MYCOBIOLOGY
2020, VOL. 48, NO. 1, 20–28
https://doi.org/10.1080/12298093.2019.1700658

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Diversity of Fungi in Soils with Different Degrees of Degradation in Germany and Panama

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ABSTRACT

Soil degradation can have an impact on the soil microbiota, but its specific effects on soil fungal communities are poorly understood. In this work, we studied the impact of soil degradation on the richness and diversity of communities of soil fungi, including three different degrees of degradation in Germany and Panama. Soil fungi were isolated monthly using the soil-sprinkling method for 8 months in Germany and 3 months in Panama, and characterized by morphological and molecular data. Soil physico-chemical properties were measured and correlated with the observed values of fungal diversity. We isolated a total of 71 fungal species, 47 from Germany, and 32 from Panama. Soil properties were not associated with fungal richness, diversity, or composition in soils, with the exception of soil compaction in Germany. The geographic location was a strong determinant of the soil fungal species composition although in both countries there was dominance by members of the orders Eurotiales and Hypocreales. In conclusion, the results of this work do not show any evident influence of soil degradation on communities of soil fungi in Germany or Panama.

ARTICLE HISTORY
Received 31 March 2019
Revised 7 September 2019
Accepted 22 November 2019

KEYWORDS
Diversity; soil degradation; species richness; soil fungal communities; environmental factors

1. Introduction

Fungi constitute an important part of the soil ecosystem, playing a central role in the biotic and abiotic interactions in this environment, participating in the decomposition of organic matter and the recycling of soil nutrients to make them available to plants [1]. Therefore, communities of soil fungi are involved in soil fertility [2] and contribute to the alleviation of soil degradation [3].

Soil fungi are an immensely diverse group of organisms. A recent study on the diversity of soil fungi revealed around 80,500 operational taxonomic units (OTUs) occurring in soils worldwide [4]. Soil fungal diversity is affected by the local environmental conditions [5], including the chemical and physical soil characteristics, which determine to a great extent the composition of extant fungal communities [6].

Soil degradation is the decline in soil quality (physical, chemical, and biological deterioration) caused by its improper use, usually due to agricultural, grazing, or industrial pressures [7]. Soil degradation can result in changes in its physical properties, such as soil texture [8]; its chemistry, often caused by the application of fertilizers and pesticides [9] that lead to soil acidification [10]; and its biological components, such as losses in vegetation cover that prevent soil erosion [11,12]. Soil degradation is triggered by human activities, which influence the biodiversity of soil [13]. Lands with different levels of soil degradation are estimated to cover between one billion to over six billion hectares worldwide [14].

Soil degradation impacts fungal diversity because soil characteristics influence the presence, distribution, and abundance of fungal species, and the soil characteristics depend on the soil degradation level. Every soil particle has a different micro-spatial composition of fungal species, which is influenced by different micro-habitats in the soil [15]. Every species of fungi requires specific conditions for development, reproduction, and propagation, including different ranges of temperature, moisture, carbon reservoirs, seasons, soil depth, or chemical factors [16]. Without bacteria and fungi, the soil degrades [9]. Soil compaction decreases soil fertility through decreasing storage and supply of water and nutrients, which entails a reduction in the activities and diversity of fungal communities [17]. Soil moisture is assumed to be very important for microorganisms, because water availability is fundamental for different processes. The soil pH has a strong

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influence on species richness of soil fungi, diversity, and community structure [10]. The composition and proportion of the soil components have appreciable effects on nutrient concentrations and soil texture, thereby influencing the community of soil fungi [18].

Analyses that include fungal morphological and molecular data in correlation with environmental conditions of soils are not extensive [19]. In Germany, the knowledge of soil fungi is more developed, but there are still many gaps. The principal biodiversity research project in the area is the German Biodiversity Exploratory (www.biodiversity-exploratories.de), which is focused on research related to forest management, on the biodiversity, and on the functions of forest ecosystems [20]. These studies focused on invertebrates in the soil, soil bacterial communities, wood-inhabiting fungi, and yeasts [21] as well as soil fungi [22]. Specifically, for Panama, research is limited. The province of Chiriquí in Panama has been recently declared as one of the four critical areas subject to soil degradation processes in the country, which affects directly the soil organisms and the biophysical system [23], making necessary the study of fungi in this region. Also, it is estimated that Panama has about 50,000 species of fungi of which only about 3.6% are known [24]. Recently, 24 species of soil fungi were identified in Western Panama including 10 new reports for the country, and 4 of these species are also new to Central America [25]. However, analyses that include soil fungal diversity data in correlation with soil environmental conditions are not extensive [19].

The objectives of this study are to (1) assess the impact of soil degradation on communities of soil fungi, (2) establish the diversity of species and their relationship with environmental factors, (3) test whether the effects of degradation are consistent across geographic and environmental conditions taking into consideration one area in Germany and one in Panama, and (4) determine which soil factors are most important for changes in fungal communities caused by degradation.

2. Materials and methods

2.1. Sampling sites

Two geographically separated study areas were selected. The first location was selected within a temperate forest in the Taunus mountain range located in the north-west of Frankfurt am Main, Hesse, Germany (50° 08′ 28.0″N, 8° 16′ 21.1″ E, ca. 360–380 m a.s.l.). The local climate comprises ranges of the temperature of 5–17 °C and of precipitation of 600–1300 mm per year [26]. The second location was within a tropical, semi-deciduous forest in the Majagua valley in the province of Chiriquí, in Panama (08° 29′ 33.5″ N, 82° 25′ 59.4″ W, ca. 120 m a.s.l.). The local climate in this province varies between ranges of temperature of 25–32 °C, and average precipitation of 3700 mm per year [27].

2.2. Collection of soil samples

Three sites were selected in each location in Germany and Panama, representing areas of dense forest (without disturbance); grasslands, indicative of biological degradation because the areas are covered by grass only [6]; and bare soils on the paths [28]. The sampling sites in each country were inside an area of less than 1000 square meters. Samplings in Germany were done across 8 months (January, March, April, May, June, September, October, and November) in 2012. In Panama, samplings were done across 3 months (February, July, and August) in 2012. At each sampling event and site, three cores were collected and pooled, resulting in a total of 24 soil samples for Germany and 9 for Panama. The collection of samples followed the protocol described by Carrasco et al. [29]. In brief, a tube was introduced in the first 5 cm of soil and this core was introduced into a plastic bag after removing roots and stones from the samples. Samples were brought to the lab for processing within 3 d. One portion of the samples was used to determine the soil characteristics.

2.3. Physico-chemical soil analysis

Physico-chemical soil characteristics were measured from one soil sample at each site. The pH of all samplings zones was determined with a litmus paper in a soluble extraction of the soil samples. Moisture content in the soil was measured by the water balance value, which was calculated from the sample weight before and after drying at 105 °C for 48 h [30]. Compaction was measured by the determination of the ratio of the mass to the bulk or macroscopic volume of soil particles plus pore spaces in a sample [Bulk density (g cm^{-3}) = Mass of dry soil (g)/Volume of core (cm^3)]. Finally, the composition of soil was measured by the sedimentation method [31]. All environmental factors measurement for each sampling event is showing in Supplementary Table S2.

2.4. Isolation of soil fungi

For the isolation of soil fungi, plates were prepared by dispersing minute quantities (of around 0.05 mg) of the different soil samples on the surface of a

sterile Petri dish with a cultivation medium following the procedure described by Rosas-Medina and Piepenbring [25]. The method is a variant of Warcup soil plates [32], and consists of spreading a minute quantity of soil in a water suspension on the surface of the agar medium. Each soil sample was cultivated in duplicate on three media: potato dextrose agar (PDA; Panreac, Darmstadt, Germany), malt extract agar (MEA; Roth, Karlsruhe, Germany), and malt yeast peptone agar (MYP, Roth), all these amended with 0.5 g l^{-1} tetracycline. The plates were incubated at 25 °C for up to 25 d in an incubation chamber until colonies developed. This process involved the observation of cultures every day, making dilutions in water to separate spores, and re-cultivating until obtaining pure cultures. Re-cultivations were done on different media (PDA, MEA, MYP, and glucose yeast peptone liquid medium) [33,34].

2.5. Identification of fungal cultures

The identification of fungal isolates by morphological characteristics was done using morphological identification keys [35–42]. Macroscopical characteristics (form, size, color, and growth rates of cultures) and microscopical characteristics (forms and sizes of hyphal cells, conidiophores, metulae, conidia, ornamentation, etc.) of the isolated fungi were compared to corresponding information in the descriptions (see examples of morphological identifications in the Supplementary material). Morphological identifications were complemented by a molecular assessment of representative isolates of each morphospecies. DNA extraction by the cetyltrimethylammonium bromide (CTAB) method, polymerase chain reaction (PCR) amplification and sequencing of the internal transcribed spacer region of ribosomal DNA (rDNA ITS1-5.8S-ITS2) with primers ITS1f and ITS4 [43,44] followed the procedure outlined by Rosas-Medina and Piepenbring [25]. The ITS sequences were compared by BLAST with other reference sequences from NCBI GenBank (http://www.ncbi.nlm.nih.gov) and the User-friendly Nordic ITS Ectomycorrhizal database group (UNITE; http://unite.ut.ee/) database for fungal ITS sequences [44]. Upon BLAST searches, sequences matching with 98% or higher of maximum identity with database records were considered as reliable identifications, while sequences with less than 98% identity were subjected to critical morphological analysis [45]. Results from BLAST were compared with morphological identification to confirm the identifications. The principal reference for the selection of currently valid names was Mycobank (http://www.mycobank.org/). Several isolates could not be identified to species level based on their ITS sequences or by their morphology. In these cases, fungi were identified morphologically to the smallest possible taxonomic category.

Fungal cultures are maintained in the Integrative Fungal Research (IPF) culture collection at Goethe University Frankfurt am Main. All the sequences obtained were deposited in the NCBI GenBank nucleotide database under accession numbers KY320587–KY320646 (Supplementary Table S2).

2.6. Data analysis

The dataset for fungal diversity analyses included the occurrence of all fungal species across sampling events and sites, expressed as the percentages of isolation per sample. Diversity analyses from the isolation dataset were performed with the community ecology library vegan version 2.4-4 [46] in the statistical program R version 3.1.1 [47]. Species richness and species accumulation curves were calculated for each treatment, including forest, grassland, and bare soil for both countries Germany and Panama. Analyses of the diversity of fungal communities were carried out with the Shannon diversity index [48]. Comparisons between richness and environmental factors were done with linear regression models. A matrix of dissimilarities in fungal community composition among samples was obtained using the Jaccard's index based on presence/absence data. Differences in species composition were compared across samples using non-metric multidimensional scaling (NMDS), and they were correlated with environmental factors using the function *envfit* of vegan.

3. Results

3.1. Fungal species diversity and systematics

A total of 764 strains of soil fungi were isolated, 533 from Germany and 231 from Panama. The isolates from Germany were distributed in three divisions: Ascomycota, Zygomycota, and Basidiomycota, the latter represented only by the order Tremellales (12 isolates). In Panama, only members of the divisions Ascomycota and Zygomycota were found. The distribution of isolates in fungal orders is shown in Figure 1.

The isolates were classified in 71 species, 47 from Germany and 32 from Panama, of which eight were shared by both countries (Supplementary Table S2). These data resulted in Shannon's index diversity values of 3.36 for Germany and 3.15 for Panama.

The number of isolates and fungal species found in each sampling event changed in both countries (Figure 2). In Germany, high variability in the number of isolates was observed between the first and

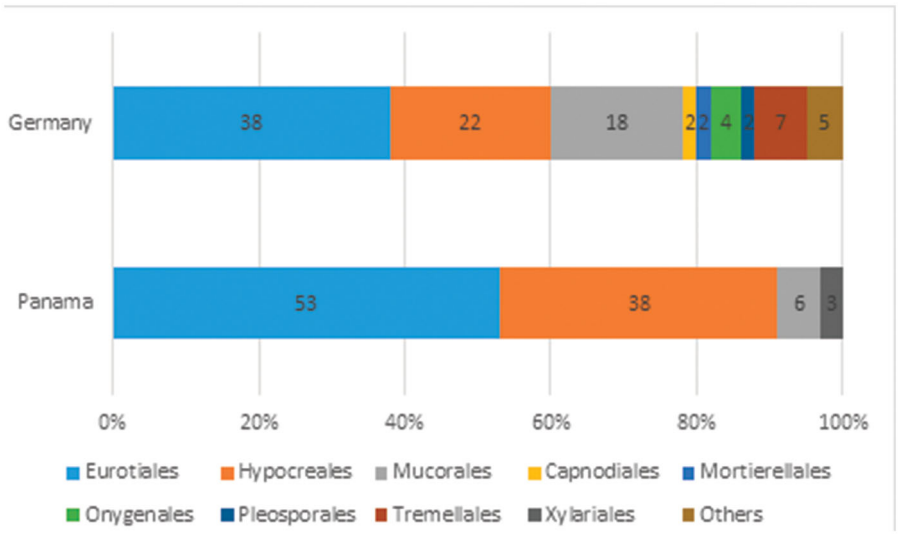

Figure 1. Affiliation of fungal strains isolated from soil in Germany or Panama to orders of fungi.

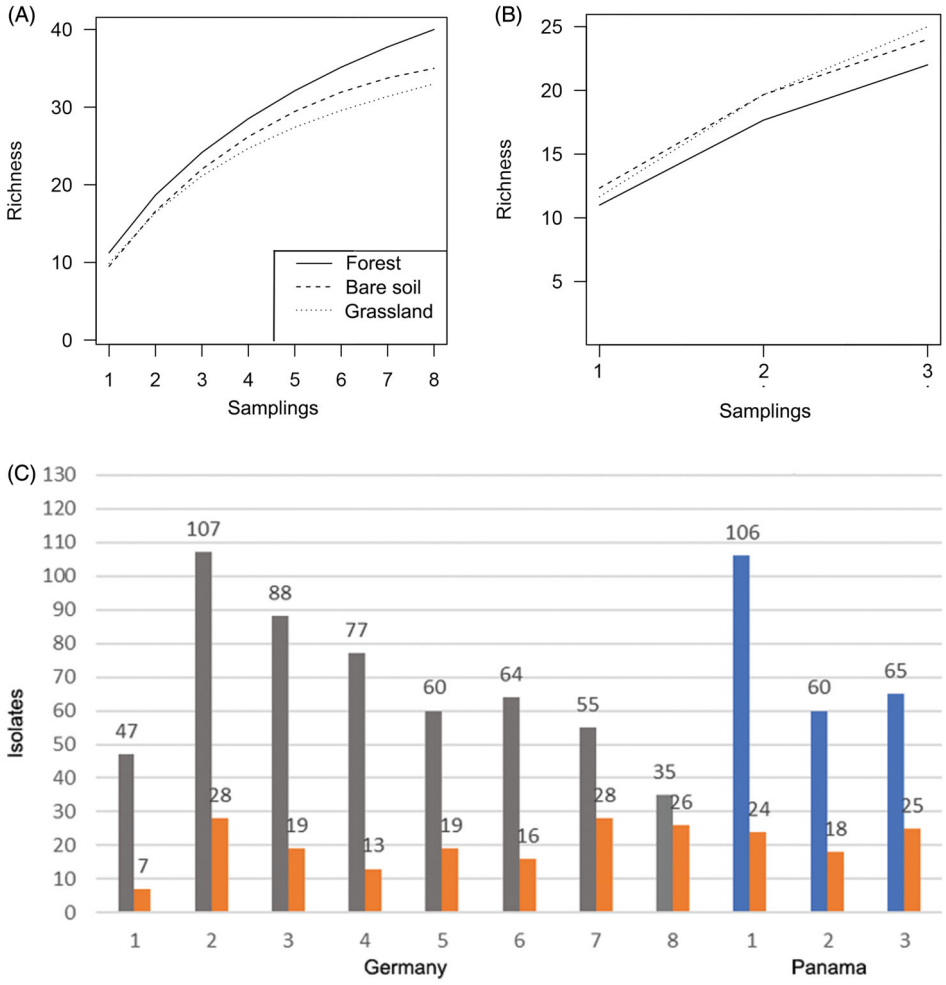

Figure 2. Species diversity and richness of soil fungi in Germany and Panama recorded during the samplings and number of isolates and species in both countries. (A) Species accumulation curves for each of the three soil types sampled in Germany, respect to sampling events; (B) Species accumulation curves for each of the three soil types sampled in Panama, respect to sampling events; (C) Numbers of isolates and numbers of species (orange bars) obtained by samplings in Germany (grey bars) and Panama (blue bars).

the second samplings, with an increment from 47 to 107 isolates. In the subsequent six sampling events, lower numbers of isolates were obtained (Figure 2(c)). The number of species ranged from 7 to 28 species (SD 3.97 bare soil, 3.22 grassland, and 3.96 forests). Two species were present in nearly all samplings, *Trichoderma hamatum* and *Mucor moelleri* (Supplementary Table S2).

In Panama, the differences between the first and second sampling are 46 isolates, and the differences between second and third sampling are 5 isolates (Figure 2(c)). The number of species ranged from 18 to 25 species (SD 3.09 bare soil, 3.56 grassland, and 2.16 forest). Three species of the order Eurotiales (*Aspergillus aculeatus, Penicillium citrinum,* and *Penicillium simplicissimum*) and three species of the order Hypocreales (*Ophiocordyceps heteropoda, Purpureocillium lilacinum,* and *Trichoderma harzianum*) were present in all sampling events. Species accumulation curves show differences in richness between Germany and Panama (Supplementary Figure S1).

3.2. Effect of soil compaction on fungal diversity

None of the environmental factors measured were significantly correlated with fungal richness or diversity ($p > .05$; Supplementary Figure S2), with the exception of soil compaction (Figure 3). This association was negative and found only in Germany ($R^2 = 0.13$, $p = .047$), whereas in Panama no effect was detected ($p > 0.5$; Figure 3).

The NMDs ordination showed clustering of samples in two groups with similar species compositions, one group for Germany and another for Panama (Supplementary Figure S2). Ordinations by countries in relationship with different vegetation covers showed no clustering pattern (Supplementary Figure S3).

4. Discussion

In this study we analyzed the fungal diversity in soils from areas in Germany and Panama with different vegetation covers, to determine which factors have an influence on the communities of soil fungi in the context of soil degradation. We did not find important effects of ecological factors related to soil degradation on fungal communities, although we found an important effect of the geographic location on fungal species composition. For both countries, however, similar orders were dominant, i.e., Eurotiales and Hypocreales.

The impact of soil degradation on fungal community composition was negligible. Previous studies have shown a similar lack of effects. For instance, Saxena and Stotzky [49] found no significant differences in the culturable fungi between soil with plants and bare soil, similar to the forest and bare soil assessed in our study. However, they mentioned that these results should be considered as preliminary, because only culturable fungi were evaluated. Our study has a similar result, considering that many fungal species dwelling in soil cannot be isolated or cultivated and hence need to be assessed with complementary methodologies [50,51]. Evaluating the effects of soil degradation on fungal communities, Samaniego-Gaxiola and Chew-Madinaveitia [52] found that in two of three cultivation areas, soil degradation had no impact on the community of soil fungi, but in the more saline area, fungal diversity was lower, showing a soil degradation effect on fungi diversity. Consequently, we cannot rule out an effect of degradation on soil fungal communities, based on results from other studies [53–55].

Species diversity of communities of soil fungi in relation to ecological factors varies according to the specific conditions of each soil. Some studies with a similar methodology to the one in this study have shown varying results. Wahegaonkar et al. [56] found 45 genera distributed in 85 species in agricultural soils. Gaddeyya et al. [57] found a total of 15 species belonging to six genera from cultivable fields, where the dominant species were *Aspergillus flavus*, *Trichoderma viride*, *T. harzianum*, *Fusarium oxysporum*, and *Fusarium solani*, that were also found in this study. In our samplings in Germany, the dominant order was the Eurotiales, with 38% of the total of isolated species. Eurotiales have a

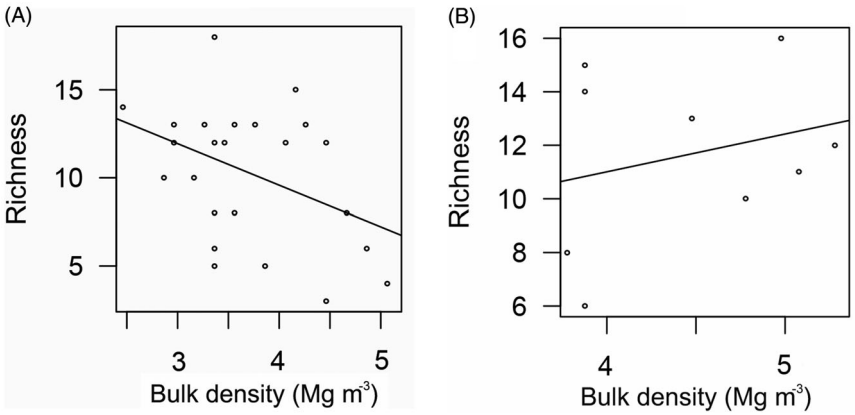

Figure 3. Richness of species of soil fungi in relation to soil compaction measured by bulk density. (A) in Germany, with 24 samples from bare soil, grassland or forest; (B) in Panama, with nine samples from bare soil, grassland, or forest.

cosmopolitan distribution with records in many habitats all over the world [37,58,59]. The second most abundant order was the Hypocreales with 22% of the total of isolated species. Species of Hypocreales can be found in different types of soil worldwide [36,41,60], and are common in all types of moist forests. In the same regions of this study in Germany and Panama, Tedersoo et al. [4] found that Eurotiales and Hypocreales were within the ten most common orders according to data obtained by environmental sequencing. Most species of Eurotiales have a pioneer colonization strategy in the soil, and are adapted to extreme environmental conditions, have cosmopolitan distribution, and are common associates of decaying plant and food material [61]. Species of Hypocreales are commonly encountered in humid tropical or subtropical forests although they also occur in arid, temperate, or boreal forests, even in the most extreme north and south latitudes, with some genera in the order being considered cosmopolitan soil fungi [36,62].

We did not find an important effect of environmental factors on species richness, except for soil compaction in Germany. Soil compaction has negative influences on soil microfungi because it reduces pore space which, in turn, affects the growth, distribution, and development of fungi [63]. Harris et al. [64] showed that soil bulk density has a negative influence on the spread and spatial distribution of *Rhizoctonia solani*.

The lack of correlation between soil factors and the diversity of soil fungi may be due to different causes. One can be that our samplings spanned a temporal but not a spatial variation, because the spatial variation implies extension in the sampling area entailing differences in environmental factors. Although the fungal communities changed across time, at the same time the values of soil factors did not vary significantly. Soil depth can influence the correlation between environmental soil factors and the biomass of soil fungi based on microclimates [65,66]. Another reason can be that fungal communities are influenced by other factors like the amount of rain per year, soil nutrient content, or total and labile organic carbon [67].

We did not find degradation effects on communities of soil fungi across geographical locations. Usually, the effects of soil degradation vary with geographic location. Goldmann et al. [68] mention that communities of soil fungi similar in one place change with increasing geographical distance. This could be driven by three main mechanisms; the first one, environmental conditions become increasingly different with increasing geographical distance, the second one is the modulation of dispersal rates of taxa influenced by the limitations of landscape heterogeneity, and the last one is the dispersal limitations of organisms in homogenous landscapes.

This study has some methodological limitations. The isolation of fungi in culture is not comprehensive because many species are not easily detected in agar media. When soil particles are scattered onto the surface of the medium, some species develop more quickly than others given their fast growth rates and, in some cases, their parasitism on other fungi [69]. The statistical analysis is also limited because it is not quantitative, as it is focused on the presence/absence of fungi. For this reason, some fungal groups are more likely to be detected than others without relationship to their abundance. However, despite the above limitations, our methodology is sufficient to find differences across soils and environmental factors. Other methodologies can be used to complement the ones in our study. For example, Tedersoo et al. [4] used 454 pyrosequencing to identify the soil fungal communities in the same areas of our study. However, with this method, 281 of the species [22] were detected by 454 pyrosequencing and cultivation, suggesting that high-throughput sequencing has its own technical biases, such as primer mismatches, differential sequence length and precision loss in the homopolymer regions [70,71]. Therefore, it is important to combine both techniques, since they provide complementary information.

In summary, our results do not show the evident effects of soil degradation on communities of soil fungi from Germany and Panama. However, our study sets the basis to develop further studies in the same direction or to test other fungal relations with more environmental factors. This study is pioneer for Panama since it is one of few studies focusing on soil mycobiota in this country. Finally, in both countries further work in this direction is needed to assess human impacts on understudied areas in terms of fungal diversity.

Acknowledgments

Authors acknowledge the support provided by Orlando Cáceres for field work in Panama, the Universidad Autonoma de Chiriquí, Panama, and the German Academic Exchange Service (DAAD). This study was supported by the LOEWE excellence initiative of the state of Hesse within the framework of the Cluster for Integrative Fungal Research (IPF).

Disclosure statement

No potential conflict of interest was reported by the authors.

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