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Redox fluctuations frame microbial community impacts on N-cycling rates in a humid tropical forest soil

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Abstract Fluctuating soil redox regimes may facilitate the co-occurrence of microbial nitrogen transformations with significantly different sensitivities to soil oxygen availability. In an upland humid tropical forest, we explored the impact of fluctuating redox regimes on gross nitrogen cycling rates and microbial community composition. Our results suggest that the rapidly fluctuating redox conditions that characterize these upland soils allow anoxic and oxic N processing to co-occur. Gross nitrogen mineralization was insensitive to soil redox fluctuations. In contrast, nitrifiers in this soil were directly affected by low redox periods, yet retained some activity even after 3–6 weeks of anoxia. Dissimilatory nitrate reduction to ammonium (DNRA) was less sensitive to oxygen exposure than expected, indicating that the organisms mediating this reductive process were also tolerant of unfavorable (oxic) conditions. Denitrification was a stronger sink for NO_3^- in consistently anoxic soils than in variable redox soils. Microbial biomass and community composition were maintained with redox fluctuation, but biomass decreased and composition changed under static oxic and anoxic soil regimes. Bacterial community structure was significantly correlated with rates of nitrification, denitrification and DNRA, suggesting that redox-control of soil microbial community structure was an important determinant of soil N-cycling rates. Specific nitrogen cycling functional groups in this environment (such as nitrifiers, DNRA organisms, and denitrifiers) appear to have adapted to nutrient resources that are spatially and temporally variable. In soils where oxygen is frequently depleted and re-supplied, characteristics of microbial tolerance and resilience can frame N cycling patterns.

Keywords ^{15}N pool dilution · Redox fluctuation · Tropical soil microbial community · Denitrification · Dissimilatory nitrate reduction to ammonium · Gross mineralization and nitrification

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Introduction

The soils of upland humid tropical forests are often characterized by fluctuating redox conditions driven in large part by high biological O_2 demand, warm temperatures, and fine textured soils that limit rates of diffusive transport (Silver et al. 1999, Schuur and Matson 2001). Rapid soil

redox fluctuations may constrain the metabolic capacity of microbes and have the potential to impact soil N cycling. Redox dynamics and associated nutrient cycling are typically studied in inundated ecosystems with relatively static low redox conditions (Binnerup et al. 1992; Matheson et al. 1997), along sediment redox gradients (Picek et al. 2000), or in ecosystems where redox shifts on daily or seasonal cycles (Eriksson et al. 2003; Satpathy et al. 1997; Twining et al. 2004). In upland humid tropical forests, large redox fluctuations have been demonstrated over short temporal scales, ranging from days to weeks (Silver et al. 1999). The microbial community adapted to such rapidly fluctuating redox conditions may be largely distinct from communities adapted to either anoxic or oxic extremes.

Ecosystems with highly variable environmental conditions have long been known to promote high functional diversity and species richness through expanded niche availability (Hutchinson 1961). Non-equilibrium community dynamics are also often linked to highly variable environments, as competitive pressures are relaxed (Weinstein and Yanai 1994) and organisms are pushed to use opportunism as a survival strategy in overcoming an unpredictable environment (Armstrong 1976). In soils where redox potential shifts between O_2 respiration and methanogenesis faster than the rate of population turnover, organisms may be expected to have evolved metabolic capacities that allow quick response (e.g. constitutive enzymes), allowing exploitation of fleeting moments of electron donor and electron acceptor availability. The impacts of rapid and large redox fluctuations on ecosystem functions (e.g. N cycling) and the microbial communities that mediate specific transformations are largely unknown.

The N cycle is particularly susceptible to changes in soil redox, because redox acts as a master switch for microbe-catalyzed processes of nitrification (oxic), denitrification (hypoxic), and dissimilatory nitrate reduction to ammonium (anoxic). Of all the reactions and microorganisms involved in the N cycle, nitrification and nitrifying bacteria may be the most specialized and are thought to be highly sensitive to redox potential and their physio-chemical environment (NH_4^+ and O_2 supply) (Bedard and Knowles 1989; Fenchel et al. 1998). Nitrification is a two stage oxidation ($NH_4^+ \rightarrow NO_2^-$ and $NO_2^- \rightarrow NO_3^-$) primarily carried out by a few genera of obligate-aerobic, slow-growing chemoautotrophs (*Nitrosomonas* and *Nitrosospira* spp.), and hence frequent low redox periods might be expected to suppress nitrifier function.

Denitrifiers are likely to be more ubiquitous in a variable redox environment since denitrification occurs as soil O_2 becomes limiting (Firestone and Davidon 1989; Tiedje 1988). Denitrification is common to a taxonomically and physiologically diverse group of facultative organisms that preferentially respire O_2, but will substitute reduction of NO_3^- and other partially oxidized N-forms (NO_2, and N_2O) when O_2 is limiting (Tiedje 1988). Well suited to fluctuating redox environments, denitrifiers couple NO_3^- respiration to electron transport chain phosphorylation, are typically characterized by a high degree of resilience (Smith and Parsons 1985), and their enzyme activity and synthesis is highly responsive to environmental changes (Parkin 1990; Rudaz et al. 1991).

The effect of highly variable redox regimes on dissimilatory nitrate reduction to ammonium (DNRA) is not known, though the necessary redox conditions for DNRA are similar to those required for denitrification (Myrold 2005). DNRA occurs as NO_3^- and NO_2^- reductase enzymes catalyze the eight electron transfer of NO_3^- to NH_4^+ (Bonin 1996; Philippot and Hojberg 1999) in order to dissipate excess reducing power or detoxify nitrite (Vanmiegroet et al. 1990). Facultative and obligately fermentative bacteria (e.g *Clostridia, Bacillus*, and most Enterobacteriacae) (Caskey and Tiedje 1979; Tiedje 1994) are thought to be primarily responsible for DNRA, as well as dissimilatory metal-reducing bacteria and sulfur reducers such as *Thiobacillus* and *Desulfovibrio* spp. (Brunet and Garciagil 1996; Dalsgaard and Bak 1994). Recent work has also demonstrated that the facultative aerobic fungus *Fusarium oxysporum* catalyzes DNRA in a fermentation process (Takaya 2002; Zhou et al. 2002). DNRA has been documented in forest soils (Bengtsson and Bergwall 2000; Silver et al. 2001; Silver et al. 2005) and highly reduced settings such as anaerobic sludge, sediments, and the rumen (Tiedje 1988).

Two other major N transformations, N immobilization and mineralization, generally do not involve either oxidation or reduction and therefore may be expected to be unaffected by rapidly fluctuating soil redox potential. Nitrogen mineralization, however, may be reduced under anaerobic conditions if organic N is associated with phenolic lignin molecules which require oxidative decomposition (Schimidt-Rohr et al. 2004).

Variable redox environments are important from the perspective of microbial physiology because life in the absence of O_2 requires enzymatic and metabolic capabilities that are distinct from those of aerobic life. In aerobic habitats, biotic communities use O_2 almost exclusively as the terminal electron acceptor in the energy-producing process of respiration, whereas in anoxic systems fermentation is ubiquitous and a wide variety of inorganic compounds (NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^2, CO_2) can be utilized as terminal electron acceptors. In an environment where O_2 availability is unpredictable, organisms with facultative physiologies that use multiple electron donors/acceptors (e.g. denitrifiers) may have a competitive advantage over specialists like obligate aerobes and anaerobes (e.g. DNRA organisms). Organisms with tolerance mechanisms to redox stress such as polyphosphate storage (Carucci et al. 1999) or antioxidant enzymes might also be promoted (Niviere and Fontecave 2004), since these microbes may be more resilient to O_2 starvation, and the toxic effects of superoxide and hydrogen peroxide (Imlay 2002).

In this study, we determined N transformation rates in soils incubated under static versus fluctuating redox regimes and evaluated these data in the context of microbial community composition. We used ^{15}N tracer experiments at three points during soil incubations (0, 3, and 6 weeks) to capture snapshots of microbial processes and community profiles. We expected that the intensity and duration of soil redox fluctuations would strongly influence the relative rates of nitrification, denitrification, and DNRA. While the effects of static redox regimes on microbial N processing may be generally predictable, the effects of fluctuating redox environments in upland forest ecosystems are not well understood.

Materials and methods

Soil collection

Soil samples were collected from the lower montane forest zone (termed Colorado forest) of the Luquillo Experimental Forest, an NSF sponsored Long-Term Ecological Research site in Puerto Rico, USA (18°18′ N, 65°50′ W). The forest occurs between 600 and 900 m above sea level and has an average of 40 tree species per hectare (Brown et al. 1983). The climate is aseasonal with a mean annual temperature of 18.5°C, relative humidity of 98%, and precipitation of 4500 mm (Weaver 1994). Soils are clay-loam ultisols rich in kaolinite, biotite and goethite derived from volcaniclastic sandstones with quartz diorite intrusions (Beinroth 1982; Kieft et al. 1998; Murphy et al. 1998). In the 0–10 cm soil depth, total N averages 0.2%, organic matter averages 8%, pH is 4.81, and bulk density is 0.55 g cm^{-3} (McGroddy and Silver 2000). Bulk O_2 concentrations measured in the 0–10 cm depth average 13 ± 0.2%, yet fluctuate between 0 and 21%. A long-term study showed that O_2 fluctuation in these soils occurs on a time scale of days to weeks; only on rare occasions do soil O_2 levels remain below 10% for longer than one month (Silver et al. 1999).

Approximately 75 soil cores were collected from 0–10 cm depth with 6 × 12 cm PVC tubes from a 10 × 10 m area. Cores were kept intact, immediately placed in sealed Ziplock™ bags, and transported to UC Berkeley at ~20°C. Within 24 h of sampling, cores were extruded and quickly homogenized in a 15-gallon container to create a single large composite sample. This large composite sample was split into two portions. The first was used immediately in a ^{15}N tracer pool dilution experiment (described below); the gross rates, pools and microbial community profiles derived from these analyses represent our 'pre-incubation' controls. The second (larger) portion was immediately divided and placed into sealed jars and subjected to one of four redox incubation treatments (described below). After 3–6 weeks of incubation, additional ^{15}N tracer experiments were conducted on these soils; we refer to the results of these

later analyses as 'post-incubation' or treatment effects.

^{15}N tracer procedures

To determine gross N-cycling rates prior to redox incubations, soils were divided into two 17 g portions (dry weight equivalent). Half received $^{15}NH_4SO_4$ at a soil concentration of 0.76 μg $^{15}N\ g^{-1}$ (raising soil enrichment to 5.8 atm% $^{15}NH_4^+$), while the other half received $K^{15}NO_3$ at 0.23 μg $^{15}N\ g^{-1}$ (raising soil enrichment to 9.9 atm% $^{15}NO_3^-$). Label addition increased the overall NH_4^+ pool size by 20–50% and had no significant effect on NO_3^- pool size. Homogenized soil was divided into jars for assays, with five replicates for each sampling time point. Jars were capped with airtight lids fitted with Hungate septa and 1 ml of label-containing solution was distributed throughout the sample with a spinal-tap needle.

Soils were incubated at room temperature for 15 min (referred to as T_0), 3, 6, or 24 h, and then extracted with 75 ml of 2 mol l^{-1} KCl. Immediately prior to extraction, a 60 ml gas sample was removed from each jar and stored in a pre-evacuated gas-tight Wheaton vial for $^{15}N_2O$ and $^{15}N_2$ analyses. For microbial biomass ^{15}N, and to distinguish between assimilatory and dissimilatory NO_3^- reduction, we removed two 10 g (oven dry equivalent) aliquots of soil from the $^{15}NO_3$ labeled T_{24} jars and extracted one aliquot immediately with 0.5 M K_2SO_4. The other aliquot was fumigated with chloroform for 5 days and then extracted in the same manner as the first. Additional sub-samples of the original homogenized soil sample were used for soil moisture determination, and 3 g sub-samples were stored in sterile microcentrifuge tubes at –80°C for subsequent molecular microbial community analysis. By summing the enrichment measured in the biomass, organic N, N_2, N_2O, NO_3^- and NH_4^+ pools, we were able to account for 112 ± 9% of the added $^{15}NO_3^-$ label.

Redox incubations

We incubated 200 g of homogenized soil in sealed jars (5 replicates × 2 time points × 4 treatments) to estimate the effects of static and fluctuating redox regimes. Jar headspace was carefully controlled to produce the following four treatments: (1) static 'aerobic' jars were constantly flushed with ambient air, (2) static 'anoxic' jars flushed with N_2 gas, (3) 'short-term fluctuation' jars with alternating flushing switched between ambient air and N_2 every 12 h, and (4) 'long-term fluctuation' jars where flushing switched between ambient air and N_2 every 4 days (Fig. 1a). Flushing with humidified gases was regulated at 66 ml min^{-1}, yielding a complete headspace turnover in 8 min. Soils were incubated at 18°C (to simulate field T) and half were harvested after 3 weeks and the remainder at 6 weeks. Redox conditions were measured using headspace trace gas analysis as an integrative index of biologically relevant bulk soil conditions (Stumm and Morgan 1981). Gas measurements were made at the end of a N_2 flushing period by temporarily sealing incubation jars and withdrawing a 10 ml gas sample every 10 min for 1 h. Gas fluxes of CH_4 and O_2 indicated that N_2 flushing had the desired effect in lowering soil redox in fluctuating and anoxic treatments (Fig. 1b). Oxygen concentrations above 0.1–5% atm. sat. indicate aerobic conditions ($p\varepsilon$ +13.75), whereas net CH_4 production indicates an anoxic system ($p\ \varepsilon$ –4.13) (Fenchel and Findlay 1995; Stumm and Morgan 1981).

At the 3 and 6-week harvest points, each jar was subdivided: a 3-g portion was frozen at –80° C for molecular community analysis and the remainder used to estimate N fluxes using ^{15}N tracer additions (as described above). At both harvests, labels were added at 0.7 μg g^{-1} ($^{15}NO_3^-$) and 3.8 μg g^{-1} ($^{15}NH_4^+$). To standardize conditions for gross rate analyses, jars from both the fluctuating treatments received a period of N_2 flushing immediately prior to the harvests.

Analytical procedures and calculations

Concentrations of N_2O, NH_4^+, NO_3^-, NO_2, soil moisture and microbial biomass N concentrations were measured and extracts were diffused according to protocols described in Silver et al. (2001) and Herman et al. (1995). We determined isotope ratios for N_2O and N_2 (run in duplicate) using a trace gas module coupled to an IRMS

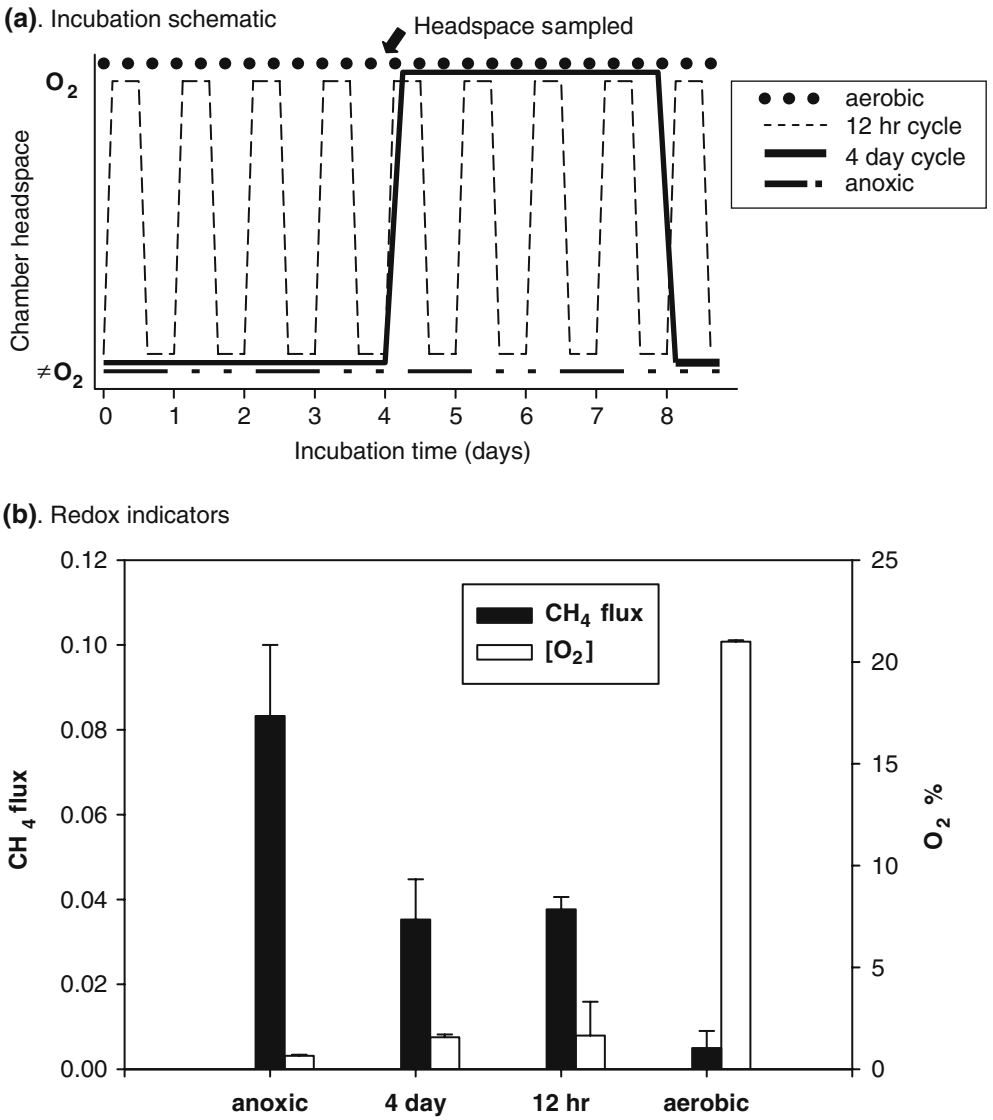

Fig. 1 (**a**) Schematic of experimental design, with two static redox treatments and two fluctuating treatments. (**b**) CH_4 gas fluxes (μg C g^{-1} day^{-1}) and O_2 (%) measured as bulk redox indicators in the headspace of incubation jars. Sampling occurred at the end of an 'anoxic' period for both fluctuating treatments as indicated in the schematic

(ANCA-IRMS, PDZE Europa Limited, Crewe, England). Calculations of atom% $^{15}N_2O$ follow those of Brooks et al. (1993). Background inorganic N pools were estimated at the beginning of each ^{15}N tracer experiment by measuring T_0 NO_3^- concentrations in soils labeled with $^{15}NH_4^+$ and T_0 NH_4^+ concentrations in soils labeled with $^{15}NO_3^-$. These pools are referred to as the 'T_0 pools' and are distinguished from the original soils collected in the field, which we refer to as ''initial'' samples.

Gross mineralization, nitrification and NH_4^+ and NO_3^- consumption were calculated according to Davidson et al. (1991). To estimate DNRA, we calculated the change in enrichment of the $^{15}NH_4^+$ pool over the interval, the change in atom percent excess (APE) of the source pool ($^{15}NO_3^-$ label), and the turnover of the product pool ($^{15}NH_4^+$) pool corrected for isotope dilution caused by mineralization during the interval. Our DNRA equation is modified from Silver

et al. (2001) in that we use the difference in $^{15}NH_4^+$ over the interval, corrected for turnover with the NH_4^+ mean residence time (MRT) and divide this by time and average NO_3^- pool atom% ^{15}N excess (Hart et al. 1994) from the sampling interval. In these calculations, we use the average 0–24 h MRT value, as this longer-term estimate minimizes transient effects and anomalies and is thus most conservative (Silver et al. 2001). We used the sum of $^{15}N_2$ and $^{15}N_2O$ fluxes (based on enrichment and gas concentrations over the interval) as an estimate of denitrification. This sum was divided by the average APE of the NO_3^- source pool to make denitrification rates comparable to DNRA rates. Three hours after label addition, $^{15}N_2O$ values were enriched above natural abundance (detection limit = 0.0006 atom% ^{15}N), however, since $^{15}N_2$ was only detectable after 24 h, we only report a denitrification rate for the 0–24 h interval. The relationship between DNRA or denitrification and substrate availability ($[NO_3^-]$) was calculated by dividing the gross rate ($\mu g\ g^{-1}\ day^{-1}$) by the average NO_3^- pool concentration ($\mu g\ g^{-1}$) in $^{15}NO_3^-$ amended soils; we refer to these ratios with the rate constants k_{DNRA} and $k_{Denitrification}$. We focus our discussion on rates from the 0–3 h time interval which are likely to be more representative of responses to nutrient pulses typical in these highly dynamic ecosystems (Lodge et al. 1994). We reference 0–24 h rates when these rates show different patterns than the 0–3 h rates.

T-RFLP analysis

Terminal restriction fragment length analysis (T-RFLP) was performed on whole community DNA extracted from 0.5 g soil with the Fast DNA Spin Kit for Soil (Bio 101 Systems, Carlsbad, CA) according to methods described in Pett-Ridge and Firestone, (2005). Briefly, we used general eubacterial primers to amplify 16S rRNA: 27F-FAM2 (AGAGTTTGATCCTGGCTCAG) and 1492-R (TACGGYTACCTTGTTACGACTT) (QIAGEN, Operon, Alameda, CA). Restriction digests were done on the purified PCR product using the restriction endonuclease *Msp*I 'C^CGG' (New England Biolabs Inc.). Digests were de-salted and prepared for sequencing according to the procedure established by Brodie et al. (2002).

Statistical analysis

We analyzed control and redox incubation treatment effects on N pools and transformations for all three ^{15}N tracer experiments (0, 3, 6 weeks). There were few differences between 3-week and 6-week results; therefore, to avoid redundancy we have focused our report on the initial and three week results. Analysis of variance (ANOVA) (Wilkinson 1990) was used to determine whether significant changes ($P < 0.05$) in pool sizes or N-cycling rates occurred among treatments and over time. We use Tukey–Kramer Honestly Significant Difference (HSD) as a multiple means comparison test, and log transformations were performed on all N process rates to correct for heteroscedasticity. Homogeneity of variance was tested using Cochran's test, normal distribution with the Shapiro–Wilk W-test (Zar 1996). Means are untransformed data and are presented ± one standard error. Paired *t*-tests were used to compare differences between gross mineralization and nitrification, nitrification and NO_3^- reduction, and mineralization and NH_4^+ assimilation. Stepwise regression was used to determine N pools and processes (moisture, inorganic N pools, MBN, NH_4^+ MRT, mineralization, NH_4^+ assimilation, nitrification, denitrification, DNRA) that best predicted patterns in microbial communities; $P < 0.05$ was set as the entry and elimination criteria.

T-RFLP data were analyzed with PCORD v4 (MJM Software Design, Gleneden Beach, OR). To account for variability in the amount of DNA digested between samples, TRF peak heights were relativized based on their proportion of total sample abundance. We analyzed fragment length and relative peak height using Principal Components Analysis (PCA), and Non-Metric Multidimensional Scaling (NMMS) (Peterson and McCune 2001). Correlations between environmental variables/N-cycling rates and microbial community structure were analyzed by stepwise regression of process rates against ordination axes.

Results

Nitrogen pools and Gross N-cycling rates

The redox treatments we imposed during incubations had substantial effects on N pools, and microbial biomass. While soil moisture was unaffected by treatment and time and averaged 56 ± 0.01%, redox treatments had a large and significant effect on soil NH_4^+ and NO_3^- concentrations ($P < 0.001$) (Tables 1, 2). Ammonium accumulated under the anoxic treatment relative to the aerobic treatment ($P < 0.001$), and in fluctuating treatments, concentrations were comparable to pre-incubation levels. Background NO_3^- concentrations exhibited the opposite pattern, accumulating in aerobic soils and remaining comparable to pre-incubation concentrations in other treatments. At the end of the experiment, microbial biomass nitrogen (MBN) in soils incubated under the 4-day fluctuation treatment was slightly higher than that of the pre-incubation soil (P=0.07) and significantly elevated relative to soils that received static aerobic or anoxic treatments ($P < 0.001$) (Fig. 2).

Twenty-four hours after adding $^{15}NO_3^-$ label, we measured the atom% ^{15}N of microbial biomass to determine whether assimilatory reduction of NO_3^- had occurred. Biomass ^{15}N was not significantly enriched above natural abundance levels, indicating no significant microbial $^{15}NO_3^-$ assimilation (Fig. 2). This is consistent with the significant NH_4^+ pool we measured, as NH_4^+ is known to repress assimilatory NO_3^- reduction to NH_4^+ (Myrold 2005). In soils sampled both pre- and post-incubation, there was no effect of treatment on biomass ^{15}N incorporation.

There were no significant differences in gross mineralization, gross NH_4^+ consumption or NH_4^+MRT among experimental treatments (Table, 1, 2). Gross mineralization and gross NH_4^+ consumption rates (data not shown) were similar in magnitude and positively correlated ($r^2 = 0.22$, $P < 0.05$). In pre-incubation soils, the turnover of the NH_4^+ pool, or MRT, was 4.7±1.9 day^{-1}. In redox incubated soils, MRT ranged from 2.0±0.7 day^{-1} to 3.1±1.0 day^{-1}.

Gross nitrification rates were significantly affected by redox treatment (Fig. 3 and Table 2).

Table 1 Effects of fluctuating redox treatments on N pools, process rates[1] and N-gas fluxes[2]

Analysis time	Redox treatment[3]	T_0 NH_4^+ (μg N g^{-1})	T_0 NO_3^- (μg N g^{-1})	Gross N process rates (μg N g^{-1} day^{-1})		Denit. NO_3^- label (μg N g^{-1} day^{-1})		Denit. NH_4^+ label (ng N g^{-1} day^{-1})
				Mineralization	DNRA	N_2O flux	N_2 flux	N_2O flux
Pre-incubation	Intial	6.1±0.4^{ab}	1.0±0.1^{b}	11.4±2.3	2.89±0.57^{a}	0.04±0.02^{a}	<0.01±0.00^{b}	0.1±0.0^{b}
3 week post-incubation	≠ O_2	12.5±2.2^{a}	0.1±0.0^{c}	12.7±4.6	0.07±0.02^{c}	0.14±0.08^{a}	1.79±0.74^{a}	7.3±2.7^{a}
3 week post-incubation	12 h	4.3±1.0^{b}	0.8±0.5^{b}	13.6±4.0	0.04±0.02^{c}	<0.01±0.00^{b}	0.31±0.19^{ab}	0.1±0.0^{b}
3 week post-incubation	4 day	6.6±0.7^{ab}	0.1±0.0^{c}	7.5±3.7	0.07±0.01^{c}	<0.01±0.00^{b}	0.14±0.08^{ab}	0.6±0.2^{b}
3 week post-incubation	O_2	4.6±1.4^{b}	11.8±0.4^{a}	8.5±0.6	0.48±0.14^{b}	<0.01±0.00^{b}	0.57±0.46^{ab}	0.1±0.0^{b}

[1]Gross rates calculated for the 0–3 h interval following label addition. Means are presented ± standard error. Different letters indicate significantly different means and are included where the overall ANOVA model is significant at $\alpha = 0.05$

[2]Nitrogen gas fluxes calculated over 24 hs following $^{15}NO_3$ and $^{15}NH_4$ label addition, determined by ^{15}N pool enrichment

[3]Soils collected from the Luquillo Experimental Forest, Puerto Rico "initial" and incubated under four redox regimes described in text

Definitions of abbreviations: $T_0NH_4^+$ background NH_4^+ pool, $T_0NO_3^-$ background NO_3^- pool, *DNRA* gross dissimilatory nitrate reduction to ammonium, *Denit.* NO_3^- *label* N-gas fluxes following the addition of $^{15}NO_3$, *Denit.* NH_4^+ *label* N-gas fluxes following the addition of $^{15}NH_4$ label

Table 2 Effects of fluctuating redox treatments on soil N pools and gross N cycling rates* determined by ANOVA

Dependent variable	Interval	*F*	*N*	df	*P*
Soil moisture		0.58	25	4	0.679
Pre-label N pools					
NO_3^-		95.45	25	4	0.000
NH_4^+		4.92	25	4	0.001
Microbial biomass					
N		13.56	25	4	0.000
^{15}N		1.76	25	4	0.177
NH_4^+ Mean residence time	0–24 h	0.92	25	4	0.474
Gross mineralization	0–3 h	1.28	25	4	0.311
Gross nitrification	0–3 h	8.93	25	4	0.000
DNRA	0–3 h	41.47	25	4	0.000
N_2O flux	0–3 h	61.65	25	4	0.000
N_2 flux	0–24 h	3.29	22	4	0.032
Denitrification	0–24 h	1.48	22	4	0.245
Principal components axis 1	–	64.61	25	4	0.000
Principal components axis 2	–	43.20	25	4	0.000
Principal components axis 3	–	5.80	25	4	0.003

*Rates were log transformed for ANOVA analysis

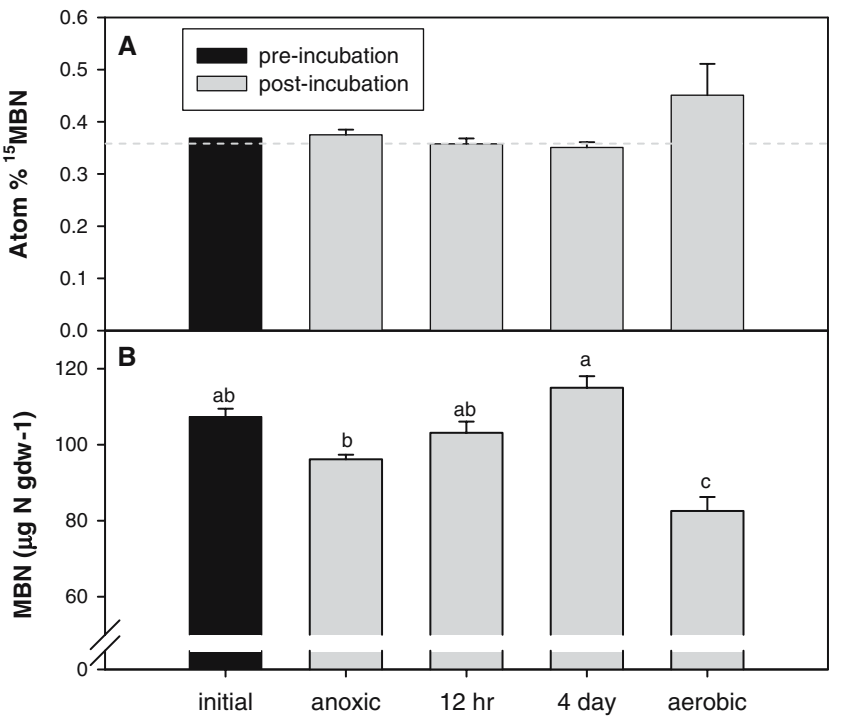

Fig. 2 (**A**) Biomass ^{15}N enrichment (atm%) (µg N g^{-1}) and (**B**) microbial biomass nitrogen pools from upland humid tropical soils. Values were measured 24 h after $^{15}NO_3$ label addition to pre-incubation (initial) soils as well as soils incubated for 6 weeks under four redox regimes (described in text). Means presented ± standard error bars. The dotted line shows pre-incubation natural abundance ^{15}N atm% for reference

Following incubation, aerobic and 4-day fluctuation soils had high rates of gross nitrification that were comparable to rates measured in pre-incubation soils (Fig. 3). We note however, that there was still measurable nitrification in anoxic soils, and that this capacity persisted at the 6-week measurement point (1.2 ± 0.1 µg N g^{-1} day^{-1}). Relative to NH_4^+ assimilation, nitrification was a much more significant fate for mineralized N in aerobic soils; in pre-incubation and variable redox soils, nitrification represented only 4–25% of mineralized N.

As mentioned above, we measured no significant $^{15}NO_3$ assimilation into microbial biomass. This confirms that the enriched NH_4^+ we measured following $^{15}NO_3^-$ addition was a result of

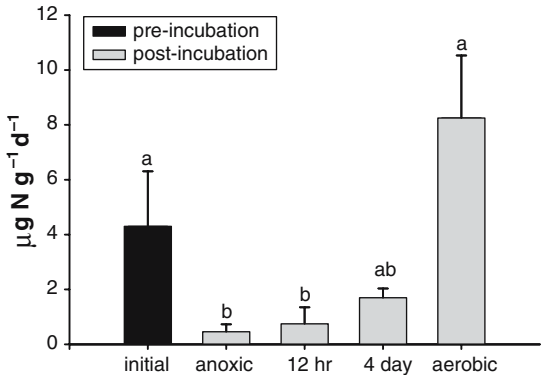

Fig. 3 Redox treatment effects on gross nitrification rates (μg N g^{-1} day^{-1}) measured between 0 and 3 h after $^{15}NO_3$ label addition in upland humid tropical soils from Puerto Rico. Treatments are described in detail in text. Means are presented ± standard error and letters indicate significant differences between treatments

DNRA. Dissimilatory nitrate reduction to ammonium occurred in all samples and was significantly higher in pre-incubation soils relative to incubated soils at the 3-week time point (Table 1). There was a significant effect of redox treatment on DNRA; interestingly, DNRA was elevated in aerobic soils relative to other redox treatments. There were no significant differences in DNRA rates between the anoxic and fluctuating treatments. By 6 weeks, DNRA in aerobic soils was no longer elevated relative to the other treatments (data not shown). DNRA rates were strongly dependent on substrate pool size, as reflected by DNRA rate constants (k_{DNRA}). DNRA rate constants (Fig. 4a) indicate that the process was more competitive for available substrate in pre-incubation, anoxic, and 4-day fluctuation soils than in the other two redox treatments ($P < 0.001$).

Nitrous oxide fluxes were substantially elevated in the 3 h immediately following $^{15}NO_3^-$ label addition; however, at this point in our assays, $^{15}N_2$ values remained indistinguishable from natural abundance levels. By 24 h post-label, substantial headspace N_2O had been further reduced to N_2, and we were able to detect enriched $^{15}N_2$ in all treatments. Therefore we only present N gas fluxes and denitrification rates from the longer (0–24 h) time interval (Table 1). Denitrification (the sum of N_2 and N_2O production) was highly variable and not significantly different among the four redox incubation treatments and pre-incubation soil (Table 2), yet tended to be higher in anoxic soils (1.93±0.76) relative to pre-incubation (0.04±0.02), 12-h (0.31±0.17), 4-day (0.14±0.08) and aerobic soils (0.57±0.46). The denitrification rate constant ($k_{denitrification}$), which reflects the magnitude of the rate relative to available NO_3^-, was significantly higher in anaerobic soils relative to all other treatments ($P < 0.001$) (Fig. 4b).

A very small portion of added $^{15}NH_4^-$ was lost from this soil as N_2O or N_2 gas during nitrification. When $^{15}NH_4^+$ label was added, $^{15}N_2O$ and $^{15}N_2$ values were near natural abundance levels, both $^{15}N_2O$ and $^{15}N_2$ fluxes were three orders of magnitude lower than comparable fluxes measured after NO_3^- labeling (Table 1). This N_2O may have resulted directly from nitrification or from nitrification followed by denitrification. Since we generally saw higher N-gas loss from more anoxic soils (as expected (Firestone and Davidson 1989)), our results support the assertion that N_2O is primarily derived from NO_3^- denitrification in humid environments (Davidson et al. 1986).

Links to bacterial community structure

The bacterial community in this soil was initially very diverse with more than 179 unique terminal restriction fragments (TRFs) detected. During 3 weeks of incubation, soil communities exposed to 12 h fluctuations or anoxic incubation changed substantially as compared to the original soil community in both principal components (Fig. 5) and NMS ordinations (data not shown). Along the first principal component (50% of variance explained), soils treated with 4-day redox fluctuation cycles retained a statistically indistinguishable bacterial community composition from the original soil, whereas anoxic and aerobic treatments moved sharply apart in ordination space (Fig. 5). The second and third principle component axes explained successively less variance in the community profile (20.3% and 9.4%, respectively).

The relationship between bacterial community composition and soil environmental or N-cycling variables was best predicted by a group of

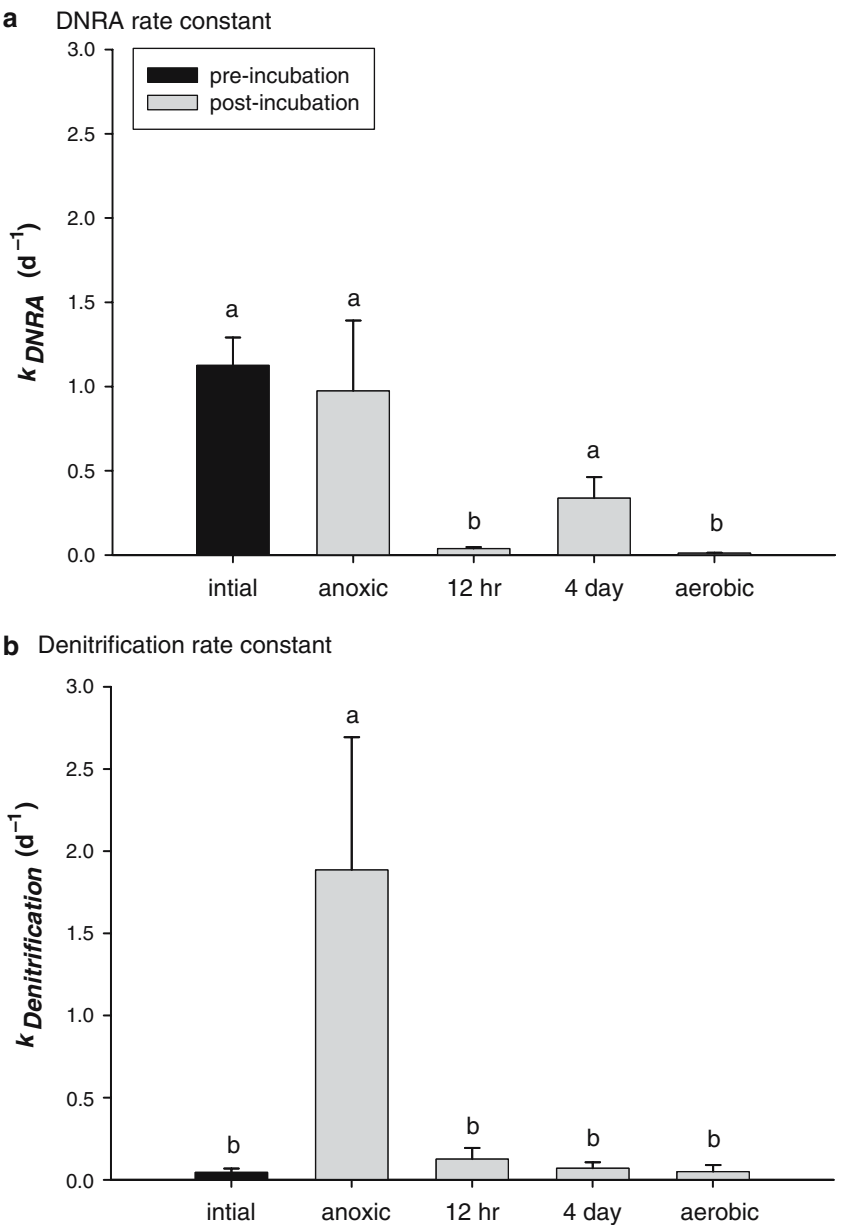

Fig. 4 Dissimilatory nitrate reduction to ammonium and denitrification rate constants (day^{-1}) for processes measured after $^{15}NO_3^-$ tracer was added to upland humid tropical soils from Puerto Rico. Rate constants (k) represent the gross rate ÷ average NO_3^- pool size. Values are means (+standard error) from pre-incubated soils (initial) and soil incubated for 3 weeks under four redox treatment regimes (described in the text). Letters indicate significant differences between treatments

variables that are all linked to production and consumption of NO_3^-. In a stepwise analysis, we noted that 0–3 h rates were generally well-correlated with 0–24 h rates. In most cases, the 0–3 h rates were removed from the analysis, as they had lower explanatory power due to their higher variability. Multiple linear regression of N-cycling rates and inorganic pools against the T-RFLP ordination indicated significant correlations between the bacterial community and denitrification, nitrification and DNRA (Fig. 5). Stepwise regression showed that a combination of inorganic N pool sizes, nitrification, and DNRA and denitrification rate constants are correlated with bacterial community structure (Table 3). For the three primary principal components axes, these variables explain half to two-thirds of overall bacterial community variability.

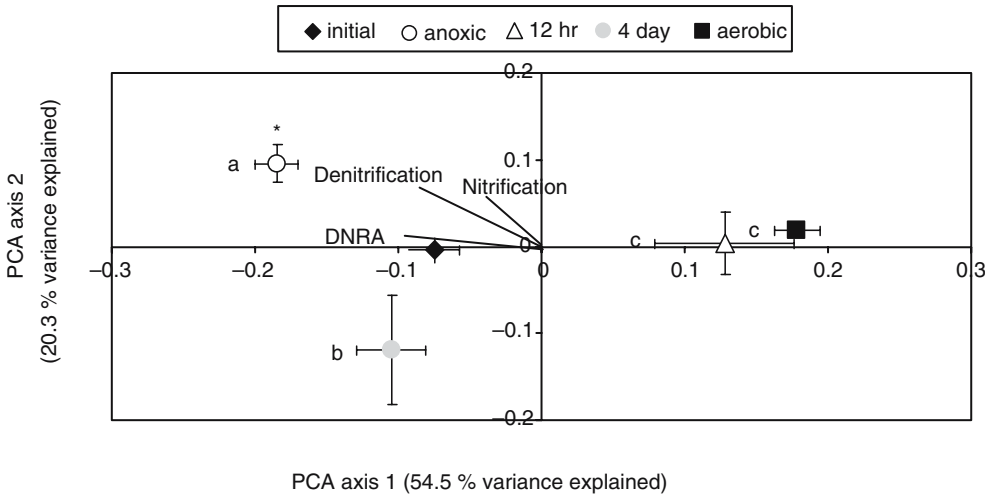

Fig. 5 Biplot of bacterial Terminal Restriction Length Polymorphism (T-RFLP) principal components ordination and correlated environmental variables measured in upland humid tropical soils from Puerto Rico. Variables regressed against microbial community ordination include those listed in Table 1 and are displayed only where $r^2 > 0.2$ and $P < 0.05$. Vector length indicates direction and strength of community-process correlation. Points are treatment means (±standard error) of five replicates, and pairwise differences between treatments are indicated by lowercase letters (PC1) or asterisks (PC2). Treatments are described in text

Table 3 Results of stepwise regressions in determining controls on microbial community fingerprints

Parameter	Regression coefficient	Partial R^2	P
PCA axis 1: R^2 for full model = 0.671, n=25			
Average $[NO_3^-]_{0\text{-}24}$	–0.100	–0.935	0.004
Average $[NH_4^+]_{0\text{-}24}$	–0.289	–0.948	0.001
0–24 h nitrification rate	0.240	0.966	0.000
$k_{\text{DNRA 0–24}}$	–0.443	–0.902	0.002
PCA axis 2: R^2 for full model = 0.693, n=25			
Average $[NO_3^-]_{0\text{-}24}$	0.093	–0.937	0.000
Average $[NH_4^+]_{0\text{-}24}$	0.167	–0.950	0.002
$k_{\text{DNRA 0–24}}$	0.241	–0.903	0.004
PCA axis 3: R^2 for full model = 0.533, n=25			
0–24 h nitrification rate	–0.022	0.262	0.034
$k_{\text{denitrifcation 0–24}}$	0.023	0.001	0.020

Results are presented only for parameters significant at $P < 0.05$. Variables tested included those listed in Table 1. PCA1, 2 and 3 are principal component axes from ordination of bacterial T-RFLP fingerprints. Nitrogen cycling rates and concentrations log transformed for analysis

Discussion

Effects of soil redox fluctuation on microbial communities

In a soil that naturally experiences rapid redox fluctuation, we found that a 4-day oxic–anoxic incubation regime best approximated microbial communities and the gross N mineralization/nitrification rates measured in field-fresh, untreated soils. The community composition of soils incubated under a 4-day fluctuation regime most closely resembled the molecular profiles of field samples. Microbial biomass patterns support a similar conclusion; fluctuating treatments appeared to maintain and/or promote biomass growth, whereas static anaerobic and aerobic treatments caused a significant loss of microbial biomass N. We note that these two results are independent measures, as a change in microbial

community structure does not necessitate nor preclude a change of total biomass. The significant decline in soil microbial biomass in samples incubated under static aerobic and anoxic conditions indicates that the constant redox treatments put considerable stress on the microbial community. Both biomass and community structure data indicate that microbial communities in these soils are adapted to rapidly fluctuating redox conditions and are clearly distinct from communities adapted to stable anoxic or oxic conditions.

How redox variability affects N cycling

Our findings indicate that the rapidly fluctuating redox conditions that characterize these upland soils could allow anoxic and oxic N processing to co-occur over short timescales. As might be expected, based on our understanding of the bacterial physiologies involved, NH_4^+ accumulated in anoxic soil, while NO_3^- accumulated in oxic soils. However, in soils incubated under fluctuating redox regimes, neither inorganic pool accumulated significantly. In the field, this pattern of redox-dependent substrate availability might select for nitrifiers, denitrifiers and DNRA organisms that respond rapidly following redox shifts in order to take advantage of new conditions before plant uptake or leaching depletes inorganic N resources.

Gross mineralization and consumption rates in this soil were unaffected by redox treatment and were comparable to rates measured in the field and in other humid tropical forests (Silver et al. 2001, 2005; Breuer et al. 2002; Neill et al. 1999). Since N-mineralization/immobilization is not directly under redox control, the fact that N-mineralization is insensitive to redox conditions is not surprising. Regardless, the literature holds conflicting evidence on this topic. Myrold (1987) reports no effect of anaerobic assays, while others suggest N mineralization is diminished in anaerobic settings (Ambus et al. 1992; Matheson et al. 1997) and it is widely accepted that organic matter mineralization is retarded under anaerobic conditions (Tiedje et al. 1984). There have been limited investigations of the mechanisms underlying these patterns (Schimidt-Rohr et al. 2004) and this area deserves further study.

Depending on the redox regime, gross NH_4^+ consumption was partitioned differently between assimilation and nitrification. In aerobic soils, nitrifiers used up to 100% of NH_4^+ generated by mineralization, and thus accounted for nearly all of NH_4^+ consumption. However, in pre-incubation and fluctuating redox treatments, nitrification accounted for only 25–50% of mineralized N; the reminder was presumably assimilated. This suggests that nitrifiers in aerobic soils compete better for NH_4^+ than nitrifiers who are responding following a period of O_2 limitation. It is not clear if the aerobic communities' advantage is attributable to differences in nitrifier community composition or physiology, or simply a higher nitrifier biomass.

In contrast to gross mineralization, gross NO_3^- production was strongly favored by aerobic conditions, although the nitrifiers indigenous to this soil appeared to be resilient to anoxic exposure. While nitrifying activity was significantly reduced by anoxic and 12-h redox fluctuation incubations, nitrifiers retained measurable activity even after 6 weeks without significant O_2. In upland soils, nitrification rates generally decline as soils become saturated (Breuer et al. 2002; Corre et al. 2003). However, research done on anaerobic lake sediments and the root zone of aerenchymatous plants shows nitrifiers have the capacity to react quickly when O_2 influxes occur, and their activity can persist in sites with periodically reduced conditions (Bodelier et al. 1996; Hall and Jeffries 1984; Jensen et al. 1993; Smorczewski and Schmidt 1991). Our results indicate that resilient ammonia oxidizers exist in tropical soils with variable redox, and appear to have substantial tolerance to low redox periods. Nitrifiers that tolerate low O_2 may have the capacity to use cellular reserve materials efficiently and at low rates in ways that are analogous to soil bacteria living under starvation conditions (Heins 1987). Alternatively, they may grow preferentially near root channels and macropores where favorable redox conditions are more likely to occur.

In this soil, the potential microbial fates for NO_3^- produced during aerobic periods are assimilation, denitrification, and DNRA. We found no evidence of NO_3^- assimilation, and observed that denitrification tended to be highest in soils that

had been anoxic for several weeks. Soils incubated under low and fluctuating redox had a substantially lower capacity for DNRA than pre-incubation soils. This could have been a delayed effect of soil homogenization (disrupting anaerobic microsites), or a loss of obligate anaerobes such fermentative *Clostridia* spp. which use DNRA as a sink for electrons in order to oxidize NADH (Caskey and Tiedje 1979). Among soil incubations, the degree of anoxic exposure had no effect on DNRA rates in these soils. While there is some indication that DNRA may be promoted by progressively more anoxic environments (Buresh and Patrick 1981; Jorgensen 1989; Sorensen 1978), other studies report that DNRA is relatively insensitive to preceding redox conditions, and more strongly controlled by NO_3^- concentration (Ambus et al. 1992; Silver et al. 2001). In ^{15}N tracer studies, Silver et al. (2001, 2005), measured rates of DNRA ranging from 0.5 to 9 mg g^{-1} day^{-1} in soils from a wide range of upland tropical forests and plantations. In the present study, DNRA rates were actually highest in aerobic soils. While this result is clearly linked to higher NO_3^- availability in the aerobic versus anoxic soils, it nonetheless suggests that the variable redox environment in this upland tropical soil selects for flexible DNRA organisms that tolerate limited O_2 exposure and may have multiple DNRA enzymatic pathways regulated by the changing availability of reductant, NO_3^- and O_2 (Cole 1996; Vanmiegroet et al. 1990). These organisms may be typically confined to anaerobic zones within aggregates (Tiedje et al. 1984). The fact that DNRA occurred in aerobic soils at 3 weeks, yet not after 6 weeks, may reflect the slow disappearance of these microhabitats.

Comparing DNRA to denitrification (the flux of $^{15}N_2O+^{15}N_2$) provides an estimate of two important fates of soil NO_3^-. In sediment and wetland soils, denitrification is often a more significant sink for NO_3^- (Ambus et al. 1992; Buresh and Patrick 1981; Matheson et al. 1997), and the same is thought to be true in upland soils. However, in the current study, we measured 70 times more DNRA than denitrification in soils freshly sampled from an upland tropical forest. This pattern reversed after soils were incubated, where DNRA rates accounted for only 4–85% of denitrification rates, regardless of soil redox regime. In addition to the disruption of anaerobic microsites mentioned previously, the explanation for these patterns may lie in the relationship between NO_3^- reduction and available NO_3^- and O_2. General rate constants for these two reductive dissimilatory processes indicate that their reduction per unit NO_3^- is roughly equivalent under consistently anoxic and aerobic conditions. However, in pre-incubation and 4-day fluctuating soils, $k_{DNRA}>k_{denitrification}$, suggesting that under highly variable redox conditions, DNRA microbes are more competitive for NO_3^- than denitrifiers. Due to its higher energy yield, denitrifiers preferentially use O_2 as an electron acceptor when possible; in a fluctuating redox soil, they may rapidly switch between O_2 and NO_3^- respiration. In contrast, DNRA microbes are much less physiologically flexible, whether they are obligate anaerobes such as *Clostridia*, or facultative organisms that can only respond to an influx of O_2 through the relatively slower transition from fermentation to respiration. These physiological traits help illuminate our results, as they indicate that denitrifiers should dominate NO_3^- use in situations where O_2 is consistently limiting, whereas microbes that mediate DNRA may be more competitive for NO_3^- in a fluctuating redox environment. Previous models of the competitive interactions between DNRA microbes and denitrifiers suggest that when C availability is held constant, DNRA will dominate when NO_3^- concentrations are elevated and in the range of zero-order kinetics (Tiedje et al. 1982). However, King and Nedwell (1985) found that lower NO_3^- concentrations promoted relatively more DNRA in anoxic sediments. Our results suggest that in upland soils the controls on these two processes are more complex than either of these model indicates, and that the dominant fate of NO_3^- depends on the periodicity of O_2 and NO_3^- availability, as well as the physical distribution of different dissimilatory NO_3^- reducers within the soil matrix.

Linkages among redox regimes, microbial communities and N processes

Our results indicate that redox regimes drive bacterial community structure which subsequently

constrains N processing and chemistry. Microbial community structure was particularly closely linked to N cycling processes that regulate NO_3^- production and consumption and soils that were stressed by static redox regimes had altered N functioning. For example, stepwise regression analysis showed that variations in gross nitrification and dissimilatory NO_3^- reduction explain a significant amount of T-RFLP community structure. Soils in which nitrification was high had different bacterial communities than soils with high denitrification and k_{DNRA}. Community differences among treatments were particularly well correlated with redox-sensitive N processes, suggesting that ecological characteristics of microbial communities may be important controllers of biogeochemistry. Interestingly, communities from soils fluctuating on 4-day vs. 12-h regimes were genetically different, though had equivalent rates of N-processing, suggesting that different communities can mediate processes in similar ways.

Because the presence/absence of soil O_2 is a critical determinant of microbial metabolism, spatial gradients of redox are expected to result in spatial stratification of microbial physiologies. Analogous patterns have been observed temporally such as seasonal wetland redox gradients where changes in aerobic vs. anaerobic phospholipids are concomitant with changes in Mn geochemistry (Keith-Roach et al. 2002). Whether spatial or temporal, this pattern infers that discrete microbial groups carry out discrete functions, and their dominance shifts in turn as redox potential changes. In contrast, the microbial communities of rapidly fluctuating environments may have evolved to be opportunistic and metabolically plastic. This second scenario is more likely under conditions in which redox change occurs faster than populations can respond via growth. Adaptation to such stochastic regimes may select for flexible microorganisms that are able to respire/ferment or use multiple electron acceptors, and have enzymatic capacities that allow rapid response, enabling them to utilize transient resource pulses before they are lost. Thus, in soils where O_2 is frequently depleted and re-supplied, characteristics of microbial tolerance and resilience may frame N cycling patterns.

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