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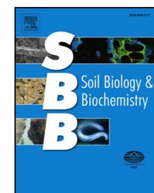

A comparison of the ability of PLFA and 16S rRNA gene metabarcoding to resolve soil community change and predict ecosystem functions

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ABSTRACT

Soil bacterial community structure has traditionally been measured using phospholipid fatty acid (PLFA) profiling. However, with the development of high-throughput sequencing technologies and metabarcoding techniques, more studies are now using 16S rRNA gene metabarcoding to measure bacterial community structure. Metabarcoding provides a much greater level of detail than PLFA profiling does, but it remains unclear whether or not the two techniques have a similar ability to answer many of the common questions asked by ecologists. We test the relative ability of the two techniques to quantify differences in bacterial community structure among five land uses (natural and planted forest, unimproved and improved grasslands, and vineyards), and to predict ecosystem functions. We also test whether PLFA- and metabarcoding-based metrics indicative of microbial growth strategies are correlated to each other. We show that both techniques showed broadly similar patterns of bacterial community composition change with land use and a remarkably similar ability to predict a wide range of ecosystem functions (carbon and nutrient cycling, and responses to drought). However, they were also complementary, as each showed different strengths in discriminating land uses and predicting ecosystem functions. PLFA metrics (i.e. the gram-positive:gram-negative ratio and fungal:bacterial ratio) were strongly correlated with the equivalent 16S rRNA gene metabarcoding metrics (i.e. the gram-positive:gram-negative and oligotrophic:copiotrophic ratios), although PLFA metrics were less well correlated with the Proteobacteria:Acidobacteria ratio. For many ecological questions the two techniques thus give broadly comparable results, providing confidence in the ability of both techniques to quantify meaningful changes in bacterial communities.

1. Introduction

Soil bacterial community structure is responsive to a myriad of factors, including management practices and global change (Clegg et al., 2003; Evans and Wallenstein, 2014; Fierer et al., 2012; Francisco et al., 2016), and has been linked to ecosystem functioning (Fierer et al., 2012; Orwin et al., 2016; Whitaker et al., 2014). Soil bacterial community structure has traditionally been measured using phospholipid fatty acid (PLFA) profiling, which gives a broad-scale, quantitative overview of the living bacterial community (Willers et al., 2015). However, the development of next-generation sequencing technologies and extensive reference databases for the V3-V4 region of the microbial 16S rRNA gene have allowed information about soil bacterial communities to be obtained at a much finer taxonomic resolution (Smets et al., 2016). Although 16S rRNA gene metabarcoding has a clear advantage where the focus of the study is on the details of bacterial ecology, the relative ability of PLFA profiling and 16S rRNA gene metabarcoding techniques to answer many of the broader questions often asked by ecosystem ecologists, and whether results can be directly compared, is largely untested. Such knowledge would help integrate the wealth of information provided by PLFA profiling-based studies with that gained from metabarcoding approaches.

One key question often asked by ecologists is how communities change in response to a given driver, with community data either analysed using multivariate tests or by calculating metrics to describe the ways in which the community has changed. A previous study showed that PLFA profiling was as sensitive as – and sometimes more sensitive than – DNA fingerprinting techniques for indicating changes in community structure (Ramsey et al., 2006). However, there are few tests of whether the greater detail available from 16S rRNA gene metabarcoding improves sensitivity (but see Cesarz et al., 2013; Chodak et al., 2013; Docherty et al., 2015; Duncan et al., 2016). One major metric used in both PLFA profiling and metabarcoding studies is estimated diversity. Diversity of bacterial PLFAs is generally a function of

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Table 1
Description of metrics based on PLFA or 16S rRNA gene results.

Ratio	Ecological interpretation	Expected relationship to other ratios	Reference
Fungal: bacterial	Fungi are associated with soils with low decomposition rates and nutrient availability, whereas bacteria are associated with soils with high decomposition rates and nutrient availability. An increase in this ratio should therefore reflect reduced nutrient availability and slower growth rates.	*Negative with:* gram-positive:gram-negative Proteobacteria:Acidobacteria *Positive with:* Oligotrophic:copiotrophic	Wardle et al., 2004
Gram-positive:gram-negative ratio	Gram-positive bacteria may typically use more recalcitrant substrates than gram-negative bacteria, but require high available N concentrations to do so. They are also thought to be more drought tolerant. An increase in this ratio may therefore reflect reduced moisture or increased N availability.	*Negative with:* fungal:bacterial oligotrophic:copiotrophic *Positive with:* Proteobacteria:Acidobacteria	Treseder et al., 2011; Yuste et al., 2014
Oligotrophic:copiotrophic ratio	Oligotrophs use more recalcitrant C, and have a lower nutrient requirement and a slower potential growth rate than copiotrophs. A high ratio value should therefore reflect lower resource availability and slower growth rates.	*Negative with:* gram-positive:gram-negative Proteobacteria:Acidobacteria *Positive with:* fungal:bacterial	Fierer et al., 2007; Collins et al., 2016
Proteobacteria:Acidobacteria ratio	High values associated with soils with a high nutrient status	*Negative with:* fungal:bacterial oligotrophic:copiotrophic *Positive with:* gram-positive:gram-negative	Smit et al., 2001

evenness as most PLFA markers are present in all samples, whereas diversity in metabarcoding reflects both evenness and taxon richness. Other metrics are more commonly used in PLFA profiling than in metabarcoding studies. For example, PLFA-based estimates of the gram-positive:gram-negative bacterial and fungal:bacterial ratios have been linked to community growth strategies (de Vries and Shade, 2013; Treseder et al., 2011; Wardle et al., 2004), and so allow an ecological interpretation of why communities have changed in a particular way (Table 1). A similar approach may help interpret results from 16S rRNA gene metabarcoding data. Three possible metrics currently exist: the Proteobacteria:Acidobacteria ratio, which is thought to reflect the nutrient status of the soil (Smit et al., 2001); the oligotrophic:copiotrophic ratio, which is based on the relative abundance of specific taxa at the phylum and class level thought to belong to each group (Collins et al., 2016; Table 1); and the gram-positive:gram-negative ratio (Treseder et al., 2011; Yuste et al., 2014; Table 1). Although gram staining does not appear to be strongly related to phylogeny, cell envelope type (i.e. monoderm vs diderm), which the gram stain largely reflects, is relatively distinct between phyla (Sutcliffe, 2010; Gupta, 2011). A gram-positive:gram-negative ratio based on cell envelope type, or the relative abundance of each of these groups, could therefore be useful metrics to use. A strong correlation between metrics based on PLFA profiling and 16S rRNA gene metabarcoding would help validate their calculation and allow easier comparison between studies based on different techniques. However, such correlations have not been explored previously.

Ecologists are also increasingly interested in the role that bacterial communities play in driving ecosystem functioning (e.g. Grigulis et al., 2013; Orwin et al., 2016; Whitaker et al., 2014). Although metrics based on both 16S rRNA gene metabarcoding and PLFA profiling have been linked to ecosystem functions such as respiration (Collins et al., 2016; Whitaker et al., 2014), nitrogen cycling (Philippot et al., 2013), and responses to disturbance (Orwin et al., 2016), no study that we are aware of has compared their relative ability to explain variation in functioning. Thus it is currently unknown how well PLFA profiling- and metabarcoding-based metrics correlate with each other, and what level of taxonomic resolution is required to detect community change and predict ecosystem functions. Here we use a broad land-use gradient to assess the commonalities and differences between PLFA profiling and 16S rRNA gene metabarcoding results. Specifically, we (i) test which technique is more sensitive to change in bacterial community composition across land uses, (ii) test whether similar metrics calculated from PLFA profiling and 16S rRNA gene metabarcoding data are correlated with each other, and (iii) examine the relative ability of each technique to predict a range of ecosystem functions (carbon and nitrogen cycling, and responses to disturbance).

2. Materials and methods

2.1. Field site

This study utilises data from two previously published studies (Orwin et al., 2016; Wood et al., in press) where both PLFA profiling and 16S rRNA gene metabarcoding techniques were applied to the same soil samples. Samples were collected from sites located in the Wairau River catchment in Marlborough, New Zealand (41°30′S, 173°50′E). Six 20 × 20-m plots were sampled in each of five land-use types: natural forest (dominated by *Lophozonia menziesii* (Hook.f.) Heenan et Smissen and *Fuscospora* sp.); planted forest (exotic *Pinus radiata* D. Don and *Pseudotsuga menziesii* (Mirb.) Franco); unimproved grassland (typically extensively managed sheep farms), improved grassland (intensively managed with irrigation, cultivation and fertilisation), and vineyards. This set of land uses encompasses both strongly different (i.e. forest vs grassland) and more similar land uses (i.e. there is strong overlap between improved grasslands and vineyards in management inputs and plant community composition). In order to sample all five land uses evenly and to minimise differences in soil type, plots were selected using a stratified random sample from points obtained by placing a 4 × 4-km grid with a random origin across the catchment. Twenty-four soil samples were taken to 15 cm depth at evenly spaced predetermined locations in each plot using a 4.75-cm diameter corer (AMS Inc. Idaho, USA) after removing the litter layer, and bulked. Further details are given in Orwin et al. (2016), and Wood et al. (in press). Soils were sieved, and subsamples stored at 4 °C for function measurements, freeze dried for PLFA analysis, or stored at −80 °C for molecular analysis.

2.2. PLFA analyses

PLFAs were extracted using the methods described by Bardgett et al. (1996), which are based on those of Bligh and Dyer (1959). Bacterial PLFAs were defined as those associated with gram-positive bacteria (i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0), gram-negative bacteria (cy-17:0, cy-19:0, 16:1ω7c, and 18:1ω7c; (Waldrop and Firestone, 2004; Zelles, 1999), and the general bacterial marker 15:0 (Bardgett et al., 1996; Orwin et al., 2016). Bacterial PLFAs were used to calculate Simpson's evenness (E) and Shannon diversity (H′), hereafter termed ‘PLFA evenness’ and ‘diversity’ respectively. Two metrics were

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