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Soil C and N as causal factors of spatial variation in extracellular enzyme activity across grassland-woodland ecotones

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ABSTRACT

Soil enzymes are a ubiquitous component of ecosystem function and play a pivotal role in key biogeochemical processes including soil organic matter (SOM) decomposition and nutrient cycling. Enzyme activity has therefore been used as a surrogate for microbial activities and an indicator of soil productivity. Although numerous studies have assessed enzyme activity individually in different ecosystems, little is known about soil factors that determine their spatial structure and associated variation in activities across ecotones. Ecotones provide unique opportunities to study how soil properties vary spatially within and across adjacent land-uses. This study used multiple approaches, including geostatistical kriging, principal component analysis (PCA) and structural equation modelling (SEM) to examine the determinants and spatial distribution patterns of six key soil enzymes across two grassland-woodland ecotones in south-eastern Australia. Our results showed significant spatial dependencies for the activities of the enzymes across ecotones, and highlighted that spatial autocorrelation patterns were controlled by soil fertility factors regardless of enzyme type. Kriging maps revealed "decomposition hotspots" across ecotones with spatial co-occurrence of high enzyme activities. The spatial range of enzymes closely resembled soil fertility level as measured by a range of soil chemical properties, indicating a high degree of spatial co-dependency. Consistent with this, the SEM analysis indicated that soil resources such as C and N levels were the causal factors of variation in enzyme activities at field-scale. Soil pH showed little variation across the landscape at both sites and thus, had no major effect on enzyme activities. Overall, this study demonstrates spatial dependency and edaphic determinants of soil enzyme activities are consistent across grassland-woodland ecotones.

1. Introduction

Soil enzymes play a critical role in the environment through facilitation of the mineralization and hydrolysis of complex carbon (C), nitrogen (N) and phosphorus (P) compounds, thus mediating soil organic matter (SOM) decomposition (Burns et al., 2013). Depolymerisation of C compounds may involve cellulolytic (e.g. β 1,4-glucosidase EC 3.2.1.21 and cellobiohydrolase EC 3.2.1.91) or ligninolytic (e.g. phenol oxidase EC 1.10.3.2, lignin peroxidase EC 1.11.1.7) enzymes in soil (Saiya-Cork et al., 2002). On the other hand, decomposition of complex N or P compounds involves enzymes such as chitinase (EC 3.2.1.14) and phosphatase (EC 3.1.3.1), respectively. Depolymerised soluble substrates are then utilized by microbial communities involved in soil C, N and P cycling (Sinsabaugh et al., 2008). Soil enzymes are therefore commonly used as indicators of soil health and functionality of biotic communities (Burns et al., 2013). Identification of the causal factors that control the distribution patterns and activities of soil enzymes has thus been the focus of considerable research (Amador et al., 1997; Saiya-Cork et al., 2002; Šnajdr et al., 2008; Sinsabaugh et al., 2008).

Soil properties such as moisture (Baldrian et al., 2010), pH (Stursova and Sinsabaugh, 2008) and organic matter content (Allison and Vitousek, 2005; Sinsabaugh et al., 2008) have been shown to be major drivers of both microbial community structure and associated enzyme activities. However, soil represents a complex multidimensional environment and most soil properties exhibit non-random and characteristic heterogeneity (Banerjee et al., 2011; Goovaerts, 1998). Consequently, microbial enzyme activities may also vary in relation to the spatial distribution of soil properties across environments (Allison, 2005). Characterisation of the spatial dependence of soil enzymes can thus provide valuable insight, not only into distribution patterns but also the underlying drivers (Banerjee and Siciliano, 2012). Moreover, it is important to determine whether functionally different groups of enzymes (e.g. cellulolytic or ligninolytic) respond similarly to

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spatial variation in soil properties, and thus contribute to "decomposition hotspots" within ecosystems (Baldrian, 2014). Several studies have examined spatial variation in the activity of enzymes individually, both within and among different land-use systems such as cropland, grassland and forest (Baldrian and Větrovský, 2012; Baldrian, 2014; Baldrian et al., 2010; Šnajdr et al., 2008). However, information on the spatial distribution of soil enzymes across two land-use types (e.g. grassland to woodland) is limited. The area across two land-uses (i.e. ecotones) is particularly interesting because it may encompass biotic and abiotic interactions occurring between adjacent ecosystems and also incorporate aspects of the spatiotemporal characteristics of each ecosystem (Gosz, 1993). Spatial patterns of enzymes in grassland-woodland ecotones can reveal how their distribution changes between two adjoining land-uses.

A recent study has shown that the spatial structure of soil enzyme activities was associated with site-specific soil abiotic factors (Boeddinghaus et al., 2015). While the zone of spatial dependency (i.e. range) may vary with site-specific factors such as local topography and microclimatic conditions, it is also important to examine whether the distribution patterns and determinants of enzyme activities are consistent across sites by distinguishing causal relationships. Structural equation modelling (SEM) is one approach that can be used to delineate complex networks involving many response and predictor variables, to identify such causal relationships, and is thus widely used in soil ecological studies (Grace et al., 2010; Jonsson and Wardle 2010; Lamb et al., 2011; McLeod et al., 2015).

In this study, we employed a multilevel approach to examine spatial patterns of soil enzyme activities and other soil properties at two grassland-woodland ecotone sites. Our objective was to assess: i) whether the spatial patterns of soil physicochemical properties and enzymes were consistent both within land-use at each site and across the two sites, ii) what edaphic factors were the causal factors of variation in enzyme activities, and iii) whether the drivers and spatial patterns for individual enzymes were consistent at both sites. We hypothesized that soil physicochemical properties and enzyme activities operated at similar spatial scales at each site, and that the drivers of enzyme activities were consistent between both land-use types and across sites.

2. Materials and methods

2.1. Study sites and sampling design

The study was conducted at two sites with native woodland adjacent to grassland in south-eastern Australia. The first site was located at Bogo (34.813°S, 148.704°E) in the Bookham-Yass district of New South Wales. The native woodland at this site was dominated by *Eucalyptus* spp. with some *Acacia dealbata* and *Acacia implexa* (de Menezes et al., 2014; Prendergast-Miller et al., 2015). Patches of native Australian and exotic grasses were also common within the woodland. The adjacent grassland was previously used for sheep grazing but had not been grazed for at least 35 years. The grassland was dominated by a mix of native grasses, primarily *Austrodanthonia* sp., and *Themeda* sp., but also contained presence of exotic species incuding *Phalaris aquatica* (phalaris) with scattered subterranean clover (*Trifolium subterraneum*). The mean annual rainfall at the Bogo site is 632.5 mm with 7.2 °C and 20.7 °C minimum and maximum mean annual daily temperature (www.weatherzone.com.au, 2016). The second site was located within Namadgi National Park (35.666° S, 148.950° E) in the Australian Capital Territory. This native woodland was similar to Bogo in terms of species composition. The adjacent grassland was dominated by native grasses only and had no history of grazing by domestic animals. The mean annual rainfall at Namadgi National Park is 777.3 mm with 4.4 °C and 17.3 °C minimum and maximum mean annual daily temperature ().

The soils at both sites were dominated by Brown Sodosols, which are typically fine sandy clay loam with 10–20% clay content (Isbell, 2002). At each site, a 50 m length × 20 m width sampling plot was established across woodland and grassland (i.e., extending 25 m into both the woodland and grassland; Fig. S1). A grid design was set up in each plot to sample soils. Each grid consisted of 55 nodes, with adjacent nodes separated by a linear or perpendicular distance of 5m. Soil samples from both sites were collected in September 2013 by sampling precisely at each node. Samples consisted of a composite of 10 individual soil cores (4 cm diameter) collected at 0–10 cm depth within a 10 cm radius of each sampling node. The corer was cleaned between sampling at each node and soil samples were placed on ice for transfer to the laboratory. For processing, plant material was first removed and each sample was homogenised and passed through a 2 mm sieve. Samples were processed and sub-sampled on the same day as collection.

2.2. Analyses of soil properties

Gravimetric soil moisture content was measured by oven-drying a 10 g sample of fresh soil at 105 °C to constant weight. Soil pH was determined on a 1:10 (w/v) ratio in distilled water using a pH meter (Denver Instruments). Dissolved organic carbon (DOC), dissolved organic nitrogen (DON), ammonium (NH_4^+), nitrate (NO_3^-) were analysed after extracting moist soils with 0.5 M K_2SO_4 at 1:5 (w/v) ratio (Weintraub et al., 2007). Concentrations of NH_4^+ and NO_3^- were determined colorimetrically on a microplate reader (SynergyMX, BioTek; Winooski, VT, USA). Dissolved organic carbon and DON were estimated using a Thermalox TOC/TN analyser (Analytical Sciences, Cambridge, UK). DON was determined by subtracting mineral nitrogen concentrations from total dissolved nitrogen. Percentage of total carbon (%C) and nitrogen (%N) were determined by LECO CN-2000 dry combustion analyser (LECO Corporation, St Joseph, MI, USA) using finely ground air-dried soil samples. Total, inorganic and organic P were measured by the ignition–extraction procedure (Olsen and Sommers, 1982).

2.3. Analysis of soil extracellular enzymes

Activities of extracellular enzymes in soil were conducted according to Saiya-Cork et al. (2002) and Sinsabaugh et al. (2003). Enzyme assays were performed within 24 h of sample collection using moist soil and substrate reagents from Sigma–Aldrich (Castle Hill, NSW, Australia). Assays for acid phosphatase, chitinase (β-*N*-acetylglucosaminidase), β-1,4-glucosidase and cellobiohydrolase were conducted using 4-methylumbelliferyl (MUB) phosphate, MUB-β-glucoside and MUB-cellobioside, respectively, whereas l-3,4-dihydroxyphenylalanine (DOPA) was used for phenol-oxidase and peroxidase assays. Sample suspensions containing 1 g soil in 125 ml acetate buffer (50 mM, pH 5) were prepared by homogenizing for 1 min in a high speed blender. Suspensions were continuously agitated before dispensing into 96-well microplates. Assays were conducted in quadruplet and each microplate contained controls for soil blanks, reagent blanks and quench controls. Plates were incubated in the dark at 25 °C for 4 h for acid phosphatase, chitinase and glucosidase or 16 h for cellobiohydrolase. A 10 μl aliquot of 1.0 M NaOH was added to each well to stop the reaction and fluorescence was determined using a microplate fluorometer (Victor 2, Multifunction plate reader, PerkinElmer, USA) at 365 nm excitation and 450 nm emission. Values were corrected for controls and quenching, and enzyme activities were expressed in units of nmol h^{-1} g^{-1}. Microplates for peroxidase and phenol oxidase activities were