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Abstract

Background The influence of outdoor green space on microbial communities indoors has scarcely been investigated. Here, we study the associations between nearby residential green space and residential indoor microbiota.

Methods We collected settled dust from 176 living rooms of participants of the ENVIR*ON*AGE birth cohort. We performed 16S and ITS amplicon sequencing, and quantitative PCR measurements of total bacterial and fungal loads to calculate bacterial and fungal diversity measures (Chao1 richness, Shannon and Simpson diversity indices) and relative abundance of individual taxa. Green spaces were estimated within 50m and 100m buffers around the residential address. We defined total residential green space using high-resolution land-cover data, further stratified in low-growing (height<3m) and high-growing green (height>3m). We used land-use data to calculate the residential nature. We ran linear regression models, adjusting for confounders and other potential determinants. Results are expressed as units change for an interquartile range (IQR) increase in residential green space and their 95% confidence intervals (CI).

Results After adjustment, we observed statistically significant associations between the indoor microbial diversity indices and nearby residential green space. For bacteria, the Shannon index was directly associated with residential nature (e.g. 0.08 units increase (CI:0.02,0.13) per IQR increase in nature within a 50m buffer). Fungal diversity was directly associated with high-growing residential green and inversely with low-growing green. For example, an IQR increase in high-growing green within a 50m buffer was associated with increases in 0.14 (CI:0.01,0.27) and 0.02 (CI:0.008,0.04) units in the Shannon and Simpson indices, respectively.

Conclusions Nearby green space determines the diversity of indoor environment microbiota, and the type of green differently impacts bacterial and fungal diversity. Further research is needed to investigate in more detail possible microbial taxa compositions underlying the observed changes in indoor microbiota diversity and to explore their contribution to beneficial health effects associated with green space exposure.

Keywords Bacteria, Fungi, indoor environment, built environment, green space.

1. Introduction

Increasing scientific evidence suggest that residential green space is generally beneficial to health. For example, living close to green spaces is associated with lower mortality rates.[1] However, the mechanisms hypothesized to explain the observed associations between green spaces and health are, in most cases, yet to be studied. Among others, environmental microbiota is a hypothesized mechanism involved in the relationships between green spaces and health.[2-7] In particular, the environmental microbiota may partly explain the inconsistent associations observed for the exposure to green spaces in relation to the development of allergic and respiratory diseases.[8] The urban environments are characterized by having less green space compared to the rural environments, but also lower microbial diversity. According the hygiene hypothesis, one of the factors explaining the growing prevalence of allergic diseases in highly urbanized areas is the indoor environmental microbiota.[9] Early life exposure to diverse microbial environments is important for the development of the immune system,[10] and several studies provide evidence for reduced allergy and asthma development when exposed to higher indoor microbial diversity.[11-17]

To better understand the role of the indoor microbiota in the relationships between green spaces and health, we need to increase our insight on how the outdoor environment shapes the indoor microbial communities. Previous research investigating the determinants of the indoor microbiota has focused on features of the indoor environment (e.g. building type, ventilation, crowdedness, or pet ownership).[18-21] However, the indoor microbial community is not a closed system and thus factors related to the residential outdoor environment may be important determinants of the indoor microbial habitat. Previous multicenter studies have reported a geographical variation in indoor fungal communities and in microbial agents like endotoxins, both on global and regional scale. They show geographical location is a stronger determinant than indoor determinants.[22-28] This suggests that factors related to the area outdoor environment, like green spaces, may be strong determinants of the characteristics of the indoor microbial environment. To date, only one study has considered green spaces as a potential determinant of the indoor microbiota. They report statistically significant

associations of surrounding greenness only with fungal diversity indices, but not with bacteria.[29] Here, we present a study within the Belgian ENVIR*ON*AGE (ENVIRonmental influence ON early AGEing) birth cohort where we investigate the nearby residential green space as a potential determinant of the indoor microbiota.

2. Methods

2.1 Study design and population

ENVIR*ON*AGE is an ongoing Belgian birth cohort that recruits mother-newborn pairs at delivery in the East-Limburg Hospital (Genk, Belgium).[30] Mothers are asked to sign the informed consent right before birth and receive a questionnaire to get information about their socio-demographic and lifestyle characteristics. When the child reaches the age of four, mother and child are invited to participate in the follow-up phase, in which anthropometric, cardiovascular and cognitive information is collected and questionnaires on lifestyle, diet and behavior are administered. The cohort participants, both at birth as well as at follow-up, are considered to be representative for the population in Flanders and are spatially distributed within the province of Limburg (Flanders, Belgium), including urban, suburban and rural areas with municipalities ranging in population density from 82 to 743 inhabitants/km^2.[30, 31] The study protocol was approved by the ethical committee of the Hasselt University, and complied with the Helsinki Declaration. More detailed information is provided elsewhere.[30]

In the present study, we included only homes of children that had already participated in the four-year follow-up up to one year before the house dust sampling, or that were going to participate within one year after the sampling. Further inclusion criteria were not moving to another home between the home visit and follow-up phase and no major indoor renovations occurring during the sampling period. We restricted the sampling period to spring months (April to June) to reduce the impact of seasonal variation[32], which resulted in sample collection being carried out in two phases: spring 2017 and spring 2018. In total, we identified 284 eligible households of which we were able to contact 233

mothers of children living in the selected households and whom we asked to participate in a home visit that included dust sampling and a questionnaire on indoor factors. Overall, 189 mothers accepted to participate, resulting in a participation rate of 81%. Due to logistic reasons we were not able to collect the Petri dishes for eight households. Two house dust samples were excluded because of irregularities during the sampling, two other samples because of insufficient amount of dust, and one sample was excluded because it exceeded the standardized number of sampling days. In total, we included dust measurements and green spaces information of 176 households.

2.2 Dust sampling, processing and DNA extraction.

Settled dust was collected passively using two sterile, open-faced Petri dishes (92x16mm) over an period of minimum 4 and maximum 9 weeks (median: 42 days) in spring 2017 and spring 2018. The Petri dishes were located at approximately two meters above floor level and a safe distance from major air flows in the household's living room.[33] Upon collection, all Petri dishes were readily sealed and stored at -20°C and eventually further processed in the summer of 2018.

In this dust processing step, each Petri dish was transferred to room temperature for a minimum of 30 minutes and a maximum of 60 minutes prior to thoroughly swabbing both petri dishes – first bottom, then lid - from one sampling location using a sterile cotton tip wettened in sterilized water + 0.05% Tween 20. The cotton tip containing the settled dust was cut into a DNA extraction tube containing glass beads and samples were transferred to − 20 °C. Samples were shipped frozen on dry ice to the Finnish Institute for Health and Welfare (Kuopio, Finland), where DNA extraction was carried out, as described in detail in the supplemental material. DNA was stored at − 20 °C until sequencing.

2.3 ITS and 16S PCR, fungal and bacterial amplicon sequencing

The DNA extracted from dust and control samples was shipped frozen to the sequencing service partner LGC Genomics (Germany), who did the library preparation and sequencing. The V4 region of the bacterial 16S rRNA gene was amplified using 515F/806R primers.[34] For fungi, the ITS1 region of

the Internal Transcribed Spacer (ITS) was amplified using ITS1F/ITS2 primers.[35] The PCR procedure is explained in more depth in the supplemental material. .

Sequencing was performed on an Illumina MiSeq with V3 chemistry resulting in paired-end reads with a length of 300 bp each. The libraries were demultiplexed and all sequence reads processed with custom Python v2.7.6 scripts to sort them by sample, removing barcode and amplicon primer sequences. Adapter sequences were removed from the 3' end of reads with a proprietary script discarding reads shorter than 100 bp.

2.4 Sequence processing and bioinformatics analyses

16S and ITS amplicon data was analyzed by standard dada2 pipeline version 1.8.[36] The pipeline inputted the forward R1 and reverse reads R2 in fastq format files and processed the data with removal of bad quality reads based on the read quality profile. Then, an ASV table with sequence count was constructed after filtering, trimming and de-replication of reads, as described in more detail in the supplemental material. Taxonomy was assigned using SILVA[37] database version 132 for bacteria and UNITE database version 7.2 for fungi[38]. For bacterial data, the phylogenetic tree was constructed in QIIME (Quantitative Insights Into Microbial Ecology) version 1.9.1[39] with the ASV sequences for the tree based diversity calculations. The downstream post processing included removal of singletons, mitochondria, eukaryote and chloroplast ASVs. In order to flag and remove potential contaminants from the sequence datasets, we included sequencing of 14 negative and reagent controls from DNA extraction (alongside bacterial and fungal mock communities), as well as DNA extracts of four Petri dish blank samples. Using decontam package version 1.2[67], a total of 81 bacterial ASVs were removed prior to downstream analyses (Supplemental Table 1). In the fungal data, four ASVs detected in samples occurred the 14 negative controls, however, only sporadically, no more than once, and none of those four ASVs were identified in any of the four Petri dish blanks (Supplemental Table 2). For these reasons, these ASVs were not considered true contaminants and were kept in the dataset. Samples with less than 1000 sequences (n=13 samples for bacteria and 19

samples for fungi, respectively) as well as mock communities were excluded from downstream analyses.

The alpha diversity within samples, including Chao1, Shannon and Simpson metrics, were calculated in QIIME (Quantitative Insights Into Microbial Ecology) software version 1.9.1[39], applying rarefaction values of 1495 sequences for bacteria and 3956 sequences for fungi, respectively. Chao1 is an estimator for the number of species within a sample, also referred to as species richness. The other two alpha diversity metrics, Simpson index, and Shannon index integrate richness and the species evenness, described as the homogeneity of species abundance but in varying degrees. Simpson puts more emphasis on evenness, whereas the Shannon index is more sensitive to richness.[40]
Relative taxa abundances were calculated using the obtained numbers of sequence reads per ASV normalized for the total number of sequence reads within a given sample. Taxa summaries were created summarizing sequence variants (ASVs) based on their taxonomic assignment to species and genus level, respectively.

2.5 Quantitative (qPCR) PCR analysis

The total Gram-positive and Gram-negative as well as fungal loads in the settled dust samples were determined via quantitative PCR (qPCR), as described in detail in the supplement. We calculated the numbers of microbial cell equivalents (CE) in the samples using relative quantification, utilizing the internal standard to adjust for presence of DNA inhibitors and/or variability in DNA extraction efficiency.[41] Results were normalized for sampling surface area and sample accumulation duration, expressed as CE per m^2 settling surface area per day, referred to hereafter as microbial load.

2.6 Residential green space

Residential addresses of the households were geocoded and green spaces around the residential address were calculated using the Geographic Information System (GIS) ArcGIS 10 software. We used

five measures of green spaces (overall green space, low-growing green, high-growing green and nature) within two radius distances around the residential address (50 m and 100 m). Overall green space was estimated based on the Green Map of Flanders (GF) 2012 (Agency for Geographic Information Flanders, AGIV).[42] This is a map using land cover data, which reflects green space in Flanders based on physical characteristics of the landscape. The land cover data is established on segment-based classification using satellite flight ortho-photos from 2012 commissioned by the Agency for Nature and Forest (ANB). The overall green space variable, including all non-agricultural vegetation, was further divided into low-growing green (i.e. all vegetation less than 3 m in height) and high-growing green (i.e. all vegetation more than 3 m in height). To estimate exposure to nature we used the Land-use Map of Flanders (LF) 2013, with a medium spatial resolution of 10x10 m.[43] This map is based on land use data, focusing on functional characteristics of the landscape of Flanders. The data contains information about the actual use of the ground cover and is divided into 22 land use classes. We created a new artificial classification "nature" that is defined as the sum of the proportions of the following 10 LF classes: thickets and bushes; poplars; deciduous, coniferous and alluvial forests, semi-natural grassland, heath, swamp, coastal dune and bay mud (Supplemental Table 3). However, none of the households included in the ENVIRONAGE birth cohort are surrounded by the last two LF classes and thus those do not contribute to the nature variable.

2.7 Covariables

Information on indoor factors such as pet ownership, ventilation system and sampling days was obtained by means of a questionnaire. In the case of six individuals who did not finish the questionnaire during the house visits, we obtained this information using the same questions asked at the follow-up phase. Pet ownership was further divided into having a furry pet or not, defined as having a cat, dog, rabbit, hamster or guinea pig. The type of ventilation system was dichotomized into the use of passive ventilation or other (i.e mechanically supported ventilation). The average outdoor temperature (°C) was provided by the Belgian Royal Meteorological Institute and was calculated as

the daily mean temperatures during the sampling period at a representative measuring station (Diepenbeek, Belgium). Black carbon (BC) concentrations (μg/m^3) were obtained using data from the Belgian telemetric air quality networks at fixed monitoring stations[44] to calculate daily interpolated exposure values during the sampling period using a spatiotemporal interpolation method.[45] This dispersion model provides high-resolution concentration values and the validation statistics explained more than 74 % of the temporal and spatial variability for BC in the Flemish Region of Belgium.[46]

2.8 Statistical analysis

2.8.1 Alpha diversity measures and normalized total microbial concentration

For the statistical analyses of the alpha-diversity measures and normalized total microbial load we used the R environment.[47] In total dust samples of 176 households were included in the analyses. We screened for outliers using a threshold of more or less than three times the standard deviation away from the mean and detected three outliers for the bacterial Simpson diversity index and two outliers for the fungal Simpson diversity index. The results concerning the total microbial concentrations were screened for outliers as described before and we detected 2 outliers for the gram-negative bacterial load, 5 outliers for the gram-positive bacterial, and 3 outliers for the fungal load. These concentrations were then log-transformed (base 10) to better comply with linear model assumptions. To investigate the relationship between the microbial indices and residential green space we ran multivariable-adjusted linear regression models adjusting for *a* priori chosen covariables including the number of sampling days and the average outdoor temperature during sampling period. Furthermore, we considered BC concentrations during the sampling period, furry pet ownership and use of passive ventilation as potential confounders and added them to the model. Results are expressed as units change for an interquartile range (IQR) increase in residential green space and their 95% confidence intervals (CI).

In a sensitivity analysis, we examined whether main findings remained after adjusting for outdoor airborne particulate matter concentrations smaller than 2.5 μm ($PM_{2.5}$), instead of BC

concentrations. Furthermore, we also investigated the associations between the indoor microbial communities and green space exposure within larger buffers (300 m , 500 m, and 1000 m) surrounding the homes.

2.8.2 Relative taxa abundances

We used ANCOM v2.1[48] for the identification of bacterial and fungal taxa (ASVs, species, genera) associated with green space indicators.[49] The analysis was initiated with pre-processing of the non-rarefied absolute abundance taxa tables to deal with different types of zeros[50] and to exclude taxa that are rare (prevalence of < 10%) across samples. For the ANCOM main function we used the pre-processed taxa tables and transformed continuous green space variables and covariables into tertiles (in the case of the land-use variable nature we used 0, <median, >= median). The analyses were adjusted for the same covariables as previously mentioned for the alpha diversity measures. Results presented are differentially abundant taxa at cutoff 0.7 as of the ANCOM main function.

3. Results

In Table 1 we provide a summary of the household characteristics, outdoor environment within 50 and 100 m buffers and indoor microbial indices. Almost half of the homes had furry pets and 82% used passive ventilation. The sampling period for dust samples ranged from 4 to 9 weeks (median=6 weeks). During this period, the median (25^{th}-75^{th} percentile) daily average outdoor temperature was 17 (15-18) °C and households had a median (25^{th}-75^{th} percentile) outdoor airborne black carbon concentration of 0.73 (0.62-0.86) μg/m^3. Overall, households had a higher percentage of low-growing green than high-growing green in their surroundings, in all buffers considered (Supplementary Table 4). We observed that the number of households with residential nature was rather low (75% had less than 6% nature in a 50 m buffer, and 25% have less than 9% nature in a 1000 m buffer).

Table 1. Description of the household characteristics, indoor microbiota and outdoor environment (within 50 m and 100 m radius) surrounding the household.

	n (%)	min	p25	p50	p75	max
Household characteristics						
Having furry pets, yes	83 (47.2)					
Use of passive ventilation, yes	142 (80.7)					
Period of sampling (days)	176	29	40	42	46	64
Average outdoor temperature over sampling period (°C)	176	13.2	15.0	17.4	18.4	19.4
Average ambient airborne black carbon concentration during sampling period (µg/m3)	176	0.43	0.62	0.73	0.86	1.14
Outdoor environment						
Total green (%)						
50 m buffer	176	6.4	36	49	62	92
100 m buffer	176	11	41	50	62	81
Low-growing green (%)						
50 m buffer	176	4.0	27	36	45	68
100 m buffer	176	4.3	28	35	42	67
High-growing green (%)						
50 m buffer	176	0.0	2.8	7.7	17	75
100 m buffer	176	0.8	6.7	11	20	68
Nature (%)						
50 m buffer	176	0.0	0.0	0.0	6.0	64
100 m buffer	176	0.0	0.0	2.9	12	51
Indoor microbiota						
Bacteria						
Chao1 richness	176	111	297	407	510	774
Shannon diversity index	176	2.36	6.58	7.12	7.63	8.57
Simpson diversity index	174	0.83	0.97	0.98	0.99	1.00
Gram-negative bacterial load (CE/m^2/day)	174	9.68	148	288	483	2787
Gram-positive bacterial load (CE/m^2/day)	171	0.44	81.9	154	266	1011
Fungi						
Chao1 estimates ASV richness	176	24.3	91.8	130	174	300
Shannon diversity index	176	0.66	2.71	3.40	4.05	5.57
Simpson diversity index	175	0.52	0.69	0.76	0.82	0.94
Fungal load (CE/m^2/day)	173	0.15	20.3	33.7	62.7	212

The correlations between the green spaces variables, in all buffers, are presented in the online supplement (Supplemental Table 5).

Regarding the microbiota measured in indoor dust, bacterial diversity indices had higher values than fungal diversity indices, and the median load of gram-positive bacteria in house dust was higher than that of gram-negative bacteria and that of fungi. Considering the relative taxa abundances of the household samples (Figure 1), we notice that the sum of the 15 most prominent bacterial genera accounts about half (44%) of the total bacterial taxa abundance in house dust, whereas this is almost 90% for the fungal taxa abundance. This is a consequence of the large abundances of the two most prominent fungal genera *Epicoccum* and *Mycosphaerella* (23% and 40% respectively), whereas the

relative abundances of the more dominant bacterial taxa only range from 1% (*Nocardiodes*) to 9% (*Staphylococcus*).

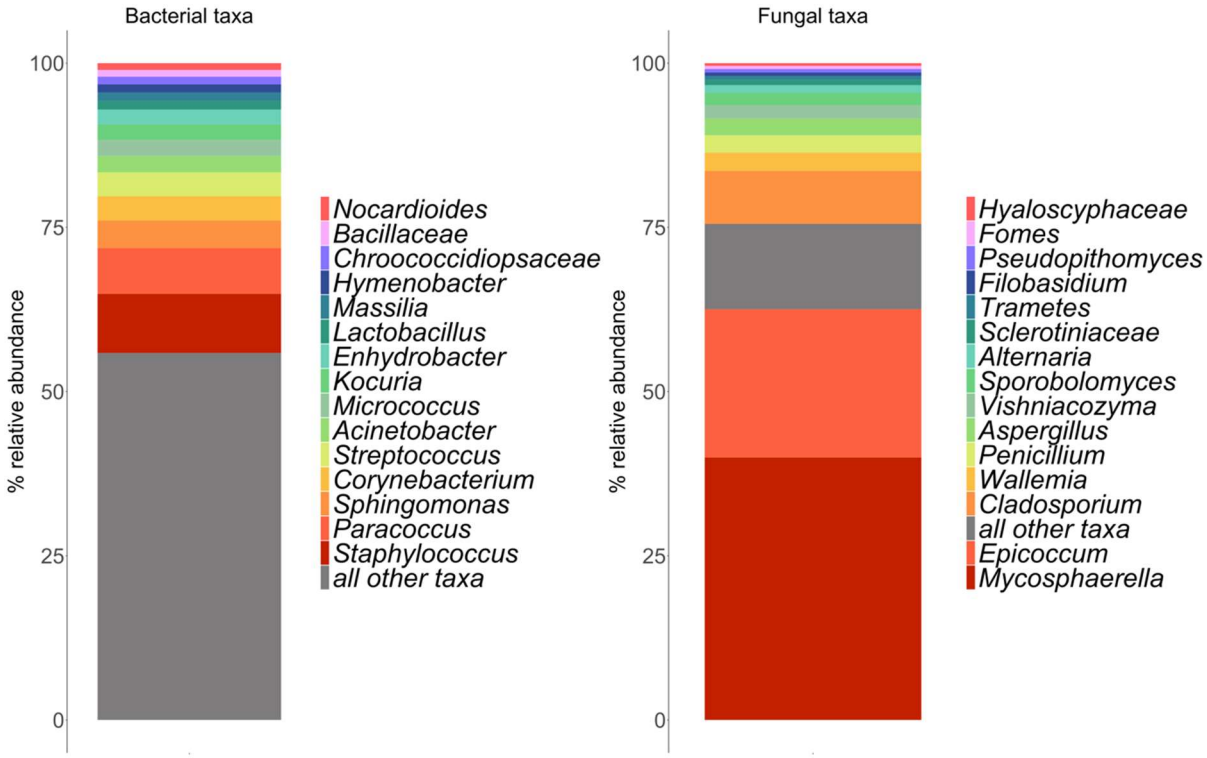

Figure 1. Overview of the relative abundances of the 15 most abundant bacterial and fungal genera in relation to all other taxa in the average household sample. The 15 bacterial and fungal genera are in increasing order from top to bottom.

Additionally, it is worth noting that the three microbial community richness and diversity measurements are generally positively and strongly correlated with each other, both for bacteria and fungi (Figure 2). The weakest correlations were observed between the Simpson and the Chao1 indices (Spearman's rho of 0.53 for bacteria and 0.56 for Fungi). Figure 2 shows the correlation matrix of the alpha diversity indices, microbial load, and residential green spaces within 50 m and 100 m buffers. The values for the correlation coefficients (rho), including all buffers are shown in the supplementary material (Supplementary Table 6). Overall, we observe weak correlations between the diversity indices and bacterial and fungal loads with residential green spaces (Spearman's rho ranging from -0.19 to 0.22). The strongest inverse correlations were observed for the fungal Shannon index with low-growing green in a 50 m buffer and Gram-positive bacterial load with nature and high-growing green. The strongest positive correlations in the smallest buffers (50 and 100 m) were observed between the bacterial Shannon and Simpson indices and high-growing green and nature, and between

the fungal Shannon and Simpson indices with high-growing green. Bacterial loads in house dust were inversely correlated with bacterial diversity indices, as are fungal load with fungal diversity indices.

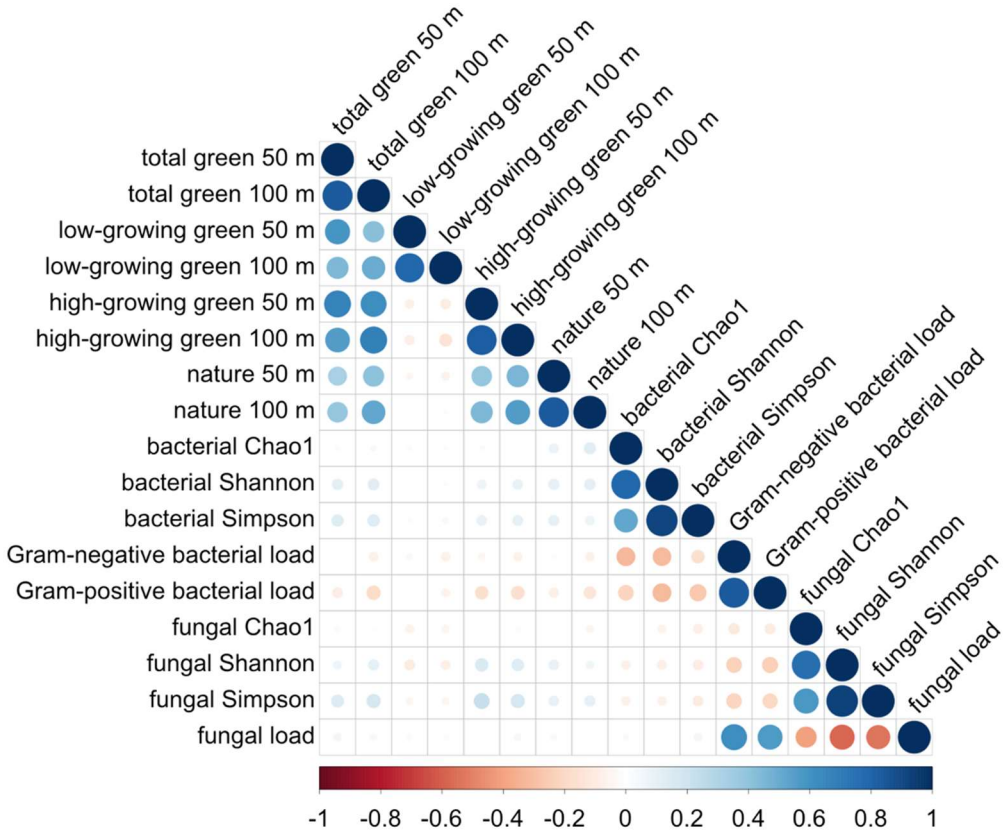

Figure 2. Spearman correlation matrix between green space variables (total green, low and high-growing green and nature) in 50 and 100 m buffers surrounding the residential address and indoor bacterial and fungal indices (Chao1 richness estimate, Shannon and Simpson diversity indices). Additionally, we included total fungal load , and Gram-positive and Gram-negative bacterial load.

After adjustment for covariables, similar trends were observed for bacteria (Table 2) and for fungi (Table 3). For bacteria (Table 2), the Gram-positive and Gram-negative bacterial loads were mostly inversely associated with residential green space, while diversity measures were mostly positively associated. However, only the positive associations between bacterial diversity indices and the nature variable were statistically significant within buffers close to the residential address (50 and 100 m). Within a 50 m buffer, an IQR increase in nature was associated with an increase of 0.08 (95% CI: 0.02 to 0.13) and 9.57 (95% CI: 0.4 to 18.7) units of the bacterial Shannon diversity index and the Chao1 taxa richness, respectively. We found the same positive association in a 100 m buffer, but that was only statistically significant for the bacterial Shannon diversity index. Here, an interquartile increase

of nature was associated with an increase of 0.15 (95% CI: 0.02 to 0.28). Neither Gram-positive nor Gram-negative bacterial loads were significantly associated with the nature variable.

Table 2 Overview of the bacterial indices (Chao1 richness estimate, Shannon and Simpson diversity indices) and Gram-negative and Gram-positive bacterial load in association with residential green space variables (total green, low-growing and high-growing green and nature) within nearby buffers (50 m and 100 m) surrounding the residential address. Models were adjusted for number of sampling days, average outdoor temperature and ambient airborne BC concentrations during the sampling period, furry pet ownership and use of passive ventilation. Results are expressed as unit change [95 % confidence interval] for an IQR increment in residential green space (estimates presented in bold are statistically significant $p < 0.05$).

	Chao1	Shannon	Simpson	Gram-negative bacterial load	Gram-positive bacterial load
Total green					
50 m buffer	3.8678[-26.9576;34.6933]	0.1079[-0.0791;0.2948]	0.0013[-0.0034;0.006]	0.0039[-0.085;0.0928]	-0.0602[-0.1542;0.0338]
100 m buffer	7.6235[-23.3737;38.6207]	0.1232[-0.0647;0.3111]	0.0028[-0.002;0.0075]	-0.0241[-0.1149;0.0667]	-0.0858[-0.1809;0.0093]
Low-growing green					
50 m buffer	-0.472[-29.4161;28.4721]	-0.0206[-0.1968;0.1556]	-0.0014[-0.0058;0.003]	0.0209[-0.0625;0.1043]	-0.0073[-0.0949;0.0803]
100 m buffer	0.267[-26.1781;26.7121]	-0.0338[-0.1947;0.1271]	0.0004[-0.0039;0.0042]	-0.0114[-0.0889;0.066]	-0.0210[-0.1000;0.0581]
High-growing green					
50 m buffer	3.734[-18.4666;25.9347]	0.1095[-0.0247;0.2436]	0.0022[-0.0012;0.0055]	-0.0115[-0.0755;0.0525]	-0.0467[-0.1134;0.0201]
100 m buffer	6.0061[-16.3665;28.3787]	0.1251[-0.0099;0.2601]	0.0021[-0.0013;0.0056]	-0.011[-0.0759;0.0539]	-0.0536[-0.1224;0.0151]
Nature					
50 m buffer	**9.5711[0.4069;18.7353]**	**0.0764[0.0211;0.1317]**	0.0011[-4e-04;0.0025]	0.0037[-0.0238;0.0312]	-0.0071[-0.0384;0.0242]
100 m buffer	20.5292[-1.1363;42.1947]	**0.1474[0.016;0.2788]**	0.0014[-0.0019;0.0048]	-0.0099[-0.073;0.0532]	-0.0372[-0.1034;0.0289]

Finally, the results of the analysis of bacterial and fungal genera versus green space indicators via ANCOM showed similar positive associations between relative bacterial taxa abundances and the green space indicators, as observed for the diversity measures (Figure 3). At increasing nature within a 50 m buffer we identified the bacterial genus *Acidothermus* to be significantly increased in relative abundance. We found the same genus significantly increased within a 100 m buffer, accompanied by genera *Kineococcus* and *Novosphingobium.* We also identified the cyanobacterial genus *Calothrix* to be significantly more abundant with an increase of low-growing green within a 50 m buffer.

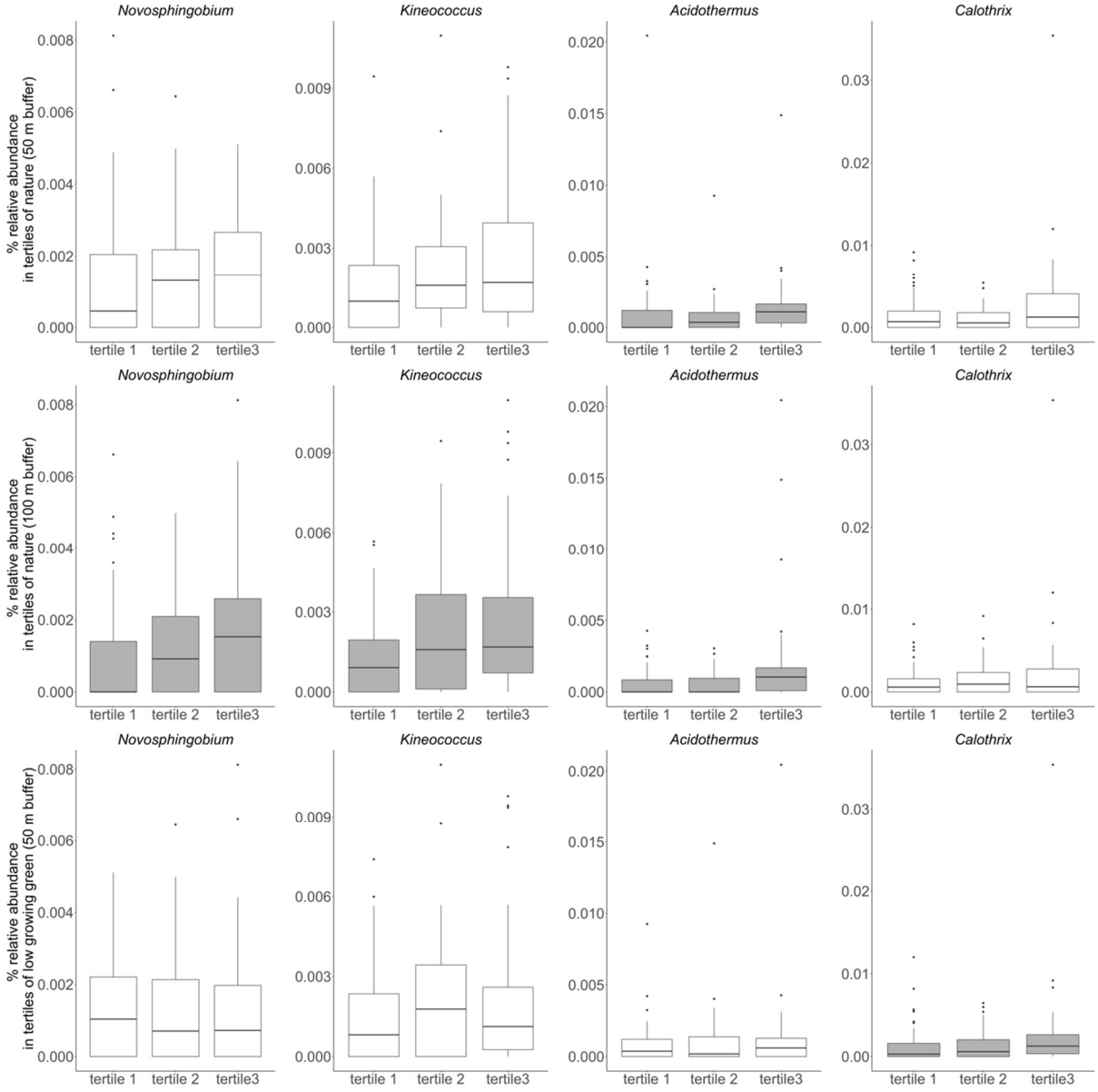

Figure 3. Boxplots (25th, 50th, 75th percentile) of relevant relative bacterial taxa abundances (*Novosphingobium, Kineococcus, Acidothermus* and *Calothrix)* identified as differentially abundant taxa at cutoff 0.7 as of the ANCOM main function associated with green space variables (nature within 50 m and 100 m buffer and low-growing green within 50 m buffer) stratified into tertiles. Models were adjusted for number of sampling days, average outdoor temperature and ambient

airborne BC concentrations during the sampling period, furry pet ownership and use of passive ventilation. Results are expressed as units change for an IQR increment in residential green space. Boxplots filled in grey indicate statistically significant differences between tertiles ($p<0.05$).

For fungi (Table 3), we found statistically significant positive, associations between fungal diversity, but not richness or total fungal load, and high-growing green and nature indices, and inverse associations between fungal diversity and low-growing green. Within a 50 m buffer, an IQR increase in high-growing green was associated with an increase of 0.02 (95% CI: 0.009 to 0.03) and 0.14 (95% CI: 0.01 to 0.27) units of fungal Simpson and Shannon diversity indices, respectively. The corresponding estimates for the 100 m buffer were 0.02 (95% CI: 0.09 to 0.03) for fungal Simpson and 0.14 (95% CI: 0.012 to 0.27) for Shannon diversity indices. Additionally, we found a significant positive association between fungal Simpson diversity and the nature variable, but only within a 100 m buffer. We found an increase of 0.01 (94% CI: 0.0004;0.03) units of fungal Simpson diversity with an IQR increase in residential nature. Regarding the inverse associations with low green, within a 50 m buffer an interquartile increase in low-growing green was associated with a decrease of 0.02 (95% CI: -0.04 to -0.001) and 0.2 (95% CI: -0.39 to -0.06) units of fungal Simpson and Shannon diversity indices, respectively. We found the same association within a 100 m buffer but found only significant decrease of 0.17 (95% CI: -0.32 to -0.02) for the fungal Shannon diversity index.

Neither fungal richness nor the total fungal load in house dust were significantly associated with any green space indicator.

Table 3 Overview of the fungal indices (Chao1 richness estimate, Shannon and Simpson diversity indices) and total fungal load in association with residential green space variables (total green, low-growing and high-growing green and nature) within nearby buffers (50 m and 100 m) surrounding the residential address. Models were adjusted for number of sampling days, average outdoor temperature and ambient airborne BC concentrations during the sampling period, furry pet ownership and use of passive ventilation. Results are expressed as unit change [95 % confidence interval] for an IQR increment in residential green space (estimates presented in bold are statistically significant $p < 0.05$).

	Chao1	Shannon	Simpson	Fungal load
Total green				
50 m buffer	-4.7623[-15.9445;6.4198]	-0.0247[-0.205;0.1555]	0.0099[-0.0085;0.0283]	0.0263[-0.0639;0.1166]
100 m buffer	-3.5815[-14.8419;7.6789]	0.0126[-0.1688;0.1940]	0.0165[-0.002;0.0350]	-0.0043[-0.0961;0.0875]
Low-growing green				
50 m buffer	-7.6855[-18.1409;2.7699]	**-0.2266[-0.3924;-0.0608]**	**-0.0182[-0.0354;0.0009]**	0.0208[-0.0631;0.1046]
100 m buffer	-6.0846[-15.6519;3.4827]	**-0.1696[-0.3222;-0.0171]**	-0.0109[-0.0263;0.0044]	-0.0125[-0.0899;0.0649]
High-growing green				
50 m buffer	1.3204[-6.7483;9.3891]	**0.1405[0.0124;0.2687]**	**0.0214[0.0086;0.0342]**	0.0078[-0.0569;0.0725]
100 m buffer	1.5615[-6.5728;9.6958]	**0.135[0.0057;0.2644]**	**0.0211[0.0083;0.034]**	0.0057[-0.0593;0.0707]
Nature				
50 m buffer	-0.7072[-4.0772;2.6627]	0.022[-0.0321;0.0762]	0.0019[-0.0039;0.0077]	0.0184[-0.0098;0.0465]
100 m buffer	-2.6045[-10.5488;5.3398]	0.0724[-0.0552;0.1999]	**0.0133[0.0004;0.0262]**	0.0185[-0.0454;0.0824]

Regarding relative taxa abundances, the same green space indicators were significantly associated with relative abundance of specific fungal taxa (Figure 4). Within a 50 m buffer of high-growing green the fungal *Rhodosporidiobolus* genus, as well as on species level *Rhodosporidiobolus colostri* and *Didymella boeremae* were significantly more abundant. With an increase of low-growing green in a 50 m buffer, the fungal *Epicoccum* and *Pseudopithomyces* genera, as well as on species level *Epicoccum nigrum*, *Pseudopithomyces* sp. and *Neoascochyta paspali*, were significantly more abundant. We found no clear associations between the nature variable and the fungal genera.

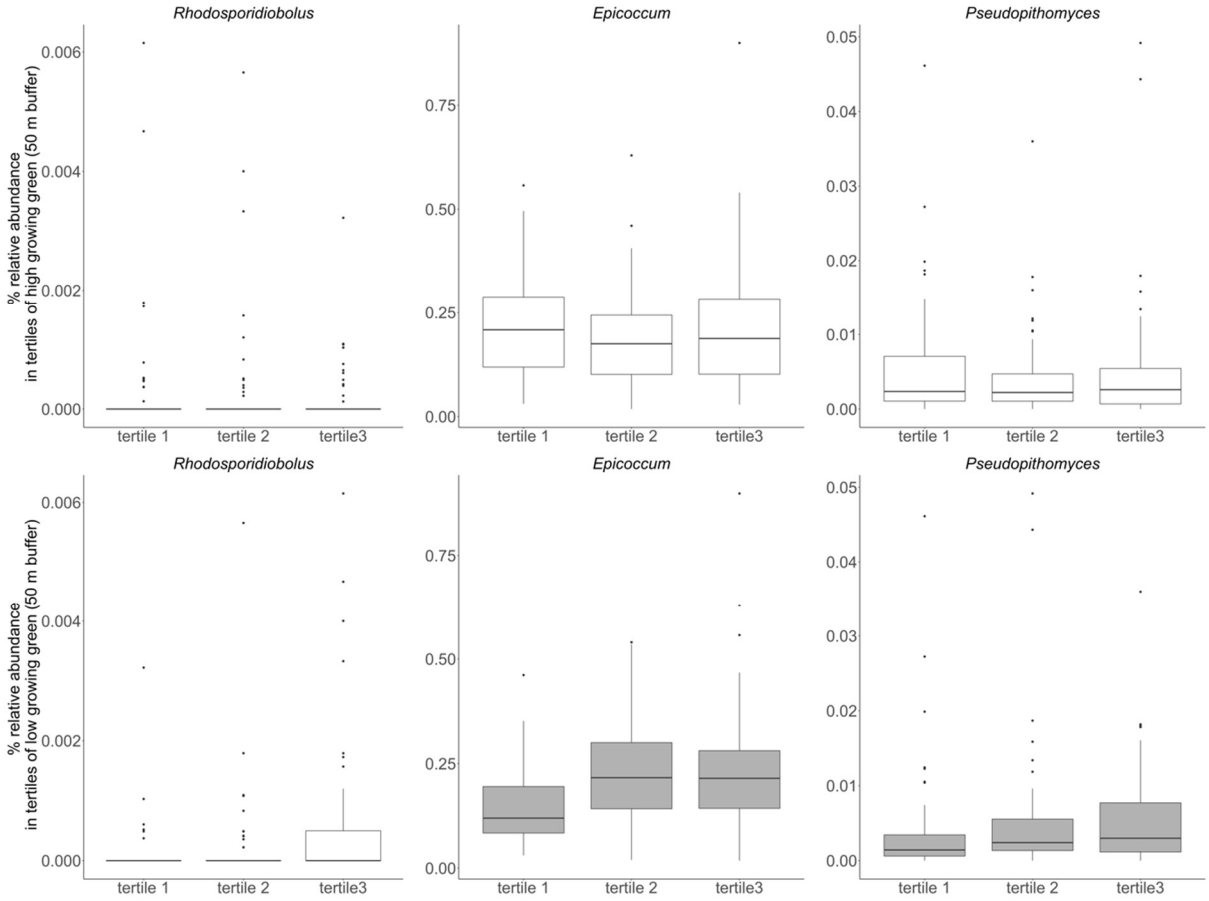

Figure 4. Boxplots (25th, 50th, 75th percentile) of relevant relative fungal taxa abundances (*Rhodosporidiobolus*,*Epicoccum* and *Pseudopithomyces*) identified as differentially abundant taxa at cutoff 0.7 as of the ANCOM main function associated with green space variables (nature within 50 m and 100 m buffer and low-growing green within 50 m buffer) stratified into tertiles. Models were adjusted for number of sampling days, average outdoor temperature and ambient airborne BC concentrations during the sampling period, furry pet ownership and use of passive ventilation. Results are expressed as units change for an IQR increment in residential green space. Boxplots filled in grey indicate statistically significant difference between tertiles ($p<0.05$).

In sensitivity analysis, adjustment of the models by outdoor concentrations of $PM_{2.5}$ instead of BC concentrations did not substantially change the estimated associations between the indoor microbial communities and the green spaces variables (Supplemental Table 7 and Supplemental Table 8). Furthermore evaluation of these associations using larger buffers for the green spaces variables (300 m, 500 m, and 1000 m) revealed no additional statistical significant associations, the increase in buffer resulted in estimates closer to the null (Supplemental Table 9 and Supplemental Table 10).

4. Discussion

In our study, we show that the amount and type of green space in the immediate surroundings, partly determines the residential indoor microbial communities. Indoor fungal and bacterial diversity are differentially impacted by outdoor environmental characteristics. Whereas the bacterial community responds solely to differences in overall residential nature, the fungal community is also affected by residential low-growing and high-growing green. These findings are comparable with results of previous studies describing bacterial communities to be commonly less responsive to outdoor characteristics compared to fungi.[26, 29] However, to date, most studies assessing determinants of the indoor microbiota describe associations with indoor factors. Only the study conducted by Weikl et al.[29] in the south of Germany explored associations of bacteria and fungi Simpson and Shannon indices and satellite data on vegetation, including the Normalized Difference Vegetation Index. They only observed statistically significant associations for fungal diversity with surrounding greenery within 100m buffer around the residential address. No significant associations were reported for Bacterial diversity in relation to their measures of green spaces.

Interestingly, we find notable associations with residential nature. The observed association with residential nature but not GV-derived green space might be explained by the difference in the type of data used to construct these green space indices. Land-use variables, such as residential nature, only consider land suitable for a specific use, signifying larger areas of connected green space and more related to qualitative green space. In contrast, land-cover variables can quantify and classify green

spaces at high resolution into low or high-growing green. Our findings suggest that the indoor bacterial community may be less sensitive to small-scale changes in the residential green but might be influenced by larger areas of qualitative green space. Apart from these spatial differences, there are also biochemical differences. Larger areas of green spaces are typically associated with certain edaphic properties, whereas the soil profile of smaller patches of urban green alongside roads and neighborhoods is different from these native ecosystems.[51-53] When looking more in-depth into the type of green spaces that we classified as nature and their distribution within this variable, we notice that thickets, forests, and semi-natural grasslands contribute the most. Interestingly, previous studies investigated the bacterial community's driving factors present in the soil of forests and grasslands and found that these edaphic parameters, such as pH levels and soil texture, are key determinants in soil bacterial diversity in forests and grasslands.[54]

For the fungal communities within residential homes, we observe that they are shaped differently depending on the type of green space surrounding the residence. We showed a lower indoor fungal diversity when the surrounding vegetation is ground-level, and a higher indoor fungal diversity when the residence is surrounded by vegetation above 3 meters in height within close proximity (50 m and 100 m). These results suggest that the presence of neighboring trees is a determining factor of indoor fungal diversity. This might also explain the observed positive association between fungal diversity and residential nature. As previously described, we notice a moderate to strong positive correlation between residential nature and high-growing green, which is likely due to the additional categorization of high-growing green as one of the several forest-type land-use variables comprising a significant portion of the nature variable. This provides more evidence that the fungal communities seems to be driven by the presence of neighboring trees. This is consistent with the knowledge that plants are significant contributors to the airborne fungi[55] and that the indoor fungal composition largely reflects the outdoor fungal communities.[23, 56] Our results also suggest that there is a difference in airborne fungal diversity and/or dispersal between different vegetation types in the surrounding environment.

As of today, several studies have investigated the impact of deforestation and forest management on fungal diversity. Both deforestation as well as forest thinning can decrease fungal diversity in several biological samples, including fungi derived from soils and plants.[57-59] Additionally, higher fungal diversity was reported in forests with more canopy cover and tree diversity.[58-60] One study specifically reported higher arbuscular mycorrhiza diversity and species richness in forests compared to grasslands.[61] Although low-growing green might include trees below 3 meters in height, these are most likely juvenile trees. Considering the fact that there are several phenological differences between juvenile and mature trees, in addition to observing a positive correlation of fungal diversity with mature trees and aged forests might account for the fact that trees below 3 meters in height do not contribute much to the airborne fungal diversity.[58, 62, 63]

Moving from more general indoor microbial community characteristics such as taxa richness and diversity to differences in abundance of specific bacterial and fungal taxa, we identified only few taxa that were associated with residential green space. This may in part be due to the rather stringent preprocessing of microbiota data excluding rare taxa, i.e. with a prevalence of less than 10% across samples, which in turn is a recommended approach to avoid reporting chance findings in the occurrence of rare taxa. We focused on taxa that were positively associated with green space and found that the majority of the bacterial taxa that increase in indoor relative abundance were associated with residential nature except for the cyanobacterial genus *Calothrix*, which was associated with low-growing green. Other bacterial genera that were more abundant in households surrounded by increased nature, include the genera *Novosphingobium, Kineococcus* and *Acidothermus.*

For fungal taxa we found that their relative abundance in house dust increased with an elevated level of low and high-growing green in the surrounding of the home. This observation is in line with our findings for fungal richness and diversity measures. Considering that we cannot determine the exact origin of the observed fungal taxa, we will not attempt to explain the difference between low and high-growing green as we did with fungal diversity. We can, however, clarify their overall observed association with residential green space. The identified fungal taxa, *Epicoccum* genus and *Epicoccum*

nigrum species should be highlighted as highly abundant in house dust samples in this study and as being strongly associated with low-growing green in the surrounding. It is a ubiquitous saprophyte involved in the decay of organic material and is frequently isolated from soils and plants.[64, 65] Similarly, *Rhodosporidiobolus*, a yeast genus, and *Pseudopithomyces* are often associated with leaves and soil, and thus their association with residential green space is well conceivable.[66, 67]

There are a number of limitations in our study that we want to acknowledge. The sample size was rather small (less than 200 households/samples were included). This could have limited the statistical power to detect statistically significant associations. Nevertheless, we were able to observe statistically significant associations between several diversity indices and residential green space, and those that were not statistically significant, were also closer to the null. In addition, we inspected QQ plots and the residuals of the observed associations to validate that the difference in the distribution between residential low-growing and high-growing green did not interfere with our results. Moreover, considering we obtain similar results before and after adjustment for covariables indicates that we do not have a strong confounders in our models, which also supports robustness in the observed associations. Furthermore, although we included several variables of green spaces and indoor microbiota, we did not adjust for multiple comparisons. The present study tested on a *a priori* hypothesis involving strongly correlated residential green space exposures and microbial indices. Therefore, the different exposure windows and outcomes do not provide a completely independent opportunity for a type I error. Instead of adjusting for multiple comparisons, we based the interpretation of our findings on their consistencies among our different measures of green spaces and our hypothesized mechanisms. Another limitation of our study is that we did not have more detailed information on the specific characteristics of the surrounding green, such as vegetational and biochemical variation. However, by sampling only during the spring months, we eliminate the potential effect of seasonal variations that interfere with the interpretation of biotic factors. Apart from these restrictions, we provide novel evidence that the type of residential green space can be an important factor contributing to shaping the indoor microbial communities. The fact that this

influence is measurable within only relatively close range of the households should provide actionable information to local policy makers as well as to residents.

5. Conclusions

Our findings suggest that the type of residential green space close by the residential address determines the indoor dust microbiota, specifically their diversity. Moreover, our results suggest that fungal and bacterial indoor communities might be differently affected by different types of residential green space. The results of this study add knowledge to one of the hypothesized mechanisms in the complex relationship between the characteristics of the built environment and health.

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