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**Understory vegetation abundance, diversity and composition in respect to N fertilization in three different stand ages of Poplar plantation in a coastal area of eastern China**

by

Daya Ram Poudel

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## Abbreviations and Acronyms

A/P	:	annual /perennial
BA	:	Basal Area
BD	:	Bulk Density
C/CK	:	Control group
C:N	:	Carbon Nitrogen
F	:	Forbs
G	:	Graminoids
IVI/SIVs	:	Important Value Index/Species important values
LF	:	Life form
N/TN	:	Nitrogen/Total Nitrogen
N0	:	Nitrogen fertilization treatment plot without nitrogen/Control
N1	:	Nitrogen fertilization treatment plot (50 kg N ha <sup>-1</sup> yr <sup>-1</sup> ).
N2	:	Nitrogen fertilization treatment plot (100 kg N ha <sup>-1</sup> yr <sup>-1</sup> )
N3	:	Nitrogen fertilization treatment plot (150 kg N ha <sup>-1</sup> yr <sup>-1</sup> )
N4	:	Nitrogen fertilization treatment plot (200 kg N ha <sup>-1</sup> yr <sup>-1</sup> )
NH <sub>4</sub> <sup>+</sup> -N	:	Ammonium nitrogen
NMDS	:	Non-metric Multidimensional scaling
NO <sub>3</sub> <sup>-</sup> N	:	Nitrate nitrogen
PerMANOVA	:	Per mutational Multivariate Analysis of Variance
r/Rho	:	Correlation coefficient
S	:	Shrubs
Sd	:	Standard deviation
SE	:	Standard error
SIVs	:	Species Important Values
SOC	:	Soil Organic Carbon
TOC	:	Total organic carbon
V	:	Vine
Yrs	:	Year



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## **Summary**

### **Significance**

Understory species account for largest proportion of floristic diversity and highest ecological significance in forest ecosystem. The herbaceous layers have considered more sensitive groups to change the environmental factors. So large scale plantations of fast growing tree species has been recognized as an important issue for long term sustainability of understory diversity in plantation ecosystem. The fast growing plantation have been said to exhaust resources and favor selective invasive that might have considerable threat to understory richness and diversity. However, little is known about how overstory stand development affect on occurrence, composition and abundance of species of herbs layer in different stand age of plantation. Consequently, proper understanding of understory dynamics of plantation forest is very vital to determine the distribution patterns and know the ecological process which ultimately helps for planning to maximize future biodiversity opportunities in plantation and address the issues for sustainable management of plantation forest.

Most of the previous studies have suggested that nitrogen (N) additions reduces plant diversity but different functional traits behave differently with different level of N and this also depends on the stand age of plantation. Although addition of Nitrogen (N) in different stand age of plantation has effect on understory herbaceous composition, diversity and abundance, but dynamics of these two factors in combination have been studied rarely.

### **Objectives**

So we compared abundance, diversity and composition of herbaceous plants in different stand age of poplar plantation in coastal region of China. So overall purpose of this study was to report abundance, diversity and composition of herbaceous in different stand age of poplar plantation and identify the environmental factors that may responsible for variation in understory diversity among the different stand age of poplar plantation. The study also reported effect of N on composition, richness and diversity of understory herbaceous in different stand age of plantation in Northern Jiangsu, China.

## **Methods**

To understand the influence of Poplar stand development on understory vegetation, we sampled 8, 12, and 18-year stand age of the plantations, each with four replicates. The experimental plots were established and N treatments were carried out in July 2012. To avoid spatial autocorrelation, stands of the same age were located at least 5 km from each other and spatially interspersed with distributed at minimum distance of 125 m for each sampling unit within each stand. In each of the sample stand, represented by twenty plots of 25×30 m size was considered as sampling unit. We systematically placed five quadrants of 1 ×1m to investigate herbs and five quadrants of 2×2m for shrubs .We examined 60 plots to measure the trees characteristics, 300 quadrates for shrubs and herbs.

Replicated Block Design was used in this experiment with five N treatment with four replicates established i.e. treatments; control (N0-without N added), N1 (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N2 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N3 (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and N4 (200 kg N ha<sup>-1</sup> yr<sup>-1</sup>) respectively in those 20 plots of 25 × 30m for vegetation survey in each stand age of plantation. The fertilization was applied 6 times per year for each treatment plots in the whole growing season and has applied since July 2012.

### **Measurement of Vegetation and Soil**

We have collected the vegetation and soil data on 2015 during the periods of growing season (June and July) for the area. Then we systematically placed five quadrants of 1m ×1m to investigate herbs and five quadrants of 2m×2m for shrubs in each plot of 25×30m. We collected the data on shrubs and herbs layer. In each plot, the diameter at breast height (DBH) and total height of each individual tree were recorded. All shrubs and herbs, we recorded with the species name, measured average height, counted the plant and estimated the cover. We classified all understory species into three groups by growth forms i.e.; shrubs (S), forbs (F), vines (V), graminoids (G). The collected data on bryophytes and woody seedling were not much considered for analysis due to their poor abundance. We measured cover of herbaceous species in each plot and percent cover of herbs which were visually estimated.

Soil samples were separately collected from two layers at the depth of 0–15 cm, 15-30cm layer for each sampling plot analyze total soil organic carbon (SOC) and total Nitrogen (TN) soil pH, soil bulk density and soil moisture.

## Results

Here, we showed understory species richness and composition differed significantly among the stand age of plantation which were strongly correlated with Soil Organic Carbon and Nitrogen (C: N) ratio but not correlated with any overstory parameter. Total herbaceous richness decreased, while total abundance increased significantly with the age; however evenness increased gradually with age of the stand but diversity did not show the any obvious trend. For the shrubs, diversity decreased significantly with stand age which was just opposite for abundance. Specifically, the richness, abundance and composition of forbs was changed significantly among the three stand age of the plantation, however these were not change significantly for vine and graminoids. The result also revealed that young plantation was more favorable for forbs and graminoids diversity while middle age plantation for the vine. The first 10 dominant species accounted for 66% Species Important Values (SIVs) for 8-years, 81 % of SIVs for both 12 and 18-years old plantation.

Result also revealed that there were no significant difference between N-treatment to diversity (Richness, Shannon, Dominance and Evenness) during the study period, however we found positive response of herbaceous diversity with increased availability of N in younger plantation and negative response in mature plantation affecting the species evenness in all stand age. In general, forbs and vine were benefitted every time, while graminoids suffered from the N treatment. The diversity of forb and vine was low at control, forbs were increased upto maximum at N3 to N4 ( $150\text{-}200\text{kg N}^{-\text{ha}}\text{yr}^{-1}$ ) treated plots where graminoids lost. Vine diversity saturated earlier than forbs at N3 ( $150\text{kg N}^{-\text{ha}}\text{yr}^{-1}$ ) consistently declined towards very high N ( $200\text{kg N}^{-\text{ha}}\text{yr}^{-1}$ ) treated plots. Non-metric Multidimensional Scaling (NMDS) exhibited obvious pattern that herbaceous groups gradually became dissimilar within plots of each stand but more similar between the different stand age of the Poplar plantations showed clear pattern of shifting the community dynamics with increased availability of N. The density of graminods and vine was varied due to N fertilization than forbs.

## **Conclusions**

Species richness decreased markedly with increased growth and age showing young plantation support more herbaceous diversity compared to the mature is comparable to many studies however this depends more on extent of competitive interaction among the herbaceous for the available resource than the direct effect of the overstory.

Ours result concluded that biodiversity increase with N addition in younger plantation and decrease in mature plantation with associated changes in soil factors. This also indicated that with increase N availability, diversity increased at area with higher soil nutrients and lower above ground woody biomass, and diversity decreased at area with lower soil nutrient content and higher above ground woody biomass and the trend was opposite for species richness. The overall decrease in biodiversity due to increased stand age of plantation was more prominent than increase in biodiversity due to N addition but this response may vary with functional groups. The study clearly indicated that the response of N on the different indices of herbs in the plantation primarily influenced by stand age of the plantation and then level of nitrogen which was determined by deliberate equilibrium between forbs and graminoids.



# 1 Introduction

## 1.1 Biodiversity and Nitrogen fertilization

Almost 6.6 % of the global forests are plantations, out of them East Asia plantation forest accounted 35 % of the total, most of these in China (FSC, 2012). These are principally established for commercial purpose to get timbers and others wood products along with variety of ecosystem services (Pawson et al., 2013). Plantation forest have been called “biological desert” (Stephens & Wagner, 2007) and some even argue that “plantation are not forests” but both cases it is misinformed (Paquette & Messier, 2009). The impact of plantation forests on biodiversity will depend on what land use they replace (Pawson et al., 2013). The numbers forest policies and laws in China have enabled the environment for rapid increase in forest cover, particularly focusing on plantation of fast growing species (Williams, 2015) and such plantation have been recognized the greatest threat to diversity (Axmacher & Sang, 2013; Weih et al., 2003). Despite their relatively small extent at global level, plantation are the focus of much debate regarding forest sustainability and biodiversity conservation (Pawson et al., 2013).

The vegetation scientist uses “herbaceous stratum”, herbaceous understory”, “ground layer”, “ground vegetation” have commonly used as synonyms of herbaceous layer (Gilliam, 2007), among them herbaceous /herb layer is most frequently use (Roberts & Gilliam, 2003). The most inclusive definition of the herbaceous basis of height as the forest layer composed of all vascular species that are 1 meter or less in height. The studies of the ecology of the herbaceous layer of forest have been carried out over nearly half a century (Gilliam, 2007). The most of earlier studies focused on structural aspect of the herbaceous layer (Zavitkovski, 1976), some others associated in ecosystem process (Siccama et al., 1970) and other many have related to experimental studies to investigate the effect of artificial nitrogen deposition on the structure and function of forest ecosystem (Dirkse et al., 1991) and recently paper have focused on the effects of N deposition on forest plant diversity (Gilliam, 2006; Xiankai et al., 2008).

Diversity comprises a broad spectrum of biotic scales and can generally be described as the number of entities, the evenness of their distribution, the differences in functional traits and their interactions (Assessment & Assessment, 2005; Díaz et al., 2006). Diversity as mathematical

measure shows the community composition as whole but it does not show how particular species behaves in particular set of species composition. Besides the numbers of individuals of species, their functional group and their life form is important to make the complete sense of species community and their relation. A functional group encompass a set of species with similar morphological, physiological and phonological traits and these groups provide similar ecosystem services and react similar to environmental changes (Hooper et al., 2005).

Experimental studies demonstrate that increase in N availability often leads to increase primarily production and decrease plant diversity (Silvertown, 1980). Changes in soil N will have considerable impact on most of soil properties and ultimate to the overall ecosystem. Excess N deposition is potential serious threat to biodiversity of many groups of organism, including diversity of plants (Clark & Tilman, 2008). Productivity-dependent biotic-homogenization hypothesis postulated that N enhancement increase  $\beta$  diversity at low-productivity sites but reduce  $\beta$  at sites of higher productivity (Chalcraft et al., 2008; Chase, 1999; Chase et al., 2000). Biodiversity is declining at unprecedented rate and on a global scale..

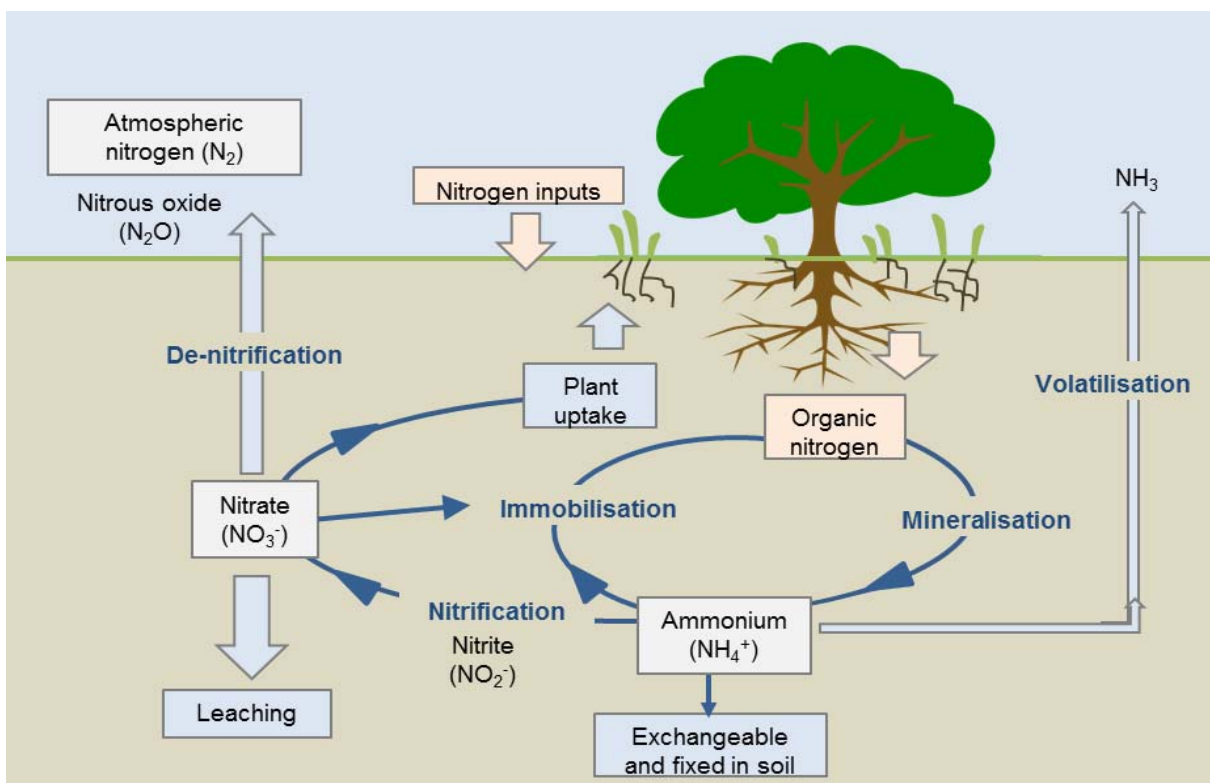


Figure 1- 1 important reaction of nitrogen on soil

Biodiversity is declining at unprecedented rate and on a global scale. Human attention in the global nitrogen (N) cycle has also been identified as a contributor to decline in biodiversity (Bobbink et al., 2010; Lu et al., 2010). The addition of N reduce plant diversity within a plot by average of 25% and most case enhance  $\beta$  diversity (Chalcraft et al., 2008). To our knowledge, there has been no report to date on the effects of N addition on forest plant diversity in tropical or subtropical areas (Bobbink et al., 2010; Xiankai et al., 2008). Currently it is still unclear how N addition affect biodiversity at larger scale (Chalcraft et al., 2008)

## **1.2 Research objective and hypothesis**

First part of this paper focused to analysis of abundance, diversity and composition of herbaceous in different stand age of poplar plantation and identify the environmental factors that may responsible for variation in understory diversity among the different stand age of poplar plantation. The study hypothesize for the poplar forest in coastal region of Northern Jiangsu china that increasing the stand age, understory species richness and abundance decrease due to growth of the stands which is depended on change in soil C: N ratio in the plantation. Furthermore, we hypothesize that the species composition varies according to stand age due to decrease in soil nutrient but expect a notable overlap particularly in widespread, generalist species among the stands of the plantation.

The second part of the report showed the effect of N on composition, richness and diversity of understory herbaceous in different stand age of Poplar plantation. We hypothesized that Nitrogen addition has been observed to change plant species composition and to reduce species richness and diversity in herbaceous vegetation as (i) it dependent on stand age (ii) level of nitrogen addition.

### 1.3 Conceptual frameworks

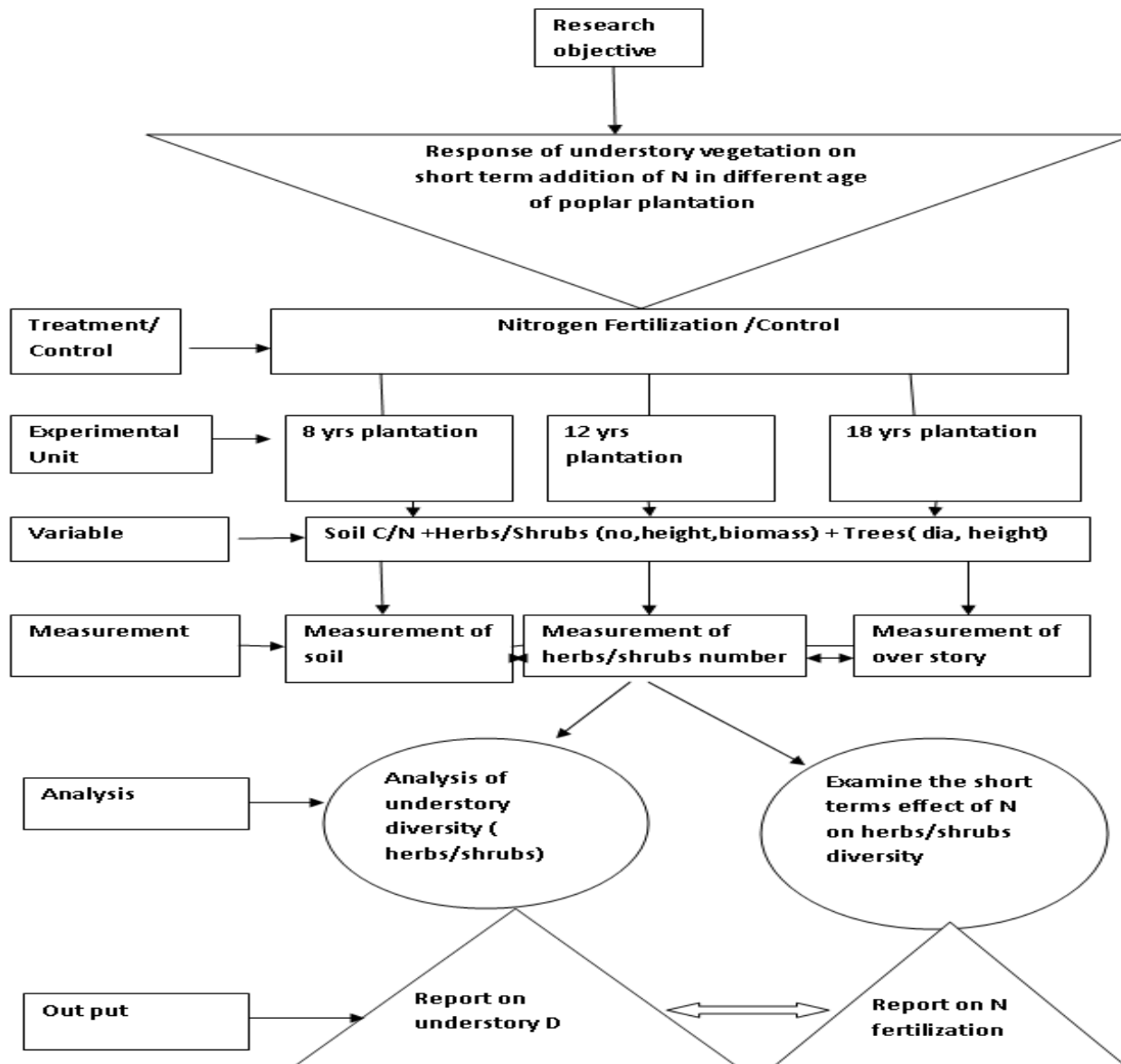


Figure 1- 2 conceptual framework

### 1.4 Limitation of the Study

The whole analysis was based on only one seasonal data might be affected the overall generalization for the entire year. Moreover, we also did not measure some others soil and overstory variables due to limited time and other resources which might be useful in herbaceous study, and likely affected the results in general.

## 2 Understory vegetation abundance, diversity and composition dynamics of Poplar plantations in east coast of China

### 苏北沿海杨树人工林林下植被丰富度、多样性及其动态

摘要：大规模的速生树种的人工林被认为是林下植被多度和多样性的威胁之一。林下植被物种占据植物多样性的大部分。大规模发展速生人工林被认为是林下植物多样性的最大威胁之一。然而，上层林冠对草本层在不同年龄人工林的影响程度被了解得很少。因此，我们比较了在中国东海岸不同年龄杨树人工林的草本群落多度，多样性和组成。结果表明，林下物种多度和组成在受土壤有机碳氮比影响的人工林中有显著的差别。总的草本多度显著下降，而总的丰富度随着立地年龄上升。而灌木，多样性随着年龄有显著差异。非禾本科草本的多度和组成在三个林龄的人工林中显著变化，而藤本和禾本科草本不显著。结果同样表明，幼林龄更有利于非禾本科和禾本科草本的多样性而中林龄有利于藤本。这个发现和一些优势草本（如 *Roegneria C* and *Duchesnea indica*）下降趋势一致。首先在八年生林龄 10 个优势物种解释了 66% 的植物重要值，12 和 18 年生的共同解释了 81% 的植物重要值。物种多度随着林龄生长而显著下降，表明幼林龄比起成熟林更有利草本多样性

#### *Abstract*

Understory species accounts for largest proportion of floristic diversity but have highest ecosystem threat than others plants species in the forest. Large scale plantations of fast growing tree species have recognized as one of the greatest threat to understory richness and diversity. However, the extent of impact of overstory on composition and abundance of species of herbs layer in different stand age of plantation is poorly understood. So we compared abundance, diversity and composition of herbaceous species in different stand age of poplar plantation in coastal region of China. Here, we showed understory species richness and composition differed significantly among the stand age of plantation which were strongly correlated with Soil Organic Carbon and Nitrogen (C: N) ratio but not correlated with any overstory parameter. Total herbaceous richness decreased, while total abundance increased significantly with the age; however evenness increased gradually with age of the stand but diversity did not show the any obvious trend. For the shrubs, diversity decrease significantly with stand age which was just opposite for abundance. Specifically, the richness, abundance and composition of forbs was

changed significantly among the three stand age of the plantation, however these were not change significantly for vine and graminoids. The result also revealed that young plantation was more favorable for forbs and graminoids diversity while middle age plantation for the vine. The finding concurs the patterns of decreasing the importance values of certain dominant herbs (eg *Roegneria C* and *Duchesnea indica*) with increasing the age of over story gradually favor some invasive species (e.g.*Erigeron annus L*, *Eupatorium andenophora Speng*). The first 10 dominant species accounted for 66% Species Important Values (SIVs) for 8-years, 81 % of SIVs for both 12 and 18-years old plantation Species richness decreased markedly with increased growth and age showing young plantation support more herbaceous diversity compared to the mature comparable to many studies however this depends more on extent of competitive interaction among the herbaceous for the available resource than the direct effect of the overstory.

Keywords: Herbaceous diversity, Invasive species, Poplar plantation, Species Important Values, Understory

## 2.1 Introduction

Understory vegetation harbors the majority of plant diversity, may contain up to 90 % of plant species in temperate deciduous forest provides a crucial role in maintaining the structure and function of forest ecosystem facilitating energy flow and nutrient recycling (Gilliam, 2007). Despite of low ( 1–2%) contribution of the understory vegetation to aboveground biomass in forest ecosystems , its role in nutrient cycling is over proportionate to its biomass (Bolte et al., 2004) due to its higher litter quality than tree foliage and decompose twice as rapidly (Muller, 2003) contributing higher nutrient concentration (Gilliam, 2007) and lower concentration of cellulose and lignin (Melillo et al., 1989) in herbaceous ecosystem. All these characteristics could make herbaceous layer more important in respect to ecosystem function.

Understory vegetation composed of herbs, ferns, shrubs and sub-canopy trees, they typically represent the largest component of temperate forest plant diversity (Hart & Chen, 2006), is highly dynamics in relation to forest stand development. Overstory stand parameters influences the dynamics of understory either by alternating light quality and quantity through shading or by altering the nutrient dynamics (Bartemucci et al., 2006; Hart & Chen, 2008; Légaré et al.,

2001; Loumeto & Huttel, 1997) more particularly by reducing the availability nutrient and water (Bartels & Chen, 2013). Higher tree layer diversity might promote greater herbaceous diversity either by increasing environmental heterogeneity (Beatty, 2003) or by creating favorable environmental condition (Vockenhuber et al., 2011) which is more obvious in younger stage of plantation. For example, deep rooted overstory plant take nutrients unavailable to shallow-rooted understory plants and deposit them on the forest floor which then readily utilized by understory shallow rooted plants (Bartels & Chen, 2013). More above ground biomass produced by poplar trees with increase stand age accumulates and storage more nutrients from the ground and it was likely to return fewer nutrients to soil (Ge et al., 2015) might increase the competition for the available resource in mature stand of poplar plantation. Understory composition shift to shade tolerant species following the canopy closure (Hart & Chen, 2006). The overstory may creates understory condition shaded, cooler and moisture which facilitate the establishment of shade tolerant plant species (Hylander et al., 2005; Pugnaire & Luque, 2001). Floral diversity declines with age, governed by competition for light and the loss of shade intolerant species (Brooks et al., 2012).

Plantation forest are becoming an increasingly important component of global forest cover (Bass, 2004; Zou et al., 2015) but our current understanding about vegetation dynamic in plantation forest remain incomplete because most of those studies of interactions between the overstory and understory have mostly limited to species of the herbaceous layer (Gilliam & Roberts, 2003) more particularly in natural forest and few studies have examined interactions among the multiple vegetation layer (Bartels & Chen, 2013). Herbaceous richness to that of overstory was found positively correlated but the relationship varies with forest types (Gilliam, 2007). The relative importance of mechanism that accounts for the effect of overstory on herbaceous diversity has often been discussed much but this mechanism rarely has been the subject of formal experimentation (Barbier et al., 2008). Furthermore, little attention has been given in China to explain the effect of environmental factors on floristic characteristics and biodiversity patterns at the local or regional scale (Liu et al., 2009) and more particularly, poor focus have given on pattern on understory diversity and it's dynamics in plantation forest.

For the poplar forest in coastal region of Northern Jiangsu china, we hypothesized that increasing the stand age, understory species richness and abundance decrease due to growth of the stands

which is depended on change in soil C: N ratio in the plantation. Furthermore, we hypothesize that the species composition varies according to stand age due to decrease in soil nutrient but expect a notable overlap particularly in widespread, generalist species among the stands of the plantation. In the entire plantation, limited resource availability with increased stand was created more competition for light and nutrient in younger plantation, may favoring to forbs reducing the competitive capability of graminoids groups (Seastedt et al., 1991; Wedin & Tilman, 1993). When the plantation increased towards the mature is associated with decrease in density of the plants and lesser suppression of forbs in this stage gradually may favor to higher richness of graminoids in mature plantation.

Each plant species is able to exist and reproduce successfully only within a definite range of environment condition in which observed range is always smaller than the potential (Good, 1931; Good, 1953). A numbers of soil properties have identified that affects herbaceous layer diversity for instances soil ph, soil moisture, nutrient availability (Vockenhuber et al., 2011) and those are subject to change with overstory tree layer diversity (Wulf & Naaf, 2009). For instance, (Laughlin et al., 2007) proposed in structural equation modeling analysis of pinus penuldata indicated that species richness in the forest was lowest where the forest overstory was densest explaining profuse indirect effect of soil organic matter, soil N and understory cover. However, many authors have indicated poor association among environmental characteristics and vegetation in the western United States because many species having their wide eco-logical amplitude (Jensen et al., 1990). Overstory was relatively insensitive to soils properties and environment gradients of heat, moisture and nutrients (Grigal & Arneman, 1970).

The questions here we attempt to answer with this study are(1) how overstory stand development affect on occurrence, compositions, abundance, distribution pattern of understory herbaceous in the plantation? (2) To what extent over story stand parameter co-related to herbaceous diversity in poplar plantations? (3) What are the major environmental factors and associated mechanism affecting the diversity and abundance in the herbaceous in different life form along with stand development in the plantation. So overall purpose of this study was to report abundance, diversity and composition of herbaceous in different stand age of poplar plantation and identify the environmental factors that may responsible for variation in



understory diversity among the different stand age of poplar plantation in Northern Jiangsu, China.

## 2.2 Materials and Methods

### 2.2.1 Study area

The study was carried out at Dongtai Forestry Farm (102 ° 49'E, 32 ° 52'N) of Jiangsu Province in eastern China (Figure 2- 1). It is located in the transition zone from a north subtropical to warm temperate climate with forest area around 2000 ha. The mean annual temperature is 13.7 °C and rainfall approximately 1051 mm and relative humidity 88.3%. It lies along the lower reaches of alluvial plain of the Yangtze River and has desalted meadow sandy loam soil. The forest coverage is around 85% dominated by Poplar plantations.

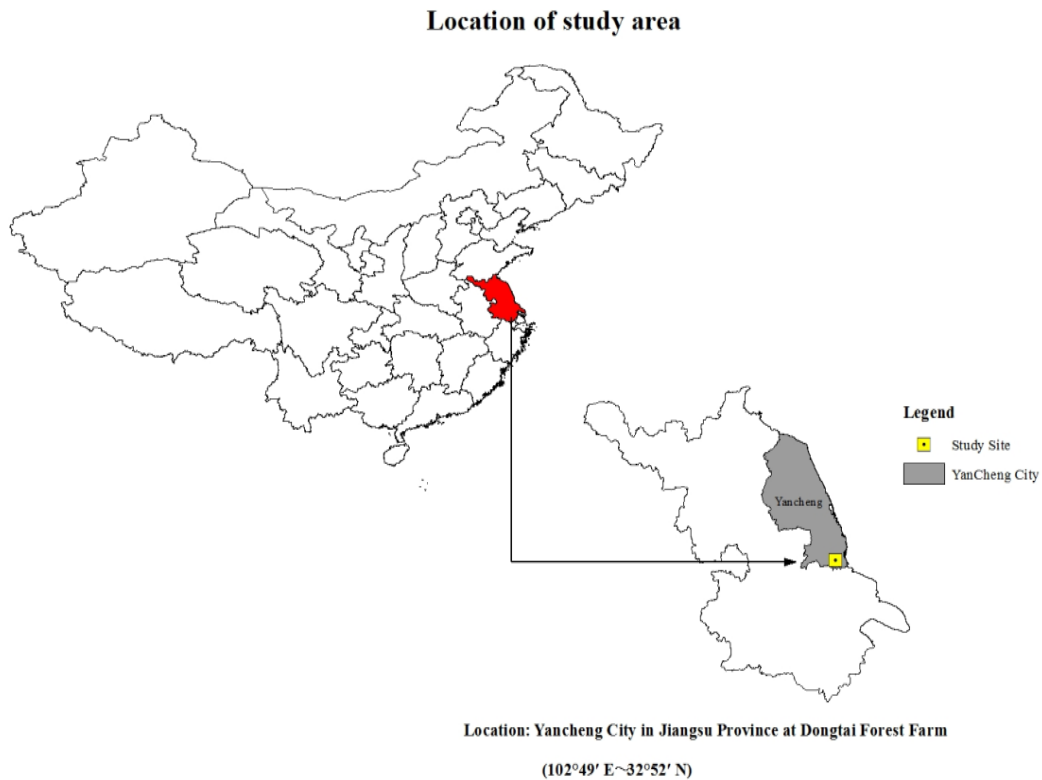


Figure 2- 1 Map of the study area

This area is nitrogen (N) limited (as less than 55 g total N/Kg of soil). The afforestation spacing of the plantation was 3 ×5m. Understory species were as follows: *Roegneria C* and *Miscanthus sinensis Anderss* (grass); *Oxalis corniculata* and *Torilis japonica* (herbs) and *Cyclosorus*

*acuminatus* (ferns). In this area, three age poplar (*Populus euramericana*) plantations i.e. 8-years old (young stage), 12-years old forest (middle age), and 18 years old (mature stage) were selected for the study. The main properties of soil and vegetation characteristic in the study area are given in Table 2- 1.

Table 2- 1 vegetation structure and soil properties of the three different ages of poplar plantations at Dongtai Forestry Farm

Dimensions	Characteristics		8-yrs	12-yrs	18-yrs	
Understory	Cover (%)	Graminoids	26.28±10.8	16.63±5.05	28±5.6	
		Forbs	37.9±9.90	34.125±4.32	39.625±4.30	
		Woody plants	.....		2	
		Vines	9.63±4.2	32.25±4.4	4.88±1.7	
		Bryophyte	.....		0.5	
		Total	74.8±12	79.25±12.3	85.5±3.71	
	Richness (5m <sup>2</sup> )	Herbs	21.64±1.45	19±0.6	15.87±1	
		Shrubs	1.25±0.10	.93±0.19	.62±0.07	
		Total	22.89±1.36	19.93±0.54	16.5±.46	
	Diversity (H)	Herbs	2.27a±0.34	2.32a±0.09	2.29a±0.23	
		Shrubs	1.47a±0.05	1.10b±0.14	0.440c±0.04	
	Density /m <sup>2</sup> )	Shrubs	2.63±0.69	3.63±0.68	2.88±0.71	
		Herbs	22.19±1.17	19.26±2.13	14.95±0.66	
	Proportions (%)	Graminoids		37.7	27	22
		Forbs(annual)		14.8	23.6	11.5
		Forbs(perennial)		44	27	47.5
		Seedling	...		1.3	3
		Vines		6	20.8	7
		Fern			0.5	8.5
		Soil (0-30cm)				
Soil properties	SOC gm/kg		14.47±0.1685	13.83±0.2005	13.08±0.37	
	BD( gm/cm <sup>3</sup> )		1.18±0.072	1.35±0.11	1.39±0.09	
	Soil pH		8.14±0.033	8.23±0.0275	8.41±0.023	
	Total N(gKg <sup>-1</sup> )		0.69±0.03	0.99±0.0265	1.23±0.08	
	Moisture (%)		0.235±0.006	0.245±0.0045	0.28±-.03	
	C:N		9.6±0.27	10.53±0.225	10.38±0.115	

BA=Basal Area; DBH=Diameter at Breast Height; SOC =Soil Organic Carbon; BD=Bulk Density; C:N=ratio of Soil Organic Carbon and total Nitrogen

### 2.2.2 Vegetation Sampling and Data Collection

To understand the influence of Poplar stand development on understory vegetation, we sampled 8, 12, and 18-year stand age of the plantations, each with four replicates. To avoid spatial autocorrelation, stands of the same age were located at least 5 km from each other. In each of the sample stand, represented by twenty plots of 25×30 m size. So we had 60 sampling units altogether and spatially interspersed with distributed at minimum distance of 125 m for each sampling unit within each stand.

Field studies were carried out in July 2015, the time of peak diversity for the region. Middle of each plantation; we recognized 5 plot of 25×30 m plot considering 4 replications. Within each 25×30 m plot; we further systematically placed five quadrants of 1 ×1m to investigate herbs and five quadrants of 2×2m for shrubs in each plot (Figure 2- 2). We examined 60 plots to measure the trees characteristics, 300 quadrates for shrubs and herbs.

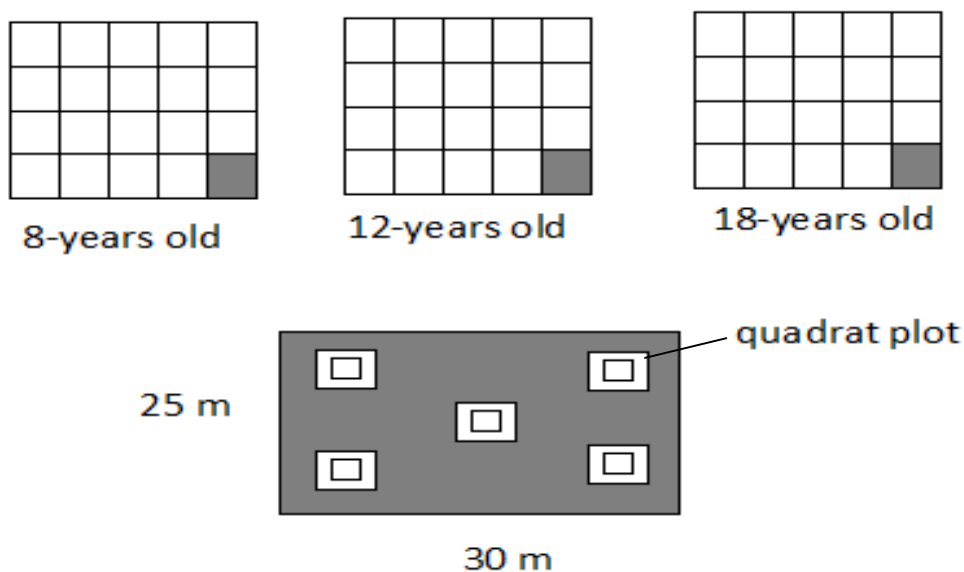


Figure 2- 2 Design of experiment for herbaceous sampling

We collected the data on shrubs and herbs layer. The understory plants including herbs is defined here as all plants less than 1.3 m in height, shrubs as any species with height between 1.3 to 4 m and tree above 4 m in height (Seedre & Chen, 2010). In each plot, the diameter at breast height (DBH) and total height of each individual tree were recorded. All shrubs and herbs presented in a quadrant, we recorded the species, name, measured its average height, counted the plant and estimated the cover. Cover of plants was estimated visually (Qian et al., 2003; Wulf & Naaf,

2009). We measure the height of herbs using a tape-ruler as these herbs most often were in groups. So, 1-3 measurement in each sub-plot was adequate to make measurement precise and accurate. The DBH of trees and shrubs were measured using Calipers and were used to calculate volume and SIVs.

After collection of data, we considered two herbaceous traits most applicable to study area; one was related to species life form (forbs, graminoids and vine) and another was life span (annual, biennial and perennial) (Verma et al., 2014; Xu et al., 2015). So we classified all understory species into three groups by growth forms i.e.; shrubs(S), forbs (F), vines (V), graminoids (G), and fern and seedling were considered in others groups (O). We then we verified traits and scientific name of some unknown specimen collected during the field (Yanqiu, 2008).

### 2.3 Data Analysis

Herbaceous species richness and diversity based on the data in the plots was expressed as species number per 5m<sup>2</sup> and 20 m<sup>2</sup> for the shrubs. The overall component of  $\alpha$ -diversity (Wu et al., 2013) species richness(S), Diversity (Shannon H), Species Dominance (D) and Pileou's evenness index (J) for each plots and also for each functional groups of herbs and for each plantation calculated as follows.

S= richness (Wang et al., 2006; Zeng et al., 2010)

$$H' = -\sum \frac{n_i}{N} \log \frac{n_i}{N} \quad (\text{Shannon, 1963})$$

J=H'/ln (S) (Guang-Long et al., 2015)

Where H = Shannon and Wiener index of general diversity,  $n_i$  = No. of individuals of the species, N = No. of individuals of all species, S=Species richness of plot and each plantation, J = Pileou's evenness

The relative importance (dominance) of species will be calculated for each herbaceous as IVs for herbs =  $(Ra+ Rh+ Rc)/3$  (Zhang et al., 2005; Zuo et al., 2012) where, Ra is relative abundance, defined as the total number of individuals of a species as a percentage of the total number of individuals of all species; Rh is relative height, defined as a species' average height as a percentage of the sum of average heights of all species; Rc is relative coverage, defined as a

species' average coverage as a percentage of the sum of average coverage of all species. Additionally, species rank–abundance plots were calculated to compare species dominance patterns of understory species between study sites. For each understory vegetation layer, species richness was calculated as the number of species recorded in each plot; the cover was the sum of all species covers for each stand age and for each life form calculated using a modification of the Braun-Blanquet approach (Gendreau-Berthiaume et al., 2015; Hnatiuk, 1969).

We have choose the NMDS ordination of herbaceous layer data to characterize difference in herbaceous community composition among the plot and correlated community composition with environmental variable (McCune et al., 2002). NMDS primarily endeavors to represent sample and species relationships as faithfully as possible in a low dimensional space (Gauch, 1982) and well suited to handle non-normal and non-continuous data (McCune et al., 2002). We used log transformation of data of plant richness, abundance and others site variables to improve normality of measured variables for the NMDS analyses. The difference in herbaceous community were calculated by per mutational Multivariate analysis of Variance ( PerMANOVA) (Hammer et al., 2001) and illustrated by NMDS of Bray Curtis distance (Bray & Curtis, 1957). Correlation coefficient (Pearson's /Rank) was calculated to compare explanatory variables (TOC, TN, C:N etc) to response variable (NMDS axis values, diversity, density, cover). All analysis was performed in statistical package SPSS 18.

## **2.4 Results**

### **2.4.1 Floristic and Forest structure**

The overall mean DBH and Height of the over story plantation were 25.23 cm and 16.30 m, respectively. In case of basal area, 18-years old plantation represented highest (48.61%) of the total, and followed by 8-years (28.97%) and 12-years old plantation (22.42%). There was significant different average basal area, average diameter and average height between the plantation of three different age ( $p < 0.05$ ) (Table 2-2).

Table 2- 1 Summary of ANOVA of over storey trees characters between three stand ages of plantation

Plantation	Mean Basal area(m <sup>2</sup> )	Mean DBH(cm)	Mean Height(m)
8- Yrs	1.84±0.082c	18.044±0.449c	13.38±0.183b
12- yrs	1.42±0.078b	25.962±0.184b	17.68±0.221a
18 -yrs	3.08±0.059a	31.7125±0.034a	17.84±0.036a
F – value	134.9	399.52	228.09

Figures with the same alphabetic suffix indicate no significance differences using multiple comparisons (Tukey,  $\alpha > 0.05$ )

A total of 113 plant species were recorded from the study plots of 4.75 ha representing 83 genera and 45 families (Figure 3B) of which 96 species were herbaceous, belonging to 34 families and 71 genera, 16 were shrub species, belonging to 10 family and 10 genera.

#### 2.4.2 Life form composition

The analysis of herbaceous species composition showed that entire plantation forbs were dominated in richness but ferns were very poorly represented in the areas (table 2- 1).

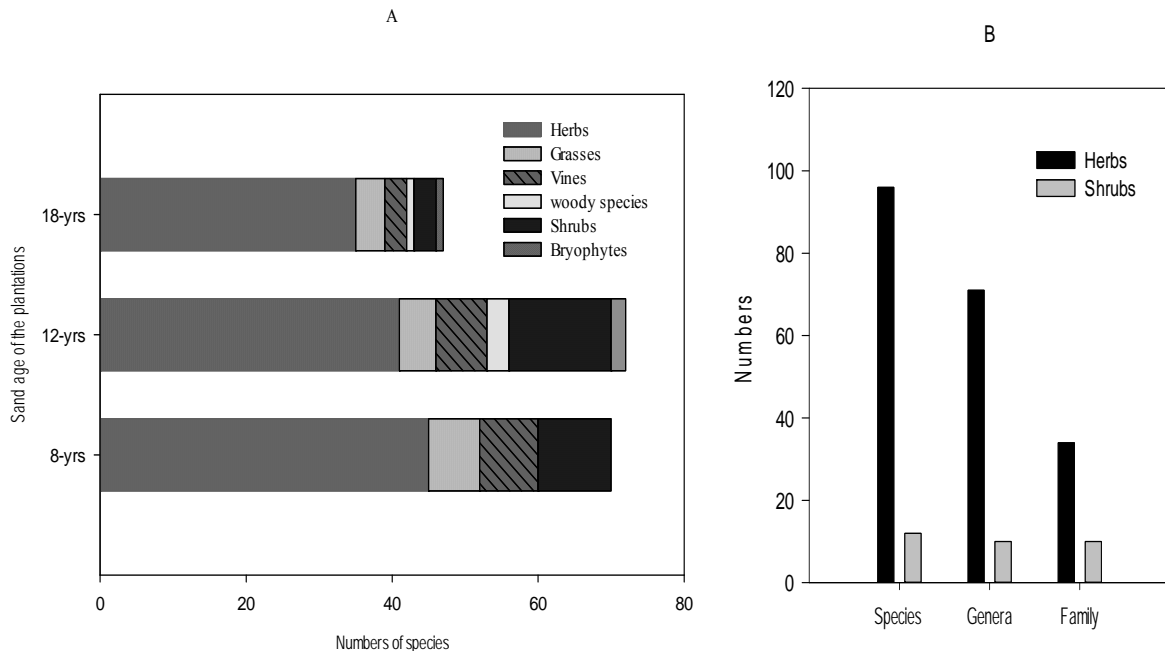


Figure 2- 3 Classification of understory species. Fig A showed the numbers of species in different life form and Fig B showed the respective Genera and Family of understory species.

As shown in the (Figure 2- 3 and Table 2-1), there was clear under-representation of shrubs and others herbaceous plant in mature plantation while in the younger plantation (both 8 and 12-years old) comparatively higher representation, and there was a more even distribution of species among life form. Species composition in the herbaceous layer among the plantation did vary significantly (PMANOVA,  $p=0.0005$ ) (Table 2- 3).

Table 2- 2 Summary of one way PMANOVA between the different stand age of the Poplar plantations (permutation N=9999)

Factors	Sum of square		F-value	p(same)
	Total	Within the groups		
Graminoids	0.165	0.064	5.152	0.0155*
Forbs	0.21	0.098	7.04	0.019*
Vines	0.564	0.398	1.872	0.191
Total herbaceous	3.28	1.718	4.11	0.0005*

Significant p value in herbs composition in different three stand age ( $p<0.05$ ) are indicated by asterisks (\*).

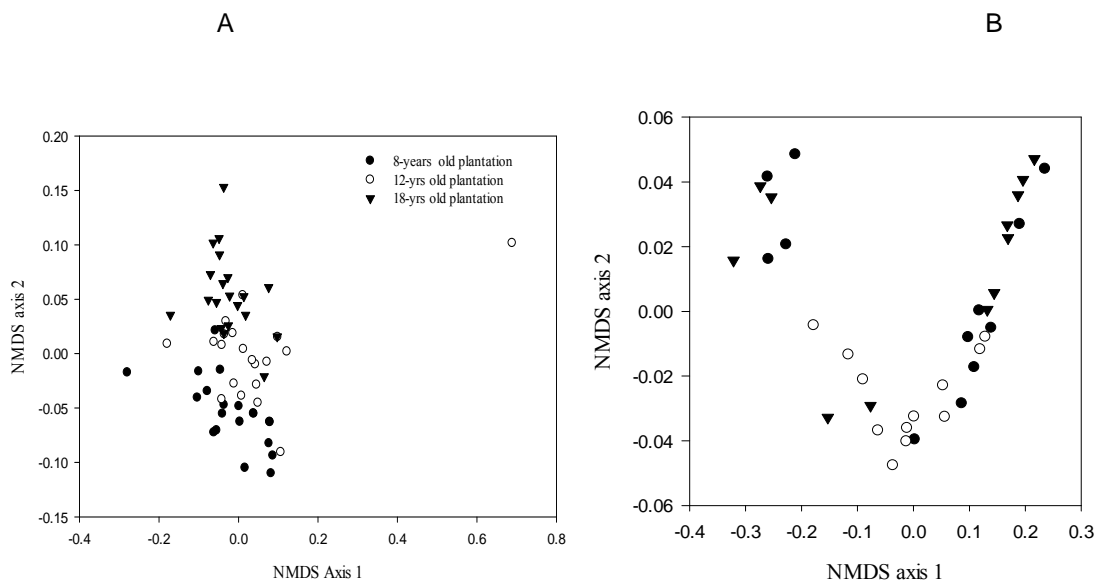


Figure 2- 4 NMDS plot ordination based on Bray-Cutis similarities Fig A showed overall herbaceous composition in different stand age and the figures B showed the response of herbaceous life form (graminoids, vine and forbs) based on density and richness in every three stand types with four replication. Plots apart have greater differences in herbaceous composition

The pattern also obvious in NMDS ordination, which reflected compositional change associated with stand age on axis 2. The proportion of density, height, cover and overall SIVs for all life form showed the similar pattern as all slightly decreased with age besides proportion of density and cover of was highest in 12- year plantation (Figure 2- 5).

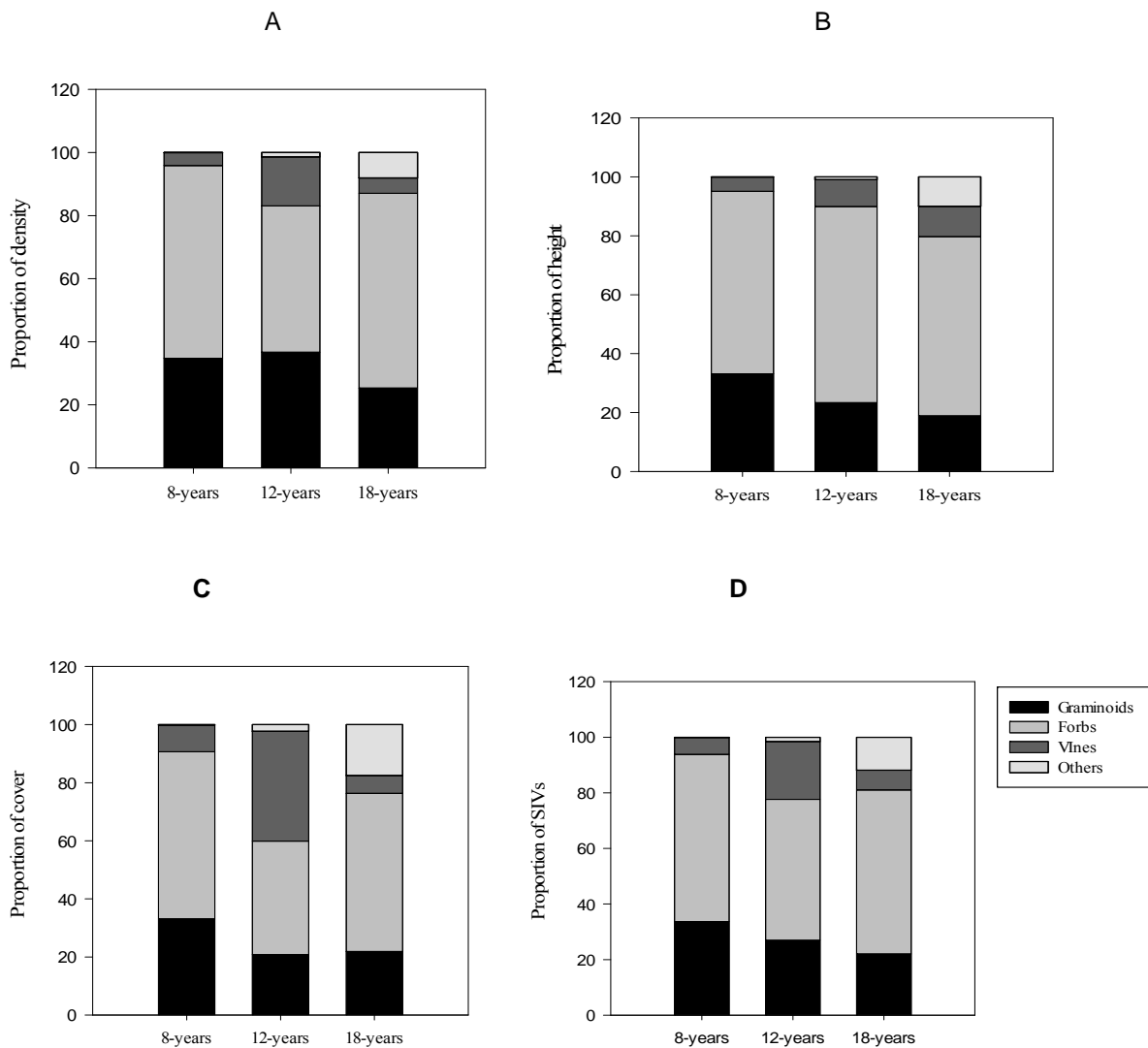


Figure 2- 5 Understory herbaceous structures in different ages of the plantation. Fig A showed the proportion of density; Fig B showed proportion of height; Fig C showed proportion of Cover and Fig D showed proportion of SIVs of different life form of the study area

The relative proportion of annual species was comparatively lower in entire plantation (less than 25%) where perennial herbs were dominated all plantation. Over 67 species of forbs were recorded in the study area dominated by Asteraceae 26 (27%) and Compositae and



planintaginacea were found 6 spp of each (7%). Leguminaceae, Violaceae, Polyonum and Commelinaceae were the families which were poorly represented (<1%). However mature plantation was dominated by single Poaceae family favoring limited numbers of invasive species. Natural regeneration of tree species was observed very poor in entire plantation.

There were several importance quantitative differences in the composition and dominance of understory herbs between three ages of plantation. The first 10 dominant species accounted for 66% and 81% of SIVs in 8 year and 12 years old plantation, respectively (appendix 2-3). Similarly, in mature plantation top 10 species were compromised 81 % of SIVs reflecting increasing dominance of selected species with age. *Roegneria C* (10.81), *Setaria viridis* (10.49), *polypogon fugax* (8.7), *Torilis japonica* (7.48), emerged as the most important species of understory in 8years old plantation. *Erigeronannuus L* (13.3), *Achyranthes bidentata BL*(12.26) were the most two forbs in 12years old plantation. *Cayratia trifolia* (9.82) was the most dominant vine in 12year plantation; still it was common to all plantation sites. *Oxalis corniculata* (21.61), *oplismentls undulatifolius follus* (11.23), *Miscanthussinensis Anderss* (10.82) and *Cyclosorus acuminatus* (8.89) were found most dominant in 18year plantation.

#### 2.4.3 Species Area and Abundance Curve

In total 113 understory plants found in entire study site, out of them 71 species were recorded in 8-years old forest and 70 were observed in 12-years old whereas 47 species were recorded in 18-year's forest. The area species curve on the basis of data measured on each plots also showed that mature forest was substantially less diverse in understory richness. The curve constructed showed the adequacy of sampling in the three plantations (Figure 2- 6 A). The curve showed plots in younger plantation (8 and 12 years) saturated early in 16 plot (12000m<sup>2</sup>) and the shape of curve of mature plantation was similar, but in later the curve saturated early as most of species encountered in 12 plots (9000m<sup>2</sup>). However, we have paid similar effort and time for sampling in all three plantations.

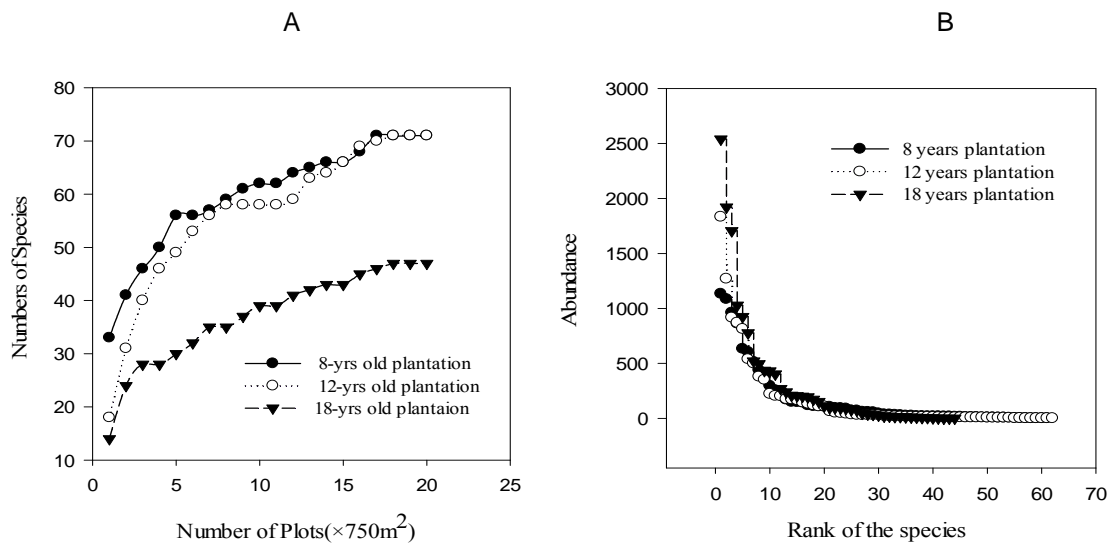


Figure 2- 6 Numbers of species and their abundance in different three stand age of poplar plantation. The fig A showed cumulative number of herbaceous species with plot and the figure B showed abundance of herbaceous species with their respective rank.

The ANOVA showed that there was significant difference in abundances of individuals of herbaceous and shrubs species between the three plantation ( $p < 0.05$ ), however we did not observe any significant different in cover of herbaceous among the three plantation. Abundance curve further showed only less 10 species were highly abundant and remaining species have shown similar pattern of abundance (Figure 2- 6 B). The much skewed pattern of the curve showed that herbaceous communities in mature plantation were poorer than the younger plantation.

#### 2.4.4 Species Diversity

We have detected  $25 \pm 3.77$  (mean  $\pm$  SD) species of herbs ( $5\text{m}^2$ ) and  $3.75 \pm 1.50$  (mean  $\pm$  SD) species of shrubs ( $20\text{m}^2$ ) each plot. The result of ANOVA showed that richness (species, Margalef) and abundance of herbs showed significant different ( $p < 0.05$ ) among the different stand age of the plantation.

However, for Shrubs in general, diversity, evenness, richness under different three plantations showed significant different ( $p < 0.05$ ). In contrast to richness, evenness of herb gradually increased from young to mature plantation, but we did not find statistically significant in our observation. The result suggested that poplar plantation result in higher understory diversity in

early and middle age of plantation, but the diversity gradually decreased with increase age and increment.

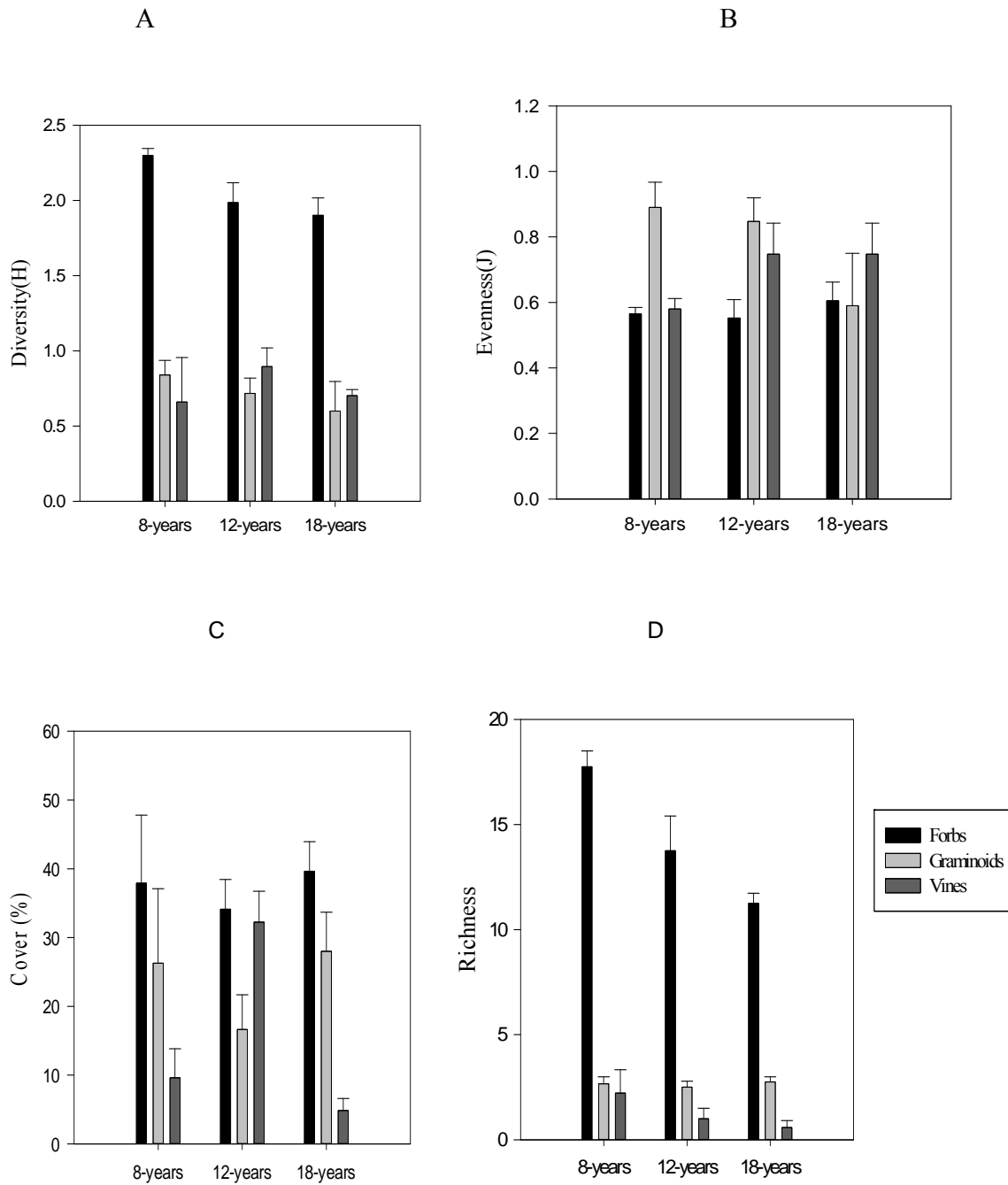


Figure 2- 7 Mean (mean± SE) univariate herbaceous responses in different three stand age of poplar. Figure A showed Shannon-Weiner index (H); Figure B showed Pielou's Evenness (J); Figure C showed mean percentage cover and Figure D showed richness for different life form of herbaceous plant of the study area.

In case of individual life form, richness of forbs found differs significantly among the three different stand age of plantation (Table 2-4).

Table 2- 3 Richness, diversity and abundance according to life form in the study site (mean  $\pm$  SE)\*

Life form	Indices	Age of the plantation		
		8-yrs	12-yrs	18-yrs
Forbs	Shannon	2.3 $\pm$ 0.045a	1.98 $\pm$ 0.13a	1.90 $\pm$ 1.12a
	Evenness	0.56 $\pm$ 0.019a	0.55 $\pm$ 0.056a	0.6 $\pm$ 0.057a
	Richness	17.75 $\pm$ 0.75a	13.75 $\pm$ 1.65ab	11.25 $\pm$ 0.48c
	Cover	37.9 $\pm$ 9.90a	34.125 $\pm$ 4.32a	39.625 $\pm$ 4.30a
Graminoids	Shannon	0.84 $\pm$ 0.097a	0.72 $\pm$ 0.2a	0.6 $\pm$ 0.196a
	Evenness	0.89 $\pm$ 0.077a	0.85 $\pm$ 0.07a	0.59 $\pm$ 0.16a
	Richness	2.67 $\pm$ 0.33a	2.5 $\pm$ 0.29a	2.75 $\pm$ 0.25a
	Cover	26.275 $\pm$ 10.8a	16.625 $\pm$ 5.05a	28 $\pm$ 5.6a
Vines	Shannon	0.66 $\pm$ 0.29a	0.89 $\pm$ 0.12a	0.7 $\pm$ 0.04a
	Evenness	0.58 $\pm$ 0.03a	0.75 $\pm$ 0.09a	0.75 $\pm$ 0.095a
	Richness	3.75 $\pm$ 1.11a	3.5 $\pm$ 0.5a	2.33 $\pm$ 0.33a
	Cover	9.625 $\pm$ 4.2b	32.25 $\pm$ 4.4a	4.875 $\pm$ 1.7b

Figures with same alphabetic suffix indicate no significant differences using multiple comparisons (Turkey,  $\alpha > 0.05$ ).

Both diversity of forbs and graminoids decreased gradually with age but we did not observe significant difference.

#### 2.4.5 Species Overlap

Pairwise Jaccards similarity on the basis of abundance of species in each site 8 and 12, 8 and 18 and 12 and 18 years old plantation were 30%, 21% and 26%, respectively, showing 8 and 12 years plantation more similar among the plantation (appendix 2-2). When we studied the overlapped species among the site 8 and 12, 8 and 18, 12 and 18 years old plantation were 10, 7 and 5 species, respectively (Figure 2- 8).

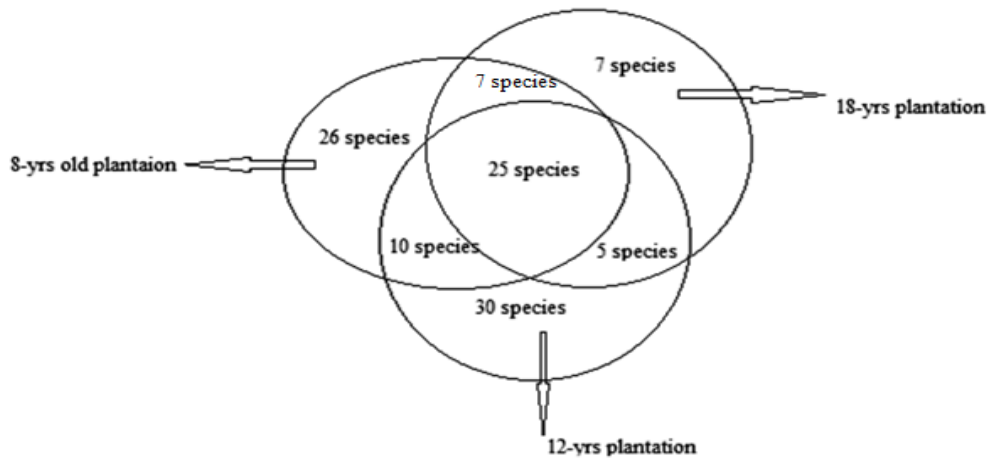


Figure 2- 8 Common and unique species

However, 25 species were found common to all plantation and others remaining 26, 30 and 7 species were found unique to 8, 12 and 18-years old plantation, respectively.

#### 2.4.6 Herbaceous vegetation relationships with environmental factor

Out of the soil variables we measured, TOC, TN C: N ratio were found different significantly in different stand age of the plantation, these variables were also found associated with diversity, abundance and composition of the area (Table2-5).

Table 2- 4 Summary of ANOVA table for Soil moisture, Soil , TOC, TN, and C:N in Dongtai Forestry Farm in Northern Jiangsu, China

Soil depth in different age		Moisture (%)	soil pH	TOC(gKg <sup>-1</sup> )	TN(gKg <sup>-1</sup> )	C:N
0-15 cm						
8 Yrs		0.24± 0.00a	8.27± 0.037a	16.43± 0.16b	1.48± 0.022a	11.08± 0.27a
12 yrs		0.25± 0.01a	8.2± 0.015a	17.89± 0.19a	1.54± 0.039a	11.37± 0.20a
18 yrs		0.25± 0.01a	8.25± 0.043a	15.92± 0.37b	1.27± 0.04b	11.03± 0.14a
F		0.142	1.17	15.32	15.142	0.762
15-30 cm						
8 Yrs		0.23± 0.01a	8.22± 0.029a	16.72± 0.17c	1.47± 0.038ab	9.14± 0.27b
12 yrs		0.24± 0.00a	8.15± 0.04a	18.37± 0.20b	1.61± 0.014b	10.2± 0.25a
18 yrs		0.31± .08a	8.28± 0.003a	15.05± 0.37a	1.27± 0.12a	9.74± 0.09ab
F		0.91	4.14	38.97	5.501	5.8

Data with same alphabetic suffix indicate no significant differences using multiple comparisons (Turkey,  $\alpha > 0.05$ ).

Additionally, richness and density of herbaceous in 8 and 18-year plantation found correlated ( $\rho < 0.05$ ). More specifically, density of vine is correlated with C: N ratio ( $r = 0.792$ ,  $p < 0.001$ ) and vine richness found correlated with soil pH ( $r = 0.590$ ,  $p < 0.05$ ). Similarly richness of graminoids found negatively correlated with TOC ( $r = 0.69$ ,  $p < 0.05$ ). The NMDS ordination plot indicates the structures of herbaceous in different three plantation area. The three soil variable that had strongest correlation with the NMDS ordination axes were TOC, TN and C:N. ( $\rho < 0.001$ ) (Table 2-6).

Table 2- 5 Correlation coefficient ( $\rho$ ) between Density, Richness and composition of herb with soil variables

Plantation		Moisture	Ph	TOC	TN	C:N
8-year	Density	0.14	0.13	$\square 0.43$	0.025	0.602*
	Richness	$\square 0.57$	0.28	$\square 0.05$	0.47	$\square 0.634^*$
12-year	Density	$\square 0.57$	$\square 0.23$	0.29	0.5	$\square 0.427$
	Richness	$\square 0.37$	$\square 0.134$	$\square 0.075$	0.23	$\square 0.455$
18-year	Density	$\square 0.09$	0.4	$\square 0.21$	0.24	$\square 0.62^*$
	Richness	$\square 0.26$	0.097	$\square 0.084$	0.34	$\square 0.644^*$
NMDS	Axis 1	$\square 0.09$	0.142	0.01	$\square 0.21$	0.07
	Axis 2	$\square 0.20$	0.269	$\square 0.61^{**}$	$\square 0.5^{**}$	$\square 0.44^{**}$

\*\* denotes correlation is significant at 0.001 and \* denotes correlation is significant at 0.05.

Neither of the variable's basal area, mean basal height, evenness, shrubs density, bulk density was correlated with neither of the NMDS axis and diversity. However, both axis in only 8-years old plantation were significantly correlated ( $\rho = 0.559$ ,  $p < 0.01$ ).

## 2.5 Discussion

### 2.5.1 Species composition

The life form species showed that flora of 8-years of Dongtai farm consist of 63.3% of forbs, 9.8% graminoids, 14% shrubs, 11.2% vines and few seedling 1.42%. Majority of understory communities were dominated by forbs in entire forest but were poorly represented by ferns. Only 3 species of ferns were recorded from which 2 of 12 years and one of 18 years plantation. The finding on basis of individual species concurs some patterns that the importance of dominant species i.e. *Roegneria* Cspecies and *Duchesnea indica* have decreased and with increase age of plantation gradually favors the more invasive species i.e. *Erigeron annuus* L, *Eupatorium adenophora* Spreng.(Yan et al., 2001) in 12years old plantation and *Oxalis* spp (Holm et al., 1991) in mature plantation.

The study supported the hypothesis that there was the significant different in species composition and abundance among the three different stand age of poplar, this is basically due to differ in composition of forbs. The proportion of forbs was much higher in the entire stand age of the plantation, so much of the result of ours study likely be affected by forbs. The ordination through NMDS, the first axis did not show much variation in herbaceous species data, showing much similarity between the plots, more specifically plots in mature plantation were much similar within than in younger plantation. Plots in mature plantation were clearly separated in composition of understory community. We also detected the skewed abundance curve which is universal feature of all communities (Verberk, 2012), however it may differ with plant communities and site interface. So in term of herbaceous composition, 8 and 12 years plantation were more similar also supported by number of overlapped species and Jaccard's similarity (fig 7). However all three plantations were different in richness and abundance was found due to significant different composition of species including particularly of forbs, age, stand structure and anthropogenic disturbance interfaced and also different in TOC and TN between the different stand age of plantation. So obviously, richness and abundance of herbaceous in the plantations might be affected by competition, succession and nutrient cycling which also supported the previous study (Chauhan et al., 2010; Yirdaw, 2001). Despite this the ordination obviously indicated that herbaceous layer also share the composition of few dominated species (25 species in all plantation). The result is consistent with the conclusion drowned from

(Archaux et al., 2010) as plant communities in young plantation showed little differences in composition according to the type of understory control. In overall 12 years plantation found much complex ecologically as it retained much layers of herbaceous and its functional diversity.

The study area was dominated with forbs especially of Asteraceae family (more than 26%). some of species under this family have found invasive (Yan et al., 2001) and the most important source family of invasive plant in China (Wu et al., 2010). As Asteraceae species often produce large quantity of small seeds which are easily dispersed by wind, also these plant could utilize the good change to grow in vacant niche (Jin et al., 2015), grow fast and invade until other resources such as N and moisture availability become limiting (Berendse, 1990; Gleeson & Tilman, 1990; Mellinger & McNaughton, 1975).

The overall performance of herbaceous in mature plantation was low could also be reflected decreased amount of TOC, TN and C:N with higher basal growth of overstory and density of shrubs. This also can be explained as the study (Ge et al., 2015) mentioned as more above ground biomass produced require more nutrients in mature stand, and it was likely to return fewer nutrients to soil. This might cause increased competition for nutrients and favor the situation of selected invasive in 18 years old plantation. So our finding of increased species value of selected species, decreased importance dominant species and decreased richness along with development of stand and age was also supported by Niche-preemption model. As the Niche-preemption model predicts that superior competitors tend to access greater amount of limiting resources such N and light and other species adapt to the presence of a superior competitor by switching to an alternative, less-used resource (Ashton et al., 2010; Hector et al., 1999; May & Mac Arthur, 1972) and finally disappearance of some of them could be expected.

### 2.5.2 Abundance and diversity

Alfa diversity can be classified into four categories; species richness, species diversity, species evenness, and species dominance (Wu et al., 2013). As species richness can be affected by either size of sample plots or overall sample size (Wang et al., 2011), so combination of indices is the precise way of measuring diversity. The result of this paper clearly reflected the different pattern on species richness which showed the plantation areas were dominated by perennial forbs



particularly flowering, but still contained considerable richness of grass and annual vines. The finding presented in this study supported the hypothesis that understory species (both herbs and shrubs) richness and abundance changed significantly in the three different age of poplar, however not showing strong effect of age to alter the herbaceous cover. The overall species richness of herb was detected consistent with as estimate of understory herbs richness for Eucalyptus plantation in Yunnan, China (Wang et al., 2011). However we observed significantly lower diversity (H) for shrubs; the richness was found very much lower than the same study.

So this study neither support fully against the conclusion (Boothroyd-Roberts et al., 2013) of young (10 years or less) hybrid poplar plantations are often devoid of most forest herb species typical of natural forests nor consistent with the finding of the study (Nagaike et al., 2003) as mentioned that the richness of herbaceous was not correlated with plantation age that was planted (Peterken & Game, 1984) between 1600 and 1947 in England.

The result was not totally consistent with that increase of age, herbaceous diversity decreased due to decreasing light intensity (Hart & Chen, 2008) and with increasing canopy coverage (Kuksina & Ulanova, 2000). In general, the result was consistent with some studies as younger poplar plantations supports higher understory plant diversity compared to mature plantation (Archaux et al., 2010; Li et al., 2014) might be due to higher intensity of light (Fang et al., 2014; Humphrey et al., 2000; Veinotte et al., 1998) and appropriate canopy shading (Archaux et al., 2010; Li et al., 2014; Pourbabaie et al., 2015; Rees & Juday, 2002) showed both species number and diversity (H) higher in younger poplar.

Moreover, accounting species richness only may be insufficient to compare the effects of fast-growing tree plantations on understory plant diversity, so proportional species composition also important factor taken to be considered (Li et al., 2014). Specifically, we observed higher diversity indices and richness of forbs in the entire forests, than other life forms classified in the forest. There was significant different in richness of forbs between different stand ages, however diversity was not differing significantly. This is consistent with understory of naturally regenerated deciduous forest and spruce plantation (Fang et al., 2014) in term of dominance of forbs but overall herbaceous diversity and richness of understory was substantially lower than this study. In the entire plantation, limited resource availability was created more competition of light and nutrient. Graminoids diversity was poorer than the forbs, may favoring to forbs

reducing the competitive capability of graminoids groups which was agreed to the study (Seastedt et al., 1991; Wedin & Tilman, 1993). This is also consistent with density hypothesis as shaded and small individual of all species lost randomly (Chu et al., 2010; Stevens & Carson, 1999). When the plantation increased towards the mature, vine, fern, forbs and shrubs diversity reduced gradually which obviously favored to graminoids richness in middle age plantation. Although it was not significant, we observed vine richness highest in 12- year plantation may be due the highest C: N ration in 12- year plantation than others.

One of the limitations of our study is that we did not measure light which is considered important in relation to biodiversity analysis as one of limiting factor in species richness measurement. However, (Pourbabaei et al., 2015) did not find any correlation between diversity indices with canopy, light and litter depth in 9 years old Masal's stand. The highest Pileou's evenness for herbaceous of 18-years old plantation (0.80) reflects that the abundance of understory species were more even within the older plantation than understory species in younger plantation (0.70).

So as the suggested by the study (Fang et al., 2014), in this forest area human activities also may severely restricted the shrubs community development resulting increased invasion by pioneer annual grass and forbs. So management of certain herbaceous and introduction of some herbs may be the better strategy to manage the understory economically and the sustainable way.

### 3 Changes in Composition of herbaceous plants in respect to N fertilization in three different ages of Poplar plantation in a coastal area of eastern China

#### 氮沉降对三个不同年龄苏北杨树人工林的影响

摘要：尽管人工林的年龄和氮肥都会对林下草本多样性及重要值产生影响，但这些因素结合在一起的研究很少被研究。在本研究中，我们建立了一个野外试验基地，共五个梯度的氮处理（对照、50、100、150、200 kg N ha<sup>-1</sup> yr<sup>-1</sup>），样地设在东台林场，有三个不同年龄层的杨树林，研究时间从 2012 到 2015。结果表明在研究期间不同的氮处理之间多样性（丰富度、香浓指数、优势度和均匀度）没有显著差异，但结果表明在幼林龄中，草本多样性(H) 随着氮浓度会呈上升趋势而在成熟林呈下降趋势，尽管草本丰富度在所有林龄都是下降的。总体上，整个人工林，非禾本科草本和藤本植物在氮处理下是受益的，但禾本科植物却遭殃了。草本物种多样性，特别是非禾本科草本和藤本在对照组生长较慢，非禾本科植物在较高和最高浓度的氮处理下(150-200kg N ha<sup>-1</sup> yr<sup>-1</sup>)达到最高值，禾本科植物却遭殃的最厉害，而藤本在较高浓度下提前达到饱和，在最高浓度下(200kg N ha<sup>-1</sup> yr<sup>-1</sup>) 却呈下降趋势。非度量多维尺度分析（NMDS）表明氮对草本种群有明显的影响，随着氮的增加在样地内，不同组别渐渐分开，而导致整个人工林的各组更接近。禾本科和藤本的密度由于氮添加更多样化。结果总结：在幼林龄生物多样性随着氮添加而增加，而成熟林是下降的。由于人工林的年龄生物多样性的总体下降得更厉害，比起因为施肥带来的增长，但这种反应在不同功能组中差异很大。本研究清楚表明人工林林下植物多样性主要受人工林的年龄影响，然后才是氮浓度。

关键词：草本多样性，氮沉降，施肥响应，物种，年龄，NMDS

#### *Abstract*

Although stand age of the plantation and nitrogen fertilizer both have effect on understory herbaceous composition, diversity and abundance, but these factors in combination have been studied rarely. We established a field experiment with five N-treatments (control, 50,100,150,200 kg N<sup>-ha</sup> yr<sup>-1</sup>) on three stand age of poplar plantation in Dongtai Forestry Farm, a coastal region in Northern China from 2012-2015. Result showed there were no significant difference between N-treatment to diversity (Richness, Shannon, Dominance and Evenness) during the study period, however we found positive response of herbaceous diversity with increased availability of N in

younger plantation and negative response in mature plantation affecting the species evenness in all stand age. In general, forbs and vine were benefitted everytime, while graminoids suffered from the N treatment. The diversity of forb and vine growing was low at control, forbs increased to maximum at N3 to N4 (150-200kg N ha<sup>-1</sup> yr<sup>-1</sup>) treated plots where graminoids lost. Vine saturated earlier than forbs at N3 (150kg N ha<sup>-1</sup> yr<sup>-1</sup>) consistently declined towards very high N (200kg N ha<sup>-1</sup> yr<sup>-1</sup>) treated plots. Non-metric Multidimensional Scalling (NMDS) exhibited obvious pattern that herbaceous groups gradually became dissimilar within plots resulting entire plantations more similar showed clear pattern of shifting the community dynamics with increased availability of N. The density of graminoids and vine was varied due to N fertilization than forbs however forbs in the entire forest was much varied between plantations of different age. Ours result concluded that biodiversity increase with nitrogen fertilization in younger plantation and decrease in mature plantation. This also indicated that with increase N availability diversity increased at area with higher soil nutrients and lower above ground woody biomass, and diversity decreased at area with lower soil nutrient content and higher woody biomass and the trend was opposite for species richness. The overall decrease in biodiversity due to increased stand age of plantation was more prominent than increase in biodiversity due to fertilization but this response may vary with functional groups. The study clearly indicated that the response of N on the different indices of herbs in the plantation primarily influenced by stand age of the plantation and then level of nitrogen which was determined by deliberate equilibrium between forbs and graminoids.

**Keyword:** Community dominance, herbaceous diversity, Nitrogen fertilizer, NMDS, stand age, Understory

### 3.1 Introduction

Plant responses to Nitrogen (N) deposition primarily have studied in temperate and boreal ecosystem (Lu et al., 2010) from where the scientific knowledge about the mechanism of response of N on plant have been documented (Gilliam, 2006). Recent studies have been focused on the effect of N addition on forest plant biodiversity (Gilliam, 2006; Lu et al., 2010). N was recognized as the primary limiting nutrient for plant growth in many terrestrial ecosystems (LeBauer & Treseder, 2008; Lu et al., 2010; Vitousek & Howarth, 1991). Past study has been shown that

rehabilitated and disturbed forests in tropical China are N limited (Mo et al., 2006). Therefore, N fertilization has been widely used to improve soil N availability (Frink et al., 1999) and increase primary productivity in terrestrial N-limited ecosystems (Frink et al., 1999; Lu et al., 2010) by increasing the leaf area index (LAI) and improving ecosystem water use efficiency (Brueck et al., 2009) and stimulate the plant growth (Xia & Wan, 2008). The overall response of understory herbaceous in different plantation to N addition remains uncertain in part because of the limited number of experimental studies in many regions.

Herbaceous layer accounts for the largest proportion of floristic diversity (Huo et al., 2014), but accounts for very low biomass, (Gilliam, 2007). So its role in nutrient cycling is over proportionate to its biomass (Bolte et al., 2004) and contribute up to 20% foliar litter to forest floor (Gilliam, 2007) which decompose two fold rapidly than tree litter (Gilliam, 2014; Neufeld & Young, 2003). It is important as a nutrient sink (Fahey et al., 1991), as its foliage have up to three time nutrient content than tree (Gilliam, 2007). So threat to forest biodiversity are most often function of threat to herbaceous species (Jolls, 2003) as it harbor 90 % of forest trees (Gilliam, 2007), the layer is also sensitive to soil fertility and site condition (Peterson & Rolfe, 1982). Over story affects the understory layer by altering light availability and fertility dynamics (Neufeld & Young, 2003). Understory vegetation in coniferous forests and mixed-wood forests (Mölder et al., 2008), temperate and boreal zone (Lu et al., 2010) well studied. In contrast, less is known on the impact of N deposition on N deposition in tropical and subtropical areas (Bobbink et al., 2010).

Changes in Input or lack of soil nutrients may affect plant performance indirectly by changing competition among species (De Keersmaeker et al., 2004). N deposition often increase above ground herbaceous productivity (Reich et al., 2001a; Reich et al., 2001b) and overall biomass which creates higher layer of herbaceous along with this reduce light and soil water availability in herbaceous vegetation (Davis et al., 1998). N addition may affect the pool of soil organic carbon (SOC) through increased litter fall because of an increase in leaf area and higher above ground biomass production (Hyvönen et al., 2008). The main effect of increase in nutrient supply might faster growth of all species of plants, which increase the competition for light (Davis et al., 1998; Pyšek & Lepš, 1991). Established species might negatively impact the performance of others species by depleting the soil resources or decreasing light availability (Neufeld & Young, 2003; Verheyen & Hermy, 2004).

Many of the studies have reported N deposition decrease plant diversity (Bobbink et al., 2010; Lu et al., 2011; Verma et al., 2014) due to loss of species that are efficient in low N and decrease of evenness caused by increasing dominance of few species (Gilliam, 2007) and some found no significant change (Bobbink et al., 2010; Gilliam, 2006). Generally it has suggested that response of increased N availability may vary with rate of N addition (Lu et al., 2010), type of land use history (Lu et al., 2011). The reduction of biodiversity with increase availability of N is the function of increase biomass (Verma et al., 2014), increase of canopy closure (Lu et al., 2011), change in dynamic of others nutrient ( eg increase soil pH, al and decrease in ca availability and fine root biomass (Lu et al., 2010), decrease of low N efficient species(Gilliam, 2006). Soil type also can affect soil fertility which ultimately affect understory abundance, composition, and diversity (Hart & Chen, 2006). It is also proposed that changes of soil properties (e.g., soil acidification, increased base cation leaching and concentrations of potentially toxic metals) induced by high-N deposition may be an important mechanism leading to the declines of diversity (Bobbink et al., 2010; Gilliam, 2006). The Mechanism underlying the decline of diversity includes competitive exclusion, disturbance factor, species invasion etc (Bobbink et al., 2010; Gilliam, 2006). Herb layer productivity might be promoted by nutrient supply and base saturation and herb-layer biomass was also positively correlated with herb-layer diversity (Mölder et al., 2008).

In natural communities response of plant to N depositions have been explained with variety of traits (Diekmann & Falkengren-Grerup, 2002). These different functional traits behave differently with different level of N. So mechanism of competition among the species sharing the same N fertilization could be explored more through the functional trait (Verma et al., 2014). The absolute measure of diversity indices has been criticized as it ignores the differences between species producing a meaningless numbers (Leinster & Cobbold, 2012) which is hard to compare percentage change in their values (Jost, 2007). Important value index (IVI) is used to determine to overall importance of each species in the community structure, which could not measure by considering absolute diversity only (Leinster & Cobbold, 2012).

The study reported effect of N on composition, richness and diversity of understory herbaceous in different stand age of plantation. The main objective of the study is to compare the effect of N on composition and diversity of understory herbaceous in Poplar plantation in Northern Jiangsu of China. We hypothesized that N addition has been observed to change plant species composition

and to reduce species richness and diversity in herbaceous vegetation as (i) it dependent on stand age (ii) level of N addition.

### 3.2 Material and Methods

#### 3.2.1 Experimental site

The study was carried out at three different age of poplar plantation (*Populus euramericana*) (8, 12 and 18-years) at Dongtai Forestry Farm (102 ° 49'E, 32 ° 52'N) located in Northern Jiangsu, China (Figure 2- 1). The area experiences north subtropical to warm temperate climate having forest area around 3000ha continues plantation dominated by poplar having covering rate 85%. Annual mean temperature, rainfall and relative humidity are 13.7°, 1051 mm, 88.3% respectively (Wang et al., 2015).

Table 3- 1 Major Soil and stand characteristics of the study area

Plantation	BA (m <sup>2</sup> /Ha)	Trees /ha	Herbs/ha	Moisture (%)	SOC gm/Kg	BD (gm/cm <sup>3</sup> )	pH	T Ng·kg <sup>-1</sup>	C:N
8-yrs	24.53	980	4600	0.24	16.22	1.18	8.24	1.67	9.2
12-yrs	18.93	360	3200	0.25	16.8	1.35	8.21	1.65	10.28
18-yrs	41.2	522	3000	0.28	16.9	4.19	8.26	1.69	10.4

BA =Basal Area of trees, BD=bulk density, N=Nitrogen, SOC=Soil Organic Carbon, C:N ration=Ratio of soil organic carbon and total nitrogen

In general soil is desalted meadow sandy loam soil, well drained with organic matter 1.4% (Wang et al., 2015), moderately fertile being N limited (as less than 55 g N/Kg of soil). The plantations were established at late 1990s and thinning was done regularly. The afforestation density of the plantation over the entire site was 3 m×5m. The three sites have same conditions in terms of physiographic factors. The area is dominated by herbs especially from Asteraceae family and also some grass, shrubs, vine and few fern species. The dominated understory species were as follows: *Roegneria C and Miscanthus* (grass); *Oxalis corniculata* and *Torilis japonica* (herbs) and *Cyclosorusacuminatus* (ferns). The mean height was 13.4, 17.68 and 17.84 m for ages of plantation respectively, 8, 12 and 18-years old. The main properties of soil and stand characteristic are given in (Table 3- 1).

### 3.2.2 Experimental treatments

Replicated Block Design was used in this experiment. Four N treatment with four replicates established i.e. control (N0-without N added), N1 (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N2 (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N3 (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and N4 (200 kg N ha<sup>-1</sup> yr<sup>-1</sup>) respectively. The fertilization was applied 6 times per year for each treatment plots in the whole growing season and has applied since July 2012. For this, there were established 20 plots of 25 × 30m for vegetation survey in each stand age of plantation and respective treatment.

### 3.2.3 Vegetation sampling

We collected the vegetation and soil data on 2015 during the periods of growing season (June and July) for the area. We systematically placed five quadrants of 1m × 1m to investigate herbs and five quadrants of 2m × 2m for shrubs in each plot of 25 × 30m. We collected the data on shrubs and herbs layer. The understory in herbs defined here as all plants ≤ 1 m in height (Gilliam & Roberts, 2003) and shrubs as any species with height between 1.3 to 4 m (Seedre & Chen, 2010). In each plot, the diameter at breast height (DBH) and total height of each individual tree were recorded. All shrubs and herbs presented in a quadrant, we recorded the species, name, measured its average height, counted the plant and estimated the cover. we considered two herbaceous traits most applicable to study area; one was related to species life form (forbs, graminoids and vine) and another was life span (annual, biennial and perennial) (Verma et al., 2014; Xu et al., 2015). So we classified all understory species into three groups by growth forms i.e.; shrubs (S), forbs (F), vines (V), graminoids (G), and fern and seedling were considered in others groups (O) for analysis. Then we verified traits and scientific name of specimen collected during the field (Yanqiu, 2008). We measured height, numbers, cover of herbaceous species in each plot and percent cover of herbs which was visually estimated (Huo et al., 2014; Qian et al., 2003; Wulf & Naaf, 2009).

### 3.2.4 Measurement of Soil

Soil samples were separately collected from two layers at the depth of 0–15 cm, 15–30cm layer for each sampling plot. For each layer, the samples from five sub quadrates were mixed completely to form a composite in each plot and stored in a bag for the measurement of soil water content by



using the classical methods of drying and weighting (Dobriyal et al., 2012). The total soil organic carbon (SOC) and total Nitrogen (TN) of soil was measured using an element analyzer (Elementar, vario ELIII Analysen Systeme GmbH, Hanau, Germany). Soil pH was measured in a 1:2.5 soil to water suspension (Huo et al., 2014; Zhang et al., 2013). Soil bulk density was determined by using the volumetric ring method.

### 3.2.5 Vegetation analysis

Species richness based on the data in the plots was expressed as species number per square meter. The values were also calculated for each functional group. Prior to computation of diversity indexes and statistical analysis, the data of plant density and diversity data were pooled per experimental plot. The overall component of  $\alpha$ -diversity (Wu et al., 2013) species richness (S), Diversity (Shannon H), Species Dominance (D) and Pielou evenness index (J) for each plots and also for each functional groups of herbs calculated. Their values were calculated as follows.

Species richness (S) (Wang et al., 2006; Zeng et al., 2010)

$$H' = -\sum \frac{n_i}{N} \log \frac{n_i}{N} \quad (\text{Shannon, 1963})$$

$$D = \sum (n_i/N)^2 \quad (\text{Hammer et al., 2001}),$$

$$\text{Evenness (Evar)} = H'/\ln(S) \quad (\text{Guang-Long et al., 2015})$$

Where H = Shannon and Wiener index of general diversity,  $n_i$  = No. of individuals of the species, N = No. of individuals of all species, S = Species richness of plot and each plantation

The relative importance (dominance) of species was calculated for each herbaceous species by summing the mean of relative cover (Rc), relative frequency (Ra) and relative height (Rh) ((Zuo et al., 2012). The dominant and co-dominant species were identified. The species having highest and second highest IVs was defined as dominant and co-dominant respectively.

### 3.2.6 Statistical analysis

One way Analysis of Variance (ANOVA) with Turkey's Post Hoc test performed to test the differences by treatment in diversity, density (mean number of plants/m<sup>2</sup> in each replication). Two way ANOVAs conducted to evaluate the effect of age and effect of N and interaction if any.

The difference in composition in herbaceous community were calculated by per mutational Multivariate analysis of Variance ( PerMANOVA) (Hammer et al., 2001) and illustrated by NMDS of Bray Curtis distance (Bray & Curtis, 1957). Rank correlation coefficient (rho) was calculated to compare explanatory variables (TOC, TN etc) to response variable (NMDS axis values, diversity and density). All analysis was performed in statistical package SPSS 16.

### 3.3 Results

#### 3.3.1 Species Composition and herbaceous dynamics

87 herbaceous plants with 33 different families were accounted in the entire study area including biannual/perennial forbs, weeds, vine and grasses. In our investigation, 17 subtropical vines (11 families), 60 species forbs (25 families), and 10 species of graminoids (2 families) were identified. Forbs accounted dominated in the entire forest representing more than 63 percent herbaceous in the study area. Apart from those, few species of ferns (4 species), 5 species of woody species also were accounted during the study but due to poor abundance we did not include those in every analysis. The species composition after the N treatment in neither of stand age of plantation found significant in comparison to control. With increased N availability, the composition of herbaceous in mature plantation affected more and the composition was least affected in 12-years plantation (Table 3- 2). As it was obvious pattern in NMDS graph, species composition among the three stand age of plantations in natural condition was differ significantly as in graph A but did not obvious in the plots of N-treatment (Figure 3- 1, Table 3- 2) .

Table 3- 2 The brief summary of PMANOVA for different stand age of plantation with pair wise comparison of selected attributes

Factors	<u>Sum of square</u>		F-value	p(same)
	Total	Within the groups		
8-years	3.27	2.57	1.03	0.4477
12-years	2.97	2.47	0.759	0.825
18-years	3.42	2.64	1.09	0.32

Data with \* (asterisk) indicates significant differences using multiple comparisons (Turkey,  $\alpha > 0.05$ ).

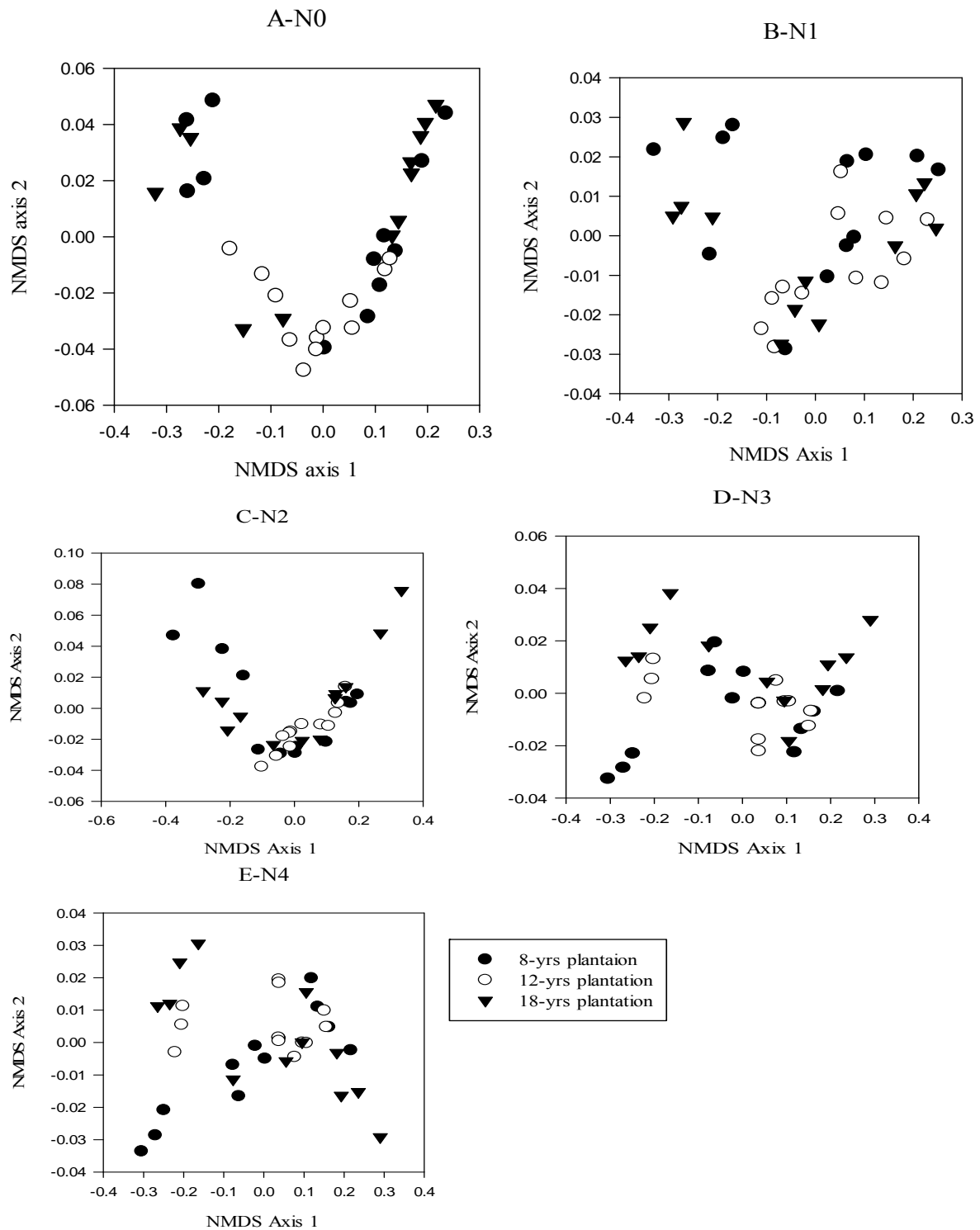


Figure 3- 1 Plot ordination on Multidimensional scaling based on Bray-Cutis similarities of the herbaceous richness and density retained by NMDS analysis. The figures showed the response of herbaceous (graminoids, vine and forbs) in every three forest types with four replication for each treatment Fig A- N0 Control (A), Fig B-N1, Fig C-N2, Fig D-N3 and Fig E-N4. Plots apart have greater differences in herbaceous composition.

The NMDS ordination plots showed (Figure 3- 1), the plots could highly separated in the plot of N1 treatment as N1 it found effective to change the composition of the herbaceous but gradually with increased availability of N composition of different plots were intermixed and it was increased upto N3 treatment to maintain the entire forest in optimum homogeneous and remained stable after that.

After the application of N3 the younger forest (8 and 12 years plantation) almost did not change in composition so much. On the basis of SIVs we investigated forbs and vine was benefitted by the N treatment. Forbs responded positively all the N-treatment in 8-years and 18-years, while in the 12-years it benefitted least. In 12-year plantation, in general, only vines were benefitted.

We found highest common species in all treatment plots of 8-years plantation which consisted forty-five percentage (*Polypogon fugax*, *Setaria viridis*, *Erigeron annuus L*, *Carpesium macrocephalum*, *Conyza Canadensis L*, etc) of total species of that forest followed by forty percentage in 18-years plantation (*Lophatherum gracile*, *Corchoropsis psilocarpa*, *Conyza canadensis L*, *Clerodendrum trichotomum Thunb.*, *Achyranthes bidentata Bl.*, etc) and lowest in 12-years which consisted twenty-four percentage (*Miscanthussinensis Anders.*, *Erigeron annuus L*, *Solidago Canadensis L*, *Carex forrestii*, *Achyranthe sbidentata Bl etc*).

On the basis of the experiment, some of these generalist herbaceous plants were found commonly productive on low level-N to very high-N levels were *Lophatherum gracile* (Poaceae), *Erigeron annuus L.*(Asteraceae), *Cayratia trifolia* (Vitaceae), *Cyclosorus acuminatus* (Fern), *Roegneria C* (Gramineae), *Corchoropsis psilocarpa* (Malvaceae), *Ixerissonchifolia* (Asteraceae), *Ipomoea purpurea* (Araceae), *Humulus scandens* (Moraceae) and those can fairly develop in all level of N but *Humulus scandens* was found highly productive in N4 plots only.

### 3.3.2 Effect of N on diversity of functional groups

During the study period, we observed forbs density increased in the every plot with N1 treatment and then decreased slightly with higher dose of N showing negligible effect of N4 (200 kg N<sup>ha</sup> yr<sup>-1</sup>). Although there was considerable different in richness of all life forms (forb, graminoids and vine) between the plots of three different stand age of plantation, over the study period, we observed significant different in forbs and vine richness and diversity due to stand age. The effect of N treatment reflected in evenness of vine only (Table 3- 3)

Table 3- 3 Summary of two ways ANOVA on herbaceous diversity due to age and N treatment levels in Dongtai Forestry Farm, China

Source	Dependent Variables		F for functional groups		
	Variables	df	Forbs	Graminoids	Vine
Age(A)	Richness	2	30.36*	0.725	19.74*
	Shannon	2	14.81*	1.255	12.96*
	Evenness	2	2.05	0.638	90.84*
Treatment(T)	Richness	4	1.83	0.652	0.89
	Shannon	4	2.4	0.645	1.01
	Evenness	4	2	0.734	2.84*
A×T	Richness	8	1.6	0.601	0.913
	Shannon	8	0.3337	0.79	1.4
	Evenness	8	0.4	1.027	2.49

F value with symbol \* indicate significant differences (Turkey,  $\alpha > 0.05$ ).

The positive response of vines diversity to N2 treatment particularly in 8-years and 12-years plantation but decreased significantly with N3 treatment and the negative trends was more pronounced in mature plantation (Figure 3-2 C) still it was again increased in N4 treated plots.

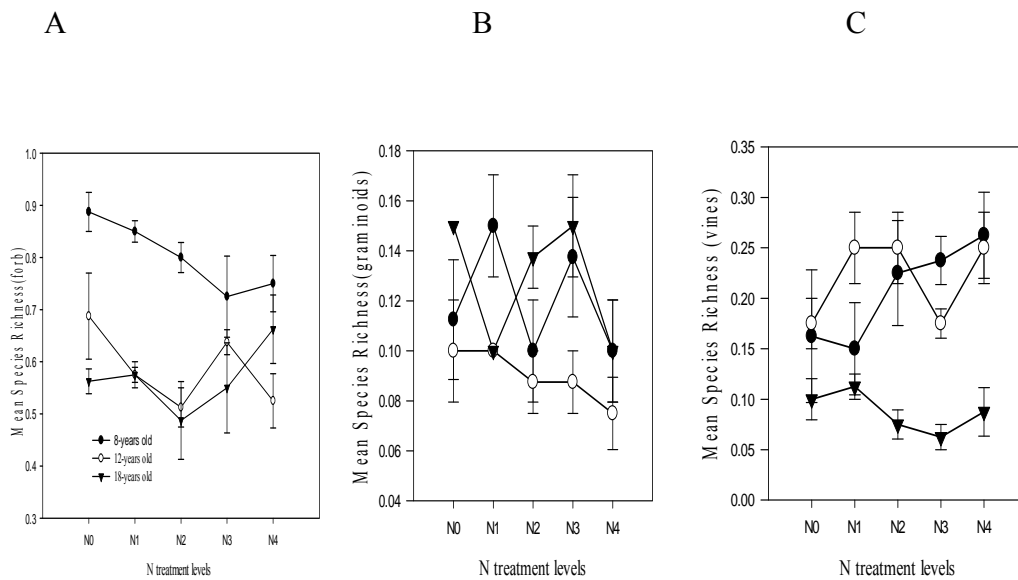


Figure 3- 2 Species richness –Forbs (A), graminoids (B) and vine (C) (mean±S.E) in different three ages of poplar plantation in Dongtai plantation

Table 3- 4 Response of different groups of herbs density (numbers/m<sup>2</sup>) to N addition

Site	Life	N0	N1	N2	N3	N4	F	Sign.
	Form							
8-years	F	11.75± 2.1a	13.45± 3.6a	11.86± 1.5a	12.59± 1.6a	14.66± 3.4a	0.27	0.925
	G	12.16± 4.2a	5.58± 1.0ab	3.23± 1.0b	5.15± 0.6ab	3.15± 0.6b	3.43	0.035
	V	0.86± 0.06a	1.06± 0.19a	1.34± 0.36a	1.74± 0.6a	1.67± 0.3a	1.21	0.344
12-years	F	8.15± 1.5a	12.6± 2.7a	8.9± 0.7a	9.21± 1.6a	8.08± 0.3a	1.2	0.349
	G	3.16± 0.6a	5.74± 2.0a	5.4± 2.2a	7.6± 0.7a	9.88± 1.8a	2.42	0.093
	V	3.95± 1.1a	3.77± 0.8a	4.2± 0.3a	3.5± 1.8a	2.64± 0.7a	0.31	0.863
18-years	F	15.36± 2.3a	17.25± 1.9a	19.78± 7.2a	17.09± 3.5a	16.6± 5.1a	0.12	0.969
	G	13.70± 3.7a	3.7± 0.4b	7.49± 1.6ab	8.73± 2.4b	4± 0.7ab	3.64	0.029
	V	0.91± 0.3a	0.80± 0.1b	1.11± 0.2ab	1.17± 0.2a	0.84± 0.1b	4.07	0.02

Data in parentheses indicate standard error. Data with same alphabetic suffix indicate no significant differences using multiple comparisons (Turkey,  $\alpha > 0.05$ ). Life form as indicated F for forbs, G for graminoids and V for vine.

The richness of forbs in 8-years old plantation was significantly higher than the rest of the others ( $p < 0.05$ ) (Figure 3-2 A). There was significant decrease of graminoids density in all N treatment plots in 8 and 18-years plantation ( $p < 0.05$ ), however in 12-years old plantation graminoids density was affected positively by N treatments but statistically was not significant ( $p < 0.05$ ) (Figure 3-2 B). In overall forbs density was increased with addition of N, although the result was not significant ( $P < 0.05$ ).

### 3.3.3 Effect of N addition on total herbaceous richness and diversity

There were no significant differences between N-treatment to any of the diversity indices (Richness, Shannon, Dominance and Evenness) measured for herbaceous plant ( $p < 0.05$ ). However, result showed positive trends of diversity (Shannon and evenness) for the younger plantation of 8 and 12-years old with increased N availability.. The response became almost reverse in mature plantation as all of the N treatments caused to decreased diversity (H) and evenness (J), with slightly increase of dominance of few species (Table 3- 2, Figure 3-3).

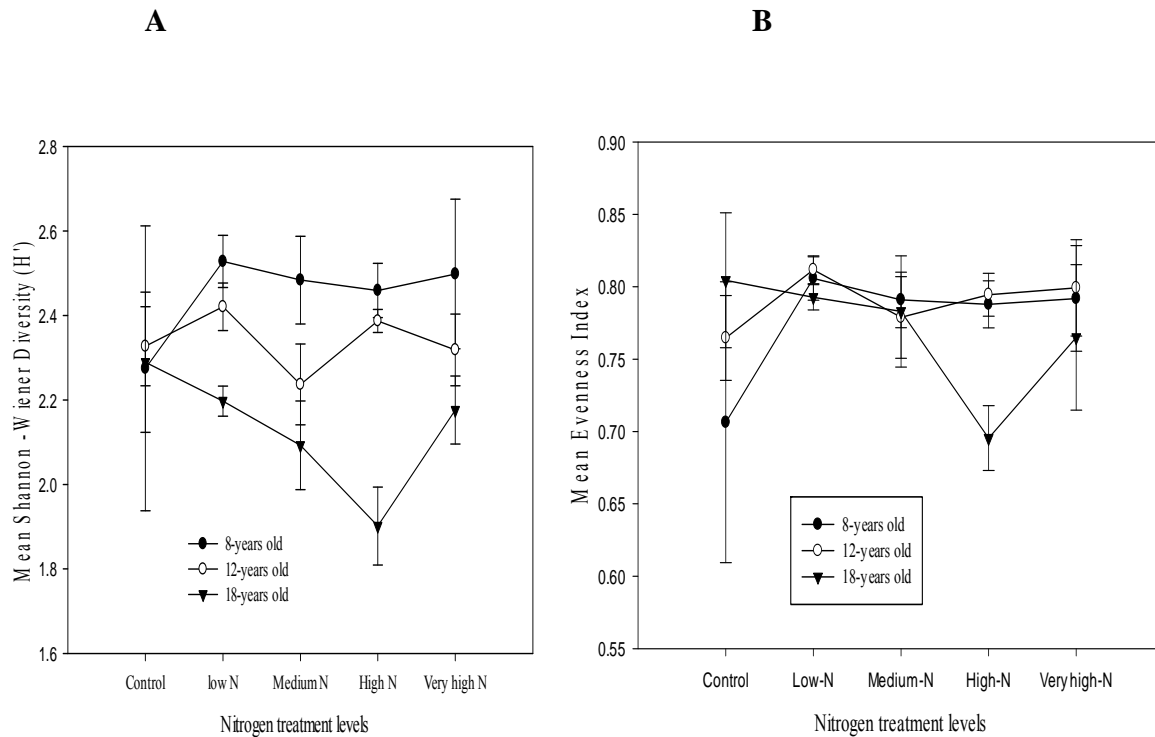


Figure 3- 3 Shannon-Wiener Diversity ( $H'$ ) Index -(A) and Pielou's Evenness Index -(B)(mean $\pm$ S.E) in different three age of poplar plantation in Dongtai plantation

The study exhibited species richness decreased consistently with increased N and was more pronounced in mature plantation, however diversity ( $H'$ ) increased and dominance decreased with increased N availability in 8-years plantation. There was negligible effect of N on both diversity and dominance in 12 years plantation. However, in the control plots, there was significant difference of species richness between plantation of different age ( $P < 0.05$ ) (Table 3-2).

Table 3- 5 One way ANOVA for response of herbaceous diversity to N addition in different age poplar Plantation (calculated in 1×1m<sup>2</sup>)

Site	Diversity	N0	N1	N2	N3	N4	F	Sig.
8 years	S	1.23± .18a	1.16± .16a	1.16± .17a	1.13± .11a	1.17± .11a	0.181	0.944
	H'	2.27± .67a	2.53± .12a	2.48± .21a	2.46± .13a	2.50± .35a	0.308	0.868
	D	.20± .21a	.11± .19a	.12± .02a	0.12± .01a	.12± .04a	0.614	0.659
	J	.70± .19a	.80± .029a	.79± .04a	.78± .03a	.79± .07a	0.684	0.614
12 years	S	1.08± .26a	.98± .07a	.88± .09a	1± .07a	.91± .04a	1.408	0.279
	H'	2.33± .19a	2.42± .11a	2.24± .19a	2.39± .05a	2.32± .17a	0.859	0.551
	D	.14± .03a	.12± .01a	.15± .04a	.12± .01a	.14± .04a	0.946	0.465
	J	.76± .05a	.81± .12a	.77± .06a	.79± .03a	.80± .07a	0.546	0.705
18 years	S	.86± 2.5a	.80± .82a	.73± 2.63a	.77± 2.52a	.88± 3.59a	0.926	0.475
	H'	2.29± .33a	2.19± .07a	2.09± .21a	1.90± .18a	2.17± .16a	1.951	0.154
	D	.15± .06a	.15± .01a	.18± .06a	.24± .05a	.16± .04a	2.193	0.119
	J	.80± .09a	.79± .01a	.78± .08a	.69± .04a	.76± .1a	1.376	0.289

Data in parentheses indicate standard deviations. Figures with same alphabetic suffix indicate no significant differences using multiple comparisons (Turkey,  $\alpha > 0.05$ ). S denotes species richness' for Shannon diversity and D for dominance and J for Evenness (pielou's) index for diversity.

In this study we analyzed soil pH, soil moisture, SOC, TN and C: N ratio and values of those variables changed significantly after application of N (Table 3-6). We found moisture was negatively correlated with density of forbs in N1, N3 and N4, while C:N and TN positively correlated with grass in N3 plots and pH was positively correlated with grass in N2 plot (Table 3- 7). Besides this, neither of the others soil variable found correlated with any life form classified in the study site.



Table 3- 6 Effect of N on of soil properties in different stand age of plantation in study area

	Soil properties	Soil depth(cm)	N0	N1	N2	N3	N4
8-yrs	Moisture	0-15	0.25±0.00a	.22±0.02ab	0.22±0.02b	0.18±0.01b	0.24±0.01a
		15-30	0.23±0.01a	.22±0.01ab	.21±0.01ab	0.19±0.0b	0.23±0.00a
	Soil PH	0-15	8.27±.04ab	8.23±.02ab	8.16±0.06b	8.32±0.02ab	8.40±0.04ab
		15-30	8.22±.03b	8.31±0.02a	8.15±0.04b	8.24±0.05ab	8.38±0.02a
	TOC	0-15	16.43±.1ab	17.89±0.2a	15.92±0.3b	16.38±.7c	12.60±0.2c
		15-30	17.02±0.3a	18.17±0.3a	13.94±.6bc	14.61±0.26b	12.20±0.20c
TN	0-15	1.48±0.2ab	1.55±0.04c	1.28±0.44c	1.32±0.07bc	0.73±0.04d	
	15-30	1.86±0.03a	1.93±.05b	1.29±0.09b	1.48±0.05b	0.77±0.02c	
C:N	0-15	11.08±.2b	11.58±0.2b	12.49±0.14b	12.56±1.09b	17.26±0.58a	
	15-30	9.15±.28b	9.41±0.28b	9.92±0.70b	10.91±0.50b	15.79±0.41a	
12-yrs	Moisture	0-15	0.25±0.01a	0.25±0.00a	0.24±0.01a	0.24±0.01a	0.24±0.03a
		15-30	0.25±0.00a	0.25±0.00a	0.24±0.00a	0.23±0.00a	0.23±0.01a
	Soil PH	0-15	8.27±0.08c	8.3±0.03ab	8.26±0.03b	8.39±0.07b	5.68±2.72a
		15-30	8.15±0.04a	8.39±0.01a	8.22±0.02a	8.28±0.08a	8.53±0.01a
	TOC	0-15	16.72±0.1b	18.38±0.2a	15.05±0.38c	12.80±0.32d	12.84±0.34d
		15-30	17.09±0.5a	18.92±0.8a	13.91±0.49b	12.15±0.28b	12.20±0.12b
TN	0-15	1.47±0.04ab	1.62±1.5a	1.27±0.76b	0.67±0.57c	.78±0.04c	
	15-30	1.67±0.01ab	1.93±0.02a	1.28±0.02b	.78±0.00c	1 ±0.00c	
C:N	0-15	11.37±0.2b	11.38±0.2b	11.96±0.94b	19±0.24a	16.72±1.58a	
	15-30	10.21±0.2bc	9.75±0.38c	10.85±0.3bc	15.59±0.72a	12.20±0.59b	
18-yrs	Moisture	0-15	0.25±0.01a	0.22±0.03a	0.28±0.02a	0.23±0.02a	0.25±0.03a
		15-30	0.31±0.08a	0.22±0.02a	0.20±0.01a	0.22±0.01a	0.23±0.02a
	Soil PH	0-15	8.25±0.04ab	8.20±0.05b	8.14±0.03b	8.23±0.09ab	8.47±0.05a
		15-30	8.28±0.0abc	8.2±0.08bc	8.38±0.6ab	8.09±0.06c	8.49±0.03a
	TOC	0-15	16.91±0.4a	14.4±0.1bc	15.8±0.03ab	13.11±0.09c	12.7±0.05c
		15-30	17.2±0.6a	13.9±0.3b	13.8±0.3b	12.1±0.23c	11.53±0.03c
TN	0-15	1.53±0.06a	1.23±0.07b	1.27±0.02ab	0.74±0.09c	0.89±0.04c	
	15-30	1.77±0.07a	1.24±0.04b	1.42±0.05b	0.76±0.03c	0.89±0.03c	
C:N	0-15	11.06±0.1b	11.76±0.2b	12.45±0.33b	18.20±1.52a	14.41±0.45b	
	15-30	9.75±0.09c	11.26±0.4c	9.77±0.14c	15.91±0.54a	12.99±0.44b	

Table 3- 7 Correlation coefficient (rho) between density of different life form in different treatment level to TN, SOC, C:N, moisture, and soil pH.

Treatment	Life form	Correlation coefficient				
		TN	TC	CN	Moisture	Ph
N0	vine	0.134	0.218	0.268	-0.042	-0.294
	Forb	-0.117	-0.233	-0.367	-0.067	0.276
	Grass	-0.017	0.283	0.067	0.217	-0.109
N1	vine	-0.066	0.124	0.448	0.464	0.086
	Forb	0.134	0.145	-0.182	-0.67*	0.102
	Grass	0.107	0.018	-0.218	-0.474	-0.433
N2	vine	0.244	-0.66	-0.598	0.424	0.428
	Forb	-0.012	0.402	0.204	-0.057	-0.504
	Grass	0.393	-0.105	-0.457	0.234	0.72*
N3	vine	-0.388	-0.257	0.368	0.463	0.26
	Forb	0.277	0.283	-0.254	-0.74*	0.207
	Grass	0.72*	0.566	0.716*	-0.603	0.069
N4	vine	0.513	-0.08	-0.538	0.618	0.4
	Forb	0.158	0.005	-0.185	-0.76*	0.499
	Grass	-0.226	0.254	0.252	0.34	-0.487

\*correlation coefficient significant at (0.05)

### 3.4 Discussion

#### 3.4.1 Influence of N on species Composition and Important Values

The effect of N on species IVs of herbs was obviously positive for younger plantation and was negative in mature plantation. In comparison to control the average IVs per species in 8 and 12 year plantation increased by 20 % and 14% respectively in N-treatment plots with but in mature plantation decreased by 8 % after treatment. So with increased N, we observed overall SIVs changes faster than species richness and diversity. This result in general agreed with the previous studies (Borer, E. T. et al., 2014; Seabloom et al., 2013; Xu et al., 2015). We also noticed that few generalist species of vines, perennial forbs were able to cope with severe N scarcity as they

can adopt variability of soil type in term of its physical and chemical composition were common in all plots of different level of N which was consistent to the study (Verma et al., 2014).

As the similar finding of this study (Davis et al., 1999), we also observed species composition changed with application of N and this may clearly varies with plantation type and characteristics of species. At the N3 and N4 treatment plot *Torilis japonica*, *Artimisia sylvatica* were dominant in 8-year plantation, *Erigeron annus L*, *Cayratia trifolia* and *Lophatherum gracile* were dominant in 12 year plantation and *Roegneria C*, *Corchoropsis psilocarpa* *Cyclosorus acuminatus* in 18-years plantation. In high-N treated plots, these 2/3 species accounted more than 30 % of SIVs and most of these species demonstrated that they have common habitat requirement for water, shade, soil pH etc. For example *Torolis japonica* is biannual invasive plant can grow in moist to dry site and withstand wide range of soil (WI DNR, 2010) and reproduce rapidly. *Roegneria C* is also share similar soil and other site requirement with *Torolis japonica*. At adequately high N level; few nitrofilious, aggressive generalists species more often invasive species (viz;*Roegneria C* *Polypogon fugax* ,*Cayratia trifolia*,*Lophatherum gracile* etc) became dominant, thereby eliminated the rare as illustrated (Verma et al., 2014). This was similar to the trend expected in the paper studied about alpine vascular plant (Alatalo et al., 2014) in relation to nutrient change which mentioned that change in nutrients might cause to shift in vascular community dominance in future. Plant species respond differently to N addition and the resultant change in species competition alter the species abundance and which ultimately accompanied by loss or increase of species (Appendix 3- 1 to 3-3).

Vine and graminoids lost their values in N-treated plots in 8-years plantation, those lost was compensated by increase of SIVs in forbs species which was inconsistent to overall conclusion of previous study in alpine communities (Capioli et al., 2012; Onipchenko et al., 2012) which reported the increase the abundance of graminoids with N addition. In mature plantation, both vine and graminoids lost their SIVs, while forbs were benefitted. So our finding did not agree with the previous conclusion (Rao et al., 2009; Xu et al., 2015) which mentioned that graminoids groups benefitted more than other herbs with increased N. We also found selected species under those groups found most sensitive and only these aggressive grass species increased and rest of others lost their important values. The study suggested that, plant species had their different

sensitivity level to different level of N and this were found depend upon the characteristic of individual and stage of plantation.

#### 3.4.2 Influence of N on herbaceous Life form diversity

We observed basically two patterns, at first in 8 and 18 years with N addition forbs and vine density increased and graminoids density decreased, and next was negligible increase of forbs and vine and with gradual increase of graminoids in 12 years plantation. The overall density of graminoids was the lowest in the plots of 12-year plantation. The increased forbs and vines, and decreased the density grass in 8 and 18-year plantation with N addition was well agreed with density hypothesis (Chu et al., 2010; Schamp & Aarssen, 2014; Stevens & Carson, 1999). According to this hypothesis, shaded and small individual of all species die and are lost from the plot randomly. Smaller species are mostly competitively inferior particularly when it related to competition for space and light (Gaudet & Keddy, 1988; Goldberg & Landa, 1991; Keddy et al., 2002; Wang et al., 2010). This is consistent with the finding with changes in soil nutrients which may affect plant performance indirectly by enhancing competition among species (Fraterrigo et al., 2009). But this decrease of graminoids diversity was compensated by increase of forbs and vine diversity however in average showed turn down the overall diversity. The changes in species number of herbs groups was responsible for turned down the overall species diversity as reported (Stevens et al., 2009) and this all phenomena particularly depended on forbs richness as proportion of forbs was highest (>60%) in entire plantation. This also indicated that contribution of graminoids and vines was little in overall diversity due to their low proportion. So diversity of understory in the plantation area was determined by deliberate equilibrium between forbs and graminoids but it differ in different stand age.

This lower vegetation density in 12-year plantation caused decrease competition for light and space provided the opportunity for obvious increase in graminoids density and still it was not significant for overall biodiversity as graminoids proportion which was low in the entire study area. In contrast to this, forbs in 8-years old, graminoids in 12-years old and vine in 18-years old plantation followed constantly decreased the in every N treated plots with different N gradient irrespective of dose which was inconsistent to general trends described in the study (Verma et al., 2014)

The N resistance of the most of forbs in the entire area was more than others. Analysis of dominant forbs in the study area showed that forbs were most highly benefitted either by low N or by high-N. With addition of N, our observation was similar as in this study (Alatalo et al., 2014) which mentioned that short forbs were benefitted over others in short term addition of N, however in the long run diversity of forbs found decreased. As forbs were higher in proportion in the entire plantation, so much of the result of ours study likely be affected by forbs.

#### 3.4.3 Influence of N on herbaceous diversity

The study concluded two pattern but opposite each other in case of richness and diversity of herbaceous in the study area. In young forest with increased N availability species richness and dominance decreased but evenness increased resulting increased the diversity in overall. In contrast to this, in mature plantation, with increased N availability species richness and dominance increased slightly but evenness decreased resulting decreasing the diversity. So in both case, evenness index found responsive measure to see the effect of fertilization as described by those in (Bowman et al., 1993). The result was found inconsistency with general trend as (Gilliam, 2006) concluded in his review that with increase N availability decrease in herb layer species richness and evenness resulting loss of diversity due to decrease of species richness and evenness. The evenness in younger plantation (8 and 12-years old) increased with increasing N-treatment but it followed opposite trend in mature plantation which resulted for overall increase in diversity in younger plantation and decrease in mature plantation.

The study suggested, for treatment in general, that species diversity was low at N0 (control plots), increases to maximum at N1 and N2 level (50-100kg N treated plots) and consistently declined towards N3 and very N4 level (150 to 200kg N treated plots). The result pattern in overall agreed with previous studies conducted in grassland in dry tropical India however dose of N treatment was different. The Shannon diversity (H) was almost equal (2.27) in control plots of 8 and 18-years old plantation and species richness was 32 and 25 respectively, with increasing N availability in 8-years plantation diversity increased (up to 2.5) but richness decreased in N-treated plots (27 species) in which trend was consistent with mature grassland and severely degraded grassland of report (Xu et al., 2015). The result also agreed to overall trend in the study (Borer, Elizabeth T et al., 2014; Isbell et al., 2013) mentioned N as primary factor to decrease species richness. The lost of species was highest in N1 plots in 8 –years plantation (19%), N2

treated plots in 12- years plantation (21%) , however it was opposite for mature plantation with highest gain was in N4 treated plots (24%). However, in mature plantation diversity decreased (up to 1.95) and specie richness increased in N-treated plot (up to 31species) which trend was consistent with extremely degraded grassland and moderately degraded grassland (Verma et al., 2014; Xu et al., 2015). However none of the diversity indices were differ significantly. The phenomenon may have been due to relatively short experimental time (Xu et al., 2015). The overall facts of response of N on species richness may be supported by the study (Vockenhuber et al., 2011) which concluded that tree diversity and environment affect the herbaceous diversity.

Ours result also concluded that biodiversity increased with N fertilization in younger plantation and decreased in mature plantation. The overall the N treatment showed obvious trend to decrease herbaceous diversity in mature age which agreed with the finding of the paper (Hautier et al., 2014; Isbell et al., 2013) that mentioned excess N decrease the herbaceous diversity in temperate forest but in young age it was quite opposite. So the increased competition for light and nutrient in younger plantation with N treatment caused to decrease the richness due to decrease of species that are efficient and adopted in low N, which was consistent with (Gilliam, 2006; Neufeld & Young, 2003; Verheyen & Hermy, 2004). Increased the biomass (as reflected in 70 percent in BA/ha than in mature age) might be related to decrease in below ground nutrient (Ge et al., 2015) in mature plantation. However, in mature plantation decreased the competition for light, space and nutrient for herbaceous along with addition of N might cause to increase the species richness. There are so many factor affect the herbaceous diversity including soil pH, nutrient availability, soil moisture, light and litter layer (Vockenhuber et al., 2011). Similarly, in this study we analyzed soil pH, Moisture, SOC, TN and C: N ratio, in most case, we did not found clear trend of relationship with density of individual life form with those measured soil variable, but we found moisture was negatively correlated with density of forbs in most of the plots (except N0 and N1 treated plot) and grass was found positively correlated TN and negatively correlated with C: N in N3 treated plot only.

But overall pattern was found clearly different with age and growth of plantation which was consistent with the conclusion of paper studied on poplar clone plantation (Birmele et al., 2015) indicated that plant community succession takes place in ground vegetation indicating species composition is age-dependent. In this study also revealed the same fact that species under the

same herbaceous groups under different stand age of same species responded differently sometime opposite with N treatment.

#### **4 Conclusion and implication**

The result showed significant differences in richness and composition among the different three stand ages of plantation in different growth stage is highly associated with C: N ratio. The diversity of both herbs and shrubs was highest in the younger stage of the plantation particularly in 12-years than in the mature stand, which was primarily the result of differences in TOC, TN and C: N ratio among the site of three stand age of plantation, and was highest in the middle age plantation. It is likely that the quantity of light was roughly equivalent to all stand age of plantation and that may be because of structure of poplar plantation ( a density of stand around 360-980/ha and all the tree pruned to 6 m above ground level) allows enough light to be transmitted to the understory in the study site. So as expected we do not find the association of overstory structure of poplar on understory herbaceous diversity. However, it might have affected indirectly influencing the nutrient cycling, which is reflected in basal growth of different stage of plantation and significant different in overstory structure among the three stage of plantation. The study findings indicated that only few species of understory were specific to particular stage of plantation and majority of them were common to the entire plantation. Among the life form we detected in the study area, the richness of forbs was highest in entire plantation and differs significantly in the three different stand age of the plantations. The contribution of 67 species of total forbs (nearly 70 %) of the total herbs list indicated that the plantation is forbs dominated particularly Asteraceae family (26 species), which was the major invasive species family in china and this showed that the understory of the plantation was high severity of invasive species.

The study concluded that N enrichment increased the herbaceous diversity in the younger plantation, while in the mature it decreased. Among the soil properties we measured TN and TOC differed significantly with age and treatment in both soil layer ( 0-15,15-30 cm) and found higher in young plantation with than the mature. Similarly there was also obvious difference in overstory parameter mainly mean basal growth, mean diameter at breast height (DBH), mean height between the three stand age of the plantation with significantly higher basal growth in mature plant. This also indicated that with increase N availability diversity increased at area with higher

soil nutrients and lower above ground woody biomass, and diversity decreased at area with lower soil nutrient content and higher woody biomass. The trend was opposite for species richness.

In general, forbs and vine were benefitted, while every time graminoids suffered from the N treatment due to significant change in soil properties and characteristics of the plant. Soil TN, TOC, C:N ratio, moisture, soil pH changed significantly with increase N availability but most often those factor were not directly associated with change in biodiversity. Only moisture found directly associated with forbs diversity in N treated plots. The overall decrease in biodiversity due to age of plantation was more prominent than increase in biodiversity due to fertilization but this response may vary with life form. The study clearly indicated that the response of N on the different indices of herbs in the study area primarily influenced by stand age of the plantation and then level of nitrogen which were determined by deliberate equilibrium between forbs and graminoids.

This research generated the baseline information. It is proposed to deal with an issue of nitrogen limitation on plantation through the artificial N addition to the level that maintain the optimum growth of herbaceous plant which provide economic gains and numbers of environmental benefits particularly through improving the nutrient cycling. This study will yield result that can be used as the base line information to monitor the understory diversity and associated parameter and ultimately helpful to formulate related policies in regional scale. The generated information from this study also expected is helpful for monitoring and evaluating sustainable management of soil nutrients in plantation and monitoring the understory in future.



## Appendix

Appendix 2-1: Pair wise t test of SIVs of important overlap herbaceous species

Comparison	R	Pair difference					
		Mean	Std Deviation	S. E	T	Df	P
8 and 12 years	-0.255	1.084	5.75	1.34	0.799	17	0.435
8 and 18 years	-0.36	2.64	7.3	2.1	-1.3	12	0.217
12 and 18 years	0.91	1.63	3.62	1	-1.56	11	0.146

Appendix 2-2 Pearson correlation coefficient with stand character with diversity (H) and Evenness and Jaccards coefficient of similarity

Plantation	Shannon (H')				Evenness						Jaccards Similarity		
	Mean Ht		Volume		Mean Ht		Volume		(H')		8- yrs	12- yrs	18- yrs
	R	P	R	P	R	P	R	P	R	P			
8-yrs	0.05	0.83	-0.24	0.29	0.18	0.42	-0.24	0.31	.91**	0	1		
12-yrs	0.11	0.62	-0.08	0.43	-0.02	.92	0.002	.99	.56*	0.	.30	1	
18-yrs	0.12	0.61	0.03	0.92	0.04	.85	-0.05	.82	.79**	0	.21	.26	1

Correlation is significant at 0.01 level indicated \*\* sign and correlation is significant at 0.05 indicated by \*

Appendix 2-3 Most abundant and rare species by life form and their Important Values

Stand Age	Abundant Species	LF	SIVs	Rare Species	LF	SIVs
8- years	<i>Roegneria C.</i> (Gramineae)	H	10.81	<i>Ipomoea purpurea</i> (Araceae)	H	0.05
	<i>Setaria viridis</i> (Poaceae)	G	10.49	<i>Fructus trichosanthis</i> (cucurbitaceae)	V	0.056
	<i>Polypogon fugax</i> (Poaceae)	G	8.7	<i>Parthenocissus quinquefolia</i> (Vitaceae)	V	0.077
	<i>Torilis japonica</i> (Apiaceae)	H	7.48	<i>Malachium aquaticum L.</i> (caryophyllaceae)	H	0.104
	<i>Miscanthus sinensis Anders.</i> (Poaceae)	G	5.41	<i>Dioscorea opposita</i> (Discoreaceae)	V	0.108
	<i>Duchesnea indica</i> (Rosaceae)	H	5.28			
	<i>Cayratia trifolia</i> (Vitaceae)	V	5.26			
	<i>Oplismenls undulatifolius folius</i> (Poaceae)	G	4.56			
	<i>Erigeron annuus L.</i> (Asteraceae)	H	4.13			
	<i>Perilla frutescens</i> (lamiaceae)	H	3.89			
	12- years	<i>Erigeron annuus L.</i> (Malvaceae)	H	13.3	<i>Polygonum hydropiper L</i> (polygonaceae)	H
<i>Lophatherum gracile</i> (Poaceae)		G	14.86	<i>Dryopteris championii</i> (Dryopteridaceae)	F	0.055
<i>Achyranthes bidentata Bl.</i> (Amaranthaceae)		H	12.26	<i>Perilla frutescens L.</i> (Lamiaceae)	H	0.08
<i>Cayratia trifolia</i> (Vitaceae)		V	9.82	<i>Viola prionantha</i> (Violaceae)	H	0.089
<i>Corchoropsis psilocarpa</i> (Malvaceae)		H	6.04	<i>Polygonum perfoliatum L.</i> (Polygonaceae)	V	0.105
<i>Oplismenls undulatifolius folius</i> (Poaceae)		G	5.37			
<i>Humulus scandens</i> (Moraceae)		V	4.51			
<i>Dioscorea L.</i> (Discoreaceae)		V	4.5			
<i>Miscanthus sinensis Anders.</i> (Poaceae)		G	3.21			
18- years		<i>Oxalis corniculata</i> (oxalidaceae)	H	21.61		
	<i>Corchoropsis psilocarpa</i> (Malvaceae)	H	5.51	<i>Plantago asiatica L.</i> (Plantaginaceae)	H	0.276
	<i>Achyranthes bidentata Bl.</i> (Amaranthaceae)	H	5.07	<i>Metaplexis japonica</i> (asclepiadaceae)	H	0.18
	<i>Plantago major Purpurea</i> (Plantaganaceae)	H	4.17	<i>Conyza canadensis</i> L. (Asteraceae)	H	0.306
	<i>Cirsium setosum</i> (Asteraceae)	H	3.68	<i>Equisetum ramosissimum Desf</i> (Equisetaceae)	H	0.287
	<i>Cayratia trifolia</i> (Vitaceae)	V	5.56			
	<i>Dioscorea L.</i> (Discoreaceae)	V	1.51			
	<i>Oplismenls undulatifolius folius</i> (Poaceae)	G	11.23			
	<i>Miscanthus sinensis Anders.</i> (Poaceae)	G	10.82			
	<i>Cyclosorus acuminatus</i> (Thelypteridaceae)	F	8.8			

Appendix 2- 4 List of herbaceous found in the study area

S.N.	Chinese Name	Scientific Name	LF	Type	Fl/No n	Family	Remarks
1	构树 阔鳞鳞毛	<i>Broussonetia papyrifera</i>	V	P	Fl	Miraceae	Invasive
2	蕨 延羽卵果	<i>Dryopteris championii</i>	F	B	Fl	Dryopteridaceae	Medicine
3	蕨	<i>Phegopteris decursivepinnata</i>	F	P	Non fl.	Thelypteridaceae	/
4	渐尖毛蕨	<i>Cyclosorus acuminatus</i>	F	P	Non	Thelypteridaceae	/
5	早熟禾	<i>Poa annua L.</i>	G	A	Fl	Poaceae	/
6	棒头草	<i>Polypogon fugax</i>	G	A	Fl	Poaceae	Invasive
7	淡竹叶	<i>Lophatherum gracile</i>	G	A	Fl	Poaceae	/
8	狗尾草	<i>Setaria viridis</i>	G	A	Fl	Poaceae	Medicine
9	求米草	<i>Oplismenites undulatifolius folius</i>	G	P	Fl	Poaceae	/
10	狗牙根	<i>Cynodon dactylon</i>	G	P	Fl	Graminoid	/
11	矛叶荩草	<i>Arthraxon prionodes</i>	G	P	Fl	Poaceae	/
12	芒=芒草	<i>Miscanthus sinensis</i> Anderss.	G	P	Fl	Poaceae	/
13	芒草	<i>Miscanthus sinensis</i> Anderss.	G	P	Fl	Poaceae	Medicine
14	一年蓬	<i>Erigeron annuus L.</i>	H	A	Fl	Asteraceae	Medicine
15	光果田麻	<i>Corchoropsis psilocarpa</i>	H	A	Fl	Malvaceae	/
16	凹头苋	<i>Amaranthus lividus L.</i>	H	A	Fl	Amaranthaceae	/
17	小窃衣	<i>Torilis japonica</i>	H	A	Fl	Apiaceae	/
18	小飞蓬	<i>Conyza canadensis.</i>	H	A	Fl	Asteraceae	/
19	灰绿苔草	<i>Carex forrestii</i>	H	A	Fl	Cyperaceae	/
20	白花益母草	<i>Leonurus sibiricus L</i>	H	A	Fl	Lamiaceae	/
21	紫苏	<i>Perilla frutescens L.</i>	H	A	Fl	Lamiaceae	/
22	翅果菊	<i>Pterocypselalaciniata</i>	H	A	Fl	Asteraceae	/
23	苦苣菜 苦苣菜=苦	<i>Sonchus oleraceus L.</i>	H	A	Fl	Asteraceae	/
24	莢菜 苦菜=苦莢	<i>Ixeris denticulate</i>	H	A	Fl	Asteraceae	/
25	菜	<i>Ixeris sonchifolia</i>	H	A	Fl	Asteraceae	/
26	莎草	<i>Cyperus rotundus L.</i>	H	A	Fl	Graminoid	/
27	水蓼	<i>Polygonum hydropiper L.</i>	H	A	Fl	Polygonum	/
28	藜	<i>Chenopodium album</i>	H	A	Fl	Amaranthaceae	/
29	贼小豆	<i>Vigna minima</i>	H	A	Fl	leguminosae	/
30	辣蓼	<i>Polygonum hydropiper L.</i>	H	A	Fl	Polygonaceae	/
31	野大豆	<i>Glycine soja</i>	H	A	Fl	Fabaceae	/
32	野老鹳草	<i>Geranium carolinianum</i> L.	H	A	Fl	Geraniaceae	/

33	鬼针草	<i>Bidens pilosa</i> L.	H	A	Fl	Asteraceae	/
34	鸭跖草	<i>Commelina communis</i>	H	A	Fl	Commelinaceae	Invasive
35	龙葵	<i>Solanum nigrum</i> L.	H	A	Fl	Solanaceae	/
36	烟管蓟	<i>Cirsium pendulum</i>	H	B	Fl	Asteraceae	Invasive
37	窃衣	<i>Torilis japonica</i>	H	B	Fl	Apiaceae	/
38	茵陈蒿	<i>Artemisiacapillaris</i> Thunb	H	B	Fl	Compositae	/
39	黄鹌菜	<i>Youngia japonica</i>	H	B	Fl	Compositae	/
40	一枝黄花	<i>Solidago canadensis</i> L.	H	P	Fl	Asteraceae	Medicine
41	三毛草	<i>Trisetum umbratile</i>	H	P	Fl	Poaceae	Poison medicinal
42	三脉紫菀	<i>Aster ageratoides</i>	H	P	Fl	Asteraceae	l
43	刺儿菜	<i>Cirsium setosum</i>	H	P	Fl	Asteraceae	Medicine
44	半夏	<i>Pinellia ternate</i> <i>Carpesium</i>	H	P	Fl	Convolvulaceae	/ medicinal
45	天名精 抱茎苦苣 菜	<i>macrocephalum</i>	H	P	Fl	Asteraceae	l
46	菜	<i>Ixeris sonchifolia</i>	H	P	Fl	Compositae	/
47	早开堇菜	<i>Viola prionantha</i>	H	P	Fl	Violaceae	/
48	泽兰	<i>Aconitum gymnandrum</i> <i>Maxim.</i>	H	P	Fl	Ranunculaceae	/
49	紫茎泽兰	<i>Eupatorium adenophora</i> <i>Spreng.</i>	H	P	Fl	Asteraceae	/
50	海州常山	<i>Clerodendrum</i> <i>trichotomum</i> Thunb.	H	P	Fl	verbenaceae	/
51	牛膝	<i>Achyranthes bidentata</i> Bl.	H	P	Fl	Amaranthaceae	/
52	白苏	<i>Perilla frutescens</i>	H	P	Fl	Lamiaceae	Invasive
53	紫叶车前	<i>Plantago major Purpurea</i>	H	P	Fl	Plantaganaceae	Invasive
54	紫花地丁	<i>Violaedoensis</i> Makino	H	P	Fl	Violaceae	Medicine
55	美洲商陆	<i>Phytolacca Americana</i> L	H	P	Fl	phytolacaceae	/
56	艾蒿	<i>Artemisia lavandulaefolia</i> <i>DC</i>	H	P	Fl	Asteraceae	/
57	节节草	<i>Equisetum ramosissimum</i> <i>Desf</i>	H	P	Fl	Equisetaceae	/
58	苧麻	<i>Boehmeria nivea</i> L.	H	P	Fl	Urticaceae	/
59	茴茴蒜	<i>Ranunculus chinensis</i>	H	P	Fl	Ranunculaceae	/
60	荩草	<i>Setaria chondrache</i>	H	P	Fl	Poaceae	/
61	萝藦	<i>Metaplexis japonica</i>	H	P	Fl	asclepiadaceae	/
62	蒲公英	<i>Taraxacum mongolicum</i>	H	P	Fl	Asteraceae	/
63	蓟	<i>Cirsium japonicum</i>	H	P	Fl	Asteraceae	/
64	蛇莓	<i>Duchesnea indica</i>	H	P	Fl	Rosaceae	/
65	车前=车前 草 车前草=车 前	<i>Plantago asiatica</i> L.	H	P	Fl	Plantaginaceae	/
66	前	<i>Plantago asiatica</i> L.	H	P	Fl	Plantaginaceae	/
67	酢浆草	<i>Oxalis corniculata</i>	H	P	Fl	oxalidaceae	/
68	野艾	<i>Artemisia lavandulaefolia</i>	H	P	Fl	Compositae	/
69	长裂苦苣	<i>Sonchus brachyotus</i> DC.	H	P	Fl	Asteraceae	/

菜							
70	阴地蒿	<i>Artemisia sylvatica</i>	H	P	Fl	Arteraceae	/
71	青绿苔草	<i>Carex breviculmis</i>	H	P	Fl	Cyperaceae	/
72	鹅观草	<i>Roegneria C.</i>	H	P	Fl	Gramineae	/
73	麦冬	<i>Ophiopogon japonicus</i>	H	P	Fl	Asparagaceae	/
74	黄花酢浆草	<i>Oxalis pes-caprae L.</i>	H	P	Fl	oxalidaceae	/
75	桑	<i>Morus alba L.</i>	Sl	P	Fl	Moraceae	/
76	甜槠	<i>Castanopsis eyrei</i>	Sl	P	Fl	fagaceae	/
77	黄檀	<i>Dalbergia hupeana Hance</i>	Sl	P	Fl	leguminosae	/
78	三裂叶绣线菊	<i>Spiraea trilobata L.</i>	Sh	P	Fl	Rosaceae	Medicine
79	白马骨	<i>Serissa serissoides</i>	Sh	P	Fl	Rubiceae	/
80	圆叶牵牛	<i>Ipomoea purpurea</i>	V	A	Fl	Araceae	/
81	杠板归	<i>Polygonum perfoliatum L.</i>	V	A	Fl	Polygonaceae	/
82	薯蓣	<i>Dioscorea L.</i>	V	A	Fl	Discoreaceae	/
83	蓼蓂	<i>Vitis bryoniifolia</i>	V	A	Fl	Vitaceae	/
84	乌莓	<i>Cayratia trifolia</i> <i>Parthenocissus</i>	V	P	Fl	Vitaceae	/
85	五叶地锦	<i>quinquefolia</i>	V	P	Fl	Vitaceae	/
86	山药	<i>Dioscorea opposite</i>	V	P	Fl	Discoreaceae	/
87	牛繁缕=鹅肠菜	<i>Malachium aquaticum L.</i>	V	P	Fl	caryophyllaceae	/
88	瓜蒌	<i>fructus trichosanthis</i>	V	P	Fl	cucurbitaceae	/
89	茜草	<i>Rubia cordifolia L.</i>	V	P	Fl	Rubiceae	/
90	莲子草	<i>Alternanthera Sessilis</i>	V	P	Fl	Amaranthaceae	/
91	葎草	<i>Humulus scandens</i>	V	P	Fl	Moraceae	/
92	蛇葡萄	<i>AmpelopsinA delavayana</i>	V	P	Fl	Vitaceae	/
93	野豌豆	<i>Vicia cracca L.</i>	V	P	Fl	Fabaceae	/
94	鸡矢藤	<i>Paederia tomentosa</i>	V	P	Fl	Rubiaceae	/
95	鹅绒藤	<i>Cynanchum chinense</i>	V	P	Fl	Apocynaceae	/
96	牛繁缕=鹅肠菜	<i>Malachium aquaticum L.</i>	V	P	Fl	caryophyllaceae	/

LF=Life form, G=graminoids; V=Vine; F=forbs; Fl=flowering; A=annual; p=perennial

Appendix 3- 1 Life form classification of 8-yeers plantation

Species	Family	Life Form	Species Importance Values				
			N0	N1	N2	N3	N4
<i>Polypogon fugax</i>	Poaceae	G	8.705	15.501	1.2336	2.1702	3.5582
<i>Setaria viridis</i>	Poaceae	G	10.5	5.6089	14.495	4.2434	4.4537
<i>Arthraxon prionodes</i>	Poaceae	G	2.029	0	2.4414	15.465	3.4054
<i>Miscanthus sinensis</i> Anderss	Poaceae	G	0	0	6.0204	3.1388	0.4449
<i>Erigeron annuus L.</i>	Asteraceae	F	4.136	2.5099	2.65	0.8537	1.1259
<i>Carpesium macrocephalum</i>	Asteraceae	F	3.406	1.317	2.9126	1.2638	0.9465
<i>Conyzac canadensis L.</i>	Asteraceae	F	0.715	2.0283	0.3333	1.1947	0.0501
<i>Ixeris sonchifolia</i>	Compositae	F	1.523	0.3663	1.3608	0.4629	2.1502
<i>Achyranthes bidentata Bl.</i>	Amaranthaceae	F	3.668	1.6438	9.0188	5.4822	7.1321
<i>Perilla frutescens</i>	Lamiaceae	F	3.893	14.629	6.8594	2.3705	4.37
<i>Torilis japonica</i>	Apiaceae	F	7.483	4.7766	3.3142	13.516	13.457
<i>Plantago major Purpurea</i>	Plantaganaceae	F	1.491	0.4905	1.8892	0.7291	0.6618
<i>Phytolacca Americana L</i>	Phytolacaceae	F	0.548	0.5935	0.125	1.4011	0.6715
<i>Artemisia lavandulaefolia DC</i>	Asteraceae	F	0.057	5.4439	0.4955	0.6162	1.4157
<i>Ixeris sonchifolia</i>	Asteraceae	F	0.43	0.5383	0.2922	0.7602	0.3855
<i>Cirsium japonicum</i>	Asteraceae	F	2.456	2.7768	1.70 02	0.8168	2.9103
<i>Duchesnea indica</i>	Rosaceae	F	5.287	11.973	9.4062	7.9449	4.4593
<i>Artemisia sylvatica</i>	Arteraceae	F	3.605	1.7514	5.315	3.765	9.5729
<i>Carex breviculmis</i>	Cyperaceae	F	1.82	1.7814	0.7394	1.367	0.3425
<i>Solanum nigrum L.</i>	Solanaceae	F	0.182	0.9368	1.7577	2.1735	2.4423
<i>Cayratia trifolia</i>	Vitaceae	V	5.261	5.0319	4.5273	3.3135	5.4137
<i>Ipomoea purpurea</i>	Araceae	V	0.05	0.2344	0.1664	0.3202	0.3332
<i>Malachium aquaticum L.</i>	Caryophyllaceae	V	0.105	0.4055	0.6613	3.2859	1.6845
<i>Fructu strichosanthis</i>	Cucurbitaceae	V	3.256	0	0.119	0.254	0.5697
Others			29.4	19.662	22.166	23.091	28.043

Appendix 3-2 Life form classification of 12-year plantation

Species	Family	Life Form	Species Importance Values				
			N0	N1	N2	N3	N4
<i>Poa annua</i> L.	Poaceae	G	0.422	0	2.907	4.0949	2.327
<i>Polypogon fugax</i>	Poaceae	G	3.082	0	0	0.4203	0
<i>Oplismenus undulatifolius</i> folius	Poaceae	G	5.377	6.325	8.522	5.0732	3.667
<i>Lophatherum gracile</i>	Poaceae	G	14.86	7.26	13.87	11.325	19.84
<i>Miscanthus sinensis</i> Anderss.	Poaceae	G	3.214	0	9.836	4.2899	0.534
<i>Erigeron annuus</i> L.	Asteraceae	F	13.3	12.38	10.37	13.212	11.78
<i>Corchoropsis silocarpa</i>	Malvaceae	F	6.041	6.863	6.796	9.3218	4.088
<i>Cirsium setosum</i>	Asteraceae	F	0.658	0.275	0.458	0.395	0.203
<i>Conyzacana densa</i> L.	Asteraceae	F	1.812	5.806	1.035	2.2222	1.59
<i>Clerodendrum trichotomum</i> Thunb.	Verbenaceae	F	0.786	0.269	0.867	0.4248	0.615
<i>Achyranthes bidentata</i> Bl.	Amaranthaceae	F	12.26	4.674	4.026	5.6676	7.037
<i>Perilla frutescens</i>	Lamiaceae	F	3.546	4.247	6.81	3.8234	3.442
<i>Setaria chondrache</i>	Poaceae	F	2.829	0	3.506	0	0
<i>Metaplexis japonica</i>	Asclepiadaceae	F	0.25	0.558	0	0.2679	0.737
<i>Duchesnea indica</i>	Rosaceae	F	1.79	3.481	3.417	0	3.713
<i>Glycine soja</i>	Fabaceae	F	0.653	1.584	2.248	0.1651	0.091
<i>Artemisia lavandulaefolia</i>	Compositae	F	1.509	1.852	1.034	4.6717	1.605
<i>Roegneria C.</i>	Gramineae	F	2.119	3.128	0.953	4.2597	1.851
<i>Cayratia trifolia</i>	Vitaceae	V	9.821	12.29	5.989	10.212	9.636
<i>Ipomoea purpurea</i>	Araceae	V	0	0.103	0.105	0.0585	1.885
<i>Fructu trichosanthis</i>	Cucurbitaceae	V	1.869	4.699	5.381	0.1702	5.564
<i>Humulus scandens</i>	Moraceae	V	4.514	4.485	2.863	6.4105	11.34
<i>Dioscorea</i> L.	Discoreaceae	V	4.507	6.387	4.034	0.8276	4.272
<i>Malachium aquaticum</i> L.	Caryophyllaceae	V	0.205	3.784	0.447	0.208	1.123
Others			4.5707	9.547	4.533	12.479	3.05

Appendix 3-3 Life form classification of 8-yrars plantation

Species	Family	Life form	Species Importance Values				
			N0	N1	N2	N3	N4
<i>Cyclosorusacuminatus</i>	Thelypteridaceae	Fn	8.892	0	9.311	14.2	8.611
<i>Oplismenltsundulatifoliusfolius</i>	Poaceae	G	11.23	7.562	12.17	4.7	4.614
<i>Arthraxonprionodes</i>	Poaceae	G	0	3.556	3.635	10.3	2.395
<i>MiscanthussinensisAnderss.</i>	Poaceae	G	10.83	4.734	13.12	4.01	3.725
<i>Erigeron annuus L.</i>	Asteraceae	F	2.448	7.141	1.929	1.81	1.754
<i>Solidagocanadensis L.</i>	Asteraceae	F	3.682	4.497	2.653	0.78	1.361
<i>Trisetumumbratile</i>	Poaceae	F	3.371	9.121	0.866	0.72	1.918
<i>Corchoropsispsilocarpa</i>	Malvaceae	F	5.518	17.63	3.295	9.34	14.51
<i>Cirsiumsetosum</i>	Asteraceae	F	3.688	6.371	3.118	5.33	1.551
<i>Carpesiummacrocephalum</i>	Asteraceae	F	2.041	0.408	0	0.42	1.839
<i>Conyzacanadensis L.</i>	Asteraceae	F	0.307	0.466	0.117	0.7	6.163
<i>Viola prionantha</i>	Violaceae	F	1.401	0	0.233	0.79	0.151
<i>Aconitum gymnantrum Maxim.</i>	Ranunculaceae	F	0	3.759	0.184	0	2.599
<i>ClerodendrumtrichotomumThunb.</i>	Verbenaceae	F	2.982	7.184	0	1.06	3.034
<i>Carexforrestii</i>	Cyperaceae	F	3.212	4.024	7.322	2.38	0.43
<i>Achyranthesbidentata Bl.</i>	Amaranthaceae	F	5.079	2.406	1.879	1.38	2.094
<i>Torilis japonica</i>	Apiaceae	F	0	0.782	0.597	0	2.085
<i>Plantago major Purpurea</i>	Plantaganaceae	F	4.177	0.176	0	0	0.446
<i>Equisetum ramosissimumDesf</i>	Equisetaceae	F	0.287	0.114	0.988	0.49	1.114
<i>Ranunculus chinensis</i>	Rununculaceae	F	0	0.169	0	0.46	0.381
<i>Plantagoasiatica L.</i>	Plantaginaceae	F	0.542	0.768	0.179	0.33	0.416
<i>Roegneria C.</i>	Gramineae	F	21.62	10.84	29.72	30.8	22.34
<i>Cayratiatrifolia</i>	Vitaceae	V	5.565	4.398	4.306	4.17	6.018
<i>Dioscorea L.</i>	Discoreaceae	V	1.517	1.129	0.558	0.83	0.305
Others			1.618	2.765	3.826	5.05	10.14



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