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AUTHOR: Timothy Grant

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Matthew Escobar	Matthew Escobar Matthew Escobar (Nov 24, 2020 17:25 PST)	11/24/2020
THESIS COMMITTEE MEMBER	SIGNATURE	DATE
Arun Sethuraman		11/26/2020
THESIS COMMITTEE MEMBER	SIGNATURE	DATE

Experimental Chronic Dry Atmospheric Nitrogen (N) Deposition Causes a Change in Soil Microbial Communities in Southern California Semi-Arid Shrublands

Timothy Grant

Department of Biological Sciences, College of Science and Mathematics

California State University San Marcos

333. S Twin Oaks Valley Rd. San Marcos, CA 92096

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Abstract

Global nitrogen (N) deposition has increased since the beginning of the Industrial Revolution due to anthropogenic influences such as the burning of fossil fuels and application of N fertilizer. Increased industrialization is expected to continue to escalate the quantity of N released to ecosystems and the atmosphere. Soil microbial communities are susceptible to this increase in N, as microbial activity can be limited by N and these communities are known to regulate biogeochemical cycles. In this study, the response of bacterial functional groups (FG) to N addition (> 50 Kg N ha^{-1} yr^{-1}) was quantified in Southern California semi-arid topsoil (0-10 cm). Soil physical-chemical properties and enzymatic activities were also quantified to further understand the direct/indirect effects of N addition on bacterial FG. Experimental N addition significantly increased the abundance N-fixing, chitinolytic, and starch degrading bacteria in semi-arid soils; while increasing N-mineralizing and denitrifying bacteria in coastal sage shrub (CSS) soil. N input also decreased the abundance metal redox bacteria in CCS soil and decreased nitrifying bacteria in chaparral soil. Furthermore, N addition increased the abundance of

copiotrophic bacteria (ie: Proteobacteria, Bacteroidetes, and Firicutes) and decreased the abundance of oligotrophic bacteria (ie: Acidobacteria) in semi-arid soils. Canonical correspondence analysis revealed that the majority of FG were influenced by soil pH and extractable nitrate, which were also significantly altered by N addition. Results indicated excessive N input directly and indirectly affected the composition of the soil bacterial community. Understanding the response of the microbial community to N additions is important in predicting ecosystem functionality and stability as anthropogenic N deposition increases.

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Introduction

Anthropogenic nitrogen emissions

Anthropogenic activities have increased atmospheric nitrogen (N) inputs and the amount of terrestrial N processed by the N cycle on a global basis. Such activities lead to the emission of reactive nitrogen (Nr), or N usable by living organisms, into the biosphere in the form of nitrogen oxides (NO_x), ammonia (NH_3), and other inorganic compounds (Cullis and Hirschler, 1982; Fahey *et al.,* 1986). Since the beginning of the Industrial Revolution, anthropogenic activities have increased N emissions to 124-132 Tg N yr^{-1}, and by 2050, N emissions are estimated to increase to 200-300 Tg N yr^{-1} due to increased industrialization (Boyer *et al.,* 2004, Fowler *et al.,* 2004; Galloway *et al.,* 2004; Galloway *et al.,* 2008; van Vuuren *et al*., 2011; Winiwarter *et al*., 2013; Kanakidou *et al.,* 2016). Two known sources of anthropogenic N emission are the use of fertilizers for agriculture and the combustion of fossil fuels (Fowler *et al.*, 2004; Galloway *et al.,* 2002; Galloway *et al*., 2004; Galloway *et al*., 2008).

Globally, the fixation of N supplies ~413 Tg N yr^{-1} to terrestrial and marine ecosystems, by which 203 ± 50 Tg N yr^{-1} is fixed biologically and ~210 Tg N yr^{-1} fixed through anthropogenic sources (Cleveland *et al*., 1999; Erisman *et al*., 2013; Fowler *et al*., 2013). From 1860 to 2005, anthropogenic N, primarily in the form of Nr fertilizer, increased terrestrial N input from 15 to 187 Tg N yr^{-1} (Galloway *et al*., 2002, 2008). The combustion of fossil fuels is also another large contributor of global anthropogenic N emissions, with an estimated 40 Tg N emitted into the atmosphere each year (Benkovitz *et al.,* 1996; Vitousek *et al*., 1997; van Vuuren *et al*., 2011; Fowler *et al*., 2013).

The increase of N from fertilizer and combustion of fossil fuels increases global N deposition (Galloway *et al.,* 2004; Liu *et al*., 2013; Li *et al*., 2016). In the United States, average

N deposition levels range from as low as 1-4 kg N ha^{-1} yr^{-1}, to as high as 30-90 kg N ha^{-1} yr^{-1} (Fenn *et al*., 2003b; Pardo *et al*., 2011; Du *et al*., 2014). Atmospheric N input increases near urban and agricultural regions due to increased anthropogenic influences (Fenn *et al*., 2003b). For example, California has both heavily populated urbanized cities and agricultural regimes that contribute to an increased N deposition (Fenn *et al.,* 2003a).

N deposition in Southern California

Southern California is home to densely populated cities and counties form the US/Mexico border to the county lines of Kern and San Bernardino County. The region is affected by elevated N deposition from agricultural farms and ranches that utilize N fertilizers, and fossil fuel use in the large urban areas, leading to a mixture of N emission sources (Riggan *et al*., 1985; Weis, 1999; Fenn *et al*., 2003a). Based on the Community Multiscale Air Quality modeling system (CMAQ), 13% (52,823 km^2) of California's total land area (423,970 km^2) is exposed to N deposition levels that exceed 10 kg N ha^{-1} yr^{-1} (Fenn *et al.,* 2010). Field studies in Riverside County have shown portions of the region receive 20-35 kg N ha^{-1} yr^{-1} (Allen *et al.,* 1998; Fenn *et al.,* 2003b). N deposition input can increase up to 45 kg N ha^{-1} yr^{-1} near urbanized landscapes such as the Los Angeles Basin and up to 145 kg N ha^{-1} yr^{-1} in high-elevation locales close to urban areas (Fenn *et al*., 2003a; Fenn *et al*., 2003b; Fenn and Poth, 2004). Since Southern California has a semiarid Mediterranean climate, a majority of its N deposition comes in the form of dry N, which accrues on vegetation and surface soil and becomes readily available after wetting events, including rainfall and agricultural watering (Davidson, 1992; Miller *et al.,* 2005).

N effects on soil microbes

The belowground microbial community plays an important role in the global N cycle (Vitousek *et al*., 1997; Francis *et al*., 2007; Falkowski *et al*., 2008). Increased N input from

anthropogenic activities can influence the microbial community and biogeochemical cycles they regulate, as one gram of soil can contain millions of microorganisms and thousands of species (Vitousek *et al.,* 1997; Torsvik and Øvreås, 2002; Galloway *et al.,* 2008; Ramirez *et al.,* 2010b). Intensified N inputs can have multiple effects on the growth, activity, and composition of soil microbes (Castro *et al.,* 2010; Ramirez *et al.,* 2012). Studies indicate that excessive N deposition and N fertilization typically decreases microbial biomass via direct inhibition from toxic osmotic potentials, leaching of Mg^{2+} and Ca^{2+}, as well as poisoning from aluminum (Al) mobilization (Baath *et al.,* 1981; Vitousek *et al.,* 1997; Compton *et al.,* 2004; Treseder, 2008; Zhou *et al*., 2017; Zhang *et al.,* 2018). Microbial biomass carbon (C) has also been reported to decrease in response to chronic N addition, but results from studies have varied based on the magnitude of N input (Zhang *et al.,* 2008; Li *et al.,* 2010; Zhang *et al.,* 2018). Correspondingly, microbial activity, such as N-mineralization and soil respiration, have been reported to have both significant and non-significant responses to additional N loads, in various ecosystems (Hatch *et al.,* 2000; Vourlitis *et al.,* 2007b; Treseder, 2008; Li *et al.,* 2010; Peng *et al.,* 2011; Ramirez *et al.,* 2012; Leff *et al.,* 2015). For example, in chaparral and coastal sage scrub (CCS), microbes exposed to long-term N deposition had higher N-mineralization rates (Vourlitis and Zorba, 2007a). Other field and lab-based studies have shown a reduction in both soil respiration and microbial biomass in response to experimental N, and the reduction in both were correlated with the duration and magnitude of N input (Treseder, 2008; Janssens *et al*., 2010). Ramirez *et al.* (2012) also reported a 12% decrease in soil respiration and a 35% decrease in microbial biomass in N amended soils sampled throughout the United States.

Microbial diversity and composition are key components in determining ecological functions (Brussaard, 1997; Nannipieri *et al*., 2003; Philippot *et al.,* 2013). Field studies have

shown that chronic N addition can cause shifts in the relative abundance of specific functional bacteria, such as ammonia oxidizing bacteria (AOB), but has an overall negative effect on microbial diversity (Shen *et al.,* 2011; Delgado-Baquerizo *et al*., 2013; Sun *et al.,* 2018; Wang *et al.,* 2018).

A number of hypotheses have been proposed to explain the N-induced decline in microbial growth, activity, and composition. For example, the *enzyme inhibition hypothesis* suggests that N directly inhibits enzymes needed for decomposition of recalcitrant C (Fog, 1988; Gallo *et al*., 2004). Conversely, the *copiotrophic hypothesis* suggests that an increase in nutrients causes a direct change in microbial activity due to a decline in the abundance of oligotrophic taxa, or taxa adapted to low nutrient availability, and an increase in copiotrophic taxa, or taxa adapted to higher nutrient availability (Fierer *et al.,* 2007; Ramirez *et al.,* 2010a). Sequencing analysis has shown consistent phylum-level changes in the bacterial communities exposed to elevated N inputs with increases in copiotrophic taxa (ie: *Actinobacteria* and *Firmicutes*) and decreases in oligotrophic taxa (ie: *Acidobacteria* and *Verrucomicrobia*) (Ramirez *et al.,* 2012). These findings are similar to those of Egerton-Warburton and Allen (2000), Nemergut *et al*. (2008), and Campbell *et al.* (2010), supporting the *copiotrophic hypothesis* and the implementation of the broad schemed *copiotrophic/oligotrophic classification model* (Cleveland *et al.,* 2007; Fierer *et al.,* 2007; Nemergut *et al.,* 2010).

A previous study by Holt (2016), at the same experimental sites for this study, was conducted to observe how experimental chronic N affected microbial biomass and soil respiration. Results showed that there was no significant difference in respiration between control plots and N-treated plots; however, there was significantly higher microbial biomass in N-treated plots. Higher microbial biomass with no difference in respiration between control and

N plots could be attributed to different soil microbial community composition in treated plots. The findings of Holt (2016) indicate a possible shift in the soil microbial community. Unfortunately, identifying changes in microbial community composition is not possible with measurements of soil respiration and microbial biomass alone (Nannipieri *et al.,* 2003), and a metagenomic study is needed to comprehensively identify microbial taxa in soil (Tringe and Rubin, 2005).

More often than not, long-term studies do not analyze N effects on microbial community composition, while the knowledge of microbial responses to chronic elevated N input could potentially help better predict consequences of anthropogenic N (Börjesson *et al.,* 2011; Ramirez *et al.,* 2012; Egan *et al.,* 2018; Zhang *et al.,* 2018). This study, therefore, aims to further the understanding of how increased chronic N affects soil microbes by quantifying abundance differences of bacterial functional groups (FG) between control and N-treated plots in Southern California's Mediterranean climate. Microbial taxa can be categorized into microbial FG based on their contribution to a specific step of a biogeochemical cycle, enzymatic properties, or microbial characteristics. For example, based on their ability to oxidize Nr during the N cycle's nitrification process, bacterial genera *Nitrobacter*, *Nitrospira*, *Nitrococcus* and *Nitrospina* can be categorized into the same functional group, nitrite (NO_2^-) oxidizing bacteria (Roux *et al*., 2016; Fierer, 2017). Bacterial taxa categorized as copiotrophic or oligotrophic are characterized into these FG by their ability to thrive in high or low nutrient level environments respectively (Ho *et al*., 2017). This study also makes use of the *copiotrophic/oligotrophic classification model*, quantifying the abundance differences of copiotrophic and oligotrophic bacteria between control and N-treated plots. Soil environmental properties and seasonal variation were also quantified, as both are known attributes that affect microbial abundance. I hypothesize that there will be a

significant change ($p \leq 0.05$) in the abundance of bacterial FG between control and N-treated soil plots. Further, I hypothesize that chronic N addition will significantly decrease the abundance of oligotrophic taxa and significantly increase the abundance of copiotrophic taxa in the soil.

Methods & Materials

Site descriptions and experimental design

Field experiments were conducted at Sky Oaks Field Station (SOFS) and Santa Margarita Ecological Reserve (SMER) California, USA. An evergreen chaparral stand, SOFS is located in NE San Diego County (33º21′ N:116º34′ W) at an elevation of 1,418 m on a 4-10º SE-SW facing slope. Its sandy loam soil (1.34 g/cm^3 bulk density) is dominated by chamise (*Adenostoma fasciculatum*) and desert ceanothus (*Ceanothus greggii*), and receives an average annual precipitation of 53 cm (Vourlitis and Zorba, 2007a; Vourlitis *et al.,* 2009). SMER is a semi-deciduous CSS stand located on a 9-11º S-SW facing slope in NW San Diego County (33º29′ N:117º09′ W) at an elevation of 338 m. The sandy clay loam soil (1.22 g/cm^3 bulk density), which receives an average annual rainfall of 36 cm (Vourlitis and Zorba, 2007a; Vourlitis *et al.,* 2009), is dominated by California sagebrush (*Artemisia californica)* and black sage (*Salvia mellifera)*.

The sites are comprised of eight 10 m x 10 m plots that are arranged in pairs, one of which was randomly assigned a N addition treatment and the other assigned as an un-manipulated control (n = 4 per treatment). Since 2003, the N addition plots at both SOFS and SMER have received a yearly treatment (50 kg N ha^{-1} yr^{-1}) of either granular ammonium nitrate (2003-2007), ammonium sulfate (2007-2009), or urea (2009-present) during the fall season (Vourlitis *et al*., 2009; Vourlitis and Hentz, 2016). CMAQ simulations predicted that the

experimental sites naturally receive 2-7 kg N ha^{-1} yr^{-1} (Fig.1; Fenn *et al*., 2010). In order to compensate for disparities in spatial N deposition, the treatment amounts of N exceeded the reported N deposition values for urban shrub lands in Southern California (Fenn *et al*., 2003; Fenn and Poth, 2004).

Field sampling and laboratory analysis

Using a T-bar soil probe, topsoil (0-10 cm) samples were randomly collected from both experimental sites in the months of May and October 2017. Since fertilization occurred during early fall, fall samples were collected in late October to prevent biased results of early urea application. Each of the control and N-treated plots were randomly sub-sampled five times, for a total of forty sub-samples per site, per season. The collected soil was sieved through a 2 mm wire mesh to remove rocks and small debris. After the soil was sieved, portions from each of the five sub-samples were combined into one soil sample to represent each plot. These samples were then analyzed for soil physical-chemical properties and soil enzyme activity. Soil DNA, needed for sequencing microbial 16S rRNA region V4, was obtained from the remaining individual sub-sample portions before further laboratory procedures continued. All soil samples were kept frozen at -20˚C until microbial DNA extractions were performed.

Soil physical-chemical properties

Soil moisture was quantified as a percentage by weight. Small crucibles were labeled and their weights then recorded. Approximately 5 grams (g) of undried (fresh) soil were added to its designated crucible and weighed. The crucibles were then oven dried at 105˚C for at least twenty-four hours, after which their weights were recorded as dry mass. Soil moisture percentage was calculated by using the following equation: ((fresh soil mass – crucible mass) – (dry soil mass – crucible mass) / (fresh soil mass – crucible mass)) x 100 = moisture percentage.

Soil pH was measured using a pH meter. Samples were prepared by weighing 15 g of fresh soil and adding it to a labeled Erlenmeyer flask. After adding 30 mL of deionized (DI) water to the soil, the solution was stirred until a slurry had formed. All of the slurry mixtures were left to stand for thirty minutes, stirring occasionally to achieve homogeneity. A calibrated pH electrode was placed into a single slurry mixture and the sample's pH was recorded after stabilization in the pH reading. This process was repeated for each solution, with the electrode being rinsed between each sample with DI water.

Inorganic N and microbial C extractions were required for microbial biomass and extractable N (nitrate (NO_3^-) and ammonium (NH_4^+)) colorimetric assays, as they allowed extractable N or C to be pulled into solution. Extractable N was derived into solution from samples by adding 40 mL of 2M KCl to 10 g of fresh soil in a labeled Erlenmeyer flask. Samples were placed on a shaker table to stir for one hour at 200 rpm. After being transferred to a 50 mL conical tube, the slurry samples were centrifuged for five minutes at 3000 rpm. The supernatant from each sample was then filtered through Fisherbrand® P8 coarse filter paper (cat. no. 09-790-12E), and the filtrate was stored in labeled 30 mL containers at 4°C. Extractable microbial C was derived following the same procedure, except 40 mL of 0.5M K_2SO_4 was used and the filtrates were stored at room temperature. Prior to any colorimetric assays, an additional filtration was performed on the extractions using 0.2 µm nylon syringe filters.

Extractable N assays were used to quantify the NO_3^- and NH_4^+ concentration (µg N/ g soil) of each soil sample. The assays used were procedurally similar, differing only in the synthesized reagents and volumes utilized. A standard procedure adopted from Miranda *et al.* (2001) was used to determine the NO_3^- concentration of soil samples, while a revised standard procedure (Nelson, 1983; Mulvaney, 1996) was adapted to determine NH_4^+ concentration. For

soil NO_3^-, 3.5 mL of 2M KCl, 7.5 mL of mixed reagent (vanadium (III) chloride, 2% sulfanilamide, 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride) and 3.5 mL of sample filtrate were placed into test tubes. These solutions were incubated in a 37°C water bath for thirty minutes. Each solution's absorbance was measured at a wavelength of 540 nm. For soil NH_4^+, 2 mL of sample filtrate, 1 mL of Ethylenediaminetetraacetic acid reagent, 4 mL of salicylate-nitroprusside reagent, 5 mL of DI water, and 2 mL of buffered hypochlorite were placed into test tubes. Absorbance of each sample was measured at a wavelength of 667 nm. NO_3^- and NH_4^+ concentrations were derived from a regression equation, generated by a standard curve of known N concentrations and their measured absorbance, then converted from units of [mg N/ L] to [µg N/ g soil].

A modified fumigation and extraction procedure (Brookes *et al.,* 1985) was performed to assess the concentration (µg C/ g fresh soil) of soil microbial biomass C. Two, 10 g samples of fresh soil from each plot were placed into a corresponding 50 mL Erlenmeyer flask and incubated at room temperature for 72 hours. Half of the samples were left to incubate in a vacuum desiccator with no chloroform (non-fumigated), while the other half were placed in a vacuum desiccator with a beaker containing 25 mL of chloroform and boiling stones (fumigated). Once the vacuum pressure caused boiling stones to boil the chloroform, the vacuum desiccator was sealed and left to incubate at room temperature for five minutes. This process was repeated three times, using a new beaker filled with boiling stones and 25 mL of chloroform after each run. The last fumigation was left under vacuumed seal to incubate at room temperature for 72 hours. After the incubation period, dissolved C was measured on the fumigated and non-fumigated samples using a modified version of the Walkley-Black method (Sims and Haby, 1971). The assay involved adding 10 mL of 1N $K_2Cr_2O_7$ and 20 mL of concentrated H_2SO_4 to 20

mL of sample extraction in a labeled 125 mL Erlenmeyer flask. Samples were incubated in a fume hood at room temperature for 20 minutes, and absorbance was measured at a wavelength of 600 nm. Biomass C concentrations were derived from a regression equation, generated by a standard curve of known C concentrations and their measured absorbance, then converted from units of [µg C/ ml] to [µg C/ g fresh soil]. Total microbial C was calculated as the C concentration of the fumigated samples minus the C concentration on of the non-fumigated samples.

Total soil N, C, and C:N ratios were measured using an elemental analysis combustion system (Model 4010, Costech Analytical Technologies Inc., Valencia, CA, USA). Samples were prepared by grinding the soil into a fine powder using a Retsch ball mill. Fifteen mg of fresh processed sample was weighed and placed into a micro tin cup, which was folded into a cube. These cubes were then placed into the elemental combustion system to be automatically processed, and the resulting percentages of total N and C were used to calculate the C:N ratio.

Enzymatic activity

The extracellular enzymatic activity (µmoles hr^{-1} g dry $mass^{-1}$) of ß-glucosidase, N-acetyl-ß-D-glucosaminidase (NAGase), phosphatase, and peroxidase were quantified by using standard protocols (Saiya-Cork *et al.,* 2002; Jackson *et al.,* 2013). The soils used for enzymatic activity assays were first air dried for 24 hours. For assays quantifying ß-glucosidase, NAGase, and phosphatase activity, methods established in Jackson *et al*. (2013) were used. Enzyme activity was calculated using the following equation: enzyme activity = final absorbance / (slope of standard curve x incubation time (hr) x sample dry mass (g)). To quantify the enzymatic activity of peroxidase, methods established in Saiya-Cork *et al*. (2002) were used. Unlike the procedure described in Jackson *et al.* (2013), the methodology defined by Saiya-Cork *et al.*

(2002) did not use a standard curve in order to calculate peroxidase activity. Instead, peroxidase activity was calculated using the following equation to convert absorbance to enzymatic activity: (final absorbance x 5 mL) / (7.9 μmoles x 0.2 mL x incubation time (hr) x sample dry weight (g)).

Soil DNA extraction

Microbial 16S rRNA V4 region sequencing was used to quantify the abundance and taxonomic composition of soil bacteria. First, DNA from each plot's five soil sub-samples was extracted using the Fisher BioReagents® SurePrep™ Soil DNA Isolation Kit (Cat.# BP2815-50, Fisher Scientific®, Fair Lawn, NJ, USA) following the manufacturer's protocol. Before further purification, gel electrophoresis was used to ensure that each isolation yielded fragments of DNA with high molecular weight. In order to run gel electrophoresis, the concentration of each DNA sub-sample was determined by processing 2-μL of sample via a nano-drop micro-volume spectrophotometer. Determining the concentration allowed for individually calculated volumes of sample to be used for each gel electrophoresis run (40 minute run at 85 volts). These calculated volumes allowed for 25 ng of DNA from each sub-sample to be loaded into a 1% agarose-gel. After gel documentation imagery conformation of isolated DNA, samples were further purified using the Monarch® PCR and DNA Cleanup Kit (5 μg) (Cat.# T1030S, New England Biolabs® Inc., Ipswich, MA, USA) with a 2:1 ratio of binding buffer to sample. Upon DNA purification, individual sample concentration and purity was reassessed using the previously described nano-drop techniques. Each sample was diluted to the lowest sub-sample's concentration using an elution buffer. From each of the five sub-samples, 4-μL was pooled to create a 20-μL composite DNA sample, representing each plot. Samples were stored at -80°C

until all samples for both sites and seasons were processed. All 32 composite DNA samples were then sent to Molecular Research LP in Shallowater, TX for sequencing.

Sequencing

Barcoded amplicon sequencing was performed using bacterial 16S-based tag-encoded FLX amplicon pyrosequencing (bTEFAP®), a trademark service of Molecular Research, LP invented by Dr. Scot E. Dowd (2007). Briefly, 16S rRNA V4 region PCR primers 515F/806R, with barcoded forward primer, were run in the 28 cycle PCR utilizing the HotStarTaq™ Plus Master Mix Kit (Qiagen®, Germantown, MD, USA). Amplification success of PCR products were assessed on a 2% agarose gel. Samples were then purified and pooled in equal proportions based on their DNA concentrations and molecular weight in preparation for construction of the Illumina® DNA sequencing library using Illumina® Nextera™ DNA Sample Preparation Kit (now called Illumina® DNA Prep). Paired-end sequencing was then performed on the Illumina® MiSeq™ (2x250 bp) platform following the manufacturer's guidelines.

Following sequencing and demultiplexing of samples, sequences were depleted of primers and barcodes; while short sequences (< 150bp) were removed from the sequence data. Q25 trimming allowed for the removal of ambiguous base calls and sequences with homopolymer runs that surpass 6 bp. Denoised sequences of operational taxonomic units (OTUs) were grouped at 99% similarity, accompanied by the depletion of singleton sequences and chimeras. Final OTUs were classified using BLASTn against the RDPII and NCBI databases. From this, OTUs were compiled into count files (counts and percentages) in Microsoft Excel and assessed on multiple taxonomic levels.

Microbial functional group abundance

Results containing microbial abundances were researched on the family taxonomic level in the primary literature, books, and the internet, in order to categorize the microbial families into FG. Microbial family sequencing counts, from both experimental sites, were placed into N-cycling and C-cycling FG. Bacterial N-cycling FG were represented by N-fixing, N-mineralizing, nitrifying, and denitrifying bacteria (Table 1 and Table 3). C-cycling bacteria were categorized into C-cycling FG, which included cellulolytic, chitin degrading, starch degrading, methanogenic, anaerobic, and metal redox bacteria (Table 2 and Table 4). Bacterial families with a minimum of five sequence counts in each plot were categorized into these respective FG. Results containing microbial abundances were further researched on the taxonomic phylum level in order to be categorized as copiotrophic or oligotrophic. Phyla representing a minimum of 3% of all sequence counts were categorized into either functional group. The copiotrophic functional group was represented by the bacterial phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Shade *et al.,* 2012; Cederlund *et al.,* 2014), whereas the oligotrophic functional group consisted of the bacterial phyla Acidobacteria, Chloroflexi, Planctomycetes, and Verrucomicrobia (Yamada and Sekiguchi, 2009; Shade *et al.,* 2012).

Data analysis

Data analyses were performed using R statistical software, with graphs constructed using both R and Microsoft Excel. A two-way repeated-measures ANOVA was used to assess the effects of the N-treatment, seasonal variation, and the possible interaction between the two factors. The two-way ANOVA was run on the physical-chemical soil properties, enzymatic activity, and microbial functional group abundance. Variables that did not pass normality (Shapiro-Wilks) and heteroscedasticity (HOV) tests were log transformed, and an ANOVA was

run on the transformed data. If log-transformation still did not correct this problem, a non-parametric, randomized permutation test was used on non-transformed data.

To relate microbial functional group abundances to environmental factors, a canonical correspondence analysis (CCA) was run with microbial FG against soil physical-chemical properties. R software's *vegan* package uses weighted average site scores as the default scores from the CCA algorithm. For the purpose of preventing an overfitted model, multiple CCA were performed with microbial FG categorized by their biogeochemical cycle and soil physical-chemical properties as different explanatory variables. Visual analysis of CCA biplots, along with proportion values for accumulated constrained CCA axes and biplot scores, were utilized to help explain the relationship between microbial FG and their environment.

Results

Soil physical-chemical properties

The effects of chronic N inputs, seasonal variation, and their possible interaction on experimental sites' soil physical-chemical properties are displayed in Tables 5 and 6. At SMER, soil moisture varied seasonally, but not in response to chronic N inputs (Table 5). This seasonal effect was observed during the fall, as soil moisture significantly (degrees freedom (df) = 1,12; p = 0.00) decreased by 65.8% (Table 5). SOFS soil moisture also experienced a significant (df = 1,12; p = 0.00) decrease in response to seasonal variation, as soil moisture in the fall was 34.9% lower than in the spring; however, N-treatment also caused a significant (df = 1,12; p = 0.00) decrease in soil moisture, by an average 29% each season (Table 6).

N-treatment induced a significant (df = 1,12; p = 0.00) decrease in soil pH at both sites (Table 5 and Table 6). SMER soil pH decreased by an average of 13.6% and SOFS soil pH

decreased by an average of 22.7% in response to N-treatment. There were no significant ($df = 1,12$; $p > 0.05$) seasonal effects on soil pH (Table 5 and Table 6).

Microbial C biomass at both sites was not significantly ($df = 1,12$; $p > 0.05$) affected by N-treatment (Table 5 and Table 6). However, microbial biomass declined significantly ($df = 1,12$; $p = 0.01$) during the fall (37.3%) at SOFS, but not at SMER (Table 5 and Table 6).

Extractable NO_3^- and NH_4^+ experienced a significant ($df = 1,12$; $p \leq 0.05$) increase in response to chronic N inputs at both sites (Table 5 and Table 6). The average increase in NO_3^- at SMER was near 3-fold, while the N amended soil at SOFS had a near 28-fold increase during the spring and 10-fold increase during the fall. NH_4^+ concentrations at SMER increased a near 3-fold compared to that of the control soil, with a near 6-fold increase during the fall. SOFS also experienced an enormous increase in its NH_4^+ concentration in response to the N-treatment, with an 11-fold increase during the spring season and a near 4-fold increase in the fall. Seasonal variation induced a significant ($df = 1,12$; $p = 0.00$) increase only for SMER NH_4^+, which increased by 2-fold from the spring to fall season (Table 5).

Total N, total C, and C:N ratio at SMER did not exhibit a significant ($df = 1,12$; $p > 0.05$) treatment or seasonal effect (Table 5). At SOFS, N-treatment had no significant ($df = 1,12$; $p > 0.05$) effect on total N, total C, and the site's C:N ratio, but total N decreased by 52.6% and C:N increased by 138% from the spring to fall season (Table 6).

Enzymatic activity

N-treatment had no significant ($df = 1,12$; $p = 0.21$) effect on SMER ß-glucosidase activity, but ß-glucosidase activity increased by 63% from the spring to the fall (Table 7). In contrast, N-treatment caused a significant ($df = 1,12$; $p = 0.00$) decrease of 16.4% in ß-glucosidase activity at SOFS, but seasonal variation was minimal (Table 8). At SMER, N-

treatment had no significant ($df = 1,12$; $p > 0.05$) effect on NAGase and phosphatase activity, but both enzymes experienced significant ($df = 1,12$; $p \leq 0.05$) declines (49.3% and 54.3% respectively) in the fall (Table 7). NAGase activity at SOFS did not vary significantly due to N ($df = 1,12$; $p = 0.61$) or season ($df = 1,12$; $p = 0.17$); however, phosphatase activity decreased significantly ($df = 1,12$; $p \leq 0.05$) due to chronic N inputs (20%) and seasonal variation (32.8%) (Table 8). N-treatment had no significant ($df = 1,12$; $p > 0.05$) effect on peroxidase activity at either site, but rates significantly ($df = 1,12$; $p \leq 0.05$) increased by 29% (SMER) and 69% (SOFS) from the spring to fall season (Table 7 and Table 8).

N-cycling bacteria

The abundance of N-fixing bacteria increased significantly ($df = 1,12$; $p \leq 0.05$) at SMER (38.6%) and SOFS (46.4%) in response to N inputs, but there was no significant ($df = 1,12$; $p > 0.05$) seasonal effect on N-fixing bacteria abundance (Fig. 1 and Fig. 2). At SMER, N-treatment caused a significant ($df = 1,12$; $p = 0.04$) increase (20.5%) in the abundance of N-mineralizing bacteria, but seasonal variations were minimal (Fig. 1), while at SOFS, neither N input ($df = 1,12$; $p = 0.39$) nor seasonal variation ($df = 1,12$; $p = 0.53$) had a significant effect on N-mineralizing bacteria abundance (Fig. 2). Experimental N inputs had no significant ($df = 1,12$; $p = 0.21$) effect on nitrifying bacteria abundance at SMER, but caused a significant ($df = 1,12$; $p = 0.00$) decline (27.4%) in nitrifying bacteria at SOFS (Fig. 1 and Fig. 2). Added N also significantly ($df = 1,12$; $p = 0.04$) increased (45.7%) denitrifying bacteria at SMER, but not at SOFS, and seasonal differences were not statistically significant ($df = 1,12$; $p > 0.05$) for either site (Fig. 1 and Fig. 2).

C-cycling bacteria

At SMER, nearly every bacterial C-cycling functional group had a significant ($df = 1,12$; $p \leq 0.05$) change in response to seasonal variation, while chronic N inputs only caused a significant ($df = 1,12$; $p \leq 0.05$) increase in abundances of chitinolytic (38%) and starch (36%) degrading bacteria and a decline (31%) in metal redox bacteria (Fig. 3). At SMER, seasonal variation had the greatest influence on chitinolytic bacteria, increasing their abundance by 70% in the fall. Significant ($df = 1,12$; $p \leq 0.05$) increases during the fall were observed in the abundance of cellulolytic (53.8%), starch degrading (36%), methanogenic (35.3%), and anaerobic bacteria (31.5%) (Fig. 3). At SOFS, seasonal variation had no significant ($df = 1,12$; $p > 0.05$) effect on the abundance of C-cycling FG, but chronic N inputs caused significant ($df = 1,12$; $p \leq 0.05$) increases in both chitinolytic (91.7%) and starch degrading (131.1%) bacteria abundances (Fig. 4).

Copiotrophic and oligotrophic bacteria

At SMER, the oligotrophic functional group did not have a significant response to N ($df = 1,12$; $p = 0.48$) or season ($df = 1,12$; $p = 0.22$), with Planctomycetes as the only oligotrophic phylum to experience a significant ($df = 1,12$; $p = 0.04$) change in response to seasonal transition, increasing in abundance by 37.4% in the fall (Fig. 5A). In contrast, SMER copiotrophic bacteria experienced a significant increase (24.3%) in response to both chronic N ($df = 1,12$; $p = 0.03$) inputs and seasonal variation ($df = 1,12$; $p = 0.00$) (33.1% in the fall). The increase in copiotrophic bacteria appeared to be due to the significant ($df = 1,12$; $p = 0.00$) increase in Proteobacteria, which was the most abundant phylum in the copiotrophic functional group and the only phylum to exhibit a significant ($df = 1,12$; $p = 0.00$) increase (30.3%) to experimental N (Fig. 5B). Increases in the fall abundances of Actinobacteria (43%) and Firmicutes (107%) were the only statistically significant ($df = 1,12$; $p \leq 0.05$) seasonal changes,

while Bacteroidetes experienced a treatment x season interaction as its abundance in N amended soil decreased in the spring, but increased in fall N (Fig. 5B).

At SOFS, oligotrophic bacteria experienced a significant (df = 1,12; p = 0.02) decrease (23.9%) with added N, mostly due to a decline in Acidobacteria, which was the most abundant oligotrophic phylum (Fig. 6A). Seasonal variation had a minor impact on SOFS oligotrophic phyla (Fig. 6A). Added N had no statistically significant (df = 1,12; p = 0.06) effect on the abundance of the copiotrophic functional group, however, Bacteroidetes and Firmicutes abundance increased (99.0% and 107%, respectively) with experimental N (Fig. 6B). None of the oligotrophic (Fig. 6A) or copiotrophic bacteria (Fig. 6B) at SOFS exhibited statistically significant (df = 1,12; p > 0.05) variations with season.

Canonical Correspondence Analysis (CCA): N-cycling bacterial

The first CCA examined the relationship between N-cycle bacteria and soil properties at SMER (Fig. 7). Axis 1 depicts total C (0.56 biplot score used to identify environmental influence on axis), microbial biomass C (0.53), and NO_3^- (-0.5) gradients contributing to 93.3% of the CCA variance, while axis 2 depicts NH_4^+ (0.63), soil moisture (-0.47), and pH (-0.53) gradients that explained another 3.8% of variance. N-mineralizing bacteria increased along an increasing soil moisture and NO_3^- gradient and a decreasing total C concentration and soil C:N ratio (Fig. 7). N-fixing and denitrifying bacteria abundances increased along an increasing NO_3^- gradient, while nitrifying bacteria abundance increased with an increase in microbial C biomass, total C, and C:N ratio (Fig. 7).

SOFS CCA axis 1 was defined by NO_3^- (0.78), soil moisture (-0.62), and pH (-0.93) gradients, which together explained 83.6% of the variation within the CCA (Fig. 8). Axis 2 depicted total C (0.67) and extractable N gradients (NO_3^- = -0.35, NH_4^+ = -0.43) as explanatory

variables, and explained an additional 11.8% of the variable in the CCA (Fig. 8). N-fixing bacteria and N-mineralizing bacterial abundance increased with increasing soil C and extractable NO_3^-, but declined with an increase in soil moisture and pH (Fig. 8). Denitrifying bacteria increased with an increase in both extractable NO_3^- and NH_4^+, while nitrifying bacteria increased with soil pH and moisture and decreased with an increase in extractable N (Fig. 8).

CCA: C-cycling bacteria

Axis 1 of the SMER CCA bi-plot (Fig. 9) explained 68% of the variation in bacterial C-cycling functional groups and was primarily explained by extractable N (NO_3^- = 0.68, NH_4^+ = 0.47), microbial C biomass (-0.68), and the soil's pH gradient (-0.7). Axis 2 explained 24% of the variation in bacterial C-cycling functional groups and was primarily explained by the soil C:N ratio (0.4), total C (0.4), and soil pH gradient (-0.21). Anaerobic bacteria, including metal redox and methanogenic, were primarily associated with higher soil moisture, microbial C and pH, although methanogenic and anaerobic bacterial were distinctly separated from metal redox bacteria. In contrast, starch degrading and chitinolytic bacteria were generally associated with higher extractable N, while cellulolytic bacteria were associated with an increase in soil N and C:N ratio (Fig. 9).

For SOFS, axis 1 of the CCA biplot for C cycling bacteria explained 72% of the variance and was primarily associated with soil pH (-0.81), total C (0.56), and NO_3^- (0.61), while axis 2 explained 13% of the variation in C cycling bacteria and was associated with microbial C (0.56), soil C:N ratio (-0.44) and total C (-0.69) (Fig. 10). As with SMER, anaerobic bacteria were primarily associated with wetter soils, but not as strongly as observed at SMER, and cellulolytic were also associated with wetter soils as well (Fig. 10). Starch degrading bacteria were strongly

associated with higher soil extractable and total N, while chitinolytic bacteria were also associated with an increase in soil C (Fig. 10).

CCA: Copiotrophic and oligotrophic bacteria

Analysis of the CCA biplot for SMER bacteria revealed that axis 1 and axis 2 explained 82% of the variation in bacterial phyla recognized as either copiotrophic or oligotrophic (Fig. 11). CCA axis 1 explained 64% of CCA variation and was represented by pH (-0.57), microbial C (-0.60), and extractable NO_3^- (0.62), while CCA axis 2 explained an addition 18% of the CCA variation and was related to variations in soil moisture (0.33), total soil C (-0.43), and the soil C:N ratio (-0.44) (Fig. 11). Most of the copiotrophic bacteria (Proteobateria, Bacteroidetes, Actinobacteria, and Frimicutes) were on the right-hand side of the CCA, which was primarily associated with an increase in extractable NO_3^-, while most of the oligotrophic bacteria (Acidobacteria, Verrucomicrobia, and Chloroflexi) were grouped on the left-hand side of the CCA, which was driven by pH, microbial C, and increase in total soil C (Fig. 11). Environments that were wetter and with a higher pH had higher abundance of Acidobacteria, Proteobateria, and Chloroflexi, while higher in soil C and extractable N had higher abundance of Verrucomicrobia, Planctomycetes, Bacteroidetes, and Frimicutes (Fig. 11).

Analysis of the CCA biplot for SOFS bacteria revealed that axis 1 and axis 2 explained 92% of the variation in copiotrophic and oligotrophic bacterial abundance (Fig. 12). CCA axis 1 explained 72% of the variation in bacteria abundance and was represented by pH (-0.97) and extractable NO_3^- (0.72), while CCA axis 2 explained an addition 20% of the CCA variation and was related to variations in total soil C (-0.52) and extractable NH_4^+ (0.58) (Fig. 12). As with the SMER CCA, copiotrophic bacteria (Proteobateria, Bacteroidetes, Actinobacteria, and Frimicutes) were on the right-hand side of the CCA, which was primarily associated with an

increase in extractable NO_3^-, while most of the oligotrophic bacteria (Acidobacteria, Verrucomicrobia, and Chloroflexi) were grouped on the left-hand side of the CCA, which was driven by pH and soil moisture (Fig. 12). Environments that were wetter and with a higher pH had higher abundance of Acidobacteria, Verrucomicrobia, and Chloroflexi, environments that had higher soil C and N had higher abundance of Proteobateria, Planctomycetes, and Bacteroidetes, and environments that had higher extractable N had higher abundance of Firmicutes and Actinobacteria (Fig. 12).

Discussion

Soil physical-chemical properties

Long-term N addition caused a significant ($df = 1,12$; $p \leq 0.05$) decline in soil pH and an increase in extractable NH_4^+ and NO_3^-; however, none of the other soil C or N variables were significantly ($df = 1,12$; $p > 0.05$) altered by N addition (Tables 5 and 6). These data are consistent with previous studies (Fenn *et al.,* 2003; Vourlitis and Zorba, 2007a; Vourlitis and Fernandez, 2012; Homyak *et al*., 2016), and while N induced changes in chemistry may be rapid, soil N and C pools are large enough to be resistant to chronic N inputs (Vourlitis and Hentz, 2016). Soil acidification may have particularly important effects on microbial abundance and activity, as microorganism growth and physiology are sensitive to changes in soil pH (Tian and Niu*,* 2015).

N- and C-Cycling Functional Classification

It was initially hypothesized that the abundances of bacterial functional groups (FG) would significantly ($df = 1,12$; $p \leq 0.05$) change in response to chronic N inputs. The results from this study supported this hypothesis, but N did not affect the abundance of all bacterial

groups. N-fixing, chitinolytic, and starch degrading bacteria were consistently affected by N addition at both sites, while the response of other bacterial FG were site specific (Fig. 1-4).

The significant ($df = 1,12$; $p \leq 0.05$) increase in N-fixing bacteria with added N is surprising, and appeared to be associated with an increase in extractable N availability and a decline in soil pH at both sites (Fig. 7 and 8). Increases in available N have long been known to reduce rates of N-fixation, because high energy demands associated with N-fixation are alleviated in N-rich environments (Bottomley and Myrold, 2007). However, it is unclear if a decline in N-fixation activity is related to a decline in bacterial abundance or activity, and it is possible that a shift from N-fixation to N assimilation in an N-rich environment would allow bacterial cells to allocate available energy towards growth instead of N-fixation. Vitousek *et al.* (2013) argue that perhaps the most effective strategy would be to fix N in an N-deficient environment but not in an N-sufficient environment, but whether such facultative N-fixation is a viable strategy depends on resource limitations (N and other non-N nutrients) (Menge *et al.,* 2009).

Other N-cycling FG responded differently to N in chaparral and CSS. In CSS, N increased the abundance of both mineralizing and denitrifying bacteria, while in chaparral, only nitrifying bacteria increased significantly ($df = 1,12$; $p = 0.00$) with N. The increase in denitrifying bacteria was related to an increase in extractable NO_3^- (Fig. 7 and 8), which is expected given that NO_3^- is the substrate for denitrification (Wang *et al.,* 2018); however, mineralizing bacteria were positively associated with soil moisture, pH and microbial C and negatively associated with extractable NH_4^+ in CSS (Fig. 7). Since increases in extractable NH_4^+ can be due to an increase in N-mineralization (Hart *et al.,* 1986; Robertson and Groffman, 2007), this suggests that the high levels of extractable NH_4^+ were due more to the N addition than to

mineralization of N from decomposing soil organic matter (SOM). N-mineralization rates generally increase with SOM quality and soil moisture (Robertson and Groffman, 2007), and why mineralizing bacteria were positively affected by N addition in CSS, but not in chaparral is unknown. It is possible that the more clay rich soil (Vourlitis *et al.,* 2009) and higher litter N enrichment (Vourlitis and Fernandez, 2012) in CSS caused mineralizing bacteria to be more responsive to N than in chaparral.

Another interesting result was the significant (df = 1,12; p = 0.00) decline (chaparral) and non-significant (df = 1,12; p = 0.59) trend towards an abundance decrease (CSS) in nitrifying bacteria exposed to N inputs (Fig. 1 and 2). One of the more important controls on nitrification is NH_4^+ availability (Robertson and Groffman, 2007), which was clearly higher in both chaparral and CSS exposed to N. However, it is possible that the nitrification rate was not lower in N exposed plots, as previous research in both chaparral and CSS indicate that N input significantly ($p \leq 0.05$) increase nitrification rates (Sirulnik *et al.,* 2007; Vourlitis and Zorba 2007a; Vourlitis *et al.,* 2009). Although nitrification rates were not quantified in this study, ^{15}N isotope pool dilution (Wessel and Tietema, 1992) could possibly be used to quantify nitrification and nitrate immobilization rates between control and treatment plots. Conversely, urea is quickly hydrolyzed into NH_3, becoming readily available to the nitrification process (Prosser, 1990; Venterea *et al*. 2012). It has been reported that members of *Nitrospira* are capable of oxidizing both NH_3 and NO_2^-, a by-product of nitrification, essentially performing a complete oxidation of NH_3 to NO_3^- (Daims *et al*., 2015; van Kessel *et al*., 2015). Together, these results may suggest a shift in the microbial community to capable nitrifiers exposed to an organic N source, decreasing nitrifying bacteria diversity (Staley *et al*., 2018). Oligotyping analyses could possibly be used to

determine the relationships between nitrifying families and nitrification gene abundances, giving insight into gene regulation by bacteria exposed to intensified N (Eren *et al.*, 2013).

The two main C cycling functional groups that responded to N addition were chitinolytic and starch degrading bacteria, both of which increased in abundance with added N (Fig. 3 and 4). Both starch degrading and chitinolytic abundance were associated with higher extractable N, and in the case of chaparral, higher soil C (Fig. 9 and 10). Chitin is a recalcitrant C and N rich compound that is a major part of fungal cell walls and exoskeletons of insects (Chapin *et al.,* 2011), and is often a dominant form of organic N entering the soil (Olander and Vitousek, 2000). Chitin often accumulates during the process of SOM formation because of the difficulty associated with breaking down recalcitrant C compounds (Horwath, 2007). In turn, fungi are often more abundant in soils of woody ecosystems (Fierer *et al.,* 2005) because they are the main group of microorganisms involved in the degradation of recalcitrant compounds such as lignin, which is abundant in chaparral and CSS litter (Schlesinger and Hasey, 1981), and chitin (Horwath, 2007). Thus, the increase in chitinolytic bacteria implies that fungal abundance also increased with N addition.

Starch is a simple plant polymer that is synthesized from glucose early in the process of photosynthetic C synthesis, and many types of bacteria and fungi can utilize starch as an energy source (Horwath, 2007). Although amylase activity was not quantified, the increase in starch degrading bacteria in chaparral was also accompanied by a decrease in β-glucosidase activity (Table 8), which is a glycoside hydrolase that is used by both bacteria and fungi to break down complex carbohydrates similar to starch (ie: cellulose and hemicellulose) (Sinsabaugh *et al.,* 1991; Keeler *et al.,* 2009). However, the increase in starch degrading bacteria implies that N inputs increased the availability of labile C in the soil, possibly from root exudation and/or fine-

root turnover (Ramirez *et al.,* 2012). Aboveground net primary production (ANPP) was observed to be stimulated by N addition in both chaparral (Vourlitis and Hentz, 2016) and CSS (Vourlitis, 2012), and typically, increases in ANPP result in increases in belowground net primary production and root exudation (Fahey *et al.,* 1998). Variation in the activity of glycoside hydrolases can also depend on the stimulation of glycosides and enzymes released from root exudation, promoting particular plant beneficial microbes (Basu *et al.,* 1999; Bais *et al.,* 2004; Tomscha *et al.,* 2004; Basu *et al.,* 2006; Bressan *et al.,* 2009).

The only other functional group that responded significantly ($df = 1,12$; $p = 0.04$) to N addition were the metal redox bacteria, but only in CSS (Fig. 3). These bacteria are often associated with anaerobic environments and typically use metals such as iron and manganese as alternate electron acceptors during anaerobic respiration (Megonigal *et al.,* 2003). While CSS soils tend to be well-drained, and thus, aerobic, the fact that the metal redox bacterial abundance was related with soil moisture indicates that periodic saturation and anaerobic conditions can develop. However, increases in NO_3^- will cause a decrease in metal reduction, and presumably, metal reducing bacteria, under anaerobic conditions because NO_3^- reduction releases more energy than metal reduction (Stumm and Morgan, 1996). This is further supported by the increase in denitrifying bacteria in CSS (Fig. 1), which would be responsible for NO_3^- reduction. Thus, the N-induced decline in metal reducing bacteria probably arose from high concentrations of NO_3^- during anaerobic periods.

Copiotrophic/Oligotrophic Functional Classification

It was initially hypothesized that chronic N inputs would induce a significant ($df = 1,12$; $p \leq 0.05$) increase in the abundance of copiotrophic bacterial and a decrease the abundances of oligotrophic bacterial, and the results from this study provided partial support for this hypothesis.

For example, oligotrophic bacterial taxa in CSS were not affected by N addition, but copiotrophic taxa, in particular Proteobacteria (which accounted for 51% of all CSS copiotrophic bacteria quantified), increased significantly (df = 1,12; p = 0.00) with added N (Fig. 5). Similarly, oligotrophic Acidobacteria, which accounted for 40% of chaparral oligotrophic bacteria, exhibited a significant (df = 1,12; p = 0.00) decline with N inputs, while copiotrophic taxa Bacteriodes and Firmicutes increased in chaparral (Fig. 6). Thus, changes in the abundance of copiotrophic and oligotrophic bacteria were not uniform within each functional group, and responses of each functional group were driven by large changes in some numerically dominant taxa.

While bacterial taxa responded differently to N inputs, CCA analyses consistently showed that the abundance of copiotrophic taxa increased in environments that had higher levels of extractable N, especially NO_3^- (Fig. 11 and 12). Multiple studies (Ramirez *et al.,* 2010b; Fierer *et al.,* 2012; Zeng *et al.,* 2016; Ling *et al.,* 2017; Dai *et al.,* 2018; Wang *et al.,* 2018; Wang *et al.,* 2020) have shown that N inputs promote the growth of copiotrophic bacteria such as Proteobacteria, Bacteroidetes, and Firicutes that have faster growth and inhibit the growth of oligotrophic bacteria, such as Acidobacteria that decompose more recalcitrant C. Some of these changes may be due to the indirect effects of N on soil acidification, as increases in extractable NO_3^- often cause a decline in pH (Vourlitis and Fernandez, 2012; Tian *et al.,* 2015). Multiple studies have shown that soil pH is a major factor in determining bacterial community composition (Lauber *et al.,* 2009; Zeng *et al.,* 2016; Ling *et al.,* 2017), and may be more important than the direct effects of N addition on bacterial abundance (Wang *et al.,* 2020). CCA analysis may support this interpretation, as the left-hand size of the CCA plots were often driven by an increase in soil pH while the right-hand side of the CCA plots were driven by an increase

in extractable N (Fig. 11 and 12). However, our data do not allow a rigorous test of whether a shift in copiotrophic/oligotrphic taxa was related to changes in soil pH or extractable N.

Shifts in oligotrophic/copiotrophic bacteria may be affected by the amount and/or duration of N input. For example, Ramirez *et al.* (2010b) found higher abundances of Proteobacteria and lower abundances of Acidobacteria in soils amended with higher N inputs. Fierer *et al.* (2012) also reported that soils receiving extremely high amounts of experimental N (>270 kg N ha^{-1} yr^{-1}) had higher relative abundances of the copiotrophic taxa Proteobacteria (*alpha*- and *gamma*-), Bacteroidetes, and Actinobacteria, and lower relative abundances of oligotrophic Acidobacteria (Fierer *et al.,* 2012). Dai *et al.* (2018) also found that shifts in copiotrophic-oligotrophic abundance were also affected by the duration of N input.

Soil moisture content, next to N availability, is another important limiting factor that influences microbial abundance in arid and semi-arid ecosystems (Zhang *et al.,* 2015; Ma *et al.,* 2019; Wang *et al.,* 2020). An increase in soil moisture content may enhance bacterial growth and decomposition (Sponseller, 2007), which may in turn impact the composition of bacterial FG (Ma *et al.* 2019). Indeed, the abundance of some bacterial taxa appeared to be related to increases in soil moisture, such as Chloroflexi and Acidobacteria at both sites, Proteobacteria in CSS, and Verrucomicrobia in chaparral. Saturated soils have been observed to sustain particular Acidobacteria subdivisions during high soil pH conditions (Jones *et al.*, 2009; Kielak *et al.*, 2016). CCA results further support these findings, as Acidobacteria were affiliated with increased soil pH (Fig. 11 and 12). Soil moisture has been found to affect extractable N availability (Homyak *et al.,* 2016), and may interact with extractable N or other minerals to affect the osmotic potential of microbes, which can alter microbial abundance (Kolb and Martin, 1988). Both CSS and chaparral soil moisture content significantly ($df = 1,12$; $p \leq 0.05$) decreased

in the fall season (Table 5 and 6), but the CSS copiotrophic functional group significantly (df = 1,12; p = 0.03) increased during the fall (Figure 5a). This evidence may further support the suggestion that shifts in the bacterial community structure may reflect different life strategies and functional capabilities (Philippot *et al.,* 2010). Wang *et al.* (2020) suggest that particular bacterial phyla are more drought resistant and an increase in these bacteria can potentially improve plant drought resistance. This shift could be essential for plants to thrive in Mediterranean or arid, semi-arid habitats.

N may also indirectly affect microbes by increasing net primary production (NPP) and in succession may increase the C quality of both vegetation litter and soil liable C, becoming readily available to microbes for growth (LeBauer and Treseder, 2008; Fierer *et al.,* 2009; Wang *et al.,* 2018). N could also potentially affect microbes by increasing available organic C via increased root C allocation (Fierer *et al.,* 2012). The *microbial N mining* hypothesis suggests that microbes shift from decomposing recalcitrant to labile C in response to intensified N (Craine *et al.* 2007). This may in part explain the increased abundance of starch degrading bacteria in chaparral, which is rich in woody, recalcitrant C, but the hypothesis does not explain the decrease in β-glucosidase activity. Fierer *et al.* (2007) also suggest that the *r*- *K*-selection continuum (MacArthur and Wilson, 1967) may be useful for classifying taxa based on tendencies of copiotrophic (r-selection) and oligotrophic (K-selection) bacteria to utilize organic C. Copiotrophic bacteria readily consume labile organic C pools and require high nutritional input to support their high intrinsic growth rates, while oligotrophic bacteria consume recalcitrant C and exhibit a slower growth rate (Fierer *et al.,* 2007). Thus, oligotrophic species may be capable of outcompeting copiotrophic species under low nutrient conditions in soils with high concentrations of recalcitrant C (Meyer, 1994; Tate, 2000). Copiotrophic-oligotrophic shifts in

the microbial community are expected to occur in soils with high C concentrations, and Fierer *et al.* (2012) indicate that these shifts are consistent across N gradients.

Conclusions

Long-term dry N inputs in chaparral and coastal sage scrub shrublands affected the abundance of some bacterial FG, and while results were not uniform across shrubland types, consistent N effects on N-fixing, chitinolytic, and starch-degrading bacteria indicate potentially important N-induced changes in ecosystem N and C cycling for these semi-arid shrublands. These data also supported the hypothesis that N enrichment would favor copiotrophic bacteria over oligotrophic; however, in the case of chaparral, the copiotrophic-oligotrophic shift was due more to the decline in oligotrophic bacteria than an increase in copiotrophic bacteria. N-induced changes in bacterial abundance appeared to be due to changes in soil N availability and/or pH, but unfortunately, the methods employed in this observational study do not allow the relative effects of N and pH to be determined. Regardless, increases in dry N deposition both increase N availability, especially NO_3, which decreases pH, so these effects tend to be part of a larger N-induced change in the soil environment.

These data indicate that the abundance of soil bacterial taxa are fundamentally altered by long-term N inputs. Understanding the mechanisms of microbial shifts to long-term N deposition is critical, as an increasing N deposition rate in semi-arid shrubland ecosystems is likely due to increased anthropogenic activity. A change in the microbial abundance could have possible consequences on ecosystem functionality and stability, as microbes help regulate C and N cycling. These data will help us understand the potential impacts of increasing N deposition on microbial abundance in these, and similar, semi-arid shrublands worldwide.

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Tables and Figures

Table 1. Quantified bacterial families from Santa Margarita Ecological Reserve categorized into N-cycling functional Groups (FG).

	N-cycle FG			
Bacterial Family	N-fixing	N-mineralizing	Nitrifying	Denitrifying
Acetobacteraceae	X			
Acidobacteriaceae			X	
Alcaligenaceae				X
Alteromonadaceae		X		
Beijerinckiaceae	X			
Bradyrhizobiaceae	X			
Caulobacteraceae				X
Chromatiaceae			X	
Clostridiaceae				X
Comamonadaceae				X
Conexibacteraceae				X
Cryomorphaceae		X		
Cyanobacteriaceae	X			
Ectothiorhodospiraceae			X	
Enterobacteriaceae				X
Frankiaceae	X			
Gemmatimonadaceae		X		
Geodermatophilaceae		X		
Hyphomicrobiaceae	X			
Micrococcaceae				X
Nitrosomonadaceae			X	
Nitrospiraceae			X	
Oscillochloridaceae	X			
Oxalobacteraceae	X			
Phyllobacteriaceae	X			
Planctomycetaceae			X	
Prochlorococcaceae	X			
Pseudomonadaceae				X
Rhizobiaceae	X			
Rhodobacteraceae				X
Rhodobiaceae	X			
Rhodocyclaceae	X			
Rhodospirillaceae	X			
Sinobacteraceae		X		
Sporichthyaceae	X			
Verrucomicrobiaceae			X	
Xanthobacteraceae	X			
Xanthomonadaceae				X

Table 2. Quantified bacterial families from Santa Margarita Ecological Reserve categorized into C-cycling functional groups (FG).

Bacterial Family	C-cycle FG					
	Cellulolytic	Chitinolytic	Starch	Methanogenic	Anaerobic	Metal Redox
Acidimicrobiaceae						X
Alicyclobacillaceae						X
Anaerolineaceae				X		
Anaeromyxobacteraceae						X
Bacillales	X					
Blastocatellaceae					X	
Blastocatellia					X	
Burkholderiales	X					
Caldilineaceae						X
Catenulisporaceae			X			
Cellulomonadaceae	X					
Chitinophagaceae		X				
Chthonomonadaceae				X		
Cyclobacteriaceae			X			
Cystobacteraceae	X					
Cytophagaceae	X					
Dehalococcoidaceae					X	
Dehalococcoidia					X	
Eubacteriaceae					X	
Ferrovaceae						X
Fibrobacteraceae	X					
Gaiellaceae				X		
Geobacteraceae						X
Gloeobacteraceae						X
Iamiaceae						X
Intrasporangiaceae				X		
Kineosporiaceae					X	
Ktedonobacteraceae			X			
Magnetococcaceae						X
Methanobacteriaceae				X		
Methanosarcinaceae				X		
Methylobacteriaceae				X		
Methylocystaceae				X		
Methylophilaceae				X		
Methylothermaceae				X		
Microbacteriaceae		X				
Micromonosporaceae	X					
Myxococcaceae						X
Nannocystaceae	X					
Nocardioidaceae		X				
Opitutaceae					X	
Patulibacteraceae	X					
Pelobacteraceae					X	

Bacterial Family	C-cycle FG					
	Cellulolytic	Chitinolytic	Starch	Methanogenic	Anaerobic	Metal Redox
Peptococcaceae				X		
Phaselicystidaceae			X			
Polyangiaceae	X					
Pseudonocardiaceae	X					
Rikenellaceae					X	
Rubrobacteraceae	X					
Sandaracinaceae			X			
Saprospiraceae			X			
Sneathiellaceae					X	
Solirubrobacteraceae					X	
Spartobacteria					X	
Sphaerobacteraceae			X			
Sphingobacteriaceae		X				
Sphingomonadaceae					X	
Streptomycetaceae	X					
Syntrophaceae				X		
Thermoactinomycetaceae					X	
Thermoanaerobacteraceae					X	
Thermomicrobiaceae					X	

Table 3. Quantified bacterial families from Sky Oaks Field Station categorized into N-cycling functional groups (FG).

	N-cycle FG			
Bacterial Family	**N-fixing**	**N-mineralizing**	**Nitrifying**	**Denitrifying**
Acetobacteraceae	X			
Acidobacteriaceae			X	
Alcaligenaceae				X
Alteromonadaceae		X		
Beijerinckiaceae	X			
Bradyrhizobiaceae	X			
Caulobacteraceae				X
Chromatiaceae			X	
Clostridiaceae				X
Comamonadaceae				X
Conexibacteraceae				X
Cryptosporangiaceae	X			
Cyanobacteriaceae	X			
Ectothiorhodospiraceae			X	
Enterobacteriaceae				X
Frankiaceae	X			
Gemmatimonadaceae		X		
Geodermatophilaceae		X		
Halomonadaceae				X
Hyphomicrobiaceae	X			
Leptolyngbyaceae	X			
Micrococcaceae				X
Motilibacteraceae	X			
Nitrosomonadaceae			X	
Nitrosopumilaceae			X	
Nitrospiraceae			X	
Oscillochloridaceae	X			
Oxalobacteraceae	X			
Phyllobacteriaceae	X			
Planctomycetaceae			X	
Prochlorococcaceae	X			
Pseudomonadaceae				X
Rhizobiaceae	X			
Rhodobacteraceae				X
Rhodobiaceae	X			
Rhodocyclaceae	X			
Rhodospirillaceae	X			
Sinobacteraceae		X		
Sporichthyaceae	X			
Synechococcaceae	X			
Verrucomicrobiaceae			X	

	N-cycle FG			
Bacterial Family	**N-fixing**	**N-mineralizing**	**Nitrifying**	**Denitrifying**
Xanthobacteraceae	**X**			
Xanthomonadaceae				**X**

Table 4. Quantified bacterial families from Sky Oaks Field Station categorized in C-cycling functional groups (FG).

Bacterial Family	C-cycle FG					
	Cellulolytic	Chitinolytic	Starch	Methanogenic	Anaerobic	Metal Redox
Acidimicrobiaceae						X
Alicyclobacillaceae						X
Alphaproteobacteria				X		
Anaerolineaceae				X		
Anaeromyxobacteraceae						X
Bacillales	X					
Blastocatellaceae					x	
Burkholderiales	X					
Caldilineaceae						X
Carnobacteriaceae					X	
Catenulisporaceae			X			
Cellulomonadaceae	X					
Chitinophagaceae		X				
Chthonomonadaceae				X		
Cyclobacteriaceae			X			
Cystobacteraceae	X					
Cytophagaceae	X					
Dehalococcoidaceae					X	
Dehalococcoidia					X	
Eubacteriaceae					X	
Ferrovaceae						X
Fibrobacteraceae	X					
Gaiellaceae				X		
Geobacteraceae						X
Gloeobacteraceae						X
Iamiaceae						X
Intrasporangiaceae				X		
Kineosporiaceae					X	
Ktedonobacteraceae			X			
Magnetococcaceae						X
Methanococcaceae				X		
Methylobacteriaceae				X		
Methylococcaceae				X		
Methylocystaceae				X		
Methylothermaceae				X		
Microbacteriaceae		X				
Micromonosporaceae	X					
Myxococcaceae						X
Nannocystaceae	X					
Nocardiaceae	X					

Bacterial Family	C-cycle FG					
	Cellulolytic	Chitinolytic	Starch	Methanogenic	Anaerobic	Metal Redox
Nocardioidaceae		X				
Opitutaceae					X	
Patulibacteraceae	X					
Pelobacteraceae					X	
Peptococcaceae				X		
Polyangiaceae	X					
Pseudonocardiaceae	X					
Rubrobacteraceae	X					
Saprospiraceae			X			
Solirubrobacteraceae					X	
Spartobacteria					X	
Sphaerobacteraceae			X			
Sphingobacteriaceae		X				
Sphingomonadaceae					X	
Sporolactobacillaceae					X	
Staphylococcaceae					X	
Streptomycetaceae	X					
Streptosporangiaceae	X					
Syntrophaceae				X		
Thermoactinomycetaceae					X	
Thermoanaerobacteraceae					X	
Thermolithobacteraceae						X
Thermomicrobiaceae					X	
Thermosporotrichaceae	X					

Table 5. Mean ± SE (n=4) soil variables under control (C) and nitrogen-induced (N) conditions at Santa Margarita Ecological Reserve during spring and fall. Also shown are results of the two-way repeated factor ANOVA (F-statistic, effect and error degrees of freedom) for effects of treatment (T), season (S), and their interaction (T:S). Log transformation of original soil data indicated by soil variable (†). *p < 0.05; **p < 0.01.

	Spring		Fall				
Soil Variable (units)	**C**	**N**	**C**	**N**	**$T_{1,12}$**	**$S_{1,12}$**	**$T{:}S_{1,12}$**
Moisture† (%)	3.9 ± 0.5	3.5 ± 0.8	1.4 ± 0.1	1.1 ± 0.1	1.6	58.6**	0.1
pH	6.7 ± 0.1	5.8 ± 0.1	6.8 ± 0.1	5.8 ± 0.2	70.9**	0.4	0.2
Microbial C† (µg/g)	162 ± 37	125 ± 67	89 ± 23	61 ± 33	1.3	2.1	0.1
NO_3^- (µg/g)	0.2 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	22.0**	0.1	1.3
NH_4^+ † (µg/g)	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	1.1 ± 0.3	33.0**	5.9*	0.5
Total N (%)	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	1.0	0.7	0.9
Total C (%)	1.4 ± 0.1	1.1 ± 0.2	1.0 ± 0.2	1.5 ± 0.4	0.1	0.1	3.3
C:N	13.5 ± 2.2	10.2 ± 2.6	12.3 ± 0.6	12.2 ± 0.5	0.9	0.1	0.9

Table 6. Mean ± SE (n=4) soil variables under control (C) and nitrogen-induced (N) conditions at Sky Oaks Field Station during spring and fall. Also shown are results of the two-way repeated factor ANOVA (F-statistic, effect and error degrees of freedom) for effects of treatment (T), season (S), and their interaction (T:S). Log transformation of original soil data indicated by soil variable (†). Permutation test (p-value) indicated by soil variable (††). *p < 0.05; **p < 0.01.

	Spring		Fall				
Soil Variable (units)	**C**	**N**	**C**	**N**	**$T_{1,12}$**	**$S_{1,12}$**	**$T{:}S_{1,12}$**
Moisture† (%)	2.0 ± 0.2	1.5 ± 0.3	1.3 ± 0.1	0.95 ± 0.1	10.8**	14.3**	0.05
pH††	6.9 ± 0.7	5.2 ± 0.2	6.8 ± 0.1	5.4 ± 0.1	p = 0.00**	p = 0.6	p = 0.14
Microbial C (µg/g)	198 ± 9	177 ± 33	131 ± 16	104 ± 30	1.0	8.5*	0.02
NO_3^- †† (µg/g)	0.03 ±0.01	0.84 ± 0.2	0.04 ± 0.01	0.38 ± 0.1	p = 0.00**	p = 0.11	p = 0.1
NH_4^+ †† (µg/g)	0.06 ± 0.06	0.62 ± 0.4	0.11 ± 0.01	0.46 ± 0.1	p = 0.01*	p = 0.91	p = 0.78
Total N†† (%)	0.09 ± 0.02	0.1 ± 0.01	0.04 ± 0.01	0.05 ± 0.003	p = 0.39	p = 0.001**	p = 0.77
Total C (%)	0.88 ± 0.06	0.99 ± 0.2	0.95 ± 0.1	1.25 ± 0.07	3.5	2.2	0.73
C:N††	10.8 ± 2.55	10.2 ± 2.07	25.1 ± 1.03	24.9 ± 1.17	p = 0.83	p = 0.00**	p = 0.91

Table 7. Mean ± SE (n=4) enzymatic activity under control (C) and nitrogen-induced (N) conditions at Santa Margarita Ecological Reserve during the spring and fall. Also shown are results of the two-way repeated factor ANOVA (F-statistic, effect and error degrees of freedom) for effects of treatment (T), season (S), and their interaction (T:S). Permutation test (p-value) indicated by soil variable (††). *p < 0.05; **p < 0.01.

	Spring		Fall				
Enzyme (units)	**C**	**N**	**C**	**N**	**$T_{1,12}$**	**$S_{1,12}$**	**$T{:}S_{1,12}$**
ß-glucosidase (µmol g^{-1} h^{-1})	50 ± 12	33 ± 2.5	71 ± 7.6	64 ± 11.6	1.7	8.2*	0.26
NAGase (µmol g^{-1} h^{-1})	13 ± 3.6	17 ± 0.6	8.5 ± 0.8	6.7 ± 0.8	0.26	15.3**	2.1
Phosphatase†† (µmol g^{-1} h^{-1})	176 ± 7.7	163 ± 6.3	78 ± 11.2	77 ± 3.4	p = 0.36	p = 0.00**	p = 0.5
Peroxidase†† (nmol g^{-1} h^{-1})	71 ± 1.5	77 ± 3.5	86 ± 4.8	104 ± 10.7	p = 0.06	p = 0.001**	p = 0.43

Table 8. Mean ± SE (n=4) enzymatic activity under control (C) and nitrogen-induced (N) conditions at Sky Oaks Field Station during the spring and fall. Also shown are results of the two-way repeated factor ANOVA (F-statistic, effect and error degrees of freedom) for effects of treatment (T), season (S), and their interaction (T:S). Log transformation of original soil data indicated by soil variable (†). Permutation test (p-value) indicated by soil variable (††). *$p < 0.05$; **$p < 0.01$.

	Spring		Fall				
Enzyme (units)	**C**	**N**	**C**	**N**	**$T_{1,12}$**	**$S_{1,12}$**	**$T{:}S_{1,12}$**
ß-glucosidase (µmol g^{-1} h^{-1})	65 ± 4.4	41 ± 7.0	72 ± 7.9	52 ± 12.2	11.4**	1.8	0.12
NAGase† (µmol g^{-1} h^{-1})	11.2 ± 0.8	11 ± 0.62	15.3 ± 3.1	10.1 ± 1.39	2.1	0.27	1.4
Phosphatase†† (µmol g^{-1} h^{-1})	162 ± 7.0	152 ± 2.3	128 ± 19.3	83 ± 13.4	p = 0.04*	p = 0.002**	p = 0.16
Peroxidase†† (nmol g^{-1} h^{-1})	64 ± 1.3	68 ± 0.2	114 ± 7.0	108 ± 4.7	p = 0.78	p = 0.00**	p = 0.24

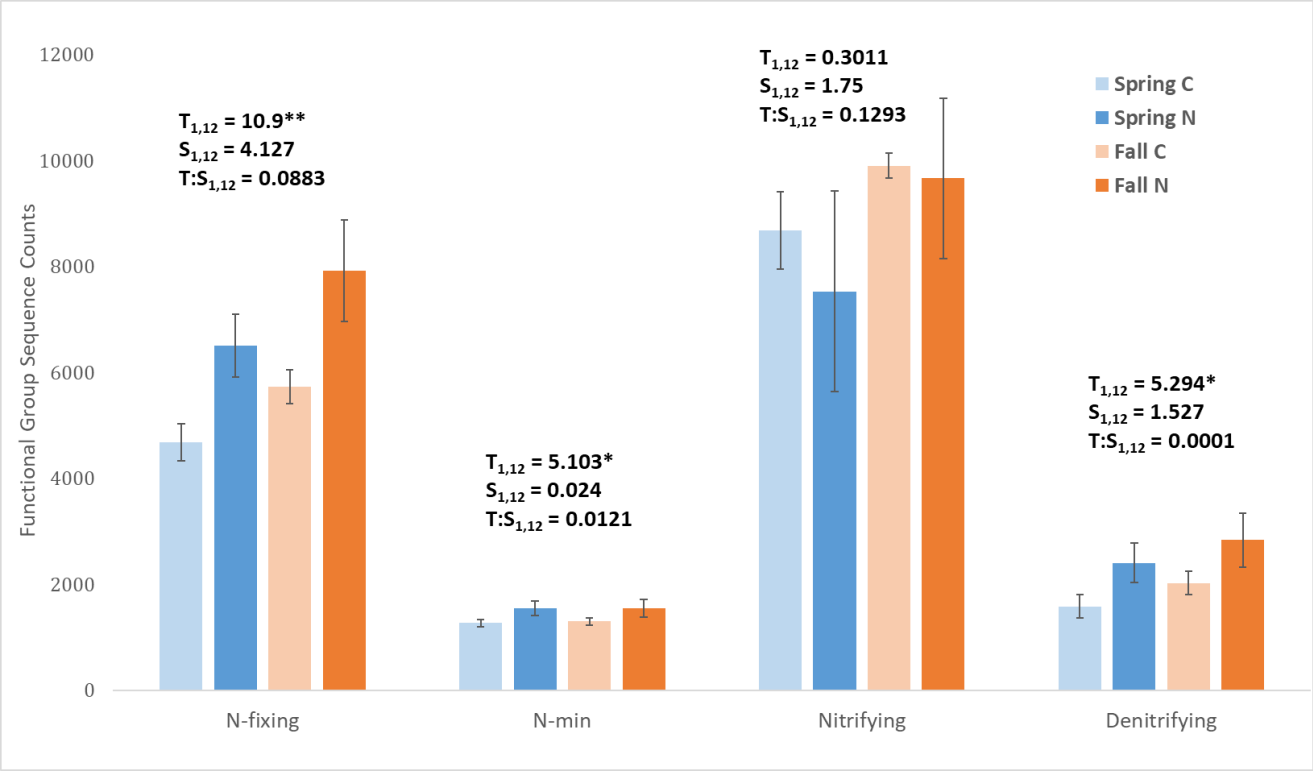

Figure 1. Mean (±SE; n = 4 plots) sequence counts for bacterial taxa arranged in N-fixing, N-mineralizing, nitrifying, and denitrifying functional groups from the Santa Margarita Ecological Reserve during the spring (blue-bars) and fall (orange-bars) of 2017 in N-amended (dark-bars) and control plots (light-bars). *$p < 0.05$; **$p < 0.01$.

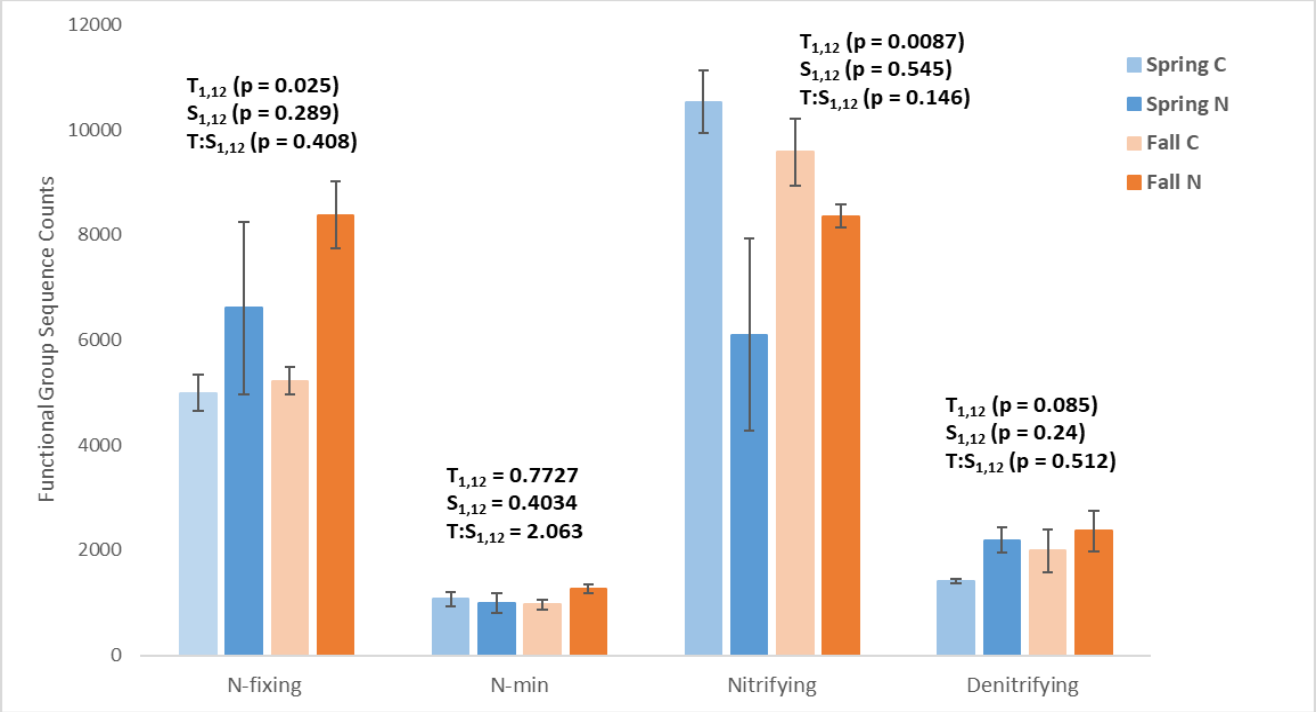

Figure 2. Mean (±SE; n = 4 plots) sequence counts for bacterial taxa arranged in N-fixing, N-mineralizing, nitrifying, and denitrifying functions groups from the Sky Oaks Field Station during the spring (blue-bars) and fall (orange-bars) of 2017 in N-amended (dark-bars) and control plots (lighter-bars). *$p < 0.05$; **$p < 0.01$.

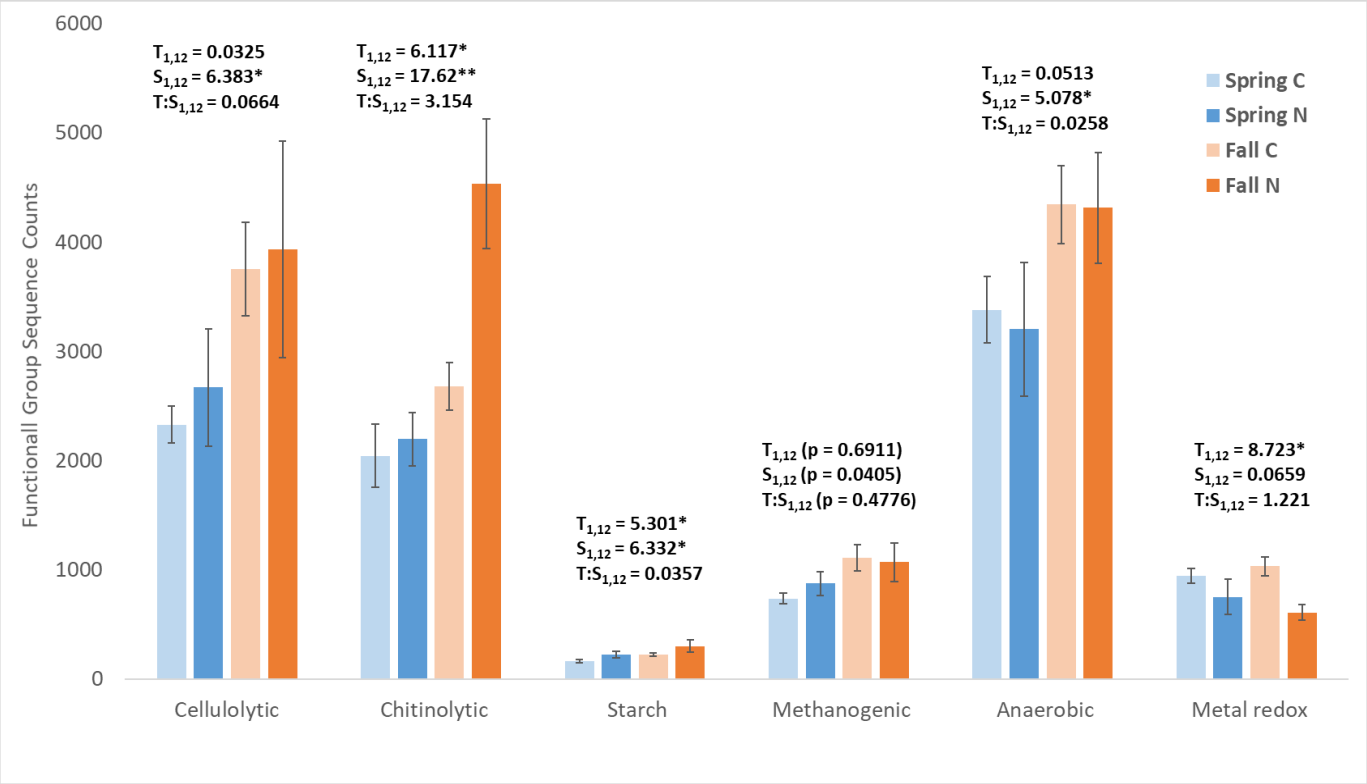

Figure 3. Mean (±SE; n = 4 plots) sequence counts for bacterial taxa arranged in cellulolytic, chitinolytic, starch, methanogenic, anaerobic, and metal redox functions groups from the Santa Margarita Ecological Reserve during the spring (blue-bars) and fall (orange-bars) of 2017 in N-amended (dark-bars) and control plots (lighter-bars). *$p < 0.05$; **$p < 0.01$.

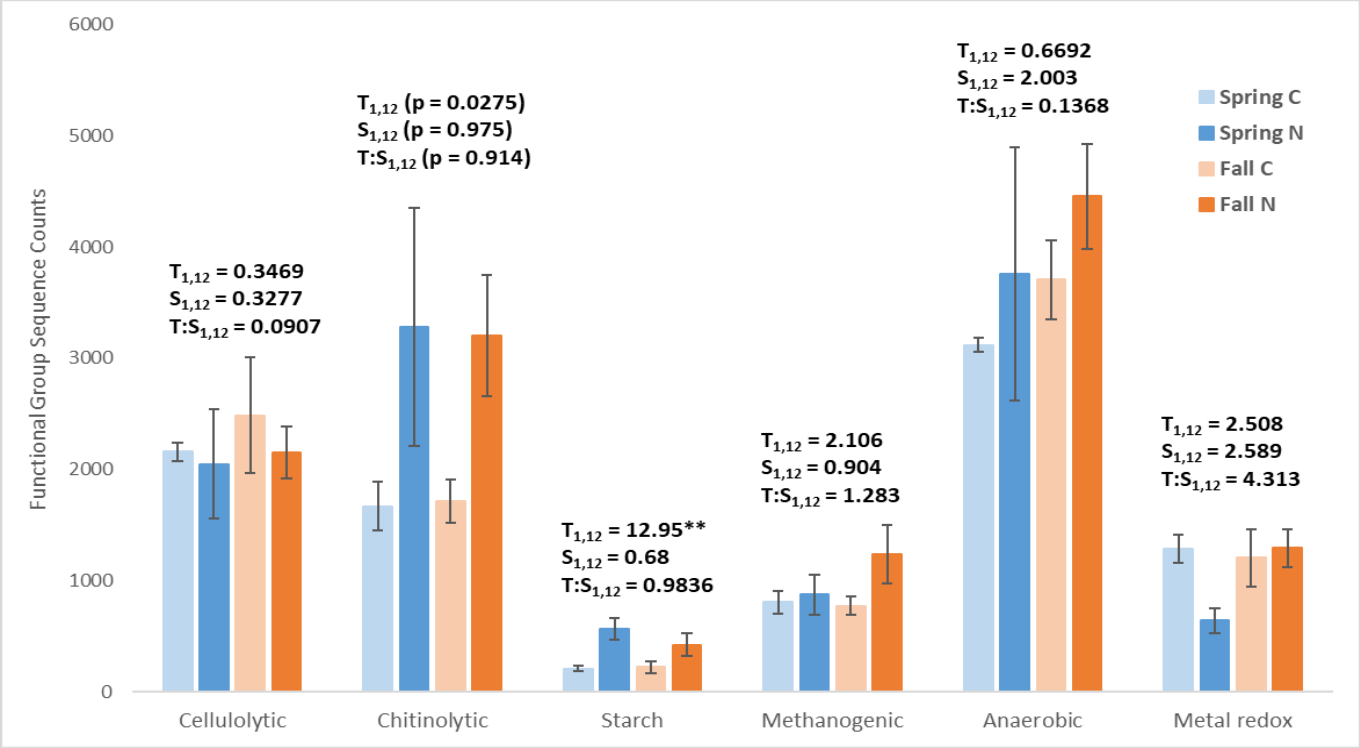

Figure 4. Mean (±SE; n = 4 plots) sequence counts for bacterial taxa arranged in cellulolytic, chitinolytic, starch, methanogenic, anaerobic, and metal redox functions groups from the Sky Oaks Field Station during the spring (blue-bars) and fall (orange-bars) of 2017 in N-amended (dark-bars) and control plots (lighter-bars). *$p < 0.05$; **$p < 0.01$.

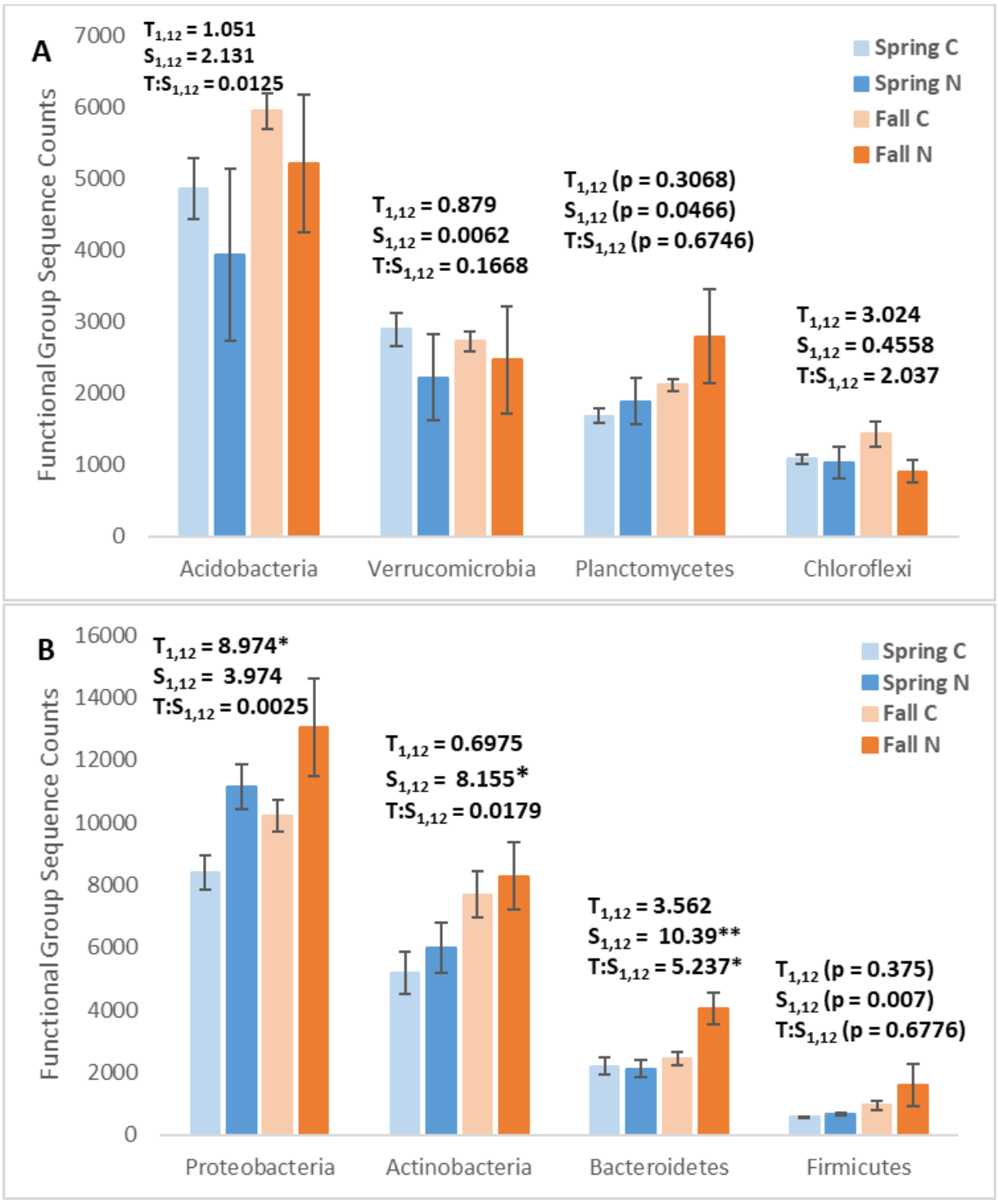

Figure 5. Mean (±SE; n = 4 plots) sequence counts for bacterial taxa arranged in (a) oligotrophic and (b) copiotrophic functions groups from the Santa Margarita Ecological Reserve during the spring (blue-bars) and fall (orange-bars) of 2017 in N-amended (dark-bars) and control plots (lighter-bars). *$p < 0.05$; **$p < 0.01$.

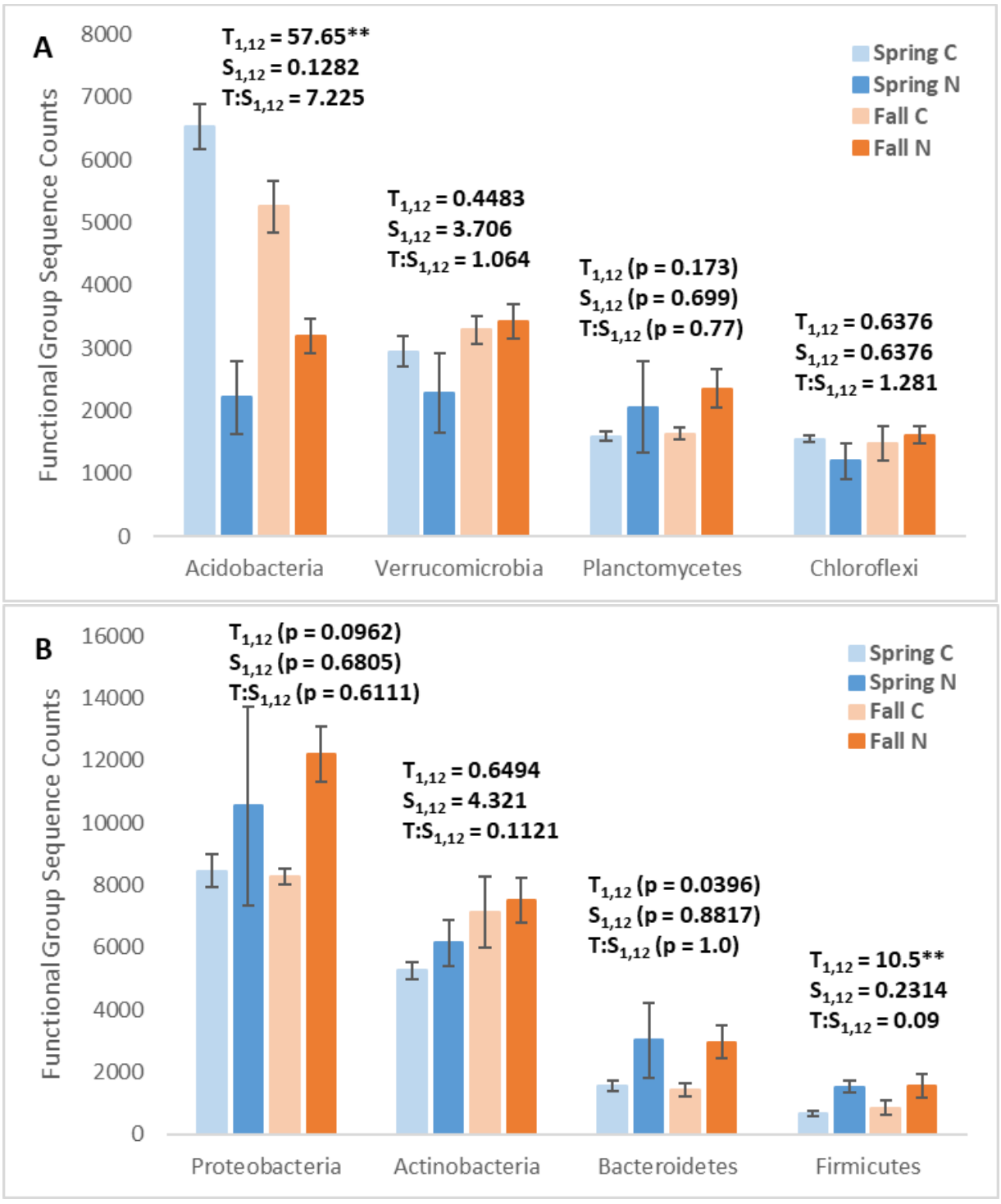

Figure 6. Mean (±SE; n = 4 plots) sequence counts for bacterial taxa arranged in (a) oligotrophic and (b) copiotrophic functions groups from the Sky Oaks Field Station during the spring (blue-bars) and fall (orange-bars) of 2017 in N-amended (dark-bars) and control plots (lighter-bars). *p < 0.05; **p < 0.01.

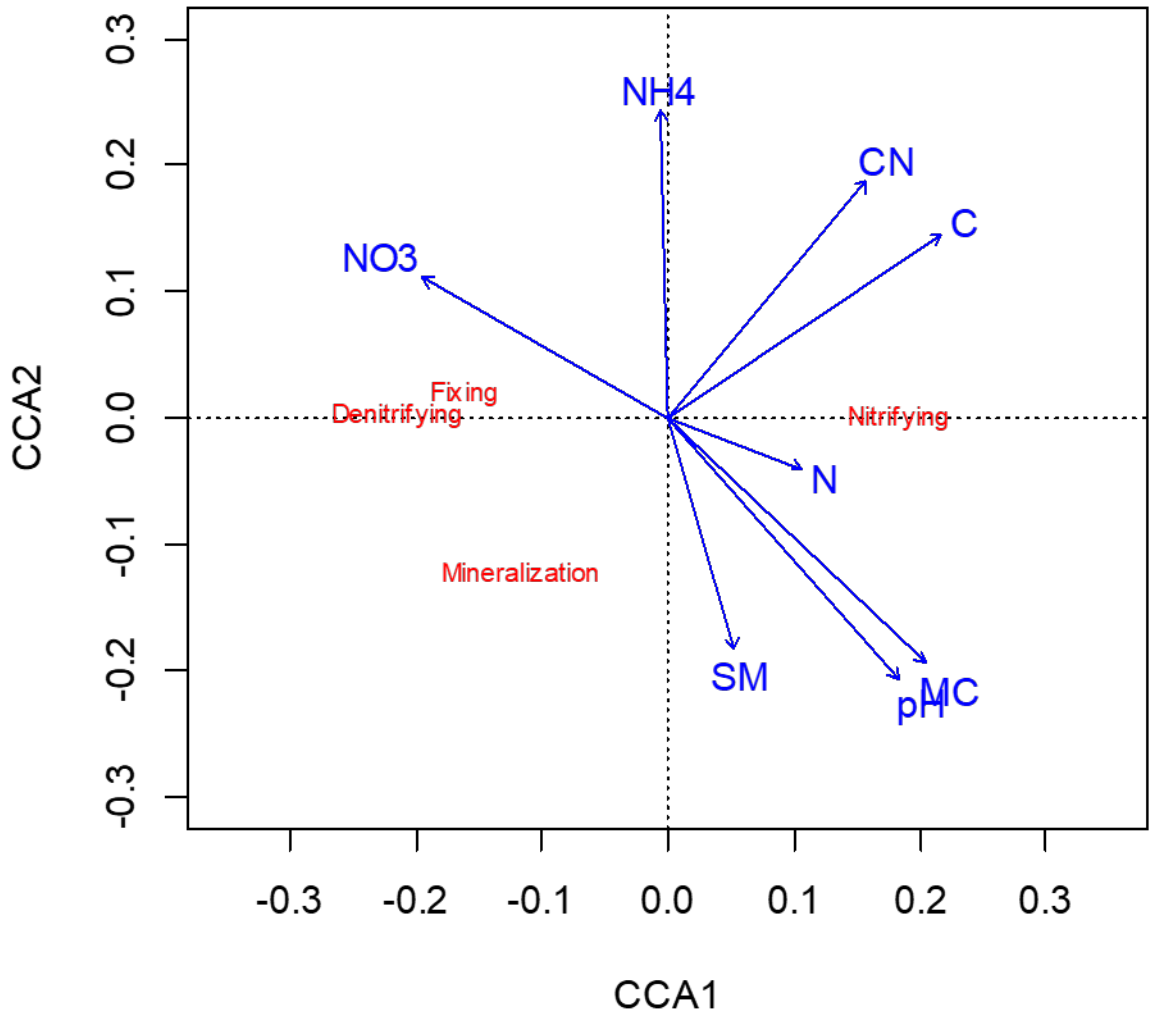

Figure 7. Canonical correspondence analysis (CCA) for bacterial taxa arranged in N-fixing, N-mineralizing, nitrifying, and denitrifying functional groups from the Santa Margarita Ecological Reserve in relation to the soil extractable nitrate (NO3) and ammonium (NH4), total soil carbon (C), nitrogen (N), and the C:N ratio, microbial C (MC), soil moisture (SM), and soil pH (pH). Axis 1 (CCA1) explained 93.3% and axis 2 (CCA2) explained 3.8% of the variance in bacterial functional groups.

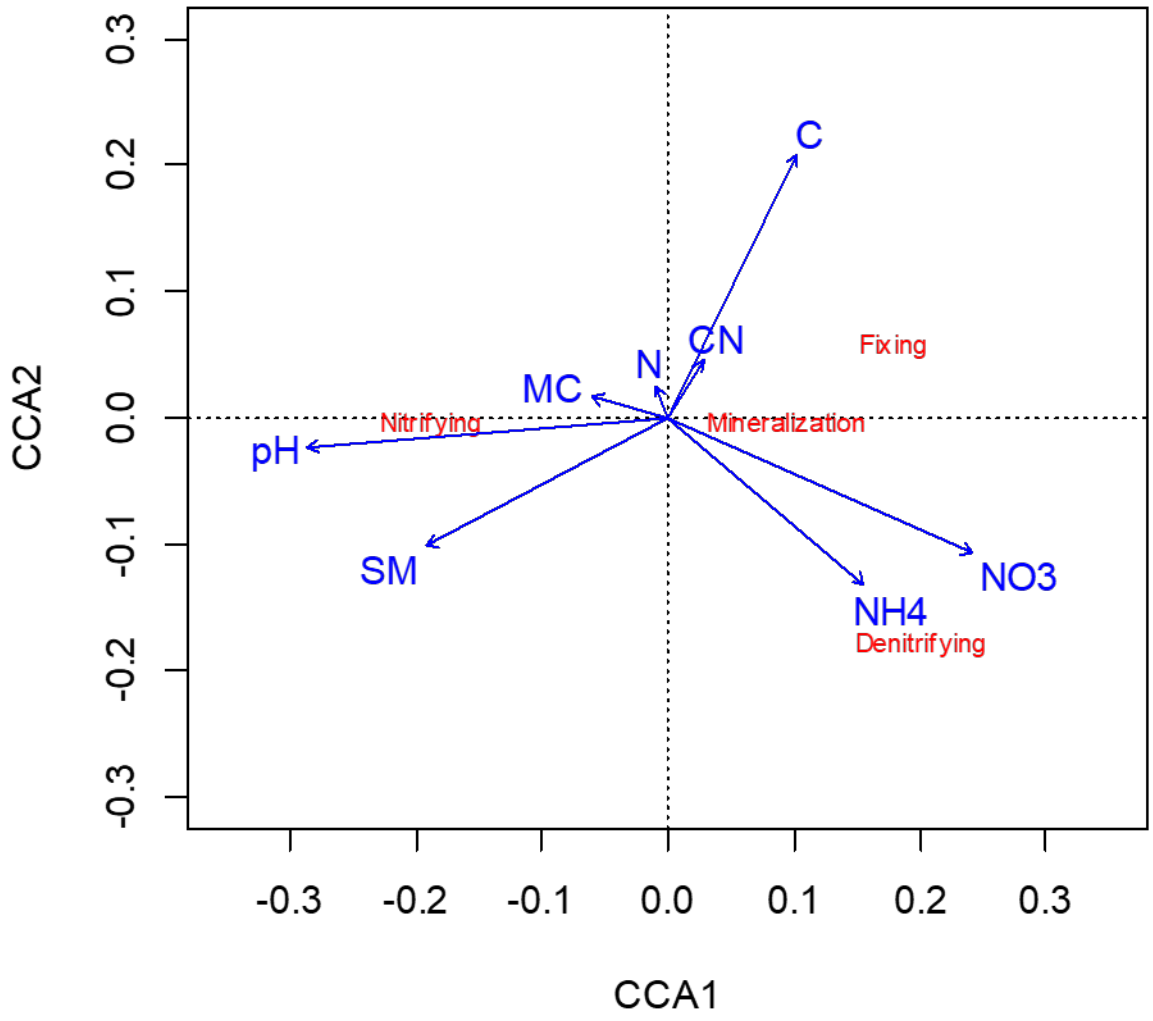

Figure 8. Canonical correspondence analysis (CCA) for bacterial taxa arranged in N-fixing, N-mineralizing, nitrifying, and denitrifying functional groups from Sky Oaks Field Station in relation to the soil extractable nitrate (NO3) and ammonium (NH4), total soil carbon (C), nitrogen (N), and the C:N ratio, microbial C (MC), soil moisture (SM), and soil pH (pH). Axis 1 (CCA1) explained 83.6% and axis 2 (CCA2) explained 11.8% of the variance in bacterial functional groups.

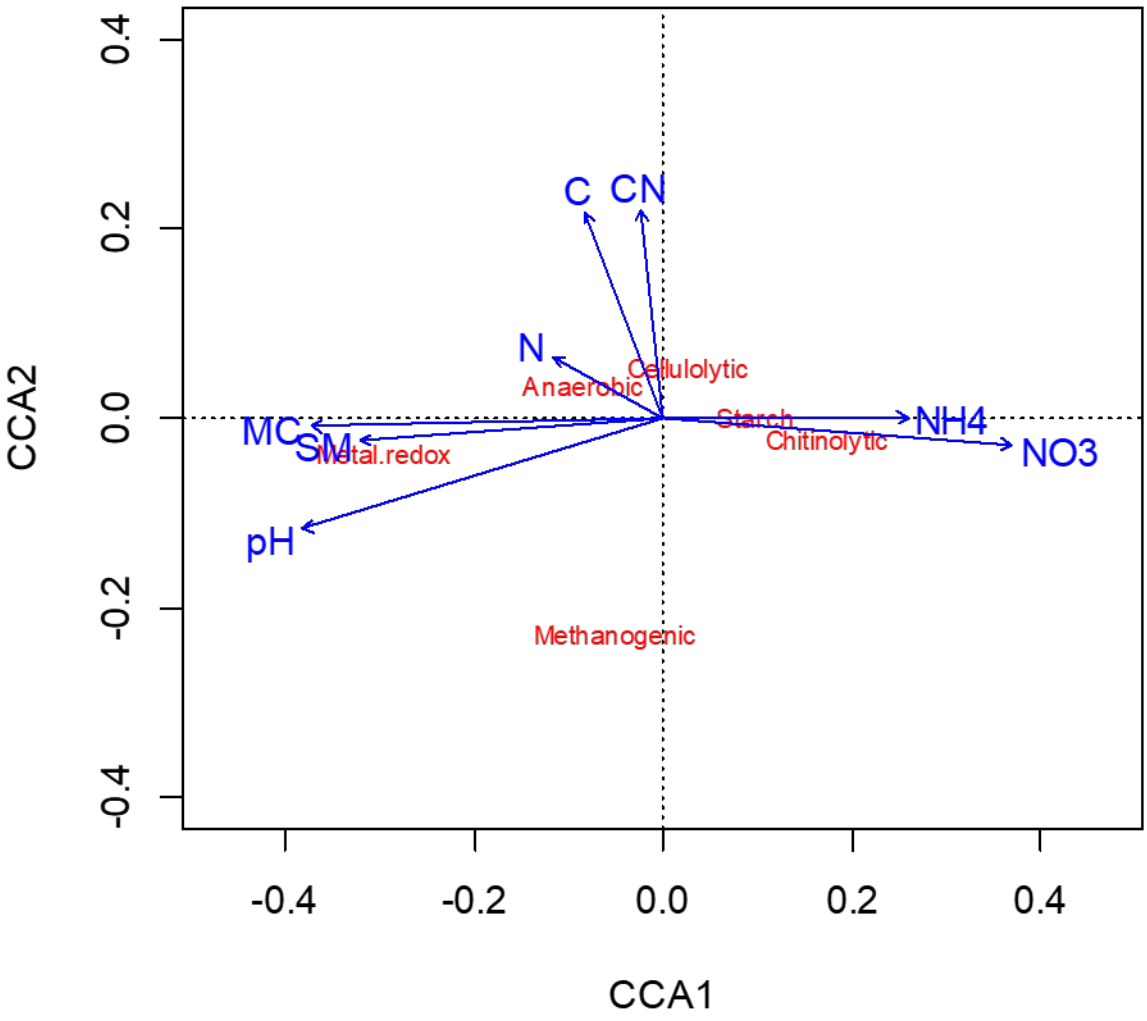

Figure 9. Canonical correspondence analysis (CCA) for bacterial taxa arranged in anaerobic, cellulolytic, chitinolytic, methanogenic, metal redox, starch degrading functional groups from the Santa Margarita Ecological Reserve in relation to the soil extractable nitrate (NO3) and ammonium (NH4), total soil carbon (C), nitrogen (N), and the C:N ratio, microbial C (MC), soil moisture (SM), and soil pH (pH). Axis 1 (CCA1) explained 68% and axis 2 (CCA2) explained 24% of the variance in bacterial functional groups.

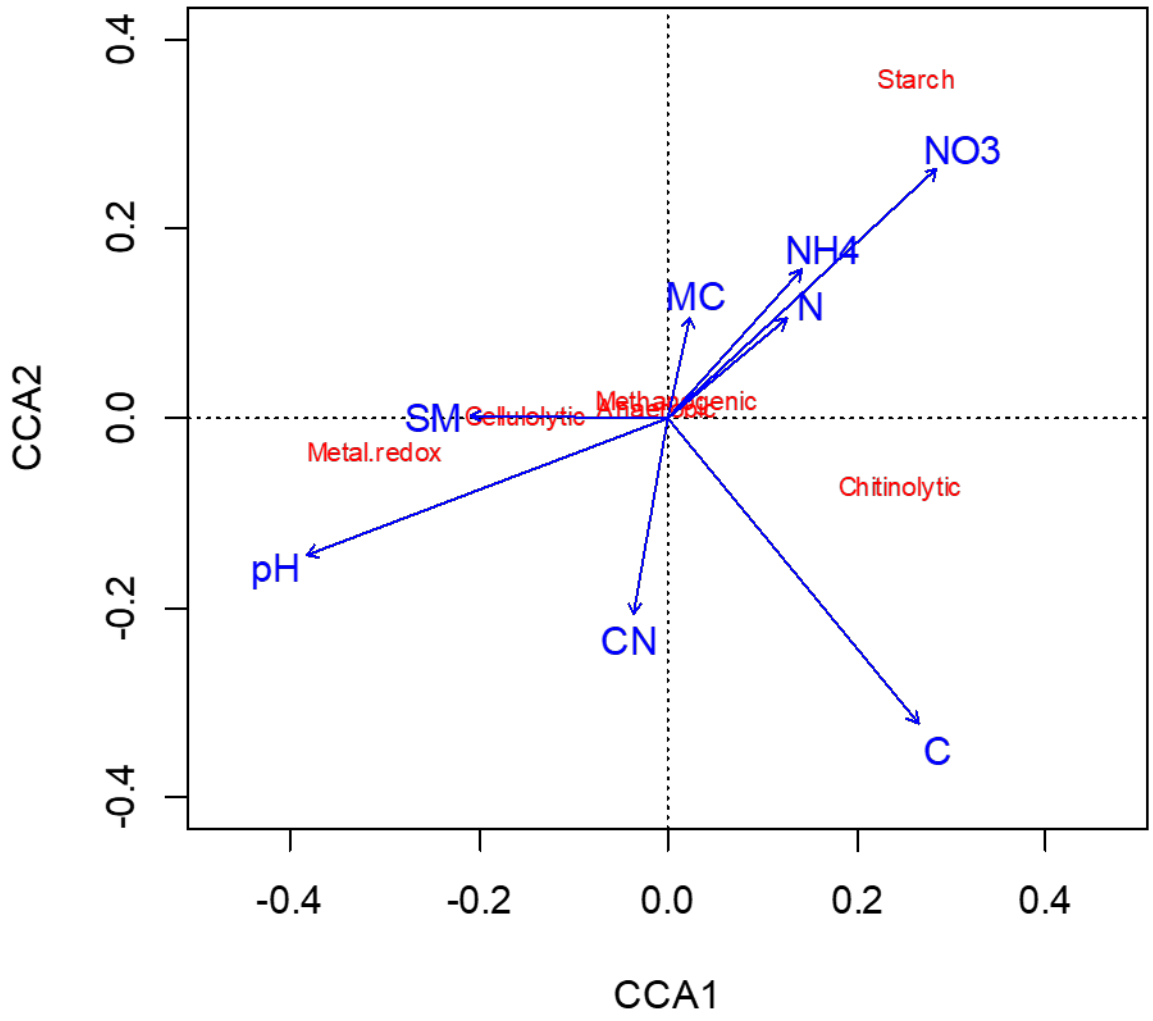

Figure 10. Canonical correspondence analysis (CCA) for bacterial taxa arranged in anaerobic, cellulolytic, chitinolytic, methanogenic, metal redox, and starch degrading functional groups from Sky Oaks Field Station in relation to the soil extractable nitrate (NO3) and ammonium (NH4), total soil carbon (C), nitrogen (N), and the C:N ratio, microbial C (MC), soil moisture (SM), and soil pH (pH). Axis 1 (CCA1) explained 72% and axis 2 (CCA2) explained 13% of the variance in bacterial functional groups.

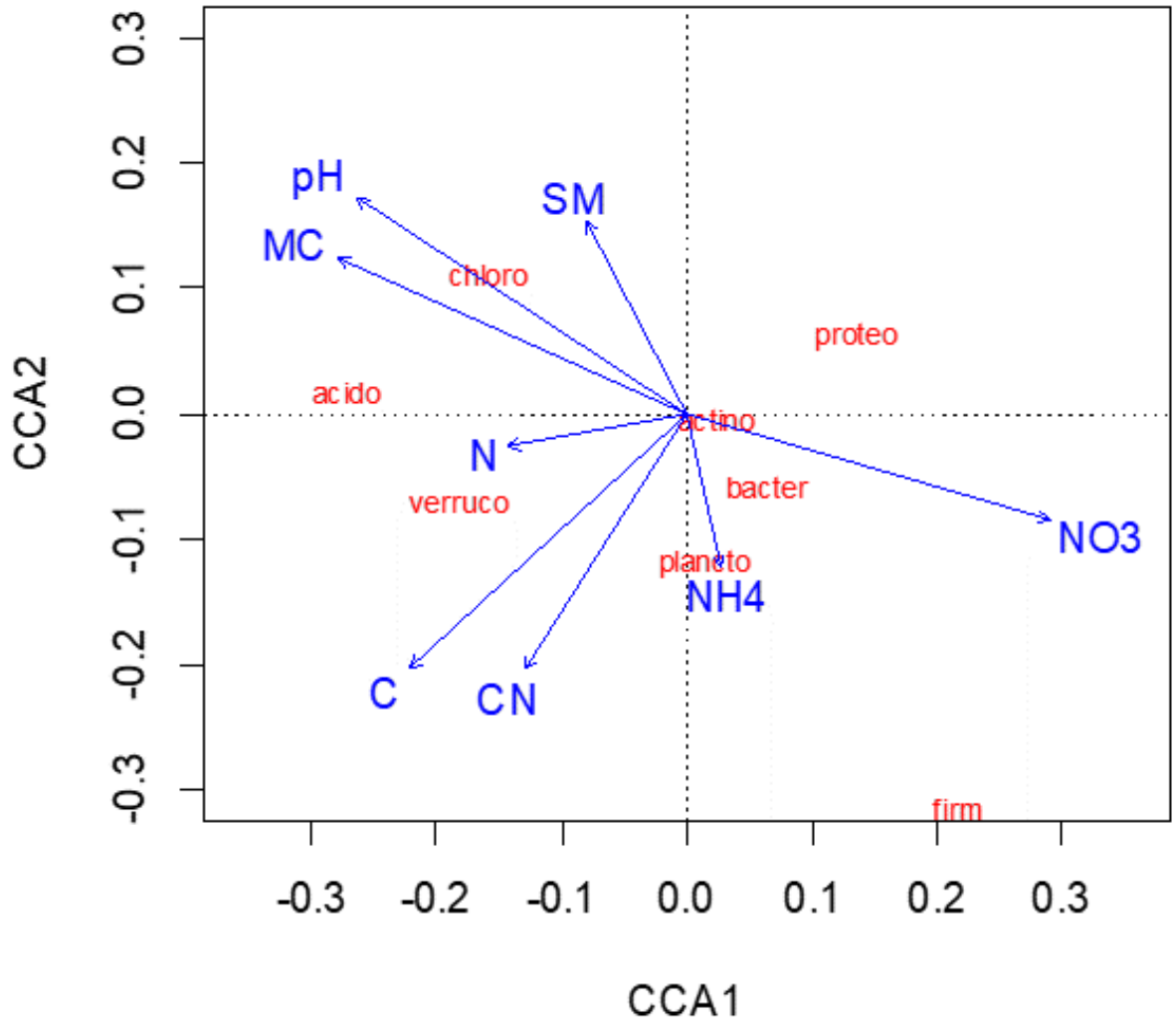

Figure 11. Canonical correspondence analysis (CCA) for bacterial phyla Acidobacteria (acido), Actinobacteria (actino), Bacteroidetes (bacter), Chloroflexi (chloro), Firmicutes (firm), Planctomycetes (plancto), Proteobacteria (proteo), and Verrucomicrobia (verruca) from Santa Margarita Ecological Reserve in relation to the soil extractable nitrate (NO3) and ammonium (NH4), total soil carbon (C), nitrogen (N), and the C:N ratio, microbial C (MC), soil moisture (SM), and soil pH (pH). Axis 1 (CCA1) explained 0.64% and axis 2 (CCA2) explained 0.18% of the variance in taxon abundance.

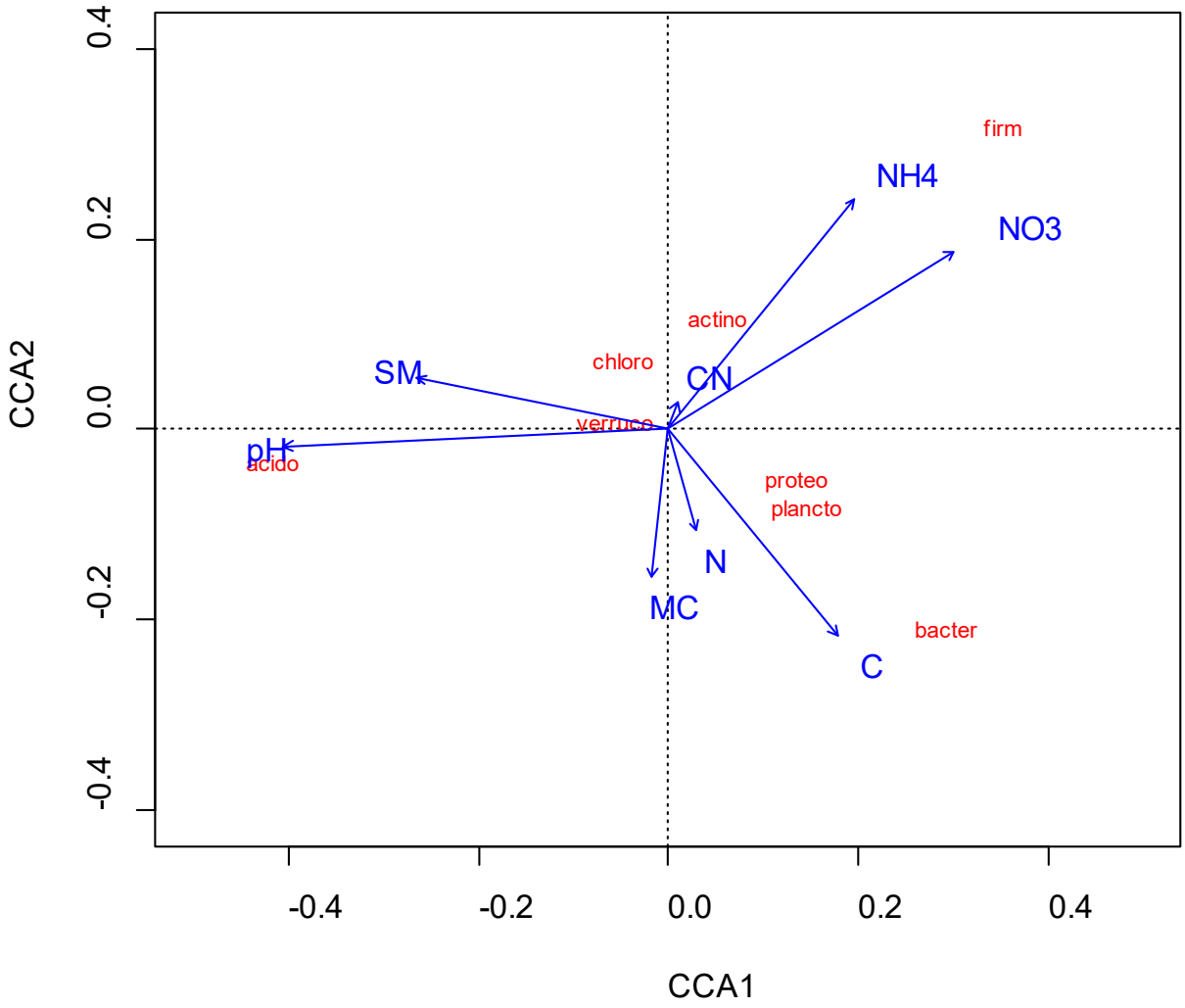

Figure 12. Canonical correspondence analysis (CCA) for bacterial phyla Acidobacteria (acido), Actinobacteria (actino), Bacteroidetes (bacter), Chloroflexi (chloro), Firmicutes (firm), Planctomycetes (plancto), Proteobacteria (proteo), and Verrucomicrobia (verruca) from Sky Oaks Field Station in relation to the soil extractable nitrate (NO3) and ammonium (NH4), total soil carbon (C), nitrogen (N), and the C:N ratio, microbial C (MC), soil moisture (SM), and soil pH (pH). Axis 1 (CCA1) explained 0.72% and axis 2 (CCA2) explained 0.20% of the variance in taxon abundance.