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Microbial communities acclimate to recurring changes in soil redox potential status

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Summary

Rapidly fluctuating environmental conditions can significantly stress organisms, particularly when fluctuations cross thresholds of normal physiological tolerance. Redox potential fluctuations are common in humid tropical soils, and microbial community acclimation or avoidance strategies for survival will in turn shape microbial community diversity and biogeochemistry. To assess the extent to which indigenous bacterial and archaeal communities are adapted to changing in redox potential, soils were incubated under static anoxic, static oxic or fluctuating redox potential conditions, and the standing (DNA-based) and active (RNA-based) communities and biogeochemistry were determined. Fluctuating redox potential conditions permitted simultaneous CO_2 respiration, methanogenesis, N_2O production and iron reduction. Exposure to static anaerobic conditions significantly changed community composition, while 4-day redox potential fluctuations did not. Using RNA : DNA ratios as a measure of activity, 285 taxa were more active under fluctuating than static conditions, compared with three taxa that were more active under static compared with fluctuating conditions. These data suggest an indigenous microbial community adapted to fluctuating redox potential.

Introduction

Soil microbial communities are dynamic assemblages of populations with different strategies for responding to changing environmental conditions. For every environmental stress there is a multitude of survival strategies for microbial populations, and one population's stress may be another's windfall (Schimel *et al*., 2007). In the face of stress, populations adjust allocation of resources and life strategy to acclimate to the new conditions. The combined outcome of all populations' successful and unsuccessful acclimation is community adaptation, resulting in changing community structure. Fluctuating environmental conditions are known to affect microbial community structure in cases where the changes are repeating and generally predictable, such as with seasonal or tidal ecosystems (Neubauer *et al*., 2005; Boucher *et al*., 2006; Waldrop and Firestone, 2006; Schmidt *et al*., 2007). Much less is understood about stochastically fluctuating systems such as redox potential in soil. Can soil microorganisms acclimate and adapt to rapid and irregular fluctuation in environmental conditions?

A change in redox status of a soil indicates changing availability of electron acceptors, requiring fundamental changes in microbial metabolic lifestyles. Therefore, systems characterized by rapid redox fluctuations present an interesting study in microbial community acclimation and adaptation. Under rapidly fluctuating conditions, microbial populations can be periodically activated and inactivated, which in turn quickly alters the nature and rate of key biogeochemical transformations (Pett-Ridge *et al*., 2006). If a redox condition persists long enough, and the regime becomes static relative to the average generation time of the organism, then community composition will change as populations adapt (Pett-Ridge and Firestone, 2005). If redox fluctuates over periods shorter than the average generation time of the organism, then members of the microbial community may become capable of exploiting a range of redox conditions through a combination of facultative metabolisms and activation or inactivation of obligate metabolisms. Under this scenario, environmental fluctuation should not result in a change in the composition of the community, but in a change in the activity of the community.

Humid tropical forest soils provide an excellent opportunity for studying the effects of environmental fluctuation on microbial community composition and activity and relationships to biogeochemistry. Tropical forests are important global sources of carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) (Vitousek and Matson,

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1992), driven largely by redox-dependent microbial respiration and fermentation processes. Dynamic redox conditions are characteristic of these ecosystems, where soils cycle between oxic and anoxic on a scale of hours to days (Silver *et al*., 1999; 2001). Constant high temperature and soil moisture coupled with high rates of net primary productivity (NPP) and substantial labile carbon (C) pools fuel considerable biological oxygen demand, leading to frequent episodes of anoxia (Silver *et al*., 1999; Schuur and Matson, 2001). Under fluctuating redox conditions, aerobic heterotrophic respiration is considered to be responsible for most soil CO_2 emissions (Subke *et al*., 2006). Low or fluctuating redox conditions drive N_2O, CH_4 and CO_2 production via anaerobic microbial respiration and fermentation (Teh *et al*., 2005; Pett-Ridge *et al*., 2006; Liptzin and Silver, 2009). Recent research has highlighted the possibility of spatially separated biogeochemical processes in sediments under fluctuating redox conditions (Nielsen *et al*., 2010), suggesting that enhanced spatial compartmentalization occurs in tropical forest soils as well. Highly weathered humid tropical forest soils are rich in amorphous and poorly crystalline iron (Fe), which is an important redox-active component in these ecosystems (Chacon *et al*., 2006; Thompson *et al*., 2006; Teh *et al*., 2008; Liptzin and Silver, 2009; Dubinsky *et al*., 2010). Microbial community structure and function are arguably two of the primary drivers of biogeochemical process in terrestrial ecosystems (Orwin *et al*., 2006). The magnitude and frequency of redox fluctuations in soils is likely to be an important selective pressure on microbial community composition and activity (Balser and Firestone, 2005; Pett-Ridge and Firestone, 2005; Wallenstein *et al*., 2006; Dubinsky *et al*., 2010).

To explore the adaptation and acclimation of an indigenous community to dynamic redox, we examined standing bacterial and archaeal communities based on 16S ribosomal RNA (rRNA) gene DNA sequences (to assess adaptation) and active communities based on rRNA (to observe acclimation), and also combine these to calculate a ribosomal RNA to DNA ratio (RNA : DNA) for each taxon as an indicator of microbial activity. Ribosomal RNA gene copies encoded in genomic DNA are generally fixed for given species, although variations in 16S rRNA gene copy number between species are at least partially responsible for differences in growth rates (Kerkhof and Ward, 1993; Muttray and Mohn, 1999; Klappenbach *et al*., 2000). As a biochemical indicator of microbial activity, the use of 16S rRNA is well established for the model bacterium *Escherichia coli* (Bremer and Dennis, 1996), and has been extended to the study of activity of other organisms due to the linear relationship between growth rate and cellular rRNA content that is generally independent of exogenous environmental factors (Dortch *et al*., 1983; Poulsen *et al*., 1993; Binder and Liu, 1998; Muttray *et al*., 2001; Schmid *et al*., 2001). Because rRNA concentrations change over shorter timescales due to changes in activity, RNA : DNA can be used as an indicator of cellular activity for single species (Poulsen *et al*., 1993; Muttray *et al*., 2001). However, there are known exceptions to the linear relationship between activity and rRNA abundance. For example, 16S rRNA abundance in marine *Planctomycetales* did not reflect growth inhibition as well as intergenic spacer regions as determined by FISH (Schmid *et al*., 2001). In another study, the marine *Synechococcus* strain WH8101 had consistently suppressed rRNA content under low growth rates, and at extremely fast growth rates the rRNA content dropped, although intermediate growth showed a linear relationship to rRNA content (Binder and Liu, 1998). While there are likely other exceptions, we use the ratio of rRNA content to rRNA gene at a taxon level to look for trends and generate hypotheses at the community level to investigate microbial community adaptation to fluctuating environmental conditions.

Our study utilizes the 16S rRNA gene microarray PhyloChip to estimate RNA and DNA 16S ribosomal copy number for thousands of taxa in parallel and then aggregate this information to examine changing activity (based on RNA : DNA) on a phylum or order level. One caveat of this approach is that the PhyloChip is an accurate measure of relative abundance, not actual abundance, so if a taxon should maintain its RNA : DNA in one treatment relative to decreasing RNA : DNA in a second, this would be construed as higher activity. However, this taxon would still be more fit in the first treatment scenario, and thus more active, although likely not reproducing actively. This approach respects taxon-level differences in rRNA gene copy number and rRNA abundance by treating each taxon's RNA : DNA as an independent variable, permitting examination of trends in soil microbial community activity as a result of soil redox potential.

To evaluate dynamics of upland humid tropical forest soil microbial communities in response to fluctuating redox compared with static redox (oxic and anoxic), we incubated soil cores for 32 days in three replicate microcosms each under either static oxic, static anoxic or fluctuating redox conditions. We measured oxidized and reduced iron (Fe) together with trace gas production as indices of the linkage between redox-sensitive soil biogeochemistry and soil microbial community adaptation and acclimation. Microarray analysis of soil communities based on ribosomal RNA genes (DNA) and ribosomal RNA (cDNA) permitted calculation of RNA : DNA for each taxon detected, and based on this we present a phylogenetic-based analysis of activity response to redox conditions.

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Results

Soil Fe concentrations

Our experimental design included subjecting soil cores to static oxic conditions (medical grade air headspace), static anoxic conditions (N_2 headspace), or fluctuating redox status conditions with 4-day fluctuations between oxic and anoxic (Fig. 1). Fluctuating redox conditions resulted in fluctuating Fe(II) concentrations (Fig. 1; Table S1), while amorphous and poorly crystalline Fe did not vary significantly among treatments or over time (Table S1), averaging 8.8 mg amorphous Fe g^{-1} dry weight soil (standard error 0.15 mg Fe g^{-1} dry weight soil). Bioavailable Fe(II) was measured as the concentration of acid extractable Fe, which varied significantly with treatment and over time. At the first sampling point, static oxic conditions kept Fe(II) consistently depressed, while static anoxic conditions led to higher Fe(II) relative to initial concentrations. Fe(II) tended to be higher at the end of anoxic periods and lower at the end of oxic periods, and at the end of the oxic period in the fluctuating redox treatment declined significantly over time ($P < 0.05$) (Table S1).

Gas production from soil incubations

Soil CO_2 fluxes were not significantly different among oxic, anoxic or fluctuating redox conditions and did not attenuate over the 32-day period of incubation (Fig. 2A). In contrast, static anoxic conditions significantly increased the fluxes of both N_2O and CH_4 into the chamber headspace compared with other conditions, while static oxic conditions had net CH_4 and N_2O consumption (Fig. 2B and C). Soils experiencing fluctuating redox had net CH_4 production rates that were intermediate to the static conditions (Fig. 2C). Fluctuating redox soils had similar net N_2O fluxes regardless of whether they were at the end of an oxic or anoxic cycle, and rates were statistically indistinguishable from the anoxic treatment. The net N_2O flux was significantly lower at the later time point (28–32 days) for oxic and fluctuating oxic treatments ($P < 0.05$) (Fig. 2B).

Microbial community analysis

PhyloChip microbial community analysis of 16S ribosomal DNA and RNA detected 2489 bacterial and archaeal taxa

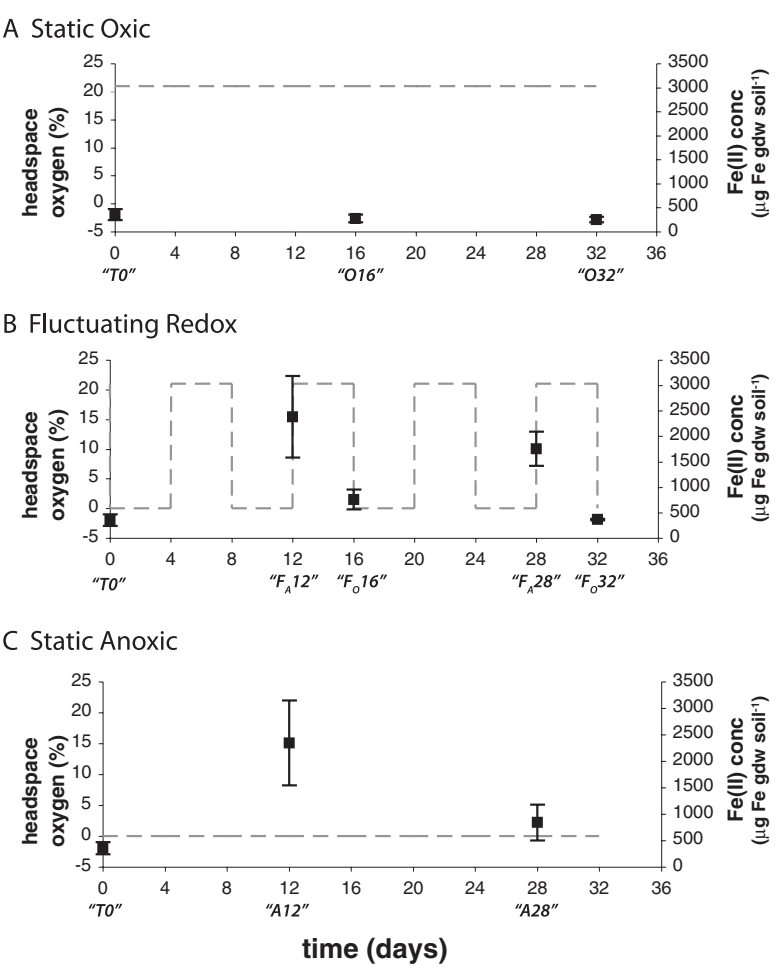

Fig. 1. Diagram of the experimental set-up for manipulating redox conditions of soil cores incubated in the lab (left-hand axis, dotted line) and the resulting Fe(II) concentrations measured in the soils (right-hand axis, squares). T-zero cores were sampled before any treatment conditions were applied. Incubations were set up as three biological replicates (jars) containing four soil cores each. Samples were taken destructively at 12, 16, 28 and 32 days. Dotted lines represent the headspace oxygen supplied by flow-through of either medical grade air or nitrogen; fluctuating redox conditions were affected using a manual switch. Soluble Fe(II) was measured as 1 M HCl extractable iron under anaerobic conditions; means for each treatment are shown with error bars denoting standard error. The concentrations of Fe(II) and significance testing are reported in Table S1.

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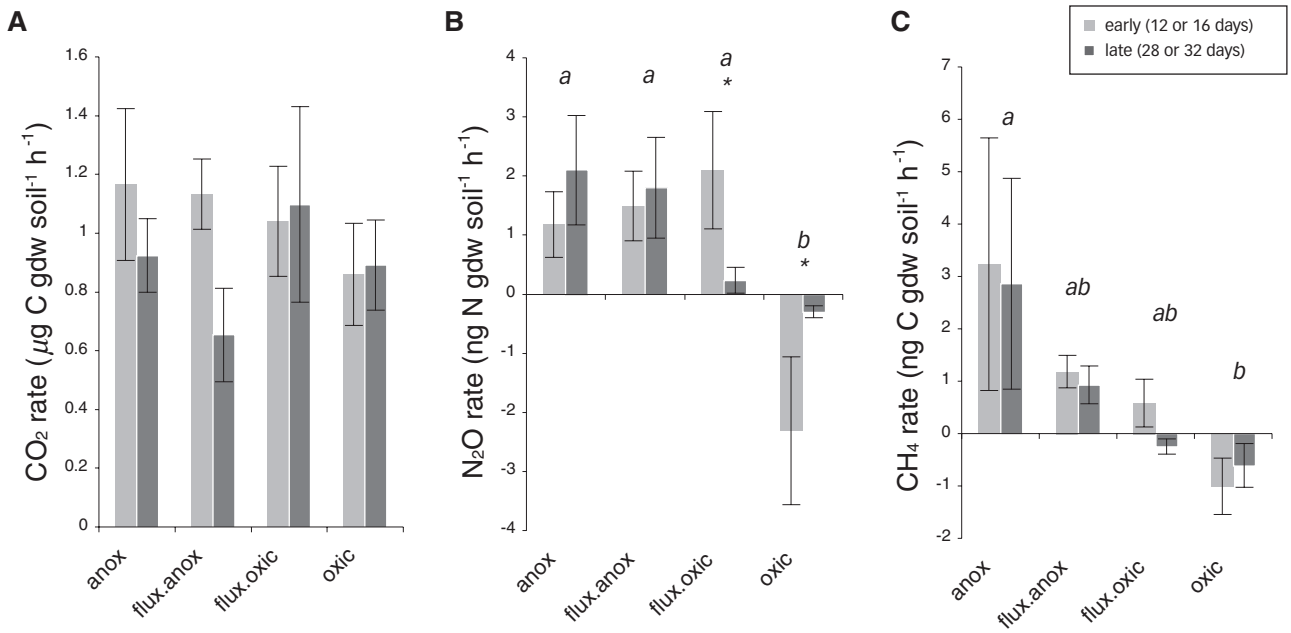

Fig. 2. (A) Carbon dioxide (CO_2), (B) nitrous oxide (N_2O) and (C) methane (CH_4) were measured from the outflow of jars just before the soil was harvested for that time point; bars show the average with standard error. Lower-case letters denote significant differences between treatments, while asterisks denote significant differences between time points within a treatment by ANOVA and Tukey's HSD test at a $P < 0.05$ cut-off. Means for each treatment are shown with error bars denoting standard error.

out of a total of 8741 possible taxa detectable at the species level; the richness of our soil samples spans 29 phyla and a summary of the taxa-level richness detected can be found in Table S2. A significant separation between the active community (RNA) and the standing community (DNA) was detected by ordination using non-metric multidimensional scaling (NMS) based on the hybridization scores of taxa detected [multi-response permutation procedure (MRPP) test, $A = 0.149$, $P < 1e\text{-}7$] (Fig. 3A). Similar effects of treatment and time were observed for the standing and the active communities, and a Mantel test supported the correlation of DNA and RNA communities ($r = 0.81$, $P < 0.001$). Static anoxic conditions had the most profound effect on the microbial community overall, while the fluctuating conditions produced communities the most similar to the initial microbial community regardless of whether it was the end of an oxic or anoxic period (MRPP test, $A = 0.3259$, $P < 0.0001$).

Multiple stepwise linear regressions were performed to create models of the environmental factors significantly correlated with standing and active microbial community structure (Table 1, Fig. S1). For the DNA community, time accounted for 77% of the variance along the primary ordination axis, and adding amorphous Fe accounted for a total of 82% ($P < 0.0001$), while N_2O, amorphous Fe, treatment and CO_2 accounted for 58% of the variance along the secondary axis ($P < 0.001$). There were no environmental variables measured that could account for the variance of the active RNA community along the primary axis, but CO_2, CH_4, Fe(II) and treatment accounted for 55% of the variance in the secondary axis ($P < 0.05$).

Because the ratio of ribosomal RNA to DNA can be a good indication of cellular activity, RNA to DNA ratios (RNA : DNA) were calculated for each taxon. Ordination of RNA : DNA ratios for the whole community was performed using scaled NMS (Fig. 3B). Overlaid on the ordination is a joint plot vector of whole-community mean RNA : DNA ratio; the joint plot vector denotes the direction and magnitude of positive correlation of mean RNA : DNA ratio to ratios of individual treatments ($R_x = 0.823$, $R_y = 0.342$, Fig. 3B). The highest RNA : DNA ratios are in the initial soils, followed by decreasing activity in the fluctuating treatment samples, then static oxic, then static anoxic.

Using the data for RNA- and DNA-based community profiles, we set out to test the hypothesis that specific taxa or populations would have higher activity and were thus acclimating to either static or fluctuating conditions in soils. To do this, the activity of each taxon based on RNA : DNA ratios was calculated, and we performed *t*-tests to evaluate different activity of a taxon under different conditions. An initial control analysis of static anoxic compared with static oxic revealed three archaeal taxa and three bacterial taxa that were more active under static anoxic conditions, which included two TM7 and a *Methanobacterium* sp.; there were 58 taxa that were more active under static oxic compared with static oxic, including 20

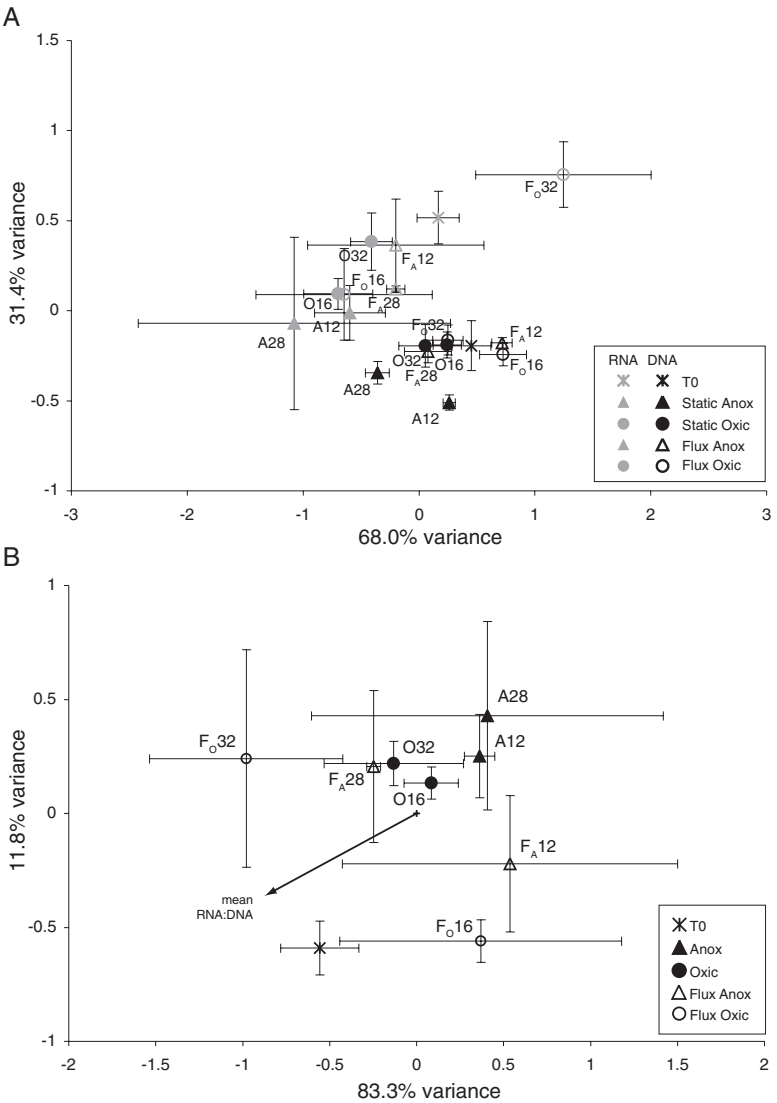

Fig. 3. Microbial community analysis of (A) the entire standing community (DNA) and active community (RNA) and (B) RNA : DNA ratios of the whole community. Ordination was performed using non-metric multidimensional scaling with the per cent variance explained by each axis printed on the axis. The joint plot of mean RNA : DNA ratio for the whole community is intended to display the magnitude and direction of highest activity.

Proteobacteria, six *Acidobacteria* and six *Actinobacteria* (Table S3). We then performed *t*-tests to compare taxon activity under any fluctuating compared with any static conditions, under fluctuating oxic compared with static oxic, and under fluctuating anoxic compared with fluctuating anoxic. The results of these comparisons are presented as the number of taxa (on the *y*-axis) significantly more active in one condition than another, and the condi-

Table 1. Least-squares model by stepwise linear multiple regression.

	PC % variance[a]	Factor	Cumulative factor R^2	Factor *P*-value	Model R^2	Model *P*-value
DNA PC1	58.2	Time	0.775	< 0.0001	0.818	< 0.0001
		Amorphous Fe	0.818	< 0.05		
DNA PC2	19.4	N_2O	0.237	< 0.05	0.579	< 0.05
		Amorphous Fe	0.404	< 0.01		
		Treatment	0.503	< 0.05		
		CO_2	0.579	0.08		
RNA PC1	53.5	n.s.		n.s.		n.s.
RNA PC2	11.4	CO_2	0.156	< 0.05	0.547	< 0.01
		CH_4	0.302	< 0.01		
		Fe(II)	0.506	< 0.01		
		Treatment	0.547	0.20		

a. This variable is the per cent of variance in the microbial community data that the principal component explains.

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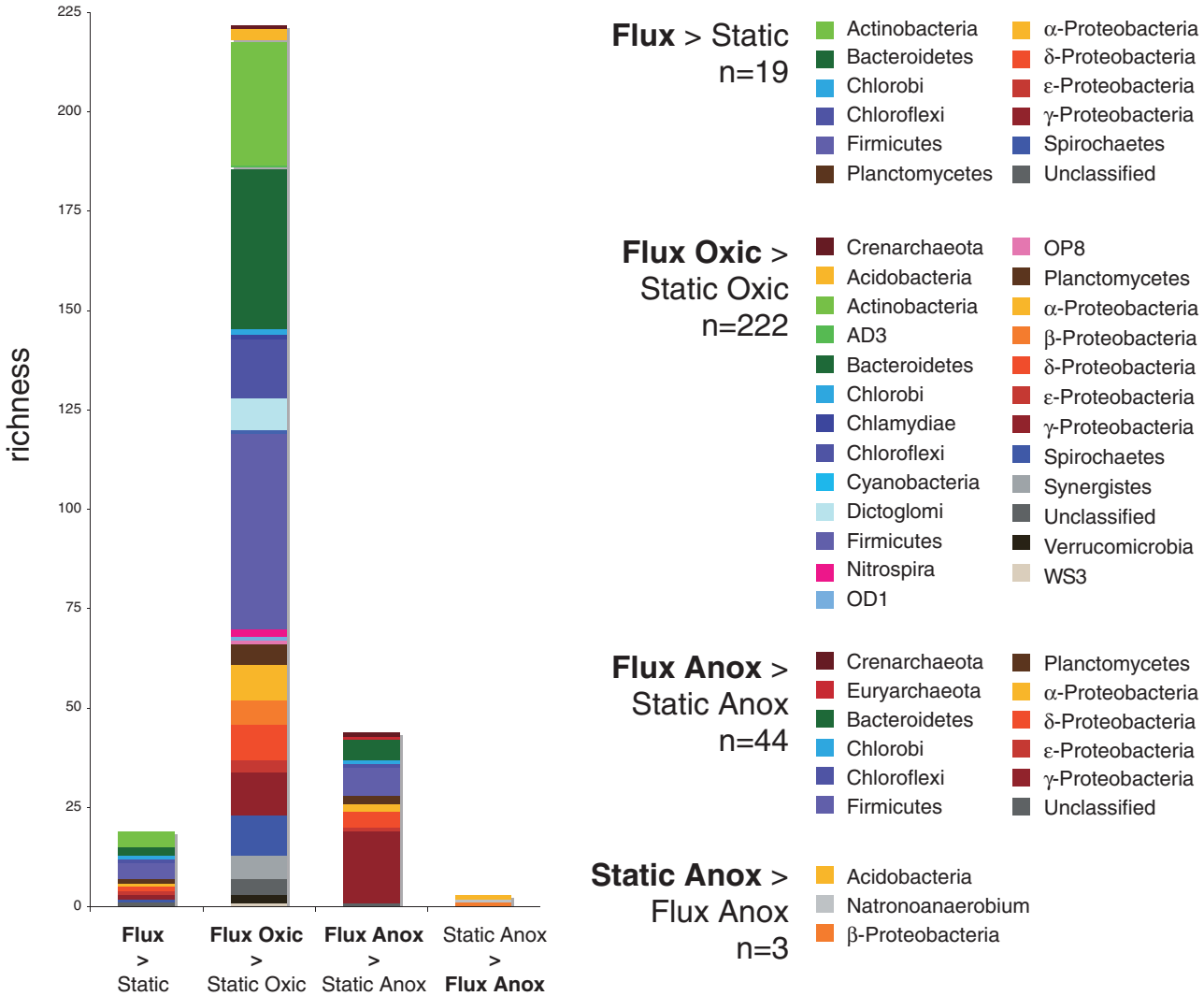

Fig. 4. The RNA : DNA ratios for each taxon were calculated and then Student's *t*-test performed to see if activity was higher or lower under static compared with fluctuating conditions (combining oxic and anoxic); under static oxic compared with fluctuating oxic; or under static anoxic compared with fluctuating anoxic. Each test was evaluated to a *P*-value of 0.05. There are separate legends for each group, but legend colors are the same for phyla across groups. No taxa were more active under static conditions compared with fluctuating or static oxic over fluctuating oxic.

tions are denoted on the *x*-axis (Fig. 4, Table 2). No taxa were significantly more active under static compared with fluctuating conditions, or under static oxic compared with fluctuating oxic conditions. There were only three taxa more active under static anoxic compared with fluctuating anoxic conditions. However, 19 taxa preferred any fluctuating condition to any static, 44 taxa preferred fluctuating anoxic to static anoxic, and 222 taxa preferred fluctuating oxic to static oxic conditions. The populations that preferred fluctuating over static redox conditions were mostly represented the archaeal phylum *Crenarchaeota* and the bacterial phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Proteobacteria* and *Spriochaetes* (Fig. 4, Table 2).

Discussion

Microbial community analysis indicated that the rapidly fluctuating redox typical of humid tropical forest soils promotes microbial communities that are specifically adapted to these conditions. There are three metabolic strategies that could explain the adaptation of microbial communities to fluctuating redox conditions. The first strategy is (i) facultative metabolism, where populations are capable of aerobic respiration as well as either fermentation or anaerobic respiration. Facultative metabolisms are generally considered to operate at maximum potential energy whenever possible, and maximum potential energy yield occurs under oxic conditions. If facultative metabolism

Table 2. Numbers of taxa with RNA : DNA ratios of hybridization scores significantly different under redox potential conditions are summarized by phylum and class; these data were used to generate the stacked bar charts in Fig. 4.

Domain	Phylum	Number of taxa	Notes[a]
Taxa significantly more active under fluctuating compared with static conditions			
Bacteria	*Actinobacteria*	4	*Rubrobacteraceae* (f)[b]
	Bacteroidetes	2	*Flavobacteraceae* (f); *Tenacibaculum ovolyticum* str., acidic forest soil clone
	Chlorobi	1	Benzene-degrading nitrate-reducing consortium clone
	Chloroflexi	1	DCP-dechlorinating consortium clone
	Firmicutes	4	3 *Clostridiales* & 1 *Symbiomacterales* (o) *Symbiobacterium toebii* str.
	Planctomycetes	1	
	Proteobacteria	4	
	Alpha-	1	*Rhizobiales* (o)
	Delta-	1	*Myxococcales* (o)
	Gamma-	1	*Enterobacterales* (o) *Dermacentor veriabillis* symbiont
	Epsilon-	1	*Campylobacterales* (o)
	Spirochaetes	1	Neutral pH mine biofilm clone
	Unclassified	1	
Taxa significantly more active under fluctuating oxic compared with static oxic conditions			
Archaea	*Crenarchaeota*	1	*C1a* (o)
Bacteria	*Acidobacteria*	3	
	Actinobacteria	31	3 *Acidimicrobiales*, 17 *Actinomycetales*, 2 *Bifidobacterales*, 9 *Rubrobacterales* (o), including *Norcardia* spp., *Gordonia* spp., *Bividobacterium* spp.
	AD3	1	
	Bacteroidetes	40	11 *Bacteroidetes*, 22 *Flavobacteria*, 1 *Sphingobacteria* (c), including *Flavobacterium* spp.
	Chlorobi	2	Benzene-degrading nitrate-reducing consortium clones
	Chlamydiae	1	*Chlamydophila pneumoniae* str.
	Chloroflexi	15	9 *Anaerolinae*, 4 *Dehalococcoides*, 1 *Thermomicrobia* (c)
	Cyanobacteria	8	2 *Chloroplasts*, 1 *Nostocales*, 1 *Oscillatoriales* (o)
	Dictoglomi	1	Marine clone
	Firmicutes	49	13 *Bacilli*, 4 *Catabacter*, 28 *Clostridia* (c)
	Nitrospira	2	
	OD1	1	
	OP8	1	
	Planctomycetes	5	4 *Planctomycetaceae*, 1 *Pirellulae* (f)
	Proteobacteria	48	
	Alpha-	9	1 *Bradyrhizobiales*, 2 *Rhizobiales*, 2 *Rhodobacterales*, 2 *Sphingomonadales* (o)
	Beta-	6	5 *Burkholderiales* (o)
	Delta-	9	2 *Desulfobacterales*, 4 *Myxococcales*, 2 *Syntrophobacterales* (o)
	Epsilon-	3	3 *Campylobacterales* (o)
	Gamma-	11	3 *Chromatiales*, 4 *Enterobacteriales* (o)
	Spirochaetes	10	8 *Spirochaetaceae*, 2 *Leptospiraceae* (f) including *Borella anserine* str.
	Synergistes	4	
	Unclassified	10	
	Verrucomicrobia	2	*Fucophilus fucoidanolyticus* str.
	WS3	1	
Taxa significantly more active under fluctuating anoxic compared with static anoxic conditions			
Archaea	*Crenarchaeota*	1	*C1a* (o) Sulyo seamount hydrothermal vent clone
	Euryarchaeota	1	*Mehanobacteriaceae* (f) *Methanobacterium curvum*
Bacteria	*Bacteroidetes*	5	1 *Bacteroidales*, 4 *Sphingobacterales* (o), including *Flexibacter* spp.
	Chlorobi	1	Benzene-degrading nitrate-reducing consortium clone
	Chloroflexi	1	Chlorobenzene-degrading consortium clone
	Firmicutes	7	1 *Bacilli*, 6 *Clostridia* (c)
	Planctomycetes	2	1 *Planctomycetaceae*, 1 *Gemmatae* (f)
	Proteobacteria	25	
	Alpha-	2	1 *Rhodobacteriaceae*, 1 *Sphingomonadaceae* (f)
	Delta-	4	1 *Desulfovibriales*, 1 *Myxococcales*, 1 *Syntrophobacterales* (o)
	Epsilon-	1	*Helicobacter hepaticus* str.
	Gamma-	18	14 *Enterobacterales* (o), including *Erwinia* spp., *Enterobacter* spp., *Serratia* spp.
	Unclassified	1	
Taxa significantly more active under static anoxic compared with fluctuating anoxic conditions			
Bacteria	*Acidobacteria*	1	
	Natronoanaerobium	1	
	Proteobacteria	1	*Burkholderiales* (f)

a. Notes include phylogeny of dominant taxa in groups, representative taxa genus and species identification when available, and exclude most singlets and unclassified taxa.
b. (c), class; (o), order; (f), family.

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was dominant in these soils, then static oxic conditions should have produced communities more active than fluctuating communities because of higher potential energy; indeed, this was the case for a study of well-aerated, grassland soils (Picek *et al*., 2000) but this was not what we observed based on RNA : DNA ratios and net CO_2 evolution. Alternative metabolic strategies for adaptation to fluctuating redox conditions could be comprised of (ii) obligate anaerobes that are favoured by episodic occurrence of oxygen, or (iii) obligate aerobes that are favoured by episodic occurrence of anoxic conditions. Obligate anaerobes could require episodic occurrence of oxygen in order to regenerate their electron acceptors, such as iron reducers, and if they had access to oxidized electron acceptors would likely also have to be aerotolerant. Episodic incursions of oxygen would also facilitate the generation of small-molecular-weight carbon substrates, which could be an advantage to some populations of methanogens. Obligate aerobes that require episodic (or nearby) occurrence of anoxic conditions do also co-occur in these soils, such as methane oxidizers (Teh *et al*., 2005). Iron cycling, methanogenesis and methane oxidation were all observed under fluctuating conditions in these soils. Previous studies on these soils have observed very fast acclimation of microbial community function with change in redox; for example, nitrification and methane production are rapidly activated once conditions permit even after lengthy periods of unfavourable redox (Teh *et al*., 2005; Pett-Ridge *et al*., 2006). Thus rapidly fluctuating redox characteristic of wet tropical forest soils produces microbial populations adapted to the stochastically fluctuating conditions, resulting in communities that are ready for change at any moment. Based on the biogeochemical observations, it is likely that all three strategies are in operation to varying degrees under fluctuating redox conditions in the field.

That tropical soil microbial communities are adapted to fluctuating redox is remarkable given that soil redox fluctuations are stochastic under natural conditions and occur at short timescales. RNA and DNA community profiling was used to observe the process of acclimation and to identify organisms highly active under fluctuating conditions. There was significant separation between RNA and DNA communities, which suggests that not all taxa present in the soils were active (Fig. 3): 55–84% of taxa that were present in the standing (DNA) community were also active as indicated by detectable RNA (Table S2). These results are consistent with prior examination of the effect of fluctuating redox on tropical forest soils, which found that the majority of taxa either adapted to fluctuating redox conditions or were tolerant of periods of unfavourable redox conditions (Pett-Ridge and Firestone, 2005). Under fluctuating redox conditions in marine sediments, changes in oxygen status of surface water cause depletion of H_2S at depths too far and rates too rapid (within hours) to be explained by a mechanism other than electron shuttling, hypothesized to be conducted by a combination of nanowires, pyrite, soluble electron shuttles and outer membrane cytochromes (Nielsen *et al*., 2010). The extent to which electric currents exist in the same manner in soils is unknown. While our tropical soils are quite wet, they may not be sufficiently saline to conduct electrons at rates comparable to those measured in marine sediments. This possibility is however an intriguing hypothetical mechanism of microbial community metabolic plasticity. Taken together, it seems that either shifts in redox on the scale of hours to days are sufficient time for populations to change gene expression in response to new conditions, or populations already have the cellular machinery ready to capitalize on new conditions.

This study uncovered a diversity of populations seemingly adapted to fluctuating redox conditions, including representatives from the phyla *Actinobacteria*, *Bacteroidetes*, *Chlamydiae* and *Firmicutes* (in classes *Bacilliales* and *Clostridia*) and *Crenarchaeota* in the C1a phylum. While the α-, β- and γ-*Proteobacteria* made up a substantial portion of the populations significantly more active under fluctuating versus static conditions (Fig. 4), relatives of the known Fe reducers *Geobacter*, *Shewanella* and *Geothrix* were detected by PhyloChip at the order level only [the orders *Desulfuromonadales* (class δ-*Proteobacteria*), *Alteromonadales* (class γ-*Proteobacteria*) and *Acidobacterales* respectively]; taxa more closely related at the family or genus level were not detected. The absence of taxa in the PhyloChip data closely related to known Fe reducers at the genus level (*Geobacter*, *Shewanella* and *Geothrix*) could be a result of the high diversity of these soils (E.L. Brodie and J. Pett-Ridge, pers. comm.), which is likely to be much greater than the diversity represented on the PhyloChip. Quantitative PCR confirmed that while total bacteria were in the range of 10^{12} cells per gram of soil, *Geobacter* and *Geothrix* were present at 10^7 and *Shewanella* at 10^6 cells per gram of soil (data not shown). These abundances approach the detection limit of the PhyloChip, which is estimated to be about 10^7 copies of 16S rRNA genes (Brodie *et al*., 2007). While we cannot discount the possibility that Fe reducers abundant in low numbers perform essential ecosystem functions even at low relative abundance, our data suggest either that canonical Fe reducers are not the main Fe reducers active in these tropical soils, or that Fe reduction as a lifestyle is a cosmopolitan metabolism among soil bacteria in these Fe-rich moist tropical forests.

It appeared that soil microsites with very different redox potentials were active at the same time under fluctuating redox conditions, where steady CO_2 fluxes accompanied

methanogenesis, N_2O production and Fe reduction (Figs 1 and 2). This suggests that during the 4-day oxic periods, microsites in the soil cores maintained sufficiently low redox for methanogenesis to occur. In contrast, a study of increasing oxidation of rice paddy soils revealed exponentially increasing soil CO_2 production with increasing Eh, accompanied by a decrease in CH_4 and N_2O production (Yu *et al*., 2007). In terrestrial ecosystems, O_2-requiring enzyme activities, such as phenol oxidases, laccases and peroxidases, are considered key in decomposition, generating labile organic C that fuels further microbial activity (Picek *et al*., 2000; Allison and Vitousek, 2004). In our study, the rates of CO_2 production under static anoxia were comparable to those under oxic conditions. This suggests that the lack of O_2-dependent macromolecular decomposition processes did not limit the availability of labile organic C substrates over this 32-day incubation. Under conditions of fluctuating O_2 availability, macromolecular decomposition processes may occur rapidly during periods of O_2 availability, suggesting that rapid changes in redox conditions permit relatively consistent rates of C mineralization.

For all treatments, the active community was more heterogeneous in response to redox compared with the standing community, resulting in greater variability in the microbial communities (Fig. 3A and Fig. S1). This is consistent with the biological basis of the different biomarkers, where rRNA may be many orders of magnitude more abundant than the encoding gene depending upon the growth rate (Kerkhof and Ward, 1993; Wagner, 1994), and may change in abundance rapidly on the order of hours (Poulsen *et al*., 1993; Muttray and Mohn, 1999). Previous studies examining standing (DNA) and active (RNA) soil microbial communities have found decreased richness in active communities which correlated with decreased microbial activity due to lack of carbon and energy (Koizumi *et al*., 2003). Our findings show no change in activity (as measured by CO_2 rates) or change in richness with treatment effect, which may be taken as an indication that energy limitation was not a major stress experienced by the microbial community in fluctuating redox soils.

Conclusions

Humid tropical forest soils are characterized by fluctuating redox conditions which provide a framework for investigating the acclimation and adaptation of microbial communities to fluctuating environmental conditions. Rapid acclimation to changing conditions suggests the presence of populations with existing physiological capacities for energy generation under a range of redox potential conditions. Taken together, our results indicate that the phylogenetically diverse bacterial and archaeal communities indigenous to these wet tropical forest soils are specifically adapted to fluctuating redox potential conditions. This apparent functional versatility allows utilization of rapidly changing resources and has a significant impact on the biogeochemical cycling of these tropical systems.

Experimental procedures

Soils

Soil samples were collected in a subtropical lower montane wet forest in the Luquillo Experimental Forest, which is part of the NSF-sponsored Long-Term Ecological Research programme in Puerto Rico (18°18′N, 65°50′W). Climate is relatively aseasonal, with mean annual rainfall of 4500 mm and mean annual temperatures of 22–24°C (Weaver and Murphy, 1990; McGroddy and Silver, 2000). The soils are clay-rich ultisols, with a mineral composition of biotite, quartz, kaolinite and Fe oxides (Murphy *et al*., 1998). The soil cores were collected at approximately 780 m a.s.l. from three 10-m-long transects established in an area approximately 100 m^2. Four sampling points were established approximately 3.3 m apart along each transect; at each sampling point nine soil cores were collected from the 0–10 cm depth using a 2.5-cm-diameter soil corer. Cores were stored intact in sealable bags at ambient temperature and immediately transported to the lab at UC Berkeley.

Laboratory incubation

One soil core from each sampling point along each transect was placed into a separate incubation chamber (mason jars), so that there were four cores per chamber and three biological replicates (chambers) which corresponded to each of the three transects. Chambers were then subjected to a continuous flow of one of three humidified gas treatments using a flow-through gas system: (i) static anoxic treated cores received continuous N_2 gas, (ii) static oxic treated cores received medical grade air, which consists of 21% oxygen, 78% nitrogen and 1% argon, and (iii) fluctuating redox treatments received 4 days of N_2 followed by 4 days of medical grade air, cycling continuously throughout the experiment. Each chamber was fitted with a Bellco Hungate septum to allow for gas sampling. The 4-day fluctuation was chosen based on earlier research that indicated that this closely mimicked field conditions (Pett-Ridge *et al*., 2006). There were two sampling points each for the static oxic (days 16 and 32) and static anoxic (days 12 and 28) treatments, and four sampling points for the fluctuating redox treatment, with two sampling points each corresponding to the oxic (days 16 and 32) and anoxic (days 12 and 28) cycles (Fig. 1). At each sampling point we destructively harvested three static and three fluctuating chambers; soil cores within a chamber were homogenized and quickly subsampled for Fe assays, and then frozen in liquid nitrogen and stored at −80°C for microbial community analysis. Microbial community analyses of the initial soils (T-0) were processed in the same way without being subjected to any treatment. The total sample size for the experiment was 27 destructively harvested chambers, including the initial, untreated samples.

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Gas sampling

We sampled gases by turning off the flow-through mechanism and sealing the chambers for 1 h. At the end of the 1 h period, 80 ml of headspace gas was sampled from each chamber through the septum for O_2, CO_2, CH_4 and N_2O concentrations. We used 50 ml of gas to measure O_2 on a YSI 51B O_2 meter fitted with a custom adapter for gas analysis (Silver *et al*., 1999). Oxygen averaged 21.0 $\pm$ 1.3% in the aerobic treatments and 2.8 $\pm$ 0.5% in the anaerobic treatments but was effectively 0%; to avoid creating negative pressure in the chamber headspace we were able to take only small gas samples for O_2 analysis and these were insufficient to completely flush the dead space volume from the O_2 electrode. Testing showed that the residual O_2 in the electrode dead space was enough to account for the slight elevation in O_2 measured. We injected approximately 30 ml of headspace gas from each chamber into a pre-evacuated 30 ml glass vial, and analysed CO_2, CH_4 and N_2O on a Shimadzu GC14A gas chromatograph (Shimadzu, Columbia, MD). Carbon dioxide was measured using a thermal conductivity detector, CH_4 with a flame ionization detector and N_2O with an electron capture detector. Trace gas fluxes were calculated as the change in headspace concentrations over the 1 h incubation and are expressed as a flux per gram of dry soil equivalent.

Fe analysis

Soil was assayed for acid-extractable Fe(II) using 1 M HCl. Samples were processed under anaerobic conditions to avoid oxidation of the Fe(II) (Chao and Zhou, 1983). Concentrations of extracted Fe(II) were then determined using a ferrozine assay (Lovley and Phillips, 1987). The resulting coloured complex was measured with a spectrophotometer (Perkin Elmer, Fremont, CA) at 562 nm wavelength. Fe(II) standards were prepared with ferrous ammonium sulfate to calculate the amount of Fe(II) in solution.

Soils were analysed for poorly crystallized Fe using a citrate-ascorbate extraction. At each time point, 2 g of soil samples were suspended in 45 ml of 0.2 M sodium citrate, 0.05 M sodium ascorbate solution (pH 6.0). Headspace was minimized in extractant tubes to avoid excessive oxidation of the reductant. Suspensions were shaken at three cycles s^{-1} for 16 h, then centrifuged for 20 min at 5500 r.p.m. Supernatant was decanted, and concentration of total Fe in solution was measured using an Optima 5300DV inductively coupled plasma atomic emission spectrophotometer (Perkin Elmer, Waltham, MA). Subsamples of all soils were dried at 105°C to a constant weight for fresh-to-oven dry conversions. All Fe values are reported on an oven dry soil basis.

Soil DNA and RNA extraction

All solutions, glassware and plastics were either certified RNase free or treated with 0.1% diethyl pyrocarbonate overnight and autoclaved. Frozen soils were ground with a mortar and pestle under liquid nitrogen and added to Lysing Matrix E tubes (Qbiogene, Irvine, CA), then extracted for DNA and RNA simultaneously using a modified version of a previously published method (Griffiths *et al*., 2000). Briefly, soils were thawed in 0.5 ml of modified CTAB extraction buffer [0.25 M phosphate buffer (pH 8) and 5% hexadecyltrimethylammonium bromide (CTAB) in 1M NaCl] and 50 μl of 0.1 M ammonium aluminium sulfate (Braid *et al*., 2003). Soil solutions were agitated with 0.5 ml of phenol : chloroform : isopropyl alcohol (25:24:1) using a FastPrep FP120 (Bio101, Vista, CA). DNA and RNA were precipitated in PEG6000 solution [30% (w/v) polyethylene glycol 6000 in 1.6 M NaCl], and then soils were extracted a second time as above. Three replicate extractions were performed for each sample, then pooled into 50 μl of TE (pH 8) and put through the Qiagen AllPrep DNA/RNA Mini kit with the DNase-free RNase Set (Qiagen, Valencia, CA). DNA and RNA were eluted twice in RNase-free water and Ambion superase immediately added to the RNA (Ambion, Applied Biosystems, Austin, TX).

Preparation of cDNA

Extracts of RNA were immediately used to make cDNA using the Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) with random primers (Invitrogen, Carlsbad, CA) as directed. cDNA was PCR amplified to check for completion, with PCR amplification of RNA extracts as a negative control to ensure no DNA contamination in the RNA or cDNA preparation.

PCR amplification of DNA and cDNA

All PCR was performed in an iCycler (Bio-Rad Laboratories, Hercules, CA). DNA and cDNA were amplified with 100 ng or 50 ng of template per reaction, respectively, with concentrations determined by gel electrophoresis and verified spectrophotometrically (Nanodrop Technologies, Wilmington, DE). PCR was performed using the primers 8F for bacteria, 4Fa for archaea, and the same reverse primer for both, 1492R (Wilson *et al*., 1990; Hershberger *et al*., 1996). Reactions were performed in a final volume of 25 μl using Takara ExTaq with 3 μM of each primer for bacteria or 6 μM for archaea, 50 μg of BSA and 2 units of DNA polymerase (Takara Mirus Bio, Madison, WI). Eight replicate PCR amplifications were performed at a range of annealing temperatures (T_m) from 48°C to 58°C with an initial denaturation (5 min) followed by 25 cycles of 95°C (30 s), T_m (25 s) and 72°C (120 s), and a final extension of 72°C (10 min). Reactions were run on 1% TAE agarose gel to check for products. Bacterial 16S rRNA PCR product was cleaned using Qiagen PCR Miniprep Kit, while archaeal 16S rRNA PCR product was gel purified using the Qiaquick Gel Purification Kit (Qiagen Sciences, Valencia, CA). When necessary, nucleic acids were concentrated using Microcon 30Y centrifugal filter devices (Millipore Corporation, Billericka, MA), which have a nominal molecular weight limit of 3000 Da.

PhyloChip bacterial and archaeal community analysis

For application onto the high-density 16S rRNA gene microarray (PhyloChip), PCR products were concentrated to 500 ng (bacteria) or 200 ng (archaea), then pooled,

fragmented, biotin labelled and hybridized as previously described (Brodie *et al*., 2007). Three biological replicates for each of nine samples (Fig. 1) were independently PCR amplified and hybridized. The PhyloChip is able to detect a possible 8741 taxa at species-level resolution, and our microbial community analysis is reported as a subset of these taxa with corresponding hybridization scores reported as arbitrary units (au). Each taxon consists of a set of 25–30 perfect match–mismatch probe pairs, and for a taxon to be detected, 90% of probe pairs in its set [probe fraction (pf) > 0.9] must have met these conditions: (i) the perfect match has an intensity of at least 1.3 times the mismatch, and (ii) both perfect match and mismatch have an intensity 500-fold above background. Hybridization scores for a taxon are reported for all samples if at least one sample out of the 12 has pf > 0.9. Hybridization scores are an average of the difference between perfect match and mismatch fluorescent intensity of all probe pairs excluding the highest and lowest. Final hybridization scores were scaled to an average of 2500 au for each PhyloChip.

Statistical analysis

All statistics were performed using JMP (SAS Institute, Cary, NC), PCOrd (McCune and Mefford, 2006) or R (R Development Core Team, 2005). For analysis of biogeochemical factors (Fe and trace gasses), ANOVA with Tukey's HSD pairwise comparison was used to examine treatment effects, while a Student's *t*-test was used to examine the effect of time within a treatment. The microbial community as defined by hybridization scores for individual taxa was analysed using NMS with a Bray distance measure; this method should be the most robust for data on discontinuous or nonlinear scales, and provides the most faithful model of relative relationships between sites (Legendre and Legendre, 1998; Legendre and Gallagher, 2001; McCune and Grace, 2002). A Mantel test was performed with 1000 permutations to examine the degree to which the two matrices, DNA and RNA microbial communities, were correlated. MRPP was used to test the null hypothesis that the ordination contained distinct subgroups that were statistically separate from one another. To explore the degree to which environmental factors (such as iron, trace gases, treatment and time) would explain in the community data, we performed principal components analysis on Hellinger-transformed PhyloChip data; because this type of ordination preserves Euclidean distances, it is appropriate for regression analysis (Legendre and Legendre, 1998; Legendre and Gallagher, 2001). Stepwise linear regression analysis of environmental variables against the ordination coordinates was performed as described in Balser and Firestone (2005). Previously, probe fraction was found to correlate well with richness patterns displayed by clone library analysis (DeSantis *et al*., 2007), so to estimate richness a probe fraction value of 0.9 was employed, below which the taxon was deemed absent. Application of an adjusted *P*-value is too strong a restriction on the community data for these purposes (Yang and Speed, 2003), and because it does not substantially change the results or the variance explained, statistics were performed using a *P*-value of 0.05. Means reported are accompanied by standard errors.

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References

Allison, S.D., and Vitousek, P.M. (2004) Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. *Biotropica* **36:** 285–296.

Balser, T.C., and Firestone, M.K. (2005) Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry* **73:** 395–415.

Binder, B.J., and Liu, Y.C. (1998) Growth rate regulation of rRNA content of a marine *Synechococcus* (Cyanobacterium) strain. *Appl Environ Microbiol* **64:** 3346–3351.

Boucher, D., Jardillier, L., and Debroas, D. (2006) Succession of bacterial community composition over two consecutive years in two aquatic systems: a natural lake and a lake-reservoir. *FEMS Microbiol Ecol* **55:** 79–97.

Braid, M.D., Daniels, L.M., and Kitts, C.L. (2003) Removal of PCR inhibitors from soil DNA by chemical flocculation. *J Microbiol Methods* **52:** 389–393.

Bremer, H., and Dennis, P.P. (1996) Modulation of chemical composition and other parameters of the cell by growth rate. In Escherichia Coli *and* Salmonella typhimurium: *Cellular and Molecular Biology*, 2nd edn. Neiderhardt, F.C., Curtiss, R., III, Ingraham, J.L., Lin, E.C.C., Low, K.B., Magasanik, B., *et al*. (eds). Washington, DC, USA: American Society for Microbiology, pp. 1553–1569.

Brodie, E.L., DeSantis, T.Z., Moberg Parker, J.P., Zubietta, I.X., Piceno, Y.M., and Andersen, G.L. (2007) Urban aerosols harbor diverse and dynamic bacterial populations. *Proc Nat Acad Sci USA* **104:** 299–304.

Chacon, N., Silver, W.L., Dubinsky, E.A., and Cusack, D.F. (2006) Iron reduction and soil phosphorus solubilization in humid tropical forests soils: the roles of labile carbon pools and an electron shuttle compound. *Biogeochemistry* **78:** 67–84.

Chao, T.T., and Zhou, L. (1983) Extraction techniques for selective dissolution of amorphous iron-oxides from soils and sediments. *Soil Sci Soc Am J* **47:** 225–232.

DeSantis, T.Z., Brodie, E.L., Moberg, J.P., and Zubieta, I.X. (2007) High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. *Microb Ecol* **53:** 371–383.

Dortch, Q., Roberts, T.L., Clayton, J.R., and Ahmed, S.I. (1983) RNA : DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organisms. *Mar Ecol Prog Ser* **13:** 61–71.

Dubinsky, E.A., Silver, W.L., and Firestone, M.K. (2010) Tropical forest soil microbial communities couple iron and carbon biogeochemistry. *Ecology* (in press): doi: 10.1890/09-1365.

Published 2010.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., and Bailey, M.J. (2000) Rapid method for co-extraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl Environ Microbiol* **66:** 5488–5491.

Hershberger, K.L., Barns, S.M., Reysenbach, A.L., Dawson, S.C., and Pace, N.R. (1996) Wide diversity of Crenarchaeota. *Nature* **384:** 420.

Kerkhof, L., and Ward, B.B. (1993) Comparison of nucleic-acid hybridization and fluorometry for measurement of the relationship between RNA/DNA ratio and growth-rate in a marine bacterium. *Appl Environ Microbiol* **59:** 1303–1309.

Klappenbach, J.A., Dunbar, J.M., and Schmidt, T.M. (2000) RRNA operon copy number reflects ecological strategies of bacteria. *Appl Environ Microbiol* **66:** 1328–1333.

Koizumi, Y., Kojima, H., and Fukui, M. (2003) Characterization of depth-related microbial community structure in lake sediment by denaturing gradient gel electrophoresis of amplified 16S rDNA and reversely transcribed 16S rRNA fragments. *FEMS Microbiol Ecol* **46:** 147–157.

Legendre, P., and Gallagher, E.D. (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia* **129:** 271–280.

Legendre, P., and Legendre, L. (1998) *Numerical Ecology. Second English Edition*. Amsterdam, the Netherlands: Elsevier.

Liptzin, D., and Silver, W.L. (2009) Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. *Soil Biol Biochem* **41:** 1696–1702.

Lovley, D.R., and Phillips, E.J.P. (1987) Rapid assay for microbially reducible ferric iron in aquatic sediments. *Appl Environ Microbiol* **53:** 1536–1540.

McCune, B., and Grace, J.B. (2002) *Analysis of Ecological Communities*. Gleneden Beach, OR, USA: MjM Software Design.

McCune, B., and Mefford, M.J. (2006) *PC-ORD: Multivariate Analysis of Ecological Data, Version 5*. Gleneden Beach, OR, USA: MjM Software.

McGroddy, M., and Silver, W.L. (2000) Variations in below-ground carbon storage and soil CO_2 flux rates along a wet tropical climate gradient. *Biotropica* **32:** 614–624.

Murphy, S.F., Brantley, S.L., Blum, A.E., White, A.F., and Dong, H. (1998) Chemical weathering in a tropical watershed, Luquillo Mountains, Puerto Rico: I. Rate and mechanism of biotite weathering. *Geochim Cosmochim Acta* **62:** 2404.

Muttray, A.F., and Mohn, W.W. (1999) Quantitation of the population size and metabolic activity of a resin acid degrading bacterium in activated sludge using slot-blot hybridization to measure the rRNA : rDNA ratio. *Microb Ecol* **38:** 348–357.

Muttray, A.F., Yu, Z.T., and Mohn, W.W. (2001) Population dynamics and metabolic activity of *Pseudomonas abietaniphila* BKME-9 within pulp mill wastewater microbial communities assayed by competitive PCR and RT-PCR. *FEMS Microbiol Ecol* **38:** 21–31.

Neubauer, S.C., Givler, K., Valentine, S.K., and Megonigal, J.P. (2005) Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. *Ecology* **86:** 3334–3344.

Nielsen, L.P., Risgaard-Petersen, N., Fossing, H., Christensen, P.B., and Sayama, M. (2010) Electric currents couple spatially separated biogeochemical processes in marine sediment. *Nature* **463:** 1071–1074.

Orwin, K.H., Wardle, D.A., and Greenfield, L.G. (2006) Ecological consequences of carbon substrate identity and diversity in a laboratory study. *Ecology* **87:** 580–593.

Pett-Ridge, J., and Firestone, M.K. (2005) Redox fluctuation structures microbial communities in a wet tropical soil. *Appl Environ Microbiol* **71:** 6998–7007.

Pett-Ridge, J., Silver, W.L., and Firestone, M.K. (2006) Redox fluctuations frame microbial community impacts on N-cycling rates in a humid tropical forest soil. *Biogeochemistry* **81:** 95–110.

Picek, T., Simek, M., and Santruckova, H. (2000) Microbial responses to fluctuation of soil aeration status and redox conditions. *Biol Fertil Soils* **31:** 315–322.

Poulsen, L.K., Ballard, G., and Stahl, D.A. (1993) Use of ribosomal-RNA fluorescence *in situ* hybridization for measuring the activity of single cells in young and established biofilms. *Appl Environ Microbiol* **59:** 1354–1360.

R Development Core Team (2005) *R: A Language and Environment for Statistical Computing, Reference Index Version 2.2.1*. Vienna, Austria: R Foundation for Statistical Computing [WWW document]. URL: http://www.R-project.org.

Schimel, J., Balser, T.C., and Wallenstein, M. (2007) Microbial stress–response physiology and its implications for ecosystem function. *Ecology* **88:** 1386–1394.

Schmid, M., Schmitz-Esser, S., Jetten, M., and Wagner, M. (2001) 16S–23S rDNA intergenic spacer and 23S rDNA of anaerobic ammonium-oxidizing bacteria: implications for phylogeny and *in situ* detection. *Environ Microbiol* **3:** 450–459.

Schmidt, S.K., Costello, E.K., Nemergut, D.R., Cleveland, C.C., Reed, S.C., Weintraub, M.N., *et al*. (2007) Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* **88:** 1379–1385.

Schuur, E.A.G., and Matson, P.A. (2001) Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. *Oecologia* **128:** 431–442.

Silver, W.L., Lugo, A.E., and Keller, M. (1999) Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. *Biogeochemistry* **44:** 301–328.

Silver, W.L., Herman, D.J., and Firestone, M.K. (2001) Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology* **82:** 2410–2416.

Subke, J.A., Inglima, I., and Cotrufo, M.F. (2006) Trends and methodological impacts in soil CO_2 efflux partitioning: a meta-analytical review. *Glob Change Biol* **12:** 1813–1813.

Teh, Y.A., Silver, W.L., and Conrad, M.E. (2005) Oxygen effects on methane production and oxidation in humid tropical forest soils. *Glob Change Biol* **11:** 1283–1297.

Teh, Y.A., Dubinsky, E.A., Silver, W.L., and Carlson, C.M. (2008) Suppression of methanogenesis by dissimilatory Fe (III)-reducing bacteria in tropical rain forest soils: implications for ecosystem methane flux. *Glob Change Biol* **14:** 413–422.

Published 2010.

Thompson, A.W., Chadwick, O., Rancourt, D., and Chorover, J. (2006) Iron-oxide crystallinity increases during soil redox oscillations. *Geochim Cosmochim Acta* **70:** 1710–1727.
Vitousek, P.M., and Matson, P.A. (1992) Tropical forests and trace gases – potential interactions between tropical biology and the atmospheric sciences. *Biotropica* **24:** 233–239.
Wagner, R. (1994) The regulation of ribosomal RNA synthesis and bacterial cell growth. *Arch Microbiol* **161:** 100–109.
Waldrop, M.P., and Firestone, M.K. (2006) Seasonal dynamics of microbial community composition and function in oak canopy and grassland soils. *Microb Ecol* **52:** 470–479.
Wallenstein, M.D., Myrold, D.D., Firestone, M., and Voytek, M. (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecol Appl* **16:** 2143–2152.
Weaver, P.L., and Murphy, P.G. (1990) Forest structure and productivity in Puerto Rico Luquillo mountains. *Biotropica* **22:** 69–82.
Wilson, K.H., Blitchington, R.B., and Greene, R.C. (1990) Amplification of bacterial-16S ribosomal DNA with polymerase chain-reaction. *J Clin Microbiol* **28:** 1942–1946.
Yang, Y.H., and Speed, T. (2003) Design and analysis of comparative microarray experiments. In *Statistical Analysis of Gene Expression Microarray Data*. Speed, T. (ed.). Washington, DC, USA: Chapman & Hall/CRC, pp. 35–92.
Yu, K., Bohme, F., Rinklebe, J., Neue, H.U., and DeLaune, R.D. (2007) Major biogeochemical processes in soils – a microcosm incubation from reducing to oxidizing conditions. *Soil Sci Soc Am J* **71:** 1406–1417.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Principal components analysis was performed on Hellinger-transformed PhyloChip hybridization intensity data for the standing DNA community (top) and the active RNA community (bottom). The points for each site are mean ordination coordinates with standard error.
Table S1. Measurements of Fe(II) and oxidized Fe availability in soils, and significance testing as a result of different treatments or over time within a single treatment.
Table S2. Richness of bacteria and archaea in microbial communities: standing (DNA), active (RNA), and both (present in both DNA and RNA). ANOVA tests were performed for each treatment across microbial community types ($n = 3$). There were no significant differences in richness within communities (DNA, RNA or both) across treatments.
Table S3. Activity of taxa in static conditions only based on RNA : DNA (ratios of hybridization scores).

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