

The impact of precipitation change on nitrogen cycling in a semi-arid ecosystem

Melissa A. Cregger^{*1}, Nate G. McDowell², Robert E. Pangle³, William T. Pockman³ and Aimée T. Classen¹

¹Department of Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, Knoxville, TN 37996, USA; ²Earth and Environmental Sciences, Los Alamos National Laboratory, MS-J495, Los Alamos, NM 87545, USA; and ³Department of Biology, University of New Mexico, Castetter Hall Rm 1480, Albuquerque, NM 87131, USA

Summary

1. Climatic change is altering ecosystem structure and function, especially in the southwestern United States where trees are near their physiological water stress threshold. In piñon-juniper (*Pinus edulis*-*Juniperus monosperma*; PJ) woodlands, increased drought is causing differential mortality of piñon resulting in an ecosystem that is becoming juniper dominated.

2. Using a precipitation manipulation, we assessed how both increased and decreased precipitation altered ecosystem function beneath piñon and juniper. We predicted that changes in precipitation would alter nitrogen (N) availability and mineralization at the site. Further, we predicted that these responses would differ beneath piñon and juniper crowns due to plant-level differences in transpiration and N uptake in response to drought.

3. We found minimal interactions between tree species and the precipitation treatments on N cycling. However, across all years measured, soil nitrate decreased with increasing soil volumetric water content; a pattern that is likely due to reduced turnover in dry plots. In contrast, potential soil net-nitrogen mineralization was reduced in water removal plots relative to water addition plots indicating that nitrogen cycling rates were slower under drought. Tree type also influenced nitrogen dynamics in this woodland. Across all 4 years, soil N availability and potential soil net-mineralization rates were higher in soils beneath piñon relative to juniper across all treatments. Interestingly, the observed shifts in N cycling were not reflected in the abundance of N in microbial biomass or in ammonia-oxidizing bacteria, which are responsible for nitrification. The observed patterns may be due to increased N leaching from the soil during periods of increased rainfall or due to decreased microbial activity or plant N uptake when conditions are dry.

4. The effect of precipitation change on N cycling may have long-term consequences on the plant community in this semi-arid ecosystem. Nitrogen concentrations are highest in the soil when water availability is low, thus when N concentrations are high, plants and microbes are relatively inactive and unable to use this resource.

Key-words: ammonia-oxidizing bacteria, climate change, nitrification, nitrogen availability, nitrogen mineralization, piñon-juniper woodland, precipitation

Introduction

Climate models predict that the frequency and severity of drought and extreme weather events will increase globally over the next century (Seager *et al.* 2007; Allison *et al.* 2009). During recent extreme droughts in the southwestern United States mortality rates of piñon (*Pinus edulis*) approached 100% in some areas (Breshears *et al.* 2005;

Shaw, Steed & DeBlander 2005). These drought-associated mortality events resulted in mixed piñon-juniper (PJ) woodlands becoming increasingly dominated by the more drought-resistant juniper (*Juniperus monosperma*; Mueller *et al.* 2005; Gitlin *et al.* 2006; Sthultz, Gehring & Whitham 2009). Changes in precipitation amounts and temporal distribution, as well as the associated shifts in plant community composition, may lead to reductions in plant productivity and nitrogen (N) cycling in PJ woodlands (Padien & Lajtha 1992; Klopatek *et al.* 1998). Further,

*Correspondence author. E-mail: mcregger@utk.edu

reductions in N cycling may interact with decreased water availability to reduce plant re-establishment in this ecosystem following mortality events.

Precipitation directly alters N cycling via its impact on soil water availability, erosion and leaching, and indirectly alters N cycling by influencing plant N uptake as well as plant productivity. Increases in precipitation, specifically in large rain events, might lead to large leaching and run-off events (Nearing *et al.* 2005), which could increase N loss and decrease N retention. For example, nitrate is readily leached down the soil profile in arid ecosystems, which could lead to N accumulation beneath the reach of plant roots (Jobaggy & Jackson 2001; Walvoord *et al.* 2003). Changes in the distribution of rainfall can lead to large rain events (Easterling *et al.* 2000). These large events and their associated run-off can redistribute N among tree types and from the intercrown space to intercrown vegetated areas leading to the loss of N beneath tree canopies (Wilcox 1994; Reid *et al.* 1999). In water-limited ecosystems, N uptake from the soil is limited by water availability, thus when precipitation and soil water availability increase, plant N uptake from the soil and overall plant productivity also increase leading to a decline in soil N availability (Stark & Firestone 1995; Austin 2002; Knapp *et al.* 2008). Similarly, N can accumulate during periods of drought. When soils are dry, decreased plant transpiration results in decreased N uptake by roots and increased N accumulation in soils (Stark & Firestone 1995; Weltzin *et al.* 2003). Piñon and juniper contrast dramatically in their hydraulic behaviour, with piñon closing its stomata and reducing transpiration rates more quickly and for longer duration during droughts than juniper (Plaut *et al.* 2012). A logical hypothesis is that the lower transpiration rates of piñon may drive increasing available N beneath piñon crowns relative to beneath juniper crowns.

Changes in precipitation can induce shifts in the structure of plant communities (Zak *et al.* 2003; Breshears *et al.* 2005; Mueller *et al.* 2005; McDowell *et al.* 2008; Allen *et al.* 2010; Kardol *et al.* 2010) that may also alter ecosystem N cycling (Hobbie 1992; Knapp *et al.* 2008; Mitchell *et al.* 2010). Plant compositional shifts may have a larger impact on N cycling in areas with low species diversity where shifts in a single species or phenotypes may cause significant shifts in input quantity or quality (Schlesinger *et al.* 1996; Murphy, Klopatek & Klopatek 1998; Rodriguez *et al.* 2011; Classen *et al.* 2013). In many arid and semi-arid ecosystems like PJ woodlands, plants form islands of fertility that result in a patchy distribution of vegetation and a heterogeneous distribution of soil water and N on the landscape (Austin *et al.* 2004). Large-scale mortality of a dominant species in these ecosystems could alter the distribution of N on the landscape. For example, during periods of stress, piñons drop their needles, which increases litter biomass accumulation beneath their crowns (Chapman *et al.* 2003). This accumulation of litter biomass may result in increased available N at a time when plants are unable to take up this limiting resource (Austin & Vitousek 1998).

Nitrogen cycling is highly dependent on the microbial community actively decomposing organic matter (Schlesinger *et al.* 1996). In dry areas, where water often limits N cycling (Sala *et al.* 1988), soil microbial communities respond to rain pulses by increasing their activity and rapidly immobilizing N (Schimel, Balsler & Wallenstein 2007; Xiang *et al.* 2008). While soil communities may mineralize N during drought, this may not translate into increases in plant uptake due, in part, to reduced water uptake by plants, resulting in increases in soil N availability (Kreuzwieser & Gessler 2010). Water availability may regulate decomposition processes and soil microbial activity in PJ woodlands to a greater extent than changes in temperature or litter quality (Murphy, Klopatek & Klopatek 1998; Classen *et al.* 2006, 2007a,b) suggesting that even small changes in precipitation may have a disproportionate impact on N cycling in these ecosystems over time.

To better understand how precipitation change altered N dynamics in a PJ woodland and what role microbial communities played in this response, we measured soil N availability, soil net-N mineralization, microbial biomass N and the relative abundance of the bacterial community involved in nitrification – a rate-limiting step in the N cycle – in a large-scale precipitation manipulation located at the Sevilleta Long-Term Ecological Research Site (LTER) in New Mexico, USA from 2007 to 2011. Plots at this site had precipitation added or precipitation removed. We predicted that changes in precipitation would alter N availability and mineralization such that increases in precipitation would increase both microbial immobilization of N and plant uptake thus reducing available N in the soil, while decreases in water availability would allow N to accumulate in the soil due to decreased microbial and plant activity. Further, we predicted that these responses would differ beneath piñon and juniper crowns due to plant-level differences in transpiration and N uptake in response to drought.

Materials and methods

SITE DESCRIPTION

To assess how precipitation change altered N inputs and cycling, we manipulated precipitation at the Sevilleta National Wildlife Refuge in central New Mexico, where the Sevilleta LTER programme is located (32°20' N, 106°50' W). The precipitation manipulation was implemented at 1911 m elevation in the Los Piños Mountains. Climate records from the Sevilleta LTER meteorological station (Cerro Montoso #42; <http://sev.lternet.edu/>) indicated a mean annual air temperature of 13 °C and mean annual precipitation of 368 mm. On average the greatest period of precipitation on the refuge occurs during the monsoon months of July, August and September. The monsoon precipitation accounts for over half of all annual precipitation inputs; but the high evapotranspiration rates in PJ woodlands prevent summer moisture from recharging soil moisture to depth (Greenland, Goodin & Smith 2003). The study area is dominated by extensive stands of intermixed piñon and juniper with an understory dominated by the C4 grass black grama (*Bouteloua eriopoda*).

We established 12 experimental plots (each 1600 m²) during the summer of 2007 that included a decreased precipitation treatment (c. 45% reduction), a precipitation-removal control treatment (cover control), an increased precipitation treatment and an ambient plot ($n = 3$ plots per treatment). Precipitation was reduced in our decreased precipitation treatment using a throughfall displacement design (Sala *et al.* 2000; Pangle *et al.* 2012). Troughs were constructed of clear UV-damage resistant acrylic sheets and installed in each drought plot at a height of c. 1 m. The cover control treatments were constructed by inverting the troughs and were instituted to control for possible unintended impacts of the troughs. The elevated precipitation treatments were created using above-canopy irrigation sprinklers. The sprinklers were tested in October 2007 and 2 mm supplemental water was added. In 2008, 57 mm of precipitation was added to each of the treatment plots (3–19 mm rain events), 69.5 mm was added in 2009 (3–19 mm rain events and 1–12.5 mm rain event), 112 mm was added in 2010 (5–19 mm rain events and 1–17 mm rain event) and 107 mm was added in 2011 (4–19 mm rain events, 1–17 mm rain event and 1–14 mm rain event; see Appendix S1 in Supporting Information for dates precipitation was added). The ambient treatments were un-manipulated plots located within the experimental plot matrix. These plots serve as ambient reference plots for the study.

Campbell scientific dataloggers continuously monitored and recorded abiotic conditions across the site. Abiotic conditions were measured at three locations under both piñon and juniper. These measurements included soil temperature at –5 cm depth and soil volumetric water content at –5 cm depth using EC-20 ECH₂O probes. Transpiration was measured as stem sap flow (J_s) using Granier heat dissipation sap-flow sensors. All target trees had two 10-mm Granier sap-flow sensors installed in the outermost sapwood. Each sensor used the two probe heated and unheated reference design (Pangle *et al.* 2012).

SOIL COLLECTION

To explore how changes in precipitation altered the N cycle, in June 2008, we randomly collected three soil cores (5 cm diameter, 0–10 cm depth) beneath the drip line of five piñon and five juniper crowns within each treatment plot using a hammer core. Three cores were collected to get a more accurate assessment of N dynamics and the soil community since the environment beneath the tree canopy can be heterogeneous (Throop & Archer 2008). After compositing the cores, a subsample from each individual tree was immediately flash-frozen in liquid N for subsequent molecular analyses. The remaining collected soil was bulked within plant species for each plot (so each plot had a piñon sample and a juniper sample), and homogenized. The soil was kept cool until it was returned to the laboratory, sieved to 2 mm and analysed as described below.

NITROGEN AVAILABILITY

Changes in precipitation can differentially alter components of the N cycle, thus we measured changes in N availability, potential net-N mineralization and net-N mineralization beneath piñon and juniper in each of our treatments. During the growing season of 2008, 2009 and 2011, we assessed N availability (nitrate and ammonium) using plant root simulator (PRS) probes (Western Ag Innovations, Inc., Saskatoon, SK, Canada). PRS probes were employed due to their ability to measure plant available N over time, their ease of use and their cost effectiveness (Qian & Schoenau 2002). Because we thought season might play a role in the data we were collecting, the first and last year of availability data assessed ammonium and nitrate availability across the peak of the growing season, while the 2009 data enabled us to look at seasonal changes (pre-monsoon, monsoon, and post-monsoon) in

availability. For all years, we placed probes randomly beneath the crowns of five piñon and five juniper in each of the treatment plots ($n = 3$). Each tree had eight PRS probes located randomly (0–10 cm depth) beneath the drip line of the tree to again account for expected heterogeneity in N availability (Throop & Archer 2008). Four probes assessed ammonium (NH₄⁺) and four assessed nitrate (NO₃⁻) availability. In 2008, the PRS probes were deployed on June 23rd and collected after 6 weeks of incubation. In 2009, we deployed PRS probes again for 6-week incubations, but this time we assessed changes in availability pre-monsoon (June 5–July 22), monsoon (July 22–September 15) and post-monsoon (September 15–October 27). In July 2011, PRS probes were deployed and incubated for 7 weeks in the field. Upon collection, PRS probes were washed with DI water and returned to Western Ag Innovations, Inc. where they were analysed for NH₄⁺ using a reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite in a buffered alkaline medium at pH 12–13 to produce a measurable green colour, and NO₃⁻ using a colorimetric reaction with a copper cadmium column reactor. The four replicate probes per tree and ammonium and nitrate values for each replicate tree were summed and averaged across each plot prior to statistical analysis yielding one value for piñon and juniper in each treatment plot. Because N supply rates are not linear over time, all data are shown as available NH₄⁺ or NO₃⁻ in µg per membrane area over the incubation period.

NET-NITROGEN TRANSFORMATIONS

Unlike N availability where plant uptake can be important, net-N mineralization assesses the accumulation, in the absence of plant uptake, of N in the soil over a given time. Thus, we were interested in exploring how shifts in precipitation altered net rates of N transformations. Net-mineralization rates were measured in an *in situ* field soil incubation using the resin-core method over the growing season (May–October) in 2009. Paired soil cores (0–15 cm) were randomly taken beneath the crowns of piñon and juniper. One core of each pair was returned to the laboratory for gravimetric water content and inorganic N analyses. The other core was incubated in a PVC pipe with an ion exchange resin bag placed at the bottom to collect inorganic N leached from the core during rain events (Binkley 1989; Robertson *et al.* 1999). Atmospheric N inputs to these ecosystems are very low (Klopatek 1987) and the ecosystem is very dry, thus we did not put a resin on the top of our cores. Additionally, because we were interested in how changes in precipitation may be influencing mineralization, we chose the open resin-core technique and not the closed core technique (Adams & Attiwill 1986). Upon removal from the field, soils were homogenized and sieved to 2 mm. Collected soils and resins were extracted with 2 M KCl and analysed for NH₄⁺ and NO₃⁻ on a Lachat Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA). The difference in inorganic N pools in the incubated soil core and inorganic N collected on the resin bag minus initial soil pools was used to estimate the rates of soil net-N mineralization over the incubation period (Binkley 1989; Hart *et al.* 1994). These data are shown on an oven dry mass basis.

POTENTIAL NET-NITROGEN MINERALIZATION

Changes in precipitation can alter N cycling by changing inputs to the soil or by changing the microclimate, for example water availability. To tease this apart we measured potential net-N mineralization, which removes the influence of environmental temperature and precipitation by incubating the soils under standard temperature and moisture in the laboratory. To assess potential net-mineralization rates, a 60-day laboratory incubation was established. Soils were brought up to field capacity and subsamples (c. 20 g) were incubated in mason jars at laboratory

temperatures (c. 22 °C) over 60 days (Robertson *et al.* 1999). Each jar contained two subsamples (one removed after 30 days, one removed after 60 days) and each jar contained a standard amount of DI water in its bottom to maintain humidity. Samples were removed and jars were flushed with air every 7 days over the course of the incubation to reduce CO₂ build-up from microbial activity. Subsamples were extracted with 2 M KCl immediately and then after 30 and 60 days of incubation. Ammonium and nitrate concentrations were analysed using a Lachat Flow Injection Analyzer (Lachat Instruments). The difference in inorganic N pools in the incubated soil minus initial soil pools was used to estimate the rates of potential net-N transformations over the incubation period (Robertson *et al.* 1999). Data are shown on an oven dry mass basis.

MICROBIAL BIOMASS NITROGEN

In June 2009, we randomly collected soil cores (15 cm depth, 5 cm diameter) beneath three juniper and three piñon crowns in each of the treatments. Samples were sieved to 2 mm and assessed for microbial biomass N using the fumigation-extraction method (Haubensak, Hart & Stark 2002). Approximately, 15 g of soil was extracted with 75 mL of 0.5 M K₂SO₄ on a shaker for 1 h. Extracts were filtered through Whatman number 1 filter paper previously leached with DI water. A second subsample was fumigated in a vacuum desiccator with CHCl₃ for 5 days. After fumigation, the sample was extracted and filtered as above. A 20-mL aliquot of each extract was digested using a micro-Kjeldahl digestion. Samples were analysed for total N using a SmartChem chemistry discrete analyzer (Westco Scientific Instruments, Inc., Brookfield, CT, USA). Microbial biomass N was determined by subtracting the initial sample N from the fumigated sample N. A K_{EN} correction factor of 0.2 was used to estimate biomass N from chloroform labile N (Davidson *et al.* 1989).

AMMONIA OXIDATION GENE ABUNDANCE

Because changes in microbial community function could result in shifts in the N cycle, we assessed the response of microbial community function to our treatments by measuring the relative abundance of the bacterial ammonia oxidation (*amoA*) gene. *AmoA* is a key gene involved in *amoA* and codes for key enzymes in nitrification. Although changes in DNA abundance do not necessarily result in a change in function, it does allow us to assess how the abundance of the microbes capable of this function respond to fluctuations in precipitation as well as the plant species present. To assay this community, we extracted DNA from c. 0.75 g of soil following the standard protocol using the UltraClean Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) collected beneath one piñon and one juniper across all experimental plots ($n = 3$) in the pre-monsoon season of 2008. DNA concentration and purity were evaluated using a microplate reader (Biotek Instruments, Winooski, VT, USA). To assess the community capable of *amoA*, we ran quantitative polymerase chain reaction (qPCR) using primers for ammonia monooxygenase (forward primer – GGGGTTTCTACTGGTGGT, reverse primer – CCCCTCKGSAAAGCCTTCTTC; Rotthauwe, Witzel & Liesack 1997). PCR mixtures contained 12.5 µL of SYBR green master mix (Life Technologies, Grand Island, NY, USA), 0.4 µmol of each primer (Eurofins mwg operon, Huntsville, AL, USA) and 1 µL of sample DNA diluted 1:10 in sterile water. All reactions were brought up to a final volume of 25 µL with sterile water. Amplification protocol consisted of an initial denaturing cycle of 95 °C for 15 min. This cycle was followed by 45 cycles of 94 °C for 30 s, 54 °C for 45 s and 72 °C for 30 s (Wallenstein & Vilgalys 2005). After completion of the cycle, a melting curve analysis was conducted to ensure purity of the amplification product. PCR

amplification was performed on a 96-well Chromo4 thermocycler (Bio-Rad Laboratories, Hercules, CA, USA).

STATISTICAL ANALYSIS

To test for seasonal effects on N availability, we used a univariate repeated measures analysis of variance (ANOVA) with a split plot design. We tested the main effect of season and the random variable tree nested within treatment on N availability in the pre-monsoon, monsoon and post-monsoon season of 2009. Two-way ANOVAs with a split plot design were used to test for the main effects of tree type, precipitation and their interactive effects on ammonium and nitrate availability, net-N mineralization, microbial biomass N and *amoA* abundance for each year separately. A Tukey HSD test was used to differentiate between treatment means when more than two levels were present (i.e. precipitation treatment differences). Because the amount of water added vs. excluded varied, we followed these analyses using soil moisture as a continuous factor. We performed an ANCOVA with the factor tree and the covariates soil temperature, soil moisture and transpiration averaged over the corresponding incubation period on N availability across all years measured. Because we did not have transpiration data on all plots before late 2009, we performed ANCOVAs using tree as a factor and the covariates soil temperature and soil moisture averaged over the corresponding incubation period on net-N mineralization, microbial biomass N and *amoA* abundance. All data were analysed using JMP 8 (Cary Institute, Cary, NC, USA). When data were not distributed normally, log and square root transformations were used prior to running all analyses. Additionally, a Brown–Forsythe's test was employed to test for homogeneity of variance (Brown and Forsythe 1974). Variances were equal across significant response variables.

Results

SOIL NITROGEN CYCLING

Across all 4 years, soil N (ammonium and nitrate combined) availability was highest in our water removal treatments (Fig. 1) and in piñon relative to juniper soils. Soil nitrate decreased with increasing soil volumetric water across all years measured (Fig. 2; $R^2 = 0.75$, $F = 14.11$, $P < 0.01$). Soil nitrate was highest in water removal treatment soils relative to the water addition soils in 2009 (Fig. 1; 2008, $F = 1.9$, $P = 0.20$; monsoon 2009, $F = 5.1$, $P = 0.03$; 2011, $F = 2.6$, $P = 0.12$). In 2011, ammonium availability was higher across both tree types in the water removal plots relative to other treatments (see Appendix S4; $F = 2.8$, $P = 0.07$). In addition, there was a precipitation treatment by tree type interaction in 2011 where ammonium availability was 6× greater in soils beneath juniper found in the water removal plots relative to soils beneath either tree across the other treatments (see Appendix S4; $F = 7.72$, $P = 0.01$). Season was important in determining the response of soil N availability to canopy and experimental precipitation. During the monsoon season of 2009, nitrate availability was 1.8× greater in the water removal soils compared with water addition soils ($F = 5.1$, $P = 0.03$). Soil ammonium availability was highest during the monsoon season, and lowest following the monsoon in 2009 ($F = 16.67$, $P < 0.01$).

Table 1. ANCOVA *F* and *P* scores testing the main and interactive effects of tree species, transpiration, soil temperature and soil moisture on nitrate availability, ammonium availability, potential N mineralization, net-N mineralization, ammonia oxidation (amoA) abundance and microbial biomass. Transpiration data were only available for four plots in 2008 and early 2009, therefore, this factor was only included in the analysis for N availability

	NH ₄ ⁺ avail.	NO ₃ ⁻ avail.	30 day NH ₄ ⁺	30 day NO ₃ ⁻	30 day Total N	60 day NH ₄ ⁺	60 day NO ₃ ⁻	60 day Total N	N-min NH ₄ ⁺	N-min NO ₃ ⁻	N-min Total N	amoA	Micro. biomass
Full model	0.87 (0.60)	5.10 (< 0.01)	0.51 (0.81)	1.59 (0.22)	2.04 (0.12)	0.52 (0.80)	1.30 (0.32)	1.57 (0.22)	1.66 (0.19)	0.70 (0.67)	0.72 (0.66)	0.32 (0.93)	1.53 (0.23)
Species	0.92 (0.35)	1.90 (0.18)	0.24 (0.63)	4.37 (0.06)	3.94 (0.07)	0.06 (0.82)	0.37 (0.55)	0.27 (0.61)	3.12 (0.10)	0.01 (0.94)	0.23 (0.64)	0.0002 (0.99)	0.09 (0.76)
Transpiration	1.30 (0.26)	1.55 (0.22)											
Species × transpiration	0.01 (0.94)	0.08 (0.78)											
Temperature	0.32 (0.58)	2.07 (0.16)	1.03 (0.33)	0.01 (0.92)	0.77 (0.40)	0.78 (0.39)	0.17 (0.69)	1.23 (0.29)	0.16 (0.69)	0.19 (0.67)	0.26 (0.62)	0.002 (0.97)	4.80 (0.04)
Species × temperature	0.001 (0.98)	0.65 (0.43)	0.47 (0.50)	0.21 (0.65)	1.49 (0.24)	1.00 (0.33)	0.36 (0.56)	0.0002 (0.99)	2.36 (0.14)	0.10 (0.76)	0.43 (0.64)	0.18 (0.68)	1.20 (0.29)
Transpiration × temperature	0.004 (0.95)	0.76 (0.39)											
Species × transpiration × temperature	1.60 (0.22)	0.11 (0.74)											
Moisture	0.02 (0.90)	14.11 (< 0.01)	0.16 (0.69)	1.66 (0.22)	1.35 (0.27)	0.45 (0.51)	2.03 (0.18)	1.44 (0.25)	0.37 (0.55)	2.39 (0.14)	1.79 (0.20)	0.75 (0.40)	3.51 (0.08)
Species × moisture	0.77 (0.39)	0.77 (0.39)	0.02 (0.90)	0.64 (0.44)	1.14 (0.30)	0.12 (0.74)	2.90 (0.11)	2.95 (0.11)	0.16 (0.69)	2.38 (0.14)	2.47 (0.14)	0.30 (0.59)	0.70 (0.42)
Transpiration × moisture	0.01 (0.94)	0.22 (0.64)											
Species × transpiration × moisture	0.04 (0.85)	0.05 (0.82)											
Temperature × moisture	0.37 (0.55)	0.26 (0.62)	0.11 (0.74)	0.09 (0.77)	0.44 (0.52)	0.05 (0.82)	0.06 (0.81)	0.18 (0.68)	0.70 (0.41)	0.55 (0.47)	0.82 (0.38)	0.03 (0.86)	0.01 (0.92)
Species × temperature × moisture	0.02 (0.89)	0.75 (0.39)	0.004 (0.95)	0.01 (0.92)	0.05 (0.82)	0.01 (0.94)	1.45 (0.25)	1.73 (0.21)	0.77 (0.39)	0.003 (0.96)	0.06 (0.80)	0.16 (0.70)	0.09 (0.77)
Transpiration × temperature × moisture	1.14 (0.30)	1.31 (0.26)											
Species × transpiration × temperature × moisture	2.27 (0.14)	0.20 (0.66)											

Bold values show significant results at $P < 0.05$.

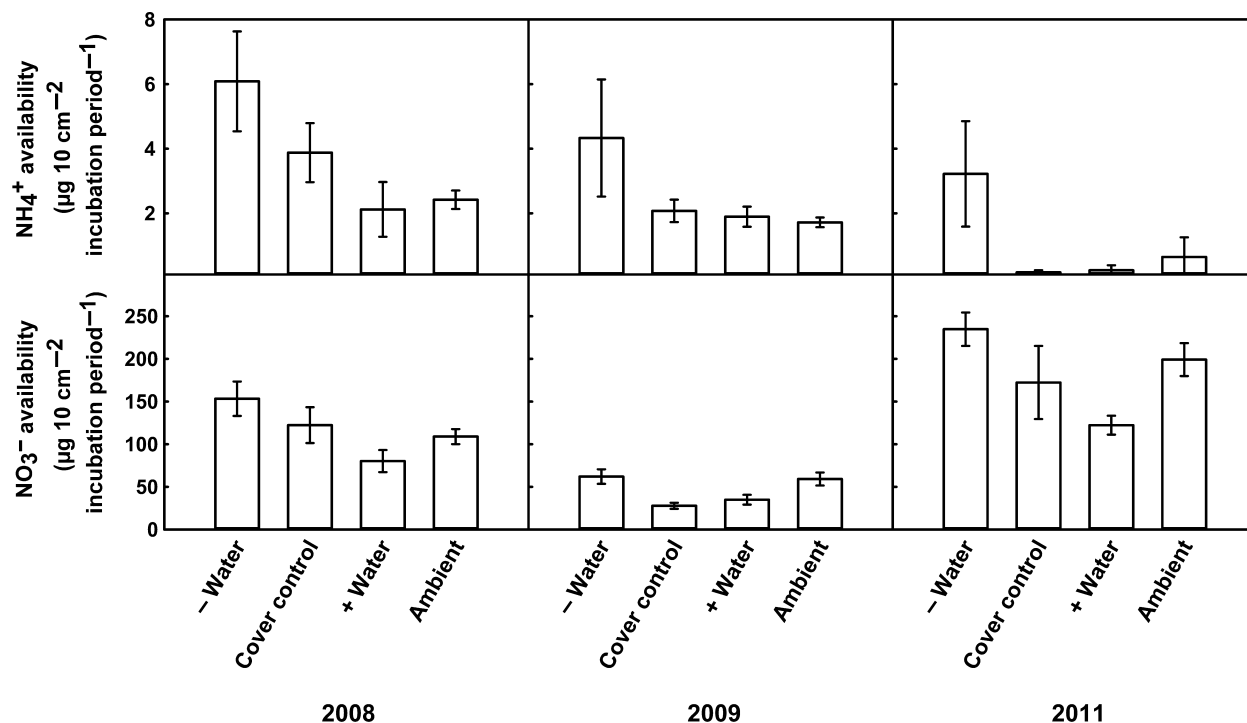


Fig. 1. Mean ammonium and nitrate availability (± 1 standard error) in 2008, monsoon season 2009 and 2011 across treatments, – water (water reduction) and + water (water addition), cover control (removal control) and control (ambient plot).

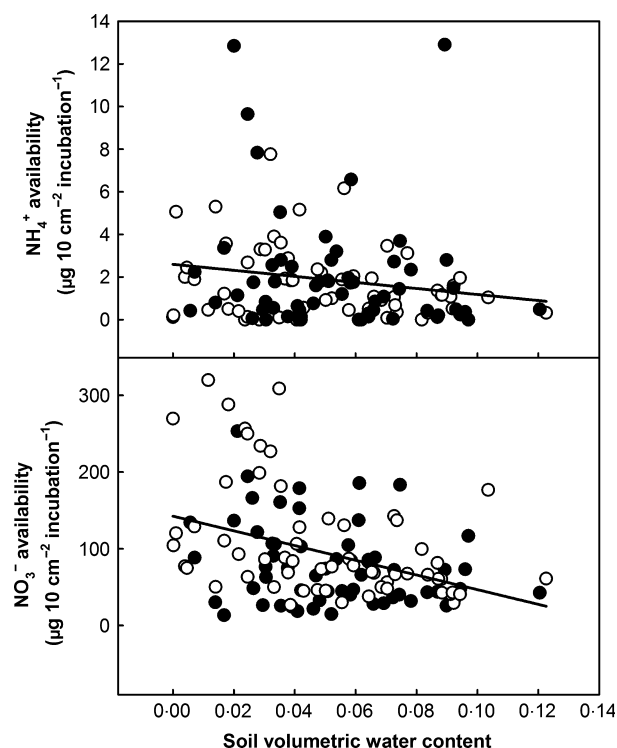


Fig. 2. Across all years measured with plant root simulator resin probes, there was no effect of soil volumetric water on ammonium availability, but nitrate availability decreased with increasing soil volumetric water content ($R^2 = 0.75$, $F = 14.11$, $P < 0.01$). Black dots denote nitrogen availability beneath juniper, while white dots denote nitrogen availability beneath piñon.

We found minimal effect of our precipitation treatments on net-N mineralization. In general, ammonium was immobilized and nitrate was released in soils across all treatments. However, there were changes in potential net-N mineralization. Potential net-N mineralization was highest in the control and water addition plots (see Appendix S4; $F = 4.45$, $P = 0.04$). Potential net-N mineralization rates, which measure N accumulation under ideal conditions, were, as suspected, higher than net-N mineralization rates.

Across all years measured, we found increased available N and increased rates of potential net-nitrification and mineralization in soils beneath piñon relative to juniper. Specifically, ammonium availability was 2 \times higher in soils beneath piñon relative to soils beneath juniper in the 2009 pre-monsoon season (see Appendix S4; $F = 14.0$, $P = 0.01$). Nitrate availability was *c.* 35% higher in soils beneath piñon relative to juniper during the 2009 monsoon and post-monsoon seasons (Fig. 3; Monsoon: $F = 29.87$, $P < 0.01$; see Appendix S4; Post-monsoon: $F = 12.5$, $P = 0.01$). Again in 2011, nitrate availability was higher beneath piñon relative to juniper (Fig. 3; $F = 3.2$, $P = 0.11$).

Across both plant species, during the laboratory incubation, ammonium was immobilized in soils and nitrate was released (Fig. 4). When measured in the field, ammonium was immobilized beneath juniper, but not beneath piñon ($F = 7.22$, $P = 0.03$). There were no differences in net-nitrification or net-N mineralization rates in soils incubated in the field; however, piñon had 1.9 \times higher potential

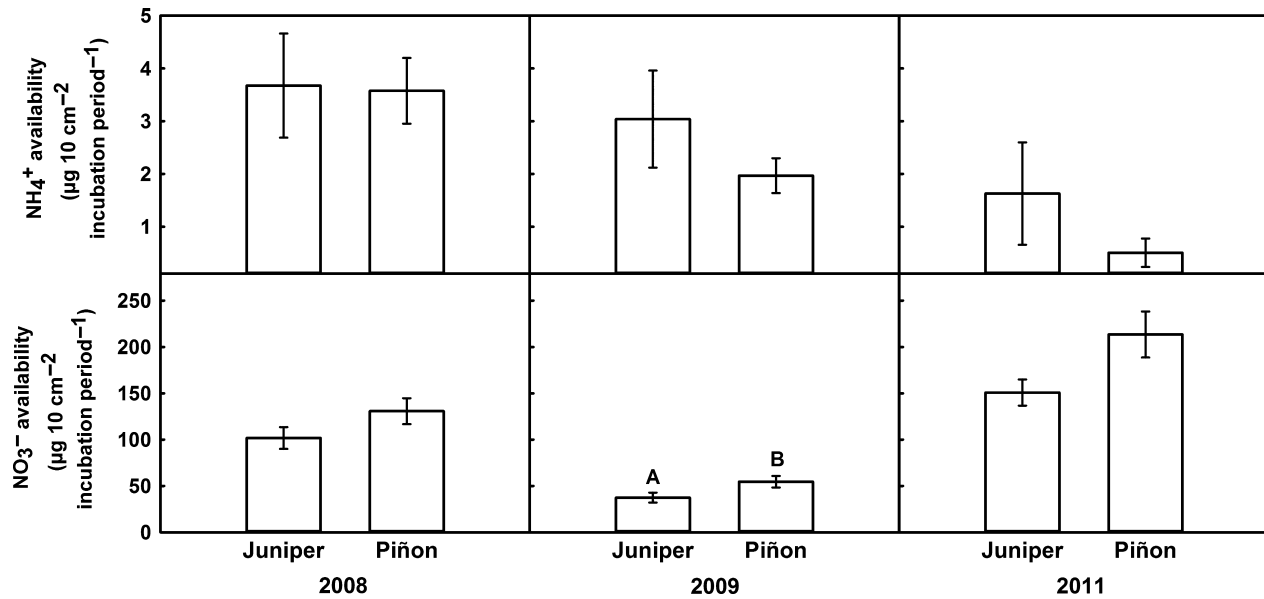


Fig. 3. Mean ammonium and nitrate availability as measured using plant root simulator probes (± 1 standard error) in 2008, monsoon season 2009 and 2011 beneath juniper and piñon. Different letters denote significant differences between piñon and juniper.

net-nitrification rates than did juniper after 30 days of incubation in the laboratory (Fig. 4; $F = 10.33$, $P = 0.01$). Potential net-nitrification after 60 days of laboratory incubation remained higher beneath piñon relative to juniper (see Appendix S4; $F = 6.14$, $P = 0.04$). In addition, potential net-mineralization rates were greater beneath piñon relative to juniper after 30 ($F = 12.50$, $P = 0.01$) and 60 ($F = 7.57$, $P = 0.03$) days of incubation.

MICROBIAL BIOMASS

We found no effect of the precipitation treatments on microbial biomass N, but we did see a trend for increasing microbial biomass as soil volumetric water increased (Table 1; $F = 3.51$, $P = 0.08$). Surprisingly, we found that soil temperature was correlated with lower microbial biomass N. As soil temperatures increased, microbial biomass

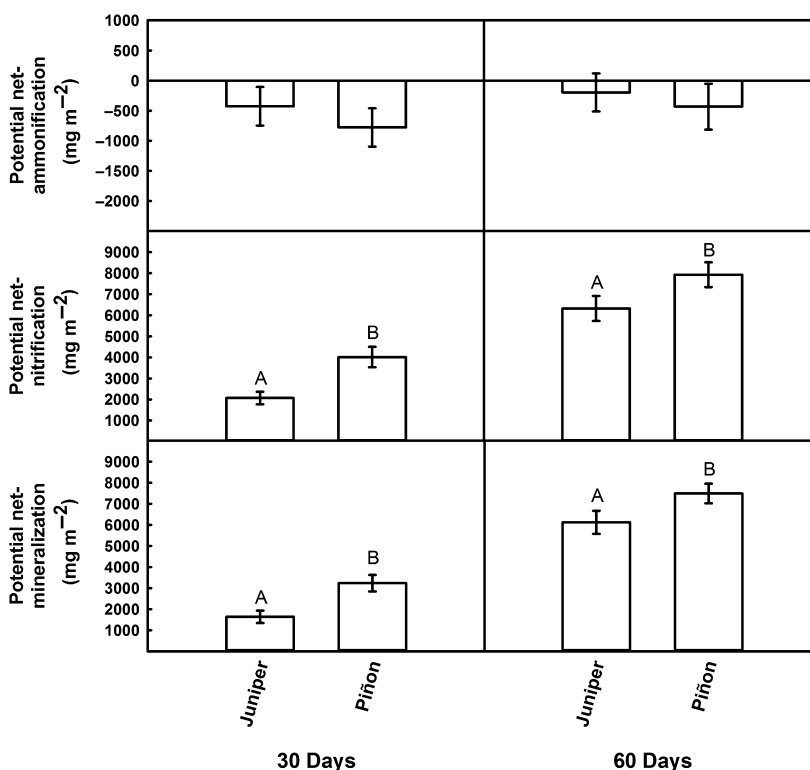


Fig. 4. Mean potential net-ammonification, net-nitrification, and net-mineralization 30 and 60 days after incubation (± 1 standard error) beneath piñon and juniper trees. Different letters denote significant differences between piñon and juniper.

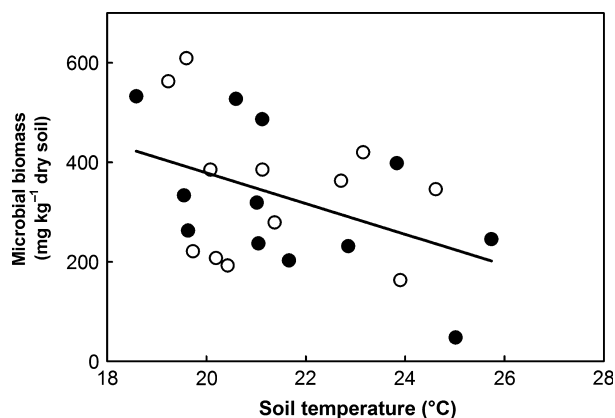


Fig. 5. Microbial biomass N decreased with increasing soil temperature ($R^2 = 0.40$, $F = 4.80$, $P = 0.04$). Black dots denote microbial biomass beneath juniper, while white dots denote microbial biomass beneath piñon.

N decreased (Fig. 5; $R^2 = 0.40$, $F = 4.80$, $P = 0.04$). We did not find an effect of tree type on microbial biomass N (see Appendix S4).

AMOA ABUNDANCE

We found no difference among our treatments in *amoA* abundance, despite its importance in nitrification and a 2× greater potential net-nitrification rate under piñon. In the pre-monsoon season of 2008, we found an average of 2792 gene copies per ng DNA of *amoA* across all treatments (see Appendix S4). This abundance is within the range of previously published results across managed and forested ecosystems (Wallenstein & Vilgalys 2005; Adair & Schwartz 2008; Hayden *et al.* 2010).

Although we found significant differences in N cycling between piñon and juniper soils, these changes were not driven by changes in the abundance of *amoA* gene copy numbers. There were on average 2891 (± 1278 SE) gene copies per ng DNA of *amoA* in juniper soils and 2692 (± 750 SE) gene copies per ng DNA in piñon soils. We did not find a significant effect of tree type on *amoA* abundance (see Appendix S4; $F = 0.02$, $P = 0.90$).

Discussion

Changes in precipitation can shape landscapes by altering the function of the plant and soil community. Here, we used a large-scale precipitation manipulation in a PJ woodland to explore how changes in precipitation altered soil N availability and N mineralization, and if these changes were a result of changes in microbial biomass N or the abundance of bacteria involved in nitrification. Further, we examined if the precipitation-driven changes in ecosystem function would be greater beneath the crowns of piñon, which are more sensitive to changing water availability than juniper (Breshears *et al.* 2009). Interestingly we found that soil N availability decreased

with increasing soil volumetric water content, but N tied up in the biomass of microbes was correlated with soil temperature, not moisture. Additionally, we found that N availability was greater beneath piñon relative to juniper, but saw no differences in microbial biomass N or the abundance of *amoA* between tree types. These results suggest that precipitation change will have both direct and indirect effects on N cycling in this semi-arid woodland.

Consistent with other studies, we found increased levels of soil inorganic N in association with decreased water availability in this semi-arid ecosystem (Jackson *et al.* 1988; Whitford, Martinez-Turanas & Martinez-Meza 1995; Reynolds *et al.* 1999; Augustine & McNaughton 2004; Yahdjian, Sala & Austin 2006; Evans and Burke 2013), but minimal effect of the precipitation treatments on nitrogen mineralization. Because nitrogen mineralization rates did not change with changing precipitation, increases in N availability during drought may be a result of decreased plant and/or microbial N uptake. This accumulation of N results in an asynchrony between N supply and microbial and plant demand where nitrogen is readily available at higher concentrations during the time plants and microbes are relatively inactive (Austin *et al.* 2004). Because N availability often regulates plant productivity in arid ecosystems, immediately following dry periods there may be large increases in plant productivity and bursts of microbial activity when water is no longer limiting (Evans and Burke 2013) resulting in lower soil N available. We aimed to assess microbial community changes that influence N cycling, however, our data indicate that multiple mechanisms, such as changes in plant and microbial uptake and leaching, may all be key drivers of N cycling.

Soil communities mineralize inorganic N from soil organic matter, thus precipitation impacts on the N cycle should be evident within the soil community. Surprisingly, we did not find a significant relationship with soil water availability and microbial biomass N or the abundance of organisms involved in nitrification. We measured these two factors during the dry, pre-monsoon season before water was added to the precipitation addition plots, thus low water availability may have limited microbial activity across all of the treatments. Interestingly, during this extremely dry season, we found that microbial biomass N decreased with increasing soil temperatures leading us to conclude that both soil moisture and temperature may limit microbial activity in this ecosystem. Other studies found that increased soil temperatures can decrease microbial biomass N and microbial activity measured as microbial respiration (Zhang *et al.* 2013). Because microbial biomass or the abundance of *amoA* genes did not change, differences in N cycling across the treatments may have been mediated by broad shifts in microbial community composition (Cregger *et al.* 2012). Alternatively, both bacteria and archaea contain *amoA* genes and this study only assessed bacterial *amoA* gene abundance. Because we did

not find differences in bacterial *amoA* gene abundance, we infer that nitrifying archaea may play a larger role than bacteria in this ecosystem. Adair & Schwartz (2008) found that ammonia-oxidizing archaea had increased abundance relative to ammonia-oxidizing bacteria across a range of arid ecosystems including PJ woodlands.

Our results suggest that plant uptake of N, leaching of N when soil water increases, as is evident during the monsoon season, or shifts in the amount of N in microbial biomass as temperatures increase could be driving the changes we observed in the N cycle. Similar to other studies in arid and semi-arid ecosystems (Yahdjian, Sala & Austin 2006), we found almost 2× more available nitrate over 4 years in the drought plots compared with water addition plots. Decreased photosynthesis and transpiration during drought are well documented (Boyer 1982; Flexas & Medrano 2002; Chaves, Maroco & Pereira 2003; Griffin, Ranney & Pharr 2004), as is increased N uptake in association with irrigation (Nilsson & Wiklund 1994). Decreased water uptake by plants, as well as reduced leaching during drought periods, could allow an accumulation of soil N (Stark & Firestone 1995; Austin *et al.* 2004). Consistent with other studies, transpiration of piñon and juniper was significantly lower in drought plots and higher in irrigation plots, relative to the controls in this experiment (Pangle *et al.* 2012; Plaut *et al.* 2012). Lowered N uptake during drought could lead to the observed increase in N availability in the water removal treatments.

We found significantly more N beneath piñon relative to juniper across all treatments. Unlike piñon, juniper trees continued water uptake during extreme drought (Pangle *et al.* 2012; Plaut *et al.* 2012; also see West *et al.* 2008 for another example) suggesting they took up more N than piñon thus reducing N availability in the soil beneath their crowns. Because no other vegetation inhabits the crown area, nitrate can accumulate beneath piñon in the soil over time. Additionally, based on a previous study at this site (Cregger *et al.* 2012), we know that microbial community richness and abundance are significantly different between these two trees, which may explain the large differences in N cycling.

Conclusion

Globally, climate-induced tree mortality is becoming increasingly prevalent and widespread (Allen *et al.* 2010). These alterations in plant community composition may have large consequences on associated biotic communities and on ecosystem processes. Our data indicate that drier conditions shift the N cycle via changes in availability. Further, the two dominant tree species in the ecosystem, which have differential mortality rates with drought, cycle or uptake N differently. Thus, differential mortality of piñon may have large implications for landscape-level nitrogen cycling. As this ecosystem transitions to becoming juniper dominated, nitrogen availability across the landscape may decrease.

Acknowledgements

We thank J. Hill and E. Yepez for their help with sample collection and processing. We are grateful to G. Newman, E. Austin, L. Souza and anonymous reviewers for their valuable comments. M. Cregger was supported by the Graduate Research for the Environment Fellowship sponsored by the Department of Energy Global Change Education Program and The University of Tennessee. The field experiment was supported by a grant from the Department of Energy, Office of Science (BER) and start-up funds to ATC from the University of Tennessee.

References

- Adair, K.L. & Schwartz, E. (2008) Evidence that ammonia-oxidizing archaea are more abundant than ammonia-oxidizing bacteria in semiarid soils of northern Arizona, USA. *Microbial Ecology*, **56**, 420–426.
- Adams, M.A. & Attiwill, P.M. (1986) Nutrient cycling and nitrogen mineralization in eucalypt forests of southeastern Australia v2 indexes of nitrogen mineralization. *Plant and Soil*, **92**, 341–362.
- Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M. *et al.* (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, **259**, 660–684.
- Allison, I., Bindoff, N.L., Bindschadler, R.A., Cox, P.M., de Noblet, N., England, M.H. *et al.* (2009) *The Copenhagen Diagnosis: Updating the World on the Latest Climate Science*, 60 pp. The University of New South Wales Climate Change Research Centre (CCRC), Sydney, Australia.
- Augustine, D.J. & McNaughton, S.J. (2004) Temporal asynchrony in soil nutrient dynamics and plant production in a semiarid ecosystem. *Ecosystems*, **7**, 829–840.
- Austin, A.T. (2002) Differential effects of precipitation on production and decomposition along a rainfall gradient in Hawaii. *Ecology*, **83**, 328–338.
- Austin, A.T. & Vitousek, P.M. (1998) Nutrient dynamics on a precipitation gradient in Hawaii. *Oecologia*, **113**, 519–529.
- Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U. *et al.* (2004) Water pulses and biogeochemical cycles in arid and semi-arid ecosystems. *Oecologia*, **141**, 221–235.
- Binkley, D. (1989) The components of nitrogen availability assessments in forest soils. *Advanced Soil Science*, **10**, 57–112.
- Boyer, J.S. (1982) Plant productivity and environment. *Science*, **218**, 443–448.
- Breshears, D.D., Cobb, N.S., Rich, P.M., Price, K.P., Allen, C.D., Balice, R.G. *et al.* (2005) Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences*, **102**, 15144–15148.
- Breshears, D.D., Myers, O.B., Meyer, C.W., Barnes, F.J., Zou, C.B., Allen, C.D. *et al.* (2009) Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. *Frontiers in Ecology and the Environment*, **7**, 185–189.
- Brown, M.B. & Forsythe, A.B. (1974) Robust tests for the equality of variances. *Journal of the American Statistical Association*, **69**, 364–367.
- Chapman, S.K., Hart, S.C., Cobb, N.S., Whitham, T.G. & Koch, G.W. (2003) Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. *Ecology*, **84**, 2867–2876.
- Chaves, M.M., Maroco, J.P. & Pereira, J.S. (2003) Understanding plant responses to drought – from genes to the whole plant. *Functional Plant Biology*, **30**, 239–264.
- Classen, A.T., DeMarco, J., Hart, S.C., Whitham, T.G., Cobb, N.S. & Koch, G.W. (2006) Impacts of herbivorous insects on decomposer communities during the early stages of primary succession in a semi-arid woodland. *Soil Biology and Biochemistry*, **38**, 972–982.
- Classen, A.T., Chapman, S.K., Whitham, T.G., Hart, S.C. & Koch, G.W. (2007a) Genetic-based plant resistance and susceptibility traits to herbivory influence needle and root litter nutrient dynamics. *Journal of Ecology*, **95**, 1181–1194.
- Classen, A.T., Overby, S.T., Hart, S.C., Koch, G.W. & Whitham, T.G. (2007b) Season mediates herbivore effects on litter and soil microbial abundance and activity in a semi-arid woodland. *Plant and Soil*, **295**, 217–227.
- Classen, A.T., Chapman, S.K., Whitham, T.G., Hart, S.C. & Koch, G.W. (2013) Long-term insect herbivory slows soil development in an arid ecosystem. *Ecosphere*, **4**, 1–14.

- Cregger, M.A., Schadt, C.W., McDowell, N.G., Pockman, W.T. & Classen, A.T. (2012) Response of the soil microbial community to changes in precipitation in a semi-arid ecosystem. *Applied and Environmental Microbiology*, **78**, 8587–8594.
- Davidson, E.A., Eckert, R.W., Hart, S.C. & Firestone, M.K. (1989) Direct extraction of microbial biomass nitrogen from forest and grassland soils of California. *Soil Biology and Biochemistry*, **21**, 773–779.
- Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R. & Mearns, L.O. (2000) Climate extremes: observations, modeling, and impacts. *Science*, **289**, 2068–2074.
- Evans, S.E. & Burke, I.C. (2013) Carbon and nitrogen decoupling under an 11-year drought in the shortgrass steppe. *Ecosystems*, **16**, 20–33.
- Flexas, J. & Medrano, H. (2002) Drought-inhibition of photosynthesis in C-3 plants: stomatal and non-stomatal limitations revisited. *Annals of Botany*, **89**, 183–189.
- Gitlin, A.R., Sthultz, C.M., Bowker, M.A., Stumpf, S., Paxton, K.L., Kennedy, K. *et al.* (2006) Mortality gradients within and among dominant plant populations as barometers of ecosystem change during extreme drought. *Conservation Biology*, **20**, 1477–1486.
- Greenland, D., Goodin, D.G. & Smith, R.C. (2003) *Climate Variability and Ecosystem Response at Long-Term Ecological Research Sites*. Oxford University Press, New York, NY.
- Griffin, J.J., Ranney, T.G. & Pharr, D.M. (2004) Heat and drought influence photosynthesis, water relations, and soluble carbohydrates of two ecotypes of redbud (*Cercis canadensis*). *Journal of the American Society for Horticultural Science*, **129**, 497–502.
- Hart, S.C., Nason, G.E., Myroid, D.D. & Perry, D.A. (1994) Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection. *Ecology*, **75**, 880–891.
- Haubensak, K.A., Hart, S.C. & Stark, J.M. (2002) Influences of chloroform exposure time and soil water content on C and N release in forest soils. *Soil Biology and Biochemistry*, **34**, 1549–1562.
- Hayden, H.L., Drake, J., Imhof, M., Oxley, A.P.A., Norng, S. & Mele, P.M. (2010) The abundance of nitrogen cycle genes amoA and nifH depends on land-uses and soil types in South-Eastern Australia. *Soil Biology and Biochemistry*, **42**, 1774–1783.
- Hobbie, S.E. (1992) Effects of plant-species on nutrient cycling. *Trends in Ecology and Evolution*, **7**, 336–339.
- Jackson, L.E., Strauss, R.B., Firestone, M.K. & Bartolome, J.W. (1988) Plant and soil-nitrogen dynamics in a California annual grassland. *Plant and Soil*, **110**, 9–17.
- Jobaggy, E.G. & Jackson, R.B. (2001) The distribution of soil nutrients with depth: global patterns and the imprints of plants. *Biogeochemistry*, **53**, 51–77.
- Kardol, P., Cregger, M.A., Company, C.E. & Classen, A.T. (2010) Soil ecosystem functioning under climate change: plant species and community effects. *Ecology*, **91**, 767–781.
- Klopatek, J.M. (1987) Nitrogen mineralization and nitrification in mineral soils of pinyon-juniper ecosystems. *Soil Science Society of America Journal*, **51**, 453–457.
- Klopatek, J.M., Conant, R.T., Francis, J.M., Malin, R.A., Murphy, K.L. & Klopatek, C.C. (1998) Implications of patterns of carbon pools and fluxes across a semiarid environmental gradient. *Landscape and Urban Planning*, **39**, 309–317.
- Knapp, A.K., Beier, C., Briske, D.D., Classen, A.T., Luo, Y., Reichstein, M. *et al.* (2008) Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience*, **58**, 811–821.
- Kreuzwieser, J. & Gessler, A. (2010) Global climate change and tree nutrition: influence of water availability. *Tree Physiology*, **30**, 1221–1234.
- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T. *et al.* (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist*, **178**, 719–739.
- Mitchell, R.J., Campbell, C.D., Chapman, S.J. & Cameron, C.M. (2010) The ecological engineering impact of a single tree species on the soil microbial community. *Journal of Ecology*, **98**, 50–61.
- Mueller, R.C., Scudder, C.M., Porter, M.E., Trotter, R.T., Gehring, C.A. & Whitham, T.G. (2005) Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. *Journal of Ecology*, **93**, 1085–1093.
- Murphy, K.L., Klopatek, J.M. & Klopatek, C.C. (1998) The effects of litter quality and climate on decomposition along an elevational gradient. *Ecological Applications*, **8**, 1061–1071.
- Nearing, M.A., Jetten, V., Baffaut, C., Cerdan, O., Couturier, A., Hernandez, M. *et al.* (2005) Modeling response of soil erosion and runoff to changes in precipitation and cover. *Catena*, **61**, 131–154.
- Nilsson, L.O. & Wiklund, K. (1994) Nitrogen uptake in a Norway spruce stand following ammonium-sulfate application, fertigation, irrigation, drought, and nitrogen-free fertilization. *Plant and Soil*, **164**, 221–229.
- Padien, D.J. & Lajtha, K. (1992) Plant spatial pattern and nutrient distribution in pinyon-juniper woodlands along an elevational gradient in Northern New-Mexico. *International Journal of Plant Sciences*, **153**, 425–433.
- Pangle, R.E., Hill, J.P., Plaut, J.A., Yepez, E.A., Elliot, J.R., Gehres, N. *et al.* (2012) Methodology and performance of a rainfall manipulation experiment in a pinon-juniper woodland. *Ecosphere*, **3**, 28.
- Plaut, J.A., Yepez, E.A., Hill, J., Pangle, R., Johnson, J., Sperry, J.S. *et al.* (2012) Hydraulic limits preceding mortality in a pinon-juniper woodland under experimental drought. *Plant, Cell, and Environment*, **35**, 1601–1617.
- Qian, P. & Schoenau, J.J. (2002) Practical applications of ion exchange resins in agriculture and environmental soil research. *Canadian Journal of Soil Science*, **82**, 9–21.
- Reid, K.D., Wilcox, B.P., Breshears, D.D. & MacDonald, L. (1999) Runoff and erosion in a pinon-juniper woodland: influence of vegetation patches. *Soil Science Society of America Journal*, **63**, 1869–1879.
- Reynolds, J.F., Virginia, R.A., Kemp, P.R., de Soyza, A.G. & Tremmel, D.C. (1999) Impact of drought on desert shrubs: effects of seasonality and degree of resource island development. *Ecological Monographs*, **69**, 69–106.
- Robertson, G.P., Coleman, D.C., Bledsoe, C.S. & Sollins, P. (1999) *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press, Inc., Oxford, NY.
- Rodriguez, A., Duran, J., Covelo, F., Fernandez-Palacios, J.M. & Gallardo, A. (2011) Spatial pattern and variability in soil N and P availability under the influence of two dominant species in a pine forest. *Plant and Soil*, **345**, 211–221.
- Rothhauwe, J.H., Witzel, K.P. & Liesack, W. (1997) The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology*, **63**, 4704–4712.
- Sala, O.E., Parton, W.J., Joyce, L.A. & Lauenroth, W.K. (1988) Primary production of the central grassland region of the United-States. *Ecology*, **69**, 40–45.
- Sala, O.E., Jackson, R.B., Mooney, H.A. & Howarth, R.W. (2000) *Methods in Ecosystem Science*. Springer-Verlag New York Inc, New York, NY.
- Schimel, J., Balsler, T.C. & Wallenstein, M. (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology*, **88**, 1386–1394.
- Schlesinger, W.H., Raikes, J.A., Hartley, A.E. & Cross, A.E. (1996) On the spatial pattern of soil nutrients in desert ecosystems. *Ecology*, **77**, 364–374.
- Seager, R., Ting, M.F., Held, I., Kushnir, Y., Lu, J., Vecchi, G. *et al.* (2007) Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, **316**, 1181–1184.
- Shaw, J.D., Steed, B.E. & DeBlander, L.T. (2005) Forest Inventory and Analysis (FIA) annual inventory answers the question: what is happening to pinyon-juniper woodlands? *Journal of Forestry*, **103**, 280–285.
- Stark, J.M. & Firestone, M.K. (1995) Mechanisms for soil-moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology*, **61**, 218–221.
- Sthultz, C.M., Gehring, C.A. & Whitham, T.G. (2009) Deadly combination of genes and drought: increased mortality of herbivore-resistant trees in a foundation species. *Global Change Biology*, **15**, 1949–1961.
- Throop, H.L. & Archer, S.R. (2008) Shrub (*Prosopis velutina*) encroachment in a semidesert grassland: spatial-temporal changes in soil organic carbon and nitrogen pools. *Global Change Biology*, **14**, 2420–2431.
- Wallenstein, M.D. & Vilgalys, R.J. (2005) Quantitative analyses of nitrogen cycling genes in soils. *Pedobiologia*, **49**, 665–672.
- Walvoord, M.A., Phillips, F.M., Stonestrom, D.A., Evans, R.D., Hartsough, P.C., Newman, B.D. *et al.* (2003) A reservoir of nitrate beneath desert soils. *Science*, **302**, 1021–1024.
- Weltzin, J.F., Loik, M.E., Schwinning, S., Williams, D.G., Fay, P.A., Haddad, B.M. *et al.* (2003) Assessing the response of terrestrial ecosystems to potential changes in precipitation. *BioScience*, **53**, 941–952.
- West, A.G., Hultine, K.R., Sperry, J.S., Bush, S.E. & Ehleringer, J.R. (2008) Transpiration and hydraulic strategies in a pinon-juniper woodland. *Ecological Applications*, **18**, 911–927.
- Whitford, W.G., Martinez-Turanzas, G. & Martinez-Meza, E. (1995) Persistence of desertified ecosystems: explanations and implications. *Environmental Monitoring and Assessment*, **37**, 319–332.

- Wilcox, B.P. (1994) Runoff and erosion in intercanopy zones of pinyon-juniper woodlands. *Journal of Range Management*, **47**, 285–295.
- Xiang, S.R., Doyle, A., Holden, P.A. & Schimel, J.P. (2008) Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. *Soil Biology and Biochemistry*, **40**, 2281–2289.
- Yahdjian, L., Sala, O. & Austin, A.T. (2006) Differential controls of water input on litter decomposition and nitrogen dynamics in the Patagonian steppe. *Ecosystems*, **9**, 128–141.
- Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D. & Tilman, D. (2003) Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology*, **84**, 2042–2050.
- Zhang, N., Weixing, L., Haijun, Y., Xingjun, Y., Gutknecht, J.L.M., Zhang, Z. *et al.* (2013) Soil microbial responses to warming and increased precipitation and their implications for ecosystem C cycling. *Oecologia*, **173**, 1125–1142.

Received 16 October 2013; accepted 28 March 2014
Handling Editor: Shuli Niu

Supporting Information

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

Appendix S1. Date and amount of water added to the water addition plots from 2008 to 2011.

Appendix S2. Mean volumetric water content beneath juniper and piñon crowns across treatments in June, July and August 2009 (published in Pangle *et al.* 2012).

Appendix S3. *F* statistics and *P* values (in parentheses) for the interactive effect of precipitation × plant species and the main effects of precipitation and plant species on N availability, potential net-N mineralization, net-N mineralization, microbial biomass N and amoA abundance.

Appendix S4. Mean (± 1 SE) for N availability, potential net-N mineralization, net-N mineralization, microbial biomass N and amoA abundance divided by treatment beneath juniper and piñon.