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Influence of repeated prescribed burning on incorporation of ^{13}C from cellulose by forest soil fungi as determined by RNA stable isotope probing

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ABSTRACT

Repeated prescribed burning is frequently used as a forest management tool and can influence soil microbial diversity and activity. Soil fungi play key roles in carbon and nutrient cycling processes and soil fungal community structure has been shown to alter with increasing burning frequency. Such changes are accompanied by changes to soil carbon and nitrogen pools, however, we know little regarding how repeated prescribed burning alters functional diversity in soil fungal communities. We amended soil with ^{13}C-cellulose and used RNA stable isotope probing to investigate the effect of biennial repeated prescribed burning over a 34-year period on cellulolytic soil fungi. Results indicated that repeated burning altered fungal community structure. Moreover, fungal community structure and diversity in ^{12}C and ^{13}C fractions from the unburned soil were not significantly different from each other, while those from the biennial burned soils differed from each other. The data indicate that fewer active fungi in the biennially burned soil incorporated ^{13}C from the labelled cellulose and that repeated prescribed burning had a significant impact on the diversity of an important functional group of soil fungi (cellulolytic fungi) that are key drivers of forest soil decomposition and carbon cycling processes.

1. Introduction

Soil fungal communities are central to decomposition processes in forest ecosystems and constitute essential components of carbon and nutrient cycles (Dighton, 2003). Forest soil fungal communities are taxonomically diverse and their structure is known to be altered by environmental disturbance (e.g. Cairney and Meharg, 1999). One such disturbance is fire which, as a result of natural wildfire or prescribed burning, can strongly influence the edaphic environment and below-ground biota (Neary et al., 1999). Although relatively few investigations of fire effects on soil fungal communities have been undertaken, it is evident that fungal community structure can be strongly influenced by single fire events, and that such effects, along with their duration, appear to be site- and/or fire-specific (reviewed by Cairney and Bastias, 2007).

Prescribed burning is used widely in forest management in order to prepare sites for planting, control pests and/or reduce the impact of wildfires on forests and neighbouring urban areas (Neary et al., 1999; Fernandes and Botelho, 2003). The benefits of fire-based forest management, however, may last for only a few years, necessitating the application of repeated prescribed burning for on-going management (Fernandes and Botelho, 2003). Such repeated burning can reduce surface and soil organic matter content, and may influence nutrient, especially nitrogen, availability (Wright and Hart, 1997; Neary et al., 1999; Guinto et al., 2001; Mao et al., 2002).

Aside from an investigation of ectomycorrhizal fungi in North America (Tuininga and Dighton, 2004), information on how repeated burning influences forest soil fungi is limited, but recent work at a long-term repeated prescribed burning experiment in a south-eastern Australian native sclerophyll forest indicates that it may have significant ramifications for soil fungal communities. Thus, direct DNA extraction from soil, coupled with denaturing gradient gel electrophoresis (DGGE) and terminal restriction length fragment polymorphism (T-RFLP) analyses, has been used to demonstrate that repeated burning over a >30-yr period significantly alters the structure of soil fungal and soil basidiomycete communities and that the effect is greater with burning at 2- than

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4-yr intervals (Bastias et al., 2006a; Anderson et al., 2007). Analysis of mycelial communities of ectomycorrhizal fungi using similar methods revealed significantly altered community structure with burning at a frequency of 2-, but not 4-yr (Bastias et al., 2006b). Although clearly demonstrating that forest management, in the form of regular prescribed burning, over a prolonged period can alter the structure of soil fungal communities and significantly decrease soil C and N content (Bastias et al., 2006a), these observations provide no information on the likely functional significance of the changes to forest nutrient and carbon cycling processes.

A functional understanding of the consequences of long-term repeated burning for decomposition processes requires knowledge of how relevant functional groups of soil fungi are affected. Cellulose is a major component of plant biomass, and a broad taxonomic range of fungi contribute to the degradation of this important polymer in soil (Lynd et al., 2002). Methods for identifying cellulolytic fungi have previously relied upon cultivation-based isolation but stable isotope probing (SIP) (Radajewski et al., 2000; Manefield et al., 2002) offers a more direct means of investigation. For example, Haichar et al. (2007) recently utilised SIP to identify cellulolytic bacteria by analysing ^{13}C-labelled DNA following incubation of soil with ^{13}C-cellulose. Since rates of RNA synthesis are higher than for DNA, RNA-SIP has been proposed as a more sensitive approach than DNA-SIP (Manefield et al., 2002). This is particularly true for fungi due to their relatively low growth rate compared with prokaryotes. RNA-SIP therefore provides a means of both determining which fungi are cellulolytic and which cellulolytic fungi are influenced by environmental change. This study therefore involved amendment of soil from the long-term prescribed burning experiment at Peachester State forest with ^{13}C-cellulose and the use RNA-SIP in combination with DGGE to determine the effect of repeated burning on incorporation of ^{13}C from cellulose by forest soil fungi.

2. Materials and methods

2.1. Site description and soil sampling

The field site used in this study comprises *Eucalyptus pilularis* Smith-dominated native wet sclerophyll forest at Peachester State Forest, situated in the Sunshine Coast hinterland of Queensland, Australia (26°50′S, 152°53′E). Since 1972, a long-term prescribed burning experiment has been maintained at the site and comprises replicated plots (30 × 27 m) subjected to biennial burning (2-yr burn plots), quadrennial burning (4-yr burn plots) or no burning (unburned plots). Further details of the site and soil characteristics are provided by Guinto et al. (2001). Previous investigations indicated that soil fungal communities in the upper 10 cm of the soil profile differed significantly between 2-yr burn plots and unburned plots (Bastias et al., 2006a,b; Anderson et al., 2007) and the current work focused on these two treatments. Ten 2.5 cm diameter soil cores (0–10 cm soil depth) were collected from two replicate plots of each treatment from two experimental blocks (a total of four plots for each treatment). To minimise the effects of within-plot heterogeneity, the ten soil samples from each plot were pooled, resulting in four pooled samples per treatment, which were sieved (2 mm mesh size) to facilitate relatively uniform mixing of ^{13}C-cellulose throughout the soil.

2.2. Amendment of soil with ^{13}C-labelled cellulose

Soil samples (four per treatment) were adjusted to 55% of field capacity by the addition of sterile milli-Q H_2O. Thirty grams of each soil sample was placed in sterile plastic specimen jars without lids, before being individually placed into 1 L glass preserving jars, containing sterile moist paper towel and sealed. Soil samples were conditioned in the preserving jars for one week at room temperature. After the conditioning period, 2 g of soil was removed from each sample for nucleic acid extraction prior to the addition of 10 mg of ^{13}C-cellulose (98 atom% ^{13}C) (*Solanum tuberosum*) (IsoLife, Wageningen, The Netherlands) which was mixed throughout the soil sample. Samples were incubated in sealed preservation jars in the dark for 35 d at 22 °C. Jars were opened on a weekly basis to remove CO_2 and replenish O_2.

2.3. Nucleic acid extraction, RNA purification and ^{13}C analysis

Following incubation, 12 × 1.0 g sub-samples were collected from each soil sample for nucleic acid extraction using the method of Griffiths et al. (2000) and bead beating for 30 s at a speed of 5 m s^{-1} in a FastPrep™ (FP120) Thermos Savant bead beating system (Bio-101, Vista, California, USA). RNA was purified from each nucleic acid extract using the RNase-free DNase set (Qiagen, Doncaster, Australia) and the RNeasy MinElute Cleanup Kit (Qiagen) following the manufacturer's instructions. The twelve replicate extracts for each soil sample were then pooled and stored at −80 °C. One microgram of RNA per sample, cut with 61.6 μg of glucose, was freeze-dried in 6 × 4 mm tin cups (Elemental Microanalysis, Okehampton, UK) and submitted for ^{13}C analyses by Isotope Ratio Mass Spectrometry (IRMS). Isotope analysis was carried out using a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser with ANCA-NT Solid/Liquid Preparation Module (Europa Scientific, Crewe, UK). When operated in the dual isotope mode, δ^{13}C is measured along with %N and %C (Scrimgeour and Robinson, 2003). ^{13}C enrichment values were compared to the international standard Pee Dee Belemnite as indicated in Rangel-Castro et al. (2005).

2.4. Density gradient separation and fractionation of RNA

RNA concentration in each purified RNA extract was determined using a Nano Drop (ND-1000) Spectrophotometer (Labtech International, Ringmer, UK). ^{13}C-labelled and unlabelled RNA were separated by isopycnic ultracentrifugation using density gradient solutions of CsTFA (Amersham Biosciences, Little Chalfont, UK) and *ca* 500 ng of RNA per sample as described by Rangel-Castro et al. (2005). Fractionation using a Fraction Recovery system (Beckman Coulter, Palo Alto, CA, USA) yielded 10 fractions (each *ca* 85 μl), of which fractions 1 and 2 were discarded as these are likely to contain labelled molecules other than nucleic acids (Manefield et al., 2002; Rangel-Castro et al., 2005). RNA from the remaining eight fractions for each sample was precipitated with ice-cold isopropanol and then re-suspended in 20 μl of RNase-free water, before being stored at −80 °C.

2.5. cDNA synthesis and PCR amplification

RNA from each fraction (for each sample) was reverse transcribed using Superscript II Reverse Transcriptase (Invitrogen Life Technologies, Paisley, UK) and the reverse primer ITS4 (White et al., 1990) following the manufacturer's instructions, except that incubation was performed at 52 °C (Rangel-Castro et al., 2005). Reverse transcription reactions included 10 μl RNA template, 1 μl 10 pmol reverse primer and 1 μl 2.5 mM dNTP. Two negative controls [no template (water only) and template without RT enzyme] were conducted for all reactions. PCR amplification was conducted using the resulting cDNA and the primers ITS1-F (Gardes and Bruns, 1993) and ITS4. Reactions were performed using a Dyad DNA Engine thermal cycler (MJ Research, Waltham, MA) in 50 μl volumes as described by Bastias et al. (2006a). A nested PCR was conducted