Biodiversity assessment and environmental risk analysis of the single line transgenic pod borer resistant cowpea (#96249)

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Biodiversity assessment and environmental risk analysis of the single line transgenic pod borer resistant cowpea

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Background: The discussion surrounding biological diversity intensified with the introduction of Nigeria's first transgenic food crop, the Pod Borer Resistant Cowpea. Concerns have arisen regarding whether the transgenic Maruca vitrata-resistant cowpea poses a threat to human health and other environmentally beneficial insects. Public apprehension, coupled with social activists' calls for the removal of this crop from the nation's food market, persists. Presently, there is a lack of data to counter the assertion that cultivating Pod Borer Resistant (PBR) cowpea may have adverse effects on biodiversity and the overall ecological system. This research has a multifaceted objective, including an examination of the environmental safety of PBR Cowpea and an assessment of its impact on biodiversity compared to its non-transgenic counterpart, IT97KN.Methods: Seeds for both the transgenic PBR Cowpea and its isoline were obtained from the Institute for Agricultural Research (IAR) Zaria before planting in various farm sites. Both transgenic and non-transgenic cowpea were cultivated following local cultural practices throughout the experiment. Elaborate taxonomic keys were employed to identify arthropods and other non-targeted organisms. Principal component analysis was used to evaluate potential modifications in all ecological niches of the crops. Diversity indices, including Shannon, Pielou, and Simpson, were analyzed using the lmer function of the R package lme4. The analysis of potential modifications in the dissimilarities of non-targeted organisms' communities was conducted using the Bray-Curtis index.Results: Examination of ecological species abundance per counting week revealed no disruption in the biological properties of non-targeted species due to the cultivation of transgenic PBR Cowpea. Analysis of species evenness and diversity indices indicated no significant difference between the fields of transgenic PBR cowpea and its isoline. Principal Component Analysis results demonstrated that planting PBR Cowpea did not create an imbalance in the distribution of ecological

species. All species and families observed during this study were more abundant in transgenic PBR Cowpea fields than in non-transgenic cowpea fields, suggesting that the transformation of cowpea does not negatively impact non-targeted organisms and their communities. Evolution dynamics of the species community between transgenic and non-transgenic cowpea fields showed a similar trend throughout the study period, with no significant divergence induced in the community structure due to PBR Cowpea planting. This study concludes that planting transgenic PBR Cowpea positively influences biodiversity and the environment.

Biodiversity Assessment and Environmental Risk Analysis of the Single Line Transgenic Pod Borer Resistant Cowpea

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Abstract

Background: The discussion surrounding biological diversity intensified with the introduction of Nigeria's first transgenic food crop, the Pod Borer Resistant Cowpea. Concerns have arisen regarding whether the transgenic Maruca vitrata-resistant cowpea poses a threat to human health and other environmentally beneficial insects. Public apprehension, coupled with social activists' calls for the removal of this crop from the nation's food market, persists. Presently, there is a lack of data to counter the assertion that cultivating Pod Borer Resistant (PBR) cowpea may have adverse effects on biodiversity and the overall ecological system. This research has a multifaceted objective, including an examination of the environmental safety of PBR Cowpea and an assessment of its impact on biodiversity compared to its non-transgenic counterpart, IT97KN.

Methods: Seeds for both the transgenic PBR Cowpea and its isoline were obtained from the Institute for Agricultural Research (IAR) Zaria before planting in various farm sites. Both transgenic and non-transgenic cowpea were cultivated following local cultural practices throughout the experiment. Elaborate taxonomic keys were employed to identify arthropods and other non-targeted organisms. Principal component analysis was used to evaluate potential modifications in all ecological niches of the crops. Diversity indices, including Shannon, Pielou, and Simpson, were analyzed using the lmer function of the R package lme4. The analysis of potential modifications in the dissimilarities of non-targeted organisms' communities was conducted using the Bray-Curtis index.

Results: Examination of ecological species abundance per counting week revealed no disruption in the biological properties of non-targeted species due to the cultivation of transgenic

PBR Cowpea. Analysis of species evenness and diversity indices indicated no significant difference between the fields of transgenic PBR cowpea and its isoline. Principal Component Analysis results demonstrated that planting PBR Cowpea did not create an imbalance in the distribution of ecological species. All species and families observed during this study were more abundant in transgenic PBR Cowpea fields than in non-transgenic cowpea fields, suggesting that the transformation of cowpea does not negatively impact non-targeted organisms and their communities. Evolution dynamics of the species community between transgenic and non-transgenic cowpea fields showed a similar trend throughout the study period, with no significant divergence induced in the community structure due to PBR Cowpea planting. This study concludes that planting transgenic PBR Cowpea positively influences biodiversity and the environment.

Introduction

Biodiversity, a term coined from the word biological diversity, is referred to as the heterogeneity and variability of the total number of biological organisms found within a given habitat or ecosystem at any given time (Roe et al., 2019; Dickson et al., 2019; Meine, 2018; Rawat and Agarwal, 2015). The concept of biodiversity is multidimensional, encompassing genetics, species, and ecology. Several studies, including Tilman et al. (2014) and Malhi et al. (2020), have revealed that the degree of variability of living organisms on earth plays a crucial role in sustaining the ecosystem and could serve as a major indicator for predicting the safety of any environment at any given time. The productivity and efficiency of any agricultural system around the world can be strongly influenced by its varietal and species diversity over an extensive scale of conditions (Pawlak and Kołodziejczak, 2020; Carpenter, 2011; Krishna et al., 2009). Biodiversity also plays crucial roles in contributing to an organism’s resiliency to stress and shocks and adaptability to new challenging environmental systems, in addition to being a vital factor in the sustainability system of production and genetic improvement (Vasiliev, 2022; Ortiz et al., 2021). With the deleterious impact of climate change in view, which is already resulting in increased crop pest infestation and decreased agricultural soil fertility globally (Pareek, 2017; Skendþić, et al., 2021; Malhi et al., 2021; Habib-ur-Rahman et al., 2022; Subedi et al., 2023), sustaining and improving the variability of crop and animal genetic resources can no longer be overemphasized as it plays a key role in ensuring the resiliency and stability of living organisms' over time.

After about thirty years of the safe use of transgenic crops with more than 3 million hectares planted across Africa (Endale et al., 2022) and their recorded benefits (Gbadegesin et al., 2022; Smyth, 2022), debate and concerns about their environmental effects have continued to persist (Gbadegesin et al., 2022; Gbashi et al., 2021; Smyth et al., 2021; Azadi et al., 2015). Critical among the issues discussed so far is its potential impact on biodiversity (Fernandes et al., 2022; Lucht, 2015). Though a number of studies, including O’Callaghan et al. (2005) and Romeis et al. (2014), have suggested that the insecticidal property of the Cry1Ab protein may be toxic to non-target species, including herbivores, parasitoids, and predators, many of these studies

examined the impact of this protein on species in non-natural systems without taking into account ecological interactions or the actual level of exposure of vulnerable stages in natural settings (Dale et al., 2002). Conducting additional studies that take into account complex systems and exposure conditions akin to those encountered in the field could offer more realistic insights into the detrimental effects of Bt crops on non-target organisms (Sears et al., 2001).

The magnitude and significance of conserving biodiversity have been emphasized in the guidance documents of the European Food Safety Authority (EFSA) (2016) as a major goal in environmental protection. Quantifying biodiversity is a prerequisite for being able to reach set targets. Since Nigeria joined the league of biotech countries after its commercialization of its first transgenic crop, insect-resistant (IR) cotton, in 2018, the general debate in Africa on the potential impact of GM crops on biodiversity has triggered (Endale et al., 2022). The introduction of her first transgenic food crop, pod borer-resistant (PBR) cowpea, in 2019 has further exacerbated these concerns among Nigeria's stakeholders. More apparent among the concerns raised about the safety of the introduction of the transgenic PBR cowpea in Nigeria is its potential to negatively impact species and ecosystem diversity, with key stakeholders speculating that its toxicity to the targeted insect, Marucca vitrata, means that there is a strong likelihood that the crop will also be toxic to non-targeted organisms (NTOs), including those that play a vital role in the ecosystem. Currently, there is a paucity of data to refute claims that this transgenic PBR cowpea supports biodiversity and is safe for our environment. The study focuses on the biodiversity assessment of the single-line transgenic pod borer-resistant cowpea, with the aim of evaluating its potential impacts on non-target organisms.

Materials & Methods

PBR Cowpea Seeds and its Isoline

Seeds of both transgenic PBR Cowpea (SAMPEA 20T) and its Isoline, IT97KN were provided by the Institute for Agricultural Research (IAR) Zaria prior to planting in the various farm site. The *Cry1Ab* event in the PBR Cowpea was confirmed using the lateral flow strip kits obtained from the Qiagen Inc. at the Mary Halaway Laboratory, Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University: 5g, each of transgenic and non-transgenic seeds were mashed separately in two different mortals, after which the extraction buffer was added to each container. The flow strip was then inserted and allowed to stay for about 10 minutes after which the lines were read **(Fig S1).**

Experimental Design and Sampling

The two cowpea lines, IT97KY and IT97KN were planted in three different farms of the National Biotechnology Development Agency (NABDA) within the period of February – May and August - October 2022 and February - April 2023 using irrigation farming method during the dry season

with three replications on each farm site (**Fig S2**). Both cowpea lines were grown following local cultural practices throughout the experiment. The two crop varieties, transgenic (IT97KT) and its non-transgenic isoline cowpea (ITN7KN) were planted in a randomized block design with 3 replications (Fig S2). The measurement of each plot was estimated at 10m by 15m encompassing eight 30cm interspaced rows with 25cm space between each plant. 3m plain boundaries were created to function as seclusion among plots (**Fig. S2**). No crop was planted on the three research farms one year prior to the research. In addition, there were no application of any herbicide or insecticide before or during the study period.

Identification of species to family and to functional groups

Arthropods and other non-targeted organisms were identified by using suitable and elaborated dichotomous taxonomic keys according to Goulet and Huber (1993), Triplehorn *et al.* (2005) and Jenny *et al.* (2017). The taxonomic grouping was done using the family level as default while in cases where classification based on family level was not obtainable, priority was given to the order and suborder to which the organism belong (Jenny *et al*., 2017). The individual organisms were further grouped into predator, parasitoid and herbivore ecological functional group. There was no unknown organism recorded all through the study period.

Non-Target Organism Community Structure

Possible moderations that may have accrued from planting the transgenic PBR Cowpea was analysed using a precise redundancy analysis (RDA) ordination method called the Principal Component Analysis (PCA) (Vanden-Brink *et al*., 2009) as recommended by Cuppen *et al*. (2000) and Moser *et al*. (2007) to be suitable for assessing the impacts of any plants or animals on the ecosystem. The PCA multivariate technique facilitates the understanding of the interaction between the organism and their environments (Moser *et al*., 2007) by analysing the possible effects of the transgenic PBR Cowpea on the community species and the resulting changes in the community structure throughout the study period.

Structural Dissimilarity analysis

The analysis for the potential modification in the dissimilarities of the non-targeted organisms' communities between the transgenic PBR cowpea (IT97KT) and its non-transgenic isoline (IT97KN) were done using Bray-Curtis index. It evaluates the degree of dissimilarity or similarity between two or more samples using a range of zero (similar) to one (dissimilar) (Krebs, 1989; Bray and Curtis, 1957). The structural dissimilarity analysis was divided into two phases. At the first phase of the analysis, the Bray Curtis (BC) Index was conducted using the data collected between all the pairs of the sample plots IT97KT and IT97KN on each sampling date. Similar procedures were repeated for the second phase of the analysis where data was collected within each cowpea plot (Collins *et al.,* 2000) and was then followed by a computation of the mean abundance for the respective taxonomic group in line IT97KT and IT97KN per sampling date.

The Bray-Curtis Dissimilarity was calculated as: $BCij = 1 – (2*Cij) / (Si + Sj)$

Where Cij = The sum of the lesser values for the species found in each site.

Si: The total number of specimens counted at site i

Sj: The total number of specimens counted at site j

The values for the mean abundance were thereafter used to estimate the BC distance between the respective treatment sampling dates. A linear regression analysis of the data obtained from the BC distance estimation was conducted versus the time-lag data.

Statistical Analysis

The total number (N) of arthropods on each plot in the three different farm sites were taken per counting week and over the entire period of the study and then divided by the total number of farm site to get the average. All statistical analyses were performed by using R version 4.2.0 (R Core Team, 2022) and excel spread sheet. The analysis of the diversity indices including Shannon (H), Pielou (J) and Simpson (D) which facilitates a comparative assessment of the community structures between different treatments in the fields (Boyle *et al*., 1990; Magurran, 2004; Pielou, 1966; Oksanen, 2013) were done using the lmer function of R package lme4 with Cowpea variety (*Bt* or non-*Bt*) and time (date of sampling) as fixed factors (Guo *et al.,* 2014). A comparison of the mean values of all the scoring parameter including H, D, J and N was done using one-way analysis of variance (ANOVA). Population less than 1% were denoted as ‘‘others’’.

A covariance analysis was used to carry out a comparative study of the slopes of the regression lines of the two treatments. The parasitoid, herbivores and predator nutritional relationships were used to classify the whole organisms into three guilds according to Heong *et al*. (1991) and Zhang *et al*. (2011). The density of the three guilds were analysed using One-way ANOVA for each variety of the cowpea and sampling date. The population of various treatments, herbivore, parasitoid and predator nutritional guild was defined by sing the formula P_i5N_i/N, where the population of the herbivore, parasitoid and predator was connoted as N_i while the treatment's entire summed abundance was connoted as N. The specie count for each community organism in the various guild was defined by the formula P_i5N_i/N, where Ni was defined as the summed ith species and N was the guild count in the respective treatment. The rare, common and dominant group were denoted by $Pi<1\%$, $1\%\leq P_i<10\%$ and $P_i\geq 10\%$ respectively (Li and Liu, 2013).

Results

Transgene Status Confirmation of the Cowpea Samples

The confirmation of the Cry1Ab event expressed in the PBR Cowpea shows a positive result as seen in **Figure S1**. Further test for the presence of the Cry1Ab gene using the event specific flow strip in the isoline of the PBR Cowpea shows negative, meaning that the isoline is not transgenic **Figure S1**.

Ecological Pattern of the transgenic and Non-Transgenic Cowpea Field

Study of the species disparities and distribution shows that there are no variations between both treatments during the first three weeks after planting in all the experimental site as seen in **Figure 1**, **Table 1**. At week 4, 5, 6 and 7, significant difference was observed between both treatment with the field of transgenic crop having higher species activities than the field of non-transgenic cowpea.

Estimated Species Diversity

From the results of the univariate analyses of both line IT97KT and IT97KN ecological niches, the estimated biodiversity indices (H, J and D) revealed that there was no significant difference between the two treatments except during the differentiated flowering time that was observed between the two cowpea lines (**Table 1 and Figure 2 a, b and c**). The habitat information provided from the Shannon diversity index analysis shows that both habitats dominated by the transgenic and non-transgenic cowpea has high specie richness and evenness throughout the counting weeks. Results obtained from the analysis using the Shanon diversity index reveals a close-range value between the transgenic and non-transgenic cowpea habitats. A higher Shannon score was observed for transgenic cowpea fields withing the counting week of 3 to 8 where flowering was peak. The diversity index score for IT97KN went slightly high at the counting week where its flowering was also peak. Result from the analysis of variance shows no significant difference at week 1, 2, 9, 10, 11 and 12 as against the subsequent counting week of 4, 5, 6 and 7 (**Figure 2a**). Analysis of the Simpson diversity indices shows similar trends in both fields of the transgenic and non-transgenic cowpea. With both fields recording their lowest Simpson score at week 1 and 2 respectively, the highest Simpson score for transgenic and non-transgenic cowpea fields were recorded at week 12 and 11 respectively (**Figure 2b**). Analysis of the Pielou Evenness Index shows that the distribution of the individual species is even across the habitat of transgenic and non-transgenic cowpea (**Figure 2c**).

Further analysis using the regression line plot between the ecological niches of transgenic and non-transgenic cowpea shows strong positive correlation with a p and r value of 1.810599e-06 and 0.9522146 respectively (**Figure 3a**). As the number of species in the ecological niches of PBR Cowpea increases, the number of species in its non-transgenic isoline, IT97KN also increases (**Figure 3a**).

Similar results were observed when the ecological niches of transgenic Cowpea (IT97KT) and its non-transgenic isoline (IT97KN) were correlated with time (figure 3b). The p and r values of 3.42862e-09 and 0.9865187 respectively were observed for transgenic cowpea vs time while p and r values of 1.535e-07 and 0.9522146 respectively were observed for non-transgenic cowpea vs time (**Table 2**).

Analysis of the Evolution Dynamics of the Transgenic and Non-Transgenic Cowpea

i. Component Analysis

Analysis using the multivariate Principal Component Technique reveals that there are no significant differences in the ecological composition of the entire study fields throughout the counting weeks (**Figure 4a and b**). The essence of the Principal Component Analysis (PCA) output is to give a clear interpretation of the specie points that have similar composition. The species scores which are represented by arrows point in the direction of increasing abundance. The angle size between a specie arrow to another specie arrow is inversely correlated, meaning that the smaller the angle size between two species arrows, the stronger the correlation and the reverse means a weaker correlation within the space. As observed from the result output, there is strong positive correlation between EI and DC in both field of transgenic and non-transgenic cowpea field. The formation of a right angle between two species arrows means that there is no correlation while the formation of opposite angle means a strong negative correlation (BioTuring, 2018; Hartmann et al., 2018). The PCA output generated below also attributes significant value to the direction of the species arrow with respect to its angle with the principal component axes within the space. The more parallel a specie arrow is to the axis of a principal component, the more weight they have on that principal component space (Hartmann et al., 2018). The PC analysis from this study shows that AC and Cs strongly influences PC1 while PP and Zv strongly influences PC2, having a heavier weight in the transgenic cowpea field. Md and SaC are the most heavily weighted in PC1, strongly influencing the PC1 of the non- while GB and PP are the most heavily weighted species of PC2 in the non-transgenic cowpea field.

The estimation of the number of statistically significant principal components for the ecological niches of both transgenic and non-transgenic cowpea is presented in **Figure 5a and 5b** below. The number of break point (10) distribution is similar for both ecological niches.

Composition of Organism Community of both the Transgenic and Non-Transgenic Species

As shown in the figure below, three major guilds, herbivores, parasitoids and predators were identified throughout the study period (**Figure 6a, b and c**). The guild analysis for both the Bt (IT97KT) and NBt (IT97KN) field reveals the identification of 12, 8 and 7 different species in the herbivore, parasitoid, and predator guild. Most of the species in both fields are herbivores while the predatory guild has the least number of organisms. SC represents the most abundant species in the parasitoid guild of both IT97KT and IT97KN ecological niches, while MB and AC are the most abundant species in the herbivore guild. CaC is the most abundant species in the predator guild. SL, LM and vf represent the least abundant species in the predator, parasitoid and herbivore guild of both ecological niches as shown in the figure. A uniform composition of the organisms in all the ecological niches were observed throughout the whole study period (**Figure 6a, b and c**).

Dissimilarity Index

The result of the Bray Curtis dissimilarity Index is presented in **Table 3** below. Bray-Curtis Dissimilarity ranges between 0 and 1, where 0 indicates that the niches have no dissimilarity while 1 indicates that the two niches have complete dissimilarity. The dissimilarity index between the ecological niches of PBR Cowpea and non-transgenic isoline is 0.2, which indicates that all the niches had similar evolutionary trends with no divergence in the community structure of the NTOs.

Discussion

In this study, the potential impact of Nigeria's transgenic Pod Borer Resistant (PBR) Cowpea, which is the first transgenic cowpea to be commercialised in the world, was assessed with the aim of evaluating the possible threats and harm that the crop may pose to the environment and the ecological niches of the diverse useful soil and plant organisms. Bray Curtis Dissimilarity Index, Pielou Evenness Index, Shannon Diversity Index and Simpson Diversity Index have been strongly recommended by Guo et al. (2014) and Clergue et al. (2005) as useful measures and indicators to gaining insight and understanding of the impact of any plant on the community structure, evolution dynamics and ecological pattern of other species and the environments where they are found.

According to Guo et al. (2014), the various functional ecological indices of the surrounding species to any newly introduced crop such as the PBR Cowpea would be significantly altered if disruption of any biological property occurs as a result of planting such crop. However, the findings from this research shows that the total species count throughout the study period are similar in values. Analysis of the various ecological indices, including Shannon Diversity index, Brays Curtis Dissimilarity Index, Pielou evenness index, Principal Component Analysis (PCA) and Renyi Diversity Silhouettes all showed a close range of values between the ecological niches of the transgenic Cowpea and its non-transgenic Isoline. Similar research study conducted at the Germany's Oderbruch European Corn Borer infestation area by Schorling and Freier (2006) on a Six-year assessment of the impact of transgenic maize expressing Cry1Ab gene on non-target organisms reported the same results. In contrast to Fernandes et al. (2022) who postulated that genetic modification of crop has the tendency of reducing crop biodiversity, research findings by Abdul et al. (2022) and Anderson (2019) has underscored that the transformation of crops for insect resistance is beneficial because it can enable plant species that are near extinction due to the heavy burden of insect infestation to be revived by improving their adaptation to diverse environmental conditions.

The Principal Component Analysis (PCA) of both the transgenic and non-transgenic Cowpea fields reveals that the distribution of the NTOs were not significantly different throughout the study period. This finding is consistent with the report of Guo et al. (2014) and Candolfi et al. (2004) where they found out that the Cry1Ab event expressed in the transgenic Corn does not affect the community structure of the NTOs. Other research study by Higgins et al. (2009)

where a three-year field monitoring of the potential impacts of Cry1F events expressed in a maize hybrid on NTOs also underscored that the community structure of the organisms remained intact. Though previous study only centred on the comparative NTO abundance between transgenic and non-transgenic plots, this study further analysed the possible evolutionary dynamics of the transgenic PBR Cowpea by carrying out a dissimilarity index analysis. The result of this study shows that there was a gradual change in the species composition of both transgenic field and non-transgenic field and this change increased with time. For instance, the specie type present during the counting week 2 of the study increased when compared to week 1. Similar occurrence was also observed when counting week 3 was compared with counting week 2. Analysis of the Bray Curtis Dissimilarity Index showed an index of 0.2 which means that the evolutionary dynamic for both transgenic and non-transgenic crops were significantly similar. Similar studies conducted by Guo et al. (2014) also recorded a similar evolutionary dynamic between non-transgenic and transgenic maize expressing CryIAc event. The potential toxicity of PBR Cowpea can also be carried out by monitoring and evaluating the exposure of the different life stages of the various species of Cowpea ecosystem (Devos et al., 2012). The assessment of the different nutritional guild of organisms identified in this study shows a rich representation of the herbivores, parasitoids and predators in all the ecological niches. Despite the high tendency of herbivores having a direct exposure to the Cry proteins expressed in the PBR Cowpea when feeding on its crop residue and pollen (Devos et al., 2012; Romeis et al., 2008), a high population density was still recorded for the PBR cowpea ecological niches when compared to the non-transgenic Cowpea. The number of herbivore species present in the ecological niches of transgenic cowpea is higher than in the non-transgenic cowpea ecological niches but the same species type including: Messor barbarous, Alydus eurinus, Eastern Lubber, variegated fritillary, Deudorix antalus, Scarabaeus satyrus, Atta cephalostes, Dysdercus cingulatus, Junonia oenone, Chorthippus biguttulus and Carausius morosus were observed for all the ecological niches. This result is in alignment with findings from Wolfenbarger et al. (2008) who carried out a study on the potential impacts of GM Crops on the functional guild of NTOs.

A further critical analysis of the population density of the predator guild in both transgenic and non-transgenic field reveals no significant difference. Assessing the population density of the predator guild can provide valid assertions on the extent of biological, as well as environmental safety of the transgenic crop since predators have multiple ways by which they come in contact with the Cry1Ab gene including direct feeding on the pollen of the PBR Cowpea, herbivores that have feed on PBR Cowpea or via the surrounding soil in which the PBR Cowpea is planted. The number of predator species present in the ecological niches of transgenic cowpea is higher than in the non-transgenic cowpea ecological niches though both had the same species type including Chilocorus stigma, Odontoponera transversa, Conozoa hyaline, Camponotus cruentatus, Pirata piraticus, Graphoderus bilineatus and Stenolophus lecontei.

Analysis of the parasitoid population can provide some very useful ecological indices because they possess the unique characteristics of having the ability to complete their life cycle by feeding on a particular host (Salama and Zaki, 1983) or a range of herbivores in a particular ecological niche (Romeis et al., 2008). They are therefore most likely to ingest the Cry protein in the host herbivore where they are found or directly from the PBR Cowpea plant (Lit et al., 2012). The analysis shows that the population density of the parasitoids in the PBR Cowpea ecological niches were not significantly different from the non-transgenic Cowpea ecological niches throughout the study period. Studies by Comas et al.
(2014) and Albajes et al. (2013) who carried out meta-analysis of the ecological impact of Bt Maize on NTOs also reported that the transgenic maize had no significant effect on the population density of the predator, herbivore and parasitoid guild throughout the study period. The result of the Principal Component Analysis (PCA) shows similar evolutionary dynamics in both the ecological niches of the transgenic and non-transgenic Cowpea. The broken stick distribution which models the number of variances by adopting a stick of unit length which is thereafter randomly broken into n pieces reveals no statistically significant difference between both ecological niches. This finding is in alignment with result obtained by Guo et al. (2014) whose research study revealed that the BtCry1Ac event expressed in the insect resistant corn caused no alteration in the community distribution of both transgenic and non-transgenic corn. The strong positive correlation between both transgenic and non-transgenic cowpea vs time shows that the increase in the species in both niches is as a result of increase in agronomic factors as the growth of both cowpea progresses. Such factors may include the onset of flowers and the steady increase, the onset of pods which followed thereafter and its steady increase, in addition to the continuous increase in the number of leaves over time. It also means that the Cry1Ab gene expressed in the PBR Cowpea had no negative impact on any of the ecological components including the non-targeted organisms. Other factors that may have played significant roles include temperature, rainfall, sunshine, nature of the soil and other surrounding elements and plants (Desneux and Bernal, 2010).

Limitation of the Current Study

The current study does not take the effect of the PBR Cowpea on egg laying capacity of non-targeted arthropods in contrast to Dang et al. (2017). Furthermore, the collection of data on the effect of PBR Cowpea on soil invertebrates over longer periods of time and the potential transfer of the Cry1Ab gene to conventional cowpea still need to be assessed.

Conclusions

The findings from this study shows that the introduction of the *Cry1Ab* transgene in the PBR cowpea did not negatively impact biodiversity and the environment. The comparative assessment of the evolutionary dynamics of the non-targeted species community of the

transgenic cowpea and that of non-transgenic cowpea recorded no significant divergence throughout the study period. The data accrued from the analysis of the species evenness and diversity indices also did not show any significant difference between the fields of transgenic PBR cowpea and its isoline. The data accrued from this research are useful in providing valuable insights that will help to shape decision-making for the regulation of the crop across the major countries where it can be grown.

References

Abdul, A.M., Brini, F., Rouached, H. and Masmoudi, K. (2022). Genetically engineered crops for sustainably enhanced food production systems. *Front. Plant Sci.* 13:1027828. doi: 10.3389/fpls.2022.1027828.

Albajes, R., Lumbierres, B., Madeira, F., Comas, C. and Ardanuy, A. (2013). Field trials for assessing risks of GM maize on non-target arthropods in Europe: the Spanish experience. *IOBC/WPRS Bulletin* 97: 1–8.

Anderson, J., Ellsworth, P., Faria, J., Head, G., Owen, M. and Pilcher, C. (2019). Genetically engineered crops: Importance of diversified integrated pest management for agricultural sustainability. *Front. Bioeng Biotechnol*. 7, 24. doi: 10.3389/fbioe.2019.00024

Azadi, H., Samiee, A., Mahmoudi, H., Jouzi, Z., Khachak, P.R., DeMaeyer, P. and Witlox, F. (2015). Genetically modified crops and small-scale farmers: main opportunities and challenges. *Critical Reviews in Biotechnology* 36(3):434-446. doi:10.3109/07388551.2014.990413.

Boyle, T.P., Smillie, G.M., Anderson, J.C. and Beeson, D.R. (1990). A sensitivity analysis of nine diversity and seven similarity indices. *Res J Water Pollut Control Fed* 62: 749–762.

Bray, J.R. and Curtis, J.T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecol Monogr* 27: 325–349.

Carpenter, J.E. (2011). Impact of GM crops on biodiversity. *GM Crops*. 2(1):7-23. doi: 10.4161/gmcr.2.1.15086.

Clergue, B., Amiaud, B., Pervanchon, F., Lasserre-Joulin, F. and Plantureux, S. (2005). Biodiversity: function and assessment in agricultural areas. A review. *Agron Sustain Dev* 25: 1–15.

Collins, S.L., Micheli, F. and Hartt, L. (2000). A method to determine rates and patterns of variability in ecological communities. *Oikos* 91: 285–293.

Comas, C., Lumbierres, B., Pons, X. and Albajes, R. (2014). No effects of Bacillus thuringiensis maize on nontarget organisms in the field in southern Europe: a meta-analysis of 26 arthropod taxa. *Transgenic Res* 23: 135–143.

Cuppen, J.G.M., Van den Brink, P.J., Camps, E., Uil, K.F. and Brock, T.C.M. (2000). Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates. *Aquat Toxicol* 48: 233–250.

Dale, P.H., Clarke, B. and Fontes, E.M.G. (2002). Potential for the environmental impact of transgenic crops. Nat. Biotechnol. 20, 567–574.

Dang, C., Zhou, X., Sun, C., Wang, F., Peng, Y. and Ye, G. (2021). Impacts of Bt rice on non-target organisms assessed by the hazard quotient (HQ), *Ecotoxicology and Environmental Safety*, Volume 207, 111214, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2020.111214.

Desneux, N. and Bernal, J.S. (2010). Genetically modified crops deserve greater ecotoxicological scrutiny. *Ecotoxicology* 19: 1642–1644.

Devos Y, De Schrijver A, De Clercq P, Kiss J, Romeis J (2012) Bt-maize event MON 88017 expressing Cry3Bb1 does not cause harm to non-target organisms. Transgenic Res 21: 1191–1214.

Dickson, A., Krishnan, U., Parisa, Z., Barbara, S. and Paul, S. (2019). The Concept of Biodiversity and its Relevance to Mankind: A Short Review. *Journal of Agriculture and Sustainability*, Volume 12, Number 2, 219-231

EFSA. (2016). Guidance to define protection goals for environmental risk assessment in relation to biodiversity and ecosystem services. *EFSA Journal*, Volume 14, issue 6: doi: https://doi.org/10.2903/j.efsa.2016.4499.

Endale, G.K., Karim, M., Joseph, G. and Muffy, K. (2022). Commercialization of genetically modified crops in Africa: Opportunities and challenges. *African Journal of Biotechnology*; Vol. 21(5), pp.188-197, DOI: 10.5897/AJB2021.17434

Fernandes, G., Silva, A., Maronhas, M., Dos Santos, A. and Lima, P. (2022). Transgene flow: Challenges to the on-farm conservation of maize landraces in the Brazilian semi-arid region. *Plants (Basel).* 11 (5), 603. doi: 10.3390/plants11050603

Gbadegesin, L.A., Ayeni, E.A., Tettey, C.K., Uyanga, V.A., Aluko, O.O., Ahiakpa, J.K., Okoye, C.O., Mbadianya, J.I., Adekoya, M.A., Aminu, R.O., Oyawole, F.P. and Odufuwa, P. (2022). GMOs in Africa: Status, adoption and public acceptance, *Food Control,* Volume 141, 109193, https://doi.org/10.1016/j.foodcont.2022.109193.

Gbashi, S., Adebo, O., Adebiyi, J.A., Targuma, S., Tebele, S., Areo, O.M., Olopade, B., Odukoya, J.O. and Njobeh, P. (2021). Food safety, food security and genetically modified organisms in Africa: a current perspective. *Biotechnology and Genetic Engineering Reviews,* 37(1):30-63. doi: 10.1080/02648725.2021.1940735.

Goulet, H. and Huber, J.T. (1993). Hymenoptera of the World: An Identification Guide to Families. *Agriculture,* Canada, Ottawa.

Guo, Y., Feng, Y., Ge, Y., Tetreau, G. and Chen, X. (2014). The Cultivation of *Bt* Corn Producing *Cry1Ac* Toxins Does Not Adversely Affect Non-Target Arthropods. *PLoS ONE* 9(12): e114228. doi:10.1371/journal.pone.0114228.

Habib-ur-Rahman M, Ahmad A, Raza A, Hasnain MU, Alharby HF, Alzahrani YM, Bamagoos AA, Hakeem KR, Ahmad S, Nasim W, Ali S, Mansour F and EL Sabagh A (2022) Impact of climate change on agricultural production; Issues, challenges, and opportunities in Asia. Front. Plant Sci. 13:925548. doi: 10.3389/fpls.2022.925548

Heong, K.L., Aquino, G.B. and Barrion, A.T. (1991). Arthropod community structures of rice ecosystems in the Philippines. *B Entomol Res* 81: 407–416.

Jenny, L., Marcel, D., Cajo, J.F.T. and Joop, J.A.V. (2017). Biodiversity analyses for risk assessment of genetically modified potato. *Agriculture, Ecosystems and Environment.* Volume 249, 1,196-205

Krebs, C.J. (1989). Ecological methodology. *Harper and Row,* New York.

Krishna, V., Zilberman, D. and Qaim, M. (2009). Transgenic technology adoption and on-farm varietal diversity. *International Association of Agricultural Economists Conference.* Beijing, China 2009.

Li, X.G. and Liu, B.A. (2013). A 2-year field study shows little evidence that the long-term planting of transgenic insect-resistant cotton affects the community structure of soil nematodes. *PLoS ONE* 8: e61670.

Lit, I.L., Caasi-Lit, M.T., Benigno, E.A., Ramal, A.F.B. and Yap, S.A. (2012). Non-target organisms on Bt corn hybrids MON89034 and MON89034/NK603: Part 2. Functional guilds of arthropods in regulated field trial sites during dry season in Luzon and Mindanao, Philippines. *Philipp Entomol* 26: 28–53.

Lucht, J.M. (2015). Public Acceptance of Plant Biotechnology and GM Crops. *Viruses*. 30;7(8):4254-81. doi: 10.3390/v7082819.

Magurran, A.E. (2004). Measuring biological diversity. *Blackwell Science*, Oxford.

Malhi, G.S.; Kaur, M.; Kaushik, P. (2021). Impact of Climate Change on Agriculture and Its Mitigation Strategies: A Review. *Sustainability*, *13*, 1318. https://doi.org/10.3390/su13031318

Malhi, Y., Franklin, J., Seddon, N., Solan, M., Turner, M.G., Field, C.B. and Knowlton, N. (2020). Climate change and ecosystems: threats, opportunities and solutions. *Phil. Trans. R. Soc.B,* 375:1794, http://doi.org/10.1098/rstb.2019.0104.

Meine, C. (2018). Biodiversity Conservation. *Reference Module in Earth Systems and Environmental Sciences,* Volume 4, Pages 205-214. Doi: https://doi.org/10.1016/B978-0-12-809665-9.10463-X.

Moser, T., Rombke, J., Schallnass, H.J. and Van, G.C.A.M. (2007). The use of the multivariate Principal Response Curve (PRC) for community level analysis: a case study on the effects of carbendazim on enchytraeids in Terrestrial Model Ecosystems (TME). *Ecotoxicology* 16: 573–583.

O'Callaghan, M., Glare, T.R., Burgess, E.P.J. and Malone, L.A. (2005). Effects of plants genetically modified for insect resistance on non-target organisms. *Annu. Rev. Entomol*. 50, 271–292.

Oksanen, J. (2013). Vegan: ecological diversity. Available at: http://cran.rproject.org/web/packages/ vegan/vignettes/diversity-vegan.pdf.

Ortiz, A.M.D., Charlotte, L., Outhwaite, C.D. and Tim, N. (2021). A review of the interactions between biodiversity, agriculture, climate change, and international trade: research and policy priorities. *One Earth*, Volume 4, Issue 1, Pages 88-101, ISSN 2590-3322, https://doi.org/10.1016/j.oneear.2020.12.008.

Pareek, N. (2017). Climate change impact on soils: adaptation and mitigation. *MOJ Eco Environ Sci.* 2(3):136-139. DOI: 10.15406/mojes.2017.02.00026

Pawlak, K. and Kołodziejczak, M. (2020). The Role of Agriculture in Ensuring Food Security in Developing Countries: Considerations in the Context of the Problem of Sustainable Food Production. *Sustainability*, 12, 5488. https://doi.org/10.3390/su12135488.

Pielou, E.C. (1966). The measurement of diversity in different types of biological collections. *J Theor Biol* 13: 131–144.

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rawat, U.S. and Agarwal, N.K. (2015). Biodiversity: Concept, threats and conservation. *Environment Conservation Journal*, 16(3) 19-28, 2015 ISSN 0972-3099

References

524 Roe, D., Seddon, N. and Elliott, J. (2019). Biodiversity loss is a development issue: a rapid review
525 of evidence. *IIED Issue Paper. IIED, London*, http://pubs.iied.org/17636IIED ISBN 978-1-
526 78431-688-4
527 Romeis, J., Meissle, M., Naranjo, S.E., Li, Y. and Bigler, F. (2014). The end of a myth – Bt (Cry1Ab)
528 maize does not harm green lacewings. *Front Plant Sci* 5, 1–10.
529 Romeis, J., van Driesche, R.G., Barratt, B.I.P. and Bigler, F. (2008). Insect-resistant transgenic
530 crops and biological control. In:, Romeis J, Shelton AM and Kennedy GG, , editors.,
531 Integration of insect-resistant genetically modified crops within IPM programs. *Springer*
532 *Science + Business Media BV*. pp. 87–117.
533 Salama, H.S. and Zaki, F.N. (1983). Interaction between Bacillus thuringiensis Berliner and the
534 parasites and predators of Spodoptera littoralis in Egypt. *Z angew Entomol*, 95: 425–429.
535 Schorling, M. and Freier, B. (2006). Six-year monitoring of non-target arthropods in Bt maize (Cry
536 1Ab) in the European corn borer (Ostrinia nubilalis) infestation area Oderbruch (Germany).
537 *J Verbr Lebensm* 1: 106–108.
538 Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R.,
539 Siegfried, B.D. and Dively, G.P. (2001). Impact of Bt corn pollen on monarch butterfly
540 populations: a risk assessment. Proc. Natl. Acad. Sci. U. S. A. 98, 11937–11942.
541 Skendžić, S., Zovko, M., Živković, I.P., Lešić, V. and Lemić, D. (2021). The Impact of Climate
542 Change on Agricultural Insect Pests. Insects. 12;12(5):440. doi: 10.3390/insects12050440.
543 Smyth, S.J. (2020). The human health benefits from GM crops. *Plant Biotechnol J*. 18(4):887-888.
544 doi: 10.1111/pbi.13261. Epub 2019 Oct 2. PMID: 31544299; PMCID: PMC7061863.
545 Smyth, S.J., McHughen, A., Entine, J., Kershen, D.R. and Parrott, W. (2021). Removing politics
546 from innovations that improve food security. *Transgenic Research*, 30, 601–612.
547 https://doi.org/10.1007/s11248-021-00261-y
548 Subedi, B., Poudel, A., Aryal, S. (2023). The impact of climate change on insect pest biology and
549 ecology: Implications for pest management strategies, crop production, and food security.
550 *Journal of*
551 *Agriculture and Food Research,* Volume 14, ISSN 26661543, https://doi.org/10.1016/j.jafr.
552 2023.100733.
553 Tilman, D., Isbell, F. and Cowles, J.M. (2014). Biodiversity and Ecosystem Functioning. *Annual*
554 *Review of Ecology, Evolution, and Systematics*, Vol. 45:471-493;
555 https://doi.org/10.1146/annurev-ecolsys-120213-091917
556 Triplehorn, C.A., Johnson, N.F. and Borror, D.J. (2005). Borror and DeLong's Introduction to the
557 Study of Insects. *Thomson Brooks/Cole, Belmont*, CA.
558 Vanden-Brink, P.J., den-Besten, P.J., bijde-Vaate, A. and ter-Braak, C.J.F. (2009). Principal
559 response curves technique for the analysis of multivariate biomonitoring time series.
560 *Environ Monit Assess* 152: 271–281.
561 Vasiliev, D. (2022). The Role of Biodiversity in Ecosystem Resilience. *IOP Conference Series Earth*
562 *and Environmental Science,* 1072(1):012012, DOI:10.1088/1755-1315/1072/1/012012
563 Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J. and Watrud, L.S. (2008). *Bt* crop
564 effects on functional guilds of non-target arthropods: a meta-analysis. *PLoS ONE,* 3: e2118.
565 Zhang, B.Y., Chen, M., Zhang, X.F., Luan, H.H. and Tian, Y.C. (2011). Expression of Bt-Cry3A in
566 transgenic Populus alba x P. glandulosa and its effects on target and non-target pests and
567 the arthropod community. *Transgenic Res* 20: 523–532.

Figure 1

Figure 1: Mean Spp Activity overview on field of Transgenic and Non transgenic Cowpea

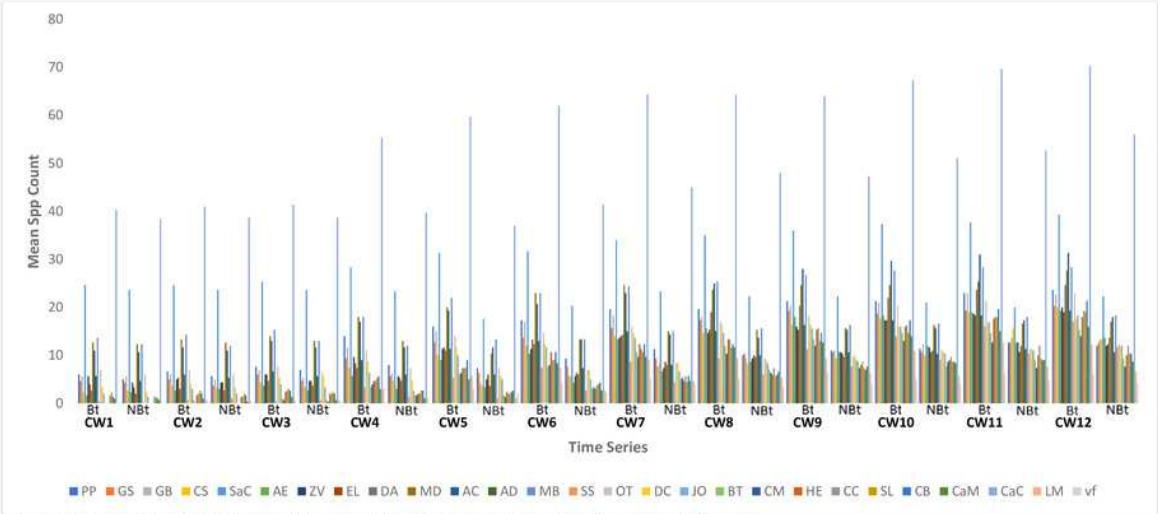

Figure 1: Mean Spp Activity overview on field of Transgenic and Non transgenic Cowpea

Where Bt: Line IT97KT; NBt: Line IT97KN; CW: Week Count; pp: Pirata piraticus; GS: Conozoa hyalina; GB: Graphoderus bilineatus; SaC: Sarcophaga crassipalpis Macquart; AE: Alydus eurinus; ZV: Zonecerus variegatus; EL: Eastern Lubber; DA: Deudorix antalus; MD: Musca domestica; AC: Atta cephalostes; AD: Apis dorsata; MB: Messor barbarous; SS: Scarabaeus satyrus; OT: Odontoponera transversa; DC: Dysdercus cingulatus; JO: Junonia oenone; BT: Bombus terrestris; CM: Chrysomya megacephala; HE: Hypolycaena erylus; CC: Conozoa carinata; SL: Stenolophus lecontei; CB: Chorthippus biguttulus; Cam: Carausius morosus; CaC: Camponotus cruentatus; LM: Liliocerис merdigera; vf: Variegated fritillary;

Figure 2

Figure 2: Mean line trend analysis of IT97KT (transgenic) vs IT97KN (non-transgenic) cowpea in a 12-week spread count using: (a) Shannon; (b) Simpson; (InvSimpson) and (c) Pielou

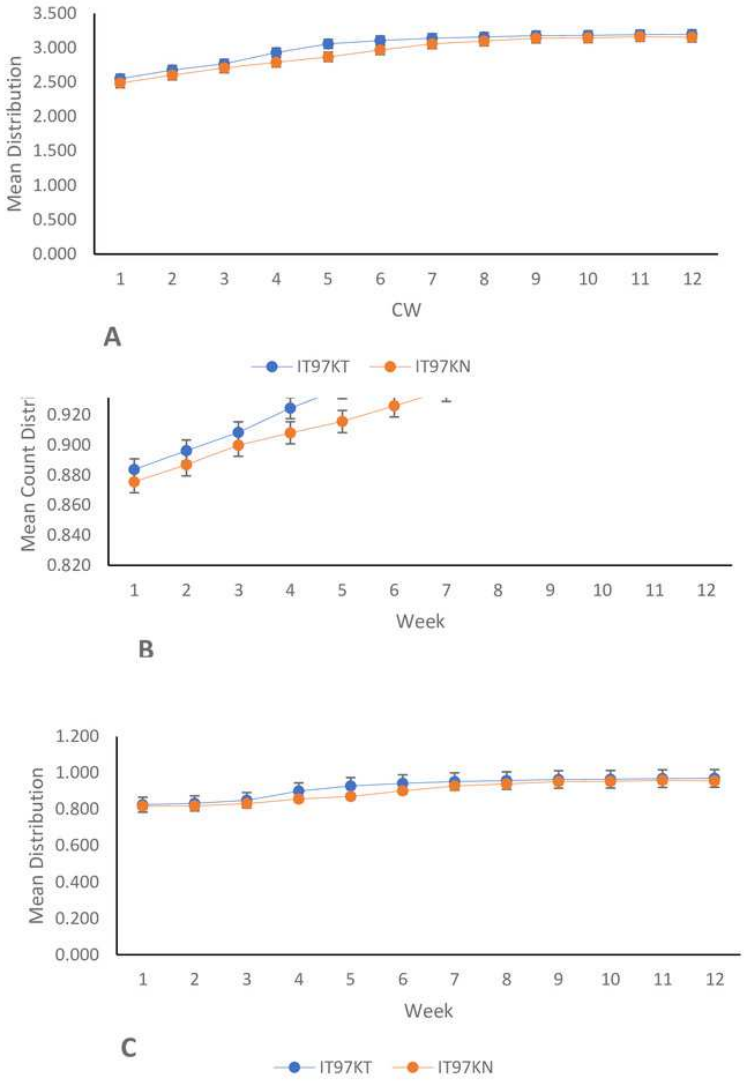

Figure 2: Mean line trend analysis of IT97KT (transgenic) vs IT97KN (non-transgenic) cowpea in a 12-week spread count using: (a) Shannon; (b) Simpson; (InvSimpson) and (c) Pielou

Figure 3

Line graph

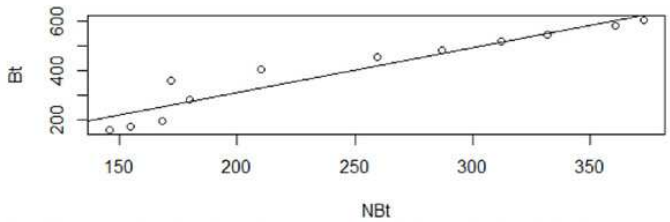

Figure 3a: Line graph showing the strong positive relationship between IT97KT and IT97KN ecological niches with p = 1.810599e-06 and r= 0.9522146

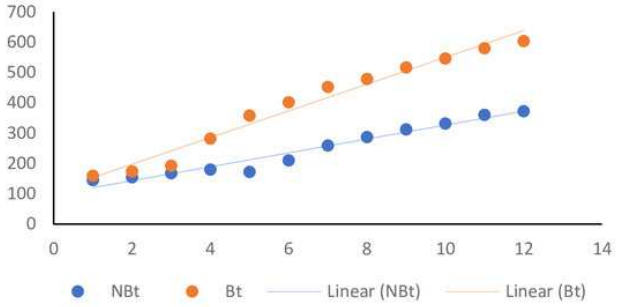

Figure 3b: Correlation of Bt vs Time and NBt vs Time: both shows significant positive correlation with time.

Figure 4(on next page)

Principal Component Plots Analysis: A. Bt PCA Plot; B. *NBt* PCA Plot

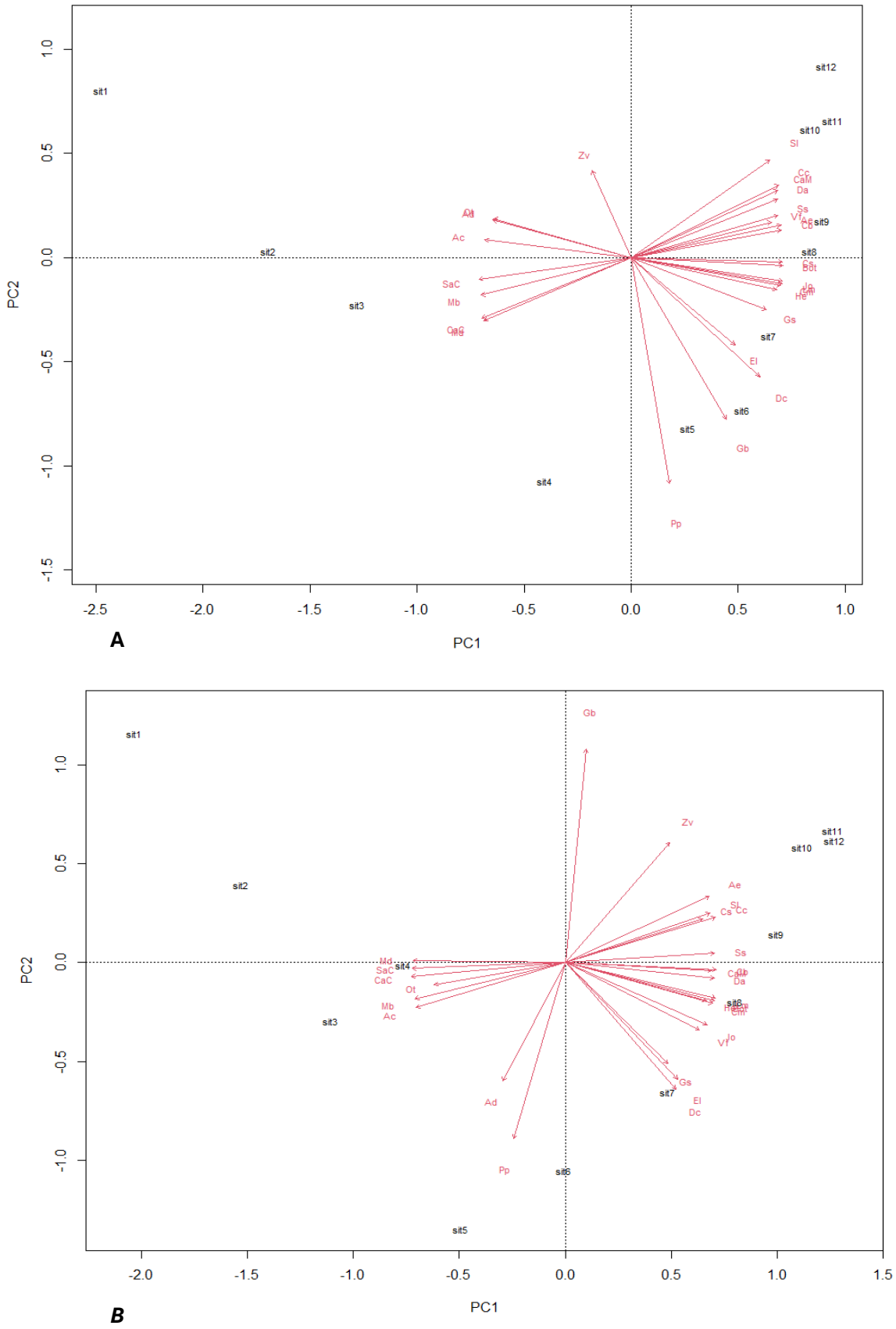

***Figure 4: Principal Component Plots Analysis: A. Bt* PCA Plot; B. *NBt* PCA Plot**

Figure 5

Broken Stick Distribution of the Principal Component between the ecological niche of transgenic PBR Cowpea and its non-transgenic isoline. A: Transgenic cowpea; B: Non-transgenic cowpea

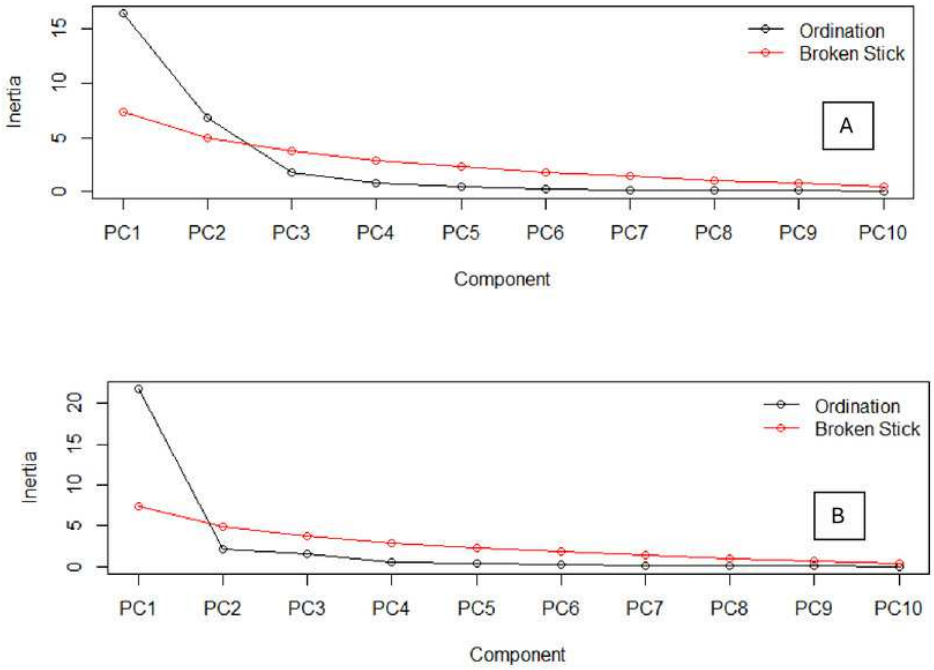

Figure 5: Broken Stick Distribution of the Principal Component between the ecological niche of transgenic PBR Cowpea and its non-transgenic isoline. A: Transgenic cowpea; B: Non-transgenic cowpea

Figure 6(on next page)

Composition of the Organism guild in both the *Bt* and N*Bt* fields

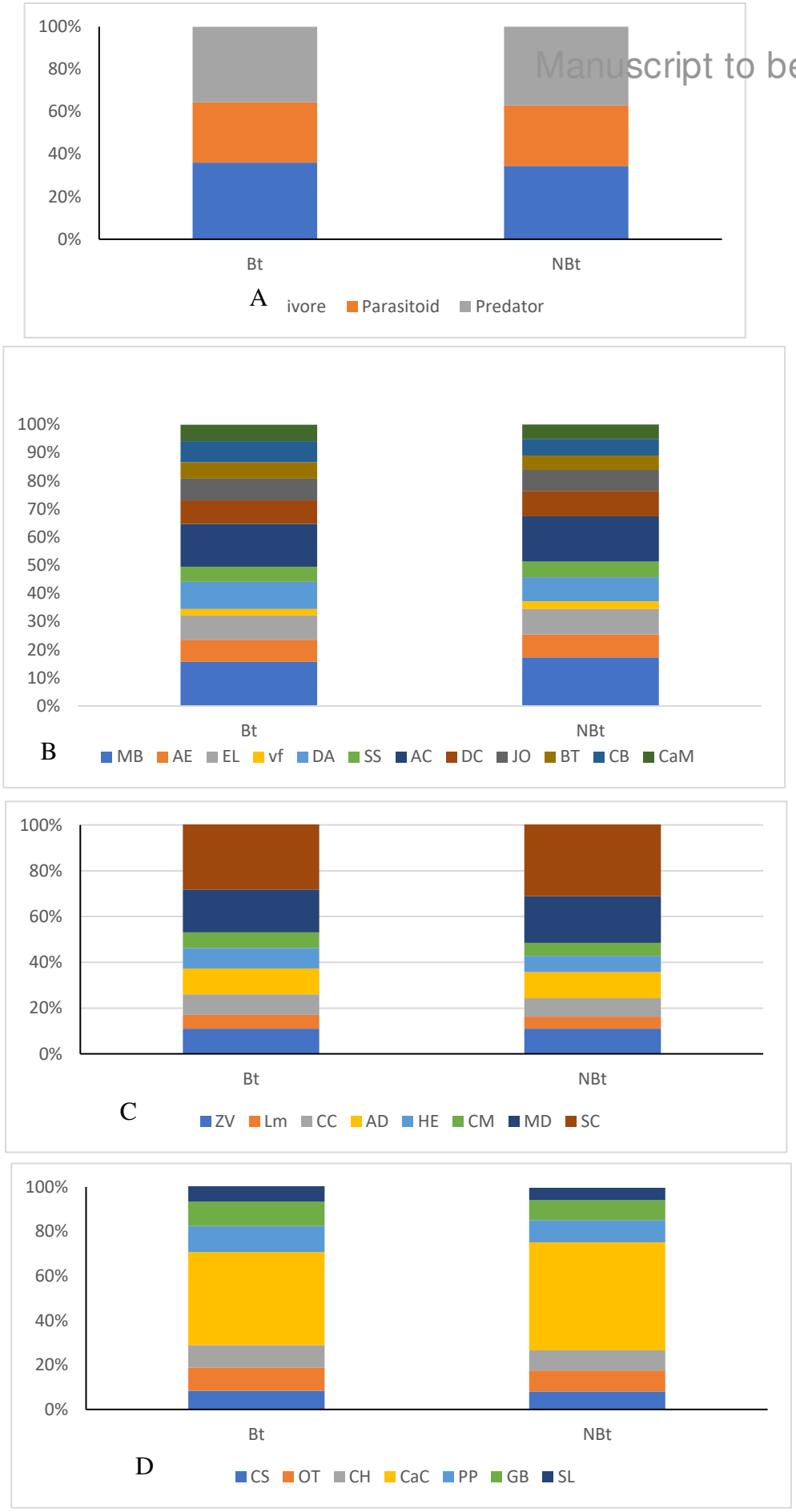

Figure 6: Composition of the Organism guild in both the *Bt* and N*Bt* fields; A: Composition of the NTO Communities; B: Composition of the herbivore guild of the *Bt* and non*Bt* Field; C: Composition of the Parasitoid guild of the *Bt* and Non *Bt* Field; D: Composition of the Predators guild of the *Bt* and Non *Bt* Field

Table 1(on next page)

Table 1: Statistical parameters of all the mean value analysis of IT97KT vs IT97KN

Table 1: Statistical parameters of all the mean value analysis of IT97KT vs IT97KN

Wk	Vr	Actual Count			Shannon			Simpson		
		Mean	P value	R^2 value	Mean	P value	R^2 Value	Mean	P value	R^2 Value
1	IT97KT	159.6667 ± 3.6742	0.11456	0.977	2.5258 ± 0.0207	0.1690	0.881	0.8823 ±0.002	0.1006	0.976
	IT97KN	145.6667 ± 3.6742			2.4641± 0.0207			0.8747 ±0.002		
2	IT97KT	173.6667±2.5495*	0.03418	0.988	2.6610 ±0.0134	0.0668	0.96	0.8951 ±0.002	0.0749	0.978
	IT97KN	154.6667±2.5495*			2.5914 ±0.0134			0.8853 ±0.002		
3	IT97KT	192.6667±2.7183*	0.02406	0.988	2.7541 ± 0.0312	0.3165	0.848	0.9067±0.003	0.18131	0.958
	IT97KN	168.3333 ± 2.7183*			2.6957 ± 0.0312			0.8989 ±0.003		
4	IT97KT	282.0000 ± 3.4238**	0.002231	0.996	2.9239± 0.0583	0.2144	0.693	0.9238±0.010	0.3374	0.562
	IT97KN	179.6667± 3.4238**			2.7760± 0.0583			0.9058±0.010		
5	IT97KT	358.3333 ± 2.3921***	0.0003295	0.999	3.0512 ± 0.0427	0.0665	0.889	0.9375±0.009	0.1345	0.806
	IT97KN	172.0000 ± 2.3921***			2.8290 ± 0.0427			0.9082±0.009		
6	IT97KT	401.6667 ± 9.6724**	0.005072	0.99	3.1006 ±0.0171	0.024 *	0.957	0.9425±0.004	0.0744	0.886
	IT97KN	210.3333±9.6724**			2.9475 ±0.0171			0.9222±0.004		
7	IT97KT	452.3333 ±8.0312**	0.003445	0.993	3.1334 ±0.0097*	0.0207	0.964	0.9464±0.002	0.05671	0.911
	IT97KN	259.3333 ±8.0312**			3.0396 ±0.0097*			0.9339±0.002		
8	IT97KT	479±11.1131**	0.006634	0.987	3.1506 ±0.011*	0.0485	0.941	0.9485±0.003	0.1051	0.882
	IT97KN	287±11.1131 **			3.0823±0.011*			0.9385±0.003		
9	IT97KT	516.6667±8.5765**	0.003505	0.993	3.1716± 0.0153	0.1703	0.85	0.9510±0.002	0.1731	0.84
	IT97KN	312.3333 ±8.5765**			3.1260±0.0153			0.9438±0.002		
10	IT97KT	546 ±8.9536**	0.003483	0.993	3.1761 ±0.0106	0.0919	0.914	0.9515±0.002	0.1445	0.856
	IT97KN	332±8.9536**			3.1303 ±0.0106			0.9444±0.002		
11	IT97KT	580.0000 ±7.728**	0.002474	0.995	3.1859 ±0.011	0.1399	0.878	0.9515±0.002	0.1445	0.856
	IT97KN	360.6667 ±7.728**			3.1489 ±0.011			0.9444±0.002		
12	IT97KT	603.3333 ±4.7317***	0.0008405	0.998	3.1913±0.0104	0.2600	0.902	0.9532± 0.002	0.1003	0.871
	IT97KN	372.6667 ±4.7317***			3.1388±0.0104			0.9458± 0.002		

Vr: variety; Wk: Week

Table 2(on next page)

Correlation analysis of Bt vs NBt, Bt vs Time (weeks) and NBt vs Time

Table 2: Correlation analysis of Bt vs NBt, Bt vs Time (weeks) and NBt vs Time

parameters	***p-value***	***r***
Bt vs NBt	*1.535e-07***	0.9522146
Bt vs Time	*3.42862e-09*	*0.9865187*
NBt vs Time (week)	*3.508742e-08*	*0.9784767*

Table 3(on next page)

Bray Curtis Dissimilarity Index Analysis

Table 3: Bray Curtis Dissimilarity Index Analysis

Descriptors	Values	Inference
C_{ij}	739	1. No divergence in NTOs community structure 2. Similar evolutionary trends
S_i	1142	
S_i	739	
BC_{ij}	0.2	

C_{ij}: the sum of the lesser values for the species found in each site; S_i: The total number of specimens counted at site I; S_j: The total number of specimens counted at site j; BC_{ij}: Bray Curtis Dissimilarity Index.