Environtropica, June 2017, Vol. 14, 63-74 ISSN 1597-815X

Seasonal variation in the diversity of anthophilous insects in three land use types on Obafemi Awolowo University, Ile-Ife, Southwestern Nigeria.

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Accepted on February 07, 2017.

Abstract
Insect diversity loss in terrestrial ecosystems is driven by land use intensification, habitat loss and other anthropogenic activities and environmental stressors. Availability of critical resources such as nectar and pollen-providing plants are crucial to the success of many anthophilous insect taxa. This could result in differences in the abundance, species richness and species diversity of anthophilous insects between seasons and across different land use types. This study investigated the effect of land use and seasonal variation on the species richness, diversity and abundance of anthophilous insects sampled on three land use types grasslands, agricultural lands and secondary forests on Obafemi Awolowo University, Ile-Ife for 12 months covering the dry and wet seasons. Higher abundance was recorded in secondary forest and grassland land use types compared to agricultural land. There was no significant difference in the species richness and diversity of anthophilous insects among the land use types. The effect of seasonal variation was more pronounced on grassland and agricultural land compared to secondary forest. Flowering plant abundance and species richness positively influenced the abundance, species richness and diversity of anthophilous insects. This supports the notion that land use change and seasonal variations influences the availability of flowering plants which directly affects the abundance, species richness and diversity of anthophilous insects.

Keywords: Landscape transformation, Flowering plants, Pollinators, Floral resources, Forest Fragmentation, Climate.

Introduction
Anthophilous insects frequently visit flowers for floral resources especially pollen and or nectar. Anthophilous is a general term for animals most especially insects that are frequent to flowering plant and they rely on different habitats because they use a variety of floral as well as specific nesting sites or nesting materials during their life cycle (Gathmann and Tscharntke, 2002). Land use change and other factors that alter the distribution of floral resources influence the composition of anthophilous insects (Potts *et al.*, 2003). The transformation of natural areas for agricultural practices, settlements and industrialization has effect on the displacement of important species inhabiting the modified habitat (Latimer *et al.*, 2004).This pattern of land use change is usually influenced by seasonal variation which affects diversity and abundance of many taxa (Tylianakis *et al.*, 2005; Miguet *et al.,* 2013; Riedinger *et al.,* 2014). The resilience of land use change across seasons is important in the distribution of anthophilous insects which rely more on structurally diverse landscapes (Gathmann and

Tscharntke, 2002). In the tropics where high composition and ecological activities of these insects are observed, it is important to understand the link between anthropogenic land use activity and insect diversity and also how seasonal changes affect this pattern.

Insect-plant interaction which is one of the important ecological relationships declines with increasing land use change and landscape transformation. Agricultural practice which is one of the dominant land use practices in the tropics reduces the complexity of plant-pollinator networks over agriculturally fragmented landscapes (Kleijn *et al*., 2006; Kehinde and Samways, 2014). The increase in spatial isolation of populations has been reported to aid the loss of floral resources and nesting sites for pollinators and in the absence of these major needs, the richness of pollinator communities decreases (Viana *et al.,* 2012). Flower-visiting insects most especially some species of bees largely depend on undisturbed habitats for nesting particularly in the tropics (Michener, 2007).Ollerton *et al*. (2011) also reported that tropical forest provides more resources in terms of nectar and pollen for the insect pollinators.

Seasonal changes determine the abundance and species richness of flowering plants which provide floral resources for anthophilous insects (Vicens and Bosch, 2000). In Nigeria, low rainfall and low humidity are characteristic features of the dry season resulting in a dry and dusty environment. Drought is usually prominent most especially in the northern part of the country. This period usually records low diversity of flowering plants and floral resources such as nectar and pollen. However, some plants have their main flowering season at some point during the dry season; this may provide some resources for insect pollinators. The dry season is immediately followed by a period of high precipitation and humidity known as the wet season. This results in higher diversity of flowering plants providing greater floral reward for pollinators in comparison with the dry season. It is however uncertain how these seasonal fluctuations affect the availability of floral resources and consequently diversity of flower-visitors in different land use types.

This study evaluated the abundance and diversity of anthophilous insects in different land use types on Obafemi Awolowo University campus located in the rainforest vegetation zone of Nigeria. The study also determined the effect of seasonal variation on the diversity and abundance of anthophilous insects within the study area.

Materials and Methods

Study Area

The study was carried out in Obafemi Awolowo University (OAU), Ile-Ife, Nigeria located between Latitudes 07^{o} 26'N and 07^{o} 32'N and Longitudes 004^{o} 31'E and 004^{o} 35'E (Fig. 1). OAU campus has a total land size of approximately 5605 hectares. The weather of the region is characterized by wet and dry seasons which last from February to September and October to January respectively. The study area is constantly influenced by human activities resulting in landscape fragmentation due to the presence of buildings, agricultural lands, as well as grassland areas.

Study Sites

Three dominant land use types were identified for this study. These include grasslands with common flowering plant species such as *Sida acuta* L., *Ageratum conyzoides* L., *Chromolaena odorata* L.,*Tridax procumbens* L. and *Aspilia Africana* Pers. The grassland habitats in the study area are constantly modified by mowing activities most especially when the grasses and flowering plants are over grown. The second land use type is agricultural lands which are managed by local farmers and the common crops planted on these lands include cassava (*Manihot esculenta* (L.) Kunth) and maize (*Zea mays L.*). Some of the grasses located on grasslands were also found around the agricultural lands. Management activities on the farms include application of agrochemicals such as pesticides and fertilizers as well as mechanical tillage and removal of weeds. The third land use type is secondary forest habitats which are characterized by high canopy and dense vegetation. The common trees found in this land use type include *Azadirachta indica* A. Juss., *Alstonia boonei* De Wild., *Hildegardia barteri* (Mast.) Kosterm, *Leucaenal eucocephala* (Lam) de Wit. The dense forest understory has patches of flowering plants most of which are found on grassland. Anthophilous insects were sampled on the understory vegetation and along forest edges. Three replicates of each land use type were selected for sampling.

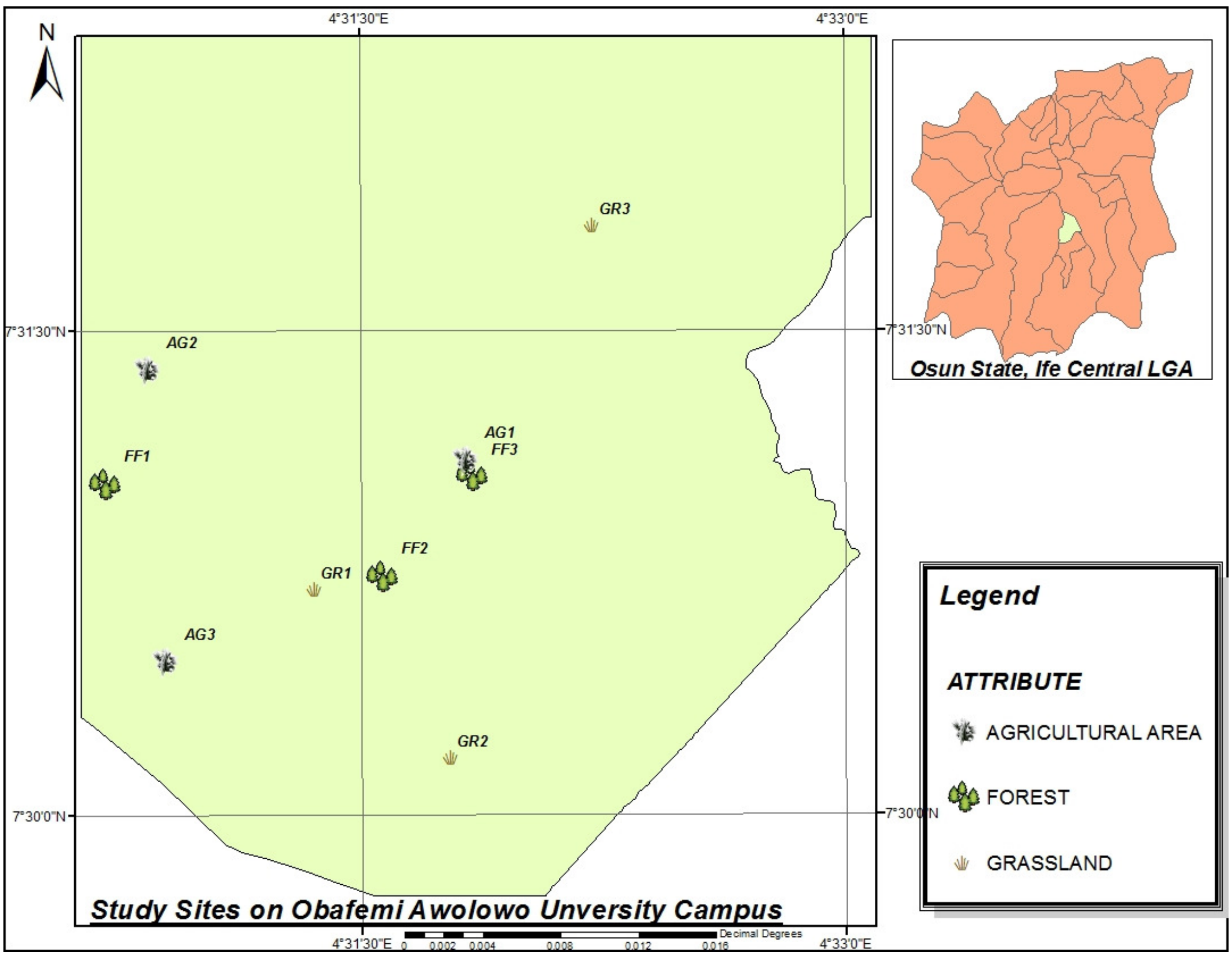

Fig 1: Study Sites and Land Use Types on Obafemi Awolowo University (OAU) Campus.
GR1 = Grassland site1, GR2 = Grassland site2, GR 3 = Grassland site3, AG1 = Agricultural land 1, AG2 = Agricultural land 2, AG3 = Agricultural land 3, FF1 = Secondary forest 1, FF2 = Secondary forest 2, FF3 = Secondary forest 3.

Sampling

Anthophilous insects were sampled for a period of 12months between May 2014 and April 2015. Sampling was conducted on days with favourable weather condition i.e. days without rainfall and with little or no cloud cover to limit bias from unfavourable weather conditions following the approach of Kehinde and Samways (2014). Reference specimens were deposited in the Entomology collections of Department of Zoology, Obafemi Awolowo University, Ile-Ife and the Entomology museum of the Agricultural Research Council, Roodeplaat, Pretoria, South Africa. Three different sampling methods were used for sampling insects on the study sites on a monthly basis as described below.

Twelve coloured bowls (each with a capacity of 1liter) comprising of four each of white, blue and yellow colours were placed randomly on the study sites following the approach described by Potts *et al.* (2005) and Wilson *et al.* (2009). Pan traps of different colours have often been used as a standard passive sampling method (Toler *et al.,* 2005, Roulston *et al.,* 2007) and are often used in biodiversity assessment (Schleuning *et al.,* 2011; Breitbach *et al.,* 2012).The bowls were half filled with water and few drops of liquid detergent were added to break the surface tension of the water and enhance insect trapping. The bowls were left on the sampling sites for a period of 48 hours after which anthophilous insects collected were removed, rinsed and stored in 70% ethanol until sorting and identification.

The second sampling method involved the observation and collection of anthophilous insects along transects on the study sites within the hours of 09:00 – 14:00 h daily. Two 100m x 5m transects were sampled on a monthly basis on each study site according to Rouslton *et al.* (2007). Insects visiting the floral part of flowering plants along the transects were observed and collected for later identification. The plants visited by the insects were also collected and identified.

The third sampling method involved the use of Modified Window Intercept Trap Method (MWIT) made with a transparent nylon material attached to poultry feeding trough. The trap was designed to intercept the flight path of anthophilous insects on the study sites. The feeding troughs were filled with water and few drops of detergent to break the surface tension of the water. Two MWIT were placed on each study site for 48 hours during the sampling period after which insects caught in the water were removed and stored in 70% ethanol until sorting and identification.

Insects sampled were sorted into different insect taxa and reference specimens of the insects were removed from the ethanol, rinsed and pinned with the use of entomological pins in an insect box. Sorting based on morphological features was done with the aid of a hand lens and dissecting microscope (Model - Zeiss, Steimi, 2000). Identification of anthophilous insects was done at Biosystematics Division of Plant Protection Research Institute, ARC – Pretoria, South Africa. Identification was done to species level and where not possible to family and genus levels. Non-anthophilous insects collected with the passive sampling methods were removed from the collection and not included in the data analysis.

The sampling of plants visited by the anthophilous insects was done for every month of sampling on the different sites. A 2m x 2m quadrat was placed at 25m interval along each 100m transect where insects were sampled. The species richness and abundance of flowering plants were estimated within the quadrats. Flowering plant specimens were identified at Ife Herbarium, Ile-Ife.

Statistical Analysis

The species diversity of insects was estimated using Shannon Wiener index of diversity. One-way Analysis of Variance (ANOVA) was used to determine the difference in the abundance, species richness and species diversity of anthophilous insect species sampled on all land use types. Mean separation was done using Tukey test. Similarly, seasonal variation in anthophilous insect abundance, species richness and species diversity on different land use types was determined using one-way ANOVA. Also, seasonal variation of flowering plant abundance and species richness on different land use types was determined with the use of one-way ANOVA. All statistical analyses were done using SPSS version 22.

Results

Diversity and Abundance of Anthophilous Insects

A total of 1,667anthophilous insects belonging to eight taxa made up of bees, wasps, beetles, butterflies, hoverflies, midges, blowflies and houseflies were collected during this study. Hoverflies had the highest percentage composition followed by bees while the least percentage composition was found for blowflies (Table 1). Anthophilous insects differed significantly in mean abundance per month among the taxonomic groups ($F_{7,280}$ = 4.544, P<0.05). Highest mean abundance per month was recorded for hoverflies followed by the bees while blowflies had the least mean abundance of the insect taxa sampled on all land use types (Fig. 2). Although, hoverflies had the highest mean abundance, however, the most species rich taxon was the wasps with a total of 21 species recorded. A total of 11 species of bees were recorded, 10 species of beetles, 8 species of butterflies, 11 species of true flies which is composed of 7 species of hoverflies.

Anthophilous insects differed significantly in mean abundance among the three land use types ($F_{2, 33}$ = 3.223, P < 0.05). Secondary forest had the highest mean abundance of anthophilous insects while the least mean abundance was recorded on the agricultural lands (Fig. 3). No significant difference was observed in the species richness ($F_{2, 33}$ = 1.967, P>0.05) and diversity ($F_{2, 33}$ = 0.540, P> 0.05) of anthophilous insects among the land use types.

Seasonal Variation

There was a significant difference in the diversity of anthophiles between seasons on grasslands ($F_{1, 10}$ = 38.166, P<0.001), agricultural lands ($F_{1, 10}$ = 11.477, P<0.05) and secondary forest ($F_{1, 10}$ = 5.551, P<0.05)with the higher mean diversity recorded in the wet season on all land use types (Table 2). Species richness of anthophilous insects also differed significantly between seasons on grasslands ($F_{1, 10}$ = 41.106, P<0.001), agricultural lands ($F_{1, 10}$ =11.481, P<0.05) and the secondary forest ($F_{1, 10}$ = 5.558, P<0.05) with the higher mean richness recorded in the wet season on all land use types (Table 2). Although abundance of anthophilous insects was significantly different between seasons on grasslands ($F_{1, 10}$ = 28.199, P<0.001) and agricultural lands ($F_{1, 10}$ = 13.785, P<0.05), abundance was not significantly different between the seasons in the secondary forest ($F_{1, 10}$ = 2.112, P>0.05).

Abundance and Species Richness of Flower

Nine species of flowering plants were identified from all the sampling sites during the study. Flower abundance differed significantly among the land use types ($F_{2, 33} = 4.172$, $P<0.05$). Highest mean abundance of flower was recorded on the secondary forest land use type while the least mean abundance of flowers was recorded on the agricultural land (Fig. 4). However, no significant difference was observed in the species richness of flowering plants among the land use types ($F_{2, 33} = 0.713$, $P>0.05$).

Table 1: Species list and percentage composition of anthophilous insects sampled

Common name	Family	Species
Bees (15.9%)	Apidae	*Apis mellifera*
		Meliponula bocandei
		Tetraloniella junodi
		Amegilla kaimosica
		Braunsapis foreata
		Xylocopa olivacea
		Xylocopa imitator
	Halictidae	*Halictus* sp.
		Lasioglossum sp1
		Lassioglossum sp2
		Pseudapis (Pachynomia) sp1
Butterfly (13.2%)	Nymphalinae	*Acraea eponia*
		Acraea sp.1
		Acraea sp.2
		Acraea lycoa
		Junonia oenone
	Papilionidae	*Papilio demodocus*
	Pieridae	*Nepheronia* sp.
	Danainae	*Danaus chrysippus*
Hoverfly (19.86%)	Syrphidae	sp1
		sp2
		sp3
		sp4
		sp5
		sp6
		sp7
Housefly (10.74%)	Muscidae	*Musca domestica*
Blowfly (6.66%)	Calliphorodae	*Chrysomya chloropyga*
Midges (6.96%)	Blephariceridae	sp1
		sp2
Wasps (11.52%)	Sphecidae	sp1
		sp2
		sp3
		sp4
		sp5
	Eumenidae	sp1
		sp2

		sp3
		sp4
		sp5
	Mutillidae	sp1.
	Formicidae	sp1
		sp2
	Pompilidae	sp1
		sp2
		sp3
		sp4
	Vespidae	*Polistes* sp1
		Polistes sp2
		Belonogaster sp
	Bethylidae	Bethylidae sp
	Ichneumonidae	Ichneumonidae sp
Beetle (15.18%)	Chrysomelidae	*Aspidimorpha dissentanea*
		Copa occidentalis
		sp1
		sp2
		sp3
	Meloidae	*Mylabris* sp1
		Mylabris sp2
		Mylabris sp3
		Phyllophaga sp.
	Scarabaeidae	sp.

Table 2: Mean difference in the abundance, diversity and species richness of anthophilous insect sampled between seasons in different land use types.

Land Use	**Grassland**		**Agricultural Land**		**Secondary Forest**	
Season	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**
Mean diversity	9.66±0.97	0.83±0.44**	5.88±0.61	2.00±1.08*	9.13±1.04	4.59±1.80*
Mean richness	29.13±2.80	2.50±1.32**	17.63±1.83	6.00±3.24*	27.38±3.11	13.75±5.39*
Mean abundance	69.25±8.31	4.75±2.42**	42.38±3.96	13.50±7.84*	73.00±7.66	46.00±22.21**ns**

Significant: * = P<0.05, ** = P<0.001, ns = Not Significant.

Discussion

Hoverflies and bees which are the anthophilous insects with the highest abundance and composition in this study are mostly obligate florivores that could outcompete other facultative flower-visitors in foraging for flower resources, hence becoming the dominant flower-visitors in flower rich habitats (Larson *et al.,* 2001). Although several reports have identified bees as the most important anthophilous insects providing the most effective pollination service in both natural and agricultural ecosystems (Klein *et al.,* 2007; Dag, 2009), however, true flies most especially hoverflies have been reported to outnumber the abundance of bees and other anthophilous insects visiting flowers for floral resources (Ssymank, 2001).

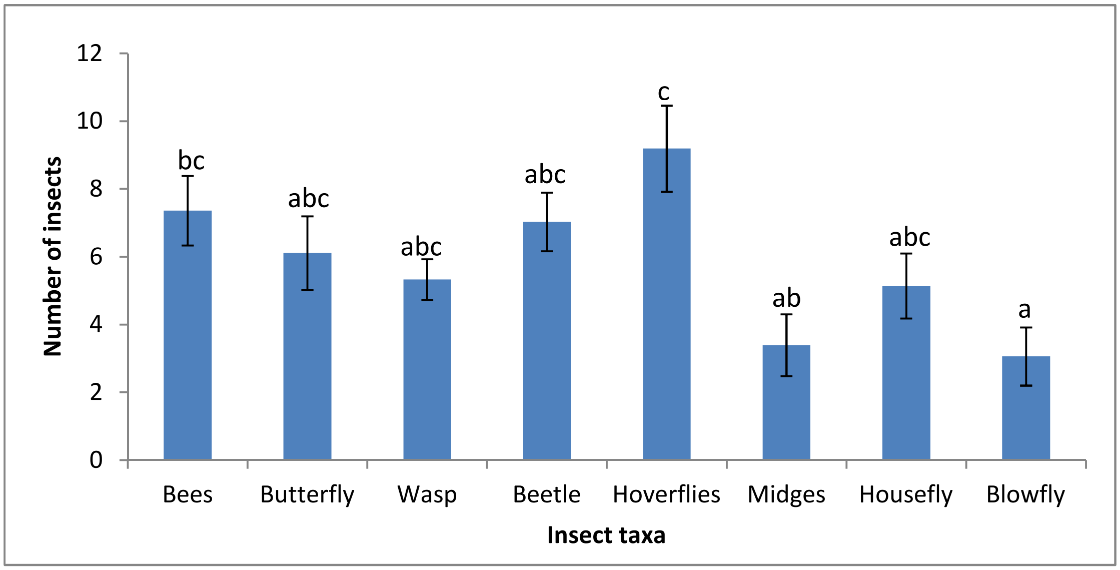

Fig 2: Mean (±SE) abundance of Insect taxa sampled per month on all study sites from all sampling methods used. Bars with the same alphabets are not significantly different at P > 0.05.

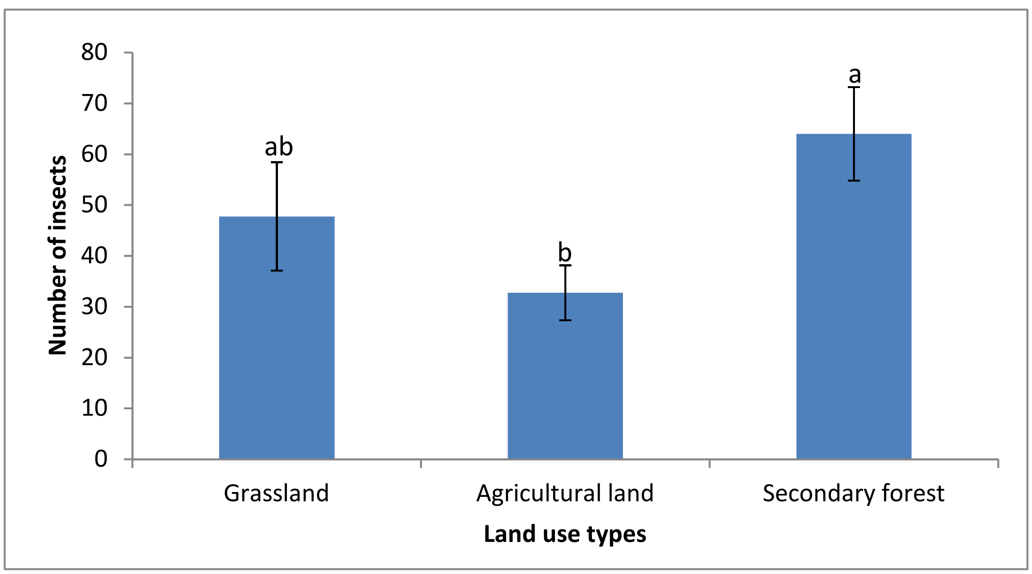

Fig 3. Mean (±SE) abundance of anthophilous insects on different land use types. Bars with the same alphabets are not significantly different at P > 0.05.

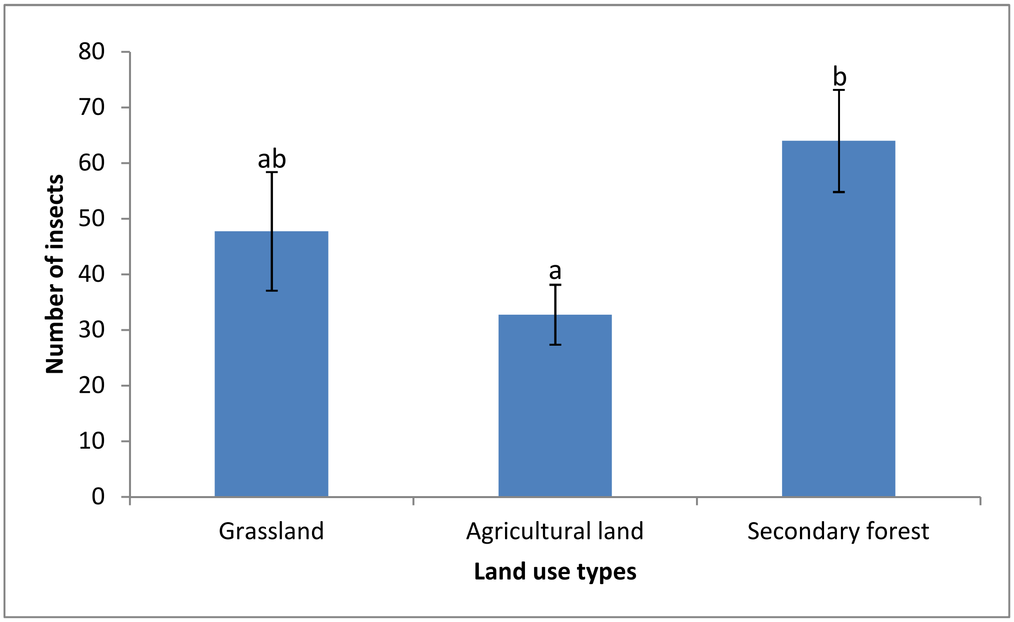

Fig 4. Mean (±SE) abundance of flowering plants on different land use types. Bars with the same alphabets are not significantly different at P > 0.05.

Land use effect is known as one of the major factors that determines flora and fauna diversity in different ecosystems. For anthophiles, reduction in their abundance, richness and diversity from the least disturbed to more disturbed habitat has been reported (Winfree *et al.,* 2011; Rickettes *et al.,* 2008). Low-level anthropogenic land use has been reported in secondary forest ecosystems which may increase heterogeneity of habitats and resources, thus increasing niche diversity (Tews *et al.,* 2004). However, some studies have shown higher abundance of anthophilous insects in moderately disturbed land use habitats (Hagen and Kraemer, 2010).The higher abundance observed in forests and grasslands in this study may be as a result of the availability of different floral resources in these land use types and the open habitat available for foraging on grasslands. Some anthophilous insects most especially bees and hoverflies have been regarded as creatures of open habitats (Michener 2007; Deans *et al.,* 2007) and this could explain the high abundance of anthophilous insects recorded on the grassland habitats.

This study reported lowest abundance, species richness and diversity of anthophilous insects on agricultural lands compared to the other land use types. Agricultural intensification in recent years has brought about expanded cultivation of annual and perennial crops at the expense of non-crop habitats such as fallows, hedges, field margins, which are known to support biodiversity by providing dispersal corridors for wildlife as well as habitat required by many species for feeding, overwintering and as refuges (Tilman *et al.,* 2001; Benton *et al.*, 2003; Stoate *et al.,* 2001; Kleijn *et al.*, 2006; Ockinger and Smith, 2006). Anthophilous insects derive little or no floral reward on agricultural lands when agricultural intensification leads to the removal of patches of weeds and flowering plants by the farmers through the application of herbicides and manual pruning (Krauss and Steffan-Dewenter, 2003, Kleijn and van Langevelde, 2006, Krauss *et al.,* 2009).

Seasonal variations affect microclimatic conditions and availability of resources which are predictors of insect population dynamics (Stubbs and Drummond, 2001). Reduction in flowering would almost certainly mean reduced food availability, which could translate into reduced reproductive output (Tscharntke *et al.,* 2005) and population densities (Westphal *et al.*, 2003) of anthophilous insects. This could explain the low abundance of anthophilous insects recorded in the dry season and the high abundance recorded in the wet rainy season. The effect of temporal variation on abundance, species richness and diversity of anthophilous insects and flowering plants was mostly observed on the grassland and agricultural lands where these anthophilous insect indices varied significantly across the sampling seasons. The open vegetation with low land cover in grasslands and agricultural lands implies that these habitats are prone to rapid fluctuations in microclimatic conditions which could have cascading effects on the flora and fauna communities in these habitats, thus making anthophilous insects to be more susceptible to the effect of fluctuations in weather condition between seasons (Stubbs and Drummond, 2001). The secondary forests have canopy cover provided by the trees that prevent rapid changes in microclimatic conditions in the under storey and could serve as a refuge to anthophilous insects under harsh weather conditions. This could explain the minimal variation in abundance, species richness and diversity of anthophilous insects and flowering plants recorded in this land use type.

Conclusion

Anthophilous insects' indices showed similar pattern to land use change with highest abundance, richness and diversity observed in secondary forests and grasslands while the agricultural land had the lowest abundance, richness and diversity of anthophilous insects. This implies that agricultural expansion and other anthropogenic activities that result in removal of forest habitats and wild flowering plants is a potential threat to the diversity of these important insects. Wildlife-friendly practices that promote flower rich non-crop vegetation in agricultural lands could possibly mitigate this threat. Furthermore, this study also shows differences in the diversity of anthophilous insects and flowering plants between seasons and this is mostly observable on the grasslands and agricultural lands. This indicates scarcity of resources for anthophiles during dry season when there is reduced abundance of flowering plants and floral resources. Furthermore, this underscores the potential of anthophiles as indicators of habitat disturbance in flower-rich land use types.

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