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Article type : Original Article

Biofiltration of airborne VOCs with green wall systems – microbial and chemical dynamics

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ina.12473

Abstract:

Botanical air filtration is a promising technology for reducing indoor air contaminants, but the underlying mechanisms need better understanding. Here, we made a set of chamber fumigation experiments of up to 16 weeks duration, to study the filtration efficiencies for seven volatile organic compounds (VOCs; decane, toluene, 2-ethylhexanol, α-pinene, octane, benzene, xylene) and to monitor microbial dynamics in simulated green wall systems. Biofiltration functioned on sub-ppm VOC levels without concentration-dependence. Airflow through the growth medium was needed for efficient removal of chemically diverse VOCs, and the use of optimized commercial growth medium further improved the efficiency compared with soil and Leca granules. Experimental green wall simulations using these components were immediately effective, indicating that initial VOC removal was largely abiotic. Golden pothos plants had a small additional positive impact on VOC filtration and bacterial diversity in the green wall system. *Proteobacteria* dominated the microbiota of rhizosphere and irrigation water. Airborne VOCs shaped the microbial communities, enriching potential VOC utilizing bacteria (especially *Nevskiaceae* and *Patulibacteraceae*) in the irrigation water, where much of the VOC degradation capacity of the biofiltration systems resided. These results clearly show the benefits of active air circulation and optimized growth media in modern green wall systems.

Keywords: indoor air bioremediation, botanical biofilter, phytotechnology, hydroculture, high-throughput sequencing, microbiome

Practical Implications: Botanical air filtration increases in popularity as a commercial solution for indoor air quality issues. Materials used in a commercially widely available botanical biofilter were verified to remove structurally diverse VOCs from actively circulated air, both at extreme and realistic VOC concentrations. Commercial granular potting medium mix supported plants equally well but outperformed soil and Leca in terms of VOC removal efficiency. Potential VOC-degrading bacteria were enriched not only in the plants roots, but also in the irrigation water. Monitoring of irrigation water is recommended for future studies for sensitive detection and identification of the botanical biofilter VOC-degrading microbial community.

INTRODUCTION

Green walls (also known as plant walls, plant-based biowalls, botanical biofilters) are vertical structures in which one or several houseplant species are grown on soil or a soilless support fabric or growth medium. In active green walls the volume of indoor air exposed to the system is increased by actively drawing air through the support, which together with the roots embedded in it is kept moist by regular or constant drippling irrigation.[1] Supplementary lighting may also be provided. Besides the reported positive effects of indoor plants on staff wellbeing, productivity and job satisfaction,[1–3] such hydroculture-based systems can effectively reduce indoor air CO_2 concentration[4] and increase humidity.[5]

Much of the research on house plants and green walls has focused on their potential to remove harmful indoor air contaminants, especially volatile organic compounds (VOCs).[1,2,6–9] VOCs are chemically diverse and detectable in indoor air as a mixture of tens to hundreds of different compounds.[1,10,11] VOCs in indoor air are often associated with sick building syndrome, and are typically released from petrochemical-derived materials such as furnishings, paints, solvents and textiles.[10,12] 2-ethylhexanol, commonly detected in indoor air, can be produced upon microbial degradation of plasticizers.[13] Besides producing,[14] microbes also consume (biodegrade) organic pollutants with seemingly unlimited catabolic potential to utilize different chemicals.[15–18] Plants have been used to inoculate and support diverse bacterial degrader communities for the clean-up of soil (rhizoremediation) or water (phyto/rhizofiltration).[18–20] Purely microbiological systems, or biofilters, have also been developed and operated to purify contaminated air even at an industrial scale, but the science of plant-assisted remediation systems for air is newer and less developed.[1] For hydroculture-based biofilters, the proposed working mechanism for air VOC removal depends on the partitioning of pollutants from the gaseous to the aqueous phase, driven by concentration difference as microbes constantly consume pollutants from the water.[15–17,21] Although biological indoor air purification systems show demonstrated potential, their microbiology is understudied,[1] as is the specific effect of plants on it.[9] According to Guieysse et al.[22], one of the major challenges of biological indoor air treatment systems is to inoculate, express and maintain a diverse microbial degrader community. Other identified knowledge gaps include the comparison of passive systems to those with active air circulation,[1] removal efficiency of VOCs in mixtures of chemically dissimilar compounds supplied in a continuous manner, and other factors that can influence the performance and stability of the process.[2,9]

Despite these gaps in knowledge about their exact functional mechanisms, several biological air cleaning products were commercially available in different countries in the early 2010s, as reviewed by Torpy et al.[1] One of the commercial active green wall systems launched on the market after that is a botanical biotrickling filter (as visualized in Soreanu et al.[9]) by Naava Ltd (Jyväskylä, Finland), which as of September 2017 is available in Finland, Sweden and the United States.[23] By utilizing or simulating the components of this commercial system, we aimed to characterize the chemical and microbial dynamics of phytotechnological VOC filtration with a set of chamber fumigation tests. Experiments were carried out to better understand the underlying mechanisms of VOC removal and the relative contributions of the different components of the system: air circulation, growth medium, and plants. Rates of removal of five to seven different VOCs were quantified by gas chromatography-mass spectrometry (GC-MS) in experiments lasting from 20 hours to 16 weeks, and bacterial succession was monitored both in the rhizosphere and irrigation water by high-throughput sequencing.

MATERIALS AND METHODS

Small chamber experiment setup: short-term effect of plant species and air circulation

Short-term small-scale experiments were carried out in airtight glass desiccators (volume 22.3 l, Duran; Figure 1, S1). The test plants were golden pothos (*Epipremnum pinnatum* cv. aureum) and white rabbit's foot fern (*Davallia fejeensis* Hook), supplied by Hydro Huisman B.V. (Rozenburg/Aalsmeer, Netherlands) and Nieuwkoop B.V. (De Kwakel, Netherlands). Plants were maintained in their original potting soil or transferred to soilless growing

medium, one plant per pot, after thorough washing of the root system with potable tap water, by Naava Ltd, Jyväskylä, Finland. The soilless growth medium used was Naava growth mix (Nmix) used in the commercial Naava Smart Green Walls, consisting of activated carbon and other granular constituents.[24] Each pot (height 14.5 cm, width 13.5×13.5 cm) was a green wall prototype with its own fan (Figure 1, S1). In experiments with air circulation, the fan (Noctua NF-A4X10FLX, DC12V, 0.6W, 0.05A; Noctua, Rascom Computer Distribution Ges.m.b.H., Wien, Austria) was positioned on the side of the plant pot and actively drew air through the rhizosphere; in reference experiments, the fan was not on. Each potted and irrigated plant was placed in a separate desiccator. An empty desiccator, with (fern experiment) or without (golden pothos experiment) air circulation by a fan, served as a negative control. Standard laboratory lamps (LIVAL Shuttle Plus Finland, Max 24 W/230V) were used for lighting during the experiments. Equal volumes of five VOCs (benzene [purity 99.7%], toluene [99.9%], *o*-xylene [97%], α-pinene [97%], and 2-ethylhexanol [99.6%]) were added upon the start of the experiment, with 1 µl of the mix (0.2 µl of each VOC) injected into a foil cup and dropped into each of the desiccators, which were sealed immediately after. The experiment was maintained and monitored for 20–21 h. Experiments with fern and golden pothos were repeated three and six times, respectively.

Large chamber experiment 1: long-term comparison of growth media

The long-term large chamber experiments were carried out in 7–8 airtight environmental chambers made of glass, size 60×60×100 cm (volume 0.36 m^3; Figure 1, S1). The test plant was golden pothos, which were transferred to growth medium, one plant per pot. Large chamber experiment 1 compared two soilless growth media: Leca® (lightweight expanded

clay aggregates; Weber Saint-Gobain Finland, Helsinki, Finland) and Nmix, both in triplicate chambers. Plants were acclimatized in the growth medium outside chambers for 12 weeks and inside chambers, with the fans on, for seven weeks prior to the start of the VOC exposure. Eight plants were placed in each of six chambers (three chambers with Nmix-filled pots, three chambers with Leca-filled pots). Approximately 25 l of fertilized liquid medium, corresponding to the solution used in the commercial green wall (0.1% Flora Micro, 0.15% Flora Gro, 0.05% Flora Bloom, General Hydroponics of Europe), was added to each chamber. Plant pots were not submerged in the water, but each plant was automatically irrigated for 15 min at noon every day. One control chamber was left empty, containing neither pots nor irrigation water. Lighting was 1500–2000 lux with metal halide lamps for 12 h d^{-1}. The humidity and temperature were monitored by data loggers (EasyLog EL-USB-2-LCD, Lascar Electronics UK) placed in two chambers. The conductivity of the liquid medium was monitored using a WTW Multi3430 Digital pH/D.O./conductivity meter for 3 digital IDS sensors; fresh fertilized medium was added when necessary to maintain the conductivity at the range of 0.8–2.5 mS cm^{-1}. The chamber ventilation rate was approx. 1 m^3 h^{-1} and the airflow rate through each green wall prototype unit 0.36 m^3 h^{-1} for Nmix and 0.78 m^3 h^{-1} for Leca, measured 2 h after irrigation. Starting from week 0, plants were exposed to a mixture of VOCs (benzene [99.7%], toluene [99.9%], octane [>99%], *p*-xylene [>99%], α-pinene [97%], decane [>95%], 2-ethylhexanol [99.6%] at a volume ratio of 1:1:2:2:2:4:6, respectively, to obtain relatively similar concentrations of each VOC due to their different evaporation rates). The VOCs were evaporated in a sealed box with the fumigated air channeled to each chamber. The chemical mixture was replaced weekly as a preliminary test with a single fumigation chamber did not detect a substantial change in the VOC concentrations in the course of a week (data not shown). The experiment was maintained

and monitored for 16 weeks. Owing to the variation in the room temperature and humidity as well as the air velocity at the fan that created the ingoing air stream, the VOC concentrations delivered to the fumigation chambers fluctuated during the experimental period from a minimum total VOC input of 1.7 ppm (parts per million, volume) at week 3 to a maximum of 4.3 ppm at week 7 (Figure S2).

Large chamber experiment 2: long-term comparison of plant effect

Large chamber experiment 2 compared the commercially used Nmix with and without golden pothos. Triplicate chambers were supplied with seven green wall prototypes with plants, and triplicate chambers with seven otherwise identical prototypes but without plants. Irrigation and other conditions were as described above for both. One control chamber was left empty, containing neither pots nor irrigation water. VOCs were supplied to the seven chambers as described above (benzene [ACS reagent >99%], toluene [technical grade], octane [reagent grade >97.5%], xylene [mixture of *p*- and *o*isomers, >99%, may contain max 25% ethylbenzene], α-pinene [FCC >97%], decane [>95%], 2-ethylhexanol [>99%]), with the exception that each chemical was supplied from a separate container. In addition, one chamber with seven green wall prototypes with plants and irrigation water had a separate supply of ambient (non-fumigated) air, to monitor microbial development in the absence of VOC input. The experiment was maintained and monitored for 8 weeks. The total input VOC concentrations varied over the course of the experiment from a minimum of 1.6 ppm at week 5 to a maximum of 5.4 ppm at week 7 (Figure S2).

VOC sampling and analysis

Chamber headspace air was pulled through stainless steel tubes packed with 200 mg of Tenax TA adsorbent (mesh 60/80; Markes International Ltd, Llantrisant, UK) at a flow rate of 200 ml min^{-1} for either 1 min (small chamber experiments) or 10 min (large chamber experiments) with vacuum pumps. For the small chamber experiments, charcoal-filtered air was supplied during VOC collection. For the large chamber experiments, air was sampled simultaneously from the inlet and outlet of the chamber before daily irrigation and plant/microbial sampling. The VOC samples were desorbed on either a PerkinElmer ATD-100 (Perkin-Elmer Ltd., Waltham, MA, USA) (small chamber experiments and large chamber experiment 1) or a Markes TD-100 (Markes International Ltd, Llantrisant, UK) (large chamber experiment 2), and analyzed by gas chromatography-mass spectrometry (GC-MS) [Hewlett-Packard 6890 GC/5973 MSD (Wilmington, DE, USA) for the small chamber experiments and large chamber experiment 1; Agilent 7890A GC/5975C VL MSD (New York, USA) for large chamber experiment 2]. For a full description of thermal desorption and GC-MS methods see Li et al.[25] The compounds were identified using both authentic standards and the Wiley mass spectral database; concentrations of individual compounds were determined according to the calibration curves of authentic standards. Data were analyzed with linear mixed models (LMM) in a repeated-measures design (SPSS 22.0 for windows; SPSS Inc., Chicago, IL), with the treatment (i.e., different growth media for the small chamber experiments and the first large chamber experiment; Nmix with and without plants for the second large chamber experiment) treated as a between-subject factor and the sampling time as a within-subject factor. Significant LMMs were followed by multiple paired

comparisons using the Bonferroni method to determine the treatment effects at each sampling time point.

Microbial sampling, DNA extraction and quantification

In large chamber experiment 1, a plant was sacrificed from each chamber after VOC collection in weeks 0, 1, 2, 4, 8 (two planted pots removed) and 12 for sampling root-associated bacteria. In large chamber experiment 2, two planted pots were removed from fumigated chambers on week 0 and one plant weekly after that for the same kind of sampling. For the single non-fumigated chamber, three plants were sacrificed on week 1, a single plant removed on week 3, and again three plants sampled on week 6. A composite root sample from one plant consisted of removing 2 cm long tips from ten roots, which were frozen at -20 °C within 2 h of sampling. Any growth medium particles attached to the roots were retained, and the frozen roots were lyophilized and ground with three 2.3-mm Chrome Steel Beads (BioSpec Products, Bartlesville, USA) at 5.5 m s^{-1} for 30 s with a FastPrep FP120 homogenizer (MP Biomedicals), which ensured the recovery of both rhizosphere and endophytic microbes. Glass beads and lysis solutions of the MoBio PowerLyzer PowerSoil Kit (MoBio) were added to the same tube and DNA extracted according to the manufacturer's instructions, starting with microbial lysis with additional beadbeating at 5.5 m s^{-1} for 15 s. Irrigation waters were sampled in large chamber experiment 2 on weeks 1, 3 and 6. Either 200 ml (week 1) or 100 ml (weeks 3 and 6) of water was filtered on 0.22-µm Express Plus Membrane Filters (Merck Millipore) and frozen at -20 °C within 2 h of sampling. DNA was extracted with the MoBio PowerLyzer PowerSoil Kit (MoBio) as described above. Extracted DNA was quantified with Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen).

Bacterial 16S rRNA gene amplification and sequencing

Preliminary tests revealed that up to 80% of 16S rRNA gene sequences from golden pothos root DNA extract, when processed with a regular bacterial amplicon sequencing pipeline, were chloroplasts, whereas the presence of mitochondria was minimal. A semi-nested polymerase chain reaction (PCR) approach was thus applied, utilizing chloroplast-discriminating bacterial reverse primer 783r (equimolar mix of variants a, b and c)[26] together with bacterial forward primer 27f (AGAGTTTGATCMTGGCTCAG) in the first amplification. The 1st PCR reaction of 25 µl consisted of Maxima SYBR Green/Fluorescein qPCR Master Mix (Thermo Scientific), 0.4 µM of each primer (Sigma Aldrich) and 20 ng of template DNA. Thermal cycling of 10 min initial denaturation at 95 °C, followed by 30 cycles of 95 °C for 30 s, 45 °C for 30 s and 72 °C for 60 s, was conducted on Bio-Rad CFX96 Real-Time System (Bio-Rad Laboratories). 1 µl of the product was used as a template in the second amplification, which targeted the V1-V2 fragment of the bacterial 16S rRNA gene with primers 27f and 338r (TGCTGCCTCCCGTAGGAGT) and added Ion Torrent PGM sequencing adapters and barcodes (IonA_IonXpressBarcode_27f and P1_338r) to the ends. The 2nd PCR consisted of five additional cycles with annealing at 52 °C, but conditions were otherwise identical to the first reaction. Products were purified with Agencourt AMPure XP (Beckman Coulter Life Sciences, Indianapolis, IN, USA), DNA quantified as described above, and pooled in equimolar quantities for 400 bp amplicon sequencing on Ion Torrent PGM. The template was prepared with the Ion PGM Hi-Q OT2 Kit and sequenced with the Ion PGM Hi-Q Sequencing Kit on Ion 314 Chip v2 (large chamber experiment 1) or Ion 316 Chip v2 (large chamber experiment 2) according to manufacturer's instructions (all Life Sciences, Thermo Fisher Scientific).

Ion Torrent amplicon data analysis

16S rRNA gene sequences were analyzed with *mothur* v.1.36.1[27] following the Standard Operating Procedure for 454 amplicon data.[28] Samples from the two large chamber experiments were sequenced and thus also analyzed separately. Reads were trimmed (maximum 1 nucleotide [nt] difference with primer and barcode, maximum homopolymer length of 8 nt, no ambiguous nt, minimum average quality of 20 in a rolling window of 10 nt [only large chamber experiment 1], minimum length of 200 nt) and aligned against the Silva v. 119 database.[29] Chimeras were searched and removed with the default commands. Remaining sequences were classified against the Silva v. 119 database using the *mothur* implementation of the "Bayesian" classifier with 1000 iterations, and chloroplasts, mitochondria, *Archaea*, *Eukaryota*, and sequences not classified on the Kingdom level were removed. The number of remaining good-quality bacterial sequences, on which taxonomic proportions are based, varied from 4300 to 12512 sequences for the large chamber experiment 1 samples, and from 1971 to 4739 sequences for the large chamber experiment 2 samples. A phylogenetic tree was constructed from the unique sequences using the *mothur* implementation of Clearcut,[30] based on which phylogenetic diversity and weighted UniFrac dissimilarity matrices[31] were calculated at a uniform sampling depth. Unique sequences were also clustered into operational taxonomic units (OTUs) at the 97% similarity level. OTUs were classified, those occurring only once in the entire dataset were discarded, and the remaining OTU table was rarefied to an equal number of observations per sample. Weighted UniFrac-based Principal Coordinate Analysis and OTU-based generalized discriminant analyses[32] were done with CAP12.[33] Figures were drawn and univariate statistical tests (One-Way ANOVA for biomass and diversity, and non-parametric Mann-

Whitney U test for differential relative abundances of bacterial taxa between substrate sources, manually Bonferroni-corrected for three comparisons in water) were performed with OriginPro 2017 (OriginLab Corporation). Bacterial 16S rRNA gene sequences with MIMARKS details have been submitted to NCBI Sequence Read Archive under BioProject NNNN (submitted, pending).

RESULTS AND DISCUSSION

Effect of air circulation through plant growth media on VOC removal

In the small chamber experiments, a potted golden pothos or fern plant in the fumigated desiccator systematically reduced concentrations of all five introduced VOCs, both immediately (1 h) as well as overnight (20–21 h) (overall treatment effects: $F>27.5$, $P\leq0.001$ for all individual VOCs; time effects: $F>34.8$, $P<0.001$ for all VOCs except 2-ethylhexanol in the fern experiment; treatment x time interaction: $F>9.9$, $P<0.001$ in all cases except 2-ethylhexanol in the fern experiment; Figure 2; Table S1). Reduction, compared to controls without plants or growth medium, was notably greater with the commercial Naava growth mix (Nmix) than with soil (Table S2). Such immediate VOC loss could be attributed to active uptake by plants,[2,6–8,15,18] or to partitioning from the gaseous phase to moist surfaces of the plants and chambers or solid phases of the growth media.[8,15] The importance of the latter mechanism was supported by the observation that active circulation of air through growth medium and rhizosphere further improved the loss; potted plants with Nmix and a fan reduced the concentrations of all added VOCs to below the detection limit in 20–21 h (Figure 2; Table S2). The superiority of the soilless growth medium compared with soil may

relate to the chemical (absorption by activated carbon) or microbiological (growth support) properties of the Nmix medium, the former being more likely at least after 1 hour of VOC exposure. In a 1-week comparison of VOC removal by plants in potting soil and hydroculture (perlite/vermiculite saturated with fertilized water) without active air circulation, Irga et al.[4] reported slightly slower benzene removal for the soilless medium. In our experiment, it is possible that the difference between the two media was partly attributable to decreased air flow through more compact potting soil compared with granular Nmix, due to which follow-up experiments were done to compare Nmix with another granular soilless growth medium.

Temporal effect of plants in soilless growth media on VOC removal

Considering the air circulation rate of a commercial green wall (40 m^3 h^{-1} for a single-office Naava Smart Clean Wall, compared with a typically threefold air volume exchanged hourly by mechanical ventilation in an office room of 20 m^2 by the Finnish indoor air regulations), the pass rate of the fumigated air through the growth medium was unrealistically high in the small chamber experiments. Large chamber experiments were thus set up with a constant flow of fumigated air through the chamber and conditions also otherwise better simulating an office environment (temperature 19.0–20.5 °C with a slight increasing trend, air relative humidity 50–80% with a decreasing trend). These experiments used golden pothos as the test plant and were monitored for weeks, allowing observation of slower biotic changes.

In large chamber experiment 1, plants in two granular soilless growth media – Nmix and Leca – showed initially identical VOC removal rates, irrespective of the VOC (Figure 3, left panel; Figure S3; Table S3, S4). This, together with the observation of removal rates not improving over time due to possible biological acclimation, implies that the immediate VOC dissipation was unlikely to be due to a biological process. As no difference was seen between the two granular media in the beginning, we hypothesize that initial removal of VOCs occurred simply by their partitioning from the fumigated air to the clean aqueous phase, efficiently taking place on the moist surfaces of both growth media and the leaf surfaces of plants. An alternative hypothesis is that even the pre-experiment conditions without fumigation had sufficiently acclimatized the plants and/or microbes to process the additional VOC input, but this seems unlikely with mean background VOC levels ranging from 2.5×10^{-5} ppm (octane) to 9.8×10^{-4} ppm (benzene). Due to the method of VOC supply, fumigation intensity fluctuated during the 16-week experiment from a minimum total VOC input of 1.7 ppm (week 3) to a maximum of 4.3 ppm (week 7) (Figure S2), total VOC removal being 1.1 ppm (removal ratio 0.65) and 1.0 ppm (removal ratio 0.59) (week 3) and 2.7 ppm (removal ratio 0.63) and 1.9 ppm (removal ratio 0.44) (week 7) for Nmix and Leca, respectively. No positive correlation was observed between removal rates and input concentrations, except for toluene in Leca chambers (Spearman rho=0.72, P=0.004).VOC removal ratios in chambers decreased upon the removal of green wall prototype units for destructive sampling. After the first weeks, Leca showed systematically lower VOC removal than Nmix, and the difference between growth media was exaggerated towards the end of the experiment, irrespective of the VOC compound (Figure 3; Table S4). Airflow through the growth medium and rhizosphere - one suspected explanation for the superiority in comparison with more compact potting soil in small chamber experiments -did not explain

the superiority of Nmix, as flow rate was higher for Leca than Nmix. However, with the flow rates applied (determined by pot and medium characteristics and fan), the airflow versus residence time may have been more optimal in the green wall prototypes with the commercial green wall growth medium. According to Torpy et al.[1], these are the key attributes for maximizing the efficiency of any biological air purification system. Other potential explanations for the more efficient VOC transfer from air to irrigation water include lower water VOC concentrations in Nmix due to better microbial biodegradation activity, or irreversible sorption of VOCs by the activated carbon. Interestingly, the efficacy of the system was relatively independent of VOC chemical structure, removal rates for Nmix ranging from 60 to 70% with two remaining prototype units and a relative humidity of <60% in week 12. Removal efficiency per remaining prototype unit approximately doubled during the 12 weeks for Nmix, and remained relatively stable for Leca (data not shown). However, after the last units were removed upon week 12 sampling, VOC dissipation dropped to negligible in chambers with just fertilized irrigation water.

Large chamber experiment 2 tested the specific effect of golden pothos, comparing green wall prototypes with plants to those without plants. The number of units without plants was kept constant to see the effects of possible microbial acclimation or exhaustion in the absence of plant-based substrates. Neither was observed; VOC removal ratios showed fluctuation but no temporal trend. No positive correlation was observed between removal rates and input concentrations. BTEX compounds (monoaromates: benzene, toluene, ethylbenzene and xylene) and α-pinene as well as octane were removed more efficiently in chambers with plants at the beginning of the experiment (Figure 3, right panel; Figure S3;

Table S3, S4). This may be attributed either to active VOC uptake by plants,[2,7,8,15,18] or to plants inoculating a more active or better acclimatized degrader community into the growth medium and/or irrigation water.[2,22] As the number of units containing plants decreased below 3 (due to plant removal for destructive root sampling), the relative effectiveness of the chambers with plants decreased below the effectiveness of the chambers without plants (but which had a constant 7 units). The most probable explanation is that with fewer green wall prototypes – fewer fans and a smaller moist growth medium surface area – VOC transfer from air to water became the limiting factor, even if removal from the aqueous phase by biodegradation and/or irreversible sorption remained high. However, even in week 6 with one prototype with a plant per chamber, removal ratios were 40–60% for different test VOCs (Figure 3, S3). In this week, the ratio "airflow through medium/airflow through chamber" roughly matched the 1/3 ratio of "hourly airflow through commercial green wall/air volume exchanged hourly by mechanical ventilation", making it the closest estimation of a real life situation reached by our chamber setup, except for the mostly exaggerated input VOC concentrations. After the last units with plants were removed, VOC dissipation was close to negligible in chambers with just irrigation water.

Effect of growth media on rhizosphere bacterial community response to VOCs

Root microbiomes analyzed from large chamber experiment 1 showed that by the start of the VOC fumigation, bacterial communities in the two different soilless growth media had diverged in composition, but their diversity was identical (Figure 4). However, under VOC exposure Nmix sustained a more diverse rhizosphere microbial community than Leca; phylogenetic diversity of root microbiomes in Leca decreased notably upon introduction of

VOCs, and never recovered. Community composition also remained distinct between the two media. Golden pothos growth, on the other hand, did not differ between the media, and the plants showed no visible symptoms of VOC stress. The observed decreases in bacterial diversity can be caused by selective death or by selective growth of certain community members – the latter is often seen upon introduction of readily degradable organic contaminants.[16,34] Apparently the bacterial community sustained by roots in Leca was less resilient to the effect of VOCs, or alternatively VOCs were more bioavailable and able to cause either toxic or growth-supporting substrate effects in the Leca medium. The latter could be explained by the capacity of Nmix to absorb VOCs. Sorption capacity was not exhausted during the 12-week experiment with the ppm-level VOC input used, which was indicated by no drop in diversity observed in Nmix.

Effect of VOCs and plants on irrigation water bacterial community succession

In large chamber experiment 2, analysis of bacterial communities from both roots and irrigation water revealed distinct microbiomes in the two locations, even though irrigation ensured daily interaction between the two niches (generalized discriminant analysis P=0.0001 with 98% samples correctly classified). The rhizosphere communities neither showed clear temporal dynamics, nor were significantly different between fumigated and non-fumigated plants in the last sampling week, supporting the potential of Nmix medium to buffer the VOC-impact in the rhizosphere observed in large chamber experiment 1 (Figure S4).

Plants had an initial positive impact on bacterial phylogenetic diversity in the irrigation water (week 1 ANOVA P=0.0006; Figure 5). This confirmed the inoculation effect of plants, which is suspected to play a role in the initially higher removal rates of some VOCs in the chambers with plants. Fumigation, on the other hand, decreased bacterial diversity during the experiment compared to the non-fumigated chambers (week 3 ANOVA P=0.0002, week 6 ANOVA P=0.01). However, at the last microbial sampling point microbial biomass was twofold higher in the fumigated chambers (with one or zero plants) compared with the non-fumigated chambers (with 3 plants) (ANOVA week 6 P=0.01; Figure 5). This confirmed the theory that VOC-associated drop in diversity was caused by selective favoring of some heterotrophic bacterial groups that could use VOCs as a source of carbon and energy.[16–18] Interestingly, the rather moderate ppm-level VOC fumigation had a greater impact on the aquatic microbial community than did the exudates from plant roots.

With the exception of 2-ethylhexanol in experiment 2, the concentrations applied in the large chamber experiments were unrealistically high compared to VOC concentrations in normal office air, which are typically at the ppb-level.[10,12] Such exaggerated concentrations were used to stress the chemical/absorptive potential of the system and to see differences more clearly; the functionality of the actual commercial active green wall at low input of a single VOC has been reported earlier.[24] More and more evidence is being accumulated on the potential of soil and plant-associated microbes to utilize extremely low atmospheric concentrations of substrates such as H_2.[18,35] In fact, uptake of gaseous compounds is not necessary, as long as concentrations in the irrigation water remain low (by microbial degradation and/or sorption) to support VOC partitioning to the aqueous phase as air is

circulated through moist Nmix. However, ppb-level VOC concentrations, even if taken up by high-affinity enzymes, will not necessarily support the growth or even long-term maintenance of a diverse catabolic microbial community – a bottleneck identified by Guieysse et al.[22] in the development of technology for biological treatment of indoor air. In the active green wall systems, plants are expected to feed and maintain a diverse and abundant microbiome even at times of low VOC supply. Indeed, microbial biomass of irrigation waters of commercial Naava Smart Clean Walls in school and office locations was comparable to that observed in large chamber experiment 2, with DNA yields ranging from 1 to 3 ng ml^{-1} (unpublished data).

Identification of potentially VOC-utilizing bacteria

Both irrigation water and rhizosphere soil were dominated by *Proteobacteria* (Figure S5). In the rhizosphere, the two most common orders were *Rhizobiales* (*Alphaproteobacteria*) and *Burkholderiales* (*Betaproteobacteria*), matching the findings of Russell et al.[21] for biowall root bacterial communities. As VOC supply did not have an impact on the rhizosphere microbiomes in the Nmix medium, OTUs positively affected by fumigation were identified in irrigation waters of week 3, when plant numbers were identical in fumigated and non-fumigated chambers. Potentially VOC-utilizing families abundant in the green wall simulation experiments included *Nevskiaceae* (*Gammaproteobacteria*), *Patulibacteraceae* (*Actinobacteria*) and *Xanthobacteraceae* (*Alphaproteobacteria*) (Figure 6). *Nevskiaceae* was the second most abundant family in the entire large chamber experiment 2 dataset, and systematically more abundant in irrigation water than rhizosphere. Within the *Nevskiaceae*, the most abundant genus *Hydrocarboniphaga* was originally characterized based on its

ability to degrade hydrocarbons such as alkanes and phenols.[36] The nearly-as-abundant *Nevskia* is often detected on the surface of freshwater environments as well as in soil.[37] *Patulibacteraceae*, on the other hand, was more abundant in the rhizosphere than irrigation water. This family was originally isolated from soil,[38] and its members have recently been shown to degrade complex contaminants such as ibuprofen and *N*-methyl-2-pyrrolidone in aquatic environments.[39] *Xanthobacteraceae*, a third VOC-favored family, consisted mostly of *Ancylobacter*, a facultative methylotroph capable of using organochlorines as the sole carbon and energy source.[40]

Very little is known about the potential VOC-degrading bacteria in green wall settings. Russell et al.[21] – to our knowledge the only earlier comparable (i.e. cultivation-independent) green wall study – identified *Hyphomicrobium* as a rhizosphere-associated genus that responded positively to both exposure to VOCs as well as to growth/maturation of the plants. In our study, VOCs caused no statistically significant increase in *Hyphomicrobiaceae* abundance in the irrigation water, when considering all the sampling times together, whereas in the rhizosphere a VOC-associated decrease was seen (Figure 6). We did notice a steeper increase from week 1 to week 6 in the fumigated chambers compared to the non-fumigated one, but interestingly, the genera *Devosia* and especially *Prosthecomicrobium* were much more abundant than *Hyphomicrobium*. Together with the study of Russell et al.,[21] our results indicate that members of the *Hyphomicrobiaceae* may be global green wall system inhabitants. Our ongoing study will map their abundance in commercial green walls at customer locations. However, experiments with ^{13}C or ^{14}C-labelled VOCs are still required to confirm their potential to utilize and degrade airborne VOCs.

The observed VOC-dependent increase in planktonic irrigation water microbial biomass and potential degrader bacteria indicate that significant VOC utilization potential resides in the aqueous phase of the active green wall system. Our results thus contradict the earlier assumption that quantitative VOC biodegradation takes place in the solid matrix of green walls: the rhizosphere and plant growth medium.[1] These findings also encourage further research into the aqueous microbial communities of hydroponic green wall systems, which may respond to external conditions and substrates (such as VOCs) more sensitively than the rhizosphere microbiome.

ACKNOWLEDGEMENTS

We gratefully acknowledge Phil Hollins, Sini Burdillat, Hobo Kukkonen, and Alvaro Cortés Molino for help with the experimental work. The study was supported by funding from the European Research Council (ERC) under the European Union's Seventh Framework Programme (FP/2007-450 2013, grant agreement no. 615146) awarded to M.T., Tekes – the Finnish Funding Agency for Innovation R&D and pilot project funding (no. 3405/31/2015) awarded to Naava Ltd.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

REFERENCES

1. Torpy FR, Irga PJ, Burchett MD. Reducing indoor air pollutants through biotechnology. In: Pacheco Torgal F, Labrincha JA, Diamanti MV, Yu CP, Lee KH, eds. *Biotechnologies and Biomimetics for Civil Engineering.* Cham: Springer International Publishing; 2015:181–210.

2. Cruz CM, Christensen JH, Thomsen JD, Müller R. Can ornamental potted plants remove volatile organic compounds from indoor air? – a review. *Environ Sci Pollut Res.* 2014; 21:13909–13928.

3. Thomsen JD, Sønderstrup-Andersen HKH, Müller R. People–plant relationships in an office workplace: perceived benefits for the workplace and employees. *HortScience.* 2011; 46:744–752.

4. Irga PJ, Torpy FR, Burchett MD. Can hydroculture be used to enhance the performance of indoor plants for the removal of air pollutants? *Atmos Environ.* 2013; 77:267–271.

5. Wang Z, Zhang JS. Characterization and performance evaluation of a full-scale activated carbon-based dynamic botanical air filtration system for improving indoor air quality. *Build Environ.* 2011; 46:758–768.

6. Sriprapat W, Suksabye P, Areephak S, et al. Uptake of toluene and ethylbenzene by plants: Removal of volatile indoor air contaminants. *Ecotoxicol Environ Saf.* 2014; 10:147–151.

7. Yang DS, Pennisi SV, Son KC, Kays SJ. Screening indoor plants for volatile organic pollutant removal efficiency. *HortScience.* 2009; 44:1377–1381.

8. Treesubsuntorn C, Thiravetyan P. Removal of benzene from indoor air by Dracaena sanderiana: Effect of wax and stomata. *Atmos Environ.* 2012; 57:317–321.

9. Soreanu G, Dixon M, Darlington A. Botanical biofiltration of indoor gaseous pollutants – A mini-review. *Chem Eng J.* 2013; 229:585–594.

10. Salonen HJ, Pasanen AL, Lappalainen SK, et al. Airborne concentrations of volatile organic compounds, formaldehyde and ammonia in Finnish office buildings with suspected indoor air problems. J *Occup Environ Hyg.* 2009; 6:200–209.

11. Wolkoff P, Nielsen GD. Organic compounds in indoor air – their relevance for perceived indoor air quality. *Atmos Environ.* 2001; 35:4407–4417.

12. Kostiainen R. Volatile organic compounds in the indoor air of normal and sick houses. *Atmos Environ.* 1995; 29: 693–702.

13. Nalli S, Horn OJ, Grochowalski AR, Cooper DG, Nicell JA. Origin of 2-ethylhexanol as a VOC. *Environ Pollut.* 2006; 140:181–185.

14. Korpi A, Järnberg J, Pasanen AL. Microbial Volatile Organic Compounds. *Crit Rev Toxicol.* 2009; 39:139–193.

15. Wood RA, Orwell RL, Tarran J, Torpy FR, Burchett M. Potted-plant/growth media interactions and capacities for removal of volatiles from indoor air. *J Horticul Sci Biotech.* 2002; 77:120–129.

16. Bouwer EJ, Zehnder AJ. Bioremediation of organic compounds – putting microbial metabolism to work. *Trends in Biotechnol.* 1993; 11:360–370.

17. Zhang H, Pennisi SV, Kays SJ, Habteselassie MY. Isolation and identification of toluene-metabolizing bacteria from rhizospheres of two indoor plants. *Water Air Soil Pollut.* 2013; 224:1648–1661.

18. Weyens N, Thijs S, Popek R, et al. The role of plant–microbe interactions and their exploitation for phytoremediation of air pollutants. *Int J Mol Sci.* 2015; 16:25576–25604.

19. Mikkonen A, Kondo E, Lappi K, et al. Contaminant and plant-derived changes in soil chemical and microbiological indicators during fuel oil rhizoremediation with *Galega orientalis*. *Geoderma.* 2011; 160:226–246.

20. Williams JB. Phytoremediation in wetland ecosystems: progress, problems, and potential. *Crit Rev Plant Sci.* 2002; 21:607–635.

21. Russell J, Hu Y, Chau L, et al. Indoor biofilter growth and exposure to airborne chemicals drive similar changes in the bacterial communities of plant roots. *Appl Environ Microbiol.* 2014; 80:4805–4813.

22. Guieysse B, Hort C, Platel V, Munoz R, Ondarts M, Revah S. Biological treatment of indoor air for VOC removal: potential and challenges. *Biotechnol Adv.* 2008; 26:398–410.

23. Naava website. https://www.naava.io/naava-service/. Accessed November 2, 2017.

24. Torpy F, Clements N, Pollinger M, et al. Testing the single-pass VOC removal efficiency of an active green wall using methyl ethyl ketone (MEK). *Air Qual Atmos Health*. 2017; https://doi.org/10.1007/s11869-017-0518-4.

25. Li T, Blande JD, Holopainen JK. Atmospheric transformation of plant volatiles disrupts host plant finding. *Sci Rep.* 2016; 6:33851.

26. Sakai M, Matsuka A, Komura T, Kanazawa S. Application of a new PCR primer for terminal restriction fragment length polymorphism analysis of the bacterial communities in plant roots. *J Microbiol Methods.* 2004; 59:81–89.

27. Schloss PD, Westcott SL, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol.* 2009; 75:7537–7541.

28. Schloss PD, Gevers D, Westcott SL. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PloS ONE.* 2011; 6:e27310.

29. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucl Acids Res.* 2013; 41:D590–D596.

30. Evans J, Sheneman L, Foster JA. Relaxed neighbor joining: a fast distance-based phylogenetic tree construction method. *J Mol Evo.* 2006; 62:785–792.

31. Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl Environ Microbiol.* 2007; 73:1576–1585.

32. Anderson MJ, Robinson J. Generalised discriminant analysis based on distances. *Aust N Z J Stat.* 2003; 45:301–318.

33. Anderson MJ. *CAP: a FORTRAN computer program for canonical analysis of principal coordinates*. Department of Statistics, University of Auckland, New Zealand; 2004.

34. Mikkonen A. *The potential of microbial ecological indicators to guide ecosophisticated management of hydrocarbon-contaminated soils*. PhD Dissertation, University of Helsinki, Helsinki, Finland; 2012.

35. Greening C, Maier RJ. Atmospheric H2 fuels plant-microbe interactions. *Environ Microbiol.* 2016; 18:2289–2291.

36. Palleroni N, Port A, Chang H, Zylstra G. *Hydrocarboniphaga effusa* gen nov, sp nov, a novel member of the *γ-Proteobacteria* active in alkane and aromatic hydrocarbon degradation. *Int J Syst Evol Microbiol.* 2004; 54:1203–1207.

37. Babenzien HD, Cypionka H. Nevskia. In: Whitman WB, eds. *Bergey's Manual of Systematics of Archaea and Bacteria.* New York: John Wiley & Sons Ltd; 2015:1–6.

38. Takahashi Y, Matsumoto A, Morisaki K, Ōmura S. *Patulibacter minatonensis* gen nov, sp nov, a novel actinobacterium isolated using an agar medium supplemented with superoxide

dismutase, and proposal of *Patulibacteraceae* fam nov. *Int J Syst Evol Microbiol.* 2006; 56:401–406.

39. Růžička J, Fusková J, Křížek K, Měrková M, Černotová A, Smělík M. Microbial degradation of *N*-methyl-2-pyrrolidone in surface water and bacteria responsible for the process. *Water Sci Technol.* 2016; 73:643–647.

40. Van den Wijngaard AJ, van der Kamp KW, van der Ploeg J, Pries F, Kazemier B, Janssen DB. Degradation of 1,2-dichloroethane by Ancylobacter aquaticus and other facultative methylotrophs. *Appl Environ Microbiol*. 1992; 58:976–983.