11 year old children. By excluding variability due to age as much as possible, the sample size can be lower.
- Realistic baseline values were taken from data in http://www.euro.who.int/__data/assets/pdf_file/0012/96996/3.1.pdf.
- 10% allergy prevalence in high biodiverse - high biodiverse landscape - school context
- an increase in allergy prevalence of + 5% in low biodiverse school context
- an increase in allergy prevalence of +10% in low biodiverse landscape context
- We assumed there is no interaction effect between landscape type (low vs high biodiverse) and school context (low vs high biodiverse).

We specified a standard deviation of 0.3 for variation between schools and for variation between school clusters (= random effects). This meant that relative to the baseline (10%) 67% and 95% of all baseline schools will have an allergy prevalence between, respectively, (7.61, 13.04) and (5.81, 16.67). The random effect for school cluster accounted for paired occurrence of a low and high biodiverse school in the same landscape context (it induces a positive correlation between a low and a high biodiverse school which share similar characteristics of possible confounding variables such as geographic location, air quality and socio-economic status).

We considered 14 scenarios with differing combinations of number of schools and number of children per school (Figure 1.3). A budget constraint suggested that we set the maximum number of children to be enrolled in the study to 1500. Each scenario was simulated 100 times.

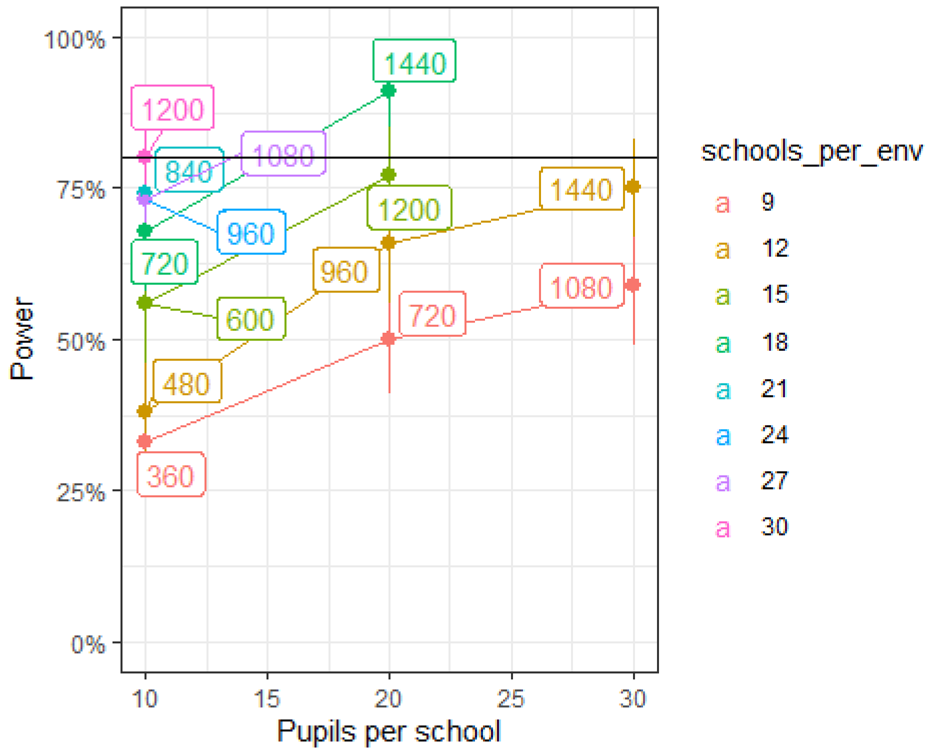


Figure 1.3 Power as a function of number of children per school and number of schools per landscape type to detect a difference of 5% between low and high biodiverse schools.

The power calculations suggested that we will have sufficient power (> 80%) to detect a difference in allergy prevalence of 5% between low and high biodiverse schools when the total number of children is 1200 or more. The scenario with 20 children per school and 18 schools in each of landscape-school context combinations was chosen as the preferred scenario when balancing operational costs (more schools increases operational costs) and statistical considerations other than power (less children per school increases risk of boundary estimation problems, which occurs when none or all children are sensitized to an allergen).

Given that the realised number of participating children and recruited schools was lower than planned, we can infer from Figure 1.3 that the power to detect a 5% difference will drop to about 50%. Larger effects sizes and more sensitive outcome variables, on the other hand, will more easily be detected. More details about the sampling design and selection schools can be found in **deliverable 1.1** - Report on sampling design & selection of schools. The following sections describe how landscape type, school context and potential confounders were determined.

Landscape Context

Land cover data from Copernicus high resolution layers were used to determine landscape naturalness:

- Imperviousness density (2018, 10 m x 10 m raster cells)
- Tree cover density (2018, 10 m x 10 m raster cells)

- Grassland vegetation probability index (2015, 20 m x 20 m raster cells)

The latter two were combined to represent semi-natural land cover classes. Mean values were calculated within a 2000 m buffer around each school. Classification as "high naturalness" or "low naturalness" was based on thresholded Euclidean distance from points representing most and least natural conditions.

School Context

The Lifewatch ecotopes classification (2 m x 2 m resolution) was used to assess school context naturalness. Proportional coverage fractions of ecotopes land cover classes were calculated in buffers of 0 m and 20 m around each school parcel. These values were used to estimate:

- Non-sealed proportional area of the school ground (excluding buildings)
- Non-sealed proportional area within a 20 m buffer ring surrounding the school grounds

Similar to the landscape classification, schools were assigned to high or low naturalness contexts using landscape-specific thresholds. Intermediate characteristics were excluded.

Air Quality

Mean annual values (2018) for outdoor air pollution levels were calculated in 100 m and 2000 m buffers around each school, considering:

- Nitrogen dioxide (NO2)
- Particulate matter smaller than 2.5 µm (PM2.5)
- Particulate matter between 10 µm and 2.5 µm (PM10 - PM2.5)
- Black carbon (bc)

Data source: https://www.irceline.be/en

Socio-economic Status

Micro-data were available for both French and Dutch-speaking communities, albeit in different formats. To ensure comparability, the data were rank-transformed and rescaled to the [0-1] range.

Task 1.2 Data centralization and management

Providing a DMP is formulated as a deliverable for Work Package 1. At the time of project proposal, a DMP was not yet an obligatory part of a BELSPO proposal. Nevertheless, we used the BELSPO template to fill out the necessary parts of a DMP. The completed DMP can be found in **deliverable 1.2 Data management plan**.

Task 1.3 Integrated data analysis

In this section we report on the methodology used for the integrated data analysis (**deliverable 1.3 Report on exploratory data analysis, model fitting and hypothesis testing**).

Calculation of derived variables: general

Derived variables were calculated based on the cleaned raw data. All derived variables were published together with the cleaned data on Zenodo (Van Calster et al. 2024).

Derived variables can be split into two groups:

- school-level varying
  - school context (high greenness versus low greenness)
  - playground biodiversity
  - indicator for local pollution based on SIRM data
  - indicator for mean SES based on education level parents, employment parents and income class
- child-level varying
  - classification of the family situation
  - classification of the cultural background
  - calculation of the attention score for the D2-test
  - calculation of Kidscreen scores for health related quality of life indicators
  - indicators for wheezing, eczema and asthma based on ISAAC questionnaires
  - latent factors derived from the attitude toward outdoor play and the nature connectedness surveys
  - latent factors derived from the risk engagement and protection survey

Calculation of derived variables: socioeconomic status (SES), family situation and cultural background

Parents of the children were asked to answer questions about their own education level (4 levels), their employment (4 levels) and their combined income (6 levels) (Reynders et al. 2005). The levels could be ordered and each level was re-coded on a scale from 0 to 1 (for instance for a 4 level factor: 0, 1/3, 2/3, 1). These scores were averaged per child to obtain a socio-economic status (SES) score at child level (with higher values reflecting higher SES). Further averaging the child-level SES per school gave us a school-level mean SES. We also calculated the difference between the child-level SES and the school-level SES (centered child-level SES), to obtain a variable that captures the child-level SES variation within schools. Both the school-level SES and centered child-level SES (further referred to as child-level SES) will be used in so-called 'within-between' multilevel model formulations (see e.g. Antonakis et al. 2021, Bell and Jones 2015).

Family situation was classified into the following categories (53% missingness):

- Original family (n = 197)
- New family situation with involvement of other parent (n = 36)

- New family situation without involvement of other parent (n = 15)
- New family situation involvement of other parent unknown (n = 1)

Parents of the children were also asked to answer about the child's nationalities, the nationalities of the mother and father, the languages they speak at home and if they consider themselves to be part of another national culture besides their nationalities. The reported countries and languages were grouped in one of the following regions: African, Asian, North-American, Central-American, South-American, Mediterranean, East-European and West-European. One child could receive multiple classifications. For example, a child with a Belgian nationality with a mother with Italian nationality would receive the classification West-European and Mediterranean.

A first exploration of the data showed that the regions that were not West-European were too small to take up further in the integrated statistical analysis. The majority of the participating children (n=310) are West-European. The second largest group was classified as West-European and Mediterranean (n=54). Other classifications were between n = 15 and n = 1.

Lastly, all other regions besides West-European were grouped together in the classification "world", resulting in the following groups: West-European n=310, West-European and world n=94, world n=3. Also this classification was not useful for further analysis as the West-European influence remains dominant.

Calculation of derived variables: school context

In this section, we explain how we calculated school context (high greenness versus low greenness). Greenness was defined as belonging to one of the following groups (see Tasks 3.1 for further details):

- 35: Grassland (managed), including intensive agricultural grassland, gardens and leisure grasslands
- 40: Open vegetation with biological interest (dry), including extensively managed grassland with biological interest, natural grassland and heathland vegetation (also peatlands if they are dry on top).
- 45: Open vegetation with biological interest (wet), including reed beds and marshes
- 48: Recently disturbed (less than 5 years before) ligneous vegetation, including young plantations and clear cuts
- 50: Needleleaved trees (>3m), isolated, in hedges or inside forests, including Christmas trees
- 51: Needleleaved shrub (<=3m), isolated, in hedges or inside forests
- 55: Broadleaved trees (>3m), isolated, in hedges or inside forests
- 56: Broadleaved trees (<=3m), isolated, in hedges or inside forests, including intensive orchards

The sum of their areas was calculated to obtain a measure for greenness. Next, we clustered these variables within each landscape type into two groups based on k-means clustering. The resulting clusters can be labelled as "high greenness" and "low greenness". We used the proportion greenness within the playground and within three consecutive bands (around the playground within school limit, up to 100m from school limit, and between 100 m and 300 m away from school limit) as variables. Because our classification is based on land-cover types and not on NDVI, seasonality has no impact on our assessment of greenness. Figure 1.4 shows

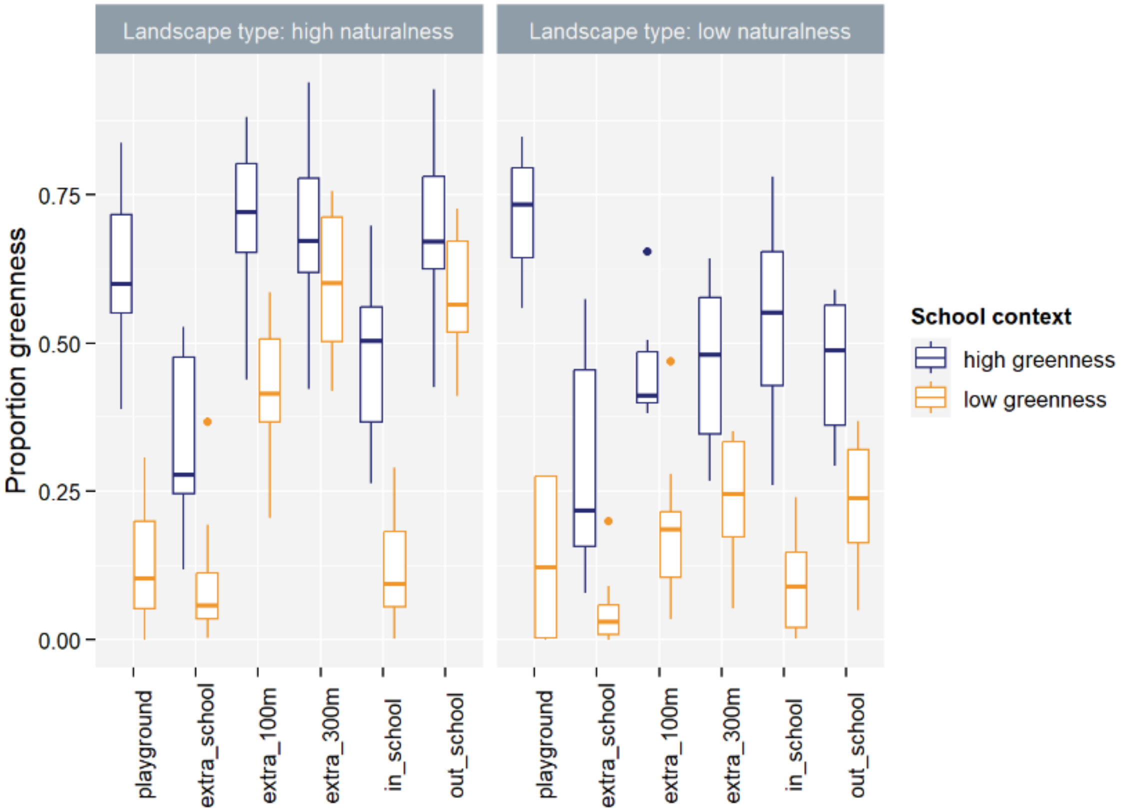


Figure 1.4 Result of k-means clustering to label schools as "high greenness" versus "low greenness" based on four spatial levels (playground = within playground(s); extra school = around playground within school limit; extra_100m = around school limit within 100m surrounding school limit; extra_300m = within 100m up to 300m surrounding school limit; in school = area-weighted sum of playground and extra school; out school = area-weighted sum of extra_100m and extra_300m.

Calculation of derived variables: confirmatory factor analysis

Several of the surveys (see Tasks 4.2, 5.1 and 5.2) used multiple questions that when taken together refer to the same underlying response (a latent factor) that the surveyor tries to measure. In these cases, we used confirmatory factor analysis (CFA) to check the questions' validity and to obtain factor scores for each of the latent factors (see Figures 1.5, 1.6 and 1.7).

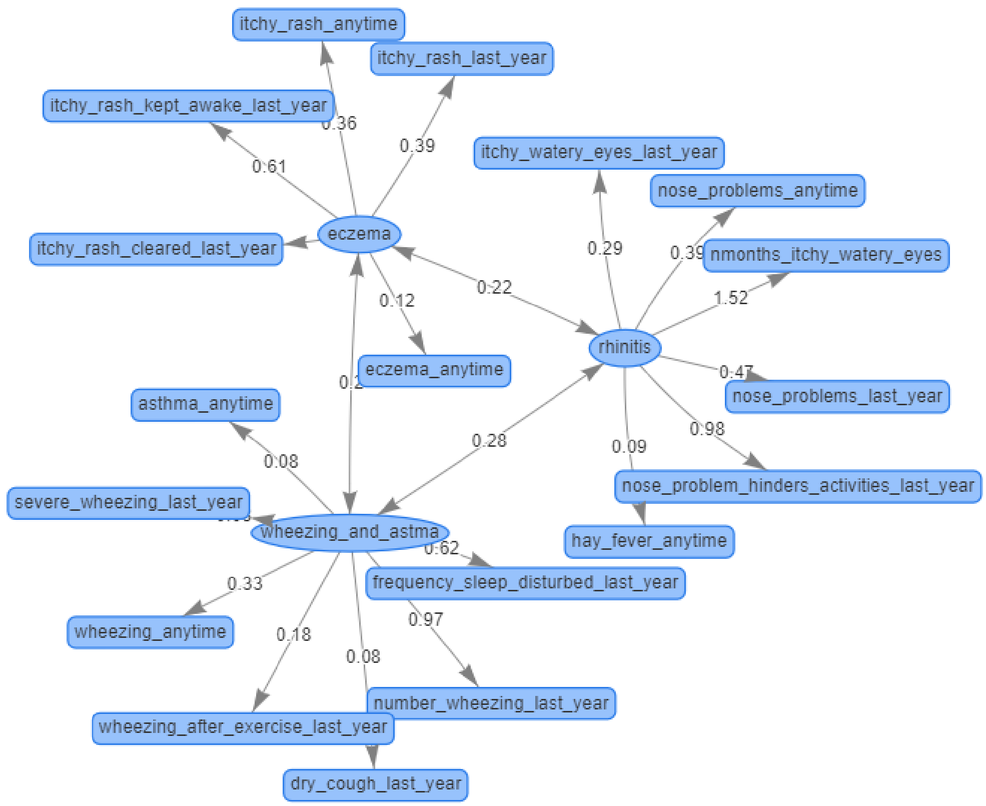


Figure 1.5 Confirmatory factor analysis result for the asthma and allergies in childhood survey (ISAAC).

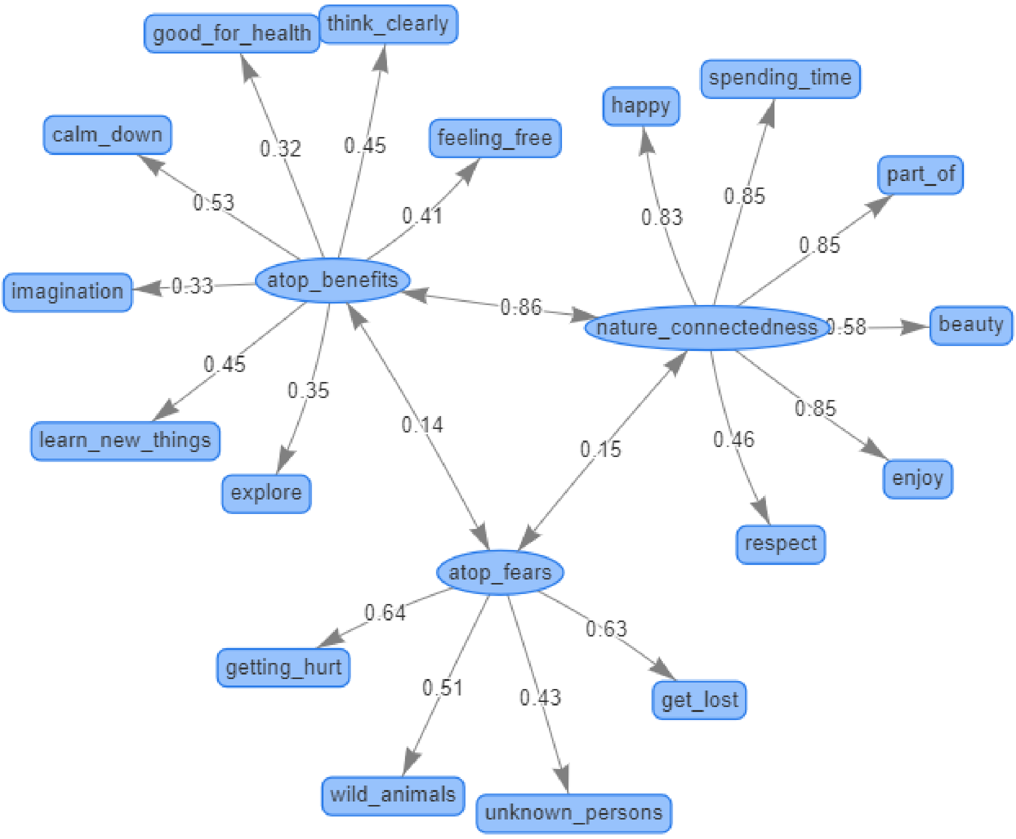


Figure 1.6 Confirmatory factor analysis result for the attitude towards outdoor play (ATOP) and nature connectedness (NC) surveys.

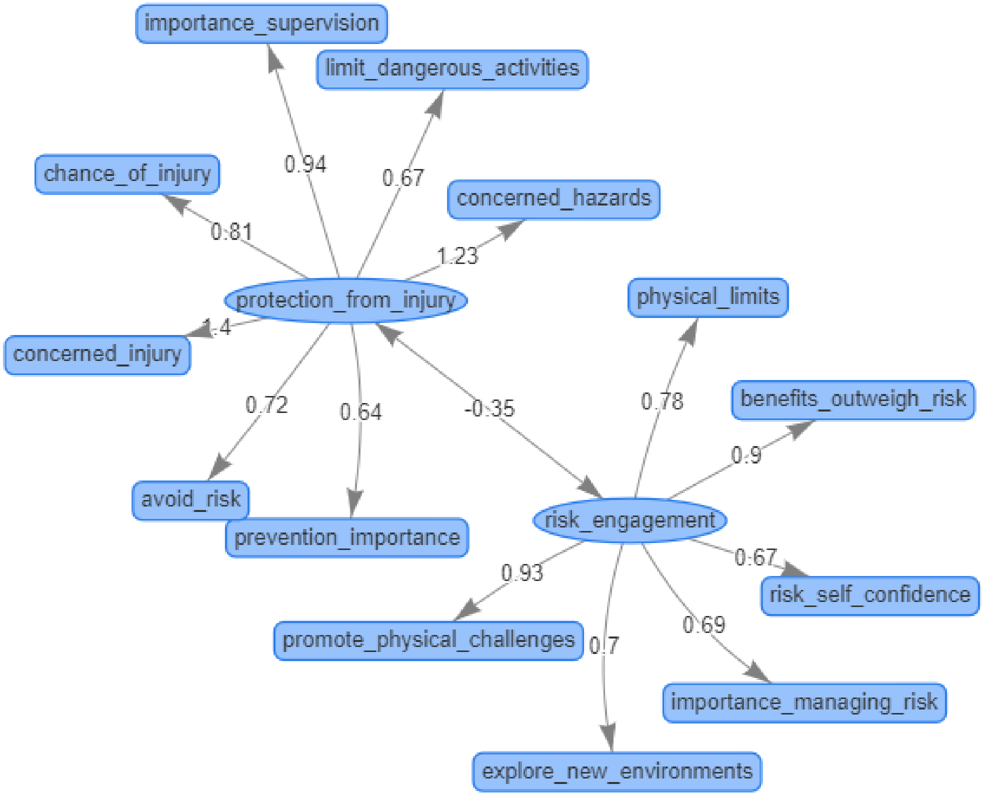


Figure 1.7 Confirmatory factor analysis result for the risk engagement and protection from injury (REPS) survey.

Statistical analysis: general

All statistical analyses were implemented in the R system for statistical computing (R Core Team, 2020). The reporting of this study contains the preferred items of the STROBE statement checklist for reports of case-control studies (von Elm et al., 2007) and the relevant items of the PRIGSHARE checklist for reports of green space health research (Cardinali et al. 2023).

The following sections contain more detailed information about the different types of analyses that were carried out.

Statistical analysis: Generalised linear multilevel models

We used generalised multilevel models based on maximum likelihood estimation to relate the outcome variables to school context and landscape type. An appropriate statistical distribution and link-function was used to relate the mean parameter of the distribution to the linear predictor. The random part of the linear predictor contained at least a random effect for school to account for the clustering of children in schools. The fixed effects structure always contained the landscape type and school context variables and their interaction. Because not all schools could be statistically matched, additional models were fitted that adjusted for possible confounding variables (e.g. within school and between school SES, scores for well-being at school and general mood and feelings, PM2.5).

All generalised linear mixed models were fitted using either the package glmmTMB (Brooks et al. 2017) or the brms package (Bürkner et al. 2017).

Statistical analysis: missing data

For some analyses, the combination of response variables and covariates had a lot of missing data. To deal with this, we extended the generalised linear mixed model approach to a multivariate analysis where for each response variable or covariate that appeared in the main regression equation and that had missing data, missing data were predicted based on relevant covariates using additional regression equations. In this approach, the missing data are declared as parameters to be estimated and they are jointly estimated with the other parameters in a Bayesian framework through the brms package (Bürkner et al. 2017). This implementation naturally results in multiple imputations of the missing data (because in a Bayesian sampling algorithm, multiple draws are taken to quantify the posterior distribution of parameters) and protects the main parameters of interest against bias resulting from the missing data. This protection is provided if we can assume that the probability of missingness is the same within groups defined only by the observed data (not the missing data).

Statistical analysis: structural equation models (based on Carmen, 2024 - with permission)

To answer research questions relating to ATOP, NC, and REPS survey data , we build Structural Equation Models (SEM), using the lavaan package (Rosseel 2012). In these models, we investigated whether there are important links between the latent factors and / or if the latent factors are related to other exogenous variables.

In the SEM, the chosen models from the CFA for each of the measurement items are repeated, including possibly correlated error terms between items. For each factor in each group, one loading is fixed to 1 to identify the model. The link between latent factors is tested by estimating the covariance between the latent factors. The link between latent factors and other exogenous variables is analysed by adding regressions to the SEM. Gender, Socio-Economic Status (SES), air pollution, naturalness of the school surroundings, greenness of the school playground, and an interaction between naturalness and greenness were included as possibly important variables. The intuition for the interaction between naturalness and greenness follows from the hypothesis that a green school playground in an environment with low naturalness may have a bigger impact than a green school playground in an environment with high naturalness. If all scales (ATOP, NC, and REPS) can reliably fit their corresponding concepts, we start with the following regressions in the SEM:

- A regression where ATOP_benefits is the endogenous variable and the exogenous variables are gender, Socio-Economic Status (SES), air pollution, naturalness of the school surroundings, greenness of the school playground, and an interaction between naturalness and greenness.
- A regression where ATOP_fears is the endogenous variable and the exogenous variables are gender, SES, air pollution, naturalness of the school surroundings, greenness of the school playground, and an interaction between naturalness and greenness.

- A regression where NC is the endogenous variable and the exogenous variables are gender, SES, air pollution, naturalness of the school surroundings, greenness of the school playground, and an interaction between naturalness and greenness.
- A regression where REPS_protection_from_injury is the endogenous variable and the exogenous variables are gender, SES, air pollution, naturalness of the school surroundings, greenness of the school playground, and an interaction between naturalness and greenness.
- A regression where REPS_risk_engagement is the endogenous variable and the exogenous variables are gender, SES, air pollution, naturalness of the school surroundings, greenness of the school playground, and an interaction between naturalness and greenness.

Non-significant exogenous variables that do not fit well and are not significant will be removed from the regressions one-by-one (backward selection procedure).

Just like in the CFA, a multi-group SEM with a grouping based on the language of the survey will be tested. If the fit is good, we will compare a model with configural, metric, and strong measurement invariance. The fit of the SEM is assessed using the same fit measures as for the CFA: CFI, TLI, RMSEA, and SRMR. For good fit, we employ the same cutoff values as for the CFA (Byrne 1994; Hu & Bentler 1999).

Statistical analysis: differential composition microbial data

Microbial data coming from DNA sequencing (see Task 3.2) are of a special nature and this needs to be taken into account when making inferences about the relative composition of the microbial communities. We used an extension of a generalised linear mixed model that can deal with this kind of data. The model that we used is published in Mangiola et al. (2023) and we used the implementation of the sccomp R package. The read counts for each microbial taxon are modelled using a beta-binomial statistical distribution where an additional sum-constrained across is enforced to get compositional properties. The latter is needed because of the special nature of the read counts. Read counts cannot be regarded as absolute counts but must be made relative to the total count in a sample, which makes the data compositional in nature (i.e. relative abundances).

Task 1.4 Definition project outcomes

This task is discussed in the "dissemination and valorisation" and "publications" section (**Deliverable 1.4 Communication of integrated assessment outcomes in scientific research papers**).

WP2 School engagement

Task 2.1 Methodological implementation

The first mission of wp2 was a support for wp1 in the preselection of the schools. GoodPlanet and MOS being in permanent contact with primary schools all over the country, both associations have some knowledge about the reality of the schools' contexts and disparities. The collaboration between wp1 and wp2 was also required to build typologies to distinguish green and grey schools. It was also fruitful when making a reality-check of the preselected schools and keeping only the schools that matched our criteria.

Preparation of recruitment schools (for task 2.2 Recruitment schools)

Wp2 created a protocol for recruitment of schools but also all the recruitment material, including (See Annexes 2.1 for samples of this material) :

- letters to direction;
- information documents for teachers and direction;
- presentation for teachers (ppt presentation);
- presentation for parents (ppt presentation);
- Declaration of consent forms for parents, teachers and supervisors;
- provide feedback on authorisation forms for children;
- creation of ideas for participation of students, teachers for supporting participation in the project.

Some educational material was uploaded on the B@SEBALL website.

- Translation and voice over of all Dutch material to French
- meetings with MOS and with GoodPlanet-colleagues.
- project-meetings with partners.
- create project website
- provide feedback on tools and methods (questionnaires, allergy test..), protocols of other WP's. Translation to French.

More details can be found in the completed dossier for Medical-Ethical Committee (**Deliverable 6.2 Request for ethical approval at the UAntwerpen medical ethical committee**)

Stakeholder and expert advisory involvement:

Contact stakeholders and invite them to the Follow-up Committee. Preparation of presentation WP2 to Follow-up Committee. All project partners contributed to the above mentioned work in preparation of the dossier of the medical ethical committee in preparation of the recruitment of school; e.g. the group of Prof. Sarah Lebeer (University of Antwerp) contributed to "WP2: School Engagement" by developing narrated and subtitled video materials in Dutch and French providing detailed information to schools, children and their parents regarding the implementation of microbial assessment. Sciensano, in close collaboration with WP2 and others of the project, initiated development of two other videos with a star from Flemish children's television: one for general advertisement for pupils, their parents and schools to take part in the project, one with regard to the skin prick test, in order to show that it is not really scary but helpful for the research.

Task 2.2 Recruitment schools

As soon as all the recruitment material was ready, we engaged the contact with the schools via mail or/and phone. Most of the time, we solicited the directors at first. But in some cases, it was easier to take contact directly with the head teacher of the concerned group of students. The first contact was essential to properly and enthusiastically present the project and the positive impact it could generate on the school, the children and the society in general. The main objective of this first interaction was to ask the school whether a group of 5th grade students could be interested in taking part in scientific research in connection with biodiversity and children' health. If they showed interest, then we could proceed to the next step and send them some information and details. This was the last step before the final engagement of the schools.

When a school agreed to belong to B@SEBALL, we provided them with an identification document (called "extra info doc") that was filled in by the director or the participating teacher(s) (for data such as: address, name of teacher, number of students in the group…). Besides, some pictures of the playground were requested from the engaged schools. Moreover, the pictures helped us to confirm the playground context. All the extra documents and pictures were stored on the team drive.

Task 2.3 Recruitment participants

Right after the extra doc was filled in and the pictures sent, we planned an appointment with the teacher in charge of the 5th primary (sometimes two groups, so two teachers) and the director (his/her presence was not compulsory but recommended). The presentation/information moment last for about 40 minutes to an hour (depending on the questions of the audience). At the end of the appointment, we planned a date for another meeting, this time with the parents.

We provided the school with a word document called "Letter for the parents" to invite them to the meeting. We let the school communicate it to the parents. This letter contained a documentation part and a little space to fill in to let us know whether they will attend the meeting or not. Most meetings were planned in the evening. Just like This information moment could last for about an hour. At the end of the presentation, we distributed the consent forms to the parents and the teacher, plus one or two envelope(s) (A4 format) with three stamps to the teacher. We asked the teacher to collect all the consent forms and send them in the envelopes to Antwerp University.

Meanwhile we decided to create a certificate for the children, acknowledging their participation in the research. This was really appreciated. (see Annexes 2.2)

Task 2.4 Collaboration with schools

Maintaining an open and easy communication with the recruited schools was essential. We therefore remain at their disposal for every question and remark. This was also done to prevent schools' drop-out. Furthermore, we designed a schedule/agenda that evolved along with the research to let them know what was next. Thus, the teachers had a clear vision of the different stages of the research and they knew when they would be particularly solicited.

Planning of the sample collection in the schools

The swab test had to be done by professionals. We planned some appointments in every school to collect the biodiversity on the cheeks of the participating students. An agenda was created to facilitate those appointments. It was also the occasion to collect the last consent forms that weren't in our possession yet. (See Annexes 2.1)

Extra effort to increase representativity and gather missing documents

The overview of the recruited schools showed some unrepresented regions. We decided to put some extra effort in the recruitment of more schools to reach a broader and more realistic diversity. This was particularly important for the reliability of the results. A selection of new schools was provided by wp1. Especially in the regions with a lack of representativity. More schools were recruited but it was so late in the research process that the engagement was more difficult. Indeed, the timeline and the deadlines for the schools were compressed and therefore harder to combine with school realities.

In collaboration with the other work packages, we have identified all the missing data from every school. Among those data we can think of : online questionnaires (parents and guardians) (WP5) and biodiversity in the playground data (WP3).

Biodiversity data were collected by WP3 directly in the schools that were missing. It was the occasion to use the workshop with the students.

The questionnaires for parents had already been sent online to the schools with several reminders to fill it in. The response rate remained low, especially from the schools in Wallonia. We decided to change our strategy. Every Walloon school was contacted by phone to organise a moment with the parents to fill in a simplified version of the questionnaire on paper (see also task 5.3). In collaboration with WP5, a kit was sent to the schools including: printed questionnaires for every parent that did not fill it in, some envelopes and stamps and an instruction document (for the teacher). Almost all the schools answered positively to this strategy and we could collect way more questionnaires.

Task 2.5 Dissemination results participants, schools

In February 2023, we decided to send to the schools a document presenting the different steps of the research and showing what we do with the samples they sent us. This document allowed us to keep contact with the school and inform them about the progress of the project.

In June 2023, in collaboration with the other WPs, WP2 sent some information about the process and the methodology of the Research to the schools. WP2 also supported the other WPs with the gathering of the last documents and questionnaires that hadn't been sent by the schools yet.

In June 2024 a document was sent to the schools. Every school that sent some bacteria samples received a specific graph showing the bacterial portrayal of its playground. Some bacteria were more abundant. We gave some little information concerning these specific bacteria. The graph, the information and a lot of other contents were provided by wp3.

We intend to send the schools a final communication in October 2024. This communication will bring together the former documents, present the results and the conclusions in a popularised way.

WP3 Biodiversity assessment

Task 3.1. Methodological implementation environmental assessment

Classroom level: air quality

An analog thermometer and hygrometer was brought to each school for in-class use, with a calendar to be filled in daily with values of temperature and relative humidity.

Playground level: biodiversity characteristics

The data collection done by children has a formal questionnaire inviting children to describe the outdoor area of the school's ground where they spent the most time. It covered, with a range of set options and one box for free input, ground surface type (water, vegetation, sand, wood chips, gravel, pavement, rubber or artificial grass, concrete or tar), vegetation type (trees, hedge or shrub, grass, flower bed, weeds between pavement, flower or vegetable boxes, vegetable garden, tree stumps, green roof, green wall, overhanging branch), tree essence (hazelnut, chestnut, oak, ash, plane tree, hornbeam, birch, alder, beech, none of these). For each of these questions, multiple choices were possible. All options were illustrated and a basic tree identification key was provided. The total number of trees was also asked. The questions on ground surface type and vegetation type (but not tree essence) were also asked about other outdoor areas of the school. Children were also provided with an A1 print out of an aerial picture depicting the school grounds. They were invited to annotate the print out with the limits of the school ground, outdoor areas ranked by frequency of use (at least once a day, at least once a week, less than once a month, never). For those areas often or occasionally frequented by children, they were invited to indicate on the map all vegetation types that were selected in the questionnaire.

The questionnaire and forms were developed in French then translated also in Dutch and tested in a French-speaking and a Dutch speaking school. Various project members visited some of the schools to help and encourage the completion of the data collection. This involved confirming school perimeters and digitising cartographic data for all schools that sent back the forms and maps.

Playground level: pollution

As described in the microbial diversity sampling section, three leaf samples were collected in paper envelopes for magnetic analysis. The saturation isothermal remanent magnetisation (SIRM) the leaf samples is measured as a proxy for exposure to particulate matter (PM) and is a valuable addition to modelled PM concentrations (Hansard et al., 2011, Kardel et al., 2012b, Mitchell and Maher, 2009, Muxworthy et al., 2003).

For this method the surface area of the leaves was first determined using a leaf area metre (LI-3100C, LI-COR), after which the samples were oven-dried (60°C). Following the protocol described by Kardel et al. (2011), the samples were tightly packed in a small sample pot using cling film and magnetised in a pulse DC magnetic field of 1 T using a Molspin pulse magnetizer (Molspin Ltd, UK). Immediately after, the remanent magnetisation was measured using a Minispin magnetometer (Molspin) with high sensitivity (∼0.1 × 10−10 A m2). The leaf area-normalised SIRM (in A) was obtained by normalising the magnetic signal by the volume of the sample pot (10 cm3) and the sample leaf area.

Playground and school-level: greenness

At the scale of the school premises itself, we calculated several land-cover based variables using the Lifewatch ecotopes raw pixel based classification product for the year 2015, which covers Belgium entirely (Lifewatch 2023). For each school, the playground(s) were delineated as well as the school perimeter. The land-cover types were reclassified into 'green' and 'not green' and the proportion of greenness was calculated within the playground (in case of several playgrounds, the areas are summed), within the school limit, but excluding the playground(s), and within 100 m surrounding the school limit, but excluding the area within the school limit, and within 300 m surrounding the school limit, but excluding the area of the 100 m buffer. Land cover types that were considered green included: grasslands, dry and wet vegetation of biological interest, recently disturbed ligneous vegetation, and deciduous and coniferous trees and shrubs (including hedges). Blue spaces were not included.

In addition, raw areas within buffers of vegetation, ploughed land, densely artificialized areas, sparsely artificialized areas, permanent bare soil and permanent water were calculated, which are aggregated groups derived from the 18 categories discerned on the Lifewatch product. Finally, the area of green patches, which were defined as spatially contiguous stretches of a single ecotope, intersecting with previously mentioned buffer distances, were calculated as well.

Landscape-level: pollution

We calculated mean values for the annual means of nitrogen dioxide (NO_2), particulate matter smaller than 2.5 µm ($PM_{2.5}$), particulate matter between 10 µm and 2.5 µm (PM_{10} - $PM_{2.5}$) and black carbon (BC) in a buffer distances of 1000 m and 2000 m around each school. Air quality data were obtained from the Belgian Interregional Environment Agency.

Landscape-level: greenness

The same Lifewatch ecotopes map derived variables that were described in section "*Playground and school-level: greenness*" were also calculated at the landscape level. For the landscape level, buffer radii of 1000 m and 2000 m were considered.

Task 3.2. Methodological implementation microbial assessment and Task 3.3. Data collection

Microbial diversity of school playgrounds and children's skin

The microbial diversity of schools was mapped by organising a sampling campaign at schools to collect samples from four environments: dust, sand, soil, and strawberry plant leaves. Additionally, the microbial diversity on the cheeks of children was also sampled. Strawberry leaves were included as these form an extensive surface that is colonised by microbes that we come into contact with, either directly via touch or indirectly via the air. Bacterial communities on plant leaves are very specific as they are shaped by the host plant. However, they also greatly depend on whatever bacteria are present in the air and by the neighbouring plants (Laforest-Lapointe 2016). Therefore, to obtain a comparable representation of the plant-associated bacteria on school playgrounds, each school was provided with three strawberry plants (*Fragaria* x *Ananassa* var. Ostara) bought from the same supplier (Agora group, Kontich, Belgium). They were placed on the school playground, at a height of approximately 1 m (e.g. on a window sill) and with an orientation towards the East/South-East, if possible. This orientation was chosen to protect the plants against prevailing winds from the West/South-West. The timing of plants placement was extensively optimized beforehand in a pilot study, resulting in 8 weeks as the optimal time necessary for microbiome stabilization. Thus, for the main B@SEBALL study the strawberry plants were placed at the schools in March 2022 and samples were collected in May 2022, allowing the microbial communities of the leaves to reflect their surrounding plant microbiome.

Four types of environmental samples were collected by children and their teacher, guided by a detailed protocol with videos suitable for children and a sampling kit that was optimized at the University of Antwerp. In brief, using disinfected gloves, approximately a teaspoon of playground sand and soil were scooped up with a sterilised spoon in a 50 ml falcon. The dust was collected with the help of a sterilised paint brush. Three samples of three strawberry plants were collected by cutting off one leaf with scissors and placing it in ziplock bags. An additional three samples from strawberry samples were sampled and placed in envelopes for magnetic SIRM analysis to quantify air pollution in collaboration with Prof. Roeland Samson (University of Antwerp). The samples were sent to the Laboratory of Applied Microbiology and Biotechnology (LAMB) at the University of Antwerp via provided mailing boxes (Bpost) and processed upon arrival.

Children's skin microbiome samples were taken by the B@SEBALL project researchers by rubbing an eNAT™ swab (Copan, Brescia, Italy) pre-wetted with phosphate buffered saline on the left cheek of a child, approximately a surface 20 cm^2 during 30s. The swab was then placed in the vial containing stabilizing eNAT buffer transported to the lab at 4°C where it was stored at -20°C until further processing.

For all samples, the bacterial composition was assessed by first extracting microbial DNA followed by *16S rRNA* gene amplicon sequencing. Microbial DNA was extracted using the DNeasy PowerSoil Pro kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. For all environmental samples, upon arrival of the samples in the lab, approx. 150 mg of the sample was transferred to DNA extraction tubes containing beads (...) and 800 µl CD1 buffer (first step in the DNeasy PowerSoil Pro kit) was added. These DNA extraction tubes were stored at -20°C until further processing. For the skin samples, 500 µL of eNAT buffer was used to start the microbial DNA extraction protocol. Secondly, the tubes containing samples and beads were shaken at max speed on a Vortex Genie (MoBio) during 10 min to lyse all cells. Next, the lysate was transferred to a 96 well plate and DNA was further extracted using the Qiacube HT robot. Next, the V4 region of the 16S rRNA gene of the extracted DNA was amplified using barcoded primers (IDT), as described by Kozich et al. The region was amplified in 30 cycles in a 20 µL reaction with Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific). For the amplification of the phyllosphere samples, peptide nucleic acid (PNA) clamps were added. These PNA clamps were designed to specifically bind and block the amplification of plastid and mitochondrial DNA (pPNA, 5′-GGCTCAACCCTGGACAG-3′, mPNA, 5′-GGCAAGTGTTCTTCGGA-3′) (24). Cycling conditions during PCR were: initial denaturation at 95°C for 2 min, 30 cycles with a denaturation step at 95°C for 20 s, a PNA clamp-binding step at 75°C for 10 s (only for phyllospere samples), a primer annealing step at 55°C for 20 s, and an extension step at 72°C for 1 min, and a final extension at 72°C for 10 min. Two PCR blanks were included in each PCR, which were also sequenced. Next, the amplicons were purified using Ampure XP (Beckman Coulter) and the DNA concentration of the purified samples was quantified using a Qubit 3.0 fluorometer (Life Technologies). These DNA concentrations were used to pool samples and blanks in equimolar concentrations, resulting in a library. The amplicon library was further purified by loading it on a 0.8% (mass/vol) agarose gel and extracting bands of approximately 380 bp with the Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel). The final library was diluted to 2 nM and sequenced on the Illumina MiSeq platform using 2 × 250 cycles at the Center of Medical Genetics Antwerp (University of Antwerp, Belgium). The sequencing data of this study were made available under study accession number PRJEB79575 in the European Nucleotide Archive (ENA).

Task 3.4. Data processing

Bioinformatics pipeline

The raw sequencing data were processed with the package DADA2 (Callahan et al. 2016) in R. Reads with more than two expected errors were removed. Forward and reverse reads were denoised per sample using the DADA2 algorithm, and reads were merged. Chimeras were removed using the removeBimeraDenovo function, and a table with sequence variants (ASVs) was constructed. The ASVs were classified using the EZBiocloud reference 16S rRNA database (Kim et al. 2012). Nonbacterial reads (i.e., plastid and mitochondrial DNA) were removed from the data set. Specific ASVs were identified as contaminants based on their presence in the blanks in combination with their low presence in the phyllosphere samples and their likelihood to be common contaminants. Further processing was performed in RStudio using the tidytacos package. Samples separated by type (e.g. skin, plant) for most analyses. Bacterial biodiversity metrics, sequence variant richness (cfr. species richness) and the Simpson diversity index, were calculated for each sample. The between-sample-diversity (beta diversity) was determined by calculating Bray-Curtis dissimilarities. These dissimilarity matrices were used in a PERMANOVA model to determine the potential impact of different factors.

Task 3.5. Data transfer

The bacterial biodiversity metrics mentioned above, sequence variant richness (cfr. species richness) and the Simpson diversity index, were incorporated in the derived data package (designed by WP1 team for easy transfer of data within the research consortium). The results of bacterial biodiversity of both the skin samples and the environmental samples were used in the integrated analyses (see below) and included in the manuscript "*Nuancing the biodiversity hypothesis – allergies in children and their bacterial exposure at schools.*" which will soon be submitted to Nature Microbiology.

WP4 Health assessment

Task 4.1 Methodological implementation Mental health and well-being and Neurodevelopment

Child-reported well-being

In the first year, the KIDSCREEN-27 questionnaire has been selected as the tool to assess self-reported health-related quality of life in children. Validated versions in French and Dutch and permission to use the KIDSCREEN-27 tool have been obtained from Chiara Jörger (KIDSCREEN Group Europe, original EC Grant Number: QLG-CT-2000- 00751, Office of Quality of Life Measures QOL@uke.de) on 27/05/2020. The KIDSCREEN-27 (Ravens-Sieberer et al. 2014) is a cross-national, validated questionnaire about health-related quality of life. Self-reporting versions for children, as well as proxy versions for parents exist in different languages. The KIDSCREEN-27 has five dimensions with 27 items in total and takes 10-15 minutes to answer. All five dimensions are Rasch scales: Physical Well-Being (5 items), Psychological Well-Being (7 items), Autonomy & Parents (7 items), Peers & Social Support (4 items), and School well-being (4 items). The KIDSCREEN-27 questionnaire was administered to the children after the d2-attention test.

Measurement of attention

To assess cognitive skills, the D2 level of attention test was selected.In the second year, the D2-scoring method for assessing cognitive skills was tested at Sciensano. We used the d2 Test of Attention, one of the most widely used measures of visual attention throughout Europe and in the US (Brickenkamp and Zillmer 1998). The d2 Test is a cancellation test to measure attention in different dimensions, including speed, accuracy (omission and commission errors) and consistency (variability). Practically, the d2-test is a trial where participants are asked to assess 47 characters on each of 14 lines. The participant gets 20 seconds to process a line. A character is either a 'p' or a 'd' with between one and four '.' (dots) placed above and/or below the 'p' and 'd'. The participant is asked to process a line from left to right and strike out (cancel) every 'd' character that has exactly two dots placed above and/or below (can also be one above and one below) the 'd' character. The final cancelled character on a line determines the number of characters that a participant could process for that line within 20 seconds.

The resulting data can be organised in a two-by-two contingency table containing (1) the number of correctly processed d2-characters (cancelled), (2) the number of correctly processed other characters (not cancelled), (3) the number of cancelled characters that were not d2-characters (commission errors, F2), and (4) the number of not cancelled characters that should have been cancelled (omission errors, F1). Based on these numbers, we calculated the corresponding contingency chi-square statistic χ^2. The theoretical maximum χ^2 statistic for the complete table is obtained for a perfect score on the d2 attention test (14 lines containing 47 characters, of which 299 are d2-characters) and equals 654. The attention score was therefore calculated as the observed χ^2 statistic divided by the theoretical maximum χ^2 statistic to obtain values between 0 and 1 (for the purpose of replicability, the R code is provided in SI). This attention score combines precision (both error types are small), accuracy (both error rates are not very different; i.e. unbiased) and speed (a high total table count). Our main models used the χ^2 statistic that was calculated for the entire test. Additional models were run to investigate changes of attention during the test (improved or reduced performance over time), and these models used line-level χ^2 statistics, based on line-by-line χ^2 statistics in three line blocks that are proposed by the original d2 protocol (the first four lines, the following six lines and the final four lines).

Task 4.2: Methodological implementation Asthma & Allergies

Child-reported asthma and allergies

To assess respiratory health and the child's sensitivity to asthma and some allergies, the ISAAC questionnaire has been selected (International Study of Asthma and Allergies in Childhood, ISAAC Phase Three Study Group core questionnaire, http://isaac.auckland.ac.nz/phases/phasethree/corequestionnaire.pdf). Versions of the questionnaire in French and Dutch were produced. For the asthma and allergies questionnaires, it was decided by the consortium to limit the questionnaires to versions in French, Dutch and English despite requests by some schools to provide information and questionnaires in other languages. ISAAC questions are grouped into questions about eczema, wheezing and asthma, and rhinitis.

Additional information on children's allergies was also collected as part of the parent questionnaires. Despite the exploration of several alternatives, we found no practical solution to perform the SPTs (skin prick test) in the participating schools, mainly due to a lack of trained persons qualified to perform the tests and interpret the results in Belgium. For the allergy component it was decided to focus on the ISAAC.

The methods described above (4.1 and 4.2) were compiled into an internal guideline, and together with the materials (questionnaires, swab material) constituted **Deliverable 4.2** (Guidelines and materials health assessment, including a) Health questionnaire ready to use in all regions involved, b) Guideline for use of the health questionnaires).

Task 4.3 Data collection

Data were collected by collaborators from all teams, coordinated by Sciensano. For all questionnaires, it was decided to use the children’s class and class number as part of the unique code. Codes would then be complemented by a number assigned to the school. Unique IDs were designed to take the form of (school number)-(class number)-(child’s number in the class). In the first half of 2022, the B@SEBALL study commenced according to the planning provided in the project proposal, including the questionnaires on health and potential allergies. A planning (order of schools) had been drafted by the consortium (**Deliverable 4.3 Field work protocol and organization plan for sampling at the schools**). In the initial planning, it was foreseen that no more than two schools could be processed per day.

Starting on 25 April 2022, several teams of the BASEBALL project performed the health assessments in the selected schools under coordination of Sciensano staff. During each visit to a participating class, the following data were collected, in this order, to limit impacts of attention waning and variation between schools: d2, KIDSCREEN, ISAAC, Nature Connectedness & Attitude Towards Outdoor Play (WP5), cheek swab (WP3). Note that no SPTs were performed.

Task 4.4 Data processing

In year three, a workflow to manually digitise completed d2 sheets of the attention test and calculate the different level of attention indicators has been established. All data were digitized by a team of Sciensano (**Deliverable 4.4 Protocol for health data base design and production database**).

Task 4.5 Data transfer

To link the data collected in the schools (coded with the unique ID described in year 2) to the data provided by the parents (not coded, labelled with the child's name), class lists with childrens' names and class numbers were provided by the teachers. In total, data were collected from over 500 pupils in 36 schools. All data were digitised and transferred to WP1 for integrated assessment (**Deliverable 4.5 Protocol for health data transfer & scientific health data paper**). All cheek swabs were first stored in a refrigerator at Sciensano and then transferred to WP3.

In year four, as part of the health assessment, the WP4 team initiated and led the development of a scientific manuscript that focuses on the attention outcome of the project

WP5 social assessment

Task 5.1 & 5.2 Methodological implementation social assessment & contact with nature/playing behaviour

The main aim of the social assessment was to collect data on the child's social profile and outdoor playing behaviour, as well as guardian responses (parents and school guardians) to outdoor play, to assess whether this influences their (health relevant) contact with nature. To gain insights in the social profile, information was collected on the Socio-Economic Status (SES), cultural background and living environment. To gain insights in the behaviour, information was collected on the child's 'Attitude towards Outdoor Play', 'Nature Connectedness' and free time activities. For the influence of guardian responses to outdoor play, we collected information on the guardian's 'Risk Engagement and Protection' during outdoor play and the allowance of outdoor 'Independent Mobility'. Data was collected through surveys, which was a combination of validated and newly developed surveys. Table 5.1 shows which data was collected from which group. The parental questionnaire also contained some general questions about i.a. the child's age, gender, allergies, years at the school.

All the surveys were available in French and Dutch. The validated surveys were only available in English so these were translated. Carmen (2024) tested if the 'Nature Connectedness', 'Attitude towards Outdoor Play' and the 'Risk Engagement and Protection' surveys were still statistically reliable after translation (see results: effects on children's behaviour). There was also an English version available for the parents who weren't fluent in Dutch or French. More details about the guardian and child surveys are presented in the following sections.

Table 5.1. List of surveys that were conducted and who were the respondents.

Variable	Survey	Collected at
Socio-economic status	Socio-economic status	Parents
Cultural background	Cultural background	Parents
Living Environment	Living Environment	Parents
Child's engagement with playing in green	Attitude towards Outdoor Play	Child
	Nature Connection	Child
	Free time activities	Parents

Guardian’s perceptions on outdoor play in green	Risk Engagement and Protection Survey	Parents
		School guardians
	Independent Mobility	Parents

Guardian-reported survey data

The survey for the parents collected most of the WP5 data. It started with a general survey with questions about the age of the parent and child, their relationship to the child, how long the child has been going to the school in question, possible allergies or chronic illnesses, and contact with animals. Next, SES (Reynders et al., 2005) and cultural background (inspired by the Belgian Health Interview Survey and Morrens et al., 2015) were questioned to assess whether there is a relation between these and other elements such as the child’s outdoor playing behaviour, their microbiome, green at school. Furthermore, the family's living environment was questioned, including questions about the presence of a garden, regular use of green elements in the neighbourhood and a typology of different living environments. Additionally, we asked the parents about their child’s outdoor play preferences (modified from Jelleyman et al., 2019). We expected that we would get more reliable answers from the parents rather than asking the children directly, as they are better to see longer term trends in the outdoor play behaviour whereas children are more likely to give their current preferences. Lastly, the Risk Engagement and Protection Survey (REPS) during outdoor play (Olsen et al., 2018; 2019) and a survey about independent mobility of the child in the living environment (Shaw et al., 2013) are included in the parents' survey to assess whether rules of parents are of influence on outdoor play behaviour of the children. The REPS was also taken with school guardians, to investigate if different responses from guardians led to different preferences or attitudes towards outdoor play during school breaks. The surveys were first tested on paper on a small sample of Dutch-speaking parents. Next, the surveys were tested using Qualtrics on a small sample of French-speaking parents. The testing resulted in minor adjustments to the questions to improve clarity and understandability.

Child-reported contact with nature

A literature search yielded several existing scales to measure Nature Connectedness (NC). For example, Salazar et al. (2020) present multiple tools to assess children’s connection to nature. We chose to use the Nature Connection Index as developed by Hunt et al. (2017). The reasons being that it was simple, developed with children in mind, tested with children in the same age group, and is statistically a valid and reliable measure of NC. Furthermore, we used a validated survey to measure ‘Attitude Towards Outdoor Play’ (ATOP) (Beyers et al., 2015) to investigate if the child values to play outdoors and its relation with e.g. school environment, SES and NC.

Both surveys were translated into Dutch and French from English. The translated questions were tested in a first round on a small sample of Dutch-speaking children. In a second round, the surveys were tested in a Dutch-speaking and a French-speaking class. In both rounds feedback was collected on whether the surveys were clear and understandable for them, which resulted in a small number of adjustments to the translated questions. WP5 surveys can be found in **Deliverable 5.2**, including the children's and school guardian questionnaires in Dutch and French, as well as the parental questionnaires in Dutch, French and English.

Task 5.3 Data collection

A data collection protocol was written and shared with the MEC before the collection of data started (**Deliverable 5.3**). The surveys were distributed from April 2022 and collected over several months. Child surveys were administered on paper in the classroom during the data collection effort coordinated by Sciensano (see task 4.3). Parent surveys were administered using the QualTrics online survey application. The same coding system was used as described in task 4.3 to guarantee anonymity. It quickly became clear that, especially the parents' survey had a very low response rate (40% participating parents / participating children). Additional efforts were made to collect the most essential data from the parents in an attempt to increase the response rate (see also task 2.4). Therefore, the parent's survey was shortened:

- Parents were no longer asked for their age.
- Socio-economic questions were reduced to the core aspects.
- Questions about areas used by the child for outdoor play in their living environment were removed.
- Answers to the questions on 'outdoor play preferences' of the child were simplified.
- REPS and the Independent Mobility survey were omitted.

The shortened questionnaire was sent on paper to schools (who agreed to contribute to this effort). The teachers would ask the parents to fill in the shortened questionnaire during a parental meeting and send them back to the researchers. Through this effort we raised the response rate of the parents to 69%. Concerning the REPS survey for the school guardians, unfortunately, multiple schools didn't fill in any surveys, while some other schools only filled in 1 or 2. The schools from which we did get a high response were too little, making any analysis impossible.

Task 5.4 & 5.5 Data processing & Data transfer

All the collected data, online and on paper, was digitised and entered in a local and secured database. The database was only accessible by the INBO-team. The data received from the parents questionnaires contained the names of the children. The names were replaced by their unique ID-code to ensure anonymity.

Next, the data was cleaned, errors were detected and solved, issues with ID-codes were identified and solved, data was standardised and code-books were created. The cleaned up and anonymised database and code-books were shared with WP1 for the integrated assessment. The cleaned data is published, together with the data of the other work packages, on Zenodo (Van Calster et al. 2024).

The questionnaires that were filled in on paper (all the children surveys and the shortered questionnaire for the parents) are stored at Sciensano.

The cleaned data was also shared with a KU-Leuven Master student in Statistics (and simultaneously an INBO-employee), who analysed the data for her Master thesis (see Carmen, 2024 and section Scientific Results: Effects on behaviour). Through a collaborative effort between WP1, 4 and 5, this will result in a manuscript that focuses on the relationships between nature connectedness, attitude towards outdoor play, and green space exposure (in preparation).

WP6 coordination

Task 6.1: Administrative duties

On 20231024 a 6 month extension of the project period was requested with the following argumentation: the final part of the project will be demanding, still lots of challenges we need to work on, partly caused by the COVID crisis which gave a severe setback in our collaborations with the schools, partly because it took longer than expected to centralize / clean the data. The extension was granted by BELSPO.

On 20240709 an embargo on the public online publication of the final report was requested with the following argumentation: as all the work has been more demanding than expected (COVID, complexity of the data)but also inspiring and relevant for scientific publications, we have the challenge to already inform you via the final report in September, whilst still after September having quite some work towards final scientific publications to be submitted. If the journals find the report online with too many overlapping content for publication in their journal, they may not want to consider our submissions, which would be very unfortunate. This embargo was granted by BELSPO until 20241215.

Task 6.2: Scientific collaboration

Internal collaboration

The B@SEBALL project developed in a very productive collaborative atmosphere with monthly full project meetings, facilitated and chaired by the coordinator in order to organize and support the challenging fieldwork, analysis and communication, and to brainstorm solutions to prominent challenges along the way. Monthly an agenda was shared for inputs, and after the meeting minutes of the meeting are shared, for all to follow progress of work, also when not able to be present at all meetings. Most meetings were online, but several meetings were physical meetings, which helped the connections between and communication among all partners. Where relevant, smaller group meetings were organized.

External collaboration

Collaborative interest beyond the project consortium crystalized in a collaborative agreement with the Airbezen project of Prof. Roeland Samson at UAntwerpen, which opens up alignment with their vast experience in working with schools, their supportive tools for schools, and air quality measurements which can be relevant for out integrated environmental health analysis. Also, the project is attracting quite some interest from students, with two group (UAntwerpen) and one individual (UGhent) master thesis projects started in 2021 in collaboration with and supportive to B@SEBALL. In 2024 the master thesis (KULeuven) of INBO researcher Raïsa Carmen resulted in a statistical evaluation of the reliability of the translated surveys in the B@SEBALL project by means of a Structural Equation Model (SEM) (Carmen, 2024).

Research dossier for the Medical Ethical Committee of Antwerp University Hospital

A very important focus of the first year of the B@SEBALL project, was aimed at preparing most research methodologies and other material needed to get started with contacting the school, for the Medical Ethical Committee of Antwerp University Hospital (**Deliverable 6.2 Request for ethical approval at the UAntwerpen medical ethical committee**). This was prepared in close collaboration with all partners and submitted in November 2020, containing the following ethics-related documents:

- Research Protocol
- Recruitment Protocol timing in Dutch and French
- Information & informed consent in Dutch and French, for the children, parents, and school staff
- Project information documentation in Dutch and French
- Project information Mail school management in Dutch and French
- Project information letter for parents in Dutch and French
- Project information Presentation schools in Dutch and French
- Project information Presentation parents in Dutch and French
- Project information video hyperlink microbial assessment
- Mental health Questionnaire KIDSCREEN-Test for the children in Dutch and French
- Asthma Questionnaire ISAAC on allergy and asthma in Dutch and French
- Social assessment questionnaire in Dutch and French
- School & participant recruitment protocols & timing
- Insurance information
- Cv's of all investigators

In 2022 updates of several components of the research approach based on further development and testing were submitted to the MEC and approved in addition to the existing and approved dossier. This was done timely before the start of the field work at the schools in April 2022.

Task 6.3: Stakeholder & expert advisory involvement

A broad variety of relevant experts were invited to take part in the B@SEBALL follow-up committee. They were contacted through the complimentary networks of the B@SEBALL partners, covering both Flemish, Brussels, Walloon and Federal Belgium organizations and experts, as well as international, from the UK and Finland. Over 30 experts agreed to participate, representing science, policy and practice, across the fields of environmental health, microbiome, education, social work and other.

Several online meetings were organized, with differing numbers and combinations of experts attending: 20200903 (21 participants), 20211001 (7 participants), 20231018 (10 participants), 20240621 (8 participants) and 20240909 (3 participants). Each meeting started with a presentation of the status of B@SEBALL work and some key issues to focus discussion on. After each meeting a draft meeting report and the meeting presentation were shared with the whole follow-up committee.

Task 6.4: Communication and dissemination

Communicating complex analytical outcomes in an understandable and useful manner is an important ambition of the project. We need careful communication to the study participants (school children, parents, the teachers and the schools): taking into account potential sensitivities, and also avoiding that 'others' (like through the media) become informed about results earlier. Also communication to (other) end-users such as policy makers and people working on greening schools and developing nature related education at schools is considered: practise relevant communications. B@SEBALL benefited a lot from relevant dialogue with the follow-up community members regarding the above mentioned ambitions and challenges. This helped us a lot in tuning both scientific analysis and communication of results.

SCIENTIFIC RESULTS AND RECOMMENDATIONS

WP1 Integrated assessment

Task 1.1 Methodological implementation sampling design

Please refer to the "Methodology" section where this is discussed.

Task 1.2 Data centralization and management Data management plan

Please refer to the "Methodology" section where this is discussed.

Task 1.3 Integrated data analysis

Integrated data analyses were always a collaborative effort with multiple work packages involved, depending on the specific topic being addressed. Results were always discussed with the steering committee and all work packages.

WP1 + WP3 Socio-economic inequality of school environmental conditions in the general population

The B@SEBALL project assumes that access to green schools may be unequally distributed in a socio-economic context. The B@SEBALL sample of schools in itself does not allow us to test if this assumption indeed holds. We did however have the necessary data as part of the steps that needed to be done to draw a sample of schools for the B@SEBALL study. We compiled a complete list of all primary schools for both communities. For each school on this list, we also collected and calculated data from publicly available resources that allowed us to test if access to green schools is indeed unequally distributed. We note that greenness of schools here is an approximation, because we do not have access to the school or playground perimeters for each of these schools. We did, however, have an latitude - longitude coordinate for each school and we used this to determine the proportional cover of vegetation within 300m from that point and between 300m and 2000m. We also had socio-economic data at school level from which a socio-economic index was calculated. The underlying data differ between communities, but we used the same calculation method to derive the indices for both communities (borrowed from how it is calculated by the French-speaking community). We also looked up the statistical sector to which each school belonged in order to be able to query the neighborhood median taxable income, which is an indicator of general affluence. We also know the number of students enrolled in each school. The breakdown of these data between both communities is given in Table 1.2.

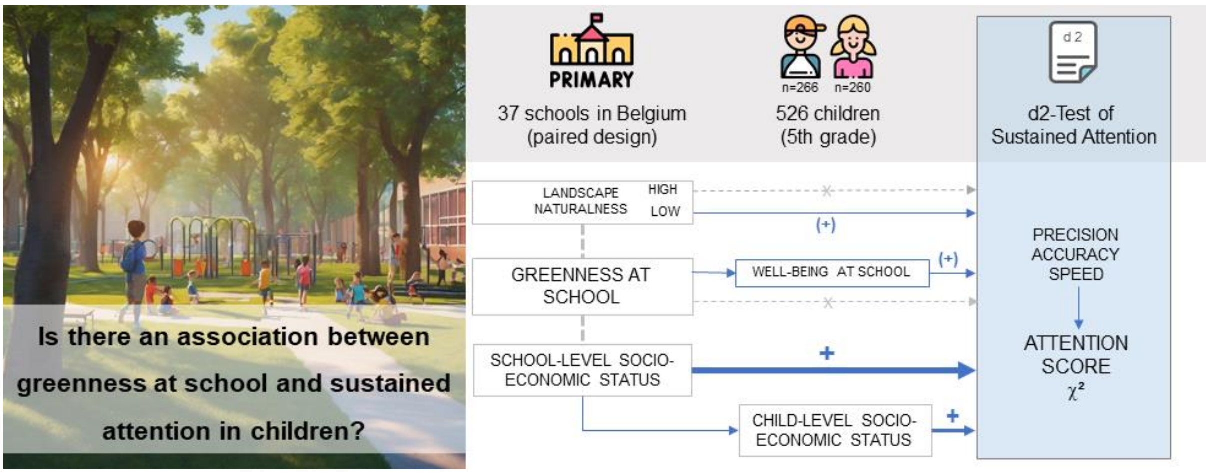
		Dutch-speaking (N=2808)		French-speaking (N=2579)			
		Mean	Std. Dev.	Mean	Std. Dev.	Diff. in Means	Std. Error
kindergarten		0.8	0.4	0.9	0.3	0.1	0.0
neighbourhood_median_income_original		26135.4	4113.8	23397.0	4654.0	-2738.4	120.1
vegetation300		0.5	0.2	0.5	0.2	0.0	0.0
vegetation300_2000		0.5	0.1	0.6	0.2	0.0	0.0
number_of_students		232.1	123.6	181.8	124.3	-50.3	3.4
		N	Pct.	N	Pct.		
territorial_typology	High density	658	23.4	763	29.6		
	Rural	427	15.2	950	36.8		
	Urban	1723	61.4	866	33.6		

Table 1.2 Summary statistics for all primary schools in the Dutch-speaking and French-speaking communities in Belgium for variables that were used to test socio-economic inequality effects on access to green schools.

We fitted a spatial generalised linear mixed model to these data to test the assumption. The model included spatial random effects to account for spatial dependence between schools. The spatial dependence model assumed a Matérn correlation function to model how dependence decreased with increasing distance between schools. Our results indicated that there is indeed an unequal distribution of the amount of green surrounding a school. Number of students enrolled (estimate in the logit-scale for Dutch-speaking community: -0.13, 95% credible interval CI [-0.16; -0.10]), vegetation cover within 300m to 2000m (1.59 [1.38; 1.80]) and neighbourhood taxable income (0.28 [015;0.41] and 0.56 [0.42;0.70] for incomes between 20k€ and 30k€, and above 30k€ compared to incomes below 20k€) all were important predictors for the amount of vegetation within 300 m with expected direction of the effect. After taking these covariates into account, the school socio-economic index still has an important positive effect on the amount of green surrounding primary schools (0.23 [0.15; 0.31] for Dutch-speaking community and median taxable income between 20k€ and 30k€). The latter corresponds to an increase in the odds of greenness around the school equal to 1.26 [1.16; 1.36] for a one standard deviation increase in socio-economic index.

WP1 + WP4 + WP5 Effects on sustained attention and well-being

The BASEBALL study provided new insights into the factors that influence student attention in schools. This project found that attention levels were higher in schools that were located in more urbanised areas and that this increase was primarily linked to socioeconomic status, rather than the amount of green space available (which was contrary to our initial hypothesis). However, further analysis revealed that in urban schools with abundant green space, students exhibited higher attention levels. Detailed statistical models suggested that the presence of green spaces indirectly supports student attention by enhancing overall well-being at school. Overall, the BASEBALL study underscores the importance of socioeconomic factors in driving attention levels among students. It also indicates that green spaces within schools can help mitigate the limited exposure to nature in highly urbanised environments, offering positive effects on attention and cognitive resilience.



WP1 + WP4 + WP5 Effects of school environment on health-related quality of life indicators

Missingness was about 10% for the Kidscreen data. The Kidscreen indices for Physical Well-Being, Psychological Well-Being, Autonomy & Parents, Peers & Social Support, and School well-being were positively correlated to each other but not to any other of the measured health outcomes (Figure 1.8).

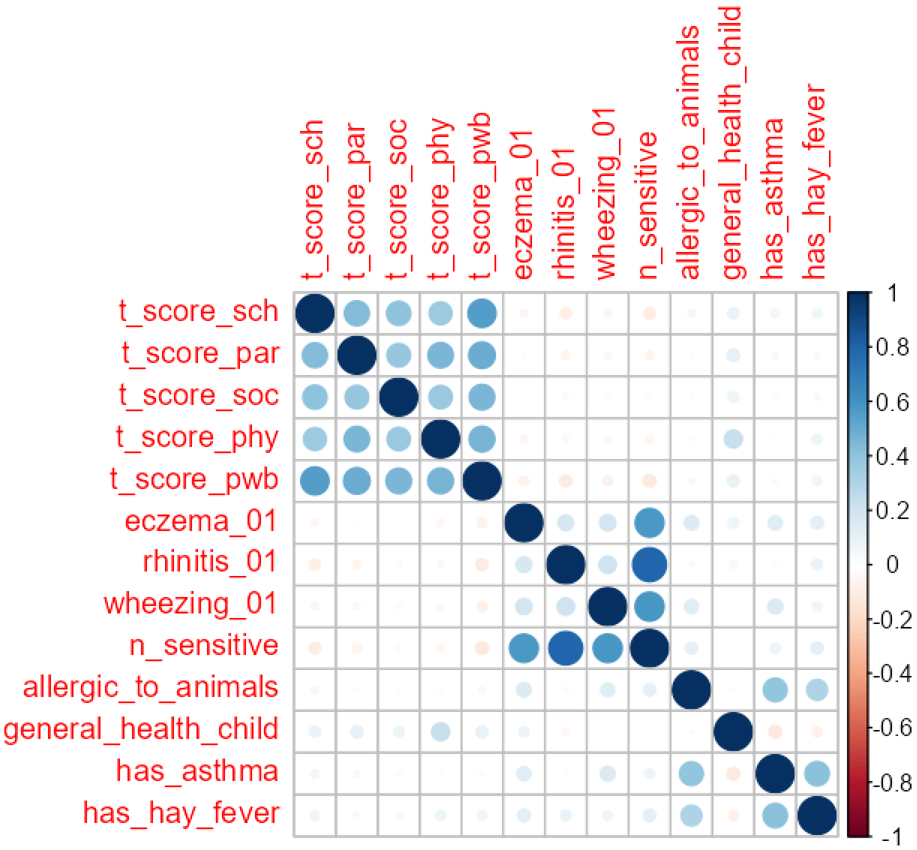


Figure 1.8: Correlogram for health outcomes of children participating in the B@SEBALL study. Colour and size of circles indicates the value of pairwise complete correlations based on Spearman's rank correlation coefficient.

Missingness of covariates indicated that covariates derived from the parent survey had most missingnes (> 30%) (Figure 1.9).

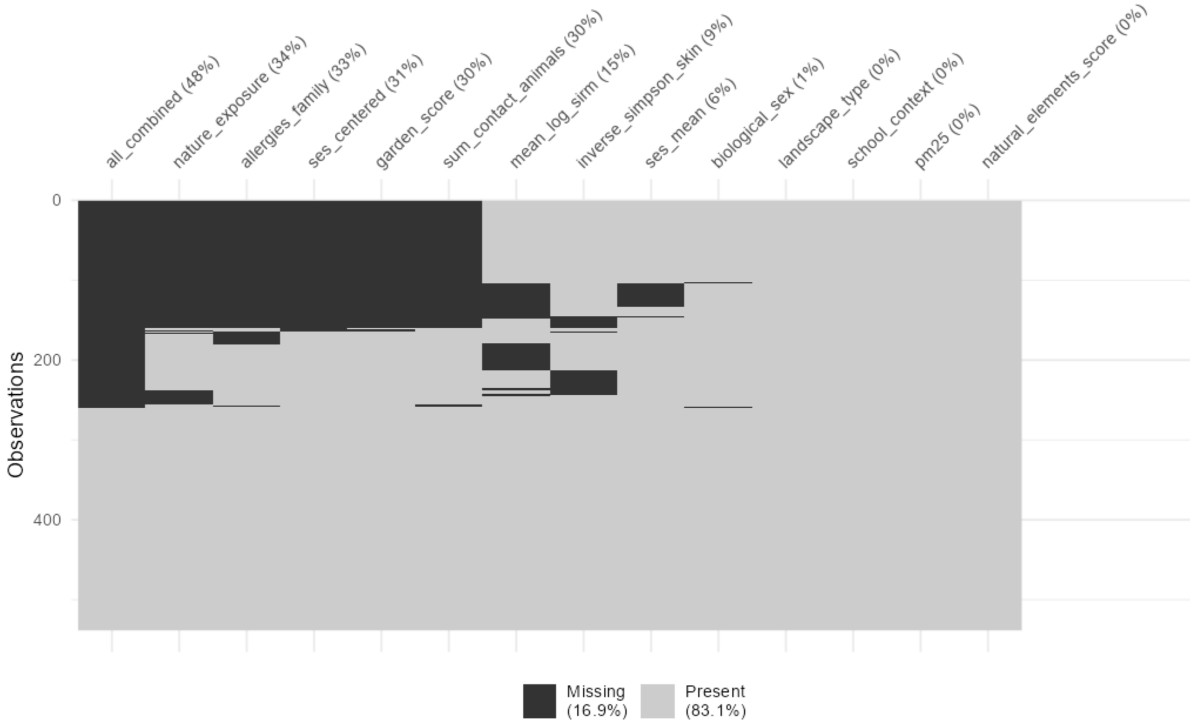


Figure 1.9 Graphical representation of the pattern of missingness among covariates. Each row represents the data for a child.

The strongest correlation between continuous covariates equals 0.52 and is between pm25 and ses_mean. This is a modest correlation and it is likely not problematic to include both covariates in a model (Figure 1.10).

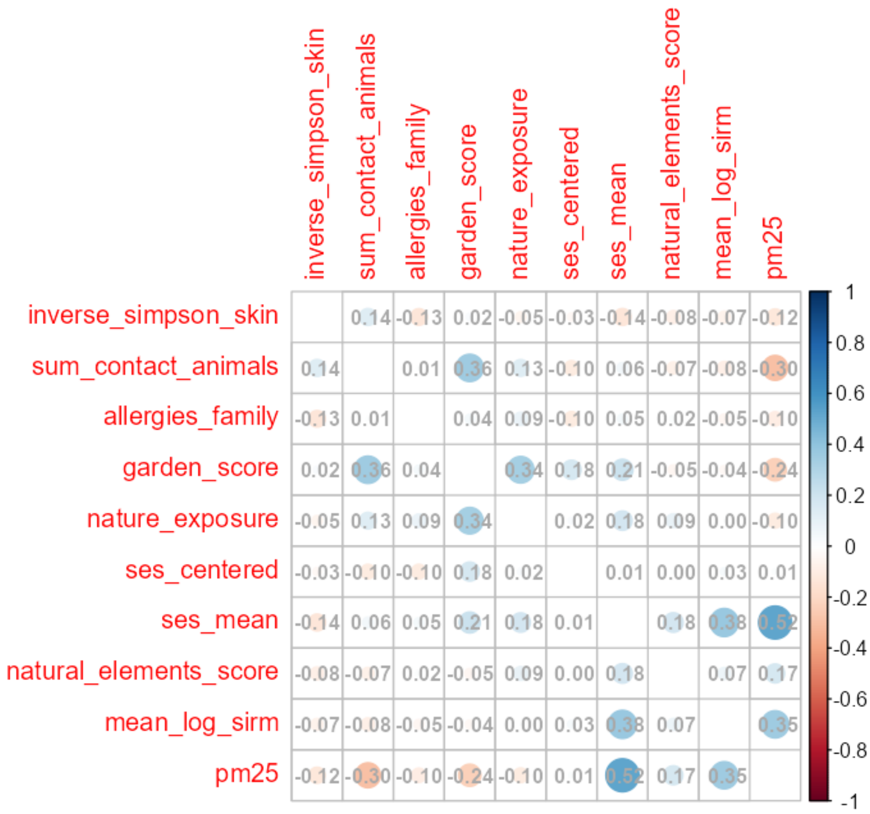


Figure 1.10 Correlogram for continuous covariates that were used to predict some of the health outcomes. Colour and size of the circles indicates the value of pairwise complete correlations based on Spearman's rank correlation coefficient.

We also looked at relationships between categorical and continuous covariates (Figure 1.11). The resulting relationships did not indicate collinearity problems. One minor concern was that the range of socio-economic status (ses_mean) values within high naturalness landscape for high greenness schools was more restricted compared to the other factor combinations.

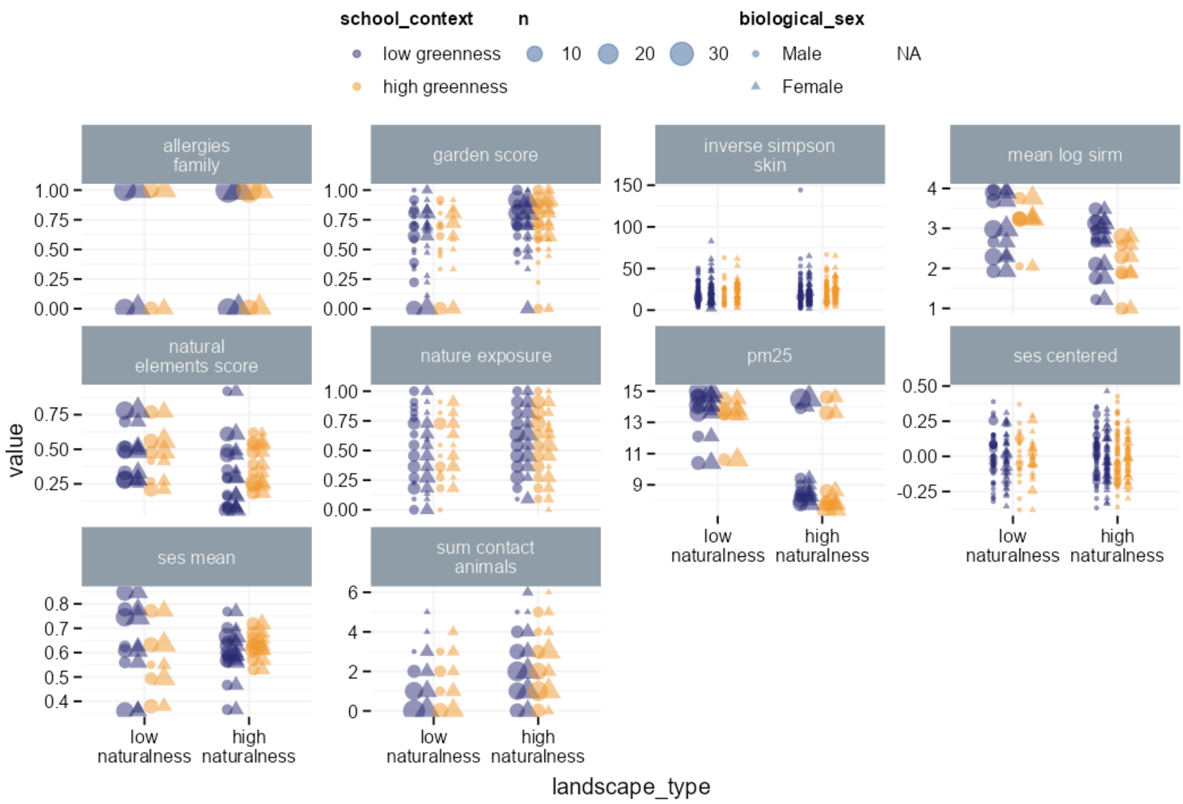


Figure 1.11 Visualisation of the distribution of the values of continuous covariates in relation to landscape type, school context and biological sex of the child. The size of shapes is proportional to the number of children represented by the data point.

The five dimensions of the kidscreen data showed fairly strong correlations with each other. The five Kidscreen indices were therefore related jointly (in a multivariate model) to the school context and landscape type, while accounting for the other potential confounders (covariates). This model took into account residual correlations and correlations between the random intercepts for school. The scores were modelled with a Gaussian distribution and an identity link. The analysis indicated an interaction between the socio-economic and the school context that was fairly consistent across all five dimensions measured by the Kidscreen health-related quality of life indicators. The interaction indicated that greener schools could compensate for lower well-being associated with lower socio-economic status. The interaction between school context and landscape type was less clear, but partly confirmed our expectation that green schools have a stronger beneficial effect on these well-being response variables in low naturalness landscapes compared to high naturalness landscapes.

WP1 + WP3 + WP4 + WP5 Effects of school environment on physical health (with focus on allergic diseases) and relation to microbiome

Analyses were performed to determine which factors related to the school environment and the measured environmental and children's microbiome have significant explanatory power of the children's allergy outcomes. To do so, the WP3 team worked together with the WP1 team in order to process the ISAAC questionnaire to obtain allergy outcomes "wheezing & asthma", "rhinitis", "eczema", and "allergic disease symptoms" and to process a questionnaire on outdoor play to obtain a metric for "nature exposure" based on children's play behaviour.

The allergy outcomes were related to school context and landscape type and their effects were interpreted after adjusting for potentially important confounding variables, which included biological sex, whether allergies were in the family or not, a garden score for home environment, a measure for playground level pollution as indicated by SIRM, the mean socio-economic index of the class and the deviation from the mean for the child (derived from parent survey questions), the before-mentioned metric for nature exposure, how much a child has contact with animals, a natural elements score for the playground, the level of mean annual air pollution at landscape level as indicated by particulate matter, and a diversity measure for a child's skin microbiome. For eczema, we found markedly higher probability of reported eczema symptoms in low naturalness landscape compared to high naturalness landscape, but no evidence for a protecting effect of high greenness at school level. For rhinitis, a protective effect of high greenness schools was found in low naturalness landscape, but not in high naturalness landscape. It is interesting to note a few of the associations with some of the covariates that were used to adjust the estimates for our design variables (school context and landscape type). For instance, having a garden at home, a higher naturalness score of the playground and higher diversity of the skin microbiome all were associated with less chance of rhinitis symptoms being reported. On the other hand, some were associated with a higher chance of reported rhinitis symptoms, such as the amount of contact with animals or the SIRM indicator for local pollution as well as the particulate matter level in the wider environment. The uncertainty around these associations was however relatively large. For wheezing, no clear differences were found. When combining the three outcomes and modelling the number of reported allergic disease symptoms, we found evidence for a protective effect of high greenness schools in low naturalness landscapes, but not so in high naturalness landscapes. Furthermore, a higher score of school ground natural elements was correlated with less reported rhinitis.

WP1 + WP3 + WP4 + WP5 Relationship between environmental and skin microbiomes and children's physical health (with focus on allergic diseases)

Soil, dust, and plant microbiomes of the schools represent the environmental microbiome, whereas the cheek microbiome represents the human microbiome that is affected by this environment. The cheek microbiome was chosen to represent the bacteria that children are exposed to in their airways and on their skin. Microbiome variation between samples was studied using beta dissimilarities in PERMANOVA models, which indicated that the microbiome variation was in the first place determined by sample type (skin vs. plant vs. dust vs. soil; $p < 0.001$, $R2 = 0.22$). When subsetting the microbiome according to sample type, PERMANOVA models showed that all school context variables contributed only slightly, but significantly (Table 1.3). School ID was the most determining factor for the microbiome within all sample types, confirming the concept of a household microbiome (Song et al, 2013) at school level. Furthermore, the plant microbiome (the bacteria on the strawberry leaves), which represents a large part of the airborne bacteria that children breathe, appeared to be most correlated with study variables and children's allergic symptoms.

Table 1.3: The following table shows results of three PERMANOVA models, which quantifies how much study variables were correlated with the microbiome of different sample types. The column name indicates the sample type, whereas each row represents a variable. A * indicates the variable contributed significantly ($p<0.05$).

		skin R^2	plant R^2	dirt/dust R^2
Air pollution metrics	$PM_{2.5}$	<0.01*	0.02*	0.01
	PM_{10}	<0.01	0.01*	0.01
	SIRM	<0.01	0.01*	0.01
	black carbon	0.01*	0.02*	0.02*
Urban vs. Rural	landscape type	<0.01*	0.01*	0.02*
School playground	school context	<0.01*	0.02*	0.02*
	natural elements score	<0.01	0.03*	0.02*
Sampling conditions	sample date	NA	0.02*	0.02*
	sample weather	NA	0.03*	0.02
Allergic symptoms	eczema	<0.01	0.02*	0.01
	wheezing	<0.01	0.02*	0.01*
	rhinitis	<0.01	0.02*	0.01
School	socio-economic status	<0.01*	0.02*	0.01
	school ID	0.096*	0.23*	0.196*

Furthermore, basic models by the WP1 team were expanded to test additional bacterial community characteristics, such as biodiversity. Bacterial biodiversity metrics, sequence variant richness (cfr. species richness) and the Simpson diversity index, were determined for all sequenced samples of the children's cheek swabs, strawberry plant leaves, sand, dirt, and dust samples. Biodiversity metrics calculated for the different microbial communities found on playgrounds and the microbiomes of children's cheeks did not show direct significant correlations with reported health or allergy-related parameters. However, when bacterial diversity was included in interaction effects, significance occurred in specific cases, implying that specific bacteria rather than the full bacterial diversity are potential drivers of allergy development in children. To study the contribution of specific bacteria, centered-log-ratio transformation was done for bacteria that were found related with green playgrounds and high naturalness locations. The transformed data of only these selected bacteria were used to extend the basic correlation models to address the potential contribution of these bacteria in improving or worsening allergic symptoms. Our models showed an association between specific bacterial taxa and health and allergy outcomes. This was observed for the children's cheek microbiomes: for example, *Prevotella* abundances typical in high-natural environments were correlated with low wheezing in children, while *Neisseria* abundances were correlated with more wheezing. For the environmental microbiomes, the correlations were context-dependent. For example, the typical plant bacterium *Massilia* was correlated with low rhinitis, wheezing and asthma symptoms of school children, but this potential positive health-effect disappeared when the school was located in a highly urbanized or polluted environment. These results will be included in the manuscript in preparation with a tentative title "*Nuancing the biodiversity hypothesis – allergies in children and their bacterial exposure at schools.*"

We observed that a biodiverse microbial environment is linked to more biodiversity of the microbiome of the children's skin. There is indeed a link between the environmental and human microbiome, as we observed at the level of specific bacterial taxa that are potentially transferred between the playgrounds and children.

The school playground microbiome composition was dependent on the naturalness of the surrounding environment and the greenness of the school playground. The link between biodiversity and the surrounding environment and the greenness of the school playground was not straightforward. Some of the bacterial taxa (such as Prevotella) found in higher abundances in more natural environments were associated with less self-reported allergic symptoms of the children, as mentioned above.

We hypothesize based on our results that the large context of the environment (e.g., landscape and pollution) can play an important role in determining the final health impact of environmental bacteria. Microbiome of school playgrounds appears highly likely to be one of the mediators in relation to allergy symptoms and general health of school children, and on the other hand it is at least in part determined by the naturalness of the school environment. Our results suggest that bacterial biodiversity on its own might not always be a comprehensive metric to understand the role of natural environments on human health. In the future, it is advised to confirm these findings in intervention studies, and include a more diverse set of health outcomes, such as immunological measurements in children and assessment of other environmental and human microorganisms such as fungi. This study is a stepping stone for follow-up mechanistic research to determine the exact mechanisms through which the environmental bacteria aid the development of children's immune systems.

- **Role of pollution**

As allergic rhinitis and asthma are known to be correlated with air pollution, we included several metrics of air pollution in our dataset. On the one hand we obtained the modelled air pollution values for particulate matter (PM2.5 and PM10), black carbon (BC), and nitrogen oxide (NO2) per school. On the other hand, in collaboration with Prof. Roeland Samson (UAntwerpen) we obtained saturation isothermal remanent magnetization (SIRM) values of the leaves of the plants we distributed to the schools. This value was previously reported as a good estimate for local air pollution. All of these pollution values were well correlated with each other, with SIRM deviating somewhat from the modelled values, indicating that local air pollution cannot always be accurately measured by modelled values. We found wheezing to be positively correlated with modelled PM2.5 values, but no other correlations between allergies and air pollution could be identified. As we lack indoor air pollution exposure data for the children, the expected correlations could be missing in our study. However, we did find that air pollution could show significant interaction effects with the abundance of some of the identified bacteria to explain variation of allergies. For example, a high abundance of the bacterial genus Massilia on school plants is correlated with less rhinitis symptoms of the school children, except under higher air pollution conditions (as measured by SIRM).

WP1 + WP4 + WP5 Effects on children's behaviour (contact with nature)

First, Carmen (2024) investigated the reliability of ATOP, NC and REPS surveys, which were standardised scale questions that were translated from English to Dutch and French. Each of these were analysed separately to determine the reliability and to check whether the translation works well. Composite reliability is checked using McDonald's ω and Cronbach Alpha (Bentler 1968; Cho 2021; McDonald 2013).

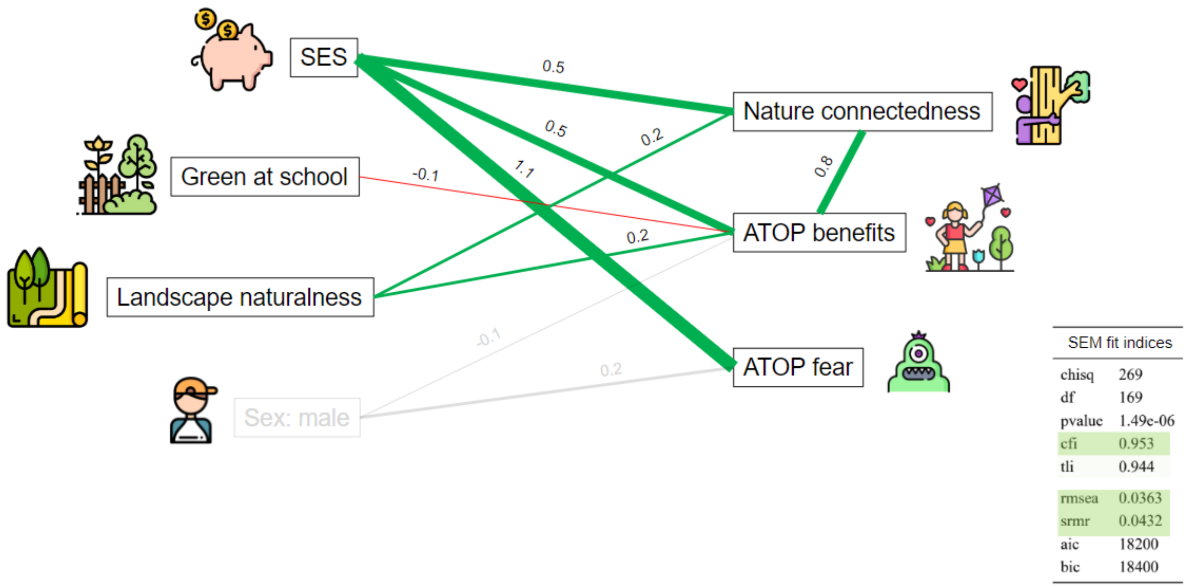
The results show the internal consistency (ω and α) of the ATOP is barely acceptable. ATOP has two subscales, ATOP_benefits and ATOP_fears, that are measured on a likert scale by seven and four statements respectively. ATOP_benefits is high if the child values the benefits that outdoor play may have and ATOP_fears is high if the child is fearful of some elements of outdoor play. The internal consistency of ATOP_fears is consistently worse than the internal consistency of ATOP_benefits. While the original scale showed a negative correlation between ATOP_fears and ATOP_benefits (Beyer et al. 2015), the CFA model shows a positive, non-significant correlation for both the Dutch and French translation. To increase the reliability, the translation of the questions should be revised, and the researchers should reconsider adding an omitted question about people with drugs ("I don't like playing outside in nature because there are people with drugs.") that was deemed inappropriate during the translation of the original survey.

The translated NC questionnaire is still reliable and valid and it measures the connection to nature well. On the other hand, for REPS the results show that it is currently not reliable. This might be due to the lower amount of data since the REPS scale was left out of the shortened questionnaire to improve the response rate. However, the results show that several statements have low reliability, especially the statement *"I encourage my child to do physical activity (with the least risk of injury)"*. Statements with low reliability need to be revised in future studies that use these translations.

Second, Carmen (2024) investigated how the child's SES relates to their NC and ATOP, as well as how the type of school environment relates to the children's ATOP and NC dimensions. REPS was excluded as this was deemed too unreliable. To investigate these relations, a Structural Equation Model was used and linked the scales to other exogenous variables. We include the ATOP and NC scales with the following additional covariances between some of the statements. Since we want to know how the three latent factors are related to each other, we include covariances between the three factors. Lastly, we add a regression for each latent factor with the following exogenous variables as covariates:

- The gender of the child (1 for male, 0 for female).
- The socio-economic status of the child as a continuous variable. We include the child-level SES, if available. Otherwise, we use the school-level SES as a proxy to avoid too much missingness.
- The school context / type of playground as a binary variable (low (coded as 0) versus high greenness (coded as 1)).
- The landscape type around the school as a binary variable (low (coded as 0) versus high naturalness (coded as 1)).

In total, 450 observations with complete data were used in the SEM. Figure 1.12 shows the model results visually, including the standardised parameter estimates, while the inset table shows that the fit of the model is overall satisfactory though there is room for improvement since the p-value of the Chi-square test is significant (which is not uncommon in SEMs based on large datasets) and the TLI could be better as well.



SEM fit indices	
chisq	269
df	169
pvalue	1.49e-06
cfi	0.953
tli	0.944
rmsea	0.0363
srmr	0.0432
aic	18200
bic	18400

Figure 12. A graphical simplified presentation of the SEM model, including standardised parameter estimates and SEM fit indices.

The SEM shows that SES is an important and significant predictor for ATOP_benefits, ATOP_fears, and NC. Higher SES will lead to a larger recognition of the benefits of playing outdoors, a stronger connection to nature, but also a larger fear of the dangers while playing outside. The latter may be slightly counter-intuitive but, as already discussed in the previous section, the validity and reliability of ATOP_fears is questionable. Next to SES, gender also plays an important role. Boys are expected to have a lower ATOP_benefits score and a higher ATOP_fears score. This is in line with a recent study that found that girls tend to have a more positive environmental attitude in children in Germany (Bucht, Bachner, and Spengler 2024).

Furthermore, the SEM shows that the direct school surroundings (the naturalness of the landscape outside the school) plays an important role in ATOP_benefits and NC, but the greenness of the playground does not. A more natural environment around the school is expected to lead to higher ATOP_benefits and NC. Many of the B@SEBALL project research questions focus on the link between the playground's greenness and the children's mental and physical health. While we did not include any health variables in the SEM for now, it does suggest that the greenness of the playground has a minimal or no impact on ATOP_benefits, ATOP_fears, and NC. The SEM shows that a green playground may even lead to slightly lower ATOP_benefits which is the opposite of the B@SEBALL project's expectations.

Task 1.4 Definition project outcomes - ***Deliverable 1.4 Communication of integrated assessment outcomes in scientific research papers***
This task is discussed in the "dissemination and valorisation" and "publications" section.

WP2 School engagement

Task 2.1 Methodological implementation
For details, see the methods section of this report.

Task 2.2 Recruitment schools
Of the total of schools that were previously preselected some of them were not keen to participate. Globally, the reasons for their refusal were the following:Too many projects at the same time

- This project can't be a priority. There's already too much to handle with the Covid situation.
- Too complicated with the parents (language barrier/sometimes because of the tests on the children (prick test and nose swab))
- Director, teachers and children are regularly absent due to Covid.
- Not interested in the project (seldom).

Although we couldn't explain this phenomenon, recruitment was more difficult inner cities compared to countryside schools. We observed the same feeling between Flanders and Wallonia. The positive answer rate was higher in the Walloon region. Once all the schools in the list of WP1 were contacted, for every school that refused to take part in the research, WP1 found a new school to replace it and added it to the list. So, WP2 could go further with the recruitment process. We received almost every document we sent to the schools (engagement documents). Those documents and pictures were a precious help for the collection of data (names of teachers, contacts, mail addresses…) and future communications.

Task 2.3 Recruitment participants
We could organize moments with the teachers and another one with the parents almost in every school. Some schools that arrived later in the recruitment process couldn't plan a meeting with the parents. The schools invited the parents with a letter we had created. Unfortunately, sometimes the communication between the school and the parents did not work well : disengaged parents, lack of time from the school to properly communicate, language barrier…for some reasons we couldn't present the project directly to the parents in a few schools. However, we made sure the parents received the information on the project. Most of the presentations with the parents occurred in the evening. Except on rare occasions, just a few of them showed up. Was there a lack of interest/time? We were still into covid or just right after in some cases. It's also a probable cause.

Task 2.4 Collaboration with schools

Our efforts to keep contact with the schools was fruitful. On the total of recruited schools we had just a few disengagements. The schools that decided to give up were overwhelmed with the quantity of daily tasks which were not linked to the research. Another challenge we met was the changing teaching team. The research underwent on 3 school years, which meant we had to make sure that the contact remained the same. Which wasn't the case in a lot of schools.

Task 2.5 Dissemination results participants, schools

The participants received the results in a 12-page document. The content was simplified and illustrated with pictures and graphs. Since all the schools did not reply to the previous communications, we decided to send it by mail (PDF) and by post mail. The schools were noticed days before that they would receive that document.

WP3 Biodiversity assessment

Task 3.1. Methodological implementation environmental assessment

Deliverable 3.1 Guideline for environmental biodiversity assessment at schools is described in the "Methodology" section.

Task 3.2. Methodological implementation microbial assessment

In this part of WP3, a dedicated methodology was developed and applied for the characterization of the playground microbiome composition and diversity at Belgian schools, and its overlap with the children's microbiome. Please refer to the "Methodology" section related to "*Task 3.2. Methodological implementation microbial assessment"* and *"Task 3.3. Data collection"* where the details of the microbiome sampling protocol are discussed. The development of the protocol has been described in detail in the intermediate annual progress reports. Hereby, the ***Task 3.3. Data collection*** with "**Deliverable 3.3 Field work protocol and organization plan for sampling at the schools**" and ***Task 3.4. Data processing*** with ***"Deliverable 3.4 Protocol for biodiversity database design and production database"*** has been accomplished.

Our results shed light on several important aspects regarding the microbial composition of the playground microbiomes in the tested Belgian schools and the related methodological considerations. A total of 184 samples of dirt, dust, sand and strawberry plant leaves were collected from 31 schools and analyzed regarding their microbiome composition, resulting in a.o. a Principal Coordinate Analysis (PCoA) plot based on a Bray-Curtis dissimilarity matrix (Figure 3.1). The microbiomes of the dirt and sand samples had a significant overlap with each other, and also showed some overlap with dust samples. The strawberry leaf microbiome was different (and thus clustered separately) from the other school playground samples.

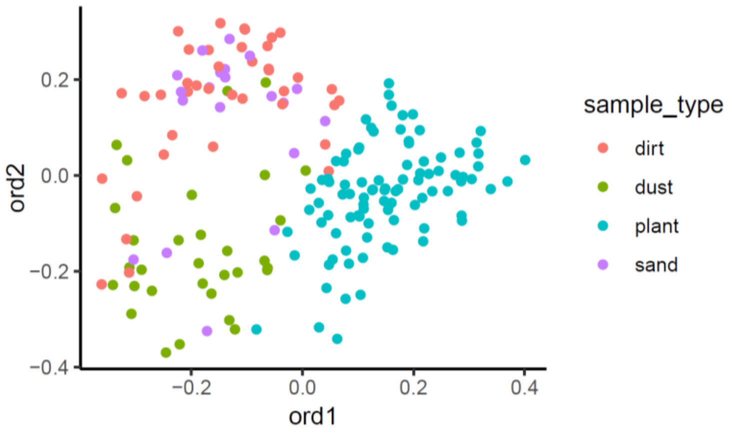


Figure 3.1. PCoA plot showing clustering of microbiome samples of dirt, dust, plant (strawberry leaves) and sand from school playgrounds based on a Bray-Curtis dissimilarity matrix.

Bacillaceae, Enterobacteriaceae, Intrasporangiaceae, Microbacteriaceae, Micrococcaceae, Nocardioidaceae, Planococcaceae, Rhodobacteraceae, Sphingomonadaceae, Staphylococcaceae and *Streptomycetaceae* were the most abundant bacterial families on the strawberry leaves. In Figure 3.2, the microbiome composition of the collected strawberry leaf samples is depicted, further zooming in on the 11 most abundant bacterial genera. The three replicates per school are depicted, and the schools are separated into schools with high greenness or low greenness. From a methodological perspective, the three leaf replicates per school show consistency in microbiome composition, with the exception of a few outlier replicates almost exclusively dominated by one bacterial genus (Figure 3.2). Figure 3.3 more specifically visualizes the leaf bacterial communities of schools with high greenness and low greenness of playgrounds, focusing on the 11 most abundant bacterial genera. According to the sparcc analysis, Sphingomonas were significantly more abundant in schools with a high greenness playground in a rural context. More genera were found to be significantly differentially abundant according to school context, landscape type, or both, but these genera are less abundant and therefore belong to “other taxa” in Figure 3.3.

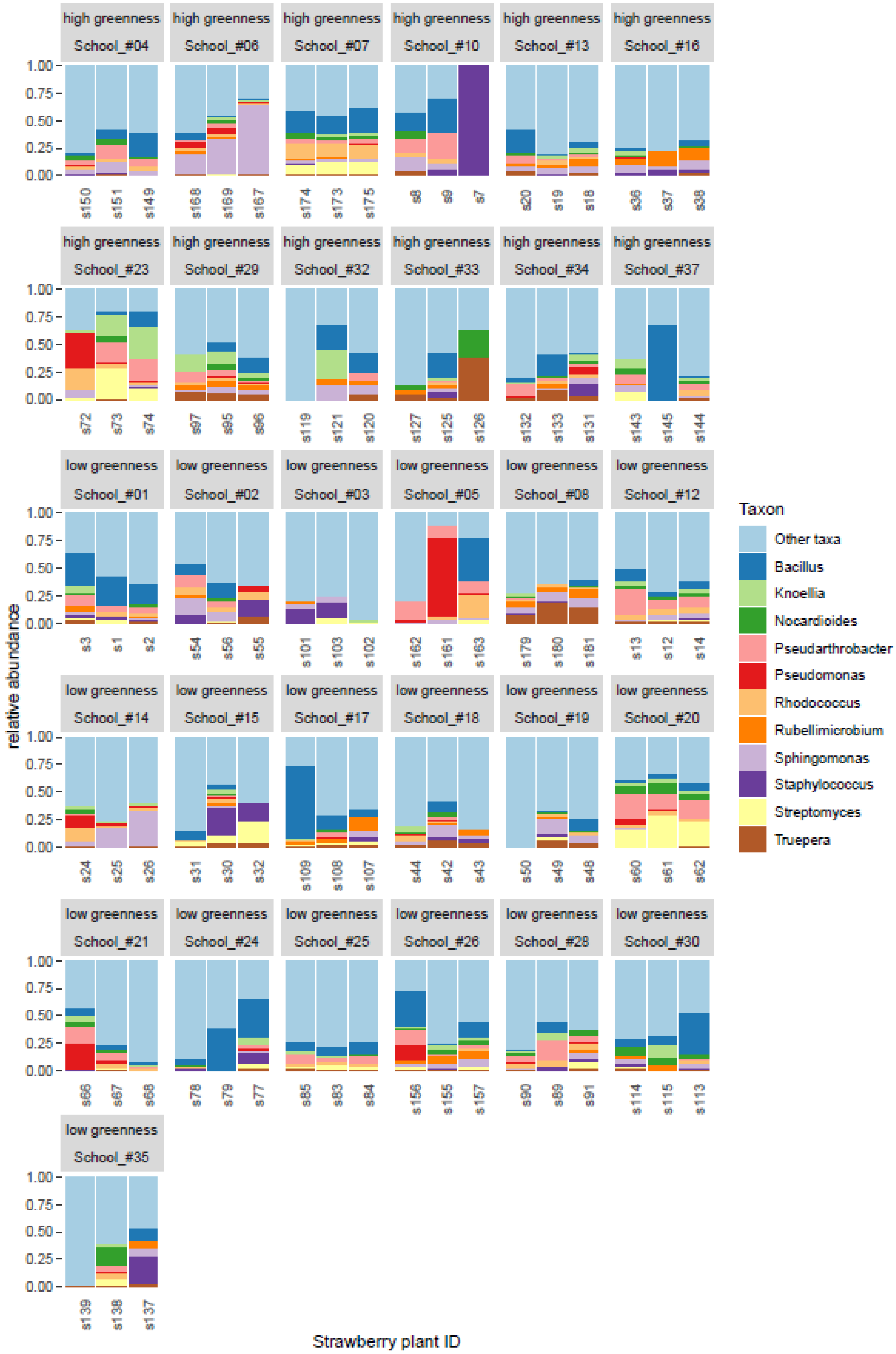


Figure 3.2. Relative abundances at genus level of bacterial genera found in samples of strawberry leaves from school playgrounds. The 11 most abundant bacterial genera are annotated. Strawberry plant ID refers to leaf samples from different schools, with 3 samples per school.

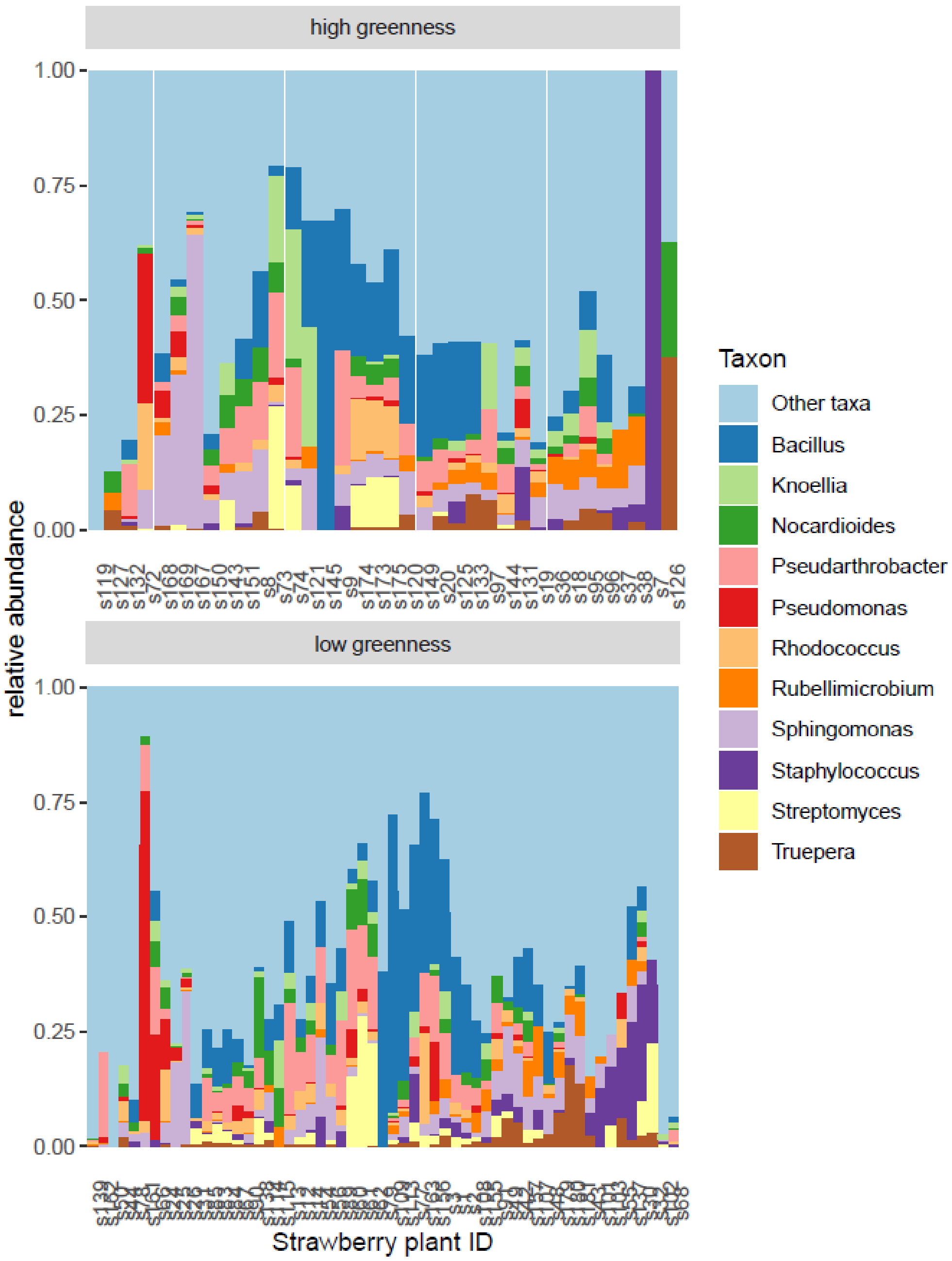


Figure 3.3. Relative abundances at genus level of bacterial genera found in samples of strawberry leaves from school playgrounds (strawberry plant ID refers to leaf samples from different schools as depicted in Figure 3.2). The schools are separated into schools with high greenness or low greenness. The 11 most abundant bacterial genera are annotated.

In addition to playground samples, also a total of 483 microbiome samples from the cheek skin of pupils from the same schools were collected and analyzed. When comparing the different microbiome samples and assessing microbial biodiversity, more diverse bacterial species were detected in the dirt, dust and sand samples compared to plant and skin samples (Figure 3.4A). The PCoA plot based on a Bray-Curtis dissimilarity matrix at bacterial family level shows a clear difference in microbiome composition of pupil's skin samples that cluster separately from all school playground samples (Figure 3.4B).

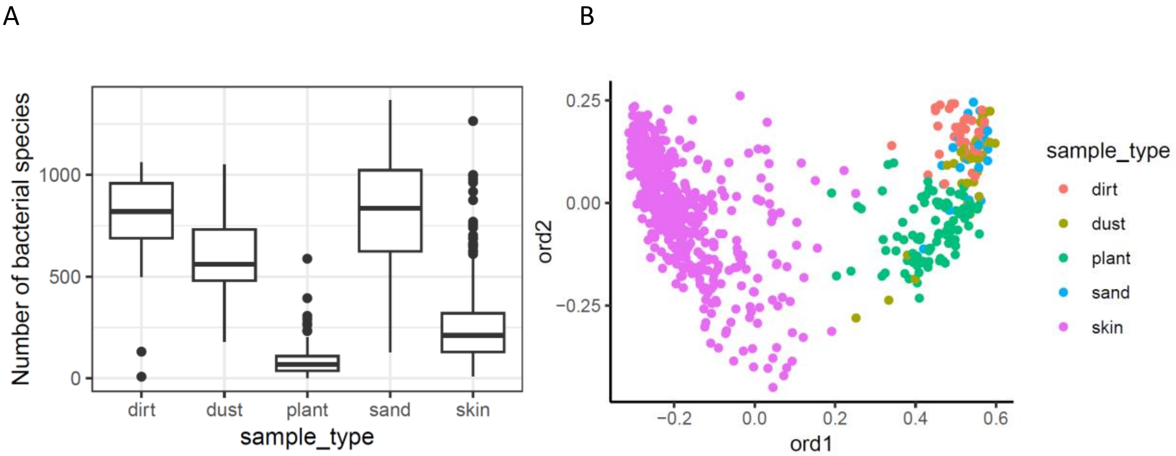


Figure 3.4. (A) Microbial biodiversity reflected in the number of bacterial species found in different types of collected samples from school playgrounds: dirt, dust, plant (strawberry leaves), sand, and from pupils (skin). (B) PCoA plot showing clustering of microbiome samples of dirt, dust, plant (strawberry leaves) and sand from school playgrounds, as well as pupil's cheek skin, based on a Bray-Curtis dissimilarity matrix.

We furthermore found a significant correlation between mean bacterial plant diversity and mean bacterial skin diversity at schools ($p = 0.03$, estimate = 0.13; Figure 3.5), indicating a connection between the children's microbiome and the environmental microbiome.

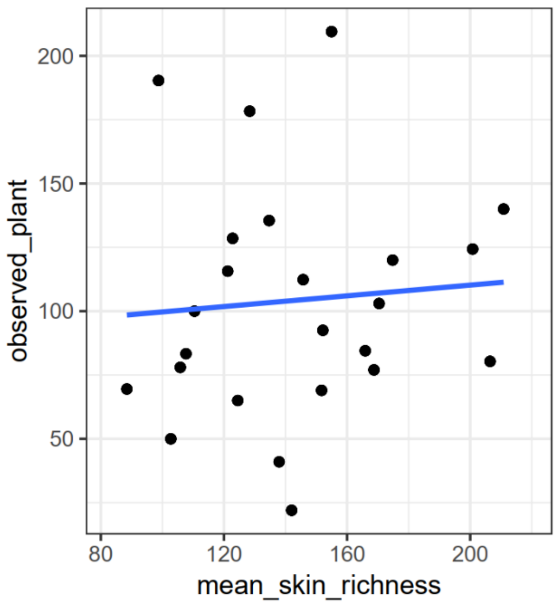


Figure 3.5: Average bacterial diversities of strawberry plant leaves and children's cheek microbiomes at each of the schools correlate positively.

In Figure 3.6, the microbiome composition of the collected pupil cheek skin samples is depicted, further zooming in on the 11 most abundant bacterial genera, and data from the schools is separated into schools with high greenness or low greenness. The most abundant bacterial genera on the skin of pupils were *Acinetobacter, Actinobacillus, Alloprevotella, Corynebacterium, Enhydrobacter, Gemella, Neisseria, Prevotella, Staphylococcus, Streptococcu*s and *Veillonella,* of which *Corynebacterium* and *Veillonella* were significantly more abundant on children in schools with high greenness playgrounds.

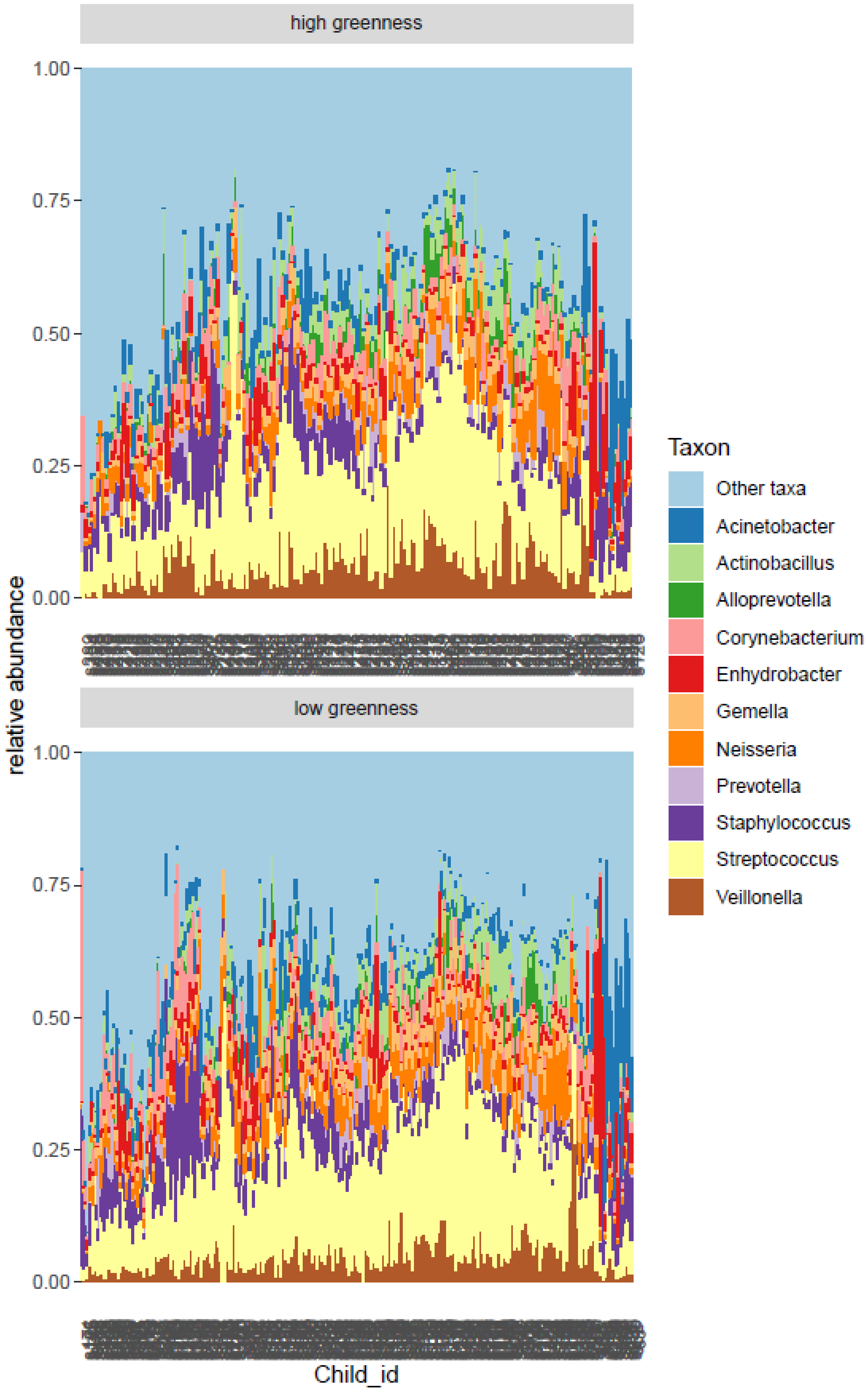


Figure 3.6. Relative abundances at genus level of bacterial genera found in samples of pupil's cheek skin. The schools are separated into schools with high greenness or low greenness. The 11 most

abundant bacterial genera are annotated. Each bar represents the microbiome

To conclude on the "**Deliverable 3.2 Guideline for microbial biodiversity assessment at schools**", we have demonstrated that playground sample collection by pupils with a teacher based on provided instructions followed by 16S rRNA amplicon sequencing of bacterial communities is a feasible and successful strategy for microbial biodiversity assessment at schools. This is an innovative result, and the developed microbiome protocol can be applied in the future to other schools, as well as to many other projects in alternative settings. Our results also show that it is important to sample different places on the same playground (soil, dust, leaves) to assess the whole diversity of playground microbial communities, and that 3 replicates of each microbiological sample are recommended. Likewise, the proposed methodology for pupil skin sampling is feasible and successful for large-scale microbiome assessment. More interpretation of the microbiome data will be published in a peer-reviewed article integrating the well-being and microbiome data, *"Nuancing the biodiversity hypothesis – allergies in children and their bacterial exposure at schools."*

Finally, we have accomplished **"Task 3.5. Data transfer"** to WP1, which was instrumental to conduct integrated analysis of biodiversity-children health linkages. This resulted in "**Deliverable 3.5 Protocol for biodiversity data transfer & scientific biodiversity data paper**" - more information on this deliverable can be found in section "Task 3.5. Data transfer" of the "Methodology" section.

WP4 Health assessment

The deliverables for WP4 are listed in the methods section.

WP5 social assessment

Task 5.1 & 5.2 Methodological implementation social assessment & contact with nature/playing behaviour

The approach that resulted in the questionnaires is described in the methods section (**deliverable 5.2**).

Task 5.3 Data collection

The protocol that was used for data collection and shared with the MEC (**deliverable 5.3**).

Task 5.4 & 5.5 Data processing & transfer

The protocol for data processing and transfer (**deliverable 5.4** and **5.5**) is described in the method section. The cleaned data is published, together with the data of the other work packages, on Zenodo (Van Calster et al. 2024). A paper based on the social data (**deliverable 5.5**) is in preparation (see Aerts et al., in preparation) and focuses on the relationships between nature connectedness, attitude towards outdoor play, and green space exposure (see results WP1, integrated assessment, section WP1 + WP4+ WP5 Effects on children's behaviour).

WP6 coordination

Task 6.2: Scientific collaboration

Research dossier for the Medical Ethical Committee of Antwerp University Hospital

Approval of the research dossier by the Medical Ethical Committee of Antwerp University Hospital B3002020000242 dated at 20201130 (Deliverable 6.2 Request for ethical approval at the UAntwerpen medical ethical committee).

Student collaboration

At UAntwerpen two student groups completed master thesis group work in relation to the B@SEBALL project: 1. Students environmental science (2020 – 2021) survey and interviews among primary education pupils, parents, teachers focusing on science communication on the type of research results B@SEBALL aims to produce. This work was finalized and is available for further uptake by B@SEBALL partners for project related work. 2. Two students medical and health science (2020 – 2024) completed a thesis on science communication about the microbial biodiversity link with asthma and allergy incidence, focusing at lay public and health professionals. At UGhent at the biology department (2020 – 2021), an individual master thesis was completed on the health of nature itself at green school playgrounds. In 2024 at KULeuven INBO researcher Raïsa Carmen completed a thesis with a statistical evaluation of the reliability of the translated surveys in the B@SEBALL project.

Task 6.3: Stakeholder & expert advisory involvement - **Deliverable 6.3 Report on policy and stakeholder organizations dialogue on end-user relevance of assessment outcomes**

The first meeting of the follow-up committee (20200903) was very well attended (20 participants) and very fruitful for introducing the research plans as they had been developed up to that timing, answering clarification questions and getting very useful feedback/advice, on (among other): clear communication on research choices (e.g. for excluding specific schools (German speaking, mentally handicapped target groups), the COVID-situation, communication of individual (pupil) results, biodiversity characterization at the schools, timing of taking microbial samples from the children, and questionnaire length. Also in this meeting, the follow-up committee helped in choosing the B@SEBALL logo from three options: see at https://www.uantwerpen.be/nl/projecten/baseball/. Most participants seemed very interested in following the progress of work during the next stages of the project.

On 20211001 B@SEBALL had its second (online) follow-up committee meeting. 7 member of the committee attended the meeting, as well as one guest: Simon Huylebroeck (both a teacher in Flemish education and a student from Environmental Sciences of Antwerp University) who represented group work that he developed with two fellow students in contribution to B@SEBALL. The status and plans of the research of B@SEBALL were presented by the various work packages and clarified when unclear, and commented by the meeting participants. Several helpful suggestions were given by committee members, e.g. on a potential collaborative/supporting role of the healthcare organizations for primary and secondary education (in Flanders called CLB) who visit the schools regularly for specific tests and monitoring of children's health, and on explaining and communicating the selection process of schools more adequately. Finally the presentation from the students was very much appreciated as very practical applications of B@SEBALL related teaching material for children in primary education, including for children with special needs.

On 20231018 B@SEBALL had its third (online) follow-up committee meeting. 10 members of the committee attended the meeting. The status of the analysis of B@SEBALL data was presented by various work packages. Several helpful suggestions were given by committee members on how to deal with the interpretative challenges considering the opportunities and limitations of the dataset. Among other, technical details of statistical approaches were discussed that were very helpful. Further, also interesting scientific publications were suggested to help interpretation. Importantly, the communication of study outcomes was discussed. We discussed specific formats, like newsletter or newsflash, but also the challenge of communicating complex analytical outcomes in an understandable and useful manner. We discussed the need for a careful communication to the study participants (school children, parents, the teachers and the schools): taking into account potential sensitivities. Also communication to (other) end-users such as policy makers and people working on greening schools and developing nature related education at schools was discussed: we aim to produce, where feasible, practice relevant communications.

At the 20240621 Follow up committee meeting with 8 participants, analysis progress, statistical challenges and intermediate results were discussed, as well as communication plans to schools, pupils and parents. As this external communication requires close dialogue with the committee members due to their scientific, policy and practice experience, an extra dialogical activity was agreed upon, at 20240909 close to the final deadline of this report (20240915), partly because of on-going work and the holiday period, partly because it was strongly suggested not to communicate to the schools in September, when schools will be very busy starting up after the Summer break, but rather do this in October. The final draft communication for the schools, pupils and parents will be sent to the committee end of August or beginning of September.

At the final 20240909 Follow up committee meeting with 3 participants, the final external communication, especially to the schools, pupils and parents, was discussed, as well as the formulation of final B@SEBALL research findings and policy & practice recommendations. In addition we received feedback via email on these subsequent documents in the finalization phase. For the outputs regarding the communication to schools see the Annex, and regarding the policy and practice recommendations, see below under recommendations.

Task 6.4: Communication and dissemination

State of the art review

The State of the art review which was delayed due to priority work load for preparation of the dossier for medical ethical committee approval and school recruitment was finalized and submitted to BELSPO. Before finalization, it was also shared with the follow-up committee for review and discussion before its second meeting: we received no crucial comments. Currently we are developing this into a scientific protocol publication.

Presentations and media coverage

See below at DISSEMINATION AND VALORISATION - Communication to broader public

Recommendations

1. *Target groups*

We aim to disseminate these policy and practice recommendations to policymakers (ministers, local authorities, city and neighbourhood councils (municipalities, regions and federal levels) and practice organizations (NGO's and local communities such as schools, parents' associations…)

2. *B@SEBALL evidence supports the importance of a green school environment for some health and well-being indicators*

Our children spend an important part of their life at school. The B@SEBALL study highlighted several positive impacts of the presence of nature and biodiversity at school on children's health and well-being. B@SEBALL contributed to a growing body of scientific evidence showing that a green environment in and around the schools can contribute to the children's mental well-being and healthy immune system development. This is especially true for the urbanised areas where exposure to pollution is increased and exposure to nature is decreased. We found that children in schools from urban environments that were greener had fewer allergic symptoms (wheezing, rhinitis or eczema) reported.

<u>Policy Recommendation</u>: Schools should be targeted for greening, especially in urban landscapes (or other types of landscapes with low naturalness), in order to contribute to children's physical and mental well-being .

<u>Practice recommendation:</u> Promotion of children's physical and mental health through greening of school environments.

3. *B@SEBALL evidence supports the importance of diverse natural elements and plant-associated bacteria on the playgrounds for some physical health parameters of children*

In addition to the general importance of a green school environment, the presence of a greater variety of natural elements such as wood chips, gravel, hedges, trees, flower beds, ponds and grass were associated with less reported rhinitis symptoms in children. In addition, some bacteria which are typically associated with plants, when they occur on the school playground, may be linked to a healthier immune development of school children reflected in less reported allergic symptoms.

<u>Policy recommendation:</u> Give the schools access to more funds and coaching to increase the level of biodiversity reflected in the presence of different natural elements at the school playground/

<u>Practice recommendation:</u> Health promotion through increase of the level of natural elements at the school playground.

4. *B@SEBALL evidence underlines the importance of tackling health inequality due to unequal access to a green environment*

Health-promoting access to nature is very much unequally distributed. Indeed, studies including this one reveal that children are not equal in terms of environmental living conditions. B@SEBALL shows that children with a high SES feel more connected to nature and have a more positive attitude towards outdoor play, possibly leading to more contact with nature and nature induced health benefits compared to children with a lower SES. Furthermore, the results of B@SEBALL show that self-reported well-being of children is higher in greener school playgrounds. Additionally, this positive association is even greater for children with lower socio-economic status (SES). This indicates that some SES inequality outcomes may be offset by greening school playgrounds. Currently, school playground greening is done for schools where teachers/volunteers have time to apply for this funding, while low-SES schools often have no resources to spare on these applications. Our results therefore imply that adapting strategies for school greening to include low-SES schools may be useful to gain more well-being with the same means and additionally decrease SES inequality outcomes.

<u>Policy recommendation</u>: target funds toward greening of schools for which socio-economic indicators are low (irrespective of landscape type).

<u>Practice recommendation:</u> Raising awareness among local communities and schools. Funding nature-education' actors such as NGOs, Universities, lifelong education services…will play an important role in empowering local councils, schools, teachers and parents.

Green up schools to increase well-being and enhance the level of attention in the classroom. Green playgrounds improve children's well-being at school and higher well-being was associated with better attention scores. A greener learning context reduces the amount of stress and offers a calmer environment. This would particularly benefit the children from very urbanised schools' context and children with low SES level.

5. *Further research needs and lessons learned:*

- Our study did not include, for instance, schools from landscapes that are dominated by intensive agricultural practices that depend on high inputs of fertilisers and pesticides. We therefore cannot easily generalise our findings to such settings. We therefore recommend that further research is needed to broaden the applicability of the B@SEBALL study.
- Due to time and logistical constraints, as well as the difficulty that we had to recruit schools after the Corona crisis, the statistical power that we aimed for from the onset of the study could not be achieved. Combined with problems due to partly missing data (i.e. cases where we do not have data on every outcome variable or confounding variable), it could very well be that some effects remained undetected. A similar, but larger study would therefore still be of scientific interest, perhaps focussed on a subset of the scientific questions that the B@SEBALL study addressed, for instance:
    - Can we gain a deeper understanding of which components of microbial diversity contribute to children's health in order to better understand the underlying ecological mechanisms?
    - Can we better understand the relative importance of socio-economic factors and school greenness on sustained attention of children and possibly other mechanisms relating to cognitive resilience - especially in urban contexts?
    - How does the relative importance of exposure to natural elements at school and at home environment for physical and mental health outcomes balance out?
    - Why was a protective effect of school greenness not found for symptoms of eczema in schools from low naturalness landscape, whereas - overall - the prevalence of these symptoms in low naturalness landscape schools was clearly higher compared to those in high naturalness landscapes?
- The planned Skin Prick Test, could unfortunately not be pursued because of a lack of skilled personnel to perform the SPTs. Future studies that include this test could give further and stronger evidence of a possible protective effect of biodiverse environments on allergies.
- Our study was a cross-sectional observational study and therefore correlative in nature. Follow-up research could be stimulated that extends such observational studies with a longitudinal time frame, preferably in combination with planned interventions (greening of schools). Furthermore, we advise to include a more diverse set of health outcomes, such as immunological measurements in children and assessment of other environmental and human microorganisms such as fungi.

DISSEMINATION AND VALORISATION

Data availability

The cleaned data are published as an open, freely-accessible data set. As such, the data can now be freely used by others to test their own hypotheses. The data is anonymized and organised according to the Frictionless Data Package standard, and is currently accessible through Zenodo (Van Calster et al. 2024). At 10 JUL 24, there were already 146 views and 36 downloads of the dataset.

Communication to broader public

B@SEBALL presentations at national and international conferences and symposia

On 10/02/2021, a presentation of the B@SEBALL project was given at the "Natuur- of milieueducatie (NME) en educatie voor duurzame ontwikkeling (EDO) netwerkdag" with Hans Van Calster, Irina Spacova, Linda Van Meersche, Anna Leonard, Sofie Heyman and Hans Keune.

Coordinator Hans Keune introduced the work of the B@SEBALL project as part of several more general presentations about nature – human health linkages:

- 20210321 symposium 'The doctor of tomorrow 2021' at KULeuven Medical Faculty
- 20210401 Expert Days PIXII on "Health and comfort"
- 20210917 Medical days University of Antwerp

Hans Keune and Irina Spacova presented the lessons learned from B@SEBALL as part of a panel discussion at the Symposium on Care and the natural living environment (Symposium Natuur en Gezondheid; Antwerp, Belgium) on May 24, 2022.

Irina Spacova and Wenke Smets presented intermediate results of B@SEBALL in a Webinar EMBL-EBI "Microbial biodiversity at schools and its link with children's health" on 20/03/2024, and at an International conference "Natural way forward" (22-24/04/2024; Hasselt, Belgium).

B@SEBALL experts from UAntwepren and GoodPlanet are asked by the Province of Antwerp to collaborate for a broad stakeholder event regarding the importance of nature contact at schools. This event is planned to happen shortly after the finalization of the B@SEBALL project.

B@SEBALL and its results were presented during lectures given by Irina Spacova to students of the Master in Bioscience Engineering: Sustainable Urban Biosciensce Engineering (course CityLab3: Human Health and Liveability) and Master of Medicine (course 'Gezonde omgeving, gezonde zorg').

B@SEBALL related press & media coverage

In several interviews, the work of B@SEBALL or related topics were highlighted:

Interview Hans Keune in De Morgen 20211117: *"We are currently investigating in Belgium whether greener school playgrounds, due to those microbes in nature, have a beneficial effect on children's mental and physical health and learning ability."*

Interview Hans Keune in Knack 20220603: *"'Contact with green is not the same for every child,' Keune knows. 'A green play environment can close that gap. That the policy supports schools in this is a good thing."*

Interview with Hans Keune and Irina Spacova in EOS 2022: *"If physical contact with nature is good for health, then you can think of it as medicine that is unevenly distributed"*

Interview Hans Keune in RAAK 20230218, magazine of KWB (big Flemish/Brussels organization for volunteer initiatives): *"research into the effect of a natural playground at school. Among other things, we do this with strawberry plants that capture environmental elements very well; that way we know exactly how biodiversity and microbiological life are doing. We compare that data with the children's skin microbiome. The study is in full swing, and we expect the results in about two years. Previous studies, including in Finland, show that allergies and asthma are significantly less common when children grow up in a more natural environment."*

Interview Hans Keune in Knack 20230823: *"'Verschillende studies laten inderdaad zien dat contact met de natuur de immuniteit verhoogt, en astma, allergieën en andere gezondheidsproblemen kan helpen voorkomen of verminderen', zegt Hans Keune, professor milieuwetenschappen en coördinator van de leerstoel zorg en natuurlijke leefomgeving (Universiteit Antwerpen). 'Er gebeuren heel veel studies op heel veel plekken, maar het veld staat nog in de kinderschoenen. Neem nu de microbioomof biodiversiteitshypothese. Die stoelt op ernstig wetenschappelijk onderzoek, maar er is nog veel onbekend – ook omdat het allemaal heel ingewikkeld is.'"*

Contributions of Raf Aerts, Hans Keune and Irina Spacova in "Natuur & gezondheid : van intuïtief verband tot meervoudige maatschappelijke kansen en uitdagingen", Natuur Focus 22 :4 (2023) , p. 159-168.

Contributions of Hans Keune and Irina Spacova in the IPBES Nexus Assessment (forthcoming 2025) on the relation between biodiversity and health, among other regarding the importance for the human microbiome.

Future dissemination plans beyond this report

We have several scientific results publications in the pipeline (see below under 'publications'), which will only get published after the deadline for this final report. We aim to publish a press release for each (forthcoming) publication, once published, highlighting subsequent policy and practice recommendations.

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B@SEBALL PUBLICATIONS

Van Calster, H., Lommelen, E., Groslambert, A., Leonard, A., Vanmeersche, L., Brulein, H., Vanwambeke, S. O., Aerts, R., De Clercq, E. M., Benchrih, R., Melike, O., Carmen, R., Lammens, L., Leone, M., Wanner, S., Lebeer, S., Legein, M., Smets, W., Spacova, I., & Keune, H. (2024). *Data from a cross-sectional study of fifth grade children in a sample of primary schools in Belgium that differ in amount of greenness at school and landscape level* [Data set]. https://doi.org/10.5281/zenodo.10527033

Aerts R., Van Calster H., Ozen M., Benchrih R., Heyman S., Swerts E., Wuyts A., Lammens L., Lommelen E., Leone M., Wanner S., Brulein H., Groslambert A., Vanmeersche L., Legein M., Smets W., Spacova I., De Clercq E.M., Lebeer S., Leonard A., Vanwambeke S.O., Keune H. (submitted, journal not disclosed) *Green playgrounds at school, well-being and sustained attention among fifth-grade children in Belgium.*

Aerts R., Carmen R., Van Calster H., Ozen M., Benchrih R., Heyman S., Swerts E., Wuyts A., Lammens L., Lommelen E., Leone M., Wanner S., Brulein H., Groslambert A., Vanmeersche L., Legein M., Smets W., , De Clercq E.M., Lebeer S., Leonard A., Vanwambeke S.O., Keune H. (in preparation) *Exposure to green space, nature connection, and attitudes towards outdoor play: a panel study among fifth-grade children in Belgium*.

Guyot M., Brulein H., Lecat A., Vanwambeke S.O., Van Calster H. + B@SEBALL collaborators (in preparation). Environmental justice: Do students have fair access to a quality environment?

Smets W., Legein M., Van Calster H., Delanghe L., Wuyts K., Aerts R., Ozen M., Benchrih R., Heyman S., Swerts E., Wuyts A., Lammens L., Lommelen E., Leone M., Wanner S., Brulein H., Groslambert A., Vanmeersche L., De Clercq E.M., Leonard A., Vanwambeke S.O., Samson R., Keune H., Lebeer S., Spacova I. (in preparation). *Nuancing the biodiversity hypothesis – allergies in children and their bacterial exposure at schools.*

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Research participants

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The follow-up committee

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B@SEBALL logo

Nicole DE GROOF (INBO)

Permission to use the KIDSCREEN questionnaire

Chiara Jörger (KIDSCREEN Group Europe, original EC Grant Number: QLG-CT-2000- 00751, Office of Quality of Life Measures QOL@uke.de)

ANNEXES

Medical ethical committee advice (including questionnaires)

Deliverable 6.2 Request for ethical approval at the UAntwerpen medical ethical committee

Communications to the schools, pupils, parents

All the illustrations here are only samples. The quantity of documents was too high to place it in the annexes. All the hereunder documents are available in French and in Dutch. For the full documentation or extra information you may send an email to GoodPlanet, Antoine Groslambert, a.groslambert@goodplanet.be

2.1 Documents for recruitment and collecting schools data

First information was sent as a Newsletter (after a first contact by phone or mail) (2 pages)

GOODPLANET.be B@SEBALL MOS

Votre école peut participer à l'étude B@seball

Qu'est-ce le projet B@seball?
B@seball est l'abréviation de "Biodiversity @ school environments benefits for all". C'est un projet de recherche qui étudie la biodiversité à l'école et l'influence positive qu'elle peut avoir sur la santé et le bien-être des enfants.

Pourquoi ce projet de recherche?
Nous avons besoin d'un système immunitaire fort pour faire face à l'augmentation des maladies chroniques.
Le **contact avec la nature est un facteur déterminant pour la bonne santé** des enfants. La nature à l'école, où les enfants passent la plupart de leur temps, peut améliorer leur santé physique et mentale. Cela a une influence positive sur la santé de CHAQUE enfant, et cela apporte ainsi plus d'égalité entre les enfants. Malheureusement, tous les enfants n'ont pas le même accès à la nature. C'est pourquoi il est si important qu'il y ait suffisamment de nature à l'école.
Bien que de nombreuses personnes pensent que le contact avec la nature est sain, **les preuves scientifiques sont encore insuffisantes.** C'est pourquoi le projet de **recherche "B@seball" a été mis sur pied.**

Pour pouvoir réaliser cela, **nous aimerions beaucoup** collaborer avec VOTRE ÉCOLE !

PWP Presentation for parents and teachers (46 slides) + video about Microbiots (2'16'')

Letter for the parents to invite them to the information moment (2 pages)

Chers parents,

La classe de votre enfant souhaite participer à la **recherche scientifique** B@SEBALL!

B@SEBALL étudie le lien entre la **nature à l'école & la santé.**

Pourquoi cette recherche ?

La nature rend les enfants heureux et en meilleure santé. En effet, en jouant dans un environnement naturel, ils développent une meilleure résistance immunitaire et se sentent davantage épanouis. Malheureusement, tous les enfants n'ont pas le même accès à la nature. Nous pensons donc qu'amener la nature à l'école pourrait remédier en grande partie à cette lacune. Intégrer plus de vert dans les écoles, permettrait à chaque enfant de bénéficier quotidiennement d'espaces naturels. Cela assurerait une plus grande égalité entre tous les enfants en termes de bien-être et de santé.

Cependant, bien que de nombreuses personnes affirment que le contact avec la nature est sain, nous ne disposons pas encore de preuves scientifiques suffisantes.

C'est la raison pour laquelle le projet B@SEBALL a été initié.

Objectif de la recherche

Grâce à B@SEBALL, nous espérons apporter les preuves scientifiques pour convaincre le gouvernement que plus de nature à l'école est vraiment nécessaire. Les écoles devraient recevoir plus de ressources pour végétaliser leur cour de récréation, afin que **TOUS les enfants** puissent profiter d'espaces naturels qui contribuent positivement à leur santé.

Moment d'information

Nous vous donnons RDV à **une séance d'information le mardi 22 mars 2022 à 18h30.**

Cette séance se déroulera **dans l'école de votre enfant et durera une petite heure.**

Nom et prénom : ....

Parent(s) de : ....

Participera ou ne participera pas à la réunion d'information (biffer la mention inutile).

Vous ne pourrez pas être présent ?

Vous pouvez m'envoyer vos questions par mail à l'adresse a.groslambert@goodplanet.be. J'y donnerai suite aussi rapidement que possible.
Vous pouvez également contacter l'école ainsi que l'enseignant(e) de votre enfant pour toute information complémentaire.

Pour GoodPlanet,
Antoine Groslambert

More detailed information about the research + timeline (7 pages)

B@SEBALL

Projet B@seball :
« Biodiversity at school environments benefits for all » -
La biodiversité en milieu scolaire profite à tous

A. Contexte et objectifs

Dans notre monde de plus en plus urbanisé, les problèmes de santé chroniques associés à la vie urbaine sont en augmentation. D'autre part, nous savons que le contact avec la biodiversité est important pour la santé humaine, en particulier pendant l'enfance.
Malgré les preuves de plus en plus nombreuses que les personnes ayant un microbiome (communauté de micro-organismes) diversifié ou qui interagissent avec des espaces verts, jouissent d'une meilleure santé, les études n'ont pas encore prouvé directement comment les espaces verts biodiversifiés pourraient modifier le microbiome humain et réduire les maladies chroniques.

Le microbiome représente tous les micro-organismes hébergés par un être humain (peau, intestin, muqueuses.....). Dans le corps humain, on retrouve 10 fois plus de cellules appartenant au microbiome que de cellules humaines !
Le microbiome et son hôte (nous) vivent en symbiose et cette relation est bénéfique pour les deux partenaires : le microbiome trouve un milieu de vie favorable en/sur nous et joue un rôle essentiel dans notre santé et bien-être. Le microbiome joue, entre autre, un rôle dans la digestion et le système immunitaire, mais il existe encore bien d'autres interactions que nous ne connaissons pas.

Dans ce contexte, le projet B@seball étudiera la biodiversité dans et autour d'écoles primaires pour évaluer comment la présence d'un microbiome varié peut être bénéfique pour :

- la santé physique : résilience de l'enfant (moins d'allergies, meilleur système immunitaire,...)
- et le bien-être des enfants (meilleure concentration, réduction du stress et de l'anxiété, émotions positives...).

Et comment cela peut-il être lié à la diversité du microbiome humain ?

Par cette étude, nous aimerions conduire à plus d'égalité entre les enfants. Chaque enfant allant à l'école, s'il est en contact avec la nature, aura de ce fait un meilleur mélange microbiologique et une meilleure santé.

En plus de cela, une cour de récréation plus diversifiée conduit à d'autres avantages :

- Une diversité de jeux pour les enfants qui développent différentes capacités et où les talents personnels sont stimulés ;
- La stimulation de la créativité et des compétences de collaboration : jouer ensemble ;
- Au développement des capacités motrices des enfants : une cours de récré dynamique ;
- Au développement de l'autonomie des enfants et de leur confiance en eux ;
- L'éducation à l'environnement et une sensibilité à la nature environnante.

Collecting data from interested pre-engaged schools (2 pages)

B@SEBALL

Votre école a exprimé son intérêt pour la participation à l'étude scientifique B@seball

Et nous en sommes très heureux !
Votre école est en phase de présélection. Afin de nous assurer que votre établissement scolaire répond bien aux critères scientifiques, nous avons besoin de quelques **informations supplémentaires (voir points 1 et 2)**.

1) **Informations sur la classe intéressée (5e) qui réalisera l'étude au cours de l'année 2021-2022. Cette étude se prolongera jusqu'en 2023, lorsque ce groupe sera en 6e.**

Veuillez nous renvoyer **les cadres ci-dessous à l'adresse a.groslambert@goodplanet.be**

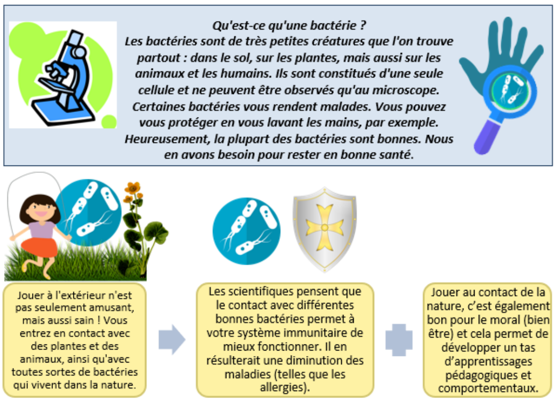
Ecole : ....
Adresse : ....

Classe 5 ....
Nombre d'élèves dans cette classe : ....

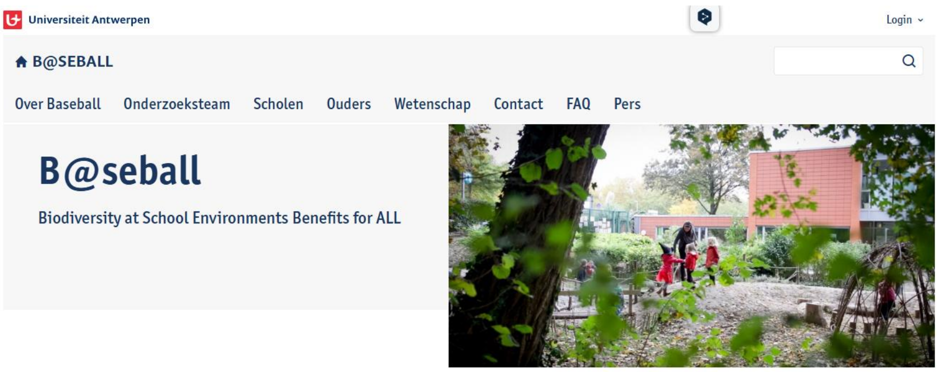
Nom de l'instituteur(trice) ou du superviseur : ....
Mail : ....
Tel : ....

Année scolaire 2021-2022	Nom de l'instituteur(rice)	Mail
Enseignant de 5ème année		
Enseignant de 6ème année		

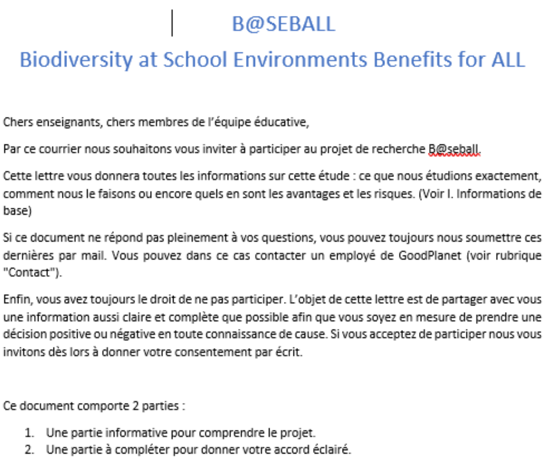
PWP presentation for children (for teachers who accepted to participate) (15 slides)



B@SEBALL Website(for extra information and educational tools)



Consent form (for teachers and parents => there was also a simplified version when suspicion of language barrier) (10 pages)



B@SEBALL

Biodiversity at School Environments Benefits for ALL

Chers enseignants, chers membres de l'équipe éducative,

Par ce courrier nous souhaitons vous inviter à participer au projet de recherche B@seball.

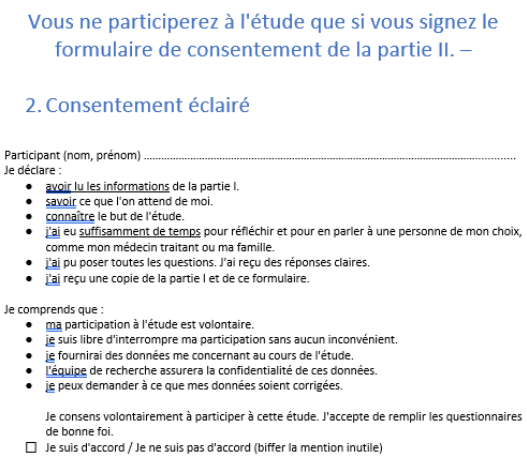
Cette lettre vous donnera toutes les informations sur cette étude : ce que nous étudions exactement, comment nous le faisons ou encore quels en sont les avantages et les risques. (Voir I. Informations de base)

Si ce document ne répond pas pleinement à vos questions, vous pouvez toujours nous soumettre ces dernières par mail. Vous pouvez dans ce cas contacter un employé de GoodPlanet (voir rubrique "Contact").

Enfin, vous avez toujours le droit de ne pas participer. L'objet de cette lettre est de partager avec vous une information aussi claire et complète que possible afin que vous soyez en mesure de prendre une décision positive ou négative en toute connaissance de cause. Si vous acceptez de participer nous vous invitons dès lors à donner votre consentement par écrit.

Ce document comporte 2 parties :

1. Une partie informative pour comprendre le projet.
2. Une partie à compléter pour donner votre accord éclairé.



Vous ne participerez à l'étude que si vous signez le formulaire de consentement de la partie II. –

2. Consentement éclairé

Participant (nom, prénom) ....
Je déclare :

- avoir lu les informations de la partie I.
- savoir ce que l'on attend de moi.
- connaître le but de l'étude.
- j'ai eu suffisamment de temps pour réfléchir et pour en parler à une personne de mon choix, comme mon médecin traitant ou ma famille.
- j'ai pu poser toutes les questions. J'ai reçu des réponses claires.
- j'ai reçu une copie de la partie I et de ce formulaire.

Je comprends que :

- ma participation à l'étude est volontaire.
- je suis libre d'interrompre ma participation sans aucun inconvénient.
- je fournirai des données me concernant au cours de l'étude.
- l'équipe de recherche assurera la confidentialité de ces données.
- je peux demander à ce que mes données soient corrigées.

Je consens volontairement à participer à cette étude. J'accepte de remplir les questionnaires de bonne foi.

☐ Je suis d'accord / Je ne suis pas d'accord (biffer la mention inutile)

Planning for swab test.

	8H45 - 12H	13H - 15H15	Namur	Liège	Hainaut	Luxembourg	Bruxelles
Lundi 2 mai			**Ecole communale fondamentale YVOIR** **082/ 61 12 26** Place du monument, 10 5530 Dir: Mme Rothelin/ Instit: Mme Bouille 18 élèves - **10 prick/ 9 cheek swab** HORAIRE:	**Ecole communale de TIEGE** **087/47 47 80** Tiège 81 4845 Sart-Lez-Spa (Jalhay) Dir: Mme Soquette/ Instit: Mme Hubert 14 élèves - ... prick/swab HORAIRE:	**Ecole fondamentale libre St François HARCHIES** **0475 / 515 . 513** Rue buissonet, 22 7321 Dir: M. Dusoulier/ Instit: Mme Desmettre 31 élèves - ... prick/swab HORAIRE:	**Ecole communale fondamentale de RENDEUX** **084 47 81 00** Rue de la roche, 22 6987 Dir: M. Boutay / Instit: M. Paquay 22 élèves - ... prick/swab HORAIRE:	**Ecole communale fondamentale l'Emeraude MOLENBEEK ST JEAN N°2** **02 / 421 10 90** Rue le Lorrain, 94 1080 Dir: Mme Schallon / Instit: Van den weghe 22 élèves - ... prick/swab HORAIRE:
Mardi 3 mai			**Ecole communale fondamentale Profondeville 2 BOIS DE VILLERS** **081/43 22 80 - 0479/18.78.36** Rue Jules Borbouse, 68 5170 Dir: Mme Jumet/ Instit: Mme vancaster et Mme Doria 26 élèves - **13 prick/ 11 cheek swab** HORAIRE:	**Ecole communale de Sart SART-LEZ-SPA** **087/47 54 89** Rue de l'école, 10 4845 Jalhay Dir: Mme Grégoire/ Instit: Mme Piqueray 26 élèves - **14 prick/ 14 cheek swab** HORAIRE:	**Ecole communale fondamentale de BLATON** **069-57 75 83** Rue de écoles, 26 7321 **(!! 2 implantations !!)** Dir: Mme Thaon/ Instit: Mme Brebant et Mme Lestrade 25 élèves (12 PS) - ... prick/swab HORAIRE:	**Ecole fondamentale libre GRAND HALLEUX** **080 21 78 40** Rue sculpteur Vinçotte, 16 6698 Dir. et instit: Mme van der Meer 16 élèves - **12 prick/12 cheek swab** HORAIRE:	**Ecole fondamentale annexée AR Leonardo Da Vinci ANDERLECHT** **0473 39 56 58** **1070** Rue Léopold Swaef 27 Dir: Mme Duron / Instit: Mauquoy 20 élèves - ... prick/swab HORAIRE:
Mercredi 4 mai			**Ecole communale fondamentale de BIEVRE** **061 51 20 25** Rue d'Houdremont 2 5555 Dir: M. Balfroid / Instit: Mme Lambot 18 élèves - ... prick/swab HORAIRE:	**Ecole communale fondamentale de POLLEUR** **087 / 22.67.31** Place Eugène Cornesse, 85 4910 Dir: M. Pungur / Instit: Mme Halin 18 élèves - **11 prick/ 12 swab** HORAIRE:	**Ecole fondamentale St François d'Assise HAUTRAGE** **065 62 19 81 / 0470 26 79 31** Place d'Hautrage, 6 7334 Dir: Mme Huant / Instit: M. Dereux 18 élèves - **11 prick/ 12 cheek swab** HORAIRE:	**Ecole communale fondamentale de LIBIN** 0475 42 29 54 Ruelle des Messes 160 6890 Dir: M. Boulard / Instit: Mme Elen et Mme ? 15 élèves (PS) classe mixte - ... prick/swab HORAIRE:	**Ecole primaire libre Saint-Charles MOLENBEEK ST JEAN** **02 410 65 73** **1080** Rue Léopold Swaef 27 Dir: Mme Rousseau / Instit: Mme Dooms 18 élèves - ... prick/swab HORAIRE:
	Ecole communale fondamentale Profondeville 2 BOIS DE VILLERS **081/43 22 80 - 0479/18.78.36** Rue Jules Borbouse, 68 5170 Dir: Mme Jumet/ Instit: Mme vancaster et			**Ecole fondamentale annexée MALMEDY** **080 / 791 125 / 0499 538 934** Route de Falize, 21 4960 Dir: ? / Instit: Mme Linze	**Ecole fondamentale institut St Joseph LA LOUVIERE** Rue Gustave Boël, 55 **7100** Dir: M. Solbreux / Instit: Mme Verlaet		**Ecole fondamentale libre Institut des Ursulines KOEKELBERG** **02/411.85.48** Boulevard Léopold II 268 1081

2.2 Communication documents

Certificates for participating children and for the participating groups

This instruction letter was sent to the schools as an attempt to collect the missing questionnaires

B@SEBALL

B@seball - Instructies voor leerkrachten om oudervragenlijsten te verzamelen

Wij verontschuldigen ons voor de vertraging bij het versturen van deze enveloppe. We hebben enkele logistieke problemen ondervonden.

Zoals u weet, hebben wij van een aantal ouders de ingevulde vragenlijsten nog niet ontvangen. Deze vragenlijsten zijn essentieel voor het succes van het onderzoek. Ons laatste redmiddel is daarom nogmaals een beroep op u te doen om de ontbrekende vragenlijsten op te halen.

Om u daarbij te helpen, hebben wij u verschillende artikelen gestuurd:

- Deze instructiekaart ;
- Een lijst van leerlingen waarvan de ouders de vragenlijst nog niet hebben ingevuld (er kunnen typefouten in de namen staan);
- Een gefrankeerde envelop gericht aan Michael Leone.

Instructies voor de distributie en inzameling van vragenlijsten :

- De vragenlijsten moeten worden overhandigd aan de ouders van de leerlingen op de verstrekte lijst;
- Het invullen van de vragenlijst duurt 5 tot maximaal 10 minuten;
- De leerkracht verzamelt alle vragenlijsten en stopt ze in de grote envelop (met adres en postzegels) en verstuurt deze naar ons.

Hartelijk dank!

Nog vragen?
a.wuyts@goodplanet.be

B@SEBALL "Nieuwsbrief"/ Le journal de B@SEBALL (sent in both languages to maintain contact with the schools and send information on the progress) => there were 2 journals (8 pages in total)

B@SEBALL

BIODIVERSITY AT SCHOOL ENVIRONMENT BENEFITS FOR ALL

JANVIER 2021 - 2023 PAGE 1

Le journal des scientifiques en herbe!

LA RECHERCHE B@SEBALL, C'EST QUOI ?

Plus de 500 enfants belges de 5e et 6e primaires ont récolté des échantillons de biodiversité dans leur cour de récréation. 36 écoles, recouvrant l'ensemble du territoire belge, ont accepté de jouer le jeu.

Cette recherche vise à déterminer s'il existe un lien entre la présence de biodiversité à l'école et la santé des enfants. Découvrez les premiers résultats de cette enquête passionnante !

Un partenariat entre UA, UCLouvain, Sciensano, INBO, MOS et GoodPlanet.

DES ÉCOLES AUX PORTRAITS (BIO)DIVERSIFIÉS

Pour assurer la fiabilité des résultats, les partenaires sur ce projet ont sélectionné des écoles présentant **des contextes environnementaux variés.**

Des écoles rurales avec des cours minérales ou encore des écoles urbaines dans un cadre verdoyant; les analyses tiennent compte des réalités diverses de notre pays.

Blottie dans un contexte très urbanisé, la section fondamentale de l'Institut Saint-Joseph à La Louvière met une belle parcelle verte à disposition des enfants. (www.oselevert.be)

Deux écoles coexistant dans un contexte similaire (rural ou urbain), peuvent abriter des environnements très différents.

PAGINA 3

BIODIVERSITEIT OP MIJN SPEELPLAATS

Uitgerust met educatief materiaal en illustraties onderzochten de kinderen de **aanwezigheid van planten** op het schooldomein. Op die manier konden kinderen planten observeren en identificeren.

In het spoor van planten (Libin School).

Onderzoek op Libin School (Provincie Luxemburg).

De kinderen waren verwonderd over de omgeving die hen elke dag omringt.

EEN FOCUS OP DE ONDERZOEKS KIT; DIE DOOR HET UCL-TEAM NAAR SCHOLEN WORDT GESTUURD :

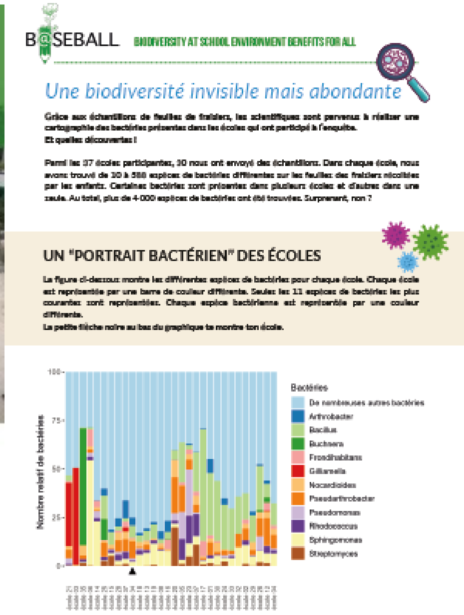
ILLUSTRATIES OM DE VERSCHILLENDE SOORTEN SUBSTRATEN IN DE TUIN TE BEPALEN.

EEN LUCHTFOTO VAN DE SCHOOL, IN GROOT FORMAAT.

VRAGENLIJSTEN EN ILLUSTRATIES OM PLANTEN TE IDENTIFICEREN.

EEN THERMO/HYGROMETER OM DE VOCHTIGHEID EN DE TEMPERATUUR IN HET KLASLOKAAL TE METEN.

A third communication was sent in June 2024. It contained some results about bacterias.
30 schools received their own bacterial profile. (5 pages)



B@SEBALL BIODIVERSITY AT SCHOOL ENVIRONMENT BENEFITS FOR ALL

Une biodiversité invisible mais abondante

Grâce aux échantillons de feuilles de fraisiers, les scientifiques sont parvenus à réaliser une cartographie des bactéries présentes dans les écoles qui ont participé à l'enquête.
Et quelles découvertes !

Parmi les 37 écoles participantes, 30 nous ont envoyé des échantillons. Dans chaque école, nous avons trouvé de 30 à 588 espèces de bactéries différentes sur les feuilles des fraisiers récoltées par les enfants. Certaines bactéries sont présentes dans plusieurs écoles et d'autres dans une seule. Au total, plus de 4 000 espèces de bactéries ont été trouvées. Surprenant, non ?

UN "PORTRAIT BACTÉRIEN" DES ÉCOLES

La figure ci-dessous montre les différentes espèces de bactéries pour chaque école. Chaque école est représentée par une barre de couleur différente. Seules les 11 espèces de bactéries les plus courantes sont représentées. Chaque espèce bactérienne est représentée par une couleur différente.
La petite flèche noire au bas du graphique te montre ton école.

The last communication includes the process and the methodology of the research + the results that may be of interest for the schools. (10 pages)

B@SEBALL BIODIVERSITY AT SCHOOL ENVIRONMENTS BENEFITS FOR ALL

Onderzoek in Belgische scholen!

Meer dan 500 kinderen van het 5e & 6e leerjaar namen deel aan dit onderzoek over de aanwezigheid van biodiversiteit op school. 37 scholen, verspreid over heel België, stemden in hieraan mee te doen.

Het doel van dit onderzoek is om na te gaan of er een verband bestaat tussen de aanwezigheid van biodiversiteit op scholen en de gezondheid van kinderen. Daarnaast bekijken we of groen op school de gezondheidsongelijkheid tussen kinderen kan helpen compenseren. De kinderen hebben hiervoor stalen genomen op hun speelplaats, planten geïdentificeerd en verschillende vragenlijsten beantwoord over hun gezondheid en speelgewoonten.

BIODIVERSI... WATTE!?

Biodiversiteit verwijst naar de verzameling aan verschillende soorten levende wezens: planten, dieren en micro-organismen zoals bacteriën. Hoe meer verschillende soorten, hoe beter de natuur bestand is tegen veranderingen zoals klimaatverandering, ziektes en plagen. Denk maar aan een blokkentoren: hoe meer verschillende blokken (soorten) je hebt, hoe steviger de toren staat. Er zijn 3 niveaus van biodiversiteit:

De wetenschappelijke methode

1. Elk wetenschappelijk onderzoek begint met een vraagstelling. Onze **onderzoeksvraag** gaat als volgt: 'Beïnvloedt de aanwezigheid van biodiversiteit op scholen de gezondheid van kinderen?'
2. Wetenschappers maken veronderstellingen om deze vraag te beantwoorden. We noemen dit hypothesen. Onze **hypothese** klinkt als volgt: 'Biodiversiteit in scholen heeft een positieve invloed op de lichamelijke en geestelijke gezondheid van kinderen.'
3. Hierna worden gegevens verzameld om de hypothese te onderzoeken. We noemen dit **gegevensverzameling**. In deze studie werden stalen genomen en vragenlijsten verzameld op de deelnemende scholen.
4. Alle verzamelde gegevens worden daarna **geanalyseerd** en **geïnterpreteerd**. Op basis hiervan wordt de hypothese bevestigd of weerlegd.
5. Ten slotte wordt er een **conclusie**, of besluit, getrokken uit deze geanalyseerde gegevens. In dit document kan je alle conclusies van onze studie lezen!

B@SEBALL BIODIVERSITY AT SCHOOL ENVIRONMENTS BENEFITS FOR ALL

Streptomyces

Deze bacteriën leven meestal in de bodem en zijn belangrijk om afgevallen bladeren en dode planten af te breken. Ze kunnen ook antibiotica produceren, bv. streptomycine, die we gebruiken tegen ziekmakende bacteriën.

Pseudomonas

Deze bacteriën zijn heel divers. Sommige leven op mensen en kunnen zelfs gevaarlijk zijn als je al een slechte gezondheid hebt. Maar veel van deze bacteriën helpen planten door ziekmakende micro-organismen te bestrijden.

Sphingomonas

Dit is een groep bacteriesoorten die verschillende dingen kunnen. Sommige kunnen vervuilende stoffen eten en afbreken. Andere moedigen planten aan om te groeien en beschermen ze tegen bacteriën die de plant ziek zouden maken. Ze komen voor op bladeren.

Wat vertellen deze resultaten ons?

Een duidelijke conclusie die we kunnen trekken, is dat de waargenomen bacteriën heel verschillend zijn van school tot school. Alle scholen samen hielpen om vele types bacteriën in kaart te brengen, zowel op de schoolspeelplaats, als op de wangen van de kinderen. Dit is belangrijk omdat een grote biodiversiteit nodig is om de leefomgeving, zelfs in steden, gezond en sterk te houden.

Een ander interessant resultaat is dat de aardbeiplanten een grotere variatie aan bacteriën herbergden wanneer het schoolplein groener en gevarieerder was.

© Ose le vert, recrée ta cour

Een aromatische spiraal biedt een toevluchtsoord voor kleine fauna.

© Ose le vert, recrée ta cour

Een mooie natuurlijke vijver die de biodiversiteit verwelkomt.

Je kan dus de biodiversiteit zelf verhogen door een grotere verscheidenheid aan ecosystemen te creëren zoals een natuurlijke vijver, stapels stenen of hout, bloeiende weiden, landelijke heggen, enz. Zo verhoog je de diversiteit aan planten en dieren en help je ook de bacteriële biodiversiteit een handje.

Er worden minder astma en allergieën gerapporteerd bij kinderen in scholen met bepaalde bacteriën op de speelplaats. Als kinderen meer in de natuur of met natuurlijke elementen spelen dan zien we deze natuurlijke bacteriën terug op hun wangen. Er is dus een transfer van de omgevingsbacteriën naar de wang van de leerlingen.