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Key Points:

- Woody legume (*Prosopis*) encroachment does not increase N trace gas emissions from upland savannas
- Less commonly measured compounds (NH_3 and NO_y) contribute 12-16% of summer N flux
- Emissions controlled by topography, temperature, and wetting, including rainfall frequency

Supporting Information:

- Supporting Information S1

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Nitrogen trace gas fluxes from a semiarid subtropical savanna under woody legume encroachment

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Abstract Savanna ecosystems are a major source of nitrogen (N) trace gases that influence air quality and climate. These systems are experiencing widespread encroachment by woody plants, frequently associated with large increases in soil N, with no consensus on implications for trace gas emissions. We investigated the impact of encroachment by N-fixing tree *Prosopis glandulosa* on total reactive N gas flux ($N_t = NO + N_2O + NO_y + NH_3$) from south Texas savanna soils over 2 years. Contrary to expectations, upland *Prosopis* groves did not have greater N_t fluxes than adjacent unencroached grasslands. However, abiotic conditions (temperature, rainfall, and topography) were strong drivers. Emissions from moist, low-lying *Prosopis* playas were up to 3 times higher than from *Prosopis* uplands. Though NO dominated emissions, NH_3 and NO_y (non-NO oxidized N) comprised 12–16% of the total summer N flux (up to 7.9 $\mu g\,N\,m^{-2}\,h^{-1}$). Flux responses to soil wetting were temperature dependent for NO, NH_3, and NO_y: a 15 mm rainfall event increased flux 3-fold to 22-fold after 24 h in summer but had no effect in winter. Repeated soil wetting reduced N flux responses, indicating substrate depletion as a likely control. Rapid (<1 min) increases in NO emissions following wetting of dry soils suggested that abiotic chemodenitrification contributes to pulse emissions. We conclude that temperature and wetting dynamics, rather than encroachment, are primary drivers of N flux from these upland savannas, with implications for future emission patterns under altered precipitation regimes.

1. Introduction

Savanna and grassland ecosystems occupy ~40% of the terrestrial surface and may contribute up to 40% of the total global flux of nitric oxide (NO) [*Davidson and Kingerlee*, 1997; *Yan et al.*, 2005]. Many savannas worldwide are undergoing significant changes in vegetation structure and function due to the encroachment of woody plants [*Eldridge et al.*, 2011]. In subtropical, semiarid savannas, encroachment is frequently driven by nitrogen (N)-fixing trees [*Archer*, 1995; *Roques et al.*, 2001; *Moleele et al.*, 2002; *Cabral et al.*, 2003; *Asner and Martin*, 2004] and contributes to large increases in quantity and transformation rates of soil N [*Archer et al.*, 2001; *Boutton and Liao*, 2010; *Blaser et al.*, 2014]. A key question is whether these changes translate into increased rates of N trace gas loss from soils, and if so, how climate and soil conditions mediate these changes. Though encroachment is widespread throughout the subtropics, few studies have explicitly investigated associated changes in trace gas emissions and there is currently no consensus on expected effects.

Woody encroachment can have variable effects on soil properties [*Eldridge et al.*, 2011], but in subtropical/tropical semiarid systems, changes often include increased soil N and organic C, decreased bulk density, increased mineralization and respiration rates, and increased microbial biomass [*Stock et al.*, 1995; *Archer et al.*, 2001; *Hibbard et al.*, 2001; *McCulley et al.*, 2004; *Liao and Boutton*, 2008; *Throop and Archer*, 2008; *Pellegrini et al.*, 2013]. *Prosopis glandulosa* (honey mesquite) encroachment in the southern United States has been shown to increase soil total N storage by twofold or greater and increase NO_3^- concentrations and net mineralization and nitrification rates [*Hibbard et al.*, 2001; *McCulley et al.*, 2004; *Throop and Archer*, 2008; *Bai et al.*, 2009]. Prior studies have reported higher rates of NO or N_2O flux from beneath *Prosopis* or other woody legume canopies, compared with between-canopy interspaces, likely an effect of the "islands of fertility" that form around encroaching trees [*Virginia et al.*, 1982; *Hartley and Schlesinger*, 2000; *McLain et al.*, 2008; *Feig et al.*, 2008a]. However, studies of trace gas flux responses to encroachment at the landscape scale have produced variable results. *Martin et al.* [2003] found a strong correlation between *Prosopis* biomass and NO production, but both *Hartley and Schlesinger* [2000] and *Feig et al.* [2008a] found higher emissions from grasslands than from N-fixing shrublands.

NO is typically the most abundant N trace gas emitted by tropical savanna ecosystems [*Bustamante et al.*, 2006]. NO affects the oxidative capacity of the atmosphere by catalyzing the formation of tropospheric ozone, a regionally important greenhouse gas and pervasive air pollutant [*Denman*, 2007]. The long-lived global greenhouse gas N_2O is also produced in savannas and encroached soils but typically at rates much lower than NO, particularly in well-drained soils [*Scholes et al.*, 1997; *Martin et al.*, 2003; *Werner et al.*, 2014]. Other gaseous N compounds including ammonia (NH_3) and non-NO reactive N oxides (NO_y, including NO_2, HONO, HNO_3, and organic oxides) can form particulates and influence the oxidative capacity of the atmosphere. Several studies report fluxes of NH_3 from desert soils [*Schlesinger and Peterjohn*, 1991; *McCalley and Sparks*, 2008] and of HONO (a component of NO_y) from desert biocrusts [*Weber et al.*, 2015], but neither are routinely measured in natural ecosystems and their fluxes remain poorly defined.

Biogenic production of N_2O and NO in soils occurs largely during the microbial processes of nitrification and denitrification, and additional NO production is also possible during abiotic chemodenitrification [*Pilegaard*, 2013]. Flux rates are linked to rates of these processes and the availability of NH_4^+, NO_2^-, and NO_3^- substrates [*Firestone and Davidson*, 1989; *Medinets et al.*, 2015]. N_2O and NO emissions from arid and semiarid soils are strongly moderated by abiotic conditions, most significantly soil moisture (which also affects O_2 availability), with secondary roles for temperature and pH [*Davidson et al.*, 1993; *Ludwig et al.*, 2001; *Meixner and Yang*, 2006; *Pilegaard*, 2013; *Werner et al.*, 2014]. Seasonal variability in plant N uptake may also influence fluxes in some arid systems [*Homyak and Sickman*, 2014]. Labile C and N availability and microbial N demand are additional controls [*Ludwig et al.*, 2001; *Pilegaard*, 2013] that may be affected by soil type, vegetation cover, and disturbance history [*Parsons et al.*, 1996; *Hartley and Schlesinger*, 2000; *Erickson and Davidson*, 2002].

Pulse dynamics, where large fluxes are associated with infrequent rainfall events, can contribute substantially to total N emissions from seasonally dry soils [*Davidson et al.*, 1991; *Austin et al.*, 2004]. During soil drying, labile N compounds accumulate in soil due to reduced diffusivity and atmospheric dry deposition. These compounds, along solutes released during cell lysis, create a substrate pool for rapid mineralization and nitrification/denitrification upon soil wetting [*Davidson et al.*, 1993; *Austin et al.*, 2004; *Borken and Matzner*, 2009; *Kim et al.*, 2012]. Large increases in NO flux are often observed in dry subtropical soils at the beginning of the monsoon [*Johansson et al.*, 1988; *Davidson et al.*, 1991, 1993]. However, subsequent fluxes in response to wetting are reduced, presumably because repeated watering reduces the accumulated substrate pool [*Johansson et al.*, 1988; *Davidson et al.*, 1991; *Hartley and Schlesinger*, 2000]. Abiotic drivers of NH_3 and NO_y fluxes in natural ecosystems remain poorly understood. Ammonia emissions, produced by dissociation of NH_4^+ ions at high pH, appear to increase with rates of mineralization and NH_4^+ availability [*Schlesinger and Peterjohn*, 1991; *Kim et al.*, 2012]. A few studies report increased NH_3 emissions in response to wetting in desert soils [*Schlesinger and Peterjohn*, 1991; *McCalley and Sparks*, 2008]. However, the mechanistic understanding of these responses is limited [*Kim et al.*, 2012].

The primary goal of this study was to determine whether changes in N cycling associated with woody legume encroachment in a subtropical, semiarid savanna translate into increased fluxes of N trace gases from soils. We also sought to determine the contribution of different N compounds to the total surface N flux, and to quantify the importance of abiotic conditions as a determinant of flux rates in this system. We measured a complete suite of reactive N trace gas (N_t, the sum of N_2O, NH_3, NO_y, and NO) emissions across a spectrum of unencroached grassland to *Prosopis*-dominated vegetation types in south Texas. We sampled fluxes in January, May, and August, replicated over 2 years, from a range of landscape positions. Fluxes were measured before and after the addition of water to simulate single or repeated rainfall events, and flux responses to wetting were considered in the context of broader rainfall patterns.

2. Materials and Methods

2.1. Study Site

Sampling was conducted at the Texas A&M AgriLife La Copita Research Area (27°40′N, 98°12′W), 65 km west of Corpus Christi, Texas, USA, on the eastern Rio Grande Plains. The climate is subtropical with typically warm, moist winters (December–February) and hot, dry summers (June–August). Mean annual temperature is 22.4°C with an average growing season of 289 days (March–November). Mean annual precipitation is

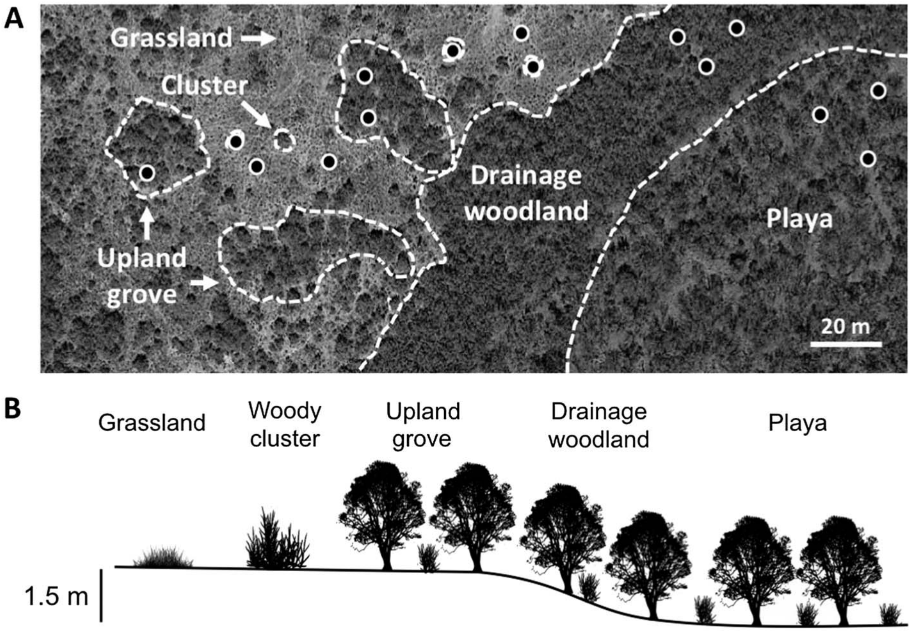

Figure 1. (a) Aerial view of study area near Ben Bolt, TX, with vegetation types delimited remnant grassland, woody clusters, and *Prosopis glandulosa*-dominated upland groves, drainage woodland, and playa. Sampling sites are indicated by white circles (each site equals one group of three collars. N = 45). (b) Site topography.

680 mm, falling year round but with maxima in May and September. During the study period, the site experienced 510, 690, and 750 mm annual rainfall in 2012–2014, respectively. Air temperature and precipitation data were measured every 10 min by a weather monitoring system on site (Nexsens Technology, Alpha, OH).

The area has experienced significant encroachment by woody plants during the past ~100 years, facilitated primarily by N-fixing *Prosopis glandulosa* (honey mesquite) [*Archer*, 1995]. The study sites lie along a 1.5 m change in elevation, from an upland matrix of remnant grasslands, woody clusters, and *Prosopis* groves through *Prosopis*-dominated drainage woodlands into *Prosopis*-dominated lowland playa (Figure 1). Upland remnant grasslands are characterized by native C4 grasses (genera *Paspalum*, *Bouteloua*, *Chloris*, and *Eragrostis*), interspersed with herbaceous cover and bare ground. Clusters are 2–4 m diameter discrete assemblages of mixed woody species, usually dominated by *Zanthoxylum fagara* and commonly including *Condalia hookeri and Berberis trifoliolata*. Clusters are hypothesized to have originally contained a *Prosopis* individual at the center [*Archer et al*., 1988], though often no evidence of these original trees remain. Upland groves are defined by two or more proximate *Prosopis* trees with a diverse woody understory. Upland soils are primarily Typic Argiustolls, usually with a subsurface argillic horizon, interspersed with patches of Typic Ustochrepts lacking an argillic horizon [*Archer*, 1995]. Drainage woodlands (Pachic Argiustolls) have a similar vegetation composition to groves, but with a closed canopy. Low-lying closed-basin depressions (playas) consist of a continuous grass layer (*Paspalum pubiflorum* and *Bothriochloa ischaemum*) with an overstory of *Prosopis* and *Acacia farnesiana*. Playa soils (Ustic Epiaquerts and Vertic Argiaquolls) are capable of supporting standing water after heavy rainfall. Soil characteristics of the five vegetation types (all sampled from the same site used in this study) are summarized in Table 1.

The site has been grazed rotationally by cattle at a low stocking rate for ~30 years, with livestock exclusion occurring for at least 2 years prior to these measurements [*Bai et al*., 2013]. There is no record of fire since at least 1950, and prior to that heavy grazing likely kept fire risk low [*Bai et al*., 2013]. Additional description of soils, climate, and vegetation elements can be found in *Archer* [1995] and *Boutton et al*. [1998].

2.2. Gas Sampling and Analysis

Trace gas flux sampling was conducted at seven time points: August 2012; January, May, and August 2013; and January, May, and August 2014. Fluxes were measured from nine collars in each of the five vegetation types (grassland, cluster, *Prosopis* grove, *Prosopis* drainage woodland, and *Prosopis* playa, $n = 45$), distributed

Table 1. Soil Characteristics of Five Vegetation Cover Types at the Study Site, the Texas A&M AgriLife La Copita Research Area[a]

Characteristic	Depth (cm)	Error Unit	Grassland	Woody Cluster	Upland *Prosopis* Grove	*Prosopis* Drainage Woodland	*Prosopis* Playa	References
Landscape position			Upland	Upland	Upland	Sloping	Lowland basin	
Soil type			Sandy loam	Sandy loam	Sandy loam	Clay loam	Clay loam	*Liu et. al.* [2013] and *Hibbard et al.* [2001]
Sand/silt/clay (%)			79/9/12	78/10/12	78/9/13	70/13/17	48/20/32	*Liu et. al.* [2013]
pH	0–15	SE	$7.1 \pm 0.1_x$	$7.1 \pm 0.2_x$	$7.2 \pm 0.1_x$	$7.2 \pm 0.1_x$	$6.0 \pm 0.1_y$	*Liu et. al.* [2013]
Bulk density (g/cm^3)	0–15	SE	$1.4 \pm 0.01_x$	$1.3 \pm 0.02_{xy}$	$1.2 \pm 0.02_y$	$1.2 \pm 0.01_y$	$1.8 \pm 0.03_y$	*Liu et. al.* [2013]
VWC[b] (%)	0–15	SE	$5.1 \pm 0.1_x$	$4.6 \pm 0.4_x$	$4.2 \pm 0.01_x$	$7.4 \pm 0.2_y$	$16.4 \pm 0.5_z$	*Liu et. al.* [2013]
Potential WHC −0.3 MPa (% volumetric)	0–10	SE	$12 \pm 1_x$	$13 \pm 2_x$	$13 \pm 2_x$		$20 \pm 2_y$	*Hibbard et al.* [2001]
Total N (g m^{-2})	0–15	SE	$123 \pm 2_x$	$143 \pm 5_{xy}$	$182 \pm 8_y$	$288 \pm 10_z$	$253 \pm 14_z$	*Liu et. al.* [2013]
SMB N (mg N kg^{-1})	0–20	SD	$39 \pm 13_z$	$51 \pm 26_y$	$59 \pm 32_{xyz}$	$90 \pm 40_x$		*McCulley et al.* [2004]
N Mineralization (mg N kg^{-1} d^{-1})	0–20	SD	$0.5 \pm 0.5_y$	$1.0 \pm 0.9_x$	$1.0 \pm 0.8_x$	$1.0 \pm 0.8_x$		*McCulley et al.* [2004]
N mineralization (mg N kg^{-1} d^{-1})	0–10	SE	$0.1 \pm 0.0_x$	$0.5 \pm 0.0_y$	$0.4 \pm 0.0_{yz}$		$0.3 \pm 0.1_z$	*Hibbard et al.* [2001]
Nitrification (mg N kg^{-1} d^{-1})	0–20	SD	$0.4 \pm 0.4_y$	$0.8 \pm 0.8_{xy}$	$1.0 \pm 0.8_x$	$1.0 \pm 0.7_x$		*McCulley et al.* [2004]
[NO_3^-] (mg N kg^{-1})	0–20	SD	$6.3 \pm 4.4_y$	$9.1 \pm 5.7_{xy}$	$21.9 \pm 17.1_x$	$19.7 \pm 15.5_{xy}$		*McCulley et al.* [2004]
[NH_4^+] (mg N kg^{-1})	0–20	SD	$4.1 \pm 5.5_x$	$5.5 \pm 6.1_x$	$8.6 \pm 8.8_x$	$7.3 \pm 6.4_x$		*McCulley et al.* [2004]
Organic C (g m^{-2})	0–15	SE	$1233 \pm 22_x$	$1482 \pm 69_{xy}$	$1925 \pm 87_y$	$3035 \pm 99_z$	$3142 \pm 155_z$	*Liu et. al.* [2013]
SMB C (mg C kg^{-1})	0–20	SD	$328 \pm 130_y$	$458 \pm 166_x$	$435 \pm 183_x$	$592 \pm 212_x$		*McCulley et al.* [2004]
C mineralization (mg C kg^{-1} d^{-1})	0–20	SD	$8 \pm 4.7_y$	$13.8 \pm 7.2_x$	$14.9 \pm 7.6_x$	$16.9 \pm 8.8_x$		*McCulley et al.* [2004]
Total soil respiration (g C m^{-2} d^{-1})		SD	$611 \pm 83_y$	$683 \pm 51_{xy}$	$780 \pm 69_x$	$771 \pm 85_x$		*McCulley et al.* [2004]

[a] xyz values indicate significant statistical differences between vegetation types within a row. Analyses by original authors. VWC, volumetric water content; WHC, water holding capacity; SMB, soil microbial biomass.

[b] Value for April. For additional values see *Bai et al.* [2008].

across 1.5 ha (Figure 1). Collars were constructed from PVC, measuring 25.5 cm in diameter, 15 cm in height, and installed 7.5 cm into the soil 6 months prior to measurement.

For the six sampling campaigns between August 2012 and May 2014, fluxes were measured twice within a 2–3 day period: once before and once 24 h after the addition of a 15 mm artificial precipitation event. Events of this magnitude or greater occurred 10–12 times per year from 2011 to 2014, represent 20% of total rainfall events, and can occur all year round. Simultaneous measurements were made of air and soil temperatures inside the collar.

To investigate short-term flux dynamics during a wetting event, one collar in *Prosopis* playa and one in grassland was measured every 10 s before and after the addition of a 3 mm artificial precipitation event in August 2013. In August 2014, six collars (three in *Prosopis* playa and three in the grassland) were measured repeatedly to determine flux response to soil wetting and rewetting over a period of 7 days. Soil fluxes were measured for 1 day before and 3 days after application of a 15 mm artificial precipitation event. Collars were then allowed to dry down for 3 days and then measured again after the application of a second event of the same magnitude. Average interval between rainfall events at the site for 2011–2014 was 5.3 days, with median interval of 2 days. Soil moisture was determined gravimetrically by taking a 10 cm depth soil core from replicate wetted collars at each measurement time point. Cores were weighed before and after drying at 105°C for 3 days.

Instantaneous flux of NO, NO_y (all other oxidized forms of N, including HONO, HNO_3, NO_2, and organic oxides), and NH_3 was measured using a chemiluminescent NO analyzer (Thermo Scientific, Waltham, MA) after selective chemical and thermal decomposition to reduce or oxidize all reactive N trace gases to NO. A detailed description of the system can be found in *McCalley et al.* [2011], with the modification that collar lids were covered in foil to reduce temperature increase during measurement. The instrument was calibrated by sequential dilution of an NO standard (Scott-Marrin, Inc. Riverside, California).

Static chamber samples were taken from the same collars as instantaneous fluxes to quantify CO_2 and N_2O flux. Chamber lids were 8 cm tall and were covered in foil to reduce temperature increase during measurement.

Chamber lids contained a septa-sealed sampling port and a vent of path length 60 cm to allow pressure equalization with the atmosphere. Using a needle syringe, 50 ml of head-space was withdrawn immediately after closing the chambers and at 30 and 60 min and placed into preevacuated glass vials fitted with rubber septa (Geo-Microbial Technologies Inc., Ochleata, OK). Samples were analyzed within 1 month of collection at Cornell University, using a gas chromatograph fitted with electron capture and thermal conductivity detectors (Shimadzu GC-2014, Shimadzu Corp. Kyoto, Japan). Chamber volume was calculated using the average of four depth measurements inside of the collar. Fluxes were calculated based on the rate of increase in concentration over time.

Table 2. Two-Way ANOVA of Vegetation Type by Sampling Month for Total Reactive Nitrogen Trace Gas Flux ($NH_3 + NO_y + NO + N_2O$, $\mu g\ N\ m^{-2}\ h^{-1}$), Wither Before Soil Wetting or 24 h After the Addition of a 15 mm Artificial Precipitation Event ($n = 206$ and 217, Respectively)[a]

Vegetation Type	Month
Prewetting	
Grassland$_{xy}$	Jan$_y$
Cluster$_y$	May$_x$
Prosopis grove$_y$	Aug$_y$
Prosopis drainage$_{xy}$	
Prosopis playa$_x$	
F 3.88	8.30
p 0.01	0.004
24 h Postwetting	
Grassland^{ns}	Jan$_z$
Cluster^{ns}	May$_y$
Prosopis grove^{ns}	Aug$_x$
Prosopis drainage^{ns}	
Prosopis playa^{ns}	
F 2.14	122.32
p 0.094	<0.001

[a] Subscript letters denote statistically significant differences between groups. Flux values are displayed in Figure 2. Interactions were not statistically significant ($p < 0.425$ and $p < 0.24$, respectively).

2.3. Statistical Analyses

Statistical analyses were performed in R (R Core Team, 2012) and JMP Pro v10.0.0 (SAS Institute, Cary, NC) with a significance value of $p < 0.05$, unless otherwise indicated. Johnson SI transformations were applied to normalize gas flux data prior to analysis and model building [*Moulin et al.*, 2014]. In cases where rainfall occurred less than 48 h prior to measurement, data were excluded from "prewetting" analyses (25 values excluded out of 506 measurements). For the August sampling seasons five very large N_t flux measurements (defined as greater than two standard deviations above the mean for that sampling season and wetting treatment) were not included in the analysis but are discussed in section 3.1. Repeated measures two-way analysis of variance (ANOVA) was used to assess the effects of vegetation type and sampling month, followed by Tukey's honest significant difference post hoc test to isolate differences between specific areas and months. Regression analysis was used to determine the relationship between flux and temperature. A mixed effects model was fitted to investigate the effects of environmental parameters on soil trace gas flux after artificial wetting. Vegetation type, temperature, time since previous rainfall, and quantity of previous rainfall were tested as fixed effects, and collar identity and year were incorporated as random effects to generate restricted maximum likelihood variance component estimates. Repeated measures *t* tests were used to compare prewetting and postwetting fluxes.

3. Results

3.1. Effect of Vegetation Cover and Landscape Position on N Gas Emissions

Under prewetting soil moisture conditions, total N gas flux ($N_t = N_2O + NO + NH_3 + NO_y$) did not differ significantly between vegetation cover types on upland soils (Table 2 and Figure 2). Specifically, total N emissions did not differ significantly between remnant grassland ($6.6 \pm 2.6\ \mu g\ N\ m^{-2}\ h^{-1}$, annual average ± SE) and adjacent upland *Prosopis* groves ($5.5 \pm 2.2\ \mu g\ N\ m^{-2}\ h^{-1}$, Table 2 and Figure 2). Within cover types dominated by *Prosopis*, there was a significant effect of landscape position/soil type low-lying, clay-rich *Prosopis* playa soils had the largest average annual emissions ($14.0 \pm 3.2\ \mu g\ N\ m^{-2}\ h^{-1}$), significantly greater than sandier upland *Prosopis* groves (Table 2 and Figure 2). Elevated playa emissions were pronounced in summer, where average emissions were twofold (May, $18.6 \pm 3.5\ \mu g\ N\ m^{-2}\ h^{-1}$) to threefold (August, $17.2 \pm 3.6\ \mu g\ N\ m^{-2}\ h^{-1}$) greater than for other vegetation types. Under postwetting conditions (24 h after the addition of a 15 mm artificial precipitation event), there was no significant difference in flux between vegetation types.

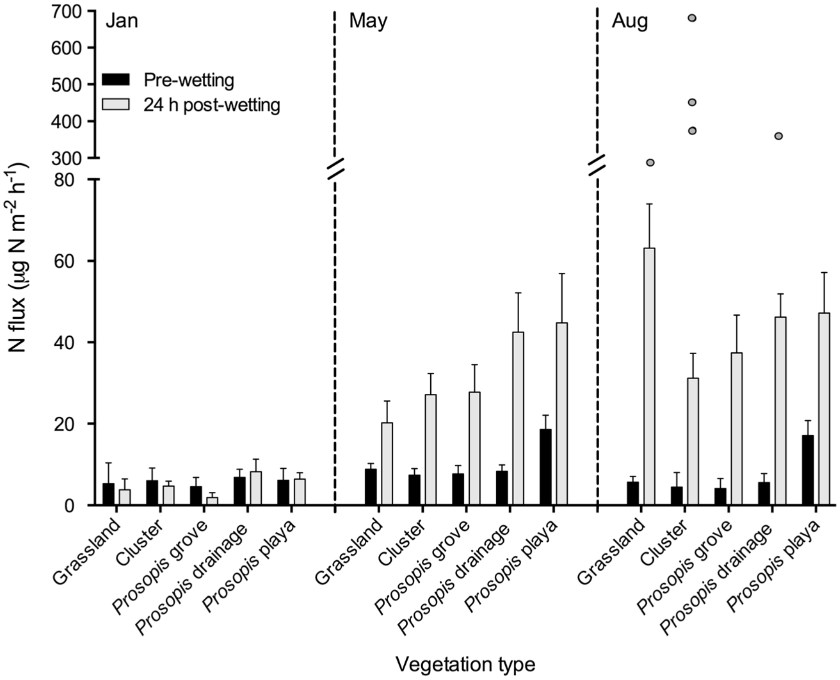

Figure 2. Reactive nitrogen trace gas flux ($NH_3 + N_2O + NO + NO_y$, $\mu g\,N\,m^{-2}\,h^{-1}$) from five vegetation types before and 24 h following the addition of a 15 mm artificial precipitation event. Values are the mean of nine collars per vegetation type sampled across 2 years ($n = 18$) ± 1 SE. Outliers not included in average calculations are indicated by grey circles. Statistical analysis is presented in Table 2.

3.2. Flux Composition

NO was the single largest component of the total N flux for all sampling dates, vegetation types, and moisture conditions (Figure 3, winter data not shown). During summer months, precipitation caused an increase in NO flux for all vegetation types ($p < 0.001$, Figure 3). NH_3 and NO_y also made up a significant portion of the total surface N flux during summer (Figure 3). For NH_3, wetting induced an increase in flux magnitude in three of the five vegetation types (grassland, cluster, and *Prosopis* grove). Overall, NH_3 made up 8.4% of the total flux measured under prewetting conditions and 9.3% postwetting, equivalent to a flux rate of 0.67–6.12 $\mu g\,N\,m^{-2}\,h^{-1}$ (Figure 3). The absolute magnitude of the NO_y flux decreased with wetting only in the *Prosopis* drainage woodland, though overall NO_y dropped from 7.7% to 2.3% as a percentage of total emissions upon wetting (0.12–1.77 $\mu g\,N\,m^{-2}\,h^{-1}$). By comparison, N_2O comprised 9.7 and 6.5% (−0.47–4.12 $\mu g\,N\,m^{-2}\,h^{-1}$) of prewetting and postwetting emissions, respectively.

3.3. Temperature Response

Temperature had a positive effect on N_t flux, particularly following soil wetting (Table 3). Under postwetting conditions, there was a positive relationship between temperature and flux rate for NH_3 and NO_y (linear) and NO (log linear, Figures 4d, 4f, and 4h), but not for N_2O (Figure 4b). NO was the only compound that also showed a substantial positive flux response to increasing temperature under prewetting conditions (linear, Figure 4g). For NO, this corresponded to an increase from 1.61 $\mu g\,N\,m^{-2}\,h^{-1}$ to 8.51 $\mu g\,N\,m^{-2}\,h^{-1}$ (prewetting) or 3.34 $\mu g\,N\,m^{-2}\,h^{-1}$ to 53.70 $\mu g\,N\,m^{-2}\,h^{-1}$ (postwetting) moving from 5°C to 35°C. For individual vegetation types, average postwetting N_t fluxes were 7-fold to 26-fold greater in August (summer) than in January (winter, Figure 2 and Table 2). N_2O, NO_y, and NH_3 all experienced instances of both net emission and net deposition to the soil surface, with consistent deposition occurring at lower temperatures (<18°C) for NO_y (Figure 4). Though temperature-flux relationships appear relatively variable when data are combined (Figure 4), temperature emerges as the strongest predictor of flux when collar identity, vegetation type, and wetting legacy are also taken into account (Table 3).

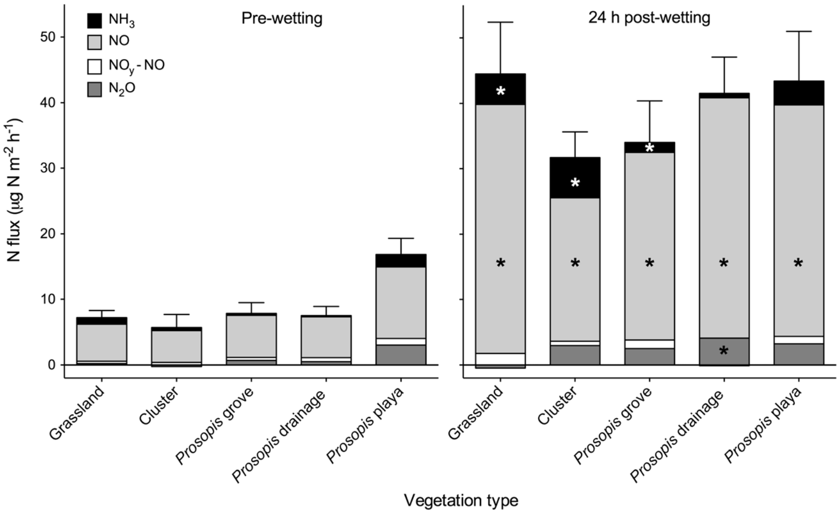

Figure 3. Flux of N_2O, NH_3, NO_y (not including NO), and NO (μg N m^{-2} h^{-1}) before and 24 h after the addition of a 15 mm artificial precipitation event for five vegetation types. Values are the mean for summer months (May and August) ± 1 SE. Asterisks indicate where the flux rate of a compound changed significantly with soil wetting ($p < 0.05$).

3.4. Variation in Response to Soil Wetting and Rainfall Patterns

Several anomalously large N trace gas pulses (defined here as more than two standard deviations above the mean pulse magnitude for that sampling season and wetting treatment) were observed 24 h after artificial wetting (15 mm) of soils under hot, dry August conditions (Figure 2). These anomalously large pulses were observed in grassland, cluster, and *Prosopis* drainage woodland sites across both measurement years and ranged in magnitude from 337 to 698 μg N m^{-2} h^{-1}. These large pulses exceeded average emissions for these vegetation types by between 4-fold and 20-fold and occurred 5 times out of 90 total postwetting flux measurements taken during August. These large pulses did not correlate with any physical factors or disturbances we observed (such as evidence of animal activity) and came from collars that did not display consistently elevated flux values at any other time points.

Total N_t fluxes showed a positive response to soil wetting during summer months (repeated measures *t* test, $p < 0.001$ for all vegetation types, Figure 2), but no response in winter. In August, water addition increased baseline N_t flux by 3-fold in *Prosopis* playa, and between 11-fold and 22-fold for other vegetation types after 24 h.

To investigate the effect of rainfall timing on soil N fluxes, a field manipulation wetting experiment was conducted in August 2014 where two artificial precipitation events of 15 mm were applied 3 days apart. For both grassland and *Prosopis* playa, the maximum flux response to wetting of dry soil was lower for the second wetting than for the first, although soil moisture had returned to prewetting levels before the second addition (Figure 5). For playa, gravimetric water content was 3.7% prior to wetting, increased to 14.4% after the first water addition, and declined to 4.8% before the second addition. For grassland, these values were 3.2%, 10.1%, and 4.0%, respectively. The maximum flux rate for the second wetting was only 42 ± 10%

Table 3. Significant Fixed Effects in a Linear Mixed Effects Model for Total N Flux Measured 24 h Postwetting[a]

Variable	*F* Ratio	Prob > *F*
Air temperature	290.6	<**0.0001**
Vegetation type	5.4	<**0.0002**
Time since previous wetting	31.7	<**0.0001**
Amount of previous wetting	33.8	<**0.0001**
Time since previous wetting × Amount of previous wetting	38.4	<**0.0001**

[a] Flux data were transformed for normality using a Johnson SI transformation prior to analysis. Collar identity and year were included as random effects to account for repeated sampling. $n = 217$.

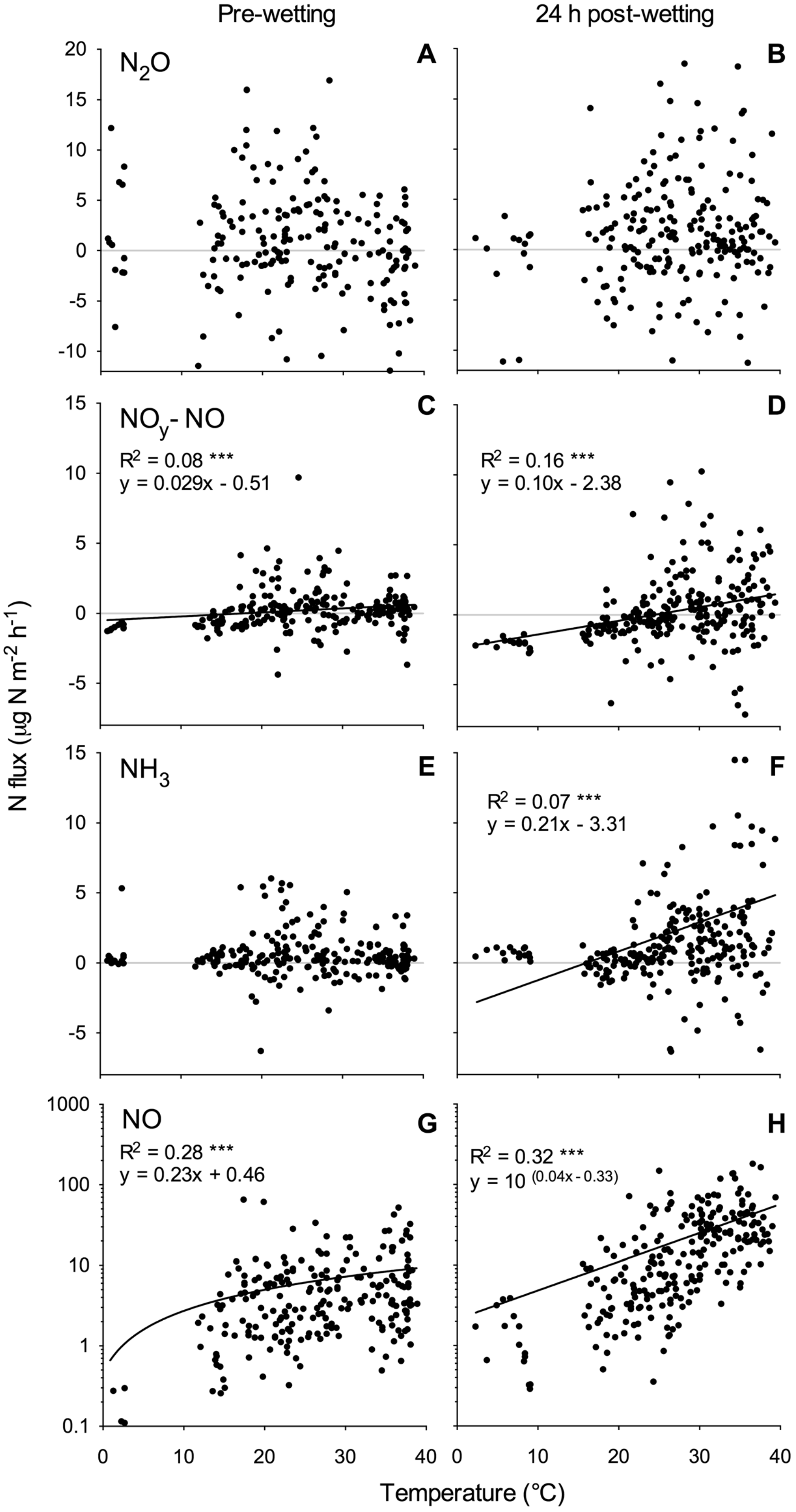

Figure 4. Relationship between reactive nitrogen trace gas flux ((a, b) N_2O; (c, d) NO_y minus NO; (e, f) NH_3; (g, h) NO, $\mu g\ N\ m^{-2}\ h^{-1}$) and temperature (Figures 4a, 4c, 4e, and 4g) before soil wetting or (Figures 4b, 4d, 4f, and 4h) 24 h after the addition of a 15 mm artificial precipitation event ($n = 233$ and 248, respectively). R^2 and p values are for normalized data. Note log scale for NO.

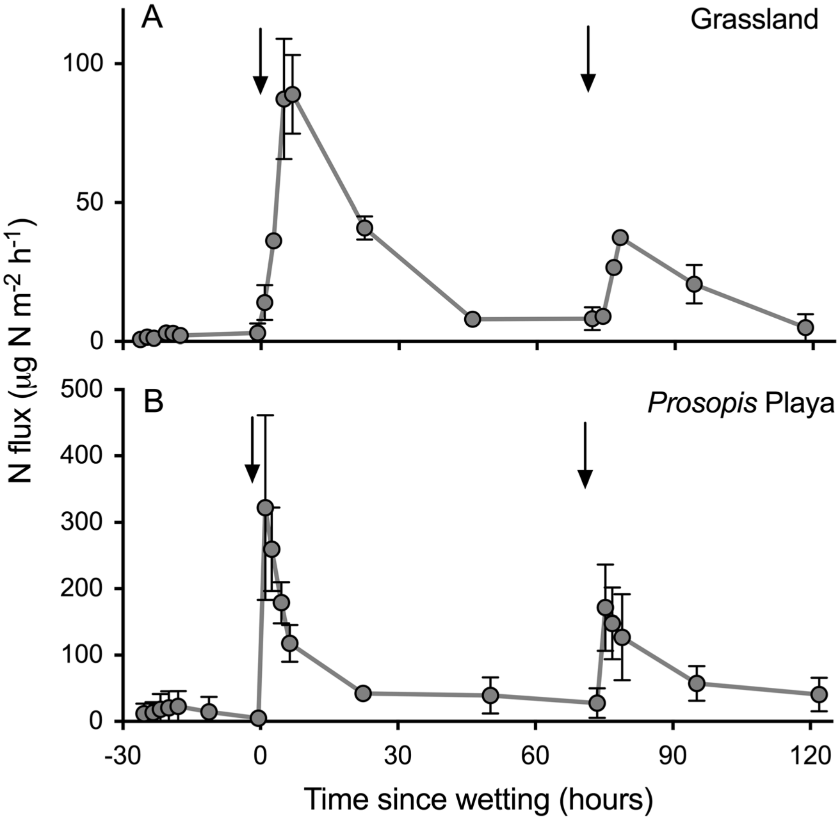

Figure 5. Reactive nitrogen trace gas flux ($NH_3 + NO_y + NO$, µg N m^{-2} h^{-1}) from (a) grassland and (b) *Prosopis* playa in response to sequential soil wettings of event size 15 mm, indicated by arrows. Values are means of three collars per vegetation type ($n = 3$) ± 1 SD, sampled in August 2014.

(grassland, mean ± 1 standard deviation (SD)) or 53 ± 25% (playa) as great as the first. As with previous measurements, there was no significant difference between playa and grassland fluxes 24 h postwetting (43 ± 4 and 49 ± 5 µg N m^{-2} h^{-1}, respectively); however, the peak flux rate immediately following wetting was markedly higher and occurred earlier for playa (322 ± 39 µg N m^{-2} h^{-1}, 1 h postwetting) than for grassland (106 ± 17 µg N m^{-2} h^{-1}, 7 h postwetting, Figure 5). When NO flux was monitored continuously following a 3 mm water addition, fluxes increased above prewetting levels within 50–80 s for both vegetation types, though the rate of increase was greater for playa (Figure 6).

A mixed effects model identified time since previous wetting and amount of previous wetting as significant predictors ($p < 0.05$) of total flux response to a wetting event, along with vegetation type, air temperature, and a significant interaction between the two (Table 3).

4. Discussion

We found no evidence that *Prosopis* encroachment increased total reactive N gas (N_t) emissions from upland soils when comparing established *Prosopis* groves with adjacent remnant grasslands (Figure 2 and Table 2). This observation is surprising given that *Prosopis* groves at this site have twofold or greater mineralization and nitrification rates, ammonium, and nitrate concentrations and 50% greater total N and microbial biomass N than adjacent grasslands (Table 1). NO (which dominates emissions at this site) is produced during microbial nitrification and denitrification. Increases in the substrate pools for these processes (NH_4^+ and NO_3^-, respectively) and the rates at which these processes occur would be expected to increase gaseous N losses [*Davidson et al.*, 2000], especially because other influencing variables such as pH and temperature are held relatively constant (Table 1). Correlations between inorganic N concentrations (particularly NH_4^+) and NO flux have been observed in similar ecosystems [*Scholes et al.*, 1997; *Martin et al.*, 2003]. However, other arid land studies have shown that inorganic N pools and cycling rates are not always good predictors of NO flux

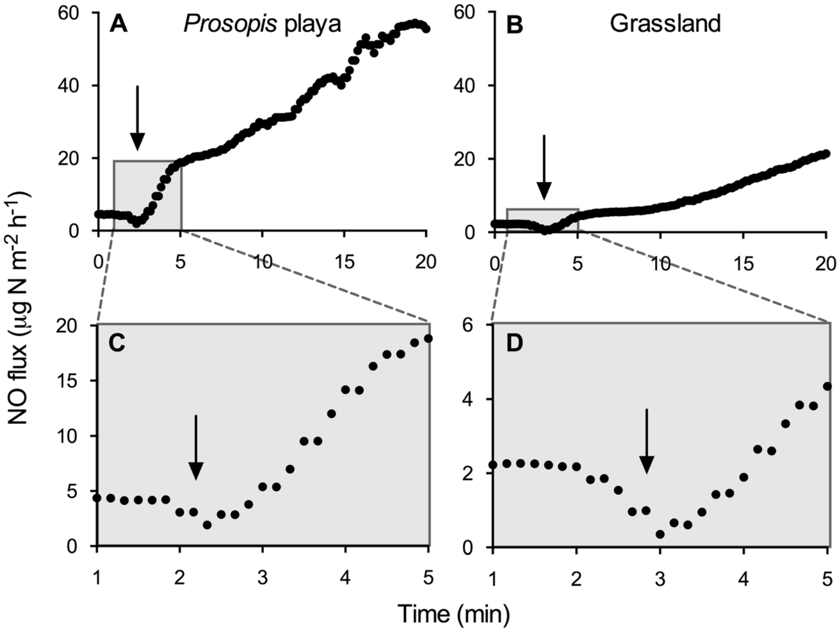

Figure 6. Nitric oxide flux (NO, μg N m^{-2} h^{-1}) from (a, c) *Prosopis* playa and (b, d) grassland soil in response to 3 mm water addition, indicated by arrow. Sampled in August 2013. Decreasing flux at ~2–3 min reflects brief removal of collar lid for water addition.

[*Hartley and Schlesinger*, 2000]. Within a *Prosopis*-dominated shrubland, *Hartley and Schlesinger* [2000] observed that short-term variation in mineralization and nitrification rates and inorganic N concentrations of up to an order of magnitude did not correspond to significant increases in NO production and vice versa. It is possible that increases in N demand during encroachment may reduce the potential for “leakiness” of NO from encroached soils [*Davidson et al.*, 2000], even in the face of measurable changes in N cycling. In *Prosopis* groves, a greater proportion of soil N occurs as microbially recalcitrant compounds and N-acquiring enzyme production increases at a greater rate than total N [*Creamer et al.*, 2013]. This can be interpreted as evidence of continued N limitation to the microbial community even in the face of greater total pools [*Creamer et al.*, 2013]. In addition, the large increase in plant biomass with encroachment almost certainly drives increased root inorganic N demand.

Several other studies have also found that woody legume encroachment does not have predictable effects on N trace gas fluxes. The range of observed responses are variable, from apparently strong relationships between *Prosopis* biomass and NO emissions [*Martin et al.*, 2003] to equivalent or greater NO flux from grasslands than legume-encroached shrublands [*Hartley and Schlesinger*, 2000; *Feig et al.*, 2008a]. With few examples to work with, and the complication of confounding factors within studies (particularly different soil types), it is difficult to parse out which features of a system determine response. *Jackson et al.* [2002] determined that precipitation was a major determinant of C and N storage or loss during shrub encroachment. We note that in the examples above, the temperate system showed a positive NO response to encroachment, while the two arid systems both showed no response. The temperate *Prosopis* system in north Texas [*Martin et al.*, 2003] has comparable rainfall to the system studied here but was somewhat cooler, with lower *Prosopis* density and a wider range of summer soil NO fluxes (1.67 to 32.5 μg N m^{-2} h^{-1}) than we observed. In practice, changes in N emissions are likely dependent on the specific attributes of a system, such as degree of C and N increase, precipitation and temperature, time since encroachment and plant density, and soil physical characteristics.

Results from this study point to temperature and wetting conditions, rather than encroachment, as the major controls over total reactive N trace gas emissions in this ecosystem. The highest N_t emissions in the landscape occurred in *Prosopis* playa soils (Figure 2) and were likely driven by soil type and topographic effects on soil moisture. Compared with upland soils (which represent >80% of the landscape), low-lying, clay-rich playa soils have elevated water holding capacity and gravimetric soil water content and receive runoff from uplands [*Bai et al.*, 2008, 2010]. Strong positive relationships between NO emissions and soil moisture (below field capacity) are common in savanna, grassland, and arid ecosystems [*Cárdenas et al.*, 1993; *Hartley and Schlesinger*, 2000; *Martin et al.*, 2003]. Thus, increased gas emissions from this soil type may be driven by more favorable moisture conditions for NO production [*Pilegaard*, 2013] under ambient conditions between rainfall events. Following a large (15 mm) simulated rainfall event, playa fluxes were comparable to those from other vegetation types, suggesting that water availability is the primary difference influencing flux under ambient conditions. While we cannot rule out an interaction between *Prosopis* encroachment and soil type/topography as a contributor to increased playa N_t fluxes, the lack of flux increase seen in upland vegetation (between remnant grasslands and encroached *Prosopis* groves) indicates that encroachment alone does not drive increases.

Temperature drives strong seasonal variability in flux response to wetting in this ecosystem (Figures 2 and 4). Water addition increased flux by 11-fold to 22-fold in August (summer, average monthly temperature 29°C) but had no effect in January (winter, 14°C). Positive relationships between temperature and NO, NH_3, and NO_y flux were stronger after wetting. More pronounced temperature dependence is frequently recognized for NO production in wet soils [*Smart et al.*, 1999; *Hartley and Schlesinger*, 2000; *Meixner and Yang*, 2006; *McCalley and Sparks*, 2008], probably driven by greater microbial activity when water availability is not a limiting factor. It is also possible that plant phenology plays a distal role in mediating seasonal flux-wetting responses, i.e., capacity for plant N uptake to respond to wetting events may be lower in August when soils are very dry and plants less physiologically active. However, hydraulic lift (which may facilitate ongoing N uptake) occurs throughout the summer in *Prosopis* [*Zou et al.*, 2005], potentially countering this effect.

NH_3 fluxes in this study increased in response to wetting (by 1 order of magnitude on average, Figure 4f) and were positively correlated with temperature. This relationship may be mediated by increasing NH_4^+ supply due to increased mineralization with temperature [*Dawson*, 1977; *McCalley and Sparks*, 2008; *Kim et al.*, 2012], or by decreased hydrologic connectivity under hotter conditions that promoted NH_4^+ accumulation [*Davidson et al.*, 1990; *Parker and Schimel*, 2011; *Homyak et al.*, 2014]. Unlike most N_2O and NO production (excluding chemodenitrification), NH_3 volatilization is an abiotic process, depending partly on the availability of NH_4^+ ions in soil solution. Though this response to wetting seems intuitive, this effect has been illustrated primarily in deserts and there are few or no reports from other ecosystems [*Kim et al.*, 2012]. This result is significant given the appreciable fluxes of NH_3 in this system, equal to or greater than N_2O (average rates up to 6.12 μg N m^{-2} h^{-1} 24 h postwetting). NH_3 volatilization is not routinely measured in natural (unfertilized) soils because this process is considered to occur primarily at alkaline pH [*Dawson*, 1977; *Schlesinger and Peterjohn*, 1991]. Fluxes here overlapped with those observed by *Schlesinger and Peterjohn* [1991] in Chihuahua shrubland at similar pH (6.7–7.4) [*McCulley et al.*, 2004] but were an order of magnitude lower than those observed by *McCalley and Sparks* [2008] in the highly alkaline (pH 9–11) Mojave Desert. Our results support the idea that NH_3 volatilization can be a significant flux even in nonfertilized soils with close to neutral pH.

Rapid NO increases in response to soil wetting, even for superficial surface events, suggest that abiotic production may play a role in NO flux. For *Prosopis* playa soils, maximum rates of gas flux occurred ~1 h following initial wetting and NO emissions began to increase within less than a minute when only the surface/litter was wetted (3 mm event, Figure 6a). These rapid rates of response, also seen in chaparral soils [*Homyak and Sickman*, 2014], have been attributed to chemodenitrification (abiotic conversion of nitrite to NO) [*Pilegaard*, 2013; *Medinets et al.*, 2015]. Microbial nitrifier response times after long periods of stasis have been reported in the order of minutes to hours [*Davidson*, 1992; *Borken and Matzner*, 2009], but not seconds. Generally, NO_2^- occurs as an intermediate product during nitrification and is metabolized by microbes, but it can also be isolated and concentrated in thin water films during soil drying [*Davidson*, 1992; *Pilegaard*, 2013]. While this abiotic NO process is more favorable at lower pH, it can occur in slightly acidic, neutral or even alkaline soils, or in acidic microsites [*Van Cleemput and Samater*, 1995; *Venterea et al.*, 2005]. Though both *Prosopis* playa and grassland soils saw a flux increase within 50–80 s of wetting, the rate of increase was much slower in the grassland. This may reflect the fact that playa soils have higher C, inorganic N and clay content, and lower pH (6.15), conditions more favorable to chemodenitrification [*Venterea et al.*, 2005; *Liu et al.*, 2010]. Small events (3 mm or less, sufficient to induce apparent abiotic NO fluxes but perhaps not enough to substantively influence plant uptake) make up more than half of all rainfall events in this region, but their contribution to annual fluxes remains unknown.

Soil wetting legacy also impacted flux magnitude, and this effect was evident both in experimental wetting manipulations and in response to natural rainfall variation. N_t flux declined with subsequent water additions under dry summer conditions (Figure 5), even when soil moisture returned to prewetting levels between events. This provides support for the hypothesis that large flux responses to initial soil wetting are driven by rapid abiotic and biotic depletion of accumulated labile N and that subsequent fluxes are lower as a result of reduced substrate availability [*Johansson et al*., 1988; *Hartley and Schlesinger*, 2000; *Butterbach-Bahl et al*., 2004; *Kim et al*., 2012; *Homyak and Sickman*, 2014]. This accumulated N may be derived from atmospheric dry deposition, microbial cell lysis, and exposure of previously protected soil surfaces, all of which increase to at least some extent with the duration or severity of a dry period [*Borken and Matzner*, 2009]. In the Chihuahua Desert, repeated wettings of creosote bush soils were shown to deplete the soil NH_4^+ pool and reduce associated NO fluxes [*Hartley and Schlesinger*, 2000].

Analysis of our complete field data set (243 measurements across 2 years) identified the duration of antecedent dry period as a significant predictor of N_t flux in response to an artificial precipitation event. Recognizing the importance of this process in arid or seasonally dry systems is important for a number of reasons. Predicted decreases in average soil moisture over the next century (particularly in the subtropics) and a likelihood of shifting precipitation patterns [*Meehl et al*., 2007] suggest that the influence of dry-wetting dynamics in controlling N trace gas flux may increase. However, many process-based biogeochemical models represent N gas flux as a function of soil moisture (or water-filled pore space), without incorporating an effect of wetting history or complex shifts in microbial substrate utilization [*Groffman et al*., 2009; *Kim et al*., 2012]. Drying-wetting pulse responses have been developed for the DAYCENT and NOE models and tested with lab data [e.g., *Li et al*., 2010; *Rabot et al*., 2014], but DAYCENT still fails to predict seasonal NO fluxes well in arid landscapes [*Homyak and Sickman*, 2014]. Collecting more data on the magnitude and mechanistic relationships behind flux responses to repeated wetting in dry ecosystems is a necessary step toward modeling and parameterizing this process.

A small number of very large NO fluxes (more than 2 SD above the mean) were measured sporadically 24 h after wetting of dry summer soils and may point to additional biotic factors affecting local gas flux (Figure 2). Though pulse emissions are commonly associated with substrate accumulation and soil wetting [*Groffman et al*., 2009], it is unclear why some collars experienced very large, sporadic pulses while adjacent collars of the same vegetation type that were indistinguishable in all other regard did not, and why repeated measurements from the same collar produced anomalously large pulses only occasionally. These large pulses were distributed across years and vegetation types and accounted for $<3\%$ of the total number of summertime measurements. Subsurface termite activity, common in this system, could be a possible cause of occasional highly elevated fluxes. Termite activity is spatially and temporally variable and has been observed to drive elevated NO emissions in southern hemisphere savannas [*Rondon et al*., 1993].

Annual NO emissions estimates for this ecosystem were generated using calculations incorporating 2 years of January, May, and August sampling data, proportional vegetation cover and rainfall records (described in the supporting information). The resulting NO flux, 0.52–0.57 kg NO-N ha^{-1} y^{-1}, is comparable to estimates for savannas globally. Other studies report NO flux rates ranging from 0.18 kg NO-N ha^{-1} y^{-1} (Chihuahuan desert *Prosopis* shrubland) [*Hartley and Schlesinger*, 2000], to 0.23 kg NO-N ha^{-1} y^{-1} (southern U.S. shrubland/bare soil) [*Davidson et al*., 1998] to 0.36–0.61 kg NO-N ha^{-1} y^{-1} (semiarid South African savanna) [*Davidson et al*., 1998; *Hartley and Schlesinger*, 2000; *Feig et al*., 2008b]. For a north Texas *Prosopis* savanna *Martin and Asner* [2005] estimated an NO flux of 0.9 kg NO-N ha^{-1} y^{-1} from field-based measurements, or 1.6 kg NO-N ha^{-1} y^{-1} incorporating remotely sensed vegetation cover distribution and pulse responses to rainfall. Though we incorporated these factors, we found no effect of encroachment on NO or total N emissions, while *Martin et al*. [2003] identified a relationship between flux and *Prosopis* biomass, a key difference in an ecosystem where *Prosopis* is the dominant vegetation cover. Including other N trace gas compounds in addition to NO increased estimated emissions by 0.06 kg N ha^{-1} y^{-1}, an increase of ~11% over NO alone on an annual basis (supporting information Table 1). *Weber et. al*. [2015] recently reported maximum fluxes of HONO (a component of NO_y) from bare desert soils that fell within the same order of magnitude as many of the NO_y fluxes measured in this study, as well as substantially higher emissions from soils with developed biocrusts.

5. Conclusions

We find no evidence that encroachment of a widespread woody legume *Prosopis* increases N trace gas fluxes from an upland semiarid subtropical savanna, despite myriad changes in soil N cycling. This contrasts with findings of some previous encroachment studies and highlights the lack of consensus on the expected effects of a globally widespread land cover change. Instead, temperature, topography, soil properties, and the occurrence and frequency of precipitation are more important determinants of flux in this ecosystem. Rapidity of flux responses to wetting provides evidence that abiotic NO production (chemodenitrification) may contribute to pulse emissions on soil wetting. Because rainfall history has an important effect independent of soil moisture, modeling efforts and supporting data collection should consider incorporating temporal distribution and dry period severity/duration. This may become increasingly relevant in the future, when precipitation patterns (quantity and temporal distribution) in arid lands of the southern U.S. and elsewhere are expected to shift as a result of global change [*Archer and Predick*, 2008]. We also found that NH_3 and NO_y, less routinely measured compounds, can comprise up to 16% of total N emissions. This adds to evidence suggesting that these compounds are an important component of savanna and arid land N fluxes and should be measured more routinely, especially when the goal is to generate estimates of total surface N flux or to estimate N budgets for arid lands.

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Supporting Information for

Nitrogen trace gas fluxes from a semi-arid subtropical savanna under woody legume encroachment

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Introduction

The following methods were used to scale seasonal N flux measurements to annual emissions estimates for the study site. The scaling results are presented in Table S1.

Text S1.

Methods

Annual N efflux estimates for this ecosystem for the ten-year period 2004-2013 were generated using a model of baseline NO and N_t flux as a function of temperature under pre-wetting (dry soil) moisture conditions, a response function for wetting events, and vegetation type cover.

Annual pre-wetting NO or N_t emissions were calculated as a function of average monthly temperature (National Ocean and Atmospheric Administration National Climatic Data Centre, NOAA-NCDC, www.ncdc.noaa.gov) fitted to vegetation type-specific linear temperature-flux regressions derived from the two-year field data set ($n = 54$ per vegetation type). Flux response to precipitation was determined for a single class of event size ≥ 15.3 mm, as there was insufficient data to determine the response to smaller events. The least squares method was used to fit gamma distribution curves to field measurements of NO or N_t flux for 72 h following a precipitation event of 15.3 mm applied in August. Separate curves were fit for *Prosopis* playa and grassland, and the grassland response was used for upland cluster, *Prosopis* grove and *Prosopis* drainage woodland vegetation types. Flux response to precipitation was set to zero in January, as field measurements indicated that this response was small or absent, and to then scale linearly with temperature according to vegetation type-specific temperature-flux response regressions. Instantaneous post-wetting flux values (24 h after the application of an artificial 15.3 mm event) were calculated as above, using temperature regressions derived from post-wetting field measurements ($n = 54$ per vegetation type). Instantaneous flux values were mapped onto gamma distribution curves and the area under the curve was calculated to determine total flux for 72 hours after each rainfall event. Precipitation data for the period 2004-2013 was obtained from the NOAA-NCDC. Fluxes were calculated separately for each vegetation type and then weighted based on percentage cover (constant over the study period), which was determined using manual classification of Google Earth satellite images (50 cm resolution) in ArcGIS 10.2.2 (ESRI INC, Redlands, USA) for an 18 ha area surrounding the study site.

Results

Seasonal patterns of N gas emissions, wetting-induced response functions, vegetation cover proportions and precipitation records for the period 2004-2013 were combined to produce annual flux estimates for this ecosystem (SI Table 1). For NO alone, this flux was 0.52-0.57 kg NO-N ha^{-1} yr^{-1}, and for all traces gases combined (N_t) was 0.59-0.64 kg N ha^{-1} yr^{-1}. Flux responses to precipitation contributed 0.04 – 0.7 g NO-N ha^{-1} yr^{-1} (for NO) or 0.02 – 0.10 g N ha^{-1} yr^{-1} (for N_t) above baseline dry fluxes, depending on number and timing of precipitation events (≥ 15.3 mm) across years.

Table S1. Estimated annual NO and N_t ($NH_3 + NO_y + NO + N_2O$) gas flux (kg N ha^{-1} y^{-1}) for a subtropical savanna ecosystem experiencing encroachment by *Prosopis glandulosa* for the years 2004-2013. Annual flux estimates include precipitation response function.

Year	Number of precipitation events ≥15.3 mm	Dry soil flux (NO)	Annual flux estimate (NO)	Dry soil flux (N_t)	Annual flux estimate (N_t)
2004	13	0.47	0.54	0.53	0.60
2005	11	0.48	0.54	0.54	0.61
2006	11	0.50	0.57	0.56	0.64
2007	14	0.47	0.54	0.53	0.61
2008	9	0.48	0.55	0.54	0.62
2009	5	0.48	0.53	0.57	0.59
2010	16	0.51	0.55	0.52	0.62
2011	6	0.46	0.52	0.55	0.59
2012	12	0.49	0.56	0.57	0.63
2013	14	0.51	0.57	0.54	0.64
Mean (± 1 S.D.)		**0.48 ± 0.02**	**0.55 ± 0.02**	**0.55 ±0.02**	**0.61 ± 0.02**