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Diversity and community assembly of moss-associated fungi in ice-free coastal outcrops of continental Antarctica

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ABSTRACT

To date, the relative roles of niche-related (e.g., environmental filtering and biological interactions) and non-niche-related (e.g., dispersal limitation) processes in the assembly of fungal communities have rarely been explored in ice-free coastal outcrops of continental Antarctica. Here, using variation partitioning, we show that the community composition of fungi associated with moss colonies sparsely distributed in ice-free coastal outcrops of East Antarctica is affected by a suite of environmental conditions more significantly than by geographic distance, indicating the primary importance of niche-related factors. This was mainly attributable to the widespread occurrence of the predominant, cosmopolitan fungal species, *Phoma herbarum*. The variance explained by spatial factors increased when the analysis was applied only to the dataset of the Lützow-Holm Bay area (by excluding the data of the Amundsen Bay area, which is 500 km from the Lützow-Holm Bay area), indicating the local importance of non-niche-related processes.

1. Introduction

Understanding how fungal communities assemble is a fundamental question in fungal ecology. Deterministic theories suggest that niche-based local processes, such as trait-mediated environmental filtering and biotic interactions, largely determine patterns of species diversity and composition (Chase, 2007; Caruso et al., 2011; Ranjard et al., 2013). In contrast, stochastic theories emphasize non-niche processes and the importance of chance colonization, random extinction, and ecological drift (Hubbell, 2001; Sloan et al., 2006; Bell, 2010). Recently, evaluation of the relative roles of deterministic and stochastic spatial processes of community assembly has advanced our understanding and provided new perspectives about the diversity and assembly of fungal communities at local, regional, continental, and global scales (Caruso et al., 2011; Hazard et al., 2013; Ranjard et al., 2013; Rodrigues et al., 2013; Kivlin et al., 2014; Põlme et al., 2014). However, little is known about the community assembly in ice-free coastal outcrops of continental Antarctica, which comprise only about 2% of the continent, and are both 'cold and arid' and 'distant and isolated', features that impose strong environmental selection pressures and dispersal limitations on the establishment of fungi.

Despite the harsh and remote environment, previous studies reported the occurrence of non-lichenized fungi in soils (Bridge and Newsham, 2009; Arenz et al., 2014) and in exotic plant substrates from Antarctica (Hirose et al., 2013). This situation appears to be in accord with the notion that many species of Antarctic fungi are cosmopolitan and have great dispersal potential (Arenz et al., 2014), and with the well-known hypothesis of Baas Becking that 'Everything is everywhere, but the environment selects' (Wit and Bouvier, 2006). A general hypothesis that can be drawn from these notions is that niche-based processes, such as trait-mediated environmental filtering associated with water availability, nitrogen level, and salinity, which characterize the environment of Antarctic terrestrial

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systems (Convey et al., 2014), play more crucial roles in the community assembly of Antarctic fungi than do non-niche, spatial processes. However, only a few previous studies have been carried out in the Antarctic Peninsula (and none in continental Antarctica) to test this hypothesis by investigating the community composition of soil fungi at multiple locations along latitudinal and environmental gradients, demonstrating the effects of environmental factors and/or latitude on the fungal communities (Yergeau et al., 2006; Dennis et al., 2012; Newsham et al., 2016). It is still unknown whether geographic patterns of the distribution of fungal communities in continental Antarctica are more strongly related to environmental conditions than to geographic location, or *vice versa*.

The purpose of the present study was to discriminate the relative roles of niche-related (environmental) and non-niche-related (spatial) processes in the spatial variation of fungal communities associated with moss colonies interspersed in coastal outcrops at 41 locations in six regions of continental Antarctica, located within 500 km of each other. Mosses are one of the dominant components of the terrestrial vegetation of Antarctica (Kanda and Inoue, 1994; Ochyra et al., 2008; Cannone et al., 2013), and harbor an array of fungi (Davey and Currah, 2006; Lindo and Gonzalez, 2010; Osono and Trofymow, 2012). Fungi have often been isolated from mosses from locations in Antarctica (McRae and Seppelt, 1999; Tosi et al., 2002; Stevens et al., 2007; Zhang et al., 2013; Yu et al., 2014). Here we assessed the α-diversity of moss-associated fungi and the relative importance of environmental and spatial factors affecting fungal β-diversity by applying variation partitioning (Peres-Neto et al., 2006) to test the hypothesis that niche-based processes, rather than non-niche processes, play dominant roles in the community assembly of fungi in continental Antarctica.

2. Materials and methods

2.1. Study area

Samples were collected at 41 locations in six ice-free regions (Fig. 1): five regions in the Lützow-Holm Bay area (East Ongul Island, Langhovde, Skallen, Skarvsnes, and Breidvågnipa) and one in the Mt. Riiser-Larsen region in the Amundsen Bay area, approximately 500 km distant from the Lützow-Holm Bay area, in East Antarctica (66°45′ to 69°40′S, 39°26′ to 50°41′E, -17 to 267 m a.s.l.). In the study regions, moss colonies are generally restricted in spatial extent and distributed sparsely and distantly separated from each other. Samples were collected during the 51st Japanese Antarctic Research Expedition (JARE-51) from December 2009 to February 2010. At each location, five moss blocks (each 2 × 2 cm, 2 cm deep from the surface) were collected, making a total of 205 moss blocks. One moss stem (2 cm in length) was chosen from within the moss block with tweezers that had been sterilized with 70% ethanol, preserved in a sterilized vial (volume 1.5 mm^3), and stored at −20 °C. The remaining moss samples were weighed to measure their fresh weight, preserved in paper bags, and dried at room temperature. They were taken back to the laboratory in Japan and used for the isolation of fungi, identification of moss species, and chemical analyses.

2.2. Isolation of fungi

Fungi were isolated from moss stems with a slightly modified washing method based on that of Osono et al. (2006). Moss stems were washed five times with 10 ml of sterilized 0.005% Aerosol-OT (di-2-ethylhexyl sodium sulfosuccinate) solution and then rinsed with sterilized water three times in a sterile test tube using a vertical type automatic mixer (S-100; Taitec Co., Ltd, Japan). The rinsed stems were transferred to a sterile filter paper in a 9 cm Petri dish and dried for 30 min. The stems were then placed on the surface of corn meal agar medium (Nissui Pharmaceutical Co., Ltd, Japan) in 100 mg/l chloramphenicol plates, and then the plates were incubated at 15 °C in the dark. The incubated plates were observed microscopically nine times at 1-week intervals. Any fungal hyphae or spores appearing on the plate were isolated, transferred to a plate containing a 1:1 mixture of corn meal agar medium and malt extract agar medium supplemented with yeast extract (Nissui Pharmaceutical Co., Ltd, Japan), incubated, and identified by observing their micromorphological characteristics.

2.3. Molecular analysis of fungi

Fungal isolates were transferred to fresh malt extract agar medium overlaid with a cellophane membrane, and genomic DNA was extracted from the mycelia following the modified CTAB method (Matsuda and Hijii, 1999). Molecular analysis was performed according to Tateno et al. (2015). Polymerase chain reactions (PCR) were performed for genomic DNA extracted from mycelia using Quick Taq HS DyeMix (Toyobo, Osaka, Japan). Each PCR reaction contained a 50 μl mixture [21 μl distilled water, 25 μl master mix, 3 μl of *c.* 0.5 ng/μl template DNA, and 0.5 μl of each primer (final concentration, 0.25 μM)]. To PCR amplify the region including the rDNA ITS and 28S rDNA D1-D2 domain, the primer pair ITS1-F (Gardes and Bruns, 1993) and LR3 (Vilgalys and Hester, 1990) were used. Each DNA fragment was amplified using a PCR thermal cycler (DNA Engine; Bio-Rad, Hercules, CA, USA) using the following thermal cycling schedule: a first cycle of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 50 °C for annealing, and 1 min at 72 °C, and a final cycle of 10 min at 72 °C. The reaction mixture was then cooled at 4 °C for 5 min. PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN, Germany) according to the manufacturer's instructions.

Purified PCR products were sequenced by FASMAC Co., Ltd. (Kanagawa, Japan). Sequencing reactions were performed using a GeneAmp PCR System 9700 (Applied Biosystems, USA) using a BigDye Terminator V3.1 Kit (Applied Biosystems), following the protocols supplied by the manufacturer. The fluorescent-labeled fragments were purified from the unincorporated terminators using an ethanol precipitation protocol. The samples were resuspended in formamide and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

The rDNA ITS sequences of the fungal isolates were compared with available sequences in the GenBank database using BLASTn searches (Altschul et al., 1997). We defined molecular operational taxonomic units (MOTUs) within the rDNA ITS sequences dataset by clustering the sequences with the BLASTclust program provided online by the Max Planck Institute (http://toolkit.tuebingen.mpg.de/blastclust#) based on 97% sequence similarity and 90% coverage criteria. We made a single count for the occurrence of a MOTU in each moss sample even when more than one isolate was detected. A MOTU was regarded as a major one when it was isolated from more than 3% (i.e. 6) of 205 moss samples tested. The sequences of MOTUs determined in this study were deposited in the DNA Data Bank of Japan (DDBJ) (LC085184–LC085206).

2.4. Identification and chemical analyses of moss

Samples were oven-dried to a constant mass at 40 °C to determine oven-dry mass. Moisture content was calculated gravimetrically according to the equations: moisture content (%) = (fresh mass - oven-dry mass)/oven-dry mass × 100. Subsamples of moss tissues were observed microscopically to identify moss species. Other subsamples were ground in a laboratory mill to pass through a 0.5-mm screen, and used for chemical analyses. Total carbon and