Plant Functional Type Specific Response to Nitrogen Addition under Different Rainfall Regimes

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Research Article

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Abstract

Grassland productivity is one of the critical characteristics which is co-constrained by nitrogen and water availability. Therefore, an understanding of how grasslands species respond to atmospheric nitrogen (N) deposition and rainfall variability needs to be systematically and quantitatively evaluated on temporal scales. In this study, we performed an experiment to evaluate the responses of aboveground and belowground biomass of grassland community and aboveground biomass of grasses, forbs and legumes at a tropical location for two years (2017–2019) using rainout shelters to simulate three rainfall conditions with three N levels. The study indicates that a higher water and nitrogen input significantly increases the aboveground and belowground biomass of the tropical grassland community in the first year but in the second year in N2 (20g N m^{-2}/yr^{-1}) plots under all the rainfall conditions, both above and belowground biomass decreased. Interestingly, rainfall had a significant effect on grasses and forbs but not on legumes while nitrogen treatment had a significant effect on all three plant functional groups. On a temporal scale, we found the significant effect of years on belowground biomass of grassland community, grasses and forbs only. Only grasses exhibited the significant interactive effect of rainfall and nitrogen. Observed plant functional type-dependent plant response to changes in rainfall regime and N input indicates that future changes in productivity of tropical grassland due to anthropogenic changes will be strongly dependent on the direction of changes in species composition.

Introduction

Climate variability has a tremendous impact on natural ecosystems and human society. The Intergovernmental Panel on Climate Change (IPCC 2018) predicts that a minor difference between mean global temperature increase of 2°C above pre-industrial ranges, compared to 1.5°C would lead to more extreme heatwaves, more intense precipitation, more frequent droughts, increased sea-level and huge loss of species and ecosystems. Researches also corroborate that precipitation has witnessed extreme variations during the last hundred years (Alexander et al. 2006; Trugman et al. 2018; Paschalis et al. 2020). Changes in water availability triggered by variation in precipitation regulate plant community dynamics and ecological characteristics (Peralta et al. 2019; Wu et al. 2016; Yang et al. 2011). For instance, modifications in the rainfall pattern impact species richness, species composition (Libalah et al. 2020; Báez et al. 2013; Cleland et al. 2013) and net primary productivity of the ecosystem (Heisler-White et al. 2009; Fay et al. 2003).

Nitrogen (N) deposition in interaction with rainfall variability has a profound impact on terrestrial ecosystems (Karl and Trenberth, 2003; Xu et al. 2012). Rainfall and N are vital ecological components in defining the structure and function of the ecological community, specifically in water and N-constrained grasslands (Epstein et al. 2002). N deposition in terrestrial ecosystems is predicted to boom to 200 Tg N yr − 1 by 2050 because of increased N-uses in industries and agriculture (Basto et al. 2018). Nitrogen enrichment will probably affect biomass production (Stevens 2019; Epstein et al. 2002; Poorter et al. 2012b). The consequences of N augmentation on forest biomass were evaluated and analyzed in preceding research (Huang et al. 2013; Qin et al. 2018). Previous studies also emphasized the effect of rainfall variability on aboveground biomass (AGB) of grasslands (IPCC 2014; Sala et al. 2015), but the consequences of N addition under different rainfall scenarios on grassland biomass still stays unknown.

In a terrestrial ecosystem, grasslands approximately cover 25% of the land surface on earth (Easterling, 2000). In comparison to forests, grasslands are more sensitive to climatic variations, even for a short duration (Maurer et al. 2020; Eziz et al. 2017), and therefore they become crucial to study in the situation of ongoing global climate change. In grasslands, biomass allocation plays a key role in determining the response of plant growth, and variation in it would lead to a substantial change in the structure and function of the grassland ecosystem (Poorter et al. 2012a,b). Changes in precipitation could affect plant productivity by altering plant physiological responses and nutrient availability over relatively short-time scales (Xu et al. 2010). Many studies have focussed on understanding the effects of N-addition or rainfall variability on aboveground biomass in grasslands (Su et al. 2013; Lu et al. 2014; Xu et al. 2015). For a long time, plant community ecologists have been interested in determining that how different plant functional groups (grasses, forbs and legumes) impact ecosystem productivity. Yet there are fewer studies that have focussed on the effect of both rainfall variability and N- addition on relative changes in AGB of different plant functional groups in grassland ecosystems. A study by Mowll et al. (2015) in a semiarid temperate grasslands report that aboveground net primary productivity (ANPP) is restrained due to N availability and climatic variables, which include rainfall and air temperature. On that account, N enrichment was found useful in increasing ANPP, but at the same time increase in ANPP with the aid of N enrichment also causes a lack of plant richness (Midolo et al. 2019; Clark and Tilman, 2008). Species which shared common functional traits also manifested similar responses to rainfall variability and N enrichment (Tian et al. 2020; Cleland et al. 2013). Nitrogen deposition increased the growth of grasses, but reduced the growth of forbs, leading to a decline in forbs richness in the grassland ecosystem (Tian et al. 2016; Tian et al. 2020). Certain plant functional groups can increase the aboveground and belowground biomass by 300% in comparison to monoculture species (Tilman et al. 2001). Low precipitation and high temperature caused the shallow and fibrous-rooted grasses to suffer (Grant et al. 2017), while deep-rooted forbs and legumes maintained their productivity. Hence, in the light of prevailing global climate change driven by rainfall variability and N-deposition, it becomes important to understand the adaptive mechanisms of different plant functional groups in tropical grassland ecosystems. Therefore, we conducted a coupled rainfall-nitrogen experiment to investigate the changes in aboveground biomass (AGB) and belowground biomass (BGB) of the grassland community along with the changes in AGB of three different plant functional groups viz. grasses, forbs and legumes due to nitrogen (N) treatments under different rainfall conditions. We addressed the subsequent questions: (1) how does the allocation of AGB and BGB vary with nitrogen treatments under different rainfall conditions? (2) How does the AGB of grasses, forbs and legumes differ in their response to nitrogen treatments under different rainfall conditions?

Materials And Methods

Study site

We performed this study in a constructed grassland (made by transplanting indigenous grassland patches) at Botanical garden situated in the campus of the Banaras Hindu University, Varanasi, Uttar Pradesh, India (25°16'3.3" N and 82°59'22.7" E) (Fig. 1a, b). The study site is characterized by a sub-tropical monsoonal climate with an annual mean temperature of 25.7˚C and mean annual precipitation of 1000 mm (80% of which falls from July to September). Rainfall mainly occurs during the growing season from July to September. The soil is assessed as inceptisol and is deep, light brown with a silty loam texture. Soil organic carbon (SOC) is 0.84% and soil total N is 0.08% at 0–10 cm soil depth.

Experimental design and treatments

We conducted a two-factor randomized block design experiment for two years (2017–2019) with four rainfall conditions (C Ambient rainfall; R1 500 mm; R2 1000 mm; R3 1400 mm) and three nitrogen treatments within them (CK no N-added; N1 10 g N $m^{-2}yr^{-1}$; N2 20 g N $m^{-2}yr^{-1}$). Overall there were 4 blocks and 36 plots. Each block referred to the four different rainfall conditions with 9 plots (1m × 1m) separated from one another by a 1m wide buffer zone, 3 plots each for three different nitrogen (N) treatments (Fig. 1d).

Three rainout shelters were established in June 2017 to simulate the three rainfall conditions. For ambient rainfall conditions, one block was left unsheltered. The rainout shelters were of the static type, made up of iron-frame supporting transparent plastic strips so that there is no limitation to the transmittance of natural light. Shelter ends and sides were left open to optimize the air movement and keep the relative humidity and air temperature quite similar to the unsheltered block (Fig. 1c).

The amount of water added to each of the three rainout shelter blocks was based on the 10-year data set (2007–2016) obtained from IMD, Department of Agronomy, Institute of Agricultural Sciences, BHU. Using the daily record of rainfall from the 10-year rainfall dataset we calculated the no. of days on which rainfall took place in each month. Based on these calculations, we framed the calendar with the number of watering days for the year 2017 and followed it for 2018 and 2019 as well. All plots in the blocks were watered manually by tapwater through a watering can. The amount of water showered over each plot was manipulated according to the surface area of the blocks. Thus, following the daily rainfall data from 10 years dataset, we used three rainfall doses viz. 500 mm(R1), 1000 mm(R2) and 1400 mm (R3) which represent the minimum, average and maximum rainfall across the 10 years.

Likewise, we also simulated N- deposition and took three N- treatments (CK no N-added; N1 10gN m^{-2} yr^{-1}; N2 20g N m^{-2} yr^{-1}) under each rainfall condition. The form of N applied was urea ($CO(NH_2)_2$), it was added in two equal split doses in N1 and N2 treated plots. Urea ($CO(NH_2)_2$) granules were added in an even distribution to the plots by hand on 20 July 2017, 5 August 2017, 23 July 2018, 8 August 2018, 20 July 2019, and 6 August 2019. We used these two nitrogen treatments by referring to the ongoing rate of N-deposition in the Europe and USA (Clark and Tilman, 2008), also the 20g N m^{-2} yr^{-1} rate is the quintessential of highest N- deposition in many areas of China (Liu et al. 2013). Besides, a study by Decina et al. (2020) reports the increased N-deposition rates in southern Asia, especially in Varanasi and Delhi (India), therefore these N-treatments are appropriate to determine the impact of N-deposition on the biomass of tropical grassland ecosystems under different rainfall conditions.

Vegetation sampling

Vegetation was sampled in the year 2018 and 2019 during late August when tropical grasslands had the highest biomass every year. Community composition for both the years during the growing season was comprised of a total of 39 species belonging to different plant functional groups (grasses, forbs, legumes and sedges) (Table 1). Aboveground biomass was determined by clipping all the aboveground vegetation at the soil surface (sedges were also included). A 50×50cm quadrat was randomly placed in each plot for the vegetation sampling but away from the edges to avoid edge effects. All living plants were oven-dried for 72 hours at 70˚C and then weighed. All living plants belonging to grasses, forbs and legumes had been taken as the aboveground biomass for grasses, forbs and legumes.

Table 1
Community composition at the grassland under this study

Species	Plant Functional groups
Cynodon dactylon	Grass
Oplismenus burmanni	Grass
Oldenlandia corymbosa	Forb
Desmodium gangeticum	Legume
Paspalidium flavidum	Grass
Commelina benghalensis	Forb
Dichanthium annulatum	Grass
Sida acuta	Forb
Abutilon indicum	Forb
Cyperus rotundus	Sedges
Cyperus kyllingia	Sedges
Euphorbia hirta	Forb
Oxalis corniculata	Forb
Digitaria sanguinalis	Grass
Eleusine indica	Grass
Anisomeles indica	Forb
Tridex procumbens	Forb
Anagallis arvensis	Forb
Ageratum conyzoides	Forb
Ludwigia adscendens	Forb
Eragrostis amabilis	Grass
Desmodium triflorum	Legume
Parthenium hysterophorus	Forb
Phyllanthus niruri	Forb
Sida rhombifolia	Forb
Cleome viscosa	Forb
Evolvulus nummularis	Forb
Hepestris rugosa	Forb

Clitoria ternatea	Legume
Vernonia cinerea	Forb
Corchorus tricapsularis	Forb
Malvestrum coromandelianum	Forb
Lindernia parviflora	Forb
Scoparia dulcis	Forb
Achyranthes aspera	Forb
Spilanthus acmela	Forb
Peristrophe bicalyculata	Forb
Bryonia laciniosa	Forb

Belowground biomass (BGB) of the grassland community was determined by sampling monoliths of 15 × 15 × 15 cm in late august during the years 2018 and 2019 from each 1 × 1 m plot. Monoliths were then transferred to mesh screens and washed under the fine jet of water.

Statistical analysis

Statistical analysis was conducted with the help of SPSS (SPSS Inc. Version 16). Multi-factor Analysis of Variance (MANOVA) was run to determine the effect of rainfall, nitrogen, year and their possible interactions on aboveground biomass, belowground biomass and aboveground biomass of grasses, forbs and legumes. Further, post hoc (Tukey) test was done to differentiate among the means. Sensitivity % was also calculated to compare the relative effect of treatment and control on biomass using the following formula:

$$\text{Sensitivity}(\backslash\%) = \frac{\text{TreatmentBiomass} - \text{ControlBiomass}}{\text{ControlBiomass}} \times 100$$

Results

Rainfall conditions and nitrogen addition in different years had a significant effect on aboveground biomass, belowground biomass, aboveground biomass of grasses and forbs but their interaction showed no significant effect on grass biomass (Table 2).

Table 2
Three-way ANOVA indicating the effect of rainfall variability(R), nitrogen treatment (N) and year (Y) on aboveground biomass(AGB), belowground biomass (BGB), grasses, forbs and legumes

	AGB		*BGB*		Grasses		Forbs		Legumes	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
R	12.276	0.000	141.776	0.000	14.832	0.000	4.622	0.006	1.159	0.335
N	7.948	0.001	22.148	0.000	9.376	0.000	8.101	0.001	21.641	0.000
Y	1.093	0.301	54.015	0.000	7.223	0.010	18.880	0.000	0.750	0.391
*R*N*	1.085	0.385	1.510	0.195	0.954	0.466	3.070	0.012	1.027	0.419
*R*Y*	18.374	0.000	7.159	0.000	2.156	0.127	1.697	0.194	0.353	0.705
*N*Y*	0.184	0.832	52.095	0.000	2.156	0.127	1.697	0.194	0.353	0.705
*R*N*Y*	3.203	0.014	3.107	0.016	1.293	0.282	4.215	0.003	3.272	0.013

Response of aboveground biomass of grassland community to N-treatments under different rainfall conditions

The total rainfall recorded during the 2019 growing season was 37.71% more than that in 2018 but the monthly distribution of rainfall was quite different between the two years. The rainfall amount at the onset of the growing season (May to June) in 2018 was 51.1mm which is 47.95% greater than that in the early growing season in 2019 (26.60 mm). The rainfall amount in the late growing season (July to September) in 2018 and 2019 was 744.40 mm and 1068.9 mm, respectively (Table 3).

Table 3
Total rainfall and monthly rainfall during the growing season (2018–2019). Ambient rainfall (C); Minimum rainfall (R1); Average rainfall (R2); Maximum rainfall (R3)

Rainfall (mm)	C		R1	R2	R3
	2018	2019			
May	15.40	0.00	21.81	43.65	61.08
June	35.70	26.60	58.16	116.40	162.88
July	245.40	296.20	130.86	261.90	366.48
August	276.00	244.70	123.59	247.35	346.12
September	223.00	528.00	87.24	174.6	244.32
Total	795.50	1095.5	290.8	843.9	1180.88

At the grassland community level, ambient rainfall condition (C) with no-added N (CK) in 2019 exhibited 87.82% higher above biomass than that in 2018 but the maximum aboveground biomass (471.23 g/m^2)

was observed in 2019 in N1 treated plots under ambient rainfall conditions (C). This value was 93.48% higher than that in 2018 for the same. Under all the rainfall conditions nitrogen treatment had no significant effect on the AGB in 2019 (Fig. 2a, b). On the other hand, belowground biomass was highest (157.31 g/ m^2) in 2018 in the N2 treatment under maximum rainfall conditions (R3). This value was 30.64% higher than that found in the N1 treatment under the maximum rainfall condition (R3). The lowest value of belowground biomass (39.36 g/m^2) was found in 2019 in N2 treatment under ambient rainfall conditions (C). This value is 8.74% lower than that found in 2018 in no-added N (CK) treatment under minimum rainfall conditions (R1). Rainfall conditions had a significant effect on belowground biomass in 2019 while in 2018 nitrogen treatment had a significant effect on belowground biomass (Fig. 3a, b).

Response of aboveground biomass of grasses, forbs and legumes to N-treatments under different rainfall conditions

During 2019 in R3 condition, grasses, forbs and legumes biomass significantly varied under all three nitrogen treatments but no legumes were found in N1 and N2 treated plots while in 2018, legumes biomass significantly varied from that of grasses and forbs under all the N- treatments (Fig. 4j, k, l)

During both the years (2018 and 2019), under R2 condition, legumes were again absent in N2 treated plots while grasses biomass was significantly greater than that of forbs in 2019. Forbs biomass in N1 treated plots under R2 condition in 2018 was more than that of grasses while the biomass of the legumes was lowest. However, in 2019, grasses, forbs and legumes biomass showed significant variation where the biomass of grasses was highest and legumes had the lowest biomass (Fig. 4g, h, i).

Likewise, in the R1 condition, no legumes were found in N2 treated plots while the biomass of grasses was more than that of forbs during both the years in N2 treated plots. Biomass of legumes increased under R1 condition with no- N treatment (CK) in 2019 from that in 2018 (Fig. 4d, e, f).

Under C rainfall conditions legumes were present in all three N-treated plots. But in N1 treated plots grasses in comparison to forbs and legumes exhibited more increase in biomass in 2019 than in 2018 (Fig. 4a,b,c).

Sensitivity (%)

AGB showed maximum sensitivity to N2 treatment under R3condition in 2018 while minimum sensitivity was observed in N1 treated plots under C condition. In 2019, AGB exhibited maximum sensitivity to N2 treatment under R1 condition while minimum sensitivity was found in N2 treated plots under C condition.

During 2018, BGB showed maximum sensitivity in N2 treatment under all the rainfall conditions. In 2018, BGB showed maximum sensitivity to N2 treatment under the R3 condition and minimum to N1 under the R2 condition. In 2019 BGB was found more sensitive to N1 treatment under R1 condition.

Among grasses, forbs and legumes, grasses were found to be more sensitive to N2 treatment under R1 conditions for both years. But legumes were found to be highly sensitive and showed a 100% decrease in biomass in N2 treatment under R1 and R2 condition in 2018 and R1, R2 and R3 in 2019. Forbs showed the highest sensitivity in 2018 to N2 treatment under the R3 condition and N2 treatment under the C condition in 2019 (Table 4).

Table 4
Sensitivity (%) of AGB, BGB, grasses, forbs and legumes to nitrogen treatments under different rainfall conditions

Rainfall condition	AGB (%)		BGB (%)		Grasses(%)		Forbs(%)		Legumes(%)	
	N1	N2	N1	N2	N1	N2	N1	N2	N1	N2
2018 C	13.51	31.66	34.18	60.61	41.98	15.63	1.01	53.51	-61.98	-76.25
R1	17.51	41.07	34.85	49.08	98.19	128.81	7.48	15.15	-11.07	-100
R2	19.01	31.79	28.74	56.33	37.93	98.73	35.35	21.82	-73.69	-100
R3	25.82	51.91	30.44	68.43	28.38	77.53	35.30	40.78	-14.33	-25.72
2019 C	16.94	-1.69	20.03	-15.79	49.16	-21.43	1.40	59.19	-76.62	-94.25
R1	37.03	42.41	68.67	31.49	99.51	112.73	22.41	35.51	-62.47	-100
R2	5.08	9.10	19.10	-12.13	8.20	-8.33	13.86	27.63	-55.59	-100
R3	4.12	-1.35	15.52	-13.68	15.68	54.92	-17.73	-30.80	-100	-100

Discussion

Many previous studies, mostly in the temperate grasslands, have indicated that atmospheric N deposition, climate change and grassland management have potential impacts on plant community productivity in grassland ecosystems (Midolo et al. 2019; Clark et al. 2018; Koerner et al. 2018; Borer et al. 2014; Collins et al. 2012). These studies have enriched our mechanistic knowledge of vegetation changes due to the changing environment and increased anthropogenic activities. However, yet it is not clear whether or not and how different levels of N- treatments under distinct rainfall conditions affect grassland productivity. These facts could have vital outcomes for grassland management under the general situation of worldwide climate change.

In this study, we observed reduced AGB of the grassland community in 2018, whilst due to better rainfall in 2019 AGB of grassland community increased (Fig. 2a,b). These effects show that accelerated rainfall will significantly increase the biomass of grassland species, while decreased rainfall significantly decreases the biomass of grassland species. This finding is consistent with long-term observations of terrestrial ecosystems around the world (Xiong et al. 2017). In addition, our results show the effects of increased ambient rainfall in 2019 on AGB, BGB and AGB of grasses, forbs and legumes (Table 2). This result is further consistent with past studies which have confirmed that the amount of rainfall determines grassland productivity (Robinson et al. 2013; Muldavin et al. 2008). Under all the rainfall scenarios, AGB initially increased with the N- dose but in the second year, AGB was found maximum only in N1 treated plots rather than in N2. This shows that the N2 dose of nitrogen has a detrimental effect on the aboveground productivity of the grassland ecosystem. This shows that the N2 dose of 20g N m^{-2} yr^{-1} would have significantly reduced the soil pH. This decreased soil pH may lead to the copious release of manganese (Mn^{2+}) and aluminium (Al^{3+}) ions and thus limiting plant growth (Tian and Niu, 2015).

Likewise, BGB increased with more rainfall and increasing rate of nitrogen-input in the first year but in the second year overall BGB decreased but BGB increased with N1 (10 g N m^{-2} yr^{-1}) input only under all the rainfall conditions. But this increase in BGB in the second year was less than that found in the first year. BGB increased with increasing rainfall amount in both the years but the increase was more pronounced in the first year (Fig. 3a,b). This result is similar to the study done by Fiala et al. (2009) where belowground biomass production decreased with low input of rainfall. But our finding is in disagreement with the optimal partitioning theory (Bloom et al. 1985) which advocates for the decreased root production in nutrient enrichment conditions.

Our study also analyses the interactive effect of both rainfall conditions and nitrogen input on plant functional groups viz. grasses, forbs and legumes. Rainfall showed a significant effect on both grasses and forbs but not on legumes (Table 2). AGB of grasses significantly increased in comparison to that of forbs during the second year due to the increased competitive ability of grasses over forbs due to increased ambient rainfall and antecedent soil moisture. These results can be interpreted by the following reasons. Firstly, N enrichment ascertains that the ecosystem is not N- constrained and promotes plant growth (Bai et al. 2010). However, with time, grasses will reduce the growth of the forbs because grasses occupy the upper layer of an ecological niche therefore they are better at capturing other resources (Fang et al. 2012), and the forbs exhibit low growth rates (Stevens et al. 2006). Secondly, with time soil acidification would also increase (Tian et al. 2016), subsequently enhancing metal toxicity and reducing forbs growth (Tian and Niu, 2015). Besides, preceding researches also illustrated that with increased study duration grasses gradually replaced the forbs (Su et al. 2013; Fang et al. 2012) which is supported by our results as well.

Conclusions

Our study explained the biomass allocation in the grassland community and relative changes in the aboveground biomass of different plant functional types (grasses, forbs and legumes) in dry tropical grassland and their relationships with rainfall variability and nitrogen addition. We found that during reduced rainfall in 2018, forbs showed increased biomass than grasses and legumes. While in 2019, both under the ambient rainfall condition which was more than that in 2018 and the other three rainfall conditions, grasses biomass increased with N-addition with a proportionate decrease in forbs and legumes biomass. In N2 treatment under both the R1 and R2 conditions, no legumes were found. Observed plant functional type-dependent plant response to changes in rainfall regime and N input indicates that future changes in productivity of grassland due to anthropogenic changes will be strongly dependent on the direction of changes in species composition.

Declarations

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Swati Mishra. The first draft of the manuscript was written by Swati Mishra. Hema Singh reviewed the manuscript and made the needful corrections as well. All authors have read and approved the manuscript.

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability statement

The data will be made available on demand.

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Figures

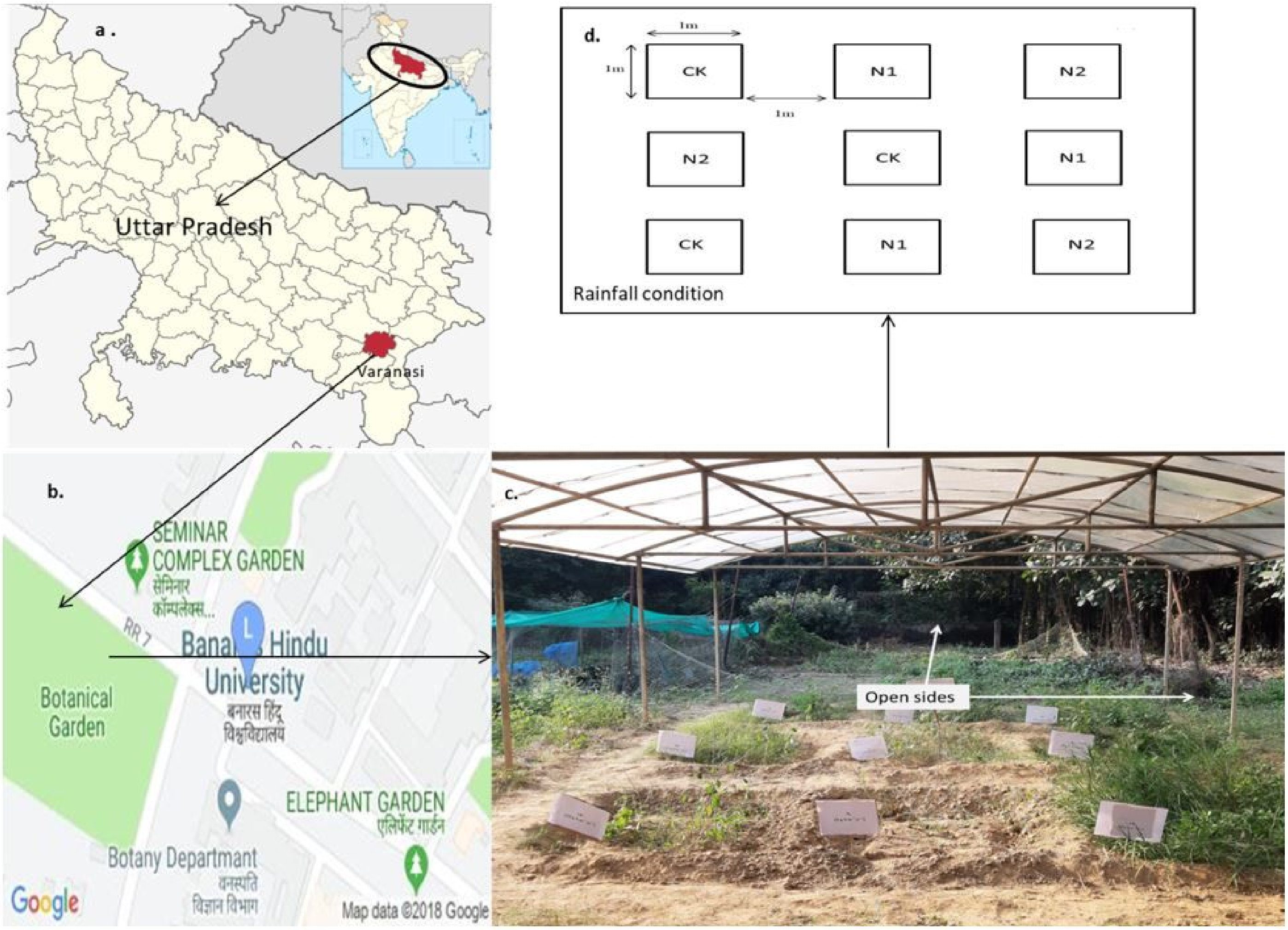

Figure 1

(**a)** Study site on the map of India (**b)** Map of BHU campus in Varanasi (Source: Wikipedia) (**c)** Rainout shelter at the study site (**d)** Layout of nitrogen treatments under a particular rainfall condition

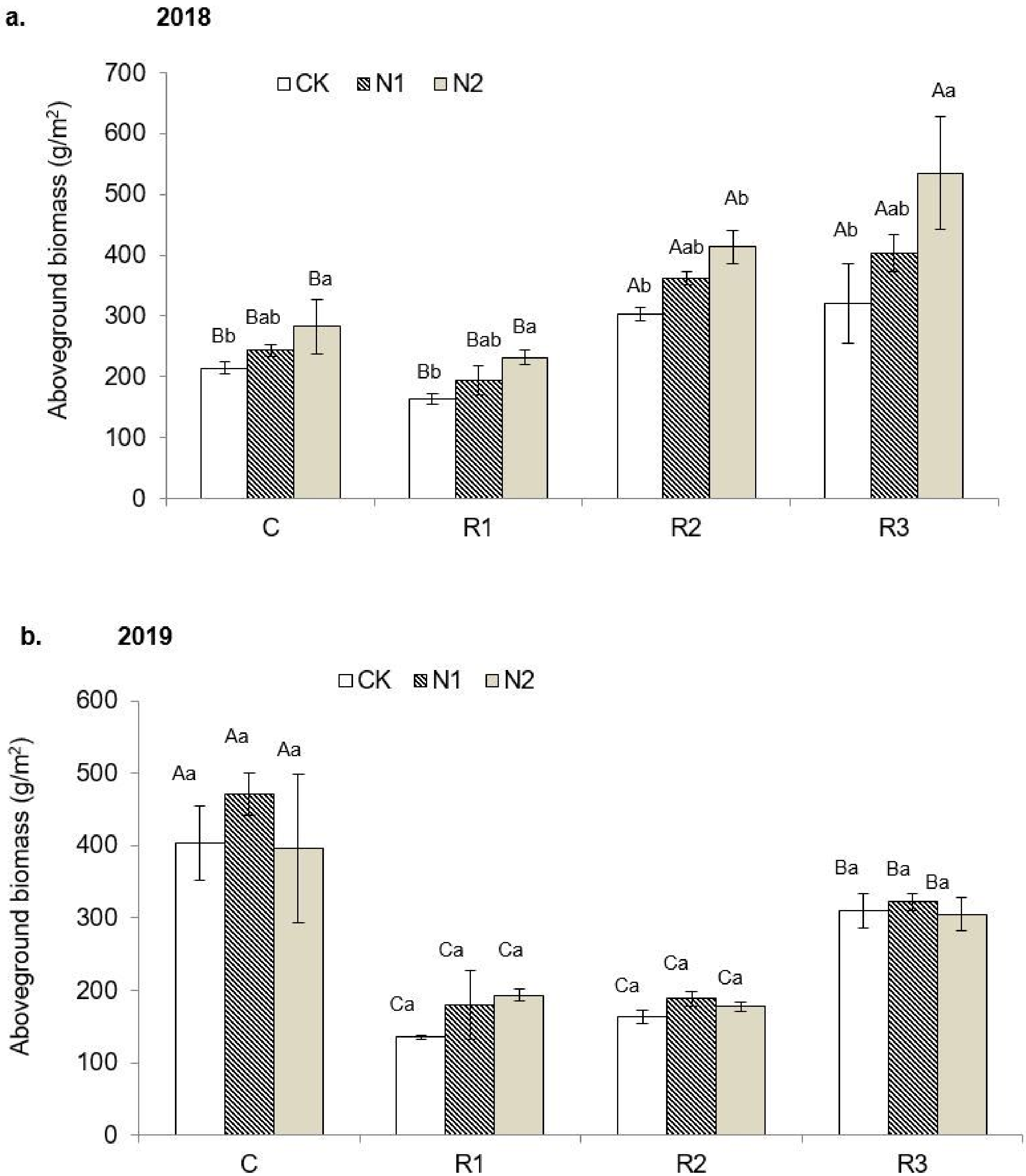

Figure 2

(a,b) Aboveground biomass for the years 2018 and 2019 among different rainfall conditions(C, R1, R2, R3) and nitrogen treatments (CK, N1, N2). Bars affixed with different combinations of letters are significantly different from each other ($P < 0.05$). The uppercase letters represent rainfall conditions and lower case letters represent nitrogen treatments

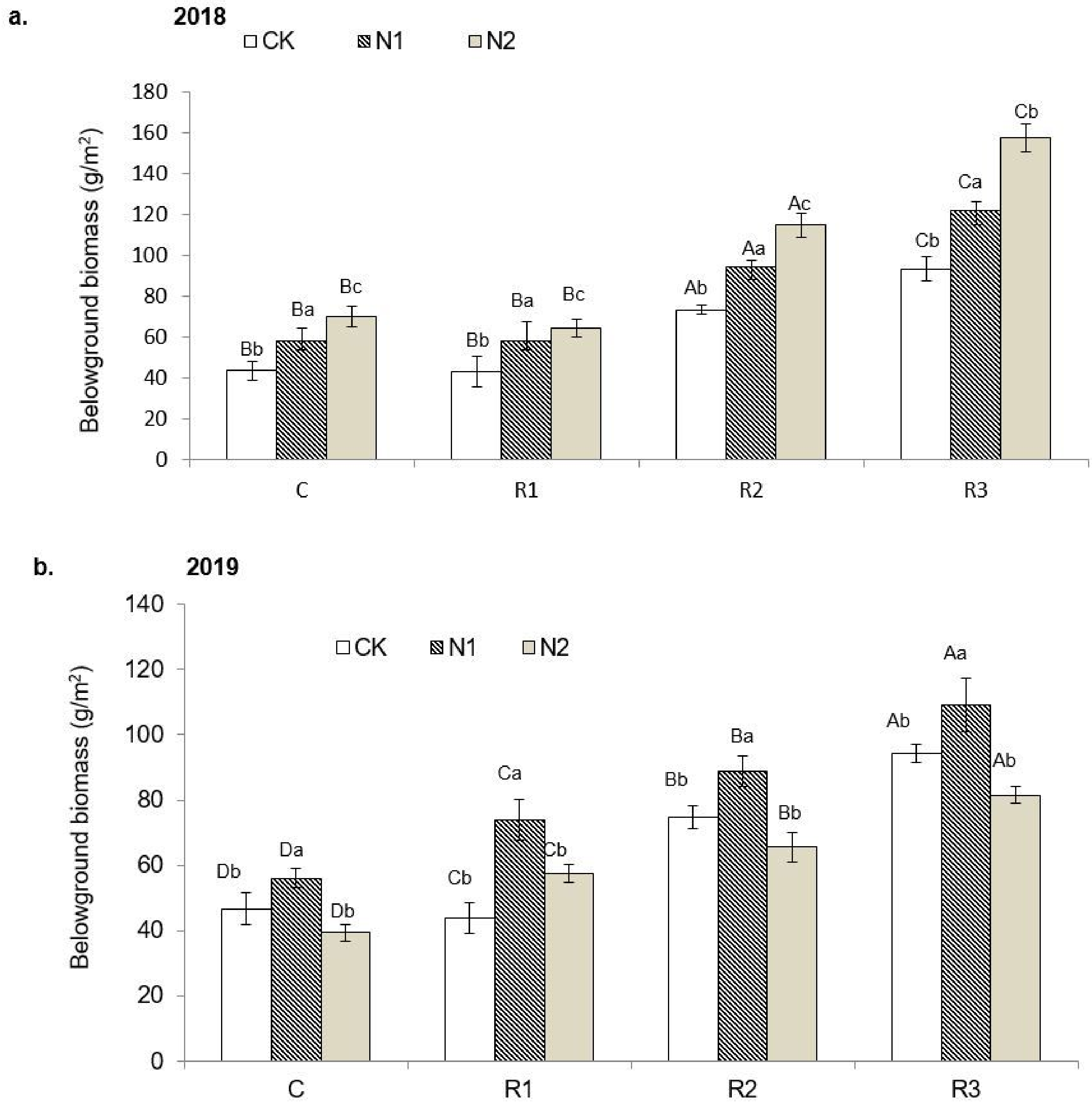

Figure 3

(a,b) Belowground biomass for the years 2018 and 2019 among different rainfall conditions(C, R1, R2, R3) and nitrogen treatments (CK, N1, N2). Bars affixed with different combinations of letters are significantly different from each other (P < 0.05). The uppercase letters represent rainfall conditions and lower case letters represent nitrogen treatments

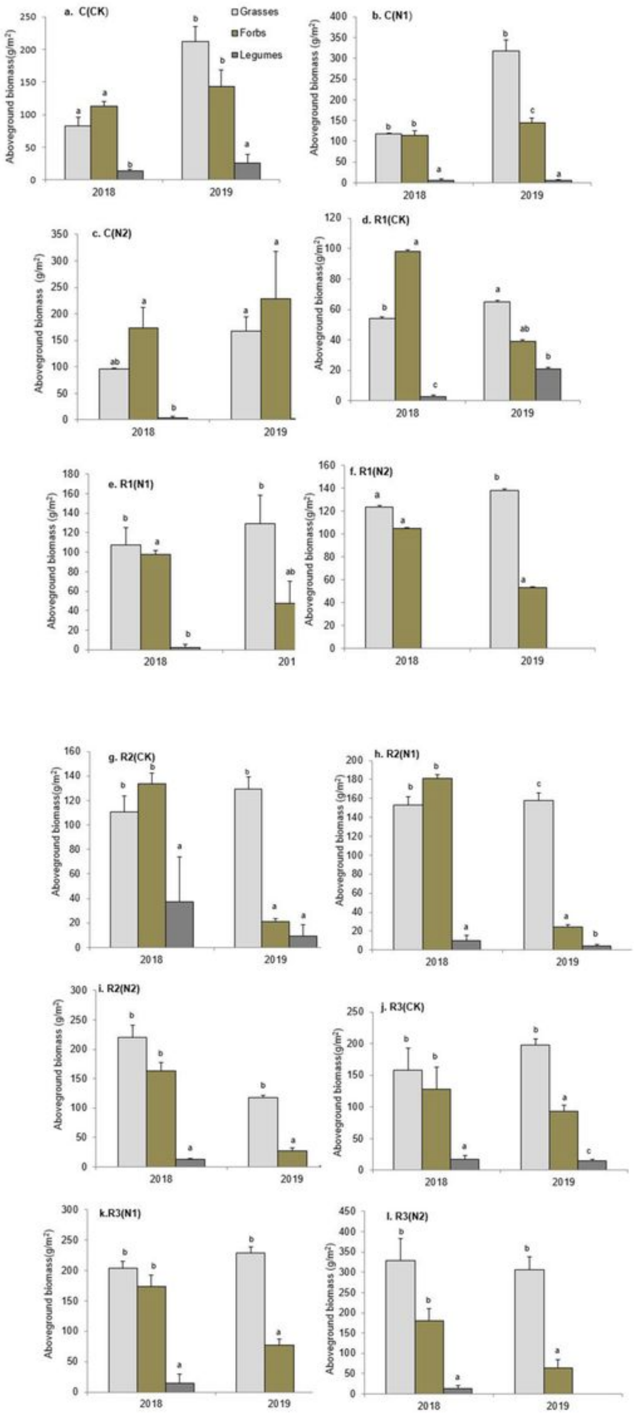

Figure 4

(a,b,c,d,e,f,g,h,I,j,k,l) Aboveground biomass of grasses, forbs and legumes in the year 2018 and 2019 for different rainfall conditions and nitrogen treatments. Bars affixed with different letters are significantly different from each other (P < 0.05). The letters represent different plant functional groups (grasses, forbs and legumes)