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Shifts in prokaryotic communities under forest and grassland within a tropical mosaic landscape

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ABSTRACT

The Anthropocene is linked to massive land use changes as a result of human activity. While aboveground changes in biodiversity are well documented, the effects on belowground microbial communities are less understood, yet could impact on many ecosystem functions. Here we aimed to identify differences in belowground microbial diversity between forest and grassland sites in a humid tropical mosaic landscape in Papua New Guinea. Using DNA-based amplicon sequencing targeting the 16S rRNA gene, prokaryotic community composition was assessed from surface soil samples. The composition of prokaryotic communities (beta diversity) differed between forest and grassland sites despite maintaining similar richness (alpha diversity) levels. Changes in community structure were small at higher taxonomic levels, but strong at the operational taxonomic unit (OTU) level but for a small subset of taxa. Changes in community composition between sites (based on Bray-Curtis distance) reflected a large rearrangement with species assemblage (OTU) differing by 68%. The results suggest that ecosystem change in this landscape leads to ecological filtering and selection at lower, but not higher taxonomic levels.

1. Introduction

The dynamics of tropical forests, grasslands and their intergrades are extremely important globally for biodiversity and biogeochemical processes. The extent and nature of these vegetation types is changing rapidly due to logging, forest clearing for agriculture or grazing, changes in fire regimes, and climate change (Murphy and Bowman, 2012). While the consequences of these changes for aboveground ecology and ecosystem services are widely appreciated, much less is known about the consequences for below-ground soil biodiversity and functions. Tropical forests and grasslands differ in their net primary productivity and also tend to differ in their soil physicochemical characteristics (Lloyd et al., 2015; Veenendaal et al., 2015) and soil organic matter dynamics (Saiz et al., 2015; Wynn and Bird, 2007). Therefore, we might expect the diversity and functions of soil microbial communities to differ between tropical forest and grassland land uses.

Soil microbial community structure is known to differ between forests and grasslands across diverse climates (Fierer and Jackson, 2006; Kaiser et al., 2016; Martiny et al., 2016; Öpik et al., 2006). The difference is related to the vegetation itself (Öpik et al., 2006) but also to soil abiotic characteristics, especially pH (Fierer and Jackson, 2006; Kaiser et al., 2016). The important influence of soil abiotic influences on microbial community structure has been demonstrated in numerous studies (e.g. Kaminsky et al., 2017; Tedersoo et al., 2014; Wakelin et al., 2016, 2008), with the effect of pH being particularly important for bacteria (Rousk et al., 2010). This relationship between microbial communities, land use and the soil environment has allowed for microorganisms to be exploited as potential indicators of soil conditions (Delgado-Baquerizo et al., 2016a; Hermans et al., 2016). Further, recent work suggests that any loss in soil biodiversity related to vegetation change is likely to have a negative impact on the ecosystem services provided by soil (Coleman and Whitman, 2005; Delgado-Baquerizo et al., 2016b; Van Der Heijden et al., 2008).

Despite the importance of microorganisms, most studies to date rely on high level observations focusing on either changes in richness/diversity or relative taxon abundances. The latter is normally presented at taxonomic levels (e.g. phylum or class) comprised of hundreds or thousands of individual species. This low level of taxonomic resolution hinders our ability to infer ecological roles since functional trait conservation can vary significantly across taxonomic ranks (Martiny et al.,

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2016; Wakelin et al., 2017). High resolution (species level or lower) classification of organisms linked to specific habitats could allow identification of keystone or marker species crucial to understanding ecosystem responses to rapidly changing aboveground conditions.

In this work we set out to compare soil microbial community structures between forest and grassland in a humid tropical environment in Papua New Guinea. A paired sampling approach was followed, taking samples from grassland and forest patches at 9 sites across a fertile coastal plain. The grassland patches are maintained by fire and the forest patches are used for shifting agriculture. These land use practices have been in place for generations, and possibly for thousands of years, as the broader region has been settled for over 30,000 years (Summerhayes et al., 2016). However, population growth and commercial agriculture are driving increasingly rapid change in the area, as elsewhere in the tropics.

2. Materials and methods

2.1. Site description and sample collection

The study site was on the coastal plain of Oro Province, Papua New Guinea (Table 1 and Fig. A1). The study area is a mosaic of grassland and forest, with a history of shifting agriculture and maintenance of grasslands by burning. The soils are vitrands formed in alluvially redeposited tephra. Annual rainfall is approximately 2380 mm, with a wet season in October–May and a dry season in June–September. In 2010, soil samples were taken from 9 paired sites, each having grassland and nearby forest. The sampling area spanned approximately 85 km^2, and the average distance between sample locations was approximately 7.5 km. A schematic of the site and sampling locations have been provided elsewhere (see Fig. 1 in Wakelin et al. (2016)). Within each pair, the forest and grassland plots were 6–480 m apart, except for Site 2, in which they were 1715 m apart. Within each vegetation type the replicate sampling plots were 5–20 m apart and > 10 m from the edge.

The forest sites had large trees at the time of sampling, but they had most likely been logged or cleared for food gardens in the past. The most common tree genera were *Alstonia, Canarium, Instia, Anisoptera, Gnetum, Artocarpus, Ficus* and *Phaleria*. The grasslands were dominated by *Imperata cylindrica* and *Saccharum* spp. The forest and grassland had been in place at the sampling sites for at least 57 years prior to sampling (Goodrick et al., 2014).

Table 1
Location of sites and field description of texture and colour of topsoil (> 15 cm thick in all cases) and subsoil (horizon at 1 m depth). S = sand, LS = loamy sand, SL = sandy loam, SCL = sandy clay loam. Colour (Munsell notation) was recorded in the moist state.

Site	Lat.	Long.	Grassland		Forest	
no.	(°S)	(°E)	Topsoil	Subsoil	Topsoil	Subsoil
1	8.79	148.39	SL, 10YR2/1	S, 10YR4/2	SCL, 10YR4/2	SCL, 10YR4/2
3	8.79	148.42	LS, 10YR2/1	S, 2.5YR5/1	LS, 10YR2/1	LS, 10YR5/3
4	8.81	148.36	LS, 10YR2/1	SL, 10YR5/6	SL, 10YR3/1	SCL, 10YR5/2
5	8.76	148.41	LS, 10YR2/1	S, 2.5YR6/1	SCL, 10YR5/3	SCL, 10YR5/1
6	8.74	148.37	LS, 10YR2/1	S, 2.5YR5/1	SL, 10YR3/3	SL, 2.5YR5/2
7	8.78	148.36	LS, 10YR3/1	S, 10YR3/2	SCL, 10YR5/1	S, 2.5YR4/1
8	8.73	148.33	SL, 10YR2/1	S, 10YR4/3	LS, 10YR2/2	S, 10YR4/4
9	8.76	148.32	SL, 10YR2/1	Gravel	SL, 10YR2/1	S, 10YR4/3
11	8.82	148.29	SL, 10YR2/1	S, 10YR5/1	S, 10YR2/1	Boulders

Soil samples (0–0.05 m depth) were collected in May–June 2010. At each site, and under each vegetation type, four samples were collected within an area of approximately 25 m diameter. Samples from the 4 locations were combined into one sample for each site and vegetation type. Where there was a significant litter layer, the soil surface was identified as the depth where plant litter fragments were < 10 mm in size. The samples were air-dried immediately after sampling and kept air-dry until analysis. It took approximately two days for the samples to reach an air-dry state. Bulk density was analysed in PNG and chemical properties in Australia, following sterilisation by gamma irradiation (50 kGy) to satisfy quarantine requirements. All samples were analysed for total C and N content, $\delta^{13}C$ and $\delta^{15}N$ using a Costech elemental analyser coupled to a Delta-Vplus stable-isotope ratio mass spectrometer. The soils contain no carbonate, so total carbon content was assumed to equal total organic carbon content. Samples were analysed for pH, electrical conductivity (EC), exchangeable cations and Colwell P using methods described by Rayment and Lyons (2011). Soil pH and electrical conductivity (EC) were measured in a 1:5 soil: water suspension. Exchangeable cations (Al, Ca, K, Mg and Na) were extracted using 0.01 m silver thiourea and analysed by inductively coupled plasma optical emission spectroscopy. Effective cation exchange capacity (ECEC) was calculated as the sum of exchangeable cations. Colwell P was extracted using 0.5 M sodium bicarbonate and analysed colorimetrically. The effects of vegetation on soil properties was analysed by a paired *t*-test.

For DNA extraction and analysis, subsamples were sent from PNG to AgResearch, New Zealand (MAF permit 2010040417). Soil DNA was extracted from duplicate sub-samples of each sample. Conducting the extractions in duplicate was not intended to reduce variability within each sample (soil samples were already mixed thoroughly) but rather to increase the total yield of DNA. Extractions were made from 0.25 g of soil using the PowerSoil extraction kit (MoBio Inc.), eluted into 50 ml of TE buffer, and the duplicate extractions for each soil sample combined. The final concentration of DNA in the samples was measured by spectroscopy (NanoDrop; ThermoFisher Inc.) and normalised across samples to 10 ng/uL prior to further processing..

2.2. Sequencing and community data processing

Barcoded amplicons were generated using primers 515F (5′-NNNNNNNGTGTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) targeting the V4 region of the 16S rRNA gene (Liu et al., 2007) as per the Earth Microbiome Project protocol (http://www.earthmicrobiome.org/emp-standard-protocols/16S/)(Caporaso et al., 2012). All barcoded samples were loaded onto a single Illumina MiSeq 2 × 151 bp run (Illumina, Inc., CA, USA). Sequences were deposited at the Sequence Read Archive (NCBI) under the BioProject ID: PRJNA374589.

Illumina output was pre-processed using a QIIME 1.9.0 open-reference operational taxonomic unit -picking workflow (Caporaso et al., 2010) (OTU = operational taxonomic unit; i.e. groups of individuals related at a defined percentage of sequence similarity). The default parameters included: minimum read length of 75 bp, min number of consecutive high quality base calls to *include* a read as a fraction of the input read length of 0.75, Phred quality score of 3, no ambiguous bases allowed, and no mismatches allowed in primer sequence (Bokulich et al., 2012). Sequences kept for analysis were all 151 bp in length. Raw sequences were demultiplexed and only forward reads were analyzed. This was done to increase the sequence depth analyzed per sample and speed of analysis, and has been shown to produce comparable results (Werner et al., 2012) to paired end data.

We used the QIIME pick_open_reference_otus.py command. Reads were clustered into OTUs (97% similarity) against the SILVA database release 119 (Quast et al., 2012) using UCLUST (Edgar, 2010), and reads not matching the reference database were clustered de novo. Taxonomic assignments were established using BLAST (Altschul et al., 1990)