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# Indoor green can modify the indoor dust microbial communities
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5 Indoor plants and home microbiota
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45 **ABSTRACT**

46 Little is known about the potential role of indoor plants in shaping the indoor microbiota.
47 Within the ENVIR*ON*AGE birth cohort, we collected settled dust and performed 16S and ITS
48 amplicon sequencing and qPCR measurements to characterize the indoor microbiota,
49 including bacterial and fungal loads and Chao1 richness, Shannon, and Simpson diversity
50 indices. For 155 households we obtained information on the number of indoor plants. We
51 performed linear regression models adjusted for several *a priori* chosen covariables. Overall,
52 an increase in indoor plants and density was associated with increased microbial diversity,
53 but not load. For example, we found an increase of 64 (95%CI:3;125) and 26 (95%CI:4;48)
54 units of bacterial and fungal taxa richness, respectively, in households with more than three
55 plants compared to no plants. Our results support the hypothesis that indoor plants can
56 enrich indoor microbial diversity, while impacts on microbial loads are not obvious.
57 Practical implications:
58 - This research provides the basis for future studies that will clarify the relation between
59 indoor plants and microbiota and explore health relevance of these indoor microbiota
60 modifications through indoor green.
61 - Indoor plants can be used in efforts to enrich and diversify indoor microbial exposure.
62
63 **Keywords** : indoor, built environment, microbiota, indoor plants, diversity

64

65 # Introduction

66

67 The importance of studying indoor air quality becomes apparent when we consider that, in
68 our current society, most of our time is spent indoors.[1] Moreover, most of our indoor
69 activities are within the comfort of our home environment. Therefore, investigating the
70 components of the indoor air and their connection to human health has been a research field
71 of emerging interest in recent years. One such airborne constituent that is of particular
72 interest is the microbial material present in the indoor air. Exposure to the indoor microbial
73 environment has been implicated in both protective and adverse health outcomes[2-10],
74 indicating heterogeneity and complexity of the underlying components and associated
75 mechanisms. Therefore, further investigation is required, including identifying which factors
76 are important in shaping the indoor microbial community, to better understand how these
77 microscopic communities affect our health.

78 Several factors have already been identified as determinants of the indoor microbiota,
79 including elements related to the indoor environment[11-15] as well as outdoor surrounding
80 green spaces.[16-18] Some have hypothesized that indoor green, represented by the presence
81 of indoor plants, might be an important contributor to the indoor microbial composition.[19,
82 20] Indeed, plants have large and exposed surface areas capable of accommodating highly
83 diverse microbial communities.[21-26] Furthermore, their leaves are in constant exchange with
84 the atmosphere, and their root-associated microbes have environmental degrading
85 properties.[27-30] There is, however, a lack of studies investigating the contribution of indoor
86 plants to the indoor microbial content, and the few available studies focus on experimental
87 research, closed and specialized high-density environments.[19, 20, 31, 32] Thus, more
88 comprehensive research is required, in particular including designs representing various real-

life conditions. The objective of our study was to explore whether indoor plants, when considered in the context of complex household environments, contribute to the total quantity and diversity of indoor bacterial and fungal communities.

# 2. Methods

### 2.1 Study design and population

The ENVIR*ON*AGE (ENVIRonmental Influence *ON* AGEing in early life) birth cohort is an ongoing longitudinal study initiated in 2010, that recruits mother-newborn pairs at delivery in the East-Limburg Hospital (Genk, Belgium). This cohort was designed to investigate interactions of environmental exposures with molecular targets of ageing, molecular signature in early life and clinical outcomes in childhood including cardiovascular and cognitive function. Further information regarding the recruitment process and eligibility criteria is provided elsewhere.[33] When the child reaches the age of four, mother and child are asked to participate in the follow-up phase, where we assess various individual health outcomes and collect information on lifestyle characteristics. The study protocol was approved by the ethical committee of the Hasselt University, and complied with the Helsinki Declaration.[33] We asked a subset of the mother-child pairs in the ENVIR*ON*AGE birth cohort to participate in an additional study between 2017 and 2018. This study required home visits, in order to collect settled dust samples, from which we measured indoor microbial communities, and questionnaires to obtain information on indoor characteristics, including the number of indoor plants. More specifically, we selected households of mother-child pairs that already participated in the follow-up study up to one year prior to the home visit or had planned follow-up in the near future. Additionally, we only included participants that did not

111 have planned indoor renovations during the sampling period and lived in the same home as
112 when they participated or would participate in the follow-up phase.
113 In total, we were able to contact 233 mothers of the 284 eligible households. Overall, 189
114 mothers accepted to participate, resulting in a participation rate of 81%. Due to logistic
115 constraints, we were not able to collect the Petri dishes for eight households. Of the Petri dish
116 samples of remaining 181 homes, two house dust samples were excluded post collection
117 because of irregularities during the sampling period, two other samples because the amount
118 of dust was too low, and one sample was excluded because it exceeded the maximum
119 sampling period of nine weeks.
120 Of the 176 households with information on the microbial measurements, we had
121 complete information on household characteristics and the number of indoor plants present
122 in the living room for 155 participants. Additionally, we investigated the association between
123 the indoor plant density, described by the number of indoor plants per square meter ($m^2$) of
124 living room, and the microbial indices within a subset of the 128 households. We excluded 27
125 households because we received no information on the average surface area of their living
126 room.
127

128 **2.2 Indoor dust microbial communities**

129 We placed two sterile, open-faced Petri dishes (91x16mm) in the household's living room to
130 collect settled dust over an average period of 6 to 8 weeks. To reduce the impact of seasonal
131 variation, we restricted the sampling period to spring months (April to June)[34], which
132 resulted in house visits being done during two years: spring 2017 and spring 2018. The Petri
133 dishes were placed distant from major air flows and at an average height of two meters.[35]
134 Upon collection, the Petri dishes were sealed and stored at -20°C, to be further processed in

135 the summer of 2018 as described in detail elsewhere.[16] After processing, samples were
136 shipped frozen on dry ice to the Finnish Institute for Health and Welfare (Kuopio, Finland),
137 where DNA extraction was conducted, as described in the supplemental material. DNA was
138 stored at −20 °C until sequencing. The DNA extracted from dust samples and empty control
139 petri dishes was shipped frozen to the sequencing service partner LGC Genomics (Germany),
140 who did the library preparation and sequencing. The V4 region of the bacterial 16S rRNA gene
141 was amplified using 515F/806R primers.[36] For fungi, the Internal Transcribed Spacer (ITS)
142 ITS1 region was amplified using ITS1F/ITS2 primers.[37] 16S and ITS amplicon data was
143 analyzed by standard dada2 pipeline version 1.8.[38] The PCR procedure and sequencing
144 protocol, as well as sequence processing and downstream bioinformatics are detailed in the
145 supplemental material and have been described in our earlier publication.[16]
146
147 The alpha diversity within samples, including Chao1, Shannon and Simpson metrics, were
148 calculated in QIIME software version 1.9.1[39], applying rarefaction values of 1495 sequences
149 for bacteria and 3956 sequences for fungi, respectively, to normalize for between sample
150 differences in sequencing depth. The Chao1 metric is an abundance-based estimator of
151 species richness within a sample, using the frequency of rare species detected to infer total
152 species richness. The other two alpha metrics, Shannon and Simpson, utilize the species
153 richness to incorporate it with the species evenness, a measurement of the homogeneity of
154 species abundance but to a different extent. Whereas the Shannon index is more sensitive to
155 species richness, the Simpson index emphasizes species evenness.[40]
156
157 We used quantitative PCR (qPCR) to calculate the total Gram-positive and Gram-negative
158 bacterial loads as well as fungal loads in the settled dust samples, as described in detail in the

159 supplement. We determined the numbers of microbial cell equivalents (CE) in the samples
160 using relative quantification, utilizing the internal standard to adjust for the presence of DNA
161 inhibitors and/or variability in DNA extraction efficiency.[41] Results were normalized for
162 sampling surface area, i.e. surface area of the Petri dishes, and sample accumulation duration,
163 and expressed as CE per $m^2$ settling surface area per day, referred to hereafter as microbial
164 load.
165

166 **2.3 Household plants**

167 Information on the number of indoor plants in the living room and surface area ($m^2$) of the
168 living room was obtained via a questionnaire. The surface area was calculated by the
169 participants. In case they had an open kitchen connected to the living room, the calculations
170 excluded the kitchen area. Because the distribution of the number of indoor plants was
171 skewed, we further stratified this variable into three categories, i.e., having no plants, having
172 one to three plants and having more than three plants. Moreover, this categorization helped
173 us to investigate the microbial communities when comparing households with a lot of plants
174 versus households with none or few plants. Additionally, we calculated the number of plants
175 per $m^2$ of living room and divided this variable into tertiles representing low (0 plants/$m^2$),
176 middle (0.02 -0.06 plants/$m^2$) and high (>0.06 plants/$m^2$) indoor plant density.
177

178 **2.4 Covariables**

179 Information on household characteristics such as the number of household members, having
180 an open kitchen connected to the living room, pet ownership and ventilation system was
181 obtained by means of a questionnaire. We divided pet ownership into having a furry pet,
182 defined as having a cat, dog, rabbit, hamster or guinea pig, or not. The type of ventilation

183 system was separated into the use of passive ventilation or other (i.e mechanically supported
184 ventilation). Previous studies have shown that air pollution can modify the indoor microbiota
185 and plant-associated microbiota[42, 43] , thus we modeled black carbon exposure (μg/m$^3$)
186 concentrations via a spatial temporal interpolation method (kriging)[44] for each household in
187 combination with a dispersion model. The interpolation method uses land-cover data
188 obtained from satellite images (CORINE land-cover data
189 set; http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-clc2006-100-
190 m-version-12-2009) and pollution data collected from a governmental stationary monitoring
191 network (http://www.irceline.be/). Coupled with a dispersion model[45, 46] that uses emissions
192 from point sources and line sources, this model chain provides high-resolution concentration
193 values, and the validation statistics explained more than 74% of the temporal and spatial
194 variability for black carbon in the Flemish Region of Belgium.[47]

195 We obtained information on nature and green space exposure within a 50 m buffer around
196 the household's addresses based on the Land-use Map of Flanders 2012 (Flanders
197 Department of Environment and Spatial Development) and the Green Map of Flanders 2012
198 (Agency for Geographic Information Flanders, AGIV), respectively, using the Geographic
199 Information System (GIS) ArcGIS 10 software. The Land-use Map of Flanders contains
200 functional information about the use of the ground cover and is divided into 22 land use
201 classes. We created a new artificial classification "nature" that is defined as the sum of the
202 proportions of the following 10 classes: thickets and bushes; poplars; deciduous, coniferous
203 and alluvial forests, semi-natural grassland, heath, swamp, coastal dune and bay mud
204 (Supplemental Table 1). However, none of the ENVIR*ON*AGE households are surrounded by
205 coastal dune or bay mud; thus these two classes did not contribute to the nature variable in

206 our study. The Green Map of Flanders contains high-resolution (1x1m) information derived
207 from a segment-based classification using aerial ortho-photographs of 2012. The overall
208 green space area, including all non-agricultural vegetation, was further divided into low-
209 growing green (less than three m in height) and high-growing green (i.e. all vegetation more
210 than three m in height).

211 In addition, we calculated relative taxa abundances on various taxonomic levels using the
212 obtained numbers of sequence reads per amplicon sequence variant (ASV) normalized for the
213 total number of sequence reads within a given sample. Subsequently, we performed a source-
214 tracking analysis to estimate the percentage contribution of human-derived bacteria within
215 the dust samples, by totaling the relative abundance of 11 family-level, human skin and oral
216 cavity indicator taxa within each sample as described earlier by Dunn et al. (2013), further
217 referred to as the human source proxy (HSP) (Supplemental Table 2).[48]

218

219 **2.5 Statistical analysis**

220 For the statistical analyses we used the R environment.[49] In total, dust samples of 155
221 households were included in the analyses. We screened for outliers using a threshold of more
222 or less three times the standard deviation from the mean of the corresponding microbial
223 measures. We detected and removed two outliers for the bacterial and three outliers for the
224 fungal Simpson diversity index, and two outliers for the bacterial Shannon index. For microbial
225 load we detected two outliers for the gram-negative bacterial load, five outliers for the gram-
226 positive bacterial, and two outliers for the fungal load. These microbial loads were then log-
227 transformed (base 10) to better comply with linear model assumptions. To investigate the
228 relationship between the microbial indices and household plants we ran multivariable-

229 adjusted regression models, adjusting for *a priori* chosen covariables including the number of
230 sampling days, the average outdoor temperature during the sampling period, furry pet
231 ownership, ventilation method, ambient black carbon exposure during the sampling period,
232 and having an open kitchen. Results are expressed as unit change [95% confidence interval
233 (CI)] compared to the corresponding reference level. For the log-transformed microbial load
234 measures, we back-transformed the estimates and expressed them as a percentage change
235 (estimates presented in bold are statistically significant $p < 0.05$).
236
237 In an additional analysis, we investigated the relationship between the HSP and indoor plants
238 and ran a multivariable-adjusted regression model, adjusting for the aforementioned
239 covariables.
240 To investigate the association between indoor microbiota and indoor plants, independent
241 from outdoor surrounding green we performed two sensitivity analyses.[16] Here, we
242 additionally adjusted the model for either residential nature or high-growing green exposure
243 in a 50 m buffer surrounding the household.
244

245 # 3. Results

246 Characteristics of the sampling and study homes are provided in Table 1. Indoor settled dust
247 sampling was performed for a minimum of 29 days and a maximum of 64 days (median = 42
248 days). During this period, the median ($25^{th}$-$75^{th}$ percentile) daily average outdoor
249 temperature was 17.1 (14.9-18.4) °C and households had a median ($25^{th}$-$75^{th}$ percentile)
250 outdoor airborne black carbon concentration of 0.71 (0.61-0.87) $\mu g/m^3$. Approximately half
251 of the household had furry pets (49.7%) and the majority used passive ventilation (82.6%).
252 Most of the households had one to three indoor plants in the living room (42.6%), while 34.8%

253 of the households had no indoor plants, and 22.6% had more than three plants. Regarding
254 the microbial communities assessed from indoor dust, bacterial diversity was found to be
255 higher than fungal diversity indices. The microbial richness and diversity metrics were overall
256 positively and strongly correlated with each other, both for bacteria and fungi. In contrast,
257 microbial load was overall negatively correlated with the corresponding bacterial or fungal
258 diversity indices (Supplemental Figure 1).
259
260 In Table 2 we provide an overview of the microbial diversity indices and microbial load per
261 category of indoor plants and plant density without any adjustment. Here, we observed a
262 dose response increase in all included diversity indices with an increasing number of plants
263 and plant density in the household living room, with those increases being partially
264 statistically significant. In contrast, the Gram-negative and Gram-positive bacterial loads in
265 house dust non-significantly decreased with increasing number of plants.
266
267 After adjustment for the *a-priori* selected covariables, we found significant positive
268 associations between bacterial and fungal diversity indices and indoor green, when
269 comparing households with more than three plants present in the living room to households
270 with no plants (Figure 1A-D and Supplemental Table 3). For bacterial and fungal Chao1
271 richness, we found an increase of 64 (95% CI: 3; 125) and an increase of 26 (95% CI: 4; 48)
272 index score units, respectively, in households with more than three plants compared to
273 households with no plants in the living room (Figure 1A). For the bacterial and fungal Shannon
274 diversity, we noticed a statistically non-significant increase of 0.32 (95% CI: -0.01; 0.65) and a
275 statistically significant increase of 0.48 (95% CI: 0.12; 0.83) index score units, respectively,
276 when comparing households with more than three plants versus with no plants (Figure 1B).

277 For the fungal Simpson diversity index, we observed a significant increase of 0.04 (95% CI:
278 0.00; 0.07) units (Figure 1C). In contrast, the microbial load measures were negatively
279 associated with indoor green, although this was not found to be significant (Figure 1D). In
280 addition, none of the microbial measures were found to be significantly associated with the
281 middle indoor plant category (1-3 indoor plants).

282

283 Regarding indoor plant density, we found a statistically significant increase in bacterial Chao1
284 richness (68 units; 95% CI: 8; 129) similar to the analysis with indoor plant numbers, when
285 comparing households with a high indoor plant density compared to households within the
286 lowest category (Figure 1E). The trends for the other bacterial and fungal diversity indices
287 were very similar to the earlier presented results for number of indoor plants, but did not
288 reach statistical significance (Figure 1F-G). In contrast, the microbial load measures were
289 negatively associated with indoor plant density, although this was not found to be significant
290 (Figure 1H). In addition, none of the microbial measures were found to be significant with the
291 middle indoor plant density category.
292 To investigate the relationship between the HSP and the indoor plants we additionally
293 performed regression models adjusting for the aforementioned covariables and found a
294 statistically significant decrease of 4.34% (95% CI: -8.23;-0.45) of HSP in households with more
295 than three plants compared to households with no plants in the living room (Supplemental
296 Table 3).

297

298 To investigate the independence of the association between indoor green, represented by
299 the indoor plants, and indoor microbiota from outdoor green we performed two sensitivity
300 analyses. In the first sensitivity analysis, we additionally adjusted for the residential nature

301 exposure (definitions see methods section) in a 50 m buffer surrounding the household. We
302 observed minimal changes to the originally observed associations (Supplemental Table 4).
303 In the second sensitivity analysis, we additionally adjusted the models for the residential high-
304 growing green (that is vegetation exceeding growth height of three meters) exposure in a 50
305 m buffer surrounding the household. In this analysis, all earlier observed associations
306 remained significant, and some of the earlier borderline non-significant associations became
307 statistically significant, specifically the increase of bacterial Shannon diversity with an
308 increasing number of plants (0.33; 95% CI: 0.00-0.66), and the increase of fungal Shannon
309 diversity with higher indoor plant density (0.37; 95% CI: 0.00- 0.73) (Supplemental Table 5).
310

## 311 5. Discussion

312 Residences are typically characterized by a complex assembly of various indoor microbial
313 determinants. The objective of this study was to investigate whether the presence of indoor
314 plants is a determinant of indoor microbial loads and diversity in household settings. We show
315 here that the presence of indoor plants was positively associated with indoor bacterial and
316 fungal diversity but not indoor microbial loads, in settled dust collected in households located
317 in Belgium.
318 Our primary finding was the increase in bacterial and fungal Chao1 richness and Shannon
319 diversity index in households with more than three indoor plants, compared to households
320 with no plants. Our basic models were adjusted for number of sampling days, average
321 outdoor temperature and ambient airborne black carbon concentrations during the sampling
322 period, furry pet ownership, use of passive ventilation, and having an open kitchen. The
323 observed associations remained significant also after adjusting for number of household

324 members (data not shown) and close-by (50 m) outdoor green exposure, reinforcing the
325 independence of the observed associations between indoor green and the microbial
326 communities found in house dust. A previous experimental study by Mahnert et al. observed
327 bacterial diversity to be increased on surfaces, whereas fungal diversity in the sampled air
328 was found to be decreased.[19] This decrease, however, was reported to be likely due to a
329 decrease in relative humidity over time within the enclosed system, which is not comparable
330 to the conditions of our home environments. Two earlier studies on indoor microbiota in
331 university dormitories in North-China explored associations of indoor microbiota
332 characteristics with having a plant in the room or not and found significantly higher bacterial
333 richness in floor dust, but not airborne settled dust, for dormitories with one or more
334 plants.[31, 32]. No significant differences in bacterial community composition (beta-diversity)
335 were reported in these studies. In contrast to the positive associations with indoor microbial
336 diversity, we did not observe any significant relationship between the bacterial and fungal
337 load measures, i.e. the amounts of microbes, in house dust and indoor green, but rather saw
338 inverse trends for microbial loads and indoor plants. One could expect higher microbial loads
339 in house dust of homes that contain one or more potted plant compared to homes without
340 plants, but this was not the case in our study. Such effect might be more pronounced and
341 measurable in floor dust, where plant and soil particulate matter might represent a more
342 quantitative addition, while the analyses of indoor microbiota in our study was done from
343 airborne settled dust collected well above floor level. This rationale is supported by the
344 above-mentioned experimental study by Mahnert et al. where they found an increase in
345 microbial abundance on surrounding floor and wall surfaces, but no increase was observed in
346 microbial abundance of indoor air.[19] Generally, we found inverse relationship between the
347 diversity and the microbial load measures. This might indicate that higher microbial loads in

348 house dust link to some extent to rather dominant taxa present at high relative abundance,
349 which in turn reduces measured indoor microbial diversity. Supporting this, our analyses
350 revealed significant positive associations between Gram-positive bacterial loads in house dust
351 and the number of household members, as well as the human source proxy, calculated as a
352 sum of eleven human-associated bacterial taxa from the sequencing data (Supplemental
353 table 6). This provides a coherent picture of the well documented effect of human occupants
354 being a major source of bacterial taxa in house dust, such as for example skin-associated
355 *Staphylococcus*, *Corynebacterium* or *Propionibacterium*.[50, 51]

356 Overall, our results indicate that indoor plants, similar to other indoor household members
357 such as humans and pets, can be considered an important dispersal source for microbial
358 communities. Moreover, our results suggest that household with more indoor plants are also
359 characterized by lower proportion of human-sourced bacteria in house dust. In our study, the
360 presence of plants indoors appeared to contribute to a gentle shift of the indoor microbiota
361 from human-sourced towards proportionally more environmentally sourced microbiota,
362 which likely is a consequence of potted plants adding plant and soil associated taxa to indoor
363 microbial communities, leading to a proportional reduction of human-source taxa. This
364 observation is particularly relevant for future research into indoor plant-associated
365 respiratory and allergic health implications, considering the contribution of either
366 environmentally-associated or human-associated taxa to the microbial environment might be
367 differentially associated with health.[31, 52-54] To support that the observed associations are
368 explained by the presence of plants and not behavioral traits connected to keeping indoor
369 plants, we considered additional potential confounding factors such as socio-economic
370 status, urbanicity, and cleaning habits but found minimal changes to the observed

371 associations (data not shown). These results – together with our earlier analyses of the impact
372 of surrounding green space[16]- help us gain insight into the relationship between the indoor
373 microbial content and green, both derived from surrounding green space and indoor plants.
374 The observed association between indoor plants and the indoor microbial diversity can, in
375 part, be explained by the permanently exposed interface of the phyllosphere and other
376 above-ground plant organs capable of accommodating highly diverse microbial
377 communities.[21-26] Besides the extensive communication between above-ground plant
378 compartments and the air, the rhizosphere, represented as the root-soil interface, similarly
379 contains many different microenvironments supporting the growth of highly diverse
380 microbial communities.[55] Moreover, research supports the potential of rhizosphere-related
381 microbial flora, similar to the microbiome of the phyllosphere, to directly or indirectly
382 contribute to the composition of the microbiome in surrounding air.[56, 57]

383 In our analysis, we identified microbial richness-sensitive indices as being associated with
384 indoor plants, whereas we did not observe an association for the amount of microbes. This
385 suggests that sourcing of plant-associated bacteria and fungi into the airborne indoor
386 microbiota appears to be a more subtle addition of bacterial and fungal taxa to the core
387 indoor microbiota, rather than a considerable increase in microbial biomass in indoor
388 airborne dust. Our study was not able to address whether the increase in indoor bacterial and
389 fungal diversity with increasing number of plants was due to merely the increase of leaf and
390 soil surface area interacting with and sourcing microbes into indoor air. An alternative
391 explanation could be the differential assembly of microbial communities in different plant
392 species, resulting in niche microbial habitats accommodating many rare microbial taxa that
393 could then be dispersed into indoor air.[58, 59]. Given that our study did not collect information

394 on size and species of indoor plants, we cannot further speculate on the predominant
395 mechanisms, but it is obvious that more research is needed to investigate the potential
396 mechanism by which indoor green can contribute to the indoor air-associated microbiota.

397 Considering the observed indoor green-related associations pertain to inhalable airborne
398 microbial agents, we hope this study will also motivate future research into potential health
399 implications. Previous research has shown that indoor microbial communities can have both
400 adverse, as well as protective effects on respiratory and allergic health. Visible mold, for
401 example, has been associated with an increased risk of chronic respiratory disease.[5, 9]
402 However, the beneficial qualities of specifically the diversity of indoor microbiota represent
403 the most reproducible finding to date, when considering associations between indoor
404 microbiota and specifically child respiratory health. For example, higher fungal diversity has
405 been associated with a reduced risk of developing sensitization in early childhood, and
406 exposure to a more diverse (farm-like) bacterial environment could protect against
407 development of allergic and atopic diseases.[10, 53, 54, 60, 61] Considering surrounding green
408 space accommodates highly diverse microbial communities, it has been proposed that the
409 microbiota act as an underlying mechanism explaining the observed associations between
410 residential green space and various health outcomes.[62, 63] Consequently, the potential of
411 indoor plants to enrich the indoor microbiota, similarly to our observations to surrounding
412 green space[16], identifies them as a potential, inexpensive and convenient tool to alter the
413 indoor microbiome and, subsequently, building occupant health, ideally with beneficial
414 outcomes. None of the latter can, however, be concluded or implied from the current
415 analysis, but we hope that the herein presented results will motivate and inform future
416 research along this pathway.

To our knowledge, we are the first study to test the hypothesis that indoor plants could be important determinants in shaping the indoor microbial communities in a natural setting, including a wide variety of residential environments. Moreover, we included both indoor-related factors as well as outdoor green in our models, allowing us to better discern the potential of indoor green in shaping the microbial communities of the indoor air. We were able to detect significant changes in the indoor microbial indices with consistent uniform associations while applying multiple models with varying covariates. However, we also acknowledge several limitations of our study. Beyond the number of plants reported by home household members, we had no additional and more specific information on the type and size of the indoor plants present in the living room, determining the actual amount of available vegetal surface area and their specific physical properties. It could be of interest to expand the analyses of the effects of indoor plants on indoor microbiota beyond diversity indices and quantity of microbes towards specific taxa contributions associated with the presence of plants. To make such analysis meaningful, a larger number of homes and more specific information on plant types would have been required, which was the reason for leaving these aspects as subject for future investigations. The location of study homes in the ENVIR*ON*AGE cohort was restricted to areas in Belgium - thus we cannot speculate on the applicability of our study findings to other climatic, geographic and cultural settings in Europe and worldwide. However, we were still able to detect significant changes in the indoor microbial indices with consistent associations while applying multiple models with varying covariables.

# 6. Conclusion

439 This study provides further insights into the complex mechanisms determining the indoor
440 microbial composition, specifically with respect to the contribution of indoor plants to indoor
441 microbial diversity. Our results show that in addition to the earlier documented factors
442 related to building, occupancy and surrounding environment and green spaces, the indoor
443 green, represented by indoor plants, can be considered as an important microbial dispersal
444 source for microbial communities in home environments. Our results suggest that indoor
445 plants could potentially represent an inexpensive and convenient tool to enrich the indoor
446 microbiota, which in turn could have health relevance. However, more research is needed to
447 better understand the specific relation between indoor plants and indoor microbial
448 communities, particularly with respect to shaping indoor microbial composition in health-
449 promoting ways considering also different age groups.

## 450 Conflict of interest

451 The authors report there are no competing interests to declare.

## 452 Author Contribution

453 **YD** was involved in writing the original draft. **YD, KW** and **MV** were involved in the investigation of the
454 study. **YD** and **BJ** performed formal analysis. **YD**, **MT** and **LC** were responsible for the methodology
455 and conceptualization and **TN** and **LC** were responsible for the supervision. All of the authors were
456 involved in writing-review and editing of the manuscript.

## 457 Data availability statement

458 The data that support the findings of this study are available from the corresponding author
459 upon reasonable request.

460

461 **References**

462


463 1. Klepeis, N.E., et al., *The National Human Activity Pattern Survey (NHAPS): a resource*
464 *for assessing exposure to environmental pollutants.* J Expo Anal Environ Epidemiol,
465 2001. **11**(3): p. 231-52.
466 2. Bonamichi-Santos, R., et al., *Microbiome and Asthma: What Have Experimental*
467 *Models Already Taught Us?* J Immunol Res, 2015. **2015**: p. 614758.
468 3. Braun-Fahrlander, C., et al., *Environmental exposure to endotoxin and its relation to*
469 *asthma in school-age children.* N Engl J Med, 2002. **347**(12): p. 869-77.
470 4. Burbank, A.J., et al., *Environmental determinants of allergy and asthma in early life.* J
471 Allergy Clin Immunol, 2017. **140**(1): p. 1-12.
472 5. Cho, S.H., et al., *Mold damage in homes and wheezing in infants.* Ann Allergy Asthma
473 Immunol, 2006. **97**(4): p. 539-45.
474 6. Dannemiller, K.C., et al., *Next-generation DNA sequencing reveals that low fungal*
475 *diversity in house dust is associated with childhood asthma development.* Indoor Air,
476 2014. **24**(3): p. 236-47.
477 7. Fishbein, A.B. and R.L. Fuleihan, *The hygiene hypothesis revisited: does exposure to*
478 *infectious agents protect us from allergy?* Curr Opin Pediatr, 2012. **24**(1): p. 98-102.
479 8. Karvonen, A.M., et al., *Indoor bacterial microbiota and development of asthma by*
480 *10.5 years of age.* J Allergy Clin Immunol, 2019. **144**(5): p. 1402-1410.
481 9. Sharpe, R.A., et al., *Variable risk of atopic disease due to indoor fungal exposure in*
482 *NHANES 2005-2006.* Clin Exp Allergy, 2015. **45**(10): p. 1566-78.
483 10. Tischer, C., et al., *Urban Dust Microbiome: Impact on Later Atopy and Wheezing.*
484 Environ Health Perspect, 2016. **124**(12): p. 1919-1923.
485 11. Adams, R.I., et al., *Airborne Bacterial Communities in Residences: Similarities and*
486 *Differences with Fungi.* PLOS ONE, 2014. **9**(3): p. e91283.
487 12. Fujimura, K.E., et al., *Man's best friend? The effect of pet ownership on house dust*
488 *microbial communities.* J Allergy Clin Immunol, 2010. **126**(2): p. 410-2, 412.e1-3.
489 13. Kembel, S.W., et al., *Architectural design influences the diversity and structure of the*
490 *built environment microbiome.* The ISME Journal, 2012. **6**(8): p. 1469-1479.
491 14. Kettleson, E.M., et al., *Key determinants of the fungal and bacterial microbiomes in*
492 *homes.* Environ Res, 2015. **138**: p. 130-5.
493 15. Meadow, J.F., et al., *Indoor airborne bacterial communities are influenced by*
494 *ventilation, occupancy, and outdoor air source.* Indoor Air, 2014. **24**(1): p. 41-8.
495 16. Dockx, Y., et al., *Residential green space can shape the indoor microbial environment.*
496 Environmental Research, 2021. **201**: p. 111543.
497 17. Adams, R.I., et al., *Dispersal in microbes: fungi in indoor air are dominated by*
498 *outdoor air and show dispersal limitation at short distances.* The ISME Journal, 2013.
499 **7**(7): p. 1262-1273.
500 18. Amend, A.S., et al., *Indoor fungal composition is geographically patterned and more*
501 *diverse in temperate zones than in the tropics.* Proceedings of the National Academy
502 of Sciences, 2010. **107**(31): p. 13748.

503 19. Mahnert, A., C. Moissl-Eichinger, and G. Berg, *Microbiome interplay: plants alter*
504 *microbial abundance and diversity within the built environment.* Frontiers in
505 Microbiology, 2015. **6**(887).
506 20. Berg, G., A. Mahnert, and C. Moissl-Eichinger, *Beneficial effects of plant-associated*
507 *microbes on indoor microbiomes and human health?* Frontiers in microbiology, 2014.
508 **5**: p. 15.
509 21. Lindow, S.E. and J.H. Leveau, *Phyllosphere microbiology.* Curr Opin Biotechnol, 2002.
510 **13**(3): p. 238-43.
511 22. Lindow, S.E. and M.T. Brandl, *Microbiology of the phyllosphere.* Appl Environ
512 Microbiol, 2003. **69**(4): p. 1875-83.
513 23. Redford, A.J., et al., *The ecology of the phyllosphere: geographic and phylogenetic*
514 *variability in the distribution of bacteria on tree leaves.* Environ Microbiol, 2010.
515 **12**(11): p. 2885-93.
516 24. Meyer, K.M. and J. Leveau, *Microbiology of the phyllosphere: A playground for*
517 *testing ecological concepts.* Oecologia, 2012. **168**: p. 621-9.
518 25. Vorholt, J.A., *Microbial life in the phyllosphere.* Nat Rev Microbiol, 2012. **10**(12): p.
519 828-40.
520 26. Rastogi, G., G.L. Coaker, and J.H.J. Leveau, *New insights into the structure and*
521 *function of phyllosphere microbiota through high-throughput molecular approaches.*
522 FEMS Microbiology Letters, 2013. **348**(1): p. 1-10.
523 27. Pegas, P.N., et al., *Could houseplants improve indoor air quality in schools?* J Toxicol
524 Environ Health A, 2012. **75**(22-23): p. 1371-80.
525 28. Kim, K.J., et al., *Efficiency of Volatile Formaldehyde Removal by Indoor Plants:*
526 *Contribution of Aerial Plant Parts versus the Root Zone.* Journal of the American
527 Society for Horticultural Science J. Amer. Soc. Hort. Sci., 2008. **133**(4): p. 521.
528 29. Sriprapat, W., et al., *Uptake of toluene and ethylbenzene by plants: removal of*
529 *volatile indoor air contaminants.* Ecotoxicol Environ Saf, 2014. **102**: p. 147-51.
530 30. Orwell, R.L., et al., *Removal of Benzene by the Indoor Plant/Substrate Microcosm and*
531 *Implications for Air Quality.* Water, Air, and Soil Pollution, 2004. **157**(1): p. 193-207.
532 31. Fu, X., et al., *Derived habitats of indoor microbes are associated with asthma*
533 *symptoms in Chinese university dormitories.* Environmental Research, 2021. **194**: p.
534 110501.
535 32. Fu, X., et al., *Associations Between Respiratory Infections and Bacterial Microbiome*
536 *in Student Dormitories in Northern China.* Indoor air, 2020.
537 33. Janssen, B.G., et al., *Cohort Profile: The ENVIRonmental influence ON early AGEing*
538 *(ENVIRONAGE): a birth cohort study.* Int J Epidemiol, 2017. **46**(5): p. 1386-1387m.
539 34. Adams, R.I., et al., *Dispersal in microbes: fungi in indoor air are dominated by*
540 *outdoor air and show dispersal limitation at short distances.* Isme Journal, 2013. **7**(7):
541 p. 1262-1273.
542 35. Hyvarinen, A., et al., *Dust sampling methods for endotoxin - an essential, but*
543 *underestimated issue.* Indoor Air, 2006. **16**(1): p. 20-7.
544 36. Caporaso, J.G., et al., *Global patterns of 16S rRNA diversity at a depth of millions of*
545 *sequences per sample.* Proc Natl Acad Sci U S A, 2011. **108 Suppl 1**(Suppl 1): p. 4516-
546 22.
547 37. Smith, D.P. and K.G. Peay, *Sequence depth, not PCR replication, improves ecological*
548 *inference from next generation DNA sequencing.* PLoS One, 2014. **9**(2): p. e90234.

549 38. Callahan, B.J., et al., *DADA2: High-resolution sample inference from Illumina*
550 *amplicon data.* Nat Methods, 2016. **13**(7): p. 581-3.
551 39. Caporaso, J.G., et al., *QIIME allows analysis of high-throughput community*
552 *sequencing data.* Nature Methods, 2010. **7**(5): p. 335-336.
553 40. Colwell, R., *Biodiversity : Concepts, patterns and measurements*, in *Princeton Guide*
554 *to Ecology*. 2009, Princeton University Press: Princeton, NJ. p. 257-263.
555 41. Haugland, R.A., et al., *Quantitative PCR analysis of selected Aspergillus, Penicillium*
556 *and Paecilomyces species.* Syst Appl Microbiol, 2004. **27**(2): p. 198-210.
557 42. Stevens, V., et al., *Ambient Air Pollution Shapes Bacterial and Fungal Ivy Leaf*
558 *Communities.* Microorganisms, 2021. **9**(10).
559 43. Zhou, F., et al., *Impact of outdoor air on indoor airborne microbiome under hazy air*
560 *pollution: A case study in winter Beijing.* Journal of Aerosol Science, 2021. **156**: p.
561 105798.
562 44. Janssen, S., et al., *Spatial interpolation of air pollution measurements using CORINE*
563 *land cover data.* Atmospheric Environment, 2008. **42**(20): p. 4884-4903.
564 45. Lefebvre, W., et al., *Presentation and evaluation of an integrated model chain to*
565 *respond to traffic-and health-related policy questions.* Environmental modelling &
566 software, 2013. **40**: p. 160-170.
567 46. Maiheu, B., et al., *Identifying the best available large-scale concentration maps for*
568 *air quality in Belgium.* Mechelen, Belgium: Flemish Institute for Technological
569 Research (VITO), 2013.
570 47. Maiheu B, V.B., Viaene P, De Rider K, Lauwaet D, Smeets N, et al. , *Identifying the*
571 *Best Available Large-Scale Concentration Maps for Air Quality in Belgium. Study*
572 *Commissioned by the Flemish Environment (MIRA) [in Dutch]*. 2013, Belgium:Flemish
573 Institute for Technological Research (VITO).
574 48. Dunn, R.R., et al., *Home Life: Factors Structuring the Bacterial Diversity Found within*
575 *and between Homes.* PLOS ONE, 2013. **8**(5): p. e64133.
576 49. R Development Core Team, *R: A language and environment for statistical computing*.
577 2019, R Foundation for Statistical Computing: Vienna, Austria.
578 50. Täubel, M., et al., *The occupant as a source of house dust bacteria.* The Journal of
579 allergy and clinical immunology, 2009. **124**: p. 834-40.e47.
580 51. Barberán, A., et al., *The ecology of microscopic life in household dust.* Proceedings of
581 the Royal Society B: Biological Sciences, 2015. **282**(1814): p. 20151139.
582 52. Pakarinen, J., et al., *Predominance of Gram-positive bacteria in house dust in the low-*
583 *allergy risk Russian Karelia.* Environmental microbiology, 2008. **10**: p. 3317-25.
584 53. Kirjavainen, P., H. Hyytiäinen, and M. Täubel, *The environmental microbiota and*
585 *asthma*. 2019. p. 216-239.
586 54. Kirjavainen, P.V., et al., *Farm-like indoor microbiota in non-farm homes protects*
587 *children from asthma development.* Nat Med, 2019. **25**(7): p. 1089-1095.
588 55. Madigan, M.T., *Brock biology of microorganisms*. 2012.
589 56. Wei, X., et al., *Phylloremediation of Air Pollutants: Exploiting the Potential of Plant*
590 *Leaves and Leaf-Associated Microbes.* Front Plant Sci, 2017. **8**: p. 1318.
591 57. Zhou, S.-Y.-D., et al., *Microbial Flow Within an Air-Phyllosphere-Soil Continuum.*
592 Frontiers in Microbiology, 2021. **11**(3325).
593 58. Dastogeer, K.M.G., et al., *Plant microbiome–an account of the factors that shape*
594 *community composition and diversity.* Current Plant Biology, 2020. **23**: p. 100161.

595 59. Fitzpatrick, C.R., et al., *Assembly and ecological function of the root microbiome*
596 *across angiosperm plant species.* Proceedings of the National Academy of Sciences,
597 2018. **115**(6): p. E1157.
598 60. Hyytiäinen, H., et al., *Microbial diversity in homes and the risk of allergic rhinitis and*
599 *inhalant atopy in two European birth cohorts.* Environmental Research, 2021. **196**: p.
600 110835.
601 61. Valkonen, M., et al., *Bacterial Exposures and Associations with Atopy and Asthma in*
602 *Children.* PLOS ONE, 2015. **10**(6): p. e0131594.
603 62. Flies, E., et al., *Biodiverse green spaces: A prescription for global urban health.*
604 Frontiers in Ecology and the Environment, 2017. **15**.
605 63. Liddicoat, C., et al., *Naturally-diverse airborne environmental microbial exposures*
606 *modulate the gut microbiome and may provide anxiolytic benefits in mice.* Sci Total
607 Environ, 2020. **701**: p. 134684.

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# Tables

610
611 **Table 1** Characteristics of the house visit

| **Microbial diversity** | n(%) | min | P25 | median | P75 | max |
|---|---|---|---|---|---|---|
| Bacteria | | | | | | |
| Chao1 richness | 155 | 111 | 296 | 406 | 505 | 774 |
| Shannon diversity index | 153 | 4.93 | 6.59 | 7.12 | 7.65 | 8.57 |
| Simpson diversity index | 154 | 0.83 | 0.97 | 0.98 | 0.99 | 1.00 |
| Fungi | | | | | | |
| Chao1 richness | 155 | 24 | 89 | 128 | 172 | 300 |
| Shannon diversity index | 155 | 0.66 | 2.70 | 3.38 | 4.05 | 5.57 |
| Simpson diversity index | 152 | 0.52 | 0.69 | 0.76 | 0.82 | 0.94 |
| **Microbial load** | | | | | | |
| Gram-negative bacterial load (CE/$m^2$/day) | 153 | 9683 | 148227 | 294609 | 486892 | 2786787 |
| Gram-positive bacterial load (CE/$m^2$/day) | 150 | 437 | 83633 | 151756 | 273459 | 1010599 |
| Fungal load (CE/$m^2$/day) | 153 | 155 | 20331 | 34762 | 62747 | 291253 |
| **Human source proxy, %** | 155 | 2.07 | 9.07 | 13.81 | 20.72 | 51.89 |
| **Characteristics of the sampling** | | | | | | |
| Period, days | 155 | 29 | 40 | 42 | 46 | 64 |
| Average Temperature, °C | 155 | 13.2 | 14.9 | 17.1 | 18.4 | 19.4 |
| Average ambient black carbon exposure, µg/$m^3$ | 155 | 0.43 | 0.61 | 0.71 | 0.87 | 1.09 |
| **Characteristics of the household** | | | | | | |
| Surface area of the living room, $m^2$ | 128 | 8.4 | 32 | 40 | 50 | 100 |
| Having an open kitchen | | | | | | |
| yes | 117 (75.5) | | | | | |
| Pet ownership | | | | | | |
| Presence furry Pets | 77 (49.7) | | | | | |
| Passive Ventilation | | | | | | |
| Passive ventilation | 128 (82.6) | | | | | |
| Number of household members | 147 | 2 | 4 | 4 | 4 | 7 |
| **Indoor plants** | | | | | | |
| Number of indoor plants | | | | | | |
| no indoor plants | 54 (34.8) | | | | | |
| 1-3 indoor plants | 66 (42.6) | | | | | |
| More than 3 plants | 35 (22.6) | | | | | |
| Number of indoor plants per $m^2$ | | | | | | |
| Low | 44 (34.4) | 0 | 0 | 0 | 0 | 0 |
| Middle | 42 (32.8) | 0.02 | 0.02 | 0.03 | 0.04 | 0.06 |
| High | 42 (32.8) | 0.06 | 0.08 | 0.12 | 0.12 | 0.37 |

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613 614 615 **Table 2** Characteristics (median (IQR)) of the microbial diversity indices (bacterial and fungal Chao1, Shannon and Simpson index) and the microbial load (Gram-negative bacterial, Gram-positive bacterial and fungal load) per category of indoor plant (no indoor plants, 1-3 indoor plants and more than 3 indoor plants) and per category of plant density (low, middle and high), with corresponding p-values for the trend.

| | Number of Plants | | | | Number of plants per $m^2$ | | | |
|---|---|---|---|---|---|---|---|---|
| | No plants (n = 54) | 1-3 plants (n= 66) | More than 3 plants (n = 35) | p-value | Low (0 plants/$m^2$) (n = 46) | Middle (0.02-0.06 plants/$m^2$) (n = 40) | High (>0.06 plants/$m^2$) (n = 42) | p-value |
| Bacterial diversity | | | | | | | | |
| Chao1 richness | 385 (231) | 399 (198) | 438 (144) | 0.06 | 367 (249) | 407 (219) | 433 (145) | 0.02 |
| Shannon diversity index | 7.11 (1.40) | 7.05 (0.87) | 7.40 (0.96) | 0.049 | 6.99 (1.42) | 7.08 (1.01) | 7.32 (0.99) | 0.05 |
| Simpson diversity index | 0.98 (0.03) | 0.98 (0.02) | 0.99(0.01) | 0.80 | 0.98 (0.03) | 0.98 (0.02) | 0.98 (0.02) | 0.29 |
| Fungal diversity | | | | | | | | |
| Chao1 richness | 115( 60) | 123 (93) | 149 (65) | 0.03 | 115(69) | 116 (79) | 142 (91) | 0.38 |
| Shannon diversity index | 3.16 (1.27) | 3.52 (1.54) | 3.67 (1.20) | 0.02 | 3.10 (1.24) | 3.31 (1.31) | 3.54 (1.45) | 0.14 |
| Simpson diversity index | 0.73 (0.10) | 0.76 (0.13) | 0.77 (0.13) | 0.03 | 0.73 (0.12) | 0.75 (0.11) | 0.76 (0.13) | 0.16 |
| Microbial load | | | | | | | | |
| Gram-negative bacterial load | 313474 (320495) | 295240 (323252) | 227125 (389420) | 0.46 | 323054 (317057) | 212840 (304560) | 288039 (323550) | 0.39 |
| Gram-positive bacterial load | 183370 (176473) | 149975 (190579) | 129805 (196076) | 0.13 | 201157 (184150) | 128700 (140640) | 168132 (179199) | 0.12 |
| Fungal load | 42963 (43866) | 32248 (39738) | 36456 (38100) | 0.38 | 42963(44254) | 28371 (25839) | 37251 (38669) | 0.58 |

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624 # Figure legend
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626 **Figure 1 (A-D) Overview of the number of plants** in the living room (no indoor plants (reference level), 1-3 indoor plants
627 and more than 3 indoor plants) in association with the microbial diversity indices (bacterial (red) and fungal (blue) Chao1
628 richness **(A)** estimate, Shannon **(B)** and Simpson diversity **(C)** indices) and the microbial load (Gram-positive bacterial (dark
629 red), Gram-negative bacterial (light red) and fungal load (blue) **D**) **(E-H) Overview of number of plants per $m^2$** divided into
630 tertiles (low (0 plants/$m^2$) (reference level), middle (0.02-0.06 plants/$m^2$), and high (>0.06 plants/$m^2$) in association with the
631 microbial indices (bacterial (red) and fungal (blue) Chao1 richness estimate **(E)**, Shannon **(F)** and Simpson diversity **(G)** indices)
632 and the microbial load (Gram-positive bacterial (dark red), Gram-negative bacterial (light red) and fungal load (blue) **H**). All
633 models were adjusted for number of sampling days, average outdoor temperature and ambient airborne black carbon
634 concentrations during the sampling period, furry pet ownership, use of passive ventilation, and having an open kitchen.
635 Results are expressed as unit change [95% confidence interval] compared to the corresponding reference level. For the log-
636 transformed microbial load measures, we back-transformed the estimates and expressed them as a percentage change