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Ecology and characterization of the phyllosphere microbiota of plants grown in a built environment

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AFFIDAVIT

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Abstract

The indigenous phyllosphere microbiome was identified as key component for plant growth and health, and for positive effects on microbial diversity within a built environment. Nevertheless, there is still limited understanding of the phyllosphere microbiome and its driving factors. To study the variability of the microbiome in relation to the plant genotype and the ambient microclimate, we investigated 14 phylogenetically diverse plant species grown under controlled conditions in the greenhouse of the Botanical Garden in Graz (Austria), using cultivation dependent analysis, and 16S rRNA gene and internal transcribed space (ITS) region amplicon sequencing on Illumina MiSeq. Furthermore, the antagonistic potential of microbes in the phyllosphere was assessed in a Two-clamps volatile organic compounds (VOCs) assay against the model pathogen *Botrytis cinerea*.

All investigated plants showed high microbial abundances. (approx. 10^2-10^6 CFU cm^{-2}) on their leaves, albeit the population density of bacteria was higher than that of the fungi. Microbial diversity was strongly plant species dependent, but comprised similar dominant phyla; i.e. *Firmicutes, Proteobacteria* and *Actinobacteria* for Bacteria, and *Capnodiales, Wallemiales* and *Tremellales* fungal communities. Non-metric multidimensional scaling and BIO-ENV analysis showed a higher correlation of community composition to plant genotype rather than the ambient microclimatic variables. It was also remarkable that the applied biopesticide *B. thuringiensis* Bt407 was also found to be well-established in all phyllospheres. The antagonistic potential of the phyllosphere microbiome towards the plant pathogen *Botrytis cinerea* determined by the production of antifungal volatile organic compounds (VOCs) was also highly different and resulted in 2 - 58% of the bacterial isolates, and 3 – 33% of the fungal isolates. Frequently isolated VOCs producers were represented by *Bacillus spp.*, *Stenotrophomonas rhizophila, Kocuria spp.*, *Penicillum spp.*, *Cladosporium spp.*, and *Cryptococcus spp*.

This study indicates that plants grown indoors feature a distinct, stable microbial diversity with a high antagonistic potential through produced bioactive VOCs in the phyllosphere irrespective of the microclimate of a room. These observations could have beneficial effects on microbial diversity and our health inside buildings.

Kurzfassung

Das heimische Mikrobiom der Phyllosphäre spielt eine Schlüsselrolle für Pflanzengesundheit und –wachstum und hat positive Auswirkungen auf die mikrobielle Diversität in Gebäuden. Trotzdem ist das Verständnis des Phyllosphäre Mikrobioms und seiner treibenden Faktoren noch sehr gering. Um die Variabilität des Mikrobioms in Bezug auf den Genotyp der Pflanze und das vorherrschende Mikroklima zu untersuchen, wurden 14 phylogenetisch unterschiedliche Pflanzenspezies, welche unter beherrschten/kontrollierten Wachstumsbedingungen im Gewächshaus des Botanischen Garten in Graz (Österreich) wuchsen, für die Studie herangezogen. Es wurden kultivierungsabhängige Analysen sowie Amplicon Sequencing der 16S rRNA Gene und sogenannter internal transcribed spacer (ITS) Regionen mittels Illumina MiSeq durchgeführt. Des Weiteren wurde das antagonistische Potential der Mikroorganismen der Phyllosphäre mittels zwei-Klammern volatile organic compounds (VOCs) Assay gegen das Pilz-Modelpathogen *Botrytis cinerea* bewertet.

Auf allen untersuchten Pflanzen konnte ein großer Zahl in den mikrobielles Vorkommen von ca. (von ca. 10^2-10^6 CFU cm^-) Blatt nachgewiesen werden, wobei die Populationsdichte der Bakterien höher war als die der Pilze. Die mikrobielle Diversität in der Phyllosphäre hing stark von der Pflanzenspezies ab, doch aus ähnlichen dominanten Phyla bestand: *Firmicutes, Proteobacteria,* und *Actinobacteria* für bakteriellen Gemeinschaften, und *Capnodiales*, *Wallemiales*, und *Tremellales* für Pilzgesellschaften. Bemerkenswerterweise konnte sich das Biopestizid *B. thuringiensis* Bt407 in allen Phyllosphären ebenfalls gut etablieren. Mittels nicht-metrischer multidimensionaler Skalierung und BIO-ENV Analyse konnte eine höhere Korrelation der Zusammensetzung der Gemeinschaften mit dem Pflanzengenotyp als mit den vorherrschenden mikroklimatischen Variablen festgestellt werden.

Das antagonistische Potential des Phyllosphäre-Mikrobioms gegen das Pflanzenpathogen *Botrytis cinerea* durch die Produktion bedingt antimykotisch wirksamer flüchtiger

organischer Verbindungen (VOCs) zeigte sich als stark unterschiedlich und reichte von 2 bis 58% der Bakterienisolate, sowie 3 bis 33% der Pilzisolate. Häufig isolierte VOCs-produzierende Mikroorganismen waren *Bacillus spp., Stenotrophomonas rhizophila, Kocuria spp., Penicillum spp.*, *Cladosporium spp.*, und *Cryptococcus spp.*.

Diese Studie zeigt, dass in Gebäuden angebaute Pflanzen eine ausgeprägte, stabile mikrobielle Diversität mit hohem antagonistischem Potential beherbergen. Die hoch bioaktiven VOCs in der Phyllosphäre, welche ungeachtet des Mikroklimas des Raumes gebildet werden, könnten sich vorteilhaft auf die mikrobielle Diversität und unser Gesundheit innerhalb von Gebäuden auswirken.

Thesis Introduction

The phyllosphere as a microbial habitat

The aboveground parts of a living plant, including leaves, stems, buds, flowers, and fruits, colonized by microorganisms is a habitat known as the phyllosphere. It represents an environment that harbours a large and complex microbial community. Of the many plant organs belonging to the phyllosphere, leaves dominate this microbial habitat with an estimated global leaf area of 10^9 km^2 (Woodward and Lomas, 2004).

The leaf surface is considered a challenging environment for microbial communities. Foremostly, it is exposed to rapidly fluctuating solar radiation. UV radiation has been established as a strong selection agent of microbial communities on plants (Lindow, 2006). The relatively high frequency of pigmented bacteria and fungi on plants were shown to result from the selecting pressure of radiation (Stout, 1960; Ayers *et al*, 1996; Sudin and Jacobs, 1999; Sundin, 2002). It was also demonstrated that filters altered bacterial composition by reducing UV flux to plants (Sundin and Jacobs, 1999; Jacobs and Sudin, 2001; Kadivar & Stapleton, 2003). In addition, in the plant canopy a higher population of bacteria was associated to increased shading (Giesler *et al*, 2000). Aside from UV radiation, microbial epiphytes are also exposed to the atmosphere and diurnal cycles of changes in temperature and humidity. Microclimatic gradients were shown to affect the growth, survival, and diversity of microbial inhabitants of the phyllosphere (Giesler *et al*, 2000, Cordier *et al*, 2012). Transient and low water availability is another characteristic of the phyllosphere. The hydrophobic waxy cuticle that covers the plant epidermis reduces water evaporation (Vorholt, 2012), and the thin laminar layer surrounding the leaf may sequester the moisture emitted through stomata alleviating water stress to the resident epiphytes (Lindow & Brandl, 2003). The phyllosphere also provides limited nutrient resources. The waxy cuticle of the leaf surface prevents leaching of plant metabolites, while the presence of veins, stomata, trichomes and hydathodes alter nutrient availability (Leveau and Lindow, 2001; Miller *et* al, 2001; Vorholt, 2012). Aside from this environmental variability, microbial inhabitants of the phyllosphere may also encounter antagonistic compounds produced by either the plant or

other microbial colonizers (Whipps *et al*, 2008; Vorholt, 2012). It is also an ephemeral environment, since some plants plants shed their leaves in accordance to their life cycle and growth seasons (Vorholt, 2012), or as an effect of abiotic factors (e.g. strong winds) and human activities.

Notwithstanding the harsh conditions of the phyllosphere diverse microbial communities including many genera of bacteria, filamentous fungi, archaea, yeast, and algae could adapt to this habitat (Lindow & Brandl, 2003; Whipps *et al*, 2008; Vorholt, JA, 2012). Most literatures describing microbial communities in the phyllosphere focused on bacterial colonizers of the leaf. This is largely due to the fact that bacteria are the most abundant microbial colonists of the phyllosphere found at an average of $10^6 – 10^7$ bacteria per square cm of leaf surface (Lindow & Brandl, 2003). However, the leaf surface also supports diverse fungal communities (Santamariá & Bayman, 2005; Kharwar *et al*, 2010, Rastogi *et al*, 2013), and smaller proportion of archaea (Delmotte *et al*, 2009; Finkel *et al*, 2011; Knief *et al*, 2012).

Potential roles of phyllosphere microbial community

Microorganisms colonizing the phyllosphere may be pathogenic, beneficial, or commensal in nature. Phyllosphere bacterial colonists were found to stimulate plant growth and inhibit or promote pathogen infection of plant tissues (Kishore *et al*, 2005; Whipps *et al*, 2008). They may also play a key role in carbon and nitrogen cycling (Delmotte *et al*, 2009; Freiberg, 1998), and help in important environmental processes such as methanol degradation (Corpe & Rheem, 1989; Van Aken *et al*, 2004), and nitrification (Papen *et al*, 2002). On the other hand, phyllosphere fungal communities were reported to impact the fitness of their host plant, and contribute to key processes in sustaining functions of plant ecosystems including nutrient cycling and water transport (Herre *et al*, 2007; Sunshine *et al*, 2009; Vujanovic *et al* 2012).

Volatile organic compounds (VOCs) produced by phyllosphere microbiota also showed potential biocontrol properties of plant pathogens inhibiting the growth and spore germination of different pathogenic fungi. VOCs produces by a strain of *Bacillus subtilis*

was identified to have antifungal activity to *Rhizoctonia solani* and *Pythium ultimum* (Fiddaman & Rossal, 1994), while volatiles produced by bacteria isolated from canola and soybean plants inhibited sclerotia and ascospore germination, and mycelial growth of *Sclerotinia sclerotiorum*, in vitro and in soil tests (Fernando *et al*, 2004). On the other hand, in vitro experiments on VOCs produced by the endophytic fungi *Muscodor albus* showed the toxicity of volatiles to peach pathogens, e.g. *Penicillium expansum, Botrytis cinerea* and *Monilinia fructicola* (Mercier & Jimenez, 2004). *M. albus* were also investigated for their potential to control molds in building s, as this fungi could significantly reduce growth of common molds (Mercier & Jimenez, 2007). Fungal VOCs were also reported to be useful indirect indicators of fungal growth in agriculture, monitoring spoilage, for chemotaxonomy purposes, for use in biofilters and for biodiesel, plant and animal disease detection, and for "mycofumigation"; the use of antimicrobial volatiles produced by fungi for the control of other organisms (Stinson *et* al, 2004; Hung *et al*, 2015). Since adhesion and aggregation on the surface of the leaves is important for successful colonization of the phyllosphere microbiota (Vorholt JA, 2013), long-distance mechanisms for antagonism are vital for their survival. Moreover, these organic compounds can serve as ideal signaling molecules in facilitating both short- and long-distance intercellular and organismal interactions (Bitas *et al*, 2013) because of their ability to move through air spaces as well as liquids (Effmert *et al*, 2012).

Aside from agricultural and economical importance of the phyllosphere microbiome, its beneficial effects on built environments have also been recently presented (Berg *et al*, 2014), and it was reported that the phyllosphere microbiome of plants have the potential to change microbial abundance and diversity in built environments (Mahnert *et al*, 2015).

Unraveling the phyllosphere microbiota diversity

All these potentials of the phyllosphere microbiome gained considerable interest over recent years and many studies aimed to get a deeper insight of the phyllosphere microbiome using culture-dependent and culture-independent techniques (Lindow & Brandl, 2003; Vorholt, 2012; Whipps *et al*, 2008). Establishment of knowledge on the phyllosphere microbial community structure and characteristics were initially based on

culture-dependent studies, however, the information it provides regarding microbial diversity were significantly limited since only minimal fractions (0.1 – 3.0 %) of environmental bacteria are culturable (Wagner *et al*, 1993). Thus, the development of culture-independent techniques, involving PCR amplification of 16S rRNA for bacteria, and internal transcribed spacer (ITS) region for fungi, provided a more complex identification of the phyllosphere bacterial community (Yang *et al*, 2001, Schoch *et al*, 2012). In recent years, different culture-independent techniques were developed to help study microbial diversity and community structure of the phyllosphere (Lindow & Brandl, 2003; Vorholt, 2012; Whipps *et al*, 2008).

Using various PCR methods, it was shown that the overall species richness in phyllosphere communities is high (Lindow & Brandl, 2003; Whipps *et al*, 2008; Vorholt, JA, 2012) and some microbial phyla, such as *Proteobacteria* and *Ascomycota,* predominate the phyllosphere of distinct plants (Whipps et al, 2008; Vorholt, 2012; Kembel & Müller, 2014). Across a wide range of agricultural crops and naturally growing plants, bacterial communities are largely composed of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes*, while *Ascomycota* and *Basidiomycota* dominate the phyllosphere fungal community at phylum levels (Rastogi *et al*, 2013; Andreote *et al*, 2014; Jumpponen & Jones, 2009, 2010; Kembel & Müller, 2014).

Drivers of microbial community structure in the phyllosphere

The growing interest on the phyllosphere microbiome extends beyond identification of the microbial community present on the leaves. Sources, drivers of community structure, and adaptation processes leading to the establishment of microorganisms on the phyllosphere have also been investigated in many studies (Vorholt, 2012; Whipps *et al*, 2008). Although successful colonization of microorganisms on the phyllosphere is a complex process influenced by many factors (Vorholt, 2012), establishment of certain antagonistic bacteria, at appropriate times during plant development, showed a strong influence on the composition of the phyllosphere bacterial community (Lindow & Brandl, 2003). Most researches on antagonistic plant-associated bacteria were exploiting the biological control potential of these microorganisms. Understanding this potential is

important in elucidating (i) the ecological role of these bacteria, (ii) their relationship with plants, and (iii) future biotechnological application (Berg *et al*, 2005).

The microbial community structure of the phyllosphere is affected by both environmental and biotic factors (Whipps *et al*, 2008; Vorholt, 2012; Rastogi *et al*, 2013). Interactions of plants with their environment controls prevailing conditions in the plant phyllosphere, determining microbial colonization and establishment of microorganisms on the leaf surface (O'Brien & Lindow, 1989; Whipps *et al*, 2008; Vorholt 2012).

Hitherto, no general conclusion has been formulated regarding major drivers of microbial phyllosphere composition, since not a single unifying factor could be identified affecting the overall phyllosphere assembly. A study on the phyllosphere microbiome of the salt excreting desert tree *Tamarix* showed that geographical location rather than plant species was found to be the major determinant of microbial community composition (Finkel *et al*., 2011). It was observed that different *Tamarix* species grown in the same geographical location haboured highly similar bacterial communities. On the other hand, a study on phyllosphere bacterial communities associated with *Pinus ponderosa* suggested that the plant drives the bacterial community composition on leaf surfaces (Redford *et al*, 2010). Regardless of the geographical location from which leaf samples were collected, the phyllosphere bacterial communities of *P. ponderosa* were relatively similar to each other. The leaves of 56 different tree species from the same location also showed plant species-specific bacterial communities. The significant correlation between plant species and bacterial community composition suggests a role of plant genetic factors in shaping the bacterial community of the phyllosphere (Redford *et al*, 2010; Whipps *et al*., 2008). Similar findings were reported on the study of spatial variation in fungal communities of European beech trees (*Fagus sylvatica*) and balsam poplar (*Populus balsamifera* L.). Host genetics were identified as a determinant of fungal community assembly on the foliage of beech leaves (Cordier *et al*, 2012), whereas pyrosequencing analysis of balsam poplar (*Populus balsamifera* L.) phyllosphere identified plant genotype as the driver of foliar fungal community composition (Bálint *et al*, 2013). The study of Bodenhausen *et al*, 2014 provided a strong evidence of the role of plant genotype in selecting specific

microbial populations. Mutations in the genes for cuticle formation, which resulted to a more permeable cuticle, on the leaves of *Arabidopsis thaliana* showed a strong effect on the associated bacteria. The mutations led to an increase in bacterial abundance and changes in the composition of the community.

Aside from environmental factors and plant genetics, management practices can also shape the microbial community of the phyllosphere. In agricultural settings practices such as organic vs. conventional farming, application of pesticides, antibiotics, and nitrogen fertilizers influence phyllosphere microbial composition (Ottesen *et al*., 2009; Zhang *et al*., 2009; Balint-Kurti & Stapleton, 2011; Ikeda *et al*., 2011). On the other hand, a study on fungal communities in the phyllosphere of oak trees (*Quercus macrocarpa*) under rural or urban management practices, showed that land use was the major driver in determining the fungal community composition, diversity, and richness on oak tree leaves (Jumpponen and Jones, 2009).

The plant samples: role in air quality improvement and botanical description

The 14 plant species used this study are classified as indoor or houseplants (Wolwerton, 1997; Orwell *et al*., 2004, Pegas *et al*., 2012). Aside from their aesthetic contribution, there have been reports that indoor plants help improved the indoor air quality by reducing air pollutants in an enclosed space (Wolverton, 1997; Orwell *et al*, 2004; Tarran *et al,* 2002; Wood *et* al, 2006; Claudi, 2011; Pegas *et al*, 2012). In the assessment made by Wolwerton (1997), 50 houseplants were ranked in order of effectiveness in improving air quality indoors using four criteria: 1) removal of chemical vapors, 2) ease of growth and maintenance, 3) resistance to insect infestation, and 4) transpiration rates. This report included *Nephrolepis spp*., *Epipremnum aureum*, *Dracaena fragrans, Dracaena marginata, Musa cavendishii* (syn: *Musa acuminata*), *Chlorophytum comosum, Aloe sp*., and *Aechmea sp*.; in order of effectiveness. *Howea fosteriana* and *Epipremnum aureum* were also reported to demonstrate the potential to remove gaseous-phase benzene from indoor air (Orwell *et al*., 2004) while *Dracaena marginata* was found to effectively remove benzene, carbon monoxide, carbon dioxide, VOC, particulate maters in the air, and soluble ions (Orwell *et al*, 2004; Tarran *et al*,

2002; Pegas *et al*, 2012). This section briefy describes the 14 plant species grown in the greenhouse of the Botanical Garden of Graz (Austia).

Sci. name: *Aechmea eurycorymbus*
Common name: Bromeliad
Description: plant large; wide-ranging roots; spectacular, tall inflorescence
Reference: Florida Council of Bromeliad Society (http://fcbs.org)

Sci. name: *Dracena marginata*
Common name: Madagascar dragon tree
Distribution: Madagascar
Description: an erect evergreen shrub to 4m, with long, sword-shaped, recurred, dark green leaves, finely edged reddish-brown
Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Sci. name: *Epipremnum aureum*
Common name: Devil's Ivy
Description: an evergreen self-clinging climber to 4m or more, with glossy bright green ovate leaves spotted and streaked with cream or yellow
Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Sci. name: *Musa x paradisiaca*

Common name: Edible banana

Description: a large herb, with succulent stem which is a cylinder of leaf-petiole sheaths, reaching a height of 20 to 25 ft (6-7.5 m) and arising from a fleshy rhizome or corm.

Reference: National Tropical Botanical Garden (http://www.ntbg.org)

Sci. name: *Dracaena fragrans*

Common name: Corn tree

Description: erect, sparsely branched evergreen shrub to 4m, the arching sword-shaped leaves with a central greenish-yellow stripe; seldom flowers

Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Sci. name: *Howea fosteriana*

Common name: Thatch-leaf palm

Distribution: Lord Howe Is., Australia

Description: single-stemmed evergreen palm to 2m in height, with dark green leaves to 1.5m in length, pinnately divided into several narrow segments. Flowers seldom produced under glass

Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Sci. name: *Malvaviscus penduliflorus*

Common name: Firecracker hibiscus

Description: a shrub up to 4 m tall with fairly dense split hairs on the stems. The leaves are oval or sword shaped, 4-10 cm long, with a pointed tip and toothed margins.

Reference: National Tropical Botanical Garden (http://www.ntbg.org)

Sci. name: *Nephrolepis cordifolia*

Common name: Erect sword ferm

Description: Stem scales spreading, concolored. Tubers present or absent. Leaves 2.5--10.7 × 0.3--0.7 dm. Petiole 0.3--2 dm, moderately to densely scaly; scales spreading, pale brown throughout.

Reference: Encyclopedia of Life (http://eol.org)

Sci. name: *Chlorophytum comosum*

Common name: Fern

Description: evergreen, perennial, clump-arching linear leaves with central creamy-white stripes, and sprays of small white flowers, often with plantlets among them

Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Sci. name: *Chlorophytum comosum*

Common name: Fern

Description: evergreen tree; sparsely branched, with terminal rosettes of sword-shaped leaves to 60cm in length and, on mature plants only, panicles of greenish-white flowers in summer followed by orange-red fruits

Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Sci. name: *Olea europaea*

Common name: Common Olive

Description: much-branched habit and slow growth, eventually 4.5-9m. Leaves are narrowly obovate or oval, to 7.5cm long, leathery, silvery beneath. Very small white flowers are borne in axillary racemes to 5cm long. Fruit rarely produced in Britain

Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Sci. name: *Aloe arborescens*

Common name: Krantz aloe

Description: many-branched, succulent shrub, with heads of green leaves arranged in attractive rosettes. These leaves are sickle-shaped and have margins armed with sharp, pale green teeth

Reference: Encyclopedia of Life (http://eol.org)

Sci. name: *Beaucarnea recurvat*

Common name: Elephant's foot

Distribution: Mexico

Description: evergreen perennial to 2m or more, forming a bulbous trunk-like stem bearing a dense rosette of spreading to recurved, sword-shaped leaves to 1m in length. Panicles of small white flowers are rarely produced

Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Sci. name: *Musa acuminata*

Common name: Dwarf Cavendish

Description: Dwarf Cavendish' is an evergreen perennial to 3m tall, with oblong leaves to 1.2m long; drooping spikes of yellow flowers with purple bracts open sporadically through the year, followed by edible yellow fruit

Reference: Royal Horticultural Society (https://www.rhs.org.uk)

Objectives and Summary of the thesis

The objective of our study was to elucidate the factors shaping microbiota composition of the phyllosphere of plants grown inside a built environment. The phyllosphere microbiome of 14 plant species (*Aechmea eurycorymbus, Aloe arborescens, Beaucarnea recurvate, Chlorophytum comosum, Dracaena draco, Dracaena marginata, Dracaena fragrans, Epipremnum aureum, Howea forsteriana, Malvaviscus penduliflorus Musa acuminata, Musa x paradisiaca, Nephrolepis cordifolia, Olea europaea*), representing phylogenetically different plant families as well as wide spread houseplants were analyzed for this purpose. All plants were grown under different controlled conditions (tropical, temperate, cold, desert microclimate) in the greenhouse of the Botanical Garden of Graz (Figure 1). To unravel the factors shaping microbiota composition – the plant genotype or the microclimate - we used the concept of the greenhouse as experimental design and analyzed the microbiomes of leaves by cultivation dependent and independent methods. In addition, to identify the functional potential of culturable bacterial strains we monitored the production of VOCs against a model pathogen *Botrytis cinerea*.

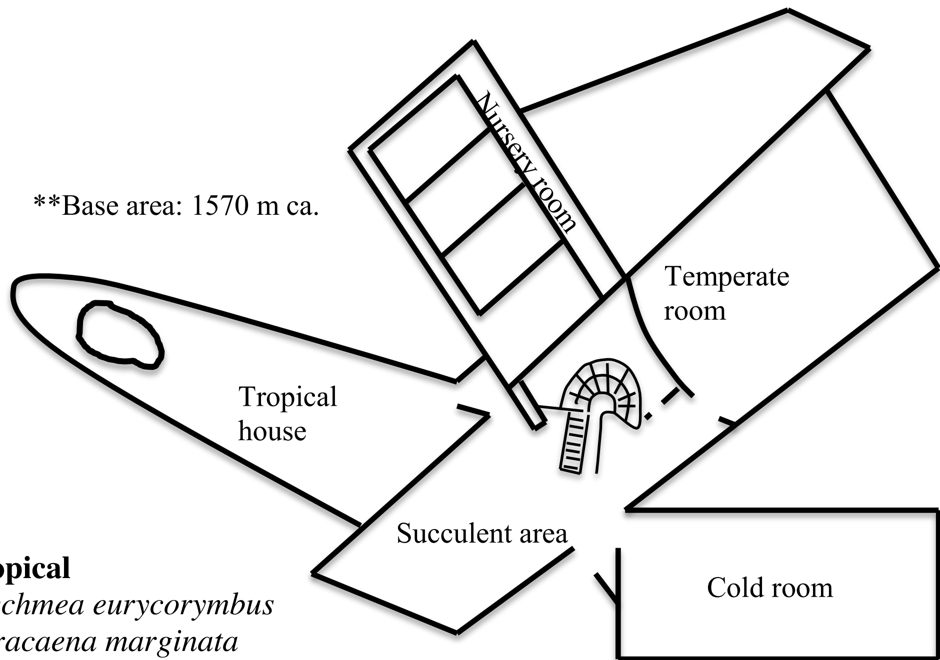
Bacterial communities in the phyllosphere of plants grown indoors exhibited genotype-specificity and highly active VOCs antagonism towards *B. cinerea* (Publication I)

This study indicates that plants grown indoors feature a distinct, stable microbial diversity with a high antagonistic potential producing diverse bioactive VOCs in the phyllosphere irrespective of the microclimate of a room that could have beneficial effects on microbial diversity and therefore our health inside buildings. All investigated plants showed high bacterial abundances (approx. 10^2 -10^6) on their leaves. Bacterial diversity and putative OTUs were strongly plant species-dependent but comprised similar dominant phyla; *Firmicutes, Proteobacteria* and *Actinobacteria*. A higher correlation of community composition was associated to plant genotype rather than the ambient microclimatic variables. The antagonistic potential of the phyllosphere microbiome towards the plant pathogen *Botrytis cinerea* measured by the production of antifungal volatile organic compounds (VOCs) was also highly different (2 to 58% of the isolates). Frequently isolated VOCs producers were represented by *Bacillus spec. div., Stenotrophomonas rhizophila and Kocuria spec. div*. Interestingly, the applied biopesticide *B. thuringiensis* Bt407 was found well-established and persistent in all sampled phyllospheres.

Plants grown indoors harbour genotype-specific fungal colonists that are active VOCs producers and antagonistic towards *B. cinerea* (Publication II)

An abundant and diverse fungal community inhabits the phyllosphere of 14 plants species grown under different controlled microclimates in the built environment of a greenhouse. All plants showed high population density of fungi ranging of approx. 10^2 -10^6. A pronounced interspecies variation within and across different rooms illustrates a strong influence of the plant species in the distribution of the fungal communities. Correspondingly, fungal diversity and putative OTUs were strongly plant species-dependent but comprised similar abundant fungi; *Capniodiales* from phylum *Ascomycota*, and order *Wallemialles* and *Tremellales* from the phylum of *Basidiomycota*. Non-metric multidimensional scaling and BIO-ENV analysis also showed correlation of

fungal community highly inclined to plant species, where the variability of the community composition is correlated to plant genotype. The phyllosphere fungal community also includes VOCs-producing species antagonistic to both the mycelial growth and spore germination of the pathogenic fungi *B. cinerea*. Along with these antagonists, some species were also found to have bipolar bioactivity against the pathogenic fungi. Frequently isolated active VOCs producers were mainly *Penicillum spec. div.*, *Cladosporium spec. div*, and *Cryptococcus spec. div*.



***Tropical**
a. *Aechmea eurycorymbus*
b. *Dracaena marginata*
c. *Epipremnum aureum*
d. *Musa x paradisiaca*

***Nursery**
e. *Nephrolephis cordifolia*

***Temperate**
f. *Dracaena fragrans*
g. *Howea fosteriana*
h. *Malvaviscus penduliflorus*

***Cold**
i. *Chlorophytum comosum*
j. *Dracaena draco*
k. *Olea europaea*

***Succulent**
l. *Aloe arborescens*
m. *Beaucarnea recurvata*
n. *Musa acuminata*

Figure 1. A) Complex plan of the Botanical Garden of Graz greenhouse and B) 14 plant samples with their respective room of origin*.

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Publication I

Plants grown indoors feature a genotype-specific and highly active microbiome towards pathogens in their phyllosphere

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Abstract

The indigenous phyllosphere microbiome was identified as a key component for plant growth and health, and for positive effects on microbial diversity within a built environment. Nevertheless, there is still limited understanding of the phyllosphere microbiome and its driving factors. To study the variability of the microbiome in relation to plant genotype and ambient microclimate, we investigated 14 phylogenetically diverse plant species grown under different controlled conditions in the greenhouse of the Botanical Garden in Graz (Austria). All investigated plants showed high individual bacterial abundances of approx. 10^6 CFU cm^{-2} on their leaves. Bacterial diversity (H'=4.1–6.8) and number of putative OTUs (501 – 1,097) were strongly plant species-dependent but comprised similar dominant phyla *Firmicutes, Proteobacteria* and *Actinobacteria*. Non-metric multidimensional scaling and BIO-ENV analysis showed a higher correlation of community composition to plant genotype rather than the ambient microclimatic variables. The antagonistic potential of the phyllosphere microbiome towards the plant pathogen *Botrytis cinerea* measured by production of antifungal volatile organic compounds (VOCs) was also highly different and ranges from 2 up to 58% of the isolates. Frequently isolated VOCs produces were represented by *Bacillus spec. div., Stenotrophomonas rhizophila and Kocuria spec. div*. Interestingly, the applied biopesticide *B. thuringiensis* Bt407 was found well-established in all phyllospheres. This study indicates that plants grown indoors feature a distinct, stable microbial diversity with high antagonistic potential producing highly bioactive VOCs in the phyllosphere irrespective of the microclimate of a room that could have beneficial effects on microbial diversity and our health inside buildings.

Keywords

Microclimate, correlation to genotypic distance of plants, plant phylogeny, indoor plants, built environments

Introduction

Plants harbour different microbial communities specific for each plant organ, for example the phyllosphere (Vorholt, 2012), rhizosphere (Berendsen *et al*., 2012; Philippot *et al*., 2013), and
60 endosphere (Hardoim *et al*., 2015). The aboveground part of a plant is dominated by the leaves with an estimated global leaf area of 10^9 km^2 km^2 (Woodward & Lomas, 2004). Although filamentous fungi, archaea, yeast, and algae are known to inhabit the leaves, bacteria are mainly the most dominant microbial colonists of the phyllosphere found at an average of 10^6 – 10^7 bacterial colonies per cm^2 of leaf surface (Lindow & Brandl, 2003).
65 However, in comparison to the well-studied plant compartment rhizosphere less is known about the drivers of phyllosphere communities. The structure of the rhizosphere communities is influenced by the soil type but has also a strong plant species-specific component (Smalla *et al*., 2001; Berg & Smalla, 2009; Bulgarelli *et al*., 2012). Extend of the latter depends on the plant family and its secondary metabolites; and was shown for several plant species up to the
70 cultivar level (Cardinale *et al*., 2015). In general, leaves have different strategies to trigger microbial colonization, for example (antimicrobial) wax layers, (antimicrobial) secondary metabolites, trichomes, and hairs, and the microbial composition seems to be highly individual but also plant-dependent (Bodenhausen *et al*., 2014; Ritpitakphong *et al*., 2016). Recently, carbohydrates such as sucrose, fructose and glucose and amino acids influencing
75 bacterial colonization were identified on the *Arabidopsis* leaf surfaces by environmental metabolomics (Ryffel *et al*., 2016). Disentangling the factors shaping microbiota composition is an important objective to improve plant growth and health (Hacquard, 2016). However, an overview of a broader range of plant phyla is still missing. The phyllosphere represents the plant-air interface; we expected an impact of both biotic and abiotic conditions on the
80 structure, diversity and function of the phyllosphere microbiome.

Phyllosphere colonizing bacteria are not only residents on and in leaves; they help stimulate plant growth and inhibit or promote pathogen infection of plant tissues (Lindow & Brandl, 2003; Kishore *et al*., 2005). In addition, phyllosphere bacteria can play a key role in carbon and nitrogen cycling (Delmotte *et al*., 2009; Freiberg, 1998), and help in important
85 environmental processes such as methanol degradation (Corpe & Rheem, 1989; Van Aken *et al*., 2004), and nitrification (Papen *et al*., 2002). Plants grown indoors provide specific conditions for microorganisms. A distinct bacterial signature was shown for lettuce grown in greenhouse and in the field (Williams & Marco, 2016) but house plants are less yet studied. Recently, beneficial effects of phyllosphere bacteria on built environments and their potential

90 to change microbial abundance and diversity in built environments have been reported (Mahnert *et al*., 2015). Due to the fact that we spent most of our lifetimes in built environments in many parts of the world, plants can be an important source for the human microbiome (Berg *et al*., 2014). Despite this potential there is limited information about the functional diversity of phyllosphere bacterial communities on the surface of the leaves of
95 plants grown inside a built environment. Plant-associated bacteria were recently shown to interact both with host plants and other microbial species through the emissions of volatile organic compounds (VOCs) that has been neglected for a long time (Ryu *et al*., 2003). VOCs have a great importance as signaling molecules in fungal-bacterial interactions (Schmidt *et al*., 2016) and are able to suppress fungal plant pathogens (De Vrieze *et al*., 2015). However,
100 nothing is known about their function on indoor plants.

The objective of our study was to analyze the phyllosphere microbiome of 14 plant species (*Aechmea eurycorymbus, Aloe arborescens, Beaucarnea recurvate, Chlorophytum comosum, Dracaena draco, Dracaena marginata, Dracaena fragrans, Epipremnum aureum, Howea forsteriana, Malvaviscus penduliflorus Musa acuminatafrom Musa x paradisiaca,*
105 *Nephrolepis cordifolia, Olea europaea*), which represent phylogenetically different plant families as well as wide spread house plants. All plants were grown under different controlled conditions (tropical, temperate, cold microclimate) in the greenhouse of the Botanical Garden of Graz. To disentangle the factors shaping microbiota composition – the plant genotype or the microclimate - we used concept of the greenhouse as experimental design and analyzed
110 the microbiomes of leaves by amplicon analysis and statistical methods. In addition, to identify the functional potential of culturable bacterial strains we monitored the production of VOCs against a model pathogenic fungus *Botrytis cinerea*.

Materials and Methods

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Site description and plant maintenance inside the greenhouse

Samples were collected from a greenhouse at The Botanical Garden of Graz. The Botanical Garden is situated in Graz, Austria at 47°04'55" N, 15°27'28" E, with an elevation of 378 m above sea level. The greenhouse complex has four different rooms simulating different
120 terrestrial climatic conditions and a nursery room where all the young plants and seedlings can be found (Figure S1). Plants-care measures for the greenhouse plants include watering, and fertilizer and microbial pesticide application. Watering of plants in different rooms vary in regularity and is dependent on the different seasons. Plants in the Cold room, for example,

are watered only in the morning during winter, while those found in the Tropical room are
125 watered more frequently. The Botanical Garden has a cistern that catches rainwater and this serves as reservoir for watering of the plants. Two types of fertilizer are used to help maintain healthy plants: 1) is a NPK liquid fertilizer for foliar application (Wuxal® Top N), and 2) is a water-soluble Phosphate and Potash nutrient (Hakaphos® Rot 8+12+24+(4)) applied in the soil. Application of these fertilizers also varies depending on the state of plant health. The
130 biological pesticide DiPel® is also used to protect the leaves of greenhouse plants from *Lepidoptera* larvae (caterpillar) that forages on them. This pesticide contains the naturally occurring bacteria *Bacillus thuringiensis* (Bt) *kurstaki* known for its toxicity on caterpillars. DiPel® application is done when there is an apparent infestation of Lepidopteran larvae, and is applied by spraying the solution on the leaves of affected plants. Along with foliar fertilizer
135 and microbial pesticide, a non-ionic surfactant (Break Thru® S240) is also applied to safeguard the effectiveness of the treatments. Both measures were done in all greenhouse rooms except the Nursery room.

Sampling design and procedure

140 Leaves of 14 species of indoor plants were collected using ethanol-washed disposable gloves and sterile instruments. They were separated from the rest of the plant by cutting from the base of the petiole avoiding any possible contact with the leaf blade. Immediately after collection samples were placed inside 25 x 32 cm freezer bags (ARO freezer bags, Düsseldorf, Germany) and stored in a portable cooler with ice packs (GIO'STYLE Colombo
145 Smart Plastics, Italy). All samples were immediately transported back to the laboratory at the Institute of Environmental Biotechnology, Graz University of Technology (TU Graz), Graz, Austria for microbial isolation and DNA extraction.

To wash the microbial cells off the leaves, 720 cm^2 of a leaf was placed inside a freezer bag (doubled as precaution from wear and tear) containing 50 ml 0.85% NaCl solution with
150 Tween 80 and was subjected to a series of washing and vortexing. Washing was done by subjecting the leaf through bag-mixer treatment (BagMixer Interscience, St. Nom, France) for 3 minutes. This step is immediately followed by vortexing, using a Transsonic Digital T910 DH sonicator (Elma™, Singen, Germany), for 3 min at 60 Hz. Right after the first sonication step, bag mixer treatment for 1 min, sonication for 3 min at 60 Hz, and a final bag mixing for
155 1 min, follows consecutively, to complete the series. The resulting microbial solution was then transferred to a 50 ml Sarstedt tube. For culture-dependent experiments, 100 µl out of the 50 ml solution was serially diluted ten-folds and plated on both R2A (Carl Roth GmbH + Co.

KG, Karlsruhe, Germany) and Nutrient Broth II (NB II) agar media (SIFIN, Berlin, Germany) in duplicates. Then, the remaining microbial solution was centrifuged (using Sorvall RC-5B

160 Refrigerated Superspeed Centrifuge; DuPont Instruments™, USA) at 6 169 g for 20 min to pellet cells. The moist pellets were then transferred to a 2.0 ml sterile Eppendorf tubes and were further centrifuged at 18 000 g for 20 minutes. Pellets obtained from this final process were then frozen at −70°C until it was used for DNA extraction.

165 **16S rRNA profiling using MiSeq Illumina Sequencing**

Genomic DNA was extracted using FastDNA® SPIN kit for soil (MP Biomedicals, Solon, OH, USA) as directed in the instruction manual with a revised first step, where pellets (from bacterial isolation) instead of soil sample was used. A total of 56 DNA samples were extracted; four replicates for each of the 14 plant samples.

170 PCR amplifications targeting the V4 region of the 16S rRNA gene were conducted for each of the 56 samples using 515f/806r primers carrying sample-specific tags (Caporaso *et al*., 2011). Using the thermocycler TC-Plus (TECHNE, Staffordshire OSA, UK), DNA was amplified in triplicate PCR reactions (50 µl each); 25 µl of 2 x Plant buffer, 0.40 µl of 1 x KAPA3G Plant DNA polymerase, 3 µl of 5 µM for each primer, 17.60 µl PCR water, and 1µl of the DNA

175 template (95°C, 3 min; 32 cycles of 95°C, 30 s; 60°C, 15 s; 72°C 12 s; and elongation at 72°C, 30s). Amplicons from three independent reactions were then pooled and purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA).

Purified amplicons were pooled in equimolar concentrations and 2 mg of total DNA was sent for sequence generation using the Illumina MiSeq platform (Eurofins Genomics,

180 Ebersberg, Germany) with chemistry version 3 (2 x 300bp).

Bioinformatics and Statistics

Illumina reads were filtered and sorted according to barcodes on 5' and 3' prime site. Raw reads are accessible under project XXX in the European Nucleotide Archive (www.ebi.ac.uk).

185 Corresponding forward and reverse reads were joined (overlap 100 bp, mismatch 0.03) and length (200 – 400 bp) and quality (phred q30) filtered in QIIME 1.8.0 (Caporaso et al., 2010; Mahnert et al., 2015). Barcodes and primer sequences were trimmed and chimeric sequences (reference: Greengenes gg_13_8) were removed with usearch (Edgar, 2010). OTUs (operational taxonomic units) were picked with Greengenes gg_13_8 as an open reference.

190 The resulting OTU table in biom format served as input for following alpha and beta diversity analysis and statistics. Adonis, ANOSIM (analysis of similarities), ANOVA (analysis of

variances), MRPP (multi response permutation procedure), BioEnv, and mantel tests were calculated in QIIME and R (vegan package) with 999 permutations (R Core Team, 2014; Fierer et al., 2010; Dixon, 2009; Oksanen et al, 2013).

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Cultivation and isolation of bacteria

Agar plates, where serially diluted 100 µl of microbial solution was plated, were incubated at room temperature for 5 days. A colony count was done on the fifth day and final counts were expressed as CFU log10 cm^{-2} leaf. Colonies with distinct phenotypes were then transferred in

200 96-well plates with NB II medium and 30% glycerol for storage. Each strain was numbered according to plant sample genus (except for *Aechmea eurycorymbus* where the common name Bromelia was used as a reference), origin, and isolation medium (e.g. Dth1N1: bacterial isolate from *Draceana* from the Tropical house grown on NB II medium). All isolates were kept in a refrigerator (-70°C) at the Institute of Environmental Biotechnology, TU Graz, Graz,

205 Austria.

Functional characterization of isolates

In analyzing the antagonistic property of the volatiles produced by the bacterial samples, Two-clamp volatile organic compounds assay (TCVA) was performed using the set-up

210 described in Cernava et al., (2015). *B. cinerea*, maintained on a Potato Dextrose Agar (PDA), from the Institute of Environmental Biotechnology, TU Graz was used as the model pathogen for this study. Fungal inoculum was prepared by growing the fungus for 6 days on fresh PDA medium. After this period, *B. cinerea* isolate was observed to have well-developed hyphae and is already sporulating.

215 A total of 1284 bacterial isolates were screened for their antagonistic activity against the pathogenic fungi *B. cinerea*. Isolates were streaked onto Nutrient Agar (NB II with agar) in 6-well plates and incubated for 24 h at 30 °C. After the 24 h incubation period, plates observed positive for growth were clamped together with newly made *B. cinerea* 6-well plates. *B. cinerea*-containing plates were prepared by cutting 5 mm plugs from a 6-day old *B. cinerea*

220 inoculum plate and placing it on the center of each well of a 6-well plate with Synthetic Nutrient-Poor Agar (SNA); pH adjusted to 5.5. Setting up of the plate-pair was done according to Cernava et al (2015) and was done in quadruplicates. The set-up is incubated at room temperature for 3 days under dark conditions to eliminate any light-induced effect on the experiment (Mares et al, 2004). Inhibition of growth was measured as percent (%) .

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Structural characterization and identification of isolates

BOX-PCR fingerprint analysis of the antagonistic isolates was done to avoid analysis of genetically similar strains. To extract bacterial DNA, stored bacterial samples were reactivated and incubated for 24 h at 30°C. After incubation, colonies that were positive for
230 growth and clear of contamination were picked for homogenization. Each colony was mixed in100 µl of double distilled water with 200 mg glass beads in a 2-µl microtube with cap (SARSTEDT, Germany), homogenized using MP FastPrep-24 sample preparation system ribolyser (Irvin, Calif., USA) (30 s; 6 ms$^-$). Homogenized samples were then frozen (30 min at -20°C), heated (100°C), and immediately centrifuged (16 000 g for 5 min at 4°C; HERMLE
235 Labor Technik, Germany). Using the Tpersonal Combi, Biometra thermocycler (Biometra GmbH, Germany) DNA was amplified in 25 µl PCR reaction mix; 1 µl of the extracted DNA, 5 µl of Taq&Go, 2.50 µl of 100 pmol ml$^-$ BOX A1R primer (5' CTA CGG CAA GGC GAC GCT GAC G 3'), and 16.50 µl PCR water. (95°C, 6 min; 35 cycles of 94°C,1 min; 53°C, 1 min, and 65°C, 8 min; with final extension at 65°C, 16 min). An aliquot of 12 µl PCR product
240 was separated by gel electrophoresis (1.5 % agarose gel in 0.5 x TBE buffer; 4 h). The gel was then stained with ethidium bromide, and photographed under UV transillumination using GelDoc 2000 (BIO-RAD,USA). The resulting BOX-PCR fingerprints were evaluated using the GelCompar program (Kortrijk, Belgium) Cluster analysis was done using unweighted pair-group average (UPGMA) algorithm.

245 A representative strain from the different BOX clusters produced was used for DNA extraction. Each strain was grown on different NA plates and incubated for 24 h at 30°C. After incubation, a colony was picked (from each strain) and diluted in 30 µl PCR water in a 96-well PCR plate half skirt with coverfoil (SARSTEDT, USA). A denaturation step was done using the thermocycler TC-Plus (TECHNE, Staffordshire OSA, UK); preheating of lid
250 at 105°C, followed by denaturation at 98.05^{o}C for 15 min, and a final hold at 10°C. This step was immediately followed by centrifugation using Centrifuge 5810 R (Eppendorf, Germany) at 3 220 g for 2 min. After DNA extraction, PCR amplification for each strain was performed using 30 µl of PCR reaction mix (6 µl Taq&Go, 1.5 µl 27f primer, 1.5 µl 1492r primer, 20 µl PCR water, and 1 µl DNA template). Amplification was done with an initial denaturation at
255 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 57°C for 30s, and 72°C for 1 min 30 s with a final extension at 72°C for 5 min using a TC-Plus thermocycler (TECHNE, Staffordshire OSA, UK). After amplification, an aliquot of 5 µl PCR product was separated by gel electrophoresis in 0.8 % agarose gel in 1 x TAE buffer for 1 h. Then the gel was stained with ethidium bromide and photographed under UV transillumination using GelDoc

260 2000 (BIO-RAD, USA). Amplicons were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA), then nucleic acid was quantitated using Nanodrop 2000c spectrophotometer (PeQlab, VWR International GmbH, Erlangen, Germany) before the template DNA solution were prepared for SANGER sequencing. A 14 µl DNA solution with 40 ng μl^{-1} concentration and specific primers (27f) were sent to LGC Genomics
265 (Berlin, Germany) for sequencing. Sequences were identified into specific bacterial species using the BLAST algorithm against the NCBI BLASTn 16S rRNA gene reference database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). SANGER sequences with a minimum length of 700 bp were considered acceptable for analysis.

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Results

The structure of the phyllosphere bacterial communities

A total of 1.04 M sequences affiliated to bacteria and archaea were generated for the 14 plant
275 samples of different species. The average sequence per sample was 18 594.68; ranging from 757 to 61 561. An average of 12 704 operational taxonomic units (OTUs) per sample were identified at 97% similarity, corresponding to the standard definition of bacterial "species". Amplicon sequencing results showed that abundant phyla include *Firmicutes* (40.4 %), *Proteobacteria* (22.5 %), and *Actinobacteria* (15.7 %) (Fig. 1). Two archaeal phyla were also
280 found, namely *Crenarcheota* and *Euryarchaeota* with 0.13 % and 0.01 % abundance, respectively.

Members of the phylum *Firmicutes* were most abundant on the phyllosphere of plants from the Tropical, Temperate and Succulent rooms of the greenhouse complex, while *Proteobacteria* and *Deinococcus-Thermus* were most abundant on the phyllosphere of plants
285 from the Cold and Nursery room, respectively (Fig. 2). It was also noted that the most abundant bacterial phylum differs for each plant. *Firmicutes* was found most abundant on the leaves of *Epipremnum aureum* (88.6 %), *Musa x paradisiaca* (61.4 %), *Dracaena fragrans* (74.7 %), *Howea fosteriana* (48.8 %), *Dracaena draco* (62.5 %), *Olea europaea* (40.5 %), and *Beaucarnea recurvata* (77.5 %), while *Proteobacteria* was observed most abundant on
290 the leaves of *Malvaviscus penduliflorus* (50.6 %) and *Chlorophytum comosum* (44.0 %). On the other hand, *Actinobacteria* was found most abundant on the leaves of *Dracaena marginata* (35.9 %) and *Musa acuminata* (56.0 %), and phylum *Deinococcus-Thermus* on the leaves of *Aechmea eurycocymbus* (40.7 %), *Nephrolepis cordifolia,* (44.1 %) and *Aloe*

arborescens (41.8 %) as shown in Fig. 3. Variability in the taxonomic structure of the
295 phyllosphere community was also observed for the different replicates of each plant sample (Fig. S2).

The relative abundance of dominant genus was also analyzed at 1% cut-off level and shown in Fig. 4. Analysis showed that abundant genus includes an unidentified genus from the Family *Bacillaceae* (30.1%), *Deinococcus* (10.4%), *Arthrobacter* (4.6%), *Sphingomonas*
300 (4.4%), and *Bacillus* (4.2%). The unidentified genus from *Bacillaceae* was observed ubiquitous in all the plant samples, while *Bacillus* was found present in all samples except on *Neprholepis cordifolia*, and *Sphingomonas* was present in all samples except *Epipremnun areum* and *Beaucarnea racurvata*.

305 **The diversity of the phyllosphere bacterial communities**

Rarefaction analysis together with Chao1 value revealed variation in the phyllosphere bacterial communities per greenhouse room and per plant sample. The rarefaction curves of phyllosphere bacterial communities from different greenhouse rooms are shown in Fig. 5. The curves showed low slopes and did not reached the saturation point. Correspondingly, the
310 number of OTUs observed covered only 26.2 % - 29.4 % of the estimated taxonomic richness (Chao1) as seen in Table 2. The computed Shannon index of diversity (H') per greenhouse room was found highest in plants inside the Cold room (6.6) and is lowest in plants inside the Tropical room (4.2). Statistical analysis using Two-sample T-Test showed that H' of bacterial communities found on the plants from the Cold room is significantly different to H' of
315 phyllosphere bacterial communities in the Tropical and Succulent rooms but not to the rest of the rooms. It was also observed that the H' of the phyllosphere bacterial community in the Tropical house is significantly different from the H' of the bacterial communities found on the plants inside the Temperate room but not to the Nursery and Succulent rooms (Table S3). Fig. 6 shows the rarefaction curves of phyllosphere bacterial community for each plant sample.
320 The curves also showed low slopes that did not reach saturation. Consequently, percent coverage ranges from 23.7 % - 33.3 % of Chao1 (Table 3). On the other hand, computation of Shannon index of diversity (H') per plant revealed a wider range of value, where *Chlorophytum comosum* showed highest at 7.2 and *Epipremnum aureum* showed lowest at 2.5. However, statistical analysis using Two-sample T-Test showed significant differences in
325 diversity per sample (Table S6).

Drivers of bacterial community structure

In order to determine the uniqueness of the associations of the phyllosphere bacterial
330 communities to room microclimate and plant host species, ordination analysis and ANOSIM were performed. BIO-ENV analysis was also done to define which abiotic and/or biotic variables have higher correlation with the dissimilarity of the communities of phyllosphere bacteria using the Euclidean distance (Clarke and Ainsworth 1993). Analysis using relative abundance-based (Bray-curtis dissimilarities) PCoA showed inconspicuous clustering of the
335 phyllosphere bacterial communities (Fig. 7A and 7B). The phyllosphere bacterial communities of the Nursery room and Succulent rooms were highly scattered with overlapping communities to Temperate and Cold rooms, while the communities in the Tropical room was distant to the rest of the room with a slight overlap to the Succulent room. Furthermore, phyllosphere bacterial communities in the Temperate and Cold rooms were
340 distinctly closer to one another than to the rest of the rooms. Nevertheless, ANOSIM results showed significant correlation between greenhouse rooms and bacterial community composition ($P=0.001$, $R=0.25$) suggesting that, although weak, there was some association between room microclimate and phyllosphere bacterial community composition.

On the other hand, ANOSIM showed a stronger correlation between phyllosphere
345 bacterial communities and plant samples ($P=0.001$, $R=0.66$), and it was observed associated to two plant leaf attributes. The first association was between bacterial communities and plant leaf-shape. *Dracaena fragrans*, *Howea fosteriana*, *Dracaena draco*, and *Beaucarnea recurvata,* having a common linear, long, non-lobed, sword-like (ensiform) leaf-shape, exhibited bacterial communities that were clustered closer to each other than to rest of the
350 plants (Fig. 7B). However, plants with similar leaf-shape but exhibited different leaf sizes showed more distant bacterial community clusters, thus revealing the second leaf attribute correlated to bacterial community composition. This was made apparent when bacterial communities of plants from the same genus were compared. The two *Musa* species shared a common oblong, long, and wide leaf-shape but *Musa x paradisiaca* have a longer leaf size.
355 Consequently, ordination analysis showed highly distant clusters of bacterial communities between the two *Musa* species. The same correlation was observed in the sample plants from the genus *Dracaena* where the phyllosphere bacterial communities of *Dracaena fragrans* and *Dracaena draco* clustered together, but that of *Dracaena marginata* were clustered distantly. Closer observation of the leaf morphology showed that the leaves of *Dracaena marginata* are
360 shorter and more narrow, thus are smaller in comparison to the leaves of *Dracaena fragrans* and *Dracaena draco* which exhibits remarkable similarity.

BIO-ENV analysis provides further evidences of the stronger correlation between bacterial community and plant species. In Fig. 8, the vectors represent the Spearman rank correlations (ρ_S) between the abiotic and biotic factors influencing the distribution of the
365 bacterial community on the leaf surface of the greenhouse plants. According to BIO-ENV analysis, "Samples" (i.e. plant species) had strong influence on the bacterial population dynamics in the phyllosphere of the greenhouse plants, being the variable that explains the distribution of the relative abundance of the bacterial community (*BEST = 0.9157*).

370 **Population densities of phyllosphere communities**

The highest abundance of culturable bacteria was found on the leaves of *Chlorophytum comosum* where 5.05×10^6 and 6.69×10^6 CFU cm^{-2} was recorded from the R2A and NA media respectively, while the lowest density was observed on the leaves of *Musa acuminata* with 336 (R2A) and 294 CFU cm^{-2} (NA) as shown in Table 1. One-way ANOVA results
375 (Table S1) showed that there are significant differences in the bacterial population densities on the phyllosphere of 14 greenhouse plants ($F\ crit=2.5;\ F=47.9;\ p\text{-}level<0.05$), and Tukeys HSD test correspondingly showed three groupings of bacterial population densities with significant differences (Table S2). Tukeys HSD grouping showed that CFU observed from *Chlorophytum comosum* is significantly different from the rest of the plant while CFU from
380 *Aechmea eurycorymbus* showed significant difference to rest of the plant except to *Dracaena marginata*, and *Musa x paradisiaca* (Table 1).

Antagonistic potential of phyllosphere bacterial community against *B. cinerea*

A total of 1284 bacterial isolates from the phyllosphere of 14 greenhouse plants were
385 screened for their antagonistic potential against *B. cinerea* using TCVA. Antagonistic effect observed includes inhibition of mycelial growth and spore germination (Fig. 9). Table 1 showed the number of isolates per plant sample and the percentage of bacterial strains that tested positive for both antagonistic effects. *Beaucarnea recurvata* showed the highest percentage of antagonistic bacterial strains where 58% of the 96 bacterial isolates exhibited
390 inhibitory effect on both mycelial growth and spore germination of *B. cinerea*.

This study focused on identifying the 233 isolates that showed optimum antagonistic potential against the model pathogenic fungi. BOX-PCR fingerprinting and analysis further divided these isolates into 49 genotypic groups at 60 % cut-off level and SANGER sequencing identified 24 species out of the 49 genotypic groups (Table 4). Frequently isolated
395 antagonistic bacterial species includes: *Bacillus thuringiensis* Bt407 (10), *Bacillus toyonensis*

BCT-7 112 (6), *Stenotrophomonas rhizophila* e-p10 (6), *Bacillus cereus* ATCC 14579 (4), *Kocuria haloterans* YIM 90716 (3), and *Kocuria sedeminis* FCS-11 (2). There was a high representation of the genus *Bacillus* with seven different *Bacillus* species identified (*B. thuringiensis, B. licheniformis, B. cerues, B. aures, B. toyonensis, B. anthrasis* and *B.*
400 *subtilis*). Three days after the initial set-up, the VOCs produced by these bacterial species decreased the fungal colony diameter by about 36% – 61% compared to the control. The mean percent inhibitions caused by each bacterial species are also shown in Table 4 and ANOVA results showed no significant differences between the means (Table S7).

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Discussion

Recent studies revealed that the ecological importance of the phyllosphere-associated microbes is also beneficial in establishing the microbiome of a built environment that
410 ultimately affects human health (Oberauner *et al*., 2013; Berg *et al*., 2014; Mahnert *et al*., 2015). This study examined the phyllosphere bacterial community profile of 14 different greenhouse plants grown under controlled greenhouse conditions. Using this unique experimental design we were able to find out that the plant genotype is the most important driver determining the phyllosphere microbiome structure and function. Moreover we find out
415 that a high proportion of phyllosphere-associated bacterial is able to produce VOCs; many of them were highly active against the fungal plant pathogen *Botrytis cineria*, which support their functional role to defend pathogens.

The plant genotype was identified as the most important driver determining the phyllosphere microbiome structure of the 14 greenhouse plants. All leaves displayed a high
420 and individual bacteria diversity but at phylum level, the composition was very similar. *Proteobacteria* and *Actinobacteria* were often reported on phyllospheres (Whipps *et al*., 2008; Zhang *et al*., 2010; Redford *et al*., 2010). However, contrary to most literatures where *Proteobacteria* was reported dominant in the phyllosphere (Vorholdt, 2012; Bodenhausen *et al*., 2013; Izhaki *et al*., 2013; Turner *et al*., 2013), the phylum *Firmicutes* was found dominant
425 in the overall bacterial community profile of the 14 greenhouse plants. There can be two reasons for the high proportion of Firmicutes: i) the controlled greenhouse conditions and ii) the application of the biopesticide Dipel®, consisting of the *Bacillus thuringiensis* strain. Until now, only short-term effects of *B. thuringiensis* on the microbial population structure were reported (Raymond *et al*., 2010). In contrast, we found the *Bacillus* strain well established in

430 all investigated leaves. Another remarkable observation was the presence of *Deinococcus-Thermus* in all of the plant samples and its high abundance in the phyllosphere of *Aechmea eurycorymbus*, *Nephrolephis cordifolia*, and *Aloe arborescens*. Members of this bacterial phylum are known for their ability to tolerate an array of environmental stress including resistance to UV radiation (Blasius *et al*., 2008; Liedert *et al*., 2012; Lin *et al*., 2016) that can
435 enable them to thrive on the hostile phyllosphere environment. These findings supports previous studies suggesting that the presence of this bacterial phylum maybe more common in the phyllosphere than previously acknowledged (Redford *et al*., 2010; Shade *et al*., 2013). The bacterial communities inhabiting the leaves of the 14 greenhouse plants also showed pronounced interspecies variation within and across different greenhouse rooms. These
440 findings support previous reports of interspecies variability in microbial phyllosphere communities (Yang *et al*., 2001; Whipps *et al*., 2008; Redford *et al*., 2010) and suggest a stronger influence of the plant species on the structure and composition of their associated bacterial communities. Further investigation on the plant species effect on the bacterial community composition revealed community assembly patterns driven by plant leaf
445 morphology. Since microbial communities on the leaves relate to a large degree on the phenotypic characteristics of the plant that is ultimately controlled by their genetic background (Whipps *et al*., 2008; Cordier *et al*., 2012; Bodenhausen, 2014), this correlation between leaf morphology and dissimilarity in bacterial assembly implies an effect of the plant genotype on the bacterial community. Thus, supportive of previous literatures, this study
450 showed a direct influence of the plant host on the phyllosphere microbial community composition correlated to the plant genotype (Redford *et al*., 2010; Cordier *et al*., 2012).

Cultivation-depended techniques also revealed that the phyllosphere of 14 greenhouse plants harbored bacterial isolates that exhibits antagonistic VOCs activity to both the growth and sporulation of *B*. *cinerea*. Bacterial isolates identified includes those that were previously
455 reported to have VOCs antagonistic activity to different pathogenic fungi including a frequent isolation of *Bacillus*, *Stenotrophomonas*, and *Kocuria* species (Fiddaman & Rosall, 1993; Sharifi *et al*., 2003; Kai *et al*., 2007; Mojica-Marín *et al*., 2008; Islam *et al*., 2012).

The results of this study provide insights into the structure and function of phyllosphere bacterial communities of plants grown inside a built environment. It was established that the
460 ambient room microclimate had little influence on the phyllosphere communities since plants species exhibited high variation of bacterial community composition within and across different greenhouse rooms. This implies that plants have a stable bacterial diversity composition regardless of the room microclimatic condition. The implication of plant species

having higher influences on the bacterial community composition of their associated microbes
465 can be beneficial in setting a healthy built environment that is ultimately favorable to human health. It presents the possibility of establishing the room microbiome by choosing plant species placed indoors (Mahnert *et al*., 2015) and possibly increase microbial diversity and beneficial microorganisms (Berg *et al*., 2014). It can also be beneficial in maintaining a healthy indoor air quality of built environments, since both plants placed indoors (Orwell *et*
470 *al*., 2004, Pegas *et al*., 2012) and their associated microbes work together in improving the air quality by absorbing, degrading, detoxifying, and sequestering air pollutants (Kim et al., 2008; Weyens *et al*., 2015). It also poses the possibility of limiting the growth of pathogenic molds and fungi, which are harmful to human health and can possibly cause "sick building" syndrome (Strauss, 2009), since the phyllosphere bacterial community also includes species
475 that produces antifungal volatiles.

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Table 1. Plate counts (CFU/cm^{-2}) and percentages of antagonistic bacteria against the plant pathogenic fungi *Botrytis cinerea*.

Greenhouse room	Plant sample	CFU ± SD		Total isolates tested	Inhibits both growth and sporulation	
		R2A	NA		No. of isolates	%
Tropical	*Aechmea eurycorymbus*	1.61E+06 ± 2.95E+04	1.48E+06 ± 1.34E+05	96	2	2
	Dracaena marginata	1.13E+06 ± 1.68E+04	9.01E+05 ± 6.14E+04	96	36	38
	Epipremnum aureum	2.99E+05 ± 1.68E+04	2.04E+05 ± 6.14E+04	96	17	18
	Musa x paradisiaca	8.13E+05 ± 3.92E+05	5.67E+05 ± 2.02E+05	96	1	1
Temperate	*Dracaena fragrans*	4.53E+04 ± 2.14E+04	8.39E+03 ± 3.75E+03	96	21	22
	Howea forsteriana	2.11E+04 ± 1.89E+04	3.32E+03 ± 1.45E+03	96	35	36
	Malvaviscus penduliflorus	2.41E+04 ± 2.06E+04	3.17E+04 ± 3.00E+04	90	2	2
Nursery	*Nephrolepis cordifolia*	1.44E+05 ± 1.04E+05	1.12E+05 ± 1.09E+05	96	23	24
Cold	*Chlorophytum comosum*	5.05E+06 ± 2.33E+06	6.69E+06 ± 3.08E+06	96	5	5
	Dracaena draco	4.79E+04 ± 3.45E+04	1.15E+04 ± 5.81E+03	96	6	6
	Oleaeuropaea	1.88E+04 ± 1.61E+04	1.39E+04 ± 1.22E+04	91	22	24
Succulent	*Aloe arborescens*	1.15E+05 ± 3.28E+04	1.74E+05 ± 4.68E+04	96	4	4
	Beaucarnea recurvata	9.89E+04 ± 6.18E+03	4.33E+04 ± 8.80E+03	96	56	58
	Musa acuminata	3.36E+02 ± 1.76E+02	2.52E+02 ± 6.24E+01	47	3	6

**Means that do not share the same letter are significantly different (alpha=0.05).*

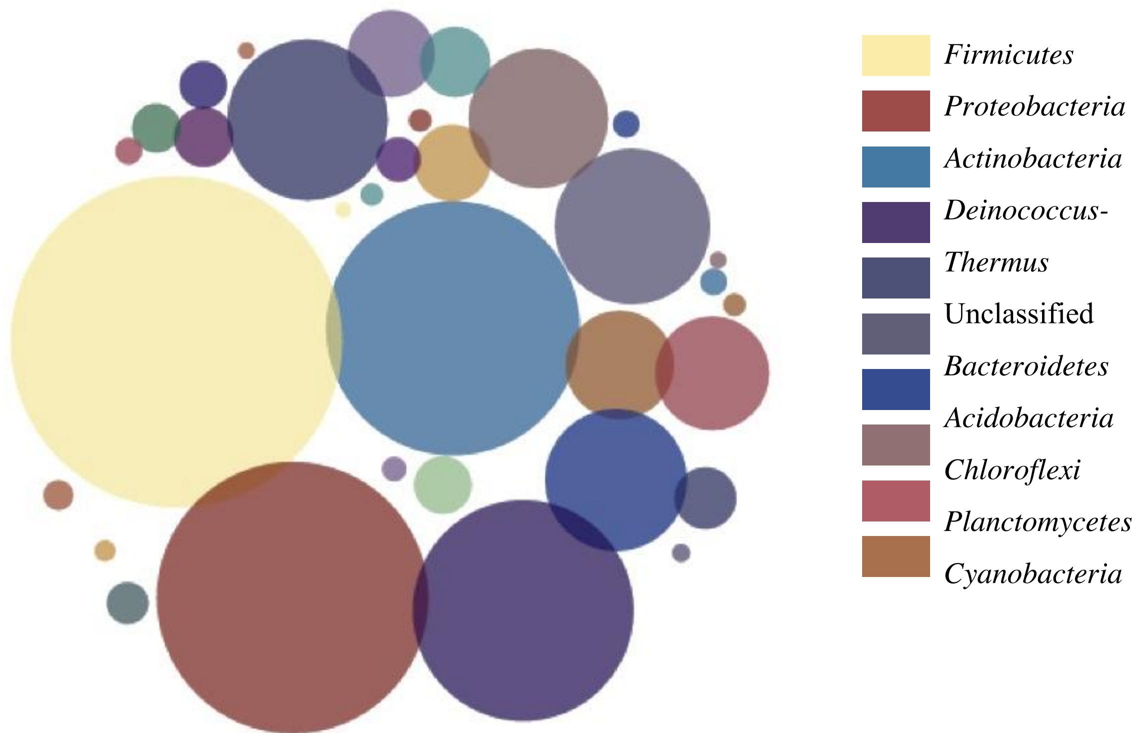

Figure 1. Overview of phyllosphere microbial communities phyla composition found on 14 different greenhouse plants. The size of the spheres shows the relative abundance of the corresponding phylum; larger spheres are mean higher abundance. Index shows the top 10 sequences generated through Illumina MiSeq platform.

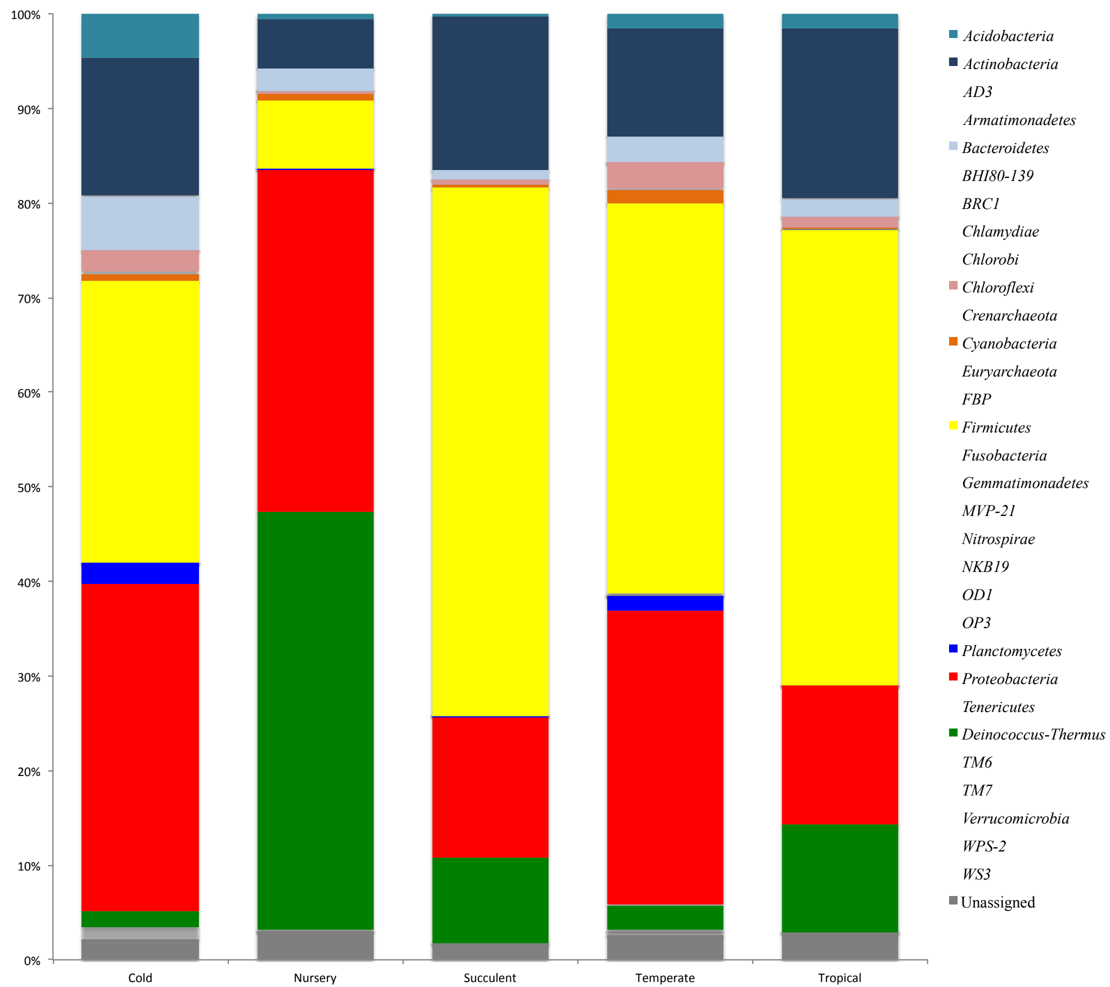

Figure 2.Phyllosphere microbial communities phyla composition found on 14 different greenhouse plants categorized per greenhouse room.

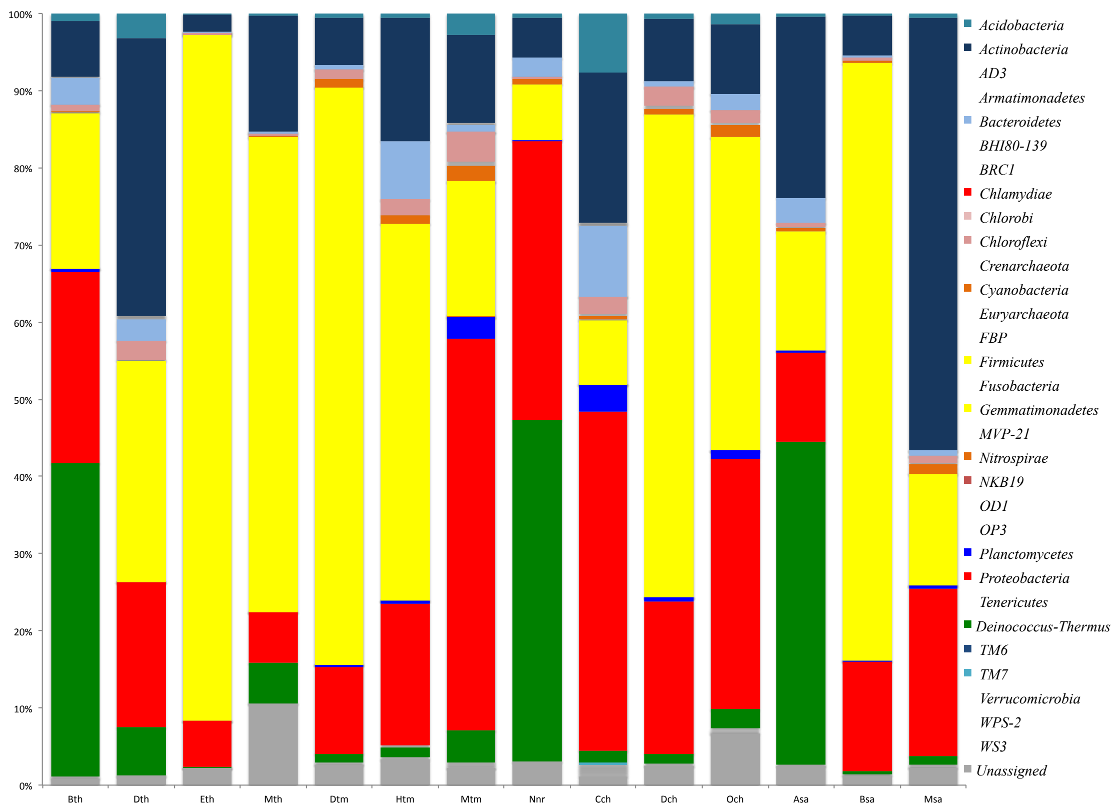

Figure 3. Relative abundance of phyllosphere bacterial community composition found on 14 different greenhouse plants categorized per plant species.

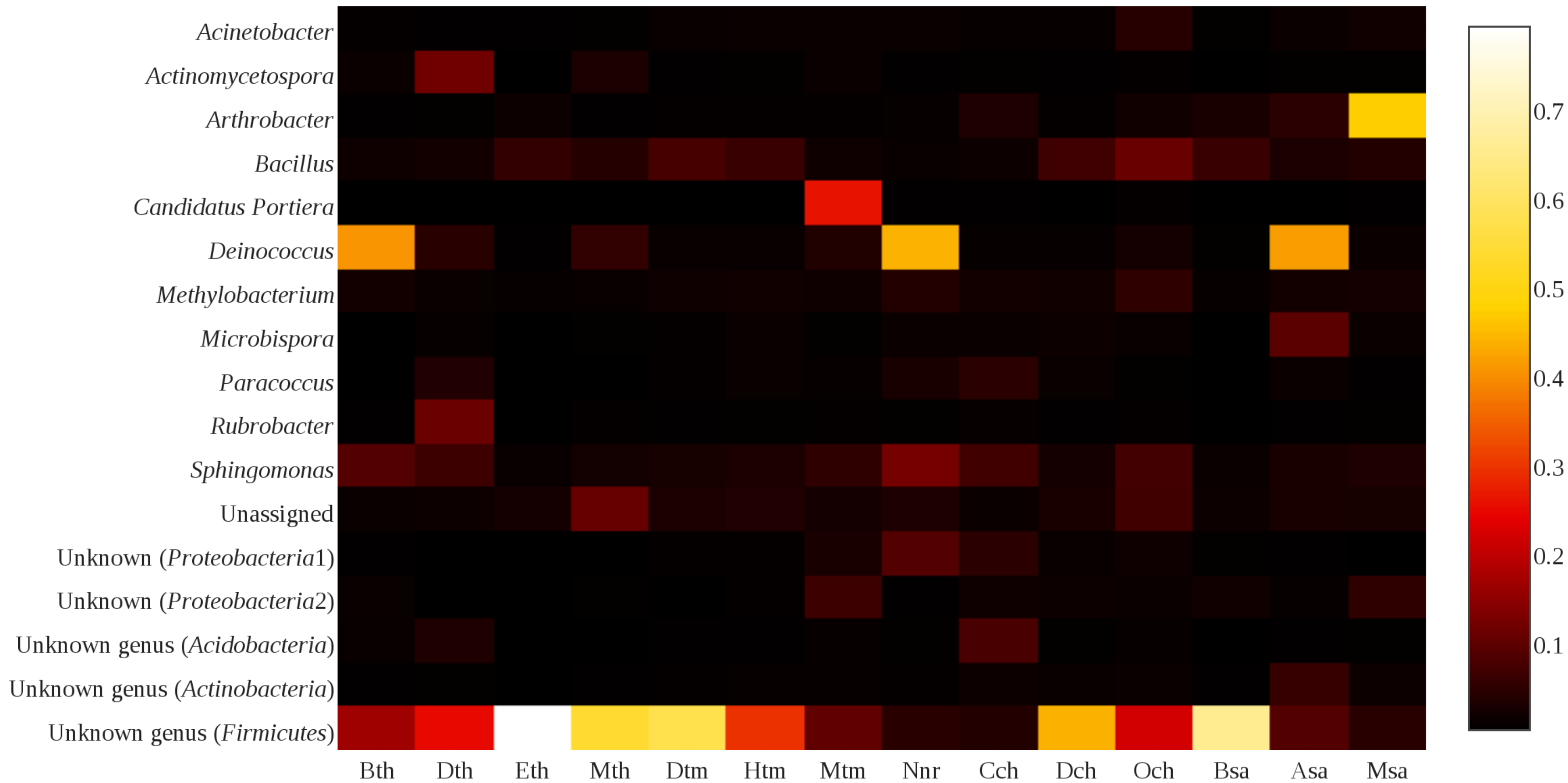

Figure 4. Relative abundance of dominant bacterial genus (cut-off 1%) found on the phyllosphere of 14 different greenhouse plants.

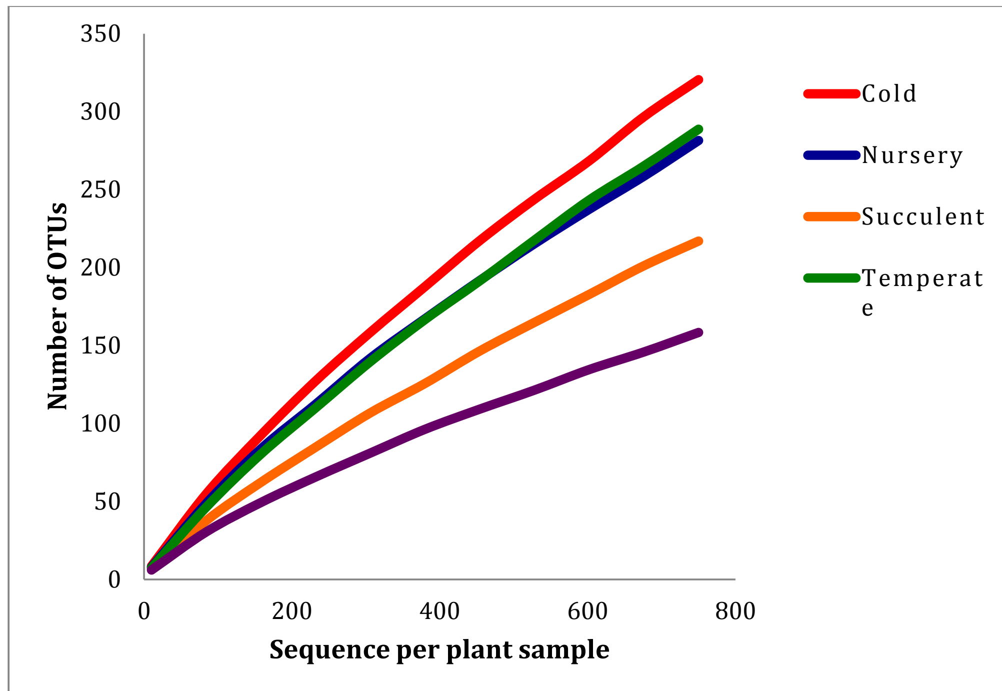

Figure 5. Rarefaction results for the diversity of phyllosphere bacterial community of plants. Diversity per room is represented.

Table 2. Species richness estimate (categorized per room) obtained at 3% genetic dissimilarity using Miseq Illumina-derived sequences of the DNA extracted from 14 greenhouse plants.

Greenhouse room	Seqs/Sample	Shannon index[a] (H')	Observed species[b] (no. of OTUs)	Chao1[c] (no. of OTUs)	Coverage (%)
Cold	714	6.6	306.7	1097.1	28.0
Nursery	714	6.1	269.5	918.0	29.4
Succulent	714	4.8	209.7	790.9	26.5
Temperate	714	5.8	279.4	1065.5	26.2
Tropical	714	4.1	152.0	510.5	29.8

[a]higher number indicates higher diversty; [b]results from the rarefaction analyses; [c]non-parametric richness estimator based on the distribution of singletons and doubletons.

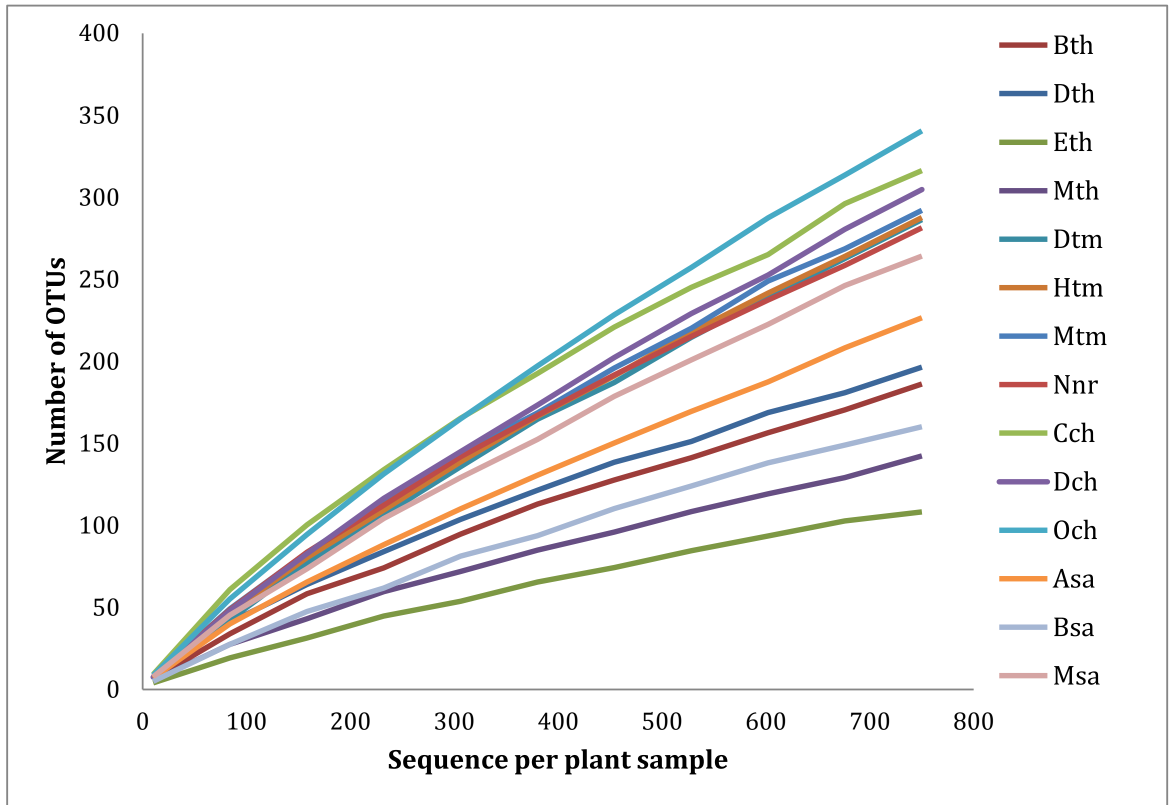

Figure 6. Rarefaction results for the diversity of phyllosphere bacterial community of plants. Diversity per plant is represented

Table 3. Species richness estimate (categorized per plant) obtained at 3% genetic dissimilarity using Miseq Illumina-derived sequences of the DNA extracted from 14 greenhouse plants.

Greenhouse room	Sample	Origin	Seqs/Sample	Shannon index (H')	Observed species (no. of OTUs)	Chao1 (no. of OTUs)	Coverage (%)
Tropical	Bth	*Aechmea eurycorymbus*	714	4.7	178.2	604.8	29.5
	Dth	*Dracaena marginata*	714	5.5	188.5	592.5	31.8
	Eth	*Epipremnum aureum*	714	2.5	104.7	344.7	30.4
	Mth	*Musa x paradisiaca*	714	3.8	136.9	499.9	27.4
Temperate	Dtm	*Dracaena fragrans*	714	5.4	276.3	1167.6	23.7
	Htm	*Howea forsteriana*	714	5.8	279.7	1046.1	26.7
	Mtm	*Malvaviscus penduliflorus*	714	6.0	282.2	982.7	28.7
Nursery	Nnr	*Nephrolepis cordifolia*	714	6.1	269.5	918.0	29.4
Cold	Cch	*Chlorophytum comosum*	714	7.2	301.4	905.3	33.3
	Dch	*Dracaena draco*	714	5.8	290.3	1114.3	26.0
	Och	*Olea europaea*	714	6.8	328.4	1271.7	25.8
Succulent	Asa	*Aloe arborescens*	714	5.2	214.8	801.3	26.8
	Bsa	*Beaucarnea recurvata*	714	3.6	155.4	536.1	29.0
	Msa	*Musa acuminata*	714	5.7	258.9	1035.2	25.0

[a]*higher number indicates higher diversty;* [b]*results from the rarefaction analyses;* [c]*non-parametric richness estimator based on the distribution of singletons and doubletons*.

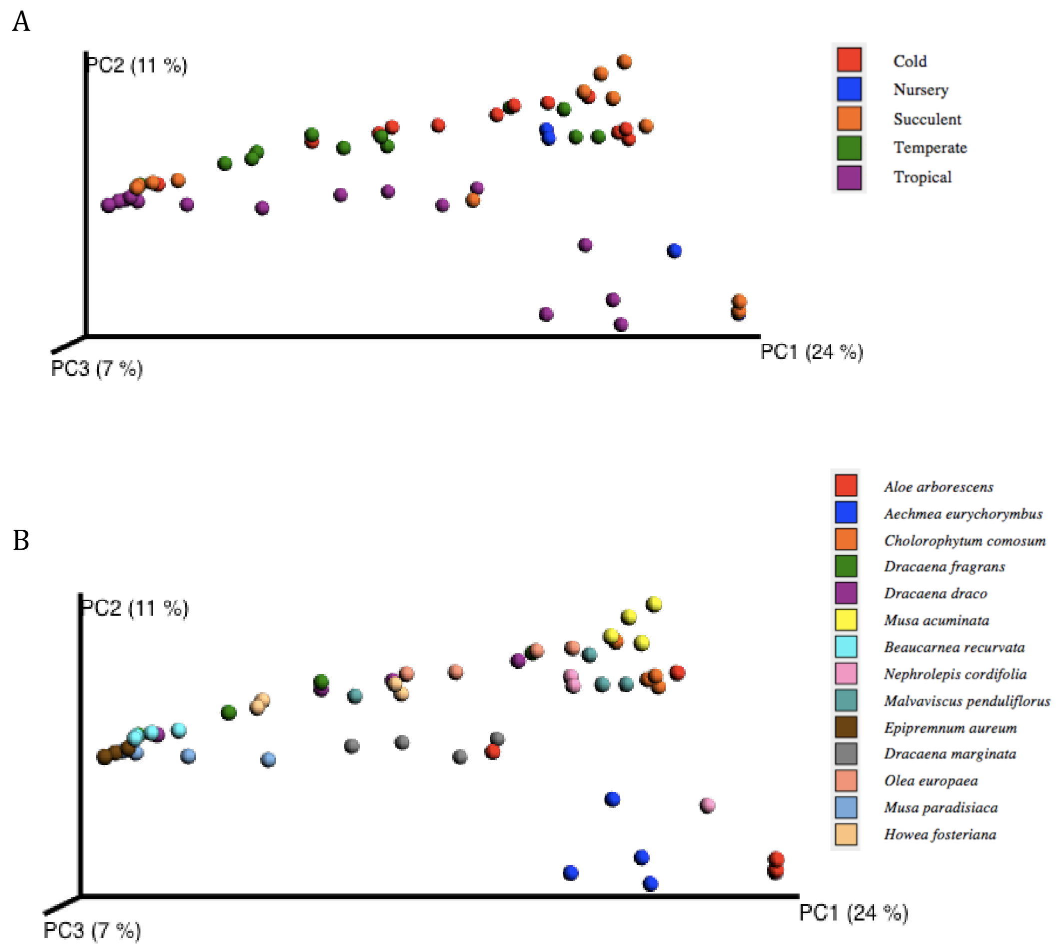

Figure 7. PCoA plots showing the clustering patterns between samples in A) greenhouse rooms, and B) plant species, based on Bray-Curtis dissimilarity.

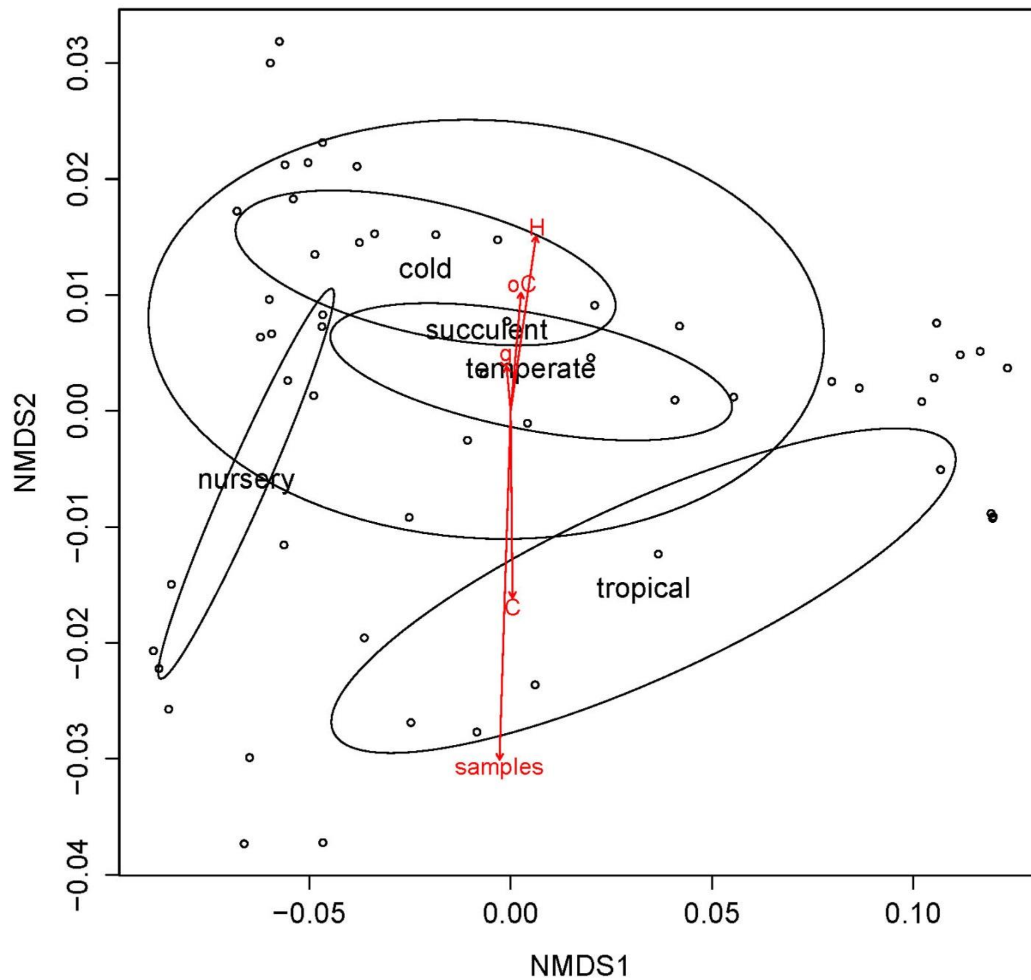

Figure 8. Non-metric multidimensional scaling (NMDS) plot derived from Bray-Curtis dissimilarity illustrating distances between bacterial community compositions. The BIO-ENV vectors of environmental variables based on Euclidean distances represent the direction along the samples of each greenhouse rooms, showing the role each of them played in explaining the distribution of the samples and its directional influence.

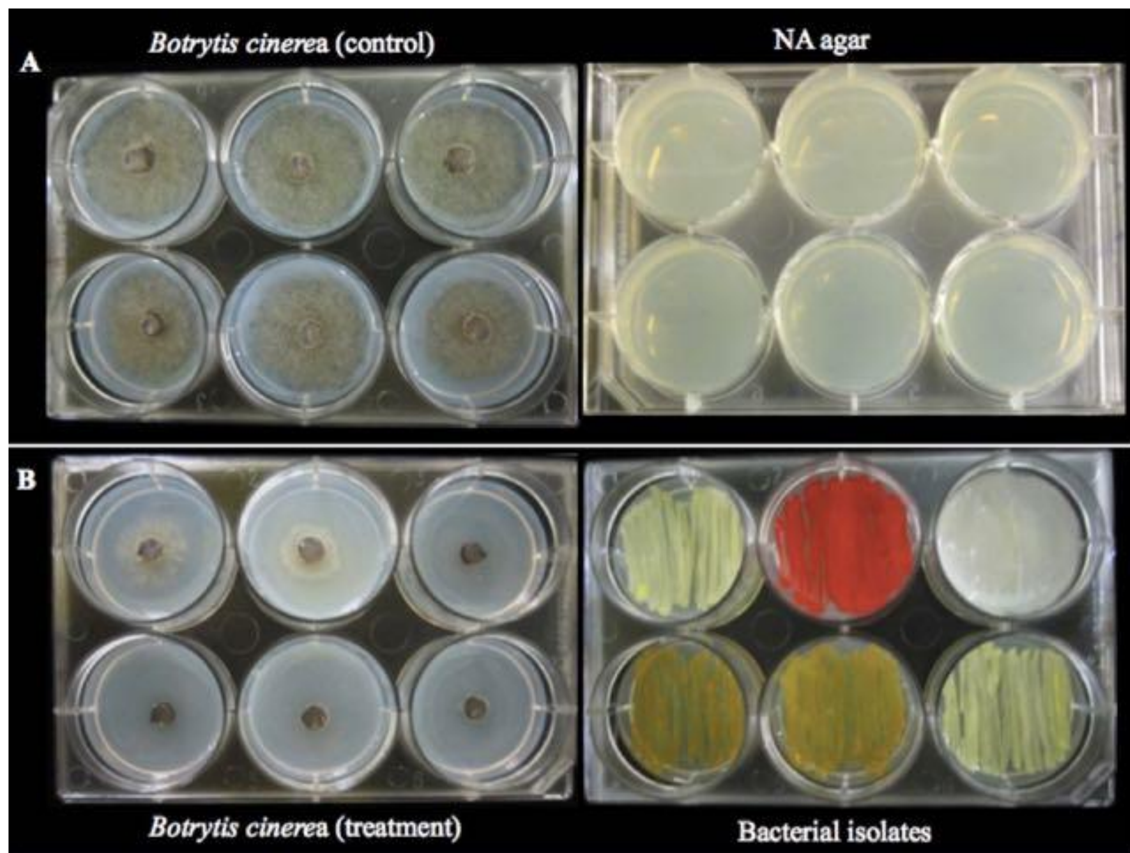

Figure 9. Two-clamp VOCs assay of bacterial isolates from 14 greenhouse plants showing antifungal volatile activity against *Botrytis cinerea*. (A) *B*. *cinerea* plugs showing mycelial growth and spore germination (left), paired with an empty NA plate (right). (B) Volatile organic compounds produced by bacteria (right) affected mycelial growth and germination of spores (left), compared to the control (above left).

Table 4. Taxonomic classification and mean growth inhibition percentage against *Botrytis cinerea* of isolated phyllosphere bacterial strains from 14 greenhouse plants.

Greenhouse room	Strains	Plant of origin	% Inhibition	Inhibition score[a]	Species	Ident (%)	Accession
Tropical	Bth1N11	*Aechmea eurycorymbus*	52,79	(++)	*Deinococcus grandis* DSM 3963	99	NR_026399.1
	Dth4N3	*Dracaena marginata*	52,14	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Dth4R1		49,93	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Dth3R4		46,99	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Dth3R5		52,79	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Dth1R12		38,86	(++)	*Staphylococcus saprophyticus* ATCC 15305	99	NR_074999.1
	Dth3R1		49,19	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Dth3N9		41,41	(++)	*Sphingobium yanoikuyae* NBRC 15102	99	NR_113730.1
	Dth4N9		48,07	(++)	*Kocuria haloterans* YIM 90716	99	NR_044025.1
	Dth1R9		40,01	(++)	*Kocuria haloterans* YIM 90716	99	NR_044025.1
	Dth2R10		61,17	(+++)	*Sphingomonas dokdonensis* DS-4	99	NR_043612.1
	Dth4N5		36,96	(++)	*Brachybacterium conglomeratum* J1015	99	NR_104689.1
Temperate	Dtm2N2	*Dracaena fragrans*	61,31	(+++)	*Bacillus licheniformis* DSM 13	99	NR_118996.1
	Dtm2R12		51,47	(++)	*Bacillus toyonensis* BCT-7 112	100	NR_121761.1
	Dtm3R12		53,13	(++)	*Kocuria haloterans* YIM 90716	99	NR_044025.1
	Dtm3N4		50,99	(++)	*Bacillus cereus* ATCC 14579	99	NR_074540.1
	Htm3N10	*Howea forsteriana*	56,18	(++)	*Bacillus aureus* 24K	100	NR_118439.1
	Htm2N10		36,76	(++)	*Bacillus toyonensis* BCT-7 112	99	NR_121761.1
	Htm4R1		53,19	(++)	*Bacillus toyonensis* BCT-7 112	99	NR_121761.1
	Mtm3R3	*Malvaviscus penduliflorus*	49,90	(++)	*Bacillus cereus* ATCC 14579	99	NR_074540.1
Nursery	Nnr2N2	*Nephrolepis cordifolia*	44,56	(++)	*Micrococcus flavus* LW4	99	NR_043881.1
	Nnr3R10		54,85	(++)	*Bacillus toyonensis* BCT-7 112	99	NR_121761.1
	Nnr4R12		50,69	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Nnr2R7		40,50	(++)	*Bacillus anthrasis* strain Ames	99	NR_074453.1

[a]*Inhibition score (+) indicates 10-30%; (++) 31-60% ; (+++) 61-80%; (++++) 81-100% growth inhibition growth inhibition effect on Botrytis cinerea.*

Table 4. Continuation..

Greenhouse room	Strains	Plant of origin	% Inhibition	Inhibition score[a]	Species	Ident (%)	Accession
Nursery	Nnr3N9	*Nephrolepis cordifolia*	39,41	(++)	*Kocuria sediminis* FCS-11	99	NR_118222.1
	Nnr2R11		49,17	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Nnr4N11		42,35	(++)	*Pseudomonas aeruginosa* SNP0614	99	NR_118644.1
	Nnr3N6		42,89	(++)	*Deinococcus xibeiensis*	99	NR_116670.1
	Nnr1R4		44,64	(++)	*Sphingobium xenophagum* BN6	99	NR_026304.1
	Nnr3R3		43,73	(++)	*Janibacter melonis* CM2104	99	NR_025805
Cold	Cch2R2	*Chlorophytum comosum*	38,21	(++)	*Pantoea vagans* C9-1	99	NR_102966.1
	Cch2R9		58,25	(++)	*Stenotrophomonas rhizophila* e-p10	99	NR_121739.1
	Cch2N9		55,84	(++)	*Stenotrophomonas rhizophila* e-p10	100	NR_121739.1
	Cch4R6		56,90	(++)	*Bacillus cereus* ATCC 14579	99	NR_074540.1
	Cch1N3		48,34	(++)	*Stenotrophomonas rhizophila* e-p10	99	NR_121739.1
	Dch3R8	*Dracaena draco*	45,88	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Dch4R3		53,97	(++)	*Stenotrophomonas rhizophila* e-p10	98	NR_121739.1
	Och1N4	*Olea europaea*	49,68	(++)	*Bacillus subtilis* SBMP4	100	NR_118383.1
	Och2R2		42,33	(++)	*Bacillus thuringiensis* Bt407	99	NR_102506.1
	Och4N12		61,15	(+++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Och2N7		51,15	(++)	*Erwinia aphidicola* X001	99	NR_104724.1
	Och4N8		43,41	(++)	*Bacillus cereus* ATCC 14579	99	NR_074540.1
	Och4R5		51,53	(++)	*Stenotrophomonas rhizophila* e-p10	100	NR_121739.1
Succulent	Asa3N6	*Aloe arborescens*	45,16	(++)	*Kocuria turfanensis* HO-9042	99	NR_043899.1
	Asa1N1		45,11	(++)	*Kocuria sediminis* FCS-11	99	NR_118222.1
	Bsa3N9	*Beaucarnea recurvata*	52,81	(++)	*Bacillus toyonensis* BCT-7 112	100	NR_121761.1
	Bsa3N2		56,29	(++)	*Bacillus thuringiensis* Bt407	100	NR_102506.1
	Bsa3R7		56,29	(++)	*Bacillus toyonensis* BCT-7 112	99	NR_121761.1
	Msa2R2	*Musa acuminata*	56,27	(++)	*Microbacterium oleivorans* BAS69	99	NR_042262.1

[a]*Inhibition score (+) indicates 10-30%; (++) 31-60% ; (+++) 61-80%; (++++) 81-100% growth inhibition effect on Botrytis cinerea*

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Supplementary figures:

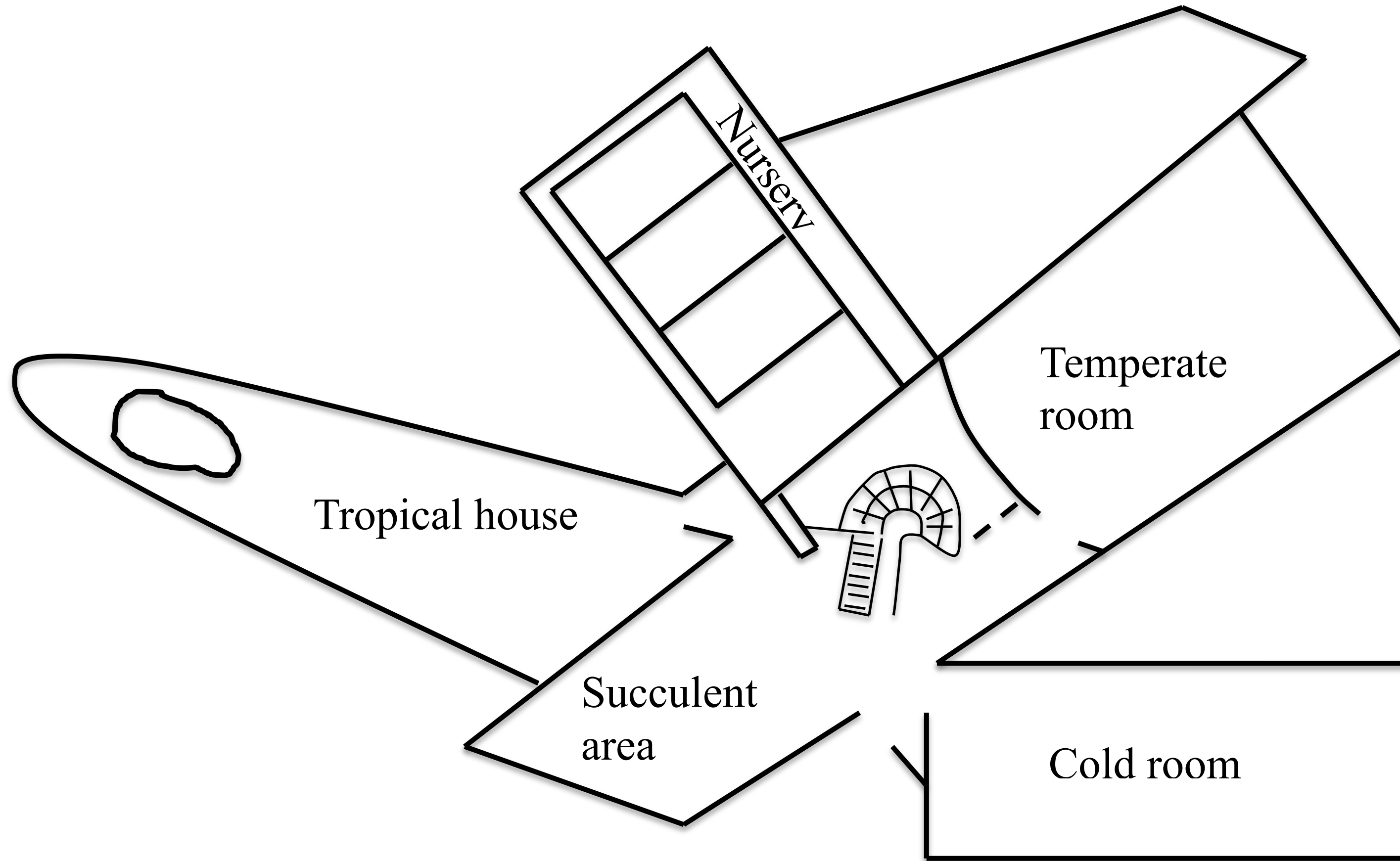

Figure S1. The complex plan of the Botanical Garden of Graz greenhouse

Table S1. ANOVA of phyllosphere bacterial CFU of 14 different greenhouse plants

Source of Variation	SS	df	MS	F	p-level	F crit
Between Groups	6.31717E+13	13	4.85937E+12	47.87393	2.64276E-09	2.50726
Within Groups	1.42105E+12	14	1.01503E+11			
Total	6.45928E+13	27				

Table S2. Tukeys test of phyllosphere bacterial CFU of 14 different greenhouse plants.

	Plant_samples	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	Msa	2	294.00		
	Htm	2	12194.40		
	Och	2	16365.00		
	Dtm	2	26848.20		
	Mtm	2	27885.00		
	Dch	2	29691.00		
	Bsa	2	71088.60		
	Nnr	2	127839.00		
	Asa	2	144513.00		
	Eth	2	251460.00		
	Mth	2	689760.00	689760.00	
	Dth	2	1016640.00	1016640.00	
	Bth	2		1545480.00	
	Cch	2			5872416.00
	Sig.		0.183	0.372	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.0

Table S3. Alpha diversity statistical analysis of the Shannon index per greenhouse room

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
cold house	tropical house	6.605629927	1.013899801	4.114037414	1.160754266	5.714483001	0.01
temperate house	succulent area	5.750714363	1.252498113	4.832221754	1.373029359	1.639129568	1.0
tropical house	succulent area	4.114037414	1.160754266	4.832221754	1.373029359	-1.442715652	1.0
cold house	temperate house	6.605629927	1.013899801	5.750714363	1.252498113	1.759563604	0.97
nursery	temperate house	6.10252267	1.199731172	5.750714363	1.252498113	0.459852192	1.0
cold house	succulent area	6.605629927	1.013899801	4.832221754	1.373029359	3.446035259	0.03
nursery	tropical house	6.10252267	1.199731172	4.114037414	1.160754266	2.887571852	0.07
nursery	succulent area	6.10252267	1.199731172	4.832221754	1.373029359	1.545345381	1.0
cold house	nursery	6.605629927	1.013899801	6.10252267	1.199731172	0.766524316	1.0
tropical house	temperate house	4.114037414	1.160754266	5.750714363	1.252498113	-3.438934953	0.02

Table S4. Alpha diversity statistical analysis of the observed species per greenhouse room

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
cold house	tropical house	306.6583333	43.52289548	152.04375	35.15995536	10.01292081	0.01
temperate house	succulent area	279.3666667	68.3166443	209.6916667	67.03340533	2.414406438	0.26
tropical house	succulent area	152.04375	35.15995536	209.6916667	67.03340533	-2.835334806	0.1
cold house	temperate house	306.6583333	43.52289548	279.3666667	68.3166443	1.117449575	1
nursery	temperate house	269.525	71.49854457	279.3666667	68.3166443	-0.2306709	1
cold house	succulent area	306.6583333	43.52289548	209.6916667	67.03340533	4.023887946	0.02
nursery	tropical house	269.525	71.49854457	152.04375	35.15995536	4.445463554	0.06
nursery	succulent area	269.525	71.49854457	209.6916667	67.03340533	1.421900655	1
cold house	nursery	306.6583333	43.52289548	269.525	71.49854457	1.158115584	1
tropical house	temperate house	152.04375	35.15995536	279.3666667	68.3166443	-6.175494178	0.01

Table S5. Alpha diversity statistical analysis of the Chao1 per greenhouse room

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
cold house	tropical house	1097.09	219.36	510.48	111.15	8.90	0.01
temperate house	succulent area	1065.48	244.16	790.87	270.12	2.50	0.19
tropical house	succulent area	510.48	111.15	790.87	270.12	-3.61	0.01
cold house	temperate house	1097.09	219.36	1065.48	244.16	0.32	1.00
nursery	temperate house	918.03	228.98	1065.48	244.16	-0.99	1.00
cold house	succulent area	1097.09	219.36	790.87	270.12	2.92	0.12
nursery	tropical house	918.03	228.98	510.48	111.15	4.85	0.01
nursery	succulent area	918.03	228.98	790.87	270.12	0.79	1.00
cold house	nursery	1097.09	219.36	918.03	228.98	1.31	1.00
tropical house	temperate house	510.48	111.15	1065.48	244.16	-7.76	0.01

Table S6. Alpha diversity statistical analysis of the Shannon index per plant sample

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Nnr	Dch	nan	nan	6.143	1.335	None	None
Nnr	Bsa	nan	nan	3.729	0.325	None	None
Msa	Bth	5.892	1.340	4.819	0.466	1.310	1
Och	Mth	7.070	0.420	3.872	0.437	9.130	1
Mtm	Bsa	6.275	1.636	3.729	0.325	2.645	1
Htm	Bsa	6.015	0.610	3.729	0.325	5.729	1
Dth	Mth	5.634	0.210	3.872	0.437	6.292	1
Dth	Nnr	5.634	0.210	nan	nan	None	None
Bth	Dtm	4.819	0.466	5.660	1.402	-0.986	1
Msa	Dtm	5.892	1.340	5.660	1.402	0.207	1
Mtm	Asa	6.275	1.636	5.377	1.280	0.750	1
Bsa	Dch	3.729	0.325	6.143	1.335	-3.045	1
Eth	Dth	2.618	0.141	5.634	0.210	-20.666	1
Nnr	Bth	nan	nan	4.819	0.466	None	None
Htm	Mth	6.015	0.610	3.872	0.437	4.942	1
Msa	Cch	5.892	1.340	7.452	0.402	-1.931	1
Htm	Asa	6.015	0.610	5.377	1.280	0.779	1
Msa	Dth	5.892	1.340	5.634	0.210	0.328	1

Table S6. Cont.

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Mtm	Mth	6.275	1.636	3.872	0.437	2.458	1
Mth	Dtm	3.872	0.437	5.660	1.402	-2.109	1
Msa	Och	5.892	1.340	7.070	0.420	-1.453	1
Dth	Cch	5.634	0.210	7.452	0.402	-6.946	1
Mtm	Htm	6.275	1.636	6.015	0.610	0.259	1
Eth	Htm	2.618	0.141	6.015	0.610	-9.393	1
Msa	Mtm	5.892	1.340	6.275	1.636	-0.314	1
Htm	Och	6.015	0.610	7.070	0.420	-2.466	1
Och	Bsa	7.070	0.420	3.729	0.325	10.899	1
Eth	Mth	2.618	0.141	3.872	0.437	-4.727	1
Dch	Dtm	6.143	1.335	5.660	1.402	0.433	1
Htm	Nnr	6.015	0.610	nan	nan	None	None
Eth	Nnr	2.618	0.141	nan	nan	None	None
Nnr	Asa	nan	nan	5.377	1.280	None	None
Dth	Bth	5.634	0.210	4.819	0.466	2.765	1
Mtm	Bth	6.275	1.636	4.819	0.466	1.483	1
Htm	Dch	6.015	0.610	6.143	1.335	-0.152	1
Htm	Cch	6.015	0.610	7.452	0.402	-3.407	1
Bsa	Cch	3.729	0.325	7.452	0.402	-12.487	1
Msa	Nnr	5.892	1.340	nan	nan	None	None
Och	Dtm	7.070	0.420	5.660	1.402	1.669	1
Msa	Mth	5.892	1.340	3.872	0.437	2.481	1
Bth	Dch	4.819	0.466	6.143	1.335	-1.623	1
Bsa	Asa	3.729	0.325	5.377	1.280	-2.162	1
Bth	Cch	4.819	0.466	7.452	0.402	-7.413	1
Eth	Dtm	2.618	0.141	5.660	1.402	-3.740	1
Dth	Bsa	5.634	0.210	3.729	0.325	8.545	1
Mtm	Cch	6.275	1.636	7.452	0.402	-1.209	1
Nnr	Mth	nan	nan	3.872	0.437	None	None

Table S6. Cont.

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Asa	Dtm	5.377	1.280	5.660	1.402	-0.258	1
Mtm	Dch	6.275	1.636	6.143	1.335	0.108	1
Eth	Bsa	2.618	0.141	3.729	0.325	-5.437	1
Dth	Dch	5.634	0.210	6.143	1.335	-0.652	1
Eth	Cch	2.618	0.141	7.452	0.402	-19.660	1
Bth	Asa	4.819	0.466	5.377	1.280	-0.710	1
Mth	Cch	3.872	0.437	7.452	0.402	-10.438	0.702
Bsa	Mth	3.729	0.325	3.872	0.437	-0.456	1
Msa	Asa	5.892	1.340	5.377	1.280	0.481	1
Mth	Dch	3.872	0.437	6.143	1.335	-2.800	1
Cch	Dtm	7.452	0.402	5.660	1.402	2.128	1
Mtm	Dtm	6.275	1.636	5.660	1.402	0.495	1
Eth	Bth	2.618	0.141	4.819	0.466	-7.831	1
Och	Dch	7.070	0.420	6.143	1.335	1.147	1
Msa	Htm	5.892	1.340	6.015	0.610	-0.145	1
Htm	Dtm	6.015	0.610	5.660	1.402	0.402	1
Msa	Eth	5.892	1.340	2.618	0.141	4.208	1
Nnr	Och	nan	nan	7.070	0.420	None	None
Mtm	Och	6.275	1.636	7.070	0.420	-0.815	1
Asa	Cch	5.377	1.280	7.452	0.402	-2.679	1
Eth	Dch	2.618	0.141	6.143	1.335	-4.550	1
Och	Bth	7.070	0.420	4.819	0.466	6.214	1
Htm	Bth	6.015	0.610	4.819	0.466	2.698	1
Mth	Asa	3.872	0.437	5.377	1.280	-1.927	1
Dth	Asa	5.634	0.210	5.377	1.280	0.344	1
Htm	Dth	6.015	0.610	5.634	0.210	1.021	1
Eth	Och	2.618	0.141	7.070	0.420	-17.391	1
Bth	Mth	4.819	0.466	3.872	0.437	2.565	1
Mtm	Nnr	6.275	1.636	nan	nan	None	None
Och	Cch	7.070	0.420	7.452	0.402	-1.137	1

Table S6. Cont.

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Nnr	Dtm	nan	nan	5.660	1.402	None	None
Dth	Dtm	5.634	0.210	5.660	1.402	-0.031	1
Bsa	Dtm	3.729	0.325	5.660	1.402	-2.325	1
Eth	Asa	2.618	0.141	5.377	1.280	-3.712	1
Dch	Asa	6.143	1.335	5.377	1.280	0.718	1
Mtm	Dth	6.275	1.636	5.634	0.210	0.673	1
Dch	Cch	6.143	1.335	7.452	0.402	-1.626	1
Bth	Bsa	4.819	0.466	3.729	0.325	3.325	1
Och	Asa	7.070	0.420	5.377	1.280	2.177	1
Msa	Bsa	5.892	1.340	3.729	0.325	2.717	1
Msa	Dch	5.892	1.340	6.143	1.335	-0.230	1
Eth	Mtm	2.618	0.141	6.275	1.636	-3.859	1
Dth	Och	5.634	0.210	7.070	0.420	-5.294	1
Nnr	Cch	nan	nan	7.452	0.402	None	None

Table S7. ANOVA of the percent inhibition of antagonistic bacterial strains against the growth of *Botrytis cinerea*

Source of Variation	SS	df	MS	F	p-level	F crit
Between Groups	5.47743	34	0.1611	0.73771	0.84357	1.54108
Within Groups	22.93	105	0.21838			
Total	28.40743	139				

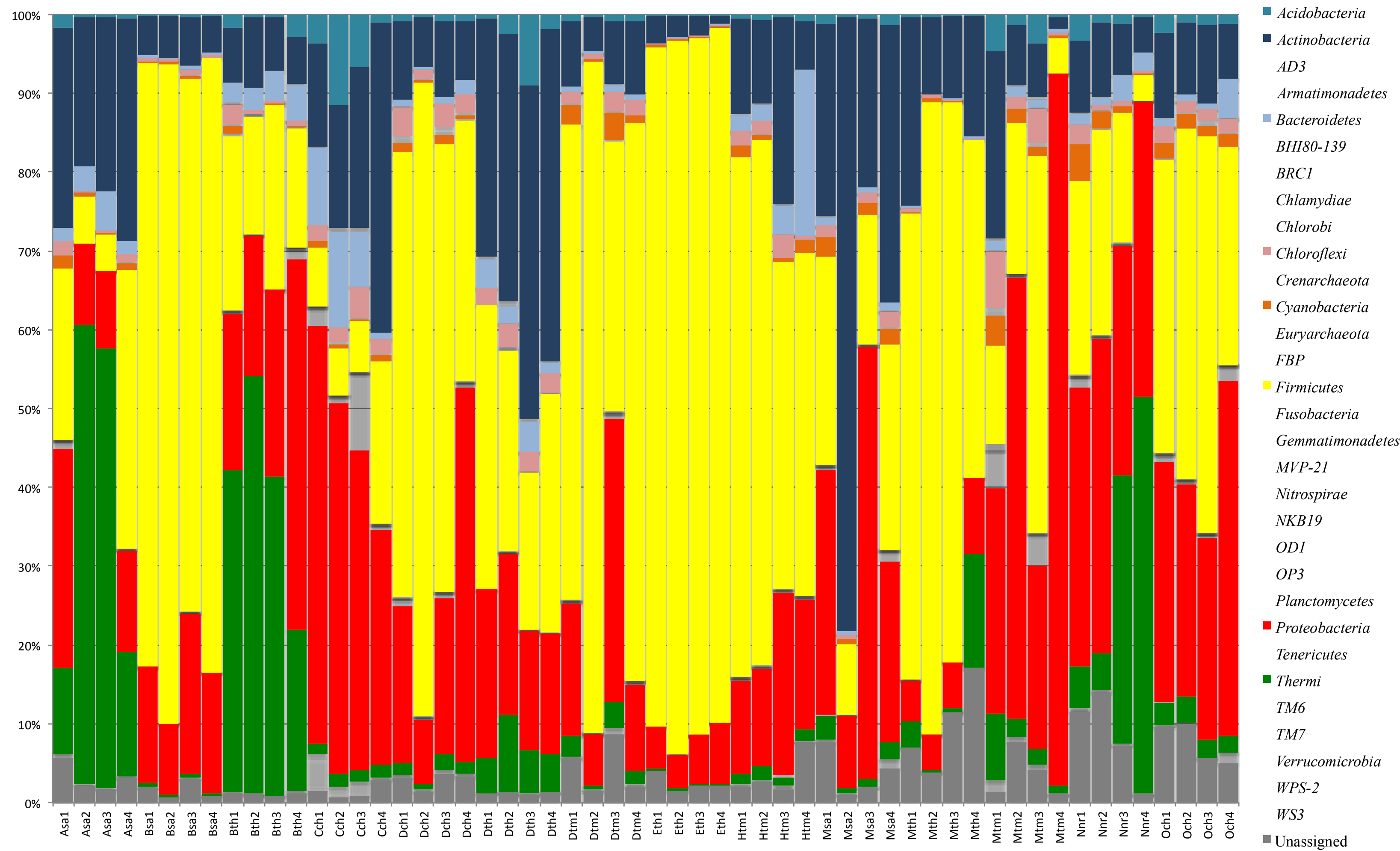

Figure S2. Relative abundance of phyllosphere bacterial community composition from four different replicates of each14 different greenhouse plants.

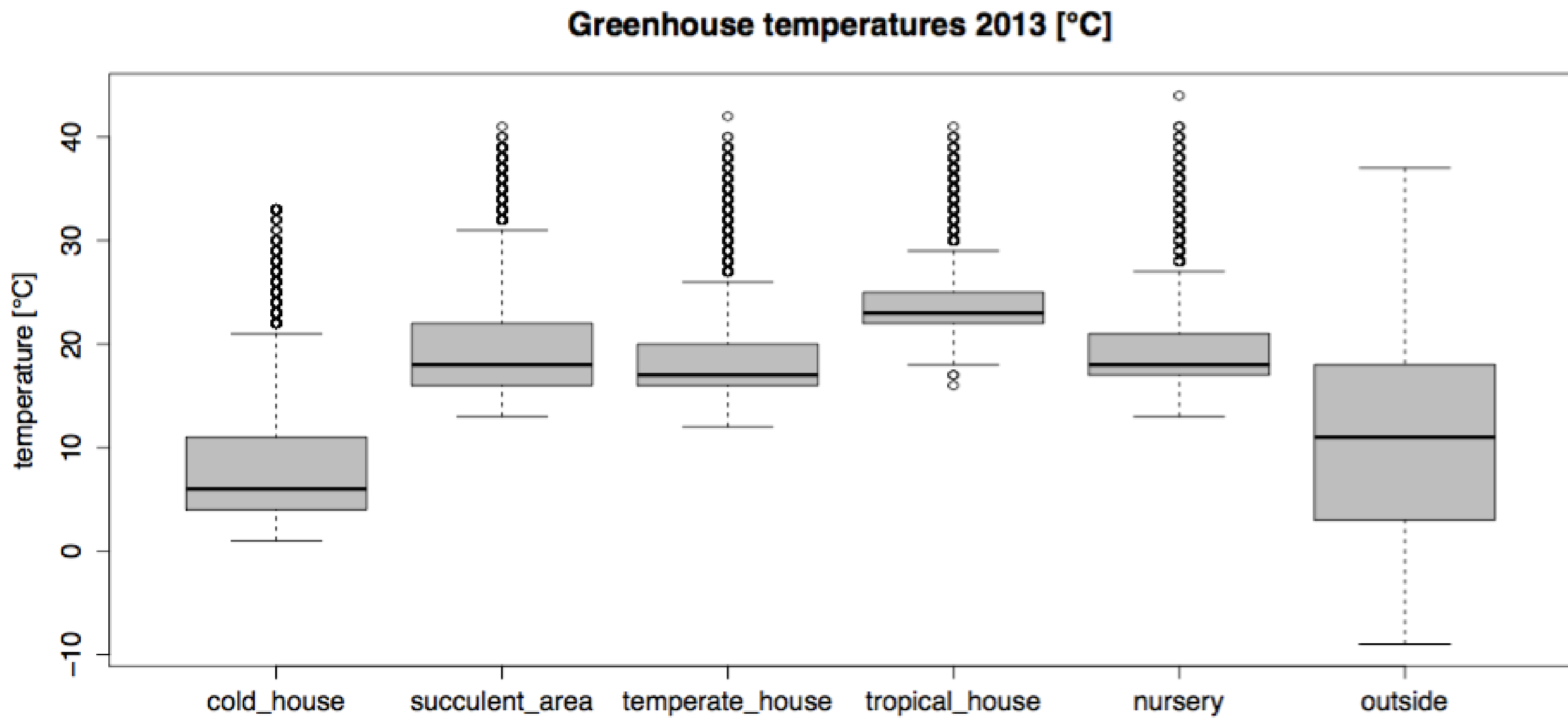

Figure S3. Mean temperature inside each room of the greenhouse complex for the year 2013

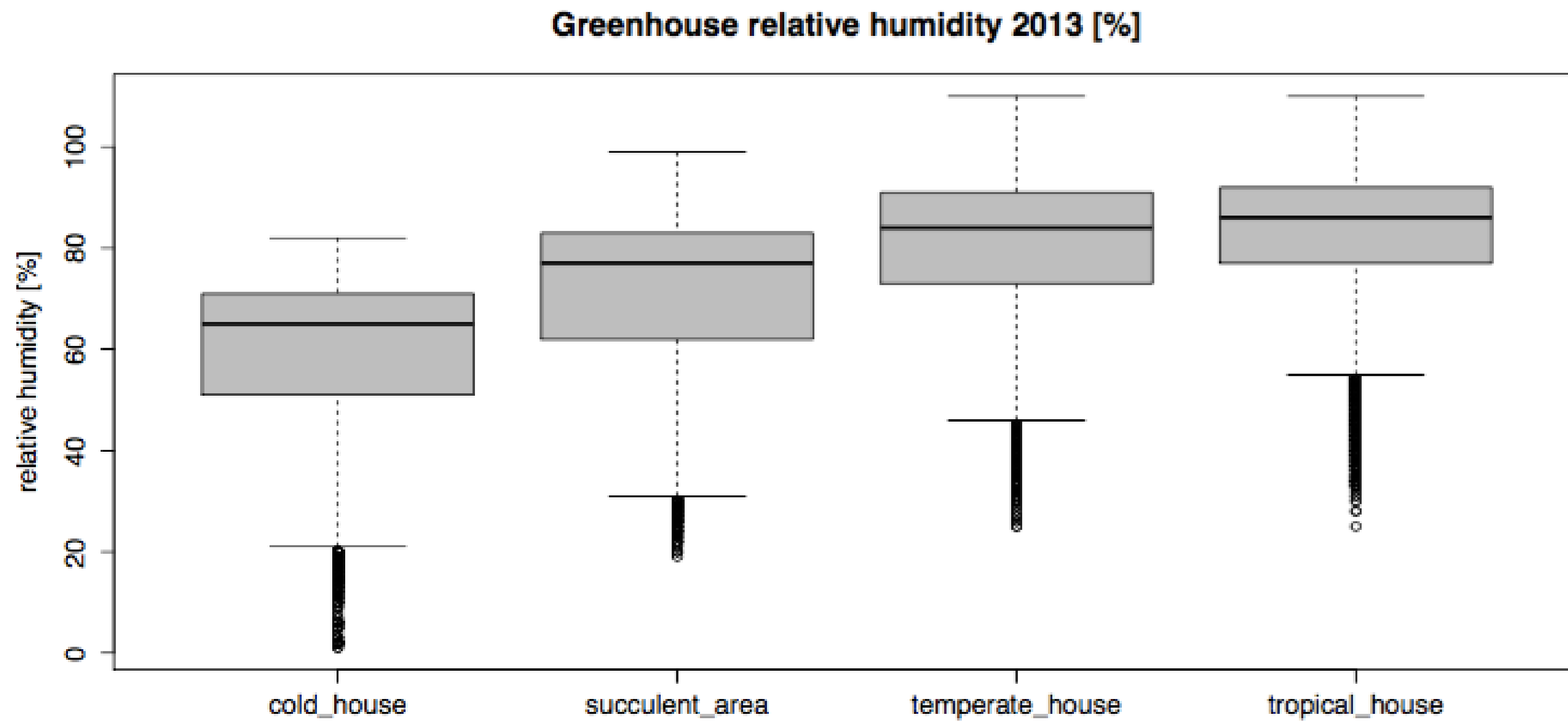

Figure S4. Mean relative humidity inside each room of the greenhouse complex for the year 2013

Publication II

Plan-host taxonomy and genotype as drivers of fungal community structure and the antagonistic potential of fungi on the leaves of greenhouse plants

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Plan-host taxonomy and genotype as drivers of fungal community structure and the antagonistic potential of fungi on the leaves of greenhouse plants

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Abstract

Exploration of the phyllosphere community revealed its many beneficial effects on plants and in built environments. Whereas most reports focus on bacterial communities on the phyllosphere a number of literatures showed that the foliar surface also hosts fungal colonists. Thus far, there is limited understanding of the fungal community structure in the phyllosphere of plants, and even less in plants found inside a built environment and the relationship of the fungal community structure to plant-host and the ambient room microclimate. This study investigated 14 common houseplants of diverse plant species and morphology grown in different controlled microclimate in the greenhouse of the Botanical Garden of Graz using cultivation dependent analysis and internal transcribed spacer (ITS) region amplicon sequencing on Illumina MiSeq. Furthermore, the antagonistic potential of fungal isolates was assessed in a Two-clamps volatile organic compounds (VOCs) assay against the model pathogenic fungi *Botrytis cinerea*.

Individual plant species showed high fungal abundance and diversity. The highest population density of culturable fungi was found on the leaves of *Musa paradisiaca* where 1.07×10^6 and 7.32×10^5 CFU cm^{-2} were recorded from Synthetic Nutrient Agar and Sabouraud growth media, respectively. Abundant fungi identified belonged to order *Capniodiales* from phylum *Ascomycota*, and order *Wallemialles* and *Tremellales* from phylum *Basidiomycota*. Non-metric multidimensional scaling and BIO-ENV analysis also showed correlation of fungal community highly inclined to plant species, where the variability of the community composition is correlated to plant genotype. Fungi isolated from the phyllosphere also exhibited VOCs-based antifungal activity; inhibiting *B*. *cinerea* mycelial growth by 32.89% - 72.23%. Frequently isolated active VOCs produces were mainly *Penicillum* species along with *Cladosporium*, and *Cryptococcus* species.

This study indicates that plants grown indoors support distinctive fungal communities that features antagonistic potential, and harbors a stable phyllosphere microbial diversity regardless of microclimate and abiotic conditions of a room. Hence, these plants maintain their microbiome independently from their surroundings that could have beneficial effects on microbial diversity and our health inside buildings in general.

Keywords: Microclimate, correlation to genotypic distance of plants, indoor plants, built environments

Introduction

The aboveground parts of a living plant provide a habitat for microorganisms known as the phyllosphere. This habitat is dominated by the leaves with an estimated global leaf area of 10^9 km^2 making it one of the largest microbial habitats on earth (Woodward and Lomas, 2004). For this reason, most studies about the phyllosphere focused mainly on plant leaves. Despite being a hostile environment with rapidly fluctuating solar radiation, temperature, humidity, and nutrient limitation, the phyllosphere supports diverse and complex microbial communities including many genera of bacteria, archaea, filamentous fungi, and yeasts (Lindow and Brandl, 2003; Whipps et al, 2008; Vorholt, JA, 2012). It has been established that the microbial community structure of the phyllosphere is affected by both environmental and biotic factors (Whipps et al, 2008; Vorholt, 2012; Rastogi et al, 2013). Plant-environment interaction controls the prevailing conditions in the plant phyllosphere, determining the establishment of microorganisms on the leaf surface, and influencing microbial colonization (O'Brien and Lindow, 1989; Whipps et al, 2008; Vorholt 2012). For example, biogeography of organisms on the plant surface distinguishes habitat zones that are differentiated based on physical environment (temperature and moisture) and availability of food (photosynthates) (Andrews and Harris, 2000).

Most literatures describing phyllosphere microbial community focus mainly on bacterial colonizers of the leaf, however the leaf surface also supports diverse fungal community (see for example: Santamariá and Bayman, 2005; Kharwar et al, 2010). Compared to their bacterial counterparts, little is known about fungal function on leaf

surfaces and fungal community structure. Although reports on the impact of phyllosphere fungal communities on the fitness of their host plant, and their contribution to key processes in the sustainable function of plant ecosystem including nutrient cycling and water transport has been established (Herre et al, 2007; Sunshine et al, 2009; Vujanovic et al 2012).

Hitherto, no general conclusion has been formulated regarding major drivers of fungal phyllosphere composition since not one unifying factor was identified affecting overall fungal phyllosphere assembly. Studies on the fungal communities in the phyllosphere of oak trees (*Quercus macrocarpa*) under rural or urban management practices showed that landuse was the major driver in determining the fungal community composition, diversity, and richness on oak tree leaves (Jumpponen and Jones, 2009). On the other hand, a study on spatial variation in fungal communities of European beech trees (*Fagus sylvatica*) identified host genetics as a determinant of fungal community assembly on the foliage of beech leaves (Cordier et al, 2012), whereas pyrosequencing analysis of balsam poplar (*Populus balsamifera* L.) phyllosphere recognized plant genotype as the driver of fungal foliar community composition (Bálint et al, 2013).

Aside from investigations of phyllosphere fungal community, there is also increasing literatures reporting fungal volatile organic compounds (VOCs) and their ecological roles. These compounds serve as ideal signaling molecules in facilitating both short- and long-distance intercellular and organismal interactions (Bitas et al, 2013) because of their ability to move through air spaces as well as liquids (Effmert et al. 2012). According to the review by Hung et al (2015), fungal VOCs are useful indirect indicators of fungal growth in agriculture, in monitoring spoilage, for chemotaxonomy purposes, for use in biofilters and for biodiesel, plant and animal disease detection, for “mycofumigation”, and with respect to plant health.

Despite the growing interest on fungal community associated to the phyllosphere, there is limited information about the diversity of phyllosphere fungal communities on the surface of the leaves of plants grown inside a built environment. Most literatures described drivers of community structure, and adaption leading to establishment of these microorganisms on the phyllosphere of field-grown plants or

forest stand tree species (see for example: Pereira et al, 2002; Rastogi et al, 2013; Coleman-Derr, 2015).

This study analyzed the fungal community composition on the leaf surface of 14 greenhouse plants from different rooms with different controlled microclimates. It aimed to show the effect of (1) room microclimate in a built system, and (2) plant-host on the phyllosphere fungal community assembly. The study also focused on characterization of a long-distance mechanism for antagonism. Using TCVA, the antagonistic potential of culturable fungal strains isolated from the 14 greenhouse plants were tested to determine their antagonistic potential against a model pathogenic fungi *Botrytis cinerea*.

Materials and Methods

Site description and plant maintenance inside the greenhouse

Samples were collected from a greenhouse at The Botanical Garden of Graz. The botanical garden can be found in Graz, Austria at 47°04'55" N, 15°27'28" E, with an elevation of 378 m above sea level. The greenhouse complex has four different rooms simulating different terrestrial climatic conditions and a nursery room where all the young plants and seedlings can be found (Figure S1).

Plants-care measures for the greenhouse plants include watering, and fertilizer and microbial pesticide application. Watering of plants in different rooms vary in regularity and is dependent on the different seasons. Plants in the Cold room, for example, are watered only in the morning during winter, while those found in the Tropical room are watered more frequently. The Botanical Garden has a cistern that catches rainwater and this serves as reservoir for watering of the plants.

Two types of fertilizer are used to help maintain healthy plants: 1) is a NPK liquid fertilizer for foliar application (Wuxal® Top N), and 2) is a water-soluble Phosphate and Potash nutrient (Hakaphos® Rot 8+12+24+(4)) applied in the soil. Application of these fertilizers also varies depending on the state of plant health.

The biological pesticide DiPel® is also used to protect the leaves of greenhouse plants from *Lepidoptera* larvae (caterpillar) that forages on them. This pesticide contains the

naturally occurring bacteria *Bacillus thuringiensis kurstaki* known for its toxicity on caterpillars. DiPel® application is done when there is an apparent infestation of Lepidopteran larvae, and is applied by spraying the solution on the leaves of affected plants. Along with foliar fertilizer and microbial pesticide, a non-ionic surfactant (Break Thru® S240) is also applied to safeguard the effectiveness of the treatments. Both measures were done in all greenhouse rooms except the Nursery room.

Sample collection

Leaves of 14 species of indoor plants were collected using ethanol-washed disposable gloves and sterile instruments. They were separated from the rest of the plant by cutting from the base of the petiole avoiding any possible contact with the leaf blade. Immediately after collection samples were placed inside 25 x 32 cm freezer bags (ARO freezer bags, Düsseldorf, Germany) and stored in a portable cooler with ice packs (GIO'STYLE Colombo Smart Plastics, Italy). All samples were immediately transported back to the laboratory at the Institute of Environmental Biotechnology, Graz University of Technology (TU Graz), Graz, Austria for microbial isolation and DNA extraction.

Fungal isolation

To wash the microbial cells off the leaves, 720 cm^2 of leaf sample was placed inside a freezer bag (doubled as precaution from wear and tear) containing 50 ml 0.85% NaCl solution with Tween 80 and was subjected to a series of washing and vortexing. Washing was done by subjecting the leaf through bag-mixer treatment (BagMixer Interscience, St. Nom, France) for 3 minutes. This step is immediately followed by vortexing, using a Transsonic Digital T910 DH sonicator (Elma™, Singen, Germany), for 3 min at 60 Hz. Right after the first sonication step, bag mixer treatment for 1 min, sonication for 3 min at 60 Hz, and a final bag mixing for 1 min, follows consecutively, to complete the series.

The resulting microbial solution was then transferred to a 50 ml Sarstedt tube. For culture-dependent experiments, 100 µl out of the 50 ml solution was serially diluted ten-folds and plated on both Synthetic nutrient agar (SNA; 0.2 g Glucose, 0.2 g Sucrose, 1 g KH_2PO_4, 1 g KNO_3, 0.5 g KCl, 0.5 g MgSO47H2O, and 22 g agar per liter distilled water; adjusted to 5.5 pH with 1 M NaOH) and Sabouraud agar media

(Carl Roth GmbH + Co. KG, Karlsruhe, Germany) in duplicates. Then, the remaining microbial solution was centrifuged (using Sorvall RC-5B Refrigerated Superspeed Centrifuge; DuPont Instruments™, USA) at 6 169 g for 20 min to pellet cells. The moist pellets were then transferred to a 2.0 ml sterile Eppendorf tubes and were further centrifuged at 18 000 g for 20 minutes (HERMLE Labor Technik, Germany). Pellets obtained from this final process were then frozen at −70°C until it was used for DNA extraction.

Determination of fungal colony forming units (CFU)

Agar plates, where serially diluted 100 µl of microbial solution was plated, were incubated at room temperature for 5 days. Colony count was done on the fifth day and final counts were expressed as CFU log10 cm^{-2} leaf.

Each colony with distinct phenotype was transferred initially into a tissue culture dish (60 x 15 mm) with Potato dextrose agar (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) medium and were incubated for 5 days to make sure the isolate was not contaminated. Cubes (1 cm^2) of samples from clean isolates were cut and transferred to 2 ml Eppendorf tubes with 1 ml fungi preservation solution (120 ml Glycerin, 40 ml 50% Glucose (stirred overnight), 20 ml Peptone (20%), 20 ml Yeast extract (10%); prepared and autoclaved separately and was numbered according to plant sample genus (except for *Aechmea eurycorymbus* where the common name Bromelia was used as a reference), origin, and isolation medium (e.g. Dth1N1: fungal isolate from *Draceana* from the Tropical house grown on NB II medium). All isolates were kept in a refrigerator (-70°C) at the Institute of Environmental Biotechnology, TU Graz, Graz, Austria.

Internal transcribed spacer (ITS) profiling using MiSeq Illumina Sequencing

DNA extraction

Genomic DNA was extracted using FastDNA® SPIN kit for soil (MP Biomedicals, Solon, OH, USA) as directed in the instruction manual with a revised first step, where pellets (from microbial isolation) instead of soil sample was used. A total of 56 DNA samples were extracted; four replicates for each of the 14 plant samples.

ITS gene amplification

PCR amplifications targeting the ITS region were conducted for each of the 56 samples using ITS1F/ITS2rP primers carrying sample-specific tags (Schoch et al, 2012; White et al, 1990; Gardes and Bruns, 1993). Using the thermocycler TC-Plus (TECHNE, Staffordshire OSA, UK), DNA was amplified in triplicate PCR reactions (30 µl each); 0.9 µl MgCl (25 mM), 6 µl Taq & Go, 1.5 µl of 5 µM for each primer, 19.1 µl PCR water, and 1µl of the DNA template (95°C, 5 min; 30 cycles of 95°C, 30 s; 58°C, 35 s; 72°C 40 s; and elongation at 72°C, 10s). Amplicons from three independent reactions were then pooled and purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA).

MiSeq Illumina sequencing

Purified amplicon samples were pooled in equimolar concentrations (520.8 ng DNA) and 50 µg of DNA was sent for Illumina MiSeq sequencing (Eurofins Genomics, Ebersberg, Germany) with chemistry version 2 (2 x 250bp). Quality controls, indexing of PCR products, sequencing, library preparations and initial filtering of raw reads were conducted by Eurofins Genomics, Ebersberg, Germany.

Bioinformatics and Statistics

After initial quality control raw reads were filtered, stiched and sorted according to respective barcodes. Raw reads are deposited as the project XXX in the European Nucleotide Archive (www.ebi.ac.uk). Stiched reads were processed according to the fungal ITS analysis tutorial in QIIME 1.9.0 (Caporaso et al., 2010; Mahnert et al., 2015). After extracting barcodes per respective lengths, reads were demultiplexed, trimmed and filtered. Chimeric sequences were identified with usearch (Edgar, 2010) providing the QIIME formatted UNITE representative sequences (sh_refs_qiime_ver7_dynamic_01.08.2015) as a reference. Subsequently, all chimeric sequences were removed from the data set. OTUs (operational taxonomic units) were picked in several steps against the UNITE reference given above using the blast algorithm and all remaining sequences were clustered de novo. The resulting OTU table was filtered for single and doubletons before it was rarefied to a depth of 10,419 sequences and served with all metadata as input for following alpha and beta diversity analysis and statistics.

Adonis, ANOSIM (analysis of similarities), ANOVA (analysis of variances), MRPP (multi response permutation procedure), BioEnv, and mantel tests were calculated in QIIME and R (vegan package) with 999 permutations (R Core Team, 2014; Oksanen, 2014; Fierer et al., 2010).

Screening of fungal strains for antagonism to *B. cinerea*

Two-clamp volatile organic compounds assay (TCVA) was done to analyze the antagonistic property of the volatiles produced by the fungal samples. *Botrytis cinerea*, maintained on a Potato Dextrose Agar (PDA), from the Institute of Environmental Biotechnology at TU Graz was used as the model pathogen for this study. TCVA was employed since it was reported a good method in screening for strain-specific antagonistic effect (Cernava et al, 2015). Fungal inoculum was prepared by growing the fungus for 6 days on fresh PDA medium. After this period, *B. cinerea* isolate was observed to have well-developed hyphae and is already sporulating.

TCVA with B. cinerea and fungal isolates from 14 greenhouse plants

A total of 629 fungal isolates were screened for their antagonistic activity against the pathogenic fungi *B. cinerea*. Samples from the stored isolates were transferred onto a new a Petri dish with PDA media and incubated for 6 days; to make sure the isolates were clean. A 5mm sample from plates that showed no contamination were cut and transferred onto a 6-well PDA plate and incubated at room temperature for 3-days. After the incubation period, plates with samples observed positive for growth were clamped together with newly made *B. cinerea* 6-well plates. *B. cinerea*-containing plates were prepared by cutting 5 mm plugs from a 6-day old *B. cinerea* inoculum plate and placing it on the center of each well of a 6-well plate with SNA media.

Setting up of the plate-pair was done based on the set-up suggested by Cernava et al (2015) and was done in quadruplicates. The set-up is incubated at room temperature for 3 days under dark conditions to eliminate any light-induced effect on the experiment (Mares et al, 2004). Inhibition of growth was measured as percent (%) inhibition and was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{diameter of fungi (control) - diameter of fungi (with VOCs)}}{\text{diameter of fungi (control)}} \times 100$$

BOX polymerase chain reaction (BOX-PCR) genetic fingerprinting

DNA extraction

BOX-PCR fingerprint analysis of the antagonistic fungal isolates was done to avoid analysis of genetically similar strains. To extract fungal DNA, stored fungal samples were transferred onto different PDA plates for reactivation and were incubated for 3 days at room temperature. After incubation, approximately 3 mm^2 of sample positive for growth and clear of contamination was cut for homogenization. After the removal of the adhered agar, each isolate sample was mixed in100 µl of double distilled water with 200 mg glass beads (0.1 - 0.25 mm) in a 2-µl microtube with cap (SARSTEDT, Germany) and was homogenized using MP FastPrep-24 sample preparation system ribolyser (Irvin, Calif., USA) (30 s; 6 ms^-). Homogenized samples were then frozen for 30 min at -20°C. Using a heat block (specs) the frozen samples were then heated at 100°C and immediately centrifuged at 16 000 g for 5 min at 4°C (HERMLE Labor Technik, Germany).

Gene amplification, fingerprint generation and evaluation

The PCR reaction mix (25 µl) that was used consist of 1 µl of the extracted DNA, 5 µl of Taq&Go, 2.50 µl of 100 pmol ml^- BOX A1R primer (5' CTA CGG CAA GGC GAC GCT GAC G 3'), and 16.50 µl PCR water. Amplification was done using the Tpersonal Combi, Biometra thermocycler (Biometra GmbH, Germany) with an initial denaturation at 95°C for 6 min, followed by 35 cycles of 94°C for 1 min, 53°C for 1 min, and 65°C for 8 min with a final extension at 65°C for16 min. After amplification, an aliquot of 12 µl PCR product was separated by gel electrophoresis in 1.5 % agarose gel in 0.5 x TBE buffer for 4 h. Then the gel was stained with ethidium bromide and photographed under UV transillumination using GelDoc 2000 (BIO-RAD,USA). The resulting BOX-PCR fingerprints were evaluated using the GelCompar program (Kortrijk, Belgium) Cluster analysis was done using unweighted pair-group average (UPGMA) algorithm.

Fungal strain identification via SANGER sequencing

DNA extraction

A representative strain from the different BOX clusters produced was used for DNA extraction. Each strain was grown on different PDA plates and incubated for 3 days at room temperature. After incubation, about 1.0 cm^2 of fungal mycel was transferred into a 2 ml microtube with cap filled with small (0.25 -0.5 mm) and medium glass beads (1.5 mm); 200 g each. In each tube, 450 µl extraction buffer (0.2 M tris-HCl (pH 8.5), 0.25 M NaCl, and 1 % (w/v) SDS) was then added and the fungal strains were homogenized using MP FastPrep-24 sample preparation system ribolyser (Irvin, Calif., USA); work done on ice. A volume of 3.0 M sodium acetate (pH 5.2) was added when the mycel appears completely homogenous and each tube was then vortexed, incubated for at least 10 min at -20°C, and centrifuged at 10 000 g for 10 min at 4°C (HERMLE Labor Technik, Germany). The supernatants were then transferred into new 2 µl Eppendorf tubes and one volume of chloroform/isoamylalcohol (24:1) was added in each tube, after which the tubes were inverted for 1 min and centrifuged at 10 000 g for 10 min at 4°C. The resulting aqueous phase for each tube was then transferred into new 2 µl Eppendorf tubes and one volume of phenol was added. Once again the tubes were inverted for 1 min and centrifuged at 10 000 g for 10 min at 4°C. After centrifugation, the aqueous phase was once again transferred to a new 2 µl Eppendorf tube and one volume of isopropanol was added before the tubes were vortexed, incubate for 5 min at room temperature, and centrifuged at 10 000 g for 10 min at 4°C. The resulting supernatant was discarded this time and the pellets were washed with 500 µl of 70% ice-cold ethanol, after which centrifugation followed (10,000 g; 10 min; 4°C). Finally the supernatant was decanted and the pellets were dried under laminar flow, then the DNA was dissolved in in 50 µl TE buffer (SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany).

Gene amplification and SANGER sequencing

After DNA extraction, PCR amplification for each strain was performed using 30 µl of PCR reaction mix (0.9 µl MgCl (25 mM), 6 µl Taq & Go, 1.5 µl of ITS1f primer, 1.5 µl of ITS4 primer, 19.1 µl PCR water, and 1µl of the DNA template. Amplification was done with an initial denaturation at 95°C for 5 min, followed by 30

cycles of 95°C for 30 s, 58°C for 35s, and 72°C for 40 s with a final extension at 72°C for 10 min using a TC-Plus thermocycler (TECHNE, Staffordshire OSA, UK). After amplification, an aliquot of 5 µl PCR product was separated by gel electrophoresis in 0.8 % agarose gel in 1 x TAE buffer for 1 h. Then the gel was stained with ethidium bromide and photographed under UV transillumination using GelDoc 2000 (BIO-RAD, USA).

Amplicons were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA), then nucleic acid was quantitated using Nanodrop 2000c spectrophotometer (PeQlab, VWR International GmbH, Erlangen, Germany) before the template DNA solution were prepared for SANGER sequencing. A 14 µl DNA solution with 40 ng $µl^{-1}$ concentration and a specific primer (ITS1f) were sent to LGC Genomics (Berlin, Germany) for sequencing.

SANGER sequencing analysis

Sequences were identified into specific fungal species using the BLAST algorithm against the NCBI Targeted Loci Nucleotide BLAST - Internal transcribed spacer region (ITS) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Population densities of fungal communities on the phyllosphere of 14 greenhouse plants

A high abundance of culturable fungi was observed in the phyllosphere of 14 greenhouse plants, with the highest abundance of CFU cm^{-2} found on the leaves of *Musa paradisiaca* with 1.07 x 10^{6} (SNA) and 7.32 x 10^{5} (Sabouraud) CFU cm^{-2} and the lowest on the leaves of *Musa acuminata* with 984 (SNA) and 450 CFU cm^{-2} (Sabouraud) as shown in Table 1. One-way ANOVA results (Table S1) showed that there are significant differences in the fungal population densities on the phyllosphere of 14 greenhouse plants (*F crit=2.5; F=13.8; p-level<0.05*) and Tukey's HSD test also showed 2 groupings of population densities per plant (Table S2) where *Musa paradisiaca* was grouped differently from the rest of the plant (Table 1).

Fungal communities associated with the phyllosphere of 14 greenhouse plants

A total of 6.01 M sequences affiliated to fungi (and few protozoa) were generated for the 14 plant samples of different species. The average sequence per sample was 101 310; ranging from 3 967 to 250 429. An average of 14 220 operational taxonomic units (OTUs) per sample were identified. The majority of the fungal sequences were assigned to Order *Capniodiales* (33.0%) from the phylum *Ascomycota*, and *Wallemialles* (20.14%) and *Tremellales* (16.71%) from the phylum *Basidiomycota* (Figure 1). Members of the *Basidiomycetuos* yeasts belonging to *Sporidiobolales* (6.30%), and *Ascomycetous* fungi from orders *Eurotiales* (5.67%) and *Pleosporales* (4.02%), were also found relatively abundant.

Across different greenhouse rooms diverse fungal community and dominant fungal taxa were observed. Order *Capniodiales*, was most abundant in the phyllosphere of plants from the Temperate (48.95%) and Cold (58.91%) rooms, *Wallemiales* in the Succulent room (49.0%), *Tremellales* in the Tropical room (25.85%), and *Sporidiobolales* in the Nursery room (58.91%) as shown in Figure 2.

Fungal abundance per plant sample also showed remarkable variation in their associated fungi. Figure 3 shows that *Capnodiales* was most abundant in the phyllosphere of *Musa paradisiaca* (72.76%) *Dracaena fragrans* (65.29%), *Howea fosteriana* (60.64%), *Malvaviscus penduliflorus* (20.91%), *Chlorophytum comosum* (55.87%), *Dracaena draco* (64.65%), and *Olea europaea* (56.22%), while class *Wallemiales* was found most abundant in the phyllosphere of *Epipremnum aureum* (79.65%), *Beaucarnea recurvata* (83.47%), *and Musa acuminata* (58.11%). On the other hand, *Tremellales* was most abundant in the phyllosphere of *Dracaena marginata* (73.17%) and *Aloe arborescens* (78.59%), whereas *Sporidiobolales* in *Aechmea eurycorymbus* (42.45%), and *Nephrolepis cordifolia* (33.45%).

Variability in the taxonomic structure of the phyllosphere community was also observed for the different replicates of each plant sample (Figure 4). In comparison to the interspecies variation in fungal diversity shown in Figure 3, Figure 4 shows there is less intraspecies variation for all plant samples, except for *Nephrolepis cordifolia*; which exhibits younger leaves compared to the rest of the plants.

The relative abundance of dominant genus was also analyzed at 1% cut-off level and shown in Figure 5. Analysis showed that dominant genera include *Cladosporium* (31.0%), *Wallemia* (20.1%), and *Cryptococcus* (16.6%). Consequently, these three genera were found ubiquitous in all the plant samples.

Alpha diversity patterns

Rarefaction analysis together with Chao1 value revealed variation in the phyllosphere fungal communities per greenhouse room and per plant sample.

The rarefaction curve for each greenhouse did not reach saturation (Figure 6) and the number of OTUs observed covered 45.8% - 53.08% of the estimated taxonomic richness (Chao1) as seen in Table 2. The computed Shannon index of diversity (H') is varied where it was highest in the phyllosphere fungal community in the Nursery room (6.5) and is lowest in the Succulent room (4.2). Statistical analysis using Two-sample T-Test showed that H' of phyllosphere fungal communities in the Nursery room is significantly different from the Cold room but not to the rest of the rooms, while the H' of the phyllosphere fungal community in the Tropical house is significantly different from the Temperate and Cold rooms, and no significant difference between the H' of the Cold and Temperate rooms was observed (Table S3).

Figure 7 shows that the rarefaction curves of phyllosphere fungal community for each plant sample did not show saturation. However, it also showed relatively high percent coverage ranging from 41.5%-53.0% of Chao1 (Table 3). On the other hand, computation of Shannon index of diversity (H') per plant revealed a wider range of value, where *Nephrolepis cordifolia* showed highest with $H' = 6.5$ and *Beaucarnea recurvata* showed lowest with $H' = 2.8$, but showed no significant differences (Table S6).

Drivers of fungal community structure - Beta diversity patterns

In order to determine the uniqueness of the associations of the phyllosphere fungal communities to room microclimate and plant host species, ordination analysis and ANOSIM were performed. BIO-ENV analysis was also done to define which abiotic

and/or biotic variables have higher correlation with the dissimilarity of the communities of phyllosphere fungi using the Euclidean distance (Clarke and Ainsworth 1993).

Analysis using relative abundance-based (Bray-curtis dissimilarities) PCoA showed inconspicuous clustering of the fungal communities (Figure 8a and 8b). Phyllosphere fungal communities in the Temperate and Cold rooms were closer to each other, while communities from the Tropical, Nursery and Succulent rooms were closer to one another, albeit relatively scattered. ANOSIM results also showed significant distances in fungal composition between different rooms (*P=0.001, R=0.40*), indicating that, although weak, there was some association between room microclimate and phyllosphere fungal community composition.

On the other hand, ANOSIM showed relatively higher correlation of fungal community diversity to plant samples (*P=0.001, R=0.89*). Similar to our previous study on phyllosphere bacterial community, the correlation of the fungal community composition was observed associated to plant leaf shape and size. This time, however, the correlation was more stringent. It was observed that only those plants with remarkably similar leaf shape and size exhibited fungal communities that were clustered closer. This was observed in *Dracaena fragrans*, *Howea fosteriana*, and *Dracaena draco*; having a common linear, long, non-lobed, sword-like (ensiform) leaf-shape. *Beaucarnea recurvata* also exhibited the same ensiform leaf-shape but was narrower than the rest of these plant species; correspondingly it also showed distant clustering of fungal communities in comparison. The same observation was apparent in the two *Musa* species shared a common oblong, long, and wide leaf-shape. *Musa x paradisiaca*, however, exhibited a longer leaf size compared to *Musa acuminata*. Consequently, ordination analysis showed highly distant clusters of bacterial communities between the two *Musa* species.

BIO-ENV analysis provides further evidences of the stronger correlation between bacterial community and plant species. In Figure 9, the vectors represent the Spearman rank correlations (ρ_S) between the abiotic and biotic factors influencing the distribution of the fungal community on the leaf surface of the greenhouse plants. According to BIO-ENV analysis, “Samples” (i.e. plant species) had strong influence

on the bacterial population dynamics in the phyllosphere of the greenhouse plants, being the variable that explains the distribution of the relative abundance of the bacterial community (*BEST = 0.9205*).

Sample plants in the Tropical room best summarized the higher influence of plant species on the fungal community composition of the phyllosphere. Despite experiencing the same ambient climatic condition clustering of the fungal communities were highly inclined to plant species (Figure 8b). This species effect was also shown in Figure 9 where the vector representing "Sample" indicates its directional influence.

Antagonistic potential of phyllosphere fungal community against *B. cinerea*

A total of 629 fungal isolates from the phyllosphere of 14 greenhouse plants were screened for their antagonistic potential against *B. cinerea* using TCVA. Antagonistic effect observed includes inhibition of mycelial growth and spore germination (Figure 10).
Table 1 showed the number of isolates per plant sample and the percentage of fungal strains that tested positive for both antagonistic effects. *Olea europaea* showed the highest percentage of antagonistic fungal strains where 33% of the total fungal isolates exhibited inhibitory effect on both mycelial growth and spore germination of *B. cinerea*.

This study focused on identifying the 85 isolates that showed optimum antagonistic potential against the model pathogenic fungi. BOX-PCR fingerprinting and analysis, further divides these isolates into 39 genotypic groups at 60 % cut-off level. These groups was further observed consist of 3 different classes including *Eurotiomycetes*, *Dothideomycetes*, and *Tremellomycetes*. Using SANGER sequencing, 17 species were identified, out of these genotypic groups (Table 4). Frequently isolated antagonistic fungal species includes: *Penicillium brevicompactum* (10), *Penicillium polonicum* (5), *Cladsporium sphaerospermum* (5), and *Penicillium crustosum* (4). It was also noted that *Penecillium* was highly represented with 13 different species namely *P. brevicompactum, P. paxili, P. rubens, P. raistrickii, P. stecki, P. corylophilum, P. commune, P. adametzioides, P. restricum, crustosum, P. polonicum, P. copticola,* and

P. nothofagi. Three days after the initial set-up, the VOCs produced by these isolates decreased the fungal colony diameter by about 32.89% - 72.23% compared to the control; albeit ANOVA showed no significant differences between the inhibition percentages. (Table S7).

During the initial screening for antagonistic potential, it was also observed that a few fungal strains promote rather than inhibit the growth of *B. cinerea*. After thorough investigation, six fungal strains showed consistent growth promotion of *B. cinerea*; albeit very minimal and did not show significant differences (Table 5). BOX-PCR fingerprinting together with SANGER sequencing assigned these strain into six different species belonging to 4 different fungal classes. *Sarocladium strictum and Fusarium circinatum*; class *Sardariomycetes, Cladosporium sphaerospermum, and Cladosporium pini-ponderosae*; class *Dothideomycetes, Penicillium steckii*; class *Eurotiomycetes,* and *Cryptococcus flavescens*; class *Tremellomycetes*

Discussion

Using cultivation-dependent and high throughput analysis, this study found high abundance and diversity of fungal community in the phyllosphere of the 14 greenhouse plants. It was also established that plants species have higher influence on fungal community composition, and plant leaf morphology was correlated to the distances in community composition of phyllosphere-associated fungi.

The documented fungal community of the phyllosphere (combining leaf samples from 14 different plant species) consists primarily, by members of the phylum *Ascomycota* and *Basidiomycota*. Fungal taxa identified were similar to those found in previous studies, including high relative abundance of *Capnodiales*, *Wallemiales*, and *Tremellales* (Jumpponen and Jones, 2009; Cordier et al, 2012; Bálint et al, 2013). The dominance of these fungal taxa in the overall phyllosphere community can be due in part to their association with the host plants since the distribution of the *Ascomycetous* and *Basideomycetous* fungal taxa was not the same for all plant samples, where some have higher abundance of *Ascomycota* while others have higher *Basidiomycota*. This association will be further discussed in the later part of this paper. The adaptive abilities of the members of these fungal taxa also facilitated for their dominance in the

phyllosphere. The phyllosphere is considered a stressful environment partly because of exposure to UV radiation and fluctuating water availability (Lindow and Brandl, 2003; Whipps et al, 2008; Vorholt, 2012). Melanization and DNA repair have been established as UV-protection response from microorganisms inhabiting the phyllosphere (Grishkan, 2011; Fernandez et al, 2012). Melanins that are responsible for the dark-green, brown, and black color of fungi are also apparently responsible for adaptive properties that enable them to survive under conditions of environmental stress, such as osmotic extremes, UV radiation, and desiccation (Sterflinger, 2006). Many melanized species belonging to order *Capnodiales* and *Pleosporales* have been isolated from environments highly exposed to UV radiation (Grishkan, 2011; Kembel and Mueller, 2014). This explains not only the dominance but also the ubiquitous nature of *Capnodiales* in the phyllosphere of the greenhouse plants observed. Aside from melanin, carotenoid pigments in yeasts has also been established to exhibit photoprotection (Moliné et al, 2010; Castiliani et al, 2014) and member of the order *Tremellales* that were identified in this study were similar to those that were previously characterized with carotenoid pigmentation (Inácio et al, 2005). This study also identified yeasts that were reported to produce and accumulate mycosporines (Fernandez et al, 2012). This fungal metabolite is known for its photoprotective effect on yeasts protecting them from UVB-induced DNA damage (Moliné et al, 2011). Aside from UV-protection microbial phyllosphere colonists also showed adaptive mechanisms that counters the fluctuating water availability in the phyllosphere (Vorholt, 2012). Members of the order *Wallemiales* that were identified in this study were also reported to be xerophilic fungi (Zalar et al, 2006). The growth of these xerophilic fungi on artificial media proved to be independent to the solute used in order to lower water activity (Vaisey 1955; Pitt and Hocking 1977; as cited by Zalar et al, 2006). The abundance of the xerophilic fungi identified in this study suggests that this mechanism is also imperative for successful colonization of the phyllosphere.

It was mentioned earlier that plant-host and fungi association also accounts for the diversity of the fungal taxa observed in this study. Fungal communities inhabiting the phyllosphere of the 14 greenhouse plants vary within and across individual plants (Figures 3 and 4). Except for *Nerphrolephis cordifolia*, a higher variation of the

fungal community was observed across than within plant species. This implies that different plant species affects the selection of respective fungal associates of their phyllosphere fungal community. The same influence was also observed in bacterial community composition of the same 14 greenhouse plant samples in our previous study on phyllosphere bacterial communities.

On the other hand, the high variation within the samples from *Nephrolephis cordifolia* may be attributed to the age of the plant. Originating from the Nursery room, *Nephrolephis cordifolia* is the only sample in this study that was relatively younger than the rest of the plants, and the high variability of associated fungi from within the leaf samples further supports previous reports that younger leaves harbors greater number of taxa than those of the older leaves (Ercolani, 1991; Thompson et al, 1993).

Plant species influence on the fungal community composition was made stronger by the variation observed in community composition within and across different greenhouse rooms. Fungal community composition was higher within than across different rooms, implying that different species found inside the same rooms harbors different fungal community composition. This was further supported by the results of BIO-ENV analysis where the BEST value was highest for “Samples” (plant samples) indicating that this factor best explains the distribution of the associated fungal community. Therefore, these evidences signify that plant species have higher effect on the phyllosphere fungal community in comparison to the ambient room temperature.

The correlation between plant leaf morphology and distances of fungal community also further strengthens the evidences of higher plant-host influence on the phyllosphere fungal community composition. *Dracaena fragrans*, *Dracaena draco* and *Howea fosteriana*, exhibiting the same ensiform leaf-shape and leaf size, harbored fungal communities that are closer to one another. The same correlation was observed in our previous study on phyllosphere bacterial community, however the correlation of the distances of fungal community to plant-leaf morphology was more stringent. Along with *Dracaena fragrans*, *Dracaena draco* and *Howea fosteriana*, *Beaucarnea recurvata* exhibited closer bacterial community composition. This difference in fungal and bacterial community correlation to leaf morphology

highlights the fact that the leaf characteristics that highly influenced the microbial community structure of the plant samples may be those that were not covered in this study. Nevertheless, these findings further supports previous reports that individual plants can have exclusive microbial associates possibly owing to their genetic make-up that ultimately controls their phenotypic characteristics and metabolism that is responsible for production of microbial attractants or defenses (Whipps, et al 2008; Hartmann et al. 2009; Cordier et al, 2012; Vorholt, 2012; Bálint et al, 2013; Kembel and Mueller, 2014).

Evidences that the phyllosphere fungal community of the samples plants consist of species that produces antifungal-volatiles were also presented in this study. Fungal species belonging to the genera *Penicillium*, *Cryptococcus*, and *Cladosporium* exhibited antagonistic effect to the pathogen *B. cinerea* by inhibiting its mycelial growth by approximately 30% to 80% with a visible reduction, if not total inhibition, of spore formation. The biocontrol activity of *Penicillium and Cryptococcus* against *B. cinerea* (Rouissi et al, 2013; Helbig 2001) and of *Cladosporium* as a mycoparasite has been well documented (Kiss, 2003) along with a comprehensive review on fungal volatiles produced by representative species from these genera (Morath et al, 2013).

Remarkably, it was also observed that VOCs produced by *Cladosporium sphaerospremum, Cladosporium pini-ponderosae* and *Penicillium steckii* have both negative and positive effects on the mycelial growth of *B. cinerea*. Apparently, fungal strains from the same lineage can have different bioactivity since they can differ significantly in their quantitative and qualitative secondary metabolite production (Dresch et al, 2015). Since fungal VOCs are produced during primary and secondary metabolism (Crespo et al, 2006) a strain-specific effect may have occurred causing the bipolar bioactivity of *Cladosporium sphaerospremum, Cladosporium pini-ponderosae* and *Penicillium steckii* against *B. cinerea* in this study. Nevertheless, additional investigation, like strain selection along with bioactivity testing, is recommended to further explain the opposing bioactivity of these three fungal species.

The results of this study provide insights into the structure, variability in distribution, and antagonistic potential of phyllosphere fungal communities of plants grown inside

a built environment. Similar to our previous study on phyllosphere bacterial community, the ambient room microclimate had little influence on the phyllosphere communities, and plant taxonomy and traits have higher correlation to the fungal community composition. This implies that plants have a stable fungal diversity composition regardless of the room microclimatic condition.

The implication of plant species having higher influences on the fungal community composition of their associated microbes can be beneficial in setting a healthy built environment that is ultimately favorable to human health. It can also be beneficial in maintaining a healthy indoor air quality of built environments, since both plants placed indoors (Orwell et al, 2004, Pegas et al, 2012) and their associated microbes work together in improving the air quality by absorbing, degrading, detoxifying, and sequestering air pollutants (Kim et al, 2008; Weyens et al, 2015). It also poses the possibility of limiting the growth of pathogenic molds and fungi, which are harmful to human health and can possibly cause “sick building” syndrome (Strauss, 2009), since the phyllosphere bacterial community also includes species that produces antifungal-volatiles. It presents the possibility of establishing the room microbiome by choosing plant species placed indoors (Mahnert et al, 2015) and possibly increase microbial diversity and beneficial microorganisms (Berg et al, 2014).

Comprehension of the driving forces of the microbial community structure is also imperative to the manipulation of plant-associated microbiome for the development of biocontrol methods that can be used as means of plant protection (Lindow and Brandl, 2003). Understanding the microbial structure of the target plant and utilization of microorganisms that occupy the same ecological niche as that of the plant pathogen can result to a more effective biocontrol that is less damaging to the environment.

Conclusion

An abundant and diverse fungal community inhabits the phyllosphere of 14 plants species grown under different controlled microclimate. Plant species strongly influence the distribution of the fungal communities as exhibited by the pronounced interspecies variation within and across different rooms and plant species. Base on plant leaf morphology, a correlation between the dissimilarity of fungal communities

to the genetic distances of the plant species was established. The phyllosphere fungal community also includes VOCs-producing species antagonistic to both the mycelial growth and spore germination of the pathogenic fungi *B. cinerea*. Along with these antagonists, some species were also found to have bipolar bioactivity against the pathogenic fungi.

Table 1. Plate counts (CFU/cm^{-2}) and percentages of antagonistic fungi against the plant pathogenic fungi *Botrytis cinerea*.

Greenhouse room	Plant sample	CFU ± SD		Total isolates tested	Inhibits growth		Inhibits sporulation		Inhibits both growth and sporulation	
		SNA	Sab		No.	%	No.	%	No.	%
Tropical	*Aechmea eurycorymbus*	[a]4.44E+05 ± 7.00E+04	[a]1.05E+05 ± 1.47E+04	78	2	3	42	54	2	3
	Dracaena marginata	[a]2.95E+05 ± 4.20E+04	[a]3.73E+05 ± 9.51E+04	78	2	3	46	59	2	3
	Epipremnum aureum	[a]1.23E+05 ± 1.16E+05	[a]6.63E+04 ± 5.94E+04	29	3	10	21	72	2	7
	Musa paradisiaca	[b]1.07E+06 ± 1.59E+05	[b]7.32E+05 ± 8.51E+04	77	13	17	37	48	13	17
Temperate	*Dracaena fragrans*	[a]1.45E+04 ± 6.68E+03	[a]2.26E+04 ± 1.27E+04	16	4	25	9	56	4	25
	Howea forsteriana	[a]3.36E+04 ± 9.81E+03	[a]3.58E+04 ± 1.30E+04	24	6	25	14	58	5	21
	Malvaviscus penduliflorus	[a]5.88E+03 ± 3.40E+03	[a]6.95E+03 ± 1.94E+03	12	1	8	8	67	0	0
Nursery	*Nephrolepis cordifolia*	[a]1.43E+03 ± 1.17E+03	[a]1.69E+03 ± 1.37E+03	24	4	17	15	63	3	13
Cold	*Chlorophytum comosum*	[a]1.81E+05 ± 7.76E+04	[a]1.94E+05 ± 8.28E+04	79	9	11	50	63	9	11
	Dracaena draco	[a]2.27E+04 ± 5.68E+03	[a]3.96E+04 ± 1.14E+04	21	5	24	13	62	5	24
	Olea europaea	[a]2.37E+04 ± 4.23E+03	[a]3.84E+04 ± 2.93E+03	18	6	33	12	67	6	33
Succulent	*Aloe arborescens*	[a]2.81E+04 ± 8.02E+03	[a]2.86E+04 ± 5.21E+03	53	16	30	23	43	15	28
	Beaucarnea recurvata	[a]6.03E+03 ± 1.18E+03	[a]3.31E+04 ± 1.58E+04	74	11	15	19	26	10	14
	Musa acuminata	[a]9.84E+02 ± 6.94E+02	[a]4.50E+02 ± 1.46E+02	46	10	22	12	26	9	20

**Means that do not share the same letter are significantly different (alpha=0.05).*

Capnodiales, 33%
Eurotiales, 6%
Pleosporales, 4%
Wallemiales, 20%
Tremellales, 17%
Sporidiobolales, 6%

Capnodiales
Pleosporales
Helotiales
Dothidiomycetes
Xylariales
Incertae sedis
Taphrinales
Incertae sedis
Erysiphales
Lecanorales
Rhizocarpales
Glomerellales
Tubeufiales
Onygenales
Microascales
Incertae sedis
Incertae sedis
Lecanoromycetes
Lichinales
Candelariales
Xylonomycetales
Orbiliales
Leotiomycetes
Chaetosphaeriales
Leotiales
Orbiliomycetes
Mycocaliciales
Peltigerales
Agyriales
Incertae sedis
Melanosporales
Umbilicariales
Patellariales
Tremellales
Polyporales
Russulales
Incertae sedis
Agaricostilbales
Filobasidiales
Hymenochaetales
Corticiales
Agaricomycetes
Trechisporales
Gloeophyllales
Incertae sedis
Ustilaginales
Trichosporonales
Sebacinales
Exobasidiomycetes
Microbotryales
Microstromatales
Agaricostilbomycetes
Phallales
Pucciniales
Entylomatales
Dacrymycetales
Geminibasidiales
Protista
Spizellomycetales
Rhizophlyctidales
Lobulomycetales
Chytridiales
Glomerales
Diversisporales
Rozellomycota
Basidiobolales
Mucorales
Fungi
Eurotiales
Ascomycota
Hypocreales
Sordariomycetes
Chaetothyriales
Sordariales
Dothideales
Arthoniales
Pezizales
Saccharomycetales
Botryosphaeriales
Lecideales
Teloschistales
Eurotiomycetes
Trichosphaeriales
Lulworthiales
Diaporthales
Venturiales
Rhytismatales
Coniochaetales
Incertae sedis
Pezizomycetes
Verrucariales
Mytilinidiales
Acarosporales
Pyrenulales
Ophiostomatales
Myriangiales
Ascosphaerales
Hysteriales
Archaeorhizomycetales
Magnaporthales
Wallemiales
Sporidiobolales
Agaricales
Incertae sedis
Cystofilobasidiales
Exobasidiales
Cantharellales
Auriculariales
Erythrobasidiales
Basidiomycota
Cystobasidiales
Atheliales
Geastrales
Boletales
Tremellomycetes
Thelephorales
Leucosporidiales
Malasseziales
Septobasidiales
Amylocorticiales
Helicobasidiales
Incertae sedis
Atractiellales
Microbotryomycetes
Wallemiomycetes
Olpidiales
Chytridiomycota
Rhizophydiales
Chytridiomycetes
Monoblepharidales
Paraglomerales
Incertae sedis
Mortierellales
Kickxellales
Endogonales
Unidentified

Figure 1. Overview of the phyllosphere fungal community composition found on 14 different greenhouse plants.

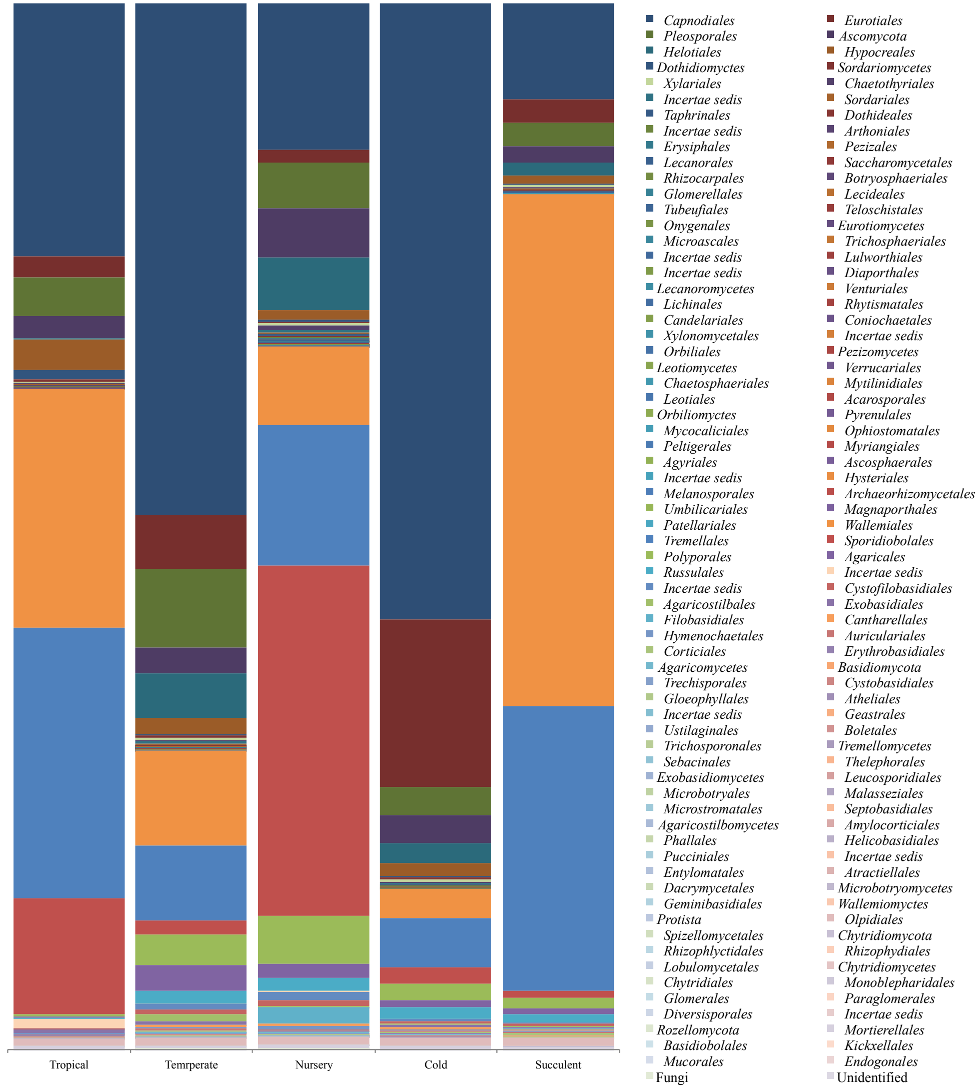

Figure 2. Relative abundance of phyllosphere fungal communities composition found on 14 different greenhouse plants categorized per greenhouse room.

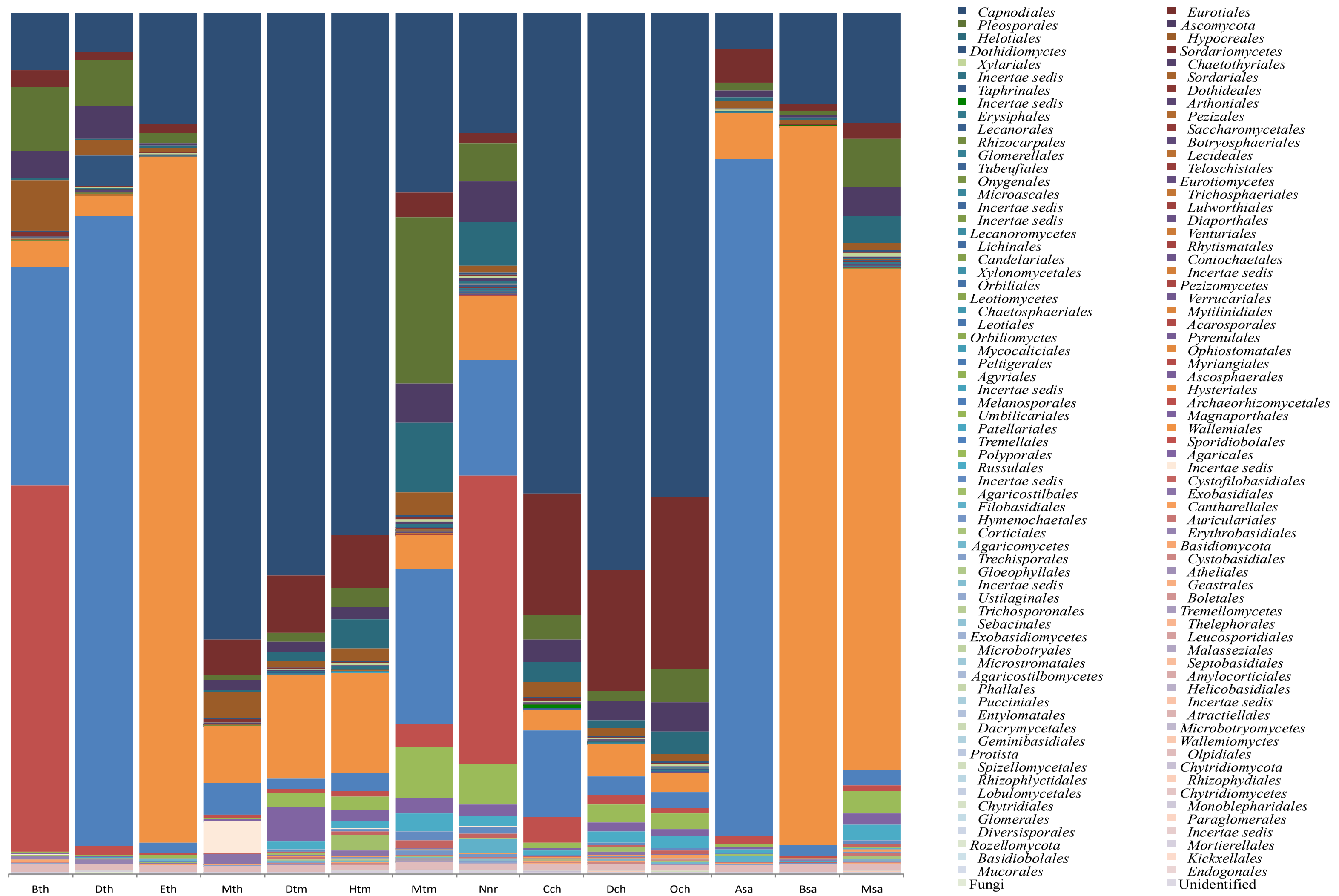

Figure 3. Relative abundance of phyllosphere fungal community composition found on 14 different greenhouse plants categorized per plant species.

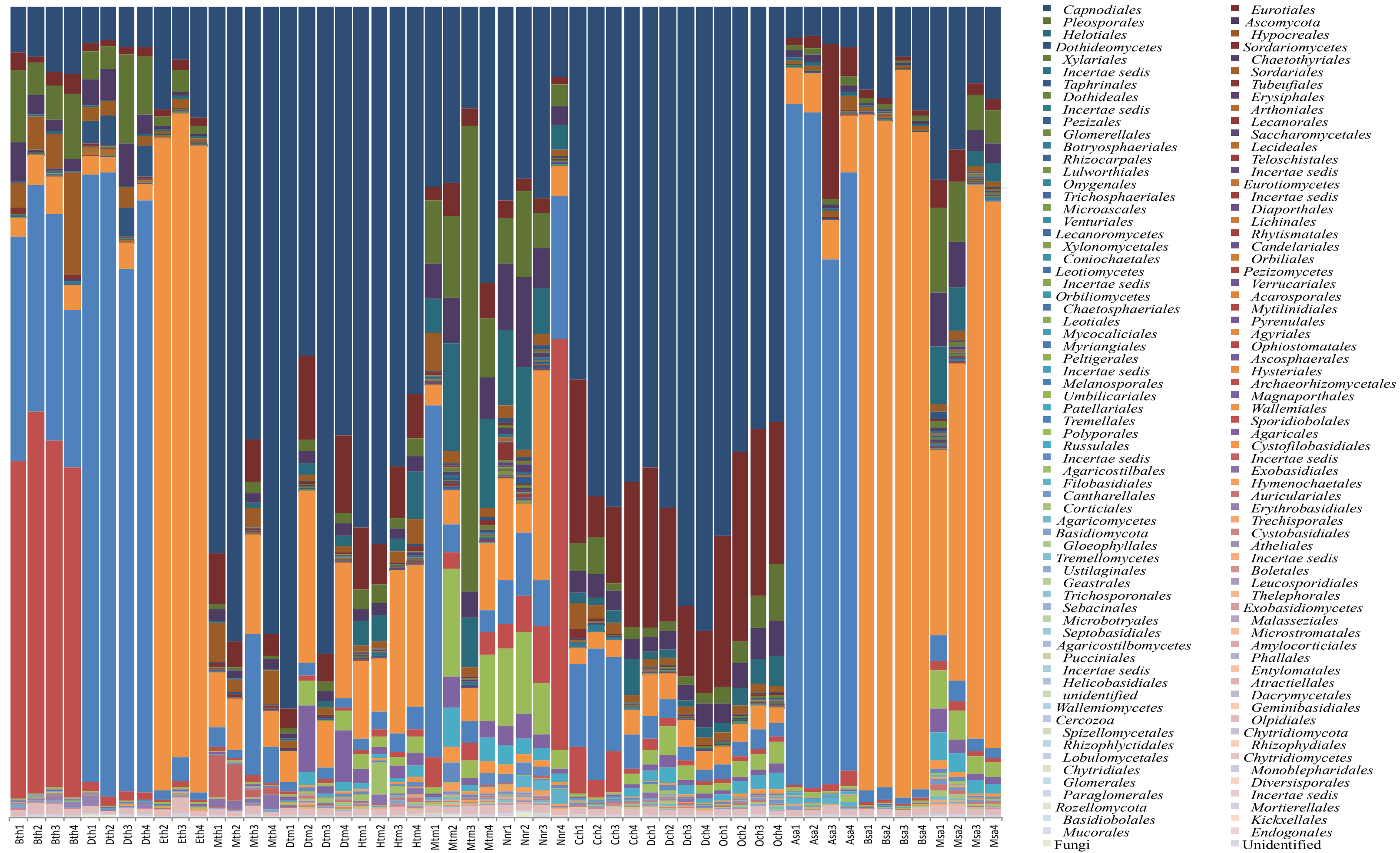

Figure 4. Relative abundance of phyllosphere fungal community composition from four different replicates of each14 different greenhouse plants.

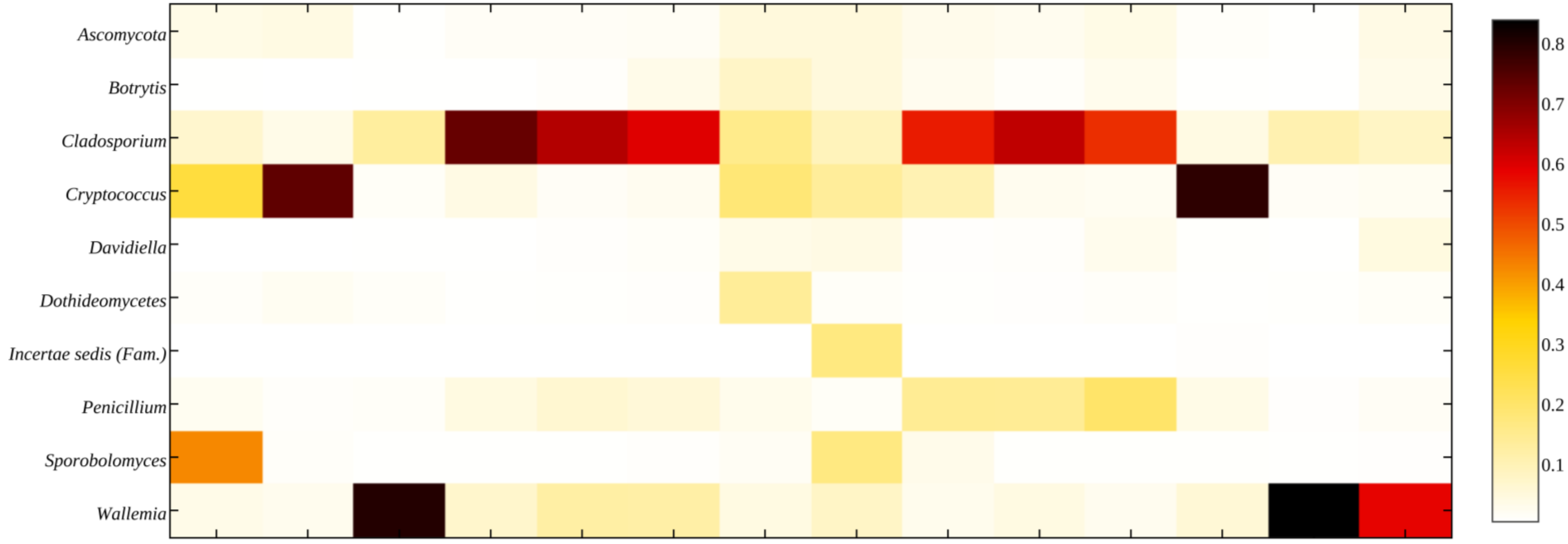

Figure 5. Relative abundance of dominant fungal genera (cut-off 1%) found on the phyllosphere of 14 different greenhouse plants.

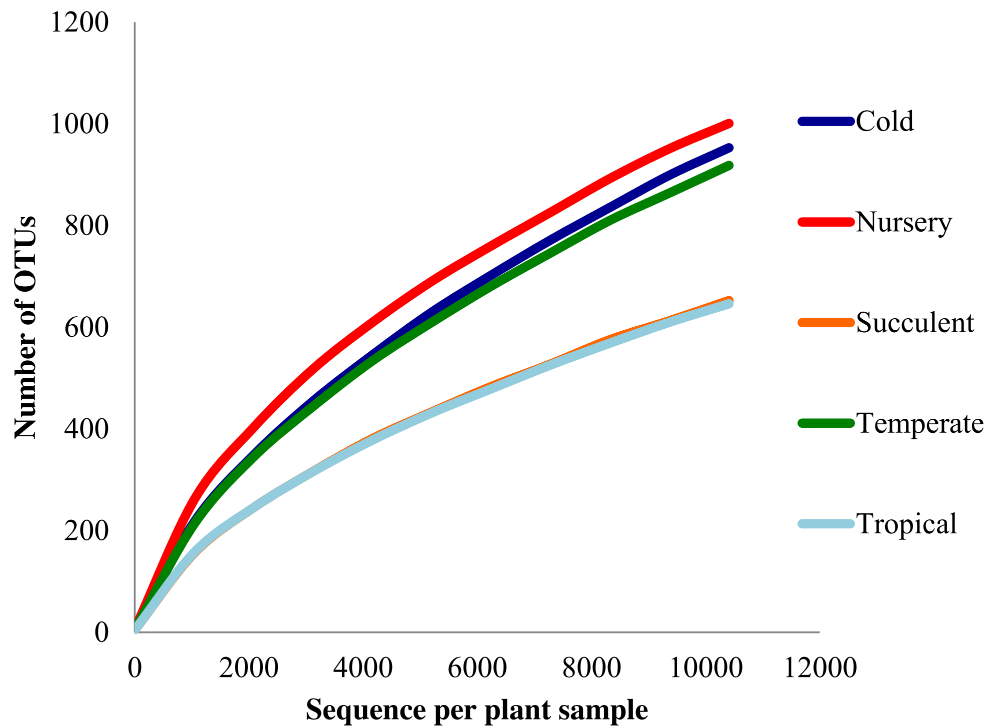

Figure 6. Rarefaction results for the diversity of phyllosphere fungal community of 14 greenhouse plants. Diversity per room is represented.

Table 2. Species richness estimate (categorized per room) obtained at 3% genetic dissimilarity using Miseq Illumina-derived sequences of the DNA extracted from 14 greenhouse plants.

Greenhouse room	Seqs/Sample	Shannon index (H')	Observed species (no. of OTUs)	Chao1 (no. of OTUs)	Coverage (%)
Cold	10410	5.3	953.0	2056.4	46.3
Nursery	10410	6.5	1000.8	1887.4	53.0
Succulent	10410	4.2	652.6	1418.2	46.0
Temperate	10410	5.4	918.2	1919.1	47.8
Tropical	10410	4.4	646.1	1409.1	45.8

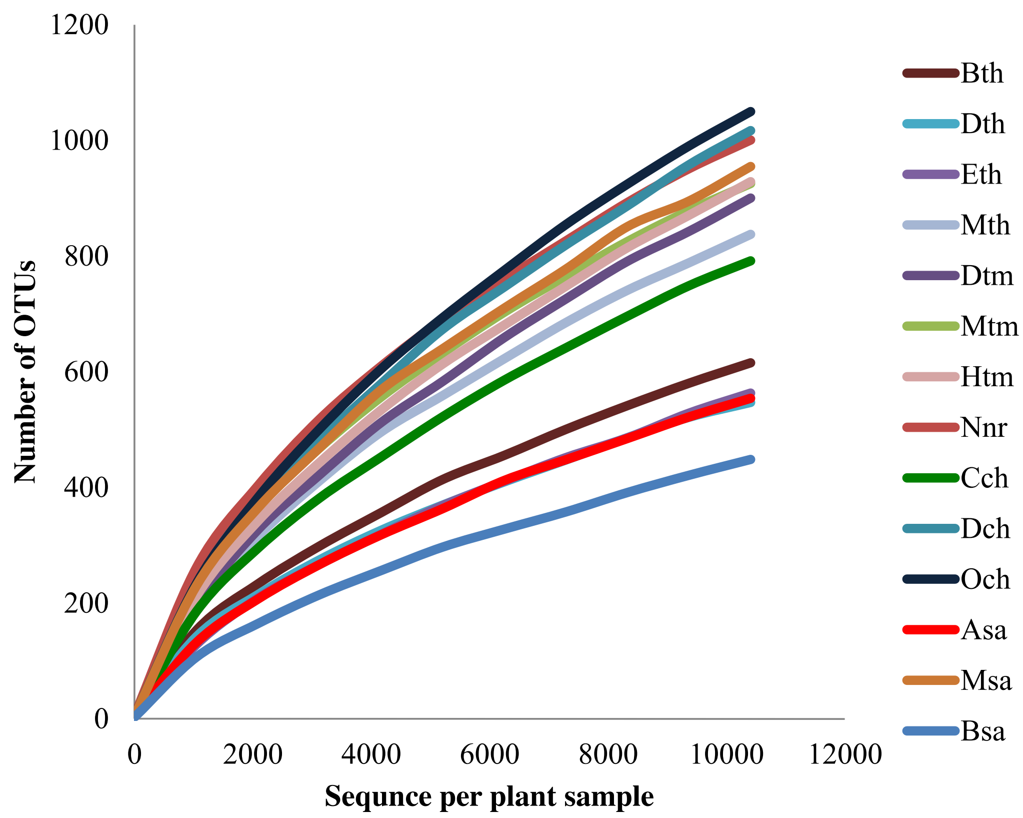

Figure 7. Rarefaction results for the diversity of phyllosphere fungal community of plants. Diversity per plant is represented

Table 3. Species richness estimate (categorized per plant) obtained at 3% genetic dissimilarity using Miseq Illumina-derived sequences of the DNA extracted from 14 greenhouse plants.

Greehouse room	Sample	Origin	Seqs/Sample	Shannon index (H')	Observed species (no. of OTUs)	Chao1 (no. of OTUs)	Coverage (%)
Tropical	Bth	*Aechmea eurycorymbus*	10410	4.4	615.5	1406.6	43.8
	Dth	*Dracaena marginata*	10410	4.5	547.1	1163.9	47.0
	Eth	*Epipremnum aureum*	10410	3.2	563.2	1358.7	41.5
	Mth	*Musa paradisiaca*	10410	5.0	837.7	1694.6	49.4
Temperate	Dtm	*Dracaena fragrans*	10410	4.9	900.4	1974.1	45.6
	Htm	*Howea forsteriana*	10410	5.3	928.7	1979.3	46.9
	Mtm	*Malvaviscus penduliflorus*	10410	6.1	925.6	1804.0	51.3
Nursery	Nnr	*Nephrolepis cordifolia*	10410	6.5	1000.8	1887.4	53.0
Cold	Cch	*Cch comosum*	10410	5.0	791.9	1753.6	45.2
	Dch	*Dracaena draco*	10410	5.5	1017.1	2190.8	46.4
	Och	*Oleaeuropaea*	10410	5.4	1050.0	2224.7	47.2
Succulent	Asa	*Aloe arborescens*	10410	4.1	554.2	1315.6	42.1
	Bsa	*Beaucarnea recurvata*	10410	2.8	448.5	1052.9	42.6
	Msa	*Musa acuminata*	10410	5.5	955.0	1886.1	50.6

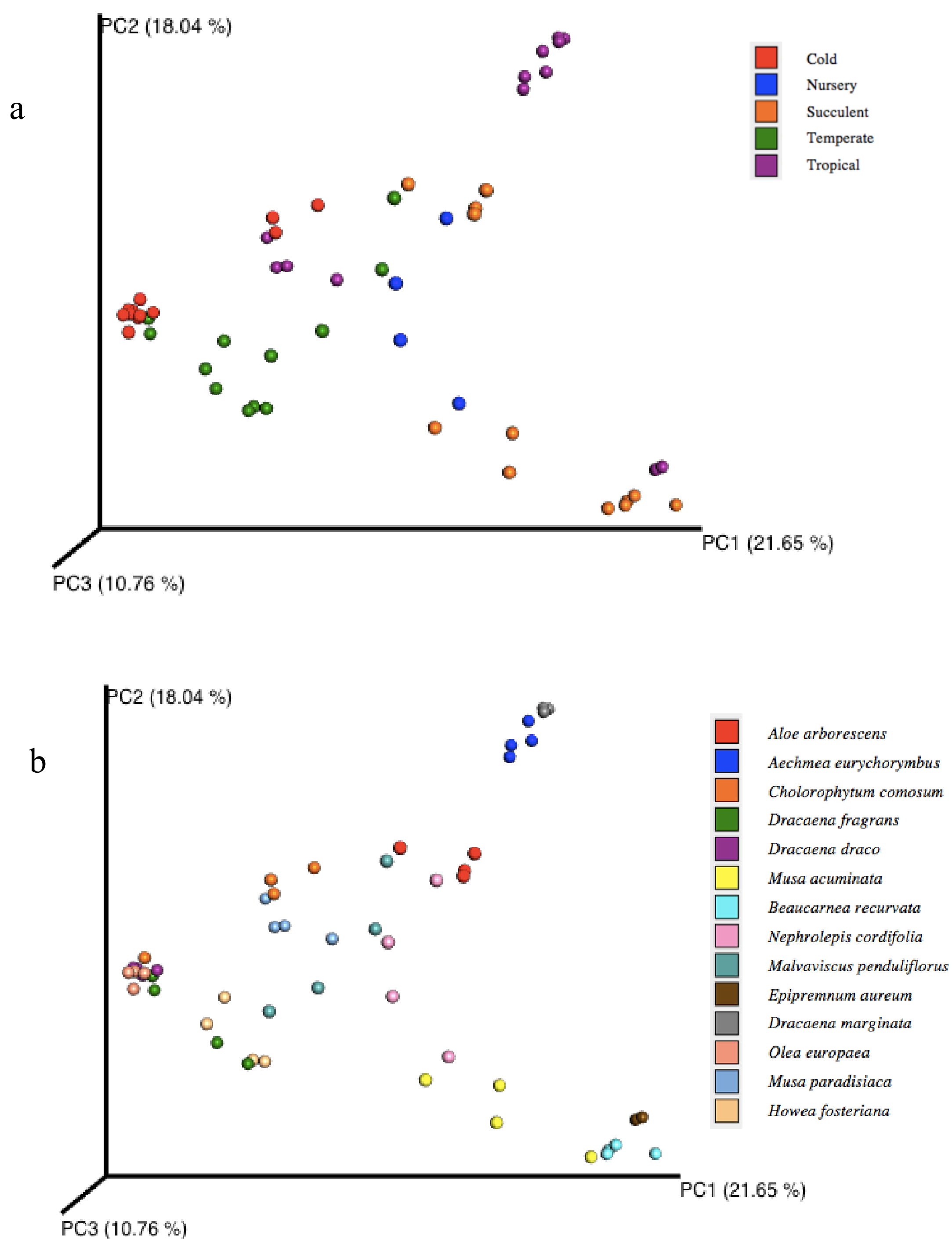

Figure 8. PCoA plots showing the clustering patterns between samples in A) greenhouse rooms, and B) plant species, based on Bray-Curtis dissimilarity.

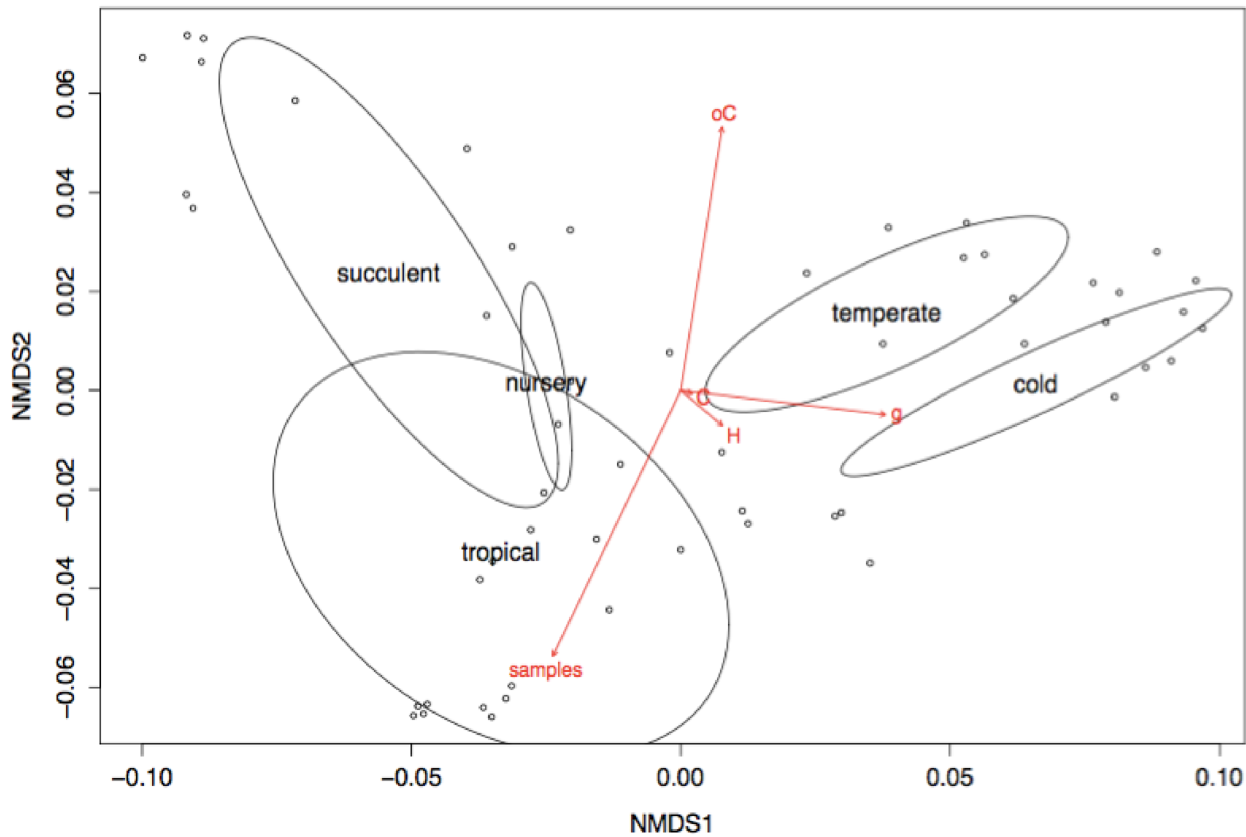

Figure 9. Non-metric multidimensional scaling (NMDS) plot derived from Bray-Curtis dissimilarity illustrating distances between fungal community compositions. The BIO-ENV vectors of environmental variables based on Euclidean distances represent the direction along the samples of each greenhouse rooms, showing the role each of them played in explaining the distribution of the samples and its directional influence.

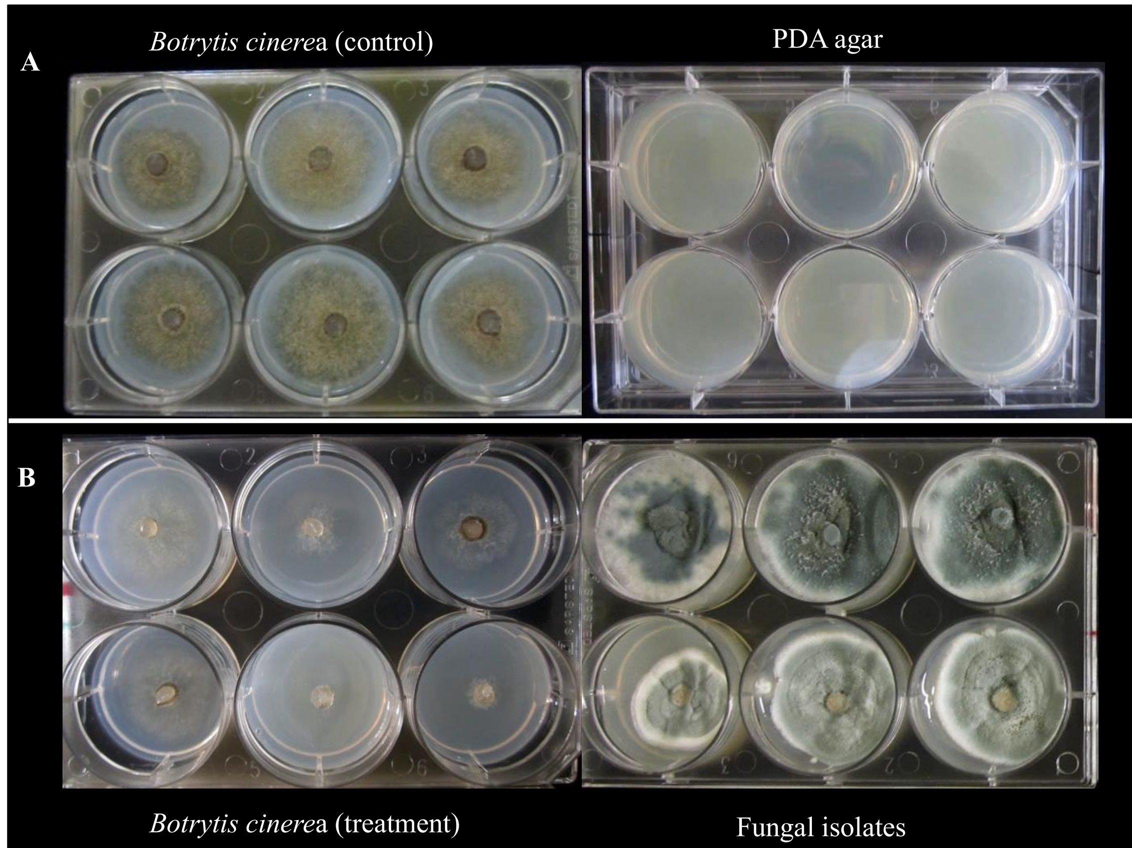

Figure 10. Two-clamp VOCs assay of fungal isolates from 14 greenhouse plants showing antifungal volatile activity against *Botrytis cinerea*. (A) *B*. *cinerea* plugs showing mycelial growth and spore germination (left), paired with an empty PDA plate (right). (B) Volatile organic compounds produced by fungi (right) affected mycelial growth and germination of spores (left), compared to the control (above left).

Table 4. Taxonomic classification of fungal strains with antagonistic effect against *Botrytis cinerea* isolated from the phyllosphere of 14 greenhouse plants.

Greenhouse room	Strains	Plant of origin	Score	Species	Ident (%)	Accession
Tropical	Bth3Sab1	*Aechmea eurycorymbus*	++	*Penicillium brevicompactum* NRRL 2011	97	NR_121299.1
	Dth2Sab10	*Dracaena marginata*	++	*Penicillium paxilli* CBS 360.48	99	NR_111483.1
	Eth1Sab2	*Epipremnum aureum*	++	*Penicillium rubens* CBS 129667	99	NR_111815.1
	Eth4Sab3		++	*Cladosporium sphaerospermum* CBS 193.54	100	NR_111222.1
	Mth3Sab3	*Musa paradisiaca*	++	*Penicillium brevicompactum* NRRL 2011	97	NR_121299.1
	Mth4Sab3		++	*Penicillium raistrickii* FRR 1044	100	NR_119493.1
	Mth4Sab9		++	*Penicillium steckii* CBS 260.55	99	NR_111488.1
Temperate	Dtm3Sab6	*Dracaena fragrans*	++	*Penicillium brevicompactum* NRRL 2011	99	NR_121299.1
	Dtm4SNA9		++	*Penicillium brevicompactum* NRRL 2011	99	NR_121299.1
	Dtn3SNA1		++	*Penicillium corylophilum* NRRL 802	99	NR_121236.1
	Htm4Sab2	*Howea forsteriana*	++	*Penicillium commune* CBS 311.48	99	NR_111143.1
	Htm4SNA7		+++	*Penicillium adametzioides* CBS 313.59	100	NR_103660.1
Nursery	Nnr4SNA1	*Nephrolepis cordifolia*	++	*Penicillium restrictum* NRRL 1748	98	NR_121239.1
Cold	Cch1Sab4	*Chlorophytum comosum*	++	*Penicillium crustosum* FRR 1669	99	NR_077153.1
	Cch1SNA2		++	*Penicillium polonicum* CBS 222.28	99	NR_103687.1
	Cch1SNA3		++	*Penicillium brevicompactum* NRRL 2011	99	NR_121299.1
	Cch3SNA8		+++	*Penicillium polonicum* CBS 222.28	100	NR_103687.1
	Cch4SNA8		+++	*Cladosporium sphaerospermum* CBS 193.54	100	NR_111222.1
	Dch3Sab5	*Dracaena draco*	+++	*Penicillium polonicum* CBS 222.28	100	NR_103687.1
	Dch3Sab8		++	*Penicillium polonicum* CBS 222.28	100	NR_103687.1
	Dch4Sab2		++	*Penicillium polonicum* CBS 222.28	99	NR_103687.1
	Och3SNA6	*Olea europaea*	++	*Penicillium brevicompactum* NRRL 2011	99	NR_121299.1

[a] *Inhibition score (+) indicates 10-30%; (++) 31-60% ; (+++) 61-80%; (++++) 81-100% mycelial growth inhibition on Botrytis cinerea.*

Table 4. cont.

Greenhouse room	Strains	Plant of origin	Score	Species	Ident (%)	Accession
Cold	Och4Sab4		+++	*Penicillium crustosum* FRR 1669	99	NR_077153.1
Succulent	Asa1SNA1	*Aloe arborescens*	+++	*Cryptococcus magnus* CBS 140	99	NR_130655.1
	Asa3SNA1		+++	*Penicillium brevicompactum* NRRL 2011	99	NR_121299.1
	Asa3SNA10		+++	*Penicillium brevicompactum* NRRL 2011	99	NR_121299.1
	Asa4Sab9		++	*Penicillium copticola* CBS 127355	99	NR_121516.1
	Asa4SNA6		+++	*Cryptococcus albidus* CBS 142	99	NR_111046.1
	Bsa2Sab3	*Beaucarnea recurvata*	+++	*Penicillium brevicompactum* NRRL 2011	97	NR_121299.1
	Bsa2Sab7		++	*Penicillium crustosum* FRR 1669	99	NR_077153.1
	Bsa2SNA4		+++	*Cladosporium sphaerospermum* CBS 193.54	99	NR_111222.1
	Bsa4Sab5		++	*Cladosporium pini-ponderosae*	97	NR_119730.1
	Bsa4SNA3		++	*Penicillium brevicompactum* NRRL 2011	99	NR_121299.1
	Bsa4SNA6		++	*Cladosporium sphaerospermum* CBS 193.54	100	NR_111222.1
	Msa2Sab7	*Musa acuminata*	++	*Penicillium nothofagi* CBS 130383	99	NR_121518.1
	Msa2SNA5		++	*Cladosporium sphaerospermum* CBS 193.54	100	NR_111222.1
	Msa3Sab6		++	*Cladosporium pini-ponderosae*	100	NR_119730.1
	Msa3SNA4		++	*Penicillium crustosum* FRR 1669	99	NR_077153.1

[a]*Inhibition score (+) indicates 10-30%; (++) 31-60% ; (+++) 61-80%; (++++) 81-100% mycelial growth inhibition on Botrytis cinerea.*

Table 5. Taxonomic classification of fungal strains with positive effect on mycelial growth of *Botrytis cinerea* isolated from the phyllosphere of 14 greenhouse plants.

Greenhouse room	Strains	Plant of origin	Score	Species	Ident (%)	Accession
Tropical	Bth2SNA6	*Aechmea eurycorymbus*	+	*Sarocladium strictum* CBS 346.70	99	NR_111145.1
	Bth2SNA7		+	*Cryptococcus flavescens* CBS 942	98	NR_130696.1
	Bth3Sab9		+	*Cladosporium sphaerospermum* CBS 193.54	100	NR_111222.1
	Bth3SNA2		+	*Fusarium circinatum* CBS 405.97	99	NR_120263.1
	Bth4SNA1		+	*Cladosporium pini-ponderosae*	99	NR_119730.1
	Mth1SNA4	*Musa paradisiaca*	+	*Penicillium steckii* CBS 260.55	99	NR_111488.1

[a]*Inhibition score (+) indicates 1-10% mycelial growth promotion on B. cinerea*

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Supplementary tables and figures:

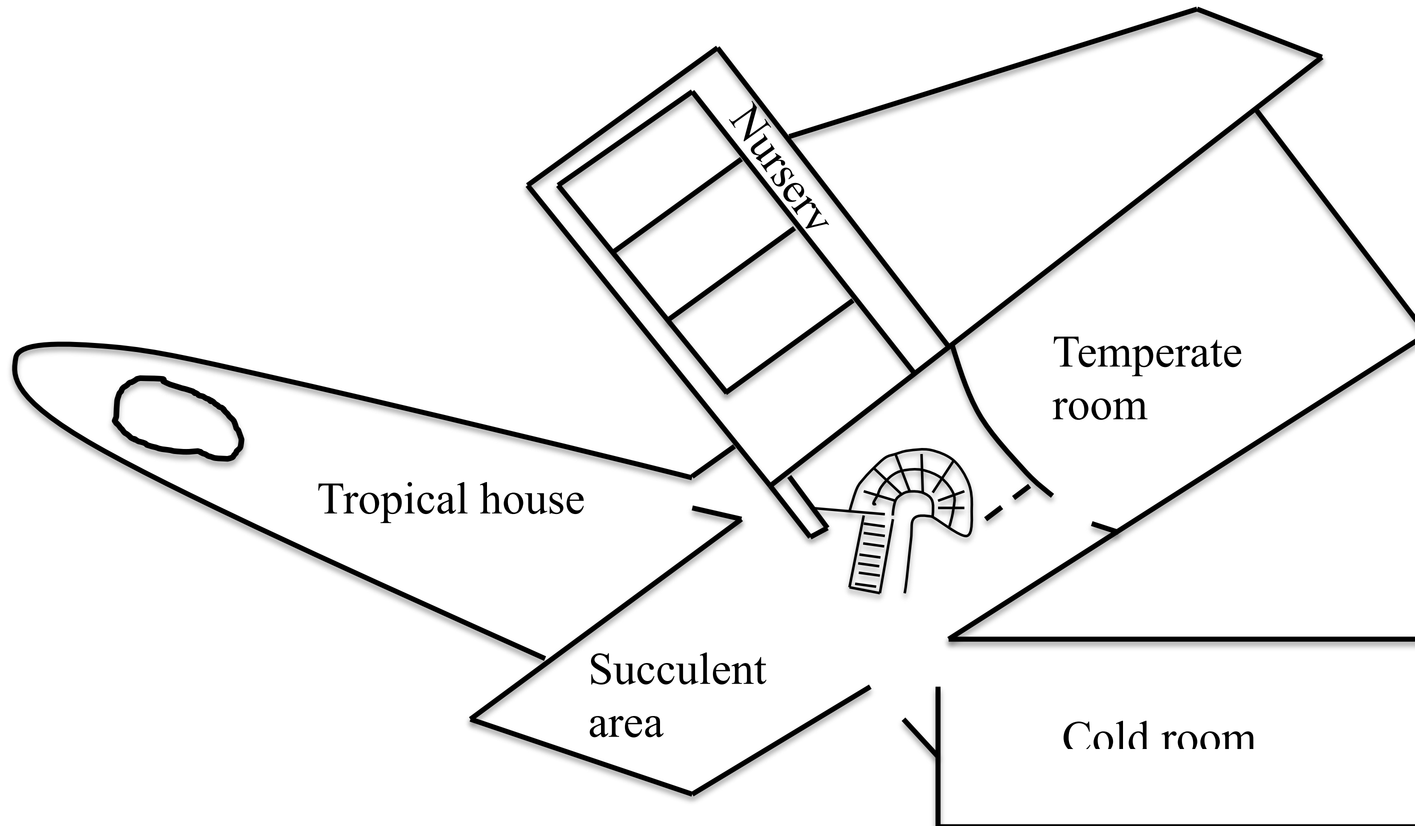

Figure S1. The complex plan of the Botanical Garden of Graz greenhouse

Table S1. ANOVA of phyllosphere fungal CFU of 14 different greenhouse plants

Source of Variation	SS	df	MS	F	p-level	F crit
Between Groups	1.5455E+12	13	1.18885E+11	13.84069	0.00001	2.50726
Within Groups	1.20253E+11	14	8.58951E+09			
Total	1.66575E+12	27				

Table S2. Tukey's test of phyllosphere fungal CFU of 14 different greenhouse plants

	Plat samples	N	Subset for alpha 0.05	
			1	2
Tukey's HSD[a]	Msa	2	717.00	
	Nnr	2	1560.00	
	Mtm	2	6414.00	
	Dtm	2	18537.00	
	Bsa	2	19566.00	
	Asa	2	28368.00	
	Och	2	31050.00	
	Dch	2	31137.00	
	Htm	2	34677.00	
	Eth	2	94566.00	
	Cch	2	187765.50	
	Bth	2	274440.00	
	Dth	2	333900.00	
	Mth	2		901440.00
	Sig.		0.10	1.00

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 2.0

Table S3. Alpha diversity statistical analysis of the Shannon index per greenhouse room

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Cold	Tropical	5.29	0.43	4.37	0.71	3.82	0.02
Cold	Temperate	5.29	0.43	5.44	0.90	-0.50	1.00
Cold	Succulent	5.29	0.43	4.16	1.29	2.75	0.08
Cold	Nursery	5.29	0.43	6.53	0.85	-3.55	0.04
Nursery	Temperate	6.53	0.85	5.44	0.90	1.98	0.73
Nursery	Tropical	6.53	0.85	4.37	0.71	4.91	0.01
Nursery	Succulent	6.53	0.85	4.16	1.29	3.20	0.07
Temperate	Succulent	5.44	0.90	4.16	1.29	2.70	0.13
Tropical	Succulent	4.37	0.71	4.16	1.29	0.52	1.00
Tropical	Temperate	4.37	0.71	5.44	0.90	-3.33	0.04

Table S4. Alpha diversity statistical analysis of the Observed Species per greenhouse room

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Cold	Tropical	952.98	153.10	646.05	129.87	5.42	0.01
Cold	Temperate	952.98	153.10	918.24	114.36	0.60	1.00
Cold	Succulent	952.98	153.10	652.56	242.09	3.48	0.02
Cold	Nursery	952.98	153.10	1000.78	136.01	-0.52	1.00
Nursery	Temperate	1000.78	136.01	918.24	114.36	1.11	1.00
Nursery	Tropical	1000.78	136.01	646.05	129.87	4.55	0.01
Nursery	Succulent	1000.78	136.01	652.56	242.09	2.56	0.29
Temperate	Succulent	918.24	114.36	652.56	242.09	3.29	0.05
Tropical	Succulent	646.05	129.87	652.56	242.09	-0.09	1.00
Tropical	Temperate	646.05	129.87	918.24	114.36	-5.49	0.01

Table S5. Alpha diversity statistical analysis of the Chao1 per greenhouse room

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Cold	Tropical	2056.37	288.44	1409.11	231.95	6.22	0.01
Cold	Temperate	2056.37	288.44	1919.12	165.13	1.37	1.00
Cold	Succulent	2056.37	288.44	1418.20	379.58	4.44	0.01
Cold	Nursery	2056.37	288.44	1887.44	199.33	1.02	1.00
Nursery	Temperate	1887.44	199.33	1919.12	165.13	-0.29	1.00
Nursery	Tropical	1887.44	199.33	1409.11	231.95	3.57	0.05
Nursery	Succulent	1887.44	199.33	1418.20	379.58	2.21	0.48
Temperate	Succulent	1919.12	165.13	1418.20	379.58	4.01	0.02
Tropical	Succulent	1409.11	231.95	1418.20	379.58	-0.07	1.00
Tropical	Temperate	1409.11	231.95	1919.12	165.13	-6.18	0.01

Table S6. Alpha diversity statistical analysis of the Shannon index per plant sample

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Asa	Dtm	4.141	0.635	4.920	0.631	-1.506	1.00
Asa	Cch	4.141	0.635	4.978	0.410	-1.919	1.00
Bsa	Dch	2.827	0.276	5.489	0.160	-14.432	1.00
Bsa	Cch	2.827	0.276	4.978	0.410	-7.536	1.00
Bsa	Asa	2.827	0.276	4.141	0.635	-3.284	1.00
Bsa	Mth	2.827	0.276	5.013	0.534	-6.290	1.00
Bsa	Dtm	2.827	0.276	4.920	0.631	-5.259	1.00
Bth	Dtm	4.394	0.256	4.920	0.631	-1.336	1.00
Bth	Dch	4.394	0.256	5.489	0.160	-6.278	1.00
Bth	Cch	4.394	0.256	4.978	0.410	-2.093	1.00
Bth	Asa	4.394	0.256	4.141	0.635	0.641	1.00
Bth	Mth	4.394	0.256	5.013	0.534	-1.808	1.00
Bth	Bsa	4.394	0.256	2.827	0.276	7.202	0.82
Cch	Dtm	4.978	0.410	4.920	0.631	0.135	1.00
Dch	Dtm	5.489	0.160	4.920	0.631	1.514	1.00
Dch	Asa	5.489	0.160	4.141	0.635	3.565	1.00
Dch	Cch	5.489	0.160	4.978	0.410	2.011	1.00
Dth	Mth	4.548	0.204	5.013	0.534	-1.407	1.00
Dth	Nnr	4.548	0.204	6.526	0.852	-3.911	1.00
Dth	Cch	4.548	0.204	4.978	0.410	-1.628	1.00
Dth	Bth	4.548	0.204	4.394	0.256	0.812	1.00
Dth	Bsa	4.548	0.204	2.827	0.276	8.673	1.00
Dth	Dch	4.548	0.204	5.489	0.160	-6.284	1.00
Dth	Asa	4.548	0.204	4.141	0.635	1.056	1.00
Dth	Dtm	4.548	0.204	4.920	0.631	-0.971	1.00
Dth	Och	4.548	0.204	5.400	0.454	-2.965	1.00
Eth	Dth	3.241	0.307	4.548	0.204	-5.705	1.00
Eth	Htm	3.241	0.307	5.333	0.253	-8.346	1.00
Eth	Mth	3.241	0.307	5.013	0.534	-4.343	1.00
Eth	Nnr	3.241	0.307	6.526	0.852	-5.386	1.00
Eth	Dtm	3.241	0.307	4.920	0.631	-3.587	1.00
Eth	Bsa	3.241	0.307	2.827	0.276	1.580	1.00
Eth	Cch	3.241	0.307	4.978	0.410	-5.204	1.00
Eth	Bth	3.241	0.307	4.394	0.256	-4.571	1.00
Eth	Dch	3.241	0.307	5.489	0.160	-10.599	1.00
Eth	Och	3.241	0.307	5.400	0.454	-6.004	1.00
Eth	Asa	3.241	0.307	4.141	0.635	-1.912	1.00
Eth	Mtm	3.241	0.307	6.066	1.139	-3.535	1.00
Htm	Bsa	5.333	0.253	2.827	0.276	11.588	1.00
Htm	Mth	5.333	0.253	5.013	0.534	0.939	1.00

Table S6. Cont.

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Htm	Asa	5.333	0.253	4.141	0.635	3.021	1.00
Htm	Och	5.333	0.253	5.400	0.454	-0.224	1.00
Htm	Nnr	5.333	0.253	6.526	0.852	-2.325	1.00
Htm	Dch	5.333	0.253	5.489	0.160	-0.902	1.00
Htm	Cch	5.333	0.253	4.978	0.410	1.277	1.00
Htm	Dtm	5.333	0.253	4.920	0.631	1.053	1.00
Htm	Bth	5.333	0.253	4.394	0.256	4.520	1.00
Htm	Dth	5.333	0.253	4.548	0.204	4.187	1.00
Msa	Bth	5.511	0.961	4.394	0.256	1.944	1.00
Msa	Dtm	5.511	0.961	4.920	0.631	0.890	1.00
Msa	Cch	5.511	0.961	4.978	0.410	0.883	1.00
Msa	Dth	5.511	0.961	4.548	0.204	1.697	1.00
Msa	Och	5.511	0.961	5.400	0.454	0.180	1.00
Msa	Mtm	5.511	0.961	6.066	1.139	-0.646	1.00
Msa	Nnr	5.511	0.961	6.526	0.852	-1.369	1.00
Msa	Mth	5.511	0.961	5.013	0.534	0.784	1.00
Msa	Asa	5.511	0.961	4.141	0.635	2.059	1.00
Msa	Htm	5.511	0.961	5.333	0.253	0.309	1.00
Msa	Eth	5.511	0.961	3.241	0.307	3.331	1.00
Msa	Bsa	5.511	0.961	2.827	0.276	4.647	1.00
Msa	Dch	5.511	0.961	5.489	0.160	0.039	1.00
Mth	Dtm	5.013	0.534	4.920	0.631	0.195	1.00
Mth	Cch	5.013	0.534	4.978	0.410	0.089	1.00
Mth	Dch	5.013	0.534	5.489	0.160	-1.478	1.00
Mth	Asa	5.013	0.534	4.141	0.635	1.819	1.00
Mtm	Bsa	6.066	1.139	2.827	0.276	4.786	1.00
Mtm	Asa	6.066	1.139	4.141	0.635	2.557	1.00
Mtm	Mth	6.066	1.139	5.013	0.534	1.450	1.00
Mtm	Htm	6.066	1.139	5.333	0.253	1.088	1.00
Mtm	Bth	6.066	1.139	4.394	0.256	2.481	1.00
Mtm	Cch	6.066	1.139	4.978	0.410	1.557	1.00
Mtm	Dch	6.066	1.139	5.489	0.160	0.869	1.00
Mtm	Dtm	6.066	1.139	4.920	0.631	1.525	1.00
Mtm	Och	6.066	1.139	5.400	0.454	0.941	1.00
Mtm	Nnr	6.066	1.139	6.526	0.852	-0.560	1.00
Mtm	Dth	6.066	1.139	4.548	0.204	2.273	1.00
Nnr	Dch	6.526	0.852	5.489	0.160	2.072	1.00
Nnr	Bsa	6.526	0.852	2.827	0.276	7.151	1.00
Nnr	Bth	6.526	0.852	4.394	0.256	4.150	1.00
Nnr	Asa	6.526	0.852	4.141	0.635	3.887	1.00

Table S6. Cont.

Group1	Group2	Group1 mean	Group1 std	Group2 mean	Group2 std	t stat	p-value
Nnr	Mth	6.526	0.852	5.013	0.534	2.606	1.00
Nnr	Och	6.526	0.852	5.400	0.454	2.019	1.00
Nnr	Dtm	6.526	0.852	4.920	0.631	2.624	1.00
Nnr	Cch	6.526	0.852	4.978	0.410	2.835	1.00
Och	Mth	5.400	0.454	5.013	0.534	0.957	1.00
Och	Bsa	5.400	0.454	2.827	0.276	8.380	1.00
Och	Dtm	5.400	0.454	4.920	0.631	1.071	1.00
Och	Dch	5.400	0.454	5.489	0.160	-0.319	1.00
Och	Bth	5.400	0.454	4.394	0.256	3.342	1.00
Och	Cch	5.400	0.454	4.978	0.410	1.195	1.00
Och	Asa	5.400	0.454	4.141	0.635	2.793	1.00

Table S7. ANOVA of the percent inhibition of antagonistic fungal strains against the growth of *Botrytis cinerea*

Source of Variation	SS	df	MS	F	p-level	F crit
Between Groups	15.6509	38	0.41187	1.35598	0.11074	1.50748
Within Groups	35.5375	117	0.30374			
Total	51.1884	155				

Table S8. ANOVA of phyllosphere fungal CFU of sample plants from the Succulent room

Source of Variation	SS	df	MS	F	p-level	F crit
Between Groups	798,225,204	2	399,112,602	3.26523	0.17661	9.55209
Within Groups	366,693,138	3	122,231,046			
Total	1,164,918,342	5				

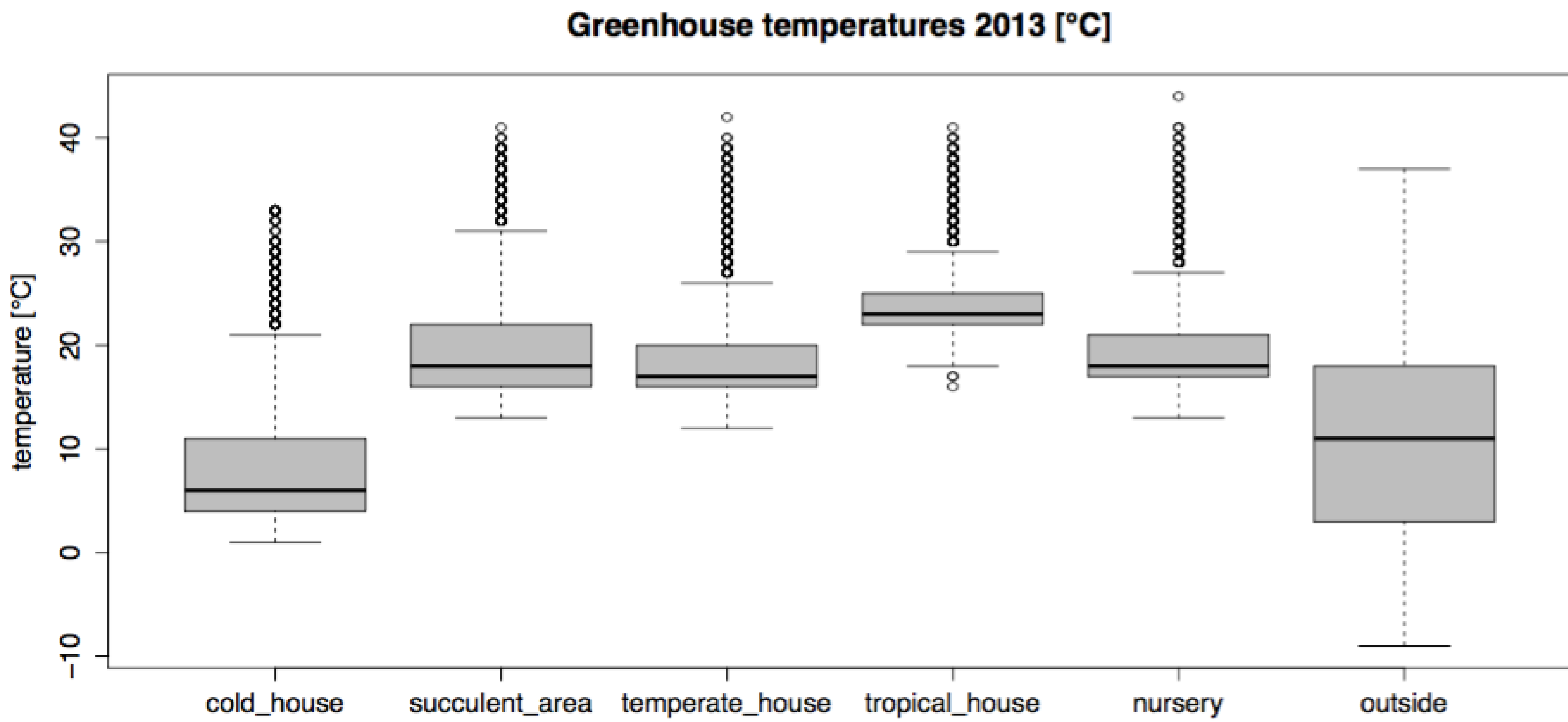

Figure S2. Mean temperature inside each room of the greenhouse complex for the year 2013

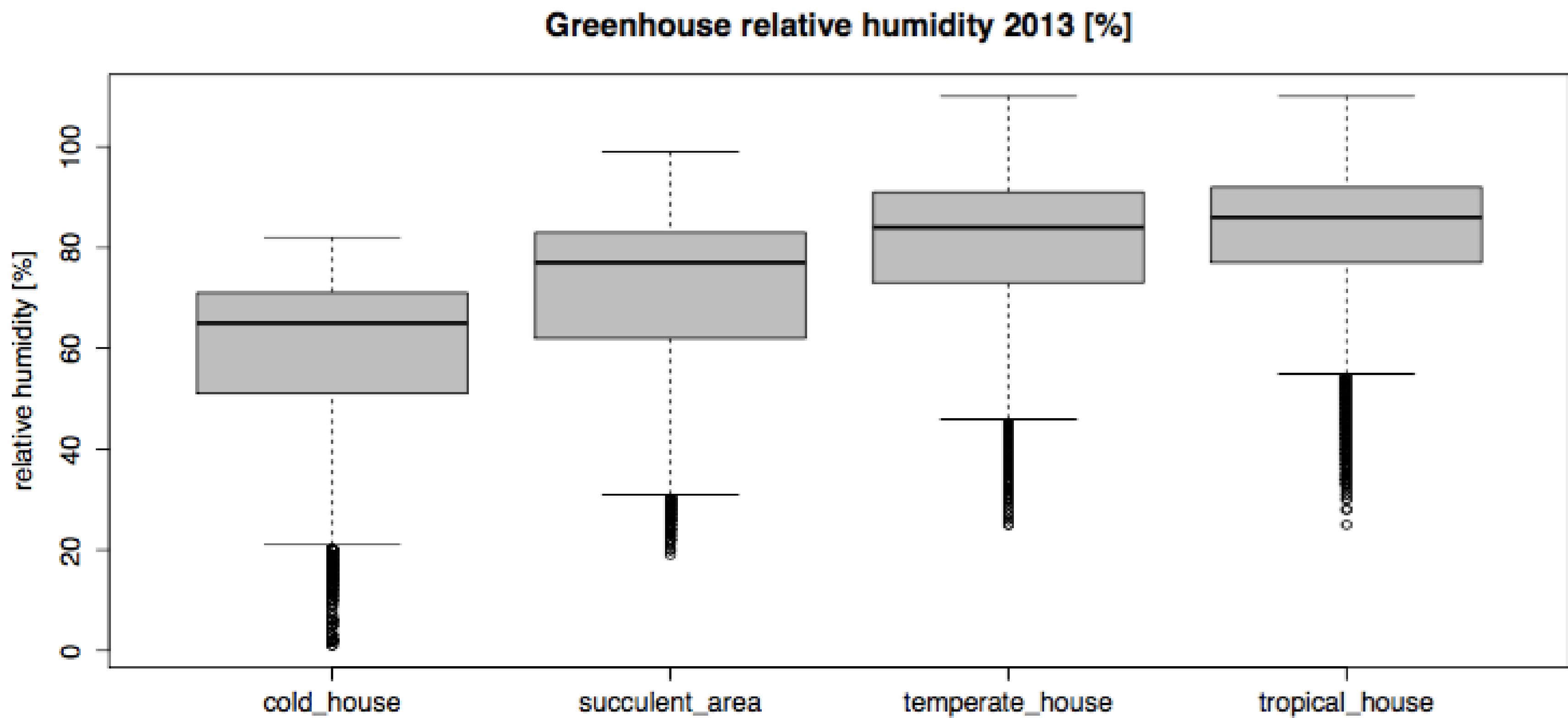

Figure S3. Mean humidity inside each room of the greenhouse complex for the year 2013

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Curriculum Vitae

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1995 – 1999 Secondary school
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Research Works

1. De Guzman, C.C., Dionisio-Sese, M.L., Ortega, R.A.P and Torreta, N.K. (2010) **Increase femaleness in the sex expression of *Jatropha curcas* L. as an effect of Ethrel treatment**. Journal of Nature Studies 10(1), May 2011

Conferences / Trainings Attended

1. **2nd National Cordillera Studies Conference: New Perspectives and Directions in Cordillera Studies**, Cordillera Studies Center, University of the Philippines Baguio, Baguio City, Benguet 18 November 2005

2. **Seminar-Workshop on Young Researcher's Involvement,Agricultural Training** Institute,Benguet State University, La Trinidad, Benguet, Cordillera Administrative Region, 8 March 2007

3. **Universiti Malaysia Sabah-University of the Philippines Los Baños Cultural Exchange Programme**, Universiti Malaysia Sabah, Kota Kinabalu Sabah, Malaysia 25 May - 1 June 2009

4. **"In-house Training Workshop on Meta-Analysis"**, Department of Mathematics and Computer Sciences, University of the Philippines Baguio, Baguio City, Benguet 26 July 2010

5. **Seminar-Workshop on Journal Manuscript Writing**, Saint Louis University as Project Cluster Implementer of the Zonal Research Program, Saint Louis University, Baguio City, Benguet 17 September 2010

6. **"Enhancing Scientific Skills Thru Research", Regional BIOTA (Biology Teachers Association of the Philippines) Convention**,University of Baguio, Baguio City, Benguet 14 November 2010

7. **Orientation for System Webinars**, UP Diliman Interactive Learning Center, University of the Philippines Diliman, Diliman, Quezon City 15 August, 2011

8. **Symposium "The Edible Microbiome"**, Technische Universität Graz, Austria. 14 June 2013

9. **1st Theodore Escherich Symposium on Medical Microbiome Research**. Medical University of Graz, Austria.17 November 2014.

10. **Inter-kingdom Interactions and Biocontrol**. Technische Universität Graz, Austria. 14-15 October 2015.

Lectures / Presentations / Talks

1. Alexander Mahnert, Rocel Amor Ortega[*], Henry Müller, Christine Moissl-Eichinger and Gabriele Berg. (2014) **Effects of indoor plants on and from their surrounding microbiome in built environments. 1st Theodore Escherich Symposium on Medical Microbiome Research. Medical University of Graz, Austria.**

2. Research Presenter on **Floral Morpho-Anatomical Development of *Jatropha curcas* L.**, Golden Jubilee Lecture, University of the Philippines Baguio, Baguio City,Benguet 17 February 2012

3. Research Presenter on **Increase in Femaleness in the Sex Expression of *Jatropha curcas* L. as an Effect of Ethrel Treatment**, 10th Annual National Convention of the Philippine Society for the Study of Nature (PSSN), University of the Philippines Baguio, Baguio City, Benguet 20-24 October 2010

4. Presenter on **International Forum on Youth and Aspirations**, Universiti Malaysia Sabah-University of the Philippines Los Baños Cultural Exchange Programme, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia 27 May 2009

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