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Effects of ecological restoration on soil microbial diversity in a temperate grassy woodland

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ABSTRACT

Soil microbial communities are often overlooked in the context of ecological restoration. Given their central role in a broad range of ecosystem processes, however, understanding their response to restoration activities is critical to predicting restoration trajectories. In this study, we quantified the response of soil bacterial and fungal communities to restoration treatments, variation in microhabitat elements and vegetation in a critically endangered Australian box-gum grassy woodland ecosystem. Restoration treatments included the addition of coarse woody debris (CWD) and reduced grazing pressure. Four years after applying restoration treatments, we found no significant effect of CWD addition on soil microbial diversity, while reduced grazing significantly affected composition of the fungal, but not the bacterial, communities. Both bacterial and fungal communities responded to microhabitat element (open ground vs. old logs and trees), overlying vegetation and soil edaphic properties, and strong aboveground-belowground linkages were observed. Plant alpha diversity was positively correlated to soil bacterial, but not fungal, alpha diversity and plant community composition was a good predictor of both soil bacterial and fungal beta diversity. Co-occurrence network analysis identified numerous complex, non-linear associations between soil bacteria, fungi, edaphic properties and overlying plants. Soil microbes affected by restoration treatments included fungal saprotrophs and Actinobacteria, likely involved in litter breakdown, as well as bacteria likely involved in soil N cycling. Although the directions of the observed plant-microbe relationships remain unclear, we demonstrated the possibility of inducing changes to soil microbial communities to enhance restoration outcomes in box-gum grassy woodland ecosystems.

1. Introduction

Soil microorganisms are key drivers of processes such as organic matter decomposition, inorganic nutrient cycling, pollutant degradation and disease (Bardgett et al., 2005; Swift et al., 1979). They play key roles in soil health, are involved in a range of symbiotic relationships, and are tightly linked to aboveground communities through trophic interactions, biogeochemical cycling and plant-soil feedbacks (e.g., Nielsen et al., 2015). As a result, soil perturbation may alter soil microbial abundance and activity, ecosystem processes and overall ecosystem performance (Bissett et al., 2013; Nielsen et al., 2015). Microbes thus represent a significant soil component, but have rarely been addressed in the context of ecological restoration (Callaham et al., 2008; Harris, 2009; Heneghan et al., 2008).

Temperate grassy woodlands are critically endangered ecosystems in Australia, and are subject to varying levels of ecological restoration management (Yates and Hobbs, 1997). Since European settlement, human-induced disturbances have led to a drastic reduction in the extent and ecological functioning of grassy eucalypt woodlands, with as little as 4% remaining (Prober et al., 2002a,b; Yates and Hobbs, 1997; Thomas et al., 2000). A range of processes, such as land clearing, livestock grazing and fertilizer addition, have resulted in changes to soil health and nutrient cycling, loss of understory vegetation, weed invasion, declining tree health, invasive exotic pest animals and decline or extinction of native fauna (McIntyre et al., 2014; Prober et al., 2002a; Yates and Hobbs, 1997).

In 2004, a long-term temperate woodland restoration experiment was established in south-eastern Australia to investigate ways of

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restoring their structure and function (the Mulligans Flat-Goorooyarroo Woodland Experiment; Manning et al., 2011; Shorthouse et al., 2012). In 2009, an 11.5 km predator-proof fence was constructed (the Mulligans Flat Woodland Sanctuary, MFWS) to allow the reintroduction of locally extinct fauna. In the restoration experiment, the following ecosystem manipulations were applied in a randomized, incomplete, block design: (i) addition of coarse woody debris (CWD), (ii) kangaroo exclusion (reduced grazing pressure), and (iii) fire (Manning et al., 2011). To date, research has shown that reduction in kangaroo grazing levels and the addition of CWD has benefited beetle and reptile diversity (Barton et al., 2011; Manning et al., 2013) by providing refuges, provision of food sources, protection from predators and shelter from changes in temperature and moisture, in particular in open areas where high kangaroo grazing occurred (Barton et al., 2011; Manning et al., 2013). Two years after addition of CWD to the woodland, localized increases in surface-soil fertility were found adjacent to CWD, demonstrating that CWD addition created sites for water infiltration and nutrient leaching (Goldin and Hutchinson, 2013, 2014).

In this study, we investigated the bacterial and fungal diversity of soil within the MFWS. Our first objective was to identify the effects of experimental restoration treatments (added CWD, reduced grazing pressure), variation in microhabitats (open ground vs. trees and logs fallen naturally from trees) and vegetation classes on (i) soil edaphic and vegetation properties and (ii) soil microbial diversity. Secondly, we aimed to elucidate drivers of soil bacterial and fungal diversity within the MFWS. Finally, we identified ecologically important connections between micro-organisms and overlying vegetation and investigated how environmental variables moderate these interactions and associated biogeochemical processes. Given the changes in soil edaphic properties with CWD addition (Goldin and Hutchinson, 2013, 2014), and likely changes resulting from reduced grazing pressure (expected increases in plant biomass and litter, for example), we anticipated that soil microbial communities in the MFWS would be altered by restoration treatments and that understanding of these microbial/above-belowground linkages will allow better prediction of restoration trajectories and management.

2. Materials and methods

2.1. Study site and sample collection

The study was conducted at the MFWS near Canberra, south-east Australia (35.191°S, 149.1817°E; Fig. 1). The MFWS covers 791 ha and comprises Yellow Box-Red Gum grassy woodland (*Eucalyptus melliodora*, *Eucalyptus blakelyi*), listed as a critically endangered ecological community in Australia (Department of Environment and Heritage, 2006). The reserve, along with the neighbouring Goorooyarroo Nature Reserve, is part of an established long-term woodland restoration project (Manning et al., 2011; Shorthouse et al., 2012). Briefly, MFWS was divided into 24 polygons, each containing four 1-ha sites (50 × 200 m). A kangaroo exclusion treatment was applied to half of the polygons to reduce grazing pressure. In October 2007, logs from *Eucalyptus* tree species were distributed across the 1-ha sites in the reserves in different CWD treatments including (a) control sites with no logs added, (b) 20 t ha^{-1} added with individual logs evenly dispersed, (c) 40 t ha^{-1} added with logs placed in both dispersed and clumped arrangements (Manning et al., 2011). Clumped arrangements aimed to mimic natural tree falls.

In 2011, 66 soil samples were collected from 18 sites within the MFWS (Fig. 1; Table S1). Six sites of each of the three CWD treatments were selected, and half of the selected sites were located within kangaroo exclusion fences. At every site, soil was collected from open ground, under a tree and adjacent to an old log, i.e., a log naturally fallen from trees. In sites where CWD was added, soil was also collected adjacent to a single dispersed log (20 t ha^{-1} treatment) or a clumped (40 t ha^{-1} treatment) arrangement of logs. GPS coordinates of each sample location were recorded. The sampling protocol was as follows: soil was taken at four points (approximately four points of a compass) as close as possible to each microhabitat element (i.e. open ground, under tree, adjacent to an old log, dispersed or clumped logs). A permanent peg was placed in each location, and a sampling frame was placed with the top left corner adjacent to the peg. Each sampling frame had five adjacent 156.25 cm^2 quadrats, and a single quadrat was randomly selected and used for sampling. First, all the vegetation attached to the soil surface (dead and alive) was cut from within the selected quadrat. Litter depth was determined using a tape measurement and all the surface litter removed. The above-ground plant material and surface litter were placed in separate bags, with all four sample points pooled, resulting in one sample (of 625 cm^2 area) of each per microhabitat element. Then, a soil core was collected within each quadrat using bulk density rings (60 mm depth, 50 mm internal diameter). The four soil samples were pooled in the field to give one soil sample per element.

2.2. Soil physicochemistry and ground layer vegetation

Soil edaphic properties (bulk density, pH, conductivity, ammonium-N, nitrate-N, total carbon, total nitrogen, resin P, moisture) were determined as follows: bulk density and moisture were determined using the approach of McKenzie et al. (2002). Five grams of field soil:25 ml DI water extract were used to determine soil pH (Method 4A1; Rayment and Higginson, 1992) and Electrical Conductivity (Method 3A1; Rayment and Higginson, 1992). Nitrate and ammonium were determined on field moist samples which were extracted on the day of sampling (1:5 2 M KCl), and analysed within 2 days colorimetrically on an Alpkem AutoAnalyser (Mulvaney, 1996). Soil plant available P was extracted using the Resin P method (Tiessen and Moir, 2007) and determined using the colorimetric molybdate-ascorbic acid method (Murphy and Riley, 1962). Total carbon and nitrogen were determined as described previously (Macdonald et al., 2015). Litter and above-ground vegetation were weighed and oven dried (60 °C) until a stable weight was reached, and the mass was recorded to represent the vegetation properties litter amount and above-ground plant biomass, respectively.

Sites were assigned to one of the four vegetation classes (densities) of the reserve (Manning et al., 2011; Table S1): high tree and high shrub cover (HTHS), high tree and low shrub cover (HTLS), low tree and high shrub cover (LTHS), or low tree and low shrub cover (LTLS). The ground layer vegetation within each site was surveyed as described previously (McIntyre et al., 2010, 2014). Briefly, all herbaceous species and woody plants (< 0.5 m height) were included and the BOTANAL method for estimating species abundance was used based on the top six abundant species in each of 30 vegetation quadrats per 1 ha site. This implies that species with < 0.2% of the biomass in a quadrat were not recorded in the survey and therefore plant species counts are relative, not absolute. We acknowledge that this might have resulted in exclusion of some rarer plant species from the dataset. However, species counts did reflect the plant diversity in the sites.

2.3. Soil microbial community analysis

Microbial DNA was isolated from each soil sample using the MO BIO Power Soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA). Bacterial and fungal communities were profiled by sequencing amplicons targeting the bacterial 16S rRNA gene (27F-519R, Lane, 1991; Lane et al., 1985) or fungal ITS region (ITS1F-ITS4, Gardes and Bruns, 1993; White et al., 1990), utilizing Roche 454 FLX titanium instruments and reagents at MR DNA (www.mrdnalab.com, Shallowater, TX, USA).

Flowgrams were trimmed and demultiplexed using MOTHUR (Schloss et al., 2009), allowing no mismatches to the primers and