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Nitrogen addition has minimal effect in Wyoming big sagebrush *(Artemisia tridentata)* communities

Running head: sagebrush nitrogen fertilization

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Abstract

Nitrogen additions are known to elicit variable responses in semi-arid ecosystems, with responses increasing with precipitation. The response of semi-arid ecosystems to nitrogen are important to understand due to their large spatial extent worldwide and the global trend of increasingly available nitrogen. In this study, we evaluated the impact of nitrogen additions on semi-arid big sagebrush (*Artemisia tridentata*) ecosystems. This is important given that sagebrush ecosystems are poorly understood, despite their prevalence in the western US. In addition, large-scale nitrogen additions have begun on sagebrush landscapes in Wyoming in

order to mitigate population declines in mule deer (*Odocoileus hemionus*). The study objectives were (1) to evaluate the effectiveness of nitrogen fertilization in increasing sagebrush biomass and forage quality, and (2) to assess effects of nitrogen addition on soil biogeochemistry and vegetation community structure. We fertilized 15 plots across 5 locations in western Wyoming using nitrogen (5.47g N m^{-2}), in the form of urea. In addition, we immobilized available nitrogen through surface hay treatments (254g hay/m^2). Nitrogen additions failed to increase growth of sagebrush, alter nitrogen content of sagebrush leaders, or alter greenhouse gas efflux from soils. However, the vegetation community shifted; nitrogen-fertilized plots were only 72% similar to the controls (Bray-Curtis). Over the two years of this study, we did not find indications of nitrogen limitation of ecosystem processes, despite a wet growing season in 2014. Thus, we have found a general lack of response to nitrogen in sagebrush ecosystems, with some shifts in the plant community composition.

INTRODUCTION

Nitrogen (N) additions are known to elicit varying responses in semi-arid ecosystems (1). Ecosystems respond to N in multiple ways, including increased net primary production and plant growth (2,3), plant community composition changes (4), and increased greenhouse gas (GHG) emissions from soils (5). Most semi-arid and arid systems (henceforth 'drylands') are thought to be primarily limited by water, though this limitation can be affected by both intra- and inter-annual variation in precipitation (6). If drylands were only restricted by water-limitation, N additions to the system should have no effect without a concurrent increase in precipitation. However, most dryland systems show a response to N additions alone, indicating at least a partial limitation by N (1,7).

In a meta-analysis of research studies on the effects of N fertilization studies in water-limited drylands worldwide, Yahdjian et al. (8) found that in almost all cases the addition of N increased net primary productivity (NPP), showing that nitrogen limitation – even if minor – is a global phenomenon. The ability to predict the effects of increased N on ecosystem process and structure is important because of the global trend of increasing N deposition (9). Given that drylands cover 40% of the Earth's terrestrial surface (10,11) and 20% of the US (12), it is critical to investigate the effects of increasing N availability in dryland systems, due to their co-limitation by both water and N. In addition, dryland systems are home to 44% of all cultivated land (13,14), making understanding the effects of increasing N particularly important due to the high use N-based fertilizers.

In the western US, big sagebrush (*Artemisia tridentata* spp.) ecosystems are the dominant dryland system (15,16), covering 48 million hectares (ha) and 13 states (17). Despite its current extent in the US, up to 60% of the historic, pre-settlement big sagebrush range has been partially or completely converted to exotic annual grasslands because of increased disturbance to these systems and conversion to grazing and agricultural lands (18). In addition, significant portions of the big sagebrush range continue to be affected by energy development, agriculture, and expansion of urban areas (19–22). These drivers of the loss of big sagebrush — unsustainable use of natural resources, population expansion, globalization, as well as climate change — are the same as those exacerbating desertification in drylands globally (13). Big sagebrush ecosystems are expected to continue to decline in coverage over the next decade with 2.3-5.5 million hectares projected to be impacted due to oil and gas development (23). While agricultural additions of N account for a majority of the increase in N availability globally, energy

development also affects N deposition locally, through the production and release of NO_x compounds (9,24,25).

The effects of nitrogen additions on big sagebrush ecosystems are poorly understood due to both the small number of studies and the complexities associated with potential co-limitation of ecosystem processes by water and N in dryland systems (26). Although current knowledge gaps exist regarding N limitation in dryland ecosystems, increased N availability is widely known to have environmental consequences in other ecosystems (5). These include: (1) increasing the amount of carbon stored in terrestrial biomass in both soil and in aboveground plant biomass (27,28), (2) increasing soil efflux of GHGs (29), and (3) changing the species composition of plant communities (30). Over the past decade, it has become increasingly important to understand the role of nitrogen in sagebrush ecosystems. Our research will help to close the gap in knowledge on the effects of N fertilization in sagebrush.

Mule Deer: A Case Study in the Nitrogen Limitation of Drylands

The Pinedale Anticline of western Wyoming is one of the most disturbed regions of big sagebrush. Land managers are particularly concerned with large population declines in mule deer (*Odocoileus hemionus*) over the 15 years since the onset of natural gas development (31). The direct loss of 615 ha of big sagebrush habitat, and the associated winter forage, appear to be associated with the increased mortality in Sublette Mule Deer Herd (32). By 2010, this population decline has been of sufficient magnitude that the Pinedale Anticline Project Office was required to initiate on-site mitigation.(26). Land managers hypothesized that fertilizing the area with N would increase habitat quality (33) through increases in big sagebrush biomass (i.e., forage production) and N content in forage (i.e., forage quality).

The Pinedale Anticline is over 12,000 ha, with 320 ha in the pilot fertilization area (26,32). Fertilization was performed by helicopter in 2010 and 2011. Each fertilization application was projected to cost US$135/ha – totaling greater than US$1.6m per year. Due to the high cost, potential environmental effects, and the expected dominance of water limitation, some ecologists argued that fertilization of big sagebrush was unlikely to be effective in offsetting forage loss for mule deer (26). The eventual monitoring report of the fertilization project reached a similar conclusion (34), however, as the study was conducted on a previously applied and unreplicated treatment, no generalizable conclusions could be reached.

Given that N deposition is likely to increase through the end of the century, the development of an N fertilization project on the Pinedale Anticline provides a rare opportunity to ask basic research questions about N limitation in drylands, while helping to inform decisions about a regional environmental issue.

Project Goals & Hypotheses

Inspired by the actions on the Pinedale Anticline, the goals of this study were (1) to evaluate the efficacy of N fertilization for increasing big sagebrush biomass and forage quality, and (2) to assess effects of N addition on soil biogeochemistry, plant biomass, and plant community composition. We hypothesized that N fertilization would increase N availability in the soil, however it would not increase growth, biomass, or forage quality of big sagebrush due to primary limitation by water. In addition, we expected changes in plant community composition and a high potential for increased GHG emission.

MATERIALS AND METHODS

Study area and site selection

We conducted this study between 2012 and 2015 in semi-arid big sagebrush plant communities in western Wyoming, near the town of Pinedale (42°51′58″ N 109°51′53″ W). The area surrounding Pinedale is consistent with sagebrush steppe in other parts of Wyoming; plant communities are co-dominated by Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) and mountain big sagebrush (*Artemisia tridentata vaseyana*). Other common plant species present include Sandberg bluegrass (*Poa secunda*), western wheatgrass (*Pascopyrum smithii*), chaffweed (*Antennaria microphylla*), squirreltail (*Elymus elymoides*), and longleaf phlox (*Phlox longifolia*).

The soils in this area are categorized as Typic Haplocryalfs, Typic Dystrocryepts, and Typic Haplocryolls with aridic moisture regimes (35). Soil textures at our sites were categorized as loam, sandy loam, and sandy clay loam. Mean annual precipitation across the region averages 290 mm and mean annual temperature is 2.6 ºC (36). The precipitation in this region falls predominantly in the spring and summer months.

We used NRCS Soil Survey data to identify five site locations across three privately owned cattle ranches, all within 25 km of the Pinedale Anticline and 10-20 km of each other. Sites were sufficiently distributed to represent variability across the region (geographic coordinates for specific sites are not provided due to privacy concerns). We used privately-owned lands because of constraints associated with imposing manipulations on public areas adjacent to the Pinedale Anticline. We identified site locations with similar soil types, textures, and landscape positions, with all sites were positioned on a slight slope. Each site had previously been moderately grazed for over a century; this grazing history is typical of the region, big sagebrush plant communities throughout the western U.S., and - most importantly – consistent

with the history of the Pinedale Anticline, for which we are trying to characterize potential effects of N fertilization.

Experimental design and nitrogen fertilization

To address our hypotheses, we applied three treatments: nitrogen fertilization, nitrogen removal through immobilization (hay addition), and a no-addition control. We created three plots (10m x 10m) at each of the five site locations. Each plot was enclosed by cattle-proof fencing and contained three subplots (2.5m x 8.5m) each randomly assigned one of the above treatments. Subplots were fertilized with nitrogen in the form of urea (4.5g N m^{-2}) and surface hay (well-aged for at least one year) was applied (referred as 'nitrogen removal' here; 250g hay m^{-2}) for the above respective treatments in September 2012. All subplots had a 0.56 - 0.625m buffer between subplots and between subplot edge and fence.

Mineralized Soil Nitrogen

Inorganic nitrogen availability, in the form of nitrate (NO_3^-), and ammonium (NH_4^+), were estimated *in situ* using Plant Root Simulator (PRS®) probes. The PRS® probes are ion exchange resin membranes held within a rigid plastic frame (Western Ag Innovations, Inc., Saskatoon, Canada) and provide an index of nitrogen availability to plants. PRS probes are placed in pairs, one anion probe (collecting NH_4^+) and one cation probe (collecting NO_3^-). Multiple pairs were placed within a single treatment. All probes were washed with DI water following the manufacturer's protocol and shipped to Western Ag Innovations, Inc., (Saskatoon, Canada) for analysis. We installed probes over winter to catch the spring snowmelt, the largest

158 pulse of nitrogen for the year (37), as plots are inaccessible from winter until soils dry out in the
159 spring.
160 During the first year of the experiment (Sept 2012 – May 2013), we placed two pairs of
161 PRS® probes within each treatment of all fifteen plots to measuring available soil nitrogen on
162 the edge (i.e., cusp) of the canopy of a big sagebrush individual, to get an integrated reading of
163 under-canopy and interspace, as N availability is known to be different in those locations (38).
164 During the first half of third year of the study (Oct 2014 – May 2015), two additional sets of
165 probes were installed, three pairs to measure soil N available under the big sagebrush canopy and
166 three pairs in the interspace.
167
168 *Sagebrush Growth & Forage Quality*
169 We estimated big sagebrush annual growth by measuring leader growth (i.e., big
170 sagebrush stems) and the ephemeral branch growth (i.e., inflorescence) in 2014. Leaders and
171 ephemeral branches were collected at the end of the growing season in late October 2014 and
172 dried in the laboratory for five days at 55°C and then at 35°C until mass remained constant over
173 a 5-day period. We then weighed and measured the length of each individual leader and branch.
174 The dried leaders were ground in a ball mill and submitted to the Stable Isotope Facility at the
175 University of Wyoming for total carbon and nitrogen analysis (39), as a proxy for forage quality.
176
177 *Plant Community*
178 We sampled the plant communities at each site over two days in August 2014. We
179 surveyed the plant community using 20 randomly placed Daubenmire quadrats (0.1 m^2) within
180 each subplot. The canopy cover of each plant species was estimated using a modified

Daubenmire (40) method with the following cover categories: <1%, 1-5%, 6-15%, 26-40%, 41-60%, and >60%. Unknown species were marked as unknown in the field and identified using the Rocky Mountain Herbarium (Univ. of Wyoming). If the unknown species were unable to be identified they were not included in the analyses of the plant community.

Soil Trace Gas Emission

We sampled soil trace gas emissions twice in July 2013; each was at least 48 hours after any precipitation because high amounts of water vapor can be can confound measurement of CO_2. All sites were sampled within a single day for each sampling period. We deployed 90 soil gas flux chambers, 2 per subplot across all 15 sites, and arranged them on the cusp of a random interspace and adjacent big sagebrush canopy, as it is well-recognized that shrubs locations and interspaces create important environmental microsites and soil heterogeneity in mountain big sagebrush ecosystems (38).

Sections of polyvinyl chloride (PVC) pipe (10 cm high x 20 cm diameter) were permanently buried in the ground to a depth of 6 ± 2cm, which then acted as bases for the chamber lid. Chamber lids were round, 20cm inside diameter and 8cm high, made of opaque PVC and vented following Hutchinson & Mosier (41). The chamber base and lid fit snuggly together and were sealed using a rubber gasket for the 30-minute sampling period. Once the chamber was sealed, 30 ml samples of air were drawn from the chamber headspace every 15 minutes (t = 0, 15, and 30 mins). Each sample was collected by drawing 60 ml of air from the headspace into a 60 ml syringe (Allison Medical, Inc.), expelling 30ml into the air outside the chamber, and then injecting as much of the sample as possible of the remaining 30ml into a 12ml flat bottomed, pre-evacuated glass vial (Exetainer, Labco Ltd.), sealed with a rubber septum.

Ambient air samples were collected at each plot following the same procedure above and stored with the sample vials to account for any systemic change during the sample period. Standards of known chemical composition were carried along on field sampling campaigns and analyzed along with the field samples to account for any systemic change or contamination. Gas samples were analyzed in the laboratory using gas chromatography on a Shimadzu GC-2014 to measure concentrations of CO_2, CH_4, and N_2O.

Data Analysis

Mineralized soil nitrogen data for each separate N species (NO_3^- and NH_4^+) and 'Total-inorganic-N' (NO_3^- plus NH_4^+) were averaged across all sites for each treatment (n=5) and a Tukey's Honest Significant Difference (HSD) test was used to evaluate differences between treatments within each of the two sampling years.

We analyzed big sagebrush growth and forage quality data by site to examine treatment effects and by individual subplots (n=45) to analyze correlation between soil N and big sagebrush growth. Tukey's HSD test was used to evaluate the site differences between treatments for four metrics of growth and three metrics of forage quality: leader length, ephemeral branch length, leader dry mass, and leader dry density, along with percent C, percent N, and C:N ratio of the big sagebrush leaders. We examined the effect of soil N availability for its ability to predict leader length with multiple models, using the 'gls' function in the 'nlme' package. Models were evaluated using Bayesian Information Criteria (BIC) and the model with the lowest BIC was selected (Table 1).

We averaged plant community data by site (n=5) for total plant cover and species richness. Total plant cover was assessed by summing the cover class midpoint for all species in

each quadrat and then averaging across all quadrate to determined mean total percent cover. We used Tukey's HSD test to evaluate differences in cover and richness between treatments. We also calculated Bray-Curtis dissimilarity using the 'distance' function within the `ecodist` package. Dissimilarity was then converted to similarity by subtracting it from 1. Specifically, we calculated average Bray-Curtis similarity of each treatment (control, N addition, N removal) to every other treatment.

We quantified soil trace gas fluxes for each treatment and plot using the rate of change for each of the gases analyzed over the 30-minute period, calculated as the slope values of a linear regression. Fluxes were then summarized by site (n=5) and evaluated using Tukey's HSD test.

We conducted all data analysis and figure creation using R version 3.4.1 (R core Team, 2017).

RESULTS

The addition of N significantly increased the availability of total inorganic N in soils ($p<0.001$; Fig 1) during the first year of the experiment, while hay addition significantly reduced the availability of N ($p= 0.049$; Fig 1). No differences were found for availability of NH_4^+ between treatments, however NO_3^- was significantly more available in the N fertilized treatments than either the control or N removal ($p<0.001$). By the end of second year of the experiment, no significant differences remained between treatments for total inorganic N, NO_3^-, or NH_4^+. During year one, NO_3^- represented 99.0% ± 0.4 of the total available inorganic N across all treatments/locations, while in year two it was 92.0% ± 1.7.

Fig 1. Mean total soil inorganic N availability within a big sagebrush plant community in Pinedale, WY.

N fertilization occurred in 2012 with soil available N measured via PRS probes in fall/winter/spring of 2012-2013 and 2014-2015 (n=5 sites). The 2013 data were collected from soil on the *cusp* of the canopy and interspace, while 2015 data were collected separately from within the *interspace* and *under the canopy*. Significant differences are indicated by different letters based on $\alpha = 0.05$. Whiskers indicate the standard error based on among-site variation.

The addition of N and the application of hay had no treatment effect on the growth of big sagebrush leaders and ephemeral branches or the C:N ratio of big sagebrush leaders (i.e., forage quality). Increased levels of nitrogen availability were significantly correlated with increases in annual leader growth across all sites ($p<0.001$, $R^2= 0.74$; Fig 2), though nitrogen availability only explained approximately 12% of the variation in leader length across all sites, with length increasing with N availability across all sites. However, ranch location explained the largest portion of the variability at nearly 62%. The model used was `total-N (2013) + ranch` (BIC=122; Table 1).

Fig 2. Relationship between sagebrush leader growth and total inorganic N with ranch as a random effect.

Different ranches and sites are indicated by colors and shapes, respectively. The model used was *leader length = total-N (2013) + ranch* ($p<0.001$, $R^2= 0.74$, n=45).

Table 1. Models and BIC values for all models considered predict length of big sagebrush leaders.

The model including additive effects of Total-N and ranch (in bold) had the lowest BIC and was selected as the best/most parsimonious model.

Model	BIC
Leader Length ~ Total-N (2013)	165
Leader Length ~ Total-N (2013) + Ranch	**122**
Leader Length ~ Total-N (2013) * Ranch	152
Leader Length ~ Total-N (2013) + Treatment	130
Leader Length ~ Total-N (2013) + Ranch * Treatment	155

We found no treatment effect on total plant cover or species richness. However, plant community composition shifted slightly between treatments; a Bray-Curtis similarity analysis showed that the largest differences were found when N was added, with a 72% similarity to both the control and N removal treatments. The N removal and control treatments were 79% similar with respect to plant community composition.

Soil trace gas emission displayed no effect from treatments for any gas species. Differences in flux among treatments types were not significant using a Tukey HSD. Fluxes were relatively consistent with other studies of gas fluxes in big sagebrush plant communities (Table 2).

Table 2. Mean fluxes for 2013 and extrapolated annual fluxes (kg N ha^{-1} y^{-1}) for different GHGs is western Wyoming.

Fluxes are reported as the mean mass of either carbon (C) or nitrogen (N) for the control treatment across both samplings in 2013. For comparison, literature referenced values for similar ecosystems are provided in the right column(s). Reference values are from Norton et al. (2008).

				Reference Values	
Flux Type	**CO_2-C**	**CH_4-C**	**N_2O-N**	**CO_2-C**	**N_2O-N**
mg m^{-2} hr^{-1}	80.26	-0.18	0.03	10.4 – 30.6	0.9 – 4.3
kg ha^{-1} yr^{-1}	1.93×10^{13}	-4.30×10^{10}	7.61×10^{9}	--	--

Discussion

N fertilization significantly increased the availability of N in soil for the first year post-treatment, however there was no significant difference in N availability by the second year. Differences in control values between year one and two are not surprising given the different locations and microsites of their installation. In addition, length of deployment, soil temperature, and soil moisture can all affect the adsorption of anion and cations to their counter-ion resins, making comparisons between years (and conditions) difficult.

The lack of significant increase in soil N availability in the second year in fertilized plots is likely due to 'normal' N losses through N mineralization, gaseous loses (volatilization and microbially-mediated), and leaching. Additions of N were conducted at 4.5 g N m^{-2} (or 45 kg N ha^{-1}), which is 150-900% of annual N mineralization (38). Trace gas emissions of N-containing gases (N_2O and NO_x) accounted for an additional 10-20 kg N ha^{-1} yr^{-1} loss from the soil, which could contribute to the lack of increase observed in inorganic soil N among treatments in year two (5,26). While we found lower fluxes of N_2O compared to these studies one year after fertilization, it may be that fluxes were greater immediately after fertilization or during the spring when water was more available, therefore not captured in our sampling period.

Increased N availability had minimal effect on the length of big sagebrush leaders over the two years of the study. Additional N did not result in an increase in forage quality; C:N ratio and mass of N were the same between the control and N fertilized plots. The minimal difference

between the N fertilized and the control subplots indicates that while N fertilization in the big sagebrush communities of the Pinedale Anticline increased the amount of N available to plants for one growing season, it did not increase forage or forage quality such that mule deer and other herbivores would benefit. Our prediction that there would be no differences between treatments was generally supported, despite the minor increases in leader length correlated with increasingly available soil N estimated in the first year.

While the plant community composition changed slightly with N addition as predicted – becoming more dissimilar with the addition of N, there was no change to either total plant cover or species richness by treatment. This is not surprising as soil N availability was only increased for the first year after treatment. In addition, drylands – and big sagebrush ecosystems – are primarily water limited, making short-lived increases of N unlikely to affect cover or richness. Interestingly, the removal of N resulted in greater differences than N addition. This is likely due to increasing the degree of N limitation within the hay treatment.

There was no effect of N additions on GHG efflux (i.e., CO_2, CH_4, and N_2O) from soil. This is similar to findings in another sagebrush studies by Norton et al. (42), which focuses on the relationship of soil N and GHGs. The lack of significant treatment effect could be due to a number of reasons: (1) trace gas measurements are highly variable and sensitive to a number of different environmental factors; (2) increased GHG fluxes occurred during a time that was not measured, potentially closer to fertilization or during the spring when water is more available; or (3) the ability of the microbial community to produce CO_2, CH_4, and N_2O may be limited primarily by water availability, not N availability.

Our findings are not consistent with other studies, which have generally found a positive response of plant productivity to N fertilization (1,8) indicating some N limitation. However

given that the mean annual precipitation of the Pinedale, WY area is 290mm, it is not surprising that we found no response of leader growth to N additions; Hooper and Johnson (1) found that the response to N was greater for areas with 450-900 mm of precipitation than for 300 mm. It is important to note that while some studies do find a primary water limitation in drylands (44), the response of dryland ecosystems to N can be expected to increase with increased precipitation (45).

Our results indicate that a one-time application of N does not affect soil N availability in the long term. We suggest that annual fertilization would be necessary to maintain high levels of soil N availability within big sagebrush systems, as the levels of soil N were the same between the fertilized and control plots by the second year. However, increases in N availability over the long term may have negative consequences, including higher volatile N losses, and increases in invasive plants (26). The increased levels of N found in the first year after fertilization only minimally increased the length of big sagebrush leaders, and continued application at that level would be unlikely to increase forage quality. Ecosystem processes in drylands are primarily water limited and do not change with short-term N additions, at least over the two-year duration of this study.

CONCLUSIONS

This study was conducted to investigate the role of N limitation in dryland sagebrush ecosystems and to consider the effect of N fertilization to increase forage and forage quality for mule deer. We evaluated responses of soil N availability, annual growth rate of big sagebrush, and forage N concentration in response to N additions. In addition, we investigated the response of trace gas production and plant community structure to increased N. This study demonstrates that N fertilization does increase N availability in soil for one year after fertilization. However, this

increased soil N availability is only loosely associated with increased growth of big sagebrush and not likely to impact forage availability and quality for mule deer. The results of this study suggest that N fertilization is likely a poor choice for mitigation of mule deer population declines on the Pinedale Anticline due to the minimal gains in forage, shifts in the plant community, and high cost.

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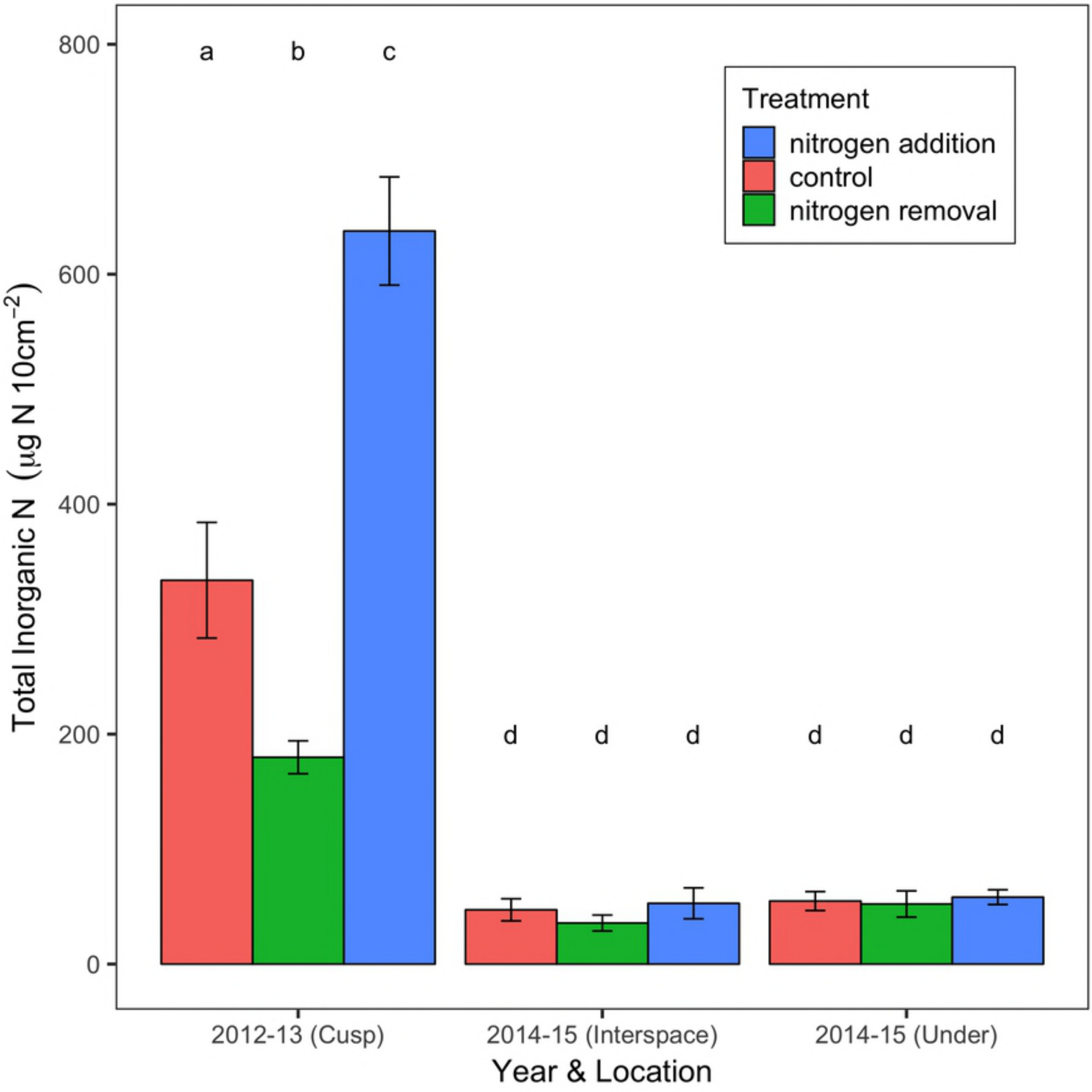
Treatment
nitrogen addition
control
nitrogen removal
Total Inorganic N (µg N 10cm^{-2})
800
600
400
200
0
a
b
c
d
d
d
d
d
d
2012-13 (Cusp)
2014-15 (Interspace)
2014-15 (Under)
Year & Location

Figure 1

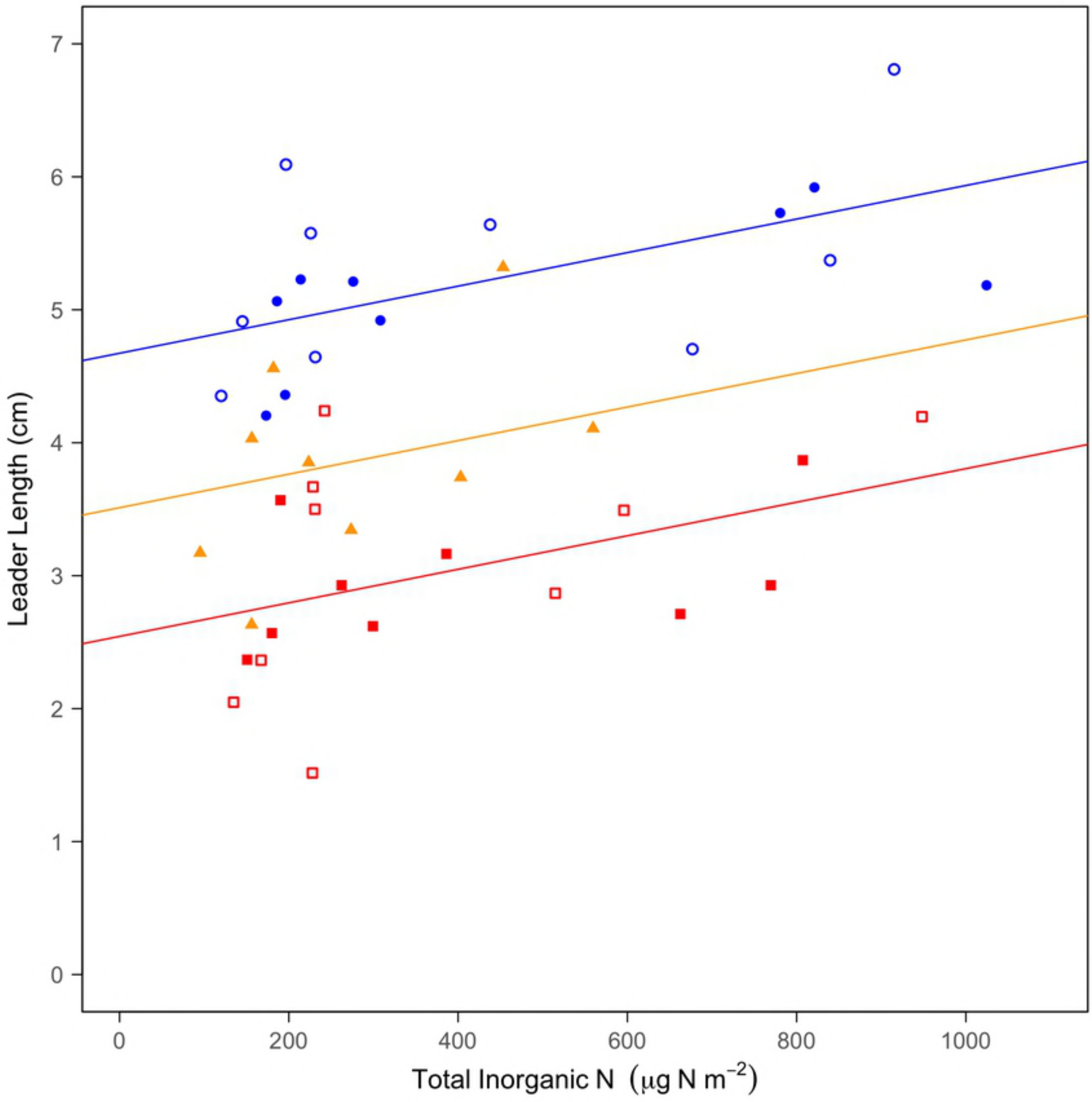
Leader Length (cm)
0
1
2
3
4
5
6
7
0
200
400
600
800
1000
Total Inorganic N (μg N m^{-2})

Figure 2