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Communities of wood-inhabiting fungi in dead pine logs along a geographical gradient in Japan

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ABSTRACT

Fungi are the main agents of coarse woody debris decomposition in forest ecosystems. We examined the associations of environmental variables with fungal community structures in dead pine logs at 12 geographically distant sites using amplicon pyrosequencing of fungal ITS rDNA. A total of 575 operational taxonomic units (OTUs) were identified based on clustering at 97% similarity. Among the known fungal ecological groups, saprotrophic fungi generally showed highest frequency of occurrence and were positively associated with mean annual temperature (MAT) and log diameter. Wood decay fungi with unknown decay type were positively associated with pine wilt disease and negatively associated with log diameter. Ordination analysis of the 42 most prevalent OTUs showed that MAT and annual precipitation significantly explained the observed fungal community structure. These results suggested that climate conditions and site history differentially effect structure fungal communities in pine logs among different ecological groups.

1. Introduction

Latitudinal gradients affect the community composition of a variety of organisms and ecosystem functions (Mittelbach, 2012). Fungi are the main agents of coarse woody debris (CWD) decomposition in forest ecosystems (Rayner and Boddy, 1988). Because CWD is a quantitatively important contributor to the annual input of dead plant matter on the forest floor (Nishioka and Kirita, 1978) and provides a variety of habitats for many organisms (Harmon et al., 1986; Stokland et al., 2012), the identification of latitudinal differences among wood decay fungal communities and the determination of their roles in CWD decomposition are important for understanding forest biodiversity, carbon budget, and nutrient cycling. However, few studies have addressed the geographical patterns of wood decay fungi (Heilmann-Clausen et al., 2014).

Global diversity and biogeography of soil fungi have been studied, using high throughput DNA pyrosequencing (Talbot et al., 2014; Tedersoo et al., 2014). Similar to plants and animals, soil fungal communities were found to be endemic to biogeographical regions, but displayed functional redundancy on a global scale. In the case of dead wood, Heilmann-Clausen et al. (2014) found that wood decay fungal communities on fallen beech logs respond to climate and were regionally endemic at the European continental scale. In contrast to the high degree of functional redundancy in soil fungal communities, the unique role of lignocellulolytic basidiomycetes in decomposing the most recalcitrant components of CWD (lignin) means that their community structure often determines decomposition processes of CWD (Hättenschwiler et al., 2005; Baldrian, 2008; Dickie et al., 2012). However, this idea has not yet been tested on a wide geographical scale. Recently, we found that the frequency of wood decay type (white, brown and soft rots) in decayed logs of *Pinus densiflora*, as a consequence of fungal wood decay activities, correlated with latitude and climatic factors on a geographical scale in Japan (Fukasawa, 2015). These observations also suggest that fungal communities inhabiting pine logs may be functionally distinct rather than convergent on a wide geographical scale.

In Japan, a severe dieback of *P. densiflora* due to pine wilt disease (PWD), caused by the North American native pinewood nematode

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Bursaphelenchus xylophilus and spread by the cerambycid beetle *Monochamus alternatus*, started in the 1970s and continues to date (Mamiya, 1988). Previous studies reported that fungal communities in dead wood caused by pests are different from those of naturally wind-fallen trees (Bače et al., 2012). Similarly, the type of wood decay occurring in pine logs reflects the PWD history of sites (Fukasawa, 2015). Because wood decay type also is affected by fungal community composition, it is expected that the structure of wood decay fungal communities will, therefore, change with PWD.

In the present study, we examined the associations of geographical location, climate, PWD, CWD treatment, and wood diameter with the fungal communities inhabiting *P. densiflora* logs. This was accomplished by constructing a dataset from 12 sites in Japan. We hypothesized that the occurrences of functionally-important wood structural decomposers (e.g., white and brown rot fungi) would be associated with geographical variables and thus endemic to geographical regions. Spatial, climatic (altitude, mean annual temperature (MAT), annual precipitation (AP)), forest (PWD damage, artificial cut down, pesticide usage), and substratum (log diameter) variables were tested. We used MAT as a temperature variable because it has previously been identified as a factor structuring fungal community composition (Tokumasu, 2001) and litter decomposition (Berg and McClaugherty, 2003) along geographical gradients.

2. Materials and methods

2.1. Study sites

Twelve forest stands were selected along a latitudinal gradient in Japan (Fig. 1, Table 1). The latitudes of the sites ranged from 31.51 (TAN; study site abbreviations are shown in Table 1) to 39.46 (KWT), the altitude ranged from 137 m (AOB) to 1113 m (SHB), the mean annual temperature (MAT) ranged from 8.2 °C (SDR) to 16.9 °C (TAN) and the annual precipitation (AP) ranged from 1167 mm (MSH) to 2750 mm (SHB). The two most distant sites (KWT and TAN) were separated by approximately 1100 km. The vegetation currently at the sites are mixed stands of *P. densiflora* and broad-leaves, except for KGM which is a plantation of commercially grown timber (*Chamaecyparis obtusa*). Most of the sites experienced PWD during the last few decades and at some stage underwent CWD management to prevent the spread of PWD (cutting down of infected trees, fumigation or spraying of pesticide). Data on the presence/absence of PWD, cut down, and pesticide treatment are based on interviews with the foresters and/or physical evidence at the sites (i.e. cut log piles covered with nylon sheets for pesticide fumigation treatment).

2.2. Field work

Five pine logs (diameter >10 cm) were selected in each stand. All logs were at decay class (DC) II, according to the five-decay-class system (Fukasawa, 2012). Logs in this class are characterized by relatively hard wood, penetrable with a knife to less than 1 cm. In DC II, bark and twigs begin to be shed but branches (diameter 1–4 cm) remain intact. We focused on DC II logs in the fungal community survey because the abundance and activities of basidiomycetes and xylariaceous ascomycetes, which are the functionally important wood structural decomposers, are known to be highest within logs of this DC (Fukasawa et al., 2010; Rajala et al., 2011, 2012). Wood chips were taken at three points along each log, at ≥20 cm intervals along the log length, using a cordless electric drill (Makita, Anjo, Japan) equipped with a 9 × 150 mm wood auger. Prior to drilling, any bark and superficial litter were removed with a knife. Holes were drilled up into the heartwood,

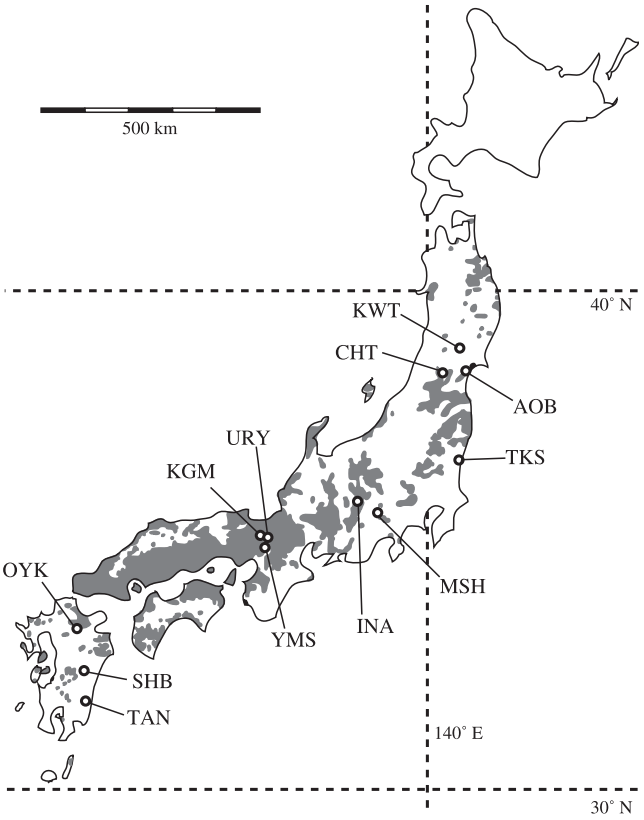

Fig. 1. Natural distribution of *Pinus densiflora* in Japan shaded areas; from Japan. Integrated Biodiversity Information System [http://www.biodic.go.jp/kiso/fnd_f.html] and locations of 12 study sites investigated in the present study. Study site abbreviations are shown in Table 1.

and the wood chip samples were collected in plastic zip-lock bags. To avoid contamination between samples, the wood auger was flamed and wiped with ethanol between each drilling. Samples from three points along each log were combined to form a single sample and kept frozen at −20 °C until DNA extraction. DNA was extracted from a total of 60 samples (12 sites × 5 logs).

2.3. DNA extraction and sample preparation for 454 pyrosequencing

In the laboratory, the wood chip samples were mixed within the zip-lock bags. Then, a 3 g subsample was placed directly into a bead tube from the PowerMax® Soil DNA Isolation kit (MoBio, Carlsbad, CA, USA). Before extraction, the samples were homogenised for 12 s using a Shake Master, ver. 1.2 (BMS, Tokyo, Japan). A semi-nested PCR protocol was used for direct 454 sequencing of the fungal internal transcribed spacer 1 (ITS1) region. The ITS region has been proposed as the formal fungal barcode (Schoch et al., 2012). In the first PCR, the entire ITS region and the 5′ end of large-subunit RNA (LSU) were amplified using the fungus-specific primers ITS1F (Gardes and Bruns, 1993) and LR3 (Vilgalys and Hester, 1990). PCR was performed in a 20.7 μl reaction mixture containing 3.0 μl of template DNA, 0.3 μl of KOD FX NEO (TOYOBO, Osaka, Japan), 9 μl of 2 × buffer, 4 μl of dNTPs, 0.4 μl each of the two primers (5 μM) and 3.6 μl of distilled water. The PCR conditions were as follows: an initial step of 2 min at 94 °C followed by 25 cycles of 10 s at 98 °C, 30 s at 55 °C for annealing and 60 s at 68 °C. The PCR products were purified using ExoSAP-IT (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and diluted by adding 180 μl of sterilised water.