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Microfungal Community Changes in Rodent Food Stores over Space and Time

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Abstract

This paper reports the changes over time in the microfungal communities that inhabit three rodent species' food stores at two climatically different locations. Results reveal that microfungal diversity values calculated from above-ground food stores are highest in the more commonly disturbed portions of the rodent dens. Interactions among food-inhabiting microbes and between the rodents and food-inhabiting microbes also appear to influence the microfungal communities within the rodent dens. For example, our data suggest that transport by animal vectors, and not by air currents, is more effective at dispersing microbial propagules. Furthermore, although fungal communities inhabiting food stores within dens varied in composition and diversity over time, standardized substrates (sorghum seeds) simultaneously placed within the food stores converged in microfungal composition the longer they were left within the dens. We hypothesize that animal vectors, including rodents, make neighboring fungal communities more alike by introducing similar communities of microbes, which in turn initiate a cascade of biological interactions that, over time, result in similar microfungal communities inhabiting newly stored food items.

Introduction

Many rodents store food items for extended periods under conditions that are amenable for microbial infection and growth [33, 34, 38], and those food stores can develop large and diverse populations of microbes, particularly fungi [11, 12, 14, 31, 33, 34]. It is likely that the diversity and species richness of microfungi present in the food stores is increased by the array of resources and disturbances within a den [14].

In contrast, microbial populations within the same den may be made more similar by various dispersal vectors. For example, air currents commonly disperse and broadly mix many microbial taxa [19, 20, 28]. Air currents also may make microfungal communities inhabiting rodent food stores more similar by transporting fungal propagules directly between food stores, particularly those above ground. Simultaneously, air currents may facilitate indirect dispersal

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of microfungi by distributing fungal spores to locations where animal vectors can more reliably transport them into their food stores (e.g., resident rodents foraging above ground interacting with microbes placed there by air currents). Furthermore, compared to air currents, animals that visit nearby dens (to steal food or interact with conspecifics) may be more reliable as microbial dispersal vectors [1, 11, 17, 21 for examples] because they are more likely to consistently come into physical contact with the microfungal communities of several food stores.

In a previous report [14] assessing the within-site (alpha) diversity of microfungal communities within rodent dens, we suggested that the high, but variable, fungal diversity and species composition over time and space [e.g., 12, 14, 36] is caused, in part, by the dynamic interplay of (i) resource variability (including disturbance events; [14, 10]) usually resulting in decreased similarity among microfungal communities, countered by (ii) fungal dispersal and succession that we believe result in increased similarity among fungal communities.

This study's purpose was to characterize the spatial distribution and beta (between-site) diversities of microfungal populations that inhabit rodent food stores and indirectly test the possibility that intraden location, wind currents, or rodent activity influence those patterns. The study examined spatial patterns of microfungal populations that inhabit dens of three rodent species (*Neotoma albigula, Dipodomys spectabilis,* and *Neotoma floridana*). Studying these food-storing rodents allowed us to examine the fungal communities inhabiting two types of food stores from the same area (food stores of *N. albigula* and *D. spectabilis* from New Mexico) and similarly cached food stores in two areas (stores of *N. albigula* from New Mexico and stores of *N. floridana* from Kansas). Analyzing spatial patterns of microfungal diversity within, among, and above (air-dispersed fungal propagules) the dens of these three rodent species also allowed us to make generalizations and additional hypotheses about the spatial and temporal dynamics of microfungal populations that inhabit food stores.

Materials and Methods

The study was conducted from 1992 through 1994 at Sevilleta National Wildlife Refuge (SNWR; Socorro County, New Mexico) and Konza Prairie Research Natural Area (KPRNA; Riley County, Kansas). *Neotoma albigula* and *D. spectabilis* are sympatric at SNWR (study Site 1 and site 2, respectively), and *N. floridana* inhabit KPRNA (study Site 3). Additional information about the study sites, rodent species, and rodent dens is described elsewhere [8, 13, 14, 39].

Food Store Sampling

Rodent dens at sites 1 and 2 were sampled during early Sep (summer) and Oct (fall) 1992, and Jan (winter) and May (spring) 1993. Rodent dens at Site 3 were sampled during early Feb (winter), May (spring), late Aug (summer), and Oct (Fall) 1994. Twelve active dens were sampled at each site. Six of the 12 dens (referred to as the "core dens") were located near each other (within 167 m). The remaining six (peripheral) dens at each site were located along a transect of geometrically increasing distance away from the core dens (100–3200 m, 14 for details).

Selection of active dens for the study was based on accessibility, distance from other dens in the study and on our ability to find food stores within the den [13]. Permanent sampling ports into the dens were established by pushing white PVC pipes (2.5 cm diameter) through the tops of the dens into locations where stored food material had been found. At least four PVCs sampling ports were placed in all core dens (locations roughly corresponded to north, south, east and west) and one PVC port was placed in each peripheral den.

PVC pipes in *Neotoma* dens at sites 1 and 3 were placed a mean of 30.4 cm (SE = 5.9) and 36.2 cm (SE = 6.9) deep, respectively. PVC pipes placed in dens of *D. spectabilis* at site 2 were a mean of 41.8 cm (SE = 1.3) deep. Because of the large size of the dens of *D. spectabilis,* two additional PVC sampling ports were placed in the center of each core den, one at least 37 cm into the den (deep port), and one between 33 and 45 cm deep (shallow port).

Food store samples were taken by pushing a sterilized hollow stainless steel rod designed to sample grain through the permanent PVC sampling port; the rod was turned open, shaken to allow food material to fall in, turned closed, and withdrawn from the den. The rod's contents then were placed into a sterile resealable bag, taken to the laboratory, stored at 4°C, and assayed for microfungi within 4 mo after they were collected (usually within 4 wk). Although Brockman et al. [3] suggest that bacterial community composition changes during storage, they also report that postsampling changes in fungal community composition appear to be minimal in subsurface soil.

Assessment of Microfungal Species Richness

Assessment of fungal communities using dilution techniques is usually biased toward those fungi that produce many fungal spores or other propagules [30]. A more accurate understanding of both the diversity and frequency of fungal species inhabiting the rodents' food stores can be obtained by using a variation of the washing scheme described by Warcup [40]. This method was used to "wash" off free propagules from a sample so that only the fungi actively growing within organic particles would be isolated. Details of the modified washing procedure are given elsewhere [13, 14]. The number of viable propagules recovered after the sample had been washed 15 times were compared using a one-way ANOVA

Table 1. Sampling dates for each of the four samples taken from each of the three sites[a]

Sample	Site 1: *N. albigula*	Site 2: *D. spectabilis*	Site 3: *N. floridana*
1	Summer sample: 11 Sep 1992 (6 d)	Summer sample: 11 Sep 1992 (6 d)	Winter sample: 5 Feb 1994 (116 d)
2	Fall sample: 26 Oct 1992 (46 d)	Fall sample: 26 Oct 1992 (46 d)	Spring sample: 8 May 1994 (93 d)
3	Winter sample: 7 Jan 1993 (73 d)	Winter sample: 7 Jan 1993 (73 d)	Fall sample: 2 Sep 1994 (118 d)
4	Spring sample: 9 May 1993 (123 d)	Spring sample: 9 May 1993 (123 d)	Fall sample: 13 Oct 1994 (43 d)

[a] Sorghum samples were placed at Sites 1 and 2 on 5 Sep 1992 and were replaced when food store samples were collected. Sorghum samples were put in at Site 3 on 13 Oct 1993 and were replaced when food store samples were collected. Sampling intervals (d) are noted in parentheses.

[44] to determine if any differences existed among the three sites. The 15th wash was chosen, because, unlike most of the other washes, the number of colonies counted were normally distributed and thus could be parametrically tested ([13] for details).

Because our primary interest centered on the dynamics and structure of microfungal communities, and not the specific identity of the fungal species inhabiting the rodent food stores, most of the cultures were identified only to genus. However, as with similar studies on microfungal ecology [6, 9, 11], each isolate (within a genus) that was morphologically different, based on microscopic study, and that exhibited dissimilar colony growth characteristics under different environmental and nutritional conditions was assigned a different accession number. When isolations had been completed for all samples, cultures representing each genus were grown on at least three different media (including malt extract, potato dextrose, cornmeal, water, glycerol nitrate and Czapek yeast agar, and sterile paper) and examined microscopically to compare morphological characteristics. This allowed comparisons to be made of all similar fungal "species" under the same conditions. This process resulted in the reassignment of some "species" and the elimination of some accession numbers. Consequently, for the purposes of this study, the term "species" will be used to denote identified and taxonomically recognized species plus morphologically and/or physiologically distinct isolates. In addition, although only culturable fungal species were used in our analyses, it is likely that those species can serve as good indicators of the community changes that affect all the fungal species in the dens.

Sorghum Seeds

It was anticipated that characteristics of microfungal community composition and dynamics could be obscured by variation within and between the food items (substrates) brought into the den (food stores). To determine the effects of substrate, season, and length of growing period, one standardized substrate, grain sorghum (*Sorghum bicolor*), was placed in all dens and removed at known intervals.

Sorghum packets were constructed by wrapping approximately 4 g of inviable seeds within a 10 × 5 cm piece of wire mesh. The mesh was large enough to allow microarthropods in but not allow sorghum seeds to fall out. One 8-kg lot was used as the source for all sorghum seeds throughout the study. All seeds in the 8-kg lot were sterilized (and made inviable) by baking them at 200°C for 0.5 h in a conventional oven. Compared with autoclaving, this procedure killed any fungi on or within the sorghum seeds, yet left the seeds' tissues and structure comparatively unmodified (J. Herrera, unpublished observation).

One seek packet was tethered to the top end of each PVC pipe and dropped into the pipe so the seeds were partially within the food store. To allow for comparisons, new seed packets were put in and old ones taken out at the same time the food stores were sampled (Table 1 for dates). Once taken from the den, the sorghum seeds were stored at 4°C and assayed for microfungi within 4 mo after they were collected (usually within 4 wk). When assayed, 10 randomly selected sorghum seeds were taken from each sample and surface sterilized by dipping them into a 5% commercial bleach solution for 30 s. The seeds were then blot-dried on a sterile paper towel and partially embedded into a plate with approximately 25 ml of malt extract agar (with 0.03% streptomycin sulfate and chlortetracycline). Colonies arising from the seeds were isolated, identified, and counted.

Spatial Diversity

Microfungal diversity over space was characterized over three spatial scales: within dens (including depth); within sites; and among sites. In addition, the dispersal ability of den-inhabiting fungi was evaluated indirectly by comparing the microfungal communities inhabiting rodent dens with those inhabiting the air currents above some of the same dens.

Intraden diversity. To determine whether fungal diversity values within a den differed, fungal community data collected from the core dens at each of the three sites were segregated by intraden location and analyzed. To determine if fungal diversity was significantly different within dens at any one site, Shannon species diversity values [24] from the intraden locations were compared for each site using a 2-way analysis of variance, with the location and den (blocked) as the main factors [44]. When the ANOVA showed locations to be different, a Newman–Keuls multiple comparison test was used to determine differences among the locations [44].

Air sampling. The number and type of fungal propagules in the air at Sites 1 and 2 were assessed using a Kramer–Collins viable spore sampler (GR Electric Manufacturing Company, Manhattan, Kansas). This volumetric, suction, slit-type sampler was constructed to impinge propagules on the surface of agar media in Petri plates placed inside the body of the sampler. All air samples were taken between 1600 hrs and sunset, and within 2 d of when food store

samples were taken from those sites (air samples taken in winter, spring, summer, and fall). One core and one peripheral den were randomly selected from each site for collection of air samples. At each of the selected dens, air samples were taken at 1.8 m above the den and at ground level next to the den entrance. Each sample included at least six moist malt extract agar plates (containing 0.03% chlortetracycline and 0.05% of streptomycin sulfate). Each of the plates was uncovered, placed within the Kramer–Collins sampler, and exposed to a constant 12 L min^{-1} for 2 min. Previous preliminary work revealed that this rate and exposure time allowed a manageable number of fungal propagules to impinge on the surface of the agar medium. After exposure, each plate was covered and sealed with Parafilm and returned to the laboratory. All plates were incubated at room temperature for at least 10 d. All fungal colonies arising from the plates were identified to genus and counted. Because the evaluation of the two air sampling locations (1.8 m above and near a den entrance) did not show obvious differences in species composition or abundance, these samples were combined for analysis and will be referred to us "air samples above the dens."

Two types of similarity indices (Morista–Horn and Sorensen's; see [24]) were used to compare the following pairs of fungal communities: (i) airborne genera isolated above two (one randomly selected core and one randomly selected peripheral) dens of *N. albigula* and two similarly selected dens of *D. spectabilis;* (ii) all airborne genera isolated above four rodent dens at sites 1 and 2, and genera isolated from within those same four gens; and (iii) all genera isolated throughout the study from food stores of *N. albigula* and genera isolated from food stores of *D. spectabilis.*

Similarity of fungal communities among and within sites. The following versions of the Morista–Horn (M-H) similarity index and Sorensen's similarity (SS) index (two measures of beta diversity [24]) were used to estimate the similarity among microfungal communities that inhabit food store and sorghum seed samples:

$$\text{M-H index} = \frac{2\Sigma\,(n_{ai}\cdot n_{bi})}{(da + db)\,N_a\cdot N_b},$$

where

N_a = no. of individuals at site A

N_b = no. of individuals at site B

n_{ai} = no. of individuals of the ith species at site A

n_{bi} = no. of individuals of the ith species at site B

$d_a = \Sigma n_{ai}^2/N_a^2$

$d_b = \Sigma n_{bi}^2/N_b^2$.

$$\text{SS index} = \frac{2j}{a + b},$$

where

a = no. of fungal species in community A

b = no. of fungal species in community B

j = no. of fungal species that communities A and B have in common.

Although the M-H index is a quantitative, robust, and commonly used index when comparing communities that differ in sample size, it also biases similarity estimates toward commonly occurring species [43]. Because previous work [14] showed that most fungal species within the rodent dens occurred infrequently, a qualitative (and unweighted) measure of similarity (SS index) was used to more thoroughly assess whether infrequent fungal species (some of which may be more biologically important to the caching rodent or to the dynamics of the microbial community) were influencing spatial diversity patterns among the fungal communities.

For each of the sites, both similarity indices (M-H and SS) were used to estimate how fungal species composition inhabiting food store samples varied along a distance gradient within all possible two-den combinations. Similar paired comparisons between fungal communities inhabiting sorghum seed samples also were performed for each of the three sites. Similarity index values were used in regression analyses to test whether fungal communities between dens were more similar to each other as interden distance decreased. Regression analyses were performed on both similarly indices for both type of samples (food store and sorghum seed) at each of the three sites (a total of 12 regression lines).

Similarity Values over Distance through Time

To determine what effect sampling periods had on both similarity indices (M-H and SS), the data were segregated by the season in which they were collected and then reanalyzed. To improve sample size, however, the fungal communities inhabiting the food store samples within core dens at each site were pooled and compared with the pooled fungal community inhabiting the food stores of the respective peripheral dens. Similar pooled comparisons at each site were performed for fungal communities inhabiting sorghum seed samples in core dens and those inhabiting the respective peripheral dens.

At different times during the study, each of our three sites had one peripheral den that was abandoned by the rodent or otherwise could not be sampled. Nevertheless, when appropriate and possible, these dens were included in the results.

Results

Assessment of Microfungal Species Richness

Food stores from *N. albigula* contained statistically more propagules after 15 washes than those from *D. spectabilis* $\bar{x}$ =

Table 2. Number of fungal isolates and various diversity measures of fungal genera and/or species[a] retrieved from stored food material, sorghum seed, and air samples from each of the three study sites

	Site 1: *N. albigula*			Site 2: *D. spectabilis*			Site 3: *N. floridana*	
Measurement	Food	Sorghum[d]	Air	Food store	Sorghum[e]	Air	Food store	Sorghum[f]
No. of colonies examined	2480	410	1352	5951	1611	1894	3818	1792
No. of species[a]	172	88	—	273	133	—	189	82
Shannon species diversity values	3.8	3.51	—	4.00	3.5	—	2.90	3.08
No. of identifiable genera[b]	31	21	14	37	28	16	41	19
Shannon genus diversity values	2.43	2.12	1.92	2.30	2.12	1.71	1.90	1.47
Mean no. of spp/genus/den[c]-(SE)	2.29(0.11)	1.81(0.14)	—	3.09(0.21)	2.29(0.18)	—	2.31(0.14)	2.14(0.13)

Note: Values represent microfungi isolated during four sampling periods (winter, spring, summer and fall) during an approximately 365-d period.
[a] "Species" here is considered the total number of identified and taxonomically recognized species and numbered isolates that were morphologically and/or physiologically distinct.
[b] Does not include isolates that were nonsporulating, overgrown, unknown, or yeasts.
[c] Mean no. of species/genus/den represents the mean number of fungal species per fungal genus (SE) isolated from the rodent dens from each of the three study sites.
[d] Of 680 sorghum seeds assayed.
[e] Of 1999 sorghum seeds assayed.
[f] Of 1833 sorghum seeds assayed.

62.1 (SE = 14.7) vs 6.5 (SE = 1.0) propagules 0.5 g^{-1} sample 0.5 mL^{-1} of water, respectively) but not *N. floridana* (F = 10.32, P = 0.0003), whose food stores contained an intermediate number of propagules ($\bar{x}$ = 33.6, SE = 5.3; Newman–Keuls test, $P < 0.0004$).

Food stores and sorghum seeds retrieved from dens of the three rodent species contained a high diversity of microfungi (Table 2 [14 for specific fungal general]). In general, food stores of *D. spectabilis* contained more genera, species and more species/genus/den than *Neotoma* food stores (Table 2).

Intraden diversity. The depth of the food samples of *D. spectabilis* (PVC pipe depth) was not related to the Shannon species diversity of the microfungal community inhabiting that sample (regression analysis, F = 0.43, P = 0.96).

Comparisons among the intraden locations (N, S, E, and W for *Neotoma* dens and N, S, E, W, shallow, and deep for dens of *D. spectabilis*) showed that fungal diversity differed within the *Neotoma* dens, but not within the dens of *D. spectabilis* (Table 3, Fig. 1). Multiple comparisons among the four intraden locations of *N. albigula* dens showed that diversity values were highest in food store samples retrieved from the northern portion of those dens, whereas similar comparisons in dens of *N. floridana* showed that samples from the northern and southern portions of those dens exhibited the highest fungal diversity (Fig. 1).

Air sampling. Fungal genera identified from air samples above four rodent dens at Sites 1 and 2 were less diverse and numerous than the fungal genera inhabiting food stores

Table 3. Results of randomized block ANOVAs comparing the Shannon species diversity values from fungal communities isolated from intraden locations of *N. albigula, D. spectabilis,* and *N. floridana,* with location and den (blocked) as the main factors[a]

	Site 1: *N. albigula*					Site 2: *D. spectabilis*					Site 3: *N. floridana*				
Source	df	SS	MS	F	*P* value	df	SS	MS	F	*P* value	df	SS	MS	F	*P* value
Den	5	0.26	0.05	0.32	0.815	5	0.94	0.19	6.30	0.001	5	1.24	0.24	2.96	0.137
Location	3	5.25	1.74	10.77	0.013	5	0.23	0.05	1.57	0.204	3	7.47	2.47	30.44	0.001
Error	15	2.44	0.16			25	0.74	0.03			15	1.22	0.08		

[a] The error term describes the pooled variance of the error and interaction commonly used in blocked designs [44].

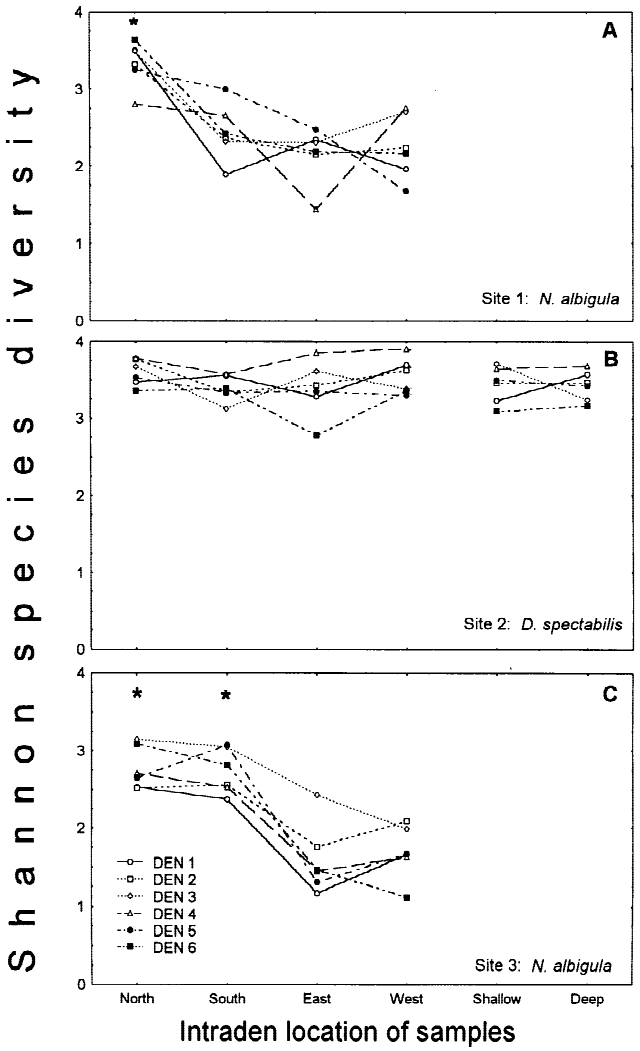

Fig. 1. (A–C) Shannon species diversity values for microfungi that inhabit rodent food store samples retrieved from different locations within the core dens of three rodent species. All locations from which samples were taken roughly corresponded to compass directions (north, south, east, and west) and/or depths (shallow and deep) within the den. Asterisk (*) marks significant differences among the locations within the site (Newman–Keuls test, $P < 0.0001$).

within the same dens (Shannon diversity for genera of all fungal isolates from air = 1.98; Tables 2, 4). Of the comparisons made, fungal genera isolated from air samples above dens of *N. albigula* and *D. spectabilis* were most similar (0.81 and 0.74 M-H and SS index values, respectively), followed closely by the comparison of the fungal communities from the food stores of the 12 *D. spectabilis* and 12 *N. algibula* dens in the study (0.76 and 0.79 M-H and SS index values, respectively); conversely, genera from the air samples were least similar to the genera isolated from the food stores in the same dens (0.30 and 0.56 M-H and SS index values, respectively).

Similarity of fungal communities among and within sites. Between-site comparisons showed that fungal communities inhabiting food stores of *N. albigula* and *D. spectabilis* were most similar regardless of which similarity index was used (Table 5). Within-site comparisons, however, showed that M-H values were higher than SS values, and unexpectedly, fungal communities inhabiting sorghum seed samples were less similar than those inhabiting food samples (Figs. 2, 3).

Although encumbered by low coefficients of determination, regression analyses suggest that fungal communities inhabiting sorghum seed samples in dens within a site are less similar the farther those dens are from each other. All three of the regression analyses of the SS similarity indices, showed that similarity between any two fungal communities inhabiting sorghum seed samples was negatively correlated to their interdistance for each site (Fig. 3). Conversely, except for site 3, intrasite regression analyses of the M-H indices comparing fungal communities inhabiting food store and sorghum samples were inconsistent (Fig. 2). At site 3, the regression analyses of the M-H and the SS indices arguably showed that fungal communities inhabiting food stores were more similar the closer they were to each other.

Similarity Values over Distance through Time

For three of the four sampling periods, fungal communities inhabiting food stores of *N. albigula* in core dens were consistently similar to those in peripheral dens (site 1, Fig. 4). The M-H index comparing fungal communities isolated from food store samples during the fall sampling period was the site's exception to this trend.

Both M-H and SS indices generally showed that fungal communities inhabiting sorghum seed samples in core and peripheral dens of *N. albigula* were more similar the longer (the more time) the sorghum seeds were left in the dens. Again, the one obvious exception was the M-H index value comparing the sorghum seed samples retrieved during the fall sampling period (site 1, Fig. 5).

The comparison between the fungal communities in food samples from core and peripheral dens of *D. spectabilis* showed that both similarity indices were constant throughout the four sampling periods (Fig. 4). Similar comparisons among the sorghum seed samples in the core and peripheral dens of *D. spectabilis,* however, showed that, with one conspicuous exception (SS index for fall), all similarity indices

Table 4. Number of fungal isolates of different genera retrieved from air samples collected using a Kramer–Collins viable spore sampler

Genera	Site 1: *N. albigula*	Site 2: *D. spectabilis*
Acremonium	2 (0.15)	1 (0.06)
Alternaria	313 (23.31)	257 (14.35)
Aspergillus	7 (0.52)	31 (1.73)
Aureobasidium	223 (16.60)	483 (26.92)
Basidomycetes[a]	—	1 (0.06)
Chaetomium	1 (0.07)	1 (0.06)
Chrysosporium	—	2 (0.11)
Circinella	—	1 (0.06)
Cladosporium	167 (12.43)	72 (4.01)
Drechslera	85 (6.33)	30 (1.67)
Fusarium	12 (0.89)	9 (0.50)
Metarhizium	—	1 (0.06)
Nigrospora	—	1 (0.06)
Nonsporulating[b]	189 (14.07)	230 (12.82)
Penicillium	33 (2.46)	135 (7.53)
Pithomyces	2 (0.15)	—
Pycnidial forms[c]	—	3 (0.17)
Rhinocladiella	1 (0.07)	—
Rhizopus	2 (0.15)	1 (0.06)
Rhodotorula	—	2 (0.11)
Sclerotial forms[d]	—	2 (0.11)
Unknowns	158 (11.76)	57 (3.18)
Verticillium	1 (0.07)	—
Yeasts	147 (10.95)	474 (26.42)
Totals	1343 (100.00)	1794 (100.00)

Note: Samples were made above one peripheral den and one core den of both *N. albigula* and *D. spectabilis* at sites 1 and 2, respectively (numbers in parentheses represent percentage of total fungal colonies that belong to that genus within each column). Values represent microfungi isolated during four sampling periods (winter, spring, summer and fall) during an approximately 365-d period.

[a] Isolates found with hyphal clamps.

[b] Identifiable isolates that did not produce spores.

[c] Isolates that produced unknown types of pycnidia.

[d] Isolates that produced unknown types of sclerotia.

increased the longer the sorghum seeds were left in the dens (Fig. 5).

Compared with Sites 1 and 2, SS indices comparing microfungi inhabiting food stores in core and peripheral dens of *N. floridana* at Site 3 showed more variability among sampling periods (Fig. 4). The sorghum seed samples at Site 3, however, followed the general trend established for the sorghum seed samples at the other sites (Fig. 5). That is, with one exception (SS index for winter), similarity values comparing fungal communities inhabiting sorghum seeds in core and peripheral dens increased the longer those seeds were left in the dens.

Table 5. Morista–Horn (below diagonal) and Sorensen (above diagonal) similarity values comparing fungal isolates that inhabit food stores of *N. albigula* and *D. spectabilis* in sites 1 and 2, respectively, and *N. floridana* in site 3

	N. albigula	*D. spectabilis*	*N. floridana*
N. albigula	—	0.80	0.66
D. spectabilis	0.21	—	0.51
N. floridana	0.11	0.11	—

Discussion

Assessment of Microfungal Species Richness

The mycological assays revealed that, compared with the surrounding air (this study) and soil [12], the food stores within the dens of the three rodent species sampled were extremely species rich and exhibited high microfungal diversity (Table 2 [14]).

Although environmental conditions did not appear to

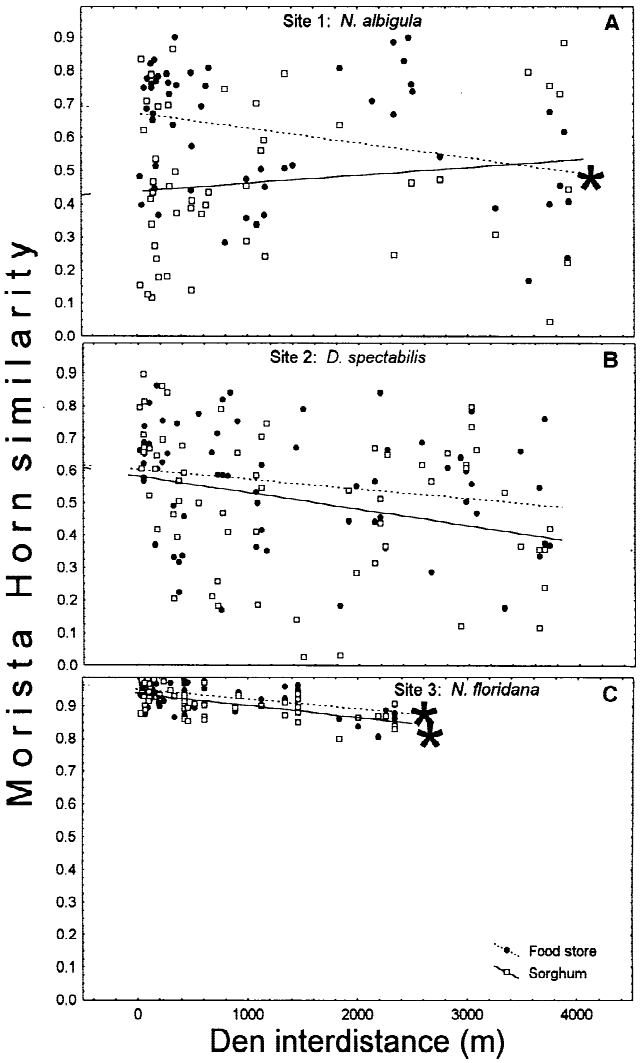

Fig. 2. Morista–Horn similarity index values comparing fungal communities inhabiting the dens of each of three rodent species. Similarity values represent comparisons among fungal species inhabiting either food store (filled circles) or sorghum seed (squares) samples retrieved from the dens. Lines represent the line of best fit for the interden similarities among all possible two-den combinations within each site using regression analysis and the least-square method (asterisk represents significantly negatively sloping line, $P < 0.01$). (A) *N. albigula.* (B) *D. spectabilis.* (C) *N. floridana.* (A) for food store, $\beta = -0.41$, $r^2 = 0.09$, $F_{1,53} = 9.70$, $P = 0.0031$; for sorghum seeds, $\beta = -0.14$, $r^2 = 0.19$, $F_{1,43} = 0.90$, $P = 0.35$; (B) for food store, $\beta = -0.21$, $r^2 = 0.04$, $F_{1,64} = 2.84$, $P = 0.10$; for sorghum seeds, $\beta = -0.29$, $r^2 = 0.09$, $F_{1,64} = 6.02$, $P = 0.17$; (C) for food store, $\beta = -0.56$, $r^2 = 0.10$, $F_{1,64} = 29.00$, $P < 0.0001$; for sorghum seeds, $\beta = -0.64$, $r^2 = 0.19$, $F_{1,64} = 44.40$, $P < 0.0001$.

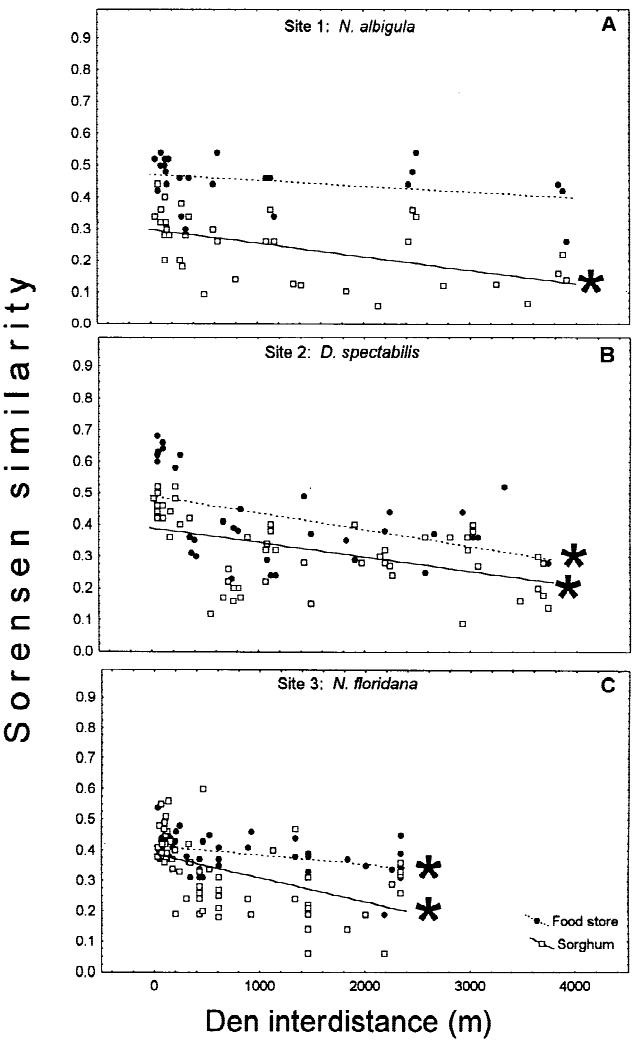

Fig. 3. Sorensen similarly index values comparing fungal communities inhabiting the dens of each of three rodent species. Similarity values represent comparisons among fungal species inhabiting either food store (filled circles) or sorghum seed (squares) samples retrieved from the dens. Lines represent the line of best fit for the inter-den similarities among all possible two-den combinations within each site using regression analysis and the least square method (asterisk represents significantly negatively sloping line, $P < 0.01$). (A) *N. albigula.* (B) *D. spectabilis.* (C) *N. floridana.* Linear regressions: (A) for food store, $\beta = -0.32$, $r^2 = 0.17$, $F_{1,47} = 2.84$, $P = 0.11$; for sorghum seeds, $\beta = -0.53$, $r^2 = 0.02$, $F_{1,47} = 9.51$, $P = 0.0049$; (B) for food store, $\beta = -0.66$, $r^2 = 0.04$, $F_{1,64} = 20.00$, $P = 0.00014$; for sorghum seeds, $\beta = -0.53$, $r^2 = 0.09$, $F_{1,64} = 10.40$, $P = 0.0034$; (C) for food store, $\beta = -0.40$, $r^2 = 0.16$, $F_{1,64} = 12.10$, $P < 0.0009$; for sorghum seeds, $\beta = -0.50$, $r^2 = 0.25$, $F_{1,64} = 21.60$, $P < 0.0002$.

influence directly the diversity values of the microfungal communities in our study, the conditions did appear to set the limits for the composition and character of those communities. For example, although sorghum seeds collected from two microenvironments (dens of *N. albigula* and *D. spectabilis,* J. Herrera, unpublished data) had very similar fungal diversity values [14], the species composition of those communities was very different (Table 5). This suggests that,

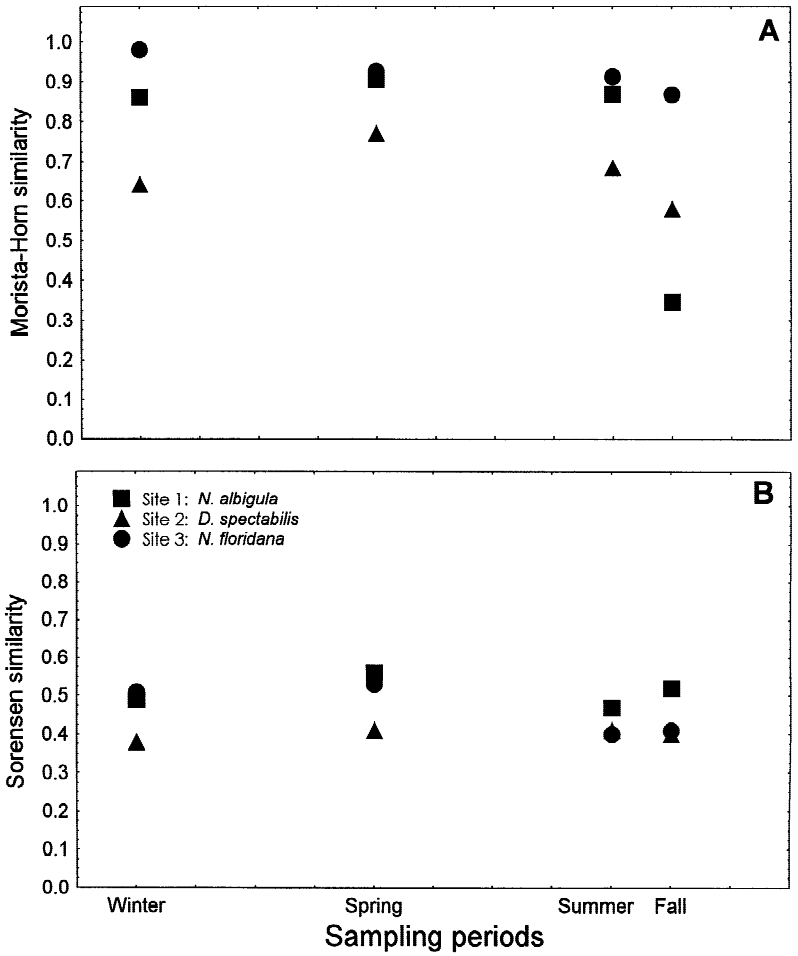

Fig. 4. Similarity index values comparing fungal communities that inhabited food store samples retrieved from core dens to those retrieved from peripheral dens. All samples were collected during four sampling periods (winter, spring, summer and fall). Samples from dens of *N. albigula* and *D. spectabilis* (sites 1 and 2) were collected in Sep and Oct 1992 and Jan and May 1993. Samples from dens of *N. floridana* (site 3) were collected during the same months as those from Sites 1 and 2, but in 1994. (A) Morista–Horn indices. (B) Sorensen indices.

although the number and distribution of fungal species were similar in different microenvironments (sites), the identity of the fungal species was not.

Intraden diversity. The comparisons among fungal diversity values within rodent dens suggests that *Neotoma* dens were influenced by environmental variables not significantly affecting the underground earthen dens of *D. spectabilis.* Such comparisons revealed that fungal diversity values were highest in samples retrieved from the northern portion of *N. albigula* dens, and highest in the samples retrieved from the northern and southern portions of *N. floridana* dens. At least two untested possibilities for those results exist. First, *Neotoma* at sites 1 and 3 may store their food items predominantly in the northern or southern portion of their den. This would be likely to increase the amount or diversity of substrates available to a wider array of fungal species. Second, and more likely, the conditions on the northern or southern portions of *Neotoma* dens were more frequently exposed to climatic disturbances (e.g., wind) or atmospheric enrichments such as rain. Thus, much like some mosses and lichens [2, 22], some fungi that inhabit woodrat dens may be gaining benefit from colonizing north-facing or windward-facing locations. By being on the windward side, lichens are though to benefit by gaining additional water inputs or by decreasing their exposure to desiccating heat by being shaded from the sun [2]. Climatic data from KPRNA indicate that prevailing winds at this site are from the north to northwest during the winter and from the southwest during the summer (John Briggs, KPRNA data set manager, Kansas State University). Similar data show that the prevailing winds at SNWR are from the northwest during the winter and from the southwest during the summer (Doug Moore, SNWR data set manager, University of New Mexico). Although untested with microfungal populations, these explanations nevertheless may be applicable here and are worthy of further examination.

Similarity of fungal communities among and within sites. Although they were assessed during different years, fungal communities inhabiting desert rodent dens (sites 1 and 2) were more similar to each other compared to those in mesic

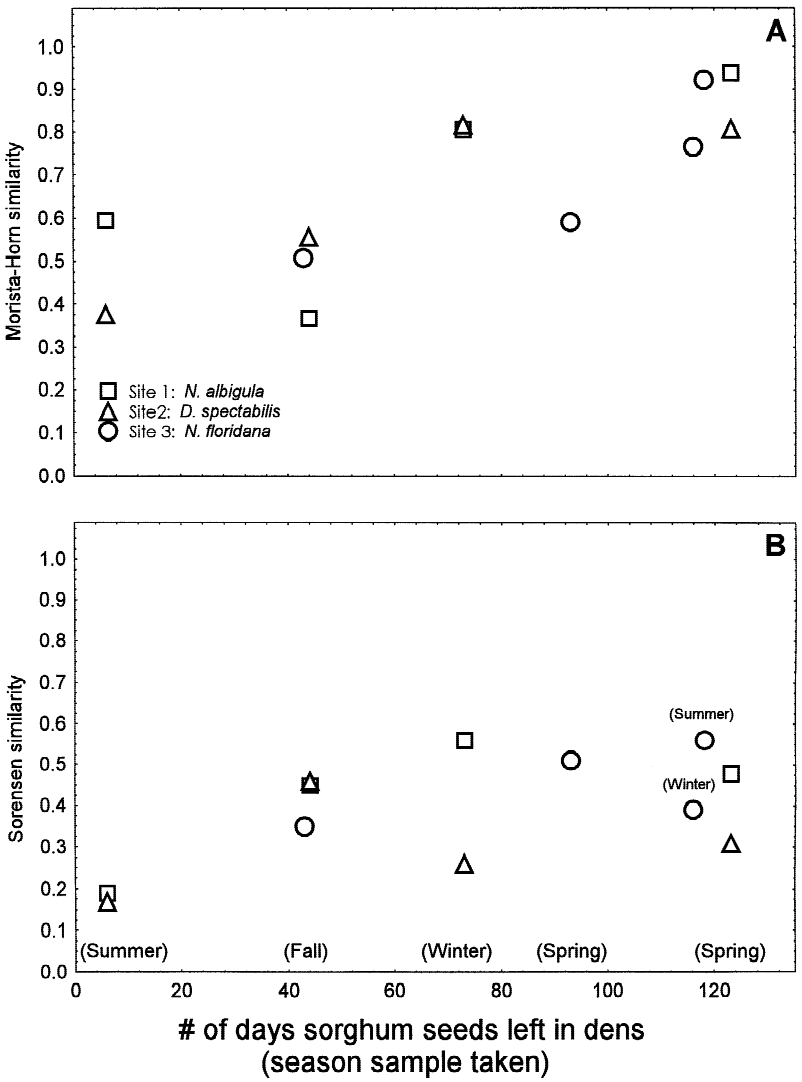

Fig. 5. Similarity index values comparing fungal communities that inhabited sorghum seed samples placed in and later retrieved from core dens to those retrieved from peripheral dens. All samples were collected during four sampling periods (winter, spring, summer and fall). Samples from dens of *N. albigula* and *D. spectabilis* (sites 1 and 2) were collected in Sep and Oct 1992 and Jan and May 1993. Samples from dens of *N. floridana* (site 3) were collected during the same seasons as those from Sites 1 and 2, but in 1994. (A) Morista–Horn indices. (B) Sorensen indices.

mixed shrub–grassland (site 3; Table 5). Nevertheless, the species composition in the fungal communities of the three sites was comparatively similar (as characterized by the SS indices), though the relative abundances of those species (as characterized by the M-H indices) were very different. Different patterns might have been observed had all the samples been taken during the same year.

Conversely, within-site comparisons among fungal communities showed that the relative abundance of each species within a site was more similar (as indicated by the higher M-H values) within these communities than the species composition. These observations suggest that (i) although many fungal species present in one site are also present in the others, differences in substrates or environmental conditions among sites were responsible for the considerable variation in relative species abundances [12]; and (ii) although commonly occurring fungal species are present in relatively equal proportions throughout any one site, the presence or absence of comparatively rare species changes the composition of the fungal community in any one den (as indicated by lower SS index values). This possibility was suggested by Hawkins [11], when she described microfungal diversity within dens of *D. spectabilis* as appearing to be influenced by a relatively cosmopolitan and consistent group of ("core") microfungi interacting with a relatively less common and constant group of microfungi. Similar explanations have been used to describe patterns of species diversity dynamics in communities of grassland plants [e.g., 5].

Furthermore, the results from the regression analyses suggest that fungal communities inhabiting food items (sorghum seeds) "stored" relatively recently are more similar in species composition (as indicated by the SS index) the closer they are to each other. Except for site 3, similarity indices comparing fungal communities inhabiting the compar-

atively older food store samples were inconsistent and do not appear to follow any obvious pattern. We suspect that food items stored in dens for longer periods have a greater probability of housing an increasingly similar pool of microbes, particularly those that are cosmopolitan and/or successional dominants.

Compared to regression analyses using M-H indices, regressions using SS indices were more likely to indicate that similarity between fungal communities decreased with increasing interden distance (3 and 5 of the 6 regressions had significant slopes, respectively; Figs. 2, 3). This difference is probably caused by the equal weight placed on rare fungal species by the SS index. The effect of rare species on similarity values is obscured when using the M-H index. Based on the biases of both indices and our results, we suggest that rare fungal species are more likely spatially clumped and are distributed to neighboring areas by the activity of animal vectors.

Other explanations for these results include (i) food items that fungi infect themselves are distributed over gradients, and (ii) air currents are more likely to distribute fungal propagules to neighboring (core) dens rather than to distant (peripheral) dens.

(i) The first explanation suggests that food items brought into a food store already possess fungal communities that vary gradually over space. Thus, a subset of this gradationally distributed fungal community also would likely infect the sorghum seed samples and result in greater dissimilarities among distantly spaced fungal communities.

Both sites 1 and 2, however, were in a dry basin on either side of the Rio Grande. The character of the vegetation and climate at both sites was similar and decreased the possibility of those sites harboring different, vegetation-specific fungal source pools. Furthermore, the slopes of the regression lines were steeper if the interden distance was less than 500 m. This suggests that the similarity among fungal communities decreases at a faster rate within a local (500 m) rodent neighborhood that contains dens that share similar climates, food items, and fungal source pools.

In addition, the pattern of similarity values does not appear to be a substrate-mediated phenomenon. Indeed, at all sites, fungal communities inhabiting the sorghum seeds exhibited lower similarity values compared with those inhabiting the food stores. This supports the contention that a wide array of microfungi (including rare species) initially infect sorghum seeds (low similarity values), but that over time, only a few microfungi successfully establish themselves within the seeds in all the dens (resulting in higher similarity values; Fig. 5).

(ii) The second possibility implies that the composition of fungal communities may be less similar over increasing interdistance because air currents are more likely to distribute fungal propagules to nearby (core) dens rather than to distant (peripheral) dens (e.g., pollen shadows [7, 32]; and seed shadows [15, 16]).

Fungal propagules identified in Table 4 or around [19, 23, 29] our sites, however, suggest that air currents do not commonly contribute to the fungal species pool that successfully colonizes rodent food stores. Compared with the food fragments within the dens, air samples taken above some of the same dens did not harbor very similar fungal genera. The vast majority of the fungal genera isolated from the air samples from sites 1 and 2 were cosmopolitan and/or specialized for air dispersal. Although the abundance of most of these fungal genera varied seasonally, over the entire year, the fungal propagules at these sites was comparatively homogeneous (J. Herrera, unpublished data). Although the air at site 3 was not assessed for fungal propagules during this study, other, more comprehensive, studies conducted by one of us (CLK) have shown similar patterns in areas surrounding site 3 (KPRNA [19, 23, 29]). In addition, some of those studies isolated fungal genera from the air not found in our *N. floridana* dens [19]. Data from this study show that air currents can mix fungal communities in the atmosphere (and perhaps on exposed substrates) and make them more similar, but the vast majority of the air-dispersed fungal species do not succeed in establishing in the den.

A third possibility is that animal vectors are primarily responsible for dispersing and making fungal communities more similar at each site. Many insects and other microarthropods transport propagules of many fungal species [4, 41] and may even make the microbial community at our sites more similar (e.g., darkling beetles (*Eleodes spp.;* Coleoptera, Tenebrionidae) move to and from dens of *D. spectabilis*).

However, rodents may more consistently frequent nearby conspecifics' dens to steal stored food or look for potential mates, particularly with the more "social" *Neotoma* species. Thus, although not systematically tested in this study, it is possible that rodents more dependably visit other conspecifics' dens and so probably function as the primary dispersal agents for fungal propagules at these sites [1, 11, 17, 21, 27, 37, 42 for examples]. For example, Hawkins [11] identified a total of 91 specimens of microfungi on *D. spectabilis* from which she isolated 87 and 60 species from cheekpouch and feet swabs, respectively.

Neotoma species in other studies also have been shown to

visit conspecifics more frequently [25, 26, 35], and so would more likely make fungal communities more similar in their neighborhood. For example, during a 4-yr study in Arizona, Newton [26] reported that on 12.4% of occasions (330 of 2426 times), he trapped *N. albigula* visiting a conspecific's den.

Our results suggest that *N. floridana* were not as effective as *N. albigula* at making fungal communities more similar within their dens. We suggest that *N. floridana* at site 3 were likely hampered from dispersing over long distances by various topographical or anthropogenic barriers (as suggested by other studies at similar sites in Kansas; MW McDonald, personal communication). Sites 1 and 2 contained few barriers that could effectively prevent either rodent species from dispersing and interacting with other conspecifics or their dens. Congruently, at these sites, results from the regression analyses of the similarity values from the fungal communities in the dens were more variable (Figs. 2, 3). The degree of variability in the similarity values used in the regression analyses, however, only allows us to provisionally claim that *D. spectabilis* do not range as far and are not as efficient as *N. albigula* at introducing fungal communities to nearby dens.

Similarity Values over Distance through Time

With two obvious exceptions, within-site comparisons between fungal communities inhabiting the sorghum seed samples from core and peripheral dens were more similar to each other the longer the sorghum seeds were left within the dens. The first exception was the M-H index comparing fungal communities inhabiting sorghum seed samples retrieved from dens of *N. albigula* during fall (Fig. 5A). During the fall sampling period (Sep–Oct), *N. albigula* bring the majority of their food items into their dens (J. Herrera, unpublished observation [26]). This enrichment of the food store is reflected in the increased fungal diversity inhabiting the food store samples during this time [14]. Thus, it is likely that because this enrichment disturbance supported additional or more abundant fungal populations, the similarity values among the various fungal communities within the dens (including the sorghum samples) experienced a transient decrease during this time. This conclusion is corroborated by the SS index (Fig. 5B), which, unlike the M-H index, stresses the presence or absence of fungal species. This suggests that though many of the same fungal species are present within all dens of *N. albigula,* a greater number of fungal species grow, sporulate, or increase their abundances during the fall sampling period.

The second exception was the SS index comparing fungal communities inhabiting sorghum seed samples collected from dens of *N. floridana* during the winter (Jan). In winter, a smaller number of fungal species were actively growing within the *Neotoma* dens (Fig. 5B). Of these, only a few cosmopolitan, cold-tolerant species dominated most fungal communities within those dens. The SS index, however, was unable to account for an increase in the abundance of these dominant species within all dens in the site and so decreased similarity estimates between fungal communities.

Although some conclusions extrapolated from our similarity data are inferred and need to be tested more rigorously, our results suggest that fungal communities inhabiting rodent dens are affected by the length of interaction among the various variables involved (other microbes, substrate chemistry, environment, etc.). Future research will focus on separating the variables and testing them individually. For example, examining the effects enrichment disturbances have on rodent food stores in a laboratory setting may more clearly define which variables most affect microfungal community structure and dynamics. These kinds of studies may work best if the microfungal community patterns were analyzed in simpler systems that include fewer fungal species. Results from such studies would help determine the effect that individual and interacting environmental variables have on microfungal community patterns.

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