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Fungal diversity and antagonistic interactions of *Stachybotrys chartarum* In Soils within Babcock University

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Abstract

Soil samples from 10 randomly selected locations within Babcock University were characterized based on texture and their fungal species diversity determined. The antagonistic activity of one of the isolates, Stachybotrys chartarum, was also studied using the diffusible metabolite bioassay method. The soils are composed mainly of sand particles and ranged from sandy through sandy-clay to the loamy-sand texture with varying pH values ranging from 5.91 to 7.56. The moisture contents of the soils ranged from 2.5% (lowest) to 12.3% (highest). Mycological analyses of the soils revealed a total of 57 isolates belonging to 14 identified genera while 9 isolates were unidentified. The identified genera are Aspergillus, Penicillium, Curvularia, Chaetomium, Fusarium, Rhizopus, Trichothecium, Trichoderma, Verticillium, Phoma, Stachybotrys, Cladosporium, Alternaria and Mucor. The Horticultural Garden soil (BUHG) emerged as the community with the highest species richness (10 species) while soils around the University Cafeteria (BUCF) were the least (3 species) with diversity index value (H') of 2.209 and 1.001, respectively. S. chartarum liberated diffusible metabolite with antagonistic activity against all tested isolates except Clad. cladosporioides (HG3) and A. niger (SQ1). The Maximum Antagonistic Rate (MAR) (mm/day) was highest for A. flavus (MC2) with a value of 1.10 but least for P. shaze (SQ2) at 0.87, as both MARs were reached within 6 d. This is the first report of P. shaze occurrence in Nigerian soil.

Keywords: *Fungal diversity, Antagonistic interactions, Stachybotrys chartarum, Soil, Diffusible metabolite*

Introduction

Fungi are a very diverse group of chemoheterotrophic eucaryotes that play important roles as decomposers in various natural ecosystems including the soil, which serves as their major reservoir (Ayanaba & Sanders, 1981; Germida, 1993; Duarte *et al*., 2006; Gadd, 2007). Some additional roles of fungi include the production of chemical bioactive compounds some of which are useful in the agricultural sector as vital biocontrol substances or in the medical and pharmaceutical industries as antimicrobials (Yuen *et al*., 1999; Bucher *et al*., 2004; Duarte *et al*., 2006; Fapohunda *et al*., 2007). Although Hawksworth (2001) estimated a commonly accepted 1.5 million total of fungal species worldwide, the actual quantity is unclear due to the continuous discovery of novel species (Arnold *et al*., 2001; Khalid *et al*., 2006; Hyde *et al*., 2007; Schmit & Mueller, 2007). However, only 5%-13% of the total estimated global fungal species have been described, as the undescribed species may be occurring in poorly studied countries, hosts, habitats, niches, etc (Hyde, 2001; Schmit & Mueller, 2007). Therefore, more information regarding species diversity in poorly studied areas and hosts are needed to assist in the determination of the accuracy of fungal estimates (Dulymamode *et al*., 2001; Hyde, 2001).

Hyde (2001) and Piepenbring (2007) suggested that the total fungal species pool in an environment might be related to the economic impact of the fungi. Also, the kind, number and distribution of fungi in soil are affected by several factors which include the soil type, texture, pH, available nutrients, moisture and aeration, organic matter, agronomic practices and other microbial groups present, as well as their intra- and inter-specific relationships (Ayanaba & Sanders, 1981; Atlas & Bartha, 1987; Molles Jr., 1999). Many

studies have associated the following genera of fungi with agricultural soils in the actively growing form or dormantly: *Alternaria*, *Aspergillus*, *Cladosporium*, *Cylindrocarpon*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Stachybotrys*, and *Trichoderma* (Qian *et al*., 1998; Obire *et al*., 2002; Kuhn & Ghannoum, 2003; Nesci *et al*., 2006; Pratt, 2008). Synergistic and antagonistic interactions existing between and within populations also influence the survival and activities of fungi in soil. Amongst the well known soil fungi, members of the aspergilli, *Cladosporium*, *Fusarium*, and *Stachybotrys* have been on record as high toxin (metabolite) producers whereby they exclude other populations (Qian *et al*., 1998; Kuhn & Ghannoum, 2003).

There has been, however, no study of the fungal diversity of the soils in Babcock University, being an agricultural community. Consequently, studies on the fungal diversity of this community may provide a basis for estimating the functional role of the fungi in relation to agriculture in this environment, the exploitation of their economic impacts and estimation of the total fungal species richness within Babcock. Therefore, this research aimed at characterizing the various soils present within Babcock University Main Campus, Ilishan Remo, based on texture. We also studied fungal diversity in each soil type as well as some specific interactions, with the view of providing relevant data to agriculturists and ecologists for the improvement of crop production and environmental maintenance.

Materials and Methods

Soil sampling

Soil samples were taken in triplicates from the top 0-15 cm of 10 randomly selected sites within Babcock University. The locations ranged from actively cultivated soils, through dormant soils to oil dumpsites, and are listed as; Main Gate (BUMG), Medical Center (BUMC), Male Resident Halls (BUMH), Female Resident Halls (BUFH), MSQ (BUMQ), Staff Quarters (BUSQ), Maintenance (BUMT), Horticultural Garden (BUHG), Water Industry (BUWI) and University Cafeteria (BUCF). The samples were placed in sterile polyethylene bags and taken to the laboratory for immediate analyses.

Soil Textural characterization

The soil type was characterized using texture, soil pH and moisture content. The Bouyoucos hydrometer method of Day (1965) as described by Sheldrick & Wang (1993) was employed in the determination of particle size of the soil samples. The triplicate samples from each location were mixed together to get the composite soil sample for each location. Fifty-one grams of each composite sample was weighed out and transferred to a big container of a high speed shaker so that 25ml of freshly prepared 5% sodium hexametaphosphate (calgon) and 400ml tap water were added to the sample in the container. The container was shaken for 2h in a mechanical shaker for particle size separation. Samples were then transferred into a 1L measuring cylinder and made to mark by adding tap water before stirring with a paddle for 1 min. The soil hydrometer (Model: ASTM-E100 152H-62, Serial number: 0252, G.H. ZEAL, UK) was introduced into the cylinder and allowed for 20sec. before taking the first reading (B) after 4min. 48 sec. (silt + clay). The second reading (A) was taken 5h later for clay. The formula below was used to deduce the sand, silt and clay percentages. Soil textural determination was by plotting the clay, sand and silt percentages onto the textural triangle for soil classification of USDA (1962).

$$\text{Clay (\%)} = [(A\ (gL^{-1}) \times 100) / 50g] - 1$$
$$\text{Silt + Clay (\%)} = [(B\ (gL^{-1}) \times 100) / 50g] - 1$$

Where 1= calgon correction

$$\text{Silt (\%)} = [(\text{Silt + Clay}) - \text{Clay}]\ \%$$
$$\text{Total sand (\%)} = [100 - (\text{Silt + Clay})]\ \%$$

The soil pH and moisture contents (%) were also analyzed following the methods of Jones (2001) and Topp (1993) respectively.

Mycological analysis

Mycological analyses of soil from the locations were carried out in 2 seasons; late wet season (August – September, 2008) and dry season (December, 2008 – January, 2009). The soil dilution technique of (James & Sutherland, 1939) was employed in the determination of soil fungal populations. To disperse the soil clumps, soil samples were mechanically shaken in a sealed bottle for 15 min. The dilution procedure involved the dissolution of 10g of each soil sample in 90ml 0.1% peptone water as diluents to get the stock solution which was further serially diluted till the 10^{-5} dilution. The spread plate method was employed in the inoculation of 1ml aliquot of soil suspension unto freshly prepared Potato Dextrose Agar (PDA) (Lab M, UK) plates. Plates were incubated at 30^{o}C for 3-7d. Average colony counts were taken at day 3 for fast growing fungi and day 6 for slow growing fungi. Pure cultures of isolates were made and preserved on PDA plates and slants, respectively. Identification of fungal species was by macroscopic and microscopic observations and comparison with descriptions, illustrations and pictures in mycological literature (Gilman, 1971;

Bulmer, 1978; Domsch *et al.*, 1980; Khalid *et al.*, 2006; Paul & Yu, 2008).

The Shannon-Wiener index, which is a combination of two components of diversity, species richness and evenness of species within a community, was computed to determine the diversity of the fungal species in the soil types using the formula below (Molles Jr., 1999).

$$\text{Shannon-Wiener index } (H') = - \Sigma [(P_i) (\log_e P_i)]$$

Where H' = the value of Shannon-Wiener diversity index

P_i = the proportion of fungal species i to other occurring species in a community (soil)

$\log_e$ = the natural logarithm of P_i

Σ = summation of all species in the community

Population Interactions

Interaction between species was determined by the level of diffusible metabolite secretion and antagonistic relationship exhibited between species. One of the isolates, *S. chartarum*, was used for the study. The bioassay involved the use of 7 test isolates; *Clad. cladosporioides* (HG3), *Rhizopus* (HG6), *A. niger* (SQ1), *P. shaze* (SQ2), *Trichod. Reesei* (SQ6), *F. semitectum* (FH5) and *A. flavus* (MC2). A Petri plate containing freshly prepared PDA was divided into quadrants using a colored pen and 3mm agar disc of a 4d old culture of the test isolate was inoculated into the center of each of the quadrants (sector) such that the test inocula were equidistant of the plate circumference and the *S. chartarum* inoculum at the center. A 3mm agar disc from a 4d old *S. chartarum* culture was also inoculated at the center of the plate which was marked by the thin intersecting quadrant lines. The inoculated plates were incubated at 30°C for 10d. Diameter zones of inhibition (mm) taken on days 2, 4, 6, 8 and 10 served as the index for studying the antagonistic relationship exhibited by *S. chartarum* toward test isolates. Diameters were gotten by measuring inhibition zones across sets of opposite inocula. Mean diameter zones of inhibition were calculated following the formula below. Maximum Antagonistic Rate (MAR), defined as the widest inhibition zone (mm) reached per number of day(s) taken to achieve this zone, was also deduced. Bioassay was repeated for each test isolate in duplicates.

$$\text{Mean diameter zone of clearance } (Dz) = \frac{D_1 + D_2}{2}$$

$$\text{MAR (mm/day)} = \frac{Dz^1}{d}$$

Where D_1 = inhibition zone across first set of opposite test inocula.

D_2 = inhibition zone across second set of opposite test inocula.

Dz^1 = widest inhibition zone (mm) reached.

d = number of day(s) taken to reach Dz^1.

Results

The results of the soil texture characterization are presented in Table 1. Samples from 5 of the location revealed that their texture was predominantly sand (i.e. BUMC, BUFH, BUMI, BUWI and BUCF) while samples from 4 locations strongly indicate that their soil textural type are of various loamy combinations (BUMG, BUMH, BUMQ and BUHG). However, samples from one of the location, BUSQ, revealed a sandy-clay texture. The soil pH is between slightly acidic to almost neutral with pH values ranging from 5.91-7.56. The soils from the Staff Quarters (BUSQ) and Female Resident Halls (BUFH) had the least (5.91) and highest (7.56) pH values, respectively. The moisture contents of the soils ranged from 2.5% (lowest) for the Female Resident Halls (BUFH) to 12.3% (highest) for MSQ.

The mycological analyses of the soils from the 10 locations revealed a total of 57 isolates belonging to 14 identified genera while 9 isolates were unidentified. The genera include; *Aspergillus*, *Penicillium*, *Curvularia*, *Chaetomium*, *Fusarium*, *Rhizopus*, *Trichothecium*, *Trichoderma*, *Verticillium*, *Phoma*, *Stachybotrys*, *Cladosporium*, *Alternaria* and *Mucor*. A higher number of actively growing fungi was gotten during the late wet season but a lower number during the dry season (data not shown). *Mucor* and *Rhizopus* were rarely isolated during the latter season. Table 2 shows an extensive compilation of the fungal diversity results in the various communities (sampled soils). The Horticultural Garden soil (BUHG) emerged as the community with the highest species richness (10 species) while soils around the University Cafeteria (BUCF) had the least (3 species). The evenness of species, the pivot upon which species diversity rests and is calculated depends on the number of isolates of each species.

Hence the most diverse community of all surveyed locations based on the Shannon-Wiener index was the Horticultural Garden soil (BUHG) with a diversity (H') value of 2.209 followed by soils from the Female Resident Halls (BUFH) with diversity (H') value of 1.928 and species richness of 8 species. The least diverse soil was that from the University

Cafeteria (BUCF) (*H'*= 1.001). Soils from the Medical Center (BUMC), MSQ (BUMQ), Water Industry (BUWI) and Maintenance (BUMT) had the same species richness (4 species) but varying diversity (*H'*) values of 1.231, 1.271, 1.313 and 1.388, respectively. Similarly, soils from the Male Resident Halls (BUMH) and Staff Quarters (BUSQ) also had the same species richness (7 species) but varying diversity (*H'*) values of 1.655and 1.769, respectively.

S. chartarum was proven to liberate diffusible metabolite that had antagonistic activity against all tested isolates except *Clad. cladosporioides* (HG3) and *A. niger* (SQ1) as shown in Fig. 1. The antagonism towards *A. flavus* (MC2) reduced after day 6 but increased significantly towards *F. semitectum* (FH5) and *Rhizopus* (HG6). The MAR (mm/day) was highest against *A. flavus* (MC2) with a value of 1.10 but least for *P. shaze* (SQ2) at 0.87, as both MAR were reached within 6 d.

Discussion

The characterized soils showed a wide range of textures ranging from mostly sand to loamy-sand although all locations had fairly high amounts of sand particles. The fairly high sandy texture observed in the soils may be explained in terms of the combined effect of the original state of the soils and the increased construction and industrial activities in these locations over the years such that more sand particles are released to the soil in addition to a higher level of erosion due to limited vegetation cover in these locations. This and many other factors such as organic matter content and ions present could have given rise to the varying pH values and moisture content.

The mycological analyses of the various soil communities indicated that there was an averagely rich diversity of fungi in these soils regardless of their texture although soils around the University Cafeteria (BUCF), Water Industry (BUWI), Medical Center (BUMC) and MSQ (BUMQ) appeared to have low species richness since they yielded 3-4 species each. The low diversity of fungi in the latter soils may not be appropriately explained but can be attributed to the low or reduced agricultural practices in these regions of Babcock University over the years. This corresponds with the reports of Nesci *et al.* (2006) who suggested that based on their research data, different agricultural practices impact soil fungal inocula; good and high practices enhance species richness and diversity while low or poor practices reduce drastically species richness. This also explains in part the high richness and diversity of fungi in the other well cultivated soils [BUHG (10 spp; *H'*=2.209), BUSQ (7 spp; *H'*=1.799), BUMH (7 spp; *H'*=1.655), BUFH (8 spp; *H'*=1.928) and BUMG (6 spp; *H'*=1.560)], some of which have received a high load of plant litter and other agricultural wastes over time. Data from Pratt (2008) also support our line of reasoning because his work suggested that waste addition to agricultural soils cause an enhancement of their fungal species richness.

The Maintenance (BUMT) soil is a peculiar one because it serves as a deposit for hydrocarbons, exhaust fumes and other heavy metals. The low species richness (4 species) seen in this soil sample is not amazing because only a few of the well known fungal populations worldwide have the potential to biodegrade oil, hydrocarbon, fume deposits and other heavy metals. Del Val *et al*. (1999) reported that contaminated soils with heavy metals have a limiting effect on the richness and diversity of fungi and the development of mycorrhizal relationship. This then shows that those naturally occurring fungal species (*Trichod. reesei*, *Clad. oxysporum*, *Clad. sphaerospermum* and *Fusarium*) in this polluted soil colonize, survive and exclude others due to their high capacity for metabolic flexibility and the possible possession of pollutant-degrading enzymes which the other fungi may lack.

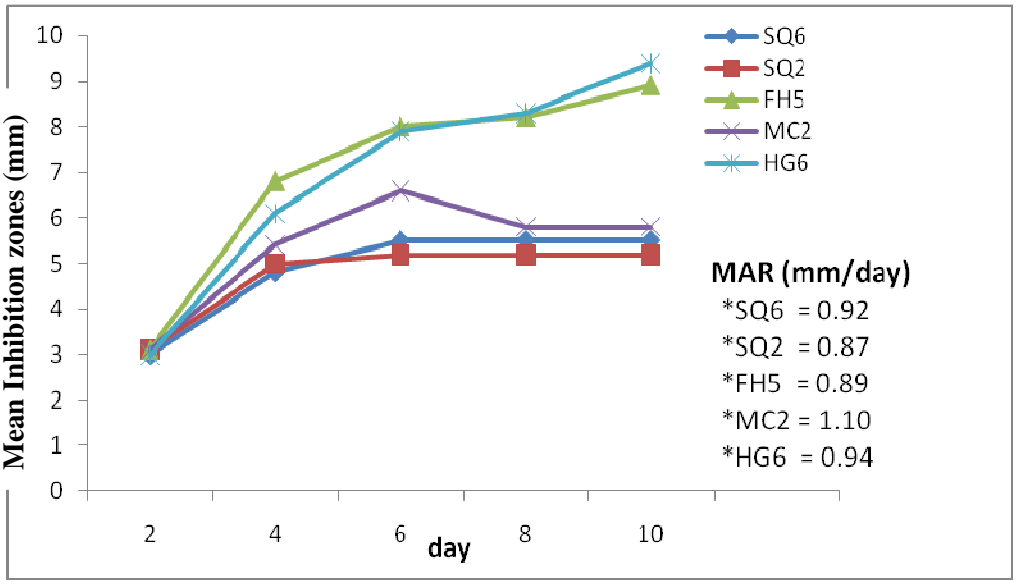
The occurrence of *Aspergillus*, *Curvularia*, *Clad. cladosporioides*, *Alternaria*, *Penicillium*, *Rhizopus*, *Mucor* and *F. oxysporum* in well cultivated soils that harbor different plants and those fed with plant litter and other agricultural wastes over the years is in line with the findings of Obire *et al*. (2002) and Wang *et al*. (2008). Many of these fungi are decomposers of plant litter in soil or plant pathogens whereby their spores are dispersed by wind or dislodged by rainfall to favorable sites where they invade and incite diseases to various plant parts.

S. chartarum is a slow-growing, naturally-occurring fungus in cellulose-rich soil reported to liberate several toxic metabolites (Kuhn & Ghannoum, 2003). Its release of toxic metabolites may be attributed to the fact that when it finds a suitable substrate (cellulose-rich material), it presents antagonistic activity against any surrounding organism, based on their susceptibility or resistance, so that it excludes the population or species for its own convenience. The above may explain the reason for *S. chartarum*'s high antagonistic interaction against *Rhizopus* (HG6) and *F. semitectum* (FH5). This shows that if any of these 2 isolates should be found in the same community with the test organism there might be a complete exclusion of the isolates from the community due to the production of diffusible metabolites. But it was noticed that *P. shaze* (SQ2) was resistant to the diffusible metabolite from *S.*

chartarum as from day 2 while *A. flavus* (MC2) and *Trichod. reesei* (SQ6) became resistant as from day 6. *A. flavus* (MC2) exhibited its resistance as a possible degradation of the metabolite by growing on the areas that had previously been recorded as zones of inhibition (shown in Fig. 2 as a decline in the line graph value) while others simply prevented the further production of *S. chartarum*'s diffusible metabolite. This result coupled with that of Fapohunda *et al*. (2007) may suggest that *Rhizopus* can serve as a positive control in bioassays for metabolite effect on fungi since it has been shown to be almost always susceptible. From the MAR (mm/day) result, it can be suggested that *S. chartarum* could possibly serve as a biocontrol agent against *Rhizopus* and *F. semitectum* over a long period, but for a short while, against *Trichod. reesei*. Our research therefore concludes that agricultural practices have a tremendous positive impact on fungal species diversity and that the release of diffusible metabolites by *S. chartarum* may serve as a competitive advantage over other isolates. This is the first report of *P. shaze* (SQ2) occurrence in Nigerian soil.

Table 1: Soil type characterization by location based on particle size, pH and moisture content (%)

S/No.	Location	Particle size				pH	Moisture Content (%)
		Clay	Silt	Sand	Texture		
1.	BUMG	5	20	75	Loamy-sand / Sandy-loam	6.04	7.5
2.	BUMC	3	8	89	Sand	7.25	12.1
3.	BUMH	27	6	67	Sandy-Clay-loam	7.08	3.4
4.	BUFH	3	3	94	Sand	7.56	2.5
5.	BUMQ	15	7	78	Sandy-loam	6.09	12.3
6.	BUSQ	49	4	47	Sandy-clay	5.91	8.7
7.	BUMT	1	2	97	Sand	6.91	9.6
8.	BUHG	7	8	85	Loamy-sand	7.36	6.6
9.	BUWI	0	1	99	Sand	7.13	5.8
10.	BUCF	0	7	93	Sand	7.07	9.6



SQ6= *Trichod. reesei*, SQ2= *P. shaze*, FH5= *F. semitectum*, MC2= *A. flavus*, HG6= *Rhizopus*.

Fig. 1: Antagonistic activity of *Stachybotrys chartarum* against selected isolates

Table 2: Diversity of fungal species in the different soil types (communities) by location

Community	Code	Species	Number	P_i	$\log_e P_i$	$P_i.\log_e P_i$	H'
BUMG	MG1	*Aspergillus niger*	8	0.44	-0.821	-0.361	
	MG2	*Penicillium*	2	0.11	-2.207	-0.243	
	MG3	*A. melleus*	3	0.17	-1.772	-0.301	
	MG4	Unidentified	2	0.11	-2.207	-0.243	
	MG5	*Curvularia*	1	0.06	-2.813	-0.169	
	MG6	Unidentified	2	0.11	-2.207	-0.243	
	Σ	**6 species**	**18**	**1.00**		**-1.560**	**1.560**
BUMC	MC1	*A. fumigatus*	5	0.38	-0.968	-0.368	
	MC2	*A. flavus*	5	0.38	-0.968	-0.368	
	MC3	*Penicillium*	1	0.08	-2.526	-0.202	
	MC4	Unidentified	2	0.16	-1.833	-0.293	
	Σ	**4 species**	**13**	**1.00**		**-1.231**	**1.231**
BUMH	MH1	*A. niger*	6	0.44	-0.821	-0.361	
	MH2	*A. flavus*	2	0.14	-1.966	-0.275	
	MH3	*Chaetomium*	1	0.07	-2..659	-0.186	
	MH4	*Fusarium subglutinans*	1	0.07	-2.659	-0.186	
	MH5	*Rhizopus*	2	0.14	-1.966	-0.275	
	MH6	*P. italicum*	1	0.07	-2.659	-0.186	
	MH7	*Trichothecium*	1	0.07	-2.659	-.0.186	
	Σ	**7 species**	**14**	**1.00**		**-1.655**	**1.655**
BUFH	FH1	*A. niger*	5	0.30	-1.204	-0.361	
	FH2	*A. flavus*	1	0.06	-2.813	-0.169	
	FH3	*A. tamarii*	2	0.13	-2.040	-0.265	
	FH4	*F. subglutinans*	1	0.06	-2.813	-0.169	
	FH5	*F. semitectum*	1	0.06	-2.813	-0.169	
	FH6	*Trichoderma reesei*	2	0.13	-2.040	-0.265	
	FH7	Unidentified	2	0.13	-2.040	-0.265	
	FH8	Unidentified	2	0.13	-2.040	-0.265	
	Σ	**8 species**	**16**	**1.00**		**-1.928**	**1.928**
BUMQ	MQ1	*A. niger*	3	0.43	-0.844	-0.362	
	MQ2	*F. semitectum*	1	0.14	-1.966	-0.275	
	MQ3	*Verticillium*	1	0.14	-1.966	-0.275	
	MQ4	Unidentified	2	0.29	-1.238	-0.359	
	Σ	**4 species**	**7**	**1.00**		**-1.271**	**1.271**
BUSQ	SQ1	*A. niger*	6	0.35	-1.050	-0.368	
	SQ2	*P. shaze*	2	0.12	-2.120	-0.254	
	SQ3	*A. tamarii*	3	0.17	-1.772	-0.301	
	SQ4	*Phoma*	2	0.12	-2.120	-0.254	
	SQ5	*Stachybotrys chartarum*	2	0.12	-2.120	-0.254	
	SQ6	*Trichod. reesei*	1	0.06	-2.813	-0.169	
	SQ7	Unidentified	1	0.06	-2.813	-.0.169	
	Σ	**7 species**	**14**	**1.00**		**-1.769**	**1.769**
BUMT	MT1	*Trichod. reesei*	1	0.25	-1.386	-0.347	
	MT2	*Cladosporium oxysporum*	1	0.25	-1.386	-0.347	
	MT3	*Clad. sphaerospermum*	1	0.25	-1.386	-0.347	
	MT4	*Fusarium*	1	0.25	-1.386	-0.347	
	Σ	**4 species**	**4**	**1.00**		**-1.388**	**1.388**
BUHG	HG1	*A. niger*	3	0.17	-1.772	-0.301	
	HG2	*Curvularia*	1	0.06	-2.813	-0.169	
	HG3	*Clad. cladosporioides*	1	0.06	-2.813	-0.169	
	HG4	*Alternaria*	2	0.12	-2.120	-0.254	
	HG5	*Penicillium*	2	0.12	-2.120	-0.254	
	HG6	*Rhizopus*	3	0.17	-1.772	-0.301	
	HG7	*Mucor*	2	0.12	-2.120	-0.254	
	HG8	*F. oxysporum*	1	0.06	-2.813	-0.169	
	HG9	Unidentified	1	0.06	-2.813	-0.169	
	HG10	Unidentified	1	0.06	-2.813	-0.169	
	Σ	**10 species**	**17**	**1.00**		**-2.209**	**2.209**
BUWI	WI1	*A. ochraceus*	3	0.30	-1.204	-0.361	
	WI2	*Mucor*	3	0.30	-1.204	-0.361	
	WI3	*Rhizopus*	3	0.30	-1.204	-0.361	
	WI4	Unidentified	1	0.10	-2.303	-0.230	
	Σ	**4 species**	**10**	**1.00**		**-1.313**	**1.313**
BUCF	CF1	*A. melleus*	3	0.43	-0.844	-0.363	
	CF2	*A. nidulans*	3	0.43	-0.844	-0.363	
	CF3	*Penicillium*	1	0.14	-1.966	-0.275	
	Σ	**3 species**	**7**	**1.00**		**-1.001**	**1.001**

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