Gene Expression by Microbial Communities in Response to Soil Wet-Up: Microbial Resuscitation Strategies, Nitrifier Response Dynamics, and N_2O Pulses

By

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Abstract

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In this dissertation I use gene expression by indigenous soil microbial communities to better understand microbial resuscitation, the biogeochemical process of nitrification, and the consequent ramifications to global nitrous oxide emissions. The first rainfall following a long dry period in arid and semi-arid ecosystems causes a large, abrupt change in water potential that can be both a severe physiological stress and a punctuated stimulus for the reawakening of soil microbial communities rendered inactive by low-water conditions. Using two California annual grassland soils collected following a typically dry Mediterranean summer, I simulated a wet-up comparable to the season's first rainfall. I extracted nucleic acids and monitored soil variables before and over a logarithmic time scale from 15 minutes through 72 hours after water addition. I looked at this experiment through three different lenses to address pressing questions in microbial ecology, soil nutrient cycling, and trace gas biogeochemistry.

To assess microbial resuscitation strategies to the wet-up event, I applied transformed RNA to a high-density (16S rRNA) microarray (PhyloChip). I identified three response strategies, rapid (within 1 hour of wet-up), intermediate (between 3 and 24 hours following wet-up), and delayed (24 to 72 hours post wet-up) and note that the taxa comprising these groups cluster phylogenetically and are relatively consistent between the two soils analyzed.

I then addressed the relationship between microbial functional gene transcription and activity. I followed the transcriptional response of functional genes in three groups of nitrifiers; ammonia-oxidizing bacteria, ammonia-oxidizing archaea, and nitrite-oxidizing bacteria of *Nitrobacter spp.* By comparing transcript abundances with soil ammonium and nitrate pools over the course of wet-up, I found strong correlations between the soil ammonium pool and functional gene transcripts of ammonia-oxidizing bacteria (*amoA*); induction of *amoA* continued until the rate of ammonia oxidation was greater than the resupply of ammonium, suggesting that transcription serves as a control point for ammonia oxidation in soil. I again measured similar time to response in both soils, this time with microorganisms grouped by metabolic function, with ammonia-oxidizing

bacteria responding first. In addition, I again discovered that microorganisms traditionally thought to be slow-growing are capable of fast response, with transcriptional response detectable within 9 hours for the slowest group, ammonia-oxidizing archaea.

Finally, I related the lag in response of nitrite-oxidizers relative to ammonia-oxidizing bacteria to a significant nitrous oxide pulse and evaluated the implications of this decoupling to global nitrous oxide emissions. I reviewed the literature in light of this observation and discovered that increases in ammonia oxidation could explain the variability in and pulsed-behavior of nitrous oxide emissions reported from unsaturated soils.

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Microbial Resuscitation Strategies Following Soil Wet-Up Are Phylogenetically Clustered

Abstract

The first rainfall following a long dry period in arid and semi-arid ecosystems provides an acute water potential change that can be both a severe physiological stress and a defined stimulus for the revival of soil microbial communities rendered inactive by low-water conditions. We followed the responses of indigenous microbial communities to the addition of water to soils taken from two California annual grasslands following a typically dry Mediterranean summer. Differential resuscitation was evident within 1 hour of wet-up using high-density microarray (PhyloChip) analysis of *16S rRNA*. We identified three response strategies based on when taxa had the highest relative ribosomal quantity: rapid-responders (within 1 hour of wet-up), intermediate-responders (between 3 and 24 hours following wet-up), and delayed-responders (24 to 72 hours post wet-up). These data suggest that the large carbon dioxide pulse produced concurrently with wet-up results from the activity of different groups of metabolically active microorganisms over time. Relative ribosomal quantity of rapid-responders was as high in the prewet dry soils as at any other time, suggesting that some organisms may be poised to respond to the wet-up event in that they preserve their capacity to synthesize proteins rapidly. Microbial response patterns clustered phylogenetically, and were primarily conserved at the phylum level, with the clustering of responses largely consistent across the two soils. Microbial resuscitation strategy following wet-up of dry soil appears to be a phylogenetically conserved ecological trait.

Introduction

By the end of a hot, dry summer in semi-arid Mediterranean ecosystems, soil microbial activity is extremely low (Chou et al 2008) as soil water potential is frequently below -20 MPa (Kieft et al 1987). The first rainfalls following dry seasons result in sudden increases in water potential and can be characterized by rapid mobilization of the microbial community and an accompanying pulse of carbon dioxide so large that it is well documented at the landscape scale (Xu and Baldocchi 2004). A number of research groups have investigated changes in soil microorganisms and biogeochemistry concomitant with wet-up of dry soil, much of it focused on the increased mineralization of carbon (Fierer and Schimel 2002, Miller et al 2005) and nitrogen (Birch 1964, Hungate et al 1997, Saetre and Stark 2005). As soil microorganisms are often considered to be carbon-limited (Madigan and Martinko 2006), the flush of available carbon at wet-up (Kieft et al 1987) may represent a significant nutrient pulse. Thus, wet-up represents both a physiological stress and a defined stimulus for microorganisms recovering from the severe drought of the summer.

Microorganisms feature a variety of states that allow them to survive harsh conditions such as the soil environment during a hot, dry Mediterranean summer. Many microorganisms produce cyst-like cells that are smaller and restructured (Suzina et al 2004). Firmicutes and some Actinobacteria produce spores. While cysts and spores are generally considered dormant states, there are other physiological responses that may provide a degree of protection from some types of stress. Exopolysaccharide production has been suggested to be a potentially widespread mechanism of protection against desiccation (Roberson and Firestone 1992) that does not require dormancy.

Microorganisms are very diverse and organisms closely related phylogenetically (as evidenced by 16S rRNA) may have very different ecological traits, ranging from habitat preference to metabolism, (Doolittle and Bapteste 2007, Philippot et al 2010). A range of phylogenetic conservatism can exist for particular ecological traits such as habitat preference (Philippot et al 2010). Since a high degree of gene coordination may be necessary for survival of water potential shock as well as for entry into and resuscitation from dormant states, patterns of response to wet-up could display clear phylogenetic signals.

In order to investigate the microbial activity of indigenous soil communities, we quantified the relative the ribosomal content of a large group of indigenous soil bacteria by extraction of RNA and analysis using a high-density microarray (PhyloChip; (Brodie et al 2006)). Ribosomes are necessary for protein synthesis, can be produced rapidly to increase cellular activity, and can be degraded rapidly when resources are depleted (Davis et al 1986, Hsu et al 1994). RNA concentration, the bulk of which is ribosomal RNA, correlates linearly with cell growth rate (Poulsen et al 1993, Schaechter et al 1958) and with protein synthesis (Schaechter et al 1958) in actively growing cells. Thus, the trajectory followed by soil microorganisms as they emerge from a desiccation-induced quiescent state following wet-up may be determined through analysis of ribosomal RNA.

In this study we investigated the responses of soil microbial communities indigenous to two California annual grasslands to an influx of water. The soils originate from two Mediterranean-type annual grasslands that are over 600 km apart; both communities experience severe drought virtually every summer, suggesting that in this ecosystem-type, microorganisms may be specifically adapted to survive drought and to respond rapidly to wet-up (Kieft et al 1987). We measured the relative abundance of 16S rRNA transcripts from 1706 detected taxa at 8 time points, before and from 15 minutes to 72 hours after water addition. To do so, we produced double-stranded cDNA from community transcripts and hybridized it to PhyloChip microarrays. We then identified resuscitation strategies by comparing the relative abundances of ribosomes from individual taxa over time following the wet-up.

Materials and Methods

Sites and collection. Soils were collected from a northern and a southern California annual grassland; both sites are characterized by a Mediterranean climate with hot, dry summers and cool, wet winters (Table 1). The northern California site (NCA) is located within Hopland Field Station, a University of California Research and Extension Center 200 km north of San Francisco. The southern California site (SCA) is located 475 km south of San Francisco, within Sedgwick Reserve, part of the University of California Natural Reserve System. Soils were collected after the summer dry season (five months without rain) and shortly before the first rain of the wet season, September in NCA and October in SCA. At each annual grassland, we collected soil cores along five transects; surface vegetation (which was dead from the summer) was pushed aside and 10-cm diameter cores of the top 10 cm of soil were removed every meter for each 8-m long transect. The cores from each of the five transects were combined, homogenized in the field, and sealed in mason jars prior to returning to the laboratory to prevent increase in water potential due to the higher humidity in Berkeley, CA. Jars were kept in the dark at room temperature until the experiments were begun, one week after collection.

Laboratory wet-up. The wet-up experiment was performed in 8-oz. glass jars. For each soil, 5 jars were allocated for destructive sampling at every time point, one jar per biological replicate (transect). On the first day, wet-up was initiated by adding 10 ml of double-distilled water to 40 g of field-dry soil in each jar, approximately equivalent to a 17 mm rainfall event being distributed through the top 5 cm. Samples taken before water addition, designated “prewet,” were weighed into jars but never received water.

Soils in the jars were destructively sampled before and at 15 minutes, 30 minutes, 1 hour, 3 hours, 9 hours, 24 hours, and 72 hours after water addition, with the decreasing frequency over time to yield a logarithmic sampling schedule. At the respective time following water addition, approximately 10 g of soil was placed in Whirlpak bags, snap frozen in liquid nitrogen, and then stored at -80°C for subsequent nucleic acid analyses.

Rates of Carbon Dioxide Production. Rates of carbon dioxide production were determined on parallel but identical jars that were not destructively sampled. Jars were sealed, water was added via a syringe through a septum in each lid, soil was mixed by shaking, and then gas phase samples were taken by syringe starting 1 minute after water addition and again at approximately 30 minutes, 1 hour, and 3 hours after water addition. In order to avoid oxygen depletion, jars were opened after the three-hour sampling point and then resealed for three 3-hour periods with gas samples taken at the beginning and end of each 3-hour assay period. Sampling endpoints of these sealed assays were at approximately 9 hours, 24 hours, and 72 hours post water addition. The measured changes in concentration of carbon dioxide between time points was divided by the time elapsed to yield rates of CO_2 production. Carbon dioxide production by the Northern California soil (NCA) was determined on a Hewlett Packard 6890 with a pulsed discharge detector and that of SCA was analyzed on a Shimadzu 14-A with a thermal conductivity detector; both instruments were calibrated from 0 to 998 ppm.

Soil Characteristics. Soil water potential was determined using soil water content data and a water retention curve developed using isopiestic equilibration (Winston and Bates 1960). Organic matter content and carbon to nitrogen ratio was determined on a CE Instruments NC2100 soil analyzer. Soil particle size analysis was determined by the UC Davis Analytical Laboratory using the hydrometer method.

Table 1-1. Soil characteristics for NCA (Hopland Field Station) and SCA (Sedgwick Reserve).

Characteristic	**NCA**	**SCA**
Location	Hopland Field Station	Sedgwick Reserve
Region	N. California	S. California
GPS of site	38º 59.5784' N / 123º 04.0469' W	34º 43.1621' N / 120º 03.424' W
Sampling Date	09/18/2008	10/29/2008
Previous Rainfall Event	04/23/2008	05/23/2008
Soil Moisture Prewet	1.0%	4.7%
pH	5.0	5.6
C:N Ratio	13	12
Total Carbon Content	2.1 %	3.1 %
Sand / Silt / Clay	60 / 27 / 13	55 / 29 / 16
Soil Series	Laughlin	Elder Loam
Parent Material	Sandstone	Alluvial
Dominant Plant	*Avena barbata*	*Avena barbata*
Mean Annual Precipitation	940 mm[1]	380 mm[2]
Mean Annual Temperature	15ºC [3]	17ºC [3]

[1] (Waldrop and Firestone 2006)
[2](Gessler et al 2000)
[3]Mean annual temperature 1970-2000 from RAND California using nearest towns, Ukiah for Hopland, CA, and Lake Cachuma for Santa Ynez, CA.

Nucleic Acid Extraction and 16S rRNA Transcript Quantification. We followed the nucleic acid extraction method reported by Placella and Firestone (in review). Subsamples of the extracted RNA were reverse transcribed to single-stranded complementary DNA (cDNA) using Qiagen's Quantitect Reverse Transcriptase kit with random hexamers. For bacterial *16S rRNA* quantification, we diluted the single stranded cDNA with water by a factor of 1000; 1 µl of the end product was used directly in each 25-µl reaction. Real-time PCR of the nucleic acids was performed in triplicate using Bio-rad's EvaGreen Supermix on a Bio-rad iQ5 thermal cycler with approximately 0.2 ng cDNA per reaction and 500 nM final concentration of each primer, eub338 and eub518 (Fierer et al 2005). Plasmid standards were prepared as in Placella and Firestone (in review). Thermocycling parameters consisted of 2 minutes at 98°C followed by 40 cycles of 5 seconds at 95°C, 10 seconds at 53°C, and 15 seconds at 65°C at which time fluorescence was measured. A melt curve was performed following every reaction to ensure results were representative of the gene in question. Average PCR efficiency was 103% with an r^2=0.99.

Microbial Community Analysis by PhyloChip. Relative abundance of *16S* transcripts over time, and relative abundance of *16S* genes before and at 72 hours after wet-up were assessed for >8700 possible taxa using a high-density 16S rRNA microarray (Affymetrix), known as the G2 PhyloChip (Brodie et al 2006). We hybridized PCR-amplified DNA to assess relative abundance at prewet and 72 hour post and we hybridized double-stranded cDNA synthesized from 16S rRNA transcripts to assess the ribosomal community at all 8 time points. For DNA PhyloChips, PCR amplification was performed for 8 50-ul reactions along a thermal gradient from 48-58°C with 0.625 units ExTaq Polymerase (Takara), 50 ng of starting DNA, 300 nM concentration of 1492R reverse primer (5'-GGTTACCTTGTTACGACTT-3') and of 27F forward primer (5'-AGAGTTTGATCCTGGCTCAG-3') for bacterial *16S*. We repeated the same protocol for archaeal *16S* only instead using the 4Fa forward primer (5'-TCCGGTTGATCCTGCCRG-3'). Thermocycling for all reactions began with 3 minutes at 95°C followed by 25 cycles of 30 seconds at 95°C, 25 seconds at the gradient temperature, and 2 minutes at 72°C, followed by a final 10-minute elongation at 72°C. The 8 bacterial reactions for every sample were combined and the 8 archaeal reactions for every sample were combined. DNA was cleaned and precipitated with isopropanol, then gel quantified. To each microarray, we applied 500 ng of bacterial 16S rRNA and 100 ng of archaeal 16S rRNA gene amplicons. For relative ribosomal abundance, or microbial activity analysis, double-stranded cDNA was synthesized from extracted *16S rRNA* transcripts according to the method of DeAngelis et al. (2010). PhyloChips were prepared and filtered as in (Brodie et al 2007) with 200 ng of cDNA applied (except for 3 samples which had less material) and the same modifications as in DeAngelis et al. (2010). Hence, the cDNA PhyloChips assessed the relative ribosomal abundances of members of the microbial community using converted transcripts of *16S rRNA*. While hybridization biases may interfere with quantitative comparison between organisms on the PhyloChip,

we can evaluate the relative abundance of 16S rRNA genes (DNA PhyloChips) or transcripts (cDNA PhyloChips) over time for each operational taxonomic unit (OTU).

For a taxon to be considered "present" at a given time point required the taxa to have a probe fraction (pf) >0.90 in 3 out of 5 replicates for that soil. Richness at a given time point was the number of taxa deemed present; because our replication was used to determine presence, we do not present analyses of variance for the richness values. Community analysis measures (by soil) included all taxa that were deemed present in that soil at any time.

Intensity analyses used all taxa that were deemed present in any sample from a given soil (pf>0.90). In order to remove noise, only taxa that passed though a statistical filter were included in the heatmap and dendrogram analysis. To be included in these analyses, taxa were required to show a change in intensity over time, from 15 minutes through 72 hours post wet-up, by Tukey's HSD (α=0.05) following ANOVA with originating soil transect as a random variable. These analyses began with the 15-minute time point because RNA present at prewet could include RNA no longer associated with living microorganisms that was protected from ribonucleases by the severely dry soil conditions. The heatmap (Fig. 4A) presents data that are normalized by taxon, the color being relative to the change in that taxon over time. The dendrogram grouped taxa based on the temporal pattern that they showed, without any information on phylogeny. We used the clustering calculated by the dendrogram and the time of maximum intensity to assign strategies to the taxa within the clusters. More background on the heatmap and dendrogram analysis is provided in Eisen et al. (1998).

Phylogenetic Tree. A phylogenetic tree was created using FastTree with -gamma, -spr 4, and -mlacc 2 –slownni modifications for greater accuracy (Price et al 2009, Price et al 2010). The input sequences were obtained from Greengenes (DeSantis et al 2006) using the prokaryotic multiple sequence alignment identifiers associated with the PhyloChip OTUs displayed in Figures 3 and 4B (taxa present at prewet, 1 hour, or 3 hours post wet-up in either soil, plus a few taxa that were assigned strategies but were not in those presence groups). The pictorial presentations of the trees with data were developed using itol.embl.de (Letunic and Bork 2007).

Data Analyses. Results from the two soils (NCA and SCA) were analyzed separately, except when specifically comparing them. Carbon dioxide was log-transformed and analyzed using ANOVA with repeated measures and Tukey's HSD (P<0.05). Bacterial 16S rRNA transcripts were log transformed and analyzed using ANOVA with transect as a random variable followed by Tukey's HSD (P<0.05). Changes in relative abundance due to wet-up were assessed using paired sample t-tests with a Benjamini-Hochberg correction. Benjamini-Hochberg-corrected correlations were performed by regression of log-normalized relative intensities and the log-transformed rate of carbon dioxide production in every sample. Community differences among time points and between soils were evaluated using anosim with Bray-Curtis dissimilarity in the vegan package in R

(Clarke 1993). If an organism was deemed "not present" in a soil, the intensity value was changed to 0. For average rank, we ranked the intensities of all taxa deemed present in any sample (by soil) in each microarray. We then averaged the rank of all the taxa in a given group for each microarray. We used the 5 replicates at every time point to make box plots of the average rank of each group over time. Using a two-tailed t-test accounting for unequal variances, we compared the prewet rank distribution to the average rank distribution of all of the other time points. The results determine if the prewet rank was higher, lower, or not statistically different from the average rank following wet-up for each group.

Results

Rapid environmental change. Addition of water to the dry soils caused soil water potential to increase rapidly and sharply in both soils, from -38 MPa in NCA and -33 MPa in SCA to -0.006 MPa and -0.03 MPa, respectively (Figure 1). After 24 hours, water potential declined to -0.01 MPa in NCA and -0.1 MPa in SCA. The decline continued more rapidly from 24 to 72 hours post water addition, to -0.2 MPa and -2 MPa in NCA and SCA, respectively.

The rate of carbon dioxide production also increased rapidly and sharply in both soils, but peaked at approximately one hour and declined gradually over time (Figure 1). In NCA, the rate of carbon dioxide production remained elevated relative to prewet at 72 hours following wet-up; in SCA, the rate of carbon dioxide production decreased within 24 hours to a rate not statistically distinguishable from the rate at prewet. The total quantity of bacterial 16S rRNA transcripts did not change significantly over time in either soil (Figure 1); we detected significantly more transcripts in NCA than SCA.

Present taxa. The communities present as DNA in the two soils were assessed before wet up and at 72 hours by PhyloChip analysis. These organisms detected by 16S rRNA genes were not necessarily active. The microbial community composition was significantly different between the soils both before and 72 hours after wet-up. However, many of the same taxa were detected in the two soil communities. We detected a total of 2742 taxa (present in 3 of 5 replicates at a given time point) by DNA between the two soils, and 95% of the taxa detected in NCA were also detected in SCA (Figure 2).

Most (97%) of the taxa detected in the ribosomal community, that is, the organisms that were detected by cDNA hybridization, were also detected by DNA analysis. Although taxa detected in the ribosomal community varied significantly with time, the change over time was primarily driven by taxa dropping below detection within 1 hour and reemerging at 3 hours. While soil origin had a significant impact on the ribosomal community, as with the DNA community, the detected taxa were generally the same in both soils but their relative intensities varied. We detected 1484 taxa as present in the ribosomal community, with 97% of those present at some time point in SCA also present in NCA (Figure 2). A summary of taxa present in the ribosomal community at any time point and their proportion to taxa detectable by PhyloChip are given in Table 3.

The richness of taxa based on the ribosomal community displayed the same characteristics over time in both soils. Namely, ribosomal taxon richness was high at prewet and at 3 hours post wet but showed two simultaneous dips in both soils. Richness gradually declined from prewet to a minimum at 1 hour post water addition. After a subsequent increase by 3 hours (Figure 3), the richness of detected taxa declined slightly at 9 hours and was then the same at 24 hours, 72 hours (Figure 1).

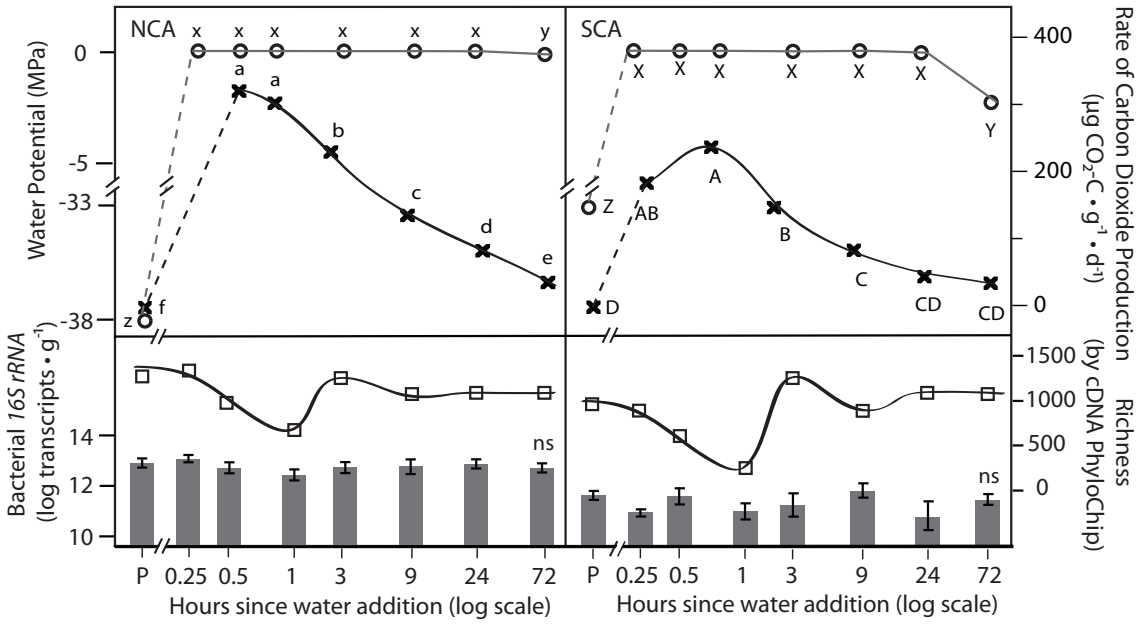

Figure 1-1. Water potential, rate of carbon dioxide production, and bacterial 16S rRNA transcript abundance before and following wet-up in NCA and SCA (n=5). P stands for prewet. Points represented by different letters are significantly different by Tukey's HSD (α<0.05).

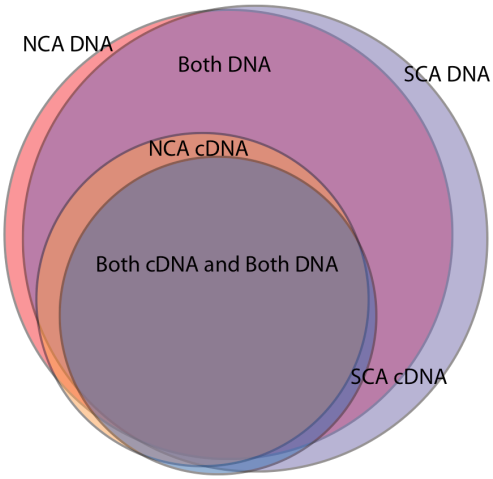

Figure 1-2. Diagram of overlap among taxa deemed present by DNA PhyloChip and by cDNA PhyloChip in NCA and SCA. Figure is approximately area-proportional.

Table 1-2. Present taxa by DNA PhyloChip.

Phylum	NCA		SCA		% NCA		% SCA	
	pre	72h	pre	72h	pre	72h	pre	72h
Acidobacteria	75	74	79	60	77%	76%	81%	61%
Actinobacteria	281	279	339	272	35%	34%	42%	34%
Aquificae	3	2	4	4	16%	11%	21%	21%
Bacteroidetes	144	154	177	139	16%	18%	20%	16%
BRC1	1	1	2	1	33%	33%	67%	33%
Caldithrix	2	2	2	2	100%	100%	100%	100%
Chlamydiae	3	2	3	3	11%	7%	11%	11%
Chlorobi	9	9	10	8	43%	43%	48%	38%
Chloroflexi	41	36	50	37	35%	31%	43%	32%
Crenarchaeota	13	13	13	13	16%	16%	16%	16%
Cyanobacteria	55	56	63	51	27%	28%	31%	25%
Deinococcus-Thermus	5	5	5	5	28%	28%	28%	28%
DSS1	2	2	2	2	100%	100%	100%	100%
Euryarchaeota	7	8	12	4	3%	4%	5%	2%
Firmicutes	435	418	469	364	22%	21%	23%	18%
Gemmatimonadetes	9	9	9	8	60%	60%	60%	53%
Lentisphaerae	3	3	3	3	38%	38%	38%	38%
Marine group A	2	2	2	2	40%	40%	40%	40%
Natronoanaerobium	4	4	4	4	57%	57%	57%	57%
NC10	1	2	2	1	25%	50%	50%	25%
Nitrospira	8	7	10	8	28%	24%	34%	28%
OP10	6	6	7	4	50%	50%	58%	33%
OP3	3	3	3	3	60%	60%	60%	60%
OP8	2	2	2	2	25%	25%	25%	25%
OP9/JS1	5	4	5	5	42%	33%	42%	42%
Planctomycetes	42	37	49	29	23%	20%	27%	16%
Proteobacteria	938	996	1101	843	30%	31%	35%	27%
α	324	332	381	290	38%	39%	45%	34%
β	185	199	207	174	46%	50%	52%	43%
δ	108	117	125	93	22%	24%	25%	19%
ε	44	45	46	42	25%	26%	26%	24%
γ	269	295	336	239	22%	25%	28%	20%
SPAM	2	2	2	2	100%	100%	100%	100%
Spirochaetes	39	36	41	29	26%	24%	27%	19%
Synergistes	6	6	6	6	32%	32%	32%	32%
Termite group 1	3	3	3	2	50%	50%	50%	33%
TM7	9	9	10	7	20%	20%	22%	16%
Verrucomicrobia	39	39	43	31	50%	50%	55%	40%
WS3	2	2	2	2	29%	29%	29%	29%
Total	2249	2283	2595	2002	26%	26%	30%	23%

Must have more than 1 taxon to be included in table.

Table 1-3. Taxa present in the ribosomal community at any time point in 3 of 5 replicates.

Phylum	# of taxa		% of PhyloChip	
	NCA	SCA	NCA	SCA
Acidobacteria	56	54	57%	55%
Actinobacteria	247	236	30%	29%
Aquificae	2	2	11%	11%
Bacteroidetes	60	56	7%	6%
BRC1	3	3	100%	100%
Caldithrix	2	2	100%	100%
Chlamydiae	3	3	11%	11%
Chlorobi	8	8	38%	38%
Chloroflexi	29	28	25%	24%
Crenarchaeota	12	12	15%	15%
Cyanobacteria	34	32	17%	16%
Deinococcus-Thermus	4	4	22%	22%
Firmicutes	255	208	13%	10%
Gemmatimonadetes	9	9	60%	60%
Lentisphaerae	3	3	38%	38%
marine group A	2	2	40%	40%
Natronoanaerobium	4	4	57%	57%
NC10	3	3	75%	75%
Nitrospira	6	5	21%	17%
OP10	5	5	42%	42%
OP3	3	3	60%	60%
OP9/JS1	4	4	33%	33%
Planctomycetes	14	12	8%	7%
Proteobacteria	574	526	18%	17%
α	211	202	25%	24%
β	121	117	30%	29%
δ	84	85	17%	17%
ε	37	32	21%	18%
γ	117	85	10%	7%
SPAM	2	2	100%	100%
Spirochaetes	28	26	19%	17%
Synergistes	5	5	26%	26%
TM7	5	4	11%	9%
Unclassified	25	24	8%	7%
Verrucomicrobia	20	17	26%	22%
WS3	2	2	29%	29%
Total	1440	1313	16%	15%

Must have more than 1 taxon to be included in table.

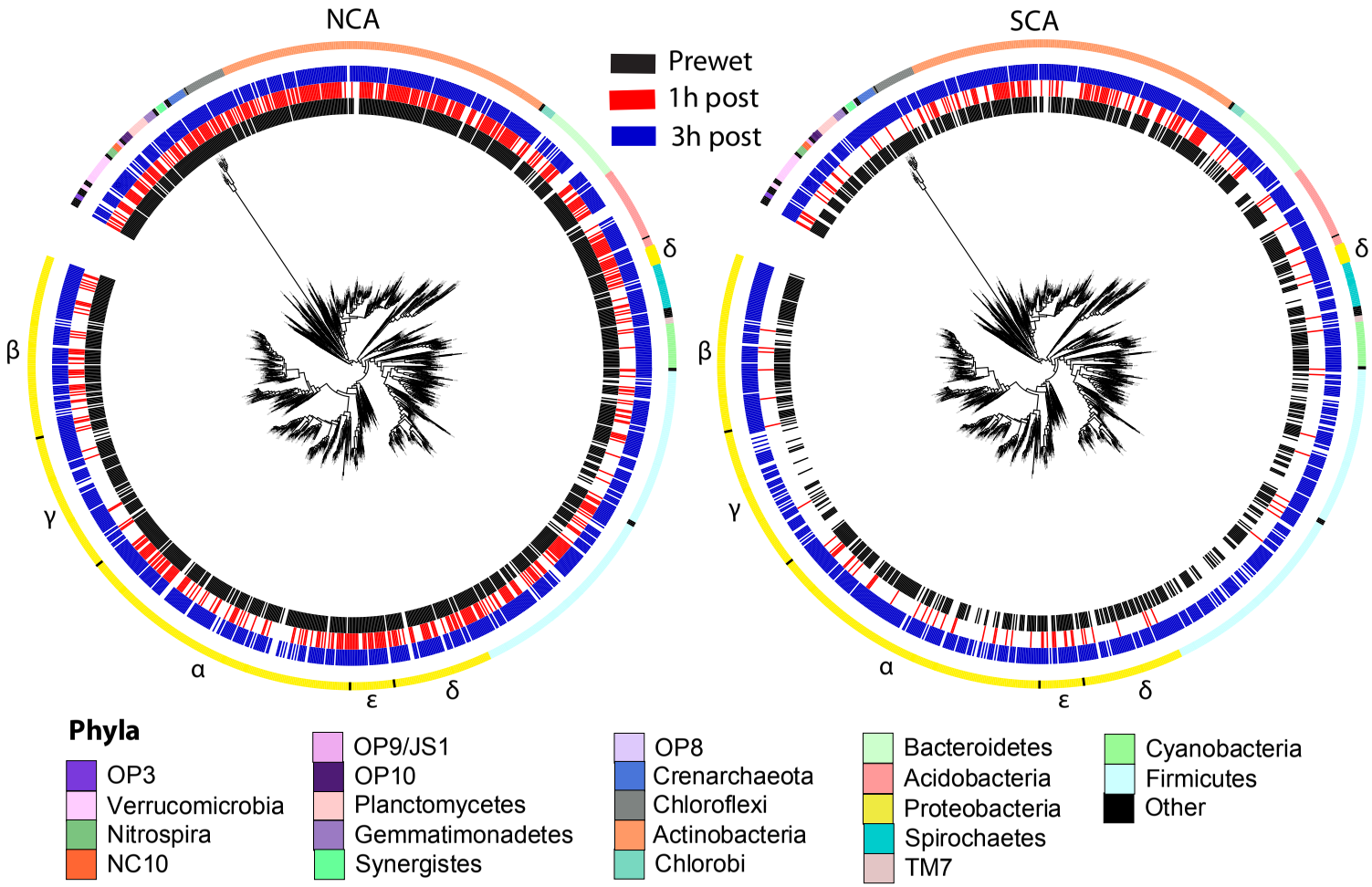

Figure 1-3. Present taxa detected in the ribosomal community at prewet, 1 hour, and 3 hours post water addition. Taxa included here were detected in 3 of 5 replicates at the given time point. The innermost ring is prewet, in black, followed by 1-hour post in red and 3-hours post in blue. The outermost ring is colored by phylum.

Microbial response trajectory. We detected significant changes in relative ribosomal abundance for many taxa, 31% and 20% of taxa present in the ribosomal community of NCA and SCA, respectively. However, taxa exhibited different trajectories, with some increasing while others decreased in relative ribosomal abundance. Because we monitored ribosomal abundance of individual taxa over a short time frame, we refer to changes in relative ribosomal abundance as changes in relative activity even though the presence of ribosomes alone does not indicate activity. In order to investigate changes in relative activity, we filtered taxa by those that had a significant change in relative intensity between any two time points after wet-up. A dendrogram made by clustering these taxa according to correlation of trajectories revealed 3 strategies in response to wet-up (Figure 4A). Some organisms displayed highest relative activity rapidly (between 15 minutes and 1 hour after wet-up), some on an intermediate time frame (between 3 hours and 24 hours), and others had a delayed response, (from 24 through 72 hours post water addition). The taxa and their assigned strategies are presented on a phylogenetically-rooted tree in Figure 4B.

Strategies generally clustered phylogenetically. In both soils, Firmicutes could largely be classified as intermediate-responders and Spirochaetes as rapid-responders. Verrucomicrobia and Actinobacteria were clearly rapid-responders in NCA. Strategies were remarkably consistent at the class and order level except within the Proteobacteria, which varied by class. Deltaproteobacteria and Epsilonproteobacteria were primarily rapid-responders. On the other hand, Alphaproteobacteria were consistently delayed-responders in both soils. In NCA Betaproteobacteria and Gammaproteobacteria were also delayed-responders but in SCA more taxa from these classes were intermediate-responders. A simplified conceptual model of our findings is presented in Figure 6. Not all taxa fit well into our three-strategy model; for instance, many Acidobacteria displayed high relative activity within an hour of water addition and then again at 72 hours. Thus Acidobacteria were assigned multiple strategies in our groupings even though they exhibited consistent response trajectories. The numbers of taxa by phylum in the three strategy groups are given in Table 5.

Ribosomes at prewet relate to strategy. In order to evaluate the role of ribosomes present in the dry soil before water addition, we compared the rank of different strategists at prewet to their average rank post wet-up. After ranking the intensities of all taxa deemed present in any replicate (by soil), we averaged the ranks of the taxa belonging to each of three strategy groups, rapid, intermediate, and delayed. The distributions of these average ranks over time by soil are shown in Figure 5. In both soils, rapid-responders had significantly more ribosomes relative to the other detected taxa at prewet than they did at the other time points averaged ($P<0.05$). Intermediate-responders in both soils and delayed-responders in SCA, on the other hand, had significantly less relative ribosomal abundance at prewet than at the other time points.

Growth. Eighteen percent and 14% of taxa in NCA and SCA, respectively, changed significantly in relative abundance (by DNA PhyloChip) from prewet to 72 hours after

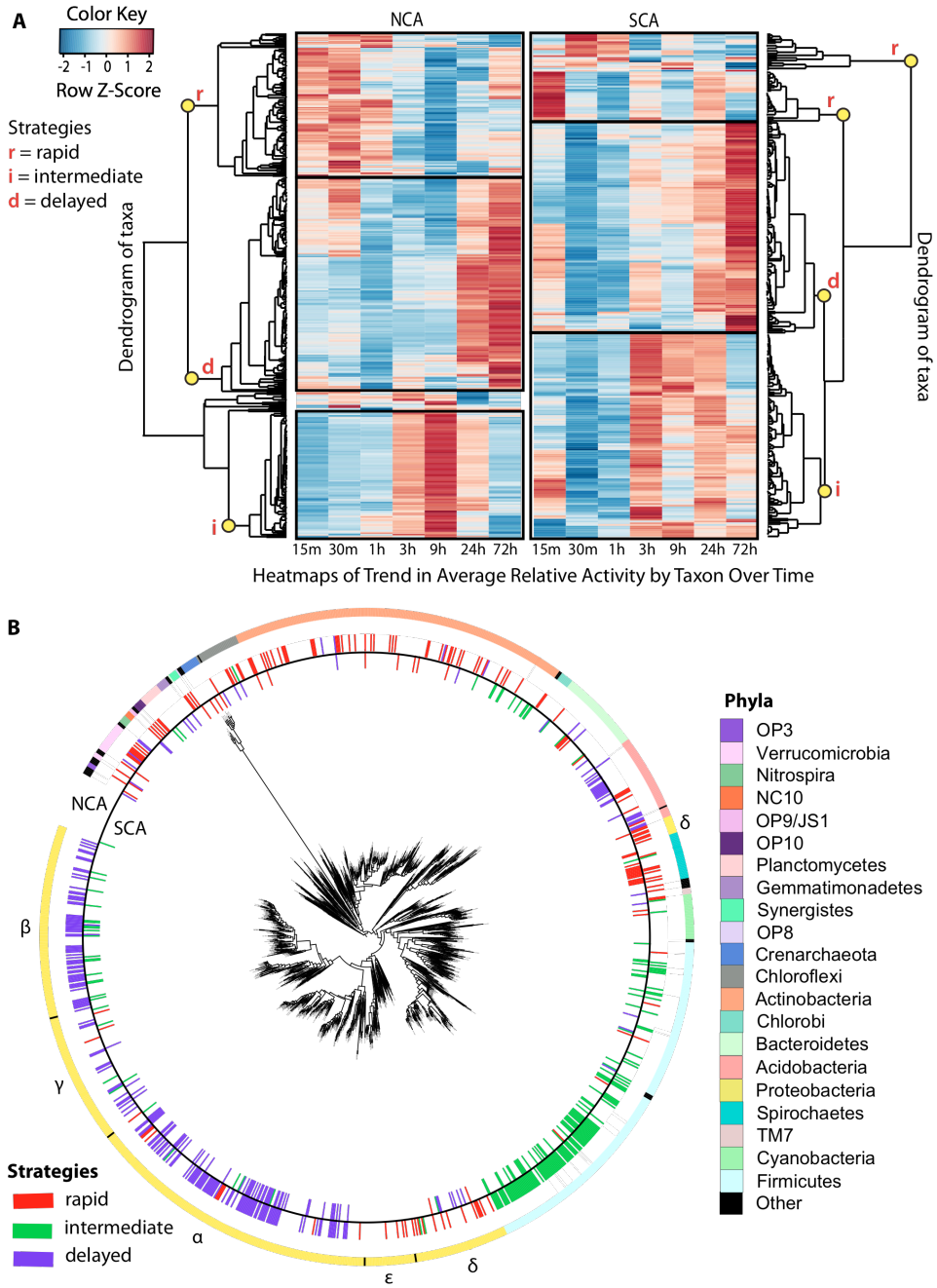

Figure 1-4. A. Heatmaps and dendrograms displaying the clustering of taxa based on response pattern over time following wetting of dry soil in NCA and SCA. Heatmaps show the trends in average relative activity (n=5) over time by taxon. Each dendrogram clusters the taxa based on the response trajectory over time; strategies were assigned to the clusters of taxa based on when relative activity was most elevated. Only taxa that passed our filter of a significant change over time included (501 in NCA and 288 in SCA). B. Phylogenetic tree of strategies assigned in Fig. 4A in NCA (outer ring) and SCA (inner ring). The outermost ring is colored by phylum. Taxa with highest relative activity rapidly (within 1 hour) are shown with a red line, intermediately (3-24 hours) with a green line, and delayed (24-72 hours) with a purple line.

Table 1-4. Number of taxa assigned early, middle, and late strategies by phylum and by soil.

Phylum	NCA			SCA		
	rapid	intermed.	delayed	rapid	intermed.	delayed
Acidobacteria	6		8			19
Actinobacteria	44		4	7	13	5
Bacteroidetes	3		2	6	4	2
BRC1				1		1
Caldithrix	1					
Chlamydiae				1		1
Chlorobi	1					
Chloroflexi	7	1		1		3
Crenarchaeota				2		
Cyanobacteria	1	2	1	2		1
Deinococcus-Thermus	1					1
DSS1	1					
Firmicutes	7	114		7	58	3
Gemmatimonadetes						2
LD1PA group	1					
marine group A	1					
Natronoanaerobium		2			2	
NC10						2
OP10	4					
OP8				1		
OP9/JS1	1					
Planctomycetes					2	1
Proteobacteria	39	5	187	5	36	76
α	8	1	98		5	62
β	1		63		20	3
δ	24	2		3	2	8
ε	4					
γ	3	2	25	2	8	4
SPAM			2			
Spirochaetes	7	1		15	1	
SR1	1					
Synergistes	3					
TM7	1					
Unclassified	2		3	1		3
Verrucomicrobia	10		2	1		
WS5						1
Total	142	125	209	50	116	121

Based on the assignments in Figure 4A.

water addition. Declines in relative abundance were common among taxa of the Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes, and Verrucomicrobia in both soils. On the other hand, taxa of the Betaproteobacteria and Gammaproteobacteria generally increased in relative abundance following wet-up in both soils. Many Bacteroidetes taxa increased in SCA despite low representation of this phylum in the ribosomal communities. However, unlike ribosomal response trajectories, changes in relative abundance varied within many phyla with responses varying by order and taxon in some cases. For instance, Alphaproteobacteria of the order Sphingomonadales increased in relative abundance while Alphaproteobacteria of the order Bradyrhizobiales declined.

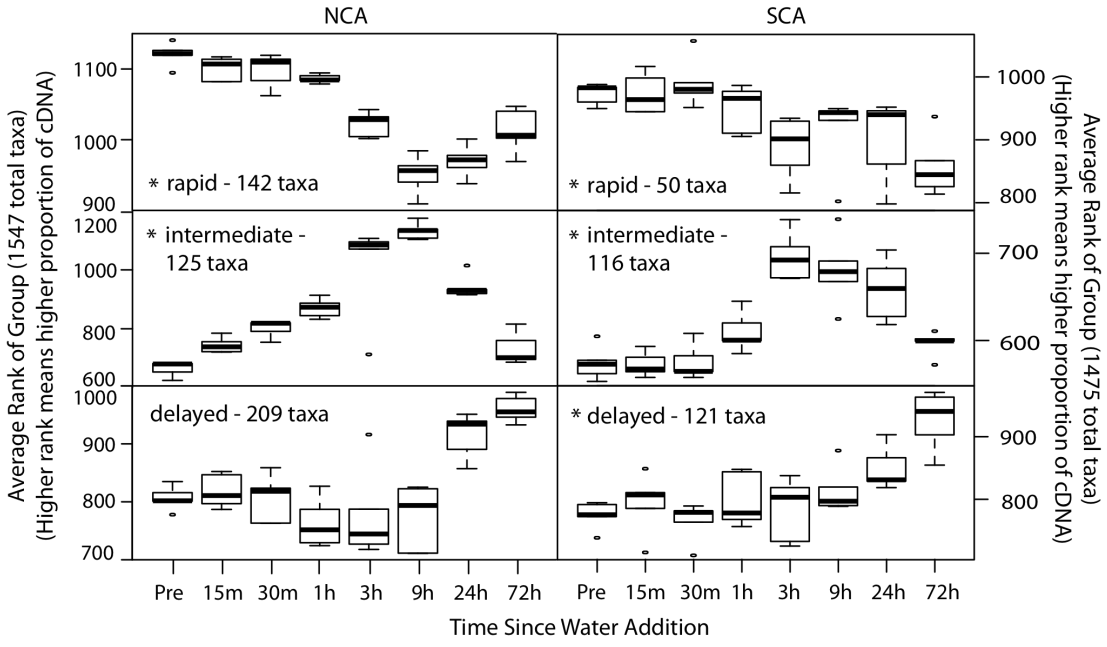

Figure 1-5. Box plots showing the average rank over time of taxa in different strategy groups in NCA and SCA. Strategy groups include always detectable in the ribosomal (cDNA) community (present in at least 3 out of 5 microarrays at all 8 time points) and early, middle, and late as determined in Fig. 4A.

Discussion

In both soils, the soil water potentials at the end of the summer (-33 and -38 MPa) were below the hydration point for nucleic acids and proteins (Potts 1994). The rapid water potential change that occurs with wet-up forces microbial cells to lyse or to actively release intracellular solutes to avoid cell lysis (Kieft et al 1987). This flush of microbial biomass carbon has been suggested to be a significant carbon source driving the carbon

dioxide pulse observed here and elsewhere following wetting of dry soil (Fierer and Schimel 2003). Following our laboratory wet-up, both soils returned immediately to conditions favorable for microbial life (Potts 1994). Our identification of three different resuscitation strategies, rapid, intermediate, and delayed, may indicate that taxa within these groups play distinct roles, physiologically, metabolically, and/or ecologically.

Table 1-5. Taxa with significant changes in relative abundance between prewet and 72 hours post water addition by phylum.

Phylum	NCA		SCA	
	-	+	-	+
Acidobacteria	5		5	1
Actinobacteria	33	3	38	4
Bacteroidetes	3	6	2	44
BRC1	1			
Chlamydiae			1	
Chlorobi	1			
Chloroflexi	10		4	2
Crenarchaeota				11
Cyanobacteria	5	13	3	2
Deinococcus-Thermus			1	
Euryarchaeota	1		1	1
Firmicutes	24	6	14	21
Order Bacillales	3	4		10
Order Clostridiales	14		7	9
Gemmatimonadetes		1	1	
Marine group A	2			
Nitrospira	2			
OP10			1	
OP8	1			
OS-K			1	
OS-L	1			
Planctomycetes	10		5	
Proteobacteria	33	231	26	156
α	20	29	18	19
β	4	82	3	50
δ	2	5	2	7
ε	1			
γ	6	115	3	80
Spirochaetes	1			2
Termite group 1	1			
TM7				3
Verrucomicrobia	3		5	
Total	137	260	108	248

Based on changes in relative abundance by DNA PhyloChip.

The ability of rapid-responders to resuscitate almost immediately following 5 months of severe desiccation and the shock of wet-up may reflect a strategy of preparedness. Rapid-responders had relatively more ribosomes at prewet than either intermediate or delayed-responders, but rather than increase, they maintained their relative activity soon after wet-up. The greater relative abundance of ribosomes at prewet could represent either a greater number of ribosomes per cell or a greater number of cells. In either case, the high prevalence of ribosomes before and soon after wet-up could allow these organisms to respond to the wet-up event with rapid protein synthesis. On the other hand, one of the main phyla of rapid-responders, Actinobacteria, are already known for another resuscitation strategy, resuscitation promoting factors, that allow cells to trigger others to reactivate (Mukamolova et al 1998). These resuscitation promoting factors seem to be abundant in soils (Lennon and Jones 2011). While not all taxa that are rapid-responders necessarily use the same mechanism, stored ribosomes, cells resistant to the physiological stress of wet-up, and resuscitation promoting factors could all contribute to rapid resuscitation.

Intermediate-responders were generally Firmicutes, a phylum well known for their ability to produce a particular type of highly resistant endospore. Although the Firmicutes strategy of sporulation is well studied, exactly how endospore outgrowth translates into recovery in soil is not well understood. In pure cultures of *Bacillus*, RNA is synthesized within minutes of transfer to favorable conditions. DNA synthesis does not begin until late outgrowth, which ranges from under 1 hour to up to 3 hours depending on the medium (Kennett and Sueoka 1971). We found increased activity of Bacilli from 3 to 24 hours after wet-up. While we do not know if the active Bacilli were in spore form before wet-up, this timeframe would have been sufficient for spore outgrowth.

Delayed-responders tended to be the microorganisms that grew. Although measured changes in relative abundance of 16S rRNA genes may in part reflect our choice of sampling end point (72h), changes may also reflect alternative strategies of rapid-response versus fast-growth as suggested for nitrifiers following wet-up (Placella and Firestone, in review). Fast-growers are known for their greater number of rRNA operons that allow them to quickly produce ribosomes during growth (Klappenbach et al 2000). However, they also rapidly degrade their ribosomes in times of stress (Davis et al 1986, Hsu et al 1994). Meanwhile, rapid-responders contained more ribosomes before wet-up. Hence, rapid-responders were able to resuscitate quickly while delayed-responders that may have had to produce ribosomes before recovering activity appeared to grow more efficiently.

Thus, our research suggests that among soil bacteria, slow-growers can be rapid-responders while fast-growers may require more time to recover their metabolic function. It may not be surprising that microorganisms function differently than larger organisms given their capacity for dormancy (Golovlev 2001, Lennon and Jones 2011). The effect of dormancy has been proposed to explain the different ecological impact of resource pulses on microorganisms relative to larger biota (Yang et al 2010). The effects of resource pulses on microbial ecology may be especially pronounced when considering ecosystem function in aridland ecosystems (Collins et al 2008). Differences in

resuscitation times among microorganisms can have implications for ecosystem processes and have been shown to impact soil nitrous oxide emissions (Placella et al in prep.).

Resuscitation strategies may reflect the ecosystem function of certain taxa and the types of carbon available over the course of wet-up. The flush of dissolved organic carbon that is associated with wet-up is generally assumed to be labile (Fierer and Schimel 2002, Kieft et al 1987), but it could include more recalcitrant materials. Using ^{13}C labeled *Avena barbata* roots, Bird et al. (2011) found rapid loss of C originating from decaying root litter following the wet-up of dry soil. Newly accessible complex carbon could explain the succession pattern following wet-up. For instance, Actinobacteria, many of which are capable of breaking down recalcitrant polymers (Goodfellow and Williams 1983), are rapid-responders. Bacilli, known to produce hydrolytic enzymes (Sharipova et al 2002), follow. Alpha, Beta, and Gammaproteobacteria, dominate the ribosomal community next; they are generally known for their abilities to use a wide variety of organic compounds but frequently lack the hydrolytic enzymes necessary to break polymers into monomers (Madigan and Martinko 2006). Thus recovery and succession may be intertwined.

Resuscitation of microorganisms following wet-up is coincident with and is likely responsible for an environmentally important CO_2 pulse (Xu and Baldocchi 2004). Our data indicate that different groups of microorganisms likely contributed to the CO_2 pulse over time. We were not able to detect a simple correlation between total 16S rRNA transcripts and rate of carbon dioxide production following wet-up, but we did measure higher CO_2 production and a greater number of 16S rRNA transcripts in NCA than in SCA. The microorganisms detected by ribosomal transcripts represent a subset of the DNA-detectable community, about half. The much greater stability of DNA than RNA implies that the DNA community should integrate portions of the soil community that have been active over some period of the past. The ribosomal community, on the other hand, may reflect only currently and recently active organisms.

Our measure of taxon richness of the ribosomal community reflected the shifts in active taxa. The increased taxon richness in the ribosomal community from 1 to 3 and again from 9 to 24 hours after wet-up may represent the "changing of the guard;" these were the time periods when one recovery strategy group declined and another emerged in both soils. Thus taxa from both the declining and the increasing strategy groups were present at these key time points. Richness was lowest at 1 hour post water addition, when the rate of CO_2 production was greatest. The decline in the richness of taxa present in the ribosomal community, from prewet to 1-hour post water addition, could have multiple causes. This time frame may allow for the RNases mobilized by cell lysis and increased aqueous phase to lead to progressive degradation of RNA from cells that were dead before wet-up or that died as a result of the drastic water potential change. Alternatively the sharp water potential increase may have differentially impacted populations; some taxa may have been more susceptible to the shock and contributed their biomass to fueling the CO_2 pulse. Or, the active community could have become dominated by a subset taxa.

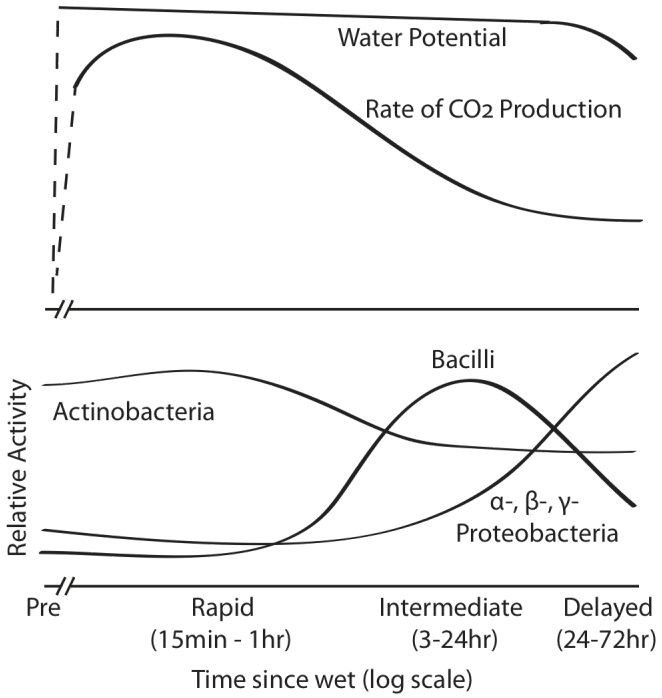

Figure 1-6. Conceptual model showing the trend in relative activity of broad phylogenetic groups over time following wet-up and their relation to water potential and the rate of CO_2 production over time.

The speed of resuscitation and dynamism of grassland soil microbial communities following wet-up may be greater than those of other microbial communities in response to favorable conditions. Following the flooding of a rice paddy, active microbial communities present in the oxic layer plummeted in richness for 9 hours (Noll et al 2005). In another study, half of inactive bacterioplankton cells became active in 6 hours following a switch to favorable conditions (Choi et al 1996). The communities present in both soils studied here were highly dynamic; approximately half of the taxa present in the prewet ribosomal community became undetectable in 1 hour but rebounded to detectability again within 3 hours.

Soil microorganisms from Mediterranean climates may be adapted to respond to favorable water conditions, in particular, in order to compete for resources and survive in this ecosystem. Soil bacteria have been suggested to be better able to survive the physiological stress of water potential fluctuations compared to microorganisms from other environments that experience less water potential fluctuation (Halverson et al 2000). Indeed, the taxa that we detected in the DNA and ribosomal communities were the expected players for a soil community, in keeping with clone library identification of abundant soil organisms being Proteobacteria, Acidobacteria, Actinobacteria, and Verrucomicrobia (Janssen 2006).

We found phylogenetic clustering of taxa assigned to rapid, intermediate, and delayed strategies. Response strategy was generally conserved at the phylum level except among Proteobacteria. Proteobacteria clustered at the class level. Thus our data demonstrate phylogenetic coherence of ecological strategy by microorganisms in response to soil wet-up. Phylogenetic coherence has previously been demonstrated for habitat preference at the class level (Hartman et al 2009, von Mering et al 2007) and genome size at the order level (Ettema and Andersson 2009). Microbial strategies for tolerating desiccation and responding to rapid wet up likely require coordinated functioning of multiple physiological traits. Such strategies may be the result of long-term evolutionary adaptation and be unlikely to be transferred by lateral gene transfer. Phylogenetic coherence of resuscitation strategies at the level of phyla may be consistent with the complexity of such resuscitation strategies.

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2

Transcriptional Response of Nitrifying Communities to Soil Wet-Up

Abstract

The first rainfall following a long dry period in arid and semi-arid ecosystems provides a large, abrupt water potential change that can be a severe physiological stress and/or a defined stimulus for the reawakening of soil microbial communities rendered inactive by low-water conditions. We followed the responses of indigenous communities of ammonia-oxidizing bacteria, ammonia-oxidizing archaea, and nitrite-oxidizing bacteria to the addition of water to soils taken from two California annual grasslands following a typically dry Mediterranean summer. We quantified the abundance of transcripts for bacterial and archaeal ammonia monooxygenases (*amoA*) and bacterial nitrite oxidoreductase (*nxrA*) in soil from 15 minutes to 72 hours after water addition. The response patterns for these three groups of nitrifiers were significantly different and were consistent in the two soils. Induction of bacterial *amoA* transcripts was detectable within one hour of wet-up and continued until the rate of ammonia oxidation was greater than the resupply of ammonium, suggesting that transcription can serve as a control point for ammonia oxidation in soil. High-density microarray analysis of 16S rRNA from the wet-up soils suggested that nitrite-oxidizing *Nitrobacter spp.*respond in tandem with ammonia-oxidizing bacteria while nitrite-oxidizing *Nitrospina* and Nitrospira may not. Ammonia-oxidizing archaea appear to have a distinct temporal niche, responding after bacterial ammonia-oxidizers but maintaining activity for a longer period. Despite months of desiccation-induced inactivation, we found that transcriptional response happens very rapidly for all three groups of soil nitrifiers. While both nitrification activity and gene transcription was rapid in the three days following wet-up, we found no evidence of nitrifier growth.

Introduction

By the end of a hot, dry summer in arid and semi-arid ecosystems, microbial activity is extremely low (Chou et al 2008) as soil water potential is frequently below -20 MPa (Kieft et al 1987). The first rainfall following a dry season results in sudden increase in water potential and can be characterized by rapid mobilization of the soil microbial community and an accompanying pulse of carbon dioxide so large that it is well documented at the landscape scale (Xu and Baldocchi 2004). This carbon dioxide pulse following wetting of dry soil has become known as the Birch effect due to its initial discovery by Birch and Friend (1956) and subsequent investigations by Birch (Birch 1958, Birch 1964). More recently, due to the importance of these carbon dioxide pulses to global carbon dioxide dynamics, researchers are again studying the carbon dioxide released from wetting of dry soils (Austin et al 2004, Davidson et al 2006, Harper et al 2005, Huxman et al 2004) as well as from freeze-thaw cycles (Grogan et al 2004, Ludwig et al 2006, Schimel and Clein 1996).

A number of groups have investigated changes in soil microorganisms and biogeochemistry upon wet-up of dry soil. Much of this work has focused on mineralization, with rapid, high rates of carbon mineralization (Fierer and Schimel 2002, Miller et al 2005) and of nitrogen mineralization (Birch 1964, Hungate et al 1997, Saetre and Stark 2005) documented. Additionally, ammonium is rapidly converted to nitrate following water pulses (Herman et al 2003, Hungate et al 1997, Saetre and Stark 2005). Very high stream nitrate concentrations following early rains have been documented, sometimes with a known nitrogen source (Baron et al 2009, Holloway et al 1998). Although ammonia-oxidizers are generally considered to be slow growers (Belser and Schmidt 1980, Konneke et al 2005), the large pulses of nitrate following wet-up suggest that nitrification may be responding relatively rapidly to wet-up events. We now have the tools to directly assess specific metabolic responses of indigenous soil microorganisms to wet-up events by following over time the production of transcripts (mRNA) for enzyme groups. Here, we use this molecular approach to monitor the response of nitrifiers to simulated rainfall wet-up of dry soil.

Nitrification is a two-step microbial process; ammonia-oxidizers convert ammonia to nitrite, and nitrite-oxidizers convert nitrite to nitrate. Ammonia-oxidizing soil bacteria have been relatively well studied and are known to belong to the Betaproteobacteria; more recently archaeal ammonia-oxidizers have been discovered and investigated (Konneke et al 2005, Leininger et al 2006). The respective roles in ammonia-oxidation for Bacteria and Archaea are still being explored; some studies suggest Archaea are more dominant (Offre et al 2009, Prosser and Nicol 2008), and others indicate bacterial ammonia-oxidizers primarily control ammonia oxidation (Di et al 2009, Jia and Conrad 2009). The only current consensus appears to be that archaeal ammonia-oxidizers may be better suited to lower ammonium availability (Martens-Habbena et al 2009). We do not know whether one of these groups has an advantage recovering from inactivity or whether the large changes in nitrogen pools during wet-up are due to the activity of one group versus the other.

In this study, we ask how long it takes for inactive soil nitrifiers to respond to a wet-up event. Do microbial response times vary by microbial function? Can insights into microbial mechanisms underlying activity pulses help us predict and potentially model ecosystem responses to changing precipitation patterns? To address these questions we studied indigenous soil microbial communities, inactive due to extended drought. Specifically we investigated nitrifier transcriptional response to wet-up and the conversion of ammonium to nitrate on a fine time scale in two soils, both from California annual grasslands. Using a logarithmic time scale from 15 minutes to 72 hours after water addition, we pinpointed differences in early response while still following the response over a 3-day period. We focused on transcript abundance of one key gene for each of three groups of nitrifiers, bacterial ammonia-oxidizers of the Betaproteobacteria, archaeal ammonia-oxidizers, and nitrite-oxidizers of *Nitrobacter spp*. We targeted *amoA*, which codes for ammonia monooxygenase, the enzyme involved in the first step of ammonia oxidation, for both bacterial and archaeal ammonia-oxidizers. We quantified *nxrA* transcripts (encoding nitrite oxidoreductase) from *Nitrobacter spp.,* one of three conserved clades of nitrite-oxidizing bacteria. We also used a high-density microarrays (PhyloChip) to identify changes in the relative activities of known nitrifier taxa in response to wet up.

Materials and Methods

Sites and collection. Soils were collected from a northern and a southern California annual grassland; both sites are coastal annual grasslands featuring a Mediterranean climate with hot, dry summers and cool, wet winters (Table 1). Our northern California site (NCA) is Hopland Field Station, a University of California Research and Extension Center 200 km north of San Francisco. Our southern California site (SCA) is Sedgwick Reserve, part of the University of California Natural Reserve System located in Santa Ynez, CA, 475 km south of San Francisco. Soils were collected shortly before the first rainfall of the wet season, September in NCA and October in SCA. At each grassland, we collected soil cores along 5 transects; surface vegetation (which was dead from the summer) was pushed aside and 10-cm diameter cores of the top 10 cm of soil were removed every meter for each 8-m long transect. For each transect, the cores were combined, homogenized in the field, and sealed in mason jars prior to returning to the laboratory to prevent an increase in water potential due to the higher humidity in Berkeley, CA. A subsample of the homogenized soil from each transect was frozen on dry ice in the field. Jars were kept in a dark room until the experiments were begun, one week after collection.

Table 2-1. Soil characteristics for Hopland Field Station (NCA) and Sedgwick Reserve (SCA).

Property	**Hopland Field Station**	**Sedgwick Reserve**
Location	N. California	S. California
Mean Annual Precipitation	940 mm[1]	380 mm[2]
Mean Annual Temperature	15ºC [3]	17ºC [3]
Soil Series	Laughlin	Elder Loam
Parent Material	Sandstone	Alluvial
Sand / Silt / Clay	60 / 27 / 13	55 / 29 / 16
Total Carbon Content	2.1 %	3.1 %
C:N Ratio	13	12
pH	5.0	5.6
Sampling Date	09/18/2008	10/29/2008
Previous Rainfall Event	04/23/2008	05/23/2008
Soil Moisture Prewet (water/dry)	1.0%	4.7%
Soil Water Potential Prewet	-38 MPa*	-33 MPa*
GPS of site	38º 59.5784' N / 123º 04.0469' W	34º 43.1621' N / 120º 03.424' W

[1] (Waldrop and Firestone 2006)
[2](Gessler et al 2000)
[3]Mean annual temperature 1970-2000 from RAND California using nearest towns, Ukiah for Hopland, CA, and Lake Cachuma for Santa Ynez, CA.

Laboratory wet-up. The wet-up experiment was performed in uncapped 8-oz. glass jars. For each soil, 5 jars were allocated for destructive sampling at every time point, one jar per biological replicate (transect). On the first day, wet-up was initiated by adding and mixing with a spatula 10 ml of double-distilled water to 40 g of field-dry soil in each jar, equivalent to a 17 mm rainfall event being distributed through the top 5 cm. Samples taken before water addition, designated "prewet," were weighed into jars but never received water.

Soils in the jars were destructively sampled before and at 15 minutes, 30 minutes, 1 hour, 3 hours, 9 hours, 24 hours, and 72 hours after water addition, with the decreasing frequency over time to yield a logarithmic sampling schedule. Between water addition and sampling, jars were kept a room temperature, open to the air, in the dark and remained moist throughout the experiment. At the time of sampling soils were divided for analysis: 6 g for soil moisture analysis, 10 g extracted with 25 ml of 2M KCl for inorganic nitrogen analysis, and 10 g in Whirlpak bags (Nasco, Fort Atkinson, WI, USA) snap frozen in liquid nitrogen and then stored at -80°C for subsequent molecular analyses.

Rates of Carbon Dioxide Production. Rates of carbon dioxide production were determined on parallel but identical jars that were not destructively sampled. Jars were sealed, water was added via a syringe through a septum in each lid, soil was mixed by shaking, and then gas phase samples were taken by syringe starting 1 minute after water addition as t0 for the water addition measurements at approximately 30 minutes, 1 hour, and 3 hours after water addition. In order to avoid oxygen depletion, jars were opened after the first three-hour time point and then sealed 3 hours prior to each subsequent sampling point, which were at approximately 9 hours, 24 hours, and 72 hours post water addition. The measured change in concentration of carbon dioxide between time points was divided by the time elapsed to yield rates of carbon dioxide production. Carbon dioxide production by the northern California soil was determined on a Hewlett Packard 6890 (Palo Alto, CA) with a pulsed discharge detector and that of SCA was analyzed on a Shimadzu 14-A (Columbia, MD, USA) with a thermal conductivity detector.

Soil Characteristics. Soil water potential was determined from soil water content values using a water retention curve based on water potential values set by isopiestic equilibration (Winston and Bates 1960). Organic matter content and carbon to nitrogen ratio was determined on a CE Instruments (Wigan, UK) NC2100 soil analyzer. Soil nitrogen pools were determined colorimetrically on a Zellweger Analytics QuikChem FIA+ Series 8000 following 2M KCl extraction. Soil particle size analysis was determined by the UC Davis Analytical Laboratory using the hydrometer method.

Nucleic Acid Extraction. We developed a larger-scale nucleic acid extraction method based on the original method of Griffiths et al. (2000) with the modifications of DeAngelis et al. (2010), by scaling the protocol up 7 times and making the following modifications. Frozen soils were ground in a Qiagen TissueLyser II with 10 ml stainless steel cups at 29 Hz for 24 seconds; the soils and the cups were surrounded by dry ice when not in the machine. Bead beating was performed in 15 ml bead tubes with an MPBiomedicals FastPrep instrument with a TeenPrep adapter (5.5 m/s for 30 seconds); this instrument was also used at 4 m/s for 5 seconds to mix in place of vortexing. We added a 5 minute incubation on ice after phenol:chloroform addition and mixing. The first centrifugation step, to separate the aqueous phase, was performed at 5,500 X *g* for 5 minutes, and the second centrifugation step, after mixing with chloroform, was performed in heavy phase-lock gel tubes at 8,500 X *g* for 3 minutes. We also added 2 µl of linear acrylamide at the start of the PEG precipitation, which we extended to 4 hours. The final centrifugation was performed at 14,000 X *g* for 10 minutes. The crude nucleic acids were frozen at -80°C after resuspension in 75 µl tris-EDTA. DNA and RNA were later separated using the Qiagen All-prep Mini kit with the RNase-free DNase Set. A few RNA samples were run on a gel and no RNA was visible. Quantitative PCR of the *16S* gene was also used to test the success of the first DNase step, with approximately 99.999% degraded. Because it was still detectable, we used a second DNase step during cDNA synthesis.

Quantitative PCR for Gene and Transcript Abundances. For gene abundances, DNA was diluted 1:12 for a final mass of approximately 25 ng per 25-µl reaction. For transcript abundances, complementary DNA was synthesized from the extracted RNA with the Qiagen (Valencia, CA, USA) Quantitect Reverse Transcriptase kit with random hexamers which included a second DNase treatment; 1 µl of the end product was used directly in each 25 µl reaction. Real-time PCR of the nucleic acids was performed in triplicate using Bio-rad's EvaGreen Supermix (Hercules, CA, USA) on a Bio-rad iQ5 thermal cycler with 500 nM final concentration for each primer, using previously designed primers (Francis et al 2005, Poly et al 2008, Rotthauwe et al 1997). Every plate included purified plasmid standards and negative controls.

Standards were purified plasmid DNA with each plasmid containing one copy of the gene of interest; the plasmid inserts were made from SCA by performing PCR and then cloning with Invitrogen's TOPO TA cloning kit. Plasmids were extracted from *E. coli* using MoBio's 6 Minute Plasmid Extraction Kit. All qPCR reactions began with 2 minutes at 98°C followed by 45 amplification cycles of the following: for bacterial *amoA*, 95°C for 5 seconds, 55°C for 15 seconds, and 65°C for 15 seconds; for archaeal *amoA*, 95°C for 5 seconds, 53°C for 10 seconds, and 60°C for 15 seconds, for *nxrA*, 95°C for 5 seconds, 55°C for 15 seconds, and 72°C for 15 seconds, and 80°C for 10 seconds. Data were collected during the last step. PCR efficiency and r^2 values for the standard curves were 85% and 0.98, 91% and 0.98, and 96% and 0.97 on average for *amoA*, archaeal *amoA*, and *nxrA*, respectively.

Relative Ribosomal Abundance of Nitrifying Taxa. Ribosomal abundance was assessed for a range of nitrifying bacteria using a high-density 16S rRNA microarray (Affymetrix, Santa Clara, CA, USA). There are 202 known nitrifying taxa represented on the G2 PhyloChip (DeSantis et al 2006). For G2 PhyloChip analysis, double-stranded complementary DNA was synthesized from extracted 16S rRNA transcripts according to the method of DeAngelis et al. (2010). Complementary DNA (200 ng in all but 3 samples which had less material) was applied directly to the microarray. PhyloChips were prepared and filtered as in (Brodie et al 2007) with the same modifications used in DeAngelis et al. (2010). Hence, the cDNA PhyloChips assessed the activities of members of the microbial community using transcripts of 16S rRNA. While hybridization biases interfere with comparison between organisms on the PhyloChip, we can evaluate the relative response of any one operational taxonomic unit (OTU), by comparing across multiple microarrays. To learn more about the nitrifying community, we selected the data from all of the OTUs representing known nitrifiers. Thus, our PhyloChip analysis is a comparison of nitrifier taxon 16S rRNA transcripts relative to the 16S rRNA transcripts of the entire detectable community. Nitrifier identity was determined using G2 PhyloChip information combined with the green genes database (DeSantis et al 2006) using both Hugenholtz and NCBI taxonomies; only consensus data are reported.

Data Analyses. The two soils (NCA and SCA) were analyzed separately. We used two different statistical analyses to address two different questions. The first significant increase in transcript abundances over the time series was determined using helmert contrasts on log-transformed data; helmert contrasts compare the second time point to the first; the third to the first and second; the fourth to the first, second, and third, etc. to determine when a time point is significantly different from all earlier time points (Crawley 2007). We used the 15-minute values as the first time point for this analysis because bacterial 16S rRNA transcript abundances followed a logarithmic decline ($p=0.02$, $r^2=0.25$) when measured by quantitative PCR at prewet and 1, 5, and 15 minutes post water addition (data not shown). This decline suggests the pool of extant transcripts rapidly degraded following wet-up; hence we selected transcripts at 15 minutes post-wet as a baseline from which to monitor response.

Significant differences among time points for soil nitrogen pools, transcripts, and relative activities were analyzed using Tukey's HSD and ANOVA with transect as a random variable ($\alpha<0.05$); for soil nitrogen pools and transcripts, data were log-transformed for the analyses. Carbon dioxide was analyzed similarly only using repeated measures. We performed an ordinary least squares regression of *amoA* against soil ammonium because we find that the error in our soil ammonium calculation is negligible in comparison to the error in quantification of transcript abundance. For correlations between transcripts, we excluded prewet and field samples from the analysis to better understand the correlations between active transcripts.

Data from the two soils were analyzed for an effect of soil using ANOVA with time as a continuous variable, transect as a random variable, and an interaction between soil and time where significant to $p=0.1$. Similarly, differences in gene abundances were determined using log-transformed data in an ANOVA with soil and time point as fixed effects, transect as a random effect, and a soil-time point interaction. A student's t-test was then used to determine soil-specific significant differences between the two time points. There were two instances of outlier data exclusion based either on Tukey's outlier criterion (Kirk 2008) or known procedural error coupled with Dixon's Q test (Rorabacher 1991).

Results

Rates of Carbon Dioxide Production. Rates of carbon dioxide production increased rapidly with the highest rate around one hour after water addition (Figure 1). In NCA, the rate of carbon dioxide production gradually declined from one hour through 72 hours, although the rate of carbon dioxide production at 72 hours was still significantly elevated relative to prewet. Rates of carbon dioxide production in SCA followed a similar trend but never reached the same magnitude as in NCA. While both NCA and SCA have approximately the same rate of carbon dioxide evolution at prewet and at 72 hours post wet, NCA was higher for all intermediate time points, resulting in a significant effect of soil on the rate of carbon dioxide production (Table 2).

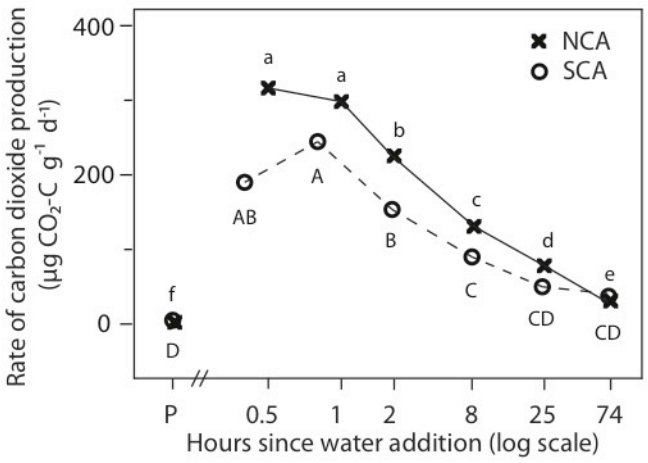

Figure 2-1. Effect of water addition on rates of carbon dioxide production by NCA (bold X symbols) and SCA (open circle symbols) soils. P stands for prewet. The points connected by lines are the average rates post water addition (n=5). Points with different letters are significantly different.

Table 2-2. Probabilities of soil and of soil-time interaction impacts on biogeochemical variables and functional gene transcript abundances following wet-up.

	Soil	Soil-Time interaction
Rate of carbon dioxide efflux	0.001	NS
Soil ammonium pool	0.004	0.004
Soil nitrate pool	0.008	0.03
Bacterial *amoA* transcript abundance	0.0003	0.001
Archaeal *amoA* transcript abundance	<0.0001	NS
Nitrobacter spp. nxrA transcript abundance	0.002	0.1

Values reported are p-values determined using ANOVA. NS represents not significant to p=0.1. In the instances where a soil-time interaction was not significant, the interaction was excluded from the ANOVA determining the probability of a soil effect.

Gene Abundances. Bacterial *amoA* and *Nitrobacter spp. nxrA* genes were more abundant in the northern California soil (Figure 2). The only significant increase in gene abundance from prewet to 72 hours after water addition was in *Nitrobacter spp. nxrA* in the NCA soil only. Gene abundance of bacterial *amoA* declined from prewet to 72 hours in the southern California soil. A change in archaeal *amoA* gene abundance was not detectable in either soil.

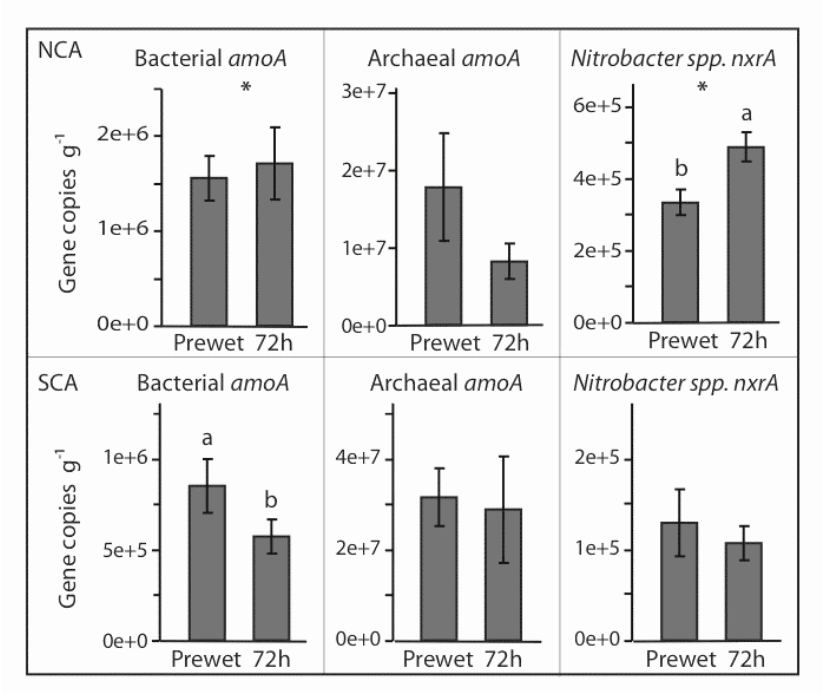

Figure 2-2. Nitrifier gene abundance at prewet and 72 hours after water addition (n=5). Error bars represent standard error. Bars represented by different letters are significantly different to p=0.05. Asterisks (*) signify NCA and SCA are statistically different to p=0.05.

Soil Nitrogen Pools. Soil ammonium and soil nitrate pools both changed rapidly after water addition, but they did not follow the same pattern (Figure 3). Soil ammonium accumulation and decline appeared remarkably similar in the two soils; soil ammonium was high at the start, increased rapidly, and declined significantly between 24 and 72 hours. However, the significant interaction between soil and time (Table 2) is consistent with a later ammonium peak in NCA than SCA. Large amounts of nitrate, on the other hand, began to accumulate when the ammonium pools reached a plateau and began to decline.

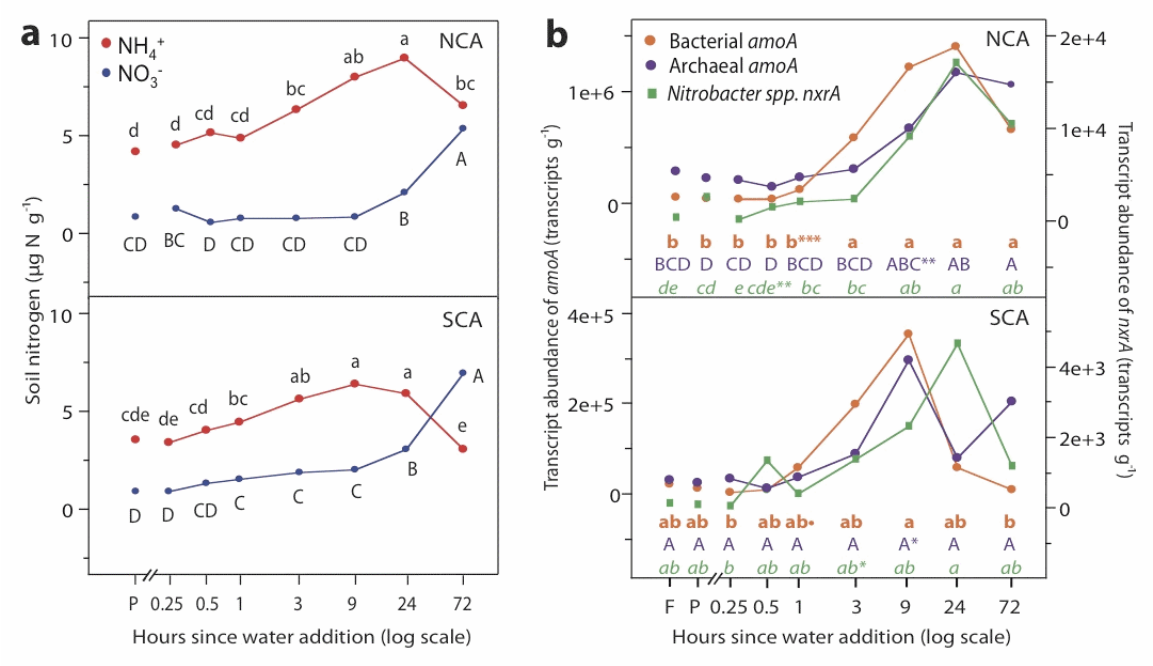

Figure 2-3. A. Soil ammonium (red circles) and soil nitrate (blue circles) pools in a northern California soil and in a southern California soil during wet-up (n=5). B. Response of nitrifier transcripts to soil water addition in NCA (upper panel) and in SCA (lower panel) soils (n=5). Bacterial *amoA* trancripts are represented by orange circles, archaeal *amoA* transcripts are represented by purple circles, and *nxrA* transcripts are shown with green squares. The transcript abundances recovered from the soil frozen on dry ice in the field are labeled F and those of the prewet soil the day of the laboratory experiment are labeled P. Letters denoting significant changes over time, or overall pattern, are in line with the points but underneath for clarity. Letters are color-coded by transcript; points represented with different letters are significantly different using Tukey's HSD on transformed data analyzed by ANOVA with transect as a random variable. The first significant change by helmert contrast is denoted according to degree of significance as follows: if $p \leq 0.001$, ***, if $p \leq 0.01$, **, if $p \leq 0.05$, *, if $p \leq 0.1$, •.

Transcript Abundances. Although significantly more transcripts for each gene were detected in NCA than in SCA, the response patterns were similar in the two soils (Figure 3). Bacterial *amoA* increased within 1 hour of water addition in both soils, although in SCA this was only significant to $p \leq 0.1$. By helmert contrast analysis of first increase, archaeal *amoA*, which was more variable, responded later (at 9 hours in both soils) than either bacterial *amoA* or *nxrA*. Transcripts for *nxrA* increased almost immediately in NCA, but a significant increase did not occur in SCA until 3 hours after water addition. The overall patterns for *nxrA* in both soils were similar to those of bacterial *amoA,* but appeared to lag behind; the best predictor of *nxrA* was bacterial *amoA,* explaining 83% of the variability in NCA (p=0.005). This relationship was not, however, significant in SCA to p=0.05.

The time from water addition to a significant increase in *amoA* transcript abundance (bacterial and archaeal) was remarkably consistent between soils. However, the amount of time bacterial *amoA* transcripts remained elevated, or the overall trend in differences over time as determined by ANOVA, varied by soil. Whereas bacterial *amoA* transcripts declined significantly from 9 hours to 72 hours post water addition in SCA, there was no significant decline in bacterial *amoA* transcripts in NCA. The difference in bacterial *amoA* transcript response pattern between the two soils was supported by the significant interaction between soil and time point.

The variability in bacterial *amoA* transcripts was best explained by the variability in the soil ammonium pool, accounting for 88% and 82% of the variability in transcripts by time in NCA and SCA, respectively. Although we detected more transcripts in NCA, the relationship between bacterial *amoA* transcript abundance and soil ammonium pools (Figure 4) was the same in both soils; the regression of bacterial *amoA* with ammonium shows a slope of 5.62 for both soils. The difference between the soils was in the intercepts, 0.99 and 0.81, in NCA and SCA, respectively, reflecting the difference in the quantity of transcripts detected.

While archaeal *amoA* transcript abundance did not show a significant correlation with ammonium, bacterial *amoA* transcripts, or *nxrA* transcripts in SCA; in NCA, it correlated with bacterial *amoA* (r^2=.78, p=.004), soil ammonium (r^2=.71, p=.008), and *nxrA* (r^2=.52, p=.04).

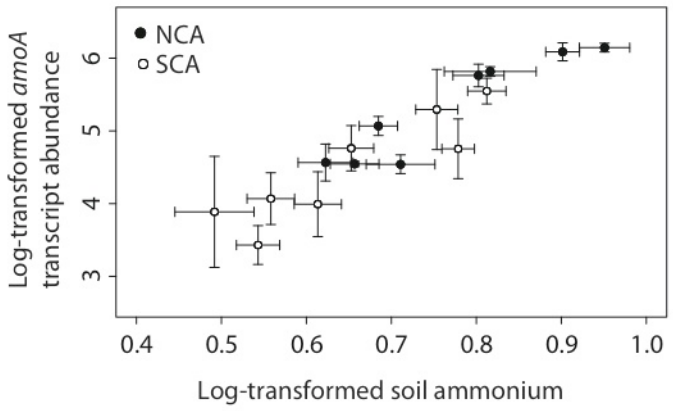

Figure 2-4. Correlation of bacterial *amoA* transcripts with soil ammonium concentration (n=5). Bars represent +/- standard error. Filled circles represent soil from the northern California site while open circles represent soil from SCA.

Relative Ribosomal Abundance of Nitrifying Taxa. We detected gene expression of 19 nitrifying taxa with the G2 PhyloChip. The 16S rRNA expression of each detected nitrifier taxon was assessed over time relative to the 16S rRNA expression of the entire detected microbial community. Among ammonia-oxidizing bacteria, we detected one

Nitrosomonas spp. and at least two *Nitrosospira spp.* including *N. briensis* and *N. multiformis.* Among nitrite-oxidizing bacteria, we found many OTUs for species within the phylum Nitrospira; we also detected at least one taxon of *Nitrobacter* and of *Nitrospina* in each soil. Due to the possibility of cross-hybridization, we grouped our results at the genus level; the statistics for each identified operational taxonomic unit are provided in Table 3.

Increases in the relative expression of 16S rRNA were consistent with trends in transcript abundances. In addition, for the same soil, we did not see different response patterns for taxa within the same genus. All detected NCA ammonia-oxidizing bacteria had significantly higher relative ribosomal content at 24 and 72 hours after water addition, including both *Nitrosospira* and *Nitrosomonas spp.* Similarly, in SCA, three bacterial ammonia-oxidizer taxa showed significantly higher relative ribosomal content at 9 hours post water addition. However, relative ribosomal content of *Nitrosospira spp*. did not decline significantly from 9 to 24 hours as expression of *amoA* did. Among nitrite-oxidizing bacteria, we presented *nxrA* transcript abundance data only for members of *Nitrobacter*. Relative ribosomal content increased significantly in one of two *Nitrobacter* taxa 72 hours after water addition in SCA. Thus, the detected increase in relative ribosomal content was delayed in comparison to the *nxrA* transcript data. In NCA we were unable to detect a significant change in *Nitrobacter spp*. ribosomal content by PhyloChip. While SCA showed a significant increase in relative ribosomal content for one taxon of Nitrospira at 72 hours, the other six detected Nitrospira taxa did not change significantly in SCA. In contrast to SCA and to *Nitrobacter spp*., in NCA the significant changes in Nitrospira and *Nitrospina* relative ribosomal content by PhyloChip were declines from prewet to 24 hours post water addition.

Discussion

Nitrifiers responded very quickly following wet-up by increasing transcript abundance for ammonia monooxygenase and nitrite oxidoreductase. Ammonia monooxygenase transcripts from ammonia-oxidizing bacteria increased significantly within one hour of wet-up in both soils. Transcripts from nitrite-oxidizing bacteria appeared to peak around 24 hours in both soils while ammonia-oxidizing archaea responded last, at 9 hours. Our PhyloChip data are based on 16S rRNA, which would not necessarily be expected to respond in tandem with transcripts for functional genes. However, our PhyloChip results were remarkably similar to our functional gene transcript results, although perhaps less sensitive temporally. At the genus level, increases in relative ribosomal content followed a similar overall pattern to increases in functional gene transcripts. The PhyloChip data also suggest that most ammonia-oxidizing bacteria respond similarly, as do the two detected *Nitrobacter* taxa. However, the decline in relative ribosomal content of *Nitrospina* and of some Nitrospira taxa in NCA indicates that these organisms may play a different functional role than *Nitrobacter spp.* at this time of year. We also note that a lack of change in relative ribosomal content could reflect a shift in the ribosomal content of the entire bacterial community following wet-up.

Table 2-3. Analysis of nitrifier taxon activity by time using relative expression of 16S rRNA by ANOVA with Tukey's HSD ($\alpha<0.05$).

Taxon	OTU/G2 ID	Soil	Prewet	0.25 h	0.5 h	1 h	3 h	9 h	24 h	72 h
Nitroso-monas spp.	7976	NCA SCA	b ns	b	b	b	b	b	**a**	**a**
Nitrosospira spp.	7931	NCA SCA	c AB	c B	c AB	c AB	c AB	bc **A**	**a** AB	**ab** **A**
	7865	NCA SCA	b ns	b	b	b	b	b	**a**	**a**
	7858	NCA SCA	bc ns	bc	bc	c	c	bc	**ab**	**a**
	7805	NCA SCA	c ns	c	c	c	c	bc	**ab**	**a**
	7796	NCA SCA	b AB	b B	b AB	b AB	b AB	b **A**	**a** AB	**a** AB
	7789	NCA SCA	abc ns	abc	bc	c	abc	abc	**ab**	**a**
	7682	NCA SCA	b ABC	b B	b BC	b ABC	b AB	b **A**	**a** ABC	**a** ABC
Nitro-bacter spp.	7438	NCA SCA	ns ns							
	6927	NCA SCA	ns AB	AB	B	B	AB	AB	AB	**A**
Nitro-spina spp.	594	NCA SCA	**a** ns	**abc**	**ab**	abcd	bcd	cd	d	abcd
Phylum Nitrospira	833	NCA SCA	**a** ns	ab	ab	ab	ab	ab	b	ab
	984	NCA SCA	ns B	AB	B	AB	AB	AB	AB	**A**
	864	NCA SCA	ns ns							
	179	NCA SCA	ns ns							
	240	NCA	**a**	ab	ab	ab	ab	ab	b	ab
	697	SCA	ns							
	542	SCA	ns							
	860	SCA	ns							

The statistics for a given taxon are shown in rows. Values represented by different letters in the same row and the same soil are significantly different across time. Letters shown in bold signify a time point that had significantly higher relative 16S rRNA expression than at least one other time point in the series. The taxa that did not change significantly are represented by "ns" in the first box. In some cases a taxon was only detected in one soil and the data for only that soil are presented.

Ammonia-oxidizers had an abundance of substrate as soon as water was added to the soil, increasing ammonium's mobility. Because soil ammonium was high in the prewet soils, we conclude that the time required to measure an increase in bacterial *amoA* transcripts is related to the amount of time required for these organisms to recover their functional capacity from the state of inactivity and dry-induced ammonium starvation. As we are not aware of any spore-forming nitrifiers, we assume that they were in a state of dormancy due to the summer condition (Roszak and Colwell 1987). Our finding of bacterial *amoA* transcript induction within one hour is on par with culture-based literature looking at induction of transcripts for ammonia monooxygenase following ammonium starvation (Sayavedra-Soto et al 1996, Stein and Arp 1998). In addition to transcript induction, studies of laboratory cultures show ammonia-oxidizing activity recovers very quickly following starvation (Bollmann et al 2005), and transcripts may be high in both growing and deprived cells (Wei et al 2006).

After wet-up of dry soil, transcription appears to serve as a control point for ammonia oxidation. Transcripts of bacterial *amoA* continued to increase until the rate of ammonium consumption was at least as great as the rate of ammonium resupply. We found this in both soils--the curves for *amoA* transcript abundance and soil ammonium pool are roughly similar, with both time courses showing decline at about the same time. Thus transcriptional control may explain the correlation between bacterial *amoA* transcript abundance and soil ammonium.

While the soil ammonium pool increases, the rate of nitrogen mineralization must be at least as great as the rate of increase in ammonium. Hence the relationship between soil ammonium and bacterial *amoA* transcripts reflects the relationship between nitrogen mineralization and ammonia oxidation. The higher rates of carbon dioxide production in NCA than in SCA could be indicative of greater gross rates of nitrogen mineralization (Schimel and Bennett 2004). Higher rates of nitrogen flux through the ammonium pool in NCA than in SCA may in part explain the higher bacterial ammonia-oxidizer biomass and *amoA* transcript abundance in NCA.

Importantly, the rapid increases in transcripts represent induction and not growth. We did not detect a significant increase in bacterial *amoA* gene copies from prewet to 72 hours in either soil (Figure 2), and laboratory cultures are known to require 10-24 hours to double (Belser and Schmidt 1980). Transcripts represent increasing ammonia-oxidizer activity, not the rate of ammonia oxidation itself. In ammonia-oxidizing bacteria, in addition to transcription, other intracellular and extracellular components, such as post-transcriptional and post-translation regulation and substrate availability, will also impact the actual rate of ammonia oxidation. However, the rate of increase in bacterial *amoA* transcripts was constant (on a linear scale) in both soils from 15 minutes to 9 hours in NCA and 15 minutes to 3 hours in SCA. The rate was twice as high in NCA, as was our estimate of the bacterial ammonia-oxidizer community by detectable *amoA* genes. This finding suggests that the maximum rate of transcript production per cell is about the same for the ammonia-oxidizing bacteria in these two soils.

Decline in *amoA* transcript abundance does not necessarily mean that bacterial ammonia-oxidizer activity declines simultaneously. In fact, nitrate accumulation

continued at approximately the same rate from peak *amoA* transcript abundance through 72 hours post water addition. A large pool of soil nitrate accumulated within 3 days of simulated wet-up; thus rapid activation of nitrification may be driving the high concentrations of stream nitrate commonly associated with the first rains.

The lack of relationship between archaeal *amoA* transcript abundance and ammonium in SCA given the strong relationship with bacterial *amoA* transcripts and ammonium in both soils could suggest a different niche for the ammonia-oxidizing archaea. In the southern California soil, archaeal *amoA* transcripts first increased significantly as soil ammonium began to decline, after 8 hours of increases in bacterial *amoA* transcripts. In both soils, a significant increase in transcription of ammonia-oxidizing archaea occurred at very high soil ammonium concentrations. The increase in transcription of archaeal *amoA* while soil ammonium was high is contrary to our knowledge of *Nitrosopumilus maritimus*, which is only capable of functioning at very low ammonium concentrations (Konneke et al 2005, Martens-Habbena et al 2009). While ammonia-oxidizing archaea could just be slow responders compared to bacterial ammonia-oxidizers, they were still able to respond in nine hours to the wet-up event after a presumed long state of inactivity.

The transcript abundance patterns of nitrite-oxidizing bacteria were similar to those of ammonia-oxidizing bacteria but lagged behind by a number of hours. These data suggest that nitrite-oxidizers of *Nitrobacter spp.* either require more time to respond than bacterial ammonia-oxidizers, or delay *nxrA* production until a steady stream of nitrite is being produced by ammonia-oxidizing bacteria, as suggested by diffusion-based models (Venterea and Rolston 2000). Indeed, based on soil nitrite concentrations, Gelfand and Yakir (2008) identify an approximately 50-hour lag between ammonia oxidation and nitrite oxidation following wetting of dry soil. In addition, laboratory culture studies show that a strain of *Nitrosomonas europaea* recovers faster from starvation than one of *Nitrobacter winogradskyi* (Tappe et al 1999).

The length of nitrifier inactivity has been suggested to correlate with the length of time needed for recovery (Tappe et al 1999). We expect that these soil microorganisms have been inactive for several months, as the previous rainfall was 5 months prior to this wet-up experiment (Table 1). In comparison to culture studies, a nine-hour recovery following such a prolonged state of inactivity is very rapid (Tappe et al 1999). Soil microorganisms indigenous to Mediterranean climates may however be adapted to annual drought. Indeed, microorganisms from semi-arid California soils have been shown to withstand large water potential increases far better than isolates from less dynamic environments (Halverson et al 2000). Thus the rapid response of soil nitrifiers may reflect a soil microbial community that is adapted to respond to profound wet-up events.

All of the nitrifier groups in this study responded within hours to wet-up, demonstrating their abilities to respond rapidly to favorable environmental conditions. While a strong positive relationship between the number of rRNA operons per genome and speed of response has been previously demonstrated (Klappenbach et al 2000), each of the groups of nitrifiers in this study, like many other soil microorganisms, contains only one 16S rRNA copy per genome (Chain et al 2003, Norton et al 2008, Starkenburg

et al 2006, Starkenburg et al 2008, Walker et al 2010). Nitrifiers were likely not the first organisms to respond to the water addition; the rates of carbon dioxide production during wet-up indicate that at least some heterotrophs respond and peak earlier than any of the nitrifier groups. Nitrifiers are commonly considered to be slow-growers. While we found fast transcriptional response, we saw little indication of growth. Together, these data suggest that the water-pulse driven characteristic of these semi-arid soils selects for fast-responding nitrifiers as opposed to fast-growing nitrifiers.

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3

Soil Nitrous Oxide Pulses Can Be Driven By Nitrifier Response Dynamics

Abstract

Nitrous oxide (N_2O) has an important role in radiative forcing as well as being a primary catalyst for stratospheric ozone destruction. Large pulses of N_2O from unsaturated soils are documented yet poorly understood. We identify a novel mechanism of N_2O generation that explains such pulses to be a result of an unbalanced increase in nitrifier activity. By following post-rainfall microbial transcription, we demonstrate that a decoupling of ammonia oxidation and nitrite oxidation occurs as a result of abrupt increases in nitrification and is strongly correlated with N_2O production. This mechanism of N_2O generation may be widespread, occurring during abrupt increases in nitrification; recognizing the environmental characteristics conducive to the production of these N_2O pulses should allow improved representation in models of atmospherically reactive trace gases.

Introduction

Soil nitrous oxide emissions are frequently episodic in nature. Both nitrification, the aerobic, two-step microbial process of ammonia oxidation through hydroxylamine to nitrite followed by nitrite oxidation to nitrate, and denitrification, the anaerobic microbial process of nitrate reduction to dinitrogen via nitrite, nitric oxide and nitrous oxide, are well known to be important sources of nitrous oxide (Firestone and Davidson 1989). High rates of nitrous oxide production are generally attributed to denitrification; however substantial pulses of nitrous oxide production often occur under conditions seemingly better suited to nitrification than to denitrification, such as unsaturated, fertilized agricultural soils (Matson et al 1998, Perez et al 2001, Venterea and Rolston 2000b) and following rain events in arid and semi-arid ecosystems (Davidson 1992, Hungate et al 1997, Rudaz et al 1991). In contrast to previous assumptions of denitrification-driven N_2O pulses, we demonstrate that a rain event that adds water to a dry soil causes a pulse of N_2O concurrent with a decoupling of ammonia oxidation and nitrite oxidation. We also report that an even greater amount of N_2O may be released after subsequent rain pulses. Understanding the underlying mechanisms that control the magnitude and duration of nitrous oxide pulses is essential to accurately estimating and predicting global nitrous oxide emissions. The two rainfall events that we simulated released approximately half of the annual estimated N_2O production for this ecosystem (Mummey et al 2000), indicating the need to incorporate pulse-driven events into regional and global models.

By examining microbial transcription of the genes encoding enzymes mediating key nitrogen transformations, we have been able to achieve temporal and mechanistic resolution of process controls that was previously unobtainable. While the origins of soil N_2O emissions have been debated for many years, investigations have been limited to a degree by the ability to distinguish nitrification and denitrification, as well as their component processes. Measurement of "potential rates" of nitrification and denitrification provide indices of enzymes available for the two processes, but measurement of actual rates of nitrification and denitrification is more difficult and generally requires addition of either a labeled substrate/product or an inhibitor. The isotopic signature of N_2O has been used to distinguish N_2O released by nitrification from that released by denitrification, but this technique has limitations (Baggs 2008). In addition, nitrification has three component enzymatic steps and denitrification four; previous methods have not allowed us to distinguish these steps in analysis of *in situ* soil N_2O production.

Pulses of N_2O production are likely due to either environmental regulation of extant enzymes mediating nitrification or denitrification or to sudden synthesis of new enzymes responsible for N_2O production. A decoupling of enzyme production could allow accumulation of reactive intermediates, such as hydroxylamine and nitrite, which are normally transient in soils. Recent advances in measuring microbial transcription of functional genes in soil communities allow us to now follow microbial transcription over short time periods. By monitoring microbial transcription of key nitrification and denitrification genes before and after simulated rainfall events, we have been able to compare the response patterns of ammonia-oxidizers, nitrite-oxidizers, and denitrifiers to

the timing of N_2O production in order to determine the contributions of nitrification and denitrification to the N_2O generated.

We set out to find the origin of large N_2O pulses produced by unsaturated soils receiving water after a substantial drought. We found large pulses of N_2O production during a lag between ammonia-oxidizer response and nitrite-oxidizer response and a strong correlation between functional gene transcripts for ammonia oxidation and the rate of N_2O production. Moreover, despite a substantial N_2O pulse, there was no evidence of denitrifier activity.

In light of these results, we reassessed published reports of N_2O pulses that included relatively complete environmental data and discovered that N_2O pulses may commonly be caused by a decoupling of ammonia oxidation and nitrite oxidation following environmental stimuli that cause abrupt increases in ammonia oxidation. We found that N_2O pulses occurring in unsaturated soils commonly correspond to rainfall events and fertilizer applications even when soils remain well below saturation.

Methods

Sites and collection. In October 2008, one week before the season's first rain, soils were collected from Sedgwick Reserve in Santa Ynez, CA, a coastal annual grassland featuring a Mediterranean climate of hot, dry summers and cool, wet winters. We collected soil from 5 transects 3 m apart and 8 m long; the dead surface vegetation was pushed aside, and eight 10-cm-diameter, 0-10-cm cores were homogenized for each transect. Soil was sealed in mason jars in the field to prevent sorption of water due to the higher humidity in Berkeley, CA. Jars were kept in the dark at room temperature until the experiment began, one week later. Soil properties are given in Table 1.

Laboratory wet-up. Laboratory wet-up was performed by adding 10 ml of double-distilled water to 40 g of field-dry soil in a glass jar, simulating a 17 mm rainfall event distributed through the top 5 cm of soil and resulting in a water potential increase from -33 MPa to -0.03 MPa. Gas production was measured in separate but identical jars using repeated measures; jars were sealed, water was added via a syringe through a septum in each lid, and then gas phase samples were taken by syringe for quantification of nitrous oxide production. To avoid oxygen depletion, jars were opened after the three-hour determination and then closed for 3-hour increments prior to each subsequent determination. The times reported are the midpoints between the measurements of headspace gas. All N_2O measurements were made on a Shimadzu 14-A gas chromatograph with an electron capture detector.

Reoccurring rainfall events during early wet season. Seasonal rainfall patterns in Mediterranean annual grassland are initiated by the first rainfall following a long drought. The first early season rain is commonly followed by shorter dry periods of weeks in duration; variability in rainfall is projected to increase during the 21st century (Easterling et al 2000) and has been shown to impact carbon cycling processes (Knapp et al 2002).

As the length of drought can impact nitrifier recovery (Tappe et al 1999), we were interested in nitrifier response to simulations of 2nd and 3rd rainfall events. Ten ml water increments were additionally added to soil incubations used in the initial wet-up experiments over two additional two week intervals; jars were kept at room temperature in the dark loosely covered with paper between water additions. To monitor nitrous oxide pulses from subsequent wet-ups we quantified N_2O production after the third water addition. The soil water potential values before and after the third simulated rainfall event were similar to the initial values: -33 MPa and -0.03 MPa, respectively. This experiment was performed with 10 jars, two jars for each of the 5 biological replicates mentioned above. One jar per replicate was used for soil nitrogen pools and another jar per replicate for gas sampling. Data were analyzed using repeated measures.

Soil Characteristics. Water potential was determined using soil water content and isopiestic equilibration (Winston and Bates 1960). Carbon content and carbon to nitrogen ratio was determined on a CE Instruments NC2100 soil analyzer. Soil nitrogen pools were determined colorimetrically on a Zellweger Analytics QuikChem FIA+ Series 8000 following 2M KCl extraction.

Table 1. Soil characteristics for Sedgwick Reserve (SCA).

Property	**Sedgwick Reserve**
Location	S. California
Mean Annual Precipitation	380 mm[1]
Mean Annual Temperature	17ºC [2]
Soil Series	Elder Loam
Parent Material	Alluvial
Sand / Silt / Clay	55 / 29 / 16
Total Carbon Content	3.1 %
C:N Ratio	12
pH	5.6
Sampling Date	10/29/2008
Previous Rainfall Event	05/23/2008
Soil Water Potential Prewet	-33 MPa
GPS of site	34º 43.1621' N / 120º 03.424' W

[1](Gessler et al 2000)
[2]Mean annual temperature 1970-2000 from RAND California using Lake Cachuma for Santa Ynez, CA.

Nucleic Acid Extraction and Quantitative PCR. Nucleic acids were extracted and cDNA synthesized as in Placella and Firestone (in review). We measured functional gene transcripts for ammonia oxidation, nitrite oxidation, and two different steps of

denitrification. For ammonia oxidation we targeted the *amoA* gene, which encodes ammonia monooxygenase subunit A (enzyme that converts ammonia to hydroxylamine in the first step of ammonia oxidation) in ammonia-oxidizing bacteria. We also targeted the archaeal *amoA* gene in ammonia-oxidizing archaea. For nitrite oxidation we quantified transcripts of *nxrA*, encoding nitrite oxidoreductase, of *Nitrobacter spp*. Quantification procedure for these transcripts is provided in Placella and Firestone (in review). We followed transcription of two functional genes in denitrifying bacteria, *nirK* for nitrite reductase and *nosZ* for nitrous oxide reductase. Thermocycling parameters for *nirK* were 98°C for 2 minutes followed by 40 cycles of 95°C for 5 seconds and 62°C for 10 seconds using the primers nirK876 and nirK1040 (Henry et al 2004). Thermocycling parameters for *nosZ* were 98°C for 2 minutes followed by 6 touchdown cycles (95°C for 5 seconds and 65°C for 15 seconds with a 1°C decrease per cycle) and 40 cycles of 95°C for 5 seconds and 60°C for 15 seconds using the nosZ2 primers (Henry et al 2006).

Total N_2O emissions. To roughly estimate the amount of N_2O produced on the landscape scale following the first and third water additions, we first calculated the total trace gas released per gram of dry soil in 75 hours of each water addition using the trapezoid rule of integration to interpolate the gas released while the jars were open. These values for the first (22 ± 7 ng N_2O-N g^{-1}) and third (110 ± 50 ng N_2O-N g^{-1}) water additions were added together and multiplied by the approximate soil bulk density (1.3 g cm^{-3}) and a response depth of 10 cm to arrive at a per-hectare estimate of 0.17 kg N_2O-N ha^{-1}.

Hydroxylamine reactivity with nitrite. Filter-sterilized (0.2 μm nylon) nitrite and hydroxylamine were combined into sterile serum vials with boiled, filter-sterilized ultra-pure water and shaken. The final concentrations were 1 mM nitrite and 100 μM hydroxylamine. Controls included 1 mM nitrite only and 100 μM hydroxylamine only. Headspace gas was measured immediately. All samples and controls were run in triplicate. We subtracted the N_2O in vials with hydroxylamine only from the nitrous oxide in the nitrite+hydroxylamine vials.

Data Analyses. Significant differences among time points were analyzed using Tukey's HSD and ANOVA with transect as a random variable and α=0.05; data were log-transformed as necessary. Rates of gas production and nitrogen pools from secondary rainfalls were analyzed similarly only using repeated measures. Our post water-addition analyses began with the 30-minute time point because this was the earliest evidence of potential transcriptional response (Figure 1).

Outliers. There is one instance of data exclusion. At 9 hours after the first simulated rainfall, all of the transcript abundance values for one biological replicate were substantially below the other four replicates. Dixon's Q test found the *nxrA* and bacterial *amoA* transcript abundances were both outliers to p=0.1. We thus excluded this sample from all transcript analyses.

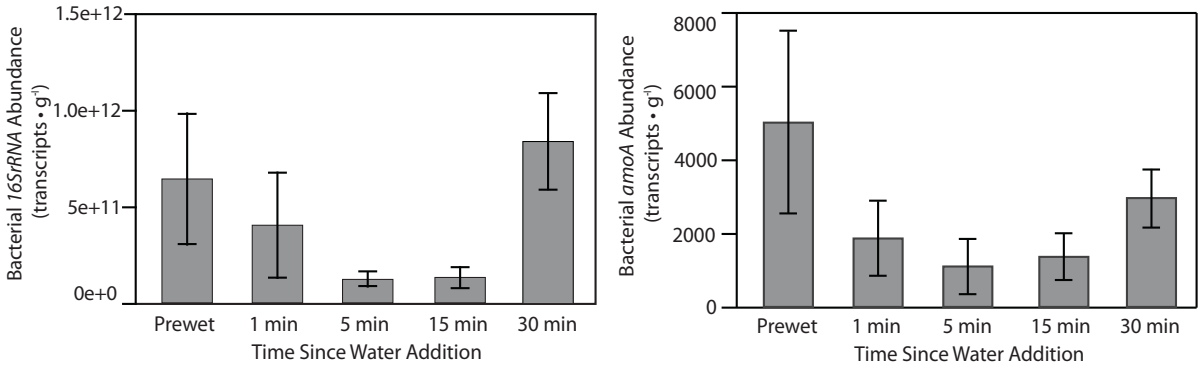

Figure 3-1. Degradation and production of RNA upon wetting of dry soil. We measure a fine time scale of bacterial 16S rRNA and bacterial *amoA* to determine when inactive transcripts at prewet may be degraded and new transcripts detected (n=5). Bars represent +/- standard error. Logarithmic fit of transformed data as a function of minutes since water addition from prewet (set as 1e-9 minutes post wet for this analysis) to 15 minutes post water addition explained 25% of the variability in 16S rRNA transcripts (p=0.02).

Results and Discussion

N_2O emissions increased substantially within 30 minutes of water addition (Figure 2), with the rate of N_2O production peaking approximately 8 hours after water addition and staying elevated for 3 days. Abundance of the *amoA* gene transcripts followed a pattern similar to that of the rate of nitrous oxide production. Transcripts of *nxrA* lagged behind transcripts of *amoA*. The prior time point's *amoA* transcript abundance explained 100% of the variability in *nxrA* transcripts for a given time point ($P<0.0001$, n=23). Archaeal *amoA* was more variable and did not show a statistically distinguishable trend over time or a correlation with other variables.

We measured transcripts of two different genes for denitrification, *nirK* for nitrite reductase and *nosZ* for nitrous oxide reductase, and found no evidence for upregulation of denitrification. We were unable to detect any transcripts of *nirK* (detection limit = 200 copies g^{-1}) despite an average of 10^6 *nirK* gene copies g^{-1} in the DNA pool. While we were able to consistently detect *nosZ* transcripts, they were always present at concentrations below that required for accurate quantification (1000 copies g^{-1}); in contrast we found 10^7 copies of *nosZ* genes g^{-1} in the extracted DNA. These low levels of transcription of N_2O reductase are consistent with pure culture studies of low constitutive expression under aerobic, non-denitrifying conditions (Beller et al 2006, Cuypers et al 1995, Philippot et al 2001).

Transcript abundance of bacterial *amoA* was the best predictor of the rate N_2O production, explaining 90% of the variability ($P=0.01$) with a simple least squares regression. These findings suggest a relationship between N_2O production and synthesis of ammonia monooxygenase; however this relationship may also reflect the observed lag in transcription of *nxrA*, coding for nitrite oxidation. The temporal lag (about 10 h)

between transcription of *amoA* and *nxrA* may allow the buildup of reactive intermediates that may be responsible for N_2O generation. Importantly, the rate of increase in the nitrate pool does not correlate with the rate of N_2O production. The steady increase in the soil nitrate pool from 9 to 72 hours post water addition (Figure 3A) suggests that nitrate production continues at a consistently high rate from 9 to 72 hours post water addition while the rate of N_2O production declines. Hence the production of the N_2O pulse coincided with the upregulation of ammonia oxidation, during a period of decoupling of ammonia oxidation and nitrite oxidation.

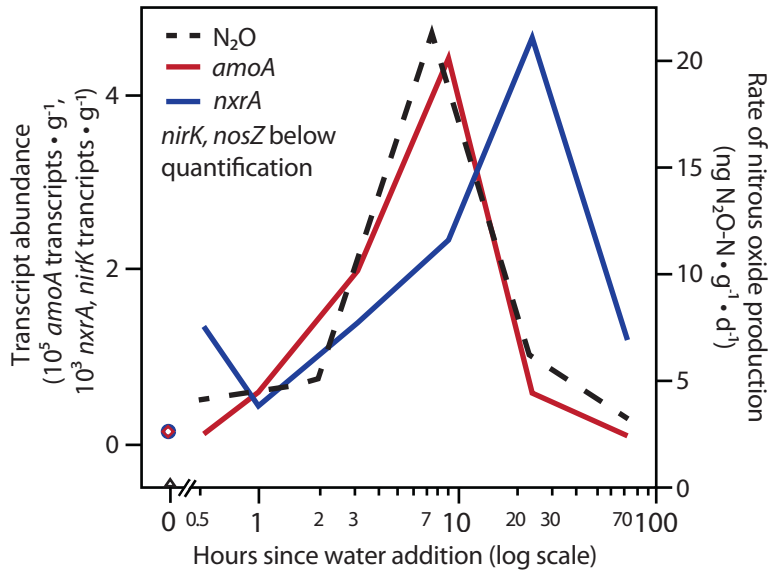

Figure 3-2. Rate of nitrous oxide production and transcript abundances for functional genes of ammonia-oxidizing bacteria (*amoA*) and nitrite-oxidizing bacteria of *Nitrobacter spp.* (*nxrA*) on a logarithmic time scale (n=5). Transcript abundances of both bacterial and archaeal *amoA* are given in the 100,000s on the left axis and shown in red in green, respectively, while those for *nxrA* are given in the 1,000s and shown in blue. Transcription of two functional genes for denitrifiers were also investigated but were either below detection, (*nirK),* or below accurate quantification, (*nosZ*). Rates of nitrous oxide production are shown with a black dashed line; corresponding values are on the right axis; rates of nitrous oxide production are shown on a linear time scale in Fig. 3. Statistics are provided in the Appendix.

Differences in the rates of response of ammonia oxidation and nitrite oxidation have been previously observed but the implication for N_2O production has not been investigated. Evidence of a decoupling between increasing ammonia oxidation and increasing nitrite oxidation following extended periods of inactivity can be found in studies of pure cultures and soil RNA and in discussion of other studies on temporal nitrite accumulation. Research involving pure cultures (Tappe et al 1999) and soil 16S rRNA (Placella and Firestone in review) suggest that ammonia-oxidizing bacteria may respond faster than nitrite-oxidizers (*Nitrobacter spp*., *Nitrospina spp.*, and taxa of the phylum Nitrospira). In addition, ammonia-oxidizing archaea appear to require more time

to respond to a stimulus than either ammonia-oxidizing or nitrite-oxidizing bacteria (Placella and Firestone in review). Similarly, temporal nitrite accumulation following the first winter rains in an Israeli semi-arid forest (Gelfand and Yakir 2008) further supports the decoupling of ammonia oxidation and nitrite-oxidation when ammonia oxidation first increases. Experimental additions of ammonium also show decoupling between ammonia oxidation and nitrite oxidation, leading to a buildup of intermediates. In a study involving ammonium addition to 116 soils, soils that showed a decline in ammonium all reported production of nitrite prior to an increase in nitrate (Morrill and Dawson 1967).

Following our observation of N_2O production resulting from nitrifier recovery after extended drought, we evaluated whether these pulses repeated with subsequent rains. To investigate a rainfall that was not associated with a long period of microbial inactivity, we continued to simulate rainfall every two weeks and monitored the third water addition pulse. We measured the rate of N_2O produced as well as ammonium and nitrate concentrations. The peak in N_2O emissions following the third simulated rainfall occurred during a decline in soil ammonium but prior to an increase in soil nitrate (Figure 3B), supporting the hypothesis that N_2O production results from the lag between ammonia oxidation and nitrite oxidation. While the temporal pattern of the rate of N_2O production was the same following both water additions, much more N_2O was produced after the third simulated rainfall than after the first (Figure 3). Nitrate levels did not decline even as the ammonium pool approached zero following the third rainfall.

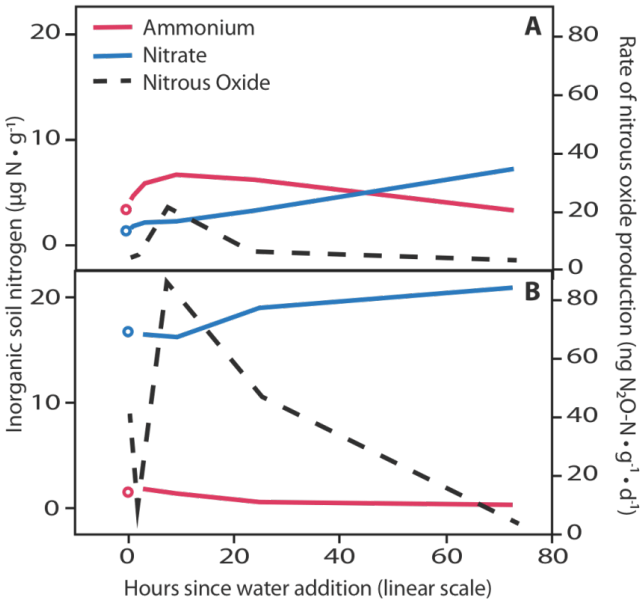

Figure 3-3. Soil ammonium and nitrate pools and rates of N_2O production following simulated rain events on a linear time scale. Soil ammonium and soil nitrate are shown on the left axis in red and blue, respectively. Rate of nitrous oxide production is given on the right axis and represented by the black dashed line. A. The first rainfall (simulated) following the dry season. Total nitrous oxide emitted from 1 minute to 75 hours was 22 ± 7 ng N_2O-N g^{-1} (n=5). B. The third rainfall (simulated) following the dry period. Total nitrous oxide emitted from this event alone, from 1 minute to 75 hours, was 110 ± 50 ng N_2O-N g^{-1} (n=5).

To assess whether abrupt increases in ammonium oxidation rates more generally cause significant pulses of N_2O production, we analyzed published soil N_2O efflux measurements where detailed soil microclimate data were reported. We found that pulses of N_2O from unsaturated soils have been reported following freeze-thaw events (Kammann et al 2008), wetting of dry soil (Davidson 1992, Hungate et al 1997, Rudaz et al 1991), addition of ammonium-based fertilizer (Kammann et al 2008), application of N-rich organic fertilizer (and irrigation) (Matson et al 1998), and urine release (Petersen et al 2004), all potential stimulants for ammonia oxidation. In addition, where nitrite has been measured along with N_2O emissions, accumulation of nitrite occurs on a similar time scale as pulses of N_2O production, supporting the importance of the buildup of intermediates (Petersen et al 2004, Venterea and Rolston 2000b). The few studies in which stable isotopes were used to analyze the source of peak N_2O pulses after fertilization and irrigation indicate that the pulses resulted from nitrification process and not denitrification (Perez et al 2001); nevertheless, most large pulses of N_2O have been attributed to denitrification, even when soils were well below saturation.

A number of studies point to the role of ammonia oxidation in N_2O production without specifically demonstrating a biological mechanism. Due to the high reactivity of nitrite (nitrous acid), nitrite accumulation due to the decoupling of ammonia oxidation and nitrite oxidation could be responsible for N_2O production via chemodenitrification, through reactions with iron minerals (Samarkin et al 2010), metals, or some organic compounds (Wrage et al 2001). While "denitrification" by ammonia-oxidizers could also contribute to N_2O emissions during nitrite accumulation, this process is only known to occur under oxygen stress (Stein 2011) and, therefore, is not expected to play a significant role in the production of N_2O that we have measured.

In light of recent data on the accumulation of hydroxylamine by ammonia-oxidizing bacteria (Schmidt et al 2004), especially during increases in ammonia oxidation (Yu and Chandran 2010), we think it may be time to revisit the "old" idea that N_2O is produced by reaction of hydroxylamine with nitrite (Arnold 1954, Bremner et al 1980, Corbet 1935). In this mechanism of N_2O generation, nitrite, the end product of ammonia oxidation, and hydroxylamine, an intermediate in ammonia oxidation, react chemically and produce N_2O. This mechanism could explain many observations including the rate of N_2O production being related to increases in the activities of ammonia-oxidizing bacteria (Yu et al 2010), declining when ammonia-oxidizing bacteria are in co-culture with nitrite-oxidizing bacteria (Kester et al 1997), and varying greatly within an ammonia-oxidizing culture (Colliver and Stephenson 2000), among others (Hooper and Terry 1979, Otte et al 1999, Ritchie and Nicholas 1972). Stein (2011) recently proposed that N_2O formation during ammonia oxidation is caused by reaction of hydroxylamine with reactive cellular constituents such as the enzyme nitric oxide reductase or cytochrome L. However, nitrite and hydroxylamine do react spontaneously to produce N_2O and under these circumstances, enzyme mediation is not necessary.

We assessed the likelihood of hydroxylamine and nitrite reacting to yield significant quantities of N_2O. Given that during the decoupling of ammonia oxidation and nitrite oxidation, local concentrations of hydroxylamine and nitrite should increase in the

vicinity of ammonia-oxidizing bacteria, we aseptically combined 1 mM sodium nitrite and 100 μM hydroxylamine hydrochloride in serum vials and immediately measured the headspace N_2O concentration. If we assume that half of the N in N_2O originated from hydroxylamine, 0.25% ± 0.01% of hydroxylamine-N would have been lost as N_2O at pH 5.8, similar to our soil pH of 5.6. The reactivity of nitrite increases as solution pH decreases; hence under more acidic conditions a greater conversion to N_2O might be expected in soil. Thus the locally reduced pH in the vicinity of ammonia-oxidizing bacteria could have a large impact on N_2O production. In our soil, 1.0% ± 0.5% and 2.0 ± 0.9% of net nitrification was lost as N_2O 3-24 h after the first season rainfall and secondary rainfall, respectively.

The N_2O pulse immediately following an increase in ammonia oxidation may depend on a combination of abiotic and biotic processes. The abiotic reaction between hydroxylamine and nitrite to produce N_2O would be significant if and when the intermediates build up during an increase in ammonia oxidation that is not yet balanced by nitrite oxidation. New techniques for quantifying the transcription of enzymes within a pathway provide the opportunity to probe the mechanisms underlying and ecological significance of such decouplings.

By simulating pulses of N_2O associated with increases in ammonia oxidation, soil N_2O models may better capture the extreme variability seen in soil N_2O fluxes. A decoupling of ammonia oxidation and nitrite oxidation after fertilizer application was proposed by Venterea and Rolston (2000a) to explain nitrite accumulation and emissions of N_2O and NO. Hence, some models of N_2O production during fertilization have attributed pulses of N_2O to abiotic decay of nitrous acid accumulating during a lag between ammonia oxidation and nitrite oxidation (Maggi et al 2008, Venterea and Rolston 2000a). Our results provide the first empirical evidence confirming a decoupling during abrupt increases in nitrification and suggesting the need for generalized incorporation into models. Most soil moisture-driven models include only a linear relationship between soil moisture and N_2O production (Parton et al 1988) and do not account for the pulsed nature of N_2O production following rapid increases in soil moisture. The reliance of N_2O pulses on changes in soil moisture as opposed to being a function of soil moisture may explain why some attempts to link N_2O production to soil moisture have been unsuccessful in the past (Billings et al 2002). Many datasets of soil N_2O emissions feature high variability that may be accounted for by correlating the high rates of N_2O production to recent rainfall events (Clough et al 1998, Maggi et al 2008, Mosier et al 2002, Venterea and Rolston 2000b).

Conclusion

Dynamic environmental conditions during which ammonia oxidation increases rapidly can cause pulses of N_2O. The results of this study indicate that rain pulses, fertilizer applications, or urine additions will likely lead to an N_2O pulse. In this study, the N_2O emitted during two simulated rain events, representing the first and third of the season,

could account for approximately one half of the annual estimate for this ecosystem. The annual estimates for grassland N_2O production may be low because pulse events are rarely identified and accurately incorporated into models. If rainfall variability increases as some models project, soil N_2O emissions may be substantially altered. Understanding of N_2O pulses, their magnitudes and durations, may allow models to better constrain global N_2O emissions and better estimate these emissions under scenarios of changing climate.

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Appendix

Table A-1. Significance of biogeochemical measurements by time point during simulated first rainfall using ANOVA with Tukey's HSD, α=0.05, n=5.

	Prewet	0.5 hr	1 hr	3 hr	9 hr	24 hr	72 hr
Rate of N_2O Production[a]	c	b	b*		**a****	b	b
Soil Ammonium	bc	b	b	**a**	**a**	**a**	c
Soil Nitrate	d	cd	c	c	c	b	**a**
Bacterial *amoA*	ab	ab	ab	ab	**a**	ab	b
Archaeal *amoA*	a	a	a	a	a	a	a
Nitrobacter spp. nxrA	a	a	a	a	a	a	a
nirK	a	a	a	a	a	a	a
nosZ	a	a	a	a	a	a	a

Time points represented by different letters are significantly different. [a]Rate of N_2O production run and evaluated with repeated measures. *Rate of N_2O production at 2 hours. **Rate of N_2O production at 7.5 hours.

Table A-2. Significance of biogeochemical measurements by time point during simulated third rainfall event using ANOVA with repeated measures and Tukey's HSD, α=0.05, n=5.

	Prewet	0.5 hr	3 hr	9 hr	24 hr	72 hr
Rate of N_2O Production	a	a	a*	a*	a*	a*
Soil Ammonium	c		**a**	b	d	d
Soil Nitrate	c		c	c	b	**a**

Time points represented by different letters are significantly different.*Rate of N2O production at 2, 7.5, 24.5, and 73.5 hours.