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Effects of N-application on the Diversity of Herbaceous Species and Growth Forms in the Dry Tropical Environment, India

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Abstract: The study examined the diversity of herbaceous species and growth forms at three N levels, differing in soil moisture intensity, in a dry tropical environment of India. A total of 45 species, distributed in 23 families, were recorded in a two year study. The study indicated that the studied herbaceous communities were spatiotemporally dynamic due to N treatment under varied moisture conditions. Further, the trait categories of year, 2009 were distinct from those of year, 2010 in the NMS (Non-metric Multidimentional Scaling) ordination diagram. In the present study, the N treatments produced a typical humped-back pattern for the diversity of species and growth forms. The study concludes that N accumulation in the soil due course of time reduces the diversity of herbaceous species as well as functional groups and moderate level of N and sufficiently high level of soil moisture are essential for maintenance of high diversity of herbaceous species and functional groups. Under ample soil moisture condition, the N accumulation will reduce the soil pH by the process of acidification and the system could be diversified by the hemicryptophytes.

Key words: Herbaceous life forms, N-deposition, non-metric multidimensional scaling, soil moisture, species diversity

INTRODUCTION

Stability and fragility of any ecosystem is characterized by the community composition, species diversity and species assemblages. A loss of biodiversity can significantly affect the structure and function of ecosystems (Ehrlich and Ehrlich, 1981; Schlapfer and Schmid, 1999) and beyond a certain threshold it could lead to completely disruption of structure and function of the ecosystem (Sagar and Singh, 1999). Habitat fragmentation, invasion, human population explosion, increase in the concentration of pollutants, overexploitation and climate change especially; atmospheric N-deposition (Heywood *et al*., 1995) due to fossil fuel combustion, industrial and agricultural activities have been identified as major threats to biodiversity loss (Sala *et al*., 2000; Bobbink *et al*., 2010). Ever increasing reactive-N in the biosphere has been cited as the third major threat to our planet after habitat fragmentation and introduction of invasive species (Sala *et al*., 2000; Giles, 2005). Evidently, during the past many decades, the structure and functioning of several ecosystems in highly populated countries have been threatened due to high atmospheric N-deposition (Van Den Berg *et al*., 2005; Zhou *et al*., 2010).

Greater annual N-deposition rates in European and North American countries had increased many folds. It is also substantially increasing in India and China due to tremendous anthropogenic activities (Galloway *et al*., 2008). On a global basis, the rate of N build-up by anthropogenic activities augmented from ~15 Tg N/year^{-1} in 1890 to ~140 Tg N-year^{-1} in 1990 (Galloway *et al*., 2008) and in Asia due to these activities the reactive N depositions radically increased from ~14.4 Tg N-year^{-1} in 1961 to ~67.7 Tg N/year^{-1} in 2000 and is expected to be 105.3 Tg N year^{-1} by 2030 (Zheng *et al*., 2002). Now N- deposition rate has been exceeds 10 kg N ha^{-1} year^{-1} in more than a few areas of the world. This is 20 times greater than the projected N-deposition of 0.5 kg N ha^{-1} year^{-1} without anthropogenic pressure (Galloway *et al*., 2008).

Globally, India is the second most population bearing country and shares major proportion of it. The ever growing population intensified the agricultural activities per unit area to fulfill the food, cloth and shelter requirements by increasing the N-based fertilizers (Shukla *et al*., 1998) and this, N-based fertilizer consumption in India has increased from 0.06 million tones in 1952 to 9.5 million tones in 1994-1995 (Braun and Roy, 1983). Besides increase in reactive-N concentration in atmosphere, human use fertilizers (Giles, 2005) also promote the emissions of NO_x, NH_3, N_2O and NO_3^- and deposition of NO_y and NH_x compounds into the biosphere (Vitousek and Matson, 1993; Vitousek *et al*., 1997; Galloway *et al*., 2008; Zhou *et al*., 2010) that cause

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variety of undesirable effects on soil, water, atmosphere and finally on the organisms of these ecosystems (Giles, 2005).

Grasslands are an integral part of the terrestrial ecosystems and occupy roughly 25% (33×10^6 Km^2) of the total land surface of the earth (Shantz, 1954). Such ecosystems are subjected to intense pressure for the production of food, fodder and fibers and thus bear high pressure for species depletion (Tilman, 1999). The emphasis on grassland in general is important because herbaceous species need less nitrogen than woody species to show their responses due to their small stature, dry biomass, less nitrogen demand and faster its turnover through litter decomposition and release (Xia and Wan, 2008). Further, the herbaceous vegetation show quick measurable response to nitrogen manipulation because most of the herbaceous species are adapted to low nutrient conditions and additional increase in N-input is expected to cause prominent variation in community structure and composition due to alteration in species richness (Smart *et al*., 2003).

Plant functional groups are defined as categories of plant species with analogous traits and functions with respect to diverse environmental factors (Skarpe, 1996; Lavorel *et al*., 1997; Sagar and Singh, 2003). Recent global changes in human induced land cover alterations, atmospheric composition and N-cycling (Vitousek *et al*., 1997) and the predictable global climatic change (Cramer and Leemans, 1993) impose an understanding of the interactions between environment and plants on a broad scale. Diverse plant functional types play unlike roles in terms of material and energy processes in ecosystems (Diaz Barradas *et al*., 1999). Study of herbaceous species assemblage into life forms provides a sound link between physiological strategies and global processes because species of a known guild shares an equivalent physiology and traits by which functional traits are categorized and have important ecosystem consequences (Chapin, 1993). The impact of N-deposition on ecosystem on a large area cannot usually be assessed on a species basis, Raunkiaer life form a substitute to species, representing ecosystem structure, may ease this job (Aguiar *et al*., 1996; Bugmann, 1996). Soil moisture is a determinant factor for the distribution of herbaceous species composition and diversity in terrestrial ecosystems (Sagar *et al*., 2008a) and soil moisture together with N amendment can affect plant performance (Marino *et al*., 1997) and hence, plant functional groups. Hence, more targeted studies are required to observe the response of these determinants on the diversity of functional types (Bobbink *et al*., 2010).

The data on the impact of N-manipulation on the herbaceous community composition, species diversity and their assemblages in different functional groups from Indian region is lacking. Therefore, in global climate change perspective, this study will make progress toward reducing N loads on sensitive ecosystems and such studies are also essential which could provide better understanding to cope up with the impact of nitrogen deposition on the distribution, composition and diversity of plant functional groups under different moisture conditions. The objective of the present study was to analyze the effects of N-manipulation on herbaceous species distribution, diversity and plant functional types under different moisture conditions in the campus of Banaras Hindu University, India.

MATERIALS AND METHODS

Experimental sites: The study was conducted at five sites in the campus of the Banaras Hindu University (24°18' N and 83°03'E and 129 m msl altitude), India. The area experiences a seasonally dry tropical monsoon climate. The year is divisible in to three seasons viz; a hot summer (April-June), a warm rainy season (July-September) and a cold winter (November-February). The month of March and October constitute transition periods between winter and summer and between rainy and winter seasons, respectively. Mean monthly minimum and maximum temperature varied between 7.3-25.4 and 25.6-35.6°C, respectively and the mean annual rainfall was 932 mm (Sagar *et al*., 2008b). The soil of the Banaras Hindu University has been characterized as Banaras Type 3 (Agrawal and Mehrotra 1952). The soil is pale brown, silty loam and inceptisol. In general, the soil is alluvial, well drained and moderately fertile being low in available nitrogen and medium in available phosphorus and potassium.

The campus of Banaras Hindu University covers more than 1300 acres of land area having extensive greenery and dense flora. From the study area, ~329 vascular plant species distributed in 80 families were reported (Singh, 2011). The possible ground vegetation of the study area are locally dominated by *Alysicarpus monilifer*, *Cynodon dactylon*, *Cyperus compressus*, *Desmodium gangeticum*, *Dicanthium annulatum*, *Evolvulus numularius*, *Imperata cylindrica*, *Malvestrum coromandelianum*, *Oplismenus burmannii*, *Sida acuta*, etc. (Sagar and Verma, 2010; Sagar *et al*., 2008b). During summer season, the grassland vegetation becomes dormant and potential species, however, under shade and mesic conditions show some green parts (Mishra, 1989; Sagar and Verma, 2010).

Experimental design: For this, five locations with varying Soil Moisture Contents (SMC) were selected to represent the entire range of variation in SMC in the campus of Banaras Hindu University. Gravimetric soil moisture of each plot for each month of a year was determined. Location-1, 2, 3, 4 and 5, respectively, had 2.70-5.21, 5.22-7.73, 7.74-10.25, 10.26-12.77 and 12.78-15.29% minimum and maximum SMC throughout the year. These locations were grouped in less (locations-1 and 2), medium (locations-3) and high moisture containing plots (location-4 and 5). Hereafter these three groups are referred to as less, medium and high Moisture Containing Plots (MCP). Selected locations substantially vary in water holding capacity and soil porosity and thus in soil moisture.

Three, 10×10 m areas were demarcated in the centre of each location. Within each 10×10 m area, nine, 1×1 m experimental plots, arranged in three parallel rows (three 1×1 m plots in each row) were established. A 1.5 m distance between two 1×1m plots was kept as buffer zone to protect against boundary effects due to migration of N out of the sampling areas. Within each moisture level three treatments of nitrogen, each replicated three times, were randomly established on the basis of lottery method: control (without N), low N (60 kg N ha^{-1} $year^{-1}$) and high N (120 kg N ha^{-1} $year^{-1}$). Thus, a total of 135, 1×1 mplot (5 moisture levels×3 treatments×9 replicates) were used in the present experiment.

N-treatment: Since March of year 2007, commercial urea fertilizer as source of nitrogen was applied to the plots in evening, at one month interval in form of split dose. In evening time, temperature is low and at this low temperature; activation energy of the urease enzyme is low that decreases the N loss by the volatilization process (Makoi and Ndakidemi, 2008). Urea was used as a source of dry N due to its relatively high N content, easy handling and price while; it has greater potential for N loss through ammonium volatilization (Jones *et al*., 2007). Published estimates of atmospheric N deposition are not available for the sites as well for the region. We used 60 and 120 kg N ha^{-1} $year^{-1}$ which is probably a relatively high dose to ensure the measurable response on the soil processes and also on species diversity because in our precedent study we applied 30 and 60 kg N $ha^{-1}year^{-1}$ in the soil, at these levels, N did not saturated in the system.

Vegetation sampling: After two years of N treatments, vegetation data were collected in August of years 2009 and 2010. At each location, 27 1×1 m plots were divided in to 108, 50×50 cm quadrats (each 1×1 m plot was gridded in to four 50×50 cm quadrats) as workable units for sampling. Thus, a total of 540, 50×50 cm quadrats were used in this study. For each quadrate, number of individuals and their herbage cover were recorded by species. Cover was measured by gridding the quadrats into 5×5 cm cells and transferring the cover outlines on a graph paper.

The collected plants were categorized into four (Raunkiaer, 1934) life forms (phanerophyte, perennating buds are located on upright shoots at least 25 cm above the surface; therophyte, seasonal plants propagating through seeds and complete their life history within a short period; hemicryptophyte, perennial plants having buds in or just below the soil surface and geophytes; buds or rhizome/bulbs are located below the soil surface.

Soil sampling: Three soil samples (0-10 cm depth) were collected from each 1×1 mplot, using a 5 cm diameter corer in year 2009. The soil samples of three cores were combined to form a composite soil sample for each plot. These composite samples were gently homogenized. Large roots, woods, litter and all fine roots were removed from the composite soil samples carefully. Soil bulk density and porosity were determined for these composite samples. Air dried soil samples, sieved through 2 mm mesh screen and were analyzed for water holding capacity, soil pH and soil nutrients.

Soil bulk density (g cm^3) was determined by using corer method (stainless steel cylinders with a volume of 100 cm^3) (Piper, 1944) and was calculated as the dry soil weight divided by the soil volume (Su and Zhao, 2003). Soil porosity was calculated by subtracting the ratio of soil bulk density and particle density (ca. 2.65) from its maximum value of 1 (Sagar and Verma, 2010). Water holding capacity of soil was determined by using perforated circular brass boxes (Piper, 1944). Soil pH was measured by using a glass electrode (1:2; soil: Water ratio). Soil organic carbon was analyzed by using dichromate oxidation and titration with ferrous ammonium sulphate (Walkley, 1947). Soil N was measured after wet digestion using macro Kjeldahl procedure (Jackson, 1958). The details of physico-chemical properties of the study plots are given in Table 1. The studied plots differed in their physico-chemical properties. Soil organic-C, Water Holding Capacity (WHC) and porosity were highest for the high MCP and minimum for the less MCP while soil pH was highest for less MCP and minimum for the high PCP (Table 1).

Data analysis: Importance Value Index (IVI) of each herbaceous species for each plot (27 plots for each location) was calculated by summing the relative frequency, relative density and relative cover (Mueller-Dombois and Ellenberg, 1974). The studied

Table 1: Variation in physiochemical properties of soil due to N-treatment under three moisture regimes

Conditions		WHC	Porosity	pH	Soil-C	Soil-N
Less	Control	38 (2.56)^{a}	45 (3.67)^{a}	7.58 (0.56)^{a}	0.75 (0.13)^{a}	0.04 (0.01)^{a}
	60	40 (2.89)^{a}	47 (4.12)^{a}	7.46 (0.25)^{ab}	1.14 (0.45)^{b}	0.11 (0.02)^{b}
	120	41 (3.45)^{a}	48 (4.31)^{a}	7.30 (0.64)^{b}	0.84 (0.24)^{a}	0.06 (0.01)^{a}
Medium	Control	41(3.32)^{a}	46 (3.83)^{a}	7.40 (0.41)^{a}	0.81 (0.22)^{a}	0.08 (0.01)^{a}
	60	45 (3.24)^{a}	48 (4.10)^{a}	7.36 (0.39)^{ab}	1.20 (0.44)^{b}	0.12 (0.02)^{b}
	120	46 (3.51)^{a}	51 (5.22)^{a}	7.25 (0.27)^{b}	0.83 (0.23)^{a}	0.09 (0.01)^{a}
High	Control	43 (3.15)^{a}	50 (4.12)^{a}	7.24 (0.21)^{a}	0.91 (0.11)^{a}	0.10 (0.01)^{a}
	60	48 (3.52)^{a}	52 (4.16)^{a}	7.16 (0.20)^{ab}	1.44 (0.17)^{b}	0.22 (0.03)^{b}
	120	50 (3.61)^{a}	54 (5.32)^{a}	7.02 (0.20)^{b}	0.99 (0.18)^{a}	0.16 (0.04)^{a}

The values are in percentage except soil pH. The values in parentheses are ±SE. Different superscripts letters within columns are significantly different at $p<0.05$

locations were further assigned by communities on the basis of their dominant and co-dominant species. The species having highest IVI was defined as dominant and that having the second highest IVI as co-dominant species. Similarly, the dominant and co-dominant functional groups of each location were recognized on the basis of Relative Importance value (RIV). The functional group having highest RIV was identified as dominant and that having the second highest RIV as co-dominant functional group.

Species composition and diversity: The α-diversity (H') and its components, i.e., Species Richness (SR) and evenness (E_w) were calculated for each plot and also for each functional group of each plot. Following equations were used to calculate the diversity parameters:

$$H' = -\sum_{i=1}^{s} p_i \ln p_i \quad \text{(Shannon and Weaver, 1949)}$$

$$SR = \frac{S-1}{\ln(n)} \quad \text{(Margalef, 1958)}$$

$$E_w = \frac{S}{\ln Ni - \ln Ns} \quad \text{(Whittaker, 1972)}$$

In the above Equations, p_i = proportion of importance value belonging to species I, S = number of species, N_i = IVI of the most important species, N_s = IVI of the least important species, n = number of individuals.

Statistical analyses: The N-treatment levels of each moisture regime based on IVI of herbaceous species and also based on RIV of functional group of years 2009 and 2010 were ordinated by Nonmetric Multidimentional Scaling (NMS) option in PC-ORD software (McCune and Mefford, 1999). Further, to determine the species composition and their distribution in different function groups of years 2009 and 2010 were also ordinated by NMS. Analysis of variance (ANOVA) procedure of SPSS package (SPSS, 1997) was used to see the effects of SMC and N-treatment on the diversity variables (Shannon index, species richness, evenness). A Tukey's HSD test was used to determine the significance of differences in mean values of diversity variables among different moisture regimes, N-treatments and also among the different trait categories. Pearson's correlation coefficients (r) were calculated to compare explanatory variables (soil properties) and response variables (NMS axes scores and indices of species diversity).

RESULTS

Community composition: A total of 45 species, distributed in 23 families, was recorded in a two year study. Family Poaceae had the maximum number of species (Appendix Table 1). Less MCP had 17 species and 12 families, medium MCP had 33 species and 19 families and high MCP had 42 species and 22 families. High MCP had the highest number of unique species (12) and Medium MCP had minimum number of unique species (Appendix Table 1). *Achyranthes aspera*, *Ageratum conyzoids*, *Atylosia marmorata*, *Blepharis repens*, *Cissampelos pareira*, *Coccinia cordifolia, Commelina benghalensis*, *Corchorus olitorus, Cayratia trifolia, Desmodium gangeticum*, *Euloliopsis binata*, *Malvastrum coromandelianum*, *Oplismenus burmannii*, *Peristroph bycalyculata* and *Triumfetta rhomboidea* were common to all moisture regimes and accounted for 33% of total species (Appendix Table 1). Further, majority of species which were present in 2009 disappeared in 2010. The species disappearance was more in less MCP (47%), compared to medium (26%) and high MCPs (19%) while in year 2010 maximum new species were immerged in high MCP (22%) compared to medium (6%) and less MCPs (6%).

In year 2009, *Oplismenus burmannii* dominated in all the three moisture regimes while; these moisture regimes differed in their co-dominant species. *Peristroph bycalyculata, Achyranthes aspera* and *Digitaria sanguinalis* co-dominated, in the less, medium high MCPs, respectively (Table 2). These data revealed that the less MCP represented *Oplismenus-Peristroph* community; medium MCP, Oplismenus-Achyranthes

Table 2: IVI value of dominant herbaceous species at three level of N-treatment under different moisture regimes

	Less				Medium				High			
Species	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
Year 2009												
Achyranthes aspera L.	23	25	24	24	16	25	24	22	18	23	30	24
Commelina benghalensis L.	6	9	3	6	7	9	7	8	27	21	10	19
Digitaria sanguinalis L.	0	0	0	0	4	33	8	15	86	34	57	59
Oplismenus burmannii Retz.	206	186	203	198	132	97	104	111	42	66	121	76
Panicum psilopodium Trin.	0	3	0	1	2	40	21	21	23	14	12	16
Peristroph bycalyculata Nees.	35	24	46	35	14	22	27	21	1	1	2	1
Salvia plebeia R.Br.	0	0	0	0	18	4	14	12	0	0	0	0
Year 2010												
Achyranthes aspera L.	33	23	26	27	5	9	19	11	27	33	27	29
Ageratum conyzoides L.	0	17	0	6	61	45	105	70	0	0	0	0
Atylosia marmorata Benth.	0	0	0	0	0	4	3	2	66	29	19	38
Clerodendrum indicum Kuntze.	14	0	0	5	53	63	31	49	0	0	0	0
Dichanthium annulatum Forssk.	0	0	0	0	0	0	0	0	45	48	29	41
Digitaria sanguinalis L.	0	0	0	0	0	31	10	14	25	41	15	27
Oplismenus burmannii Retz.	250	237	274	254	117	93	72	94	3	21	10	11
Panicum psilopodium Trin.	0	0	0	0	3	0	0	1	76	50	139	88

A= Control, B = 60 kg N ha^{-1} year^{-1}, C =120 kg N ha^{-1} year^{-1}

community and high MCP, *Oplismenus-Digitaria* community. In year 2010, the dominant species of year 2009 also dominated in medium and less MCPs but in high MCP *Panicum psilopodium* was a dominant species. Interestingly, the SMC caused changes in co-dominant species, viz; *Achyranthes aspera*, *Ageratum conyzoids* and *Dicanthium annulatum* co-dominated in less, medium and high MCPs, respectively (Table 2). Further, these data suggested that the less MCP exhibited *Oplismenus-Achyranthes* community; medium MCP, *Oplismenus-Ageratum* community and high MCP, *Panicum-Dicanthium* community (Table 2).

In year 2009, the dominant species of each moisture regime did not differ with increased dose of N-application. However, in general, the co-dominant species of each MCP differed due to increased dose of N-application, except in high MCP (Table 2). In less MCP, *Peristroph bycalyculata* co-dominated in control and 120 kg N ha^{-1} year^{-1} treated plots and *Achyranthes aspera* in 60 kg N ha^{-1} year^{-1} treated plot. In medium MCP, *Salvia plebeia, Panicum psilopodium* and *Peristroph bycalyculata* co-dominated, respectively, in control, 60 and 120 kg N ha^{-1} year^{-1} treated plots. In high MCP *Oplismenus burmannii* co-dominated in control plots and *Digitaria sanguinalis* co-dominated in 60 and 120 kg N ha^{-1} year^{-1} treated plots (Table 2). In year 2010, similar to year 2009 the dominant species of each moisture level did not vary due to increased dose of N-application. Only dominant species of high MCP (*Panicum psilopodium*) of year 2010 varied with those of year 2009. The co-dominant species of N-treated plots within each moisture regimes differed from the control plots. Hence, the data suggested that the N-treatment transformed the community composition of the studied plots by showing modification in co-dominant species (Table 2).

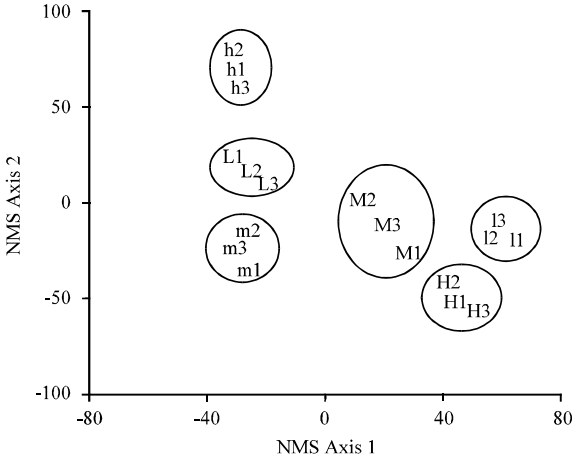

Fig. 1: NMS (Nonmetric Multidimentional Scaling) ordination of the N-treated plots under different moisture regimes on the basis of IVI of herbaceous species of years 2009 and 2010. L, l = less MCPs, M, m = medium MCPs and H, h = high MCPs (upper case represents data for year 2009 while lower case for year 2010). 1, 2 and 3, respectively represent control, 60 and 120 kg N ha^{-1} year^{-1}

NMS ordination of the N-treated plots under different moisture regimes on the basis of IVI of herbaceous species is illustrated in Fig. 1, indicated three prominent groups, each group having three plots of N-treatments, suggesting differences in community composition due to soil moisture and N treatments. NMS axes 1 ($r = -0.82$, $p = 0.01$) and 2 ($r = 0.81$, $p = 0.01$) represented gradients of soil pH. NMS axis 2 was also related to water holding capacity ($r = -0.70$, $p = 0.04$) and soil porosity ($r = -0.68$, $p = 0.04$).

Species diversity: Multivariate Analysis of Variance (MANOVA) showed that the α-diversity and its components varied notably due to year, moisture andN-application (Table 3). The union of year and moisture exhibited alteration in Shannon diversity while year and N-application modified evenness. The N-application coupled with moisture regimes and also all interaction caused remarkable difference in the α-diversity and its components (Table 3). Generally, the values of all the diversity indices were less in year 2010 compared to year 2009. In both years, diversity parameters increased due to increased SMC (Table 4). Furthermore, in years 2009 and 2010 diversity parameters were greater in 60 kg N ha^{-1} treated dplots compared to zero and 120 kg N ha^{-1} treated plots.

Functional group composition: The number of species among the trait categories, across the moisture regimes in years 2009 and 2010 varied from 1 to 19 and from 1 to 15. As expected, the number of species of all the trait categories for each MCP were less in year 2010 than the year 2009, except for phanerophyte which had greater species in less MCP of year 2010 than the year 2009 (Appendix Table 1). Therophyte and hemicryptophyte had maximum number of species in 60 kg N ha^{-1} $year^{-1}$ treated plots in less MCP of year 2009. This pattern was retained in year 2010 for hemicryptophte but values were less in year 2010 for both the traits compared to year 2009.Therophyte harboured greater number of species in 60 kg N ha^{-1} $year^{-1}$ treated plots in all the MCPs (Appendix Table 1).

Table 3: Analysis of variance for Shannon diversity, species richness and evenness using year, moisture and N-treatment and all interactions

Independent variables	Df	Shannon index	Species richness	Evenness
Year	1	31.50***	16.28***	1217***
Moisture	4	71.41***	43.108***	6.23**
N-Treatment	2	1.83*	3.12**	1.89*
Year×Moisture	4	2.68*	0.08^{ns}	0.73^{ns}
Year×N-treatment	2	0.37^{ns}	0.24^{ns}	1.84*
Moisture×N-treatment	8	2.93*	1.60*	2.30*
Year×Moisture×N-treatment	8	3.56**	2.80*	2.92*
Error	240			

*p<0.05, **p<0.01, ***p<0.001; ns: Not significant

Table 4: Species diversity of different functional groups of years 2009 and 2010 under three N treatment levels in a moisture gradient

		Less				Medium				High			
Years	Functional types	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
Shannon-wiener index													
2009	Therophyte	0.82	0.86	0.77	0.82	1.02	1.43	1.12	1.19	1.36	1.57	1.18	1.37
	Hemicryptophyte	0.06	0.07	0.03	0.05	0.10	0.35	0.23	0.23	0.49	0.51	0.23	0.41
	Phanerophyte	0.31	0.40	0.31	0.34	0.20	0.39	0.26	0.28	0.39	0.41	0.27	0.36
	Geophyte	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.01	0.05	0.11	0.06	0.07
	Mean	0.30	0.33	0.28	0.30	0.33	0.55	0.41	0.43	0.57	0.65	0.44	0.55
2010	Therophyte	0.44	0.66	0.29	0.46	0.65	0.97	0.75	0.79	1.09	1.32	0.99	1.13
	Hemicryptophyte	0.00	0.00	0.00	0.00	0.06	0.07	0.05	0.06	0.5	0.53	0.52	0.52
	Phanerophyte	0.05	0.07	0.04	0.05	0.27	0.39	0.37	0.34	0.43	0.6	0.23	0.42
	Geophyte	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00
	Mean	0.12	0.18	0.08	0.13	0.25	0.36	0.30	0.30	0.51	0.61	0.44	0.52
Species richness													
2009	Therophyte	0.57	0.57	0.53	0.57	1.06	1.36	1.04	1.15	1.70	1.85	1.19	1.58
	Hemicryptophyte	0.00	0.00	0.00	0.00	0.13	0.14	0.07	0.11	0.44	1.3	1.02	0.92
	Phanerophyte	0.30	1.20	0.21	0.56	0.47	0.63	0.25	0.45	0.80	0.89	0.52	0.74
	Geophyte	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.30	0.00	0.16
	Mean	0.22	0.44	0.19	0.28	0.42	0.53	0.34	0.43	0.78	1.09	0.68	0.85
2010	Therophyte	0.31	0.36	0.35	0.34	1.09	1.32	0.82	1.08	1.17	1.26	1.22	1.22
	Hemicryptophyte	0.00	0.00	0.00	0.00	0.00	0.64	0.00	0.21	0.79	0.86	0.49	0.71
	Phanerophyte	0.00	0.00	0.00	0.00	0.00	0.00	0.69	0.23	0.42	0.47	0.45	0.45
	Geophyte	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean	0.08	0.09	0.09	0.09	0.27	0.49	0.38	0.38	0.60	0.65	0.54	0.59
Evenness													
2009	Therophyte	1.49	1.57	1.42	1.49	2.03	2.82	2.19	2.35	3.26	3.66	2.31	3.08
	Hemicryptophyte	0.00	0.00	0.00	0.00	0.63	1.21	0.63	0.82	1.57	2.73	1.6	1.97
	Phanerophyte	2.83	3.67	1.81	2.77	2.15	3.29	2.02	2.49	5.29	10.97	0.83	5.7
	Geophyte	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean	1.08	1.31	0.81	1.07	1.20	1.83	1.21	1.41	2.53	4.34	1.19	2.69
2010	Therophyte	1.01	1.05	0.85	0.97	2.49	3.19	1.63	2.44	2.48	2.88	2.64	2.67
	Hemicryptophyte	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.38	2.3	1.41	1.7
	Phanerophyte	0.00	0.00	0.00	0.00	0.00	2.47	1.28	1.25	1.64	3.03	2.43	2.37
	Geophyte	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean	0.25	0.26	0.21	0.24	0.62	1.42	0.73	0.92	1.38	2.05	1.62	1.68

1= 0 kg N ha^{-1} $year^{-1}$, 2 = 60 kg N ha^{-1} $year^{-1}$, 3 = 120 kg N ha^{-1} $year^{-1}$

Table 5: Effect of N treatment on the Relative importance values distributed in different life forms under different moisture conditions in year 2009 and 2010

		Life forms			
Moisture gradient	N levels kg N $ha^{-1}year^{-1}$	T	H	P	G
Year 2009					
Less	0	61	16	19	4
	60	72	11	17	0
	120	75	9	16	0
	Mean	69	12	17	1
Medium	0	14	68	18	1
	60	30	55	14	1
	120	39	50	10	1
	Mean	28	58	14	1
High	0	28	62	10	0
	60	34	58	8	0
	120	42	52	6	0
	Mean	35	57	8	0
Year 2010					
Less	0	46	30	24	0
	60	57	26	17	0
	120	68	21	11	0
	Mean	57	26	17	0
Medium	0	39	45	16	0
	60	32	42	26	0
	120	31	40	27	2
	Mean	34	42	23	1
High	0	24	58	18	0
	60	31	47	22	0
	120	46	37	17	0
	Mean	34	47	19	0

T = Therophytes, H = Hemicryptophytes, p = Phanerophytes, G =Geophytes

Relative Importance Value (RIV) of each trait across the N-treatment under different moisture regimes of year 2009 and 2010 are presented in Table 5. Results indicated that the therophyte dominanted in less MCPs, whereas hemicryptophytes dominated in medium and high MCPs in both years. The RIV of therophytes increased with increased dose of N in all the MCPs of years 2009 and 2010, except medium MCP of year 2010. Interestingly, the RIV of hemicryptophytes decreased consistently due to increased dose of N in all the MCPs during the studied period. Phanerophytes co dominated in less MCP of year 2009. Similar to hemicryptophytes, the RIV of phanerophytes also decreased due to increased dose of N in all the MCPs, except for medium and high MCPs of year 2010 (Table 5).

MS ordination of the trait categories of the years 2009 and 2010 indicated that each trait of year 2009 soundly segregated in ordination diagram. Similarly, the trait categories of year 2010 also conspicuously alienated in the ordination diagram. Each trait category of the year 2009 markedly segregated with the corresponding trait category of the year 2010 (Fig. 2). Thus, the data revealed that the trait categories were temporally independent. Further, NMS ordination of the N-treated plots under different moisture regimes on the basis of RIV of different trait categories of years 2009 and 2010 are presented in Fig. 3, yielding three outstanding groups for each year. Among these groups, each group had three plots of

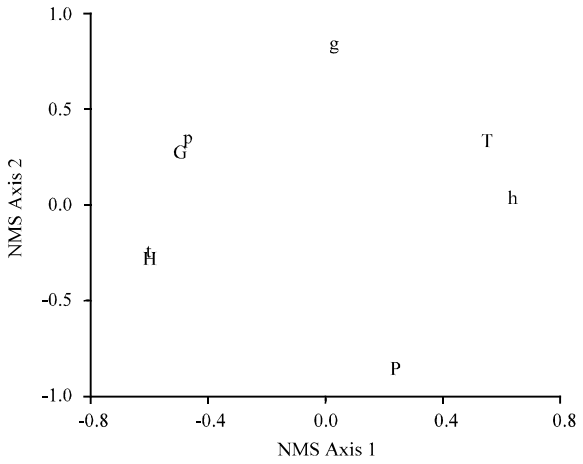

Fig. 2: NMS (Nonmetric Multidimentional Scaling) ordination of the trait categories on the basis of RIV of the years 2009 and 2010. T, t = therophytes; H, h = hemicryptophytes; P, p = phanerophytes; G, g = geophytes (upper case represents data for year 2009 and lower case for year 2010)

N-treatments and these groups of each year were distinctly different from each other in ordination diagram (Fig. 3). NMS axis 1 of the year 2009 was significantly related to soil pH ($r = -0.84$, $p = 0.01$), organic carbon ($r = 0.70$, $p = 0.04$), water holding capacity ($r = 0.68$,

p = 0.04) and soil porosity (r = 0.72, p = 0.03). NMS axis 2 was also related to soil pH (r = - 0.83, p = 0.01), organic carbon (r = 0.67, p = 0.04), water holding capacity (r = 0.71, p = 0.03) and soil porosity (r = 0.67, p = 0.04).

Functional trait diversity: MANOVA indicated that in general the diversity indices of all the trait categories differed significantly due to SMC. Year exhibited statistically variation in all the diversity indices of only hemicryptophytic trait and it was phanerophytic richness and evenness which varied due to N treatment. The interactions of year, moisture and N treatment caused variation in all the indices of therophytic trait only (Table 6).

Table 4 indicated that over two years of study, the mean values of Shannon index, Margalef (1958) index and Whittaker index among the trait categories, across the moisture regimes varied from 0.01 to 1.37, 0.11-1.58 and 0.24 to 5.70, respectively. The values of these indices were less in year 2010 than the year 2009. The therophytes had greater indices of diversity in both the years, except for the phanerophytic evenness of year 2009. In general, all the trait categories had greater indices of diversity in high MCP compared to less and medium MCPs. In both the years, generally all the trait groups produced humped back pattern for the indices of diversity in relation to N treatment (Table 4). Pearson correlation analysis indicated that among the studied soil parameters of year 2009, soil pH negatively influenced the Shannon (r = -0.79, p = 0.02), Margalef (1958) (r = - 0.78, p = 0.01) and Whittaker indices (r = - 0.70, P = 0.04) of hemicryptophytes. For therophytes, soil pH inhibited the Margalef (1958) (r = - 0.81, p = 0.01) and Whittaker indices (r = 0.74, p = 0.02) of diversity while for geophytes, only Margalef (1958) index (r = -0.67, p = 0.04) was reserved by the soil pH. Accordingly, it was

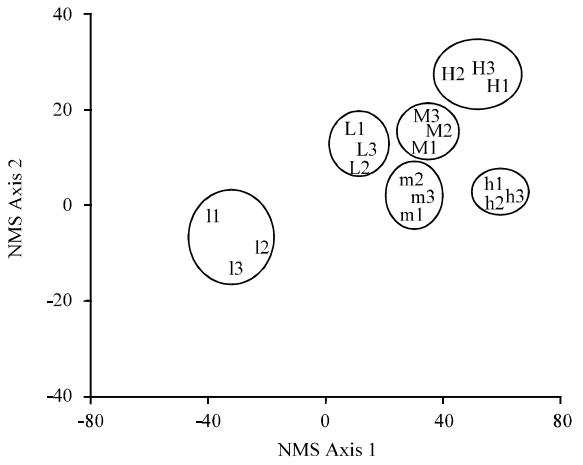

Fig. 3: NMS (Nonmetric Multidimentional Scaling) ordination of the N-treated plots under different moisture regimes on the basis of RIV of different trait categories of years 2009 and 2010. L, l = MCPs, M, m = medium MCPs and H, h = high MCPs (upper case represents data for year 2009 and lower case for year 2010). 1, 2 and 3, respectively represent control, 60 and 120 kg N ha^{-1} $year^{-1}$

Table 6: Analysis of variance for each functional group using Year, moisture, N-treatment and all interactions

Treatments	Df	T	H	P	G
Shannon index					
Year	1	27.03***	62.89***	2.06^{ns}	7.68**
Moisture	4	26.26***	21.52***	14.73***	5.90**
N-Treatment	2	1.03^{ns}	0.18^{ns}	0.52^{ns}	0.76^{ns}
Year×Moisture	4	5.69**	9.66***	6.67**	7.17**
Year×N-treatment	2	0.10^{ns}	0.38^{ns}	1.97*	0.97^{ns}
Moisture×N-treatment	8	2.07*	3.00*	2.27*	0.78^{ns}
Year×Moisture×N-treatment	8	2.05*	2.99*	2.54*	0.45^{ns}
Species richness					
Year	1	6.31**	53.20***	6.68**	2.22^{ns}
Moisture	4	42.11***	23.35***	4.09*	2.23*
N-Treatment	2	0.89^{ns}	2.46*	2.34*	0.70^{ns}
Year×Moisture	4	0.91^{ns}	23.35***	1.51*	2.23*
Year×N-treatment	2	0.13^{ns}	2.46*	0.31^{ns}	0.70^{ns}
Moisture×N-treatment	8	0.56^{ns}	1.68*	1.66*	0.70^{ns}
Year×Moisture×N-treatment	8	2.14*	1.68*	1.20^{ns}	0.70^{ns}
Evenness					
Year	1	3.42^{ns}	22.19***	5.46*	1.51^{ns}
Moisture	4	41.85***	6.02**	0.15^{ns}	1.51*
N-Treatment	2	2.01*	0.08^{ns}	3.09*	0.80^{ns}
Year×Moisture	4	1.57*	6.02**	2.40*	1.51*
Year×N-treatment	2	0.45^{ns}	0.08^{ns}	2.65*	0.80^{ns}
Moisture×N-treatment	8	1.28*	0.54^{ns}	2.43*	0.79^{ns}
Year×Moisture×N-treatment	8	3.98**	0.54^{ns}	2.47*	0.80^{ns}
Error	240				

*p<0.05, **p<0.01, ***p<0.001; ns: Not significant, T = Therophytes, H = Hemicryptophytes, p = Phanerophytes, G = Geophytes

the soil pH that influenced the diversity indices of the selected trait categories under the N treatment along the soil moisture gradient and hemicryptophytic diversity was the most sensitive group towards the change in soil pH under studied system.

DISCUSSION

Community composition: IVI analysis represented three distinct communities due to different combinations of dominant and co-dominant species. It was also supported by NMS ordination. The significant relationships between NMS axes scores 1 and 2 with soil pH and NMS axis score 2 with soil pH, water holding capacity and soil porosity indicated that soil pH was a major determinant for the distribution of herbaceous flora. However, water holding capacity and soil porosity were substantially governed the constitution and distribution of herbaceous communities of the experimental plots. It is well established fact that higher amounts of pores present in the soil system reduce the soil compaction and increase the availability of soil moisture which supports the variety of herbaceous species (Brady and Weil, 2002). Other studies also agreed that soil porosity, soil moisture and water holding capacity are essential for organization and determination of herbaceous floristic composition (Singh *et al*., 1998; Sagar *et al*., 2008a, b; Sagar and Verma, 2010).

Occurrence of generalist species (*A. aspera*, *A. conyzoids*, *A. marmorata, B. repens*, *C. pareira*, *C. cordifolia, C. benghalensis*, *C. olitorus*, *C. trifolia*, *D. gangeticum*, *E. binata*, *M. coromandelianum*, *O. burmannii, P. bycalyculata* and *T. rhomboidea*) at all the moisture regimes suggested their wide niche and ecological amplitude and their ability to resist against the soil moisture. Constantly, presence of dominant species along increased dose of N-application at each of the moisture level, suggested their strong fitness against N-application in their respective moisture amplitude. A generalist species may stick with in a landscape due to its capability to utilize numerous location types where one location is prevalent but excessively low in quality to hold persisting populations while the other is fit but too sparse to allow ample dispersal among locations (Harrison, 1999).

Species diversity: Over a two year of N-application, net species loss was greater in less MCP compared to high MCP, on the other hand, occurrence of new and unique species were comparatively greater in high MCP. These conditions suggested that dry habitats are less suitable for the accumulation of species diversity. Under fertilizer experiment the dry locations may support less species due to less seed germination and seedling growth (Parker and Oliver, 1938). In present study, we assumed that soil moisture played substantial role for the seed germination and seedling establishment which facilitated significant species diversity buildup at moisture rich locations (Pausas and Austin, 2001). The soil pH in present study was reduced due to the process of acidification caused by N deposition. Another water molecule further reacts with urea, during this process; urea hydrolysis again reduces the soil pH. This reduction in soil pH provided suitable condition for the seed germination and seedling growth of several characteristic species. This could be the additional reason for greater species diversity in high MCPs compared to less and medium MCPs. It is well known that a large number of species enjoy and perpetuate in the soil pH rage of 6.1-6.5 and below or above this range the occurrence of species will be low (Grime, 1979). Thus, the study suggested that N depositions at dry locations could have more severe impacts than the moisture rich locations.

In this study, year moisture and N treatment independently altered the diversity indices of overall herbaceous vegetation. The interaction of year with soil moisture revealed the variation in Shannon index and the interaction of year with N treatment altered the evenness. The coupling of soil moisture and N treatment exhibited reasonable response on all the diversity parameters which was also supported by the pooled effects. Thus, the study suggested that time, soil moisture and N treatment either in identity or jointly could influence the species diversity parameters in the studied system.

In general, each moisture regime experienced a typical humped-back pattern for indices of species diversity in relation to N-treatment. This means species diversity is low at low N-levels, increase to peak at moderate levels decreases gradually at high N-levels. This trend can be interpreted as; a few species are competent to bear intense situation of N deficit. As N increases, more species can stay alive and as a result species diversity increases. At sufficiently high N-levels a few high aggressive species become dominant, suppressing other species. This aggressive elimination can turn down species diversity (Bakelaar and Odum, 1978; Tilman, 1999; Richardson *et al*., 1999). In this study, 60 kg N ha^{-1} $year^{-1}$ is sufficient to permit co-existence of many species while at sufficiently high N levels the system tended to become N-saturated and beyond this threshold level the survival of many species could be hampered (Bracken and Nielsen, 2004). Plant species diversity is suggested to be a maximum under moderate, rather than high or low, levels of fertility. A decrease in diversity with fertilization is expected in fertile sites, where further addition of nutrients limits the coexistence of species (Tilman, 1999; Jonasson, 1992; Pausas and Austin, 2001). Usually, very

high concentrations of nutrients inhibit the root growth of several species by killing their root tips (Parrish and Bazzaz, 1982) which might be the reason for less species diversity at sufficiently high N dose.

Functional groups composition: Life forms categorization is attributed for the adaptational and evolutional diversification of flora in response to the climate (Smith, 1980). The interaction of functional groups and climate provide the direction of evolution of a characteristic physiognomy of the vegetation by favoring a few species to dominate the flora, or in the direction of the evolution of flora itself by encouraging those elements to survive and occur which are adapted to the given climatic condition (Saxena *et al*., 1982), for example; community with high proportion of phanerophytes is a feature of hot climates, a community consisting mostly of hemicryptophytes is a trait of moisture rich habitat (Danin and Orshan, 1990) and a community dominated by therophytes is attribute of deserts (Ricklefs, 1979; Smith, 1980) and disturbed habitats (Kleyer, 1999). In grasslands the prevalence of therophytes due to heavy grazing is a common phenomenon (Yadava and Singh, 1977; Cain, 1950) because biotic perturbation and relative dryness is beneficial for the establishment of therophytes (Cain, 1950; Daubenmire, 1968; Kleyer, 1999).

The predominance of the therophytes in less MCP and hemicryptophytes in medium and high MCPs perhaps due to the competition among the life forms for the soil moisture (Danin and Orshan, 1990). The predominance of the therophytes in less MCP might be used as indicator of dry habitat (Daubenmire, 1968; Danin and Orshan, 1990) experiencing high biotic interferences (Yadava and Singh, 1977; Cain, 1950; Kleyer, 1999). The differential responses of therophytes and hemicryptophytes due to N-treatment indicated that the habitat receiving greater dose of N may allow therophytes to dominate by suppressing the hemicryptophytes and also relatively trigger the habitat towards the dryness (Ricklefs, 1979; Smith, 1980; Danin and Orshan, 1990).

The significant relations of NMS axes scores with soil pH, organic carbon, water holding capacity and soil porosity in year 2009, suggested that the studied soil variables had sound effect on the distribution and composition of herbaceous plant functional groups due to differential resource use strategy. Other studies, reported that soil organic carbon (Sagar *et al*., 2008a), soil moisture and water holding capacity (Singh *et al*., 1998; Sagar *et al*., 2008a, b; Sagar and Verma, 2010) are essential for the regulation of floristic composition. The corresponding plant functional groups of year 2010 segregated with year 2009 suggesting temporal variation in trait composition due to split (slow) but continuous N application (Nordin *et al*., 2005).

Functional group diversity: In general, the study reflected that the diversity indices for all the trait categories were greater in year 2009 compared to 2010 indicating temporal effect of N on the species diversity of trait categories. Majority of trait categories had greater species diversity in 60 kg N ha^{-1} $year^{-1}$ treated plots, supporting humped-back pattern of species diversity along the resource gradient (Tilman, 1999). The soil moisture availability enhanced the species diversity of all the trait categories. Among the studied functional traits, only hemicryptophytes experienced the negative response towards the soil pH for all diversity indices. Hence, the study may imply that if such a decline in soil pH due to N-accumulation will continue under the heterogeneity of soil moisture, the system would be diversified by the hemicryptophytes. Moreover, significant reduction in therophytic diversity due to year×soil moisture had provided the platform to the hemicryptophytes for better utilization of available resources which had helped them in their diversification. In Central Europe, the hemicryptophytes were well suited in their climatic rhythm because their buds were directly in contact with earth and buds were thickly wrapped around by leaves which posse's higher longevity due to clonal propagation and the germinating buds helped them to sustain under extreme conditions. On these bases Raunkiaer defended for more diversification of hemicryptophytic flora in Europe (Ellenberg, 1988).

Ecological implications: In dry environments, the small but continuous N-deposition will amount over time in lower soil horizons within and below the rooting zone because of its low leaching rate (Walvoord *et al*., 2003; Wood *et al*., 2006). This N-accumulation lingers a significant trouble for many habitats (NEGTAP, 2001) and recovery may often not be possible without active management and restoration measures (Cunha *et al*., 2002). This experiment is an initiation step for setting a critical load which can provide information about maximum diversity of species and functional group. In addition, the information derived from this study will add new towards the global climatic change from the Indian perspective. Since soil perturbation is one of the most imperative selection factor for vegetation (Zanin *et al*., 1997) and also linked to nutrient and lethal element availability, therefore, reduction in soil pH due to N-application played a substantial role in the structure, composition and diversity of species as well as functional groups. As a consequence, N-application along with soil moisture on alkaline soil could be used as a strategy to minimize the soil pH up to a certain level which in turn may support greater herbaceous diversity. Further, hemicryptophytic diversity was negatively correlated with soil pH, for this reason, the establishment of

hemicryptophytic functional trait might be an alternative tool for restoration of degraded ecosystems experiencing high soil pH to achieve the ecosystem processes and services because in such ecosystems soil pH will act as a filter to remove the species that lack traits for persisting under this meticulous set of situation (Keddy, 1992).

ACKNOWLEDGMENTS

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APPENDIX

Appendix Table 1: Herbaceous species composition under three different moisture regimes

Species	Family	Life form
Achyranthes aspera L.[1,2,3,4,5,6]	Amaranthaceae	T
Ageratum conyzoids L.[1,3,4,6]	Asteraceae	T
Ammannia baccifera L.[1,4]	Lythraceae	T
Anagallis aevensis L.[1,2,3]	Primulaceae	T
Anisomeles obata R. Br.[1,2,3,4]	Lamiaceae	T
Atylosia marmorata Benth.[1,2,3,4,5]	Fabaceae	P
Blepharis repens[2,3,4,5,6]	Acanthaceae	T
Cissampelos pareira Linn[2,3,5]	Menispermaceae	P
Clerodendrum indicum L. kuntze.[3,4,6]	Verbinaceae	P
Coccinia cordifolia L. Cong[2, 3,5]	Cucurbitaceae	P
Commelina benghalensis L.[1,2,3,4,5,6]	Commelinaceae	H
Commelina L.spp[1,4]	Commelinaceae	H
Corchorus olitorus L.[1,3,4,5]	Tiliaceae	T
Corchorus tridens L.[1,4]	Tiliaceae	T
Cayratia trifolia L. Domin[1,2,3,4,5,6]	Vitaceae	P
Cyperus fuscus L.[1,6]	Cyperaceae	G
Cyperus kyllingia Endl.[1,3,4]	Cyperaceae	G
Cyperus rotundus L.[1]	Cyperaceae	G
Desmodium gangeticum L. DC.[1,2,3,4,5]	Fabaceae	H
Dicanthium annulatum Forssk [2,3]	Poaceae	H
Digitaria sanguinalis L.[1,2,3,4]	Poaceae	T
Eulaliopsis binata (Retz) C.E. Hubb.[1,3,4,5]	Poaceae	T
Eragrostis tenella L.[1,3]	Poaceae	T
Euphorbia hirta L.[1,2]	Euphorbiaceae	T
Euphorbia pulcherima Willd. ex Klotzsch[2]	Euphorbiaceae	P
Herpestis monniera L.[1,2,3,4]	Scrophulariaceae	T
Hyptis suaveolens Poit.[2,3]	Lamiaceae	T
Ipomoea quamoclit L.[2]	Convolvulaceae	T
Malvastrum coromandelianum L.[1,2,3,4,5,6]	Malvaceae	T
Nicotiana plumbaginifolia Viv.	Solanaceae	T
Oldenlendia ambellata L.[1]	Rubiaceae	T
Oplismenus burmannii Retz.[1,2,3,4,5,6]	Poaceae	T
Panicum psilopodium Trin.[1,2,5]	Poaceae	H
Peperomia pellucida L.[1,3,4]	Piperaceae	T
Peristroph bycalyculata Nees.[1,2,3,4,5]	Acanthaceae	T
Phyllanthus urinaria L.[1,2,3]	Euphorbiaceae	T
Physalis minima L.[3]	Solanaceae	T
Rungia parviflora Retz.[1,6]	Acanthaceae	H
Salvia plebeia R.Br.[3,4]	Lamiaceae	P
Scoparia dulsis L.[1,2,3,4]	Scrophulariaceae	T
Scrofularia nodosa L.[1]	Scrophulariaceae	H
Sida acuta Burn.F[2]	Malvaceae	H
Triumfetta rhomboidea Jacq.[1,2,3,4,5]	Tiliaceae	P
Tylophora indica Burm.F.Merr.[2,3]	Asclepidaceae	P
Urena lobata L.[3]	Malvaceae	T

Abbreviation used: 1-less MCP-2009, 2- less MCP-2010, 3-medium MCP-2009, 4-medium MCP -2010, 5-high MCP-2009, 6-high MCP 2010. T: Therophyte, P: Phanerophyte, H: h emicryptophyte, G: geophyte. F: Forb, G: Grass, L: Legume, S: Sedges

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