EFFECT OF GRAZING EXCLUSION ON SOIL PROPERTIES AND VEGETATION CHARACTERISTICS IN *PARTHENIUM HYSTEROPHORUS* L. INVADED GRASSLAND OF HETAUDA, CENTRAL NEPAL

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RECOMMENDATION

This is to certify that M.Sc. Dissertation entitled **"Effect of grazing exclusion on soil properties and vegetation characteristics in *Parthenium hysterophorus* L. invaded grassland of Hetauda, Central Nepal"** has been carried out by **Miss Prativa Paneru** under our supervision. This work has been completed by candidate's original research work based on field sampling and lab work. To the best of our knowledge this work has not been submitted to any other academic degree. We recommend this dissertation work to be accepted as a partial fulfillment for Master's Degree in Botany at Institute of Science and Technology, Tribhuvan University.

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LETTER OF APPROVAL

The M.Sc. Dissertation entitled **"Effect of grazing exclusion on soil properties and vegetation characteristics in *Parthenium hysterophorus* L. invaded grassland of Hetauda, Central Nepal"** submitted by **Miss Prativa Paneru** has been accepted for the partial fulfillment of her Master's Degree in Botany (Specialization: Ecology and Resources Management) from Tribhuvan University.

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ABSTRACT

Grasslands outside Protected Areas (PAs) in Nepal are much exploited ecosystems since these areas are surrounded by heavy settlement and grazing activity in these areas is entirely human controlled rather than being natural. Intense grazing practices without any management effort are making these grasslands likely to be invaded by IAPS and deteriorating the quality of the grasslands. Therefore this study was aimed to find out the impact of grazing exclusion in a highly grazed *Parthenium hysterophorus* invaded grassland. Three plots of 10 m × 10 m had been fenced since 2015 in the grassland to exclude grazing for research purposes. Three plots adjacent to those permanent plots were established in 2017. Effect of grazing exclusion on soil properties, relative abundance of weed species and plant species diversity of both Above Ground Vegetation (AGV) and Below Ground Vegetation (BGV) were compared between Freely Grazed (FG) and Grazing Excluded (GE) plots. The finding demonstrated that grazing exclusion of even three years showed some noticeable difference in some of the soil physico-chemical properties and vegetation characters of FG and GE plots. Soil bulk density and organic carbon were reduced while soil pH and electrical conductivity were enhanced by grazing exclusion. However, no apparent impact of livestock exclusion was observed for soil nitrogen, phosphorus and potassium. Grazing exclusion substantially altered the species composition of the grassland and enhanced the growth of much diverse plant species in AGV of GE plots. Similarly, grazing exclusion reduced relative abundance of *Parthenium hysterophorus* in germinable seed bank but it has no effect in AGV. However, since grazing exclusion showed opposite effect on species diversity of AGV and BGV no conclusion could be drawn about its effect on species diversity. Therefore more studies with longer period of grazing exclusion are required to fully understand the impact of grazing exclusion on soil properties and vegetation structure of grassland in order to use it as management practice in invaded grasslands.

Key words: grassland management, grazing, IAPS, physico-chemical properties, species diversity

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ABBREVIATIONS

AGV	Above Ground Vegetation
BGV	Below Ground Vegetation
CCA	Canonical Correspondence Analysis
DCA	Detrended Canonical Analysis
DHM	Department of Hydrology and Meteorology
BD	Bulk Density
EC	Electrical Conductivity
FAS	Ferrous Ammonium Sulfate
FG	Freely Grazed
GE	Grazing Excluded
GoN	Government of Nepal
H'	Shannon-Wiener's Index
HCl	Hydrochloric Acid
H_2SO_4	Sulfuric Acid
IAPS	Invasive Alien Plant Species
IP	Importance Percentage
KATH	National Herbarium and Plant Laboratories, Godawari
$K_2Cr_2O_7$	Potassium Dichromate
masl	meter above sea level
MoFSC	Ministry of Forest and Soil Conservation
NBSAP	National Biodiversity Strategy and Action Plans
nm	nanometers
OC	Organic Carbon
p	Level of Significance
SB	Seed Bank
SE	Standard Error
S/m	Siemens per meter
$SnCl_2$	Stannous Dichloride
sp.	species
TUCH	Tribhuvan University Cetral Herbarium, Kirtipur, Kathmandu

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1. INTRODUCTION

1.1 Background

1.1.1 Grasslands

Grasslands comprise open spaces covered with grasses, shrubs and sometimes even small trees along with different microbes, insects and animals depending on it. These ecosystems are considered as a unique type of ecosystem since they are often exposed to disturbances and the dominant plant community has more below ground biomass than above ground (Sala *et al*., 1996; Ma *et al*., 2008). Disturbances such as drought, fire and grazing are the integral components of grasslands which plays prime role in determining structure of grassland communities (Anderson 1982 as cited by Sala *et al*., 1996).

Grasslands have great ecological, economic and social importance. They are very important areas in terms of biodiversity as it hosts all types of plant species having different life history strategies i.e. annuals, perennials, herbs, grasses, sedges, shrubs as well as few tree species along with different animal species depending upon those plant species. Grasslands provide a huge annual ecosystem services of worth $ 906 billion per year globally (Costanza *et al*., 1997). They are solitary source of forage for most of herbivores. Grasslands act as sole source of forage for animal husbandry and support people for their livelihood. In addition, grasslands also have social significance as they are often used as place for gathering during festive, playgrounds and place of outdoor amusements (Koshravi *et al*., 2017).

Globally, grasslands and rangelands occur from tropical to alpine region and cover an area of 45 million square kilometer comprising 41% of the total land surface (Zhang, 2006). In Nepal, natural grasslands cover approximately 7.90% of total land and occur from tropical to alpine region (Uddin *et al*., 2014). Many of the world's ecosystems are degraded as a consequence of global environment changes and human utilization. Among all, grasslands are one of the most exploited ecosystems due to anthropogenic activities (Zhan *et al*., 2007). These ecosystems have been often unseen in terms of productive lands and thus converted to other forms of land use. Land use change occurs in a variety of forms including both change in area and changes in intensity of use (Houghton, 1994). In case of grasslands, the land use has been changed in both the forms i.e. area and

intensity. On the one hand grassland areas had been converted to agricultural and settlement areas resulting to reduction in the area of natural grasslands and on the other hand overstocking of livestock on already shrunken area of grassland has increased the intensity of use (Modernel *et al*., 2016). The intensive use of grasslands is reducing the quality as well as the carrying capacity of grasslands in present context. Continuous exposure of grasslands up to disturbance level makes them more likely to be invaded by exotic plant species globally (Davis *et al*., 2000; Seabloom *et al*., 2015). The invasion of less palatable exotic species in grasslands are deteriorating the grassland's quality (Hejda 2009; Davies, 2011; Seabloom *et al*., 2015).

1.1.2 Impact of grazing on grassland

Grazing is the most conspicuous and important feature of grasslands. Grazing by herbivores is the most common use of grasslands that presents an amalgam of different effect in the grassland ecosystem. It is considered as a natural ecological process which alters both biotic and abiotic components of the grassland ecosystem (Sala *et al*., 1996). Grazing plays an important role in shaping the community structure and ecosystem functioning of grasslands as continuous grazing is responsible for changing the appearance, productivity, and composition of grasslands.

Grazing by herbivores alters soil properties of the grasslands and modifies the habitat conditions. It affects physical (i.e. the soil structure and soil compactness), chemical (i.e. soil pH, soil electrical conductivity, nutrient contents) and biological (i.e. soil biota) condition of the soil (Mofidi *et al*., 2012; Blair *et al*., 2014). Animal treading during grazing exerts huge pressure on the top soil of the grasslands which results in increase in bulk density of the soil (Mullen *et al*., 1974). Similarly, grazing plants from a larger area and depositing the nutrients in relatively small area by defecation results in alteration of nutrient concentration of the grassland soil (Sala *et al*., 1996). Change in nutrient concentration further changes the soil pH and electrical conductivity. In this way grazing impacts the soil properties and alters the habitat condition leading to the modification of vegetation structure in grasslands.

Grazing presents enigmatic situation regarding conservation issues. On the one hand, grazing has been related to the ‘intermediate disturbance hypothesis’ which suggests that grazing at intermediate level promotes diversity of an area as it reduces dominance of few species (Conell, 1978). While on the other hand, grazing has been found to induce plant

invasion *via* reducing competition by feeding on dominant palatable plants, compacting soil by trampling and altering its nutrient contents, etc and creating micro sites for invasive alien plant species (IAPS) supporting 'fluctuating resource hypothesis' (Davis *et al*., 2000). Thus the role of herbivores in controlling plant species richness is a critical issue in the conservation and management of grassland biodiversity.

Soil seed bank, which represents a stock of regeneration potential in plant communities, is an important component of ecosystem resilience (Rice, 1989). It is responsible to determine the future vegetation composition of a plant community. Therefore, understanding of soil seed bank gives an insight into the mechanisms maintaining natural community dynamics. Grazing also affects the seed bank in grasslands. Feeding upon above ground plant parts during herbivory affects seed production of plant species directly as well as indirectly (Sternberg *et al*., 2003). Defoliation of plant leaves implies indirect effect on seed production as it affects the allocation of plant resources for reproduction due to reduction of photosynthetic surface. Whereas feeding on reproductive structures such as flowers and seeds reduces the seed amount and impacts seed production directly. However, only palatable plant species faces this negative impact of grazing. This further affects size and composition of soil seed bank. Thus grazing presents a crucial role in determining the seed bank composition of a community.

1.1.3 Plant invasion in grasslands

Plant invasion and community homogenization by IAPS has degraded the grassland quality world widely (Wick *et al*., 2016; Baggio *et al*., 2018). Although grazing is considered as a natural ecological process, overgrazing by cattle has been found to induce plant invasion in grassland communities. Studies had shown that excessive grazing had made grasslands likely to be invaded by exotic plant species globally (Davis, 2000; Brenda *et al*., 2014). Plant invasion has been considered as one of the most difficult threat to be considered for maintaining global biodiversity in present context (Powell *et al*., 2011). Although there is no evidence of global extinctions of native plants solely because of plant invasions, there is evidence that native plants have reached other thresholds along the extinction trajectory due to the impacts associated with plant invasions (Downey and Richardson, 2016). Invasion by IAPS may add to the number of species in a given area which results into initial increase in species richness of the area (Thomas and Palmer, 2015). However, soon the IAPS spreads in the invaded community in such a way that it

leads to declines in abundance of the native plant species of that area (Seabloom *et al*., 2015). Community homogenization by invasive species leads to the reduction in quality of the grasslands which further intensifies the grazing pressure on grasslands. Thus, plant invasion in grasslands has been a major challenge for the ecologists in recent context. Despite of the fact that grazing has been found to have both positive and negative impact on grasslands, overgrazing by overstocking of livestock has degraded the grassland quality by promoting plant invasions. Nevertheless grazing by livestock is also helping the local people in uplifting their economic status *via* animal husbandry. Therefore, productivity of plants and animals, grassland composition and ecosystem services are three key variables to be considered while managing the grasslands.

1.2 Hypothesis

The hypothesis of this study is:

- Grazing exclusion in grassland will lead to decline in relative abundance of herbaceous invasive plant species (IPS).

1.3 Objectives

The main objective of the study is to find out the impact of grazing exclusion on soil properties and vegetation characters in a grassland invaded by *Parthenium hysterophorus* situated at Hetauda.

The specific objectives of this study are:

- To compare the soil physico-chemical properties between Freely Grazed (FG) and Grazing Excluded (GE) plots.
- To compare species diversity and composition of plant species between FG and GE plots.
- To compare the soil seed bank of plant species between FG and GE plots.

1.4 Justification

Grasslands ecosystems are one of the most exploited ecosystems of Nepal as people are still ungrateful about the immense ecosystem services being provided by it. Most of the grasslands in Nepal are either converted to cultivation land or settlement areas. Natural

grasslands occur only inside protected areas (PAs) in the plains of Nepal (Richard *et al*., 1999). However there are still some patches of fallow lands which are being utilized for grazing as common property. The condition of these grasslands outside PAs is worst since these areas are surrounded by heavy settlement and grazing activity in these areas is human controlled rather than being natural. Since people are less interested in grassland management, these areas are facing the problem of 'the tragedy of the commons'. These grasslands are being threatened by the serious problem of overgrazing followed by plant invasion. Excessive grazing is inducing the encroachment of grasslands by IAPS. Since invasive species are less palatable in nature, increase in number of such species is reducing the carrying capacity of grasslands (Sapkota, 2007).This is further increasing grazing pressure on the grasslands and affecting even the animal husbandry practice as well. The problem is much severe in heavily settled areas mostly in tropical region. Acknowledging the possible harmful effects of IAPS in national biodiversity, the management of invasive plant species has been kept as one of the action plan in Nepal National Biodiversity Strategy and Action Plan 2014-2020 (GoN/ MoFSC, 2014). Among the different IAPS of Nepal, *Parthenium hysterophorus* is considered as a noxious weed which adversely affects the grass species (Shrestha *et al*., 2015). Invasion by *P. hysterophorus* has been shown to be highly responsible for decreasing the number of species as well as altering soil properties in invaded regions (Timsina *et al*., 2011). Although biological control and manual processes such as uprooting are used to remove this unwanted weed species, they are much laborious and can be practiced only in small areas. Numerous studies have shown that grazing exclusion in grasslands has reduced the abundance of exotic plant species (Seabloom *et al*., 2015; Nuzzo *et al*., 2017). Thus grazing exclusion could be used as an ecosystem based management effort of invaded grasslands in Nepal as well. Therefore there is a strong need of this research to be conducted.

1.5 Limitation

The limitation of this study is:

- This study addressed the exclusion of only large grazers.

2. LITERATURE REVIEW

2.1 Grasslands and Grazing Practice

According to Owen and Wiegert (1981) and Herrera and Donana (1982) the relationship between grazers and grasslands has developed over millions of years, and it is likely that grazers and grasslands ecosystems coevolved. From this it becomes clear that grazing was the main and obviously most common use of grasslands since their evolution. However, earlier, grazing was once a natural activity, and then pastoral activity that involved people moving with their herds from place to place, but now it has become a far more sedentary utilization (Alkemade, 2013). This sedentary utilization of grassland has been considered to be responsible for degradation of grassland ecosystems worldwide (Blair *et al*., 2014).

Grazing is an important process in grasslands which acts as a key factor in affecting species composition and biomass production in grassland ecosystems (McNaughton *et al*., 1988). The negative impacts of livestock grazing are often the result of misuse of the grasslands. Anthropogenic activities which involve modification of disturbance regime such as land conversions, change in grazing regimes, change in grazing intensities, etc have pushed grazing activity to detrimental level (Hobbs and Huenneke, 1992). Further, continuous use of grassland ecosystems without any management efforts has made the condition of these ecosystems much worse.

2.2 Impacts of Grazing on Grasslands

Grazing has been considered as major factor altering different soil properties in grasslands (Blair *et al*., 2014). One potentially degrading effect on soil condition is that of soil compaction by animal treading (Mullen *et al*., 1974). Since soil is a complex system of biotic and abiotic components, soil compaction affects several properties of soils that may affect rangeland vegetation (Mofidi *et al*., 2012). These include changes in root growth, availability and movement of air and water, and microbial activity. Therefore, grazing regime and intensity has been found to alter habitat condition and nutrient content of the grasslands.

Grazers promotes heterogeneity in grasslands by selectively consuming some species while leaving others, through trampling, soil compaction, soil tunneling, and

redistribution of nutrients (Hobbs and Huenneke, 1992). Similarly grazing and browsing, nutrient harvest over a large area accompanied by deposition in small areas due to foraging, defecation and urination by grazers, etc causes disturbances in grassland community (Sala *et al*., 1999).

According to ' fluctuating resource hypothesis' by Davis *et al*. (2000), fluctuation in resource availability along with any kind of disturbances in any community makes it highly susceptible to invasion by exotic plant species. The reduction in competition, creation of open canopy and alteration of soil nutrient concentrations in grassland due to grazing activity often makes grasslands easily invaded by IAPS. Plant invasion in grasslands is decreasing the capacity of grassland to feed animals and most of the grasslands are being overgrazed (Zhang, 2006).

Overgrazing followed by plant invasion leads to complete deterioration of the quality of grasslands. Davies (2011) had reported decline in plant community diversity and native plant abundance with an increasing abundance of an exotic annual grass in a shrub-bunch grass plant community. The study had also demonstrated a negative correlation between the exotic grass density and plant species diversity and species richness in the study.

The problem of overgrazing followed by plant invasion is also common in grasslands of Nepal. Sapkota (2007) has reported that invasion of grassland by *Mikania micrantha* had deteriorated its quality in Chitwan National Park. Invasion of fallow land particularly in tropical and sub-tropical region is a recent threat for Nepal (Timsina *et al*., 2011). In Shuklaphanta National Park, invasion by woody plant species and poor management practices had detrimental effect on the grassland (Bhattarai, 2012). One of the IAPS rapidly spreading in grasslands of Nepal with detrimental effects is *Parthenium hysterophorus* (Shrestha *et al*., 2015).

2.3 Restoration of Degraded Grasslands *via* Grazing Exclusion

Grassland degradation is the main challenge for conservationists and grassland managers in this century globally (Zhang, 2006; Koshravi *et al*., 2017; Baggio *et al*., 2018). Biodiversity in rangelands is decreasing, due to intense utilization of these areas for livestock production (Alkemade *et al*., 2013). Thus an approach to mitigate these impacts is to conduct a restoration program following the cessation of the grazing activity.

Numerous studies with ban of grazing has been carried out in different degraded grasslands around the world to improve the condition of those areas (Wu *et al*., 2010; Koshravi *et al*., 2017; Baggio *et al*., 2018). However the effect of grazing exclusion has been found to be different in different grassland ecosystems. Most of the researches have shown that grazing exclusion has been useful in improving the condition of degraded grassland by reducing abundance of exotic unpalatable species and increasing native plant cover (Seabloom *et al*., 2015; Nuzzo *et al*., 2017) and improving soil conditions (Su *et al*., 2005; Bi *et al*., 2018). Grazing exclusions have impact on soil physical and chemical properties, species diversity, abundance of invasive species and soil seed bank size and composition.

2.3.1 Impact of grazing exclusion on soil properties

Grazing exclusion has always been found to improve soil condition by reducing the soil bulk density (Mullen *et al*., 1974; Yong-Zhong *et al*., 2005; Wu *et al*., 2010; Medina-Roldan *et al*., 2012 and Li *et al*., 2014). Cessation of the animal treading and trampling activities in grazing excluded areas has been reported to be responsible for decline in bulk density in those areas.

Soil pH and EC are the measure of total soil nutrient contents. Grazing exclusions have contrasting result in regard of total soil nutrient content. Some studies have shown increase in total nutrient content due to grazing exclusion (Wu *et al*., 2010; Koshravi *et al*., 2017) whereas others reported slower nutrient cycling and thus decline in total nutrient content of the soil due to grazing exclusion (Bol *et al*., 2000). Thus areas where nutrient content of soil was enriched by grazing exclusion, soil pH and EC has been lowered by grazing exclusion (Haynes and Williams, 1992; Lu *et al*., 2015) while in the areas where grazing exclusion lead to decline in nutrient content soil EC and pH has been increased (Wu *et al*., 2010; Li *et al*., 2014).

Similarly, grazing plants from a larger area and depositing the nutrients in relatively small area by defecation results in alteration of nutrient concentration of the grasslands (Sala *et al*., 1996). Bol *et al*. (2000) in a study carried out in a temperate grassland found that grazing exclusion lead to decline in total nutrient content of grasslands. The author found that in absence of cattle dung and urine which acts as stimulant for microbial activity, soil nutrient cycling becomes slower and thus the total nutrient content of grazing excluded

areas gets declined. Similarly McNaughton *et al*. (1988) pointed that litter accumulation in large amount due to absence of herbivory in grazing excluded areas can reduce microbial activity and thus reduce the nutrient content of the grazing excluded regions.

However, another study on effect of continuous grazing and livestock exclusion in a degraded sandy grassland has suggested that excluding grazing livestock on the desertified sandy grassland in erosion prone region has a great potential to restore soil fertility, sequester soil carbon and improve biological activity (Su *et al*., 2005). Similarly, Wu *et al*. (2010), Zhu *et al*. (2016) and Koshravi *et al*. (2017) observed that soil organic carbon increased when grazing was excluded and suggested that grazing exclusion can be used as soil restoration method for improving the quality of degraded grasslands.

2.3.2 Impact of grazing exclusion on AGV traits

Grazing exclusion can reverse the detrimental effects of grazing on plant communities in grasslands. It has been found to increase the abundance of native palatable species and improve the quality of grasslands (Kimball and Schiffman, 2003; Seabloom *et al*., 2015; Nuzzo *et al*., 2017).

Li *et al*. (2014) had suggested that the long term fencing on alkaline degraded grassland ecosystems can restore the vegetation. The study also suggested that change in grazing regime i.e. few years of grazing followed by some years of resting can prevent the further degradation of the degraded grasslands.

Exotic species are more likely to invade grassland ecosystems globally and the addition of nutrients results in increase in the cover and richness of exotic species while it decrease cover and richness of native species (Seabloom *et al*., 2015). However, herbivore fencing can lead to increase in cover of native species.

Species richness and diversity is maintained by selective mortality at some equilibrium level while random mortality prevents the establishment of community equilibrium by preventing the dominance of one superior competitor and the exclusion of other species (Hobbs and Huenneke, 1992).

Impact of grazing exclusion on species richness and species diversity vary with locations. Proulx and Mazumder (1998) had reported that plant species richness decreased with high grazing in nutrient-poor ecosystems, while it increased with high grazing in nutrient rich-

ecosystems. According to Lunt *et al*. (2007) has reported that effect of grazing and its exclusion is determined by grazing history, grazer's size and site productivity.

Some filed experiments in grassland communities had shown that grazing exclusion reduce plant diversity (Belsky, 1992; Baggio *et al*., 2018) however some other studies showed just reverse effect (McCauley and Briand, 1979; Bi *et al*., 2018). Therefore, the impact of grazing exclusion on plant species diversity has been found to be different according to different type of grassland ecosystem. This difference might be due to the different factors including ages of grazing exclusion period (Satkamp *et al*., 2017), environmental condition such as climate, precipitation, etc (Bat-Oyun, 2016), vegetation type (Holecheck *et al*., 2018) and the grazing history (Wu *et al*., 2014) in the study area.

In studies where grassland is invaded by IAPS, grazing exclusion has found to reduce the species diversity (Baggio *et al*., 2018). In invaded communities, cessation of grazing leads to enrichment in abundance of native competitive plant species (Seabloom *et al*., 2015) and increases the competition among the plant species of the grazing excluded area. This resists invasive plant species to colonize the habitat (Davies *et al*., 2011) and thus its immediate result seems to decline the species diversity of the area.

2.3.3 Impact of grazing exclusion on soil seed bank

Mixed results have been observed in different studies regarding impact of grazing exclusion on soil seed bank. Grazing exclusion has been found to change the species composition and size of soil seed banks in most of the studies while some other studies has shown either neutral or negative effect (Osterheld and Sala, 1990; Meissner and Facelli, 1999; Chaidefteftau *et al*., 2008).

The cessation of grazing releases the plant species from disturbance and predation which consequently improves the soil seed bank size and alters composition. Osterheld and Sala (1990) reported significant decline in abundance of an invasive species *Leontodon taraxacoides* by grazing exclusion for seven years. However, Meissner and Facelli (1999) reported that there was no any effect of grazing exclusion in abundance of invasive species.

Ma *et al*. (2015) found a significant increase of species richness in soil seed bank of an area subjected to grazing exclusion for ten years. Likewise, Chaideftau *et al*. (2008) also

reported higher species diversity in soil seed bank of non-grazed deciduous Oak forest. Similarly, Jutila *et al*. (1998) and Benjamin and Sanderson (2000) had also demonstrated comparatively higher species diversity in ungrazed sites than in grazed sites. However, Navie *et al*. (1996) found that the peak species diversity of seed banks was at a high level of stocking intensity.

Therefore, grasslands has been degraded due to overgrazing and most of the field studies have shown that fencing on grasslands had increased the cover of native plant species and improved the condition of degraded grasslands (Kimball and Schiffman, 2003; Seabloom *et al*., 2015; Nuzzo *et al*., 2017). This has evolved the idea of using enclosures on grassland for some years as management practice of grasslands. However no any research work has been carried out in larger scale to document the impact of grazing exclusion on grasslands till now. Thus there is a strong need of this research work to be carried on to address the effect of grazing exclusion on grasslands of Nepal so that it could be used as a grassland management practice.

3. MATERIALS AND METHODS

3.1 Study Area

3.1.1 Geographic location

The study was carried out in a grass land of Hetauda municipality located in an Inner tarai valley of Makwanpur district in the southern central Nepal (Figure 1). The study area (27° 24' N, 85° 04' E, elevation: 475m) is surrounded by Mahabharat range on its northern side and Siwalik hills to the south (Acharya *et al*., 2015). The city is traversed by three major rivers: Rapti, Samari and Karra from West, North and South direction respectively.

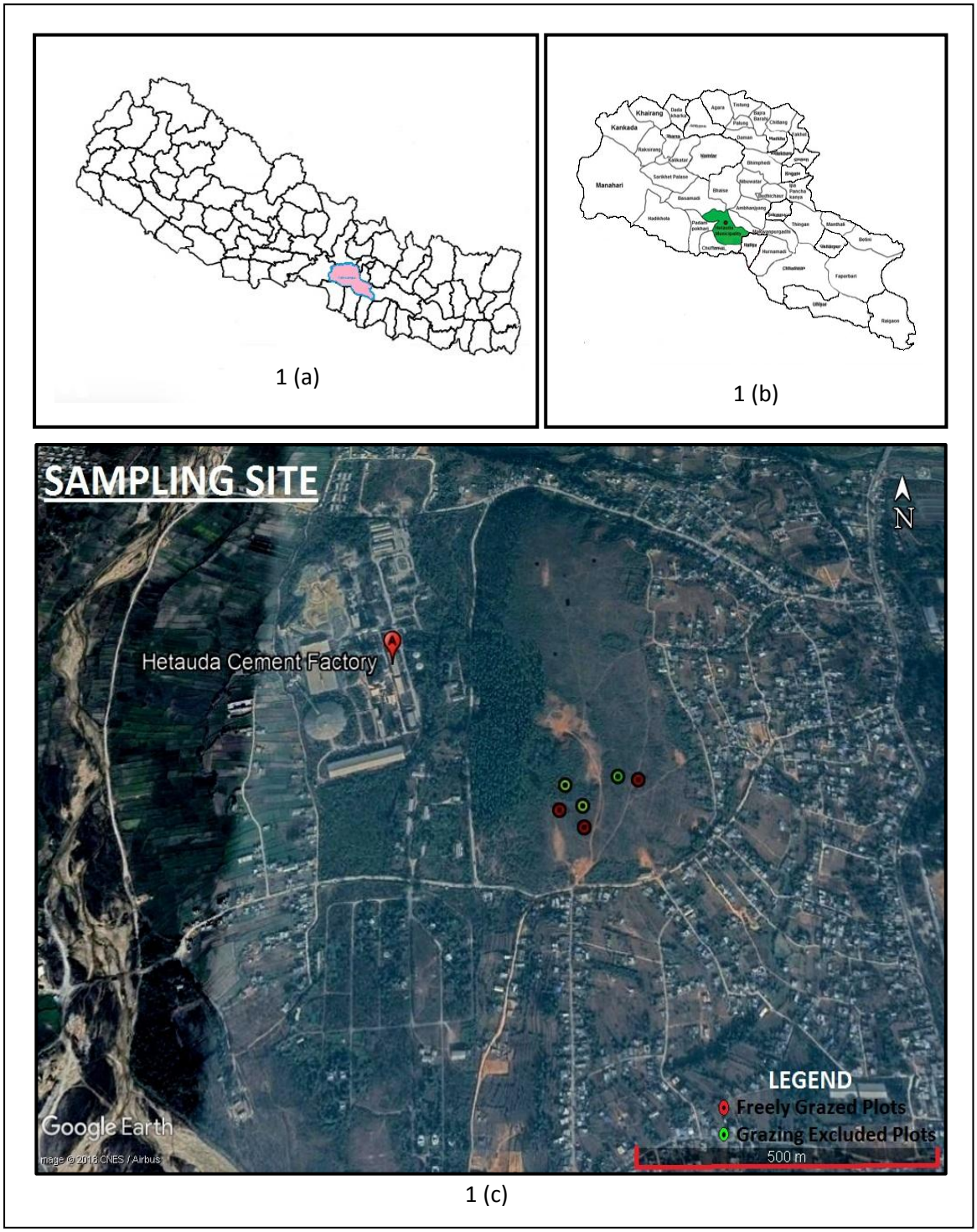


Figure 1: Map of (a) Nepal showing Makwanpur district (b) Makwanpur district showing Hetauda municipality and (c) Sampling sites in Hetauda (made by using Google Earth Pro and Arc GIS).

3.1.2 Climate and vegetation

Hetauda is characterized by tropical climate with mild and generally warm condition. The mean yearly temperature of the area ranges from 29.67° C (maximum) to 17.26° C (minimum) and makes an annual mean temperature of 23.47° C (Figure 2).The area experiences the highest temperature during April and lowest during January with an average minimum temperature of only 7.74° C. Wet season in Hetauda starts from May and it lasts till September. The average annual precipitation of the area is 2206 mm and the area receives the highest precipitation in July (602 mm).

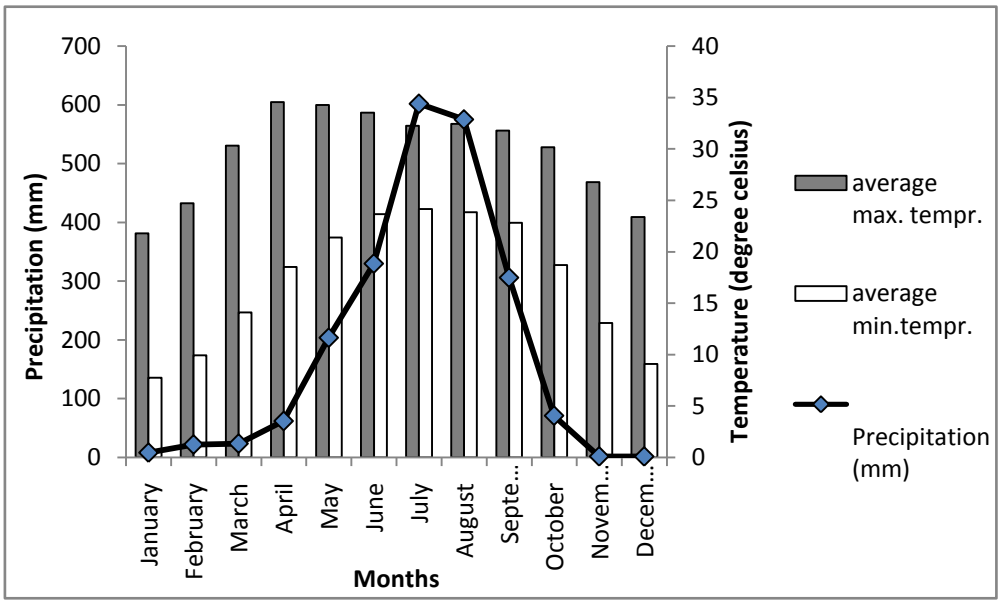


Figure 2: Ten years (2007- 2016) climatic data of Hetauda recorded at Institute of Forestry Hetauda; 474 masl which is about one kilometer north from the study site (Source: DHM).

The vegetation of this region is characterized by Sal and Riverine forest. The sal forest is comprised of *Shorea robusta* C.F. Gaertn., *Terminalia chebula* Retz., *Terminalia bellirica* (Gaertn.) Roxb., *Adina cordifolia* (Roxb.) Brandis, *Terminalia alata* Roth, *Lagerstroemia parviflora* Roxb. and *Dillenia pentagyna* Roxb.. In riverine forest, *Acacia catechu* (L.f.) Willd., *Dalbergia sissoo* Roxb. ex DC., *Mallotus nudiflorus* (L.) Kulju & Welzen, *Bombax ceiba* L. and *Albizia julibrissin* Durazz. were dominant (GoN/MFSC, 2016).

3.1.3 Sampling site

The study was conducted in a slightly raised (476 m at the base to 497 m at the top) fallow grazing land named Lamsure Dada of Ratmate, Hetauda which was owned by Hetauda Cement Factory. The land is surrounded by road and dense settlement area following the road. Untill 1974, the land was used for cultivation and settlement by the locals. In 1974, the land was purchased by Hetauda Cement Factory and left abandoned, leading to development of grassland. Since then, the land has been used by the local people of Ratmate as grassland for grazing and forage collection. The area had red soil.

The vegetation of the sampling site consisted of *Chrysopogon aciculatus* (Retz.) Trin., *Clerodendrum infortunatum* Vent., *Cynodon dactylon* (L.) Pers., *Desmodium triflorum* (L.) DC., *Euphorbia hirta* L., *Evolvulus nummularius* (L.) L., *Imperata cylindrica* (L.) P. Beauv., *Lantana camara* L., *Mimosa pudica* L., *Parthenium hysterophorus* L., *Senna tora* (L.) Roxb., *Sida cordata* (Burm.f.) Bross.Waalk., *Sida rhombifolia* L., etc. Among all, *P. hysterophorus, I. cylindrica, L. camara, M. pudica, C. aciculatus* and *C. infortunatum* were the most abundant plant species of the sampling site.

Animal husbandry has been considered the most common use of this area. The total land extends up to 1,71,239 square meters and supports about 50 large domestic cattle (i.e. Buffalos and Cows) along with 100-120 goats entirely depending on it for grazing. The local people depend significantly on the grassland to maintain their livestock needs.

3.2 Study Design

3.2.1 Plot design

Three permanent fenced plots were established in the study site in 2015 by *Parthenium* research group of Tribhuvan University led by Dr. Bharat Babu Shrestha for research purpose. Each plot was of 12 m × 12 m size with an inner buffer zone of 1m in each side of the plot. Thus the size of working plot was 10 m × 10 m. Altogether forty small pieces of green PVC pipes of 0.5 inch diameter and 15 cm were dug into soil with upper 2 cm of pipe visible on surface of soil in every 1 m distance along the buffer zone boundary in order to make it easier to divide the working plot into 100 small subplots of 1 m × 1 m.

In order to carry out a comparative study, new temporary plots with same area were made adjacent to each fenced plots (Figure 3). These new plots differed from the previous one in that; these areas were freely grazed throughout the year. Thus the fenced plots were named as Grazing Excluded (GE) plots and the unfenced ones as Freely Grazed (FG) plots (Figure 4). The distance between the GE and FG plot was 30 m in the two pairs and 20 m in the third one to exclude trail within the plot. Forty small pieces of PVC pipes (15 cm length and 0.5 inch diameter) were dug along four boundary of the grazed plot keeping above 2 cm of the pipe above the surface of soil to demarcate the area of plot just as in fenced plot.

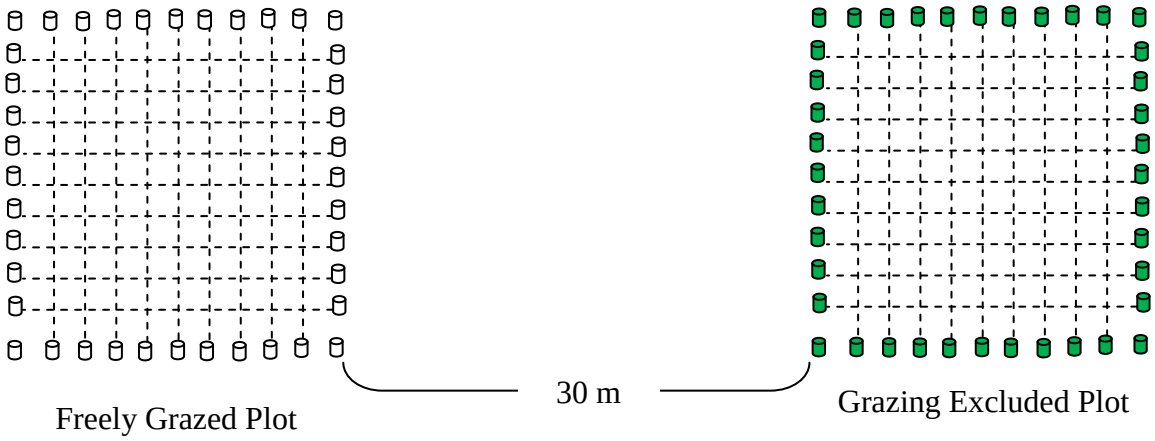


Figure 3: Diagrammatic representation of 10 m × 10 m plot design of both FG and GE plots showing demarcation of subplots of 1 m × 1 m within each plot.

4(a)

4(b)

Figure 4: Photographs of sampling site showing (a) Freely Grazed (FG) and (b) Grazing Excluded (GE) plot.

3.2.2 Field sampling and data collection

Three field visits were carried out from June, 2017 to October, 2017. The first preliminary visit was carried out in June to access the information about topographic features and vegetation of the study area. The second visit was in July, 2017 during which vegetation and soil sampling (for analysis of physico-chemical properties) were done. The final field visit was done in October, 2017 during which the soil samples for germinable soil seed bank analysis were collected.

During sampling, each working plot of 10 m × 10 m were subsequently divided into 100 subplots of 1 m × 1 m. A unique number ranging from 1 to 100 was assigned to each subplots starting from any one corner of the working plot. Afterwards, out of total 100 subplots, ten subplots were selected randomly for sampling with the help of random numbers generated from MS Excel. Since there were three replicates of each FG and GE plot, altogether data from 30 subplots of FG and 30 subplots of GE plot were collected and used for further analysis.

Soil samples were collected from the center of each subplots. Core sampling method was used for collecting soil samples for BD and seed bank analysis. Core samplers of 6.1 cm height, 4.2 cm diameter and 10 cm height, 10 cm diameter were used for collecting soil samples for BD and germinable seed bank respectively. However for physico-chemical parameters, soil was simply collected from the layer of 5-10 cm by using diggers. Only single soil sample was collected for each purpose (bulk density analysis, physico-chemical analysis, germinable seed bank analysis) from each subplot in order to minimize disturbance in the site. Thus collected soil was kept in plastic zip lock bags and transported to laboratory within 1-4 days of collection.

Vegetation sampling was done along with soil sampling on July, 2017. Vascular plants present in each subplots were recorded along with their percentage coverage. The areal cover of each species was measured independently so that summed cover could exceed 100% for multi-layered canopies. Daubenmire cover class was assigned to the visually estimated percentage cover of each individual species (Zoebel *et al*., 1987) and midpoint of cover class percentage was used as cover for the further calculations. Topographical features such as latitude, longitude and altitude were recorded for each plot.

3.2.3 Herbarium specimen collection

Voucher specimens of each species of vascular plants were collected from the study site along with their field notes during vegetation sampling. The study area was revisited in October, 2017 to collect herbarium specimens of those plants which were collected in vegetative stage during vegetation sampling in July, 2017. Thus collected plant species were tagged, pressed and dried by standard herbarium preparation technique and finally herbarium specimens were prepared. Identification of those voucher specimens was carried out by following standard literatures (Siwakoti and Varma, 1999), online database (www.efloras.com), expert consultation and comparing with specimens deposited at KATH herbarium at Godawari, Lalitpur. For accepted names, author citation, family names and life history traits online database such as 'Encyclopedia of Life' (www.eol.org), 'The Plant List' (www.theplantlist.org), 'Plants of the world online' (www.plantsoftheworldonline.org) and 'Catalogue of Life' (www.catalogueoflife.org) were used. The identified voucher specimens will be deposited in TUCH, Kirtipur, Kathmandu.

3.3 Soil Analysis

3.3.1 Bulk density

The dry mass of soil in a given volume has been considered as the bulk density (BD) of soil. Soil BD was estimated by using undisturbed core method following the procedure described by Gupta *et al*. (2000). The undisturbed soil cores were transferred from plastic zip lock bag to paper envelop (photo 7) and oven dried in 105° C for 17 hours (time period was determined according to volume of soil core) and afterward, the dry weight of core was recorded. Volume of soil core was assumed to be equal to the volume of the soil core sampler used to collect the soil sample and was calculated by using following formula:

$$Volume\ of\ soil\ core = Volume\ of\ \ iron\ core$$

$$= (\pi r^2 h\ )$$

Where,

r = Radius of soil core

h = Height of soil core

Finally, the bulk density of soil was calculated by using following formula:

$$Soil\ bulk\ density = \frac{Dry\ weight\ of\ soil\ core}{Volume\ of\ soil\ core}$$

3.3.2 Soil pH and electrical conductivity

Soil pH of samples were measured using a pocket sized digital pH meter "model – PH 009"- (photo 9) having an accuracy of $\pm$ 1. Soil to water suspensions of 1:2.5 ratios were made (Gupta *et al*., 2000) by mixing 10 g of soil with 25 ml distilled water. Thus made suspension was stirred for 3 minutes and allowed to settle for half an hour. Then the digital pH meter was dipped in the mixture to measure pH. The pH meter was calibrated with buffer solution of pH 7 and 9 and rinsed through distilled water repeatedly after taking each reading. The pH of two replicate suspensions of each soil sample was averaged to obtain the final pH value of the soil sample.

$$Soil\ pH = \frac{pH\ of\ I^{st}\ replicate + pH\ of\ II^{nd}\ replicate}{2}$$

Similarly, electrical conductivity (EC) of the soil extract at 25° C (EC25) was used as the measure of salinity of soil. Soil salinity was determined by using conductivity method (Bado *et al*., 2016). A soil to water suspension of 1:5 was made by adding 15ml distilled water to 3 g of soil. Thus prepared soil suspension was stirred continuously for three minutes with the help of glass rod. Then the reading of Electrical Conductivity (EC) was taken by using a digital analysis kit named "Deluxe Water and Soil Analysis Kit model - 191" and recorded as EC_t. The temperature of the soil suspension was also measured on every 10 minutes and finally the soil electrical conductivity was calculated by using following formula:

$$EC_{25} = EC_t \times ft$$

Where,

EC_{25} = Electrical conductivity of soil sample at 25° C

EC_t = Measured electrical conductivity of soil sample at 't' temperature

Ft = Corrected value of electrical conductivity at 't' which was obtained from table (Bado *et al*., 2016)

3.3.3 Soil nutrient analysis

3.3.3.1 Soil organic carbon

Organic carbon (OC) is a measurable component of soil organic matter which enters the soil through decomposition of plant and animal residue, microorganism and soil biota. The organic carbon of soil was estimated by using Walkely and Black rapid titration method (Gupta *et al*., 2000; photo no. 11). The 0.5 g of soil was taken into a conical flask (250 ml) and 5 ml of $K_2Cr_2O_7$ and 10 ml of conc. H_2SO_4 was added to it one after another. The mixture was gently stirred to ensure proper mixing and then kept for 30 minutes for digestion. Afterward 100ml of distilled water and 5ml of ortho-phosphoric acid was added correspondingly to the mixture. Lastly 0.5 ml of diphenylamine indicator solution was added to the mixture, stirred for few seconds till the whole mixture becomes dark blue colored and then titrated against 0.5 N Ferrous Ammonium Sulphate (FAS) solution. The end point of titration was noted when the dark blue color of the mixture turned to bright green color. The initial and final reading of FAS solution in burette was noted and the amount consumed by the samples was determined as:

$$Amount\ of\ FAS\ consumed = (Final\ reading\ - Initial\ reading) of\ FAS$$

A standardized blank without soil was also run in the same way as control. Finally, the percentage of OC in soil was calculated by using formula shown below:

$$\%\ of\ OC\ in\ soil = N\frac{(B-C)}{weight\ of\ soil(g)} \times 0.003 \times 100$$

Where,

N= Normality of ferrous ammonium sulphate

B= Blank reading

C= Titration reading

Lastly, the organic carbon percentage was multiplied by a factor 1.3 based on a assumption that there was 77% recovery of organic matter in this procedure and total organic carbon measure was obtained as:

$$Total\ Organic\ Carbon\ \ (\%) = estimated\ organic\ carbon\ \% \times 1.3$$

3.3.3.2 Soil nitrogen

The nitrogen content of soil samples was estimated using micro Kjeldahl method (Kalra 1998). The process comprised of three steps i.e. digestion (photo no. 12), distillation (photo no.13) and titration (photo no. 14). The 500 mg of soil sample was taken in a Kjeldahl digestion flask and 3.5 g of Potassium sulphate plus 0.4 g of Copper sulphate along with 6ml of conc. H_2SO_4 was added to the soil sample one by one. Then the flask was kept in mantle and heated gently at 30° C till the bubbles disappear from the mixture. Later on, the temperature was raised to 70° C - 100° C for about an hour and the soil sample was digested completely. During digestion, the color of sample changed from black to brownish and attended to be greenish. The digested material was allowed to cool for 15-20 minutes and then 50 ml of distilled water was poured to it and stirred for few seconds for proper mixing of digested material with water.

Afterward the digested material was transferred to Kjeldahl distillation flask and heated at first in 30° C - 40° C temperature till the digested material becomes lukewarm. Once the mixture became warm, 30 ml of 40% sodium hydroxide solution was added to it and then temperature of mantle was increased to 70° C - 100° C. The distillate containing ammonia was collected in a conical flask containing 10 ml of boric acid indicator. The distillation process was continued till the total amount of distillate reached about 50 ml.

After that, the distillate with boric acid indicator was titrated against 0.1 N HCl solution. The end point of titration was noted when the bluish color of sample turned bright pink in color. Same process was repeated for a blank solution without soil after set of every 10 soil samples. Finally the percentage of Nitrogen in soil sample was calculated by using following formula:

$$Nitrogen\ \%\ in\ soil = \frac{(14 \times N \times (S - B) \times 100)}{M}$$

Where,

N= Normality of HCl

S= Volume of HCl consumed by soil sample (ml)

B= Volume of HCl consumed by blank (ml)

M= Mass of soil sample taken (mg)

3.3.3.3 Soil phosphorus

Available phosphorus of soil samples was estimated by using procedures as described in Trivedy and Goel (1986). Turger's extract was made by mixing 2 g of soil with 200 ml of 0.002 N H_2SO_4 and the mixture was shaken (photo 15) in a vibrator (model: KCH-VIBRAX-VXR) at speed of 1200/min for half an hour. Then the total suspension (200 ml) was filtered by using Whatmann No.1 filter paper to get a clear soil solution (photo 16). Filtration was repeated until the filtrate was clear. Then after, 50 ml of the filtrate was taken in a clean beaker and 2 ml of ammonium molybdate solution was added to it followed by 5 drops of $SnCl_2$ solution. A blue color was observed in the mixture after addition of $SnCl_2$ solution. Then the reading of the solution was taken at 690 nm on a spectrophotometer using distilled water as blank solution with same amount of chemicals (photo 17). The reading was taken after 5–12 minutes of the addition of $SnCl_2$.

Similarly, for standard curve, various dilutions of the standard phosphate solution at the interval of 0.1 mg P/L were made and their absorbance at 690 nm was noted. Finally, a curve of absorbance and concentration of various dilutions of phosphorus was made and the equation for the curve was estimated by using MS Excel (Figure 5).

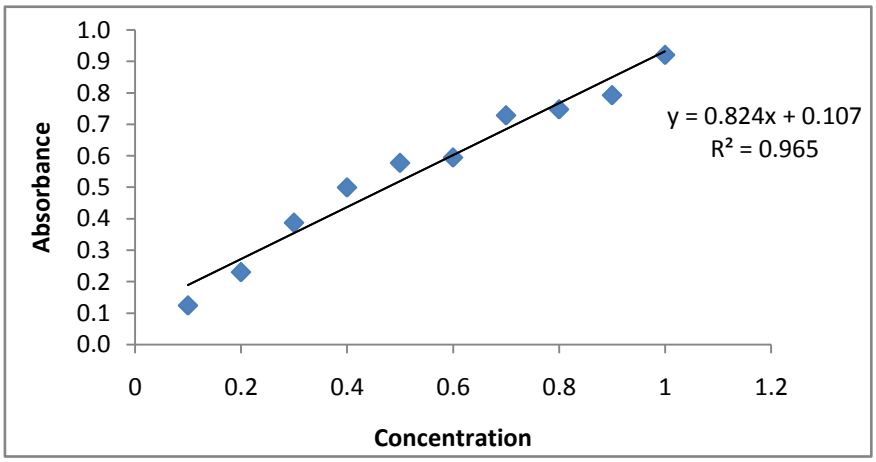


Figure 5: Line graph showing calibration curve of available phosphorus

Thus obtained equation from standard curve was used to estimate the concentration of available phosphorus. Finally, the percentage of available phosphorus in soil was calculated by using following formula:

$$\%\ available\ phosphorus = \frac{mg\ P/L\ \ soil\ solution}{50}$$

Where,

mg P/L soil solution was obtained with the help of standard curve.

3.3.3.4 Soil potassium

Exchangeable Potassium content of soil samples was determined by flame photometer method (Trivedy and Goel, 1986). Soil extract was prepared by mixing 2 g of soil sample with 20 ml of 1 N ammonium acetate solution. Then the mixture was shaken in a vibrator of KCH-VIBRAX-VXR model at speed of 1200/min for fifteen minutes. Afterwards, the total suspension of 20 ml was filtered by using whatmann no.1 filter paper to get a clear soil solution. Filtration was repeated until the filtrate was clear. Thus obtained clear filtrate was transferred to a clean rinsed test tube and its flame photometer reading was noted at filter of 768 nm (photo 18).

Likewise, the calibration curve of potassium was made by diluting the standard phosphate solution to different level and their absorbance at 768 nm was noted. Finally, a curve of absorbance and concentration of various dilutions of potassium was made and the equation for the curve was estimated by using MS Excel (Figure 6).

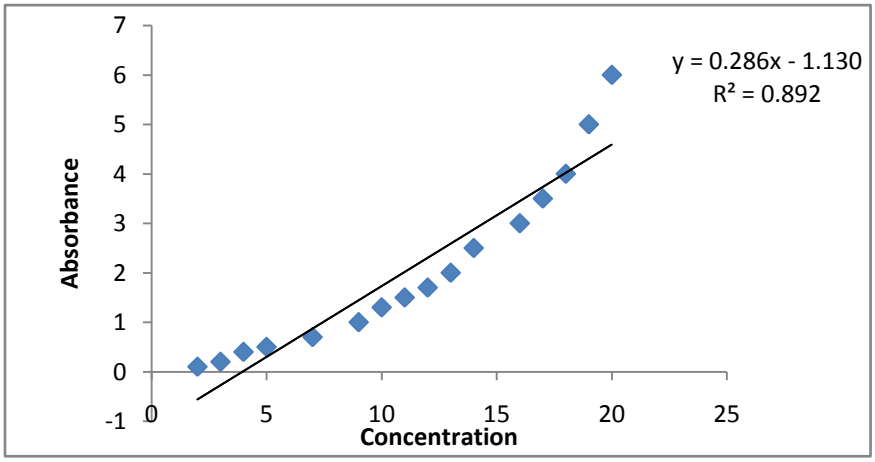


Figure 6: Line graph showing calibration curve of potassium

The concentration of potassium was determined by equation obtained from pre-prepared calibration curve of potassium. Finally the percentage of exchangeable potassium was determined by using following formula:

$$Potassium\ (\%) = \frac{mg\ K/L\ of\ soil\ extract\ \times V}{10000 \times S}$$

Where,

V= Total weight of soil extract prepared (20 ml)

S= Weight of soil taken (2 g)

3.4 Vegetation Analysis

3.4.1 Frequency and relative frequency

Frequency and relative frequency were calculated using the following formula:

$$Frequency\ (\%) = \frac{No.\ of\ sampling\ plots\ in\ which\ individual\ species\ occured}{Total\ number\ of\ sampling\ plots} \times 100$$

$$Relative\ Frequency\ (\%) = \frac{Frequency\ of\ individual\ species}{Total\ frequency\ of\ all\ species} \times 100$$

3.4.2 Coverage and relative coverage

Coverage and relative coverage of plant species were calculated as:

$$Coverage\ (\%) = \frac{Total\ midpoint\ cover\ percent\ of\ individual\ species}{Total\ number\ of\ sampling\ plots}$$

$$Relative\ Coverage\ (\%) = \frac{Coverage\ of\ individual\ species}{Coverage\ of\ all\ the\ species} \times 100$$

3.4.3 Importance percentage

Importance percentage (IP) of each species was calculated to determine the overall importance of individual species in each Freely Grazed (FG) and Grazing Excluded (GE) plots. It was calculated as the function of relative frequency and relative coverage of each species in the two contrasting sites (FG and GE) such that the IP value of any species in a community ranges between 0-100. Importance percentage of each species was calculated as:

$$Importance\ Percentage\ (\%) = \frac{RF + RC}{2}$$

Where,

RF = Relative frequency of individual species

RC = Relative coverage of individual species

3.4.4 Similarity index

Similarity indices are the indicators of the degree of resemblance between the two communities represented by the samples. The higher the index value the lower will be the difference in vegetation composition between the communities. Sorensen's Similarity Index (Qs) was determined as the measure of similarity index in order to know the species compositional difference of the two contrasting sites .The index of similarity (Qs) between two communities as proposed by Sorensen (1948) was calculated by using following formula:

$$Sorensen^{'}s\ Similarity\ Index\ (Q_s) = \frac{(2C \times 100)}{(A + B)}$$

Where,

C = Species common to both FG and GE plots

A = Total species of FG plots

B = Total species of GE plots

3.4.5 Relative abundance of *Parthenium hysterophorus* (RaPh)

Relative abundance of a species is the percent composition of a species relative to the total species in the area. The relative abundance of *P. hysterophorus*, the most dominant Invasive Plant Species (IPS) was determined in each sampling plot (1 m × 1 m) in order to test our proposed research hypothesis i.e. grazing exclusion will lead to decline in abundance of herbaceous IPS. The relative coverage of *P. hysterophorus* in each sampling plot was used as the measure of relative abundance of *Parthenium* in that sampling plots. It was determined by using following formula:

$$RaPh\ (\%) = \frac{Coverage\ of\ Parthenium}{Total\ coverage\ of\ plant\ species\ in\ that\ plot} \times 100$$

3.4.6 Species richness and diversity

In this study, species richness has been determined in 1 m × 1 m subplot level as well as in 10 m × 10 m plot level. The number of species per unit area of interest has been used as measure of species richness of that particular area i.e.

$$Species\ Richness\ (R) = S$$

Where,

S= Number of species within the area of interest

Species diversity refers to the number of species and their relative abundance in a particular location. Shannon Diversity Index (H') was used as measure of species diversity in this study. Species diversity has also been determined in 1 m × 1 m subplot level as well as in 10 m × 10 m plot level. The diversity index was calculated by following formula given by Shannon, (1948).

$$Shannon - Weiner\ \ Index\ (H^{'}) = -\sum_{i=1}^{n}(pi \times lnpi)$$

Where

pi = Relative coverage of individual species in unit area of interest

3.5 Germinable Seed Bank Analysis

Germinable soil seed bank was determined for both FG and GE plots in a greenhouse at Kirtipur, Kathmandu. Soil samples brought for seed bank analysis were spread in well perforated plastic plates (to avoid flooding effect) containing double layer of blotting papers kept on it to maintain the moisture level and the soil were watered regularly with equal amount of water (i.e.150 ml) on each plate and allowed for germination (photo plate III). Two control plates with sun dried sand were kept as control plates which were watered regularly along with other plates to observe contamination by air borne seeds. Identified germinated seeds were removed from the plates to reduce the competition for newly emerging seedlings. The sample of unidentified plant species were allowed to grow till adult for identification (Savadogo *et al*., 2016).

3.5.1 Seed bank density (SBD)

Seed bank is defined as the reserve of seeds present in soil and seed bank density refers to the total number of seeds present in unit area of soil. Germinable seed bank density was calculated as the qualitative measure of assessing abundance of individual species in soil seed bank of FG and GE plots. It was calculated by following Tessema *et al*. (2017) as:

$$SBD = \frac{No.of\ total\ seedlings\ germinated\ from\ each\ soil\ sample}{Area\ from\ which\ the\ soil\ sample\ was\ taken\ (m^2)}$$

3.5.2 Relative abundance of *Parthenium hysterophorus* (RaPh)

Relative abundance of *Parthenium hysterophorus* in the seed bank of FG and GE plots was determined in each soil sample in order to know the effect of grazing exclusion on seed bank of *P. hysterophorus* using following formula:

$$RaPh\ (\%) = \frac{Density\ of\ Parthenium}{Total\ density\ of\ all\ plant\ species\ in\ that\ plot} \times 100$$

3.5.3 Species richness and diversity

Furthermore, species richness and diversity was also estimated and compared between the seed bank of FG and GE to know the below ground impact by grazing. The species richness (R) and Shannon-Weiner index (H') were calculated as:

$$Species\ Richness\ (R) = S$$

Where,

S= Total number of species germinated from seed bank of each soil sample

$$Shannon - Weiner\ Index\ (H^{'}) = -\sum_{i=1}^{n}(pi \times lnpi)$$

Where,

pi= Relative density of individual species on each soil sample

3.6 Statistical Analysis

3.6.1 Comparison between traits of FG and GE plots

All statistical analysis was performed in Rstudio (Mangiafico, 2016). The normality and homogeneity of variance for soil physico-chemical parameters (BD, pH, EC, OC, N, P, K), above ground vegetation characters (species richness, diversity, relative abundance of IPS), and germinable seed bank traits (SD, relative abundance of IPS, species richness and diversity) were tested prior to choosing the type of statistical analysis tool i.e. parametric tool or non-parametric tool. Shapiro-Wilk test was used to determine the normality of the data. The data which did not show normal distribution were subjected to different transformations (log transformation, square root transformation and logit transformation) according to the nature of data for normalizing the data (Mangiafico, 2016). Similarly homogeneity of variance of the data was determined by using Levene's test. In this study all the data type i.e. normal data, transformed data and non-normal data showed homoscedasticity.

In view of the fact that there were only two grouping variables (i.e. Freely Grazed and Grazing Excluded) and altogether 30 observations for each grouping variables in this study, independent sample t-test was chosen as analysis tool. Both parametric (t-test) and non-parametric (Wilcoxon U-test) statistical tests were performed to analyze the data. The comparison of mean difference of normally distributed homogenous data was carried out by independent sample t-test assuming equal variance (parametric test) whereas,

complementary non- parametric test i.e. Wilcoxon U-test was chosen for comparing the mean difference of non normal data. The mean of species diversity (both AGV and SB), soil pH, soil bulk density, soil organic carbon, soil nitrogen and soil potassium of FG and GE were compared by independent sample t-test. Species richness (both AGV and SB), and soil phosphorus were compared by Wilcoxon-U test.

3.6.2 Ordination

The species compositional difference and species distribution along environmental gradient in the FG and GE plots were evaluated by ordination. Detrended Correspondence Analysis (DCA), an unconstrained indirect gradient analysis was done in order to know the nature (either linear or hump shaped) of species response curve. The gradient length of DCA I^{st} axis was used as determiner of further analysis. DCA analysis revealed a gradient length of first axis as 2.94, which indicated the unimodal nature of species response curve. Since the species response curve was unimodal, Cannonical Correspondence Analysis (CCA) was performed (ter Braak, 1995) to demonstrate the species compositional difference along environment gradient.

4. RESULT

4.1 Effect of Grazing Exclusion on Physico-Chemical Properties of Soil

Grazing exclusion of even three years showed some noticeable difference in some of the soil properties of FG and GE plots. Soil bulk density and organic carbon were higher in FG plots than in GE but pH, electrical conductivity and phosphorus were higher in GE plots (Table 1). There was no significant difference in nitrogen, phosphorus and potassium between FG and GE plots.

Table 1: Soil physical and chemical properties in FG and GE plots

Soil attributes	FG	GE	t-stat	p-value
Bulk Density (g/cm3)	**1.37**±0.03	1.18±0.03	5.1744	0.00**
pH	7.79±0.03	**7.92**±0.03	-3.0657	0.00**
Electrical Conductivity	0.07±0.00	**0.10**±0.00	-4.9357	0.00**
Organic Carbon (%)	**3.47**±0.08	2.73±0.06	7.3979	0.00**
Nitrogen (%)	0.21±0.01	0.20±0.01	1.0965	0.28
Phosphorus (%)	0.01±0.00	0.02±0.00	329	0.06
Potassium (%)	0.18±0.02	0.17±0.02	0.3660	0.72

(Note: Results are expressed in terms of Mean ± Standard Error. p-value with '**'indicates statistical significance of 0.01 at the confidence level of 95%.Stat value for Phosphorus is W- value of Wilcoxon U-test.)

4.2 Effect of Grazing Exclusion on Above Ground Vegetation

4.2.1 Floristic composition

A total of 50 species (12 Monocots and 38 Dicots) belonging to 19 families and 45 genera were recorded from the study area (Appendix 3). Highest number of species belonged to Asteraceae and Poaceae which together accounted for 44% of the total recorded species (Figure 7).

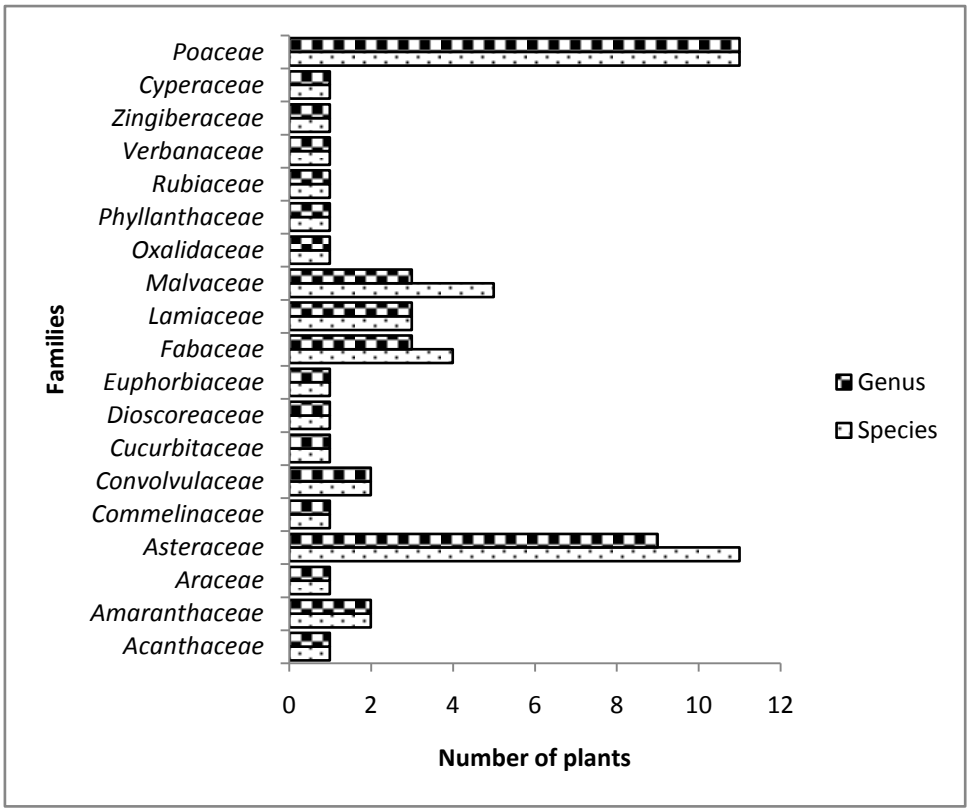


Figure 7: Total number of genus and species belonging to different families in above ground vegetation of study site

Out of total 50 species, 23 species were common to both Freely Grazed (FG) and Grazing Excluded (GE) plots, while 15 species were present only in FG plots and 12 species were only in GE (Appendix 4). Annual plants comprised more than 60% of the total species in both FG and GE plots (Figure 8).

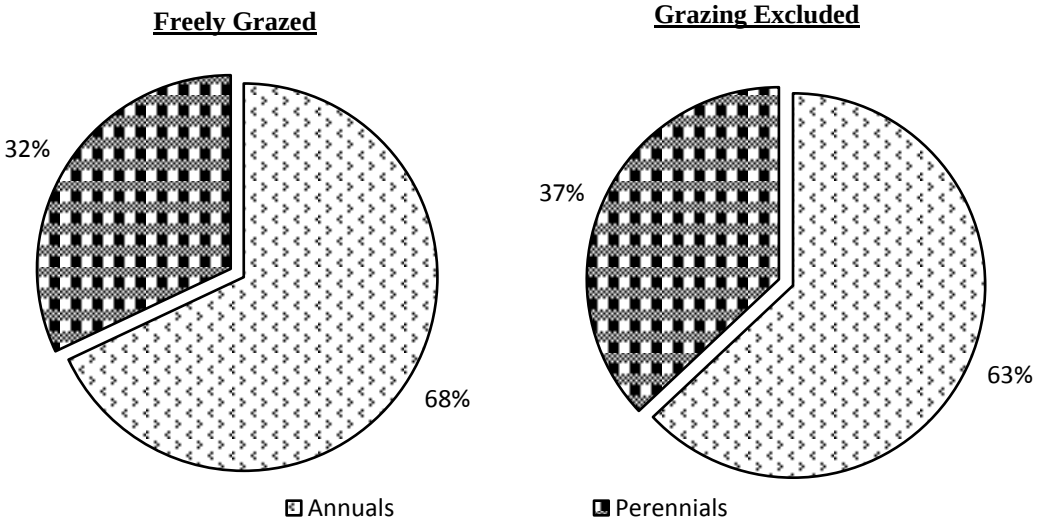


Figure 8: Percentage composition of life history of plant species in FG and GE plots.

Growth forms shown by plant species of GE plots were more diverse than that of FG plots (Figure 9). The percentage of forbs and grasses were higher in FG plots whereas percentage of shrubs and plant species with other growth forms like climbers, sedges, etc were higher in GE plots. Sorensen's Similarity Index showed that only 59% of the floristic composition of the FG and GE plots was similar.

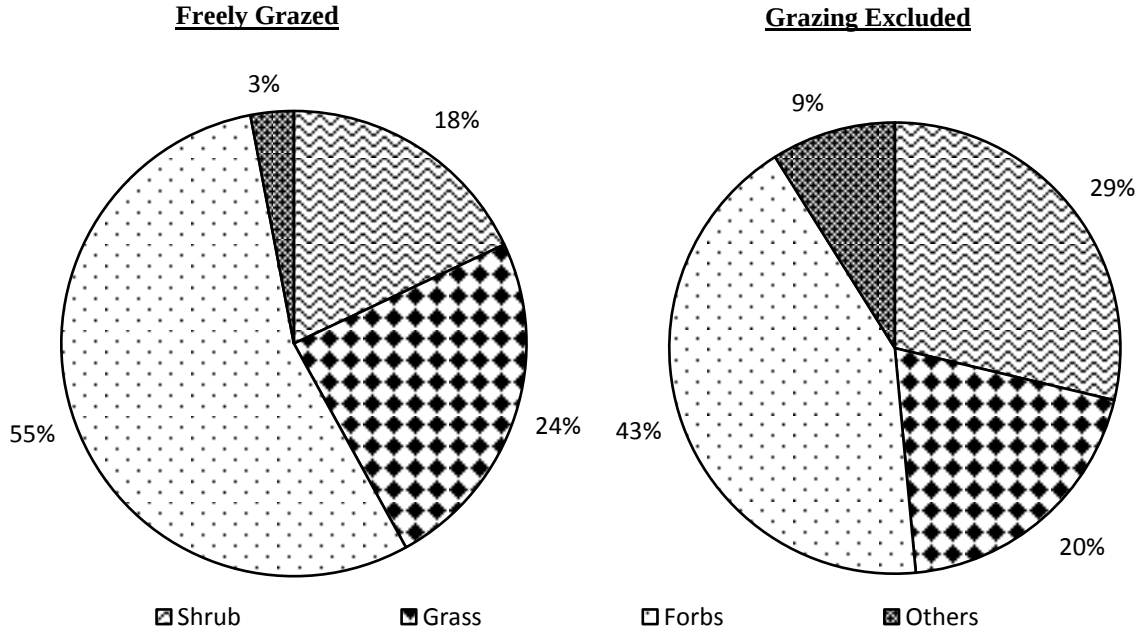
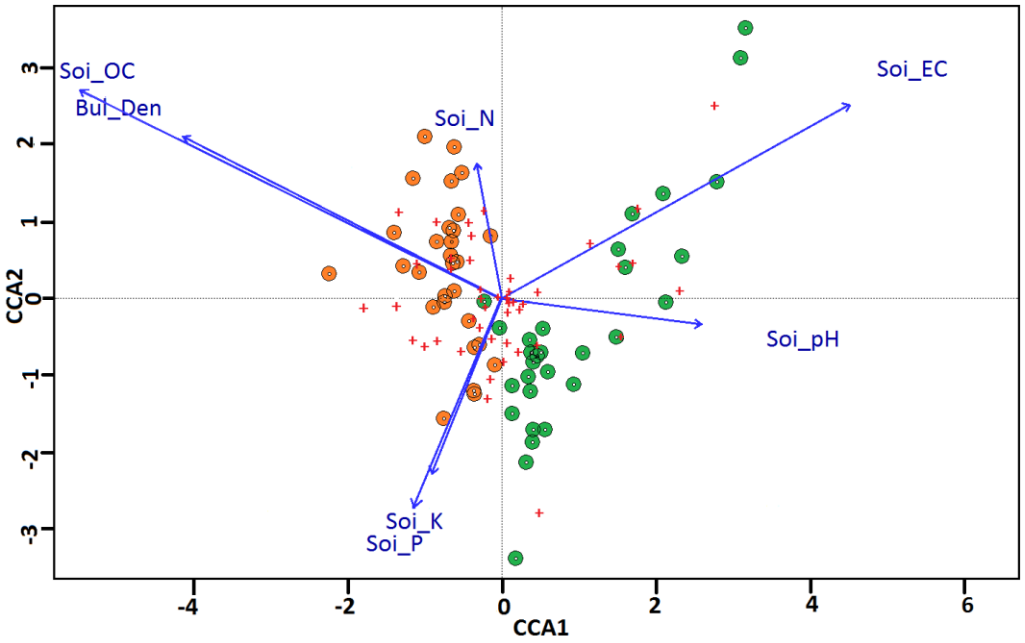
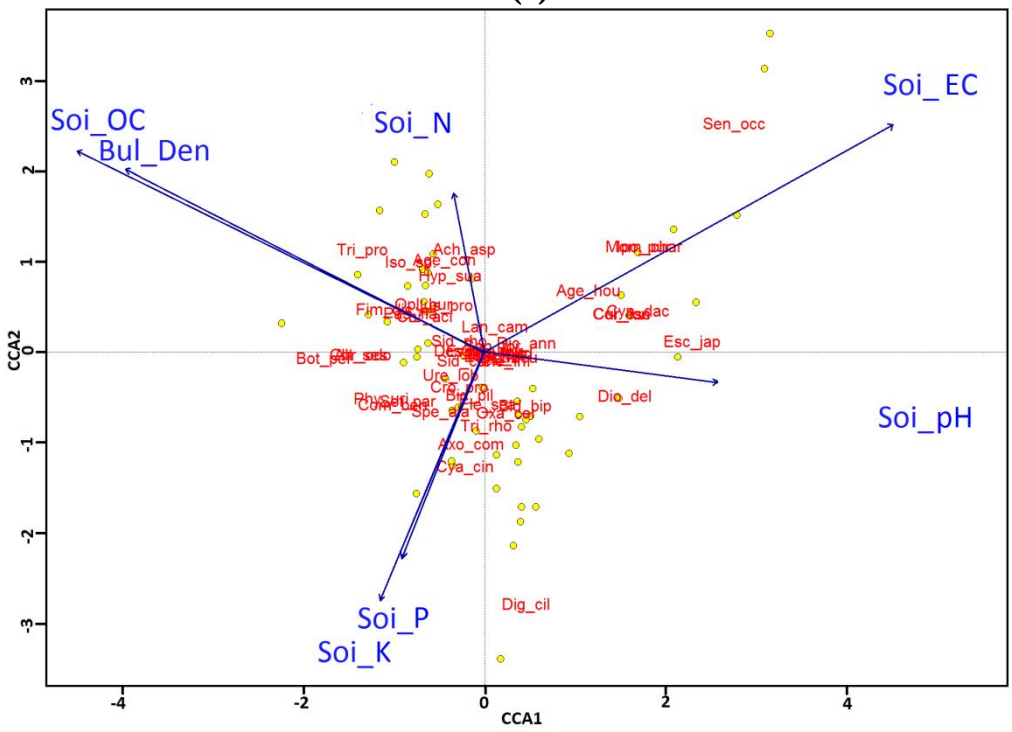


Figure 9: Percentage composition of growth forms of plant species in FG and GE plots.

Plotting of CCA ordination revealed segregation of sampling plots of FG and GE plots which indicated that grazing exclusion had substantially altered the species composition as well as soil properties of the grassland (Figure 10). GE subplots had relatively higher pH and EC but lower bulk density, soil organic carbon and nitrogen.



10(a)



10(b)

Figure 10: CCA plots showing (a) sampling plots of FG and GE plots (green colored & orange colored points representing GE & FG plots, species are represented as red colored '+' signs and environmental parameters are in blue colored abbreviated text forms) (b) species compositional difference between FG and GE plots (plots are represented as yellow colored points and names are represented in abbreviated forms; full names are in appendix 5).

Further, the test of importance of environmental variables in species distribution revealed that species distribution was significantly affected by the soil bulk density and soil organic carbon content, each of which explained the 12% variance in shaping vegetation pattern in response to fencing (Table 2). Beside these two environmental parameters, species distribution was also impacted by the soil EC which explained 8% of total variance.

Table 2: Relative importance of environment variables on species composition based on the CCA analysis. The statistical significance (p-value) of the variables was obtained from Monte Carlo permutation test with 999 permutations.

Environmental variables	Abbreviation	Variance explained	F	p-value
Bulk Density (g/cm^3)	Bul_Den	0.1246	1.9723	0.01**
Soil pH	Soi_pH	0.0736	1.1660	0.15
Soil Electrical Conductivity (S/m)	Soi_EC	0.1042	1.6502	0.06*
Soil Organic Carbon (%)	Soi_OC	0.1208	1.9123	0.01**
Soil Nitrogen (%)	Soi_N	0.0457	0.7241	0.82
Soil Phosphorus (%)	Soi_P	0.0836	1.3233	0.04*
Soil Potassium (%)	Soi_K	0.0661	1.0469	0.38

(p-value with '**' and '*' signs indicates statistical significance at 0.01 and marginal significance at 0.05 level respectively)

Bulk density and soil organic carbon was lower in grazing excluded areas due to which some species which prefer lower bulk density and carbon such as *Dioscorea deltoidea, Bidens bipinnata, Oxalis corniculata,* etc were exclusively present only in GE plots (Figure 10.b). However species such as *Tridax procumbens, Fimbristylis dichotoma, Chrisopogon aciculatus, Paspalidium flavidium, Oplismenus burmanii,* etc were found to prefer higher bulk density and were present only in FG plots. Similarly, species like *Cyanthium cinerium, Axonopus compressus, Triumfetta rhomboidea, Spermacoce alata,*

Bidens pilosa, etc were found to prefer higher soil phosphorus. Likewise, species such as *Senna occidentalis*, *Momordica charantia*, *Cynodon dactylon*, *Eschencia japonica*, etc were found to prefer higher soil pH and were present only in GE plots.

Importance percentage

The importance percentage (IP) value showed that four plant species i.e. *Clerodendrum infortunatum*, *Imperata cylindrica*, *Mimosa pudica* and *Parthenium hysterophorus* were the dominant species of the study site (Appendix 6). Ten most frequently recorded species of FG and GE plots along with their IP value are listed below in Table 3. Three of ten most frequently encountered species were IAPS.

Table 3: List of ten most frequently encountered species along with their Importance Percentage value in FG and GE plots. Appendix 6 has complete list of species with their importance percentage.

S.N.	Name of species	Frequency		IP	
		FG	GE	FG	GE
1	*Chrysopogon aciculatus* (Retz.) Trin.	77	-	5	-
2	*Clerodendrum infortunatum* Vent.	67	93	7	13
3	*Cynodon dactylon*(L.) Pers.	-	33	-	2
4	*Desmodium triflorum* (L.) DC.	90	-	4	-
5	*Euphorbia hirta* L.	-	40	-	3
6	*Evolvulus nummularius* (L.) L.	67	40	4	3
7	*Imperata cylindrica* (L.) P. Beauv.	97	93	13	19
8	*Mimosa pudica* L.	93	**100**	8	12
9	*Parthenium hysterophorus* L.	**100**	**100**	**21**	**20**
10	*Senna tora* (L.) Roxb.	60	50	5	4
11	*Sida cordata* (Burm. fil.) Borss. Waalk.	80	40	5	3
12	*Sida rhombifolia* L.	**100**	33	6	3

(Highest values are indicated with bold letters; '-' represents lower/zero frequency and importance percentage of the plant species in that particular plot)

Among the ten most frequent plant species of FG and GE plots, *P. hysterophorus* was the most frequent species present in all subplots. Not only that, the highest IP value of *P. hysterophorus* in both FG and GE plots indicated that it was the most dominant species

of both the plots. Likewise, *I. cylindrica* was the second most abundant species of both plots however it's IP value was higher in GE (19%) than in FG (13%).

4.2.2 Relative abundance of *Parthenium hysterophorus*

The relative abundance of *P. hysterophorus* expressed in terms of relative coverage was slightly higher in FG than in GE plots but the difference was not statistically significant (Figure 11).

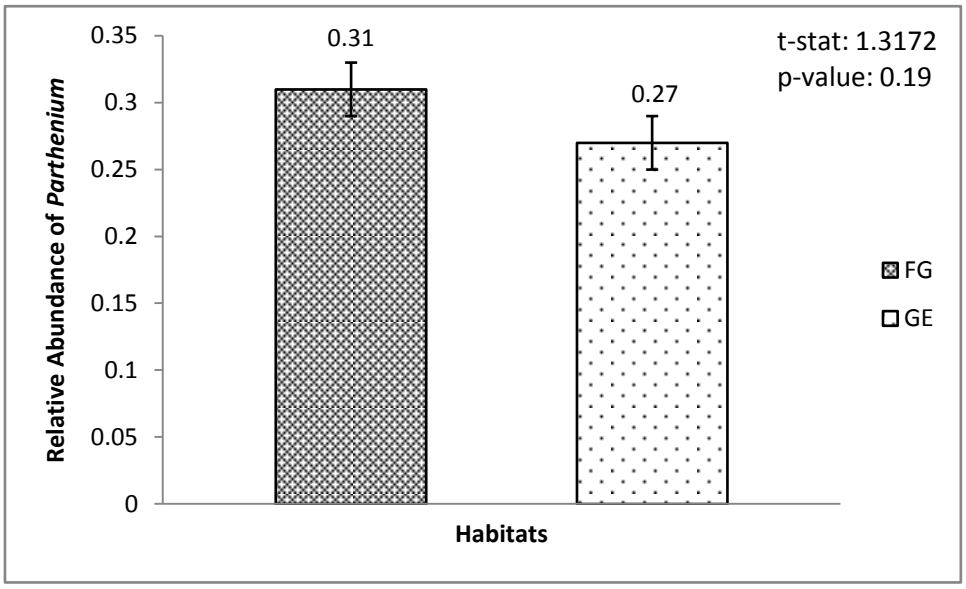
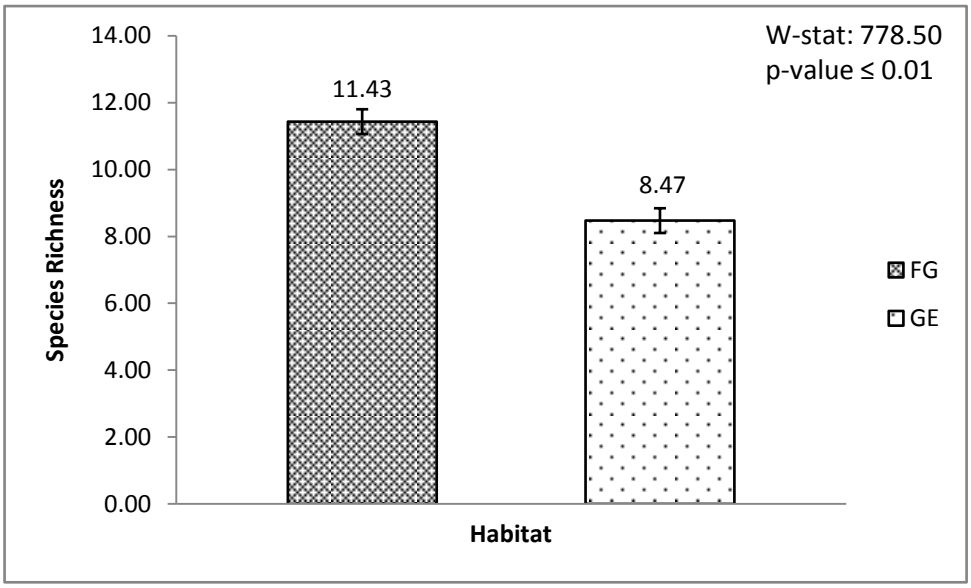


Figure 11: Comparison of relative abundance of *P. hysterophorus* in Freely Grazed (FG) and Grazing Excluded (GE) plots along with t-stat and p-value at confidence level of 95% shown in top right corner of graph. Error bars are Mean ± SE.

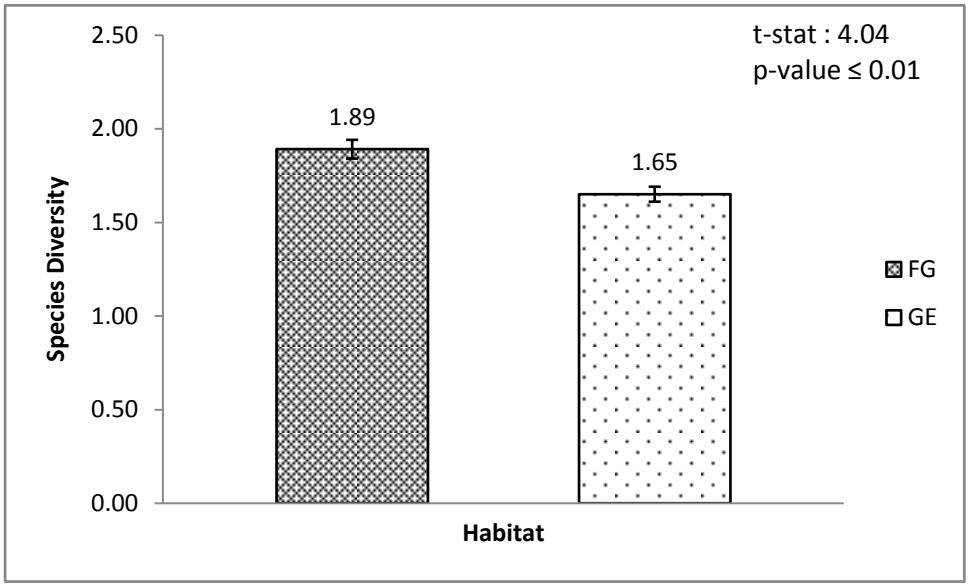
4.2.3 Species richness and diversity

Species richness in subplot level was significantly higher in FG plots than in GE (Figure 12. a). In plot (10 m × 10 m) level too, the species richness was higher in FG (26 ± 1.53) than in GE (21.67 ± 1.67).

Likewise, species diversity (Shannon-Weiner Index) of FG was significantly higher (at $p \leq 0.01$) than of GE at subplot level (Figure 12. b). When analyzed at plot level, the species diversity was again higher in FG (2.25 ± 0.07) than in GE (2.06 ± 0.05).



12(a)



12(b)

Figure 12: Comparison of (a) species richness and (b) species diversity between Freely Grazed (FG) and Grazing Excluded (GE) plots along with stat & p-value at confidence level of 95% showed in top right corner of graph. Error bars are Mean ± SE.

4.3 Effect of Grazing Exclusion on Germinable Soil Seed Bank

4.3.1 Seed bank composition

No seedlings were found in the control plots which indicated that there were no airborne seed contaminants. Altogether 13,855 seedlings belonging to 14 families, 21 genera and 25 species germinated which resulted in total seed bank density of 29,401 seeds m^{-2} (Appenxix 7). Out of 25 species, 21 species were identified to species level, 1 species to genus level and 2 species to family level. One species i.e. Unknown sp. 3 (photo 42) could not be identified even up to family level so it was omitted from the further analysis. Five of total 24 species were monocots while remaining 19 species were dicots. Half of the total species belonged to Asteraceae and Poaceae family (Figure 13).

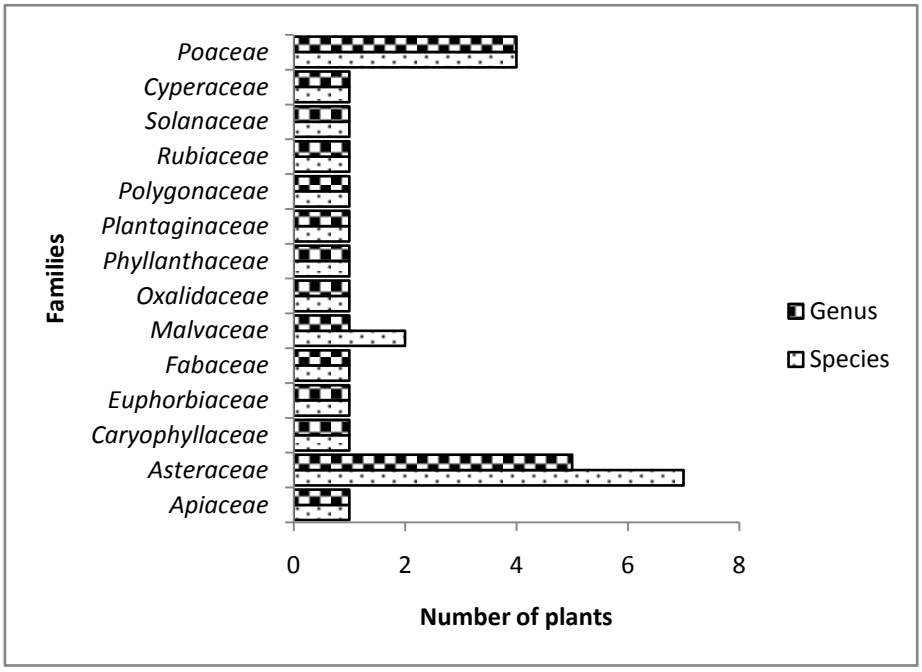


Figure 13: Total number of genus and species of plant germinated from seed bank belonging to different families.

Out of total 24 species observed in germinable seed bank study, 17 species were common to both plots while three species were unique to FG and four species were unique to GE (Appendix 8). Plant species composition of both FG and GE site was almost similar with plants having same life history strategies (Figure 14). Sorensen's similarity index showed that 83% of species were similar in between FG and GE plots.

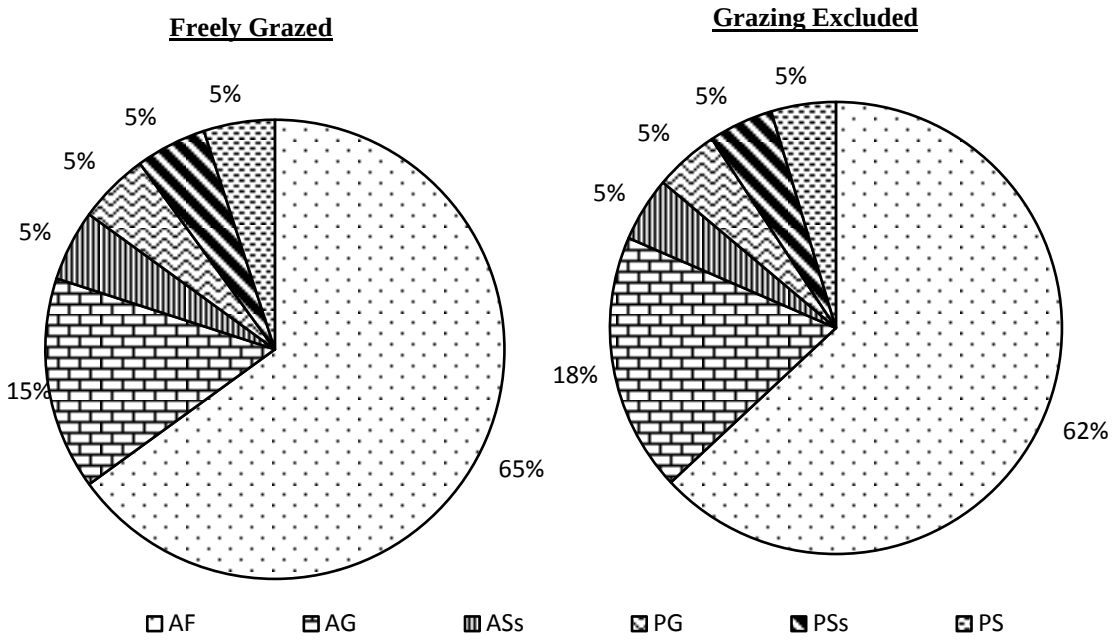


Figure 14: Percentage composition of plant species having different life history strategies observed in germinable seed bank of FG and GE plots. AF: Annual Forbs; AG: Annual Grasses; ASs: Annual Sub-shrubs; PG: Perennial Grasses; PS: Perennial Shrubs; PSs: Perennial Sub-shrubs.

Annual plant species comprised the bulk (80-83%) of total plant species observed in the germinable seed bank study. Most of the species were annual forbs and annual grasses which together made 80% of total seed bank composition. The proportion of annual forbs was slightly higher in FG while proportion of annual grasses was higher in GE. Plant species belonging to perennial grasses, sub-shrubs and shrubs were similar in both FG and GE plots.

Seed bank density

The average seed bank density in FG was 1525 seeds m^{-2} while that in GE was 925 seeds m^{-2}. *Parthenium hysterophorus* showed the most dominant soil seed bank in both FG and GE plots (Appendix 9). Besides *P. hysterophorus*, *Ageratum houstonianum*, *Oxalis corniculata* and *Mimosa pudica* were the species that contributed the highest to total seed bank. The 15 of total germinated species were present in AGV as well while 9 of them were unique to the seed bank only. The list of ten most frequent species observed in seed bank of FG and GE have been tabulated below along with their seed bank density (Table 4).

Table 4: List of ten most frequently encountered species along with their germinable seed bank density in FG and GE plots

S.N.	Name of Species	Frequency		Density (Seed/m2)	
		FG	GE	FG	GE
1	*Ageratum houstonianum* Mill.	10	23	51	102
2	*Bidens pilosa* L.	17	20	42	30
3	*Bidens bipinnata* L.	-	13	-	34
4	*Cynodon dactylon* (L.) Pers.	23	-	55	-
5	*Fimbristylis dichotoma* (L.) Vahl.	17	17	25	51
6	*Gnaphalium polycaulon* Pers.	-	20	-	25
7	*Mimosa pudica* L.	50	27	85	42
8	*Oplismenus burmanni* (Retz.) P. Beauv.	27	33	55	59
9	*Oxalis corniculata* L.	83	70	569	798
10	*Parthenium hysterophorus* L.	**100**	**100**	**35494**	**20907**
11	*Phyllanthus urinaria* L.	10	-	38	-
12	*Senna tora* (L.) Roxb.	20	23	25	64

(Highest value is indicated with bold letters, '-' sign indicates lower/zero frequency and seed bank density of the species in that particular plot).

Parthenium hysterophorus was the most frequent species observed in seed bank of both FG and GE plots forming the highest seeed bank density in both plots. Seed bank density of *P. hysterophorus* alone comprised 96% of the total seed bank density. Similarly, *Oxalis cornicualata* and *Mimosa pudica* were the second and third most frequent species in FG seed bank whereas *Oxalis corniculata* and *Oplismenus burmanii* were the second and third most frequent species in GE.

4.3.2 Relative abundance of *Parthenium hysterophorus*

Relative abundance of *Parthenium hysterophorus* expressed in terms of relative density was higher in FG than in GE (Figure 15).

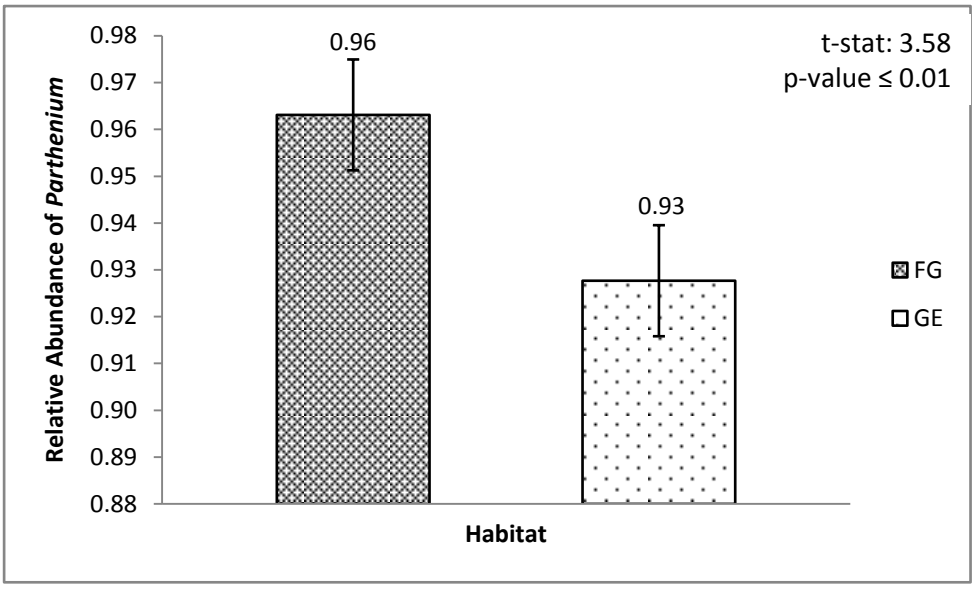
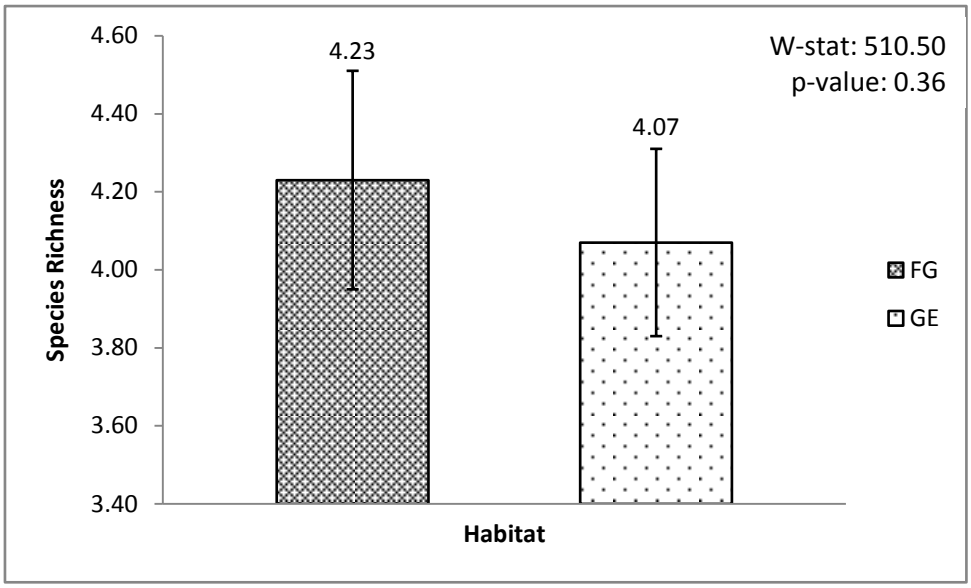


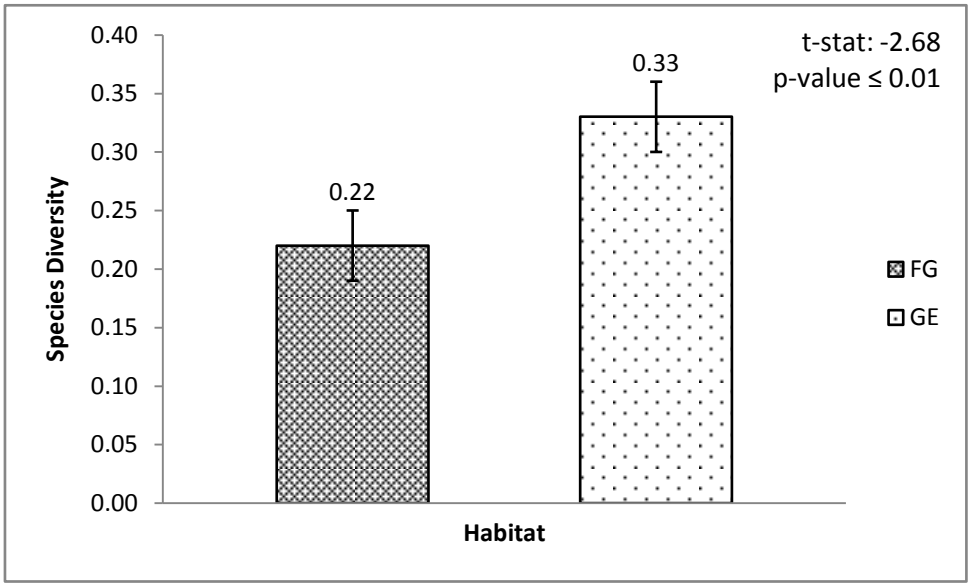
Figure 15: Comparison of relative abundance of *Parthenium* in seed bank of Freely Grazed (FG) and Grazing Excluded (GE) plots along with t-stat and p-value (at confidence level of 95%) showed in top right corner of graph. Error bars are Mean ± SE.

4.3.3 Species richness and diversity

There was no significant difference in species richness of soil seed bank between FG and GE (Figure 16. a) but the species diversity (Shannon-Weiner index) of GE was significantly higher than in FG (Figure 16. b).



16(a)



16(b)

Figure 16: Comparison of species richness (a) and species diversity (b) of seed bank between Freely Grazed (FG) and Grazing Excluded (GE) plots along with t-stat and p-value (at confidence level of 95%) showed in top right corner of graph. Error bars are Mean ± SE.

5. DISCUSSION

5.1 Effect of Grazing Exclusion on Soil Properties

Trampling, altering nutrient content via defecation and release of nutrient from damaged plant parts during grazing has been found to affect soil physico-chemical properties of the grassland (Sala *et al*., 1996). Soil bulk density is often considered as an important indicator of soil degradation in grazed ecosystems, as it can further alter soil properties, such as water infiltration and retention, and thereby restrain plant growth (Li *et al*., 2014). Soil bulk density was significantly higher in FG plots than in GE (Table 1). The higher soil bulk density in grazed site was also observed by Yong-Zhong *et al*. (2005), Wu *et al*. (2010) and Medina-Roldan *et al*. (2012). Higher soil bulk density in FG plots might be due to livestock trampling (Mullen *et al*., 1974; Holt, 1997). In contrast, the livestock were banned to enter the GE plots for three years, which might have reduced the soil bulk density. Soil compaction may destroy soil structural units and change pore distribution, thereby slowing water infiltration and gaseous diffusion (Taylor and Brar 1991).Thus significantly higher bulk density of soil in FG plots suggested that soil condition in grazed areas has been degrading.

Soil pH was significantly higher in plots where grazing was banned than in continuously grazed areas (Table 1). The relatively lower soil pH in FG plots might be due to hydrolysis of urine urea (Haynes and Williams, 1992). Lu *et al*. (2015) had also reported higher soil pH in ungrazed plots in alpine grassland of Tibet. In the same way, soil EC was found to be higher in GE plots than in FG plots. The higher EC value in GE might be due to comparatively higher available nutrient concentration in soil solution in that area. However, this finding contradicted with the findings of Wu *et al*. (2010) and Li *et al*. (2014) who reported increased nutrient content when grazing was excluded. This contradiction in observation may be explained by the fact that natural decomposition and nutrient sequestration are slower processes. Grazing exclusion period of three years in this study might have been insufficient to observe the effect of natural decomposition and nutrient sequestration in GE plots.

Similarly, soil organic carbon was statistically greater in FG than in GE (Table 1). The comparatively higher amount of soil organic carbon in FG plots might be due to the dung

of cattle (Bol *et al*., 2000). In addition to it, the release of plant exudates due to herbivory might also have accounted for some increase in organic carbon content of soil in FG (Sala *et al*., 1996). This finding was contradictory with the findings made by Wu *et al*. (2010), Zhu *et al*. (2016) and Koshravi *et al*. (2017) who reported that soil organic carbon increased when grazing was banned from grassland. Natural soil nutrient sequestration is a long term process and the reduced microbial activities in GE plots might have resulted in its lower soil organic carbon content (Medina-Roldan *et al*., 2012). The lack of input of animal excreta, which acts as stimulate for microbial activity and nutrient cycling in grassland soils might have reduced microbial activities in GE plots (McNaughton *et al*., 1988). The shorter grazing exclusion period might also have been insufficient for organic carbon to accumulate naturally in GE plots.

Lack of significant difference in soil nitrogen, phosphorus and potassium of FG and GE plots in this study could be due to a short period of grazing exclusion. Often, soil processes takes much time to show the prominent changes (Lu *et al*., 2015; Chen and Tang, 2016) and the three years grazing exclusion period might not have been sufficient enough for obtaining statistically significant differences in these properties of soil in FG and GE plots.

5.2 Effect of Grazing Exclusion on Above Ground Vegetation

5.2.1 Species composition

Altogether 50 species were recorded in AGV of the study site belonging to 19 families with 12 of them being Monocots and 38 of them being Dicots. Similar finding was observed by KC (2012) in the same study site who reported altogether 55 plant species belonging to 18 families among which 36 were dicots and 19 were monocots. Most of the plant species recorded from both the FG and GE plots belonged to Asteraceae and Poaceae families which comprised 44% of total species richness. The observed dominance of Asteraceae family may be attributed to its mass seed production capacity and its efficient seed dispersal mechanism (Rastogi *et al*., 2015). Similarly, the dominance of Poaceae family can be explained by its capacity of long-distance dispersal, effective establishment biology, ecological flexibility, resilience to disturbance and the capacity to modify environments by changing the nature of fire and mammalian herbivory

(Linder *et al*., 2018). Dominancy of Asteraceae and Poaceae families was also observed in grassland studies of Pinto *et al*. (2013) and Koshravi *et al*. (2017).

Out of total 50 species, 23 species were common to both the FG and GE plots while 15 species of FG and 12 species of GE were unique to each of them. Species composition of GE was much diverse with plant species having various life history strategies than in FG. Whereas in FG plots, only annual forbs and perennial grasses formed the dominant vegetation structure which comprised more than 70% of total vegetation. Some cultivated species such as *Curcuma longa, Colocasia esculenta*, *Momordica charantia*, etc were observed exclusively in vegetation of GE plots which might have resulted in diversity in plant species composition in GE plots. The reason for presence of cultivated species in grassland lies in the land use history of the study site. The site used to be used as cultivation land earlier so it might have some propagules of cultivable species which sprouted in GE plots when the habitat condition was less disturbed. Sorensen's Similarity Index revealed that only 59% of the vegetation of FG and GE plots was similar. These values indicated a reasonably low level of similarity in species composition of FG and GE plots considering that the plots were so close to one another.

CCA ordination plot demonstrated segregation of sampling plots based on species composition which suggested that grazing exclusion had resulted in difference in species composition of the grassland. Palatable plant species such as *Dioscorea deltoidea, Cynodon dactylon, Digitaria ciliaris*, etc as well as shrubby species such as *Bidens bipinnata*, *Sida acuta*, *Triumfetta rhomboidea*, etc were found to prefer grazing exclusion as these were present exclusively in GE plots. The comparatively less disturbed habitat and release from herbivory might be responsible for the growth of these species in GE plots. Whereas, unpalatable exotic plant species: *Ageratum houstonianum*, *Hyptis suaveolens, Spermacoce alata*, etc and grass species: *Axonopus compressus, Bothriocloa pertusa, Chrysopogon aciculatus*, etc were found to love grazing activity. The continuous disturbance and more saline habitat in grazed plots might have been responsible for the growth of exotic species in FG plots. Similarly, formation of open canopy due to grazing might be responsible for growth of different grass species rather than dominance of single grass species in FG plots. The ordination scatter was best explained by bulk density and soil organic matter. Difference in species composition of grazed and fenced site was also observed in study of Koshravi *et al*. (2017).

Since this study was carried out in a *Parthenium hysterophorus* invaded grassland, *Parthenium* was found to be the most important plant species of both FG and GE plots with highest Importance Percentage (IP) value than other plant species. The observed strong dominance of *P. hysterophorus* in both FG and GE plots could be attributed to its efficient dispersal mechanism, high growth rate and short life cycle which enable it to quickly colonize in sites leading to its strong dominance in invaded habitats (Timsina *et al*., 2011). Despite of its dominance in both the sites, the IP value of *P. hysterophorus* was comparatively lower in GE (20%) than in FG (21%). The comparatively higher IP value of *P. hysterophorus* in FG plots may be due to comparatively higher relative abundance of *Parthenium* in those plots. The relatively higher nutrient concentration and less competition from competitor species might be the reason behind its higher abundance in FG plots. The heavy grazing has often been found to enhance abundance of invasive species (Brenda *et al*., 2014; Wu *et al*., 2015; Baggio *et al*., 2018).

Similarly, *Imperata cylindrica* was found to be the second most dominant species of both FG and GE plots with second highest IP value. However IP value of *I. cylindrica* was relatively higher i.e. 19% in GE plots than in FG plots i.e. 13% which may be explained by its palatable nature. In FG plots due to continuous grazing the relative coverage of *I. cylindrica* was comparatively lower as a consequence its IP value became lower than that in GE plots. The trend was similar for *Mimosa pudica* as well which showed relatively higher abundance in GE plots in comparison to FG plots and this may be because goats feeds on *M. pudica* when they are young so in FG plots their relative coverage was lower in comparison to that in GE plots. A similar study of 35 years livestock exclusion by Al-Rowailey *et al*. (2015) also observed similar finding i.e. abundance and richness of palatable species was increased when protected from herbivores.

In contrast, species such as *Senna tora*, *Sida cordata* and *Sida rhombifolia* were comparatively more important species for FG plots which may be attributed to their relatively higher coverage and frequency in FG plots than in GE plots. The higher frequency and abundance of *S. tora*, *S. cordata* and *S. rhombifolia* in FG site might be due to their exotic nature since exotic species often colonize disturbed habitat. As a disturbance factor, grazing might have created suitable microhabitat for these species.

5.2.2 Relative abundance of *Parthenium hysterophorus*

There was no statistically significant difference in relative abundance of *Parthenium hysterophorus* although the mean relative abundance of Parthenium for FG site was found to be higher than in GE site. This result contradicted the finding obtained by Davies (2011), Brenda (2014), Seabloom *et al*. (2015) and Koshravi *et al*. (2017) who reported that grazing exclusion significantly decreased the abundance of invasive plant species in fenced areas. The reduction in dominant competitors and enrichment in nutrient concentration due to grazing activity might have enhanced the persistence of *P. hysterophorus* in FG plots (Olff and Ritchie, 1998; Davis *et al*., 2000). However the observed statistical insignificance in mean difference of relative abundance in this study may be due to short exclusion period because of which the effect of grazing exclusion might not have prominently seen. The effects of enclosure age on vegetation characters have been found to be systematically stronger for older compared to younger enclosures (Chen and Tang, 2016; Satkamp *et al*., 2017).

5.2.3 Species richness and diversity

The species richness and diversity in GE was lower than in FG supporting "intermediate disturbance hypothesis". The presence of comparatively higher number of IAPS and their relatively higher coverage in FG site might have lead to the higher species richness and diversity of the area (Hobbs and Huenneke, 1992). Baggio *et al*. (2018) also came up with similar finding in regard of species richness and diversity in a study of grassland invaded by *Eragrostis plana* in southern Brazil. Similarly, Wu *et al*. (2009) also reported comparatively higher species richness and diversity in grazed areas than in grazing excluded areas. However, findings of Zhang and Zhao (2015) and Zhu *et al*. (2016) contradicted the finding of this study which demonstrated a significant increase in species richness as well as diversity in the fenced areas. This dissimilarity may be due to different environmental conditions as well as different ages of grazing exclusion period and the grazing intensity among the different study areas (Bork, 1998; Satkamp *et al*., 2017). The studies which showed comparatively higher species richness and diversity in fenced areas were demonstrating grazing exclusion effect of longer exclusion period i.e. 5-15 years while this study was dealing with effect of only 3 years grazing exclusion. Similarly, finding of this study was demonstrating the impact of GE in invaded grassland as in Baggio *et al*. (2018) while this was not the case for Zhang and Zhao (2015) and Zhu *et al*.

(2016). However there was no any significant difference in species richness and diversity of FG and GE site in larger scale i.e. plot level (10 m × 10 m) which might be due to inadequate sample numbers (Sullivan *et al*., 2012) or due to scale effect (Whittaker, 2001). Local processes are much prominent in 1 m × 1 m plots as it is the resolution where species interactions are mostly likely to unfold (Davies *et al*., 2005).

5.3 Effect of Grazing Exclusion on Below Ground Vegetation

Seed bank of a plant community represents regeneration potential and future composition of the community and thus this study intended to observe the impact of grazing exclusion in the below ground vegetation structure of two contrasting FG and GE plots via qualitative measure i.e. by observing the germinable seed bank of two contrasting (FG & GE) areas.

5.3.1 Seed bank composition

In total, 24 species belonging to 14 families were recorded in germinable seed bank study. Similar finding was observed by KC (2012) who reported 27 species belonging to 12 families from seed bank study carried out in same study area. Out of 24 total species observed in seed bank study, 15 species were also present in above ground vegetation while nine species were completely missing in above ground vegetation. Similar findings have been also reported by Rice (1989), Vila & Gimeno, (2007) and Davies *et al*. (2018) where species composition in the seed bank flora and the above ground plant community were dissimilar.

The total seed bank density of this study was 29,401 seeds/m^2. The majority of seedlings observed in seed bank belonged to annual species similar as in the findings of germinable seed bank studies by Meissner and Facelli (1999) and Shabbir (2015). Most of the species observed in seed bank belonged to Asteraceae and Poaceae which showed consistency with the finding of Navie *et al*. (1996). *Parthenium hysterophorus* alone was found to form the 96% of total germinable seed bank density. This extreme dominance of *P. hysterophorus* in seed bank might be because of its high seed producing capacity. *P. hysterophorus* is a plentiful seed producer which can produce 15,000 to 25,000 seeds per single mature plant (Haseler, 1976). This weed's high seed production capability and high viability of its seeds enables it to form large soil seed bank in invaded regions (Navie *et*

al., 2004). The seed bank composition of FG and GE plots was found to be similar with Sorensen's similarity index value of 83%. However, the mean seed bank density of FG plots was found to be higher than that in GE plots. The comparatively higher seed bank density of *P. hysterophorus* in FG plots might have consequently resulted in higher mean seed bank density of those plots. Ma *et al*. (2015) in a study carried out in a saline alkaline grassland of Northeast China had also reported highest seed bank density in areas subjected to grazed treatment than in other treatments.

5.3.2 Relative abundance of *Parthenium hysterophorus*

There was statistically significant difference in relative abundance of *P. hysterophorus* between FG and GE plots. The relative abundance of *P.hysterophorus* was lower in GE plots (0.93) than in FG plots (0.96). Absence of grazing must have increased the competition for *P. hysterophorus* which resulted into lower abundance of adult *P. hysterophorus* species in AGV structure of GE plots. This must have further affected the seed rain and consequently reduced the relative abundance of *P.hysterophorus* in seed bank of GE plots. The decrease in abundance of invasive species after grazing exclusion is also evident in study by Osterheld and Sala (1990) who reported significant decline in abundance of an invasive species *Leontodon taraxacoides* by grazing exclusion of seven years period. However, a study by Meissner and Facelli (1999) had reported that there was no any effect of stock exclusion in abundance of invasive species. This difference among the finding of the different study might be due to differences in grazing exclusion periods, intensity of invasion and nature of invasive plant species.

5.3.3 Species richness and diversity

Grazing exclusion did not have effect on the species richness in soil seed bank. However, Ma *et al*. (2015) had reported that grazing exclusion period of ten years resulted in significant increase of species richness in soil seed bank. The insignificant change in species richness in seed bank of this study may be due to the short grazing exclusion period. The comparative study of species diversity in soil seed bank revealed significantly higher species diversity in seed bank of GE plots than in the seed bank of FG plots. This finding was supported by finding of Chaideftau *et al*. (2008) who reported higher species diversity in soil seed bank of non-grazed deciduous Oak forest. Similarly, this finding also showed consistency with finding of Jutila *et al*. (1998) and Benjamin and Sanderson

(2000) which demonstrated comparatively higher species diversity in ungrazed sites than in grazed sites. However, Navie *et al*. (1996) found that the peak species diversity of seed banks was at a high level of stocking intensity. The dissimilarities among these findings may be due to different vegetation type and different level of grazing intensity in different studies.

6. CONCLUSION

6.1 Conclusion

Grazing exclusion had some significant changes in composition of both above ground as well as below ground (seed bank) vegetation along with few of soil physico-chemical properties. Soil organic carbon and bulk density was enhanced by grazing while soil pH and EC were enhanced by exclusion of grazing activity. No significant change was observed in soil nitrogen, phosphorus and potassium content of FG and GE plots. Grazing exclusion considerably altered the species composition of grasslands. However, the species composition of AGV was much affected by grazing exclusion than the species composition of BGV. Species richness and diversity were statistically higher in FG than in GE plots in AGV but the result was just opposite in seed bank analysis. These contrasting findings of AGV and BGV presented a conundrum in making any core conclusion regarding impact of grazing exclusion in plant species diversity. However, the increase in species richness and diversity in AGV of FG plots might be due to presence of relatively more number and coverage of invasive species in those areas. Since seed bank often illustrates the regenerating potential of the community the relatively higher species diversity in seed bank of GE plots suggested that grazing exclusion results in vegetation enrichment. Likewise, the comparatively less relative abundance of *P. hysterophorus* in GE plots in both above and below ground vegetation studies (though the differences was significant only in BGV) indicated that grazing exclusion can also be used as an ecosystem level management practice of herbaceous invasive plant species such as *P. hysterophorus* and help to improve the condition of invaded grazing lands. The proper management of grasslands in order to increase production and restore degraded grassland requires sufficient information on the grassland management. Therefore, a proper evaluation of effects of livestock grazing in order to discover the correct management strategy is essential in degraded grasslands. Thus more studies with longer period (at least of 5 years, or a decade or more) of grazing exclusion are required in order to fully understand the impact of grazing exclusion on soil properties and vegetation structure of grassland and to come up with solid results.

6.2 Recommendations

Based on the results of this study following recommendations are made:

- Studies with longer exclusion periods might be useful to make solid conclusions about effect of grazing exclusion on different soil and vegetation traits.
- Chronological studies with different grazing exclusion period might be useful to determine the suitable period of exclusion for maintaining diversity in grassland.

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APPENDICES

Appendix 1: Geographic information of the studied Freely Grazed (FG) and Grazing Excluded (GE) plots with their latitude, longitude, altitude and distance between the adjacent contrasting plots.

S.N.	Blocks	Treatments	Latitude	Longitude	Altitude	Distance in between
1	4A	GE	27° 24' 23.91"	85° 01' 08.81"	476	20 m
2	4A'	FG	27° 24' 23.68"	85° 01' 10.29"	472	
3	5A	GE	27° 24' 22.85"	85° 01' 06.06"	487	30 m
4	5A'	FG	27° 24' 20.40"	85° 01' 06.08"	484	
5	6A	GE	27° 24' 23.29"	85° 01' 04.85"	497	30 m
6	6A'	FG	27° 24' 21.56"	85° 01' 04.35"	495	

Appendix 2: DCA table showing the eigen value and gradient length of DCA1 to DCA 4 axis.

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.1568	0.2036	0.1870	0.1718
Decorana values	0.3662	0.2771	0.1914	0.1622
Axis lengths	2.9487	3.0493	2.2491	2.1298

Appendix 3: Summary of above ground vegetation composition of the study site showing total number of orders, families, genera and species.

S.N.	Orders	Families	Genus	Species
1	Dicotyledons	Acanthaceae	1	1
		Amaranthaceae	2	2
		Araceae	1	1
		Asteraceae	9	11
		Commelinaceae	1	1
		Convolvulaceae	2	2
		Cucurbitaceae	1	1
		Dioscoreaceae	1	1
		Euphorbiaceae	1	1
		Fabaceae	3	4
		Lamiaceae	3	3
		Malvaceae	3	5
		Oxalidaceae	1	1
		Phyllanthaceae	1	1
		Rubiaceae	1	1
		Verbanaceae	1	1
		Zingiberaceae	1	1
2	Monocotyledons	Cyperaceae	1	1
		Poaceae	11	11

Appendix 4: List of plant species, their family name, life history strategy along with their presence/absence data in FG and GE plots.

S.N	Name of plant species	Family	L.h.	P/A	
				FG	GE
1	*Achyranthes aspera* L.	Amaranthaceae	A/F	+	-
2	*Ageratum conyzoides* L.	Asteraceae	A/F	+	-
3	*Ageratum houstonianum* Mill.	Asteraceae	A/F	+	+
4	*Alternanthera sessilis* (L.) DC	Amaranthaceae	A/F	+	-
5	*Axonopus compressus* (Sw.) P. Beauv.	Poaceae	A/G	+	+
6	*Bidens bipinnata* L.	Asteraceae	A/Ss	-	+
7	*Bidens pilosa* L.	Asteraceae	A/F	+	+
8	*Bothriocloa pertusa* (L.) A. Camus	Poaceae	P/G	+	-
9	*Cenchrus purpureus* (Schumach.) Morrone.	Poaceae	P/G	+	+
10	*Chromolaena odorata* (L.) R.M. King & H. Rob.	Asteraceae	A/F	+	-
11	*Chrysopogon aciculatus* (Retz.) Trin.	Poaceae	P/G	+	-
12	*Clerodendrum infortunatum* Vent.	Lamiaceae	P/S	+	+
13	*Colocasia esculenta* (L.) Schott.	Araceae	P/F	-	+
14	*Commelina benghalensis* L.	Commelinaceae	A/F	+	-
15	*Crotalaria prostrata* Rottler ex. Willdenow	Fabaceae	A/F	+	+
16	*Curcuma longa* L.	Zingiberaceae	P/F	-	+
17	*Cyanthillium cinereum* (L.) H. Rob.	Asteraceae	A/F	+	-
18	*Cynodon dactylon* (L.) Pers.	Poaceae	P/G	-	+
19	*Desmodium triflorum* (L.) DC.	Fabaceae	A/F	+	+
20	*Dichanthium annulatum* (Forssk.) Stapf.	Poaceae	P/G	+	+
21	*Digitaria ciliaris* (Retz.) Koeler	Poaceae	A/G	-	+
22	*Dioscorea deltoidea* Wall.ex Griseb	Dioscoreaceae	P/C	-	+
23	*Elephantopus scaber* L.	Asteraceae	A/F	+	+
24	*Eschenbachia japonica* (Thunb.) J. Kost.	Asteraceae	A/F	-	+
25	*Euphorbia hirta* L.	Euphorbiaceae	A/F	+	+
26	*Evolvulus nummularius* (L.) L.	Convolvulaceae	A/F	+	+
27	*Fimbristylis dichotoma* (L.) Vahl.	Cyperaceae	A/Se	+	-
28	*Hyptis suaveolens* (L.) Poit.	Lamiaceae	A/F	+	-
29	*Imperata cylindrica* (L.) P. Beauv.	Poaceae	P/G	+	+
30	*Ipomoea purpurea* (L.) Roth	Convolvulaceae	A/C	-	+
31	*Isodon* sp.	Lamiaceae	A/F	+	-
32	*Justicia procumbens* var simplex L.	Acanthaceae	A/F	+	+
33	*Lantana camara* L.	Verbanaceae	P/S	+	+
34	*Mimosa pudica* L.	Asteraceae	A/Ss	+	+
35	*Momordica charantia* L.	Cucurbitaceae	A/C	-	+
36	*Oplismenus burmanni* (Retz.) P. Beauv.	Poaceae	A/G	+	+

37	*Oxalis corniculata* L.	Oxalidaceae	A/F	-	+
38	*Parthenium hysterophorus* L.	Asteraceae	A/Ss	+	+
39	*Paspalidium flavidum* (Retz.) A. Camus	Poaceae	P/G	+	-
40	*Phyllanthus urinaria* L.	Phyllanthaceae	A/F	+	-
41	*Senna occidentalis* (L.) Link	Fabaceae	A/Ss	-	+
42	*Senna tora* (L.) Roxb.	Fabaceae	A/Ss	+	+
43	*Setaria parviflora* (Poir.) Kerguélen	Poaceae	P/G	+	-
44	*Sida acuta* Burm. fil.	Malvaceae	P/Ss	-	+
45	*Sida cordata* (Burm. fil.) Borss. Waalk.	Malvaceae	P/F	+	+
46	*Sida rhombifolia* L.	Malvaceae	P/Ss	+	+
47	*Spermacoce alata* Aubl.	Rubiaceae	A/F	+	+
48	*Tridax procumbens* L.	Asteraceae	A/F	+	-
49	*Triumfetta rhomboidea* Jacq.	Malvaceae	P/S	+	+
50	*Urena lobata* L.	Malvaceae	A/F	+	+

(Note: A/C: Annual Climbers; A/F: Annual Forbs; A/G: Annual Grass; A/Se: Annual Sedge; A/Ss: Annual Sub-shrub; P/C: Perennial Climbers; P/F: Perennial Forbs; P/G: Perennial Grass; P/S: Perennial Shrub; P/Ss: Perrenial Sub-shrub; '+' indicates presence of species and '-' indicates absence of species.)

Appendix 5: Full names of abbreviated forms of plant names and soil parameters used in CCA ordination plot.

S.N.	Abbreviated names of plant species	Full names of plant species
1	Ach_ asp	*Achyranthes aspera*
2	Age_ con	*Ageratum conyzoides*
3	Age_hou	*Ageratum houstonianum*
4	Alt_ ses	*Alternanthera sessilis*
5	Axo_com	*Axonopus compressus*
6	Bid_ bip	*Bidens bipinnata*
7	Bid_ pil	*Bidens pilosa*
8	Bot_ per	*Bothriocloa pertusa*
9	Cen_ pur	*Cenchrus purpureus*
10	Chr_ odo	*Chromolaena odorata*
11	Chr_ aci	*Chrysopogon aciculatus*
12	Cle_inf	*Clerodendrum infortunatum*
13	Col_esc	*Colocasia esculenta*
14	Com_ben	*Commelina benghalensis*
15	Cro_ pro	*Crotalaria prostrata*
16	Cur_lon	*Curcuma longa*
17	Cya_cin	*Cyanthillium cinereum*
18	Cyn_ dac	*Cynodon dactylon*
19	Des_ tri	*Desmodium triflorum*
20	Dic_ann	*Dichanthium annulatum*
21	Dig_cil	*Digitaria ciliaris*
22	Dio_del	*Dioscorea deltoidea*
23	Ele_ sca	*Elephantopus scaber*
24	Esc _jap	*Eschenbachia japonica*
25	Eup_hir	*Euphorbia hirta*
26	Evo_num	*Evolvulus nummularius*
27	Fim_dic	*Fimbristylis dichotoma*
28	Hyp_sua	*Hyptis suaveolens*
29	Imp_cyl	*Imperata cylindrica*
30	Ipo_pur	*Ipomoea purpurea*
31	Iso_ sp.	*Isodon* sp.
32	Jus_pro	*Justicia procumbens*
33	Lan_ cam	*Lantana camara*
34	Mim_pud	*Mimosa pudica*
35	Mom_cha	*Momordica charantia*
36	Opl_ bur	*Oplismenus burmanni*
37	Oxa_ cor	*Oxalis corniculata*
38	Par_ hys	*Parthenium hysterophorus*
39	Pas_fla	*Paspalidium flavidum*
40	Phy_ uri	*Phyllanthus urinaria*

41	Sen_ occ	*Senna occidentalis*
42	Sen_tor	*Senna tora*
43	Set_par	*Setaria parviflora*
44	Sid_ acu	*Sida acuta*
45	Sid_ cor	*Sida cordata*
46	Sid_ rho	*Sida rhombifolia*
47	Spe_ ala	*Spermacoce alata*
48	Tri_ pro	*Tridax procumbens*
49	Tri_ rho	*Triumfetta rhomboidea*
50	Ure_ lob	*Urena lobata*

	Abbreviated names of environmental parameters	Full names of environmental parameters
1	Bul_Den	Bulk Density
2	Soi_OC	Soil Organic Carbon
3	Soi_EC	Soil Electrical Conductivity
4	Soi_pH	Soil pH
6	Soi_N	Soil Nitrogen
7	Soi_P	Soil Phosphorus
8	Soi_K	Soil Potassium

Appendix 6: List of plant species along with their relative frequency (RF), relative coverage (RC) and Importance Percentage (IP) values in aboveground vegetation of FG and GE plots.

S.N.	Name of plant species	RF		RC		IP	
		FG	GE	FG	GE	FG	GE
1	*Achyranthes aspera*	0.88	0.00	0.20	0.00	1	0
2	*Ageratum conyzoides*	0.29	0.00	0.08	0.00	0	0
3	*Ageratum houstonianum*	0.29	1.20	0.08	0.19	0	1
4	*Alternanthera sessilis*	0.29	0.00	0.09	0.00	0	0
5	*Axonopus compressus*	0.29	0.40	0.07	0.04	0	0
6	*Bidens bipinnata*	0.00	2.81	0.00	1.47	0	2
7	*Bidens pilosa*	1.75	2.41	0.46	2.20	1	2
8	*Bothriocloa pertusa*	0.29	0.00	0.09	0.00	0	0
9	*Cenchrus purpureus*	0.88	0.80	0.27	1.10	1	1
10	*Chromolaena odorata*	0.29	0.00	0.09	0.00	0	0
11	*Chrysopogon aciculatus*	6.73	0.00	4.20	0.00	5	0
12	*Clerodendrum infortunatum*	5.85	11.24	7.82	14.36	7	13
13	*Colocasia esculenta*	0.00	0.40	0.00	0.93	0	1
14	*Commelina benghalensis*	0.29	0.00	0.07	0.00	0	0
15	*Crotalaria prostrata*	0.58	0.40	0.12	0.07	0	0
16	*Curcuma longa*	0.00	0.40	0.00	0.37	0	0
17	*Cyanthillium cinereum*	0.29	0.00	0.06	0.00	0	0
18	*Cynodon dactylon*	0.00	4.02	0.00	0.68	0	2
19	*Desmodium triflorum*	7.89	3.21	3.04	1.08	4	2
20	*Dichanthium annulatum*	0.58	0.80	0.53	0.09	1	0
21	*Digitaria ciliaris*	0.00	0.40	0.00	0.07	0	0
22	*Dioscorea deltoidea*	0.00	1.20	0.00	0.45	0	1
23	*Elephantopus scaber*	2.92	2.41	3.04	0.69	3	2
24	*Eschenbachia japonica*	0.00	0.40	0.00	0.05	0	0
25	*Euphorbia hirta*	3.51	4.82	0.88	0.79	2	3
26	*Evolvulus nummularius*	5.85	4.82	2.49	1.00	4	3
27	*Fimbristylis dichotoma*	0.58	0.00	0.60	0.00	1	0
28	*Hyptis suaveolens*	0.58	0.00	1.03	0.00	1	0
29	*Imperata cylindrica*	8.48	11.24	18.36	26.79	13	19
30	*Ipomoea purpurea*	0.00	0.40	0.00	0.43	0	0
31	*Isodon* sp.	0.29	0.00	0.07	0.00	0	0
32	*Justicia procumbens*	2.05	0.80	0.54	0.14	1	0
33	*Lantana camara*	1.46	0.80	0.64	0.76	1	1
34	*Mimosa pudica*	8.19	12.05	7.96	11.17	8	12
35	*Momordica charantia*	0.00	0.40	0.00	0.43	0	0
36	*Oplismenus burmanni*	2.34	0.40	1.81	0.09	2	0
37	*Oxalis corniculata*	0.00	1.61	0.00	0.23	0	1

38	*Parthenium hysterophorus*	8.77	12.05	32.76	27.10	21	20
39	*Paspalidium flavidum*	3.80	0.00	1.75	0.00	3	0
40	*Phyllanthus urinaria*	0.58	0.00	0.15	0.00	0	0
41	*Senna occidentalis*	0.00	0.40	0.00	0.06	0	0
42	*Senna tora*	5.26	6.02	4.54	2.21	4	4
43	*Setaria parviflora*	0.58	0.00	0.14	0.00	0	0
44	*Sida acuta*	0.00	1.20	0.00	1.48	0	1
45	*Sida cordata*	7.02	4.82	3.91	1.09	5	3
46	*Sida rhombifolia*	8.77	4.02	3.06	1.82	6	3
47	*Spermacoce alata*	0.58	0.40	0.15	0.05	0	0
48	*Tridax procumbens*	0.29	0.00	0.07	0.00	0	0
49	*Triumfetta rhomboidea*	0.29	0.80	0.06	0.46	0	1
50	*Urena lobata*	0.29	0.40	0.05	0.06	0	0

Appendix 7: Summary of below ground vegetation (germinable seed bank) composition of the study site showing total number of orders, families, genera and species.

S.N.	Orders	Families	Genus	Species
1	Dicots	Apiaceae	1	1
		Asteraceae	5	7
		Caryophyllaceae	1	1
		Euphorbiaceae	1	1
		Fabaceae	1	1
		Malvaceae	1	2
		Oxalidaceae	1	1
		Phyllanthaceae	1	1
		Plantaginaceae	1	1
		Polygonaceae	1	1
		Rubiaceae	1	1
		Solanaceae	1	1
2	Monocots	Cyperaceae	1	1
		Poaceae	4	4

Appendix 8: List of plant species observed in germinable seed bank study along with their family name, presence/absence in above ground vegetation and life history strategies.

S.N.	Name of plant species with author citations	Family	P/A in AGV	L.h.
1	*Ageratum conyzoides* L.	Asteraceae	+	A/F
2	*Ageratum houstonianum* Mill.	Asteraceae	+	A/F
3	*Bidens bipinnata* L.	Asteraceae	+	A/F
4	*Bidens pilosa* L.	Asteraceae	+	A/F
5	*Centella asiatica* (L.) Urb.	Apiacea	-	A/F
6	*Cynodon dactylon* (L.) Pers.	Poaceae	+	P/G
7	*Drymaria cordata* (L.)Willd. ex Roem. & Schult	Caryophyllaceae	-	A/F
8	*Euphorbia hirta* L.	Euphorbiaceae	+	A/F
9	*Fimbristylis dichotoma* (L.) Vahl.	Cyperaceae	+	A/G
10	*Gnaphalium polycaulon* Pers.	Asteraceae	-	A/F
11	*Mazus pumilus* (Burm. f.) Steenis	Plantaginaceae	-	A/F
12	*Mimosa pudica* L.	Asteraceae	+	A/F
13	*Oldenlandia diffusa* (Willd.) Roxb.	Rubiacea	-	A/F
14	*Oplismenus burmanni* (Retz.) P. Beauv.	Poaceae	+	A/G
15	*Oxalis corniculata* L.	Oxalidaceae	+	A/F
16	*Parthenium hysterophorus* L.	Asteraceae	+	A/F
17	*Polygonum* sp.	Polygonaceae	-	A/F
18	*Phyllanthus urinaria* L.	Phyllanthaceae	+	A/F
19	*Senna tora* (L.) Roxb.	Fabaceae	+	A/Ss
20	*Sida cordata* (Burm. fil.) Borss. Waalk.	Malvaceae	+	P/Ss
21	*Sida rhombifolia* L.	Malvaceae	+	P/S
22	*Solanum nigrum* L.	Solanaceae	-	A/F
23	Unknown sp 1	Poaceae	-	A/G
24	Unknown sp 2	Poaceae	-	A/G

(Note: P/A in AGV: Presence/Absence of the individual species in Above Ground Vegetation; '+' indicates presence of species in AGV; '-' indicates absence of species in AGV; L.h.: Life history; A/G: Annual Grass; A/F: Annual Forbs; A/Ss: Annual Sub-shrubs; P/G: Perennial Grass; P/S: Perennial Shrub; P/Ss: Perennial Sub-shrub)

Appendix 9: List of plant species observed in germinable seed bank study along with their total number of germinated seedlings and seed bank density in FG and GE plots.

S.N.	Name of plant species	No. of seedlings		Density (Seed/m^2)	
		FG	GE	FG	GE
1	*Ageratum conyzoides*	1	2	4	8
2	*Ageratum houstonianum*	12	24	51	102
3	*Bidens bipinnata*	0	8	0	34
4	*Bidens pilosa*	10	7	42	30
5	*Centella asiatica*	8	0	34	0
6	*Cynodon dactylon*	13	4	55	17
7	*Drymaria cordata*	1	2	4	8
8	*Euphorbia hirta*	3	1	13	4
9	*Fimbristylis dichotoma*	6	12	25	51
10	*Gnaphalium polycaulon*	2	6	8	25
11	*Mazus pumilus*	1	0	4	0
12	*Mimosa pudica*	20	10	85	42
13	*Oldenlandia diffusa*	4	1	17	4
14	*Oplismenus burmanni*	13	14	55	59
15	*Oxalis corniculata*	134	188	569	798
16	*Parthenium hysterophorus*	8363	4926	35494	20907
17	*Polygonum* sp.	0	1	0	4
18	*Phyllanthus urinaria*	9	6	38	25
19	*Senna tora*	6	15	25	64
20	*Sida cordata*	3	1	13	4
21	*Sida rhombifolia*	2	1	8	4
22	*Solanum nigrum*	0	2	0	8
23	Unknown sp 1	0	1	0	4
24	Unknown sp 2	11	1	47	4

PHOTO PLATE I: FIELD WORK & HERBARIUM TALLY

1. Working site

2. Grazing in study site

3. Making temporary FG plot

4. Vegetation sampling

5. Soil sampling

6. Herbarium tally

PHOTO PLATE II: LABORATORY ANALYSIS OF SOIL

7. Soil packed for oven dry

8. Weighing soil for bulk density

9. Soil pH analysis

10. Soil electrical conductivity analysis

11. Soil organic carbon analysis

12. Soil digestion for nitrogen analysis

13. Distillation for nitrogen analysis

14. Titration for nitrogen analysis

15. Shaking of soil extract in vibrator

16. Filtration of soil extract

17. Soil phosphorus analysis

18. Soil potassium analysis

PHOTO PLATE III: GERMINABLE SEED BANK STUDY

19. Soil spread for germination

20. Thermometer for temp. record

21. Start of plant germination

22. Watering in soil samples

23 Some matured plants from seed bank

24. Soil disturbed for further germination

PHOTO PLATE IV: SPECIES SEEN IN SEED BANK STUDY

25. ***Ageratum houstonianum***

26. ***Bidens bipinnata***

27. ***Bidens pilosa***

28. ***Centella asiatica***

29. ***Cynodon dactylon***

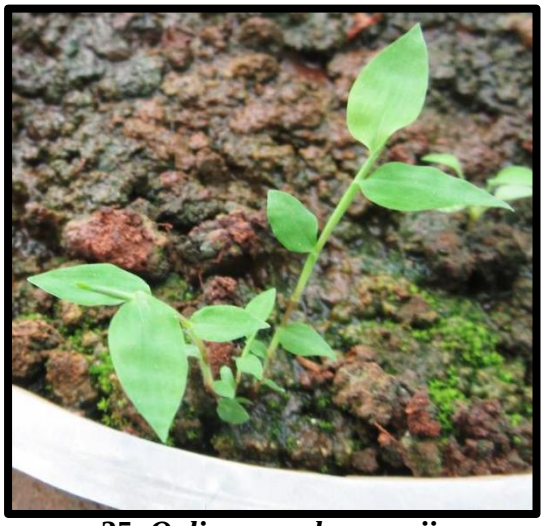
30. ***Fimbristylis dichotoma***

31. ***Gnaphalium polycaulon***

32. ***Mazus pumilus***

33. ***Mimosa pudica***

34. ***Oldenlandia diffusa***



35. ***Oplismenus burmanii***

36. ***Oxalis corniculata***

37. *Parthenium hysterophorus*

38. *Polygonum* sp.

39. *Senna tora*

40. *Sida cordata*

41. *Solanum nigrum*

42. Unknown sp. 3