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# Monitoring Indoor Exposure to Combustion-Derived Particles using Plants

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## 20 Abstract

21 Indoor plants can be used to monitor atmospheric particulates. Here, we report the label-free 22 detection of combustion-derived particles (CDPs) on plants as a monitoring tool for indoor 23 pollution. First, we measured the indoor CDP deposition on Atlantic ivy leaves (*Hedera hibernica*) using two-photon femtosecond microscopy. Subsequently, to prove its effectiveness for using it as 24 25 a monitoring tool, ivy plants were placed near five different indoor sources. CDP particle area and 26 number were used as output metrics. CDP values ranged between a median particle area of  $0.45 \times 10^2$  to  $1.35 \times 10^4 \,\mu\text{m}^2$ , and a median particle number of  $0.10 \times 10^2$  to  $1.42 \times 10^3$  particles for the 27 28 indoor sources: control (greenhouse) < milling machine < indoor smokers < wood stove < gas 29 stove < laser printer. Our findings demonstrate that Atlantic ivy, combined with label-free 30 detection, can be effectively used in indoor atmospheric monitoring studies.

# 31 Main finding

32 Two-photon femtosecond microscopy can be used to selectively measure the deposition of
 33 combustion-derived particles on indoor plants at different exposure levels.

# 34 Keywords

35 Indoor pollution; Combustion-derived particles; Monitoring; Ivy

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## 40 **1. Introduction**

41 Indoor air quality is an essential determinant of healthy life and well-being, especially since 42 people are spending a large amount of time indoors. Indoor concentrations of air pollutants can 43 significantly increase when important sources of pollutants are present (Bott, 2000; Myers and 44 Maynard, 2005). Particulate matter (PM) is an important indoor pollutant of particular concern 45 with regard to adverse health effects. The EU Directive 2008/50/EC recognized that there is no 46 identifiable threshold for PM exposure below which it would not pose a risk to human health 47 (European Parliament Council of the European Union, 2008), and the 2013 recommendation of 48 the International Agency for Research on Cancer (IARC) identified the PM mixture as a group 1 49 carcinogen (IARC, 2013). Indoor PM includes both particles of outdoor and indoor origin. Apte 50 and Salvi noted more than 60 sources of indoor air pollution (Apte and Salvi, 2016). The most 51 significant indoor PM sources include fuel used for cooking (Stabile et al., 2014) as well as heating 52 practices (Apte and Salvi, 2016; United States Environmental Protection Agency, 2019), and 53 indoor tobacco smoking (Gerber et al., 2015). Additionally, printers have become common indoor 54 electronic equipment and are high emitters of ultrafine particles (He et al., 2007; Morawska et al., 55 2019) but also low levels of PM<sub>10</sub> and PM<sub>2.5</sub> are emitted (Tang et al., 2012). These sources 56 generally generate combustion-derived particles (CDPs). CDPs comprise both engineered carbon 57 black (CB) used in and emitted by numerous consumer products such as printer toners, and black 58 carbon (BC) particles that are emitted as an unwanted by-product during the incomplete 59 combustion of fossil fuels, biofuels and biomass (Center for Climate and Energy Solutions, 2010; 60 Climate and Clean Air Coalition, 2016; Long et al., 2013). Those particles are considered one of 61 the most toxic components of PM (Janssen et al., 2011; Krzyzanowski et al., 2005). Recently, our 62 research group has demonstrated that in real-life conditions BC particles, as part of the CDPs,

translocate from the lungs to different organs as shown by their presence in urine (Saenen et al.,
2017) and placental tissue (Bové et al., 2019).

65 Approaches for monitoring air pollution were expensive and required complex equipment, 66 limiting large-scale applicability and accessibility. However, this has changed with the availability 67 of low-cost and easy-to-use air pollution sensors (Snyder et al., 2013). While these detection 68 devices are currently available to monitor the concentrations of gas-phase species or PM in general, 69 no method is readily available for the accurate determination of the CDP fraction in the air 70 pollution mixture. To overcome these challenges, we evaluated the feasibility of the label-free 71 detection of CDPs on indoor plants as a monitoring tool. Already numerous studies have 72 demonstrated the successful use of plant leaves as a monitoring tool of atmospheric PM because of their ability to scavenge and accumulate significant amounts of particulates (Baldacchini et al., 73 74 2017; Capozzi et al., 2019; Di Palma et al., 2017; Dzierżanowski et al., 2011; Hofman et al., 2017; 75 Popek et al., 2013; Sæbø et al., 2012). In addition, using plants leaves has been pointed as a rapid, 76 yet reliable approach that enables the collection of site-specific PM. The deposition and 77 accumulation of atmospheric particulates is generally higher with vegetation than with other 78 surfaces such as artificial substrates (Pugh et al., 2012). The accumulation efficiency of leaves 79 varies between plant species, influenced by their phenology (deciduous vs. evergreen) and their micro-morphological characteristics, e.g. wax layer properties, microsurface roughness and 80 81 presence of trichomes (Popek et al., 2013). For this study, Hedera hibernica or Atlantic ivy was 82 selected as test plant because of its known high capacity to scavenge ambient particulates, 83 evergreen foliage, robustness both indoors and outdoors, presence of stomata and trichomes, and 84 resistance to air pollution (Metcalfe, 2005; Sternberg et al., 2010). By combining these 85 advantageous features with the label-free detection of the deposited CDPs using two-photon

86 femtosecond microscopy, as recently developed by Bové et al. (Bové et al., 2016), a very unique 87 monitoring approach is presented. This approach allows the direct visualization of CDPs without 88 the need for sample/particle labeling and/or pretreatment, meaning we are not adding fluorophores 89 and, thus, only fluorescent signals from endogenous compounds are possible. In addition, it allows 90 the specific detection of all carbon-based particles including all CDPs independently of their 91 origin/source. After optimizing and validating the quantification of indoor CDP deposition on the 92 indoor green, our developed monitoring tool was employed to evaluate five different indoor 93 sources producing varying CDP concentrations.

94 **2. Materials and Methods** 

#### 95 2.1 Experimental steps for CDP detection

96 Per location (see section 2.3), four exposed leaves from one plant were selected and three 97 biopsies per leave were taken on distinct locations between the largest veins, as shown in Figure 98 1A, using a sterile scalpel and forceps in a clean room with filtered air (Genano 310/OY, Finland, 99 particle filtration cut-off  $> 0.003 \mu m$ ) to prevent any external particulate contamination. Each 100 biopsy was placed and taped on a coverslip (Menzel-Gläser, 24'55 mm, 1.5 mm) with the abaxial 101 side facing the glass for inverted imaging (Figure 1B). The CDP deposition on the ivy leaves was 102 identified using a specific and sensitive detection method based on the white light (WL) generation 103 of the particles under two-photon femtosecond illumination, see Figure 1B (Bové et al., 2016). 104 Bové et al. (Bové et al., 2016) observed the WL generation for four different carbon-based particles 105 with diameters ranging from 13 to 500 nm, suggesting that the WL emission under femtosecond 106 near-infrared illumination is a general property of carbon-based particles. Images were collected 107 using a Zeiss LSM 510 (Carl Zeiss, Jena, Germany) confocal laser scanning microscope suitable 108 for non-linear optical imaging, equipped with a two-photon femtosecond pulsed laser (MaiTai

109 DeepSee, Spectra-Physics, USA; 110 femtoseconds, 80 MHz, 10 mW average laser power on the 110 sample or a power density of  $1 \times 10^5$  W/cm<sup>2</sup>) tuned to a central wavelength of 810 nm. WL from 111 the BC particles was acquired in the non-descanned mode, meaning the emission light is directly 112 reflected on the detector, after spectral separation with a 442 nm dichroic beam splitter and 113 emission filtering employing a 400 - 410 nm band-pass filter. The two-photon excited 114 autofluorescence (TPAF) from the leaves was captured using a short pass dichroic 650 nm beam 115 splitter and a 450 - 650 nm bandpass filter to additionally filter the emission light. Within every 116 biopsy, three spots were chosen randomly and a z-stack throughout the whole leaf (intervals of 117 6.62  $\mu$ m) was made for every spot using a 10x/0.3 (Plan-Neofluar) objective (Figure 1C). Per 118 location, four biological repeats and nine technical repeats were made, resulting in 36 z-stacks, 119 each with a size of 898.20 x 898.20 x 6.62 µm<sup>3</sup> (1.76 x 1.76 x 6.62 µm<sup>3</sup> voxel size) and recorded 120 with a 3.09 µs pixel dwell time.

121 To quantify the CDPs in the acquired z-stacks, a customized and automated Matlab 122 program (Matlab R2017b (9.3.0.713579), MathWorks, Eindhoven, the Netherlands) was used. The 123 program calculates a maximum projection of the z-stack followed by a peak-find algorithm that 124 detects connected pixels with an intensity above a certain threshold value which was set here 125 0.03% lower than the highest pixel intensity value of the images (Figure 1D). This threshold 126 resulted in reproducible results without false positive and/or negative values, which was checked 127 using Fiji (ImageJ v2.0, Open source software, http://fiji.sc/Fiji). The output metrics 'particle 128 area', the total area of the particles in the field of view, and 'particle number', the total number of 129 particles in the field of view, were used for further analysis (Figure 1E).

130 2.2 Optimization and validation experiments of CDP detection

131 To evaluate the differences in CDP deposition in relation to the leaf topography at the 132 abaxial and adaxial side, the deposited CDP areas of both sides were analyzed after exposure.

133 The emission fingerprints of the detected CDP particles on the leaves and TPAF from the 134 leaves were recorded using a Zeiss LSM 880 confocal laser scanning microscope suitable for non-135 linear optical imaging equipped with the same two-photon femtosecond pulsed laser as described 136 for the LSM 510 system. This setup allowed accurate detection of the emission fingerprint of the 137 particles. Gain and laser power were changed to avoid saturation of the emission signal so that the 138 WL signal could be observed over the range from 410 to 650 nm: signals were collected in 9.7 nm 139 bins of a QUASAR thirty-two channel GaASP spectral detector (Carl Zeiss, Jena, Germany). The 140 resulting  $1024 \times 1024$  lambda image with a pixel size of 0.104 µm was detected with a pixel dwell 141 time of 2.05 µs. The emission fingerprint of commercially available CB nanoparticles (CCB; US 142 Research Nanomaterials, USA) was recorded as a reference using identical settings.

143 Following femtosecond pulsed laser illumination, the temporal responses of the emitted 144 signals originating from the CDPs on the leaves, from the ivy leaf cells themselves and from 145 reference particles dried on a coverslip were detected using the BiG.2 GaASP detector of the LSM 146 880 microscope. The detector was coupled with an SPC 830 card (Becker and Hickl, Germany), 147 which was synchronized to the pulse train of the MaiTai DeepSee laser. Recordings of  $256 \times 256$ 148 images with a pixel size of  $0.346 \,\mu\text{m}$  were acquired using a pixel dwell time of  $8.19 \,\mu\text{s}$ . The 149 instrument response function (IRF) was determined by detecting the temporal response of the laser 150 pulse using potassium dihydrogen phosphate crystals. The obtained IRF was used for the analysis 151 of all other temporal measurements for curve fitting. Time-correlated single photon counting 152 measurements were captured using SPCM 9.80 software and analyzed using SPCImage 7.3 153 software (Becker and Hickl).

154 In Raman spectra, the carbon fingerprint of the deposited particles was much weaker than 155 and indistinguishable from the autofluorescence of the leaves, the leaves were chemically 156 quenched for 2 h in 0.5% Sudan Black in methanol. Raman measurements were performed on an 157 inverted optical microscope (TiU, Nikon, Japan) equipped with a piezoelectric stage on a home-158 built optical platform. Continuous-wave laser light from 488 nm Argon Ion laser (Spectra-Physics, 159 USA) with an average power of 10 to 15 mW was reflected by a dichroic mirror (Chroma, 160 ZT488rdc, USA) and focused onto the sample with the objective (60x, Plan Fluor, N.A. 0.85, 161 Nikon, Japan). Raman scattered light from the sample was collected using the same objective and 162 directed to a CCD camera (Newton 920, Andor, UK) equipped with a blazed grating 163 monochromator (IHR-320, Horiba, Japan) with a grating of 1200 g/mm. The Raman signal passed 164 through the 500 nm long-pass filter (Chroma, HQ500LP, USA) after a 100 µm pinhole for confocal 165 detection. The slit width was set to 2000  $\mu$ m. The acquisition time was set to 1 s with averages of 166 50 acquisitions to increase the signal to noise ratio. The data were analyzed and fitted using 167 OriginPro (version 2018b (9.55), USA) and Fityk (version 0.9.8, open-source software, 168 https://fityk.nieto.pl/) (Wojdyr, 2010). The background was corrected for the ivy leaf tissue and 169 reference particles following Cadusch P.J. et al. (Cadusch et al., 2013). The Raman spectra of CB nanoparticles were recorded on dry powder as a reference using identical settings. 170

## 171 2.3 Study design indoor sources

To assess our monitoring tool, five indoor sources with varying CDP concentrations and one control were selected: (1) gas stove (Diepenbeek, Belgium), (2) wood stove (Beringen, Belgium), (3)laser printer (Diepenbeek, Belgium), (4) milling machine (Bilzen, Belgium), (5) indoor smokers (Leopoldsburg, Belgium), (6) control (greenhouse of Hasselt University, Diepenbeek, Belgium). Two plants were placed near each source to ensure a sufficient amount of

177 leaves usable for analysis, *i.e.* fully-developed and undamaged (Supplementary Information (SI), 178 Figure S1). Plants were all placed close to (approximately 1 m) the CDP source and were exposed 179 for 46 days (19/03/2018-03/05/2018). The exposure period was based on the advice of Hofman et 180 al. (Hofman et al., 2017, 2014) and Hauke et al. (Hauke and Schreiber, 1998) about the minimum 181 and maximum exposure period taking into account leaf senescence as well as the exposure period 182 applied in the study of Gawronska & Bakera (Gawrońska and Bakera, 2015). The plants were all 183 placed on a spot with indirect sunlight at a height of 1.0-1.5 m. Participants were asked to water 184 the soil of the plants every week and to not touch, dust nor move them. They were also requested 185 to avoid cross-contamination, meaning CDP contribution from the other sources under study. For 186 example, active and/or passive smoking was only allowed for the location where they smoked 187 indoors and this was also the case for the use of gas and/or wood stoves. The living room with the 188 wood stove had a volume of approximately 45 m<sup>3</sup>, and the joinery where the milling machine was 189 located was approximately 625 m<sup>3</sup>. The locations of all other sources had a volume of 190 approximately 25 m<sup>3</sup>. In none of the locations air conditioning nor forced ventilation was used. 191 The gas stove was used daily and is located in a half-open kitchen with the exhaust system right 192 above the cooking stove. The wood stove was used once a week for approximately 4 h meaning 193 on average 7 times or 28 h of active burning during the study period. In the joinery, the plants were 194 placed on top of a computer numerically controlled milling machine, which was used daily during 195 working hours. For the location with the indoor smokers, two people smoked each a package of 196 20 cigarettes per day. The laser printer (Canon iR Advance C5540i) was frequently used, on 197 average 632 pages were printed each working day during the study period.

198 2.4 Leaf sampling

199 Atlantic ivy plants (*Hedera hibernica*, ø pot 90 mm, all of the same age) were kept in a 200 greenhouse at Hasselt University under controlled conditions (Diepenbeek, Belgium; 60% air 201 humidity, 15h photoperiod, temperature: day 23°C and night 18°C) prior to use. On the first day 202 of the experiment, the leaves of the plants were carefully rinsed with sterile magnesium sulfate (10 203 mM, Sigma Aldrich, Belgium) in order to remove as much as possible of the pollution present on 204 the leaves. For each plant, one leaf was removed and taped into a Petri dish with the abaxial side 205 facing up, using sterile forceps (SI, Figure S2). Petri dishes were stored in an airtight box in the 206 dark, at constant temperature and humidity until further analysis, *i.e.* ten days after collection of 207 the leaves at the locations. These control leaves were used to study the variations in the initial CDP 208 loading on each plant, which was found to be insignificant (data not shown).

At the end of the experimental period, four leaves in the middle part of the vertical axis of the plant were removed from one of the two plants, randomly chosen in the case that the leaves of both plants were intact, using sterile forceps and placed in separate Petri dishes. Each leaf was fixed with tape in a Petri dish with the abaxial side facing up, to avoid curling and excessive movement of the leaves, and adherence of particles to the bottom of the Petri dish. Samples were then stored as described previously until further analysis, *i.e.* ten days after the collection .

215 2.5 Statistical analysis

216 Data was analyzed with GraphPad Prism (version 5.00 for Windows, GraphPad Software,

USA). Images were analyzed using Fiji (ImageJ v2.0, Open source software, http://fiji.sc/Fiji).

For each indoor source, the CDP particle area and number were expressed as the median and interquartile range (25<sup>th</sup>, 75<sup>th</sup> percentile) obtained from the CDP values of the 36 corresponding z-stacks.

| 221 | Data were not normally distributed, therefore to compare the CDP particle area and number            |
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| 222 | on the indoor plants of the five different indoor sources, a Kruskal-Wallis rank-sum test was        |
| 223 | applied followed by pairwise comparisons using the Wilcoxon rank-sum test. Multiple testing was      |
| 224 | accounted for by using a Bonferroni correction (significance level = $0.003$ ) ensuring a control of |
| 225 | the family-wise error rate below 0.05. Robustness was analyzed after removal of extreme values       |
| 226 | identified by being 1.5 times lower or higher than the first and third quartile respectively.        |
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| 232 | 3. Results and Discussion  |
| 233 | 3.1 Optimization of the experimental steps for label-free CDP detection                              |
| 234 | Our previously established method based on the white-light (WL) generation (i.e. signal              |
| 235 | ranging over the whole visible spectrum) of CDPs under two-photon femtosecond pulsed laser           |
| 236 | illumination for the sensitive and specific detection of CDPs was used to study indoor particulate   |
| 237 | deposition on ivy leaves (Bové et al., 2016). Besides the WL signals originating from the deposited  |
| 238 | CDPs, the plant tissue generates two-photon excited autofluorescence (TPAF) under the two-           |
| 239 | photon illumination, which was detected simultaneously. The latter provides useful information       |

on the leaf surface and thickness for determining the spot locations while avoiding veins and the
number of z-stack images throughout the leaf tissue. The CDP particles were analyzed based on
two features: (i) the WL signals saturate compared to TPAF allowing thresholding of the CDP

243 particles (Figure 1D) and (ii) the WL emission by the CDPs was only captured in a narrow

emission window (400 - 410 nm) in which interference from other signals is unlikely. As output
metrics, both particle number and particle area were defined (Figure 1E). In summary, a flowchart
of the experimental steps is shown in Figure 1. Every step in the experiment was designed and
monitored to exclude any external contamination.

248 Figure 2 clearly shows that the CDPs tend to aggregate/agglomerate on the leaf surface 249 instead of being localized individually. This influences the metric particle number, which should 250 be taken into account when interpreting the data. The CDPs taken up by the leaves/plant also 251 aggregate/agglomerate in the veins of the leaves. Therefore, biopsies were taken between the 252 principal veins of the leaves and other large veins were avoided during imaging. Leaves containing 253 spider webs and/or an excessive amount of dust were identified by visually examining the leaves 254 through the ocular of the microscope and excluded from the study to avoid any influence on the 255 results. The heterogeneity of CDP deposition on the leaves was taken into account. From 256 optimization measurements (data not shown), we found that the acquisition of at least 36 z-stacks 257 per indoor source (three spots in three biopsies of four exposed leaves) were necessary to obtain 258 reproducible results.

259 The particle aggregates/agglomerates on the adaxial and abaxial leaf surfaces were 260 analyzed. At every site, both the particle number and area on the adaxial surface were significantly 261 higher than on the abaxial surface (SI, Figure S3). This may be explained by the distinct 262 morphological traits of both sides of the Atlantic ivy leaves. Atlantic ivy leaves have an undulated 263 topography and their epicuticular wax structures are characterized as platelets. While similar 264 micromorphology and wax structure are observed on both leaf surfaces, a high stomatal density 265 on the abaxial side and absence of stomata on the adaxial side are observed. Also, the abaxial 266 surface contains more trichomes (SI, Figure S4), hair-like structures proven to enhance the

accumulation of ambient particles, than the other side of the leaves (Burkhardt, 2010; Castanheiro
et al., 2020; Hofman et al., 2017; Li et al., 2019; Metcalfe, 2005; Muhammad et al., 2019). Due to
these differences in morphological traits of both surfaces of the ivy leaves and corresponding
dissimilarities in particle deposition, the leaf biopsies were imaged from the abaxial towards the
adaxial side.

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#### 273 3.2 Validation experiments of CDP detection

274 Various validation experiments were conducted to confirm the carbonaceous nature of the275 identified particles on the Atlantic ivy leaves.

276 First of all, the characteristic features of the emitted WL produced under two-photon 277 femtosecond pulsed illumination and generated by the identified CDP aggregates on the indoor 278 green were verified. As previously described and checked for specificity and sensitivity by Bové 279 et al., and Saenen et al. (Bové et al., 2016; Saenen et al., 2017): (i) the emission fingerprint should 280 stretch over the whole visible spectrum and (ii) the temporal response should be instantaneous. 281 First, the recorded emission fingerprint of the identified CDPs (Figure 3A) shows that indeed the 282 emission signal ranges over the various wavelengths of the visible spectrum. As a reference, the 283 WL signal of commercially CB particles was measured, which confirms the WL emission profile. 284 On the other hand, the emission fingerprint of the TPAF of the leaf consists of a distinct emission 285 peak. Subsequently, the temporal responses of the identified CDP particles, reference particles and 286 TPAF (Figure 3B) were recorded to be 350, 320 and 1470 ps, respectively. The temporal responses 287 of the reference and CDP particles are instantaneous and non-resolved from the instrument response function. These results are consistent with previously obtained results and validate the
carbonaceous nature of the identified CDP particles (Bové et al., 2016).

Additionally, to confirm the carbonaceous nature of the identified CDPs, Raman spectra from the CDPs on the ivy leaves (Figure 4) and from reference particles (SI, Figure S5) were acquired. Raman spectra of both the CDP and REF particles displayed broad D- and G-peaks typically for carbon-based particles and were located at comparable frequencies (Table S1) (Robertson, 2002).

From the performed validation experiments it can be concluded that the identified CDPs are indeed carbon-based particles and thus the particles of interest. In addition, the proposed technique is specific and sensitive for particles, thereby excluding carbon-containing aromatic compounds. Hence, the developed experimental steps present a unique label-free monitoring tool for the screening of CDPs on indoor leaves.

### 300 *3.3 Evaluation of the developed monitoring tool for five sources*

The second general aim of our study was to evaluate the developed monitoring tool comprising the label-free detection of CDP deposition by testing it on Atlantic ivy leaves following indoor particulate pollution.

The obtained results from the different indoor sources are summarized in Figure 5, Table 1 & 2, and Table S2 & S3. The median CDP particle area (Figure 5A) of the leaves from the control plant (greenhouse)  $(0.05 \times 10^3 \ \mu\text{m}^2)$  differed significantly (p<0.001) from all other sources. The highest CDP particle area was found on the leaves collected from the plant located near the laser printer, where a median particle area of  $1.35 \times 10^4 \ \mu\text{m}^2$  was found. The particle area measured near

the laser printer differed significantly from the particle areas measured near the wood stove (4.10x10<sup>3</sup>  $\mu$ m<sup>2</sup>, p<0.05), indoor smokers (2.64x10<sup>3</sup>  $\mu$ m<sup>2</sup>, p<0.01) and the milling machine (1.60x10<sup>3</sup>  $\mu$ m<sup>2</sup>, p<0.001). The median CDP particle area of 7.03x10<sup>3</sup>  $\mu$ m<sup>2</sup> found in the kitchen with the gas stove differed significantly from the indoor smokers (p<0.01) and the milling machine (p<0.001).

314 Similar results were found for CDP particle number (Figure 5B). The median CDP particle 315 number detected on the leaves of the control plant (greenhouse)  $(0.01 \times 10^3 \text{ particles})$  differed 316 significantly (p<0.001) from all other sources. The highest median CDP particle number was found 317 on the leaves from the printer room  $(1.4 \times 10^3 \text{ particles})$ , which differed significantly from the 318 number detected near the milling machine  $(2.5 \times 10^2 \text{ particles}, p < 0.001)$ , where the lowest number 319 of particles was detected. The median particle CDP particle number found in the kitchen with the 320 gas stove (6.6x10<sup>2</sup> particles) differed significantly from the number detected near the milling 321 machine as well (p<0.001). There were no significant differences found between the other sources 322 (Figure 5B, Table 2 and SI, Table S3).

Robustness analysis was performed removing extreme values, but this did not change the conclusions for particle area (SI, Figure S6A and Table S4) and particle number (SI, Figure S6B and Table S5). In summary, for both particle area and number, the indoor sources can be ranked according to increasing CDP deposition on ivy plants as follows: control (greenhouse) < milling machine < indoor smokers < wood stove < gas stove < laser printer.

Our obtained ranking of the different indoor sources based on the identified CDP deposition on the analyzed indoor plants confirmed our expectations. The median CDP particle area and number were the highest for the laser printer, where the printer was intensively used in

331 an office setting with limited ventilation. Laser printers indeed have been reported to be high 332 emitters of CDPs including CB, which is an important component of printer toner (He et al., 2007; 333 Mitsubishi Chemical, n.d.). In 2007, He et al. (He et al., 2007), demonstrated that approximately 334 40% of the laser printers tested, did emit submicrometer particles and 27% of them were high 335 particle emitters. They defined high emitters as printers having a ratio > 10 for particle 336 concentrations measured immediately after the first printed page, compared to the control, i.e. the 337 background office concentrations. We found that the median CDP particle area detected from the 338 printer was 2 times higher than from the gas stove, which was scored as being the second highest 339 source of CDPs in our ranking. Compared to the control, the median particle area detected for the 340 printer was even 300 times higher. For particle number, the median of the printer was 1.2 and 140 341 times higher than of the gas stove and the control, respectively. Both the gas stove and wood stove 342 include the incomplete combustion of gas and wood, respectively. While higher CDP 343 concentrations are expected from wood burning compared to gas cooking, the slightly higher level 344 in the latter might be due to the fact that the gas cooking stove was used on a daily basis, while the 345 wood stove was only used 7 times for a total of 28 h during the experimental period. In addition, 346 the living room with the wood stove was larger in volume than the kitchen with the gas stove (45 347 vs. 25 m<sup>3</sup>), which can explain the ranking as well (SI, Figure S7 and Tables S6 and S7). In the case 348 of indoor smoking, high concentrations of CDPs are expected since cigarette smoke contains high 349 amounts of BC, which rapidly increase over the background with statistically significant difference 350 (Ruprecht et al., 2017). Yet, the lower ranking of this source may be attributed to the large volume 351 and well ventilation on a daily basis of the living room in which the plant was located. The plant 352 near the milling machine captured a low amount of CDPs. Although during wood shaving large 353 quantities of PM are produced (Barbosa et al., 2018), it does not include a combustion process and almost no CDPs are generated and emitted. Normalizing for the volume of the living room with the wood stove, only changed the ranking with the gas stove (SI, Figure S7 and tables S6 and S7), future leaf monitoring studies should take into account the volume and the ventilation of the room wherein the source is located.

358 While the ranking of the indoor sources was in line with our expectations, we 359 acknowledge that a limitation of our study is that this ranking is based on only one plant at one 360 location for each CDP source. However, our aim was to demonstrate the effectiveness of our 361 proposed monitoring tool and therefore the ranking is only indicative. A second limitation is that 362 the obtained results for the different indoor sources were not validated using real-time measuring 363 equipment that can sample the indoor concentration of BC aerosols. Examples of such real-time 364 equipment include optical measurements using filter-based absorption photometers, such as 365 Aethalometers (Weingartner et al., 2003), particle soot absorption photometers (Bond et al., 1999), and multi-angle absorption photometers (Petzold et al., 2005). Photometers can provide real-time 366 367 measurements and are therefore highly desirable for detecting short-term peaks in concentrations 368 and tracking sources. However, this method is not uniquely sensitive to BC and only provides an 369 estimation of its mass (Petzold et al., 2013). The light absorption coefficients determined from 370 these methods are often biased since the scattering and absorption properties of particles on the 371 filter are not the same as in the atmosphere (Watson et al., 2005). The conversion of these aerosol 372 light absorption coefficients into a light-absorbing carbon mass concentration requires precise 373 knowledge of the mass-specific absorption cross section which can vary significantly in time and 374 space. The application of this conversion also assumes that BC is the only light-absorbing 375 particulate species present, but cross-sensitivity to mineral dust and organic carbon compounds 376 can influence the outcome (Petzold et al., 2013; Schwarz et al., 2010; Sharma et al., 2017). In 377 addition, all filter-based optical methods exhibit a filter loading effect that decreases the 378 photometer sensitivity and obtained data require a lot of post-processing to accurately determine 379 the CDP fraction in polluted air (Backman et al., 2017; Drinovec et al., 2014). To avoid additional 380 bias, it is also recommended to use a new filter strip for each sampling campaign, typically one 381 per day. Thus, although various techniques exist to determine CDP content in the air, there is no 382 standard method that generates a consistent and accurate determination of CDPs in polluted air, 383 one of the most toxic fractions of PM. In this study we suggest the use of ivy leaves, which have 384 already shown to be a reliable bio-indicator for PM, as a monitoring tool for CDPs. The deposition 385 efficiency of atmospheric particulates is generally higher to vegetation than to other surfaces due 386 to the micro-morphological attributes of plant leaves that promote the deposition and accumulation 387 of atmospheric particulates on their surface (Pugh et al., 2012). By combining the advantageous 388 features of ivy plants with the label-free detection of the deposited CDPs, we provide a unique tool 389 that can detect and quantify the CDP fraction in the air pollution mixture in a specific and sensitive 390 manner.

#### **4.** Conclusions

392 Our results demonstrate that two-photon femtosecond microscopy can be used to selectively 393 determine the CDP deposition on indoor plants at different exposure levels. Using plants as a 394 biological monitoring tool does not require sophisticated and high maintenance equipment and is 395 particularly suitable for long-term monitoring over large areas. In addition, the ease of sampling, 396 the absence of any necessary expensive technical equipment, and the possibility of determining 397 spatial and temporal trends make plants a very suitable tool. By combining this novel detection 398 approach with the advantageous characteristics of Atlantic ivy in terms of CDP scavenging and 399 accumulation, a plant-based monitoring approach is presented that can discriminate between

400 different levels of CDPs related to the different user sources. This study can contribute to providing

- 401 a solution to the demand to improve air quality monitoring thereby enhancing the ability to study
- 402 the adverse health effects related to indoor air pollutants.

# 403 **5. Artwork and Figures**

404 **Table 1**. The median,  $25^{\text{th}}$ , and  $75^{\text{th}}$  percentile of the area ( $\mu$ m<sup>2</sup>) of the CDPs deposited on the ivy 405 leaves near the different sources.

|                      | 25 <sup>th</sup> percentile                  | Median                                       | 75 <sup>th</sup> percentile                  |
|----------------------|--|--|--|
| Control              | 0.01x10 <sup>3</sup>                         | 0.05x10 <sup>3</sup>                         | 0.26x10 <sup>3</sup>                         |
| Milling machine      | 0.68x10 <sup>3</sup>                         | 1.60x10 <sup>3</sup>                         | 3.67x10 <sup>3</sup>                         |
| Indoor smokers       | 0.93x10 <sup>3</sup>                         | 2.64x10 <sup>3</sup>                         | 8.91x10 <sup>3</sup>                         |
| Wood stove           | 1.16x10 <sup>3</sup>                         | 4.10x10 <sup>3</sup>                         | $1.01 \times 10^4$                           |
| Gas stove            | 4.92x10 <sup>3</sup>                         | 7.03x10 <sup>3</sup>                         | 1.27x10 <sup>4</sup>                         |
| Printer              | 3.14x10 <sup>3</sup>                         | 1.35x10 <sup>4</sup>                         | 2.55x10 <sup>4</sup>                         |
| Gas stove<br>Printer | 4.92x10 <sup>3</sup><br>3.14x10 <sup>3</sup> | 7.03x10 <sup>3</sup><br>1.35x10 <sup>4</sup> | 1.27x10 <sup>4</sup><br>2.55x10 <sup>4</sup> |

406 The number of analyzed z-stacks: control (greenhouse) n=22, all other sources n=36.

407

Table 2. The median, 25<sup>th</sup>, and 75<sup>th</sup> percentile of the number of CDPs deposited on the ivy leaves
 near the different sources.

|                 | 25 <sup>th</sup> percentile | Median               | 75 <sup>th</sup> percentile |
|-----------------|-----------------------------|----------------------|-----------------------------|
| Control         | 0.04x10 <sup>2</sup>        | 0.01x10 <sup>3</sup> | 0.33x10 <sup>2</sup>        |
| Milling machine | 1.26x10 <sup>2</sup>        | $2.48 \times 10^2$   | $4.88 \times 10^2$          |
| Indoor smokers  | $1.82 \times 10^2$          | $3.74 \times 10^2$   | $1.04 \times 10^3$          |
| Wood stove      | 1.37x10 <sup>2</sup>        | $4.30 \times 10^2$   | $1.20 \times 10^3$          |
| Gas stove       | 5.21x10 <sup>2</sup>        | 6.59x10 <sup>2</sup> | 1.13x10 <sup>3</sup>        |
| Printer         | 3.41x10 <sup>2</sup>        | $1.42 \times 10^3$   | 2.35x10 <sup>3</sup>        |
|                 |                             |                      |                             |

#### 411



413 Figure 1. Flowchart of the experimental steps for CDP detection on ivy leaves. (A) From four 414 exposed leaves per indoor source, three standardized biopsies were taken on distinct locations 415 between the largest veins of each leaf. (B) The biopsies were taped on cover slides with the abaxial 416 side facing downwards for inverted imaging. The samples were illuminated using a two-photon 417 femtosecond pulsed laser tuned to a central wavelength of 810 nm (red, 10 mW radiant power at 418 the sample) using a 10x/0.3 objective at room temperature. (C) WL and TPAF signals generated 419 by the CDPs (red) and leaf tissue (green), respectively, were detected (see materials and methods for detailed experimental steps). In total, 36 z-stacks throughout the leaf tissue were taken per 420

421 location; three different spots randomly chosen in the three biopsies from four leaves per indoor 422 source. (D) For CDP analysis, a peak-find algorithm counting connected pixels above a threshold 423 value, i.e. 0.03% lower than the highest pixel intensity value of the images, was used. (E) The 424 output metrics were defined as 'number of particles' and 'particle area'. **2-column fitting image,** 425 print in color

426



427

428 Figure 2. Label-free detection of CDP aggregates deposited on the surface an Atlantic ivy leaf 429 using WL generation under femtosecond pulsed laser illumination (10x/0.3 (Plan-Neofluar) 430 objective, excitation 810 nm, 80 MHz, 10 mW laser power on the sample). (A) Correlative bright 431 field imaging showing the large CDP aggregates as dark spots in the middle of the analyzed abaxial 432 leaf biopsy. (B) Simultaneous detection of the WL signals from the CDP aggregates and the TPAF 433 from the leaf tissue observed at 450-650 nm. (C) Confined detection of the WL signals from the 434 CDP aggregates and single particles observed at 400-410 nm. 2-column fitting image, print in 435 color



438 Figure 3. Confirmation of the WL characteristics of the identified CDP particles on the ivy leaves. 439 (A) Emission fingerprint of identified combustion-derived particles (CDP), reference carbon black 440 (REF) particles, and two-photon excited autofluorescence (TPAF) under femtosecond pulsed 441 illumination. (B) Temporal response of CDP and REF particles and TPAF measured by time-442 correlated single-photon counting. The instrument response function (IRF) of the employed 443 microscopic system is shown in blue. Presented data are from one particle (aggregate/agglomerate) 444 measured in one technical and experimental repeat, and representative for the three experimental 445 repeats performed on three randomly chosen samples. 2-column fitting image, print in color



Figure 4. Raman spectra (black solid line) from CDPs on an ivy leaf generating an autofluorescent
background (blue dotted line). Inset shows the spectra of the CDPs on the leaves (black line), triple
Lorentzian line fit (red), spectral components (purple, green and blue lines) fit results for D-(green)
and G-bands (blue), and background correction (red dotted line). Summary of fit results can be
found in Table S1. 1.5-column fitting image, print in color



455 Figure 5. Box plots (median, first and third percentiles, interquartile range, and whiskers
456 indicating 95% of the data) showing (A) the analyzed CDP particle area and (B) the analyzed CDP
457 particle number for the control (greenhouse) (n=22), milling machine (n=36), indoor smokers

458 (n=36), wood stove (n=36), gas stove (n=36), and laser printer (n=36). N indicates the number of
459 analyzed z-stacks per indoor source as a result of four biological and nine technical repeats. Black
460 solid dots represent outliers. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. 2-column fitting image, print in</li>
461 black-white
462

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