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Climatic and Edaphic Controllers of Soil N Transformations and Microbial Community Composition in Coast Redwood Forests

By

Damon Charles Bradbury

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Environmental Science, Policy and Management

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Mary K. Firestone, Chair
Professor Whendee L. Silver
Professor Steven E. Lindow

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Abstract

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University of California, Berkeley

Professor Mary K. Firestone, Chair

Coast redwood forests comprise one of the most cherished ecosystems in California, if not the world. There is concern that changes in climate, particularly in summer fog frequency and duration, may negatively impact redwood forests; however, little is known about how climate change will impact soil nitrogen (N) transformations and soil microbial community composition in these forests. These topics deserve further study because soil microbes perform critical ecosystem functions, including decomposition and nutrient transformations, and N is an essential macronutrient that often limits growth in temperate forests. In this dissertation, I examine the influence of climatic and edaphic factors on both soil N transformations and microbial community composition in coast redwood forests, particularly with respect to potential impacts of future changes in climate.

In Chapter 1, I report the results of an initial study conducted to measure rates of gross N mineralization and nitrification in redwood forests, as well as the potential for the dissimilatory reduction of nitrate to ammonium under low redox conditions and at warmer temperatures. Soils were collected in late winter, during the wettest time of year. The two northern redwood forests studied have high gross rates of N mineralization and nitrification as well as ammonium and nitrate consumption and cycle inorganic N tightly during the cool, moist winter. Gross nitrification rates were close to 4 $\mu g\ N \cdot g\ soil^{-1} \cdot day^{-1}$ in both sites at field temperature; these relatively high rates indicate that nitrification is not inhibited in redwood forests. Exposing the soils to low redox conditions stimulated DNRA and $^{15}N_2O$ production in these forests, suggesting that both processes can be important in areas of low redox soil, especially at warmer temperatures.

Next, I conducted a reciprocal transplant experiment across the latitudinal gradient of coast redwoods to study the potential for climate change to alter rates of gross N mineralization and nitrification and soil microbial community composition in redwood forests. Soils were reciprocally transplanted among three sites and collected after one and three years. This transplant experiment focused on soil conditions at the end of the summer because this is a time of year when climate can differ dramatically across the north-south gradient of coast

redwoods, especially in terms of fog, which is a defining characteristic of coast redwood forests during the otherwise dry summer. Results from analyses of the impact of climate change on soil N transformations and soil microbial community composition are presented in Chapters 2 and 3, respectively.

After one year, rates of gross N mineralization and nitrification, the abundances of ammonia oxidizing bacteria (AOB) and archaea (AOA), and several edaphic characteristics, including soil water availability, were measured (Chapter 2). While rates of gross N mineralization varied among soils of different origins, they did not differ in response to transplanting. In contrast, rates of gross nitrification changed significantly in response to transplantation into a new climate. Gross nitrification was sensitive to water availability, and rates of gross nitrification were very low below a soil water potential of -0.05 MPa. Above this soil water potential, rates varied widely. Rates of gross nitrification were correlated with the abundances of AOA and AOB, which were correlated with water availability. The results suggest that the abundances of AOA and AOB and rates of gross nitrification (and nitrate availability) are likely to be more influenced by changes in summer climate (fog frequency) in coast redwood forests than rates of gross N mineralization (and ammonium availability).

For Chapter 3, the impacts of transplant-induced changes in climate on soil microbial communities were examined. Soil bacterial and fungal communities were examined after one year by terminal restriction fragment length polymorphism (T-RFLP), and bacterial communities were also examined with a high-density 16S rDNA oligonucleotide microarray (G2 PhyloChip) after one and three years. Both fungal and bacterial communities changed in composition after one year, and the patterns in compositional change persisted for bacteria after three years. Soil characteristics interacted with climate to frame the magnitude and character of these changes in community composition; the variability in community composition was correlated with edaphic as well as climatic variables for both bacterial and fungal T-RFLP. Of the over 2,000 bacterial taxa detected on the G2 PhyloChip, 3.2% differed in relative abundance between transplants after one year, while 12.2% differed after 3 years. The bacterial taxa responding to transplant-induced climate change showed strong phylogenetic clustering by net relatedness analyses after both 1 and 3 years. There appear to be taxonomic, and phylogenetic, patterns in the speed of the soil bacterial response to changing climate, and the patterns of community change indicate that the impact of climate change on microbial community composition should be assessed using reasonably long-term (multi-year) experiments.

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Chapter 1

Soil N dynamics in coast redwood forests: inorganic N production and consumption and the potential for dissimilatory reduction of nitrate to ammonium and nitrous oxide

Abstract

Coast redwood forests are a highly cherished ecosystem and the focus of intensive conservation efforts. However, little is known about soil N transformations in these forests. This study provides the first measurements of gross rates of soil N-cycling processes in coast redwood forests, including N mineralization, nitrification, and dissimilatory nitrate reduction to ammonium (DNRA), which has rarely been measured in temperate conifer forests yet has the potential to act as an important N conservation mechanism. Two northern Californian coast redwood forest sites were sampled in late winter in March 2002, during a wet time of year. In laboratory incubations, soils were exposed to two temperatures (7 °C and 23 °C) and two headspace conditions (ambient air and N_2 headspace). Incubating soils at lab temperature (23 °C) as opposed to field temperature (7 °C), generally stimulated process rates, except for nitrification. Rates of N mineralization were higher in the site with the higher organic matter content and correlated with the concentration of soluble organic nitrogen. Gross nitrification rates were around 4 μg $N \cdot g\ soil^{-1} \cdot day^{-1}$ in both sites at field temperature and thus do not appear to be inhibited in redwood forests. Gross consumption rates of ammonium and nitrate were significantly related to gross rates of N mineralization and nitrification, respectively; coast redwood forests cycle inorganic N tightly during the cool, moist winter. Exposing the soils to low redox conditions stimulated DNRA and $^{15}N_2O$ production, suggesting that both processes can be important in areas of low redox soil. Rates of both DNRA and $^{15}N_2O$ production were strongly correlated with CO_2 production rates under low redox conditions, but only DNRA under the ambient atmosphere. Higher rates of DNRA than $^{15}N_2O$ production under the ambient headspace and the stronger response of $^{15}N_2O$ production than DNRA to low redox conditions indicate that denitrification may be more sensitive to oxygen than DNRA.

Introduction

Coast redwood forests are cherished ecosystems in California that may be endangered by harvesting and climate change. However, there is little information concerning nitrogen (N) cycling in these forests (Noss et al. 2000, Ewing et al. 2009). An understanding of controls of soil N transformations is important because the availability of N can limit productivity in temperate forests (Vitousek and Howarth 1991), while fluxes of nitrous oxide (N_2O) contribute to the greenhouse effect. Early studies of inorganic N concentrations in coast redwood forest soils reported very low concentrations of nitrate (NO_3) and concluded that

there was virtually no nitrate production (Bollen and Wright 1961, Florence 1965). Monoterpenes from redwood needles were shown to inhibit nitrification of an ammonia-oxidizing bacteria, *Nitrosomonas europea* (Ward et al. 1997), and the production of these compounds by redwoods was assumed to be a mechanism of allelopathic inhibition. Since this time, however, the use of stable isotopes to measure gross rates of N mineralization and nitrification has greatly increased our general understanding of soil N transformations in coniferous forest ecosystems (Davidson et al. 1992, Hart et al. 1994, Stark and Hart 1997). In mature conifer forests, rates of gross nitrification can be as high as those found in any ecosystem, as is true for gross N mineralization and ammonium (NH_4) and nitrate (NO_3) consumption (Stark and Hart 1997, Booth et al. 2005).

Much of the NO_3 produced by nitrification can be immobilized by soil microbes under conditions of high carbon (C) and oxygen availability, such as those found in old-growth forests (Stark and Hart 1997). Nitrate is also subject to several other fates including leaching losses (Vitousek et al. 1979), and dissimilatory reduction to dinitrogen (N_2, denitrification) or ammonium (NH_4, DNRA) by microorganisms (Tiedje 1988). The dynamics of the production and consumption of inorganic N greatly influence the magnitude and form of N loss from forests.

Dissimilatory reduction of nitrate to ammonium (DNRA) is widespread, and it can be an important fate of nitrate in soils under low redox conditions (Silver et al. 2001, Burgin and Hamilton 2007). Yet, little is known about rates of DNRA in high-rainfall old-growth conifer ecosystems. DNRA has been previously shown to reduce NO_3 to NH_4 in highly reducing anaerobic sludge digesters and anoxic sediments (Sorenson 1978, Tiedje 1988), and the importance of DNRA has been evaluated in tropical forest soils (Silver et al. 2001, 2005, Huygens et al. 2007, Templer et al. 2008, Rutting et al. 2008) and aquatic ecosystems (Burgin and Hamilton 2007). DNRA has also been found to occur in temperate wetlands (Matheson et al. 2002), old grassland soils (Muller et al. 2004) and a fertilized spruce forest (Bengtsson and Bergwall 2002).

Rates of DNRA and the partitioning of NO_3 between DNRA and denitrification depend on several environmental factors, including the redox status of the immediate environment and the supplies of organic C and NO_3 (Tiedje et al. 1982, Tiedje 1988). Conditions of high C availability relative to NO_3 could favor DNRA because DNRA has a higher capacity to dissipate electrons per mol of nitrate than denitrification (Tiedje et al. 1982). With an ample supply of electron donor (carbon, C), anaerobic heterotrophs may be limited by the availability of electron acceptors. When the availability of C relative to NO_3 declines, denitrifiers could have a greater advantage than DNRA-capable organisms because they can generate more energy per mol of nitrate reduced. Many studies have found that increasing C availability favors DNRA in soils (Ambus et al. 1992, Yin et al. 2002, Fazzolari et al. 1998, Silver et al. 2005). The roles of C and nitrate availability in the partitioning of nitrate between DNRA and denitrification appear to vary somewhat across ecosystems (Fazzolari et al. 1998, Silver et al. 2001, 2005, Ambus et al. 1992). The results of some studies could suggest that DNRA may be less sensitive to oxygen concentrations in the soil than denitrification (Fazolarri et al. 1998, Silver 2005, Pett-Ridge et al. 2006). The relative influence of C, NO_3 and oxygen availability on the relative importance of DNRA and denitrification is not fully understood.

The two primary objectives of this study were to: 1) explore N-cycling dynamics in coast redwood forests using ^{15}N isotopic methods and 2) determine the potential for DNRA to be a mechanism of NO_3 consumption in old-growth coniferous forests receiving high rainfall in the winter. Old-growth coast redwood forest soils in far northern California were chosen because they receive 1.5 to 2.5 meters of rainfall a year, almost entirely from November through May, and have very high rates of productivity and soil organic matter accumulation. These wet productive temperate forests may be locations of high rates of DNRA, nitrous oxide production, and N-cycling in general.

Methods

Site selection and soil sampling

Samples were collected from two locations in old-growth coast redwood (*Sequoia sempervirens*) forests in far northern California, Prairie Creek Redwoods State Park (PCSP) (41.41º N / -124.02º W) and Jedediah Smith State Park (JSSP) (41.78º N / -124.10º W) (Table 1). Sites were located under coast redwood overstory with an understory of sword fern (*Polystichum munitum*). The soils at these sites have high concentrations of organic C and N and are moderately acidic (Table 1). The coast redwood forest soils in this region are mainly classified as Typic Haplohumults or Paleohumults (J. Seney, USDA Soil Survey, personal communication). Mean annual precipitation in these sites is ~1,700 mm, and mean annual temperature is ~11 °C, ranging from 5 °C to 16 °C (obtained from NOAA National Climatic Data Center weather station data for Orick Prairie Creek Park, Station 046498, 41° 22′ N and 124° 01′ W, and Crescent City 3 NNW Station 042147, 41° 48′ N and 124° 13′ W). Soil temperature ranges from 7 °C to 15 °C throughout the year, an isomesic soil temperature regime.

Soils were sampled from both sites on 4 March 2002 after a recent rain; soils were moist and the soil temperature was ~7°C (Table 1). In each site, a 15-meter long transect was established and 7 samples were collected at 2-meter intervals. For each sample, the O horizon was removed and ~2 kg of soil were collected from the 0-10 cm depth of the A horizon. Soil samples were stored in a cold room at field temperature (7 °C) overnight and processed the following day. The samples were mixed well and a 10-gram subsample was taken for the measurement of gravimetric soil moisture and total soil C and N analysis. Each sample was then divided into 8 approximately 150-gram samples. The 8 sub-samples were then divided between 4 treatments: 1) 7 °C and ambient atmosphere, 2) 23 °C and ambient atmosphere, 3) 7 °C and N_2-flushed atmosphere and 4) 23 °C and N_2-flushed atmosphere. For each treatment, one sub-sample was labeled with $^{15}NH_4$ and one was labeled with $^{15}NO_3$ as described below.

A laboratory experiment was conducted similar to that of Silver et al. 2005. After a pre-incubation period of ~15 hours (overnight), half of the 150-g samples were labeled with 1 ml of 27.2 μg ^{15}N as $(^{15}NH_4)_2SO_4$, and the other half with 1 ml of either 22.7 or 87.2 μg ^{15}N as $K^{15}NO_3$ (for JSSP and PCSP, respectively) for a targeted increase of 15 atom % in the NH_4 or NO_3 pools. The ^{15}N-labeled soil was mixed well for 3 minutes and then split into: 1) an initial 50-g sample that was extracted in 150 ml of 2M KCl after 15 min and 2) a 100-g sample that was incubated for 3 hours in a pint-size mason jar sealed with a lid with a septum.

After the 3-hour incubation, two 30-ml gas samples were taken from the headspace and stored in evacuated serum vials. Then the 100 g of soil were divided 4 ways: 1) 50 g of soil (~15-20 g dry weight) was extracted in 150 ml of 2M KCL, 2) 20 g was extracted in 80 ml of 0.5M K_2SO_4, 3) another 20 g was fumigated with ethanol-free chloroform for 5 days and then extracted in 80 ml of 0.5M K_2SO_4, and 5) the last 10 g were used to determine gravimetric water content. The pre-incubations for the ambient and N_2-flushed headspace samples were done the first and second nights after sampling, respectively. The N_2-flushed samples were placed in an N_2-flushed glove bag at either 7 °C or 23 °C for the pre-incubation period, and the headspaces of the mason jars were flushed with N_2 before the 3-hour incubation.

Soil analysis

Soil moisture was determined gravimetrically after drying at 105 °C for over 24 hours. Soil pH was measured on a 1:1 solution of soil:0.01M $CaCl_2$. Total C and N were measured by combustion on a CN analyzer (CE Elantech 2500, Lakewood, NJ). Soil was extracted in 2M KCl using standard methods, and NH_4 and NO_3 + NO_2 concentrations were determined colorimetrically using an autoanalyzer (Lachat Quik Chem flow injection analyzer, Lachat Zellweger Instruments, Milwaukee, Wisconsin, USA). Ammonium and NO_3 were diffused from the extract solution (Herman et al. 1995), and N isotope ratios were measured using an automated N and C analyzer coupled to an isotope-ratio mass spectrometer (ANCA-IRMS; PDZ Europa Limited, Crewe, UK). Concentrations of N_2O and CO_2 were determined by gas chromatography using an electron capture ^{63}Ni detector and thermal conductivity detector, respectively (GC8610c, SRI Instruments, Torrance, CA, USA). Nitrogen gas isotope ratios of N_2O were determined using a trace gas module coupled to the IRMS. Total N in the 0.5M K_2SO_4 extracts was measured by Kjeldahl digestion and colorimetry. Total organic carbon in the K_2SO_4 extracts was measured on a TOC analyzer (OI Analytical 1010 TOC analyzer, College Station, TX). Microbial biomass C and N were calculated as the difference between the samples fumigated with chloroform and the unfumigated samples. A correction factor of 0.54 was applied (Brookes 1985). Kjeldahl-digested samples were also diffused and run on the ANCA-IRMS to determine microbial biomass atom% ^{15}N, calculated as the difference in ^{15}N between the fumigated and unfumigated sample extracts divided by the difference in total N in the extracts multiplied by 100. The concentrations of organic C and N in the initial unfumigated 0.05M K_2SO_4 extracts were used as indices of soluble organic C (SOC) and N (SON)

Rate calculations

Gross rates of N mineralization, nitrification, and NH_4 and NO_3 consumption were calculated according to Kirkham and Bartholomew (1954). Rates of N_2O and CO_2 production were calculated as the change in concentration (vol/vol) over the course of the experiment, converted to mass using the ideal gas law and expressed as ng or μg $N \cdot g^{-1} \cdot day^{-1}$ or μg $C \cdot g^{-1} \cdot day^{-1}$ to compare to other process rates. Rates of DNRA were calculated according to Silver et al. 2005. The increase in $^{15}NH_4$ atom% over the incubation period was multiplied by the average ammonium pool size and divided by the average atom % of $^{15}NO_3$ for the incubation. The increase in $^{15}NH_4$ atom% was corrected for turnover of the ammonium pool by adding the average ^{15}N in the NH_4 pool multiplied by the length of the incubation and divided by the mean residence time of the ammonium pool to the measured increase in $^{15}NH_4$. A mean

residence time of 1 day was used for all samples to standardize across treatments and because 1 day has been observed to be the typical MRT for the NH_4 pool in soil (Booth et al. 2005). The rate of $^{15}N_2O$ production from denitrification was calculated from the $^{15}NO_3$ labeled samples as the increase in $^{15}N_2O$ per gram of soil per day divided by the average atom% of the nitrate pool.

Rates of gross N mineralization, NH_4 consumption, and CO_2 production were calculated using the NH_4 labeled samples. Gross nitrification, NO_3 consumption, DNRA, denitrification, and $^{15}N_2O$ production rates were calculated using the NO_3 labeled samples. Microbial biomass C and N, SOC, and SON are averages of samples for both labels. Microbial biomass ^{15}N was analyzed separately for each label. Results for gross rates of N mineralization, nitrification, and NH_4 and NO_3 consumption were evaluated for the ambient headspace treatments only, while rates of DNRA and $^{15}N_2O$ production were examined for both the ambient and N_2-flushed headspace atmospheres.

Statistical analysis

All statistical analyses were performed using SAS JMP Version 5.1.2 (SAS Institute Inc., Cary, NC) or the R statistical environment (R version 2.10.0). Data were root or natural log (ln) transformed to improve normality and reduce heteroscedasticity when necessary. Analysis of variance was used to determine if pools or rates differed by site, temperature, headspace, or any interaction thereof. Relationships among pools and process rates were explored by pairwise correlations (Pearson r). Simple linear regression was used to further examine relationships between process rates, or process rates and soil N pools. There were 7 samples collected from each site, but one sample from the PCSP site was removed as an outlier because it had values for process rates and pools that were much more than 3X the standard deviation greater than the mean. After the removal of this outlier, a total of 52 samples were used for statistical analysis, 26 per site or headspace, and 13 per site x headspace combination.

Results

Soil C and N pools

Total soil C and N, microbial biomass C and N, soluble organic C (SOC) and N (SON), and pools of nitrate were all significantly higher in the PCSP soils than those of JSSP ($p \leq 0.01$ for site effect for all; Table 1). There were no significant differences between the two sites in pH or pools of NH_4, and the ratios of total C:N, microbial biomass C:N and SOC:SON were very similar between sites.

Impact of temperature and headspace treatments on soil C and N pools

Microbial biomass C and N did not differ among treatments after incubation at the two temperatures or atmosphere conditions; however, pools of SOC and SON did ($p < 0.05$). The pools of SOC and SON were tightly correlated with each other, as were microbial biomass C and N, and total C and N ($r = 0.86$, 0.94, and 0.96, respectively, all $p < 0.001$). All of these pools were correlated to each other as well ($0.64 < r < 0.90$, all $p < 0.001$). Microbial biomass ^{15}N measurements were not significantly enriched above background levels in samples with

$^{15}NO_3$ added. Microbial biomass ^{15}N was slightly enriched following $^{15}NH_4$ addition to JSSP samples incubated under the $^{15}N_2$ atmosphere at both temperatures and PC samples incubated at 7 °C under both atmospheres.

Nitrogen transformations under the ambient atmosphere

Gross N mineralization and gross NH_4 consumption differed significantly between sites ($p = 0.0002$) and temperatures ($p = 0.011$) under the ambient atmosphere; rates were higher in PCSP than JSSP, and rates were higher for lab temperature incubations than at field temperature (Table 2A). Net ammonium production did not differ by site ($p = 0.16$), but the interaction of site x temperature was significant ($p = 0.0003$; Table 2A).

Gross nitrification was not affected by site ($p = 0.97$) or temperature ($p = 0.40$). At 7 °C, the rates were almost identical in the two sites and together averaged 3.8 +/- 1.3 $\mu g\ N \cdot g^{-1} \cdot day^{-1}$ (Table 2A). Nitrate consumption was not significantly affected by site ($p = 0.52$) or temperature ($p = 0.52$). Net rates of nitrate production tended to be higher in PCSP than JSSP but the difference was not significant ($p = 0.18$), and temperature had no effect on net nitrate accumulation ($p = 0.85$; Table 2A).

Rates of DNRA and $^{15}N_2O$ production were orders of magnitude lower than rates of gross N mineralization and nitrification under the ambient headspace, but rates of DNRA were higher than rates of $^{15}N_2O$ production ($p < 0.0001$; Fig. 2A). DNRA was significantly higher in PCSP than JSSP ($p = 0.001$) under the ambient headspace, but $^{15}N_2O$ production did not differ between the two sites ($p = 0.61$). The production of $^{15}N_2O$ differed significantly between temperatures ($p = 0.015$), but the differences in DNRA between temperatures were only weakly significant ($p = 0.078$; Table 2).

Response of DNRA and $^{15}N_2O$ production to low redox conditions

Rates of DNRA and $^{15}N_2O$ production were much greater under the N_2 atmosphere than the ambient atmosphere ($p < 0.0001$; Table 2, Fig. 2) and were between 11 to 51% of the rates of gross N mineralization and gross nitrification measured under the ambient atmosphere. Both DNRA and $^{15}N_2O$ production were greater in PCSP than JSSP ($p < 0.0001$) and higher at lab temperature ($p < 0.0001$; Table 2B). In contrast to the ambient atmosphere incubations, NO_3 concentrations decreased over the course of the incubation at lab temperature. The decrease in NO_3 was not significantly different between sites ($p = 0.25$), but it was greater for lab temperature incubations ($p < 0.0001$). Net NH_4 production differed by temperature ($p = 0.02$) but not site ($p = 0.36$); the increase was much greater at lab temperature than field temperature (Table 2B).

Relationships between soil N transformations and other processes or nutrient pools

Gross N mineralization was positively correlated to the pools of SOC and SON as well as the rate of CO_2 production (Table 3A). Gross NH_4 consumption was positively correlated to gross N mineralization and CO_2 production, as well as SOC. Gross nitrification did not correlate well to any potentially explanatory variable that was measured, but NO_3 consumption was positively correlated to gross nitrification (Table 3A). Regression analysis showed that the relationships between 1) gross N mineralization and SON, 2) NH_4 consumption and gross N mineralization and 3) NO_3 consumption and gross nitrification were all highly significant ($p<0.0001$ for all, and $r^2 = 0.63$, 0.84, and 0.79, respectively, Figs. 1A,

1C and 1D). The relationship between gross N mineralization and CO_2 production was also significant ($r^2 = 0.61$, $p<0.0001$; Fig 1B), but CO_2 production was not significant when included with SON concentration ($p = 0.007$ for SON and $p = 0.25$ for CO_2 production).

Relationships of DNRA and $^{15}N_2O$ production with substrate pools were analyzed separately for ambient and N_2-flushed atmosphere incubations because of the order(s) of magnitude differences in these rates between the two headspace atmospheres. Regression analysis showed that, under the N_2 atmosphere, the relationships of the rates of DNRA and $^{15}N_2O$ production with the rate of CO_2 production were highly significant ($p<0.0001$) with $r^2 = 0.76$ and 0.82, respectively (Fig. 3B). The relationships of DNRA and $^{15}N_2O$ production with the ratio of CO_2 production:NO_3 concentration ($r^2 = 0.31$ and 0.26, respectively, and $p \leq 0.01$) were also significant but weaker than those found with CO_2 production alone. Neither DNRA nor $^{15}N_2O$ production were correlated with NO_3 concentrations alone in the samples under the N_2 atmosphere ($p > 0.22$, Table 3B).

Under the ambient atmosphere, DNRA was also highly positively correlated to CO_2 production, as well as the concentration of NO_3 (Table 3A). The correlations between $^{15}N_2O$ production and these variables were not significant (Table 3A). Thus, the regression between DNRA and CO_2 production ($r^2 = 0.56$, $p<0.0001$) was highly significant, but not that between $^{15}N_2O$ production and CO_2 production ($p = 0.11$, Fig. 3A). The regression of DNRA versus NO_3 was also significant under the ambient atmosphere ($r^2 = 0.47$, $p = 0.0002$), but not the ratio of CO_2 production:NO_3 concentration ($p = 0.52$). Nitrate concentrations were correlated to CO_2 production under the ambient atmosphere ($r = 0.79$, $p<0.0001$), but were not under the N_2-flushed atmosphere ($p = 0.31$)

Discussion

Gross N mineralization, nitrification and NH_4 and NO_3 consumption in coast redwood forests
As might be expected from other studies on coniferous forests (Stark and Hart 1997, Davidson et al. 1992, Pedersen et al. 1999), rates of gross N mineralization and gross nitrification are high in these moist, organic rich coast redwood forests. Higher concentrations of NO_3 than NH_4 in the PCSP soils are indicative of high nitrification rates consuming much of the mineralized NH_4. While this is not too surprising given the findings from other temperate conifer forests, it is still noteworthy considering that coast redwood forests had been long thought to be sites of very low nitrification. Measurements of low or no NO_3, no net nitrification, and the potential for monoterpenes from redwood needles to inhibit ammonia-oxidizing bacteria promoted this belief (Bollen and Wright 1961, Florence 1965, Ward et al. 1997). However, nitrate concentrations collected in tension lysimeters in the surface soil of a coast redwood site have been suggested to indicate that nitrification occurs in redwood forests (Ewing et al. 2009), and the results from this study indicate that coast redwood forests appear to be similar to other conifer ecosystems with high C availability and moist soils in that they have high rates of gross N mineralization, nitrification and NH_4 and NO_3 consumption.

Gross N mineralization has been found to positively correlate with microbial biomass and soil C and N concentrations across a range of studies (Booth et al. 2005), and this was true in our study as well. The soils from the site with the higher organic matter content had a higher microbial biomass and activity, higher concentrations of soluble organic carbon (SOC)

and nitrogen (SON) and higher rates of CO_2 production and gross N mineralization. Across sites and temperatures, gross N mineralization was highly correlated to the pool of SON and CO_2 production. These correlations demonstrate the influence of differences in organic matter content and availability between sites, as well as the influence of an increase in microbial activity with temperature, on rates of gross N mineralization.

The correlation between gross N mineralization and the SON pool could be an indication of a limitation of gross N mineralization by the break down of complex N-containing organic polymers into smaller molecules (Schimel and Bennet 2004). It is hard to know what portions of the SON pool are substrates for further depolymerization or for direct N mineralization, but a large portion of the N-containing molecules are likely available amino acid N (proteins and peptides; Mengel et al. 1999, Yu et al. 2002). In a study of rhizosphere and bulk soil from a California annual grassland, the patterns in the pool of SON mirrored those of rates of exoenzyme activity and gross N mineralization (DeAngelis 2008). Soil organic N pools have been reported to be correlated to total N, microbial biomass N, and net N mineralization in studies conducted in coniferous forests in Germany and mature red oak forests in rural New York as well (Zhong and Makeschin 2003, Zhu and Carreiro 2004).

Rates of gross nitrification in coast redwoods are at the high end of those reported for coniferous forest mineral soils (Stark and Hart 1997). Across terrestrial ecosystems, as rates of gross N mineralization increase, rates of nitrification plateau and decrease as a percentage of gross N mineralization (Booth et al. 2005). This is consistent with the results from the JSSP soils that had greater rates of gross N mineralization at 23 °C than 7 °C but no difference in gross rates of nitrification. However, for the extremely organic rich PCSP soils, the rates of both gross N mineralization and gross nitrification were higher at 23°C than 7°C, though nitrification rates varied greatly at 23°C. Overall, these results demonstrate significant rates of nitrification and nitrate cycling in coast redwood forest soils.

DNRA and $^{15}N_2O$ production under ambient headspace conditions

Under the ambient headspace condition, rates of DNRA and $^{15}N_2O$ production account for only a small portion of nitrate consumption. Rates of DNRA are 1-2 orders of magnitude less than gross nitrification, while rates of $^{15}N_2O$ production are 1-2 orders of magnitude lower than DNRA. At field temperature (7 °C), rates of DNRA averaged 0.14 and 0.03 $\mu g\ N \cdot g^{-1} \cdot day^{-1}$ in the PCSP and JSSP soils, respectively, while rates of $^{15}N_2O$ production were less than 1 $ng\ N \cdot g^{-1} \cdot day^{-1}$. Although DNRA was only around 1% of gross N mineralization and 1-2 % of gross nitrification under the ambient atmosphere, the rate of DNRA measured in the more organic rich redwood forest (PCSP) approached those of tropical forest soils in Puerto Rico, Costa Rica and Chile (Silver et al. 2001, 2005, Huygens et al. 2007, Rutting et al. 2008). The tropical forest rates are 1.7-8.6X higher, however, ranging from 0.24 to 1.2 $\mu g\ N \cdot g^{-1} \cdot day^{-1}$ in the field or at field conditions, and 8-40X greater than the rate of DNRA in JSSP. DNRA is also a greater percentage of gross N mineralization and nitrification in these tropical forest studies.

While the immobilization of NO_3 appears to be the most important fate of NO_3 in coast redwood soils, rates of DNRA are still greater than or comparable to the fluxes of NO_3 subject to leaching and N_2O losses. As mentioned above, rates of DNRA were greater than $^{15}N_2O$ production under ambient conditions. The rates of $^{15}N_2O$ production measured in these coast redwood soils ($< 1\ ng\ N \cdot g^{-1} \cdot day^{-1}$ or $< \sim 1.0\ g\ N \cdot ha^{-1} \cdot day^{-1}$) are comparable to the low

end of the range of denitrification (0.1 to 40 g $N \cdot ha^{-1} \cdot day^{-1}$) measured in forest soils of Oregon and elsewhere (Vermes and Myrold 1991, Barton et al 1999). With rates of gaseous loss so low, the leaching of soluble organic and inorganic N during and following intense periods of rain in the winter could account for much of the N loss from these forests. In a coast redwood site about 200 miles to the south where mean annual precipitation is ~1.4 meters (~300 mm lower than that of these forests), the flux of inorganic N into gravity lysimeters 70 cm deep in the soil was 6.2 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (Ewing et al. 2009). Some of this flux may not make it out of the soil. In other coniferous forests of the northwestern U.S. with similar lysimeter concentrations of inorganic N, fluxes of NO_3-N out of the system were lower and ranged from 0.3 to 3.9 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (Vitousek et al. 1979, Bockheim and Langley-Turnbaugh 1997). Thus, even though DNRA probably does not account for a large percentage of internal NO_3 cycling in temperate conifer forest, it does constitute a significant fate of nitrate compared to the amounts lost through leaching and N_2O production.

Influence of low redox on DNRA and $^{15}N_2O$ production

Exposing the soils to low redox conditions stimulated DNRA and $^{15}N_2O$ production, which suggests both can be important processes in low redox areas, such as saturated soils below pools of water that can occur in depressions in coast redwood forest in the winter. Under the N_2 atmosphere, rates of DNRA increased by 1-2 orders of magnitude and ranged from 0.43 +/- 0.06 $\mu g \, N \cdot g^{-1} \cdot day^{-1}$ to 4.8 +/- 0.6 $\mu g \, N \cdot g^{-1} \cdot day^{-1}$ in JSSP at 7 °C and PCSP at 23 °C, respectively, accounting for 10-50% of the rate of gross nitrification (measured under the ambient atmosphere). Rates of $^{15}N_2O$ production also increased dramatically, even more so than DNRA (up to 7.4 +/- 1.5 $\mu g \, N \cdot g^{-1} \cdot day^{-1}$ in PCSP at 23 °C), and under an N_2 atmosphere, rates of DNRA were 50% to 75% of the rate of $^{15}N_2O$ production regardless of temperature. The dissimilatory reduction of NO_3 caused concentrations of NO_3 to decline rapidly under the N_2 atmosphere at lab temperature, as opposed to the increases observed under the ambient headspace. When soils experience low redox conditions, DNRA competes with denitrification for NO_3 and is extremely important for preventing gaseous losses of N_2O and N_2 in organic-rich conifer forests as well as tropical forests.

While denitrification in soils is extremely sensitive to oxygen (Firestone et al. 1979, Parkin and Tiedje 1984), the results from some studies suggest that DNRA may be less sensitive to the presence of oxygen (Fazzolari et al. 1998, Silver et al. 2005, Pett-Ridge 2006, this study). For example, incubations of soils from Puerto Rico and Costa Rica under an N_2 atmosphere did not stimulate rates of DNRA, but did stimulate $^{15}N_2O$ production (Silver et al. 2001, 2005, Pett-Ridge et al. 2006). In addition, rates of DNRA are greater than denitrification or $^{15}N_2O$ production under oxic headspace conditions in these preceding studies. In this coast redwood study, both DNRA and $^{15}N_2O$ production increased greatly when incubated under N_2, but the relative magnitudes of the two processes differed when comparing the ambient and N_2 atmospheres; i.e., DNRA was much higher than $^{15}N_2O$ production under the ambient headspace while the rates of the two processes were similar and $^{15}N_2O$ production was slightly higher under the N_2 atmosphere.

Differences in the oxygen sensitivity of the organisms capable of DNRA and denitrification could explain the differences in the magnitudes of DNRA and $^{15}N_2O$ production measured under the ambient and N_2 atmospheres. As facultative anaerobes, denitrifiers use oxygen when available rather than NO_3. While many of the microorganisms

responsible for DNRA in soils could be facultative, it is likely that many are obligately anaerobic fermentative microbes (Tiedje 1988). In oxygen-tolerant obligate anaerobes, the DNRA operon and enzymes may not be subject to the same level of regulation by oxygen as denitrification. Hence, higher rates of DNRA under more oxic conditions could occur because denitrifiers are using oxygen in the bulk soil environment while many of the obligately anaerobic fermentative organisms capable of DNRA are not. In addition to the dramatic impact of oxygen on the dissimilatory reduction of NO_3, the availability of C and NO_3 can also strongly influence the rates of DNRA and denitrification.

The impact of substrate availability (C and NO3) on DNRA

The impact of C availability on rates of DNRA and N_2O production is very apparent under the N_2-flushed atmosphere. Rates of DNRA and $^{15}N_2O$ production are highly correlated to CO_2 production rates and soluble organic carbon (SOC) concentrations, as well as NO_3 consumption. Once oxygen has been depleted, the availability of C exerts a strong influence on the rates of dissimilatory NO_3-reducing processes. While this relationship is expected by theory, the strength of the correlation in these soils under the N_2 atmosphere is striking. The increase in C mineralization due to higher activity under the lab temperature provided the primary basis for this observed relationship. The supply of C itself, the respiratory consumption of any oxygen remaining in microsites after the N_2 flush, or the combination of the two could cause the strength of this relationship. The availability of C appears to exert a stronger control on the of rates DNRA in the short term after the onset of anaerobisis than availability of NO_3 or the ratio of CO_2 production:NO_3 concentrations in these coast redwood soils. Rates of DNRA have been found to be correlated to the available C:NO_3 ratio in amended soils from cultivated fields (Fazzolari et al. 1998) and the ratio of total C:NO_3 concentrations in Costa Rican forest plantation soils (Silver et al. 2005), but it is difficult to determine if it is the availability of C alone or the ratio of C:NO_3 that drives these relationships.

Under the ambient atmosphere, rates of dissimilatory nitrate reduction were much lower than under the N_2 atmosphere, but DNRA was still related to C availability. However, it is hard to determine the relative importance of C, NO_3, or the ratio of C to NO_3 availability because C and NO_3 availability were also correlated in these samples. Regardless, substrate availability is clearly important in determining the rate of DNRA under the oxic soil conditions as well as the low redox conditions. The correlation between DNRA and C availability could indicate that DNRA found in oxic conditions is due to heterotrophic activity, while the tight correlation between DNRA and CO_2 production under the N_2 atmosphere (when CO_2 and NO_3 are not correlated) strongly indicates that the majority of this flux is due to heterotrophic activity under low redox conditions. On the other hand, neither the availability of C nor NO_3 appeared to be as important in determining rates of $^{15}N_2O$ production under oxic conditions, which were very low and not correlated to CO_2 production or the ratio of CO_2 production:NO_3 concentration. Finally, the correlation between DNRA and C availability under oxic conditions and lack thereof between $^{15}N_2O$ production and C availability reinforces the hypothesis that DNRA is less sensitive than denitrification to oxygen.

Conclusions

While coast redwood forests are truly magnificent and unique in many respects, coast redwood soils are similar to those of other organic rich conifer forests in terms of gross N mineralization and nitrification. High rates of gross N mineralization and consumption cycle N rapidly internally maintaining low gaseous and leaching losses under oxic conditions. Gross N cycling under low redox conditions has not been well studied in temperate conifer ecosystems, and this study demonstrates the importance of DNRA in microsites or during periods of low oxygen availability. Substrate availability is an important controller of DNRA under both oxic and suboxic conditions, but rates of DNRA are 1 to 2 orders of magnitude greater under low redox conditions in these old-growth conifer forests.

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Table 1.	Prairie Creek S. P.	Jedediah Smith S. P.
Mean Annual Precip. (mm)	1677	1697
Mean Annual Temp. (range) (°C)	10.7 (5.1 to 16.2)	11.2 (7.1 – 15.2)
Soil grav. water content (%)	77 (7) [a]	52 (2) [b]
Soil temperature (%)	7.4 (0.04) [a]	6.9 (0.05) [a]
Soil pH	5.1 (0.1) [a]	4.9 (0.1) [a]
Soil %C ($\mu g\ C\ g^{-1}$ soil d.w.)	10.6 (1.0) [a]	5.0 (0.3) [b]
Soil %N ($\mu g\ N\ g^{-1}$ soil d.w.)	0.58 (0.04) [a]	0.28 (0.01) [b]
MBC ($\mu g\ C\ g^{-1}$ soil d.w.)	3,643 (290) [a]	1,887 (65) [b]
MBN ($\mu g\ N\ g^{-1}$ soil d.w.)	306 (32) [a]	189 (15.3) [b]
SOC ($\mu g\ C\ g^{-1}$ soil d.w.)	388 (38) [a]	280 (13) [b]
SON ($\mu g\ N\ g^{-1}$ soil d.w.)	17.0 (2.0) [a]	9.0 (0.5) [b]
Soil NH_4 ($\mu g\ N\ g^{-1}$ soil d.w.)	1.1 (0.4) [a]	1.3 (0.3) [a]
Soil NO_3 ($\mu g\ N\ g^{-1}$ soil d.w.)	5.1 (0.6) [a]	1.8 (0.2) [b]

Table 1. Site mean annual precipitation and temperature and soil characteristics at time of sampling, March 2002. Soil temperature was measured at the time of sampling. Concentrations of soil ammonium (NH_4) and nitrate (NO_3) come from soils extracted immediately after sampling. Microbial biomass C (MBC) and N (MBN) and soluble organic C (SOC) and N (SON) are reported for the samples incubated closest to field conditions (7 °C and ambient atmosphere). Lowercase letters denote significant differences between sites (all $p < 0.01$).

2A. Ambient headspace		Prairie Creek S.P.		Jedediah Smith S.P.	
	ANOVA	7 °C	23 °C	7 °C	23 °C
Gross N mineralization	site, temp	10.3 (2.0)	18.2 (2.9)	4.4 (1.2)	7.3 (1.1)
Gross NH_4 consumption	site, temp	4.3 (1.8)	19.9 (3.4)	2.0 (0.9)	5.3 (1.0)
Gross nitrification	n.s.	3.9 (2.4)	9.5 (5.8)	3.8 (1.4)	3.7 (0.9)
Gross NO_3 consumption	n.s.	3.4 (2.2)	6.8 (4.5)	0.9 (0.4)	2.2 (1.1)
CO_2 production	site, temp	25.5 (3.8)	70.3 (10.1)	13.1 (1.4)	35.4 (5.9)
Net NH_4	temp, int.	5.7 (1.0)	-3.3 (1.6)	2.9 (0.4)	2.1 (0.4)
Net NO_3	n.s.	10.0 (3.9)	10.8 (7.0)	3.7 (1.2)	3.4 (1.7)
DNRA	site	0.14 (0.05)	0.21 (0.05)	0.03 (0.004)	0.074 (0.023)
$^{15}N_2O$ production	temp	.0002 (.0002)	.0015 (.0006)	0 (0)	0.002 (0.001)

2B. N_2-flushed headspace		Prairie Creek S.P.		Jedediah Smith S.P.	
	ANOVA	7 °C	23 °C	7 °C	23 °C
DNRA	site, temp, int.	0.73 (0.11)	4.8 (0.5)	0.43 (0.06)	1.8 (0.3)
$^{15}N_2O$ production	site, temp	1.2 (0.3)	7.4 (1.5)	0.74 (0.08)	3.5 (0.2)
CO_2 production	temp	17.6 (1.4)	33.7 (4.4)	12.2 (1.4)	21.7 (1.9)
Net NH_4	temp	5.7 (3.7)	11.9 (1.6)	2.4 (2.0)	10.0 (3.3)
Net NO_3	temp	5.4 (3.4)	-12.6 (2.4)	1.6 (2.4)	-5.3 (0.6)

Table 2. Rates of soil N transformations for 3-hour incubations done at 7 °C and 23 °C under either (A) an ambient headspace or (B) an N_2-flushed headspace. Rates (mean (1 s.e.)) of soil N transformations are given in μg $N \cdot g^{-1} \cdot day^{-1}$ and CO_2 production is given in μg $C \cdot g^{-1} \cdot day^{-1}$. The ANOVA column lists significant terms for ANOVAs by site x temperature for each headspace: s: site; t: temperature; int: site x temperature. Insignificant terms are not listed.

Table 3A.	Gross N mineralization	NH_4 consumption	Gross nitrification	NO_3 consumption	CO_2 production
NH_4 consumption	0.91^{***}				
Gross nitrification	0.21	0.39^{*}			
NO_3 consumption	0.21	0.36^{ns*}	0.89^{***}		
CO_2 production	0.78^{***}	0.81^{***}	0.10	0.15	
MBC	0.61^{***}	0.37^{ns*}	$0.002^{n.s.}$	0.03	0.37^{*}
MBN	0.52^{**}	0.33^{ns*}	0.05	0.09	0.25
SOC	0.71^{***}	0.68^{***}	0.09	0.14	0.70^{***}
SON	0.79^{***}	0.61^{***}	0.03	0.04	0.67^{***}
NH_4	0.38^{*}	0.53^{**}	-0.12	0.20	0.46^{*}
NO_3	0.28	0.84^{***}	0.34^{ns*}	0.37^{ns*}	0.79^{***}

Table 3B.	Ambient headspace		N_2-flushed headspace	
	DNRA	$^{15}N_2O$	DNRA	$^{15}N_2O$
$^{15}N_2O$ production	0.26		0.90^{***}	
CO_2 production	0.75^{***}	0.32	0.87^{***}	0.90^{***}
NO_3	0.68^{***}	0.24	-0.26	-0.22
CO_2 : NO_3	0.14	0.10	0.56^{**}	0.51^{**}
Net NO_3	0.73^{***}	0.04	-0.74^{***}	-0.75^{***}

Table 3. Correlations between process rates and other process rates or soil nutrient pools for (A) gross N mineralization and gross nitrification under the ambient headspace and (B) DNRA and $^{15}N_2O$ production under both the ambient headspace and N_2-flushed headspace. Significance is indicated by: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns* $0.05 < p < \leq 0.01$. Correlations without any superscript are not significant, $p > 0.10$.

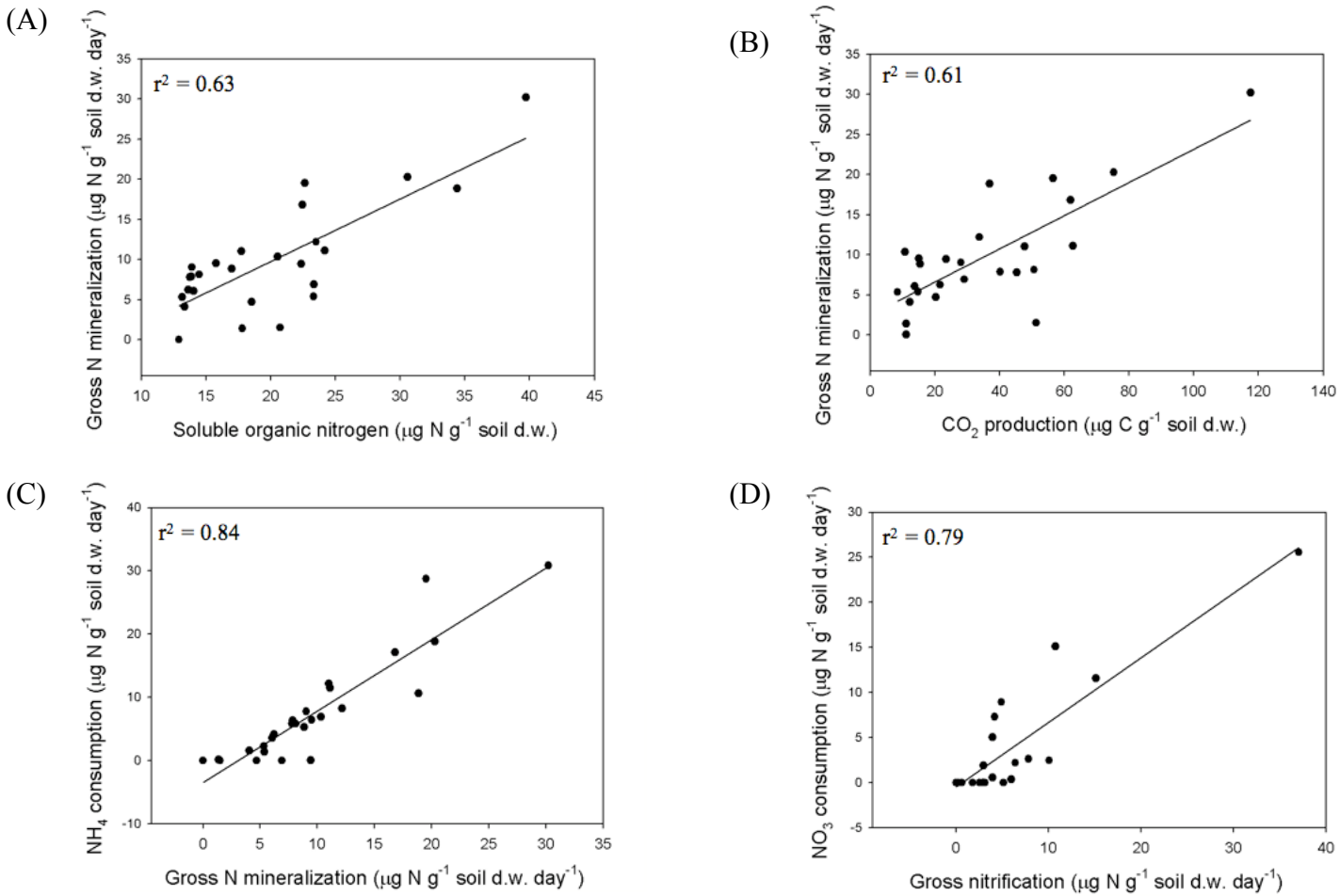
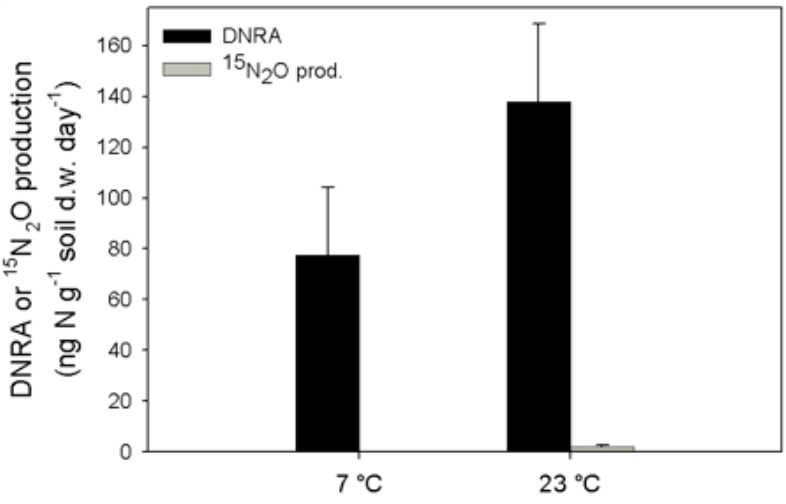


Figure 1. Relationships between (A) gross N mineralization and soluble organic nitrogen, (B) gross N mineralization and carbon dioxide production, (C) NH_4 consumption and gross N mineralization and (D) NO_3 consumption and gross nitrification. All regressions include both sites and both temperatures under an ambient headspace; all are significant at $p < 0.0001$.

(A)



(B)

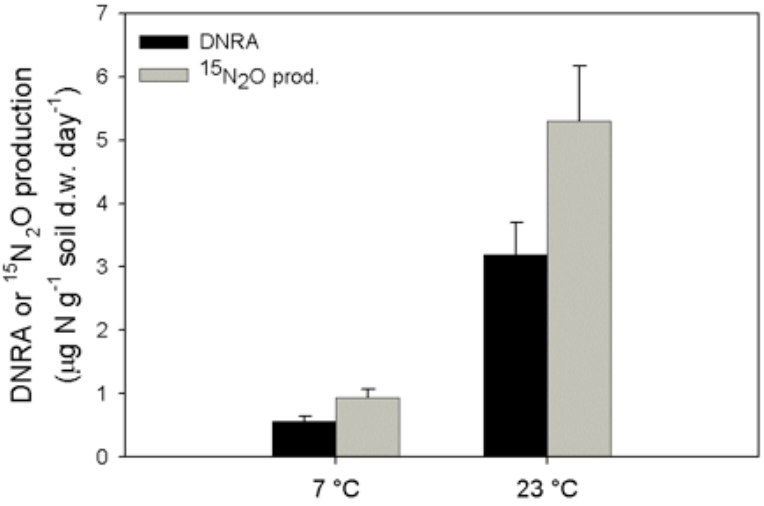


Figure 2. Rates of DNRA and $^{15}N_2O$ production at 7 °C and 23 °C under (A) an ambient headspace atmosphere and (B) an N_2-flushed headspace. Rates were averaged for the two sites. Note the difference in units in the y-axis between the two graphs.

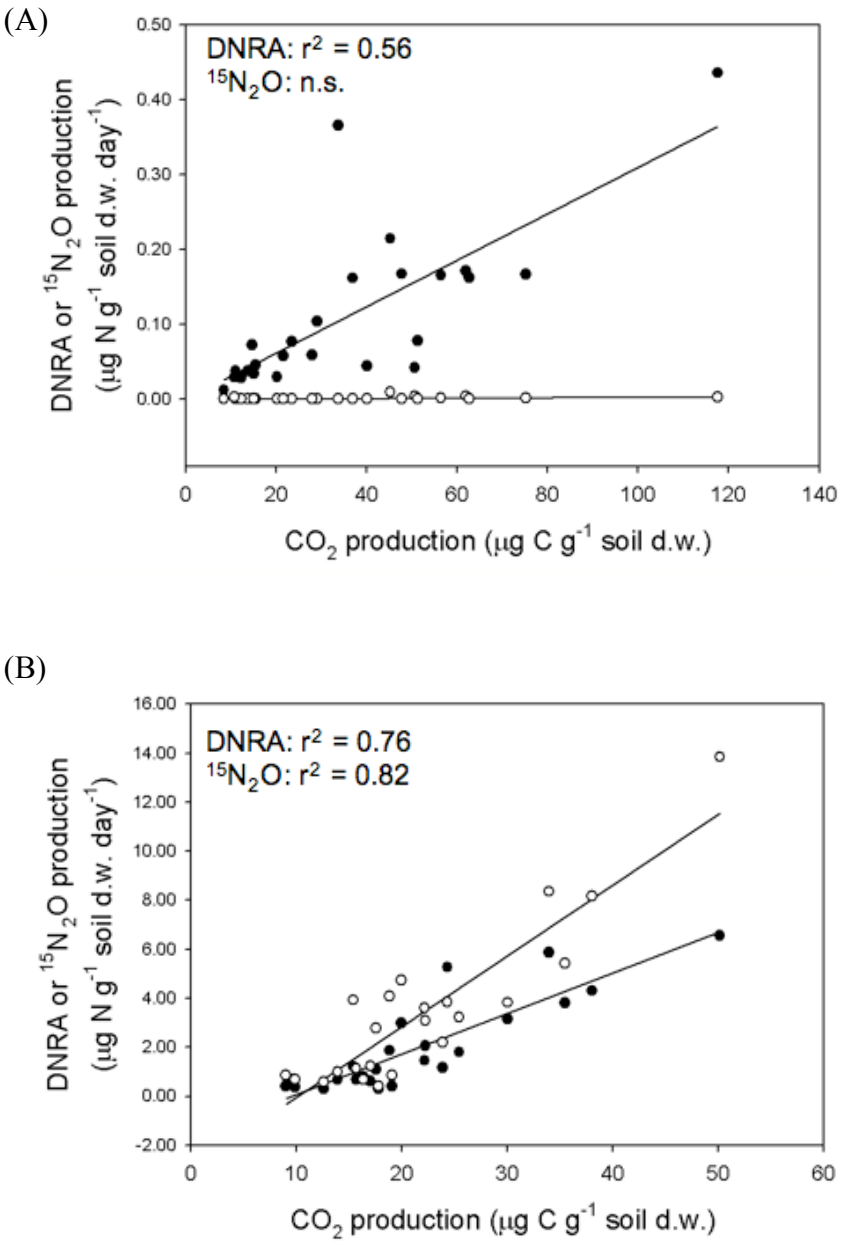


Figure 3. The relationships of rates of DNRA (closed circles) and $^{15}N_2O$ production (open circles) with rates of CO_2 production under (A) an ambient headspace atmosphere and (B) an N_2-flushed headspace. Both sites and temperatures were included in the regressions.

Chapter 2

Climatic and edaphic controllers differentially affect gross nitrification and gross N mineralization in coast redwood forest soils

Abstract

The potential impacts of climate change on rates of soil N mineralization and nitrification were examined by means of a one-year long, three-way reciprocal transplant across a latitudinal gradient from Prairie Creek State Park in northern California to Big Basin State Park in central California. The three coast redwood forests (North, Middle, and South) differed in climate and edaphic characteristics. Laboratory incubations varying soil moisture and temperature were also conducted for two sites (North and Middle). While rates of gross N mineralization in soils differed between sites of origin, they did not differ in response to transplanting or moisture and temperature manipulations. In contrast, gross nitrification differed by transplant site, but not site of origin. Gross nitrification responded differently than gross N mineralization, in part, because of differences in the effect of soil water availability on the two processes. Gross nitrification was sensitive to water availability. Below a water potential of -0.05 MPa, rates of gross nitrification were very low, whereas rates varied widely above this water potential. There was also a significant relationship between gross nitrification rates and the abundances of ammonia-oxidizing bacteria (AOB) and archaea (AOA) *amoA* gene copies. Therefore, both ammonia oxidizer abundance and water availability strongly influence gross nitrification rates. Abundances of AOA and AOB and rates of gross nitrification (and nitrate availability) are likely to be more influenced by changes in summer climate (fog frequency) in coast redwood forests than rate of gross N mineralization (and ammonium availability).

Introduction

The availability of inorganic nitrogen (N), i.e., ammonium (NH_4) and nitrate (NO_3), in soil is primarily controlled by microbial transformations of N. Organic N is mineralized to ammonium by a wide variety of organisms, and ammonium can subsequently be converted to nitrate (nitrification). The first, generally rate-limiting step of nitrification is the oxidation of ammonium to nitrite, which is carried out by ammonia-oxidizing bacteria (AOB) and archaea (AOA). Together these nitrogen transformations determine the amounts of N available for plant uptake as well as the amount of nitrate available for leaching from soil and reduction to gaseous species, including the greenhouse gas nitrous oxide. How gross rates of N mineralization and nitrification will change in response to climate change is thus an important topic for study.

While N mineralization and nitrification have been studied since the late 1800s, our understanding of these processes is still developing. Rates of net N mineralization have been studied as an index of plant available N, and the C:N ratio of plant litter and soil organic matter has been recognized as a controller of net N mineralization for several decades (Sylvia et al. 1998). More recently, the use of isotope pool dilution to measure gross rates of N mineralization and nitrification as well as ammonium and nitrate consumption has furthered our understanding of these processes (Davidson et al. 1991, Stark and Hart 1997, Schimel and Bennet 2004, Booth et al. 2005) A review of patterns and controls of gross N mineralization and gross nitrification across a variety of ecosystems by Booth et al. (2005) found support for the influence of microbial biomass and substrate quantity, and to an extent quality, on rates of gross N mineralization. Gross N mineralization was also correlated with soil moisture, but moisture and concentrations of soil organic matter were correlated with each other and thus their effects can be difficult to separate. Studies that compare the effects of changes in soil moisture on soils with different organic matter concentrations could help disentangle this relationship.

Nitrification requires soil moisture, oxygen and a supply of ammonium, as well as the presence of ammonia-oxidizing organisms. In the last decade, an entirely new group of ammonia oxidizers (in the Crenarcahaea) has been discovered (Treusch et al. 2005, Könneke et al. 2005) and found to be widespread and abundant in marine and soil environments (Francis et al. 2005, Leninger et al. 2006). Studies have highlighted the potential for soil moisture, temperature, pH and oxygen availability to influence rates of nitrification (e.g., Stark and Hart 1995a, Stark and Hart 1995b, Tourna et al. 2008, Nicol et al 2008, Gleeson et al. 2010), as well as the abundance and activity of ammonia-oxidizing bacteria and archaea (e.g., Jia and Conrad 2009, Beman et al. 2008, Tourna et al. 2009, Gleeson et al. 2010). Across a variety of ecosystems, gross nitrification has been found to have a log-linear response to gross N mineralization; at lower rates of gross N mineralization, nitrification increases rapidly, but the increase in nitrification drops off substantially at higher rates of N mineralization (Booth et al. 2005). Clearly, the abundance and activity of ammonia-oxidizing organisms as well as environmental factors influence nitrification in soils and other environments.

Recently, much attention has been paid to the relative abundances of ammonia-oxidizing archaea (AOA) and bacteria (AOB) in the environment. There is some evidence for higher abundances of AOA than AOB in marine, estuary and some soil environments (e.g., Wuchter et al. 2006, Beman et al. 2008, Leninger et al. 2006, He et al. 2007, Nicol et al. 2008,) and shifts between AOA and AOB dominance based on environmental factors, such as salinity in subterranean estuaries (Santoro et al. 2008), but AOB have been found to be more abundant than AOA in estuary sediments (Wankel et al. 2011), some agricultural soils (Di et al. 2009, Gleeson et al. 2010) and Alaskan soils (Peterson et al. in review) While the abundances of AOA and AOB gene copies can differ within environments, AOB abundance often tends to vary more than that of AOA and responds more to changes in environmental factors in some studies (Leninger et al. 2006, Santoro et al. 2008, Mertens et al. 2009, Gleeson et al. 2010, Peterson et al., in review). In various studies, either AOB or AOA have been suggested to be mainly responsible for rates of nitrification (Wuchter et al. 2006, Tourna et al. 2008, Beman et al. 2008, Jia and Conrad 2009, Di et al. 2009, Gleeson et al. 2010).

More research is needed to fully understand the relationship between AOA and AOB abundances and rates of nitrification in soils (Nicol and Prosser 2008).

Due to the influence of temperature and moisture on microbial activity, changes in gross N mineralization and nitrification are likely to occur due to climate change. There is evidence that differences in temperature can influence net and gross N mineralization rates (e.g., Rustad et al. 2001, Melillo et al. 2002, Andersen and Jensen 2001, Cookson et al. 2007), but in a review of many studies, the impact of soil moisture on gross N mineralization could not be separated from that of organic matter quantity (Booth et al. 2005). The activity of nitrifying communities is influenced by the impact of soil water availability on both the diffusion of ammonium and physiological stress (Stark and Firestone 1995), and manipulations of temperature have also been found to effect nitrification rates (e.g., Stark and Firestone 1996, Avrahami et al. 2003, Hoyle et al. 2006). Soil moisture, in particular, has been suggested to be one of the main factors that will influence rates of soil N mineralization and nitrification in response to changes in climate, especially in seasonally dry ecosystems (Jamieson et al 1999, Gomez-Rey et al. 2010). It has been proposed that N mineralization may not be as affected by changes in moisture as gross nitrification because general N mineralization reactions are carried out by a number of different prokaryotic organisms and fungi and nitrification is carried out by only two groups, archaeal and bacterial nitrifiers (Paul and Clark 1996, Gleeson et al. 2010). Yet, it is unclear how regional changes in climate will affect the relationship between gross rates of N mineralization and nitrification.

This study examined the impact of climate change on rates of gross N mineralization, gross nitrification and the abundances of AOB and AOA in coast redwood forests. Soil were sampled at the end of the dry, Mediterranean-climate summer, a time of year when the presence of fog is a defining characteristic of coast redwood forests (Noss 2000, Azevedo and Morgan 1974). While it had been suggested that increased coastal upwelling due to climate change could result in an increase in summer fog (Bakun 1990), the warming of the Pacific Ocean can result in a reduction in the formation of fog because fog forms as warm air passes over cool ocean water. Indeed, long-term records of cloud ceiling height and temperature in northern California suggest that the frequency of fog has declined over the last century (Johnstone and Dawson 2010). Soils were transplanted across the latitudinal range of coast redwood forests in northern California (Fig. 1) to examine interactions among the native soil characteristics, changes in climatic exposure and gross rates of N mineralization and nitrification. The results elucidate the potential of climate change to alter rates of these important N-cycling processes, while also considering how soil characteristics can modulate the impact of climate change on these processes.

Methods

Study sites

Study sites were established in three old-growth coast redwood forests across a North-South gradient (Fig 1) in the summer of 2004. Old-growth coast redwood (*Sequoia sempervirens*) forests occur within a narrow fog belt across an 800-km long latitudinal gradient in precipitation and temperature extending from the southwestern tip of Oregon (42º09' N. latitude) to southern Monterey County in CA (35º01' N. latitude). Redwood forests experience a Mediterranean climate with cool, wet winters and warm, dry summers. The

presence of fog in the summer is a defining characteristic of coast redwood forest (Noss 2000). Almost all of the rain falls between September and April. It rains extremely little if at all in the summer. In August 2004, soils were transplanted between three old-growth coast redwood forest sites that differ in climate and edaphic characteristics (Fig. 1A, Table 1). The sites were located in Prairie Creek State Park, CA (Humboldt County), the Grove of the Old Trees, Occidental, CA (Sonoma County) and Big Basin State Park, CA (Santa Cruz County). These sites are referred to as the North, Middle and South sites, respectively.

All three of the sites were located in stands of old-growth coast redwood forest with an understory of mainly sword fern (*Polystichum munitum*) with some tanoak (*Lithocarpus densiflorus*) and sorrel (*Oxalis oregana*). The biomasses of the trees and understory are much greater in the northern site and the rates of primary productivity and decomposition are higher there as well (Table 1). The annual average precipitation increases with latitude and temperature decreases with latitude. Soil characteristics (e.g., soil C and N, pH, % silt and sand, and water retention curves) also differ between the sites (Table 1). As described below, soils from each site were transplanted into all three sites allowing for the examination of the potential for a change in climate to alter gross rates of N mineralization and nitrification in soils as well as the capacity of soil characteristics to buffer or enhance changes in response to climate.

Experimental design – 3-way reciprocal transplant

In August 2004, two types of intact soil cores were transplanted between each pair of sites: 1) 10-cm diameter, 15-cm deep solid schedule 40 PVC cylinders and 2) flexible 2-mm-mesh shade cloth sown into cylinders of the same dimensions with nylon thread. For five (3 m x 3 m) plots at each site that were located ~30-400 m apart, each type of soil core was removed from its site of origin and transplanted into both of the other sites. Plot pairs between sites were chosen randomly, and cores were randomly located within a plot. Soil cores were also returned to their plots of origin to serve as controls. Ninety cores were harvested after one year in early September 2005 (45 mesh and 45 PVC; 5 of each from 3 sites of origin x 3 transplant sites). The PVC cores were originally intended to prevent roots from infiltrating the cores, but roots were able to easily grow up into the cores. The two types of cores were analyzed together because they did not differ significantly. A "fresh" previously undisturbed soil core was also collected from adjacent to each site (3 sites x 5 plots = 15 fresh cores).

Climate and soil characteristics

Several climatic and edaphic variables were measured for this study. Monthly precipitation data was obtained from the closest NOAA National Climatic Data Center weather station data available for each site. The stations used for the North and Middle, respectively, were Orick Prairie Creek Park (Station 046498, 41° 22′ N and 124° 01′ W) and Occidental (Station 046370, 38° 23′ N and 122° 58′ W), and for the South, the average of Ben Lomond #4 and Felton (Station 040673, 37° 05′ N and 122° 05′ W; Station 043004, 37° 03′ N and 122° 05′ W) was used. Soil temperatures were recorded at the time of sampling and in February, May and November as well as to determine the annual ranges in soil temperature. Gravimetric water content (GWC) was determined by drying soil (collected at the same times) at 105 °C. Measurements of bulk density made for each site (on 10 cm diam. x 15 cm deep cores) were used to transform GWC into volumetric water content (VWC) and water-filled pore space

(WFPS). Samples were sent to the UC Davis Analytical Lab for determinations of soil texture (% sand, % silt and % clay in soil suspension by the hydrometer method; Sheldrick and Wang, 1993) and water retention curves (by the pressure plate system; Klute 1986). These water retention curves were used to transform GWC values into soil water potentials. The other edaphic variables measured included: pH (1:1 soil : 0.01M $CaCl_2$), soluble organic carbon and nitrogen (extracted in 0.05M K_2SO_4), and microbial biomass C and N (by chloroform fumigation – direct extraction; Brookes et al. 1985). The total organic C concentration in extracts was determined on a TOC analyzer (OI Analytical 1010 TOC analyzer, College Station, TX). The dissolved N in extracts was converted into NO_3 by persulfate digestion (Cabrera and Beare 1993), and the NO_3–N concentrations were determined colorimetrically using an autoanalyzer (Lachat Quik Chem Flow Injection Analyzer, Lachat Zellweger Instruments, Milwaukee, Wisconsin, USA). The concentrations of organic C and N in the initial unfumigated 0.05M K_2SO_4 extracts were used as indices of soluble organic C (SOC) and N (SON).

Rates of gross N mineralization and nitrification

Gross rates of N mineralization, nitrification, and ammonium and nitrate consumption were measured by ^{15}N-isotope pool dilution (Herman et al. 1995, Kirkham and Bartholomew 1954). Transplanted soils and fresh soils were sampled one day and sieved the next day. Incubations were begun the day following sieving. Soils were labeled with ^{15}N as either $(^{15}NH_4)_2SO_4$, for gross N mineralization or $K^{15}NO_3$ for gross nitrification; 1 ml of labeled solution was added to 50 g d.w. of soil to increase the appropriate pool to approximately 50 or 25 atom percent ^{15}N, respectively. Initial samples were taken after approximately 2 hours and final samples 16 hours later. Concentrations of NO_3 and NH_4 were determined after a one-hour extraction in 1 M KCl. Atom% ^{15}N was determined using an automated N and C analyzer coupled to an isotope-ratio mass spectrometer (ANCA-IRMS; PDZ Europa Limited, Crewe, UK) after diffusing out the NH_4 or NO_3 onto filter disks (Herman *et. al.* 1995). Gross rates of N mineralization, nitrification, and ammonium and nitrate consumption were calculated according to Kirkham and Bartholomew (1954). Soils were kept cool and stored at 12 °C between collection, sieving and incubations; incubations were also performed at 12 °C to measure rates that were reasonably representative of field conditions.

Laboratory manipulations of soil temperature and moisture

Soils from the North and Middle sites were subjected to manipulations of temperature and soil moisture in short-term laboratory incubations. For temperature manipulations, moist soils were collected in mid-May 2005 during a wet spring with late rains in northern California. Two soil cores (10 cm diam. X 15 cm deep) were collected from areas adjacent to each transplant plot in each of the two sites. The soils were sieved (< 2 mm) and all of the cores were mixed together. The soils were then split into three temperatures (5, 12 and 25 °C) and pre-incubated for a period of three days. After pre-incubation, rates of gross N mineralization and nitrification were measured as described above for transplant samples. For moisture manipulations, soils were collected in early November 2005, after a period of over five months without rain. Soils were sampled and processed (sieved and mixed) similarly to those collected for the temperature manipulation. Soils from the middle site were split into samples that were: left at field moisture, or had their GWC increased by 10, 19 or 26%. For the North

soils, one subset was air dried (at 12 °C) so that GWC decreased by 14%, one left at field moisture, one increased by 16% GWC and one increased by 31% GWC. The moisture experiment was also conducted at 12 °C (as opposed to lab temperature) in order to keep soils relatively close to field temperature. Rates of gross N mineralization and gross nitrification were measured as described above.

Abundances of ammonia-oxidizing bacteria (AOB) and archaea (AOA)
Soil samples were kept at field temperature until they were sieved (< 2 mm) and mixed within 24 hours. At this time, five-gram subsamples were frozen and stored at –80 °C until community analysis could be performed. Bulk soil DNA was extracted from 3 replicates of each soil origin-transplant combination (3 origin x 3 transplant = 27) as well as replicates of fresh undisturbed soil from each site (3 sites x 3 fresh undisturbed = 9). For each sample, 500 mg of soil was extracted using the Bio101 Fast DNA Spin Kit for Soils (Q Biogene, Carlsbad, CA) according to the manufacturer's instructions. The abundances of amoA genes from AOB and AOA were measured using the primer pairs of amoA1F/amo2R (Rottahue et al. 1997) and Arch-amoAF/Arch-amoAR (Francis et al. 2005), respectively. Quantitative PCR was performed using the iQ SybrGreen super mix on an iCycler QPCR system (Bio-Rad, Hercules, CA) with 1 μl of 25 ng DNA in a total volume of 20 μl μg using the PCR thermal profile as in Santoro et al. (2008). The purity and size of the PCR products were verified by melting curves and gel electrophoresis.

Statistical analyses
All of the statistical analyses were preformed in the R statistical environment (version 2.10.0). Analysis of variance was used to determine if there were differences in abundances of AOB and AOA *amoA* gene copies, process rates, soil nutrient pools or other edaphic characteristics between sites of origin, transplant sites or the interaction between the two. Simple linear regressions were performed to determine if there were any relationships among either process rates or *amoA* gene copy abundances and climatic or edaphic variables. The stepAIC function in the MASS packages was used to perform stepwise multiple regressions.

Results

Background soil characteristics and gross N mineralization and nitrification rates
Rates of gross N mineralization and soil ammonium concentrations did not differ significantly between the three sites in August 2005 (Table 2). Gross nitrification rates, soil nitrate concentrations and the concentrations of microbial biomass carbon and nitrogen and soluble organic nitrogen and carbon were all significantly higher in the North soils than in the other soils. Soil pH was significantly lower in the North than in the South, and the Middle soils were intermediate. The abundances of ammonia-oxidizing bacterial (AOB) and archaeal (AOA) *amoA* genes were greater in the North than in the South. The abundances of both groups were intermediate in the Middle soils but not significantly different from those in the South soils; the AOB abundance was significantly lower in the Middle than in the North.

Rainfall across sites and soil moisture in transplanted soils

While 30-yr average total annual rainfall decreases from the North to the Middle to the South site, it rained as much in the South as in the North from September of 2004 until August 2005. However, it still rained little to not at all from June 1 through the end of August (< 8.4 cm; and not at all after July 1 except for 3 mm in July in the North site). The soils in the South were expected to dry out the most over the summer because it is the warmest site with the least fog. Indeed, there was a significant effect of site of harvest on soil moisture, and the mean gravimetric (and volumetric) soil moisture and soil water potential was lower in soils incubated in the South site than in the Middle or North site (Fig. 2A). However, there was also an effect of site of origin on soil water availability; soils from the North had higher soil moisture and water potential than the other two sites. Soils from the North had high soil water potentials at all sites, even after spending the summer in the much warmer South site. The soils from the North were able to maintain higher water contents (and water potentials) than the other two soils due to their much higher organic matter content and slightly higher clay content (Fig. 2B).

Soil characteristics: soil and climate controls

Many soil characteristics that originally differed between sites did not change significantly in response to spending a year in another location along the North-South gradient (Table 3). Those that differed very little or did not change at all included soil pH, microbial biomass C and N, and soluble organic N. Soluble organic C differed significantly among both soils from different origins and soils from different transplant locations (harvest sites), but the differences among soil origins were much greater. Patterns in soil ammonium and nitrate concentrations between sites differed from those of fresh soil. Site of origin had a significant effect on NH_4 and NO_3 concentrations; soils from the South site had higher NH_4 and NO_3 concentrations. There was also a significant effect of site of transplant on soil NO_3 concentrations (Table 3). Soils incubated in the Middle site had the highest nitrate concentrations on average; the NO_3 concentrations in the North soils that spent the year in the South site were the highest of any soil-climate combination. Soils transplanted into the North site also had higher NO_3 concentrations than soils transplanted into the South site.

Gross N mineralization: soil and climate controls

The main factor impacting gross rates of N mineralization was the origin of the soil. In transplanted soils, rates of gross N mineralization differed significantly by site of origin, but not by transplant site or the interaction of site and origin (Fig 3). Soils with a South origin tended to have higher rates of gross N mineralization on average than soils from the other two sites (Fig. 3A), but were only significantly higher than soils of Middle origin. Across all soils, the only significant linear regressions between gross N mineralization and the individual soil characteristics that were measured (i.e., MBC, MBN, SOC, SON, %GWC, %VWC, WFPS, MPa, NH_4, pH) were with SOC concentration ($p = 0.0001$, $r^2 = 0.17$) and pH ($p = 0.05$, $r^2 = 0.05$). The relationship with SOC was driven by one sample point that had a high gross N mineralization rate and high SOC concentration. Without this point, the relationship was not significant ($p = 0.60$). In a stepwise regression, SOC, SON, MPa, and pH produced the best-fit model ($p < 0.0001$); together they can explain 38% of the variation in gross N mineralization rates.

Neither laboratory manipulations of soil moisture nor temperature significantly affected rates of gross N mineralization in the North and Middle soils (Fig. 4). Soil collected in May after a wet spring had average rates that ranged up to 19.7 μg N g soil^{-1} day^{-1} in the North soils and up to 11.4 μg N g soil^{-1} day^{-1} in the Middle soils across all temperatures; although not significant, rates did tend to be higher at 25 °C (Fig 4). For soils collected in Nov (before the first rain) and incubated at different moistures, mean gross N mineralization rates were close to 2 μg N g soil^{-1} day^{-1} at all soil moistures for the North soils and varied between 1 and 2 μg N g soil^{-1} day^{-1} in the Middle soils.

Gross nitrification: soil and climate controls

Rates of gross nitrification differed significantly among soils harvested from different sites (of transplantation), but not among soils from different origins. Gross nitrification rates were higher when transplanted soils had been located in the North site compared to the other two sites (Fig 5A). In terms of soil origin, soils from the South tended to have lower rates of gross nitrification than the Middle and North, but the differences were not significant (Fig. 5B). There were no significant correlations between gross nitrification and gross N mineralization or between gross nitrification and any of the individual environmental variables measured; although the regression between gross nitrification and MBN + VWC was significant ($p = 0.0001$) and explained 21% of the variability in rates of gross nitrification. More strikingly, there appeared to be a minimum soil water potential below which nitrification was seriously impaired (Fig 6). Below -0.05 MPa, rates of gross nitrification were always below 0.8 μg N g soil^{-1} day^{-1}. Above this water potential, rates varied widely, ranging between zero and 9.5 μg N g soil^{-1} day^{-1}. Linear regression analysis between rates of gross nitrification and AOB or AOA *amoA* gene copy abundance found significant relationships between the rates of gross nitrification and the log of AOB abundance ($p = 0.05$, $r^2 = 0.13$) and log of AOA abundance ($p = 0.02$, $r^2 = 0.19$).

Regression run with only the samples with soil water potentials above -0.05 MPa were performed as well to look for factors that explain the high variability in gross nitrification rates at high water potentials. The results for regressions of gross nitrification against rates of gross N mineralization and other soil variables mirrored those for the full dataset; regressions with gross N mineralization or any of the individual environmental variables measured were not significant, but the regression against MBN + VWC was significant ($p = 0.001$) and explained 18% of gross nitrification rates. For samples that had measurements of AOB and AOA *amoA* gene copy abundance values, there were again significant regressions between the rate of gross nitrification and the log of AOB abundance ($p = 0.05$, $r^2 = 0.15$) and log of AOA abundance ($p = 0.015$, $r^2 = 0.22$).

When examining the impact of moisture on dry soil samples collected in mid-Nov before any fall rain, nitrification rates did not differ significantly between soil moistures for either the North or Middle site. In the North soils, the two wetter treatments tended to be higher than the two drier treatments, but the differences were not significant (Fig. 7A). The driest soil water potential of the North soils (34.8% GWC) was, however, only -0.2 MPa; it was -0.09 MPa (18.4% GWC) for the Middle soils. Moist soils (from both the North and Middle sites) collected after a relatively warm and wet May did not differ in rates of gross nitrification among laboratory manipulations of temperature (5, 12 and 25 °C; 7C and 7D).

Abundances of ammonia-oxidizing bacteria (AOB) and archaea (AOA): soil and climate controls

For the abundances of AOB and AOA *amoA* gene copies, there was again a significant effect of the site of origin, but not the site of transplant (incubation/harvest site) or the interaction (Figs. 8A-D). The abundances of AOB and AOA differed significantly between all of the sites of origin and were highest in the North, intermediate in the Middle and lowest in the South (Figs. 8A and 8B). The mean abundances of *amoA* gene copies of AOB were somewhat greater than those of AOA in all transplant (soil-climate) treatments, and the ratio of AOB:AOA was greater in soil of South origin than in the North soils. The abundances of AOB and AOA are much more similar in the North soil, in which the abundance of AOB *amoA* genes is only ~2x greater than that of AOA. In contrast, they are close to 20X and 50X more abundant on average in the Middle and South soils, respectively.

The abundances of AOB and AOA *amoA* gene copies were regressed against environmental variables to look for factors that influenced ammonia oxidizer abundance. Soil water availability explained a significant amount of variation in the abundances of AOB and AOA *amoA* gene copies. The regressions between AOB and AOA abundance and VWC were highly significant ($p = 0.0001$ and $r^2 = 0.35$ for AOB and $p = 0.005$ and $r^2 = 0.22$ for AOA). Similar but slightly lower r^2 values were obtained for the other measures of water availability (GWC, WFPS and MPa), though the regressions with WFPS and MPa were only significant at $p = 0.06$ and $p = 0.09$ for AOA. Regressions between the abundance of AOB and concentrations of SON ($p = 0.02$ and $r^2 = 0.15$) and MBN ($p = 0.04$ and $r^2 = 0.12$) were also significant alone but not when included with VWC, and regressions with variables such as gross N mineralization and concentrations of NH_4, MBC, and SOC were not significant. For AOA, regressions between the abundance of AOA and concentrations of NH_4 ($p = 0.001$ and $r^2 = 0.27$) and SON ($p = 0.0003$ and $r^2 = 0.33$) were significant, and VWC and NH_4 together ($p < 0.0001$) explained 58% of the variability in the abundance of AOA *amoA* gene copies.

Discussion

The responses of gross N mineralization and gross nitrification to imposed changes in climate differed. While rates of gross N mineralization differed among soils with different sites of origin, rates of gross nitrification differed among transplant sites (regardless of origin), that is, the sites at which the soil cores spent the preceding year. Consequently, rates of gross nitrification were not correlated with rates of gross N mineralization. Instead, the impact of transplanting on gross nitrification seems to be due to the relationships between gross nitrification and water availability and the abundances of ammonia-oxidizing bacteria and archaea.

Rates of gross nitrification in coast redwood forests are clearly affected by soil water availability at the end of the summer when north-south differences in fog frequency can cause substantial differences in climate between sites. It has been previously shown in laboratory studies that the impacts of soil water availability on nitrification result from two primary factors: the effects of soil water content on the diffusion of NH_4 to ammonia oxidizers and the

physiological impacts of the energetic availability of water (Stark and Firestone 1995). In this field study, there was a striking relationship between gross nitrification and soil water potential. Rates of nitrification were always low (< 0.8 μg N g soil^{-1} day^{-1}) below a water potential of -0.05 MPa. This water potential, however, is an order of magnitude greater than the -0.6 MPa at which Stark and Firestone (1995) found physiological stress to become more important than diffusion in terms of limiting nitrification.

The lower rates of nitrification below -0.05 MPa are likely then due to diffusional limitation of ammonium or some other factor besides physiological stress. Ammonia-oxidizing bacteria are known to be able to carry out ammonia oxidation down to below -1.0 MPa in soils (Paul and Clark 1996, Chen et al. 2011), but little is known about the physiology of archaeal nitrifiers because few have been cultured (Konneke et al. 2005, de la Torre et al. 2008) and none from soil. Soils in coast redwood forests are well suited to maintain relatively high water availability throughout the summer. The lowest water potential in this study was -0.5 MPa. The high organic matter contents (and associated high water holding capacity) and the presence of fog (which reduces evapotranspiration) can both contribute to this characteristic.

The abundances of AOB and AOA *amoA* gene copies were the only variables measured that independently explained a significant amount of variation in gross nitrification rates in linear regressions, both across all samples (r^2 = 0.13 and 0.19 for AOB and AOA, respectively) and in the samples above -0.05MPa (r^2 = 0.15 and 0.22 for AOB and AOA, respectively). It is clear that both water availability and the abundance of ammonia oxidizers are important in determining rates of gross nitrification. Other studies have also found relationships between *amoA* gene copy abundances and nitrification rates for both AOB and AOA in soil (He et al. 2007), and either AOB in soil (Jia and Conrad 2009, Di et al. 2009) or AOA in marine (Beman et al. 2008) or soil (Tourna et al. 2008) environments, but *amoA* copy abundances and gross nitrification rates are not always correlated (Wankel et al. 2011). While some previous studies have suggested either AOB or AOA to be mainly responsible for nitrification in various environments (Beman et al. 2008, Tourna et al. 2008, Jia and Conrad 2009, Di et al. 2009, Mertens et al. 2009, Wankel et al. 2011), the results discussed above indicate that both bacterial and archaeal nitrifiers could play a significant role in nitrification in coast redwood soils.

In the soils from this study, AOB were always more abundant than AOA, but the ratio of AOB:AOA varied between sites. Recently, Petersen and coworkers (in review) reported abundances of AOB *amoA* gene copies that exceed those of AOA by 2 to 3 orders of magnitude in five moist-to-wet Alaskan soils that had low pH values (4.3-4.8) and high organic contents. Higher abundances of AOB than AOA have also been measured in semi-arid agricultural soils (Gleeson et al. 2010) and estuary sediments in California's Elkhorn Slough (Wankel et al. 2011). However, many other studies have found greater abundances of AOA than AOB in soils (e.g., He et al. 2007, Leninger et al. 2006, Nicol et al. 2008, Jia and Conrad 2009). The *amoA* gene abundances of AOB and AOA in coast redwood soils were greatest, and most similar, in soils from the North site, while the ratio of AOB:AOA *amoA* gene copies was greatest in the South. Soil volumetric water content explained a fair amount of variation in the abundances of AOB and AOA *amoA* gene copies (r^2 = 0.35 and 0.22, respectively). The increase in the coefficient of determination to 0.58 when including ammonium concentrations with VWC may indicate a more direct relationship between AOA

abundance and NH_4 availability, though there are more AOB than AOA *amoA* gene copies at all sites. Both AOB and AOA *amoA* gene copy numbers varied across soils (by site of origin), while some soil other studies have found that AOB *amoA* abundances vary but AOA do not (Leninger et al. 2006, Di et al. 2009, Gleeson et al. 2010).

In contrast to gross nitrification and *amoA* abundances, rates of gross N mineralization in coast redwood forests were relatively insensitive to any variable measured, edaphic or climatic. In general, there were not significant relationships between rates of gross N mineralization and any of the individual variables measured (rainfall, soil moisture, soluble organic carbon or nitrogen). However SOC, soil water potential and pH together were able to explain 38% of the variation in gross N mineralization rates. Other studies have reported direct relationships between gross N mineralization and substrate availability (Booth et al. 2005), and a significant relationship between gross N mineralization and concentrations of SON was previously found for two northern coast redwood sites (Chapter 1). However, the study reported in Chapter 1 was conducted during a very wet time of year in late winter March 2003, while this transplant study concentrated on a drier time of year at the end of summer. At the end of the dry summer period, it may be impossible to separate substrate and water controls of gross mineralization. In general, rates of N mineralization were relatively consistent across sites of origin and transplant sites. Hence, it appears unlikely that changes in summer climate will significantly affect rates of gross N mineralization in coast redwood forests at this time of year.

Studies conducted in pasture and grassland soils have found significant decreases in gross N mineralization at water potentials lower than those measured in these coast redwood soils (-1.5 MPa in Murphy et al. 1997 and -1.5MPa in Jamieson et al. 1998), and a decrease in water potential from -0.1 MPa to -1.0 MPa did not cause a substantial decrease in gross N mineralization in the EA horizon of an acid coniferous forest soil (Chen et al. 2011). Thus, rates of gross N mineralization in forest mineral soils may not be highly influenced by changes in soil water availability down to at least -0.5 or -1.0 MPa.

Implications for climate change in redwoods

Changes in summer fog frequency could have important effects on coast redwood forests. The importance of fog to coast redwood ecosystems in terms of supplying water and reducing transpiration during the otherwise dry summer has been demonstrated for coast redwood trees and their understory species (Dawson 1998, Simonin et al. 2009, Limm et al. 2009). By examining the impact of differences in climate on gross N mineralization and nitrification, this study has helped to elucidate the importance of water availability for these N-cycling processes that can control plant N availability at this critical time of year. While rates of gross N mineralization seem to be relatively insensitive to differences in summer climate across coast redwood forests, rates of gross nitrification are significantly affected by differences in summer climate and water availability. Therefore, continued reduction in fog frequency or amount, as has been observed over the last 100 years (Johnstone and Dawson 2010), may affect populations of ammonia-oxidizing bacteria and archaea and cause a significant reduction in rates of gross nitrification and resulting nitrate availability.

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	North	Middle	South
Location	Prairie Creek S.P., Orick, CA	Grove of the Old Trees, Occidental, CA	Big Basin S.P., Boulder Creek , CA
(Latitude, distance to coast, elevation)	41° 41' N, 6.8 km, 54 m.a.s.l.	38° 24' N, 6.5 km, 148 m.a.s.l.	37° 10' N, 6.4 km, 128 m.a.s.l.
ANPP ($kg \cdot ha^{-1} \cdot yr^{-1}$)*	7,138	4,807	4,710
Decomposition (k; yr^{-1})*	0.28	0.14	0.13
Mean annual rainfall (mm)	1,677	1,419	1,211
Summer fog	Foggy, but little fog drip	Foggy, and a few big drip events	Very little fog
Air temperature (mean / range)	11 °C (2 °C – 21 °C)	14 °C (4 °C – 29 °C)	15 °C (2 °C – 30 °C)
Soil moisture range (0-15 cm, vol. water content)	29.7% to 65.9%	15.2% to 38.4%	11.1 % to 34.4%
Soil temperature (range at 7.5 cm deep)	5 °C – 14 °C	7 °C – 16 °C	8 °C – 17 °C
Soil texture (0-15 cm)	Sandy Loam	Sandy Loam	Sandy Loam
% sand (mean +/- 1 s.e.)	42 +/- 3.8	53 +/- 0.6	32 +/- 2.8
% silt (mean +/- 1 s.e.)	41 +/- 3.5	35 +/- 0.7	55 +/- 3.0
% clay (mean +/- 1 s.e.)	16 +/- 0.3	12 +/- 0.9	13 +/- 0.6
Soil pH (0-15 cm; mean +/- 1 s.e.)	4.9 +/- 0.1	5.3 +/- 0.2	5.7 +/- 0.2
Soil %C (0-10 cm) mean +/- 1 s.e.	10.7 +/- 0.9	4.6 +/- 0.4	4.5 +/- 0.5
Soil %N (0-10 cm) mean +/- 1 s.e.	0.58 +/- 0.04	0.27 +/- 0.07	0.25 +/- 0.03

*The ANPP (aboveground net primary productivity, i.e., litterfall) and steady-state decomposition values are from V. Boukili and T. Dawson (unpublished data).

Table 1. Site characteristics. The mean annual rainfall and air temperature are the 30-year averages (1970-2000) and ranges from the NOAA weather stations identified in the methods section. The soil moisture and temperature are from samples collected during the period from August 2004 – September 2007. The soil texture, soil pH, soil %C and soil %N are data from samples collected in August 2005.

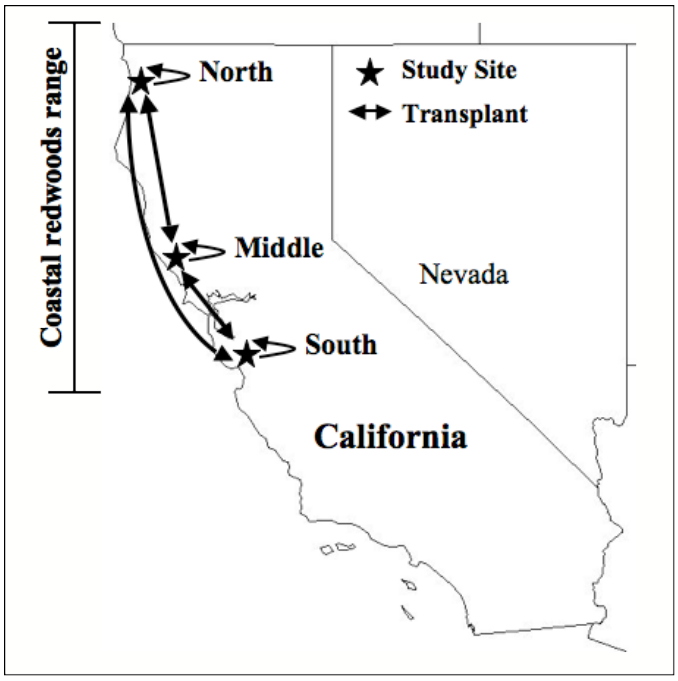
Pool / Process	North	Middle	South
Gross N mineralization $^{n.s.}$ (μg N g soil^{-1} day^{-1})	3.9 ± 1.1	2.1 ± 1.0	3.8 ± 0.1
Gross nitrification ** (μg N g soil^{-1} day^{-1})	2.3 ± 0.5 ^{a}	0.5 ± 0.3 ^{b}	0.3 ± 0.1 ^{b}
Soluble organic C ** (μg C g soil^{-1})	56.4 ± 8.1 ^{a}	24.2 ± 5.0 ^{b}	31.2 ± 2.4 ^{b}
Soluble organic N **** (μg N g soil^{-1})	15.4 ± 5.1 ^{a}	3.4 ± 0.8 ^{b}	4.4 ± 2.04 ^{b}
Microbial biomass C **** (μg C g soil^{-1})	812 ± 53 ^{a}	287 ± 55 ^{b}	514 ± 29 ^{c}
Microbial biomass N **** (μg N g soil^{-1})	193 ± 16 ^{a}	53.9 ± 13 ^{b}	91.9 ± 17 ^{b}
pH *	4.9 ± 0.3 ^{a}	5.3 ± 0.4 ^{ab}	5.7 ± 0.4 ^{b}
Soil ammonium $^{n.s.}$ (μg NH4-N g soil^{-1})	2.8 ± 0.4	3.2 ± 1.0	3.0 ± 0.7
Soil nitrate **** (μg NO3-N g soil^{-1})	6.6 ± 0.5 ^{a}	1.1 ± 0.5 ^{b}	0.3 ± 0.1 ^{b}
AOB abundance ** (# amoA copies g soil^{-1})	691 ± 94 ^{a}	144 ± 66 ^{b}	48 ± 9.6 ^{b}
AOA abundance * (# amoA copies g soil^{-1})	416 ± 150 ^{a}	118 ±110 ^{ab}	9.0 ± 2.8 ^{b}
AOB:AOA $^{n.s.}$	2.1 ± 0.6	14.3 ± 11.0	6.8 ± 2.3
% Gravimetric water content ****	48 ± 2.0 ^{a}	22 ± 1.8 ^{b}	24 ± 1.6 ^{b}
% Vol. water content ****	38 ± 1.2 ^{a}	23 ± 2.1 ^{b}	20 ± 1.7 ^{b}
Water potential (MPa) ****	0.0002 ± 0.0001 ^{a}	0.039 ± 0.026 ^{ab}	0.18 ± 0.12 ^{b}

Table 2. Process rates and soil characteristics for undisturbed soils freshly sampled in August 2005. Different letters indicates significant differences among sites. The levels of significance are: n.s. - not significant, * $p < 0.05$, ** $p < 0.01$, $p < 0.001$, $p < 0.0001$

Pool / Process	Origin	Harvest	Origin x Harvest
Gross N mineralization	**0.008**	0.14	0.30
Gross nitrification	0.18	**0.007**	0.32
Soluble organic C	**<0.0001**	**0.016**	0.88
Soluble organic N	**<0.0001**	0.22	0.50
Microbial biomass C	**<0.0001**	0.27	0.35
Microbial biomass N	**<0.0001**	.36	.30
Soil pH	**<0.0001**	0.25	0.87
Soil ammonium	**<0.0001**	0.73	0.10
Soil nitrate	**<0.0001**	**<0.0001**	0.93
AOB *amoA* abundance	**<0.0001**	0.54	0.67
AOA *amoA* abundance	**<0.0001**	0.53	0.34
AOB:AOA	**0.004**	0.29	0.36
GWC	**<0.0001**	**0.0004**	0.93
VWC	**<0.0001**	**<0.0001**	0.96
MPa	**<0.0001**	**<0.0001**	0.25

Table 3. Results of ANOVA tests for transplanted samples. The p-values are given for ANOVAs for process rates and soil characteristics by site of origin, site of transplanting (incubation) and their interaction. Significant values are in bold. The units for the pool and process measurements that were used for the ANOVAs are the same as those given in Table 2.

(A)



(B)

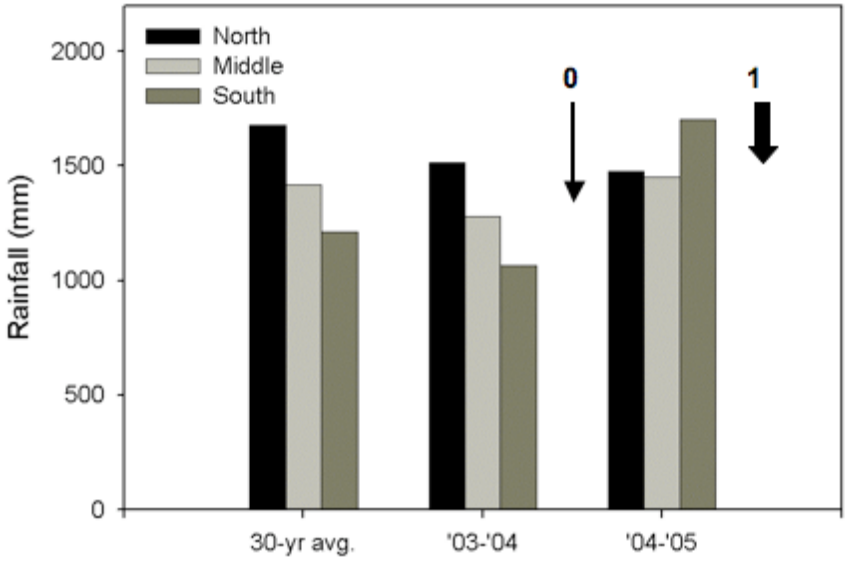
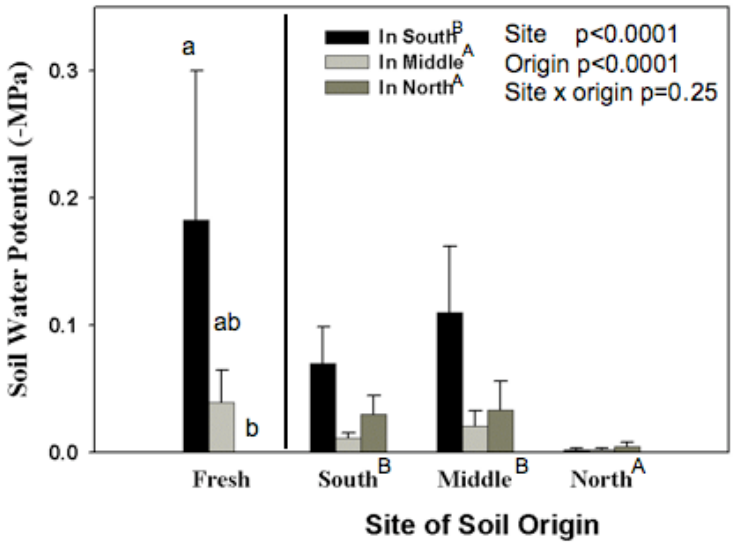


Figure 1. (A) Schematic of coast redwood sites and reciprocal transplants between the three sites (North, Middle and South). The circular arrows at each site indicate the transplant controls. (B) Annual rainfall (as the total from September – August) at the three study sites. The 30-yr average is for the years 1970 – 2000. The arrow at the top labeled 0 indicates when soils were transplanted, and the thicker arrow labeled 1 denotes when they were collected after 1 year.

(A)



(B)

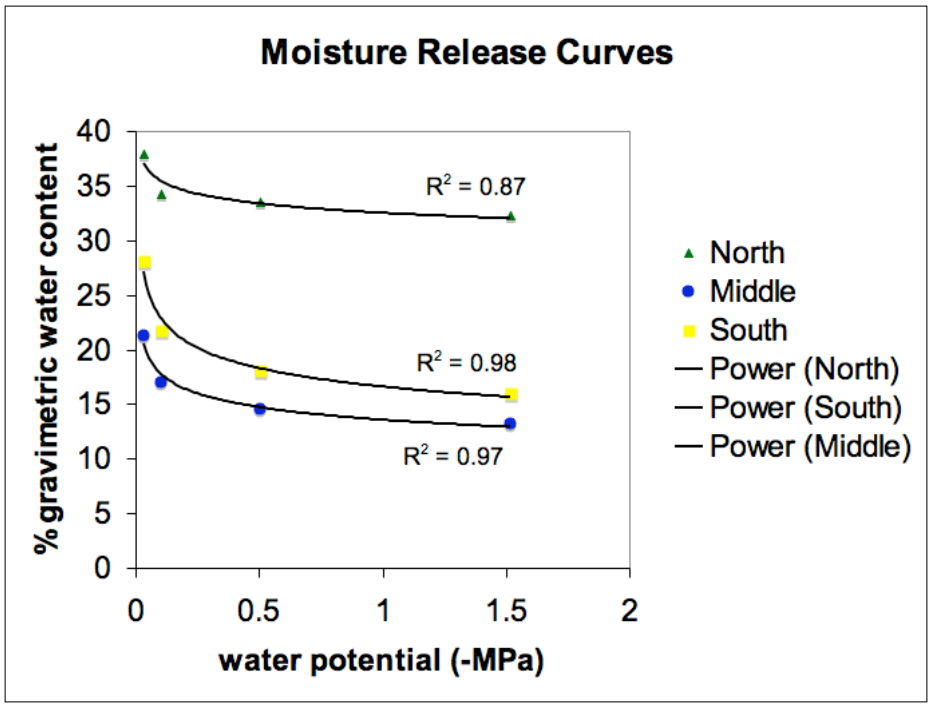


Figure 2. (A) Soil water potential in undisturbed (fresh) samples collected in August 2005 (to the left of the vertical line) and transplanted samples grouped by soil origin. Lowercase letters indicate significant differences in undisturbed samples and uppercase letters indicate significant differences by either site of origin or transplant. (B) Moisture release curves determined for undisturbed soil collected from the three sites (sieved < 2 mm).

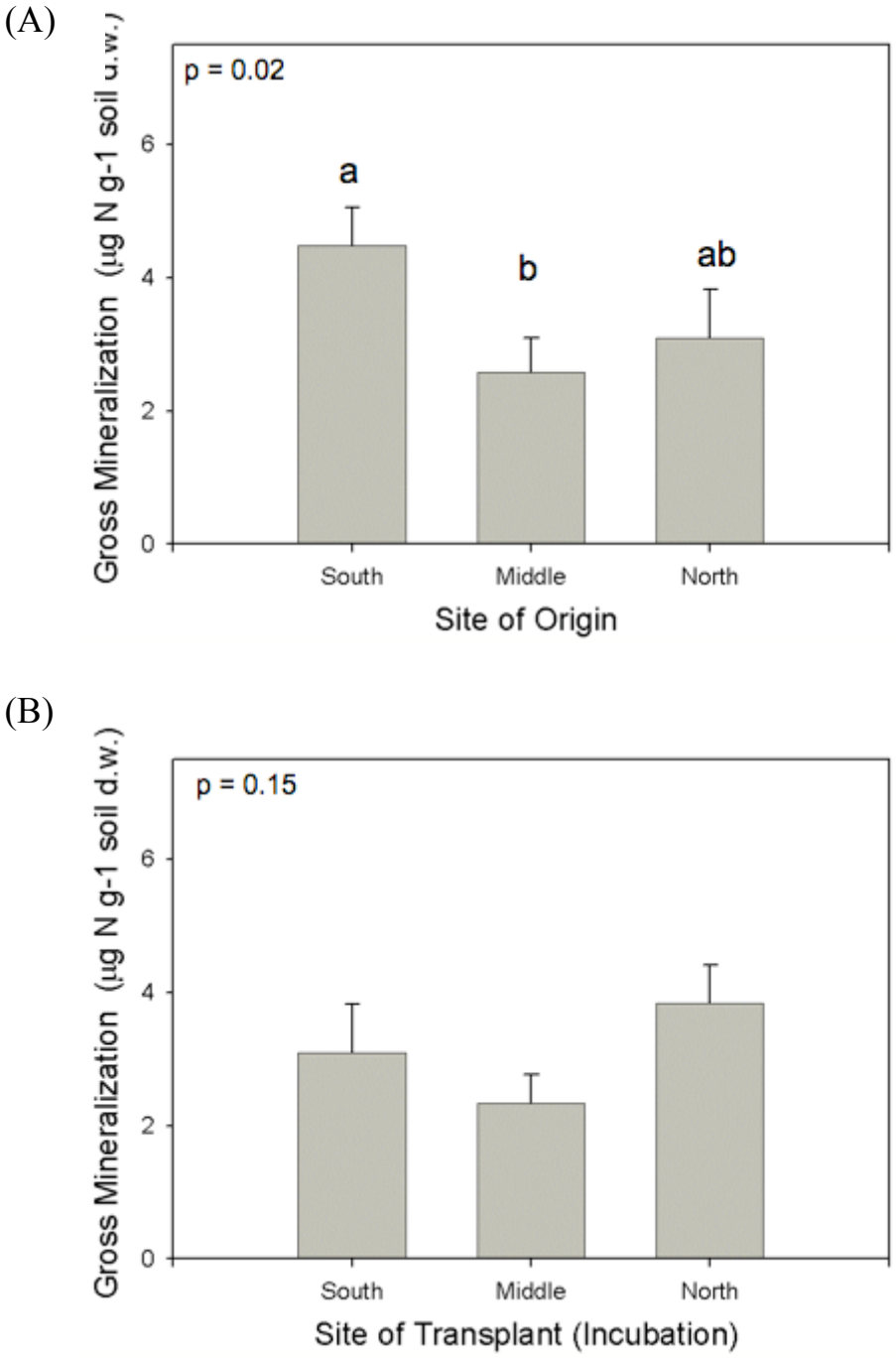


Figure 3. Gross N mineralization graphed by (A) site of origin and (B) site of transplant (incubation) for transplanted samples collected after one year. ANOVA p-values are in the upper left-hand corner and significant differences are denoted by lowercase letters.

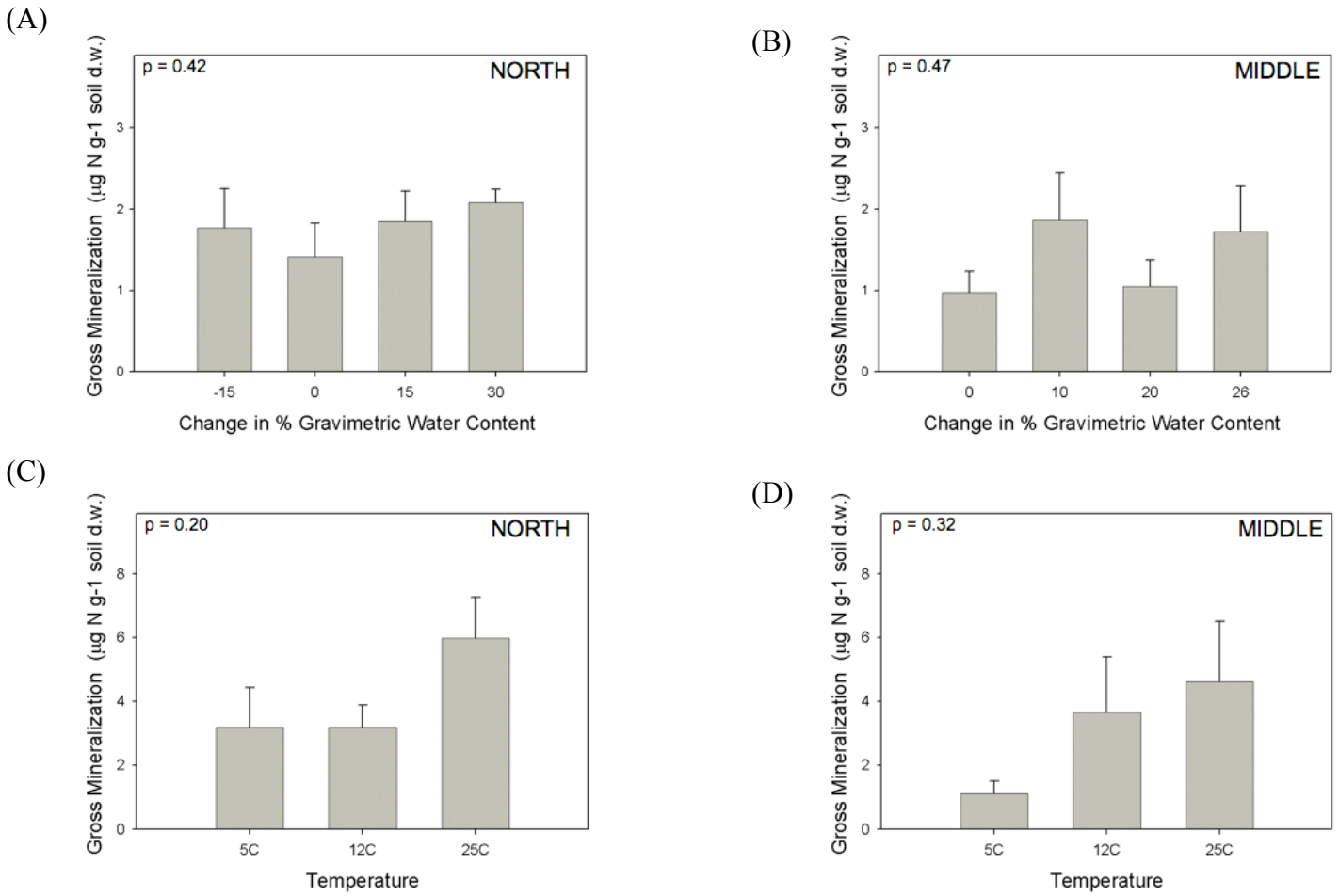


Figure 4. Impact of manipulations of (A and B) soil moisture and (C and D) soil temperature on gross N mineralization rates in soils from the North (A and C) and Middle (B and D) sites. Soils were collected when moist (in May 2005) for temperature manipulations and when dry (in November 2005) for moisture manipulations.

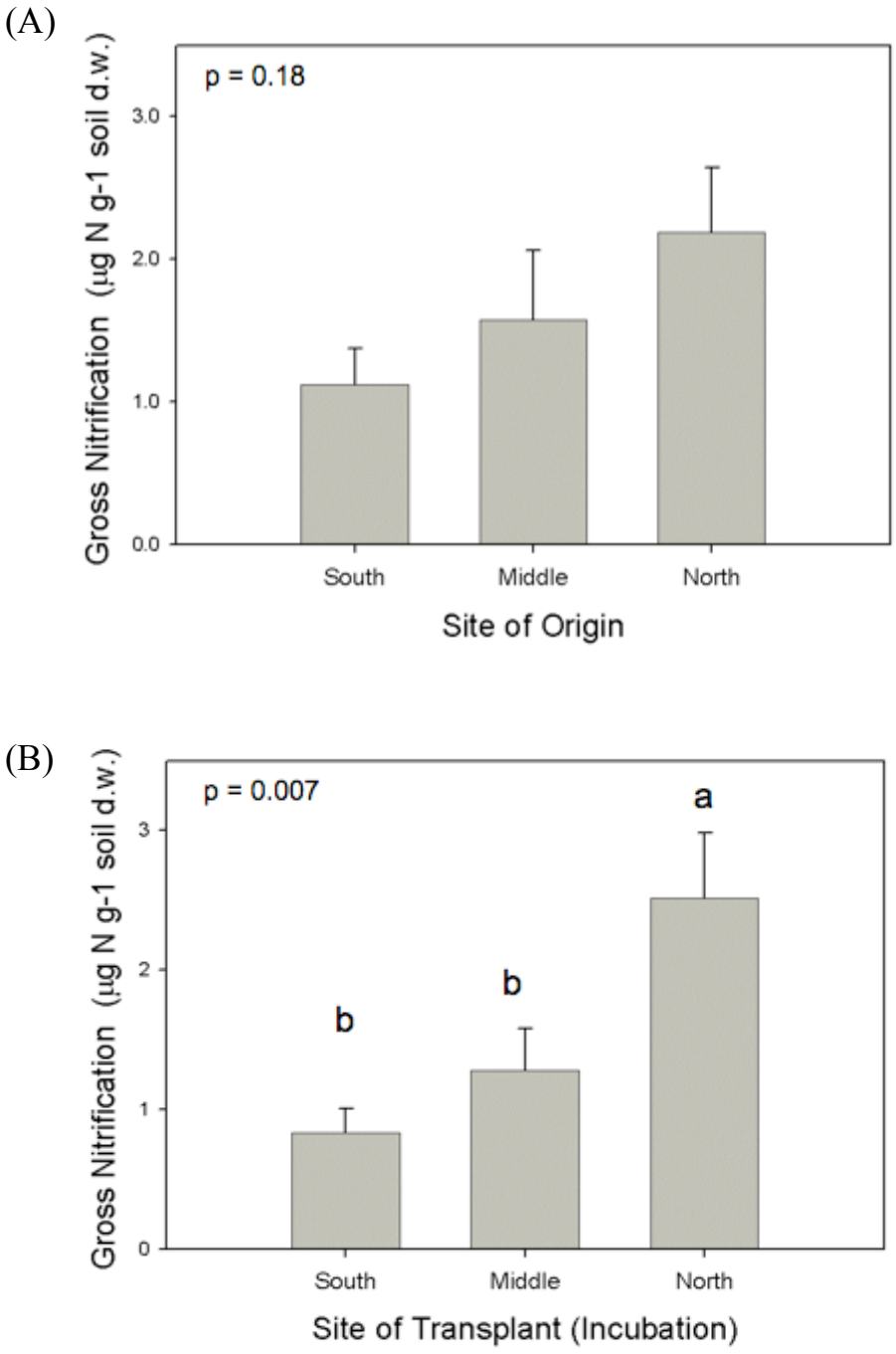


Figure 5. Gross nitrification graphed by (A) site of origin and (B) site of transplant (incubation) for transplanted samples collected after one year. ANOVA p-values are in the upper left-hand corner and significant differences are denoted by lowercase letters.

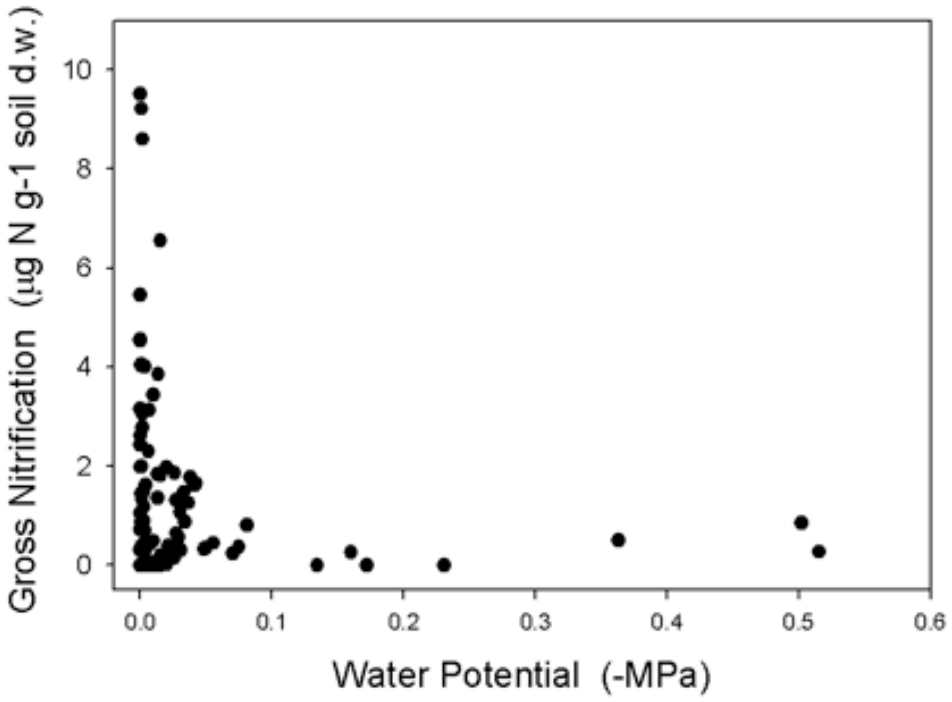


Figure 6. Rates of gross nitrification graphed against soil water potential for transplanted samples collected after one year.

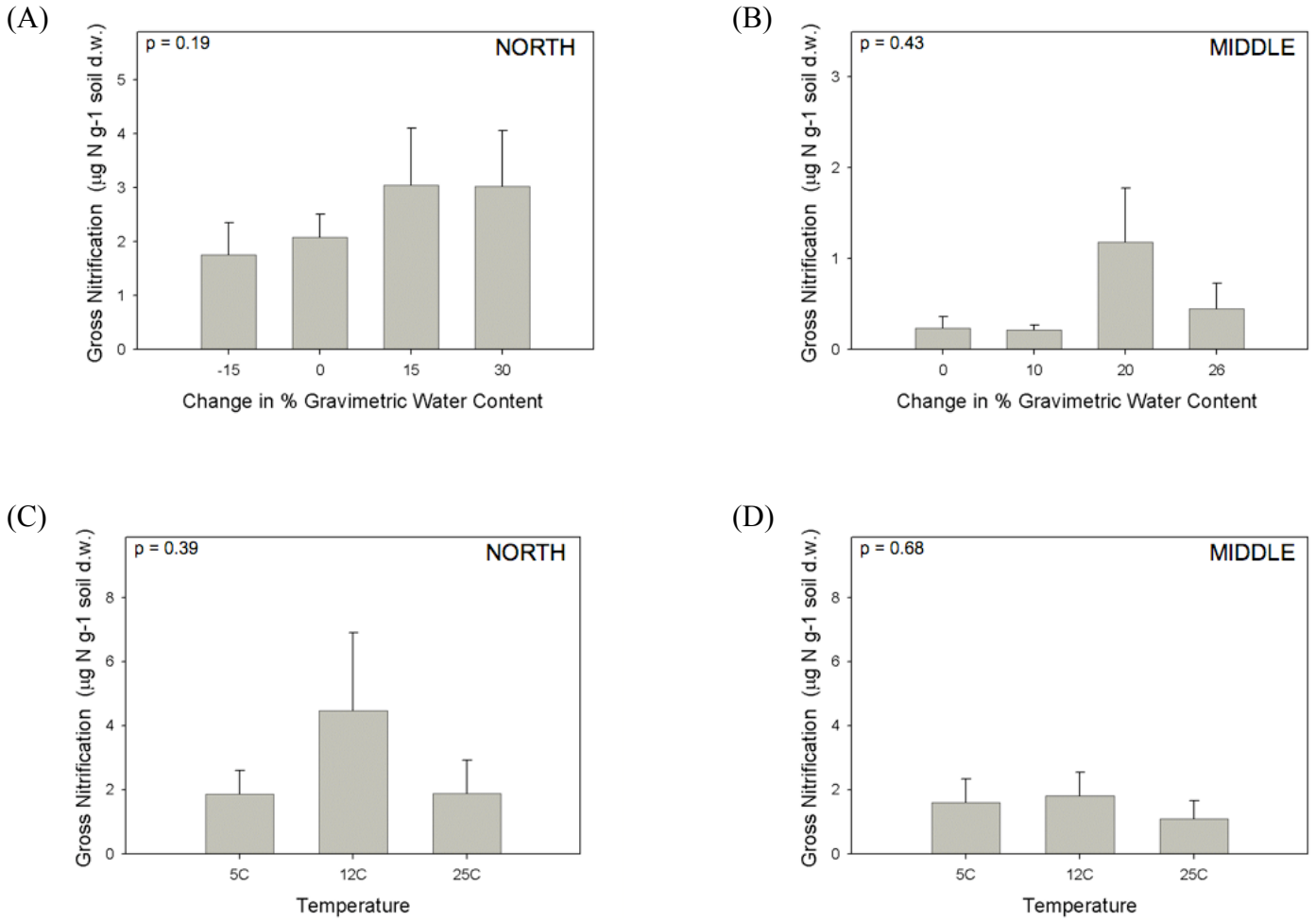


Figure 7. Impact of manipulations of (A and B) soil moisture and (C and D) soil temperature on gross nitrification rates in soils from the North (A and C) and Middle (B and D) sites. Soils were collected when moist (in May 2005) for temperature manipulations and when dry (in November 2005) for moisture manipulations.

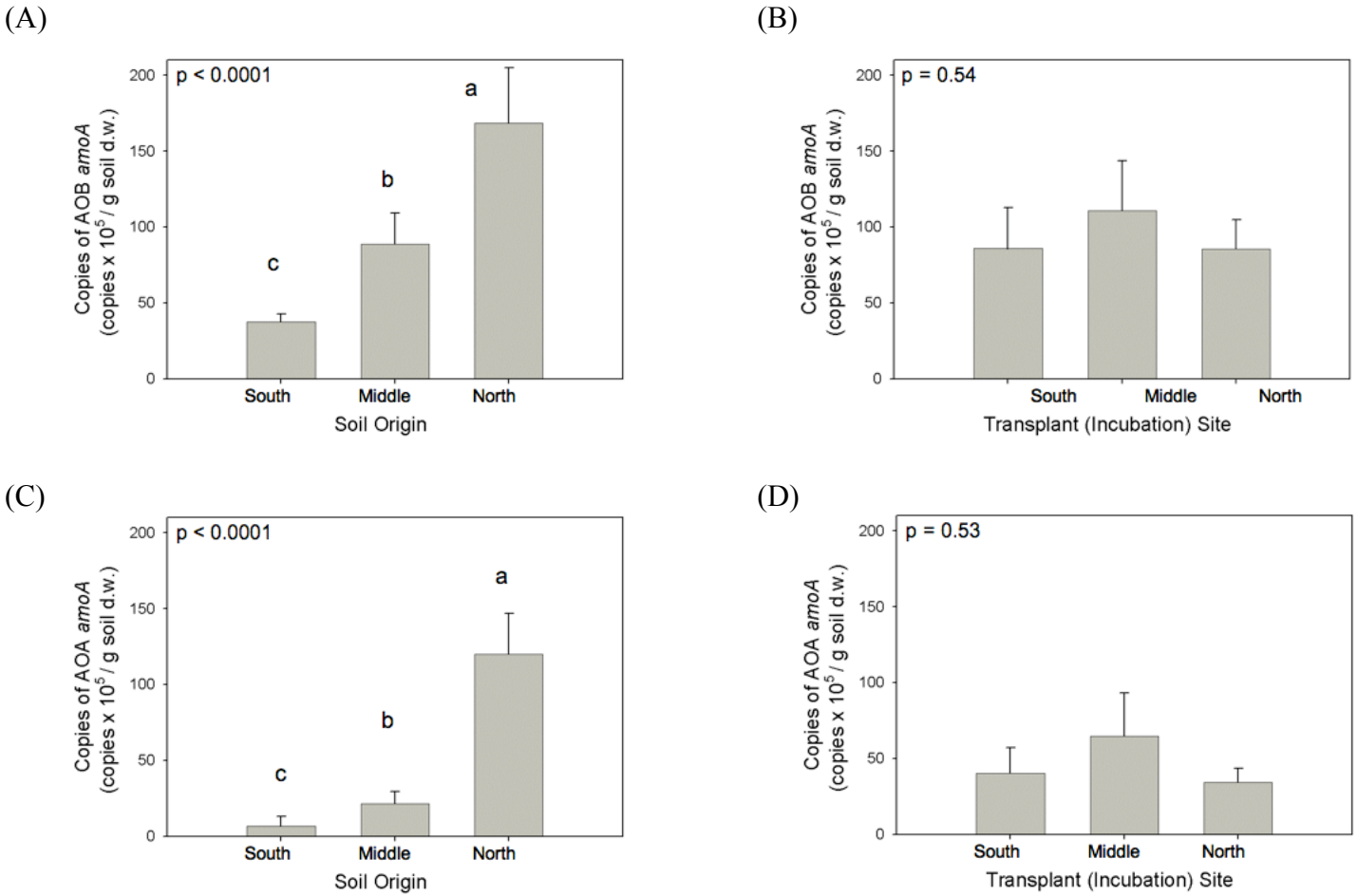


Figure 8. The abundances of ammonia-oxidizing bacteria (AOB) and archaea (AOA) *amoA* gene copies graphed by site of origin and site of transplant (incubation) for transplanted samples collected after one year. ANOVA p-values are in the upper left-hand corner and significant differences are denoted by lowercase letters. (A) AOB by origin; (B) AOB by transplant site; (C) AOA by origin; (D) AOA by transplant site.

Chapter 3

Response of soil microbial community composition to regional climate change in coast redwood forests

Abstract

The impact of changing climate on soil microbial community composition in coast redwood forests was assessed after one and three years of field exposure to a new climate. Soils were reciprocally transplanted between three coast redwood sites that differ in climate and fog frequency. Analysis by terminal restriction fragment length polymorphism (T-RFLP) demonstrated that both fungal and bacterial communities changed in composition after one year. Soil characteristics interacted with climate to frame the magnitude and character of these changes in community composition; the variability in community composition was correlated with edaphic as well as climatic variables for both bacteria and fungi. After both one and three years, a high-density 16s rDNA oligonucleotide microarray (PhyloChip) was used to examine bacterial community composition. Of the 2,339 bacterial taxa detected, 3.2% differed in relative abundance between transplants after one year, while 12.2% differed after 3 years. Taxa primarily within the phyla Proteobacteria and Firmicutes responded within one year to the changed environment. After three years, taxa from many common soil phyla differed in abundance, including Actinomyctetes, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetales and Spirochaetes. The bacterial taxa responding to transplant-induced climate change showed strong phylogenetic clustering by net relatedness analysis after both 1 and 3 years (NRI values of 4.8 and 2.4, respectively). Thus, there appear to be taxonomic, and phylogenetic, patterns in the speed of the soil bacterial response to changing climate. The patterns of community change reported here indicate that the impact of climate change on microbial community composition should be assessed using multi-year experiments.

Introduction

Scenarios of global change predict alterations in regional patterns of precipitation and temperature (IPCC 2007). An understanding of how soil microbes respond to alterations in climate is important because these organisms determine many aspects of ecosystem structure, function and services. While patterns have been proposed for the manner in which macrobiota can respond to shifts in climate (e.g., shifts in species range with latitude or altitude; Parmesan and Yohe 2003, Colwell et al. 2008), we have limited knowledge of microbial responses to climate change.

Soil microbial community composition often differs across gradients in environmental variables, including precipitation and temperature as well as edaphic characteristics (e.g., Angel et al. 2010, Waldrop and Firestone 2006a, Dubinsky 2008). Differences in microbial

community composition can impact a number of key ecosystem processes including community respiration (Balser and Wixon 2009), denitrification (e.g., Cavillegi and Robertson 2000, Holtan-Hartwig et al. 2002, Rich et al. 2003), nitrification (e.g., Stark and Firestone 1996, Horz et al. 2004, Martens-Habbena et al. 2009) and methane oxidation (Bengtson et al. 2009). Therefore, microbial responses to alterations in climate may be critically important in terms of how they affect ecosystem processes, particularly in their feedbacks to concentrations of important greenhouse gases: carbon dioxide, nitrous oxide and methane.

Studies that have examined the response of microbial community composition to changes in climate have found a range of responses in terms of the timing and magnitude of change. While it may seem reasonable for soil microbial community composition to change rapidly in response to climate change due to microbial capacity for rapid growth and succession (on lab media), rapid change does not seem to be the rule. Bacterial community composition has been reported to change rapidly, slowly or very little to not at all (Waldrop and Firestone 2006a, Balser and Firestone 2005, Cruz-Martinez et al. 2009, Rinnan et al. 2007, and Castro et al. 2010). For fungi, experimental warming was found to cause significant shifts in the structure and diversity of the active fungal community in black spruce forest (Allison and Treseder 2008), but warming did not cause a change in the overall fungal community composition in an upland boreal ecosystem that had experienced a severe fire seven years earlier (Allison et al. 2010). Differences in the results of these studies may be due to differences in: the sensitivity of the molecular methods used to determine microbial community composition and structure, the degree of similarity between the historical range of climate experienced by different soil microbial communities and the climatic regimes of experimental treatments, the length of time to which soils were exposed to a change in climate, or some combination of factors.

Many of the reported studies have been done by manipulating the environment at a site or transplanting soils between two different environments located close to one another. Studies that examine the impact of regional differences in climate within the same ecosystem type on microbial community composition will fill an important gap in our knowledge because the magnitude and character of these changes are generally within potential changes predicted by climate models.

Coast redwood forests, which are highly valued for their aesthetic, cultural and economic attributes, could be impacted by future changes in climate in coastal northern California, particularly the presence of fog. Native redwood forest is only found within a narrow fog belt extending eastward up to 60 km from the ocean and stretching 725 km from north to south, mainly in northern California (Noss 2000). While increased coastal upwelling could result in an increase in summer fog (Bakun 1990), warming of the Pacific Ocean can result in a reduction in the formation of fog because fog forms as warm air passes over cool ocean water. Long-term records of cloud ceiling height and temperature in northern California suggest that the frequency of fog has declined over the last century (Johnstone and Dawson 2010).

In order to test the response of indigenous fungal and bacterial microbial communities to changes in ambient climate, we carried out a three-site reciprocal transplant of soil cores across a latitudinal climate gradient in coast redwood (*Sequoia sempervirens*) forests, from near the northern limit (coolest and wettest) to the southern extent (warmest and driest) of

these forests (Figure 1). Soils were reciprocally transplanted between three coast redwood forest sites that differ in climate and fog frequency. Our research examines the timing and extent of the impacts of these transplants on bacterial and fungal community composition.

We hypothesized three general scenarios for the magnitude and pattern of the response of soil microbial community composition to exposure to a new climate (Figure 2): 1) The composition and structure of soil microbial community changes little and still resembles the community of its origin (2A). 2) Community composition changes to closely resemble that of the community native to the new climate (2C). 3) Community composition changes to something intermediate between the two extremes (2B). An intermediate composition is likely to occur if the new climate and the native soil edaphic or biological characteristics interact to influence soil microbial community composition.

Methods

Study sites

Study sites were established in three old-growth coast redwood forests across a north-south gradient in the summer of 2004. Old-growth coast redwood forests occur within a narrow fog belt across an 800-km long latitudinal gradient in precipitation and temperature extending from the southwestern tip of Oregon (42º09' N. latitude) to southern Monterey County in CA (35º01' N. latitude). Redwood forests experience a Mediterranean climate with cool, wet winters and warm, dry summers. The presence of fog in the summer is a defining characteristic of coast redwood forest (Noss 2000). Almost all of the rain falls between September and April, and it rains extremely little if at all in the summer. In August 2004, soils were transplanted between three old-growth redwood forest sites that differ in climate and edaphic characteristics (Fig. 1A, Table 1). The sites were located in Prairie Creek State Park, CA (Humboldt County), the Grove of the Old Trees, Occidental, CA (Sonoma County) and Big Basin State Park, CA (Santa Cruz County). These sites are referred to as the North, Middle and South sites, respectively.

All three of the sites were located in stands of old-growth coast redwood forest located in flat areas with an understory of mainly sword fern (*Polystichum munitum*) with some tanoak (*Lithocarpus densiflorus*) and sorrel (*Oxalis oregana*). The biomasses of the trees and understory plants are much greater in the northern site and the rates of primary productivity and decomposition are higher there as well (Table 1). The annual average precipitation increases with latitude while temperature decreases with latitude. Soil characteristics (e.g., soil C and N, pH, % silt and sand, and water retention curves) also differ between the sites (Table 1). As described below, soils from each site were transplanted into all three sites allowing for the examination of the potential for a change in climate to alter the microbial community composition in soils as well as the capacity of the native soil characteristics to buffer against changes in community composition in response to climate.

Experimental design – 3-way reciprocal transplant

In August 2004, two types of intact soil cores were transplanted between each pair of sites: 1) 10-cm diameter, 15-cm deep solid schedule 40 PVC cylinders and 2) flexible 2-mm-mesh shade cloth sown into cylinders of the same dimensions with nylon thread. For five (3 m x 3 m) plots at each site that were located ~30-400 m apart, each type of soil core was removed

from its site of origin and transplanted into both of the other sites. Plot pairs between sites were chosen randomly, and cores were randomly located within a plot. Soil cores were also returned to their plots of origin to serve as controls. All community changes in transplanted microbial communities were determined by comparing the transplanted community to the comparable control community remaining at the site of origin. In total, 180 cores were transplanted or replaced (as controls). After one year, 90 cores were harvested (30 cores per site consisting of 10 cores from each site, i.e., 20 transplants and 10 controls); after 3 years an additional 45 were harvested (15 cores per site consisting of 5 cores from each site, i.e., 10 transplants and 5 controls). A core of previously undisturbed soil was also collected adjacent to each plot (five per site) at each sampling time. Both the PVC and mesh cores were sampled after one year (early Sep. 2005), before the first rain. As expected, roots grew easily into the mesh cores; coast redwood roots were also able to easily colonize the PVC cores within the first year by growing up into them from the bottom. The two types of cores did not differ significantly in microbial community composition (by bacterial T-RFLP) at this first collection time, and thus, only the remaining PVC cores were collected along with another set of undisturbed cores at the three-year time point (early Sept. 2007).

Microbial community analyses

Soil samples were kept at field temperature until they were sieved (< 2 mm) and mixed within 24 hours. At this time, five-gram subsamples were frozen and stored at –80 °C until community analysis could be performed. Bulk soil DNA was extracted from 500 mg of soil using the Bio101 Fast DNA Spin Kit for Soils (Q Biogene, Carlsbad, CA) according to the manufacturer's instructions. The DNA extracts were then diluted (7.5X or 10X) to approximately 40 ng / μl. The PCR primers (from Sigma-Genosys, The Woodlands, TX) used for bacteria were 27F and 1492R (Giovanni 1991), and the ITS1F and ITS4 primers were used for fungi (Gardes and Bruns 1993). The forward primers were labeled with 6-FAM for the terminal restriction fragment length polymorphism (T-RFLP) PCR reactions. For the bacterial and fungal T-RFLP, three PCR reactions were bulked together before PCR clean up and digestion. For the PhyloChips, eight PCR reactions from across an annealing temperature gradient (48-58 °C) were bulked together before PCR clean up and PhyloChip fragmentation, labeling and hybridization. The PCR cycles used were: for fungi, 94 °C for 3 min; 30 cycles of 94 °C for 1 min, 51 °C for 1 min and 72 °C for 3 min; 72 °C for 8 min, and for bacteria: 95 °C for 3 min; 27 cycles of 95 °C for 30 s, 53 °C for 25 s and 72 °C for 1 min; 72 °C for 7 min, except the PhyloChip PCR reactions had an annealing temperature gradient from 48 °C to 58 °C. All of the PCR products were subject to the MoBio UltraPure Clean Up Kit and eluted in 50 μl of elution buffer before their downstream use.

Fungal and bacterial communities in soils collected one year after transplanting were analyzed by terminal restriction fragment length polymorphism (T-RFLP; Liu et al. 1997). All ten replicates of transplanted samples and controls and five replicates of fresh samples were analyzed by bacteria T-RFLP, and four samples (two mesh and two PVC cores) were analyzed for fungal T-RFLP. After PCR clean up, approximately 400 ng of bacterial 16s PCR product was digested with *MspI*. For fungi, 100 ng of fungal ITS PCR product was digested with *HhaI*. The tubes were incubated at 37 °C for 18 hours to ensure complete digestion and then ethanol precipitated and washed twice before they were resuspended in 10 μl of fresh

formamide. The abundance of different terminal restriction fragments (TRFs) was measured using an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, CA) run in the GeneScan mode. Each TRF was converted into its relative abundance (% abundance) by dividing each individual TRF peak height by the total sum of peak heights for all of the TRFs found in a sample and multiplying by 100.

For PhyloChip analysis, the PCR product was fragmented, labeled and hybridized to the PhyloChip following the manufacturer's instructions. Three replicate samples of transplanted soils (from PVC cores) and fresh soils were analyzed. For each sample, five hundred nanograms of 16s rDNA was labeled and hybridized to a PhyloChip. The details of this procedure and a description of the design and initial processing of the PhyloChip output are given in Brodie et al. (2006) and DeSantis et al. (2007). The PhyloChip has probes for 8,434 bacterial operational taxonomic units (OTUs). There is a set of 11 or more specific 25-mer probes for each OTU; the average number of probes for each OTU is 24. A pF (positive fraction of probes for a given OTU) value of 0.90 was used as the cutoff to determine the presence or absence of a species in a sample. The hybridization scores (intensities) for OTUs have previously been found to have a linear relationship with the amount of 16S ribosomal gene copies hybridized (Brodie et al. 2007). Hence, OTU intensities are interpreted as indices of taxa relative abundance. Statistical analyses were performed using the relative abundances (intensities) for all of the taxa (OTUs) detected.

Climate and soil characteristics

For both the one-year and three-year sampling times, the amount of precipitation, soil temperature and soil water availability were determined. Several other edaphic variables were also measured for the one-year sampling time point. Monthly precipitation data was obtained from the closest NOAA National Climatic Data Center weather station data available for each site. The stations used for the North and Middle, respectively, were Orick Prairie Creek Park (Station 046498, 41° 22′ N and 124° 01′ W) and Occidental (Station 046370, 38° 23′ N and 122° 58′ W), and for the South, the average of Ben Lomond #4 and Felton (Station 040673, 37° 05′ N and 122° 05′ W; Station 043004, 37° 03′ N and 122° 05′ W) was used. Soil temperatures were recorded at the time of sampling and in February, May and November as well as to determine the annual ranges in soil temperature. Gravimetric water content (GWC) was determined by drying soil (collected at the same times) at 105 °C. Measurements of bulk density made for each site were used to transform GWC into volumetric water content (VWC) and water-filled pore space (WFPS). Samples were sent to the UC Davis Analytical Lab for determinations of soil texture (% sand, % silt and % clay in soil suspension by the hydrometer method; Sheldrick and Wang, 1993) and water retention curves (by the pressure plate system; Klute 1986). These water retention curves were used to transform GWC values into soil water potentials. The other edaphic variables measured on the one-year samples included: pH (1:1 soil : 0.01M CaCl2), soluble organic carbon and nitrogen (extracted in 0.05M K_2SO_4), and microbial biomass C and N (by chloroform fumigation – direct extraction; Brookes et al. 1985). The total organic C concentration in extracts was determined on a TOC analyzer (OI Analytical 1010 TOC analyzer, College Station, TX). The dissolved N in extracts was converted into NO_3 by persulfate digestion (Cabrera and Beare 1993), and the NO_3-N concentrations were determined colorimetrically using an autoanalyzer (Lachat Quik Chem Flow Injection Analyzer, Lachat Zellweger Instruments, Milwaukee, Wisconsin, USA).

Statistical analysis

Non-metric multidimensional scaling (NMDS) was used to visualize the similarity in community composition between samples (McCune and Grace 2002). In NMDS ordinations, the closer samples are in proximity the more similar they are in composition. The NMDS ordinations were done in PCOrd 4.0 following the recommendations of McCune and Grace (2002) to produce a final NMDS ordination. The final stress of an ordination provides an indication of how well the relationship between the ranked distances of the samples in the NMDS ordination reflects their distances from each other in their original n-dimensional space; the lower the stress the better the ordination. The coefficient of determination (r^2) between the Euclidean distances between samples in ordination space, for each axis, and the Bray-Curtis distances between samples in the original p-dimensional (number of taxa) space provide an indication of how well the distance between samples for each NMS axis correlates with their original distances.

Permutational multivariate analysis of variance (PERMANOVA) was performed to determine significant differences in composition between soil-climate groups (Anderson 2001, 2005). PERMANOVA tests the response of one or more variables to one or more factors in an ANOVA experimental design on the basis of a distance measure by using permutation methods (Anderson 2005). Pair-wise differences between groups are determined *a posteriori* using a multivariate version of the t-statistic (based on distances). The Bray Curtis distance was used, and the tests were done using the distance ranks.

The results from the NMDS ordinations and PERMANOVA tests were compared to the hypothesized general response scenarios described in Figure 2 to determine if the transplanted redwood microbial communities had a strong response, intermediate response or no response to the change in climate. Transplanted samples were always compared to the control samples of both the site of origin and the new destination/climate. In the 3-way reciprocal transplant, there were six transplant scenarios that could be examined for an effect of climate change: North soil into South climate, North soil into Middle climate, Middle soil into North climate, Middle soil into South climate, South soil into North climate, and South soil into Middle climate.

The variability in fungal and bacterial community composition was correlated with environmental variables by performing Mantel tests. The Bray-Curtis distance was used for the taxa abundance matrix and the Euclidean distance for the environmental variable matrix. The standardized Mantel statistic (r) is equivalent to the Pearson's correlation (r) statistic. Mantel tests were performed to examine correlations between fungal and bacterial community compositions and several climatic and edaphic variables, including: gravimetric water content, volumetric water content, water filled pore space, water potential, total annual rainfall, spring rainfall (MJ), summer rainfall (JA), temperature, maximum mean monthly temperature, soluble organic carbon, total soluble nitrogen, microbial biomass carbon, microbial biomass nitrogen, pH, % sand, % silt and % clay and pH. All of these variables were tested independently, all together and as combinations of individually significant variables for their correlations with bacterial and fungal community composition one year after transplanting. Three years after transplanting, only bacterial composition by PhyloChip and precipitation, temperature, soil water availability and soil texture variables were available.

Each OTU has an associated phylogenetic identity (prokMSA id), and they are hereafter referred to as taxa. An OTU's taxon is the closest neighbor from GenBank to the OTU sequence that was used for the G2 PhyloChip design (from the greengenes taxonomy, 15 March 2002). To determine which taxa had a significant difference in relative abundance among transplanted and control samples, analysis of variance (ANOVA) was performed for each taxon with the site-climate transplant combination as the independent variable (n = 27; 9 groups x 3 replicates). The taxa with a significant result ($p < 0.10$) are referred to as "dynamic taxa."

To assess the phylogenetic relatedness and clustering of the dynamic taxa within all of the taxa detected in coast redwood soil (across both years), the net relatedness index (NRI) and nearest taxon index (NTI) were calculated for the dynamic taxa for each sample time (year 1 and 3) using the *picante* package in R (Webb 2000, Webb et al. 2002, Kembel et al. 2010). The NRI value quantifies the overall clustering of a subset of taxa within a tree by comparing the mean phylogenetic distance between the taxa found in the subset to a null model. The NTI is a measure of the mean distance between a taxon and its nearest neighbor and quantifies the extent of local clustering on terminal branches. Positive values of NRI and NTI indicate overall or local clustering, respectively, while a value near zero indicates no pattern; the more positive the NRI or NTI, the greater the degree of clustering. It is possible to have a high NRI and low NTI if the dynamic taxa are clustered within the overall tree, but there is not significant clustering on the terminal branches of the tree. For the NRI and NTI analyses, a phylogenetic tree based on the G2 PhyloChip bacteria greengenes taxonomy that was constructed using RAxML was pruned in R to include only the taxa detected in these coast redwood forest soils. After pruning, this tree was rooted using the first tree tip label as the new outgroup. Then, the *picante* package (Kembel et al. 2010) was used to calculate the NRI and NTI values for the year one and year three dynamic taxa from the mean phylogenetic distance (MPD) and mean nearest taxon distance (MNTD), respectively, as described by Webb et al. (2009), using the "taxa.labels" null model for presence-absence data and 999 runs.

Results

Patterns of precipitation and soil water availability for the years prior to sample collections
The 30-year average annual rainfall (1970-2000) and the total annual rainfalls from 2003 to 2007 are shown in Figure 1B. Since it rains extremely little to not at all in the summer months (JJA), the annual rainfall is presented as the total rainfall from September of one year through August of the next year. This also reflects the timing of soil sampling (at the end of the summer in early September, before the first rain). On average, it rains the most in the North, the least in the South, and an intermediate amount in the Middle site. While rainfall totals were similar to the 30-year averages in the year prior to transplanting the soils, it was an extremely wet winter in the South in 2004-2005, the year prior to the year 1 sampling. In contrast, the rainfall totals for the year prior to the year 3 sampling and pattern among sites were more similar to the long-term averages.
Differences in soil microbial community composition between sites

Fresh, undisturbed soils were sampled for community analysis in early September 2005 and 2007. The NMDS ordinations of fungal and bacterial community composition by ITS and 16S T-RFLP, respectively, show differences in the communities among the three sites in Sep. 2005 (Figs. 3A and 3B). Ordinations of the bacterial communities by PhyloChip analysis were more variable with the South and North communities being indistinguishable but different from the Middle after 1 year (Fig. 3C), while the only South and North communities were significantly different after 3 years and the community composition of the Middle soils varied greatly (Fig. 3D). After 1 year, the community compositions of samples from control cores generally differed from those of previously undisturbed (fresh) samples (Fig. S1). However, the patterns in compositional differences between sites were similar for the control samples and fresh samples. After three years, the PhyloChip composition of fresh and control samples for a given site did not differ significantly from each other according to PERMANOVA analysis ($0.14 < p < 0.45$).

Effect of transplant/climate change on soil microbial community composition

Fungal ITS T-RFLP: Visual inspection of the NMDS ordination for fungal communities (Fig. 4) reveals that, while the fungal community compositions of the transplanted samples shifted to more closely resemble those of the controls of the soil native to the new site/climate after one year, they did not become indistinguishable from those of the new site/climate. All community responses were classified as "intermediate" by the ranked PERMANOVA (Table 2).

Bacteria T-RFLP: Transplanting soil into a new climate also generally caused an intermediate change in bacterial community composition when examined by 16S T-RFLP (Fig. 5). Five of the six transplanted soils changed in composition but still differed from the control samples of the new site according to the PERMANOVA analysis (Table 2). One transplant scenario did not cause a community response, North soil into the South climate.

Bacteria PhyloChip: Bacterial community analysis using PhyloChips also indicated that the transplant generally caused an intermediate change in soil bacterial community composition after one year. Four of the six transplant scenarios resulted in intermediate responses (Figs. 6A and 6C, Table 2). In the other two scenarios (the samples reciprocally transplanted between the North and South, Fig. 6B), the community compositions of the transplanted samples differed from their site controls, but the soil controls from the two sites were similar to each other, as were the compositions of the samples transplanted between the two sites (North soil into South climate, and South soil into North climate) These two responses were classified as "alternate" responses (Table 2).

NMDS ordinations of the PhyloChip-defined bacterial communities three years after transplanting show results similar to the one-year results, with a few differences (Fig. 7, Table 2). The North-Middle and Middle-South reciprocal transplants again resulted in mainly intermediate responses to climate (Figs. 7A and 7C), but the Middle soil transplanted into the North was classified as exhibiting a "strong" response to the transplant-induced change in climate (Fig. 7A, Table 2). The North and South controls were again similar in composition to each other, but in year three the South soil transplanted into the North climate did not differ in composition from its South origin control and was thus classified as having "no response" (Fig. 7B, Table 2).

Taxonomy and phylogeny of taxa responding to reciprocal transplants

An analysis of variance (ANOVA) was conducted for each PhyloChip taxon detected across all transplanted samples after one and three years to determine if there was a significant difference in relative abundance among the nine site-climate transplant and control groups (six transplants of soil from one site to another and three transplant control sites, n = 27). There were 2,339 taxa detected across all of the one-year samples and 2,620 taxa detected in the three-year samples, and the taxa that had ANOVA p-values ≤ 0.10 were considered as "dynamic taxa". The number of dynamic taxa increased from 3.2% after one year to 12.4% after three years. The number of phyla that contained at least one dynamic taxon increased from 7 to 25. After one year, the only phyla that had more than two dynamic taxa were the Proteobacteria and Firmicutes (5.4% and 3.9% of their detected taxa, respectively; Table 4). After three years, however, twenty percent or more of the taxa detected for Acidobacteria, Chloroflexi and Spirochaeteas were significantly different among soil-climate combinations, as well as between 8.8 and 15.8 % of Firmicutes, Proteobacteria, Actinobacteria and Planctomycetales, and 4.3% of Bacteriodetes.

The degree of phylogenetic relatedness of the dynamic taxa was assessed by calculating the NRI and NTI values for the dynamic taxa after one and three years (Table 4). The significant positive NRI values (4.8 and 2.3 for years one and three were, respectively, p = 0.001 and 0.015) indicate that the dynamic taxa were clustered within the phylogeny of coast redwood bacteria. The dynamic taxa were more highly clustered after one year, but the relatively greater number and diversity of dynamic taxa were still significantly clustered after three years. The NTI values indicate that there was terminal clustering after one year (NTI = 6.4, p = 0.001), but not after three years (NTI = 1.0, p = 0.16), when more dynamic taxa from a greater number of phyla were more spread out across terminal branches.

Correlation of environmental variables with variability in community composition

After one year, fungal community composition (by T-RFLP) correlated significantly with temperature, precipitation, pH and soil texture values. The strongest correlations were found with the mean maximum daily temperature (r = 0.62), mean temperature (r = 0.56), the amount of summer rainfall (r = 0.56) and the total annual rainfall of the previous year (r = 0.37; Table 3). When examining the correlations between bacterial community composition by T-RFLP and environmental variables, measures of precipitation and soil water availability had weaker correlations (r = 0.15 to 0.20). Measures of soil texture and soil pH had correlations between 0.28 and 0.39, and total dissolved nitrogen (r = 0.18), microbial biomass nitrogen (r = 0.20) and microbial biomass carbon (r = 0.23) all had lower but significant correlations with bacterial T-RFLP community composition (Table 3). In contrast, after both one and three years, the only significant correlations found between environmental variables and bacterial community composition by PhyloChip were with variables associated with precipitation, soil water availability and temperature (Table 3). The total annual rainfall of the previous year was the only variable that was significantly correlated with PhyloChip bacterial community composition in both years. For all years and community metrics, neither all of the variables together nor any combination of individually significant variables resulted in higher correlations than those of the single highest individual correlations (not shown).

Discussion

Response of bacterial and fungal community composition to a change in climate

Climate change resulting from transplanting soil cores across a 500-km latitudinal gradient in climate caused detectable changes in bacterial and fungal community compositions within one year. Under most of the transplant scenarios in this study, the community composition shifted to more closely resemble that of the site into which it was transplanted. However, the community compositions of the transplanted samples were usually still distinguishable from those of the new sites. Given more time, the composition of a microbial community may continue to change, but it may never fully resemble that of the new site. While climate is an important determinant of microbial community composition, it is certainly not the only significant controller.

The varying impacts of climate change on microbial community composition that have been found across studies could be related to the strength of the change in climate to which soils are exposed. For example, in California, Waldrop and Firestone (2006a) also found that soil microbial community composition (by PLFA) changed when soils were transplanted from beneath oak canopies into an open grassland environment, but the reverse was not true (grassland soil microbial community composition did not change when transplanted beneath an oak canopy). They suggested that the exposure of communities to climatic conditions outside of the more recent range in historical climate that they had experienced caused a rapid change in community composition. In a coastal California grassland study, bacterial community composition (also examined by PhyloChip) differed little in response to increased rainfall after five years of rainfall manipulations (Cruz-Martinez et al. 2009). In that study, all of the communities had experienced the same historical range in climate, and the climatic regime likely encompassed the conditions experienced under the rainfall manipulations. Castro et al. (2010), however, observed changes in fungal and bacterial abundances (by q-PCR) and community composition (by sequencing and cloning) after three years in response to manipulations of climatic drivers (temperature, precipitation and CO_2 concentration) in old-field ecosystems. They found that precipitation had the largest impact on composition.

The climatic conditions of our three redwood forest sites overlap. The mean values for temperature and precipitation at all sites are within the ranges historically experienced by the indigenous communities. However, the extremes in precipitation and temperature (maxima and minima) occurring in the "new" sites post-transplanting may have provided sufficient stimulation for a change in community composition to occur. The relative importance of mean temperature and rainfall values compared to maxima and minima (and their duration) in determining community composition is an important but largely unexplored question

Response of bacterial taxa to climate change

While overall bacterial community composition shifted in response to a transplant-induced change in climate after 1 year, only a small portion of the taxa detected by the PhyloChip had significant differences in relative abundance across soil-climate combinations. It is notable that the differences between treatments took longer to occur (three years vs. one year) for some bacterial taxa than others and that changes in the abundance of bacterial taxa were still occurring three years after being exposed to new climatic regimes. The number of bacterial

taxa that exhibited a difference in abundance between soil-climate scenarios ("dynamic taxa") increased from 3.2% of the detected taxa after one year to 12.4% after three years, and the number of phyla that contained dynamic taxa also increased from 8 phyla to 26 phyla between one and three years. These results suggest that changes in the abundance of many taxa across many common soil phyla will continue to occur for a number of years. This study examined community composition based on ribosomal DNA. While changes in microbial community composition may occur somewhat slowly in response to a change in climate, it is likely that changes in activity and physiological responses to climate occur much more quickly.

The two phyla that were most responsive to climate change after one year were the Proteobacteria and Firmicutes, which contained 52 and 16 dynamic taxa, respectively. All of the Firmicutes taxa were *Bacilli* except one *Clostridium*, and most (17 of 21) of the β-Proteobacteria were *Burkholderia*. Both the Firmicutes and Proteobacteria are commonly found in soil (e.g., Buckley and Schmidt 2003, Fierer et al. 2005, Janssen 2006), and they are considered to contain "weedy species" because they can outcompete other more common soil microorganisms and grow rapidly on lab media. This trait could enable their rapid response to transplanting.

In contrast to the one-year results, the dynamic taxa represented a larger proportion of the taxa detected from many common soil phyla after three years. A number of dynamic taxa were present among the Acidobacteria, Actinobacteria, Bacteroidetes, Choloroflexi, Planctomycetes, and Spriochaetes as well as the Proteobacteria and Firmicutes. Some of these phyla are among the most abundant groups found in soil (e.g., α-Proteobacteria, Acidobacteria and Actinobacteria), and others (Bacteroidetes, Firmicutes, Chloroflexi, Planctomycetes and Spirochaetes) belong to phyla well represented in soil but often occurring in abundances lower than 5% of the bacterial community (e.g., Buckley and Schmidt 2003, Fierer et al. 2005, Janssen 2006). Many soil bacterial phyla, including the most abundant ones, seem to respond to climate change over a period of several years.

The capacity of bacterial groups to respond quickly to a change in climate could depend in part on their capacity for rapid growth in soils in response to changes in resource availability. Fierer et al. (2007) found that β-Proteobacteria and Bacteroidetes tended to respond rapidly to increased C availability, while Acidobacteria tended to respond more slowly, and Firmicutes and Actinobacteria showed no response. In our study, β-Proteobacteria were the group with the most dynamic taxa after one year, and only one taxa of Acidobacteria differed significantly in abundance between soil-climate scenarios. These results lend support to the idea that β-Proteobacteria are capable of a more rapid response, while Acidobacteria grow more slowly and respond slower to environmental change. There were 17 dynamic taxa of Acidobacteria (20% of those detected) after three years, however. The numbers of dynamic Bacteroidetes taxa were low (only one and eight dynamic taxa after one and three years, respectively), but did increase over time. In this study, Firmicutes were the only other group besides Proteobacteria to have a number of dynamic taxa after one year, and this number more than doubled after three years. In contrast, there were no dynamic Actinobacteria taxa after one year, but 38 (13% of those detected) differed significantly among soil-climate scenarios after three years. Capacity for rapid growth could impact bacterial responses to climate change, but other physiological adaptations that impact optimal growth temperatures and responses to water availability are likely to also be important.

Taxonomic coherence and phylogenetic relatedness of dynamic taxa

The net relatedness index (NRI) and nearest taxon index (NTI) values for the dynamic taxa for years one and three provide insight into the phylogenetic clustering of the dynamic taxa. After both one and three years, the dynamic taxa were significantly clustered within the phylogenetic tree of the detected coast redwood soil bacteria (Table 4). This suggests a degree of phylogenetic coherence in the ability of bacteria to respond to a transplant-induced change in climate. The NRI value decreases between years one and three (from 4.8 to 2.3), indicating that the dynamic taxa are less clustered in year three. This decrease reflects the increase in the number of phyla that contain several dynamic taxa. The dynamic taxa were not terminally clustered at the tree branch tips after three years, though they were after one year. The insignificant NTI value for the three-year dynamic taxa reflects the spread of the bacterial response over time across the terminal branches of a number of phyla that contain dynamic taxa. This result indicates that very closely related groups of soil bacteria respond rapidly to climate change within one year, and a greater phylogenetic diversity of bacteria, which are still related at a higher taxonomic level, respond by three years after exposure to a new climate.

The role of edaphic factors in determining changes in community composition in response to climate

While climate clearly influences community composition in this study, it is not the sole determinant of microbial community composition. The predominance of intermediate changes in microbial community composition, even after three years, suggests that biological and/or edaphic factors also influence the trajectory and magnitude of the community response to climate. The composition of some communities may be slowly changing toward that of the new site, but in other instances, the transplanted community may never closely approach the composition of the new site's native community. The native soil characteristics may buffer against the effects of climate; additionally, biological interactions or environmental stochasticity could cause the change in community composition to take a different trajectory. In this study of coast redwood soils, the strongest correlations between environmental variables and both PhyloChip-based bacterial community composition and T-RFLP fungal community composition were found with climatic variables, not edaphic variables (Table 3). However, there were strong correlations between edaphic variables, especially soil texture and pH, and both the bacterial and fungal community compositions by T-RFLP. There are substantial differences between the soils from the South and North sites in pH, and the proportions of sand, silt, and clay, as well as total soil carbon, which differs by a factor of two. Differences in soil organic matter content have been previously proposed to influence microbial community composition across soil depths as well as across different cultivations histories (Krave et. al. 2002, Buckley and Schmidt 2003), and soil pH has been found to be a strong environmental driver of bacterial community composition at a continental scale (Fierer and Jackson 2006, Chu et al. 2010).

The variations in T-RFLP community composition correlate with edaphic characteristics, but the PhyloChip bacterial community composition (year 1) does not. This finding reflects the differences in the patterns in community composition evident when using the two methods. Community composition differed between all three sites by T-RFLP, but the North and South sites had similar compositions by PhyloChip. Thus, some edaphic

characteristics such as pH and soil texture are more strongly correlated with T-RFLP composition because of the strong differences in these edaphic characteristics between sites. This could indicate that the composition of some of the more dominant taxa (picked up by T-RFLP) are more closely linked to edaphic characteristics such as pH and soil texture than more rare members of communities (only detected by PhyloChip and not T-RFLP). The PhyloChip is much more sensitive for detecting taxa than T-RFLP and provides data of an order of magnitude greater taxonomic detection. Deeper sampling of the more rare members of soil bacterial communities may have obscured relationships of some of the more abundant taxa with strong edaphic controllers like pH, especially if the rarer taxa vary more with differences in climate than other edaphic characteristics.

Conclusions

A regional-scale transplant-induced change in climate caused changes in fungal and bacterial community composition within one year. These changes occurred within the framework of the native soil characteristics, possibly in response to the initial community's exposure to conditions outside of its historical range in climate. The relative importance of climate maxima and minima compared to climatic means requires further exploration. The number of bacterial taxa that differed across the various soil-climate combinations increased between one and three years, but these taxa still showed strong phylogenetic clustering indicating a level of taxonomic coherence among groups that respond to climate change within three years. Results from this research suggest that studies with longer (multi-year) time-scales are required to understand the full extent to which climate change will ultimately alter microbial community composition.

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	North	Middle	South
Location	Prairie Creek S.P., Orick, CA	Grove of the Old Trees, Occidental, CA	Big Basin S.P., Boulder Creek , CA
(Latitude distance to coast, elevation)	41° 41' N, 6.8 km, 54 m.a.s.l.	38° 24' N, 6.5 km, 148 m.a.s.l.	37° 10' N, 6.4 km, 128 m.a.s.l.
ANPP ($kg \cdot ha^{-1} \cdot yr^{-1}$)*	7,138	4,807	4,710
Decomposition (k; yr^{-1})*	0.28	0.14	0.13
Mean annual rainfall (mm)	1,677	1,419	1,211
Summer fog	Foggy, but little fog drip	Foggy, and a few big drip events	Very little fog
Air temperature (mean / range)	11 °C (2 °C – 21 °C)	14 °C (4 °C – 29 °C)	15 °C (2 °C – 30 °C)
Soil moisture range for 0-15 cm (vol. water content)	29.7% to 65.9%	15.2% to 38.4%	11.1 % to 34.4%
Soil temperature (range at 7.5 cm deep)	5 °C – 14 °C	7 °C – 16 °C	8 °C – 17 °C
Soil texture (0-15 cm)	Sandy Loam	Sandy Loam	Sandy Loam
% sand (mean +/- 1 s.e.)	42 +/- 3.8	53 +/- 0.6	32 +/- 2.8
% silt (mean +/- 1 s.e.)	41 +/- 3.5	35 +/- 0.7	55 +/- 3.0
% clay (mean +/- 1 s.e.)	16 +/- 0.3	12 +/- 0.9	13 +/- 0.6
Soil pH, 0-15 cm (mean +/- 1 s.e)	4.9 +/- 0.1	5.3 +/- 0.2	5.7 +/- 0.2
Soil %C, 0-10 cm (mean +/- 1 s.e.)	10.7 +/- 0.9	4.6 +/- 0.4	4.5 +/- 0.5
Soil %N, 0-10 cm (mean +/- 1 s.e.)	0.58 +/- 0.04	0.27 +/- 0.07	0.25 +/- 0.03

*The ANPP (aboveground net primary productivity, i.e., litterfall) and steady-state decomposition are from V. Boukili and T. Dawson (unpublished data).

Table 1. Site Characteristics. The mean annual rainfall and air temperature are the 30-year averages (1970-2000) and ranges from the NOAA weather stations identified in the methods section. The soil moisture and temperature are from samples collected during the period from August 2004 – September 2007. The soil texture, soil pH, soil %C and soil %N are data from samples collected in August 2004 or August 2005.

Transplant	Strong response	Intermediate response	No response	Alternate response
North into South		F_1	B_1	P_1 , P_3
North into Middle		F_1 , B_1 , P_1 , P_3		
Middle into North	P_3	F_1 , B_1 , P_1		
Middle into South		F_1 , B_1 , P_1 , P_3		
South into North		F_1 , B_1	P_3	P_1
South into Middle		F_1 , B_1 , P_1	P_3	

Note: F_1 = one-year fungal TRFLP composition; B_1 = one-year bacterial TRFLP composition; P_1 = one-year PhyloChip bacterial composition; P_3 = three-year PhyloChip bacterial composition

Table 2. Summary table of the strengths of the responses of soil microbial community composition to changes in climate due to transplanting between sites. The degree of the response for a transplant pair was determined by examining the NMDS ordinations and PERMANOVA results shown in Figs. 4-6.

Environmental variable	r	p-value
Fungi T-RFLP, after one year		
Annual rainfall	0.37	0.0001
Late spring rain	0.32	0.0001
Summer rain	0.56	0.0001
Temperature	0.56	0.0001
Max. temperature	0.62	0.0001
pH	0.20	0.006
% sand	0.12	0.010
% silt	0.12	0.018
% clay	0.14	0.0045
Bacteria T-RFLP, after one year		
Annual rainfall	0.20	0.0005
Late spring rain	0.15	0.001
Gravimetric water content	0.10	0.001
Water-filled pore space	0.13	0.0002
pH	0.39	0.0001
% sand	0.28	0.0001
% silt	0.28	0.0001
% clay	0.28	0.0001
Total dissolved N	0.18	0.002
PhyloChip bacteria, after one year		
Annual rainfall	0.19	0.013
Late spring rain	0.19	0.019
PhyloChip bacteria, after three years		
Annual rainfall	0.17	0.009
Summer rain	0.19	0.007
Gravimetric water content	0.18	0.040
Temperature	0.13	0.047

Table 3. Significant standardized Mantel statistics (r) for the relationships between soil microbial community composition of transplanted samples and environmental variables.

Dynamic taxa		**After 1 year**		**After 3 years**	
Phylum/Division	*Class*	*Number*	*(percentage)*	*Number*	*(percentage)*
Acidobacteria		1	(1.2%)	17	(19.8%)
Actinobacteria		0		38	(13.4%)
Bacteroidetes		1	(0.6%)	8	(4.3%)
Chlorflexi		0		14	(28.0%)
Firmicutes		16	(3.9%)	39	(8.8%)
Planctomycetlaes		0		9	(15.8%)
Proteobacteria		52	(5.4%)	143	(12.7%)
	Alpha-	11	(3.3%)	104	(28.3%)
	Beta-	21	(10.8%)	15	(7.1%)
	Delta-	0		11	(8.2%)
	Gamma-	20	(6.5%)	13	(3.2%)
Spirochaetes		1	(2.4%)	18	(41.9%)
Other		3	(3.3%)	39	(12.5%)
TOTAL		74	(3.2%)	325	(12.4%)
Phylogenetic relatedness		*After 1 year*		*After 3 years*	
NRI		4.8^{***}		2.4^{**}	
NTI		6.3^{***}		$1.0^{n.s.}$	

Table 4. Numbers of PhyloChip bacterial taxa that differed significantly in abundance (intensity) among site-climate transplant scenarios (at $p < 0.10$), and indices of their phylogenetic relatedness. An ANOVA was performed for each taxa using the intensities for the transplanted and control samples (n = 27; 9 groups x 3 replicates). The numbers of significant taxa (dynamic taxa) are summarized by phyla. The percentage of the total taxa detected in that year is given in parentheses. In year one, the 3 "other" dynamic taxa were spread across 2 phyla, and the 39 "other" dynamic taxa were spread across 18 phyla in year three. After one year, 2,339 taxa were detected in total across all of the samples, and 2,620 taxa were detected after three years. For the NRI and NTI values: ** indicates $p < 0.01$; *** indicates $p = 0.001$; n.s. indicates not significant,

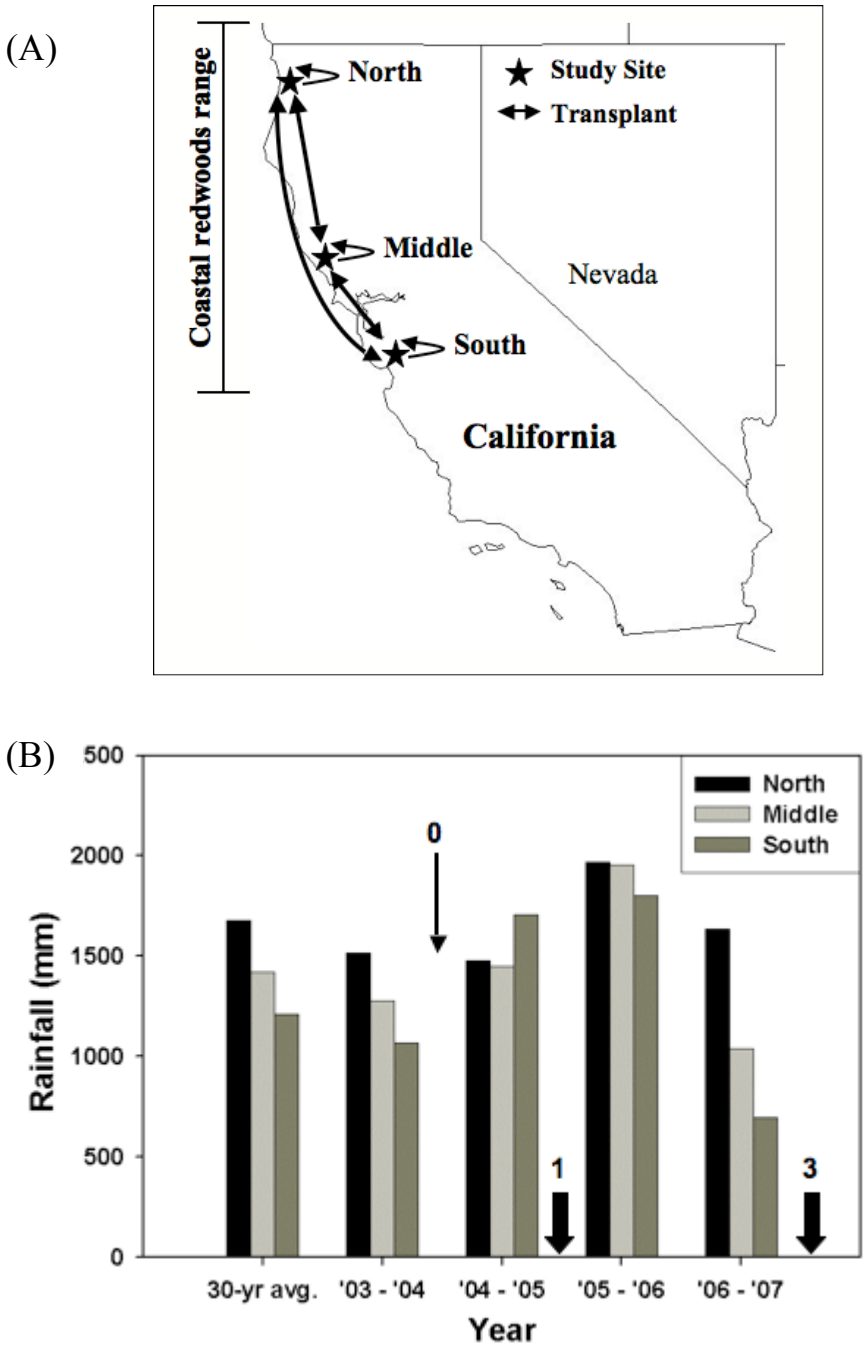

Figure 1. (A) Schematic of coast redwood sites and reciprocal transplants between the three sites (North, Middle and South). The circular arrows at each site indicate the transplant controls. (B) Annual rainfall (as the total from September – August) at the three study sites. The 30-yr average is for the years 1970 – 2000. The arrow at the top (0) indicates when soils were transplanted, and the thicker arrows at the bottom (1 and 3) denote the 1-year and 3-year sampling time points.

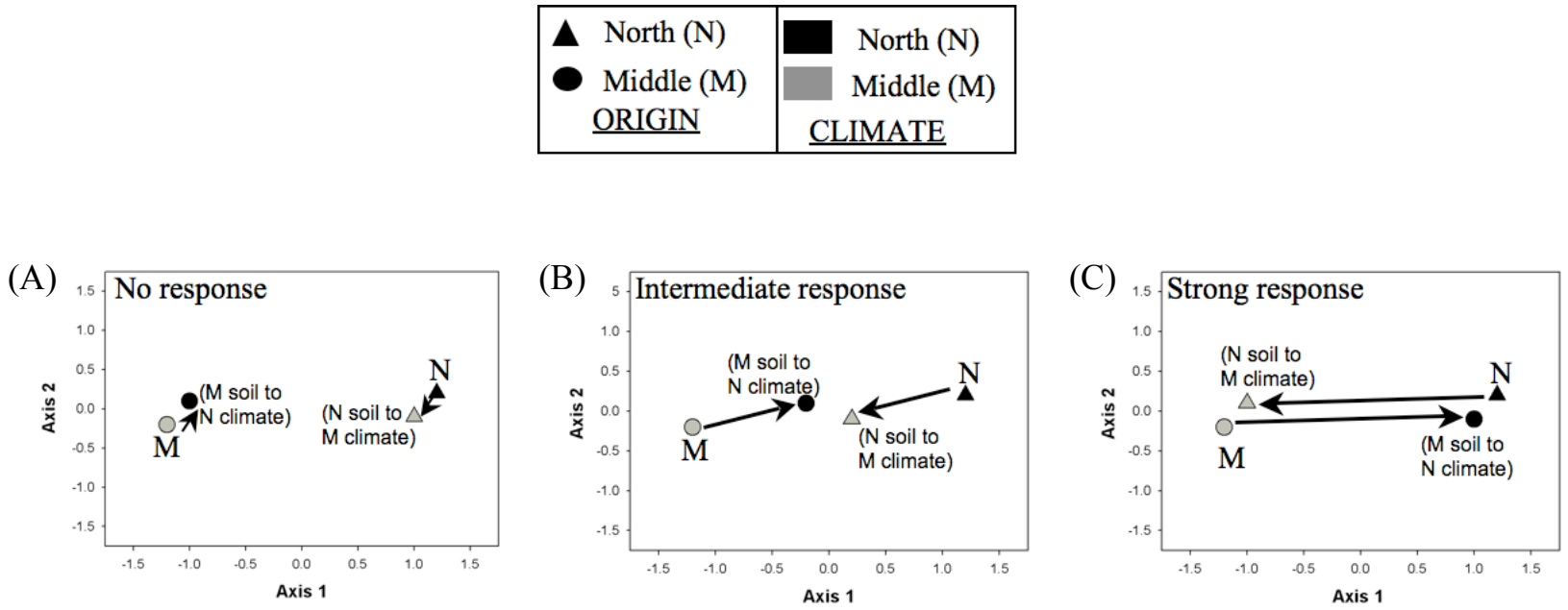

Figure 2. Hypothetical responses of soil microbial communities to climate change. The graphs show theoretical ordination results for samples reciprocally transplanted between two sites. Transplanted samples are compared to their site of origin and their destination site. The shape of a symbol indicates the origin of the soil, and the color its climate (transplant site). The control samples are labeled with a letter. The distance between samples is indicative of the magnitude of the difference in community composition.

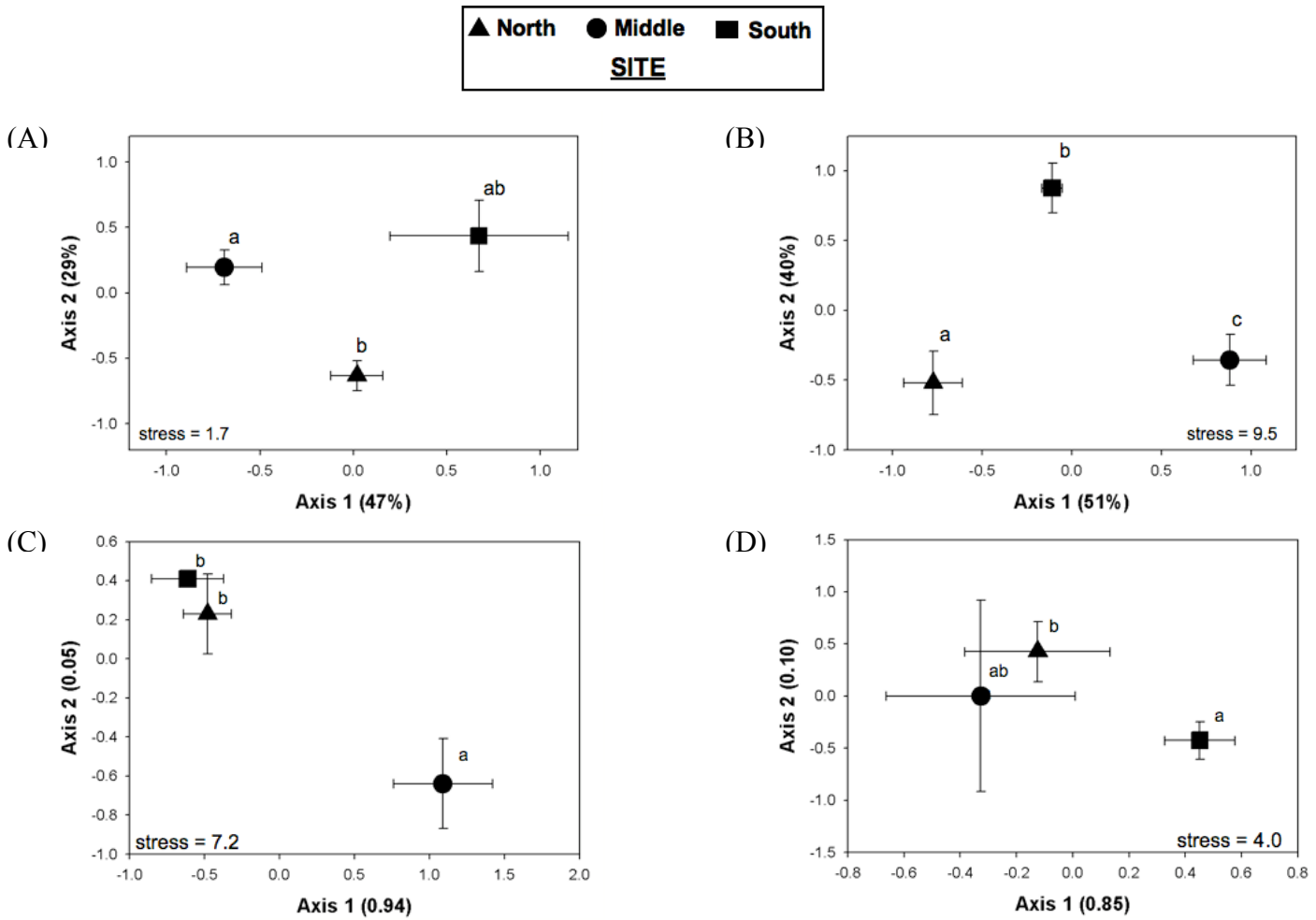

Figure 3. NMDS ordinations for (A) bacterial 16s T-RFLP, (B) fungal ITS T-RFLP, (C) bacteria composition by PhyloChip of fresh samples collected in early Sep. 2005 and (D) early Sep. 2007. The shape of a symbol indicates the origin of the soil. The coefficients of determination between the Euclidean distances between samples in the ordination space and the Bray-Curtis distances between samples in the original p-dimensional (number of taxa) space are shown in parentheses next to the axis labels. Communities with different letters are significantly different by PERMANOVA.

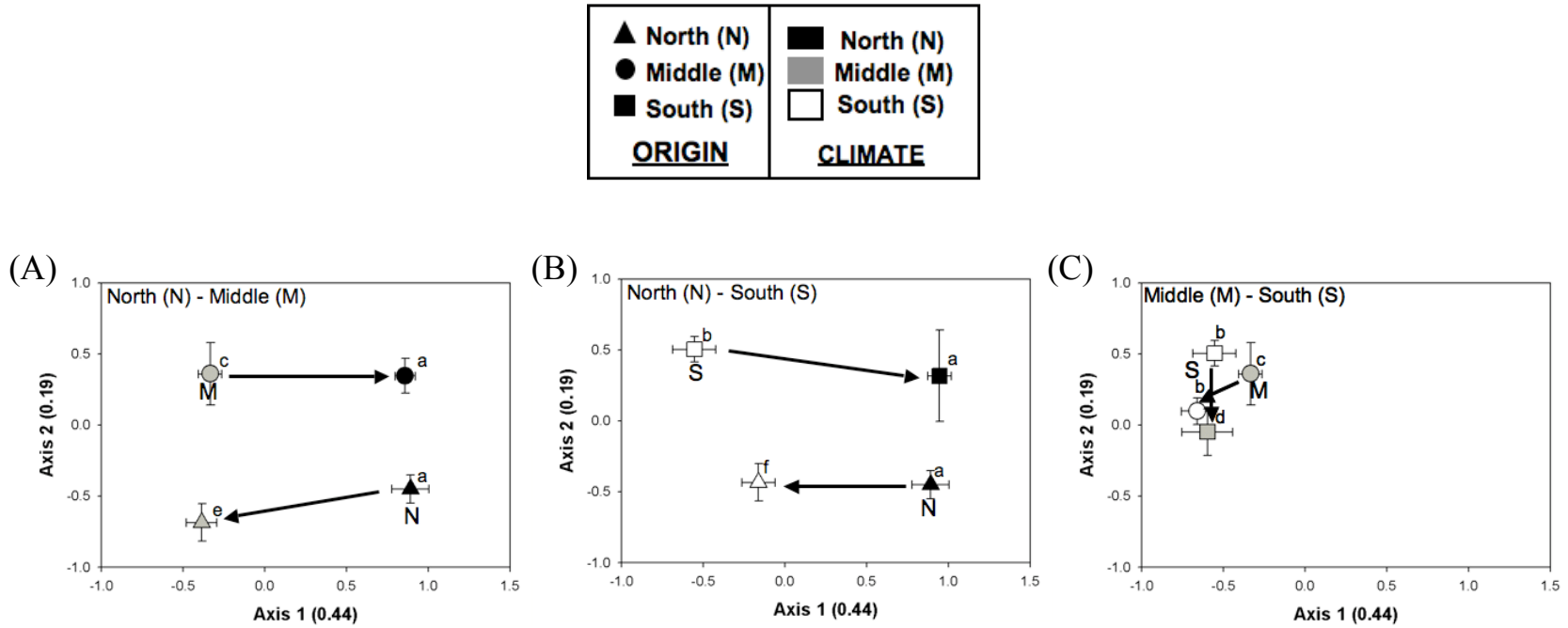

Figure 4. Fungal Communities after 1 year by ITS T-RFLP: NMDS ordinations comparing transplanted samples to transplant controls. The symbol shape indicates soil origin, and the color designates climate (transplant site). The graphs all show the results from a single ordination done with all of the samples, with a stress = 13.1. The coefficients of determination between the Euclidean distances between samples in the ordination space and the Bray-Curtis distances between samples in the original p-dimensional (number of taxa) space are shown in parentheses next to the axis labels. Communities with different letters are significantly different by PERMANOVA. The "strengths" of the responses are summarized in Table 2.

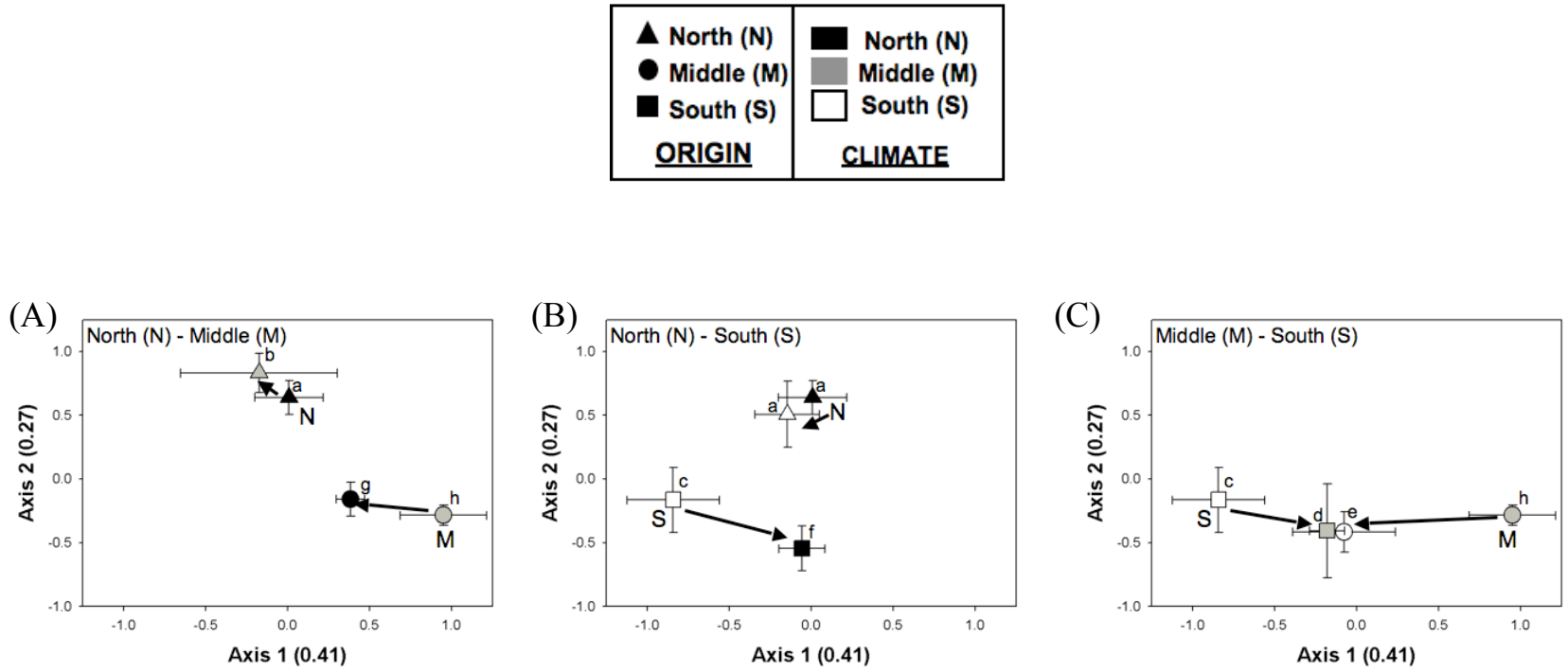

Figure 5. NMDS ordinations of 16S T-RFLP bacterial OTUs comparing transplanted samples to transplant controls one year after transplanting. The symbol shape indicates soil origin, and the color its climate (transplant site). The graphs all show the results from a single ordination done with all of the samples, with a stress = 13.1. The coefficients of determination between the Euclidean distances between samples in the ordination space and the Bray-Curtis distances between samples in the original p-dimensional (number of taxa) space are shown in parentheses next to the axis labels. Communities with different letters are significantly different by PERMANOVA. The strengths of the responses are summarized in Table 2.

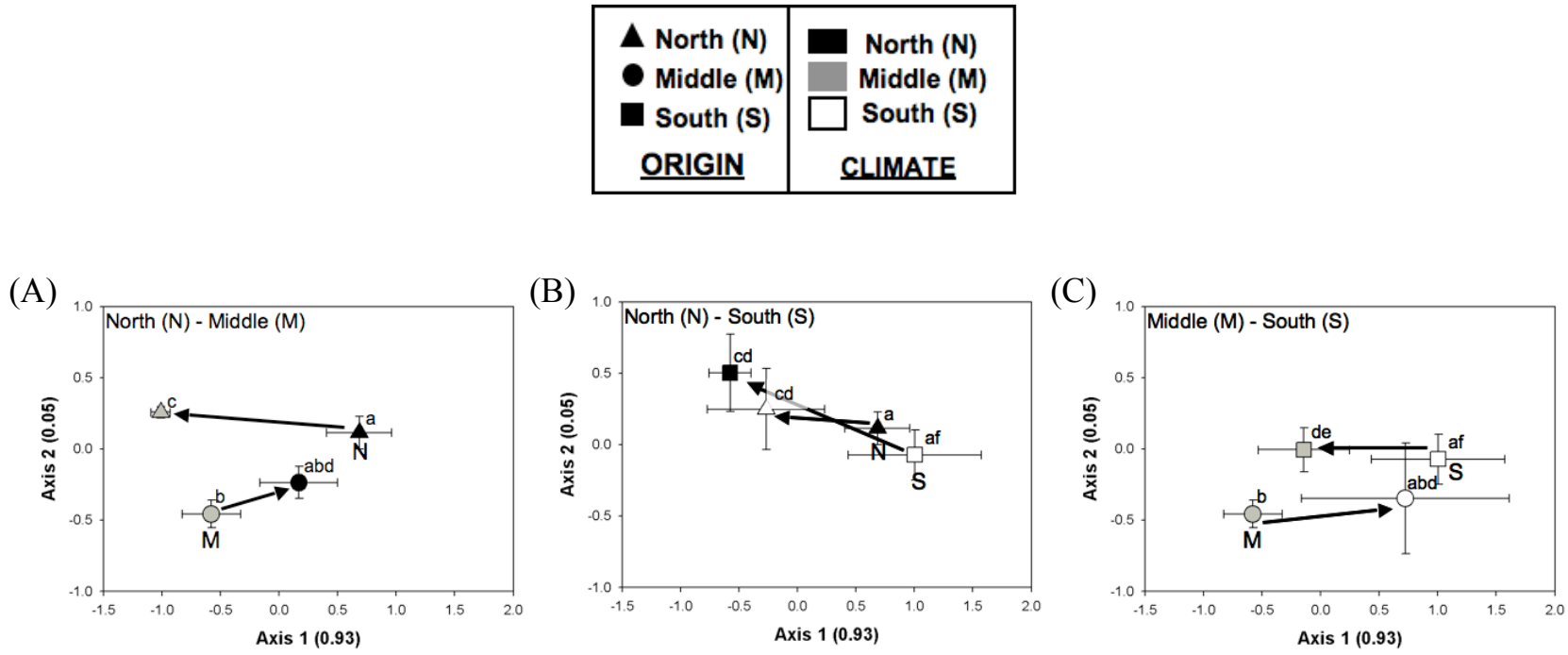

Figure 6. Bacterial communities after 1year by PhyloChip: NMDS ordinations comparing transplanted samples to transplant controls. The symbol shape indicates soil origin, and the color designates climate (transplant site). The graphs all show the results from a single ordination done with all of the samples, with a stress = 5.9. The coefficients of determination between the Euclidean distances between samples in the ordination space and the Bray-Curtis distances between samples in the original p-dimensional (number of taxa) space are shown in parentheses next to the axis labels. Communities with different letters are significantly different by PERMANOVA. The strengths of the responses are summarized in Table 2.

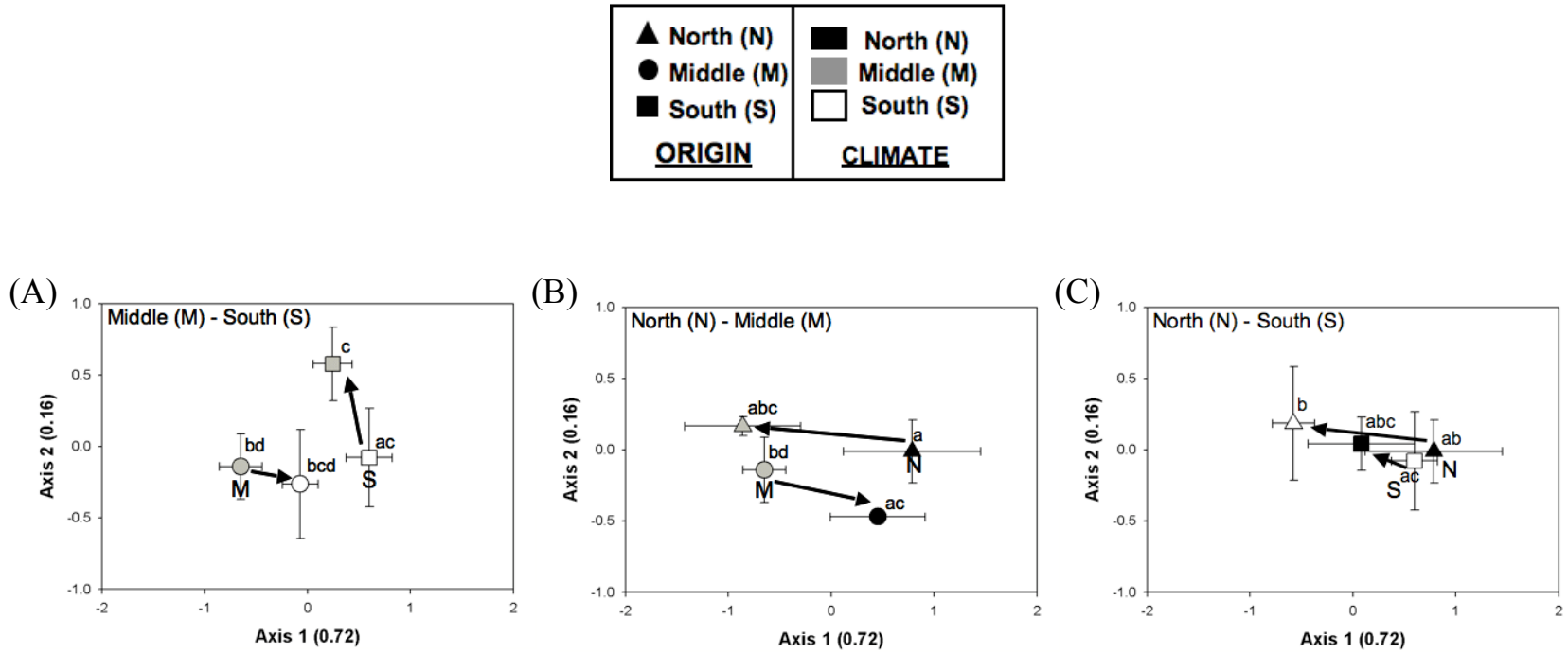

Figure 7. Bacterial communities after 3 years by PhyloChip: NMDS ordinations comparing transplanted samples to transplant controls. The symbol shape indicates soil origin, and the color designates climate (transplant site). The graphs all show the results from a single ordination done with all of the samples, with a stress = 6.1. The coefficients of determination between the Euclidean distances between samples in the ordination space and the Bray-Curtis distances between samples in the original p-dimensional (number of taxa) space are shown in parentheses next to the axis labels. Communities with different letters are significantly different by PERMANOVA. The "strengths" of the responses are summarized in Table 2.

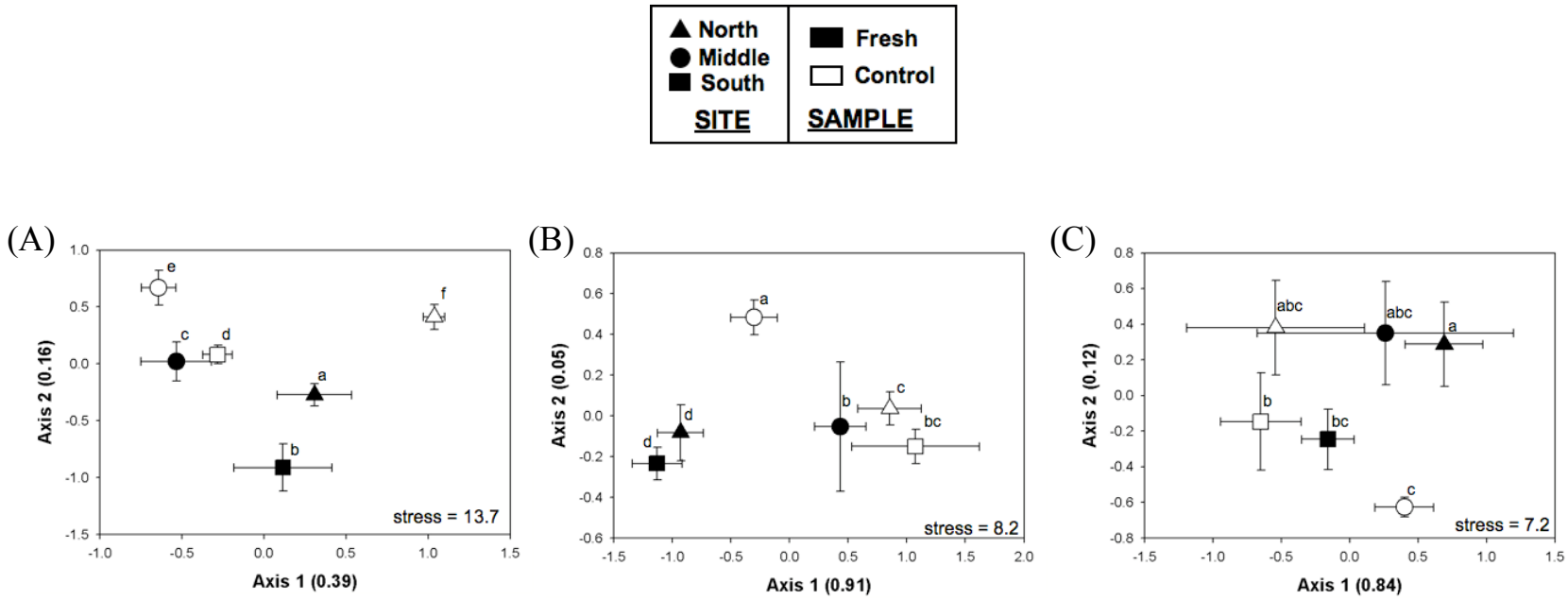

Figure S1. NMDS ordinations of fresh samples and transplant controls for (A) fungal ITS T-RFLP and (B) bacterial PhyloChip community composition one year after transplanting (Sep. 2005) and (C) bacterial PhyloChip community composition after three years (Sep. 2007). The shape of a symbol indicates the origin of the soil. Fresh samples are in black while transplant controls are white. The coefficients of determination between the Euclidean distances between samples in the ordination space and the Bray-Curtis distances between samples in the original p-dimensional (number of taxa) space are shown in parentheses next to the axis labels. Communities with different letters are significantly different by PERMANOVA.