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Bacterial community structure of contrasting soils underlying Bornean rain forests: Inferences from microarray and next-generation sequencing methods

Sabrina E. Russo [a,*], Ryan Legge [b], Karrie A. Weber [a,c], Eoin L. Brodie [d], Katherine C. Goldfarb [d], Andrew K. Benson [b], Sylvester Tan [e]

[a] School of Biological Sciences, Manter Hall, University of Nebraska-Lincoln, Lincoln, NE 68588-0118, USA
[b] Food Science and Technology Department, University of Nebraska-Lincoln, NE, USA
[c] Department of Earth and Atmospheric Sciences, University of Nebraska-Lincoln, NE, USA
[d] Ecology Department, Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA
[e] CTFS-AA Asia Program, Center for Tropical Forest Science – Arnold Arboretum of Harvard University, Cambridge, MA, USA

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ABSTRACT

Soil microbial diversity is vast, and we lack even basic understanding of how this diversity is distributed ecologically. Using pyrosequencing and microarray methods, we quantified the structure of bacterial communities in two contrasting soils underlying Bornean rain forest (clay and sandy loam) that differ markedly in soil properties, aboveground tree flora, and leaf litter decomposition rates. We found significant soil-related taxonomic and phylogenetic differences between communities that, due to their proximity, are independent of climate. Bacterial communities showed distinct compositional and taxon-abundance distributions that were significantly correlated with the structure of the overlying tree community. Richness of bacteria was greater in the more resource-rich clay soil. Phylogenetic community analyses suggested that environmental filtering may be an important mechanism of community assembly in clay, compared to niche-competition in sandy loam. The Acidobacteria were the most abundant group in clay, but the Proteobacteria dominated in sandy loam. Of the ten most abundant classes, the Actinobacteria, Betaproteobacteria, Clostridia, Bacilli, and Gammaproteobacteria were more abundant in sandy loam than clay. Our study, which is the first to quantify edaphic variation in bacterial communities using high-throughput methods in soils underlying one of the most tree species rich forests on Earth, indicates an important role of plant–soil feedbacks linking the community structure of the trees and the underlying soil microbiome. We suggest the biochemical composition of carbon and nutrient resources in plant litter and soil pH and oxygen availability as important determinants of the distribution of bacterial diversity.

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1. Introduction

Microbial diversity in soils is vast: a recent estimate suggests that a ton of soil may harbor at least one million species (Curtis et al., 2002). Of these, the microorganisms that decompose organic litter are a crucial component of global biogeochemical cycles (Burgin et al., 2011; van der Heijden et al., 2008), breaking down complex organic carbon and releasing CO_2 to the atmosphere via cellular respiration and making nutrients available to support primary production (Bardgett et al., 2009, 2008; Dubinsky et al., 2010; Nielsen et al., 2011; Reynolds et al., 2003; Zak et al., 2003). Unlike higher organisms, broad surveys of microbial diversity, including Bacteria, Fungi, and Archaea, have historically been technologically infeasible, especially in soils, but recently developed molecular and genomic tools now open a window on the diversity and structure of these communities (Caporaso et al., 2011; Fierer et al., 2007b).

Nowhere are soil microbial communities likely to be more complex than under tropical rain forests, which house the majority of plant diversity on Earth (Dirzo and Raven, 2003). However, the factors that control the structure and composition of soil microbial communities are not well understood (Fierer and Lennon, 2011), and there is some evidence that well-established patterns of plant diversity are not necessarily predictive of those for microorganisms (Bryant et al., 2008; Fierer et al., 2011; Green et al., 2004), despite their obvious trophic linkages (Zak et al., 2003). In many plant communities, litter principally defines the resources available to decomposer microorganisms in and above the soil (Hobbie, 1992; Waldrop et al., 2006; Wieder et al., 2008). Tropical rain forests

* Corresponding author. Tel.: +1 402 472 8387; fax: +1 402 472 2083.
E-mail address: srusso2@unl.edu (S.E. Russo).

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contribute some of the highest levels of primary production on the planet (Lewis et al., 2009; Sitch et al., 2008), much of which ultimately falls as leaf litter. The amount and diversity of leaf litter and its quality can have strong impacts on microbial community composition and function (Chapman and Newman, 2010; Nemergut et al., 2010). Leaf litter from different plant species can present dramatically different growth substrates for microorganisms (Strickland et al., 2009; Ushio et al., 2008; Wardle et al., 2009; Wu et al., 2011), due to variation in plant species' functional traits, such as leaf litter carbon and nutrient concentrations (Cornwell et al., 2008; De Deyn et al., 2008). As a result, soil microbial community composition often associates with the composition of the plants in the overlying vegetation (Slabbert et al., 2010; Yarwood et al., 2010). Abiotic environmental properties can also sharply define the composition and function of soil microbial communities. Factors such as soil temperature (Fierer et al., 2005), moisture (Hollister et al., 2010), pH (Rousk et al., 2010), nutrient availability (Cleveland et al., 2004; Cusack et al., 2011), and redox potential (DeAngelis et al., 2010) are recognized as important determinants of microbial growth and survival, and as such, can influence the structure of soil microbial communities. Despite recent advances revealing the incredible diversity of microorganisms belowground, even basic ecological identification and interpretation of these patterns, such as the associations between soil microbial community composition and the characteristics of the plant communities that these soils support, remains rudimentary at best (Fierer et al., 2007a).

As a first step towards understanding the biotic and abiotic factors that contribute to variation in microbial communities in tropical soils, we quantified the structure and composition of bacterial communities in two contrasting soils underlying Bornean rain forest. These soils share similar amounts of litterfall, but differ markedly in their rates of leaf litter decomposition (Baillie et al., 2006; Palmiotto, 1998), and represent the extremes of an edaphic gradient that strongly influences tree species composition (Davies et al., 2005). One of the soils is sandstone-derived, nutrient-depleted, and well-drained (sandy loam), and the other is shale-derived, less nutrient-depleted, and less well-drained (clay) (Table 1) (Baillie et al., 2006; Tan et al., 2009). The half-life of aggregated leaf litter on the sandy loam soil is three times longer than for litter on clay (Palmiotto, 1998). The slower rates of decomposition on sandy loam cause standing forest-floor necromass to be threefold larger than on clay (Baillie et al., 2006), resulting in higher total soil carbon (Table 1).

In addition to their disparate soil properties, the variation in decomposition rates of these soils may be due to differences in the leaf litter resources available, resulting in selection of distinct microbial communities. Supporting this hypothesis are the findings that the sandy loam and clay soils support dramatically different assemblages of tree species (Davies et al., 2005), resulting in large shifts in their leaf functional traits that are relevant for leaf litter decomposition (Russo et al., 2010; S.E. Russo, unpub. data). Relative to tree species typical of clay, fresh leaves of sandy loam specialists are significantly tougher and thicker, likely due to their total carbon contents and higher cellulose and lignin contents per unit area (S.E. Russo, unpub. data). Leaves of sandy loam specialists also have lower concentrations of N and P and greater C:N and C:P ratios (S.E. Russo, unpub. data). These differences are also likely to characterize recently fallen leaf litter, as fresh leaf and leaf litter traits are strongly correlated at this site (Kurokawa and Nakashizuka, 2008), and aggregated leaf litter on sandy loam has significantly lower concentrations of every nutrient examined (Table 1).

As a result of the strong variation between clay and sandy loam soils in the abiotic environment and the consequent effects on leaf litter resources available for microbial colonists, we predicted that soil bacterial communities would show covariation with soil type and, ultimately, the effects of soil type on vegetation, between the clay and sandy loam soils. Given the between-soil differences in carbon and nutrient resources, we anticipated the variation to reflect the copiotrophic–oligotrophic resource-use spectrum proposed by Fierer et al. (2007a), in which bacterial strategies exist on a continuum from copiotrophic bacteria that are abundant in nutrient-rich environments with high carbon availability to oligotrophic bacteria that are abundant in environments with low carbon availability. This would translate into communities dominated by Proteobacteria and Bacteriodetes in the more resource-rich conditions in the clay soil, compared to communities dominated by Acidobacteria in the sandy loam. Here, we explore these hypotheses by examining the community structures of sandy loam and clay soils using both taxonomic and phylogenetic analyses of 16S rRNA by pyrosequencing and microarray-based approaches. Our results show clear associations of soil bacterial community structure with soil type and the overlying tree community.

Table 1
Properties of surface soil (0–10 cm) and leaf litterfall and decomposition for two soil types underlying Bornean rain forest. Significant differences among soil types are indicated by different lowercase letters, with standard errors following the means, when available. Abbreviations are as follows: CEC, cation exchange capacity and BS, base saturation.

Property	Soil type		
	Sandy loam	Clay	Source
Soil			
pH	4.64 ± 0.01 a	4.43 ± 0.04 b	Davies et al. (2005)
Bulk density	0.83 ± 0.04 a	0.95 ± 0.02 b	Palmiotto (1998)
CEC (cmol+/kg)	7.61	7.21	Baillie et al. (2006)
BS (%)	7.0 a	11.8 b	Baillie et al. (2006)
Total C (%)	1.90 ± 0.10 a	1.49 ± 0.12 b	Davies et al. (2005)
Total N (%)	0.093 ± 0.001 a	0.107 ± 0.003 b	Davies et al. (2005)
C:N	14.20 ± 0.80 a	10.60 ± 0.80 b	Palmiotto (1998)
Total P (mg/kg)	43.7 ± 0.7 a	133.6 ± 4.1 b	Davies et al. (2005)
Available P (mg/kg)	1.4	1.4	Baillie et al. (2006)
Exchangeable Mg (cmol+/kg)	0.12 ± 0.01 a	0.70 ± 0.04 b	Davies et al. (2005)
Exchangeable Ca (cmol+/kg)	0.21 ± 0.01 a	0.52 ± 0.01 b	Davies et al. (2005)
Exchangeable K (cmol+/kg)	0.12 a	0.14 b	Baillie et al. (2006)
Residual P (mg/kg)	90 a	129 b	Baillie et al. (2006)
Residual Ca (mg/kg)	133	170	Baillie et al. (2006)
Residual Mg (mg/kg)	733 a	1421 b	Baillie et al. (2006)
Residual K (mg/kg)	2356 a	4231 b	Baillie et al. (2006)
Residual Fe (mg/kg)	7808 a	14564 b	Baillie et al. (2006)
Leaf litter			
Litterfall (kg/ha-y)	6260	6550	Palmiotto (1998)
Half-life of leaf litter (y)	1.24 a	0.48 b	Palmiotto (1998)
Leaf litter nutrient input			
N (kg/ha-y)	57.00 ± 6.60	74.00 ± 3.00	Palmiotto (1998)
P (kg/ha-y)	0.89 ± 0.10	1.83 ± 0.07	Palmiotto (1998)
K (kg/ha-y)	14.30 ± 1.60	18.40 ± 0.70	Palmiotto (1998)
Ca (kg/ha-y)	15.80 ± 1.80	50.20 ± 2.00	Palmiotto (1998)
Mg (kg/ha-y)	11.30 ± 1.30	13.30 ± 0.50	Palmiotto (1998)

2. Materials and methods

2.1. Study system

Soil bacterial communities were characterized in Lambir Hills National Park, Sarawak, Malaysia (4°11′ N, 114°01′ E). The Park encompasses 6800 ha of lowland mixed dipterocarp forest with the highest tree species richness recorded in the Palaeotropics (Ashton and Hall, 1992; Lee et al., 2002). The forest is old-growth forest that has never been logged and experiences only natural forms of disturbance. Based on mortality rates, the longevity of many of the shade-tolerant tree species in this forest likely exceeds several

hundred years. Rainfall is ca. 3000 mm/y, lacking a well-defined dry season, with all months averaging >100 mm (Watson, 1985). Monthly mean temperatures vary little, ranging from 26 to 28 °C, and diurnal temperature shifts are <10 °C (Watson, 1985). In 1991, a 52-ha plot (hereafter, Lambir) was established in the Park following methods used in similar studies by the Center for Tropical Forest Science (Condit, 1998).

The soils within the Lambir plot lie on an edaphic gradient and range from coarse loams that are sandstone-derived, leached, nutrient-depleted, and well-drained, with substantial raw humus, to clays that are shale-derived, less nutrient-depleted, and less well-drained, with little raw humus (Baillie et al. 2006; Tan et al. 2009). Four soil-habitats were defined along this gradient, based on variation in nutrient contents (total C, N and P and exchangeable K, Ca and Mg) and elevation, and each 20 × 20 m grid square with in the Lambir plot was categorized as one of these soil habitats (Davies et al. 2005). Here, we focus on the soils at the extremes of the gradient, sandy loam and clay, that are the most divergent in soil properties, including total carbon and decomposition rate (Table 1). Both soils are acidic, but surface soil volumetric water content is significantly greater on clay than sandy loam in all months, with values on clay reaching >30% (Russo et al., 2010). Relative to other tropical soils, the clay soil at Lambir has low to very low Ca- and P-fertility, but is moderately fertile for K and Mg, although the latter are not in immediately accessible forms. The sandy loam is less fertile for all nutrients, with reduced cation exchange capacity, but greater organic carbon content (Baillie et al. 2006; Tan et al. 2009).

The high tree species richness at Lambir is at least partly attributable to high beta-diversity, arising from substantial turnover of species composition and congeneric replacements between soil-defined habitats along the edaphic gradient (Lee et al. 2002; Davies et al. 2005). Among the 764 tree species within the Lambir plot tested, 73% were soil specialists, having distributions significantly aggregated on one or two soil habitats, and only 13% were generalists, having a completely neutral distribution with respect to the edaphic gradient (Davies et al. 2005). These dramatic shifts in tree species composition between forests on sandy loam and clay (Table S1, Fig. S1) are associated with shifts in tree functional traits potentially influencing the resources available to microbial decomposers. Leaf litter falling on clay is higher in all measured nutrient concentrations, compared with sandy loam, although total mass of leaf litter input is similar (Table 1).

2.2. Field sampling and DNA isolation

Surface soils were sampled on two rain-free days during the early monsoon season, which represents a period of active leaf litter decomposition, from the centers of 17 randomly selected 20 × 20 m squares within the 52-ha Lambir plot that had been classified as either clay ($n = 10$ locations) or sandy loam ($n = 7$ locations). Leaf litter was removed from the soil surface, and three 2-cm wide, 10-cm deep soil cores were taken at each location and homogenized within a sterile Whirl-pack bag (Nasco, Fort Atkinson, WI, USA). DNA was isolated immediately from 0.25 g of soil from each homogenized sample using Power Soil DNA Isolation kits (MO BIO Laboratories, Inc., Carlsbad, CA, USA). DNA was concentrated using 5 M NaCl and 100% cold ethanol.

2.3. PhyloChip processing, scanning, probe set scoring and normalization

Genomic DNA from soil samples was used as template in PCR reactions for amplification of 16S rRNA gene sequence as described in (Ivanov et al., 2009). Briefly, eight replicate polymerase chain reactions were prepared for each sample containing final concentrations of ∼1 ng gDNA template, 0.02 U/μL ExTaq (Takara Bio Inc.), 1X ExTaq buffer, 0.2 mM dNTP mixture, 1 μg/μL Bovine Serum Albumin (BSA), and 300 pM each of universal bacterial primers: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT- 3′). To minimize PCR bias due to variable template annealing efficiencies and random effects, PCR was performed on a BioRad iCycler with an eight temperature annealing gradient (48–58 °C) and the following conditions: 95 °C (3 min), followed by 30 cycles of 95 °C (30 s), annealing (30 s), 72 °C (2 min), and a final extension at 72 °C (10 min). Reactions were combined for each sample and concentrated with 0.8 volumes isopropanol, washed twice with ice cold 70% ethanol and resuspended in 50 μL nuclease-free water.

2.3.1. PhyloChip microarray analysis of 16S rRNA genes

A mass of 500 ng of pooled PCR amplicons of each sample were spiked with known concentrations of amplicons derived from yeast and bacterial metabolic genes. This mix was fragmented to 50–200 bp using DNase I (0.02 U μg^{-1} DNA, Invitrogen, Carlsbad, CA, USA) and One-Phor-All buffer (GE Healthcare, Piscataway, NJ, USA) following the manufacturer's protocols. The mixture was then incubated at 25 °C for 20 min and 98 °C for 10 min before biotin labeling with a GeneChip DNA labeling reagent kit (Affymetrix, Santa Clara, CA, USA) following the manufacturer's instructions. Next, the labeled DNA was denatured at 99 °C for 5 min and hybridized to custom-made Affymetrix GeneChips (16S rRNA genes PhyloChips) at 48 °C and 60 rpm for 16 h. PhyloChip washing and staining were performed according to the standard Affymetrix protocols described previously (Masuda and Church, 2002).

Each PhyloChip was scanned and recorded as a pixel image, and initial data acquisition and intensity determination were performed using standard Affymetrix software (GeneChip microarray analysis suite, version 5.1). Background subtraction, data normalization and probe pair scoring were performed as reported previously (Brodie et al., 2007; DeSantis et al., 2007, 2006). The positive fraction (PosFrac) was calculated for each probe set as the number of positive probe pairs divided by the total number of probe pairs in a probe set. Taxa were deemed present when the PosFrac value exceeded 0.90. Intensities were summarized for each taxon/probe-set using a trimmed average (highest and lowest values removed before averaging) of the intensities of the perfect match probes (PM) minus their corresponding mismatch probes (PM).

2.3.2. Normalization of PhyloChip data of bacterial community composition

To correct for variation associated with quantification of amplicon target (quantification variation), and downstream variation associated with target fragmentation, labeling, hybridization, washing, staining and scanning (microarray technical variation), a two-step normalization procedure was developed. First, for each PhyloChip experiment, a scaling factor best explaining the intensities of the spiked control probes under a multiplicative error model was estimated using a maximum-likelihood procedure as follows. PhyloChip design contains control probes targeting amplicons of bacterial metabolic genes and synthetic 16S rRNA genes (spike-in probes). These are spiked in known quantities into the final hybridization mix. To take advantage of the spiked probesets, an optimization procedure similar to the one suggested in Hartemink *et al.* (Hartemink et al., 2001) was implemented in R software environment (http://www.r-project.org). This was previously described in detail in Ivanov et al. (2009).

2.4. Pyrosequencing and processing

Recovered DNA was amplified using barcoded 16S rRNA Pyrosequencing tags to achieve parallel sequencing of samples. The

V1–V2 region of the 16S rRNA gene was amplified using bar-coded fusion primers that contain the Roche-454 A or B sequencing adapters (shown in italics), followed by a unique barcode sequence (*N*) and the 5′ end of primer: A-8FM 5′-*GCCTCCCTCGCGCCAT-CAG*NNNNNNNAGAGTTTGATCMTGGCTCAG-3′ and B-357R 5′-*GCCTT GCCAGCCCGCTCAG*CTGCTGCCTYCCGTA-3′ (FLX chemistry primers). All PCR reactions were quality-controlled for amplicon saturation by gel electrophoresis; band intensity was quantified against standards using GeneTools (Syngene) software. All PCR reactions were quality-controlled for amplicon saturation by gel electrophoresis; band intensity was quantified against standards using GeneTools (Syngene) software. For each region of a two-region PicoTitre Plate, amplicon reactions were pooled in equal amounts based on the GeneTools outputs to achieve to achieve ~5000 reads per sample, and the resulting pooled sample was gel-purified. Recovered products were quantified using picogreen (Invitrogen) and a Qubit fluorometer (Invitrogen) and sequenced using Roche-454 GS FLX chemistry.

Raw read output was quality filtered by discarding reads with <150 bp, reads >500 bp, or reads with more than two base miscalls (*N*); and average quality scores of $Q \geq 20$ were achieved for the full length of each read (Benson et al., 2010; Kunin et al., 2010). Filter-pass reads were parsed into their respective sample-specific barcode bins only if they matched the entire forward primer and barcoded sequence. Forward, reverse primers and barcodes were removed after binning. Data were filtered for chimeric sequences using ChimeraSlayer (Caporaso et al., 2010). After initial filtering, <0.5% of sequences were identified as chimeras.

Data from each sample was clustered based on k-mers using a threshold of 97% sequence similarity with the CD-HIT-EST algorithm (CD-HIT) (Li and Godzik, 2006). OTUs with <10 reads summed across all samples were discarded. For each soil sample, the numbers of reads in each OTU for each sample were standardized by the total number of reads in that sample to obtain a measure of relative abundance. Representative sequences from OTUs were extracted and aligned with the Ribosomal Database Project (RDP) aligner and phylogenic reconstruction based on fasttree maximum likelihood (Price et al., 2009). Perl scripts were developed to create the necessary inputs for UniFrac (see Statistical analysis). Samples were also subjected to a RDP analysis (Cole et al., 2009), using the web-based RDP Pyro Pipeline (http://pyro.cme.msu.edu/) as previously described (Benson et al., 2010). The RDP classifier assigns taxonomic rank to sequence reads by matching distributions of nucleotide substrings to a model defined from sequences of known microorganisms. Sequences were separated by soil type (sandy loam or clay) and aligned using the RDP aligner. They were then clustered using RDP complete linkage clustering at a maximum distance of 3% (corresponding to 97% sequence similarity). Rarefaction curves for each soil type were created based on the aligned sequences.

2.5. Statistical analysis

Three data sets were analyzed to identify differences between clay and sandy loam in the community structure of soil bacteria: (1) OTUs from the PhyloChip microarray data and OTUs defined from sequence data analyzed using (2) CD-HIT and (3) RDP classifier. Analyses were performed using the statistical software, R (R Core Development Team, 2009).

Differences between soils in the richness of OTUs were tested using rarefaction (Gotelli and Colwell, 2001) for sequence data analyzed using RDP and CD-HIT. Rarefaction curves were generated with the RDP online platform. Rarefaction analysis of OTUs from CD-HIT was performed using the *vegan* R package (Oksanen et al., 2011). Student's *t*-tests were used to test for differences in richness for RDP-analyzed sequence and microarray data on a taxonomic rank-specific basis.

Differences between soils in their bacterial community structure were analyzed using principal components (PCA) and non-metric multidimensional scaling (NMDS) analyses (Legendre and Legendre, 1998). PCAs used OTUs organized by taxonomic rank for RDP-analyzed sequence data and all OTUs for CD-HIT-analyzed sequence data, weighted by relative abundance, which was taken to be the number of amplicons of an OTU divided by the total number of amplicons present in a sample. NMDS analyses used rank-specific OTUs for microarray data, with the Mountford distance metric (presence/absence only) (Mountford, 1962), three dimensions, and 100 randomly chosen starting parameter values; optimizations were run until convergence. Permutational multivariate analysis of variance (pMANOVA) (Anderson, 2001; Zapala and Schork, 2006), which is analogous to redundancy analysis, was used to describe how variation in metric distance matrices was attributed to the predictor variable, soil type. Matrices of the community-level distances between sampling locations were constructed based on metrics of relative abundance of OTUs using the Jaccard distance metric (Magurran, 2004) and based on presence/absence of OTUs using the Mountford distance metric and were subjected to pMANOVA to test the hypothesis that composition of bacterial OTUs differed between the clay and sandy loam using CD-HIT- and RDP-analyzed sequence data (weighted and unweighted) and microarray data (unweighted only). Tests with the latter two data sets were by taxonomic rank. Weighted and unweighted distance matrices based on the UniFrac distance metric (Hamady et al., 2009; Lozupone and Knight, 2005), which accounts for phylogenetic relatedness of taxa in addition to compositional differences, were created using the *phyloseq* R-package (McMurdie and Holmes, 2012). In all analyses, unweighted analyses reflect differences in composition only, whereas weighted analyses reflect differences in composition and abundance. The most important OTUs determined from RDP-analyzed sequence data at taxonomic ranks from genus to phylum were identified in clay and sandy loam based on the sum across all samples within a soil type of the relative abundances of an OTU within a sample.

Quantifying the phylogenetic structure of communities can aide interpretation of how the composition of communities depends on the evolution of phenotypic traits, related to two processes involved in the assembly of communities: environmental filtering and competitive exclusion. Environmental filtering occurs when composition is limited to taxa that can coexist in a community on the basis of their tolerance of the abiotic environment, whereas niche-competitive interactions among taxa limit their long-term coexistence (Weiher and Keddy, 1999). Assuming that closely related taxa share similar phenotypes, these two processes make opposing predictions about the phylogenetic relatedness of taxa co-occurring in a community (Webb et al., 2002). If closely related species share similar physiological limitations, then environmental filtering will tend to cause closely related taxa to co-occur (phylogenetic clustering). On the other hand, competitive exclusion should limit the coexistence of closely related taxa if they share limiting resources, leading to a pattern in which closely related taxa tend not to co-occur (phylogenetic evenness). For sequence data analyzed using CD-HIT, phylogenetic structure of soil bacterial communities was quantified using the *picante* R package (Kembel et al., 2010). Phylogenetic diversity (Faith, 1992) was estimated for each sample, and differences between soils were tested using Wilcoxon rank sums test. The average phylogenetic relatedness of OTUs and amplicons on each soil was estimated using the mean phylogenetic distance (MPD), which is the average distance between two OTUs (or amplicons, for abundance-weighted analyses) randomly chosen from a community, and using mean nearest

taxon distance (NTD), which is the mean distance of each species to its closest relative in the community (Webb et al., 2002). Net relatedness index (NRI) and the nearest taxon index (NTI) were calculated as in Webb et al. (2002). They represent measures of effect size and are calculated as the difference between the phylogenetic distances (MPD or NTD, respectively) of observed and null communities, standardized by the standard deviation of distances in the null community. Mean pairwise distance tends to be more sensitive to patterns of evenness or clustering across the entire phylogeny, whereas NTD tends to be more sensitive to variation in at the tips of the phylogeny. Probabilities of two-tailed tests were calculated as the minimum of either twice the number of MPD or NTD values higher or lower than the observed value divided by the number of randomizations. Whether OTUs (or amplicons) were more closely or distantly related to each other than expected by chance was tested by comparison of observed MPD values with the distribution of MPD values from randomly assembled communities. Null communities were assembled using the independent swap algorithm (Gotelli, 2000), which maintains the taxonomic richness and abundance distribution, but shuffles the identities of the OTUs occurring in each community. The pairwise probability of OTUs co-occurring in the same soil type or in the same sampling location was estimated using Jaccard's co-occurrence index (Magurran, 2004). The co-occurrence probabilities were correlated with pairwise phylogenetic distance of OTUs in each soil type or sample. Whether observed correlation coefficients were different from those expected by chance was tested by comparison with the distribution of p-values of correlations from communities randomly assembled using the null model described above.

A Mantel test (Mantel, 1967) was used to quantify the multivariate correlation between tree and bacterial communities. Basal area was calculated for each tree species in the 20 m by 20 m area surrounding each location where bacterial communities were quantified, based on the 2003–2004 Lambir plot tree census data. The size of this area was chosen based on the crown diameter of canopy trees near the soil sampling location that could contribute leaf litter to the location. Bacterial OTUs and abundances were based on CD-HIT-analyzed pyrosequencing data. Statistical significance of the correlation coefficient was based on 10,000 random permutations of the distance matrices calculated using the Jaccard metric.

3. Results

3.1. Taxonomic richness and composition in sandy loam and clay soils

The richness of bacterial operational taxonomic units (OTUs) differed between soils (Fig. 1, Table 2). Based on RDP classifier (RDP), 18% of 16S rRNA amplicons were unclassified with respect to any taxonomic rank. Of those that were classified, richness of all taxa and taxa unique to a soil type were higher in clay, but this difference was only statistically significant at the rank of phylum (Table 2). Rarefaction curves of OTUs defined at 97% sequence similarity through the RDP pipeline showed significantly greater richness in clay, but neither curve reached an asymptote (Fig. 1). At 97% sequence similarity, an analysis using CD-HIT identified a total of 1983 OTUs. Ninety-nine percent (1963) of these OTUs were observed in clay, compared to only 78% (1549) observed in sandy loam. A core set of 1529 of the OTUs (77%) could be found in both soils. Twenty-two percent (434) and 1% (20) of OTUs were unique to clay and sandy loam, respectively. As is typical of complex microbial communities, many of the OTUs were rare and sparsely distributed across samples. A total of 62% of all amplicons were from OTUs that occurred at a total abundance of <100 reads, summed across all samples (Fig. S2). In keeping with the higher

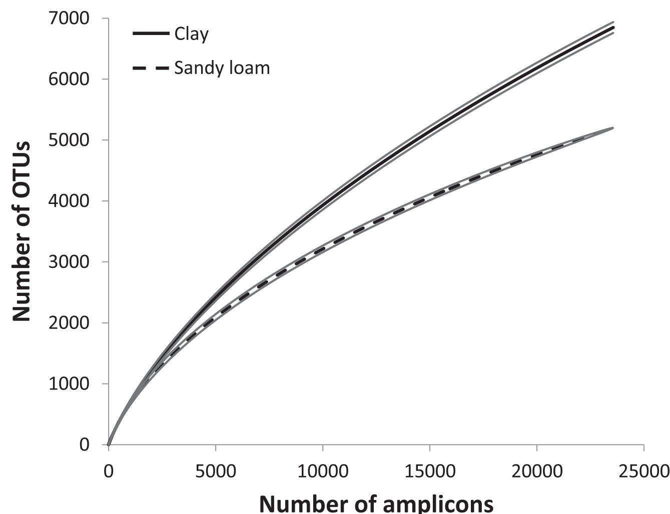

Fig. 1. Richness of bacterial operational taxonomic units (OTUs) in two soils, sandy loam and clay, underlying Bornean rain forest: the accumulation of OTUs with the number of sequences sampled, based on rarefaction analysis of pyrosequencing data at 97% sequence similarity of 16S rRNA amplicons using the Ribosomal Database Project Classifier pipeline. Fine gray lines show upper and lower 95% confidence intervals.

number of OTUs in clay, this soil also had significantly greater OTU richness than did sandy loam based on rarefaction ($p < 0.05$). In contrast to the pyrosequencing data, the PhyloChip analysis showed that richness of all OTUs and OTUs unique to a soil type were more similar across soils, with no significant differences in richness at any taxonomic rank (Table 2).

Multivariate analyses of OTUs, weighted by relative abundance, revealed significant differences between the structure of bacterial communities in clay and sandy loam soil, based on both sequence and microarray data (Fig. 2A–C, Figs. S3, S4). For CD-HIT-defined OTUs, pMANOVA showed significant differences in structure based on composition only (presence/absence) and based on abundance-weighted composition ($F_{(1,16)} = 13.83$, $p = 0.014$ and $F_{(1,16)} = 2.44$, $p < 0.001$, respectively; Fig. 2A–C). Based on sequence data analyzed with RDP, differences between soils in composition were significant at the ranks of genus and order (genus: $F_{(1,16)} = 1.49$, $p = 0.017$; family: $F_{(1,16)} = 1.29$, $p = 0.142$; order: $F_{(1,16)} = 3.47$, $p = 0.037$; class: $F_{(1,16)} = 5.48$, $p = 0.058$; phylum: $F_{(1,16)} = 6.46$, $p = 0.194$). The result based on presence/absence contrasted somewhat with those for abundance-weighted composition of bacterial communities, which displayed statistically significant soil-related structure at the ranks of order, class, and phylum (genus: $F_{(1,16)} = 1.22$, $p = 0.191$; family: $F_{(1,16)} = 1.40$, $p = 0.150$; order: $F_{(1,16)} = 1.90$, $p = 0.047$; class: $F_{(1,16)} = 1.96$, $p = 0.026$; phylum: $F_{(1,16)} = 2.49$, $p = 0.012$; Fig. S3). In contrast, based on microarray data, composition of bacterial communities was significantly different between soils only at the family level (family: $F_{(1,16)} = 1.86$, $p = 0.037$; order: $F_{(1,16)} = 1.54$, $p = 0.216$; class: $F_{(1,16)} = 0.86$, $p = 0.468$; phylum $F_{(1,16)} = 1.06$, $p = 0.414$; Fig. S4).

Based on RDP-analyzed sequence data, clay and sandy loam were dominated by three and four phyla, respectively, and these phyla comprised >90% of the sequences from each soil (Fig. 3). Bacteria in clay were dominated by Acidobacteria (54% of the amplicons identified to Phylum in clay), whereas Proteobacteria was the most abundant phylum in sandy loam (43% of the amplicons identified to Phylum in sandy loam). The relative abundance of taxonomic groups within the Acidobacteria was similar in the two soils (Fig. 4), but orders of Proteobacteria were more equitably distributed on sandy loam than on clay (Fig. S5). Of the top ten most abundant classes, the following were more abundant on sandy

Table 2
Numbers of bacterial operational taxonomic units (OTUs) at different taxonomic ranks identified in two soil types underlying Bornean rain forest using the Ribosomal Database Project Classifier. Total values reflect all OTUs present across both soils or on each soil; unique values reflect OTUs present on only one soil; shared values reflect OTUs present on both soils. Probabilities are from a Student's *t*-test of the difference in mean OTU richness between soils, with statistically significant tests in bold typeface.

Taxonomic rank	Total no. OTUs	Clay		Sandy loam		OTUs shared no. (%)	Probability
		All OTUs no. (%)	Unique OTUs no. (%)	All OTUs no. (%)	Unique OTUs no. (%)		
RDP classifier – 16S rRNA amplicons							
Genus	197	171 (87%)	56 (28%)	141 (72%)	26 (13%)	115 (58%)	0.376
Family	94	87 (93%)	16 (17%)	78 (83%)	7 (7%)	71 (76%)	0.608
Order	40	37 (93%)	5 (13%)	35 (88%)	3 (8%)	32 (80%)	0.280
Class	24	22 (92%)	5 (21%)	19 (79%)	2 (8%)	17 (71%)	0.060
Phylum	19	16 (84%)	6 (32%)	13 (68%)	3 (16%)	10 (53%)	**0.018**
PhyloChip							
Family	164	161 (98%)	4 (2%)	160 (98%)	3 (2%)	157 (96%)	0.416
Order	100	98 (98%)	3 (3%)	97 (97%)	2 (2%)	95 (95%)	0.204
Class	51	51 (100%)	0 (0%)	51 (100%)	0 (0%)	51 (100%)	0.674
Phylum	42	42 (100%)	0 (0%)	42 (100%)	0 (0%)	42 (100%)	0.845

loam than on clay (Fig. 5; values indicate the percent of amplicons in sandy loam versus clay): Actinobacteria (6% versus 4%), Betaproteobacteria (7% vs. 3%), Clostridia (6% vs 1%), Bacilli (4% vs. 1%), and Gammaproteobacteria (5% vs. 1%). More broadly, although relative abundances of OTUs were strongly correlated between soil types at each taxonomic rank, there was still considerable variation (Fig. S6). In both RDP-analyzed sequence and microarray data sets, abundant taxa tended to be abundant on both soil types, although not identically ranked in abundance. The taxa unique to a soil type were limited to clay (Table S2). The eighth most abundant phylum

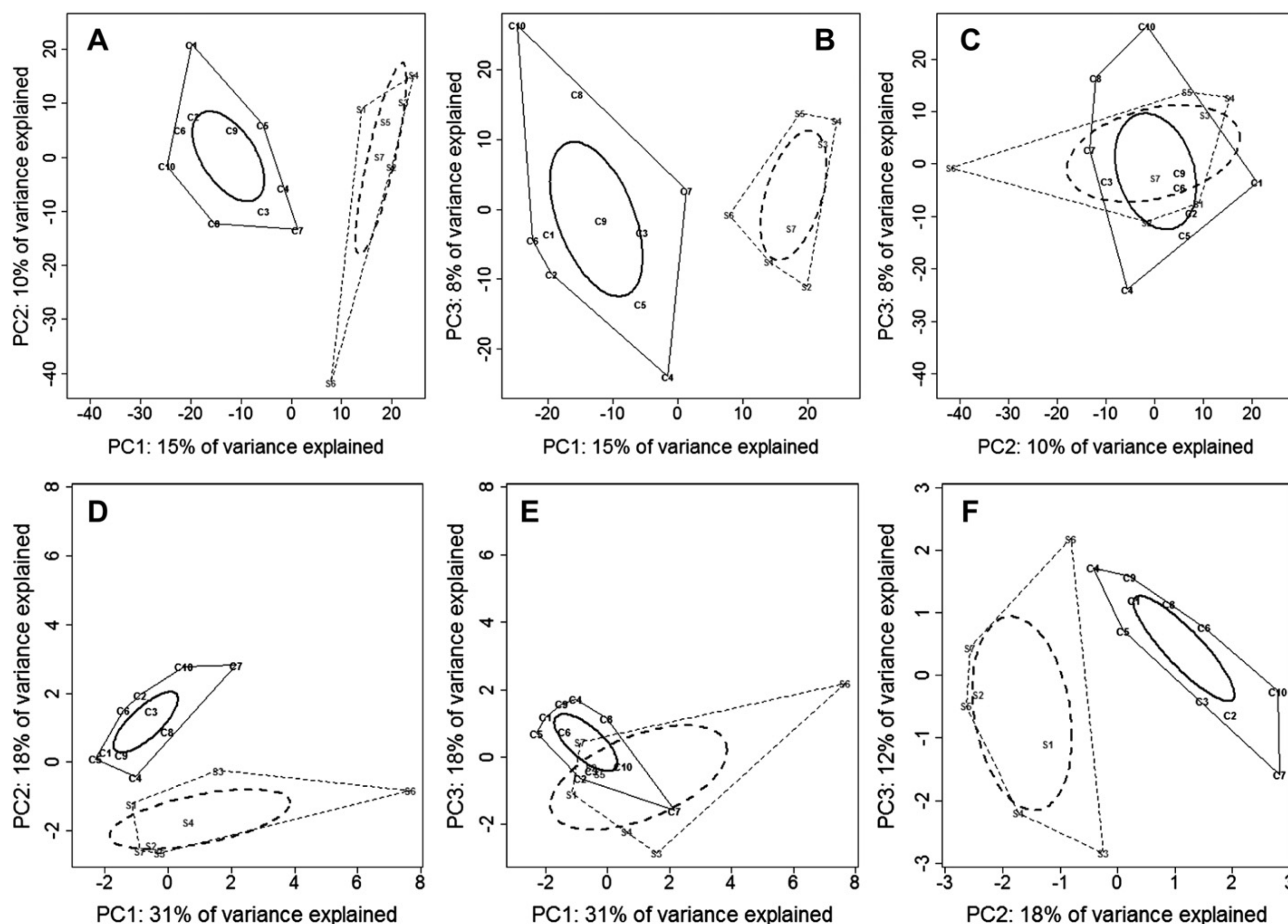

Fig. 2. Principal components analysis of soil bacterial communities in two Bornean rain forest soils based on 16S rRNA amplicons from pyrosequencing (clay: solid lines, sandy loam: dashed lines). The fine line gives the minimum convex hull, and the bold line gives the 95% confidence ellipse for the centroid for each soil type. (A–C) depict an abundance-weighted taxonomic analysis of OTUs defined using CD-HIT using a threshold of 97% sequence similarity. (D–F) Phylogenetic beta-diversity of soil bacterial communities in two Bornean rain forests based on 16S rRNA amplicons: an analysis of phylogenetic distances unweighted by abundance (UniFrac) of OTUs defined with CD-HIT using a threshold of 97% sequence similarity.

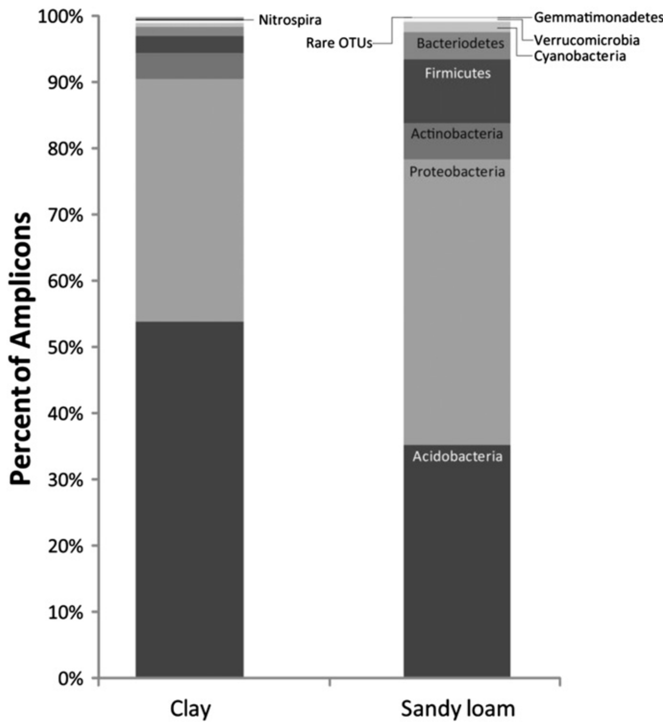

Fig. 3. Relative abundance of bacterial phyla in two Bornean rain forest soils based on 16S rRNA amplicons from pyrosequencing analyzed using RDP. The percent of all amplicons on a soil type that were classified to each phylum is indicated by the stacked bars. The order of phylum names follows the labels for sandy loam, except for Nitrospira, which was uniquely found on clay. Rare OTUs include phyla with <100 amplicons summed across all samples on a soil type.

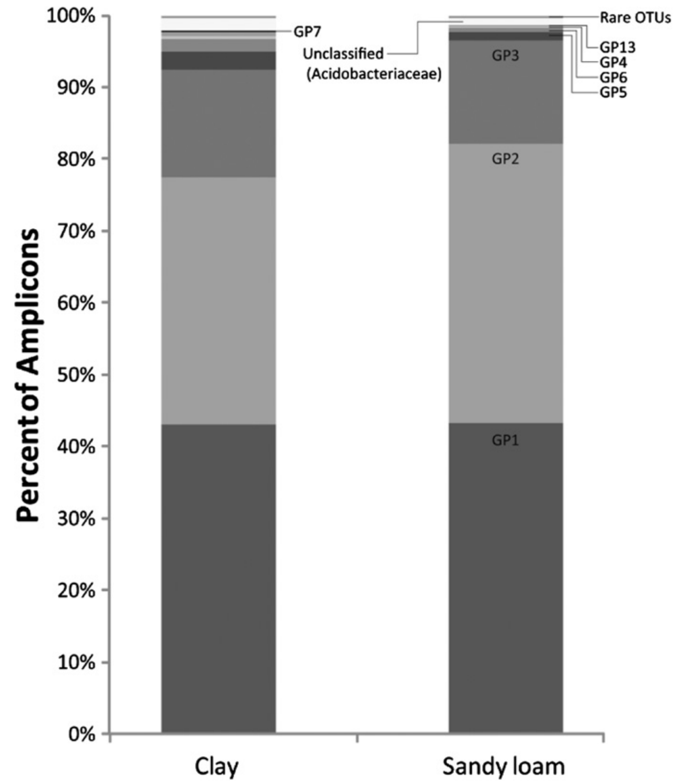

Fig. 4. Relative abundance of bacterial taxonomic groups (approximately genera) within Acidobacteria in two Bornean rain forest soils based on 16S rRNA amplicons from pyrosequencing analyzed using RDP. The percent of all amplicons on a soil type that were classified to each group is indicated by the stacked bars. The order of taxonomic names follows the labels for sandy loam, except for GP7, which was uniquely found on clay. Rare OTUs include those with <100 amplicons summed across all samples on a soil type.

in RDP-analyzed sequence data, Nitrospira, was only found on clay (Fig. 3). The relative abundance of this group also caused higher taxonomic levels including them to show numerical dominance, with the genus *Nitra* ranked as the 18th most abundant and its corresponding family, the Nitrospiraceae, ranked as the 23rd most abundant family. The microarray data identified the family Nitrospiraceae in both soils and failed to yield significant quantitative differences. Chloroflexi was the 12th most abundant phylum in the RDP-analyzed sequence data and uniquely found in clay. The 19th most abundant class, Anaerolineae, was unique to clay. Paenibacillaceae, the 53rd most abundant family, was also unique to clay, as was the 25th most abundant genus in the RDP-analyzed sequence data, GP7, in the Acidobacteriaceae.

The significant variation in soil bacterial community structure between clay and sandy loam was also related to the tree community structure near the sampling location. Based on CD-HIT-defined OTUs, the structures of the tree and bacterial communities were significantly correlated (Mantel test; $r = 0.20$, $p = 0.013$). This correlation was, however, due to the differences between soil types in tree species composition and abundance, since within-soil correlations between the tree and bacterial communities were not statistically significant (clay: $r = 0.15$, $p = 0.185$; sandy loam: $r = -0.40$, $p = 0.934$).

3.2. Patterns in phylogenetic community assembly

Based on CD-HIT-analyzed sequence data, there were no significant differences between bacterial communities in phylogenetic diversity, as measured by Faith's index (Wilcoxon $W = 45$, $p = 0.364$). Phylogenetic multivariate analyses of OTUs revealed differences between the composition of bacterial communities in

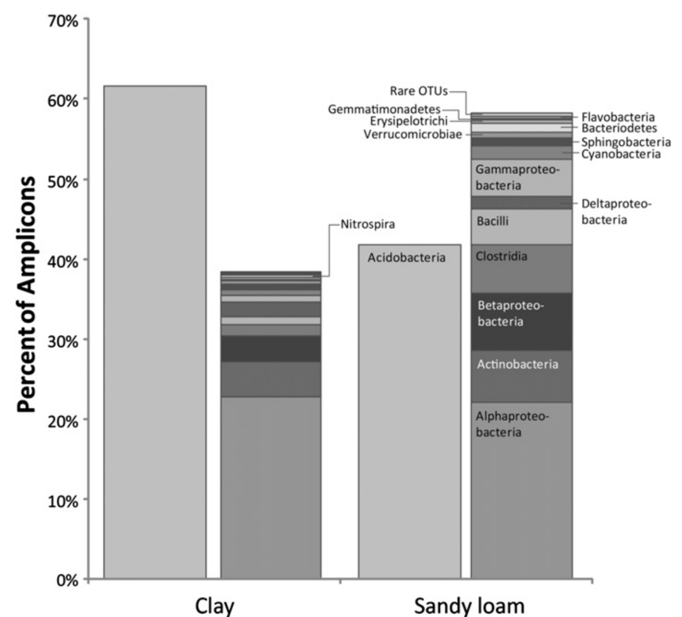

Fig. 5. Relative abundance of bacterial classes in two Bornean rain forest soils based on 16S rRNA amplicons from pyrosequencing analyzed using RDP. The percent of all amplicons on a soil type that were classified to each class is indicated by the stacked bars. The order of taxonomic names follows the labels for sandy loam, except for Nitrospira, which was uniquely found on clay. Rare OTUs include those with <100 amplicons summed across all samples on a soil type.

clay and sandy loam soil (Fig. 2D–F), and these differences were statistically significant when analyses were both weighted and unweighted by relative abundance (weighted, $F_{(1,16)} = 2.00$, $p = 0.001$; unweighted, $F_{(1,16)} = 3.22$, $p = 0.002$). Analyses that were weighted and unweighted by abundance showed similar phylogenetic community structure based on mean phylogenetic distance (MPD) (Table 3). Both OTUs and individuals (sequences) in clay had significantly lower MPD, indicating that they were more closely related to each other than in randomly assembled communities. However, OTUs and individuals in sandy loam were significantly less related to each other compared with randomly assembled communities. Analyses using nearest taxon distance (NTD) (Table 3) were consistent with those using MPD for clay in that NTD was significantly lower than in null communities for both weighed and unweighted analyses. However, for sandy loam, OTUs and individuals showed similar phylogenetic clustering based on NTD compared to that in null communities.

There was a slightly negative, statistically significant correlation between the probability of OTUs co-occurring in the same soil type and the phylogenetic distance between them ($r = -0.05$, $p < 0.001$). This correlation coefficient was smaller than that for all randomly assembled communities ($p < 0.001$), indicating that more closely related taxa had a very slight tendency to co-occur more often than more distantly related taxa. Among sampling locations on clay, there was a negative, statistically significant correlation between the probability of OTUs co-occurring in the same soil sample and the phylogenetic distance between them ($r = -0.12$, $p < 0.001$), and this correlation coefficient was smaller than that for all randomly assembled communities ($p < 0.001$), indicating that closely related taxa had a higher probability of occurring together in the same sample, compared to distantly related taxa. Among sampling locations on sandy loam, there was also a slightly negative, statistically significant correlation between the probability co-occurrence in the same sample and phylogenetic distance. However, the coefficient for sandy loam was closer to zero than for samples on clay ($r = -0.06$, $p < 0.001$), but still smaller than that for all randomly assembled communities ($p < 0.001$), indicating that closely related taxa had only a very weak tendency to co-occur in the same location.

4. Discussion

Based on both pyrosequencing and microarray data, we found significant soil-related taxonomic and phylogenetic differences in the structure of soil bacterial communities in two Bornean soils. These differences were also significantly correlated with the structure of the overlying tree community in this rain forest. This is the first study to link soil and vegetation types to the soil bacterial community using high-throughput sequencing methods in one of the most tree species-rich tropical forests on Earth. Due to plant–soil feedbacks, we might expect the bacterial communities underlying tree species-rich forests to be similarly diverse, and our observed taxon-accumulation curves provide supporting evidence for this prediction. Although several studies outside of tropical forest have found differences in bacterial communities of soil underlying different vegetation types (*e.g.*, Bezemer et al., 2006; Hackl et al., 2004; Pennanen et al., 1999; Wardle, 2002), fewer have dissociated the effects of climate from soil abiotic and vegetation properties (Mitchell et al., 2010). Because, in our study, these two soils are located less than a few hundred meters from each other at a single site, the differences that we observed can be attributed primarily to soil and vegetation properties.

Both the clay and sandy loam soils were dominated by two Phyla, Acidobacteria and Proteobacteria, groups commonly found in soil (Lauber et al., 2009; Nemergut et al., 2010). Specifically, the soil with the faster decomposition rate and lesser total carbon, clay, had greater richness of bacterial taxa and was dominated by the Acidobacteria (*ca.* 54% of amplicons), whereas the sandy loam soil, with the greater total carbon and slower decomposition rate was dominated by the Proteobacteria (*ca.* 43% of amplicons). These findings are inconsistent with predictions based on the copiotrophic–oligotrophic resource-use spectrum (Fierer et al., 2007a). Because these soils contrast dramatically in their abiotic environmental properties and the composition of the forest overstory, it is apparent that these sources of variation significantly affect the underlying microbiome, and together, they are likely to contribute to the observed variation in the decomposition rates of organic matter.

4.1. Ecological inferences from community differences: possible links to litter quality and anoxia

Greater richness of bacterial OTUs was observed in clay than sandy loam with rarefied pyrosequencing data. Rarefaction curves did not, however, level off, which is indicative of both the substantial undiscovered diversity of microbial communities in soil and the intense sampling required to characterize them (Fierer et al., 2007b). Indeed, the shape of the abundance distribution of OTUs across both soils suggests Preston's veil line (Preston, 1948), and, despite our relatively deep sampling, further diversity of less abundant taxa may yet be unveiled. Nonetheless, the more abundant taxa sampled by our methods still served as reliable estimates of the associations between soil and vegetation type and the underlying microbiome. The higher richness in clay can be expected due to the greater surface area for microorganisms provided by the smaller sizes of clay particles. Analyses of the PhyloChip data showed no differences in richness. However, this contrasting result is likely due to the fact that it is not possible to rarefy hybridization-intensity data, causing biases in comparisons of richness, if the samples compared differ in the number of individuals (sequences) per sampling unit or in taxon-abundance distributions (Gotelli and Colwell, 2001), as was the case in our study. In addition, a more limited set of OTUs is quantified using microarray, compared with pyrosequencing, methods, as novel diversity cannot be detected. We recognize that it is possible that a portion of the differences

Table 3
Phylogenetic structure of bacterial communities in clay and sandy loam soils underlying Bornean rain forest. Mean phylogenetic distance (MPD) and nearest taxon distance (NTD) were estimated for observed (Obs.) and null communities, and net taxon index (NTI) and net relatedness index (NRI) were calculated, respectively, based on 9999 randomizations. See main text for details of null community construction. Probabilities are from two-tailed tests based on the number of randomizations with MPD or NTD values greater or less than the observed values for each community (rank).

	Clay					Sandy loam				
	Obs.	Null	NRI or NTI	Rank of Obs. parameter	*P*-value	Obs.	Null	NRI or NTI	Rank of Obs. parameter	*P*-value
MPD, unweighted	1.796	1.805	−3.62	22	0.004	1.788	1.777	3.60	9978	0.004
MPD, weighted	1.845	1.862	−5.73	2	<0.001	1.827	1.810	5.53	9999	<0.001
NTD, unweighted	0.100	0.102	−2.92	250	0.050	0.110	0.108	1.38	9286	0.142
NTD, weighted	0.086	0.092	−5.92	1	<0.001	0.105	0.100	3.63	9700	0.056

between soil types that we observed could be attributable to sequence variability resulting from PCR bias (*e.g.*, Engelbrektson et al. 2010). Our study design incorporated the use of multiple techniques specifically designed for PCR-based analysis of microbial communities in soils and maintained the use of the same primer pairs across all samples in order to compare relative abundances of dominant OTUs across soil types.

Despite the differences in the sensitivity of the two platforms, both the pyrosequencing and PhyloChip approaches showed soil-specific clustering of bacterial communities in multivariate analyses, indicating that these communities are indeed distinct in terms of composition and taxon-abundance distributions. Moreover, soil bacterial community structure was significantly related to that of the surrounding trees, indicating tree species compositional effects on the soil microbiome. These effects were directly related to differences between soils in tree species composition and abundance, not within-soil, local spatial variation in tree assemblages, and may be due to the disparate biochemical composition of fresh leaves, and hence, leaf litter, of the tree species that are dominant on each soil type, tree-species-specific root exudates, or other indirect effects involving tree species. Although the correlation between tree and bacterial communities was significant, the coefficient was not large, suggesting that other factors are also likely to influence the soil microbiome. The soil and vegetation properties that available data show to be the most disparate between these soils and that are most likely to affect bacterial growth and survival, and hence community structure, are soil particle size, moisture, and nutrient availability and the biochemical composition and structure of plant litter.

An ecological classification of soil microbial communities was proposed by Fierer et al. (2007a), in which bacterial metabolic strategies exist on a spectrum, paralleling the *r*- vs. *K*-selection continuum, from copiotrophic bacteria that are abundant in nutrient-rich environments with high carbon availability to oligotrophic bacteria that are abundant in environments with low carbon and nutrient availability. Using sugar mineralization rates, these authors demonstrated that in environments with ample available carbon and nutrient resources, the abundance of copiotrophs, such as Betaproteobacteria and Bacteriodetes, was positively correlated with high rates of carbon mineralization. In contrast, in environments with lower rates of carbon mineralization and low resource availability, oligotrophs, such as Acidobacteria, predominated. Based on this classification, we would anticipate that clay, which has higher nutrient availability and more rapid leaf litter decomposition rates (Table 1), would harbor more Betaproteobacteria and Bacteriodetes than sandy loam, which is relatively nutrient poor with a slower decomposition rate. Conversely, the Acidobacteria would be more abundant on sandy loam. However, in our study, Acidobacteria were more abundant on clay than on sandy loam. The contrasting result was also observed for the Betaproteobacteria and Bacteriodetes, which were more abundant in sandy loam than clay. Our results are inconsistent with those from a litter manipulation study in Costa Rica that found greater abundances of Acidobacteria in soil plots from which leaf litter had been removed (Nemergut et al., 2010). In our study, the higher total soil carbon in sandy loam is likely to be more a reflection of the accumulated products of decomposition, rather than the carbon resources available to support it. While we have not measured carbon mineralization rates in these soils, their differences in total soil carbon and forest floor necromass indicate that sandy loam stores more carbon, likely as a consequence of longer residence time of organic matter due to slower decomposition and carbon mineralization rates (Palmiotto, 1998; Baillie et al. 2006). The slower decomposition rate of leaf litter on sandy loam could also be explained by lower microbial biomass, which would be expected if soil particle size is negatively correlated with microbial biomass.

Leaf litter of trees typical of sandy loam provides more total carbon, but less nitrogen and other nutrients, to the indigenous soil microbial community than does clay (Table 1). However, that carbon is likely to be of more complex, potentially more recalcitrant, forms, given the higher lignin and cellulose contents of the fresh leaves of tree species typical of sandy loam (S.E. Russo, unpub. data). While abiotic soil properties and nutrient availability have direct effects on decomposition (Hobbie and Gough, 2004), the biochemical and structural properties of leaves reflect their decomposability (Hobbie, 2000; Lovett et al., 2004; Swift et al., 1979; Wardle, 2005). Plant species with leaves exhibiting higher photosynthetic rates and lower structural investment costs are more decomposable (Cornwell et al., 2008; Santiago, 2007), especially in abiotic environments favorable to decomposition, such as wet tropical systems (Couteaux et al., 1995; Wieder et al., 2009) like this Bornean forest, which receives *ca.* 3000 mm annual rainfall. The rapid leaf litter decomposition rate and lower C:N ratios observed in clay soil in our study indicate that the structural carbon present may be of simpler forms and likely more available to decomposer microbiota. Whether more recalcitrant forms of carbon, such as lignin, slow decomposition, as has been observed in some tropical systems (Hirobe et al., 2004; Hobbie, 2000), but not others (Raich et al., 2007), may depend on the local abundance of fungal decomposers that can convert the most complex carbon forms to the simpler ones that are subsequently made available to the majority of bacteria (Couteaux et al., 1995; Dix and Webster, 1995; Hattenschwiler et al., 2005; Wu et al., 2011), as well as, on the availability of nutrients (Scott and Binkley, 1997) and more labile carbon sources (Klotzbücher et al., 2011). Although our study cannot disentangle these complex plant–soil feedbacks, it provides a basis for developing hypotheses to explain important mechanisms affecting edaphic variation in the community structure of soil bacteria.

In addition to fungal degradation of structural organic carbon, bacteria such as Actinobacteria, abundant on sandy loam, could solubilize complex carbon substrates, such as lignin (Lynd et al., 2002; Pasti et al., 1990; Ralph, 2005). Further studies are required to test this hypothesis, as cellulolytic activity is characteristic of several taxa higher in abundance on sandy loam, such as some members of the Bacteriodetes, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, and Firmicutes (Lynd et al., 2002). Much less is known with regard to degradation of structural organic carbon by Acidobacteria, as very few cultured representatives of this Phylum are available for physiological studies. However, sequenced genomes of three cultured Acidobacteria revealed sequences encoding for proteins capable of degrading structural carbon, such as cellulose and hemicellulose (Ward et al., 2009). A recent Acidobacterial isolate from peatland soils was capable of degrading cellulose, albeit at a slow rate (Pankratov et al., 2011).

Decomposition and mineralization of organic carbon results in the consumption of oxygen via respiratory processes, thereby reducing oxygen availability in the soil. Thus, readily utilized organic matter will result in rapid oxygen loss, contributing to the generation of anaerobic microsites, especially in soils with higher bulk density, such as the clay soil at Lambir. The resulting environment would favor microaerophilic and facultative or obligately anaerobic microbiota. Redox conditions and fluctuations within tropical forest soils have been demonstrated to play a role in shaping the microbial community structure (DeAngelis et al. 2010). Thus, the microbial community identified in clay could also be a reflection of hypoxic conditions favoring microaerophilic and the facultative or obligate anaerobic bacteria, resulting in environmental filtering of the bacterial community, as our phylogenetic community analyses suggest (see below). Hypoxic conditions have been described as a physiological requirement for *Nitrospira* spp.

(Lücker et al., 2010), which was specific to clay, and the Veillonellaceae (Saddler and Bradbury, 2005), which was more abundant in clay (Table S3), thus supporting the hypothesis of lower oxygen availability in clay. Conversely, aerobic conditions would be more likely to prevail on the well-drained sandy loam. Consistent with this hypothesis, is the increased abundance of the Xanthomonads, obligate aerobic cellulolytic bacteria (Lynd et al., 2002; Saddler and Bradbury, 2005), suggesting greater oxygen availability.

Although both clay and sandy loam are acidic, the pH of the clay soil is lower. The relative abundances of major bacterial classes on each soil type match predictions based on this difference in soil pH (Lauber et al., 2009), in that Acidobacteria were less abundant, Actinobacteria, Beta- and Gammaproteobacteria, and Bacteriodetes were more abundant, and Alphaproteobacteria were similarly abundant on sandy loam, compared with clay, soil.

4.2. Soil-related differences in phylogenetic community structure

In clay, OTUs and individuals tended to be more phylogenetically related to each other than expected in randomly assembled bacterial communities, and more closely related OTUs co-occurred more often than more distantly related ones. Such phylogenetic clustering suggests that environmental filtering, a reduction in the range of successful strategies among coexisting taxa on the basis of tolerance of the abiotic environment (Weiher and Keddy, 1999), is an important ecological process in the assembly of bacterial communities on clay. This interpretation is valid provided that closely related bacterial taxa are more likely to share similar functional traits than are distantly related taxa, which is not well-known due to the prevalence of horizontal gene transfer. One possible filter on microbial composition may be induced by periodic soil hypoxia (DeAngelis et al., 2010), since this would select for taxa that can tolerate hypoxia. As noted above, the clay soil, due to its greater water holding capacity, higher bulk density, lower porosity (Baillie et al., 2006), and higher moisture content (Russo et al., 2010), is more likely than the sandy loam to contain anoxic microsites. In contrast, on sandy loam, OTUs and individuals were more distantly related than in randomly assembled communities, and although there was a statistically significant negative correlation between co-occurrence and phylogenetic distance, the coefficient was very close to zero. This result suggests that niche-driven competitive interactions, in which inferior functionally similar taxa are extirpated from the community due to competitive exclusion, are important in bacterial community assembly, assuming that closely related bacterial taxa are more likely to share phenotypes relevant for resource-competition than are distantly related taxa. Consistent with our findings, a phylogenetic community analysis of several bacterial communities also found strong evidence of phylogenetic clustering (Horner-Devine and Bohannan, 2006). However, they found phylogenetic structure to vary along a productivity gradient in a direction contrasting with our results, namely a pattern of decreasing relatedness associated with increasing plant productivity. In our study, the more productive clay soil exhibited increased relatedness of bacterial taxa, which could be a function of the more likely production of anoxic microsites. Along gradients of total soil organic carbon, their analyses revealed decreased relatedness associated with increased total organic carbon, in accordance with our findings.

5. Conclusions

Despite having lineages in common, we found that the structure of bacterial communities varied significantly between two contrasting Bornean rain forest soils that share the same climate, but differ strongly in vegetation and soil properties. This finding is consistent other studies demonstrating that microbial communities of dissimilar environments can show taxonomic distinctiveness, yet also exhibit co-occurrence of diverse lineages (Chaffron et al., 2010; Nemergut et al., 2010). Moreover, the variation between Bornean soil bacterial communities was significantly associated with differences between soil types in tree community structure, indicating direct and indirect trophic links between overstory trees and the soil microbiome. Our analyses also suggest that the assembly processes determining community structure also vary between these soils. In clay, habitat-filtering processes may predominate, which is consistent with our observations that obligate and facultative anaerobes were more abundant on this soil than on the likely better-aerated sandy loam. On the other hand, niche-competitive processes appear more important in assembly on sandy loam. We hypothesize that, in addition to the direct effects of soil properties, especially oxygen availability and pH, the variation in bacterial composition that we observed between soil types also reflects differences in the biochemical composition of litter, in terms of structural organic carbon and mineral nutrients, but which factors are the best predictors of the composition and structure of soil bacterial communities remains to be identified.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.soilbio.2012.05.021.

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