Reductions in tree performance during hotter droughts are mitigated by shifts in nitrogen cycling

Short running title: Mitigation of hotter droughts

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Abstract

Climate warming should result in hotter droughts of unprecedented severity in this century. Such droughts have been linked with massive tree mortality and data suggest warming interacts with drought to aggravate plant performance. Yet, how forests will respond to hotter droughts remains unclear, as does the suite of mechanisms trees use to deal with hot droughts. We used an ecosystem-scale manipulation of precipitation and temperature on piñon pine (*Pinus edulis*) and juniper (*Juniperus monosperma*) trees to investigate nitrogen (N) cycling-induced mitigation processes related to hotter droughts. We found that while negative impacts on plant carbon and water balance are manifest after prolonged drought, performance reductions were not amplified by warmer temperatures. Rather, increased temperatures for five years stimulated soil N cycling under piñon trees and modified tree N allocation for both species, resulting in mitigation of hotter drought impacts on tree water and carbon functions. These findings suggest that adjustments in N cycling are likely after multi-year warming conditions and that such changes may buffer reductions in tree performance during hotter droughts. The results highlight our incomplete understanding of trees' ability to acclimate to climate change, raising fundamental questions about the resistance potential of forests to long-term, compound climatic stresses.

Keyword index: acclimation, climate change, *Juniperus monosperma*, forest ecosystems, ^{15}N, nitrogen allocation, *Pinus edulis*, warming.

Introduction

Great concern has emerged in recent years over large-scale climate-induced reductions in forest growth and survival. In particular, the increased frequency of droughts occurring at warmer temperatures (Weiss et al., 2009) (also called hotter droughts or global-change-type droughts) has been linked to massive tree mortality in forests around the globe (Breshears et al., 2005, 2009; Van Mantgem et al., 2009; Anderegg et al., 2013; Allen et al., 2015). Compared to typical droughts (defined here as periods of low precipitation and thus reduced soil water availability), hotter droughts are characterized by soil drought superimposed on atmospheric drought as high temperature increases atmospheric evaporative demand (Williams et al., 2013). In already dry and warm regions, the additive effects of warming and soil moisture limitation are therefore expected to exacerbate reductions in tree performance relative to ambient droughts, and bring forests closer to or beyond their tolerance thresholds (McDowell et al., 2015; Adams et al., 2017).

Because of the critical role of forests in climate regulation, their ability to maintain processes such as photosynthesis and transpiration at sustainable levels during hotter droughts has become a major research frontier (Breshears et al., 2009; Allen et al., 2015). A warming atmosphere is expected to amplify soil moisture limitation impacts on plants during hotter droughts through direct stimulation of evapotranspiration (Williams et al., 2013; McDowell et al., 2015). However, we rarely consider the stimulating effect of temperature on biogeochemical cycles beyond carbon and water, particularly on soil nutrient cycling and plant uptake (Gessler et al., 2017). Assuming soil moisture does not drop below the lowest thresholds for microbial activity during hotter droughts, long-term temperature increase has the potential to stimulate soil microbial activity and thus increase rates of nutrient cycling (Rustad et al., 2001; Sardans et al., 2008). Warming could then theoretically have a positive feedback on plant performance by stimulating nutrient mineralization and thus nutrient

availability and uptake by plants. On the contrary, if soil moisture conditions are extremely dry, warming-related benefits for plant nutrition may not occur due to a drought-induced reduction in microbial activity (Cregger et al., 2014). While tree nutritional status is known to influence tree responses to drought stress via effects on photosynthesis and water use rates (see Gessler et al., 2017 for potential nitrogen [N]-related mechanisms impacting drought responses of trees), effects of nutritional status on tree performance (e.g., photosynthesis, water use, growth, or survival) during hotter droughts remains unexplored (Gessler et al., 2017; Kreuzwieser & Gessler, 2010).

In addition to potential shifts in soil nutrient cycling, plants can undergo compensatory shifts in their physiology to maintain physiological functions in response to environmental variability (Nicotra et al., 2010; Franks et al., 2013). For instance, plants can shift allocation of resources such as carbon (C) or N among different plant tissues and processes in response to varying climate (De Boeck et al., 2008; Ruehr et al., 2009; Hommel et al., 2016). Under drier climate, previous work highlighted that trees allocate a higher proportion of C and N belowground to support root integrity and function, and maintain efficient water uptake during droughts (Bloom et al., 1985; Poorter et al., 2012; Brunner et al., 2015). Yet, whether such compensatory shifts in resource allocation and use, and their associated feedbacks to plant processes, could help buffer the additive adverse effects of warming and drought on plant performance remains largely theoretical (Gessler et al., 2017).

To address the potential for mitigation of tree performance during hotter droughts, particularly the role of nutrient-based soil and plant mechanisms, we used an experimental manipulation of precipitation and temperature on mature trees. The forest type, piñon-juniper woodlands, is one of the most widely distributed forests in the southwestern USA (West et al., 1999) and has been identified as particularly threatened by hotter droughts in the decades to come (Allen et al., 2010). Different vulnerabilities to drought in cohabiting piñon pine and

juniper trees, including water use regulation (relatively isohydric *vs*. anisohydric species, respectively) (Garcia-Forner et al., 2016) and rooting depths (shallow *vs*. deep rooted species, respectively) (Grossiord et al., 2017a), have made these woodlands a useful system for understanding how trees with contrasting survival strategies respond to drought (McDowell et al., 2008). These recent studies have highlighted a functional and structural advantage of juniper over piñon during droughts, suggesting lower mortality risk during upcoming hot droughts. This semi-arid system is usually considered N-limited because of low inherent soil N availability (Bobbink *et al*., 2010), thus we could expect shifts in soil and plant N cycling to have relatively large impacts on plant functions in this ecosystem. Experimental trees (i.e., piñon pine and one-seed juniper) were exposed to warming ($\approx$ +4 °C), drought (-45% incoming precipitation), or both (warming + drought; i.e., hotter drought conditions) to assess the occurrence of N-related mitigation processes and the drivers of forest responses to future climate. Measurements were conducted after the trees had been subjected to climate manipulation for five years, allowing compensatory shifts to occur and providing more realistic responses compared to short-term studies (Leuzinger et al., 2011). Measurements included assessment of fundamental soil N cycling processes (potential ammonification and nitrification rates, soil microbial biomass C and N concentrations, and "available" soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations), internal plant N allocation strategy (^{15}N incorporation in fine roots, coarse roots, twigs, and leaves using a ^{15}N-labeling approach), and three major physiological processes related to tree C fixation and water balance: leaf-level photosynthesis, canopy-level stomatal conductance and predawn leaf water potential. We hypothesized that:

(1) multi-year drought would reduce rates of soil N cycling while warming would result in increased rates of soil N cycling. Increased rates of soil N cycling would also occur under hotter drought conditions if soil moisture did not drop below the lowest threshold for

microbial activity.

(2) Trees would increase their N allocation to belowground compartments in response to prolonged drought and warming, but piñon would allocate a higher N proportion to roots compared to juniper because of higher drought stress exposure.

(3) Increased soil N cycling and N allocation belowground in response to hotter droughts would result in smaller reductions in photosynthesis, stomatal conductance and leaf water potential relative to drought acting alone.

Material and methods

Site description and manipulative treatments

The study was conducted at the Los Alamos Survival-Mortality (SUMO) experiment located in Los Alamos County, New Mexico (35.49°N, 106.18°W, 2175 m a.s.l). The site is characterized by Hackroy clay loam soils with a typical profile of 0-10 cm of sandy loam (60-80% sand, <20% clay, <20% silt), 10-55 cm of clay loam (55-80% clay, 20-40% silt, 20-40% sand) and bed rock derived from volcanic tuff (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, http://websoilsurvey.nrcs.usda.gov) with soil depths of approx. 55 cm or less. The vegetation is dominated by piñon pine (*Pinus edulis* Engelm.) and one-seed juniper (*Juniperus monosperma* Engelm. Sarg.). Grasses and other tree species such as Gambel oak (*Quercus gambelli* Nutt.) can be found in inter-canopy spaces but they do not contribute significantly to total stand biomass (< 5%). The site covers an area of approximately 0.4 ha. The climate is semi-arid, with a mean annual temperature of 10.1°C (21.3°C during the growing season) and a mean annual precipitation of 360 mm, with about 50% falling during the North American

Monsoon season from July to August (1987-2016 mean, Los Alamos Weather Machine, http://environweb.lanl.gov/weathermachine). The active growing season for piñon pine and juniper takes place between March and September with a significant dry period in June followed by a recovery period in July, August and September (Grossiord et al., 2017b).

In June 2012, active warming and passive precipitation reduction infrastructures were installed on site. For warming treatments, 3-7 m tall (depending on tree height) open-top chambers covered with thermoplastic polymer sheets (Makloron SL Polycarbonate Sheet, Sheffield Plastics, Sheffield, MA, USA) were used to chronically increase air temperature by ca. 4.0°C (Figs. 1 & S1). Atmospheric temperature and relative humidity were measured in all chambers using CS215 Campbell sensors (Campbell Scientific, Logan, UT, USA) at two positions (1m height and 2/3 canopy height) and were used for controlling industrial-scale air-conditioning units located outside and connected to the chambers using aluminum ventilation pipes (Fig. S2). The units regulated chamber temperature by blowing either warm or cool air inside the chambers as needed to keep the chamber temperature within 0.5 degrees of the target. For drought manipulation, precipitation exclusion structures (total area of 500 m², ca. 1.2 m above ground) consisting of clear polymer troughs that intercepted and reduced incoming precipitation to the soil by 45% (by area) were installed. Five trees of each species were randomly assigned to each of the five treatments: 1) ambient, for trees under ambient temperature and precipitation; 2) chamber control, for trees situated inside chambers with temperature regulated to match ambient temperature; 3) warming, for trees inside chambers where the temperature was maintained at 4.0°C above ambient temperature; 4) drought, for trees located within the precipitation exclusion structure; and 5) warming+drought, for trees where both treatments were applied simultaneously, in other words, the hotter drought treatment. Chamber footprints ranged from 7 m² to 20 m² and contained between one and five trees located at a minimum distance of 1.5 m from the chamber boundary ($n = 12$ chambers).

The selected trees in the drought treatment were located at least 10 m from the border of the precipitation exclusion structure (equivalent to two times the height of the tallest tree in the treatment). Mean tree age was 56 ± 5 years and 79 ± 7 years for piñon and juniper, respectively (estimated from tree cores on nearby trees). Ambient meteorological parameters were measured continuously by two weather stations (Climatronics, Bohemia, NY, USA) at the site (Fig. S2). Soil water content (%) and soil temperature were measured every 10 min at the base of each tree between 0 and 15 cm depth using TDR probes (CS625, Campbell Scientific, Logan, UT, USA) and homemade thermocouples (Fig. S1, S2 & Table S1).

Previous studies conducted over the 2012-2016 period found no differences between trees in the ambient and chamber control treatments for both climatic and edaphic conditions (air temperature, relative humidity, vapor pressure deficit, air temperature, Table S1) and tree physiological responses such as photosynthesis, transpiration, phenology, foliar structure, or water uptake depth (Adams et al., 2015; Garcia-Forner et al., 2016; Grossiord et al., 2017a, b, c), suggesting no indirect effect of the chambers. To avoid repetitive destructive measurements, we only used trees in the ambient treatment as controls for the purpose of this study (n = 40 trees, i.e. 5 trees per treatment and per species). No tree mortality occurred over the full study period (2012-2016).

Soil biogeochemistry

On August 19, 2016, soil samples were collected around each focal tree for assessment of components of soil biogeochemical cycling. Two soil cores were collected from the drip line of each tree using a 3 cm diameter soil corer to 20 cm depth (i.e., location of the majority of soil microbes and roots). Each core was split into 0-10 cm and 10-20 cm depth segments composited by depth for each tree. Soil samples were returned to the laboratory and

processed the same day. Soils were sieved to 4 mm and were analyzed for extractable inorganic N concentrations, potential N mineralization and nitrification rates, microbial biomass C and N concentrations, total soil N concentrations, and total, inorganic, and organic C concentrations. Extractable inorganic N concentrations (NO_3^- and NH_4^+) were determined by adding 35 ml of 2 N KCl to ~ 8 g of soil, shaking for 1 hour, and incubating at room temperature for 18 hours. Soil extracts were vacuum filtered using Whatman #1 filter paper, and NO_3^- and NH_4^+ concentrations were determined using a SmartChem autoanalyzer (Westco, Inc., Brookfield, CT, USA) (Robertson et al., 1999). Potential N mineralization and nitrification rates were assessed by moistening soil to 10% of water holding capacity and incubating at 22°C for seven days. Samples were analyzed for NO_3^- and NH_4^+ as described above: potential N mineralization rates were estimated as the difference in inorganic N (the sum of NO_3^- and NH_4^+) between time points (i.e., before and after incubation), accounting for incubation duration. Potential nitrification rates were calculated in the same way using only NO_3^-. Microbial biomass C and N concentrations were determined by using two soil sub-samples. For the first, ~8 g of soil was extracted in 35 ml of 0.5 M K_2SO_4, shaken for one hour, and filtered through Whatman #1 filter paper. For the second sub-sample, ~8 g of soil was placed into a 125 ml Erlenmeyer flask with 2 ml of ethanol-free chloroform. Flasks were tightly stoppered and incubated in the dark for 20 hours. Samples were extracted with 0.5 M K_2SO_4 as described above, and both sets of solutions were analyzed for C and N on a total organic C/total dissolved N analyzer (TOC/TDN; Shimadzu, Inc., Columbia, MD, USA). The difference in concentrations between chloroform-fumigated and unfumigated samples was used to estimate microbial biomass C and N. Total soil C and N concentrations were determined using an elemental analyzer (Elementar Americas, Mt Laurel, NJ, USA) on soils dried at 60 °C for 48 hours. Many dryland soils contain carbonates and thus we also assessed soil inorganic C concentrations using a modified Pressure-Calcimeter method (Sherrod et al.,

2002). Organic C was calculated as the difference between total C and inorganic C concentrations. Soil moisture was determined by weighing and drying soils at 105 °C for 48 hours. For all measured parameters, average values from the two soil depths (0-10 and 10-20 cm) per tree were used for data analyses.

Tree N allocation strategy

To determine internal N allocation patterns in tree compartments involved in important supporting functions such as photosynthesis, reproduction, storage, primary growth and water uptake (i.e. in the leaves, twigs, fine- and coarse roots) a $^{15}NO_3^{-15}NH_4^+$ solution was applied to the soil surface around all focal trees. We selected the amount of $^{15}NO_3^{-15}NH_4^+$ applied to be high enough to avoid strong dilution in the soil and plant material but we also limited $^{15}NO_3^{-15}NH_4^+$ amounts to avoid fertilization effects. Following recommendations from previous labeling experiments, we applied a total of 75 g of the $^{15}NO_3^{-15}NH_4^+$ solution (corresponding to ≈160 mg/m^2 of soil surface) in order to increase soil extractable N concentrations by no more than 15% (Fotelli et al., 2004). This calculation was based on mean NO_3^- (0.44 μg/g of soil) and NH_4^+ (0.72 μg/g of soil) concentrations in the topsoil (0-25cm) at the site (n= 34 measurements throughout the site). Based on root distribution assessments conducted on nearby piñon and juniper trees, we estimated that the most active fine roots would be distributed within a 25 m^2 area around the trunk (Foxx & Tierney, 1987). The solution was thus applied to that area around each tree to optimize root uptake. The $^{15}NO_3^{-15}NH_4^+$ solution was diluted in 1500 L of dechlorinated potable water before being applied uniformly on August 23, 2016 (i.e. two days after the soil biogeochemistry measurements). Two liters of the diluted solution was applied per m^2 of soil in order to represent a very small precipitation event (2 mm) and avoid treatment disturbances. Although differences in rooting structure between species could affect the amount of $^{15}NO_3^{-15}NH_4^+$

solution taken up by the trees, we don't expect this to affect our findings as we did not intend to compare the actual amount of N taken up by trees but instead the allocation of N inside the different tree compartments.

To determine the natural ^{15}N abundance in the soil, soil cores were collected one day before applying the $^{15}NO_3^{-15}NH_4^+$ solution using a hammer drill at five positions close to the experimental trees (n = 5 cores per treatment) over the whole soil profile. The soil cores were then separated in three depths (0-10, 10-20, 20-55 cm). Similarly, tree tissues (current-year leaves, twigs, fine- and coarse roots) were randomly collected at two heights (samples from 1/3 and 2/3 of the canopy were pooled together), one depth (first 1/3 of the twig length corresponding to sun exposed leaves and twigs) and one cardinal direction (south facing) from each focal tree on the same date to determine their natural ^{15}N abundance. Thereafter, soil cores and tree tissues were collected at five dates following the solution application over a period of two months (+ 2 days, + 7 days, + 14 days, + 30 days and + 60 days) to allow sufficient solution uptake by trees. All samples were dried at 65 °C (tree tissues) and 100 °C (soil samples) for a minimum of 48 h, before being bulked (i.e., for samples originating from the same compartment) and ground to a fine powder. Between 2-20 mg (tree tissues) and 50-64 mg (soil samples) of the powdered material was placed into tin capsules for analysis of nitrogen isotope composition (δ^{15}N, ‰) and total N (%). The samples were sent to the Stable Isotope Facility of UC Davis (CA, USA) and analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Specific ^{15}N incorporation (μmol N g^{-1} dry weight) in each tissue and at each date following application of the solution was calculated using the following equation (Fotelli et al., 2004):

$$^{15}\text{N incorporation} = \frac{(15N_t - 15N_c) \times [N] \times 10^4}{MW}$$

where $^{15}N_t$ is the ^{15}N abundance (atom %) in tree tissues after applying the $^{15}NO_3^{-15}NH_4^+$ solution and $^{15}N_c$ is the ^{15}N abundance (atom %) in tree tissues before applying the solution (i.e. natural abundance), [N] is the N concentration in the measured tissue (%N g^{-1} dry weight), and MW is the molecular weight of ^{15}N (g mol^{-1}). We used the mean ^{15}N incorporation over the two month period (i.e., mean of the five sampling dates following solution application) for each tree and each tissue for statistical analyses to account for treatment effects on both the total amount incorporated and the rate of ^{15}N incorporation.

Photosynthesis

Leaf-level photosynthesis at light-saturating levels (A_{sat}, µmol m^{-2} s^{-1}) was measured monthly on one current-year sun-exposed shoot on the southern hemisphere of all focal trees in 2016 (i.e., March to August). A_{sat} was measured using a LI-6400 infrared gas-exchange analyzer system (Li-Cor Inc., Lincoln, NE, USA). The measurements took place in the morning with the following settings using the 2×3 LED chamber: 400 ppm reference CO_2 concentration, 1500 µmol m^{-2} s^{-1} light-saturating photosynthetic photon flux density, block temperature at 20°C or 25°C to match ambient air temperature, and relative humidity on full scrub. Measurements were conducted with relative humidity on full scrub because relative humidity was very low (4-15%) during the measurements. As a consequence the ambient VPD values were very close to conditions in the chamber (Grossiord et al., 2016a). Projected leaf area of the measured foliage was determined using a Li-Cor LI-3100C area meter and was used to correct A_{sat}. More details on the methods are provided by Grossiord et al., (2017c). Mean 2016 A_{sat} per tree was calculated as the mean of A_{sat} over the full measurement period and was used for statistical analyses.

Canopy-level stomatal conductance

      The thermal dissipation method (Granier et al., 1987) was used to measure the sap flux density of all trees (F_D, L dm^{-2} h^{-1}) in 2016 (i.e. March to August). Two 20 mm-long probes (Plant Sensors, Nakara, Australia) were installed under the bark and the cambium of trees at 0.8 to 1.3 m aboveground with a 10 cm vertical spacing between probes. This height was necessary to minimize thermal disturbance from ground heating. All sensors were covered with reflective insulation to reduce the risk of direct sunlight causing thermal gradients. Each sensor was supplied with 0.2 W of constant power. Data were collected every 10 s with a CR1000 data logger (Campbell Scientific Inc, Leicestershire, UK), and 30 min averages were recorded. F_D was calculated following the equation proposed by Granier et al., (1987). The empirically derived coefficients in the Granier equation may introduce error in F_D calculations, although errors resulting from non-species-specific calibration are generally greater for ring-porous than diffuse-porous or tracheid-bearing species such as piñon and juniper (Bush et al., 2010). Measurement errors associated with differences in sensor length and sapwood length (i.e. conductive tissue transporting water in the trunk) were corrected by using direct measurements of sapwood thickness on each tree taken just above the sensors (Clearwater et al., 1999). Whole-tree transpiration per unit leaf area (E_L, kg H$_2$O m^{-2} leaf s^{-1}) of each tree was calculated by multiplying F_D by the total sapwood area, and dividing by total tree leaf area. The methods for calculating total leaf and sapwood area are presented in the *SI* and fully described in McBranch et al., (2018).

      E_L was used to calculate canopy-level stomatal conductance (G_s, m s^{-1}) for individual trees using a simplified inversion of the Penman-Monteith model (Monteith & Unswoth, 1990) (see Fig. S3 for F_D results):

$$G_s = \frac{K_G(T_A)E_L}{VPD}$$

where K_G (T_A) is the conductance coefficient as a function of temperature (115.8 ± 0.4236T_A, kPa m^3 kg^{-1}), and T_A is the air temperature (°C) (Phillips & Oren, 1998). G_s was converted to mmol m^{-2} s^{-1} using site-specific atmospheric pressure (77.1 kPa) and T_A. G_s was calculated as the mean daily G_s per tree over the whole measurement period (i.e., i.e. March to August) and was used for statistical analyses.

Predawn leaf water potential

To determine the drought stress intensity experienced by trees, we measured predawn leaf water potential (Ψ_{PD}, MPa) monthly on all trees in 2016 (i.e. March to August). The measurements were carried out on the same day as A_{sat} measurements. Two twig samples from each tree were taken before sunrise, placed in plastic bags and stored in a refrigerator until they were measured at the study site within one hour of collection with a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA). Mean Ψ_{PD} for each date was determined as the mean of the two branch samples for each tree. Mean 2016 Ψ_{PD} per tree was calculated as the mean of Ψ_{PD} over the March-August period and was used for statistical analyses.

Statistical analyses

All analyses were performed using the software R (3.2.1, R Development Core Team 2015) with an α of 0.05. We analyzed responses in soil biogeochemistry, plant ^{15}N incorporation and physiological functions (photosynthesis, stomatal conductance and predawn leaf water potential; averaged over the 2016 measurement period) to drought, atmospheric warming, and the combination of the treatments for each species and in each compartment (i.e., for plant ^{15}N incorporation) using mixed linear, random-intercepts models

where heating (yes or no) and drought (yes or no) were used as fixed effects. For all tests, the individual trees nested in the chambers (i.e. warming treatments) were input as random effects in the following model: lme(function) ~ warming*drought, random=~1|chamber/tree, method="ML"). Similar mixed models were used to determine the differences between treatments in environmental variables (air temperature, soil temperature and soil water content), with treatments (i.e., ambient, drought, warming and warming+drought) used as a fixed effect. Post-hoc analysis to determine differences between treatments was performed with Tukey's HSD post-hoc test. Statistical analyses were performed using the package *nlme* for Linear mixed effects models. Prior to data analysis, soil biogeochemistry, ^{15}N incorporation and physiological data were checked and inconsistent data (i.e., values out of range of the natural variability and reflecting measurement errors) were removed. When data were not distributed normally, log transformations were used prior to running the analyses.

Results

Environmental conditions

During the experiment (2012 to 2016), atmospheric temperatures were significantly higher in the warming (+ 3.49°C, $P < 0.001$) and warming+drought treatments (+ 4.24°C, $P < 0.001$) compared to ambient conditions (Fig. S1 & Table S1). Soil temperature (0-15 cm) was significantly higher in the drought (+ 1.2°C, $P = 0.001$), warming (+ 3.9°C, $P < 0.001$) and warming+drought (+ 4.8°C, $P < 0.001$) treatments compared to ambient conditions (Fig. S1 & Table S1). Soil water content (0-15 cm) was significantly higher in ambient conditions than in the warming (+ 7%, $P < 0.001$), drought (+ 7.8%, $P < 0.001$) and warming+drought (+ 8.9%, $P < 0.001$) treatments (Fig. S1 & Table S1).

Soil biogeochemistry

Multi-year warming increased the rates of several metrics of soil N cycling for both species: mixed models revealed a significant increase in soil microbial biomass N for both species, and potential nitrification rates and soil NO_3^- concentrations for piñon in response to warming (Figs. 2 & S4, Table S2). A significant increase in NH_4^+ concentrations in response to drought relative to ambient conditions was also observed for piñon (Figs. 2 & S4, Table S2). The majority of temperature- or drought-mediated changes in soil biogeochemistry (i.e., potential nitrification rates, soil NO_3^- and NH_4^+ concentrations) were maintained when warming and drought acted together under hotter drought conditions for piñon trees (Figs. 2 & S4, Table S2). No changes in soil N cycling were observed for juniper in the warming+drought treatment compared to ambient conditions (Figs. 2 & S4, Table S2).

Tree N allocation strategy

Warming and drought had no impact on soil $\delta^{15}N$ ($P < 0.05$), suggesting that trees had access to similar amount of $^{15}NO_3^-{}^{15}NH_4^+$ solution during the two months following the label application in all treatments (Fig. S5). No differences in total N content were found between treatments in leaves, twigs, coarse roots and fine roots (Fig. S6). For both species, N allocation significantly changed in response to warming and drought acting separately or combined, but the two species adopted contrasting strategies. For juniper trees, mixed models showed a significant decrease in ^{15}N incorporation in coarse and fine roots in response to all treatments (i.e., warming, drought and warming+drought) relative to ambient conditions, but a significant increase in ^{15}N incorporation in twigs and leaves only when exposed to warming+drought (Figs. 3 & S7, Table S3). In contrast, for piñon trees, drought and warming+drought significantly decreased ^{15}N incorporation in leaves but the drought

treatment increased ^{15}N incorporation in coarse and fine roots (Figs. 3 & S7, Table S3). The observed tendency for increased ^{15}N incorporation in roots was maintained for piñon trees under warming+drought conditions but was not significant when considering all dates (P = 0.10). Increasing ^{15}N incorporation in coarse and fine roots in the warming+drought treatment for piñon only occurred significantly on sampling + 7 and + 14 days (Fig. S7). Warming alone had no effect on piñon ^{15}N incorporation. Statistical analyses revealed that these N allocation responses to hotter droughts were apparently driven by a combination of temperature and soil moisture effects for juniper (significant warming and drought effects, Table S3), and entirely by soil moisture limitation for piñon (significant drought effect only, Table S3).

Tree physiology

Drought significantly reduced canopy-level stomatal conductance (G_S) and predawn leaf water potential (Ψ_{PD}) for both species, and photosynthesis (A_{sat}) for piñon (Fig. 4, Table S4). Warming significantly reduced G_S for juniper but had no other effect acting alone on physiological functions (Fig. 4, Table S4). For both species, similar reductions in G_S, A_{sat} and Ψ_{PD} were found under single stresses (i.e., warming or drought) as under multi-year hotter drought conditions (i.e., warming + drought, Fig. 4, Table S4) suggesting no aggravation of additive warming and drought effects.

Discussion

The additive effects of warming and drought during hotter droughts are expected to exacerbate reductions in tree performance in the future (McDowell et al., 2015; Adams et al.,

2017). Contrary to many observations and predictions (Breshears et al., 2005, 2009; Van Mantgem et al., 2009; Anderegg et al., 2013; Williams et al., 2013; Allen et al., 2015; McDowell et al., 2015; Adams et al., 2017), we showed that hotter droughts might not impair performance of tree C and water processes to a larger extent than current droughts without additional stressors, such as insect or pathogen outbreaks, at least in the system studied here. The effects of five years of continuous soil drought acting separately on photosynthesis and stomatal conductance were comparable to combined drought and warming (Fig. 4). This unexpected response can be partially explained by long-term warming-induced mitigation processes related to soil N cycling and plant N allocation (Figs. 2 & 3). Overall, our results suggest that nutrient-related mitigation processes may help compensate the compound effects of temperature and soil moisture limitations on tree function in these semi-arid woodlands.

Changed soil N cycling during hotter droughts

Although trees were exposed to extremely low soil moisture in the hotter drought conditions imposed by the experiment, atmospheric warming and subsequent soil warming (Table S1) increased soil N cycling rates and nutritional status. In particular, when warming had an effect, the increased temperature resulted in increased soil nitrate and microbial biomass N concentrations, as well as potential nitrification rates. Over the short-term these changes could be expected to increase N availability and these findings support previous observations that reported a stimulating role of temperature on soil biogeochemical processes (Rustad et al., 2001; Sardans et al., 2008). They also suggest that persistent temperature rise over several years could improve the nutritional status of forest soils in the future, independent of precipitation change. However, it must be noted that the chambers used in the warming treatments may have reduced windspeed, and thus vapor pressure deficit under warming conditions. Therefore our findings mostly reflect impacts of temperature rise and

not necessarily increased evaporative demand as projected for the future (Williams *et al.*, 2013). Furthermore, due to the mobility of NO_3^- and its potential to be lost via leaching and denitrification, warming-induced increases in nitrate and nitrification rates could also potentially result in reduced soil N pools over the long-term (Peterjohn & Schlesinger, 1990).

Interestingly, most treatment shifts in soil biogeochemistry were found under piñon trees but not under juniper (Fig. 2), indicating that warming-induced benefits in soil N availability are not universal across species. Among other processes, differences in microbial communities and root exudates (Cregger et al., 2014; Allen et al., 2010) may have contributed to these species-specific differences. Alternatively, these differences could be related to varying moisture content at the tree base emerging from differences in tree transpiration (Robinson et al., 2010) and tree N uptake rates (Cregger et al., 2014). Indeed, in similar piñon-juniper woodlands, Cregger et al., (2014) showed that reduced water uptake during drought increased soil N availability for piñon pine. Nevertheless, the ^{15}N-labeling approach clearly demonstrated that all trees, even those with low canopy stomatal conductance and thus lower water use, were able to take up and incorporate some of this additional warming-induced nutrient supply (Fig. S7). Nitrogen incorporation is directly linked to many metabolic and maintenance functions such as photosynthesis, growth, reproduction, and defense (Reich et al., 1999), and thus increased availability and uptake of N speaks to specific plant adjustments to temperature and soil moisture stress. Among other underlying mitigation mechanisms, higher N plant uptake has been suggested to modify tree foliar C:N ratio (Gressler et al., 2017). Thereby, trees could modify their palatability for biotic agents and reduce the risk of insect and pathogen attacks during hotter droughts. Improved nutrient status and plant N uptake could also support the regrowth of tissue lost during drought (e.g., roots, foliage) resulting in improved resilience of trees to hotter droughts (Gessler et al., 2017).

Overall, our findings suggest that forest nutrition may play a significant role in the maintenance of tree and forest performance during hotter droughts, with temperature stimulating nutrient cycling for some species at least. Moreover, the results highlight the need to further explore and integrate nutrients in the drought-induced tree mortality framework (Gessler et al., 2017), particularly the underlying processes involved in nutrient-induced mitigation of hotter droughts.

Trees adjust N incorporation during hotter droughts

Species with contrasting functional water resource use strategies (i.e., iso- and anisohydric species) were both able to adjust their resource use in response to long-term warming and drought (Fig. 3). However, they favored the sustainability of different processes: trees with anisohydric regulation strategies for stomatal conductance, like juniper, are known to be "risk-taking" plants during dry periods by commonly maintaining water uptake and photosynthesis even under severe soil moisture stress (Tardieu & Simonneau, 1998). In contrast, isohydric species like pines are known to regulate water use and photosynthesis depending on available soil moisture (Tardieu & Simonneau, 1998) by minimizing major functions (e.g., photosynthesis, water use and growth) during extended drought events (Garcia-Forner et al., 2016). Our results indicate that species' N-cycling responses to global change are similar to the long-discussed C and water use strategies, suggesting that the differences in N cycling are traits coupled to C and water strategies in order to deal with environmental variability.

More specifically, we show that anisohydric species are likely to maintain a more risky strategy with global warming by investing nutritional resources in the maintenance of photosynthesis and growth processes rather than in their rooting system (Figs. 3 & S7). While

this approach could be considered maladaptive at first, it probably contributed to the maintenance of photosynthesis in juniper trees (Fig. 4), and thus demonstrates a mitigation mechanism favoring the maintenance of C-dependent plant functions during hotter droughts. Increased N allocation to aboveground compartments can modify multiple physiological functions such as lower hydraulic risks during drought induced by changes in leaf area:sapwood area ratio, reduced drought effects on photosynthesis, reduced stomatal sensitivity resulting in later stomatal closure during drought (Ghashghaie & Saugier, 1989), lower protein degradation in leaves (Fotelli et al., 2002) and increased foliar enzyme and membrane integrity (Ashraf & Foolad, 2007). We observed no shifts in whole-tree leaf area:sapwood area ratios at our site (McBranch et al., 2018), suggesting that higher N allocation aboveground did not modify the ratio of evaporative and conductive structures for juniper. However, whether increased N allocation to aboveground tissues may have buffered drought effects through the improvement of other physiological processes cited here above remains unknown and is a subject for future work. Conversely, this study showed that the N-allocation strategy of the isohydric species during persistent hotter droughts was to reduce nutrient investment in aboveground tissues and, to a certain extent, increase allocation to their rooting system, probably to maintain active water uptake for longer periods and/or avoid irreversible damage to root hydraulics. Although the underlying processes by which altered N-allocation in piñon may have impacted plant physiology remains unclear, this strategy helped maintain photosynthesis and water use to similar values as when trees where subjected to single stress levels (Fig. 4), suggesting N-related buffering mechanisms.

Whether the differential strategies of juniper and piñon pine (i.e. support of aboveground *vs*. belowground organs and functions) may favor the survival of one species over the other during future hot droughts remains unclear as no mortality occurred for both species. Furthermore, these two mitigation mechanisms appeared insufficient to fully

overcome relative reductions in water resources and temperature stress during persistent hotter droughts for both species as reductions in photosynthesis and water use still occurred (Fig. 4).

Conclusions and implications of N-related mitigation processes during hotter droughts

To summarize, our findings show that atmospheric warming could increase soil N cycling rates and tree nutritional status during hotter droughts. We also observed large reductions in performance (i.e., photosynthesis and/or stomatal conductance) for both studied species under combined warming and drought conditions. However, the experimental design used here exposed trees to severe hot drought conditions for several years, largely exceeding levels observed during recent extreme events in this region (Breshears et al., 2009) (Fig. 1) and reflecting conditions expected for the future (Allen et al., 2010). Although the already low soil moisture conditions in the drought treatment alone probably resulted in no exacerbation of soil drought under additional warming (Fig. S2), plants in the hot drought treatment were subjected to an additive temperature stress as warming should have altered plant functions independently of soil moisture stress. Indeed, measurements at this site suggested that air temperature above 32°C, which occurred frequently during the 2012-2016 growing seasons, could significantly impact the photosynthetic activity of trees (Grossiord et al., 2017a). Furthermore, predawn leaf water potential values below –2.4 MPa and -5.3 MPa for piñon pine and juniper, respectively, corresponding to the stomatal closure point of these species have been associated with high mortality rates in this region (Williams et al., 2013; McDowell et al., 2015). Some trees reached such values at several occasions during the experiment (McDowell et al., unpublished). Yet, we observed no exacerbation of drought-sensitive processes with superimposed warming and no vitality threshold (i.e., no tree mortality) was crossed by either species because trees were able to, at least partially, buffer

the additive effects of drought and warming through mitigation processes. This study thus demonstrated that forest ecosystems may be more resistant to hotter droughts than previously thought, highlighting our incomplete understanding of trees' ability to acclimate to climate change.

Among other mitigation mechanisms previously assessed in this system, which may be dependent on shifts in N cycling (e.g., shifts in tree water sources in the soil, hydraulic anatomy and foliar structures, Grossiord et al., 2017a, b, c, McBranch et al., 2018), we show that temperature increase may result in nutrient cycling differences that help create some resistance in the face of drought even if the potential role of N-status in determining the trajectory of communities following mortality remains relatively unexplored (Gessler et al., 2017). Future studies will need to address the impact and nature of the underlying processes by which shifts in soil N and plant N allocation may have altered plant responses to hotter drought, and investigate how these processes may vary throughout the growing season and among sites. Long-term N acclimation to elevated temperature may have reduced the additive effects of warming and soil drought on plant photosynthesis and respiration (Way & Yamori 2014; Slot & Kitajima 2015), resulting in lower C losses for trees and ultimately maintenance of plant functions. For instance, some studies have suggested that shifts in N allocation to Rubisco could underlie photosynthetic and respiration acclimation (Scafaro et al 2017; but see Tarvainen et al 2018; Crous et al 2017). Understanding the underlying N-related buffering mechanisms is particularly relevant for global forest mortality predictions and underscores the importance of accurate knowledge on long-term plant acclimation to global warming, as well as interactive effects of temperature and soil moisture on plant nutritional status. Improving our understanding of forest responses to prolonged hotter drought conditions can help policy makers and land management agencies anticipate and prepare for long-term impacts. Moreover, understanding the linkages between soil nutrient cycling and

plant responses for precipitation changes beyond drought, such as with increased frequency of extreme precipitation events, could be of significant value as we work towards predictive frameworks of future dryland function. Finally, an important feature to consider is that our experimental manipulation may have not impacted temperature and vapor pressure deficit simultaneously as expected during future hotter droughts (Williams et al., 2013). Therefore, how the combination of temperature and evaporative demand may impact plant function together during hotter droughts remains an important research topic for future research and will require the development of alternate experimental designs.

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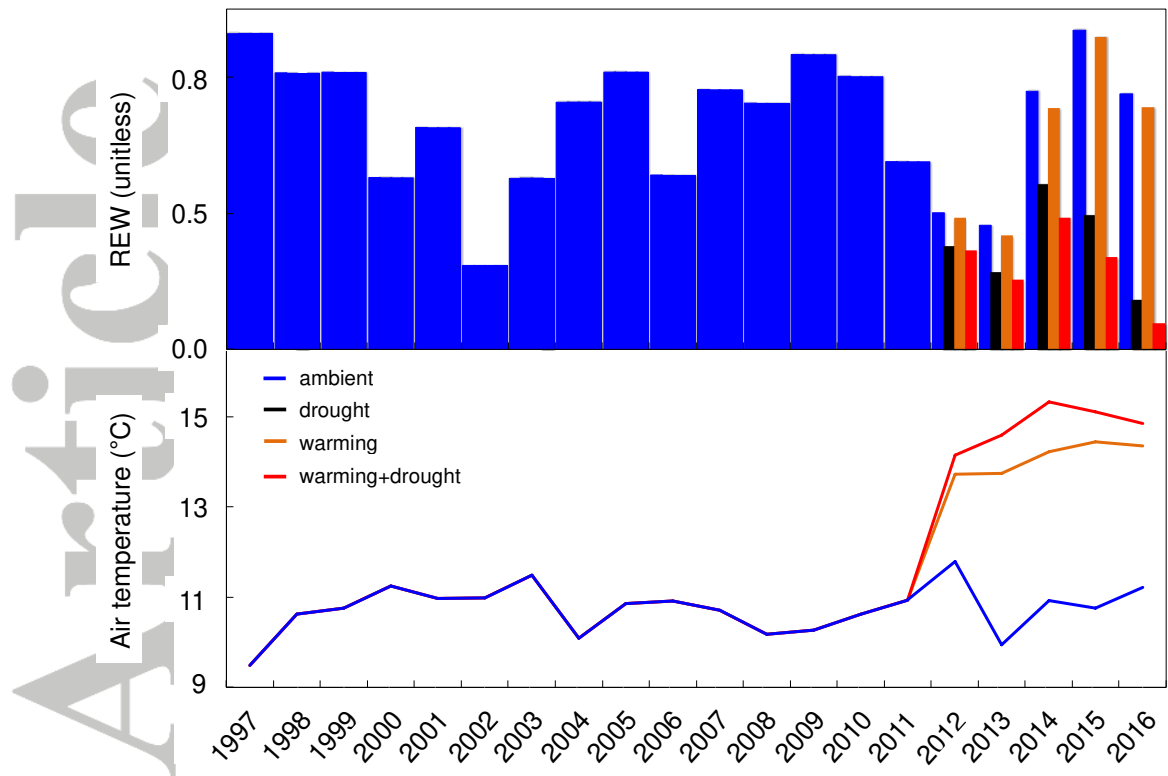
Figure 1. Mean yearly relative extractable water (REW, unitless) calculated using a soil water balance model (Grossiord *et al*., 2017a) and mean yearly air temperature (°C) over the 1997-2016 period at the study site. Starting in 2012 (when treatments started), REW and air temperature are given for each treatment. Air temperatures in the drought treatment are not presented, as they are equal to air temperatures in ambient conditions.
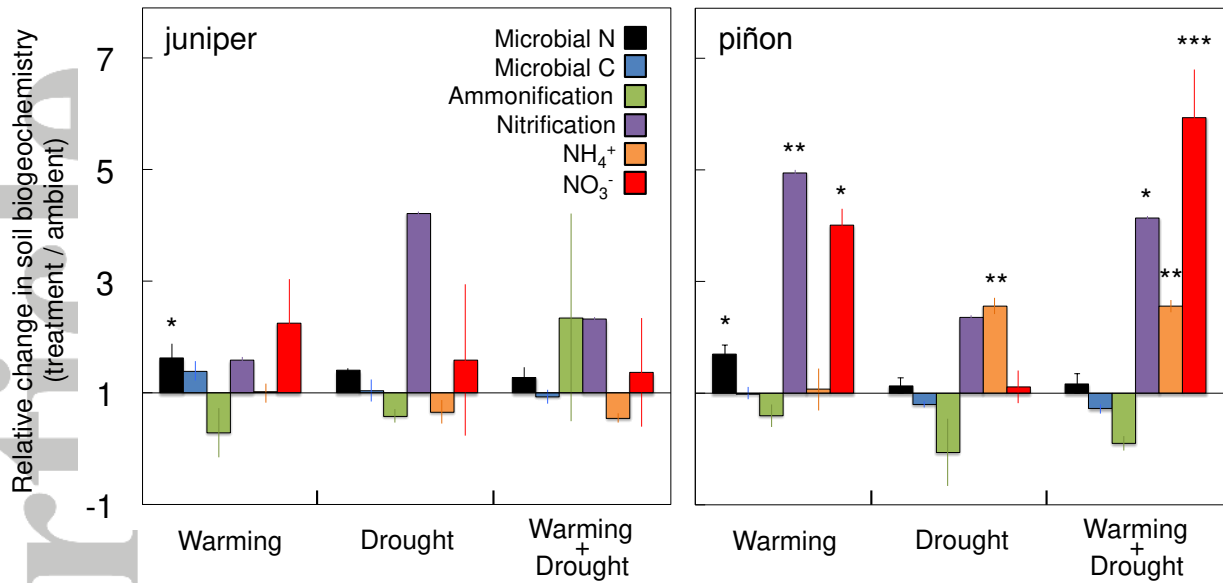
Figure 2. Relative changes in biogeochemistry metrics are calculated as individual tree values divided by the average value of ambient (control) trees (i.e., treatment values equal ambient values when the relative change equals one). Data are means (± SE) and asterisks denote significant differences among treatments on actual values (drought, warming and warming+drought) and control (ambient) determined from post-hoc tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, n= 5 trees per species and per treatment.
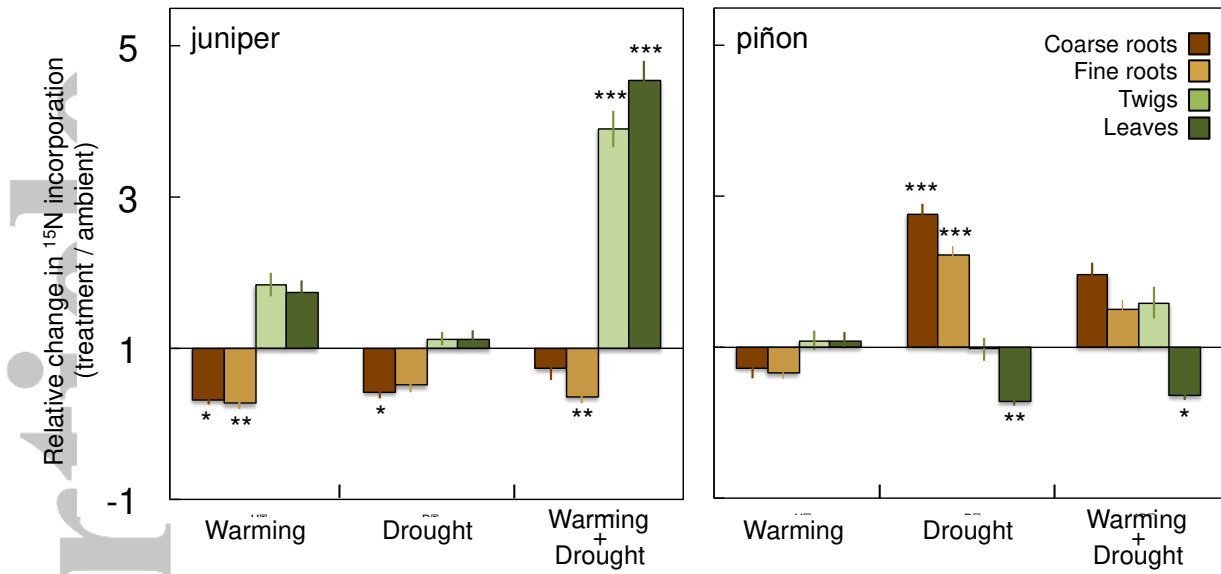
Figure 3. Relative changes in ^{15}N incorporation are calculated as individual tree values divided by the average value of ambient (control) trees (i.e, treatment values equal ambient values when the relative change equals one). Data are means (± SE) and asterisks denote significant differences among treatments on actual values (drought, warming and warming+drought) and control (ambient) determined from post-hoc tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, n= 5 trees per species and per treatment.
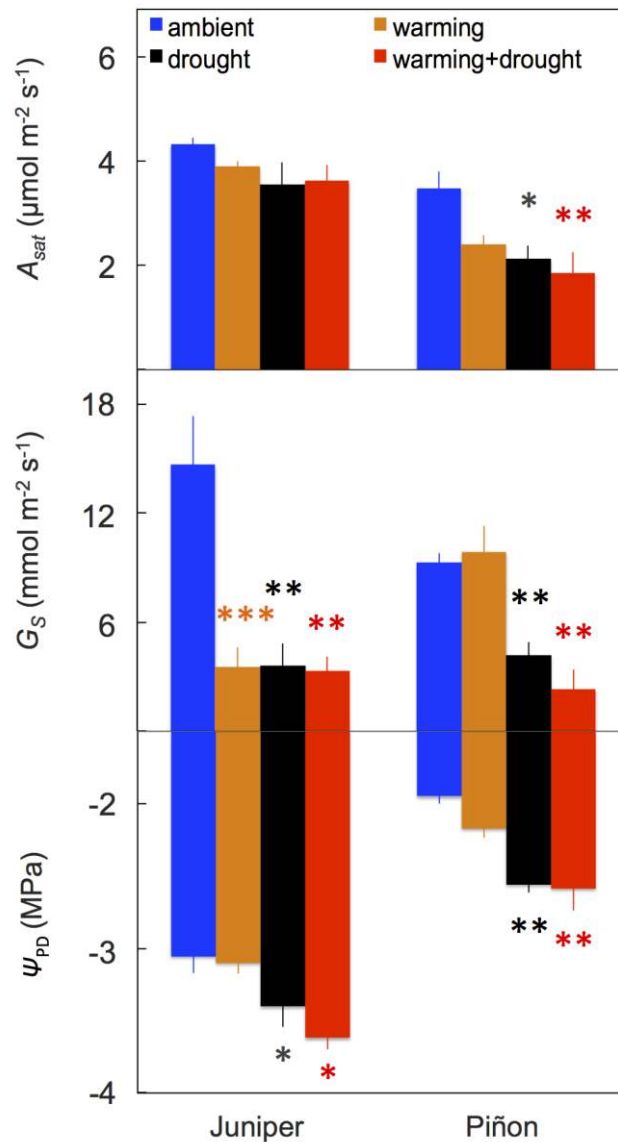
Figure 4. Means of light-saturating photosynthesis (A_{sat}), canopy-level stomatal conductance (G_S) and predawn leaf water potential (Ψ_{PD}) for each treatment (ambient, warming, drought and warming+drought) for juniper and piñon trees (± SE). Asterisks denote significant differences between treatments (drought, warming and warming+drought) and control (ambient) determined from post-hoc tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$, $n = 5$ trees per species and per treatment.