



Metacommunity analyses of Ceratocystidaceae fungi across heterogeneous African savanna landscapes



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ABSTRACT

Metacommunity theory offers a powerful framework to investigate the structure and dynamics of ecological communities. We used Ceratocystidaceae fungi as an empirical system to explore the potential of metacommunity principles to explain the incidence of putative fungal tree pathogens in natural ecosystems. The diversity of Ceratocystidaceae fungi was evaluated on elephant-damaged trees across the Kruger National Park of South Africa. Multivariate statistics were then used to assess the influence of landscapes, tree hosts and nitidulid beetle associates as well as isolation by distance on fungal community structure. Eight fungal and six beetle species were recovered from trees representing several plant genera. The distribution of Ceratocystidaceae fungi was highly heterogeneous across landscapes. Both tree host and nitidulid vector emerged as key factors contributing to this heterogeneity, while isolation by distance showed little influence. Our results are consistent with a model of metacommunity dynamics combining species sorting and patch dynamics processes.

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1. Introduction

Fungal plant pathogens play an influential role in driving the dynamics of natural vegetation landscapes. This is via direct and indirect effects on the fitness of their hosts, which help to maintain intra and interspecific diversity and facilitate succession in plant communities (Dobson and Crawley, 1994; Castello et al., 1995; Alexander, 2010; Mordecai, 2011). Fungal pathogens also occasionally cause rapid decline in populations of particular host plants, leading to dramatic structural and compositional changes in vegetation landscapes (Dobson and Crawley, 1994; Castello et al., 1995; Alexander, 2010; Mordecai, 2011). In turn, the patterns and processes inherent to natural plant communities influence the abundance and distribution of fungal plant pathogens. For example, the dilution effect hypothesis that links reduced disease risk with

increased diversity of host community (Keesing et al., 2006) is well illustrated in fungal plant disease systems (Mitchell et al., 2002; Haas et al., 2011). There is also a substantial body of literature, both theoretical and empirical, supporting a correlation between the genetic diversity of plants and the genetic diversity of their fungal pathogens in nature, which may in part be linked to frequency-dependent selection in the context of a co-evolutionary arms race (e.g., Damgaard, 1999; Thrall and Burdon, 2003; Ravensdale et al., 2011). However, most previous studies have focused on simplified model systems involving single fungal pathogens or fungus-plant pathosystems. The nature and outcomes of interactions involving fungal pathogens in larger microbial communities and/or multi-host systems is still largely overlooked.

Community ecology theory provides a framework to investigate the mechanisms underlying patterns of species diversity and distribution in complex biological systems (Vellend, 2010; Verhoef and Morin, 2010). Metacommunity theory (Leibold et al., 2004) in particular has been broadly exploited during the course of the past decade or so to investigate how dispersal processes at a regional scale, and ecological interactions at a local scale, may affect the

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diversity and composition of biological assemblages (Cottenie, 2005; Logue et al., 2011; Alexander et al., 2012; Costello et al., 2012; Eros et al., 2012). Metacommunity theory posits that spatially distinct patches occupied by multiple interacting species are connected by species dispersal (Leibold et al., 2004). In practice, metacommunity studies involve assessing how well empirical community patterns fit in with theoretical models of community dynamics that incorporate variable contributions of local versus regional underlying driving processes (Logue et al., 2011). Four principal models, 'neutral-dynamics', 'patch-dynamics', 'mass-effect' and 'species-sorting', are at the basis of metacommunity theory (Leibold et al., 2004).

There is growing interest in utilising metacommunity concepts to further our understanding of pathogen dynamics and pathogen-host interaction as they relate to disease emergence and spread (Costello et al., 2012; Seabloom et al., 2015; Borer et al., 2016). Here, we follow the same approach to explore the incidence in a natural ecosystem of tree-infecting fungi in the ascomycete family Ceratocystidaceae. These fungi infect their host trees through open wounds and are characterized by a wide range of aggressiveness from species that can be lethal pathogens, causing stem cankers, rots and wilt diseases (e.g. most *Ceratocystis* spp., *Thielaviopsis* spp. and *Chalaropsis* spp.) to those that have little negative impact on their hosts (e.g. most *Huntia* spp.) (De Beer et al., 2014). All of these fungi thrive on newly exposed wood surfaces and subcortical spaces of freshly wounded trees. This makes it feasible to use natural or artificially created tree wounds as substrates to study their diversity and ecology in woody ecosystems (Kamgan et al., 2008; Van Wyk et al., 2011; Mbenoun et al., 2015).

Tree-infecting fungi in the Ceratocystidaceae present the additional advantage that their mode of dispersal is fairly well known, principally via fungal-feeding insect vectors, especially nitidulid beetles (Coleoptera: Nitidulidae) (Moller and DeVay, 1968; Cease and Juzwik, 2001; Hayslett et al., 2008; Heath et al., 2009; Mbenoun et al., 2015). Nitidulid beetles are attracted to infected plant tissues by volatile aromatic metabolites produced by the fungi (Hanssen, 1993) where they come into contact with sticky fungal propagules which adhere to their bodies (Moller and DeVay, 1968; Heath et al., 2009). Fungal transmission from tree to tree is enhanced by the fact that the same insects are also sap feeders (hence their common name sap beetles), and are attracted by the volatiles emanating from fresh tree wounds, which they visit and infect shortly after wounding occurs (Moller and DeVay, 1968; Juzwik et al., 2004; Mbenoun et al., 2015). This system provides an opportunity to consider the dispersal of the Ceratocystidaceae via studies of their associated nitidulid communities.

Previous studies have highlighted the importance of indigenous communities of Ceratocystidaceae fungi in Africa as a source of potentially harmful new pathogens of tree crops (Roux et al., 2007; Kamgan et al., 2008; Mbenoun et al., 2015). These studies have particularly sought to provide knowledge that might be used to predict and prevent new disease outbreaks in agroforestry systems, as exemplified by the wattle wilt disease affecting plantation forests based on non-native, Australian *Acacia* tree species, and caused by the indigenous fungus *Ceratocystis albifundus* (Morris et al., 1993; Wingfield et al., 1996). In the present study, we considered the factors that determine the incidence and abundance of Ceratocystidaceae fungi in a natural African ecosystem. Specifically, we investigated the diversity of the Ceratocystidaceae on elephant-damaged trees across four contiguous and heterogeneous savanna landscapes of the Kruger National Park (KNP) in South Africa. We then applied multivariate statistics in a metacommunity framework to examine whether geographic isolation, landscape characteristics, including the composition of tree host and nitidulid associate communities, may influence the distribution and

abundance of these fungi across the park. We posit that: (i) a strong tree host effect would support environmental filtering by the landscape on Ceratocystidaceae fungi; (ii) a strong nitidulid vector effect would indicate differential dispersal rates among fungal species; and (iii) significant spatial autocorrelation of the Ceratocystidaceae diversity would be consistent with limited overall dispersal ability for these fungi at the scale of the park.

2. Materials and methods

2.1. Study site

The KNP straddles the Limpopo and Mpumalanga Provinces in the northeast of South Africa (Supplementary material – Fig. S1). It stretches between 22°20'S–25°32'S and 30°53'E–32°02'E, covering a surface area of 19 485 km² (Grainger et al., 2005). The prevailing general climatic conditions are semi-arid to subtropical, characterized by hot and humid summers contrasting with mild and dry winters (Venter and Gertenbach, 1986). The park receives approximately 400–700 mm rainfall per year, and has a range of daily average temperatures from 27 to 32 °C between the winter and summer seasons (Venter and Gertenbach, 1986). Although embedded in the broader Zambezi and Mopane woodlands ecoregion (<https://www.worldwildlife.org/ecoregions/at0725>), the KNP forms a complex mosaic of local ecotypes, varying from dense woodlands to shrubby and open grasslands. Gertenbach (1983) recognized 35 landscapes in the KNP, representing unique combinations of geomorphological, soil and microclimatic conditions supporting distinctive plant communities and associated fauna. Expanding on the Gertenbach landscape scheme, the vegetation of the park was summarized in a map circumscribing 15 ecozones (Anonymous, 1999), based on the distribution and density of some 1968 plant species, including 457 trees and shrubs found across the park (Venter and Gertenbach, 1986). The KNP harbors a diverse community of large herbivores, including a substantial population (13 050 at the 2007 census) of elephants, whose browsing activity represents a major source of mechanical damage to trees. This makes it an ideal natural site to investigate fungal pathogens such as Ceratocystidaceae fungi that infect fresh open wounds on trees.

2.2. Field methods and fungal collection

Four of the park districts, relatively distantly located, were selected to represent distinctive landscapes according to the Jacana ecozone map of the KNP (Anonymous, 1999; Supplementary material – Fig. S1). These included Punda Maria (Tree Mopane Savanna), Letaba (Mopane Shrubveld/Bush willow), Satara (Knob thorn/Marula savanna) and Skukuza (Thorn thickets). Three pilot samplings were conducted in the northern region of the park, around Punda Maria and northwards, during summer and winter of 2009 and summer 2010. This made it possible to identify the winter period as the most effective time to collect fungi infecting fresh tree wounds across the park. This result emerged from the fact that there was substantially greater browsing activity and damage by elephants on trees during that period, consistent with seasonal patterns in the feeding behavior of African elephants in semi-arid savanna woodlands (Baxter, 1996).

Building on the preliminary observations of pilot field visits, an extensive survey of recently (less than one month) elephant-damaged trees was undertaken across the four selected park districts during winter 2010. Every wounded tree encountered was carefully inspected for signs of infection by Ceratocystidaceae fungi. Samples including wood and bark material were collected from infected trees, placed in paper bags and transported to the laboratory for fungal isolation. Where nitidulid beetles were found

associated with wounds, these were collected using an aspirator and placed in glass vials. The insects were later enumerated and sorted by morphospecies. Representatives of each morphospecies were preserved in 90% ethanol for identification and the remaining individuals were used for fungal isolation. At each sampling point geographic coordinates as well as the species identity of sampled trees were recorded.

2.3. Fungal isolation and identification

Fungal isolation from plant samples and insects was performed using direct isolation and carrot-baiting techniques. Plant material was incubated in moist chambers for a few days to induce the development of fungal structures. Small pieces from the same material were sandwiched between carrot discs to facilitate isolation of slow-growing *Ceratocystis* spp. and *Chalaropsis* spp. Isolation from nitidulid beetles was also achieved using carrot-baiting. The insects were individually crushed on the surface of carrot discs, which were then maintained in moist conditions. Isolates were obtained by lifting a few mycelial strands or single ascospore droplets from the tips of perithecia developing on the surfaces of plant tissues and carrot baits. The resulting cultures were purified by subculturing from single hyphal tips or ascospore droplets. Isolates obtained from a single sampled tree were initially grouped based on morphocultural characteristics, and when possible two representatives of each morphotype were retained for further analyses.

The procedures leading to fungal identification have previously been described (Mbenoun et al., 2014). Briefly, all isolates were characterized by ITS rDNA gene sequencing (for those morphologically related to *Ceratocystis* and *Chalaropsis*) or β -*tub* gene sequencing (for those related to *Huntia*). These genes were selected for their good taxonomic resolution in the respective fungal groups and used for species delineation, applying the General Mixed Yule Coalescent (GMYC) method (Pons et al., 2006). Complete specific identification was achieved with a subset of isolates from each GMYC cluster, using multigene phylogenetic analyses. These included sequences of the ITS, β -*tub*, and *tef1- α* gene regions. For the purpose of this publication initial fungal identifications (Mbenoun et al., 2014) were updated according to the taxonomic revision of the Ceratocystidaceae by De Beer et al. (2014).

2.4. Statistical analyses

Fungal diversity and community assembly analyses were performed with R statistical software version 2.14.1 (<http://www.R-project.org/>) using sample-based incidence (presence/absence) data and sampled trees as observational units. A fungal community matrix was constructed in which every species of Ceratocystidaceae recovered was recorded as present or absent on each sampled tree. Another matrix included explanatory variables, including the species identity of sampled trees, location (site and geographic coordinates), as well as the identity and numbers of nitidulid beetles collected from them. The package BiodiversityR (Kindt, 2012) was used to describe alpha diversity at each study site and gamma diversity across the park. This was achieved graphically using species accumulation curves and Rényi diversity profiles, as well as numerically using the Chao 1 index [$S_{Chao1} = S_{obs} + n1(n1 - 1)/2(n2 + 1)$, where S_{obs} is the observed number of species, $n1$ is the number of singletons and $n2$ is the number of doubletons] and Shannon-Weiner index ($H = -\sum p_k \ln p_k$, where p_k is the frequency of the k th species; typically, $H = 1.5$ – 3.5 in most ecological studies). Gamma diversity

was derived from the pooled 2009–2010 data set, while alpha diversities were derived from data collected during 2010 in order to standardize the sampling period for all study sites for comparative purposes. The 2010 data set was also used to quantify beta diversity (variation in species composition among sites) by measuring the dissimilarity between all possible pairs of sampling sites using the Bray-Curtis dissimilarity index ($d_{ij} = 1 - \frac{2\sum C_{ij}}{S_i + S_j}$, where C_{ij} is the smaller number of records for each species shared among both sites and S_i and S_j are the total numbers of fungal records at each site respectively; d ranges from 0 for identical communities to 1 for completely dissimilar communities). Total beta diversity (variation across the park) was determined based on the equation $\hat{\sigma}^2 = \frac{1}{N-1}(\sum d_{ij}^2/N)$, where N represents the number of sites and d_{ij} pairwise dissimilarity between the i th and j th sites (Anderson et al., 2011). For $N = 4$, $\hat{\sigma}^2$ ranges from 0 (when all communities are identical) to 0.5 (when all communities are completely dissimilar). Pairwise dissimilarity (d_{ij}) was calculated using cumulative incidence data that were square root transformed to better accommodate rare species.

Factors influencing alpha and beta diversities were considered using multivariate ordination analyses. To identify putative assembly patterns in the distribution of Ceratocystidaceae fungi across the park, a multiple correspondence analysis (MCA) was performed involving fungal records as observations and fungal identities and sampling sites as active categorical variables, with host trees treated as supplementary categorical variables and nitidulid beetle vectors as supplementary quantitative variables. This was achieved using the package FactoMineR (Lê et al., 2008) and visualized using dynGraph (available at <http://dyngraph.free.fr/index.html>). The influence of landscape types, tree hosts and nitidulid vectors on fungal community assembly was assessed collectively and individually using Pearson's chi-square test (on cumulative fungal records for the collective effect of vectors) and partial redundancy analyses (RDA) based on Bray-Curtis distance with BiodiversityR. RDAs were followed by F tests, based on 1000 Monte Carlo permutations to assess the significance of the results. Host factors were defined at the genus level, while vector factors were defined based on insect morphospecies. The effect of isolation by distance was evaluated by measuring the correlation between the matrix of pairwise geographic distances and the Bray-Curtis dissimilarity matrix using Mantel tests with BiodiversityR. To suit the requirements of chi-square statistics, rare fungal species (<5 records) were pooled together when taxonomically closely related or excluded. Unidentified sampled trees were also excluded from the analyses.

3. Results

3.1. Host trees

Samples for fungal isolation were obtained from 300 trees over the two sampling periods (2009–2010), of which 114 yielded fungal isolates representing species in the Ceratocystidaceae. These trees belonged to 25 species in 17 genera and 10 botanical families. During the winter of 2010, isolates were obtained from 65 trees that included eight plant genera (Supplementary – Fig. S1). The proportion of samples collected on different tree species generally reflected the composition of the plant communities, with the most abundant trees providing the greatest numbers of samples. For instance, samples from *Colophospermum mopane* were predominant in Letaba and Punda Maria, while *Combretum* spp. were most commonly sampled in Skukuza.

Table 1
Numbers of nitidulid beetles collected from different sites and tree species in the Kruger National Park during winter 2010.

Site	Host tree	Number of nitidulid beetles			
		<i>Ca. dimidiatus</i>	<i>Ca. apicipennis</i>	<i>B. ater</i>	<i>Ca. hemipterus</i> s.l.
Letaba	<i>Acacia tortilis</i>	5		3	12
	<i>Colophospermum mopane</i>	5	1		23
	<i>Combretum hereroense</i>				
	<i>Philenoptera violacea</i>			2	8
Punda Maria	Unidentified				
	<i>Acacia nigrescens</i>	95		13	2
	<i>Colophospermum mopane</i>	75	1	13	4
	<i>Combretum apiculatum</i>	12			
	<i>Combretum zeyheri</i>	14	1		5
	<i>Terminalia sericea</i>	12	3		
	<i>Ziziphus mucronata</i>	22			
Satara	Unidentified		1	1	72
	<i>Acacia nigrescens</i>	8	5	3	67
	<i>Combretum hereroense</i>				
	<i>Combretum imberbe</i>		2	1	32
	<i>Lannea stuhlmannii</i>				
Skukuza	<i>Philenoptera violacea</i>		1		1
	<i>Schotia brachypetala</i>			8	
	<i>Acacia grandicornuta</i>	18			
	<i>Combretum apiculatum</i>	47		2	
	<i>Combretum hereroense</i>	6			
	<i>Combretum molle</i>	44		4	33
	<i>Combretum zeyheri</i>	30			
	<i>Lannea</i> sp.	22	1		
	<i>Terminalia sericea</i>	52		1	
<i>Ziziphus mucronata</i>	148			1	

3.2. Nitidulid vectors

2000 nitidulid beetles were collected in association with Ceratocystidaceae fungi, including 942 that were obtained during the winter of 2010 (Table 1). They represented two genera of the Nitidulidae, namely *Brachypeplus* and *Carpophilus*. The genus *Brachypeplus* was represented by the single morphospecies, *Brachypeplus ater*, while five morphospecies of *Carpophilus* were recovered, including *Carpophilus apicipennis*, *Carpophilus bisignatus*, *Carpophilus dimidiatus*, *Carpophilus hemipterus* and one unidentified morphospecies (Table 1). Variable numbers of insects were collected from different tree hosts and sites (Table 1). Overall, one species, *Ca. dimidiatus*, was dominant and accounted for 65% of all the beetles collected during winter 2010. The second most abundant (28%) nitidulid group included *Ca. bisignatus*, *Ca. hemipterus* and the unidentified *Carpophilus* sp. These three species generally occurred together and were, at the time of data collection and fungal isolation, mistakenly identified to represent the single species, *Ca. hemipterus*, because of their morphological resemblance. For this reason, they were treated as a single morphospecies in the statistical analyses and are hereafter referred to as *Ca. hemipterus* s.l.

3.3. Fungal community composition

A total of 308 isolates of Ceratocystidaceae fungi were obtained from damaged trees and associated nitidulid beetles over the study period, including 187 that were collected during the extended survey of winter 2010. Isolation was successful with 35% of sampled plant material, while the rate of successful isolation from insects was ~85%. Identification using multigene phylogenies revealed that these fungi represented three genera and eight distinct species in the Ceratocystidaceae, including *Ceratocystis albifundus*, *Ceratocystis thulamensis*, *Ceratocystis zambeziensis*, *Huntiaella cryptoformis*, *Huntiaella oblonga*, *Huntiaella savannae*, as well as *Chalaropsis thielavioides* and an undescribed *Chalaropsis* sp.

(Supplementary – Fig. S2). Among these, only *Ch. thielavioides* was not recovered during the winter of 2010. The number of fungal species recovered from a single sampled tree with active infection by Ceratocystidaceae fungi during this latter period ranged from one to four (average = 1.53), corresponding to a total of 100 fungal records across 65 trees. *C. albifundus* was the most prevalent species encountered on wounds and insects ($n = 38$) followed by *H. cryptoformis* ($n = 24$). These two species were recovered across all four study sites (Table 2, Fig. 1). *H. oblonga* and *H. savannae* were also commonly encountered ($n = 17$ and $n = 15$ respectively), but they were only found at three sites each, whereas *C. thulamensis* ($n = 2$), *C. zambeziensis* ($n = 3$) and *Chalara* sp. ($n = 1$) were rare and found at only one site each (Table 2, Fig. 1). These values generally correlated with the overall relative abundance of the different fungal species calculated over the two sampling years across the KNP (Fig. 1).

3.4. Fungal diversity

Using the Chao 1 index, the total species richness for Ceratocystidaceae fungi estimated across the KNP was $S_{Chao1} = 8$, while the species richness estimated per site was $S_{Chao1} = 4–5$. These values were generally consistent with the actual numbers of species recovered (Table 3) and the shape of species rarefaction curves. These displayed a well-defined saturation plateau for the Letaba and Punda Maria sites as well as the KNP as a whole, while approaching saturation for the Satara and Skukuza sites (Fig. 2). The Shannon-Weiner index indicated a relatively low species diversity according to the biological diversity standard throughout the park ($H = 1.55$) and to a greater extent at each local site ($H = 1.02–1.40$) (Table 3). This reflected a generally low species richness (eight species in total), but also an uneven incidence among species within communities, as illustrated by the steepness of Rényi diversity spectra (Fig. 3).

Total beta diversity for Ceratocystidaceae fungi across the KNP was relatively low ($\hat{\sigma}^2 = 0.07$), while pairwise comparisons based

Table 2
Fungal records per tree species across study sites in the Kruger National Park.

Site	Host tree	Number of fungal records						
		<i>H. cryptoformis</i>	<i>H. savannae</i>	<i>H. oblonga</i>	<i>C. albifundus</i>	<i>C. zambeziensis</i>	<i>C. thulamensis</i>	<i>Ch. basicola</i>
Letaba	<i>Acacia tortilis</i>				1			
	<i>Colophospermum mopane</i>	4	5	5	4			
	<i>Combretum hereroense</i>		1					
	<i>Philenoptera violacea</i>		1	1	1			
	Unidentified		1					
Punda Maria	<i>Acacia nigrescens</i>		1	1	2			
	<i>Colophospermum mopane</i>	1	4	7	5		1	
	<i>Combretum apiculatum</i>				1			
	<i>Combretum zeyheri</i>				2		1	
	<i>Terminalia sericea</i>				1			
	<i>Ziziphus mucronata</i>				1			
	Unidentified	1	1	1				
Satara	<i>Acacia nigrescens</i>	3			3		1	
	<i>Combretum hereroense</i>				1			
	<i>Combretum imberbe</i>	2	1		1		1	
	<i>Lannea stuhlmannii</i>	1						
	<i>Philenoptera violacea</i>				1			
Skukuza	<i>Schotia brachypetala</i>						1	
	<i>Acacia grandicornuta</i>	1		1	1			1
	<i>Combretum apiculatum</i>	1			1			
	<i>Combretum hereroense</i>				1			
	<i>Combretum molle</i>	1			1			
	<i>Combretum zeyheri</i>	5			3			
	<i>Lannea</i> sp.	1			1			
	<i>Terminalia sericea</i>	2			4			
<i>Ziziphus mucronata</i>	1		1	2				

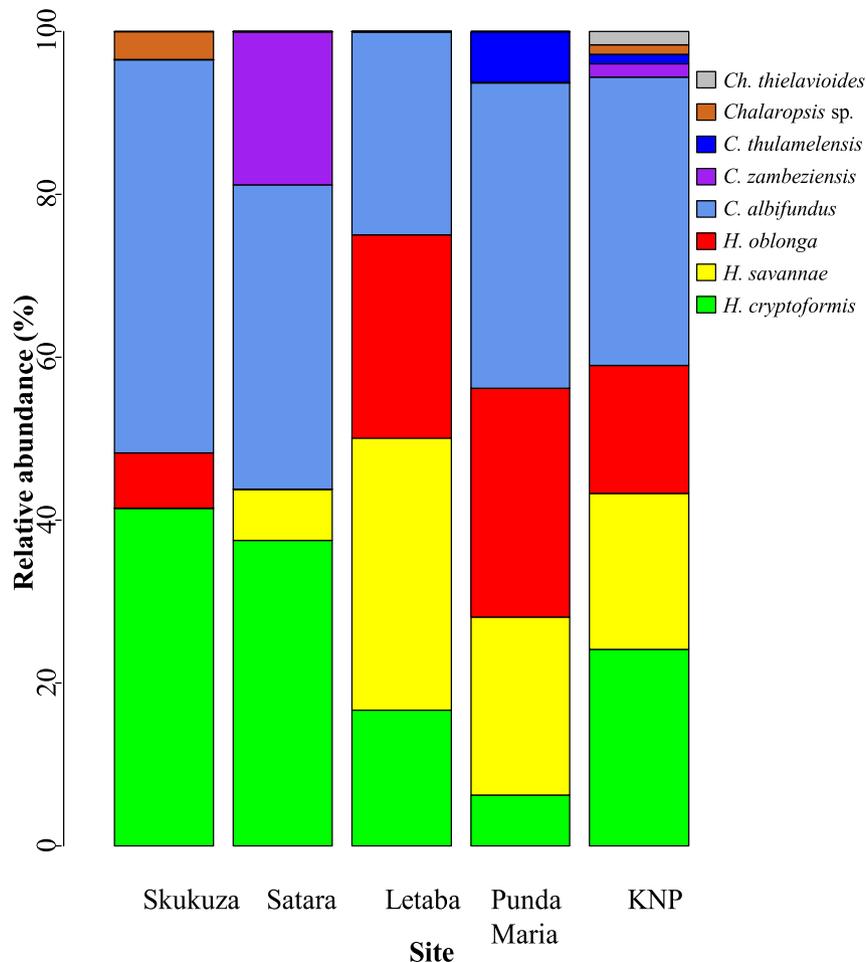


Fig. 1. Community composition of Ceratocystidaceae at each study site based on collections undertaken during winter 2010 and across the Kruger National Park as a whole (KNP) based on cumulative, 2009–2010 collections.

Table 3
Alpha and gamma diversity of Ceratocystidaceae infecting tree-wounds in the Kruger National Park.

Site	Observed richness	Chao index	Shannon-Weiner index
Skukuza	4	4.5	1.02
Satara	4	4	1.22
Punda Maria	5	5	1.40
Letaba	4	4	1.36
KNP	8	8	1.55

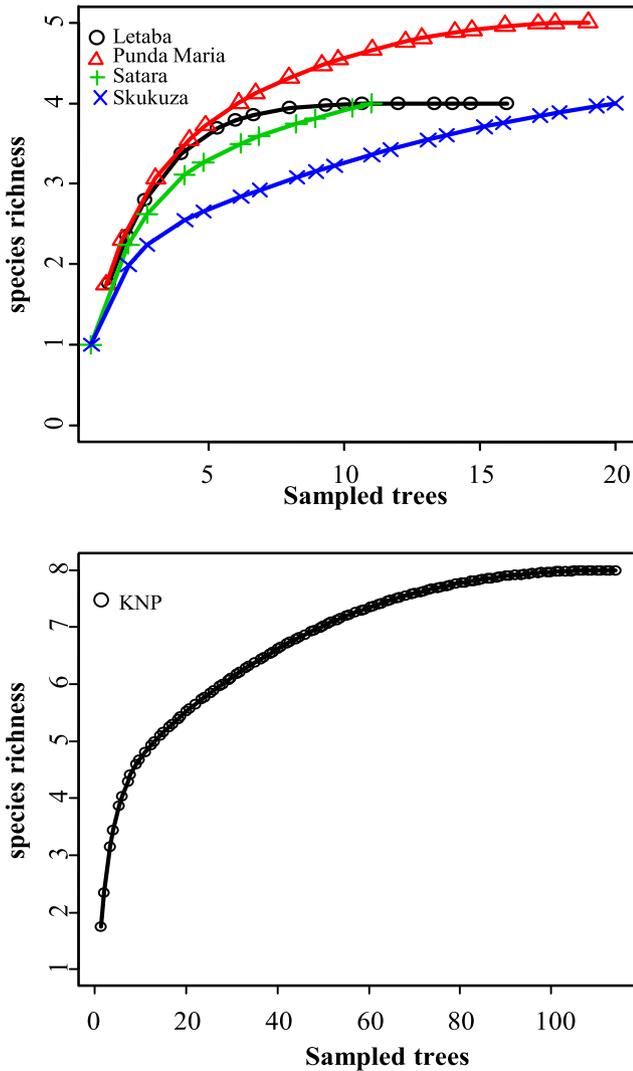


Fig. 2. Species accumulation curves of Ceratocystidaceae at each study site and across the Kruger National Park (KNP).

on Bray-Curtis dissimilarity metrics indicated low to moderate variations among communities (Table 4). Punda Maria and Letaba exhibited the lowest dissimilarity ($d = 0.2$), having only one unshared species, while Punda Maria and Skukuza were moderately dissimilar ($d = 0.47$), with 3 unshared species. All other site combinations included between 2 and 4 unshared species (Table 4).

3.5. Fungal community assembly

The MCA projection of fungal species around the first two ordination axes showed that four species (*C. albifundus*,

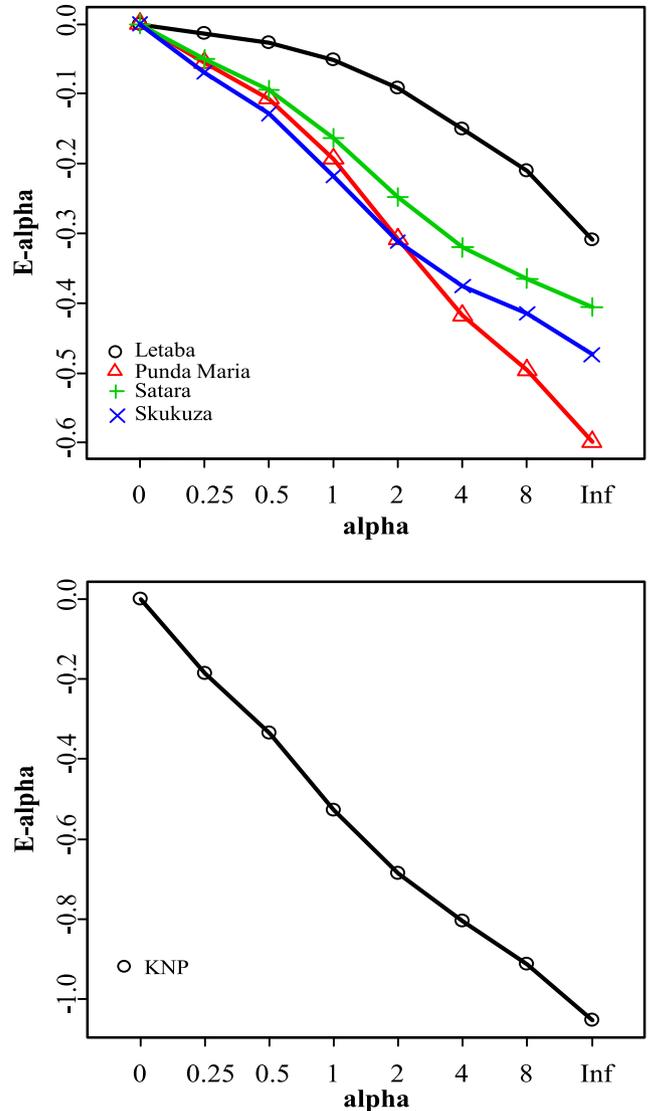


Fig. 3. Rényi diversity profiles of Ceratocystidaceae at each study site and across the Kruger National Park (KNP).

Table 4
Beta diversity of Ceratocystidaceae fungi in the Kruger National Park based on the number of unshared species (above diagonal) and Bray-Curtis dissimilarity index (below diagonal).

	Skukuza	Satara	Punda Maria	Letaba
Skukuza	–	4	2	2
Satara	0.34	–	3	2
Punda Maria	0.47	0.46	–	1
Letaba	0.38	0.28	0.20	–

H. cryptoformis, *H. oblonga* and *H. savannae*) clustered away from *C. zambeziensis* (Fig. 4). Furthermore, two putative associations were displayed, one involving *C. albifundus* and *H. cryptoformis* and another involving *H. oblonga* and *H. savannae*. The position of the four sampling sites in the graph showed some overlap between Punda Maria and Letaba, apparently driven by the predominance of *H. oblonga* and *H. savannae* in these sites. These two sites appeared to diverge from Skukuza and Satara that were dominated by *C. albifundus* and *H. cryptoformis*. There was also a divergence

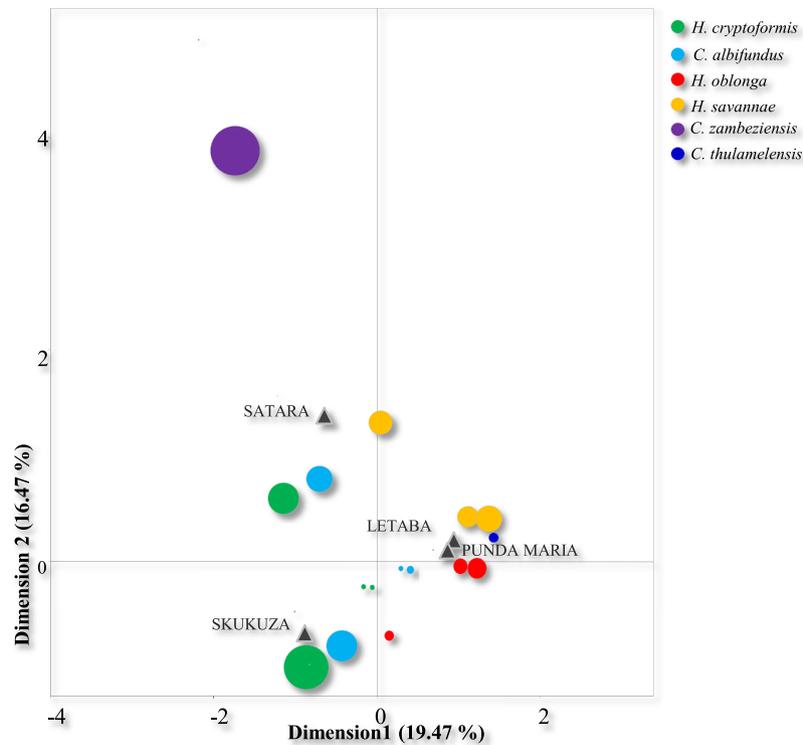


Fig. 4. Patterns of community assembly of Ceratocystidaceae in the Kruger National Park depicted from the first two ordination dimensions of Multiple Correspondence Analyses involving fungal species and study sites. The circles representing fungal species are weighed by their contribution to ordination dimensions.

between the latter two sites, due to the outlying position of *C. zambeziensis*. This pattern was supported by the description of factors significantly contributing to each dimension (Supplementary – Table S1). For instance, *C. zambeziensis*, *H. cryptoformis*, Satara and Skukuza were positively correlated with the first dimension, contrasting with *C. thulamelensis*, *H. oblonga*, *H. savannae* and Letaba and Punda Maria that were negatively correlated with that same dimension. On the other hand, Satara and *C. zambeziensis* were positively correlated with the second dimension, whereas Skukuza, *C. albifundus*, *H. cryptoformis*, *H. oblonga* and *H. savannae* were in a negative correlation with that same dimension. The cumulative contribution of the two ordination dimensions to total variance was 36%.

Constrained ordination analyses based on RDA supported the heterogeneous pattern of MCA for the distribution of Ceratocystidaceae fungi with a strong site (or landscape) effect ($F = 4.03$; $df = 1$; $P < 0.001$) on the incidence of these fungi. As components of the landscape, the composition of host trees ($F = 2.10$; $df = 1$; $P < 0.002$) and associated nitidulid beetles ($\chi^2 = 215.3$; $df = 9$; $P < 0.001$) were also shown to be strongly influential on the Ceratocystidaceae. When considering different levels of these factors (i.e. individual tree and nitidulid species), two beetle species, *Ca. dimidiatus* and *B. ater*, were found to have a significant effect. The effect of *Ca. dimidiatus* was directed towards *C. albifundus*, while that of *B. ater* was directed towards *C. zambeziensis*, *H. savannae* and *H. oblonga* (Table 5, Fig. 5). Similarly, a significant effect was found among the hosts from *Colophospermum* spp. towards *H. oblonga* and *H. savannae*, and from *Combretum* spp. towards *H. cryptoformis* (Table 5, Fig. 5). Mantel tests based on Pearson's ($r = -0.46$, $P = 0.82$) as well as Spearman's ($r = -0.23$; $P = 0.68$) coefficients showed no correlation between the fungal dissimilarity matrix and the matrix of geographic distances,

Table 5

Contributions of factor levels to community assembly of Ceratocystidaceae in the Kruger National Park based on partial redundancy analyses.

Factors	F	Pr (>F) ^a
Landscape		
Letaba	3.35	0.016*
Punda Maria	3.90	0.002**
Satara	2.45	0.05*
Skukuza	4.89	0.002**
Tree host		
<i>Acacia</i> spp.	0.84	0.509
<i>Colophospermum</i> spp.	6.24	0.001**
<i>Combretum</i> spp.	2.73	0.026*
<i>Lannea</i> spp.	1.16	0.305
<i>Philenoptera</i> spp.	0.98	0.447
<i>Schotia</i> spp.	1.62	0.118
<i>Terminalia</i> spp.	1.75	0.125
<i>Ziziphus</i> spp.	0.80	0.559
Nitidulid vector		
<i>Carpophilus apicipennis</i>	1.55	0.168
<i>Carpophilus dimidiatus</i>	3.60	0.003**
<i>Carpophilus hemipterus</i> s.l.	2.02	0.064
<i>Brachyepelus ater</i>	2.24	0.042*

Level of significance: * ($p < 0.05$), ** ($p < 0.01$).

^a Based on 1000 permutations.

suggesting that there was no isolation by distance between sites.

4. Discussion

The diversity of the Ceratocystidaceae recovered on tree wounds, and in particular those made by elephants, in the KNP included eight species, varying in their incidence from rare to

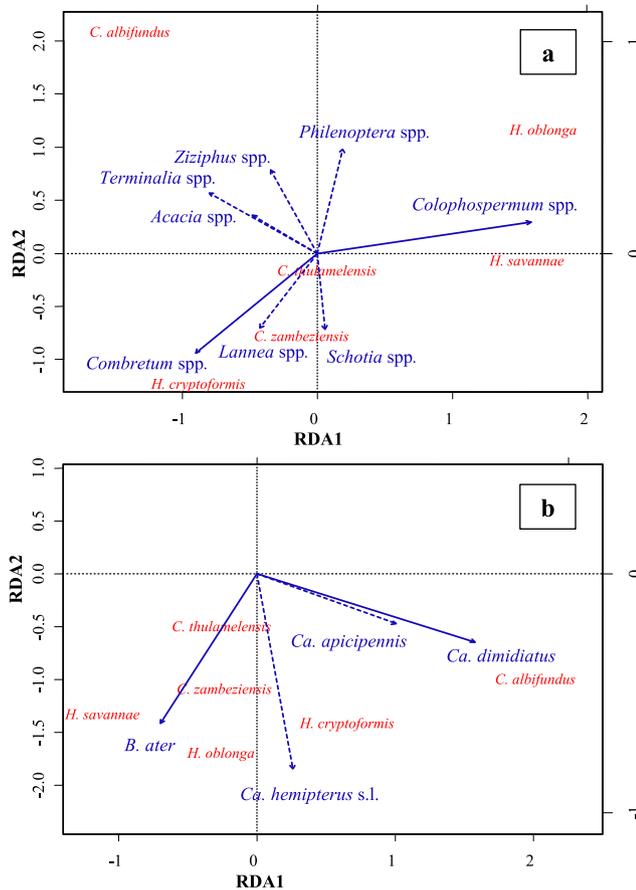


Fig. 5. Influence of tree hosts and nitidulid vectors on the Ceratocystidaceae community assembly in the Kruger National Park based on partial redundancy analyses. Arrows indicate the strength and direction of the effect exerted by individual factors. Solid lines indicate significant effect based on 1000 Monte Carlo permutations ($P < 0.05$).

relatively abundant. Multivariate statistics indicated that this diversity was heterogeneously distributed, supporting its compartmentalization into local community assemblages consistent with the landscapes of the park. These fungi, which represent three genera with variable ecological characteristics, from pathogens to saprobes (De Beer et al., 2014), exhibited selective associations with their host trees and nitidulid beetle vectors; though there were insufficient data to support robust conclusions regarding fungus-host and fungus-insect interactions with rare species. Furthermore, isolation by distance was not a good predictor of fungal community structure at the scale of the park. Therefore, neither of the underlying processes of metacommunity dynamics, i.e., environmental filtering, dispersal and species interaction could be discounted *a priori* for the observed spatial pattern of Ceratocystidaceae fungi in the KNP (Leibold et al., 2004; Biswas and Wagner, 2012).

4.1. The filtering effect of the landscape

The conjunction of strong site effect and negligible effect of isolation by distance on the distribution of Ceratocystidaceae fungi was indicative of the important role that landscape characteristics play in determining the incidence of these fungi. Similar environmental filtering effects of the landscape have been reported for other fungi, including endophytes (Zimmerman and Vitousek,

2012), mycorrhizae (Kernaghan and Harper, 2001), as well as some plant pathogens, such as rust fungi (Mitchell et al., 2002; Mundt et al., 2011) and oomycetes (Haas et al., 2011; Gómez-Aparicio et al., 2012). Consistent with the findings in those studies, our results suggested that the composition of the community of tree hosts on which the Ceratocystidaceae occurred was one component of the landscape that could explain the influence it exerts on these fungi.

Partial redundancy analyses supported a strong collective host effect on fungal structure and singled out some of the trees, most significantly contributing to this effect. Among these were *Colophospermum spp.*, which appeared to be strongly correlated with *H. oblonga* and *H. savannae*. This association may explain the overlapping fungal diversity measured between Punda Maria and Letaba, since the two sites are not the closest geographically, but lie in contiguous landscapes dominated by *C. mopane*. Similarly, the correlation between *Combretum spp.* and *H. cryptoformis* may explain the abundance of this fungus at both the Satara and Skukuza sites.

4.2. Dispersal by nitidulid beetles

Because fresh tree wounds provide ephemeral substrates, especially in the dry and hot environmental conditions of African savanna ecosystems where they may desiccate rapidly, it is of crucial importance for Ceratocystidaceae fungi to be able to move efficiently to new substrates when available. It appeared that nitidulid beetles provide these fungi with this capacity in the KNP, as it has been observed in other systems (Moller and DeVay, 1968; Cease and Juzwik, 2001; Hayslett et al., 2008; Heath et al., 2009; Mbenoun et al., 2015). These include at least five species in the genus *Carpophilus* and one species in the genus *Brachypeplus*.

Evidence from this study suggests that *Brachypeplus* and *Carpophilus* nitidulids contribute variably to the dispersal of different species of Ceratocystidaceae fungi in the KNP. First, co-occurrence of fungi and insects on tree wounds was shown to be non-random. This was illustrated by two strong fungus-insect associations, the one involving *Ca. dimidiatus* and *C. albifundus* and another involving *B. ater* and one or all of three fungi, including *H. oblonga*, *H. savannae* and *C. zambeziensis*. There was also a clear disjunction in fungal associations between the most abundant nitidulid beetles, *Ca. dimidiatus* and *Ca. hemipterus s.l.* The latter occurred most commonly along with *Huntia* spp.

Consistent interspecific associations between Ceratocystidaceae fungi and nitidulid beetles could be driven by the insects' selective fungivory and linked to their ecological fitness. Nitidulid beetles transmitting the oak wilt and aspen canker diseases in the USA feed and reproduce on *Ceratocystis fagacearum* and *Ceratocystis fimbriata* s.l., the causal agents of these diseases respectively (Moller and DeVay, 1968; Hinds, 1972). Interestingly, *Colopterus truncatus* is listed as a vector of both fungal species, but the population dynamics of this insect appears to be most strongly influenced by the oak wilt disease cycle, suggesting a stronger ecological association with *C. fagacearum* (Juzwik, 1999; Cease and Juzwik, 2001). In the present study the mats of Ceratocystidaceae fungi on elephant-damaged trees were generally infested by various nitidulid adults and larvae (unpublished data). This suggests that fungivory may be a common behavior among *Brachypeplus* spp. and *Carpophilus* spp. associated with Ceratocystidaceae fungi in the KNP. A direct implication of selective fungivory is that nitidulid beetles would have a filtering effect on the Ceratocystidaceae, driven by differences in transmission frequencies of the fungi by different insects, which could also be reflected on the spatial pattern exhibited by these fungi.

4.3. Interspecific interactions

The successful infection of an open wound on a tree by a Ceratocystidaceae fungus ultimately depends on the suitability of the environment as well as biotic interactions occurring on the wound surface. In this regard, the very low frequencies of the two *Chalaropsis* species recovered in our study may mean that tree wounds represent secondary and/or sub-optimal habitat for these fungi, as compared to the soil environment where they commonly occur in association with plant roots (Weber and Tribe, 2004). It is also interesting that all four of the most abundant Ceratocystidaceae species in the KNP (*C. albifundus*, *H. cryptoformis*, *H. oblonga* and *H. savannae*) grow optimally around 30 °C and/or can survive beyond 35 °C (Wingfield et al., 1996; Kamgan et al., 2008; Heath et al., 2009; Mbenoun et al., 2014). This illustrates greater compatibility with the thermal conditions prevailing in the KNP for these fungi as compared to the less prevalent species (*Chalaropsis* spp., *C. thulamensis* and *C. zambeziensis*), which require cooler temperatures (Weber and Tribe, 2004; Mbenoun et al., 2014). Such differences in fitness may modulate fungal interspecific competition for spatial resources.

However, biotic interactions on the wound surface may be more diverse, involving a cohort of other microbial wound-colonists and mobilising a variety of antagonistic and cooperative mechanisms (Galiana et al., 2011). For instance, the Ophiostomataceae, another family of ascomycete fungi commonly isolated from tree wounds in the KNP (Kamgan et al., 2008; unpublished data from this study), when pioneers on the wound surface, can preclude subsequent infection by Ceratocystidaceae fungi (Gibbs, 1980; Heath et al., 2010). Similar relationships may also exist among species of Ceratocystidaceae fungi in the KNP as their low estimated co-infection rate (1.53 species/tree) appears to suggest.

4.4. Evaluation of theoretical metacommunity perspectives

The features of ecological processes driving metacommunity dynamics, as they relate to the Ceratocystidaceae in the KNP, suggest that the spatial pattern displayed by these fungi is more the result of deterministic mechanisms rather than random ecological drift characterizing neutral dynamics. The active dissemination by nitidulid beetles supports potentially high dispersal rates for their fungal associates, especially when the high incidence of tree wounds sustains large populations of these insects. This would have promoted mass-effect mechanisms, driven by substantial dispersal between patches. However, because of the selective nature of Ceratocystidaceae-Nitidulidae interactions, dispersal appears also as a filtering factor acting in favor of species-sorting. Species-sorting is further reinforced by differences in host quality among fungal and probably vector species, which add up to the observed landscape filtering effect on fungi. On the other hand, regardless of their tree composition, woodlands are primarily and equally inhospitable in the absence of wounded trees. Episodic tree wounding events, mostly emerging from elephant browsing activities, bring about ephemeral patches of suitable habitats, and all the dynamics of Ceratocystidaceae fungi revolve around these patches. In sum, as expected for most natural communities (Leibold et al., 2004), the structure and dynamics of Ceratocystidaceae fungi in the KNP are probably determined by a combination of mechanisms from all theoretical metacommunity models, but with substantial contributions from species sorting and patch dynamics processes.

5. Conclusions

Fungal pathogens are increasingly being recognized to pose a

serious hazard to natural plant communities as well as cultivated plant systems due to the rising incidence of virulent emerging and re-emerging infectious diseases associated with global environmental change and other anthropogenic drivers (Anderson et al., 2004; Fisher et al., 2012; Wingfield et al., 2015). Responding effectively to these threats can be very challenging, especially in natural ecosystems because of the multiplicity and complexity of factors that may contribute to disease emergence and spread in these systems. The present study, focusing on tree-infecting fungi in the Ceratocystidaceae, demonstrates that metacommunity theory can provide a useful framework to decipher the patterns of fungal pathogen incidence and abundance in natural ecosystems. It can, therefore, assist epidemiological studies and disease risk assessment by identifying putative, predictive risk factors for pathogens and diseases. In the present case, such indicators would include extensive tree wounding activities and the presence of some tree and nitidulid species. The latter appear to play a central role in the community ecology of Ceratocystidaceae fungi, by enhancing their dispersal capacity, acting as ecological filters and probably mediating competition between community members via vector competitive exclusion. Further research should focus on better understanding the strength and drivers of associations between nitidulid beetles and members of the Ceratocystidaceae as these could provide new insights and opportunities for the control of numerous plant pathogens residing in this fungal group.

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Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funeco.2016.09.007>.

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