RESEARCH ARTICLE

The Impact of N Input on Plant Functional Traits Such as C_3, C_4, Native, and Non-native in Terms of Abundance, Frequency, and Density

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Abstract

This study investigates the ecological changes in grassland vegetation at Banaras Hindu University's horticulture field. Caudate methods were used for vegetation analyses. In 72 experimental plots of $1 \times 1m^2$, repeated quadrat sampling yielded 176 herbs. Plant functional types such as legumes, non-legumes, grasses, forbs, and sedges were classified as C_3, C_4, native, and non-natives traits based on their frequency, abundance, and density. From the rainy season of 2016 to the summer season of 2019, the mean values of frequency, abundance, and density against the N gradient were calculated. Therefore the results showed that, nitrogen is essential for the competitive equilibrium of C_3 and C_4 species. This study suggests that N deposition-induced changes in competitive interactions may be disadvantages to native species that thrive in low-nutrient environments, such as N_2-fixers, ultimately leading to changes in the composition of plant communities. In comparison to N_2 fixers, non-N_2 fixers appear to be more effective at using extra N for growth. Our findings show that the diversity of grasslands has dramatically shifted from native to introduced species, proving that non-native space invaders are destroying the rich grassland ecosystems around the world. By changing the dominant species and its response to which species dominates the response at the community level, this change in abundance may alter the ecosystem functions. Based on our study, the vegetation was found to be heterogeneous up to N dosage of 60 kg/ha/year.

Keywords: Nitrogen, Natives, Non-natives, C_3 and C_4 plant species, Tg-teragram, Frequency, Abundance, Density.

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Introduction

Nitrogen deposition has been discovered to be a serious threat to the functioning of ecosystems all over the world. N deposition has been identified as a significant threat to the health of fragile ecosystems worldwide (Waldrop *et al.,* 2004; Zhaohui *et al.,* 2012). The primary contributors to atmospheric N depositions have been identified as the combustion of fossil fuels, the combustion of biomass, the modification of land use patterns, and the application of fertilisers. These N deposition sources have multiplied the global N cycle by two over the last century (Fowler *et al*., 2013). According to studies, reactive N deposition in terrestrial ecosystems increased fourfold between 1860 and the beginning of the 1990s, from 15.88 Tg $year^{-1}$ to 63.5 Tg $year^{-1}$ (Galloway *et al.,* 2008 and Zhou *et al.,* 2016). In recent years, nitrogen (N) deposition rates have routinely exceeded 100 Kg N $ha^{-1}yr^{-1}$. By 2050, this rate could increase to 125.2 Tg $year^{-1}$. It is anticipated that the deposition will increase by a factor of 2.5 over the next century (Lamarque *et al.,* 2005). Reactive-N deposition in Asia is anticipated to increase by more than 1.5 times compared to 2000 by 2030 (from 67.7 Tg $year^{-1}$ to 105.3 Tg $year^{-1}$), and by 2020, it is anticipated to surpass the sum of North American and European emissions (Galloway *et al.,* 1995). Deposition of nitrogen (N) has a significant impact on many ecosystems' structure and operation and contributes significantly to global warming. Future community composition may be influenced by how species react to N deposition. Each community is distinguished by its diversity of species, growth forms, structures, and dominance successional trends. The numerical data emphasizes the dominant species in the communities. To assess a species' dominance, certain quantitative traits of the species are expressed, such as frequency, density, and abundance. Analytical characters are served by a variety of techniques, including the quadrat method, line transect method, and point frame method. Even though many ecologists have already made contributions to research on ecological diversity. (Aerts *et al.,* 2006; Wassie *et al.,* 2010; Gotelli and Colwell 2011; Graham and Duda 2011.; Erenso *et al.,* 2014; Cadott *et al.,* 2002; Chiarucci *et al.,* 2001). However, only a few studies have looked into the connection between tropical nitrogen (N)-deposition gradients and PFTs (plant functional traits). The majority of research has been done in neotropical (Stevens *et al.,* 2003; Diaz *et al.,* 1999; de Bello *et al.,* 2005; de Bello *et al.,* 2006) and temperate climates (Condit *et al.,* 1996; Kraft *et al.,* 2008). Furthermore, the performance of individual species at various life stages may have an impact on how plant communities react. As a result, pinpointing the precise functional and/or phenological traits impacted by N may be necessary to ascertain which species and plant communities

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Table 1: Effect of N- input (kg-N/ ha/yr) on the frequency of C_3, C_4, natives and non-natives species (Seasonal data from 2016-2019 were used).

Frequency of PFTs	*Season*	*0 (kg-N/ ha/yr)*	*30 (kg-N/ ha/yr)*	*60 (kg-N/ ha/yr)*	*90 (kg-N/ ha/yr)*	*120 (kg-N/ ha/yr)*	*150 (kg-N/ ha/yr)*
	Winter	100	100	100	100	100	100
Native	Summer	100	100	100	100	100	100
	Rainy	100	100	100	100	100	100
	Winter	100	100	100	100	100	100
Non-native	Summer	100	100	100	100	100	100
	Rainy	100	100	100	100	100	100
	Winter	100	100	100	100	100	100
C_3	Summer	100	100	100	100	100	100
	Rainy	100	100	100	100	100	100
	Winter	100	100	100	100	100	100
C4	Summer	100	100	100	100	100	100
	Rainy	100	100	100	100	100	100

Table 2: Effect of N-input (kg-N/ ha/yr) on the density of C_3, C_4, natives and non-natives species (Seasonal data from 2016-2019 were used).

Density of PFTs	*Season*	*0 (kg-N/ ha/yr)*	*30 (kg-N/ ha/yr)*	*60 (kg-N/ ha/yr)*	*90 (kg-N/ ha/yr)*	*120 (kg-N/ ha/yr)*	*150 (kg-N/ ha/yr)*
	Winter	5.5	5.61	7.02	3.75	3.19	3
Native	Summer	3.69	3.97	4.08	4.11	3.11	3.05
	Rainy	5.36	5.91	5.25	5	3.33	3.5
	Winter	5.44	4.91	7.22	6.16	5.25	6.66
Non-native	Summer	3.55	3.22	3.83	5.66	4.44	6.08
	Rainy	5.47	5.61	6.75	7.94	3.41	5.86
	Winter	4.16	4.55	5.77	3.36	3.38	3.52
C_3	Summer	3.36	3.30	3.80	3.55	3.36	3.38
	Rainy	4.47	4.83	5.58	4.52	4.02	4.02
	Winter	5	6.72	6.25	4.72	4.80	4.63
C_4	Summer	3.38	4.13	4.77	4.58	5.47	4.61
	Rainy	5.27	6.36	6.61	6.27	8.02	5.52

Table 3: Effect of N- input (kg-N/ ha/yr) on the abundance of C_3, C_4, natives and non-natives species (Seasonal data from 2016-2019 were used).

Abundance of PFTs	*Season*	*0 (kg-N/ ha/yr)*	*30 (kg-N/ ha/yr)*	*60 (kg-N/ ha/yr)*	*90 (kg-N/ ha/yr)*	*120 (kg-N/ ha/yr)*	*150 (kg-N/ ha/yr)*
	Winter	5.5	5.61	7.02	3.75	3.19	3
Native	Summer	3.69	3.97	4.08	4.11	3.11	3.05
	Rainy	5.36	5.91	5.25	5	3.33	3.5
	Winter	5.44	4.91	7.22	6.16	5.25	6.66
Non native	Summer	3.55	3.22	3.83	5.66	4.44	6.08
	Rainy	5.47	5.61	6.75	7.94	3.41	5.86
	Winter	4.16	4.55	5.77	3.36	3.38	3.52
C_3	Summer	3.36	3.30	3.80	3.55	3.36	3.38
	Rainy	4.47	4.83	5.58	4.52	4.02	4.02
	Winter	5	6.72	6.25	4.72	4.80	4.63
C_4	Summer	3.38	4.13	4.77	4.58	5.47	4.61
	Rainy	5.27	6.36	6.61	6.27	8.02	5.52

are most susceptible to chronically increased N deposition. A complex, manipulative experiment that takes into account these various factors helps to clarify where N management efforts should and should not be focused.

Materials and methods

Site Description

Our study took place in a horticulture field between 24°18′N and 83°30′E, 129m above sea level, on the Banaras Hindu University campus in Varanasi, India. Summer (April–June), warm rainy season (July–September), and winter were the three distinct seasons at this study site, which experienced a dry tropical monsoon climate (November-February). The transitional months between winter and summer, as well as between the rainy and winter seasons, are March and October. The annual average rainfall was 932 mm, and the monthly minimum temperatures ranged from 7.3–25.4°C to 25.6–35.6°C (Singh and Singh 1994). The Banaras Hindu University campus has been given Banaras Type III soil (Piper 1944). A light brown silty loam, the soil has a neutral reaction. Alluvial, well-drained, and moderately fertile are the general characteristics of the soil, with low nitrogen availability and medium phosphorus and potassium availability

(Sagar and Verma 2010). According to Sagar *et al.* (2008), the soil's pH ranges from neutral to alkaline).

Plot Design

In the horticulture field, 72 experimental plots each of 1×1m^2 with a 1.5 m buffer strip between every adjacent plot were established in the year 2016. The plots were set up in six parallel rows (12 plots in each row). Within these plots, six N treatments were established with 12 replicates at random: 12 plots received no treatments, 12 plots received 30 kg-N/ha/yr treatments, 12 plots received 60 kg-N/ha/yr treatments, 12 plots received 90kg-N/ha/yr treatments, 12 plots received 120kg-N/ha/yr treatments, and 12 plots received 150kg-N/ha/yr treatments. Treatments were given in the form of urea at the one-month interval in the evening time because the low temperature in the evening, results in a lower loss of N through volatilization (Makoi and Ndakidemi 2008). The chemical formula of urea is NH_2CONH_2 and it contains 46% of nitrogen. So 19.56g and 32.604g urea in 1×1m^2 plot/yr is equivalent to 90kg-N/ha/yr and 150kg-N/ha/yr As a source of dry N urea was taken because of its easy handling; comparatively high N content (46%) and low price.

Vegetation Sampling and Analysis

A 50×50cm quadrat was used to sample grassland vegetation in this study because it is a workable unit for sampling. Each plot was randomly assigned a quadrat, and the total number of species and individuals of each species were recorded monthly. All of the species discovered in each quadrat were identified and numerically counted from the rainy season of 2016 to the summer season of 2019. To learn more about the species found on campus, repeated quadrat samples were collected. This study contributes to the identification and occurrence of dominant functional traits at the study site. To identify the herbaceous flora in the field for this study, we used the Flora of Bombay Presidency (Cooke, 1901). The percentage frequency, density, and abundance were then calculated based on the names of the species and the number of individuals of each species present in each quadrat.

Statistical Analyses

Analysis of variance (ANOVA) was used to determine the effects of N treatments on the functional trait diversity. All statistical parameters were analyzed using SPSS 16.

Abundance and Density

Both words refer to the number of species within a community. Individual species abundance is reported as a percentage of the total number of species present in the community and is a relative statistic. By applying the calculations listed below, we were able to determine the abundance, density, and frequency. Understanding the link between frequency and abundance is essential for understanding how a community is set up.

$$\text{Abundance} = \frac{\text{Total no.of individual of the species}}{\text{No.of quadrate per units in which they occur}} x100$$

$$\text{Density} = \frac{\text{Total no.of individual of the species}}{\text{No.of quadrat per units studied}} x100$$

$$\%\ \text{Frequency} = \frac{\text{No.of units in which the species occurred}}{\text{Total no.of unit studied}} x100$$

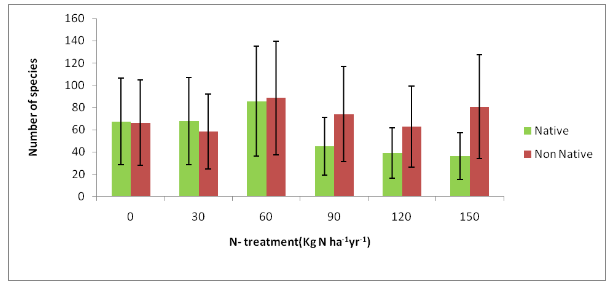

Fig. 1: Effect of N-treatment gradient on a number of species (m^{-2}) of native and non -native during winter season.

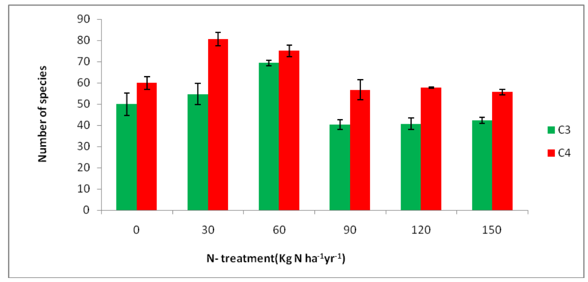

Fig. 2: Effect of N-treatment gradient on a number of species (m^{-2}) of C_3 and C_4 plants during the winter season.

Table 4: Summary of MANOVA for the effect of the year (Y), season (S) and N-treatment (NTRT)on native, non-natives, C_3 and C_4 from rainy 2016 to summer 2019. (Significant level at $p \leq 0.05$*; $p \leq 0.01$**; $p \leq 0.001$***; NS = not significant; df= degree of freedom; F value(ANOVA); Sig=Significant).

Tests of Between-Subjects Effects				
Source of variation	*Dependent variables*	*df*	*F*	*Sig.*
Year	C3	2	6.19	**
C4		2	3.42	*
Natives		2	0.63	NS
Non natives		2	12.19	***
Season	C3	2	26.42	***
C4		2	44.32	***
Natives		2	22.75	***
Non natives		2	43.46	***
NTRT	C3	5	6.85	***
C4		5	5.89	***
Natives		5	14.17	***
Non natives		5	12.18	***
Y*S	C3	4	4.03	**
C4		4	3.25	**
Natives		4	3.65	**
Non natives		4	19.19	***
Y*NTRT	C3	10	.37	NS
C4		10	.22	NS
Natives		10	.90	NS
Non natives		10	2.08	*
S*NTRT	C3	10	1.17	NS
C4		10	2.12	*
Natives		10	2.57	**
Non natives		10	8.98	***
Y*S*NTRT	C3	20	.47	NS
C4		20	.47	NS
Natives		20	1.50	NS
Non natives		20	1.98	**

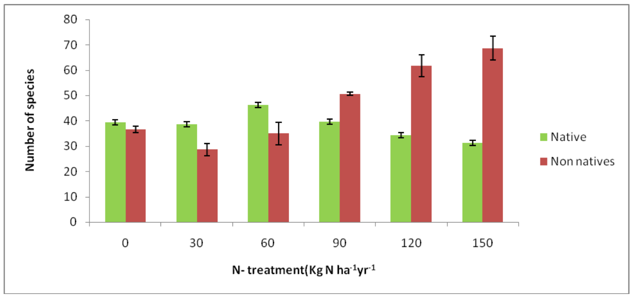

Fig. 3: Effect of N-treatment gradient on the number of species (m^{-2}) of native and non-native during the summer season.

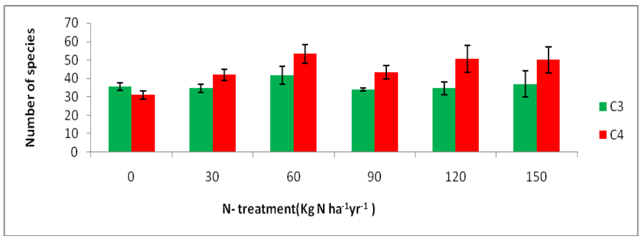

Fig. 4: Effect of N-treatment gradient on the number of species (m^{-2}) of C_3 and C_4 plants during the summer season.

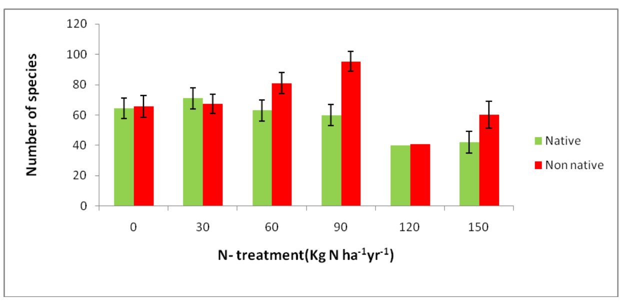

Fig. 5: Effect of N-treatment gradient on a number of species of native and non-native during the rainy season.

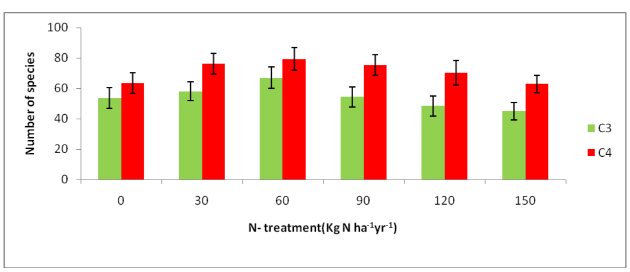

Fig. 6: Effect of N-treatment gradient on number of species of C3 and C4 plants during the rainy season.

Results

Moderate levels of N amendment (60 kg-N/ha/yr) increased the diversity of herbaceous species, and high levels of nutrients decreased it. Therefore, the establishment and maintenance of India's species-poor dry tropical grassland may require 60 kg-N/ha/yr of N fertilization.

The frequency of native, non-native, C_3 and C_4 species did not change either across the seasons or the N-gradient. Frequency is the number of sampling units or quadrat in which a given species occurred(Table 1).

Based on 3-year seasonal data obtained for density, among all the plant functional traits, both native and non-native's density was found maximum in 60 Kg-N/ha/yr treated plots and minimum in 150 Kg-N/ha/yr treated plots. C_3 and C_4 plant species also showed different distribution patterns across the six N-treatments during the three seasons of the year- winter, summer and rainy. During the summer season, C_3 and C_4 species showed a significant relationship with N-treatment. No. of C_4 species was more than C_3 species in all the N-treated plots. The Sum of total no. of C_3 and C_4 species was maximum in 60 kg N/ha/yr treated plots and minimum in 150 kg N/ha/yr treated plots. However, both C_3 and C_4 showed a decreasing trend from 90 kg N/ha/yr treatment to 150 kg N/ha/yr. C_3 and C_4 species density increased across N treatment to 60 kg-N/ ha/ yr. The number of C_4 species was more than C_3 species under all the N-treatments(Table 2).

Based on seasonal mean data of abundance, among all the plant functional traits, natives and non-natives abundance were found maximum under 60 Kg-N/ha/yr treated plots. The abundance of C_3 was maximum under 60 Kg-N/ha/yr N treatment and while C_4 abundance was maximum under 30 Kg-N/ha/yr N treatment and minimum in 150 Kg-N/ha/yr treatment (Table3).

Like other functional traits, invasiveness was also an important trait determined by the N-treatment gradient across the plots On the basis of the winter season, among all the plant functional traits, natives and non-natives were found maximum under 60 Kg-N/ha/yr treated plots. Native species were maximum under 60 Kg-N/ha/yr. N treatment and minimum native species found in 150 Kg-N/ha/yr (Fig 1).

In winter season number of species of C_3 and C_4 plants showed substantial variation across six different – level of N- treatment (Control- 0 Kg N $ha^{-1}yr^{-1}$, 30 Kg N $ha^{-1}yr^{-1}$,60 Kg N $ha^{-1}yr^{-1}$,90 Kg N $ha^{-1}yr^{-1}$,120 Kg N $ha^{-1}yr^{-1}$, 150 Kg N $ha^{-1}yr^{-1}$). A maximum number of species of C_3 plants was found in 60 Kg N $ha^{-1}yr^{-1}$N- treatment while the least number of species of C_3 was found in 150 Kg N $ha^{-1}yr^{-1}$. As C_4 plants have more nitrogen use efficiency (NUE) than C_3 plants so even at low doses of N-treatment viz. are 30 and 60 Kg N $ha^{-1}yr^{-1}$, the number of species of C_4 plants was maximum. While the number of species of C_4 plants was minimum in 150 Kg N $ha^{-1}yr^{-1}$treatment (Fig. 2)

On the basis of the summer season, among all the plant functional traits, natives were found maximum under 60 Kg-N/ha/yr treated plots. Non-native species were maximum under 150 Kg-N/ha/yr. N treatment and minimum native species found in 150 Kg-N/ha/yr (Fig. 3).

In the summer season, a maximum number of species of C_3 plants was found in 60 Kg N $ha^{-1}yr^{-1}$ N- treatment while the least number of species of C_3 was found in 90 Kg N $ha^{-1}yr^{-1}$. A maximum number of species of C_4 plants was found in 60 Kg N $ha^{-1}yr^{-1}$Ntreatmen (Fig. 4).

In the rainy season, N-treatment gradient on a number of native species was found maximum in 0 Kg N $ha^{-1}yr^{-1}$and 30 Kg N $ha^{-1}yr^{-1}$ and a minimum number of non-natives species were found in 120 Kg N $ha^{-1}yr^{-1}$ (Fig. 5).

In the rainy season, a maximum number of C_3 and C_4 species were found in 60 Kg N $ha^{-1}yr^{-1}$and minimum number of C_3 and C_4 species were found in 60 Kg N $ha^{-1}yr^{-1}$(Fig 6).

N-treatment and season had a significant effect on the no. of C_3 and C_4 species and native and non-natives species ($p < 0.05$). The trend of occurrence of C_3 and C_4 species in all the N-treated plots was the same as obtained during winter, summer and rainy i.e. maximum in 60 kg N/ha/yr treated plots and minimum in 150 kg N/ha/yr plots. N treatment and year-wise C_3 and C_4 species and native and non-natives species had no significant effect (Table 4).

Discussion

C_4 species were found to be more competitive for N than C_3 species in this N- manipulative experiment, and thus appeared in greater numbers across all treatment plots. C_4 species have a higher NUE by definition than C_3 species (Yuan et al., 2007) Because of the abundant soil moisture, the number of C_3 and C_4 plants increased in all plots during the rainy and winter seasons. C_4 demonstrated dominance over C_3 across all six treatments. As a result, our findings suggest that both soil moisture and the N input threshold value increase the number of C_4 species. Previous research has shown that N fertilization reduces C_3 plant growth and N_2-fixation. The differences in functional traits and reproductive allocation strategies between native and invasive plants may be closely related to the success of the latter since co-occurring native and invasive plants are subject to similar environmental selection pressures (in this case, N-deposition). Additionally, nutrient additions result in novel environmental conditions where some species may gain an advantage over native species, resulting in a loss of diversity and a decline in species richness (Harpole *et al.,* 2016). By changing which species dominates the community-level response, this change in abundance may cause a change in how the ecosystem functions. As a result, after treatment, the number of invasive species rose from 0 to 60 kg N/ha/yr. This study suggests that changes in competitive relationships caused by N deposition may disadvantage native species that thrive in low-nutrient environments, such as N_2-fixers, eventually contributing to changes in plant community composition. Non-N_2 fixers, in particular, appear to be more efficient than N_2 fixers at utilizing additional N for growth. Non-native 'space invaders' are destroying the world's valuable grassland ecosystems, according to our findings, which show that grassland biodiversity has shifted significantly from native to introduced species. The current ecological study demonstrates that the vegetation was heterogeneous in nature up to a treatment of 60 kg-N/ha/yr. Overall, the study discovered that high levels of nutrients reduced the diversity of herbaceous species while moderate levels of N amendment (60 kg-N/ha/yr) increased it. Therefore, 60 kg-N/ha/yr of N fertilization may be required for the establishment and maintenance of India's species-poor dry tropical grassland.

Conclusion

The study found a strong correlation between plant functional attributes and species responses to nitrogen perturbations. A more robust theory has to be developed because the few features that did respond to manipulations did not do so consistently. This shows that the traits' predictive ability to respond to N-deposition has some limitations.

The photosynthetic pathway-C_3 and C_4 are related to species functional qualities such as species sensitivity to N addition, which are linked to strategies for species adaptation to varying nutritional conditions. In the competitive balance of C_3 and C_4 species, nitrogen is crucial. In humid temperate grasslands, experimental N additions have often favoured C_3 grasses and forbs over C_4 grasses (Wedin and Tilman, 1996). As compared to C_3 plants, the maximal carbon accumulating capability of C_4 plants might occur at a lower NUE due to their higher photosynthetic efficiency. The significance of these two physiological pathways on the ecology of the plants possessing them, has been clarified by studying the large-scale distributional patterns of C_3 and C_4 plants. As a result, the present study suggests that nitrogen is crucial to the competitive equilibrium of C_3 and C_4 species. Therefore, changes in competitive interactions brought on by N deposition may be disadvantageous to native species that flourish in low-nutrient conditions, like N_2-fixers, ultimately causing changes in the composition of plant communities. Particularly non-N_2 fixers seem to be more effective at using extra N for growth than N_2 fixers.

Our results demonstrate that grassland biodiversity has shifted dramatically from native to introduced species, indicating that non-native space invaders are ruining the world's rich grassland ecosystems. This shift in abundance may result in a change in the ecosystem functions by altering the dominance of species and their response at the community level. As a result, invasive species increased from 0 to 60 kg N/ha/yr.

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