Water controls on nitrogen transformations and stocks in an arid ecosystem

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Abstract.   Following water, nitrogen (N) is the most frequent limiting factor to aboveground net primary production in arid ecosystems. Increased water availability can stimulate both plant nitrogen uptake and microbial nitrogen mineralization, but may also stimulate losses from the ecosystem. Here, we assess the effect of water availability on nitrogen stocks and transformations in an arid ecosystem. We conducted a field experiment with five levels of precipitation input (-80%, -50%, ambient, $+50\%$, $+80\%$) and two levels of N fertilization (ambient or 10 g·m^{-2}·yr^{-1} NH$_4$NO$_3$) in a desert grassland of the Chihuahuan Desert. We measured in situ net N mineralization, plant N uptake, foliar N, N leaching under grass-rooting zone, and soil N availability during two years.

Our results showed that increased water availability did not affect net N mineralization, but there was higher plant N uptake than with drought. Soil inorganic N pools were 2–4 times lower with increased water availability compared to drought conditions. N leaching below grass-rooting zone was higher in dry than wet conditions because of higher available N. Increased water availability differentially affected N species significantly reducing the NO$_3$:NH$_4$ ratio. The accumulation of inorganic N during drought was the result of a decoupling between microbial and plant activity, and suggests that the cycling of N is more open in dry years than in wet years.

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Introduction

Nitrogen (N) and water availability both directly control the functioning of arid ecosystems (Lauenroth et al. 1978, Yahdjian et al. 2011). In addition, water and N availability interact with each other so that water availability affects the N cycle; and N availability affects the water cycle. Water directly affects the activity of plants and soil microorganisms that are responsible for N processes, and affects the diffusion of different inorganic N forms (Paul 2007). During moist periods, nutrients with enhanced solubility could be assimilated by plants, immobilized or mineralized by microbes, or leached through the soil profile. High water availability may also increase denitrification rates by displacing oxygen and creating anoxic conditions in the soil that

stimulate anaerobic nitrate transformation to N_2 and N_2O (Schlesinger 1997). The extent to which biological demand and availability of N are in synchrony may determine the degree of N conservation within the plant-soil system.

Studies in arid-semiarid ecosystems yield inconsistent results of the effects of water availability on N cycling. Biogeochemical simulation models predict that net N mineralization increases with increasing moisture along precipitation gradients in semiarid to mesic ecosystems (Burke et al. 1997), mostly driven by climatic controls and increasing N pools. At the local scale, some studies report that mineral N concentration decreases with increasing water availability (Fisher et al. 1987) and some report the opposite pattern (Whitford et al. 1995). Other studies show that net N mineralization does not differ between dry or wet soils (Schimel and Parton 1986, Reynolds et al. 1999, Yahdjian et al. 2006), or across precipitation gradients in the Great Plains (McCulley et al. 2009). A meta-analysis of aboveground net primary production (ANPP) responses to N fertilization across arid to subhumid ecosystems showed that the importance of N limitation increases with annual precipitation from arid to subhumid regions (Yahdjian et al. 2011). Water and nutrient uptake, in some cases, occur from deep soil layers in dry grasslands (McCulley et al. 2004, Sala et al. 2012), suggesting that significant N leaching may occur under certain circumstances. This variety of outcomes indicates that we are still not able to fully understand the response of the N cycle to changes in water availability.

Current global change models predict a transition throughout the century to a more arid climate in Southwestern North America that could match Dust Bowl conditions of the 1930s (Seager et al. 2007). The IPCC A1B scenario, for example, suggests a decrease in precipitation of 5–10% for the Southwestern US. Perhaps more importantly, an increase in PPT interannual variability and frequency of extreme events is very likely to occur as well (Solomon et al. 2007). Drier sites along precipitation gradients have shown a more "open" N cycle than sites receiving higher precipitation (Handley et al. 1999), which means that the loss relative to N cycled between plants and soil is larger than in wetter sites. Thus, the indirect effect of precipi-

tation through changes in the openness of the N cycle has significant impacts on ecosystem functioning, and has been proposed to maintain water and N co-limitation in semi-arid ecosystems (McCulley et al. 2009). Within this context, we were interested in looking at the degree of synchrony between N availability and demand and how this relationship changes in dry and wet years in a desert grassland.

In this study, we focus on the effects of water availability on N stocks and transformations in the context of inter-annual variability of precipitation at the local scale. Arid ecosystems experience intermittent wet periods that stimulate biological activity, but plants and soil microbes may have different thresholds for using and cycling available resources (Schwinning and Sala 2004). We hypothesized that increased water availability would: (1) increase net N mineralization, which may result from stimulating soil microbial gross N production relative to microbial immobilization; (2) increase N uptake as a result of enhanced plant growth; (3) increase leaching losses below the 0–25 cm soil layer where 70% of root biomass, in this ecosystem, concentrates (Jackson et al. 1996); and (4) would affect the relative abundance of N species in the top layer as a result of plant preference for nitrate and/or differential rates of movement in the soil of inorganic N forms, leading to a decreased nitrate: ammonium ratio in the grass-root dominated layers.

To test our hypotheses, we conducted a field experiment in the Chihuahuan Desert, which is the largest desert in North America, where we manipulated annual precipitation (PPT) and N. We created a precipitation gradient with fixed-location rainout shelters and supplemental watering that was crossed with a N fertilization treatment. The large range of our water manipulation, from 20% to 180% of ambient, is consistent with the range of observed variability in this site during the last century (Wainwright 2006). These five levels of water input during two years of experiments generated a range of soil moisture conditions that would otherwise require at least 30 years of observational studies. We measured soil nitrate (NO_3^-) and ammonium (NH_4^+) concentration, estimated in situ net N mineralization, N allocated to aboveground net primary production (ANPP-N) and leaching

below the grass-rooting zone during two years of manipulations.

MATERIALS AND METHODS

Site description

Our research took place at the Jornada Basin LTER (32.5° N, 106.8° W, 1188 m asl), which is located in the Northern Chihuahuan Desert, ca. 40 km NNE of Las Cruces, NM. Mean annual precipitation from 1915–1995 was 245 mm. Most rainfall (>53%) occurs in local, convective thundershowers between July and September. Average annual temperature recorded during the same period was 14.7°C, with an average of 3.8°C in January and 26°C in June. Vegetation at the study site is a mixture of grasses and shrubs co-dominated by *Bouteloua eriopoda* (Torr.) Torr. (black grama) with *Prosopis glandulosa* Torr. (honey mesquite), which jointly account for 65% of plant cover. Topography is flat (1–3% slopes) and soils are coarse-textured, well-drained, sandy loams (Typic Paleorthids) (Soil Survey Staff 1999). A layer of calcium carbonate is often found at depths from 64–76 cm (Herbel et al. 1972, Gibbens et al. 1986).

Experimental design

We conducted a replicated manipulative experiment of precipitation reduction and supplementation with and without N fertilization for two years. Our treatments were 80% and 50% reduction, ambient control, and 50% and 80% enhanced precipitation; whole-plots were either unfertilized or fertilized with NH_4NO_3. Rainfall manipulation treatments had 12 replicate plots per fertilization level, whereas ambient precipitation treatments used 18 plots per fertilization level, resulting in a total of 132 plots. Plots were established in three blocks, each within a 2 ha livestock-fenced exclosure in the fall of 2006. We used a randomized complete block design and randomly assigned one of ten treatment combinations to each plot within each block. We centered 2.5 × 2.5 m plots on mesquite shrubs of similar size, so that initial cover of dominant shrub and grass species was not statistically different (p > 0.05) among the three blocks. Mesquite shrubs selected for this study were 0.50 m in average height, and <1 m^2 of canopy cover, which was similar to the average shrub size in this area (Drewa 2003).

To exclude precipitation from the plots, we installed 48 2.5 × 2.5 m fixed-location rainout shelters in November of 2006 that passively blocked incoming rainfall following the design by Yahdjian and Sala (2002). Similar rainout shelters are being used in many studies from the Patagonian Steppe, the Arctic Tundra, the short grass steppe, the Mediterranean and Californian grasslands to the southwestern rangelands of the US (Heisler-White et al. 2008, Adler et al. 2009, Fiala et al. 2009, Levine et al. 2010, Rao and Allen 2010, Matías et al. 2011, Talmon et al. 2011). The shelter design consists of a metal structure that supports V-shaped clear acrylic bands ('shingles'). The number of shingles per rainout shelter was adjusted to obtain different levels of rainfall reduction. Shelters intercept a fraction of incoming precipitation, which is then routed outside the plot by a gutter. Shingles were molded from ACRYLITE FF with high light transmission (>92% PAR transmitted) and less than 3% change in light transmission over a 10-yr period (CYRO Industries, Parsippany, NJ). Shelters have minimal effects on the microenvironment, and only a small edge effect on soil water content of approximately 20 cm (Yahdjian and Sala 2002), hence we excluded the edge area from our sampling.

For the increased precipitation treatments, a PVC-pipe irrigation system was used to water the plots with sprinklers. Irrigation occurred the day after a precipitation event >2 mm with the addition of 50% or 80% of that event. Rainfall was collected off-site and pumped through the irrigation system. Water supplementation started with the monsoon rains in July of 2007 and continued throughout December of 2007, then resumed by July 2008 until the end of November 2008. Only one precipitation event of 7 mm was missed during 2007–08 winter. Ambient control precipitation plots had neither shelters nor irrigation systems.

We applied ammonium nitrate fertilizer every growing season at a rate of 10 g $N \cdot m^{-2} \cdot yr^{-1}$, half in mid-July and the other half at the end of July. Prior to application, we dissolved NH_4NO_3 in water to decrease N loss to the atmosphere due to wind erosion or ammonia volatilization. We applied the same amount of water, equivalent to a 2-mm rain, to the control N plots.

Response variables

In situ net N mineralization was measured at two temporal scales: growing season and annual. Net N mineralization was estimated as the difference between initial and final inorganic N content (NO_3^- + NH_4^+) in tubes that prevented plant uptake (Raison et al. 1987). This is a standard soil method in ecological research (Robertson et al. 1999, Stark 2000), although it has some drawbacks, such as assuming similar N concentration for adjacent cores at the beginning of the incubation. To estimate growing season mineralization, cores were left in the field for fifty consecutive days in the summer of 2007, and for ten and twenty-six days in the summer of 2008. We calculated daily net mineralization by dividing the difference in N content at the beginning and at the end of the incubation, by the duration of the incubation period. Annual rates of N mineralization were estimated by taking repeated samples throughout the first year of manipulations (Pastor et al. 1984). These additional incubations took place in the fall (90 days), winter (85 days) and spring (85 days). We used half of the plots (n = 66) for each incubation period to minimize plot disturbance. We buried 5 cm diameter × 10 cm long incubation-cores next to a grass patch and at least 20 cm from mesquite shrubs to a depth of 10 cm. Cores consisted in PVC cylinders open on both sides so that soils experienced the altered precipitation treatments. Cores were oriented vertically, and were leveled with the soil surface. After we removed the cores from the soil, we transferred the samples to plastic bags and transported them to the laboratory for immediate (within 8 hours) extraction of NO_3^- and NH_4^+. We extracted a 10-g subsample of fresh, sieved soil in 50 ml 2 M KCl and filtered the extracts within 2–4 days. Another subsample was placed in a drying oven at 105°C for 48 h for estimating gravimetric soil water content and correcting soil N concentration for soil water content (Jarrell et al. 1999). We analyzed soil extracts using a 2-channel QuikChem 8500 flow injection (Lachat Instruments, Hach Company; Loveland, CO), which uses a colorimetric method for inorganic N concentration analysis in a KCl matrix. To account for leaching losses, we placed one UNIBEST PST-1 ion exchange resin capsule at the bottom of each incubation core. Resin capsules were not reused, and consisted in a 2-cm diameter polyester mesh bag filled with a 50/50 mixture of cation and anion resin (Amberlite IR-150, Rohm & Haas Company). We also collected cores adjacent to N mineralization cores at the beginning of each incubation period for initial nitrate and ammonium stocks. Our estimates of net N mineralization from field incubations represent the balance between gross mineralization and microbial immobilization corrected by leaching losses (Robertson et al. 1999).

We estimated growing-season N uptake by multiplying N content in senescent leaves (% weight) by ANPP ($g \cdot m^{-2} \cdot yr^{-1}$) (Berendse and Aerts 1987) of mesquite and black grama. Leaves were dried at 60°C, ground, and analyzed for N content on an elemental analyzer (Costech Analytical, ECS 4010; Valencia, CA). Gold, standing dead leaves were used as opposed to green leaves because their N was not internally recycled to perennial organs. This is the N lost per year that equals the annual N uptake in a steady state system (Schlesinger et al. 2006).

ANPP was equated to green biomass at peak of growing season (Sala and Austin 2000; Reichmann et al. in review). To estimate biomass, we used a non-destructive technique that was calibrated for the study site following the methodology used by Flombaum and Sala (2007). We chose twenty 20 × 100 cm plots for each species located nearby our experimental plots, measured plant cover, and harvested aboveground biomass. Vegetation cover was evaluated with two parallel lines per plot, where we recorded green and standing dead interception per species for all plants present in the plot. We harvested whole-plot aboveground biomass, sorted and weighted it after drying at 70°C. Then, we performed linear regression analysis between green vegetation cover and green aboveground biomass for grasses, and green cover and green leaves for shrubs. Our biomass-cover regressions gave comparable estimates of ANPP to those from the LTER-IBP exclosure, a grassland with similar characteristics to our study site where ANPP was calculated using a different method (Huenneke et al. 2001) (i.e., 173 ± 24.23 g dry biomass·m^{-2}·yr^{-1} estimated in our control plots versus 191.8 g dry biomass·m^{-2}·yr^{-1} at the IBP site in 2008; data available at http://jornada-www.nmsu.edu/datacat.php). The rela-

tionship between cover and biomass was significant at P < 0.001 (grass biomass [g/m^2] = 264.56 × grass percent cover, N = 20, r^2 = 0.68, percent cover ranged between 0.09 and 0.80; shrub biomass [g/m^2] = 184.61 × shrub percent cover, N = 20, r^2 = 0.88, cover ranged between 0.08 and 0.75). These equations were used to estimate black grama ANPP and mesquite leaf productivity in our experimental plots. Within each plot, we measured plant cover with three parallel lines per plot, each of 250 cm length, evenly spaced from the east border. We recorded green interception per species for all plants present in the plot, and used the regressions to obtain an estimate of biomass by life-form (Huenneke et al. 2001, Flombaum and Sala 2007).

N movement below the grass rooting zone was estimated by incubating UNIBEST PST-1 ion exchange resin capsules at 25 cm depth for 43 days in the summer of 2008 (July 29th to September 10th). The rate of inorganic N accumulation in the resin represents the rate of N leaching below 25 cm. Giving the porous nature of soils at the study site, we assumed that flow paths were basically vertical and that the intercepting area ranged from a minimum of 3.14 cm^2, which is the resin capsule top area, to a maximum of 31.4 cm^2 as suggested by Kramer et al. (2006). The average of minimum and maximum leaching rates results in a conservative estimate to compare with N mineralization and uptake rates, because it assumes an area up to 10 times greater than the resin surface area. Grasses have 70% of their root biomass in the top 30 cm of soil (Jackson et al. 1996), therefore resins at 25 cm captured N that was likely to be lost from grass-root absorption. We used one resin capsule per plot in 66 plots. Resins were inserted at a 75° angle from the soil surface and placed below a black grama patch. We were very careful to keep the soil structure above the resin, and hence water infiltration and roots, intact. Resin capsules from the mineralization and leaching experiments were extracted in a 2 M KCl solution. When removed from the soil, we rinsed soil particles from the resins with deionized water and placed them in individual clean containers. Because desorption is time and diffusion dependent, we extracted resins in 25 ml 2 M KCl for 24 h twice. Extracts were analyzed following the same methodology as for soil KCl extracts.

Volumetric soil water content (VWC) was measured in 30 plots (n = 6 plots/precipitation treatment in unfertilized treatments) to evaluate the effectiveness of the water manipulation treatments. Soil water content was monitored at two depths using ECH$_2$O EC-5 moisture sensors (Decagon Devices, Pullman, WA) at 5–10 cm depth, and with ECH$_2$O EC-20 moisture sensors at 30–50 cm depth. We used an ECH$_2$O check handheld to take VWC measurements the 1st, 3rd and 5th days following a precipitation event >2 mm, and every 2 to 3 weeks during the period in between rainfall events. Probes were calibrated for soils at the study site following instructions from the manufacturer (calibration r^2 = 0.98, n = 5). A wireless datalogger located <300 m from our experiment recorded daily precipitation data.

We summarized available long-term soil moisture data and compared them to soil moisture measured during our incubations to evaluate the probability of N leaching below the grass-rooting zone. Long-term monthly soil-water content measurements were made at the Jornada-IBPE LTER-II grassland site, approximately 9 km west of our study site and with similar vegetation and soil characteristics. Soil moisture was measured for 19 years (1989–2010) at ten depths (where possible) at each of ten access tubes using a neutron probe (Campbell Model 503DR Hydroprobe). We analyzed soil moisture data for the month of August from 30-cm depth, which matches the insertion depth of the ECHO probes at our study site and the time period of our resin capsule study. Data sets were provided by the Jornada Basin Long-Term Ecological Research (LTER) project.

Statistical analyses

We used general linear model analysis (JMP v.5.0.1, SAS Institute Inc.) to study how N stocks and transformations (net N mineralization, N uptake, N leaching under 25cm, and soil NO$_3^-$ and NH$_4^+$ availability) change with changes in PPT. The replicated regression approach was appropriate given our experimental design consisting in a continuous PPT gradient (Cottingham et al. 2005). We used N fertilization as a qualitative predictor variable in an ANCOVA design. The ANCOVA model includes precipitation, a factorial combination of fertilization as

whole plot effects and block as a random effect.

Net N mineralization, N uptake, and available soil N were regressed against PPT and fertilization as explanatory variables. Nitrate and ammonium leaching were regressed against the PPT received during the incubation period. We used water-year PPT as the amount received between October 1st of the previous year and September 30th of the current year. We calculated PPT input in each water addition treatment by adding the % increase in amount to that recorded at the weather station. Precipitation input in each interception treatment was calculated by subtracting 80% or 50% to the precipitation received in the study site. We log transformed N leaching loss and N stocks data to meet statistical assumptions, and then back transformed the linear fits for simpler presentation of results. Values greater than 1.5 times the interquartile range were removed as outliers before analysis and statistical significance was determined at $\alpha = 0.05$.

RESULTS

No PPT effects were detected on net N mineralization along the experimental precipitation gradient that ranged from 62 to 511 mm/yr, neither in the unfertilized nor in the fertilized plots (Fig. 1; Appendix: Table A1; and see Appendix: Fig. A1 for soil moisture response to PPT treatments). Ammonification and nitrification rates were not affected by PPT either (Appendix: Table A1). A similar lack of trend was obtained when years were considered separately (results not shown). Fertilization had a negative effect on net mineralization (Fig. 1; Appendix: Table A1) with no significant interaction between precipitation and fertilization. As a result, ambient N treatments showed a net mineralization of inorganic N ($N_{min} = 5.01 \pm 1.94$ mg N·m^{-2}·day^{-1}, Fig. 1A) while fertilized plots had a net immobilization of N ($N_{min} = -97.53 \pm 21.36$ mg N·m^{-2}·day^{-1}, Fig. 1B). Net N mineralization varied among seasons (Table 1), but the overall annual trend still showed net N production in unfertilized treatments of 1.87 g N·m^{-2}·yr^{-1} and net N immobilization in fertilized treatments of -3.22 g N·m^{-2}·yr^{-1}.

Black grama N uptake showed a positive response to incoming precipitation and to fertil-
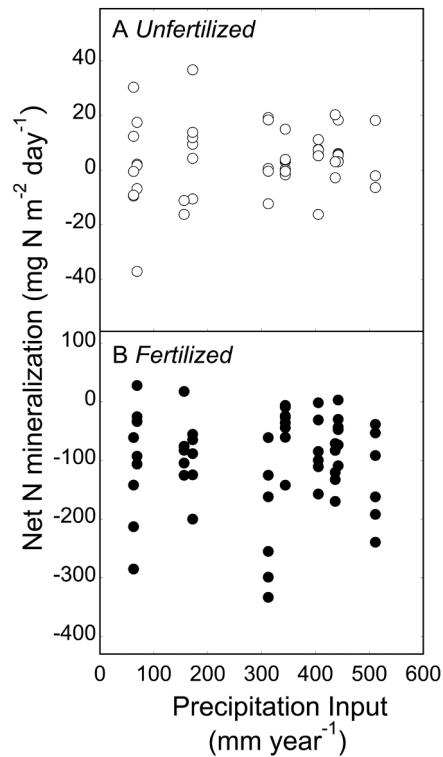

Fig. 1. Effect of annual precipitation on net N mineralization in unfertilized (A), and fertilized (B) treatments. Each data point represents an individual-plot measurement. The x axis represents water-year precipitation input in the five precipitation treatments from 80% reduced PPT to 80% enhanced PPT for 2007 and 2008. Mineralization was not affected by PPT input, but responded negatively to fertilization. Note different y axis scales.

ization (whole model $r^2 = 0.48$, Appendix: Table A2). Nitrogen uptake increased linearly along the experimental rainfall gradient (unfertilized, N uptake [mg N·m^{-2}·yr^{-1}] = 38.37 + 0.67 × PPT [mm]; Fig. 2A). Fertilization had a positive effect on N uptake on an annual basis as well, and grasses from fertilized plots immobilized one order of magnitude more N than grasses from unfertilized plots (fertilized, N uptake [mg N·m^{-2}·yr^{-1}] = 533.47 + 0.67 × PPT [mm]; Fig. 2A). The increase in N uptake due to fertilization was similar across the water availability gradient (PPT × N interaction NS), and was explained by the response of grass ANPP and N concentration in leaves. ANPP significantly increased with precipitation and N addition (ANPP [g·m^{-2}·yr^{-1}]

Table 1. Seasonal and annual net N mineralization (mg N·m^{-2}·day^{-1}) under ambient and N-fertilized (10 g N·m^{-2}·yr^{-1}) treatments.

Treatment	Summer	Fall	Winter	Spring	Annual
Ambient N	4.48	2.54	7.92	6.42	1.87
	(0.99)	(0.95)	(0.95)	(0.97)	(0.32)
Fertilized	−61.33	−9.86	10.11	5.08	−3.22
	(6.17)	(5.64)	(5.52)	(5.46)	(1.66)

Notes: In situ net N mineralization for different seasons and overall annual net mineralization under ambient and N-fertilized treatments. The effect of precipitation treatment was not significant, whereas the effect of time was significant at P < 0.001. Mean values for n = 33, 1 SE in parentheses. Annual mineralization rates were calculated for the first year of experiments as the weighted average of seasonal rates by the incubation length, multiplied by 365 days.

$= 27.29 + 0.10 \times PPT$ [mm] $+ 12.43 \times$ treatment(0–1)N; $r^2 = 0.45$, PPT $p < 0.0001$, N $p = 0.0003$, n = 132). Black grama leaf N content increased only with fertilization (whole model $r^2 = 0.42$; Fig. 2B).

Mesquite N uptake did not respond to changes in water availability or fertilization, and was 3x greater than grass N uptake from control plots (Appendix: Table A2, mesquite N uptake = 685.08 ± 127.10 mg N·m^{-2}·yr^{-1}, Fig. 2C). N concentration in mesquite leaves slightly increased with fertilization and water availability (whole model $r^2 = 0.32$; Fig. 2D), but this did not extend to N uptake because mesquite ANPP was insensitive to precipitation ($F_{1, 127} = 0.018$; p = 0.89).

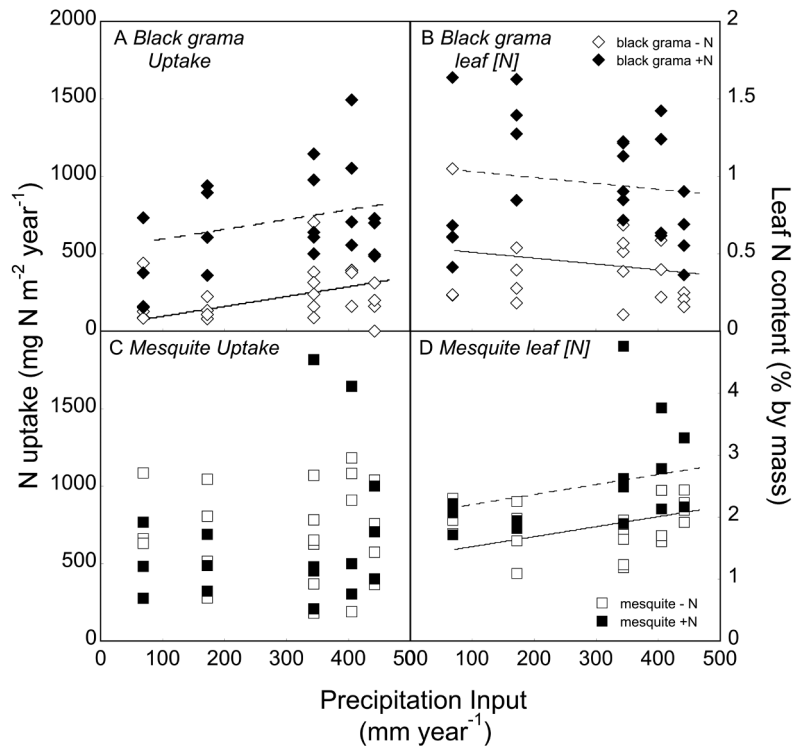N movement below 25-cm depth was inversely

Fig. 2. Precipitation and fertilization effect on black grama (A, B) and mesquite (C, D) nitrogen uptake and leaf N content. Nitrogen uptake was calculated as the product between ANPP and the amount of N in gold-color standing dead leaves for each species. Open symbols represent unfertilized plots and filled symbols represent fertilized plots. The *x* axis represents water-year precipitation input from October 2006 to September 2007. There was an additive effect of precipitation and fertilization on black grama N uptake and the model explained 48% of the variance. Black grama leaf N content increased with fertilization (P < 0.001) and was not affected by precipitation (P = 0.25). Mesquite N uptake was not affected by water availability or fertilization. Fertilization and precipitation increased leaf N content in mesquite leaves (additive effect, ANCOVA, $r^2 = 0.32$). Lines correspond to significant linear relationships with P < 0.05.
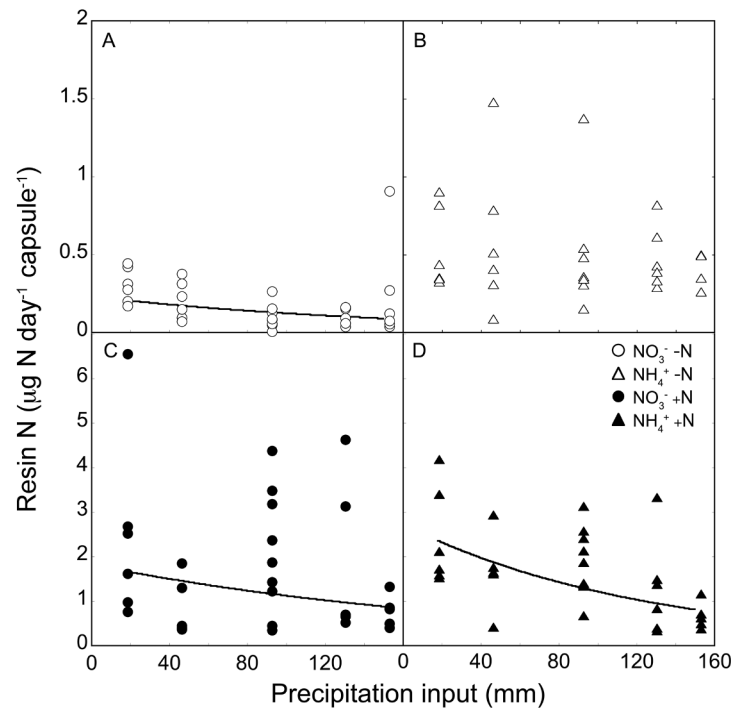
Fig. 3. Effect of incoming precipitation and fertilization on inorganic N in resin capsules at 25 cm depth. Nitrate (A, C) and ammonium leaching (B, D) are expressed as μg N·resin^{-1}·day^{-1}. The x axis represents the precipitation received during in situ incubations in each of the five precipitation treatments. Nitrate leaching (filled and open circles) decreased with precipitation and increased with fertilization (ln Nitrate loss $= -0.432 - 0.006 \times$ PPT $+ [1_{fert}; -1_{unfert}] \times 1.04$; additive effect, ANCOVA, $r^2 = 0.62$). Ammonium loss (open triangles) did not change with PPT in the unfertilized treatment, but decreased with water availability in fertilized plots (filled triangles) (ln Ammonium loss $= -0.09 - 0.003 \times$ PPT $+ [1_{fert}; -1_{unfert}] \times 0.52 + 0.004 \times$ (PPT $\times$ N); ANCOVA, $r^2 = 0.55$). Curves correspond to significant relationships with $P < 0.05$ and were back-transformed for easier interpretation of results. Note different y-axis scales.

related to PPT input (Fig. 3), the opposite trend observed for N uptake. Significant exponential relationships were found between NO_3^- leaching and PPT input in either fertilized or unfertilized treatments (whole model $r^2 = 0.62$, Fig. 3A, C); and between NH_4^+ leaching and PPT input only in fertilized treatments (whole model $r^2 = 0.55$; Fig. 3B, D). Overall, movement of inorganic N to 25-cm depth was greater in plots from drought treatments than in plots with increased water availability, and more N reached 25-cm depth in fertilized than in unfertilized ones (Appendix: Table A3). Only ammonium movement from ambient N treatments was not related to water inputs (Fig. 3B). The conservative estimate of mean total inorganic N ($NO_3^- + NH_4^+$) daily leaching at 25-cm depth from ambient-unfertilized plots was 1.05 ± 0.26 mg N·m^{-2}·day^{-1},

representing 20% of the net mineralization rate in unfertilized treatments of 5.01 mg N·m^{-2}·day^{-1} (Fig. 1A). Fertilized plots loss of total inorganic N below 25 cm depth was 6.92 ± 1.20 mg N·m^{-2}·day^{-1}.

During the time period when we measured N movement below 25-cm depth, which is the depth where the majority of black grama roots are located (Jackson et al. 1996), soil water content in our driest treatment (-80%) ranged between 16% and 12% (Fig. 4B). These are values above the wilting point of these soils (5% [Duniway et al. 2010]) and reflect precipitation events that occurred during July before the incubation started, but that set initial wet conditions for all our treatments (140 mm of accumulated monsoon rainfall in ambient treatments, Fig. 4B). Precipitation during incubations
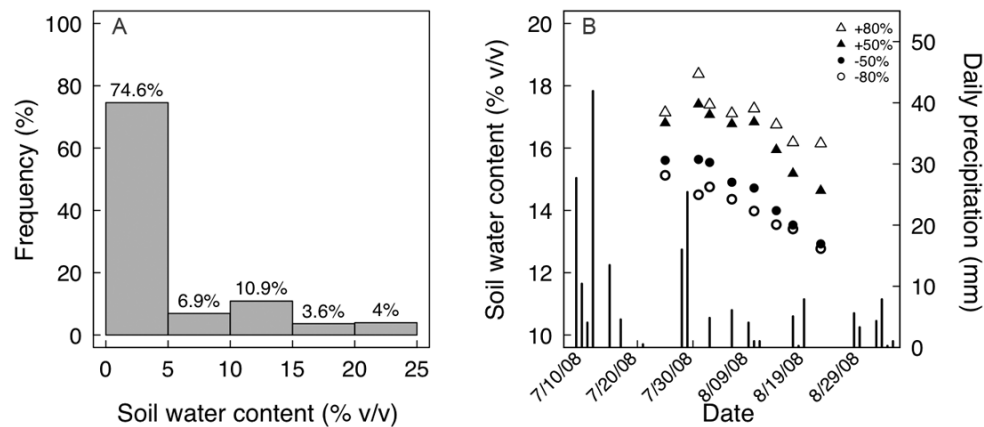
Fig. 4. Frequency distribution of monthly soil water content at the Jornada-IBPE LTER-II grassland site (A), and soil water content 30–50 cm depth from precipitation manipulation treatments at the study site (left y axis, symbols represent treatment means, n = 6) along with distribution of daily precipitation (right y axis, bars) during the experiment of resin incubation at 25-cm depth (B). The IBPE site is located 9 km west of our study site. We synthesized soil moisture data collected by the Jornada Basin LTER (http://jornada.nmsu.edu/lter) at 30 cm depth recorded every August for 19 years (1989–2010) using a neutron probe (Campbell Model 503DR Hydroprobe). Soil moisture is expressed as percent volumetric water content.

was only 92 mm, which resulted in soils drying down towards the end of the incubation. Long-term monthly estimates during a 19-year period showed that soil water contents above 5% occurred in these sites with frequency of 26% (Fig. 4A).

Soil inorganic N availability was inversely related to precipitation input (whole model $r^2 =$ 0.88 for nitrate, $r^2 = 0.87$ for ammonium; Fig. 5; Appendix: Table A3). Nitrate concentration at the beginning of the growing season was highest in drought treatments and decreased exponentially with PPT input (Fig. 5A, D). Ammonium concentration in the soil also decreased with PPT input, but in a less steep fashion than nitrate (Fig. 5B, E). Fertilization increased available soil N and interacted with PPT, because the decrease in NO_3^- stocks with precipitation was steeper in fertilized than in ambient N plots (Appendix: Table A3). Nitrate to ammonium ratio in the soil decreased with increasing PPT (unfertilized, $NO_3^-:NH_4^+ = 1.9844 - 0.0032$ mm^{-1} × PPT [mm], $r^2 = 0.28$, Fig. 5C; fertilized, $NO_3^-:NH_4^+ =$ $1.2299 - 0.0009$ mm^{-1} × PPT [mm], $r^2 = 0.09$, Fig. 5F). The estimated contribution of nitrate to total N with conditions of average PPT (245 mm) would be 55%, which is much larger than reported for other arid-semiarid ecosystems

(Austin and Sala 2002).

DISCUSSION

Our results do not support the hypothesis that increased water availability increases net N mineralization. No treatment effects were detected in in situ net N mineralization to changes in water availability imposed by our rainfall manipulation in two consecutive growing seasons (Fig. 1). This result agrees with findings from the Patagonian Steppe and the Chihuahuan Desert (Reynolds et al. 1999, Yahdjian et al. 2006, Yahdjian and Sala 2008) but disagrees with regional modeling results (Burke et al. 1997) and local experiments (Whitford et al. 1995). There are three compatible explanations for this phenomenon; substrate limitation, location in the soil profile of the bulk of mineralization in the upper layers, or the simultaneous increase of gross mineralization and immobilization along the precipitation gradient. (1) In the long term (months to a year), organic N, which is the substrate of gross mineralization, may constrain mineralization in arid ecosystems regardless of precipitation amount (Yahdjian and Sala 2008). N mineralization would be limited by water availability and microbial activity for only very short
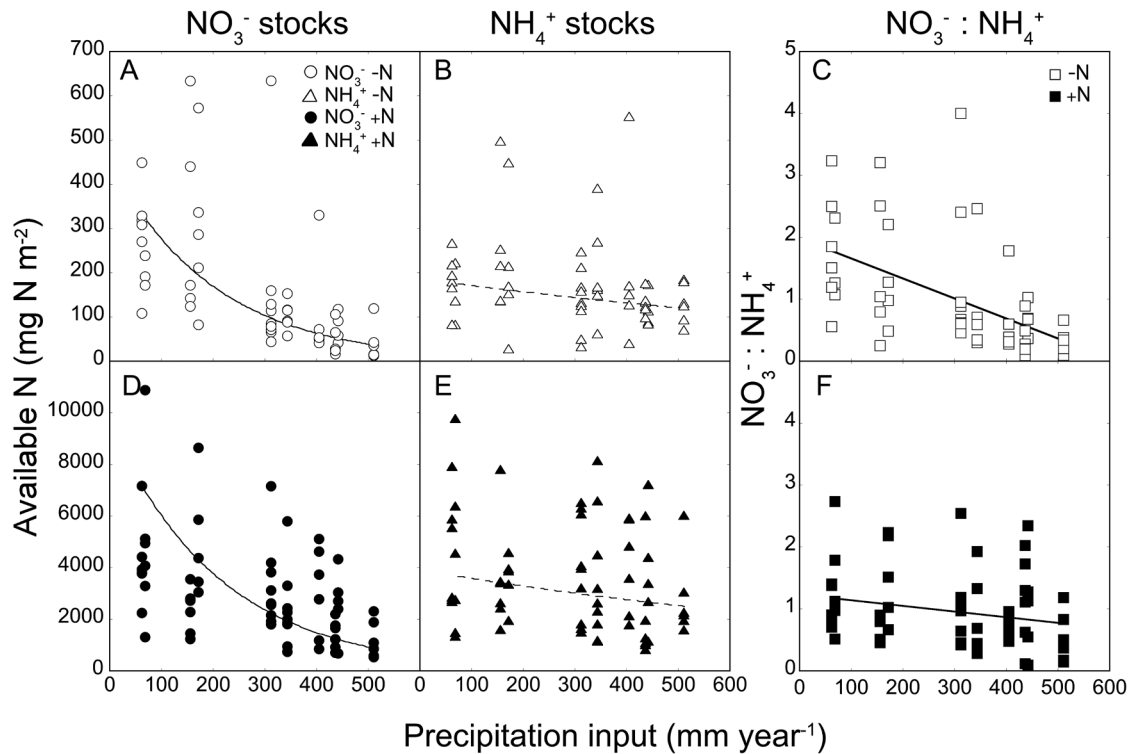
Fig. 5. Effect of annual precipitation and fertilization on available N at the beginning of the growing season. Available nitrate, circles (A, D), was inversely related to precipitation input and was higher in fertilized than in unfertilized plots (ANCOVA, $r^2 = 0.88$). Similarly, available ammonium, triangles (B, E), decreased with precipitation input and was higher in fertilized than unfertilized plots (ANCOVA, $r^2 = 0.87$). The ratio of nitrate to ammonium decreased with water availability (C, F) (ANCOVA, $r^2 = 0.28$). Each data point represents an individual plot measurement for 2007 and 2008 years pooled ($n = 132$). Curves correspond to significant relationships with $P < 0.05$. Note different y-axis scales.

periods of time until it is limited by substrate availability. (2) Most mineralization occurs in the top 2.5 to 5 cm of soil (Schimel and Parton 1986, Norton et al. 2004). This layer reaches field capacity with small rain events (2 to 3 mm), whereas events larger than 6 mm reach to 10-cm depth or more (Duniway et al. 2010). The duration of favorable conditions for mineralization in the uppermost layer would be affected more by frequency than by size of events. Our rainfall manipulations modified the total amount of precipitation by altering the size of events without changing their frequency, which did not change net mineralization patterns. Thus, changes in frequency or amount would yield very different results for net N mineralization in arid ecosystems. (3) It is possible that N immobilization and gross mineralization increase with water

availability at the same rate yielding not a significant effect of water availability on net N mineralization. The increase of gross mineralization and immobilization would produce a counterbalancing effect on net N mineralization. The design of the current experiment does not provide information to evaluate the three potential mechanisms of the net N mineralization insensitivity to changes in water availability. In addition to these ecological explanations, no treatment effects could result from the inherent variability of field estimates of net N mineralization and the heterogeneous spatial distribution of resources in arid ecosystems. Mineralization rates usually exhibit high spatial variability in the field, with rates that span an order of magnitude within a few meters (Robertson et al. 1999). Rates of variability in N mineralization

rates similar to ours have been reported for many ecosystem types (Austin and Vitousek 1998, Barrett et al. 2002, Augustine and McNaughton 2006).

Our results support the hypothesis that plant N uptake increases with increasing water availability (Fig. 2), which improves plant physiological status, increasing photosynthetic rate and thereby growth rate, increasing the N-plant sink. Enhanced soil water availability also results in increased plant water status, stomatal conductance, transpiration and water absorption (Sala and Lauenroth 1982, Sala et al. 1982) that would lead to increased absorption of N. Desert plants can rapidly deploy new roots in response to precipitation events (Lauenroth et al. 1987, Throop et al. 2012), which will also stimulate N uptake by plants. Grass N uptake and leaf N were responsive to N fertilization even in the driest treatment. Mesquite shrubs had higher rates of N uptake than black grama but were not affected by changes in water availability nor fertilization. Mesquite is a N-fixing shrub and N fixation may provide up to 48% of N uptake (Lajtha and Schlesinger 1986, Geesing et al. 2000, Schlesinger et al. 2006). Thus, it is not surprising that mesquite maintains constant N uptake regardless of soil N availability. We are aware that our estimates of N uptake from ANPP do not reflect changes in the belowground biomass N pool, or N increments in mesquite wood tissue. Still, we think that our estimates reflect overall trends because leaf N generally doubles root N concentrations (Reich and Oleksyn 2004, Yuan et al. 2011). The increase of N uptake with PPT among years and treatments was similar to the results found by McCulley et al. (2009) across a precipitation gradient of the Central Great Plains.

In contrast with our third hypothesis, increased rainfall reduced N leaching. N captured in resin capsules at 25-cm depth, which is a proxy of leaching of inorganic N below the horizon explored by grass roots, was inversely related to precipitation input (Fig. 3). We found that N loss was highest in drought plots with lowest plant N uptake, and lowest in plots with enhanced water availability and highest N uptake (Figs. 2 and 3). Leaching was related to N concentration in the soil that in turn was controlled by N uptake since mineralization was insensitive to water availability. An indirect effect of precipitation on N leaching occurred through the effect of precipitation on plant N uptake. Moreover, N loss was higher in fertilized plots than in unfertilized ones (Fig. 3), showing that N loss was positively related to N concentration in the soil (Appendix: Figure A2). The highest N loss occurred with drought and accounted for 31% of N mineralization in unfertilized treatments. This would represent a significant loss of N for grasses in this ecosystem that have 70% of their root biomass in the top 30 cm (Jackson et al. 1996, Gibbens and Lenz 2001). Our experimental results in conjunction with analysis of soil and precipitation characteristics of the Chihuahuan desert (Fig. 4A, B) suggest that N leaching during dry years is a general phenomenon. Given the soil characteristics at the study site, water infiltrates to at least 30 cm in a dry soil with precipitation events $\geq$18 mm (AWHC 0–30 cm = 0.061 (v/v) [Duniway et al. 2010]). Single rain events of 20 mm or more are common during the monsoon season (Fig. 4B, right y axis) and current climate models predict an increase in extreme events (Solomon et al. 2007). Once field capacity is reached, N would be leached below the grass-rooting zone. One key characteristic of this monsoon driven desert grassland is that there is no significant relationship between the maximum daily rainfall and the mean average rainfall (Wainwright 2006), thus extreme events of >30 mm may occur with the same probability in dry as well as wet years. Our comparison between soil-water conditions during the experiment and long-term soil water content measured monthly during a 19-year period further supports the generality of the N leaching phenomenon in dry years (Fig. 4A, B). We found that 20% of the soil-water content measurements taken at 30 cm depth within 1989 and 2010 had soil moisture values greater than 10% (Fig. 4A). In synthesis, long-term water content and our soil water measurements support the idea that deep percolation of N is a phenomenon that may occur even in dry years (Walvoord et al. 2003). The increase of leaching during dry years (Fig. 3), gives additional support to the idea that N conservation decreases with drought.

In agreement with our fourth hypothesis, there were major changes in the proportion of N species in the soil as a result of changes in water availability. Nitrate to ammonium concentration

was close to one under ambient precipitation conditions but shifted from nitrate dominated in drought treatments to ammonium dominated in wet treatments (Fig. 5). We identified three non-exclusive mechanisms that could result in changes in the ratio of inorganic N forms: differential movement of nitrate over ammonium under wet conditions; differential plant preference for ammonium and nitrate; and differential moisture sensitivity of nitrifying over ammonifier bacteria. (1) Ions like nitrate, which have negative charge, move easily in the soil matrix in contrast to ammonium, a cation that binds easily with negatively charged clay particles and consequently has limited movement in the soil profile (Paul 2007). (2) Plant species preference for nitrate relative to ammonium has been shown for grasses in several ecosystems (Gherardi et al. in review; Streeter et al. 2000, Bardgett et al. 2003, Weigelt et al. 2003, Weigelt et al. 2005). (3) It has been proposed that nitrification is sensitive to moisture and that is more inhibited during wet conditions than is ammonification (Schimel and Parton 1986).

Overall, our results revealed that, during dry conditions, there exist a temporal decoupling between microbe-mediated N availability and plant activity that made the system susceptible to nitrate leaching to deep soil layers after nitrate accumulated. The effect of water availability on N metabolism led to a change in inorganic N availability in the soil (Fig. 5), so that N accumulated during drought conditions and decreased with increasing water availability. N net mineralization was insensitive to changes in water availability whereas plant N uptake and leaching were tightly related to changes in moisture although in opposite directions with high water availability enhancing uptake and reducing leaching. Dry conditions have the opposite effect. The differential response of uptake and leaching explains the patterns of N stocks with water availability. Assuming a growing season length of 90 days, leaching below 25 cm would be equal to 94.5 mg N/m^2 over the growing season in an average PPT year in ambient N treatments. This would represent about 20% of N uptake in an average-PPT year, that results from 105 mg N/m^2 from grass uptake plus half of mesquite N uptake not attributable to N fixation (Latjha and others (1986) estimated

that 48% of mesquite N comes from N fixation), totaling 447 mg N/m^2 of N uptake annually. The decrease in N uptake with drought overshadowed the increase in leaching and resulted in N accumulated in the soil. Similarly, in wet years, the increase in plant N uptake results in lower N availability relative to dry years.

Climate-change scenarios predict reductions in soil water availability in the Southwest US, either as a result of warming, or warming and reduced precipitation (Seager et al. 2007). Our work suggests that a shift to drier conditions would result in a more open N cycle. Dry conditions result in accumulation of nitrate in shallow soil layers and make the ecosystem vulnerable to significant N losses. When dry conditions are followed by a wet year, N losses during the dry year may constrain ecosystem response to increased water availability.

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SUPPLEMENTAL MATERIAL

APPENDIX

Table A1. ANCOVA results for effects of precipitation and fertilization on N-cycle. Effects with $P < 0.05$ are shown in bold. Only significant interaction effects are included in the table; non-significant interactions were dropped from the corresponding model.

| Response variable	F	prob $> F$
Mineralization†		
Treatment		
PPT	1.42	0.24
N	**73.08**	**<0.0001**
Model, error df	4, 103	
Ammonification		
Treatment		
PPT	0.10	0.75
N	**49.94**	**<0.0001**
Model, error df	4, 103	
Nitrification		
Treatment		
PPT	0.13	0.72
N	**31.26**	**<0.0001**
Model, error df	4, 103	
NO_3^-:NH_4^+		
Treatment		
PPT	**25.47**	**<0.0001**
N	0.19	0.65
PPT $\times$ N	**7.06**	**0.009**
Model, error df	5, 111	

†Unfertilized: year 1 vs. year 2 NS, p = 0.78; Fertilized: year 1 vs. year 2 S, (yr1 > yr2), p = 0.0007.

Table A2. Summary of ANCOVA statistic parameters for effects of precipitation and fertilization on plant N-uptake and leaf N concentration.

	Species			
	Black grama		Mesquite	
Response variable	F	prob $> F$	F	prob $> F$
N uptake				
Treatment				
PPT	**4.54**	**0.04**	0.53	0.47
N	**32.62**	**<0.01**	0.11	0.75
Model, error df	2, 41		2, 34	
Leaf N				
Treatment				
PPT	1.85	0.18	**5.47**	**0.025**
N	**30.47**	**<0.0001**	**11.60**	**0.0017**
Model, error df	2, 41		2, 34	

Table A3. Summary of ANCOVA statistic parameters for effects of precipitation and fertilization on N loss below 25 cm and soil N stocks 0–10 cm. With one exception (NH_4^+ leaching loss; $F_{2,55} = 4.94$, $P = 0.011$), block effect was not significant and therefore is not shown for clarity.

Response variable	NO_3^-		NH_4^+		Total inorganic N	
	F	prob > F	F	prob > F	F	prob > F
N loss						
Treatment						
PPT	**4.28**	**0.043**	0.28	0.60	**4.67**	**0.03**
N	**84.71**	**<0.0001**	**45.58**	**<0.0001**	**77.67**	**<0.0001**
PPT × N			**8.22**	**<0.01**		
Model, error df	4, 56		5, 55		4, 56	
N stocks						
Treatment						
PPT	**74.66**	**<0.01**	**5.71**	**0.02**	**34.41**	**<0.0001**
N	**745.09**	**<0.01**	**779.86**	**<0.01**	**1230.51**	**<0.0001**
PPT × N	**10.40**	**<0.01**				
Model, error df	5, 113		4, 114		4, 113	
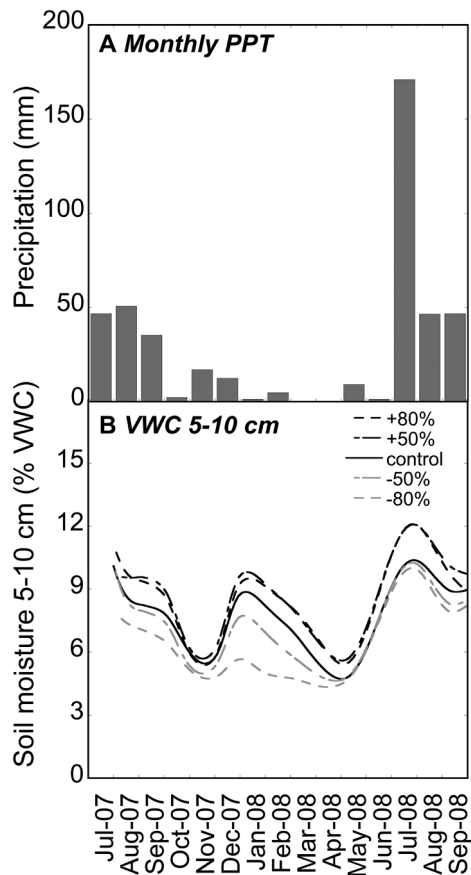
← Fig. A1. Monthly precipitation at the study site from July 2007 to September 2008 (A), and soil moisture expressed as volumetric water content (VWC, monthly average) (B) in the following treatments: 80% enhanced precipitation treatment; the 50% enhanced precipitation treatment; the control represents the ambient precipitation treatment; the 50% reduced incoming precipitation (−50%); the 80% reduced precipitation treatment (−80%). Annual precipitation at the Jornada Experimental Range was very similar during the two years of rainfall manipulation (2007: 344 mm; 2008: 312 mm). Rainfall manipulations changed soil water content: soils from the +80% PPT plots were 23% wetter on average than soils under the 80% reduced PPT treatments throughout the duration of the study ($F_{1,12} = 3.879$, $P < 0.005$).
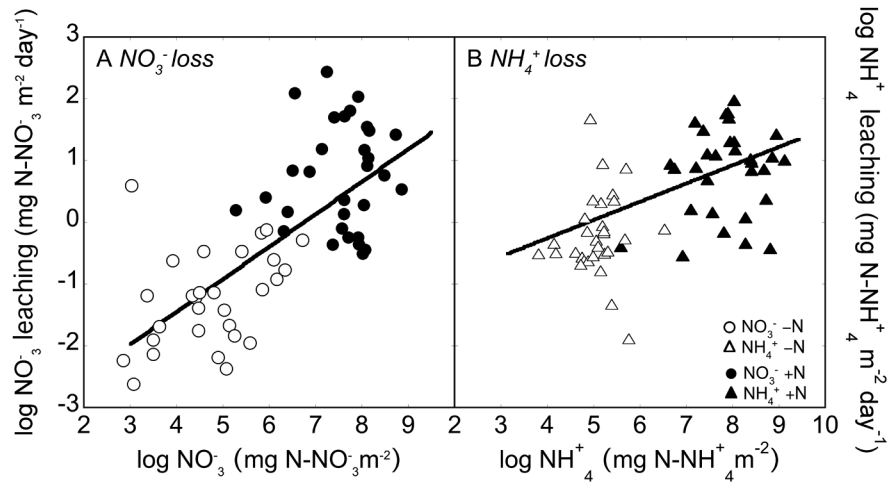
Fig. A2. Log-log relationship between available nitrate and nitrate loss (A) and available ammonium and ammonium loss (B) below the grass-rooting zone. The x axis represents the concentration of nitrate or ammonium at the beginning of the growing season. Nitrate loss (circles) and ammonium loss (triangles) were proportional to the available nitrate and ammonium in the soil respectively (log NO_3^- loss $= -3.55 + 0.53$ log$[NO_3^-]$, n $= 61$, $r^2 = 0.43$; log NH_4^+ loss $= -1.85 + 0.34$ log$[NH_4^+]$, n $= 61$, $r^2 = 0.44$). Leaching rates are based on the assumption that the 3.14-cm^2 resin capsule absorbed reactive N from a soil column not >31.4 cm^2 and not <3.14 cm^2, giving a range for N leaching. The values shown are the average of minimum and maximum leaching rates. Curves correspond to significant linear relationships with P < 0.0001.