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Developing design criteria for active green wall bioremediation performance: Growth media selection shapes plant physiology, water and air flow patterns

Phoebe Mankiewicz [a,d,*], Aleca Borsuk [b], Christina Ciardullo [a,d], Elizabeth Hénaff [c,e], Anna Dyson [a,b,d]

[a] Center for Ecosystems and Architecture (CEA), Yale University, New Haven, CT, United States
[b] Yale School of the Environment, Yale University, New Haven, CT, United States
[c] New York University, Tandon School of Engineering, New York, NY, United States
[d] Center for Architecture Science and Ecology (CASE), Rensselaer Polytechnic Institute (RPI), Troy, NY, United States
[e] Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY, United States

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ABSTRACT

Emerging data indicates that incumbent mechanical/physio-chemical air handling systems inadequately address common indoor air quality (IAQ) problems, including elevated CO_2 levels and volatile organic compounds (VOCs), with compounding negative impacts to human health. Preliminary research suggests that active plant-based systems may synthetically address these challenges. However, in order to design system performance parameters, the significance of species selection and biogeochemical mechanisms of growth media design need further characterization towards an effective bioremediative interface with air handling systems. Here, through three different species across three different growth media designs, we investigate trade-offs between CO_2 sequestration through photosynthesis and CO_2 production by metabolically active root-zones (that may remediate VOCs). Across the species, hydroponic media produced 61% greater photosynthetic leaf area compared to organic media which produced 66% more root biomass. CO_2 concentration changes driven by differing plant and growth media (organic vs. hydroponic) treatments were measured within a semi-sealed chamber. Repeated estimations of net CO_2 concentrations throughout plant development revealed decreasing influxes of CO_2 within the chamber over time, indicating evolving photosynthesis/respiration balances. Multivariate analysis indicates growth media design, through impacts to water availability, air flow rates and plant development, was a more significant driver of bioremediation performance metrics than species selection.

1. Literature review

1.1. Precedent Literature: Air Quality & Health

Air pollution "is the biggest environmental risk to [human] health" according to the World Health Organization [1]. While air-pollution related deaths are strongly associated with a person's age and their country of origin's economic status, poor indoor air quality (IAQ) has been correlated with health impacts ranging from transient symptoms such as difficulty concentrating [2,3], and headaches [2–4], to chronic, more serious symptoms such as asthma [5] and cancer [5,6] in both developing and developed nations. IAQ diminishes as levels of carbon dioxide (CO_2), volatile organic compounds (VOCs) and particulate matter (PM) increase, each with measurable impacts to human health. Anthropogenic VOCs have had demonstrable negative impacts to human health since the 1980s in concentrations ranging from 25 mg/m^3 to 5 mg/m^3 and beyond [7,8]. PM consists of liquid droplets and solids [9,10], the smallest of which can infiltrate bronchi within the lungs, moving through capillary surfaces into the blood stream, causing many long term health impacts [11]. Finally, CO_2 levels at relatively low concentrations commonly measured in indoor spaces across multiple building types (1,000–2,500 parts per million (ppm)) have been connected to a range of stress indicators such as increased breathing rate [12], heart rate, blood pressure [13],

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Abbreviations: HAC, Hydroponic Activated Carbon; *HBC*, Hydroponic Biochar; *GS*, GaiaSoil; *PCA*, Principal Component Analysis; *CA*, Cluster Analysis; *DA*, Discriminant Analysis; *IAQ*, Indoor Air Quality; *VOCs*, Volatile Organic Compounds; *CO_2*, Carbon Dioxide; *PM*, Particulate Matter.

* Corresponding author.
E-mail address: phoebe.mankiewicz@yale.edu (P. Mankiewicz).

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headaches, irritability, sleepiness [14,15], as well as heart rate variability, focus [16], cognitive function and the ability to perform tasks [17]. Climate change patterns, rapid global urbanization [18] and urban activities are all factors of elevated levels of indoor CO_2 [19,20], concurrently creating a growing public health concern while acting as a proxy for IAQ problems from indoor sources [19].

1.2. Precedent Literature: Energy Use & IAQ

Due in part to the consequences of poor IAQ, residential, commercial, and industrial buildings in the United States allocate 30–40% of the over $1.0x10^{20}$ joules consumed each year towards heating ventilation and air conditioning (HVAC) systems [21,22]. Despite such large energy inputs, high concentrations of pollutants (e.g., VOCs, CO_2) are commonly measured within mechanically ventilated airstreams, indicating a need for researching novel approaches towards ameliorating IAQ. Contemporary HVAC systems condition IAQ under the assumption that outdoor air contains fewer pollutants, which is problematic within many urban and exurban environments [23], and can further increase specific VOC, PM and biological contaminants within indoor spaces [24,25].

1.3. Precedent Literature: IAQ Bioremediation

Vegetation-based air quality remediation systems have been proposed as a strategy to remediate IAQ, representing an approach with potential to reduce HVAC energy use and ventilation requirements while passively humidifying indoor air. A 1989 NASA research report is commonly cited to demonstrate the depth of research published in this area [26], however subsequent analysis of this and more recent data has been highly controversial with outstanding disagreement as to the demonstrated and/or potential efficacy of this approach [27,28]. While initial hypotheses and research assumed IAQ bioremediation was primarily plant and substrate mediated, focusing on pollutant adsorption, absorption, and chemisorption to plant surfaces and interstitial spaces within the growth media [29–31], developments within the field have since identified that plant photosynthetic capacity, alongside the more varied metabolic activity of extremely ecologically diverse microorganism communities associated with plant root system surface areas (rhizospheres) [32], may more efficiently break down pollutants such as VOCs [33,34], sequester particulate matter [33,35], and alter indoor CO_2 [33,36].

Many questions remain, however, as to the impacts of plant species selection [37] or basic mechanisms of rhizosphere microbial metabolisms on air bioremediation system performance [38]. In addition, questions remain as to the potential rates of pollutant reductions of such systems and their subsequent viability given air quality requirements and volumes of indoor spaces [27,28]. Questions have also been raised as to the viability of allocating expensive urban indoor space towards large volumes of photosynthetic surface areas necessary for significant indoor CO_2 remediation indoors [27,28,39]. Nevertheless, increasing urban air quality challenges alongside the rising popularity of urban agricultural systems have led to a resurgence of interest in harnessing plant growth to remediate IAQ [40–42] and reduce building energy consumption profiles [29,43,44]. To that end, hybrid prototypes have emerged that pull indoor air through or around plant growth media, allowing contact between the air, vegetation, and biogeochemical components in growth media [45–47].

Reviews of studies testing the efficacy of active IAQ bioremediation have reported statistically significant pollutant reductions [29,33], as well as positive psychological and physiological impacts to human inhabitants [33], however the viability of the calculated rates of such remediation in the context of indoor environmental control systems are continually scrutinized by interdisciplinary parties [27,28]. Some active air flow bioremediation systems have grown plants in felt-based media [48], while others have used mineral hydroponic gravel-like media [46,49], or some combination of potting soil and coconut coir and additives [29,50]. While many studies have been published since the NASA work, they include both active and passive air flow designs, as well as a variety of growth media, plant species, VOC concentrations, light intensities, and air flow rates, leading to a wide range in calculated removal efficiencies of CO_2, VOCs, and PM [29,33,51].

2. Introduction

2.1. Questions Concerning CO_2 Bioremediation

While evolving research continues to illustrate aspects of active green infrastructure's potential to improve energy efficiency and remediate components of poor IAQ, the relative impacts of design criteria such as growth media design, plant species selection and cascading impacts to performance such as water availability and plant growth have not yet been characterized in the context of these systems. In addition, while vegetation can be a significant photosynthetic CO_2 sink under the right environmental conditions including adequate light, water, and air flow conditions [52], plant and rhizosphere respiration can also be significant producers of CO_2 under different conditions [53]. Studies have measured CO_2 emanating from urban/suburban areas where landscape vegetation is reduced (e.g. mowed lawns) [54], as well as when photosynthesis does not occur such as urban forests at night [55]. This CO_2 production by plant and rhizosphere respiration could be counterproductive indoors under certain environmental factors such as the low levels of irradiance that exist in indoor environments (less than 1,000 lux [56]), or during prolonged diurnal dark phases. While rhizosphere metabolism should be theoretically maximized for VOCs degradation, respiration related to VOC metabolism would likely alter net CO_2 uptake and indoor green infrastructures' overall carbon mass balance, potentially limiting the capacity for plant/rhizosphere systems to reduce indoor CO_2 concentrations.

2.2. Study Contribution

In the research presented, we investigate relationships between plant species choice, growth media design (hydroponic versus organic), and factors of design-related performance such as weight, water content, and air flow rate through growth media as they relate to resulting patterns of CO_2 flux under low levels of light representative of indoor lighting environments. The proposed methodology was designed to improve upon the methods of previous studies. For instance, chamber experiments were limited to a time period of 1.5 h in order to address concerns as to the applicability of long term (e.g. 36 h and longer) chamber experiments to volumes of air relevant to building use [28] in order to improve the applicability of the produced data to future work. In addition, although previous literature has investigated the impact of growth media [49,50,57] or plant species [37,41,58] on indoor air bioremediation pollutant removal rates, few have included growth media utilizing organics as fertilizer [59,60] and none to the authors knowledge have included both growth media impacts to plant development and metrics of system performance such as water retention and air flow rates through the growth media.

Due to the preliminary nature of this study, the methodological design remained broad in order to determine how many factors may vary in relation to hydroponic versus organic growth media design and plant species selection. For example, if the intention of this study were to focus only on CO_2 balance, pulling air through the growth media would be counterproductive due to rhizosphere respiration. Testing the CO_2 balance *in the context of potential VOC remediation* required pulling air past the leaves of the plant, through the growth media and root system (and amongst potentially VOC remediating microorganisms). While VOC concentrations are of interest in this context, such specificity was beyond the scope of this preliminary study due to the inherent complexity of air quality chemistry, anthropogenic versus biogenic VOC production and concentrations, as well as rhizosphere interactions involved in VOC metabolic pathways. In addition, in order to determine if airflow rate was dependent on other factors such as growth media design (i.e., density) and water content, airflow rate was not kept constant between trails, therefore pressure drop, partial pressures, leakage rates, and thus exact CO_2 concentration changes were not accounted for in this preliminary investigation.

With these assumptions in mind, plant/growth media pairs were tested during early stages of growth, representing a critical yet overlooked phase of phytoremediation due to low leaf areas and largely underdeveloped rhizospheres. Plant treatment, growth media design, system weight, growth media water content, air flow rate, and measures of CO_2 were then analyzed alongside root and leaf size estimations in order to determine if plant or growth media design criteria, or some combination, may explain significant portions of variation in measures of system CO_2 performance. Plant species selection [46,49], growth media design [46,61], testing chamber design and protocols [42], and plant physiology data collection protocols [62] were developed based on precedent peer reviewed literature. Results from this study begin to characterize the quantity and quality of impacts plant and growth media choice may have on the performance of indoor air bioremediation systems containing developmentally young plant/rhizosphere arrays, and may assist in future attempts to reduce building energy use.

3. Materials and Methods

The main novelties and objectives of the presented work are to explore the potential and development of growth media and/or plant treatments' carbon balance between photosynthetic CO_2 reduction and rhizosphere CO_2 production in the context of HVAC ventilation (energy use), IAQ, bioremediation system performance, and rhizosphere VOC remediation hypotheses. In order to begin answering these questions, plants in early vegetative stages of development grown in differing growth media (Fig. 1, panel 1) were placed in a semi-sealed chamber for 1.5 h experiments utilizing fan-driven air flow directionally from leaves through roots to measure alterations to chamber CO_2 levels and associated variables such as growth media water content (Fig. 1, panel 2). Once the testing chamber data collection was complete, leaf area and root lengths were measured for each plant in each module in ImageJ (Fig. 1, panel 3).

3.1. System Design: Growth Media

Three growth media options representing a range available for indoor applications were tested: (1) a purely mineral expanded clay hydroponic media [46,49] augmented with activated carbon (HAC) (a common practice in indoor air bioremediation systems design [26,40,49]), (2) an identical hydroponic media augmented instead with biochar (HBC), and (3) a growth media utilizing organic fertilizer GaiaSoil™ (Gaia) [61], patented by the Gaia Institute® utilizing shredded recycled polystyrene foam and a proprietary mixture of organics as a nutrient source. Equal volumes (4,000 cm^3) of each growth media were used for each module. The hydroponic systems (HAC and HBC) received fertilizer (Foxfarm© Hydroponic Plant Food®) at identical concentrations (2 teaspoons per gallon of tap water). Modules including the GS growth media were irrigated with tap water only.

3.2. System Design: Plant Species Choice

Plant species choices for this experiment were based on previous research and characteristics that could contribute to a green wall installation's performance: (1) *Epipremnum aureum*, known as golden pothos has high water-stress tolerance and is often used in indoor green walls [42,49], (2) *Brassica narinosa* or tatsoi, an agricultural plant with a high nutrient density [63] with potential for agricultural applications, and (3) *Oxalis stricta,* known commonly as wood sorrel, is both invasive (nearly cosmopolitan in North America), with benefits in common with golden pothos (hardiness, stress tolerance) and tatsoi (edible). The three species were sprouted in peat moss plugs before being transplanted. While tatsoi and sorrel were sprouted from seed, pothos was propagated from cuttings of adult plants.

3.3. System Development

Seedlings were sprouted for 41 days, after which plugs of all three species were planted in the following treatments: 3 pothos,

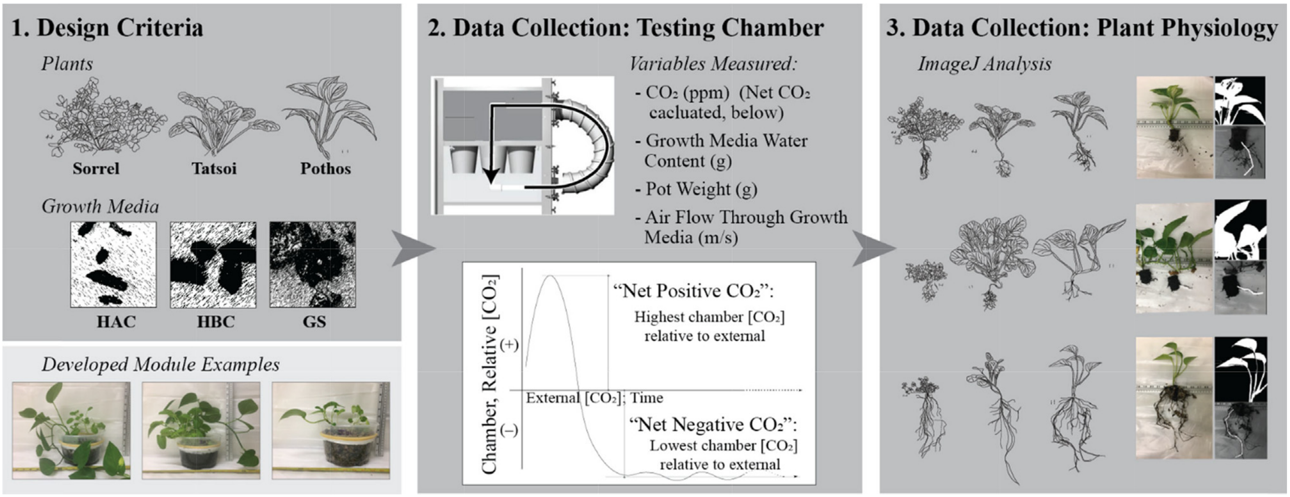

Fig. 1. Methods Flow Chart outlining the methods for the study including plant and growth media treatments, and two phases of data collection.

3 tatsoi, 3 sorrel, 1 of each, and control (empty plugs only). Each treatment was applied to each of the three growth media with three replicates for a total of 45 pots (see Fig. 2. Growing conditions between treatments were kept as constant as possible: pots were placed in identical acrylic boxes (6 pots each) in a windowless room with gravity fed irrigation systems and automated lighting (16/8 h light/dark schedule) and watering schedules driven by timers and solenoid valves. Watering differed depending on growth media but within growth media treatments were consistent: GaiaSoil dried more slowly, and so was watered less frequently. Three weeks after transplanting the fans pulling air through the pots came on for two 4-hour cycles per day to acclimate the plants to air flow through root systems and limit shock during testing.

3.4. Testing Chamber Design

In order to measure the impact each plant-based ecosystem had on CO_2 levels in a confined volume of air under active air flow conditions, a semi-sealed testing chamber was designed to circulate air using a similar approach to those used in precedent literature [42]. A chamber (volume 0.3 m^3) lit with light-emitting diodes (LEDs) positioned 0.15 m above the plants producing 1,000 lux (approximately 20 μmol/s photosynthetic photon flux) was designed to approximate indoor light levels [56] while circulating air through and around the biomass in a continuous fashion was built per Fig. 3.

3.5. Data Collection: Trial Data

One week following fan initiation, chamber experiments commenced. Each identical treatment was tested on 4 separate occasions, one to two weeks apart for a total of 60 trials. The relationship between changes in chamber CO_2 concentrations relative to external levels were used to determine how much and in what direction the plant-media pair altered CO_2 concentrations. Each trial followed the same protocol:

(1) A CO_2 sensor was placed in the open chamber, measuring external room CO_2 concentrations,
(2) Plant system was placed, chamber sealed, fans initiated and air flow through the growth media was measured. CO_2 sensor continued to record changes following air flow initiation,
(3) Fans are turned off, chamber opened, plant system removed, CO_2 sensor monitored room concentrations,
(4) Pot weight and growth media water content were measured.

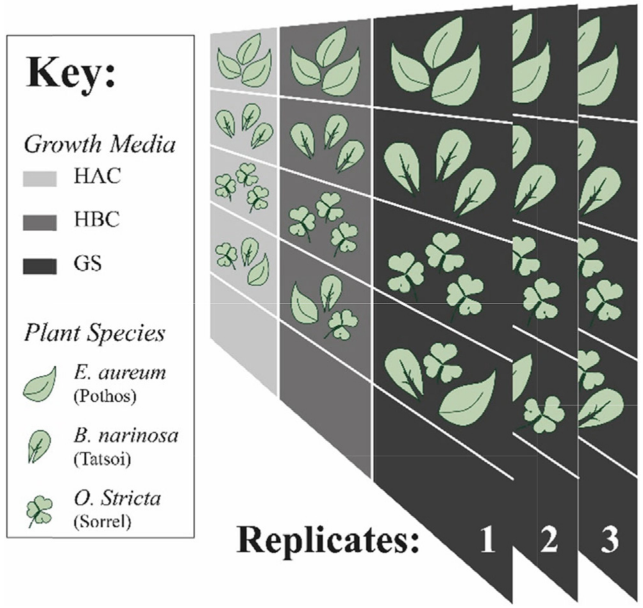

Fig. 2. Module Design Matrix of Variables depicting plant treatment and growth media variables in 45 experimental pots. Unique treatments were replicated three times.

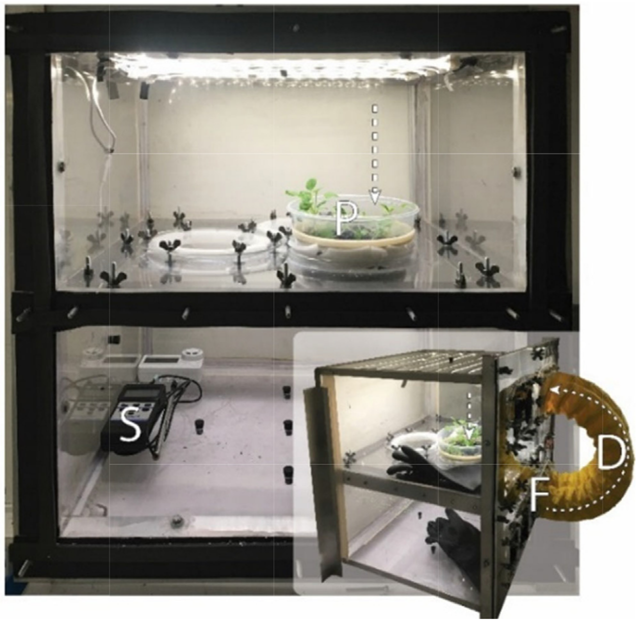

Fig. 3. Testing Chamber Design depicting the chamber ducts (D), sensors (S), plants (P), fans (F), and air flow (arrows).

CO_2 concentrations were measured in parts per million (ppm) with a Hobo® MX1102 CO2 Data Logger. A VWR® Traceable® Hot Wire Anemometer-Thermometer was used to measure air flow through the growth media, as well as test the chamber for leaks before each trial. Water content of the growth media was measured using the Dynamax Inc.® SM150™ Soil Moisture Sensor. The Dynamax sensor measurements were calibrated to known volumes of water in each growth media.

3.6. Data Collection: Plant Physiology

Following the final trial, leaf area and root length for each unique plant/growth media pair were calculated. Plants were uprooted and photographed alongside rulers (Fig. 4, right). Leaf area and maximum root length were calculated in ImageJ based on precedent protocols [62]: a reference length drawn over the reference ruler allowed the area of pixels of a specified color (Fig. 4, right, upper images), and the length of the longest root researchers were able to trace (Fig. 4, right, lower images) were calculated. Maximum root length was measured in this way due to the variability, cross-connection, and growth media integration of much longer roots illustrated in Fig. 4.

3.7. Net CO_2 Summary Variable Analysis

Due to the variability in CO_2 levels in the room where the trials were held, a mathematical model for CO_2 levels external to the chamber during each trial was calculated from baseline CO_2 data collected before and after each trial. The impact the plant system in question incurred on the air in the chamber was calculated by subtracting interpolated values of the room CO_2 model from experimental data. Net differences could then be directly compared

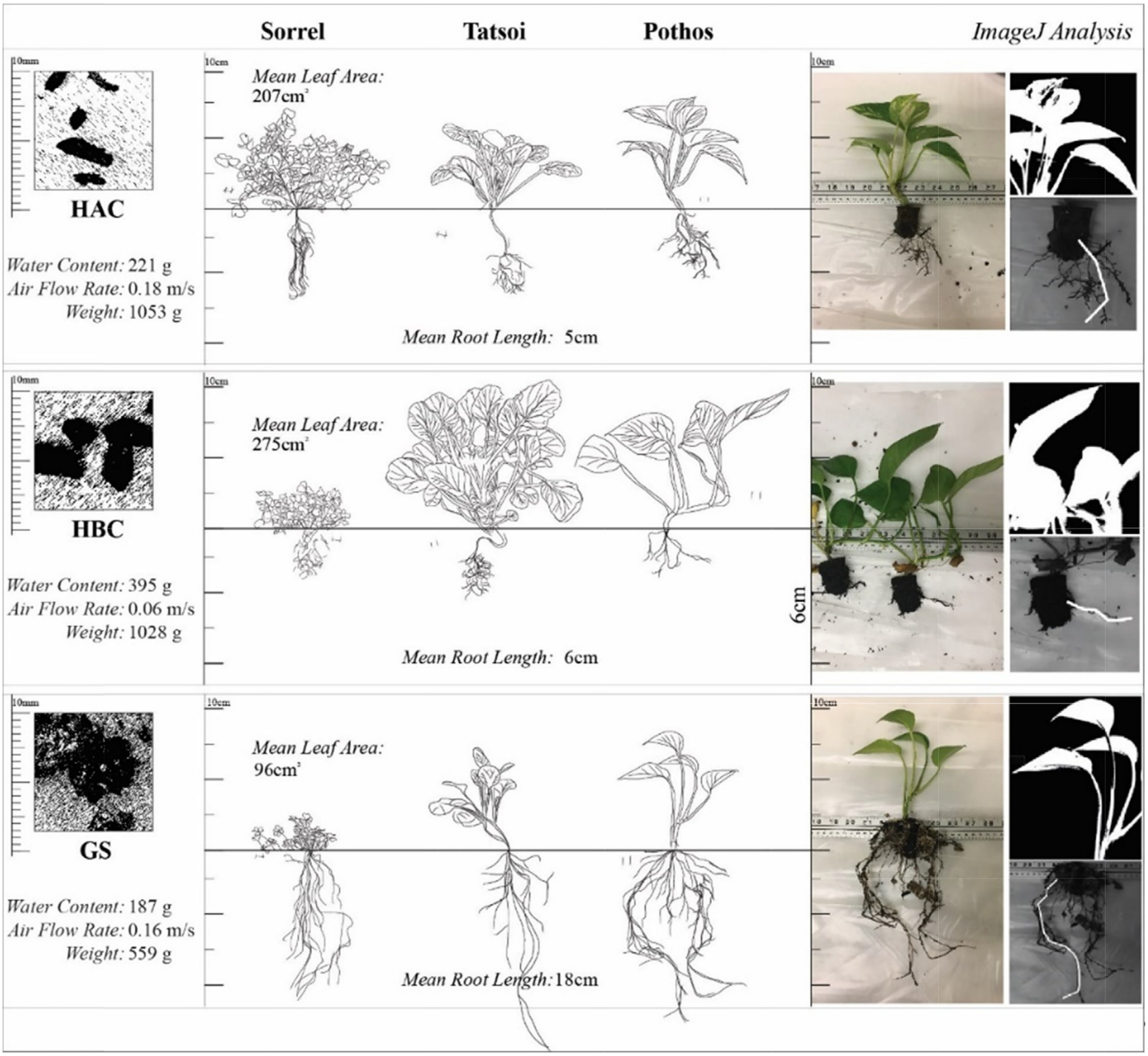

Fig. 4. Root/Leaf Analysis and Results. Select examples of leaf area and root length measurements alongside differences in growth media related variables (left) and ImageJ calculation outputs (right). Leaf area and root length units are respectively: cm^2, and cm.

between trials. While each trial produced many data points, they consistently followed a similar pattern, therefore two data points were used to represent the behavior of each plant/growth media combination (see Fig. 1, panel 2) in order to allow for multivariate statistics. Each of the 60 trials was summarized as a maximum (Net Positive CO_2) and minimum (Net Negative CO_2) value in order to illustrate relative CO_2 production and reduction rates respectively. While the maximum (Net Positive CO_2) and minimum (Net Negative CO_2) values varied through time and between trials, each trial began with a net increase in CO_2 concentrations, which then diminished throughout the rest of the trial to a level below that of the room, therefore such summary values were deemed appropriate.

3.8. Dataset Analysis

The resulting trial and plant physiology datasets were analyzed in R version 3.5.2© [64] using analysis of variance (ANOVA), multivariate ANOVA (MANOVA), as well as multivariate statistical tests: Principal Component Analysis [65,66], Parallel Analysis[67], Cluster Analysis [66,68] and Discriminant Analysis [68]. Preceding PCA, the data were standardized due to differences in scale between especially CO_2 (hundreds of ppm) and air flow (tenths of a meter per second). Principal Component Analysis (PCA) was used to identify the principal drivers of variation between continuous variables and directions in which most of the variability in the trial dataset occurred. Parallel Analysis was utilized to determine how many principal components to keep. Cluster Analysis (CA) was used to determine if there are groups within a data set that are more or less similar: euclidean distance utilizing a wards agglomeration method is emerging as standard in data analysis pertaining to biological and interrelated systems [66,68], so these methods were identified as suitable distance measurement methods. Finally, Discriminant Analysis (DA) was used to determine differences between data grouped by pre-defined qualitative variables (i.e. growth media and plant treatment). Due to the outcomes of PCA and CA, there was reason to believe that the individual classes of growth media would exhibit distinct covariances, making DA an appropriate test and illustration of those differences. The packages used during this analysis were Corrplot [69], PerformanceAnalytics [70], vegan [71], tidyverse [72] ggplot2 [73], cluster [74], MASS [75], DiscriMiner [76], and klaR [77].

4. Results

Statistical analysis revealed significant differences between environmental factors and measures of biological function between growth media, but not between plant treatments. While the relationships between the measured environmental factors during each trial were not all statistically significantly correlated as bivariate relationships (see Section 4.2), multivariate analysis techniques allowed for significant relationships to emerge when the datasets were considered holistically.

4.1. Univariate & Multivariate Normality

Plots of bivariate relationships between all quantitative variables were visually analyzed preceding any statistical test. Net Positive CO_2 and air flow variables showed slightly skewed distributions while many of the other variables are bi-modal (following patterns outlined in Table 1, however Chi Square Quantile plots indicate the data within each quantitative variable fall within a 95% confidence interval boundary indicating that (1) no transformations were necessary and (2) that the data have an approximately normal distribution. Multivariate analysis of normality yielded similar results.

4.2. Univariate Data Summary

Single factor ANOVA revealed significant differences between leaf area by growth media (p = 0.046) and plant treatment (p = 0.02), while root length varied significantly by growth media (p = $2.08e^{-5}$), but not by plant treatment (p = 0.1). Average air flow, weight, and water content by growth media are reported in Table 1. Single factor ANOVA returned significant p-values for combined trial data between growth media for water content, air flow, and weight, 1.36x10-19, 2.27x10-9, and 1.81x10-38 respectively. Similar analysis between plant treatments returned p-values larger than 0.05.

CO_2 concentrations within the room and the testing chamber ranged from 295 ppm to 1,257 ppm. Fig. 5 provides an overview of the ranges of CO_2 concentrations measured during each segment of each round of trial data collection. Each bar represents a culmination of the data collected for every module.

Trial CO_2 concentrations were corrected for modelled CO_2 concentration changes within the room according to the methods outlined in section 3.7 “Net CO_2 Summary Variable Analysis". Following these corrections, testing chamber concentrations ranged from 628 ppm over those measured in the room (indicating CO_2 concentrations within the chamber were higher than external) to 207 ppm under those measured in the room (indicating CO_2 concentrations within the chamber were lower than external). Single factor ANOVA comparing Net Positive and Negative CO_2 variables between growth media and plant treatment qualitative variables returned p-values larger than 0.05.

Following the final trial, leaf area and root length for each unique plant/growth media pair were calculated. Plants were uprooted and photographed alongside rulers (Fig. 4, right). Leaf area and maximum root length were calculated in ImageJ based on precedent protocols [62]: a reference length drawn over the reference ruler allowed the area of pixels of a specified color (Fig. 4, right, upper images), and the length of the longest root researchers were able to trace (Fig. 4, right, lower images) were calculated. Maximum root length was measured in this way due to the variability, cross-connection, and growth media integration of much longer roots illustrated in Fig. 4

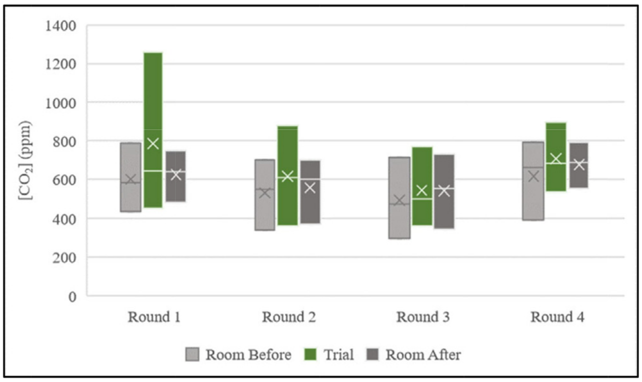

Fig. 5. Trial CO_2 Results Overview. Box plots depicting the measured CO_2 concentration ranges as maximum, minimum and median quantities taken during each round of testing.

R values for significant bivariate relationships between quantitative variables are summarized in Table 2, including a significant indirect relationship between Net Negative CO_2 and water content. One-way MANOVA analyzing combined differences between growth media for water content and Net Negative CO_2 returns a statistically significant Wilks test p-value less than 0.0001, and a lambda of 0.278.

4.3. Principal Component Analysis (PCA):

Following Parallel Analysis, two principal components were kept; The first principal component was far above both Longman and Allen methods for randomly generated 95th percentile eigenvalues. The second principal component fell between the Longman and Allen methods, however a corresponding elbow in the scree plot led to its inclusion. Together these components explained 58% of the data variance.

The first principal component included four variables with high loadings, two with negative loadings: Net Negative CO_2 (-0.35), air flow (-0.53), and two with positive loadings: water content (0.63) and weight (0.45). The second principal component included only two variables with high loadings, experimental day (0.7), and Net Positive CO_2 (−0.71). The relationships between these variables are illustrated in a PCA biplot in Fig. 6. Relative directionality indicates direct (same direction, positive) or indirect (opposing direction, negative) relationships

4.4. Cluster Analysis (CA)

Clusters within the trial data set resulting from CA were labeled according to the qualitative variables of interest: growth media

Table 1
Trial data summary table; Averages by growth media.

	Air Flow (m/s)	Weight (g/pot)	Water Content (g/pot)	Net + CO_2 (ppm)	Net - CO_2 (ppm)
HAC	0.18	1053	221	159	−20
HBC	0.06	1028	395	149	−35
GS	0.16	559	187	135	−28

Table 2

Statistically significant bivariate correlations found between variables preceding multivariate analysis.

	Net Positive CO_2 (ppm)	Net Negative CO_2 (ppm)	Weight (mL/pot)	Air Flow (m/s)
Experimental Day	−0.32			
Water Content (g/pot)		−0.39	0.52	−0.69

and plant treatment. As illustrated in Fig. 7, the only qualitative variable tested in this way that yielded significant clustering was growth media. This indicates that growth media is significantly correlated to the multivariate distances between data points taking Net Positive and Net Negative CO_2 estimates, air flow, water content, and weight into consideration.

4.5. Discriminant Analysis (DA)

DA was utilized to attempt to discriminate between the two categorical variables of interest, growth media and plant treatment for first the trial data set (Net Positive and Net Negative CO_2 estimates, air flow rate, water content, and weight), and then for the plant physiology dataset (leaf area and root length). Similar to the outcome of CA, DA of both data sets based on growth media was a more significant indicator of clustering within the data set than plant treatment in both cases.

Box's M analysis returned p-values of 8.333e-08 and 8.7e-5 for the plant physiology and trial datasets respectively, which calls for a rejection of the null hypotheses (that the covariance matrices are the same), meaning quadratic DA was required for both data sets. Based on matrix plots and the outcome of the chi-square quantile plots, transformations were not necessary.

The results of the average longest root length (cm) and leaf area (cm^2) DA were the clearest outcome of the study, and are illustrated in Fig. 8 and Table 3. The results of DA of the leaf area and root length analysis indicate that organic media resulted in higher root lengths and lower leaf areas than either hydroponic media, while the hydroponic media (both HAC and HBC) did not differ significantly from each other. This is supported by the distinct groupings of organic ("G") and hydroponic ("H") growth media by discriminant function illustrated in Fig. 8. These conclusions are supported by the confusion matrix in Table 3 which indicates that quadratic DA discriminates between the GaiaSoil and hydroponic medias with very little error, while the hydroponic media overlap significantly.

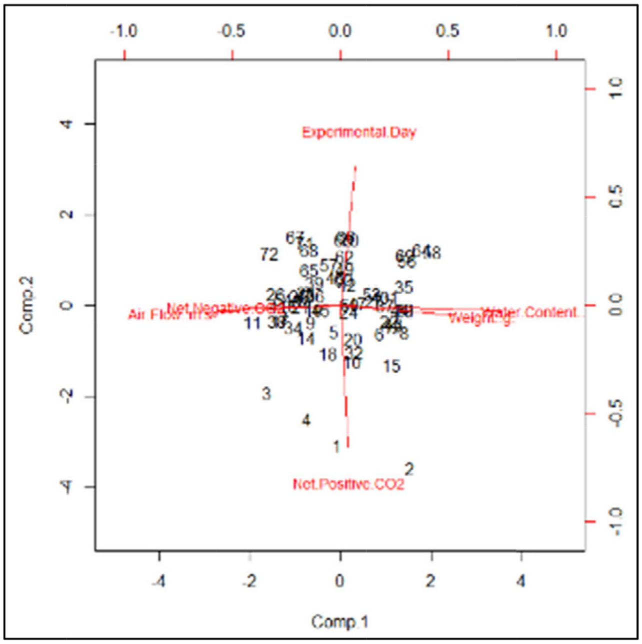

Fig. 6. Trial Data PCA Biplot. The biplot illustrates the relationships between significant variables in relation to the significant principal components. Red lines indicate the directionality and magnitude of relationships.

Discriminant analysis of the trial data returned both pot weight and water content as significant discriminating functions. The relative discriminating power of pot weight is 0.738; the addition of water content brings the discriminating power to 0.969. With regular classification, the misclassification error rate was approximately 0.015, whereas with cross-validated classification, the misclassification error rate was 0.400, both acceptably small values. While the misclassification error and discriminant scores for each observation differed slightly between classification schemes, only one observation was misclassified in each case.

Considering the output of the Wilks Lambda analysis, the direct proportion of the variance unaccounted for by the grouping variable, F-statistics (a standardized coefficient which, when significant, indicates that the means in question are not equal) were calculated to determine which variables were the best discriminators amongst the three groups. These F-statistics indicate that weight (365.81), water content (137.04), and air flow (25.783) all vary significantly between growth media. However, taken together with the stepwise discriminant analysis, adding air flow to the model does not result in more than a 5% increase in discrimination power, so this variable was excluded.

5. Discussion

The described experiments were carried out during early stages of vegetative growth representing a critical yet largely overlooked phase of IAQ bioremediation due to low leaf areas and largely underdeveloped rhizospheres. While most bioremediation systems assume developed plants, theoretically maximizing photosynthetic capacity and rhizosphere development, understanding system function during early developmental stages in which CO_2 balances within the system may be anticipated to tip towards net positive concentrations could be critical for long-term planning, maintenance schedules, and plant replacement strategies in the context of cofactors such as water availability and air flow rates.

5.1. Growth Media and Plant Development

The results of the leaf area and root length DA indicate that these variables are significantly impacted by growth media choices. Specifically, the organics-based GaiaSoil media resulted in higher root lengths and lower leaf areas than either hydroponic medium, while the hydroponic media (both HAC and HBC) did not differ significantly from each other. Interestingly, these differences did not seem to correspond with significantly higher relative respiration or photosynthesis estimates, in spite of lower leaf areas and higher root lengths grown in GaiaSoil (see section 5.2). While these relationships may evolve as plants grow and differences between plants grown in differing growth media become larger, within the context of this experiment insignificant single factor ANOVA results between growth media and the Net CO_2 variables do not support such conclusions. However, confounding factors such as

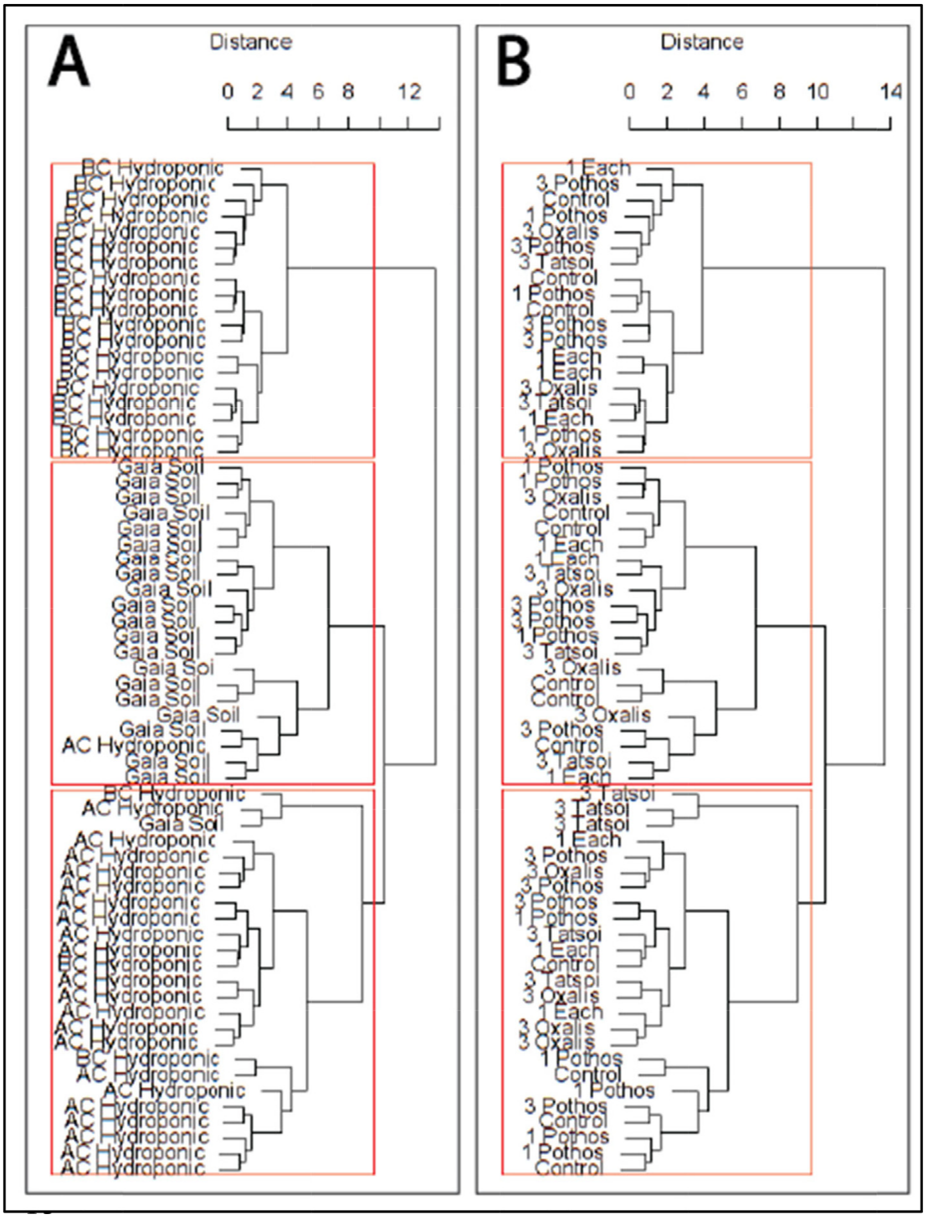

Fig. 7. Trial Data CA. CA labeled by qualitative variables indicates that growth media (panel A) rather than plant treatment (panel B) is a key factor in trial data clusters.

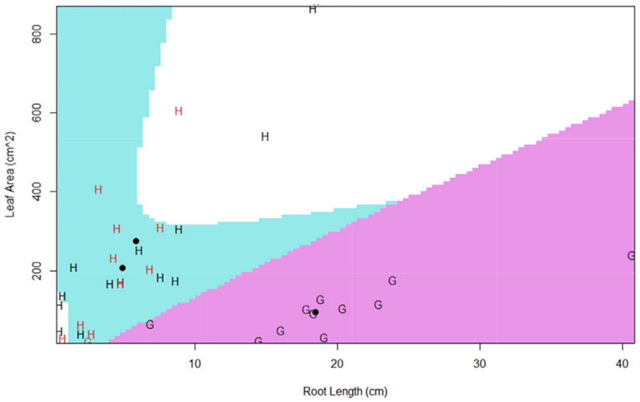

Fig. 8. Leaf/Root Discriminant Analysis Results between HAC (red H), HBC (black H), and GS (black G). The resulting discriminant functions are shaded in purple (Gaia), blue (HBC), and white (HAC).

Table 3
The confusion matrix for root and leaf cross-validated discriminant analysis. Diagonal boxes are correctly predicted instances, all others are incorrectly predicted instances.

		Predicted HAC	HBC	GS
Original	HAC	9	2	0
	HBC	8	4	0
	GS	1	0	11

photosynthetic rate were not taken into account, and likely impacted the relationship between CO_2 release and uptake between growth media groups.

More research is required to deepen our understanding of how growth media choice can be used to inform indoor air bioremediation system design and optimize rates of photosynthesis, respiration, or air flow through rhizospheres, as these processes involve complex biophysical and biogeochemical processes. Differing physiological patterns in root length and leaf size between organic and hydroponic growth media choice indicates that plants grown in organic growth media with longer roots may develop more extensive rhizospheres, potentially prioritizing system function for microbial respiration and potential benefits such as the reformation of VOCs, while plants grown in hydroponic media with more extensive leaf area may predispose such systems towards photosynthesis and preferential CO_2 removal and oxygenation processes during later developmental stages.

Depending on the design priorities of building integrated systems and design criteria such as growth media, both targeted VOC and CO_2 remediation strategies may be simultaneously possible within bioremediation infrastructure, however this work indicates that in order to have both functions operating within a single installation, environmental factors such as growth media should be given careful consideration.

5.2. Temporal Trends in Net CO_2 Flux

The results of bivariate and principal component analysis of CO_2 flux within the testing chamber indicated two things: Net Positive CO_2 decreased with time, and Net Negative CO_2 was not correlated significantly with time, positively or otherwise. In the context of the experimental trials, this implies that Net Negative CO_2, a variable used as an indicator of the balance between active respiration and active photosynthesis, was more closely correlated with other factors such as growth media (see section 5.3).

During experimentation it was immediately apparent that CO_2 production relative to photosynthesis could be significant in systems early in plant development immediately following fan initiation: Net increases in CO_2 concentrations within the chamber were measured at the beginning of each and every trial (i.e., the Net Positive CO_2 variable). This phenomenon is likely less an indication of active respiration, but is rather likely a culmination of previously respired root/rhizosphere CO_2 flushed from growth media air pockets, CO_2 production through active respiration (a common occurrences in soils [78]), context dependent CO_2 reduction through active photosynthesis [79], and chamber leakage. According to PCA, and with an R-value of -0.32 (Table 2) experimental day (i.e., time) and Net Positive CO_2 were inversely correlated. This indicates that the phenomenon of increases in chamber CO_2 concentrations decreased temporally, indicating a shift in one or all of the factors influencing this variable. Within the context of the measurements taken during the trials, it wasn't possible to determine which factor(s) were responsible for the pattern, or if the pattern might disappear if larger volumes of air, pressure drop, air flow rate, or other variables were taken into account as they can during meta-analyses that include new variables [28]. However, within the context of this study, all treatments developed temporally towards CO_2 balances that could be beneficial for IAQ.

This temporal aspect in the development of patterns in CO_2 sequestration and production may be critically important, if this pattern is confirmed at scale, for designers and implementors of active building-integrated green infrastructure to account for during the design process, especially if a primary goal of the system is to act as an indoor carbon sink and to improve IAQ through net uptake of CO_2. While previous studies have identified 5 m^2 of growing area as the required system size to offset the CO_2 produced by one person [42], this study indicates that this estimate may change over time.

5.3. Growth Media Impacts on Function

Bivariate and multivariate analysis results of this study indicate that growth media choice is a likely driver of the relationship between photosynthesis and respiration during early plant/rhizosphere developmental stages through its impact on water content. This is illustrated by the significant relationships between variables in ANOVA (growth media, weight, water content, air flow, leaf area, and root length) MANOVA (growth media, water content, Net Negative CO_2) the first principal component of PCA (weight, water content, Net Negative CO_2, and air flow), as well as the significance of the CA clusters by growth media. These relationships were further illustrated and defined by the results of DA between growth media treatments.

The first principal components of PCA included water content, weight, air flow, and Net Negative CO_2, which can all be connected to water content and availability within the growth media. Water content and weight had positive loadings, while air flow and Net Negative CO_2 had negative loadings. This indicates that, as would be expected, water content and weight are positively correlated, while water content, air flow, and intra-chamber CO_2 are negatively correlated; As water content increased, overall system weight increased, which likely lead to increased resistance to air flow while plants may have had access to more water, potentially increasing photosynthetic rates [52] leading to a reduction in Net Negative CO_2.

While the results of CA are by nature highly variable according to methodology, in this case the combination of quantitative cluster analysis with qualitative data provide compelling evidence that growth media is a significant overall factor driving quantitative measurements, while plant treatment had less of an impact on outcomes. Taken together, the described analyses and results indicate that growth media, more so than plant species selection, with emanating impacts on water content, air flow, leaf area, and root lengths during early plant development may be critical drivers of active green infrastructure performance and carbon balance at scale.

6. Conclusions

As society faces ever more intractable IAQ challenges with increasing evidence of disproportionate exposures to pollution indoors, it is becoming increasingly critical to investigate the potential for indoor air bioremediation methods to supplement the limitations of existing mechanical/physio-chemical air handling systems. However, the complexity of bioremediation in this context demands careful consideration of constituent components in order to continue to develop evidence-based design criteria towards impactful performance at scale.

The results of this experiment point to two critical considerations that may significantly impact active indoor air bioremediation design and performance. First, growth media selection should be considered a primary design criterion, with potentially significant implications for the ultimate CO_2 balance and biological function of installations especially as it relates to patterns of plant development and water availability. This is a departure from much of the precedent literature focusing instead on plant species impacts [37,41,58,80]. Secondly, influxes of CO_2 concentrations during the initiation of active air flow and early plant/rhizosphere development may have to be accounted for if the patterns of measured CO_2 fluxes are found to persist at scale. Physiological differences driven by growth media type and respiration by plants and associated microbes are not new concepts in research related to plant rhizosphere development [81,82]. However, in the context of active air flow systems and indoor air pollutant bioremediation (CO_2 included), relative rates of CO_2 production and sequestration as they relate to potential VOC remediation rates become critical for short term IAQ and implications for HVAC energy use.

The interdisciplinary arena in which indoor air bioremediation resides includes the complexity of indoor patterns of population, pollutant loads, HVAC ventilation, and human respiration rates by building type and use. Such complexity brings to light the significance of the potential impact growth media design may have towards deliberately designing specific functional outcomes of active green infrastructure. This is especially true in contained indoor occupant environments where the mass balance between humans, plant/rhizosphere ecosystems, and pollutant loads are paramount. However, once specific remediation goals have been identified, such as rates of VOC versus CO_2 remediation, this study also illustrates that basal design decisions such as growth media could be an important factor in optimizing indoor carbon balance conditions. The impacts growth media selection has on plant physiology, such as leaf area and root length, and system performance, such as water content and air flow rate, have important implications for more involved variables relevant to HVAC energy use and design, such as resistance to air flow through growth media, systemic phytoremediation rates, and rhizosphere development. Once the influence growth media design could have on these variables become more well understood, these design criteria could compound the ability for designers of active green infrastructure systems to evolve the carbon balance of indoor air streams more intentionally.

7. Future Work

Interrelationships between building energy use and the process of indoor air bioremediation involve complex biogeochemical relationships between plant, rhizosphere, growth media, human, urban air quality and HVAC factors. Individually, and especially together, these are complex and interrelated metabolisms. As such, although the presented study indicates that system performance evolved over time, the experimental timeline was likely insufficient in the length. Moving forward, more precise documentation of system performance over longer timescales covered by this preliminary study would be required to determine rates at which such systems can develop into net pollutant sinks towards the design of impactful building integrated indoor bioremediation systems at scale.

Due to the limited scope of this preliminary study, many pertinent variables were not directly measured. Although we acknowledge the complexities of the variables involved, beginning to include comprehensive VOC analysis (i.e. both biogenic and anthropogenic VOCs, not just one compound) could vastly benefit this area of research. In addition, future studies that include measurements of photosynthetic rate would be able to determine if growth media impacts to plant physiology impact system-wide photosynthetic rate. For example, systems grown in organic media may have lower leaf area overall, however longer root lengths may give the plants access to more resources such as water spatialized within the growth media, leading to higher relative photosynthetic rates.

Moving forward, if bioremediation infrastructure designs not only take growth media into account as a factor but begin to more systematically outline differences in biological functionality driven by growth media choice, system performance models may become more accurate and pollutant remediation rates may become more reproducible. Advances in these areas could greatly benefit resulting HVAC ventilation requirement and energy use models towards applications research, and developing healthier indoor air streams.

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CRediT authorship contribution statement

Phoebe Mankiewicz: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Aleca Borsuk:** Data curation, Formal analysis. **Christina Ciardullo:** Visualization. **Elizabeth Hénaff:** Methodology. **Anna Dyson:** Funding acquisition, Project administration, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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