

## COMPOSITION, DIVERSITY, AND BIOMASS OF HERBACEOUS SPECIES AND FUNCTIONAL GROUPS IN A GRADIENT OF NITROGEN AMENDMENT IN A DRY TROPICAL ENVIRONMENT OF INDIA

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*Abstract.* Nitrogen depositions due to anthropogenically induced disturbances are adding more reduced N to the biosphere, and have had considerable impacts on soil and vegetation. The objectives of the present study were to investigate the effects of N application on the diversity and biomass of herbaceous functional groups in a dry tropical environment of India. For this, a total of 135 1-m<sup>2</sup> plots distributed in five locations were established in the year 2007 on the campus of Banaras Hindu University, Varanasi, India. Each plot received a randomly chosen dose of N (0 kg N ha<sup>-1</sup> yr<sup>-1</sup> [control], 60 kg N ha<sup>-1</sup> yr<sup>-1</sup>, or 120 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Vegetation samples were collected in 2009 and 2010. The species diversity of each functional group in each 1-m<sup>2</sup> plot was calculated using the Shannon-Wiener index, and peak shoot biomass of the same was established by harvesting. The data were subjected to appropriate statistical analyses. NMS ordination suggested that soil moisture and N amendment caused changes in species and functional group composition and diversity. Location, year, and N amendment all contributed to significant differences in species diversity and biomass. Species diversity was maximum in the 60 kg N ha<sup>-1</sup> yr<sup>-1</sup> treated plots, while herbaceous above-ground biomass further increased due to the increase in N dose. The increase in total herbaceous biomass along the N amendment was mainly due to an increase in the biomass of grasses as there was a decline in forbs and legumes.

*Key words:* biomass, functional groups, herbaceous vegetation, nitrogen, soil moisture, species diversity.

### INTRODUCTION

Nitrogen (N) deposition in certain ecosystems due to immense agricultural activities (Vitousek *et al.* 1997, Bobbink *et al.* 2010, Kros *et al.* 2011), fossil fuel combustion, biomass burning, and changes in land use patterns (Waldrop *et al.* 2004) is a much discussed subject under the umbrella of global climate change (Cramer & Leemans 1993, Vitousek *et al.* 1997, WRI 1998, Jones *et al.* 2007, Bobbink *et al.* 2010, Jensen *et al.* 2011). In this context, to meet global food requirements humans have increased many-fold the use of chemical fertilizers, which has generated around 40% of the net annual

release of fixed N by total anthropogenic sources (60% of the global sources of biologically available or fixed N) (Vitousek *et al.* 1997). This surplus N in the atmosphere has started to overwhelm the normal N cycle, causing great and abnormal effects on soil (reduction in soil fertility), water (eutrophication), air (increase in concentrations of nitric and nitrous oxide, respectively causing acid rain and the greenhouse effect), and vegetation (decline in biodiversity). There is a limit to the amount of N that natural ecosystems can absorb, and beyond this threshold severe destruction can be expected. Certain natural ecosystems in South Africa and Australia that are very low in N supply show high species diversities (WRI 1998, Bobbink *et al.* 2010).

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Global estimates suggest that due to the continuous intensification of agriculture and energy use, global N deposition would be more than double in year 2025 (Asner *et al.* 1997), and that its consequences would be very diverse, ranging from positive to negative. Studies have indicated that in sensitive terrestrial ecosystems N saturation has affected soil chemistry by causing the loss of soil nutrients and ultimately reducing soil fertility (Vitousek *et al.* 1997, WRI 1998). Further, it has been observed that excess N has disrupted the structure and functioning of certain ecosystems due to a reduction in species richness and an increase in vegetation biomass (Reich *et al.* 2001). Gilliam (2006) described a decline in species richness caused by the loss of many N-efficient species, a reduction in species equitability as a result of increasing dominance by a few high-N-demanding species, and finally a decline in species diversity as a consequence of a decrease in both species richness and evenness due to increased N depositions (Bernhardt-Römermann *et al.* 2010).

We used a plant functional type approach (groups of plant species with similar traits and functions with respect to multiple environmental factors) to understand the reaction of herbaceous vegetation to changes in N availability, since current global changes in atmospheric composition, N cycling, and anthropogenically induced land-cover modifications, along with projected global climatic change (Cramer & Leemans 1993), necessitate an understanding of the interactions between environment and plants on a large scale. For such purposes it may be more constructive to work with a functional as opposed to a species classification of plants (Skarpe 1996). As the impact of climatic change on ecosystems over large areas cannot be assessed on a species basis, plant functional types may ease this task (Bugmann 1996). Using this approach, we can determine which functional trait composition – which can cause impacts on plant conditions through its effects on growth, perpetuation, and existence – is likely to result from variation in N availability (Bernhardt-Römermann *et al.* 2010).

Nitrogen is the fundamental component determining soil quality and vegetation type (Sierra 1997, Vourlitis *et al.* 2007). Prolonged and continuous N application in the soil makes the system N saturated (N saturation is the availability of ammonium and nitrates in excess of total combined plant and microbial nutritional demand), which will have adverse impacts on both soil and vegetation (Vitousek *et al.*

1997, WRI 1998, Reich *et al.* 2001). Thus the effects of continuous N fertilizer application as a surrogate of N deposition on the composition, diversity, and biomass of herbaceous functional groups in dry tropical environments of India, which were ignored for so long, must be understood.

Therefore the objectives of the present study were to assess the temporal impacts of different levels of N application on the distribution, composition, diversity, and biomass of herbaceous species as well as functional groups, at different locations on the campus of Banaras Hindu University, Varanasi, India. In addition, the study was also intended to note the relationship between net change in species diversity and biomass over two years, which will improve our knowledge about species diversity and ecosystem function under a variety of N applications. We selected herbaceous flora due to its higher species richness, more easily accessibility and rapid response compared with the arboreal flora following alterations in N deposition (Bernhardt-Römermann *et al.* 2010).

## MATERIALS AND METHODS

*Study area.* The experimental plots were located within the campus of Banaras Hindu University (24°18'N, 83°03'E; altitude 129 m a.s.l.), Varanasi, India. The study area experiences a seasonally dry tropical monsoon climate with three distinct seasons: a hot summer (April to June), a warm rainy season (July to September), and a cold winter (November to February). The months of March and October comprise transition periods between winter and summer and between rainy and winter seasons respectively. Mean monthly minimum and maximum temperatures varied from 7.3 to 25.4°C and 25.6 to 35.6°C respectively, and mean annual rainfall was 932 mm (Sagar *et al.* 2008). The soil is pale brown silty loam and inceptisol. In general, the soil is alluvial, well drained and moderately fertile, being low in soil carbon and nitrogen, medium in water holding capacity (WHC) and porosity, and moderately high in soil pH (Table 1).

*Study design.* The study was conducted at five locations differing in gravimetric soil moisture within the study area during February-March 2007 (Table 1). In the center of each location three homogeneous 10×10-m areas were demarcated for the experimental setup. Within each 100-m<sup>2</sup> area, nine 1×1-m experimental plots were arranged in three parallel rows, hence three plots in each row. A 1.5-m distance be-

TABLE 1. Initial mean values of soil characteristics of the experimental sites located in the campus of Banaras Hindu University. Values in parentheses are  $\pm$  1 SE. All the values are percentages except soil pH. Different superscript letters within rows are significantly different at  $p = \leq 0.05$ .

| Parameters             | Location 1      | Location 2       | Location 3      | Location 4        | Location 5       |
|------------------------|-----------------|------------------|-----------------|-------------------|------------------|
| Soil moisture          | 3.96a<br>(0.04) | 6.46ab<br>(0.03) | 9.01b<br>(0.08) | 11.56bc<br>(0.02) | 14.04c<br>(0.06) |
| Water holding capacity | 37a<br>(0.06)   | 41b<br>(0.05)    | 42b<br>(0.09)   | 43bc<br>(0.08)    | 45c<br>(0.04)    |
| Porosity               | 43a<br>(1.23)   | 44ab<br>(1.38)   | 46b<br>(0.81)   | 47bc<br>(0.85)    | 52c<br>(01.28)   |
| pH                     | 7.82a<br>(0.07) | 7.49a<br>(0.05)  | 7.54a<br>(0.05) | 7.22a<br>(0.03)   | 7.36a<br>(0.06)  |
| Soil C                 | 0.73a<br>(0.09) | 0.75a<br>(0.06)  | 0.79b<br>(0.01) | 0.88c<br>(0.06)   | 0.90c<br>(0.32)  |
| Soil N                 | 0.02a<br>(0.00) | 0.04ab<br>(0.01) | 0.07b<br>(0.02) | 0.08bc<br>(0.04)  | 0.10c<br>(0.03)  |

tween two 1-m<sup>2</sup> plots was kept as a buffer zone to protect against boundary effects due to migration of N out of the sampling areas. For each site (three 100-m<sup>2</sup> areas per site), three treatments of nitrogen, each replicated three times, were randomly determined on the basis of a lottery method: control (without N or zero kg N ha<sup>-1</sup> yr<sup>-1</sup>), low N (60 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and high N (120 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Thus a total of 135 1-m<sup>2</sup> plots (5 locations  $\times$  3 treatments  $\times$  9 replicates) were used in the experiment.

*Initial soil characteristics.* Prior to N treatment, soil moisture, WHC, porosity, pH, soil C and soil N of the experimental plots were determined. For this, three soil samples (0-10 cm depth) were collected from each 1-m<sup>2</sup> plot, using a 100-cm<sup>3</sup> stainless steel cylindrical corer. The three soil cores were combined to form a composite soil sample for each 1-m<sup>2</sup> plot. These composite samples were gently homogenized. Large roots, wood fragments, litter, and all fine roots were carefully removed from the composite soil samples. One part of each sample was weighed and oven-dried at 105°C to a constant weight to determine the gravimetric moisture content, the differences in fresh and dry weight of the soil divided by its dry weight (Black 1965). One part of each soil sample was air-dried, sieved through a 2-mm mesh screen, and analyzed for determination of soil bulk density, porosity and WHC, and soil nutrients C and N.

Soil bulk density (g/cm<sup>3</sup>) was also measured by using the corer method (Piper 1944) and determined as soil dry weight divided by soil volume. Soil porosity was determined by subtracting the ratio of soil

bulk density to the particle density (*ca.* 2.65) from its maximum value of 1. The constant 2.65 is the assumed particle density of the soil (Sagar & Verma 2010). Soil pH was determined by using a glass electrode (1:2, soil:water ratio). Soil C was analyzed using dichromate oxidation and titration with ferrous ammonium sulphate (Walkley 1947). Soil N was measured after wet digestion using the micro Kjeldahl method (Jackson 1958).

The selected locations differed in their initial soil physicochemical characters, except for pH (Table 1). The percentage soil moisture, WHC, and porosity ranged from 3.96 to 4.04, 37 to 45, and 43 to 52 respectively. These values were minimum for Location 1 and maximum for Location 5. Soil pH was highest at Location 1 (7.82) and lowest at Location 4 (7.22). Percent soil C and N contents were highest at Location 5 (0.90 and 0.10) and lowest at Location 1 (0.73 and 0.02).

*N treatment.* The application of a commercial urea fertilizer (100 kg urea is equivalent to 46 kg of N) as source of N to the plots was started in year 2007. The fertilizer was applied to the plots in the evening at one-month intervals in the form of a split dose (60 kg N ha<sup>-1</sup> yr<sup>-1</sup> corresponds to 10.87 kg urea ha<sup>-1</sup> mo<sup>-1</sup> or 1.087 g urea m<sup>-2</sup> mo<sup>-1</sup>). In the evening temperature is low and at this temperature the activation energy of the urease enzyme remains low, preventing N loss by volatilization (Makoi & Ndakidemi 2008). Urea was used as a source of dry N on the basis of its relatively high N content, easy handling, and price, though it has a greater potential for N loss through

ammonium volatilization (Jones *et al.* 2007). We used 60 and 120 kg N ha<sup>-1</sup> yr<sup>-1</sup>, which is probably a relatively high dose, to ensure a measurable response in the soil and vegetation, since in a previous study we added 30 and 60 kg N ha<sup>-1</sup> yr<sup>-1</sup> to the soil, and at these levels N did not saturate the soil system.

*Vegetation sampling and analyses.* After two years of N treatment, vegetation data were collected in 2009 and 2010. All 135 1-m<sup>2</sup> plots equally distributed at five locations (45 1-m<sup>2</sup> plots per location) were divided into 540 50×50-cm quadrats as workable units for sampling. For each quadrat, peak above-ground biomass, number of individuals, and their herbage cover were recorded by species. Cover was measured by gridding the quadrats in 5×5-cm cells and transferring the cover outlines to graph paper (Sagar *et al.* 2008). The above-ground peak biomass of each species for each quadrat was determined by clipping the shoots of individual species at the ground surface and drying them at 80°C until their weight was constant (Odum 1960). The present work was confined to above-ground biomass, since it was difficult to sample below-ground biomass precisely on a species basis.

The Importance Value Index (IVI) of each herbaceous species for each 1-m<sup>2</sup> plot was calculated by summing the relative frequency, relative density, and relative cover (Mueller-Dombois & Ellenberg 1974). The Shannon & Weaver (1949) equation

$$H' = - \sum_{i=1}^s p_i \ln p_i$$

was used to calculate species diversity ( $H'$ ) for each 1-m<sup>2</sup> plot on the basis of the IVI of the component species in both years. In this equation,  $p_i$  is the proportion of importance value belonging to species  $i$ .

*Statistical analyses.* The 135 plots distributed over five locations were ordinated using the mean IVI of the

years 2009 and 2010 of the component species by Non-metric Multidimensional Scaling (NMS) with PC-ORD software (McCune & Mefford 1999). The relationships of NMS axes scores with soil moisture were established by correlation using the SPSS statistical software package (SPSS 1997). The three N levels of before and after N amendment were ordinated to establish the variation in species composition due to the N treatment. In the case of N treatment, the mean IVIs of component species in 2009 and 2010 were used. The four functional groups based on the IVIs of the component functional groups were also ordinated by NMS, and the variation in functional group composition according to year under N treatment calculated. MANOVA (Multivariate Analysis of Variance) was used to calculate the effects of year, locations, N treatment, and functional group on the diversity and biomass of the herbaceous vegetation. A paired T-test was used to determine the significance of differences in the mean values of diversity and biomass of total vegetation between different locations and N treatments. This test was also used to determine the differences in species diversity and biomass between pairs of functional groups at each location and N level. The mean species diversity and biomass of each functional group in the two study years, as well as total vegetation, were linearly regressed with mean soil moisture.

## RESULTS

The entire 135 1-m<sup>2</sup> plots (before and after N treatment) yielded a total of 56 species distributed in 23 families. Over the two years of the N treatment study, a total of 47 species in 23 families was recorded. The family Poaceae had the maximum number of species (6), and 12 families were characterized by a single

TABLE 2. Total and unique species (present only in the respective treatment) numbers for herbaceous vegetation exhibited by each of the three N treatment levels in years 2009 and 2010 and their percentage change over two study periods. The negative signs represent the corresponding loss in total number of species and unique species.

| N levels                                   | Year 2009 |        | Year 2010 |        | % change |        |
|--|-----------|--------|-----------|--------|----------|--------|
|  | Total     | Unique | Total     | Unique | Total    | Unique |
| 0 kg N ha <sup>-1</sup> yr <sup>-1</sup>   | 30        | 0      | 29        | 0      | -3       | 0      |
| 60 kg N ha <sup>-1</sup> yr <sup>-1</sup>  | 39        | 5      | 36        | 3      | -8       | -40    |
| 120 kg N ha <sup>-1</sup> yr <sup>-1</sup> | 33        | 2      | 30        | 1      | -10      | -50    |
| Overall                                    | 45        | 10     | 37        | 2      | -18      | -80    |

species each. In addition, the year 2009 enumeration had 45 and the year 2010 had 37 species (Appendix Table 1). In both the years, the numbers of species were maximum in the 60 kg N ha<sup>-1</sup> yr<sup>-1</sup> plots and minimum in the control plots. The percentage disappearance in total, as well as of unique (present only in the respective treatment) species, between 2009 and 2010 were maximum in the 120 kg N ha<sup>-1</sup> yr<sup>-1</sup> plots. Overall, 80% of unique species disappeared between 2009 and 2010 (Table 2).

The segregation of the 135 plots into five locations is shown in Fig. 1a. The NMS axis 1 accounted for 39% of variation in species composition and NMS axis 2 accounted for 22% of variation. Soil moisture was positively related with NMS axis 1 ( $r^2 = 0.65$ ,  $p = \leq 0.05$ ) and negatively with NMS axis 2 ( $r^2 = 0.46$ ,  $p = \leq 0.05$ ). Fig. 1b shows the NMS ordination diagram of before and after N treatment of plots. In the ordination diagram, post-N treatment plots occupy a different position than the pre-treatment plots. Further, among the N-treated plots, each N treatment level had a distinct location in the ordination space, suggesting that N amendment caused an alteration in species composition. The NMS ordinations of the functional groups based on the IVIs of the component functional group of the years 2009 and 2010 are presented in Figure 1c (where the functional groups are distinguished from each other for 2009 and 2010) suggesting compositional change in the functional group of herbaceous vegetation due to N amendment over the two years. Thus the study suggests that both N amendment and soil moisture caused changes in species as well as functional group composition in this study.

Analysis of variance suggested that year, location, and N treatment individually, as well as in combination, caused variations in species diversity and biomass (Table 3). The mean values of herbaceous species diversity and biomass of the functional groups for the years 2009 and 2010 at the five locations (Table 4) and three N treatment levels (Table 5) indicated that in both sampling years total species diversity and biomass were maximum at Location 5 and minimum at Location 1. Species diversity was greater in 60 kg N ha<sup>-1</sup> yr<sup>-1</sup> plots compared with both control and 120 kg N ha<sup>-1</sup> yr<sup>-1</sup> plots in both years, yielding a peak of diversity at a moderate level of N, while biomass further increased with increased dosage of N (Table 5). Interestingly, in 2010 there were lower species diversity and higher biomass than in 2009.

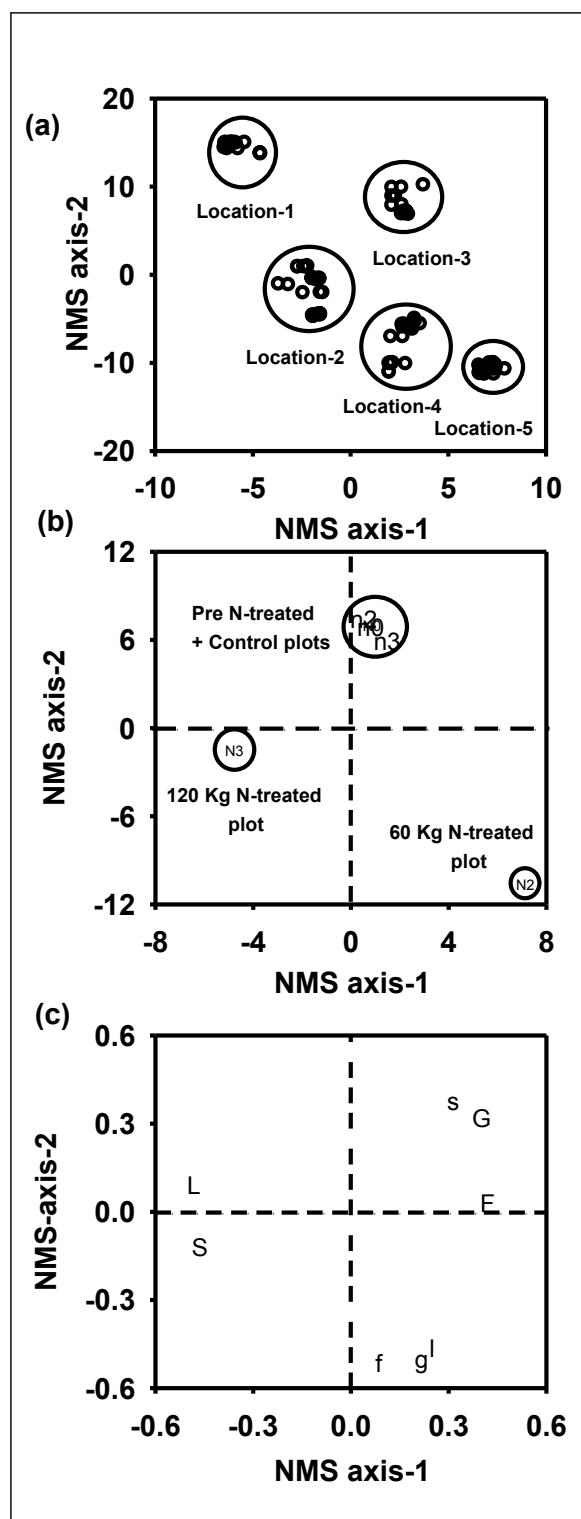


FIG. 1. NMS ordination at five locations (a); three N treatment levels (b); and four functional groups in a dry tropical grassland (c). In Figure 1c the upper-case letters represent the functional groups in 2009, and lower-case letters the functional groups in 2010. The letters in the diagram are the initials of the functional groups: forbs, grasses, legumes, and sedges.



TABLE 3. Summary of MANOVA indicating the effects of year, site, and N treatments and their combinations on the species diversity and biomass of different functional groups and total herbaceous vegetation in experimental plots located in the campus of Banaras Hindu University, India. \* =  $\leq 0.05$ , \*\* =  $\leq 0.01$ , \*\*\* =  $\leq 0.001$ , NS = Not significant.

| Parameters    | Year (A)           | Site (B)           | Nitrogen (C)       | A × B              | A × C              | B × C              | A × B × C          | Error |
|---------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------|
| Shannon index |                    |                    |                    |                    |                    |                    |                    |       |
| <i>Df</i>     | 1                  | 4                  | 2                  | 4                  | 2                  | 8                  | 8                  | 240   |
| Forbs         | 7.47*              | 13.34***           | 0.36 <sup>NS</sup> | 1.99*              | 0.28 <sup>NS</sup> | 2.07 <sup>NS</sup> | 1.42 <sup>NS</sup> |       |
| Grasses       | 5.78*              | 94***              | 3.97*              | 9.74***            | 1.62*              | 3.64*              | 1.58*              |       |
| Legumes       | 0.03 <sup>NS</sup> | 50***              | 0.62 <sup>NS</sup> | 1.90*              | 2.31*              | 5.26**             | 3.58*              |       |
| Sedges        | 0.91 <sup>NS</sup> | 2.21 <sup>NS</sup> | 0.57 <sup>NS</sup> | 4.74*              | 1.96*              | 1.65*              | 0.79 <sup>NS</sup> |       |
| Total         | 335***             | 1220***            | 13***              | 1.19 <sup>NS</sup> | 0.01 <sup>NS</sup> | 2.27*              | 0.95 <sup>NS</sup> |       |
| Biomass       |                    |                    |                    |                    |                    |                    |                    |       |
| Forbs         | 0.04 <sup>NS</sup> | 8.61***            | 2.29*              | 0.87 <sup>NS</sup> | 0.98 <sup>NS</sup> | 0.93 <sup>NS</sup> | 0.36 <sup>NS</sup> |       |
| Grasses       | 0.01 <sup>NS</sup> | 26***              | 7.72**             | 0.88 <sup>NS</sup> | 0.23 <sup>NS</sup> | 5.92**             | 0.24 <sup>NS</sup> |       |
| Legumes       | 6.07*              | 27***              | 1.39 <sup>NS</sup> | 6.98**             | 1.97*              | 1.48 <sup>NS</sup> | 1.99*              |       |
| Sedges        | 3.32 <sup>NS</sup> | 3.29*              | 2.50*              | 3.29*              | 2.50*              | 2.40*              | 2.35*              |       |
| Total         | 40***              | 1060***            | 16***              | 0.06 <sup>NS</sup> | 0.62 <sup>NS</sup> | 2.25*              | 0.02 <sup>NS</sup> |       |

TABLE 4. Species diversity and biomass of different functional groups at five locations arranged in a gradient of soil moisture (less: Location 1 to high: Location 5) in the years 2009 and 2010. Different lower case superscripts letters within column of each location are significantly different at  $p = \leq 0.05$ , and different uppercase superscripts letters within the column are significantly different at  $p = \leq 0.05$ .

| Locations  | Functional groups | Year 2009         |                              | Year 2010         |                              |
|------------|-------------------|-------------------|------------------------------|-------------------|------------------------------|
|            |                   | Shannon index     | Biomass (g m <sup>-2</sup> ) | Shannon index     | Biomass (g m <sup>-2</sup> ) |
| Location 1 | Forbs             | 0.29 <sup>a</sup> | 36 <sup>a</sup>              | 0.11 <sup>a</sup> | 34 <sup>a</sup>              |
|            | Grasses           | 0.42 <sup>b</sup> | 58 <sup>b</sup>              | 0.53 <sup>b</sup> | 71 <sup>b</sup>              |
|            | Legumes           | 0.03 <sup>c</sup> | 8 <sup>c</sup>               | 0.02 <sup>c</sup> | 2 <sup>c</sup>               |
|            | Sedges            | 0.00 <sup>c</sup> | 0 <sup>d</sup>               | 0.00 <sup>c</sup> | 0 <sup>d</sup>               |
|            | Total             | 0.74 <sup>A</sup> | 102 <sup>A</sup>             | 0.66 <sup>A</sup> | 107 <sup>A</sup>             |
| Location 2 | Forbs             | 0.33 <sup>a</sup> | 47 <sup>a</sup>              | 0.26 <sup>a</sup> | 42 <sup>a</sup>              |
|            | Grasses           | 0.48 <sup>b</sup> | 69 <sup>b</sup>              | 0.56 <sup>b</sup> | 100 <sup>b</sup>             |
|            | Legumes           | 0.07 <sup>c</sup> | 10 <sup>c</sup>              | 0.05 <sup>c</sup> | 2 <sup>c</sup>               |
|            | Sedges            | 0.04 <sup>c</sup> | 0 <sup>d</sup>               | 0.02 <sup>c</sup> | 0 <sup>d</sup>               |
|            | Total             | 0.92 <sup>B</sup> | 126 <sup>B</sup>             | 0.87 <sup>B</sup> | 144 <sup>B</sup>             |
| Location 3 | Forbs             | 0.46 <sup>a</sup> | 74 <sup>a</sup>              | 0.33 <sup>a</sup> | 70 <sup>a</sup>              |
|            | Grasses           | 0.72 <sup>b</sup> | 84 <sup>a</sup>              | 0.79 <sup>b</sup> | 142 <sup>b</sup>             |
|            | Legumes           | 0.12 <sup>c</sup> | 120 <sup>b</sup>             | 0.05 <sup>c</sup> | 40 <sup>c</sup>              |
|            | Sedges            | 0.04 <sup>c</sup> | 0 <sup>c</sup>               | 0.02 <sup>c</sup> | 0 <sup>d</sup>               |
|            | Total             | 1.34 <sup>C</sup> | 170 <sup>C</sup>             | 1.19 <sup>C</sup> | 214 <sup>C</sup>             |
| Location 4 | Forbs             | 0.58 <sup>a</sup> | 90 <sup>a</sup>              | 0.39 <sup>a</sup> | 88 <sup>a</sup>              |
|            | Grasses           | 0.88 <sup>b</sup> | 100 <sup>b</sup>             | 0.92 <sup>b</sup> | 147 <sup>b</sup>             |
|            | Legumes           | 0.19 <sup>c</sup> | 35 <sup>c</sup>              | 0.12 <sup>c</sup> | 18 <sup>c</sup>              |
|            | Sedges            | 0.07 <sup>c</sup> | 1 <sup>d</sup>               | 0.03 <sup>c</sup> | 5 <sup>d</sup>               |
|            | Total             | 1.72 <sup>D</sup> | 226 <sup>D</sup>             | 1.46 <sup>D</sup> | 258 <sup>D</sup>             |
| Location 5 | Forbs             | 0.67 <sup>a</sup> | 106 <sup>a</sup>             | 0.49 <sup>a</sup> | 99 <sup>a</sup>              |
|            | Grasses           | 1.13 <sup>b</sup> | 120 <sup>b</sup>             | 1.25 <sup>b</sup> | 152 <sup>b</sup>             |
|            | Legumes           | 0.27 <sup>c</sup> | 41 <sup>c</sup>              | 0.14 <sup>c</sup> | 19 <sup>c</sup>              |
|            | Sedges            | 0.09 <sup>c</sup> | 1 <sup>d</sup>               | 0.05 <sup>c</sup> | 6 <sup>d</sup>               |
|            | Total             | 2.16 <sup>E</sup> | 268 <sup>E</sup>             | 1.93 <sup>E</sup> | 276 <sup>E</sup>             |

TABLE 5. Species diversity and biomass of different functional groups distributed in three N treatment levels in 2009 and 2010. Different lower case superscripts letters within column of each location are significantly different at  $p = \leq 0.05$ , and different uppercase superscripts letters within the column are significantly different at  $p = \leq 0.05$ .

| N-levels                                   | Functional groups | Year 2009         |                              | Year 2010         |                              |
|--|-------------------|-------------------|------------------------------|-------------------|------------------------------|
|  |                   | Shannon index     | Biomass (g m <sup>-2</sup> ) | Shannon index     | Biomass (g m <sup>-2</sup> ) |
| 0 kg N ha <sup>-1</sup> yr <sup>-1</sup>   | Forbs             | 1.46 <sup>a</sup> | 112 <sup>a</sup>             | 1.43 <sup>a</sup> | 107 <sup>a</sup>             |
|  | Grasses           | 1.49 <sup>a</sup> | 117 <sup>a</sup>             | 1.47 <sup>a</sup> | 118 <sup>a</sup>             |
|  | Legumes           | 0.42 <sup>b</sup> | 37 <sup>b</sup>              | 0.38 <sup>b</sup> | 37 <sup>b</sup>              |
|  | Sedges            | 0.03 <sup>c</sup> | 1 <sup>c</sup>               | 0.10 <sup>c</sup> | 3 <sup>c</sup>               |
|  | Total             | 3.40 <sup>A</sup> | 267 <sup>A</sup>             | 3.38 <sup>A</sup> | 265 <sup>A</sup>             |
| 60 kg N ha <sup>-1</sup> yr <sup>-1</sup>  | Forbs             | 1.88 <sup>a</sup> | 125 <sup>a</sup>             | 1.65 <sup>a</sup> | 118 <sup>a</sup>             |
|  | Grasses           | 2.11 <sup>b</sup> | 140 <sup>b</sup>             | 2.24 <sup>b</sup> | 202 <sup>b</sup>             |
|  | Legumes           | 0.12 <sup>c</sup> | 35 <sup>c</sup>              | 0.06 <sup>c</sup> | 8 <sup>c</sup>               |
|  | Sedges            | 0.18 <sup>c</sup> | 1 <sup>d</sup>               | 0.09 <sup>d</sup> | 4 <sup>c</sup>               |
|  | Total             | 4.43 <sup>B</sup> | 302 <sup>B</sup>             | 4.17 <sup>B</sup> | 333 <sup>B</sup>             |
| 120 kg N ha <sup>-1</sup> yr <sup>-1</sup> | Forbs             | 1.61 <sup>a</sup> | 115 <sup>a</sup>             | 1.48 <sup>a</sup> | 108 <sup>a</sup>             |
|  | Grasses           | 1.78 <sup>b</sup> | 173 <sup>b</sup>             | 1.78 <sup>b</sup> | 241 <sup>b</sup>             |
|  | Legumes           | 0.08 <sup>c</sup> | 34 <sup>c</sup>              | 0.04 <sup>c</sup> | 0 <sup>c</sup>               |
|  | Sedges            | 0.11 <sup>c</sup> | 1 <sup>d</sup>               | 0.13 <sup>c</sup> | 4 <sup>c</sup>               |
|  | Total             | 3.58 <sup>A</sup> | 323 <sup>B</sup>             | 3.43 <sup>A</sup> | 353 <sup>B</sup>             |

The study revealed that the functional groups of the studied vegetation showed significant differences in diversity ( $F_{3, 960} = 172$ ,  $P = \leq 0.05$ ) and biomass ( $F_{3, 960} = 63$ ,  $P = \leq 0.05$ ). Therefore it is essential to investigate the effects of year, location, N and their interactions on the diversity and biomass of individual functional groups, as well as the patterns of these variables for each functional group with respect to year, location and N, because each functional group has a specific role with respect to multiple environmental gradients. The results showed that among the functional groups, the grasses had higher species diversity and biomass in both years at all locations (Table 4) and in each N treatment class (Table 5). On the other hand, the diversity and biomass of forbs and legumes were less in 2010 than in 2009 at all the locations in each N treatment class (Tables 4, 5). These parameters for legumes declined due to N amendment, but diversity of grasses and forbs produced a humped pattern in relation to N treatment. The biomass of grasses and forbs increased due to N treatment, but this increase was less consistent in forbs than in grasses (Table 5).

The linear and positively significant relationships during the study period between average species diversity and soil moisture (Fig. 2a), and between average biomass and soil moisture (Fig. 2b), for each functional group and also for the pooled data indicated that soil moisture is essential for the rise in species diversity and biomass of the herbaceous vegetation. The relationship between herbaceous productivity (g m<sup>-2</sup> yr<sup>-1</sup>), and temporal diversity is presented in Fig. 2c. To identify the key functional group responsible for the determination of existing ecosystem functions, the data of individual functional groups were deleted separately from the pooled data. The results indicated that independent removal of forbs, grasses, and sedges did not reveal a significant change in the existing relationship. The removal of the functional group legumes resulted in a statistically insignificant relationship between diversity and productivity ( $r^2 = 0.02$ ,  $p = 0.07$ ). Thus these relationships suggested that if the current trend of N deposition under varying environmental conditions continues in the near future, ecosystem services will be determined by legumes and the re-

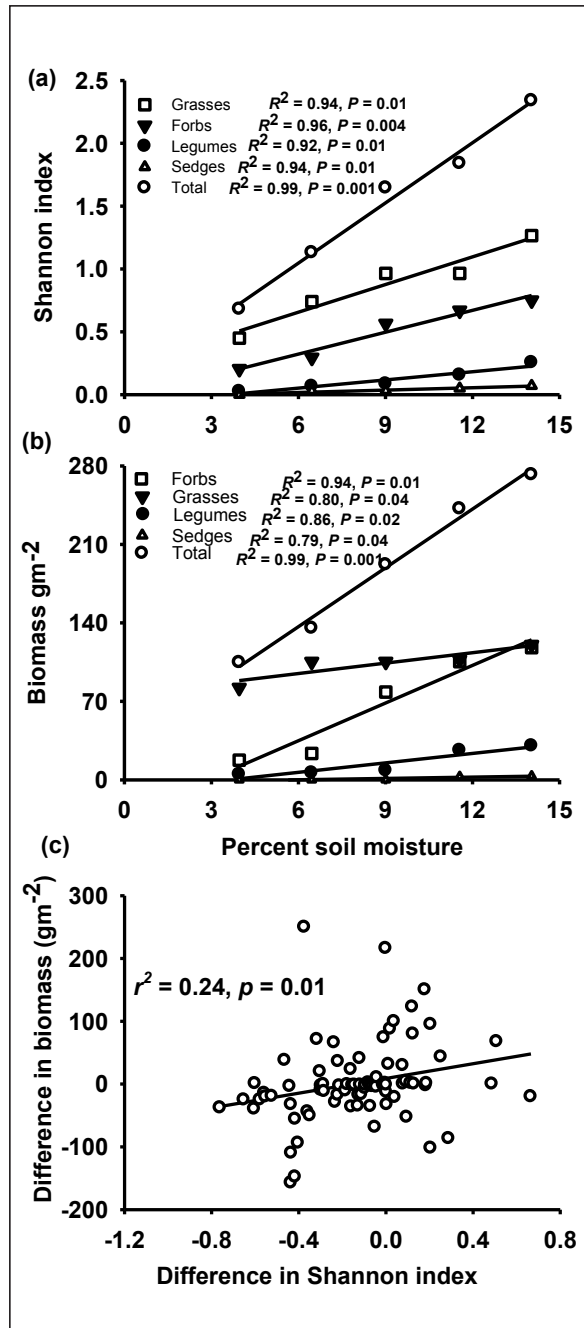


FIG. 2. Linear relationships between soil moisture and Shannon index for total as well as each functional group (a); between soil moisture and biomass of total as well as individual functional group (b); and between the total herbaceous productivity  $g\ m^{-2}\ yr^{-1}$  (difference in biomass between 2009 and 2010) and temporal diversity (difference in Shannon index) (c).

removal of this particular functional group from the system could disrupt the functioning of the ecosystem.

## DISCUSSION

In the present study, species diversity and biomass of total herbaceous vegetation, and of forbs, grasses and legumes functional groups, varied owing to location, which could be due to variability in soil moisture as suggested by positive and linear relationships between species diversity and biomass and soil moisture. Our previous study also supported the idea that greater herbaceous diversity was due to increased soil moisture (Sagar & Verma 2010). The availability of soil moisture determines the structure and functioning of certain ecosystems and supports high species diversity and biomass (Liste & White 2008) by regulating soil temperature, the metabolic activity of soil microbes (Anonymous 2011), soil N-mineralization (Bernhard-Reversat 1988, Mazzarino *et al.* 1998, Chen *et al.* 2005), and by serving as a solvent to dissolve the salts that can carry nutrients for the growth and survival of plants (Anonymous 2011).

Sala *et al.* (1992) have argued that different functional groups present in the shortgrass steppe exploited water from different soil layers according to their rooting characteristics. Succulents, having the shallowest root system, exploited the uppermost soil layers, forbs and shrubs the deepest soil layers, and the grasses the intermediate soil layers. Since grasses have a higher nutrient (particularly nitrate) acquisition ability (Berendse & Aerts 1987) than forbs due to differences in root length and specific root length (Leonard *et al.* 2008), perennial grasses are therefore the dominant species at dry sites due to well aerated root systems that allow them to cope with drought and low N availability (Pan *et al.* 2011).

Inorganic N can be available to plants in an oxidized form in nitrates  $NO_3^-$  or in the reduced form as ammonium  $NH_4^+$ . Well aerated agricultural or ruderal soils are generally considered rich in  $NO_3^-$  N and poor in  $NH_4^+$  N. Nitrification can proceed at high rates in agricultural, perturbed, or early successional soils (Likens *et al.* 1969). For this reason, late successional species preferentially utilize ammonium as their inorganic N source, whereas species indigenous to agricultural, perturbed, or early succession are believed to prefer nitrates (Stewart *et al.* 1990). Thus the changes in species composition and diversity in this study due to the differential soil N utilization capacity of species could not be ignored.

Our study sites exhibited a characteristic hump-shaped trend for species diversity in relation to N



application. This emphasizes that species diversity is low at low N levels, increases to a maximum at moderate levels, and decreases gradually at high N levels. This trend can be interpreted as showing that only a few species are capable of thriving in a situation of N deficit (particularly legumes). As N increases, more species – rare as well as nitrophilic – can survive and as a result species diversity increases. At adequately high N levels a few nitrophilic and invasive species (*Ageratum conyzoides*, *Hyptis suaveolens*, *Physalis minima*, *Urena lobata*) and grasses (*Digitaria sanguinalis*, *Eragrostis tenella*, *Oplismenus burmannii*, *Panicum psilopodium*) become dominant, eliminating rare (*Anisomeles ovate*, *Commelina benghalensis*, *Oldenlandia ambellata*), perennial forb (*Euphorbia hirta*, *Scoparia dulcis*), and leguminous (*Alysicarpus vaginalis*, *Atylosia marmorata*, *Clitoria ternatea*, *Rhynchosia minima*) species. Thus species diversity decreased with increased doses of N amendment. Similar trends have also been observed in other studies (Tilman 1982, Bobbink *et al.* 1998). In this study, 60 kg N ha<sup>-1</sup> yr<sup>-1</sup> was sufficient to permit the coexistence of more species despite the fact that at suitably high levels of N the survival of many species could be vulnerable (Bracken & Nielsen 2004).

Plant species diversity has been 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, and so diversity declines (Pausas & Austin 2001). N deposition will improve conditions for the growth and survival of nitrophilic species (Eckert 2009). Plant species require limited amounts of N, but due to increased N in the soil some species will proliferate and dominate over rarer species with low N requirements (Goulding *et al.* 1998). Since N is a limiting nutrient for the growth and survival of plants in many terrestrial ecosystems, an enhanced amount of N beyond a critical threshold is responsible for a reduction in species diversity by favoring invasive and nitrophilic species (Pan *et al.* 2010). At intermediate N levels, niche differentiation, facilitation, and frequency-dependent growth all increase species diversity by enhancing the population growth rate of rare, invasive, or nitrophilic species (Lambers *et al.* 2004). In this study, both species diversity and biomass of forbs declined with increasing N amendment in contrast to the grasses, as also reported by Song *et al.* (2011) for a temperate steppe ecosystem of Mongolia and Stevens *et al.* (2004, 2006) in the

United Kingdom. Generally the forbs in the latter study were prostrate or of rosette form and hence not adapted to compete for light, so they may become light-limited due to shading by taller nitrophilous plants with more vigorous growth rates (Stevens *et al.* 2006). According to Pan *et al.* (2011), the random death of small individuals of all species after N enrichment leads to community-level thinning and the extinction of rare species, because competition for light could lead to the loss of short-stature plant species after N enrichment. As in our study, a decline in forb and legume diversity followed by a fertilization-driven increase in annual grass production within two years was also reported in Californian (Zavaleta *et al.* 2003) and other early successional temperate grasslands (Foster & Gross 1998). Thus decline in overall species diversity following N amendment could be due to the decline in diversity of forbs and legumes (Zavaleta *et al.* 2003) or rare and short-stature plants (Stevens *et al.* 2004). The increased total biomass with increased N dose observed here and noted in many similar studies (e.g. Vitousek *et al.* 1997, Reich *et al.* 2006, Li *et al.* 2010, De Schrijver *et al.* 2011) could be due to the increased biomass of only a few functional groups, especially grasses and invasive species. A possible explanation for such a phenomenon might be that grasses, as well as invasive species, have greater photosynthetic efficiency in the use of N than forbs and legumes, resulting in faster growth than in forbs and other native species, as available N in the soil increases with increased N amendment (Peppler-Lisbach & Petersen 2001, Rao *et al.* 2009). In one study, Dukes (2001) also reported annual grasses having a greater biomass than forbs and perennial grass seedlings. In addition, a majority of studies suggest that increased N fertilization have an effect, directly or indirectly, on the population of decomposers that determines the availability of N to nitrophilic species for their protein and biomass synthesis in adequate moisture condition (Rao *et al.* 2009).

The diversity-disease hypothesis (Elton 1958) also suggests that a decline in species diversity augments the severity of diseases caused by specialist pathogens, because the proliferation of both diversity and diseases is related to host species abundance. The reduced diversity allows remaining species to increase in abundance, which then facilitates the spreading of pathogens by increasing their chances of reaching a specific host (Mitchell *et al.* 2003).

Increased foliar N concentration in C<sub>3</sub> and C<sub>4</sub> grasses due to increased atmospheric N deposition causes foliar pathogen infections, whose reproduction and growth in grassland ecosystems poses a serious risk through changes in plant community composition that reduce overall species diversity (Mitchell *et al.* 2003, Stevens *et al.* 2006, De Schrijver *et al.* 2011). Moreover, at very high levels of N in the soil system, ammonium is toxic to plants and even at lower levels negative effects on herbaceous vegetation have been widely reported (Stevens *et al.* 2006). Such effects may prevail because of soil acidification and eutrophication, both of which have the potential to modify soil microbial processes, resource availability, and the performance of both individual species and functional groups (Stevens *et al.* 2004, 2006). Such variation in functional group composition has also been revealed by NMS ordination in the present study.

The occurrence of linear and positive diversity-productivity relationships in our study may follow the diversity-stability hypothesis of Elton (1958), suggests that plots having greater species diversity could have greater herbage biomass and productivity for the proper functioning of the ecosystem (Craine *et al.* 2003), though this argument has been opposed by many ecologists (see McCann 2000 for greater detail). Among them, one school of thought suggested that the productivity of an ecosystem and its response to N deposition depends on the diversity and composition of the plant functional groups in contrast to species diversity (Reich *et al.* 2001, Tilman *et al.* 2001). To validate this argument, the data of the legumes functional group has been deleted from the pooled data. This manipulation resulted in a diversity-productivity relationship that was not statistically significant. Thus the present study also favors the opinions of Reich *et al.* (2001) and Tilman *et al.* (2001). Therefore as a consequence, the maintenance of a legumes functional group could be important for the proper functioning of a dry tropical environment.

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APPENDIX TABLE 1. List of herbaceous species with their families, life forms and life span during study period, in the campus of Banaras Hindu University, India. 1 = Species present before initiation of the experiment, 2 = Species present only in year 2009, 3 = Species present in both years, 4 = Species present only in year 2010. F = Forbs, G = Grasses, L = Legumes, S = Sedges.

| Species  | Family           | Life forms | Life span |
|--|------------------|------------|-----------|
| <i>Achyranthes aspera</i> L. <sup>3</sup>                    | Amaranthaceae    | F          | Perennial |
| <i>Ageratum conyzoides</i> L. <sup>3</sup>                   | Asteraceae       | F          | Annual    |
| <i>Alysicarpus vaginalis</i> L. <sup>1,2</sup>               | Fabaceae         | L          | Perennial |
| <i>Amaranthus spinosus</i> L. <sup>1</sup>                   | Amaranthaceae    | H          | Perennial |
| <i>Ammannia baccifera</i> L. <sup>1,3</sup>                  | Lythraceae       | F          | Annual    |
| <i>Anagallis arvensis</i> L. <sup>1,2</sup>                  | Primulaceae      | F          | Annual    |
| <i>Aneilema nudiflorum</i> R. Br. <sup>1</sup>               | Commelinaceae    | H          | Perennial |
| <i>Anisomeles ovata</i> R. Br. <sup>1,3</sup>                | Lamiaceae        | F          | Perennial |
| <i>Atylosia marmorata</i> Benth. <sup>1,2</sup>              | Fabaceae         | L          | Perennial |
| <i>Blepharis repens</i> L. <sup>1,3</sup>                    | Acanthaceae      | F          | Perennial |
| <i>Cayratia trifolia</i> L. Domin. <sup>1,3</sup>            | Vitaceae         | F          | Biennial  |
| <i>Cissampelos pareira</i> L. <sup>1,3</sup>                 | Menispermaceae   | F          | Perennial |
| <i>Clerodendrum indicum</i> L. Kuntze. <sup>1,3</sup>        | Verbinaceae      | F          | Perennial |
| <i>Clitoria ternatea</i> L. <sup>1,2</sup>                   | Fabaceae         | L          | Perennial |
| <i>Coccinia cordifolia</i> L. Cong. <sup>1,3</sup>           | Cucurbitaceae    | F          | Perennial |
| <i>Commelina nudiflora</i> L. <sup>1,3</sup>                 | Commelinaceae    | F          | Annual    |
| <i>Commelina benghalensis</i> L. <sup>1,3</sup>              | Commelinaceae    | F          | Annual    |
| <i>Corchorus olitorius</i> L. <sup>1,3</sup>                 | Tiliaceae        | F          | Annual    |
| <i>Corchorus tridens</i> L. <sup>1,3</sup>                   | Tiliaceae        | F          | Perennial |
| <i>Cyperus fuscus</i> L. <sup>1,3</sup>                      | Cyperaceae       | S          | Annual    |
| <i>Cyperus kyllingia</i> Endl. <sup>1,2</sup>                | Cyperaceae       | S          | Annual    |
| <i>Cyperus rotundus</i> L. <sup>1,3</sup>                    | Cyperaceae       | S          | Annual    |
| <i>Desmodium gangeticum</i> L. DC. <sup>1,3</sup>            | Fabaceae         | L          | Perennial |
| <i>Desmodium triflorum</i> L. <sup>1</sup>                   | Fabaceae         | L          | Perennial |
| <i>Dichanthium annulatum</i> Forssk. <sup>1,3</sup>          | Poaceae          | G          | Perennial |
| <i>Digitaria sanguinalis</i> L. <sup>4</sup>                 | Poaceae          | G          | Annual    |
| <i>Eclipta alba</i> Hask. <sup>1</sup>                       | Astraceae        | F          | Perennial |
| <i>Eragrostis tenella</i> L. <sup>4</sup>                    | Poaceae          | G          | Annual    |
| <i>Eulaliopsis binata</i> (Retz) C.E. Hubb. <sup>1,3</sup>   | Poaceae          | G          | Perennial |
| <i>Euphorbia hirta</i> L. <sup>1,2</sup>                     | Euphorbiaceae    | F          | Perennial |
| <i>Euphorbia pulcherima</i> Willd. ex Klotzsch. <sup>3</sup> | Euphorbiaceae    | F          | Perennial |
| <i>Euphorbia thymifolia</i> L. <sup>1</sup>                  | Euphorbiaceae    | H          | Perennial |
| <i>Herpestis monniera</i> L. <sup>1,2</sup>                  | Scrophulariaceae | F          | Perennial |
| <i>Heylandia latebrosa</i> DC. <sup>1</sup>                  | Fabaceae         | L          | Annual    |
| <i>Hyptis suaveolens</i> Poit. <sup>3</sup>                  | Lamiaceae        | F          | Perennial |
| <i>Ipomoea quamoclit</i> L. <sup>3</sup>                     | Convolvulaceae   | F          | Annual    |
| <i>Lindernia ciliata</i> (Colsm.) Pennell. <sup>1</sup>      | Scrophulariaceae | H          | Annual    |
| <i>Malvastrum coromandelianum</i> L. <sup>1,3</sup>          | Malvaceae        | F          | Perennial |
| <i>Nicotiana plumbaginifolia</i> Viv. <sup>1,3</sup>         | Solanaceae       | F          | Annual    |



| Species   | Family           | Life forms | Life span |
|---|------------------|------------|-----------|
| <i>Oldenlandia ambellata</i> L. <sup>1,2</sup>        | Rubiaceae        | F          | Perennial |
| <i>Oplismenus burmannii</i> Retz. <sup>1,3</sup>      | Poaceae          | G          | Annual    |
| <i>Panicum psilopodium</i> Trin. <sup>1,3</sup>       | Poaceae          | G          | Annual    |
| <i>Peperomia pellucida</i> L. <sup>1,2</sup>          | Piperaceae       | F          | Annual    |
| <i>Peristrophe bycalyculata</i> Nees. <sup>1,3</sup>  | Acanthaceae      | F          | Annual    |
| <i>Phyllanthus urinaria</i> L. <sup>1,3</sup>         | Euphorbiaceae    | F          | Annual    |
| <i>Physalis minima</i> L. <sup>1,3</sup>              | Solanaceae       | F          | Annual    |
| <i>Rhynchosia minima</i> DC. <sup>1</sup>             | Fabaceae         | L          | Annual    |
| <i>Rungia parviflora</i> Retz. <sup>1,3</sup>         | Acanthaceae      | F          | Annual    |
| <i>Salvia plebeia</i> R. Br. <sup>1,3</sup>           | Lamiaceae        | F          | Perennial |
| <i>Scoparia dulcis</i> L. <sup>1,2</sup>              | Scrophulariaceae | F          | Perennial |
| <i>Scrophularia nodosa</i> L. <sup>1,3</sup>          | Scrophulariaceae | F          | Perennial |
| <i>Sida acuta</i> Burn. F. <sup>1,3</sup>             | Malvaceae        | F          | Perennial |
| <i>Spilanthes acmella</i> Murr. <sup>1</sup>          | Astraceae        | H          | Perennial |
| <i>Triumfetta rhomboidea</i> Jacq. <sup>1,3</sup>     | Tiliaceae        | F          | Perennial |
| <i>Tylophora indica</i> Burm. F. Merr. <sup>1,3</sup> | Asclepidaceae    | F          | Perennial |
| <i>Urena lobata</i> L. <sup>1,3</sup>                 | Malvaceae        | F          | Perennial |