

Natural Enemies of Invasive Plants in the Peruvian Amazon: Identifying Insect Consumers and Characterizing Insect Communities of Invasive Kudzu and *Brachiaria spp.*

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Abstract

Invasive plants are a threat to ecosystem biodiversity and native habitat conservation and restoration. Traditional strategies of invasive plant control face trade-offs in terms of time, monetary, and environmental costs, leaving the need for improvements in invasive plant control methods. Biological control methods are one possible strategy with great potential for exploration. Previously, local herbivorous insect species have been documented adapting to and consuming invasive plants and have been used as biological control methods. Additionally, comparing the biological characteristics of insect communities across invasive and native plant habitats in a region can be used to learn about the effects of invasive plant presence in that ecosystem. In a region of the Southeastern Peruvian Amazon, comparing insect communities' compositions in invasive and native plant habitats depicts the adaption and reaction of local insect communities to invasive *Brachiaria spp.* and kudzu. These findings reaffirm past observations of herbivorous insects quickly adapting to invasive plants and being promising candidates for use as biological control agents, as well as invasive plant presence having negative effects on ecosystem biodiversity. The results of this study provide further knowledge on the characteristics of insect communities in invasive and native habitats in the Southeastern Peruvian Amazon and identify herbivorous insects that consume invasive *Brachiaria spp.* and kudzu. The results demonstrate how local insect communities respond to invasive plants and implicates the potential of local herbivorous insects as biological control agents of invasive plants.

Introduction

Conservationists have regarded invasive plants as a major issue facing natural areas for centuries (Henderson et al. 2006). Recorded impacts of invasive plants include altered vegetation communities and nutrient cycling (Weidenhamer et al. 2010, Martijn Bezemer et al. 2014), decreased biodiversity (Powell et al. 2011, Litt et al. 2014, Crystal-Ornelas et al. 2020), and decreased habitat for

native plants and wildlife across the globe (Pyšek et al. 2010). Out of 680 extinct animal species in the IUCN Red List database, the effects of invasive species were listed as a cause of 54% of extinctions (Clavero et al. 2005). Additionally, the economic costs of alien weeds are great, with yearly economic damages in the United States alone estimated at \$US 120 billion (Pimental et al. 2005). For

these reasons, invasive plants should be considered a topic of great importance for research and natural resource management. There is a great demand for methods to manage invasive plant species (D'Antonio et al. 2016), but many methods, such as the use of chemicals, have significant side-effects including damage to the environment (Rinella et al. 2009, Peterson et al. 2020, Waltham et al. 2020), contamination of food and water (Orlando et al. 2014, Alengebawy et al. 2021), and high time and money costs (Reid et al. 2007, Klein 2011, Clewley et al. 2012, Aurambout et al. 2018). When implemented correctly (Harms et al. 2021), biological control methods are regarded as a relatively safe and cost-effective strategy in the long-term for controlling invasive plant species (Page et al. 2006, Seastedt et al. 2007, Chalak et al. 2011). Research has shown that invasive grasses, like *Brachiaria spp.*, and kudzu are good candidates for the use of biological control methods due to the existence of specialized natural enemies to kudzu and invasive grasses (Imai et al. 2010, Li et al. 2011, Sutton et al. 2019). Additionally, herbivorous insect communities have been shown to adapt relatively quickly to invasive plant presence, making them promising candidates as biological control agents (Siemann et al. 2006, Schilthuizen et al. 2016, Falcón et al. 2017). Globally, total insect populations are shown to be declining due to a variety of possible causes, including the impact of invasive plants (Wagner 2020, Tallamy et al. 2021). It is important to understand the effects and interactions of invasive plants in their surrounding ecosystem, and specifically in surrounding insect communities, to determine the best management practices for native

biodiversity conservation (Sunny et al. 2015). Data on the impacts of invasive plants on insect community composition can give valuable insight to the likely impacts of invasive plants on the greater ecosystem, due to the important role of insects in ecosystem functioning (Siemann et al. 2008). The development and exploration of biological control agents for the management of invasive plant species is a topic of great importance for the preservation and restoration of natural areas and native species (Ellison et al. 2004, de Lange et al. 2010, Van Driesche et al. 2016, Van Driesche et al. 2017).

The tropical rainforest restoration and conservation efforts at Finca Las Piedras in Peru are undermined by the growth of several invasive plant species, particularly kudzu (*Pueraria montana*) and African cattle grass (*Brachiaria spp.*). Kudzu is a vine native to Asia that was introduced to the Americas in the 1800s for use as an ornamental plant and for erosion control before spreading rapidly and becoming one of the most aggressive introduced vines in the Americas (Forseth et al. 2004). African cattle grass was introduced to the region for use in cattle pasture (Ellison et al. 2004). These invasive non-native plants grow quickly and densely, outcompeting native plants and leading to a noticeable loss of plant biodiversity in affected lands, as is typical of invasive plant species (Padmanaba et al. 2014). Past research has demonstrated the potential for using herbivorous insect biological control agents to reduce the competitive superiority of invasive kudzu (Frye et al. 2012). If natural enemies of invasive kudzu and *Brachiaria spp.* can be identified, then such species could be used as biological control agents to assist in the eradication of the alien plants and the restoration of native plant communities.

In this study I examined the herbivorous insects feeding on the invasive plants kudzu and African cattle grass at Finca Las Piedras as a first step towards creating a biological control strategy for managing invasive plants and promoting forest restoration at the site. Additionally, I examined and identified to morphospecies the insects present at each site to characterize and compare the insect community's abundance and diversity across invasive kudzu and *Brachiaria spp.* habitats, and native plant habitats.

Methods

Study plots containing dense growth of kudzu and/or African cattle grass were established in which plants and insects were monitored for 15 minutes per plot during the morning and the evening. Morning monitoring of the plots took place between the times of 8:35am and 11:35am, while night monitoring took place between 7:35pm and 10:30pm. Sites were 1x1 meter each on abandoned field habitat for invasive treatment plots and regenerating secondary forest habitat for native treatment plots, where these plants are known to be abundant. 10 replicate plots were established for each treatment, making a total of 30 plots. The plot sites were chosen randomly by establishing sites at ten-meter minimum distance in areas chosen based on the following criteria:

Site 1 (control): Mixed species native vegetation in regenerating secondary forest, without a dominant invasive species presence (natives making up at least 80% of sites' plant cover).

Site 2 (test): Regenerating secondary forest edge habitat with kudzu plants dominating

sites' plant cover (kudzu forming at least 80% of sites' plant cover).

Site 3 (test): Abandoned pasture habitat with *Brachiaria spp.* plants dominating sites' plant cover (*Brachiaria spp.* forming at least 80% of sites' plant cover).

During each monitoring session plants were checked for the presence of herbivorous insects by carefully and closely visually scanning the leaf surface, underside, stems, and ground of the plots from multiple vantage points. Lepidoptera and Coleoptera larvae found on a plant were assumed to be feeding on it; other insects (e.g., Orthoptera, adult Coleoptera, Hemiptera) were counted as feeding on a plant if had they had previously been seen consuming the plant or if they were known to be herbivorous. Prior to collection, insects present were photographed at the site. After being trapped and collected by hand using small ethanol-filled plastic containers, herbivorous insects were photographed against a white background, (save for Lepidoptera and Coleoptera larvae), depending on the size of the individual, and stored in plastic vials filled with 70% ethanol labeled with the site ID, treatment, collection date and time. Some specimens were later transferred into plastic bags and frozen to reuse ethanol and vials.

Morphospecies identifications were created based on differing morphological features of the insects and were assigned to individuals based on in-field observations, photographs, and inspection of collected individuals. A reference library of the morphospecies was created, which contained the morphospecies or scientific names, morphological descriptions, order, and photos. Data was entered in an Excel spreadsheet relating the morphospecies' abundance to host

plant species and site location, and noting the date and time of observance, behavior, type of specimen voucher (ethanol container), and miscellaneous observations. The number of total morphospecies and individuals of each treatment were calculated in Excel, along with the Species-Richness Index and Shannon-Wiener Index of each treatment. A Correspondence Analysis and NMDS visualizations were conducted using CA and Vegan packages in R software (R Core Team, 2020) to find the similarity of species composition among sites and find out which species co-occur together among sites. Visualizations were made using ordiplot and ordihull for polygons. An ANOVA test was conducted on the final data using R, to determine if the variance of the abundance and diversity of herbivorous insects consuming the invasive versus native plants was statistically significant. Variances of species count were

assumed to be equal among treatments ($F = 0.08$, $p = 0.92$, n.s.) and species data to be normally distributed ($W = 0.95$, $p = 0.22$). For individual's data, homogeneity of variances ($F = 0.73$, $p = 0.49$, n.s.) and normally distributed data ($W = 0.99$, $p = 0.94$) were analyzed. Therefore, ANOVA assumptions were met.

Results

The total number of insects observed over the course of the study was 1469 individuals. The invasive treatments (kudzu and *Brachiaria spp.*) had a higher number of total observed individuals, with 526 and 470 individuals observed, respectively, than did the Native treatment, with 473 individuals observed. A total of 191 unique morphospecies were observed during the study. The Native treatment had a higher overall number of observed morphospecies, with 110 total species, compared to the 89 and 98 total species

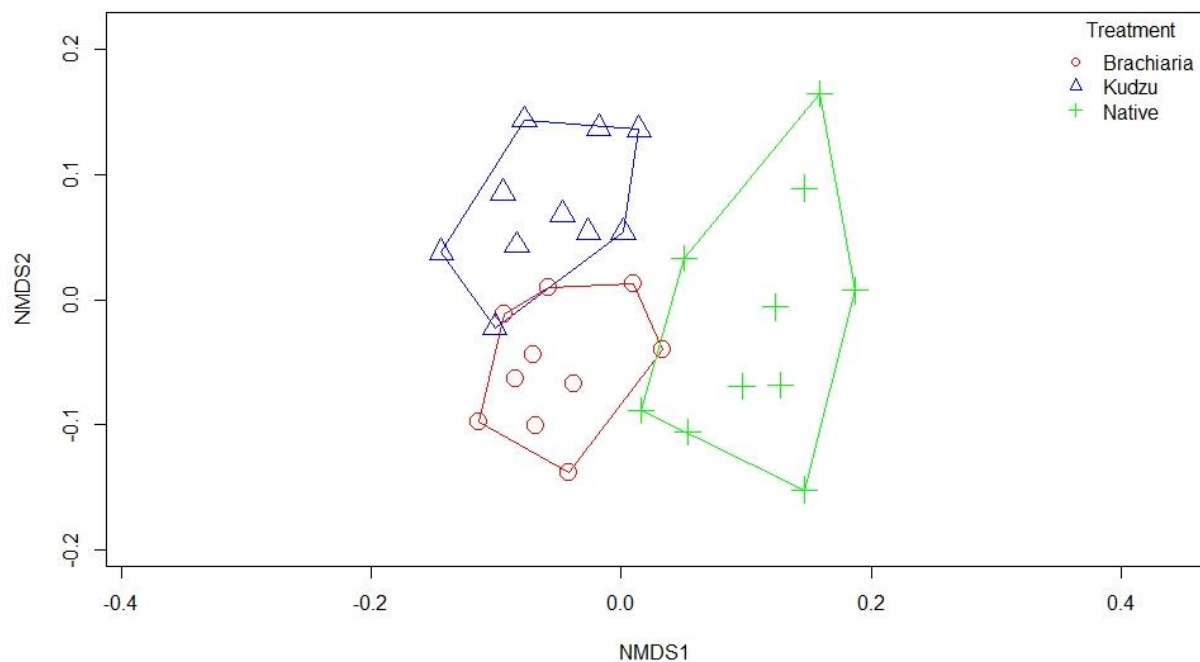


Figure 1: NMDS of species composition and abundances in insect communities among three different treatments at Finca Las Piedras. Invasive plants: *Brachiaria spp.* (red circles), kudzu (blue triangles), and Native (green crosses).

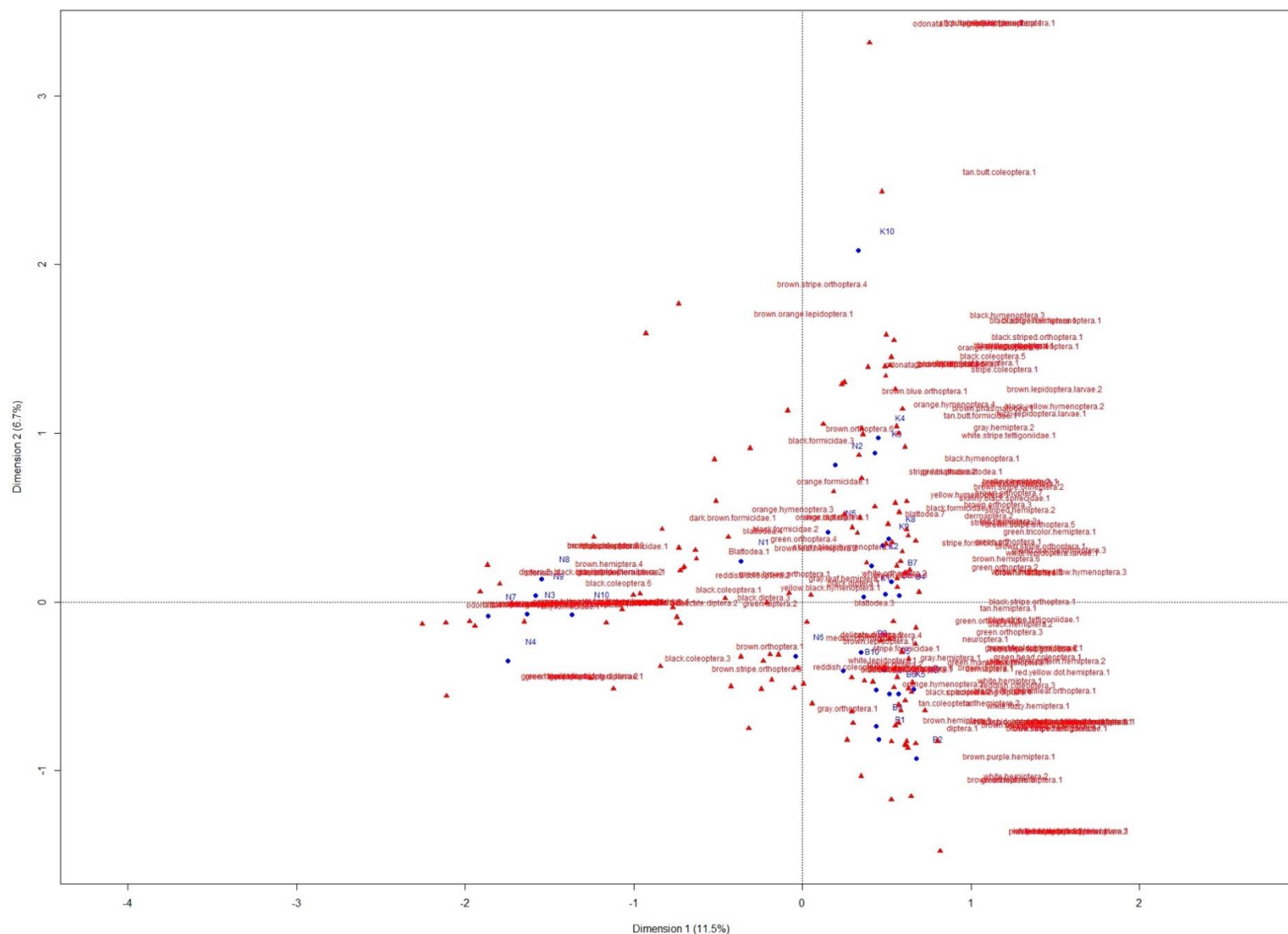


Figure 2: Correspondence analysis of species composition and abundances in insect communities (red triangle=morphospecies) among three different treatments (blue circle=plot).

of the *Brachiaria spp.* and kudzu treatments, respectively (Table 1).

Table 1: The number of unique morphospecies and individuals across three different treatments at Finca Las Piedras.

| Treatment | Species Count | Individual Count |
|------------------------|---------------|------------------|
| <i>Brachiaria spp.</i> | 89 | 470 |
| Kudzu | 98 | 526 |
| Native | 110 | 473 |
| Grand Total | 191 | 1469 |

Marked clustering was found between treatments, particularly between kudzu and *Brachiaria spp.*, and distinct separation was found between the Native treatment and invasive treatments (Figure 1 and 2). The NMDS showed a remarked difference between invasive and Native plant insect communities, as well as a greater variation of species composition in the Native treatment compared to the invasive treatments.

The correspondence analysis showed the association of certain insect species to particular treatments. Such species included the strong association of the morphospecies “tan butt coleoptera 1” with kudzu treatments, the association of “brown hemiptera 4” to Native treatments, and the association of “white hemiptera 2” and “brown purple hemiptera 1” with *Brachiaria spp.* treatments.

Brachiaria spp. had the highest level of variation in the number of species present at each plot (Minimum=12, Maximum=26), while kudzu had the least (Minimum=16, Maximum=30), and Native had an intermediate level of variation (Minimum=15, Maximum=24). Kudzu had the highest mean number of species per plot at 22, followed by *Brachiaria spp.* and Native treatments which

had equal mean species counts (19.7). The number of species per plot across the three treatments was found to not be significantly different ($F_{2, 27} = 1.03$, $p = 0.37$, n.s.).

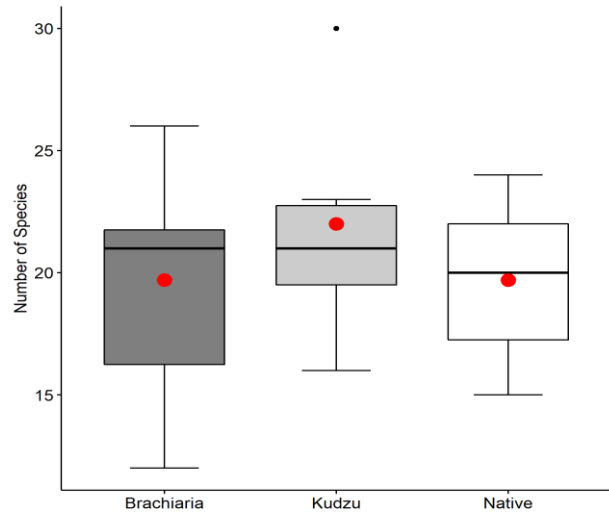


Figure 3: Boxplot representing the number of species per plot across three treatments at Finca Las Piedras (red circle=mean).

Native treatment had far greater variation in the number of individuals found per plot than did the invasive plots. *Brachiaria spp.* had the least amount of variation in the number of individuals found per plot. Kudzu had the highest mean count of individuals per plot with 52.6 individuals, while Native and *Brachiaria spp.* had similar counts of 47.3 and 47.0 individuals, respectively. The number of individual insects per plot across three separate treatments was found to not be significantly different ($F_{2, 27} = 0.7$, $p = 0.50$, n.s.).

Brachiaria spp. was calculated as having a Species Richness Index of 4.11 and a Shannon-Wiener Index of 3.42. The kudzu treatment was found to have a Species-Richness Index of 4.27, and a Shannon-Wiener Index of 3.66. The Native treatment had a Species Richness Index of 5.06 and a Shannon-Wiener Index of 3.19.

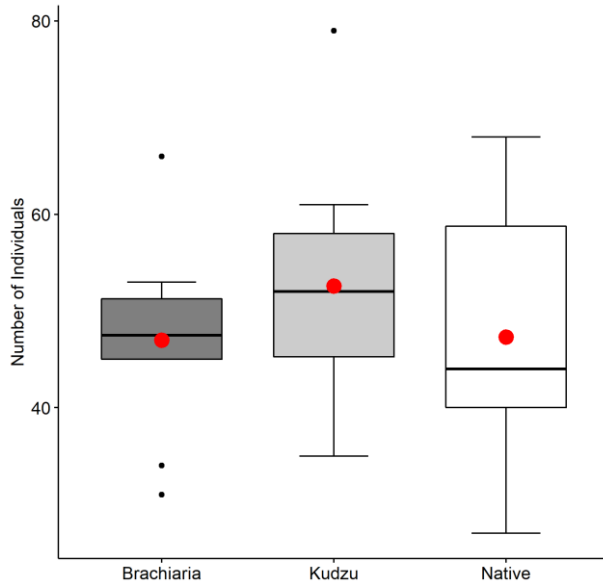


Figure 4: Box plot of the number of individual insects present per plot across three treatments at Finca Las Piedras (red circle=mean number of insects per plot).

The number of individuals observed in each order across separate treatments was recorded and analyzed. The abundance of insect orders varied across order type and treatments. Six orders saw their highest abundance in Native treatments and lower

Table 2: Table of the Species Richness Index and Shannon-Wiener Index across three treatments at Finca Las Piedras.

| Treatment | Species Richness Index | Shannon-Wiener Index |
|-------------------|------------------------|----------------------|
| <i>Brachiaria</i> | 4.10526388 | 3.41957893 |
| <i>spp.</i> | | |
| Kudzu | 4.27300306 | 3.65825311 |
| Native | 5.05780539 | 3.1910609 |

abundances in invasive treatments (Blattodea, Coleoptera, Hymenoptera, Lepidoptera, Odonata, and Phasmatodea). Five orders saw their highest abundance in *Brachiaria spp.* treatments (Dermaptera, Diptera, Hemiptera, Mantodea, and Neuroptera). Only one order had its highest abundance in kudzu treatments (Orthoptera). Two orders were present in only one treatment type (Mantodea and Neuroptera in *Brachiaria spp.*). Two individuals that were not able to be identified to order were found in the Native treatment.

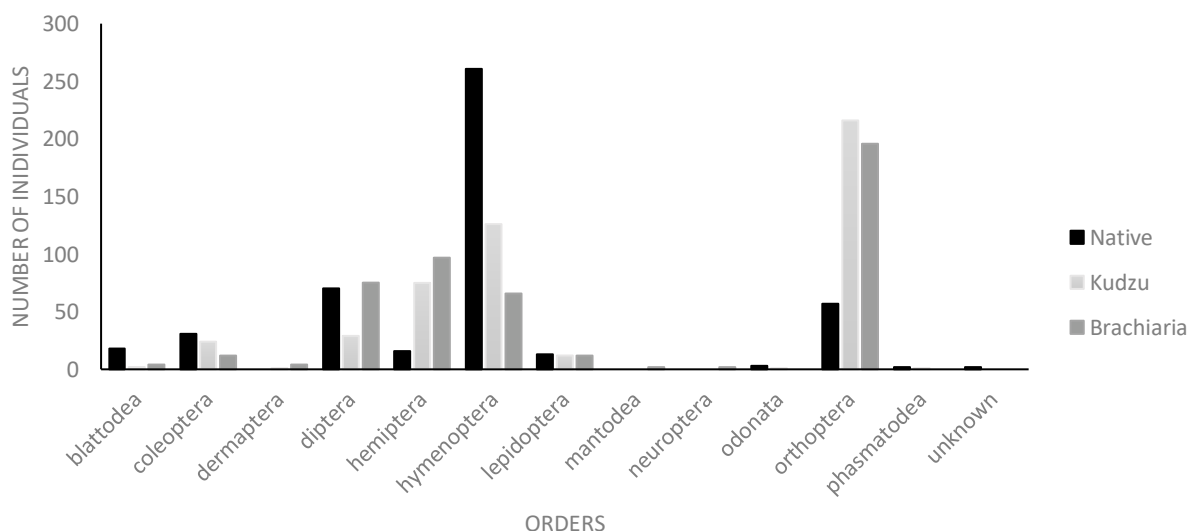
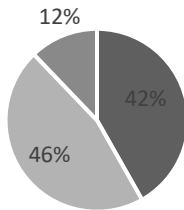
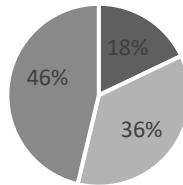


Figure 5: Histogram of the number of individuals per order across three treatments at Finca Las Piedras.

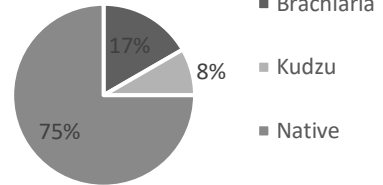
Orthopterans



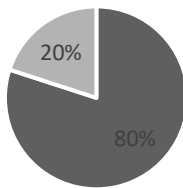
Coloeptera



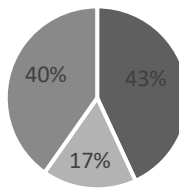
Blattodea



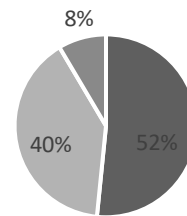
Dermaptera



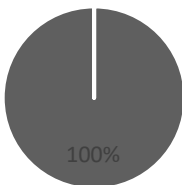
Diptera



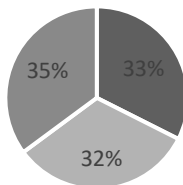
Hemiptera



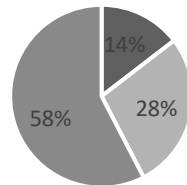
Mantodea



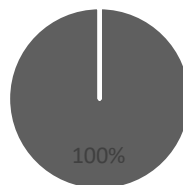
Lepidoptera



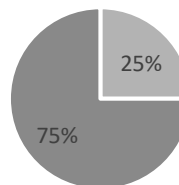
Hymenoptera



Neuroptera



Odonata



Phasmatodea

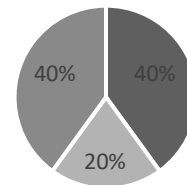


Figure 6: Pie graphs of the distribution of individuals in each order across the three treatments at Finca Las Piedras.

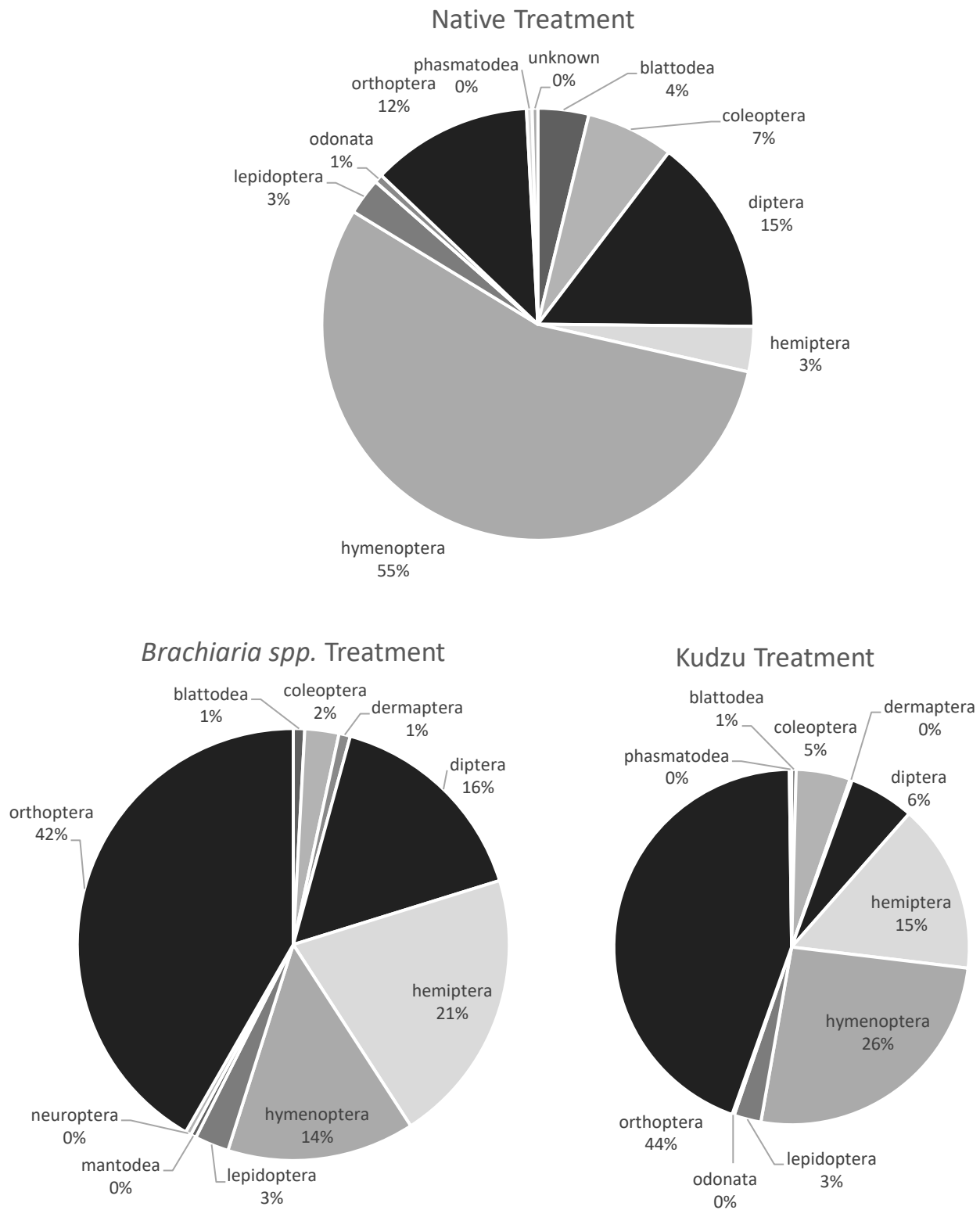


Figure 7: Pie graphs of the order composition of the individuals found in Native, *Brachiaria spp.*, and kudzu treatments at Finca Las Piedras.

Analysis of the number of individuals in each order across each of the three treatments showed that the orders with the most variation in abundance across treatments included Blattodea (18 in Native, 2 in kudzu, 4 in *Brachiaria spp.*), Hemiptera (16 in Native, 75 in kudzu, 97 in Hemiptera), Hymenoptera (261 in Native, 126 in kudzu, and 66 in *Brachiaria spp.*), and Orthoptera (57 in Native, 216 in kudzu, and 196 in *Brachiaria spp.*).

Analysis of the insect communities found at each of the three treatments showed that the overall order composition varied across the treatments, particularly between the Native treatment and the more similar invasive treatments. Hymenoptera made up 55% of the insect community in the Native treatment, 14% in *Brachiaria spp.*, and 26% in kudzu. Orthoptera made up only 12% of the community in the Native treatment, while in the *Brachiaria spp.* and kudzu treatments it made up 42% and 44% of the communities, respectively. Hemiptera made up only 3% of the Native treatment community, versus 21% in the *Brachiaria spp.* treatment and 15% in the

kudzu. Blattodea was responsible for 4% of the Native treatment community but just 1% of both invasive treatment communities. In other instances, the invasives and Native treatments shared more similar order compositions than the invasives did with one another, particularly so in the instances of Diptera and Coleoptera. 15% of the Native treatment was formed by Diptera. Similarly, 16% of the *Brachiaria spp.* treatment order composition was formed by Diptera, and the 6% Diptera composition in the kudzu treatment was the least similar. The Native treatment insect community was made up of 7% Coleopterans, and the kudzu treatment community was made up of 5% Coleopterans, while the *Brachiaria spp.* treatment was made up of just 2% Coleopterans. On the other hand, Lepidoptera made up 3% of the insect community in each of the treatments.

Finally, considering Orthopteras as focal group, the number of species and individuals found per treatment were significantly different (species: $F_{2,27}=3.67$, $p=0.03$, individuals: $F_{2,27}=12.47$, $p<0.001$) (Figure 8).

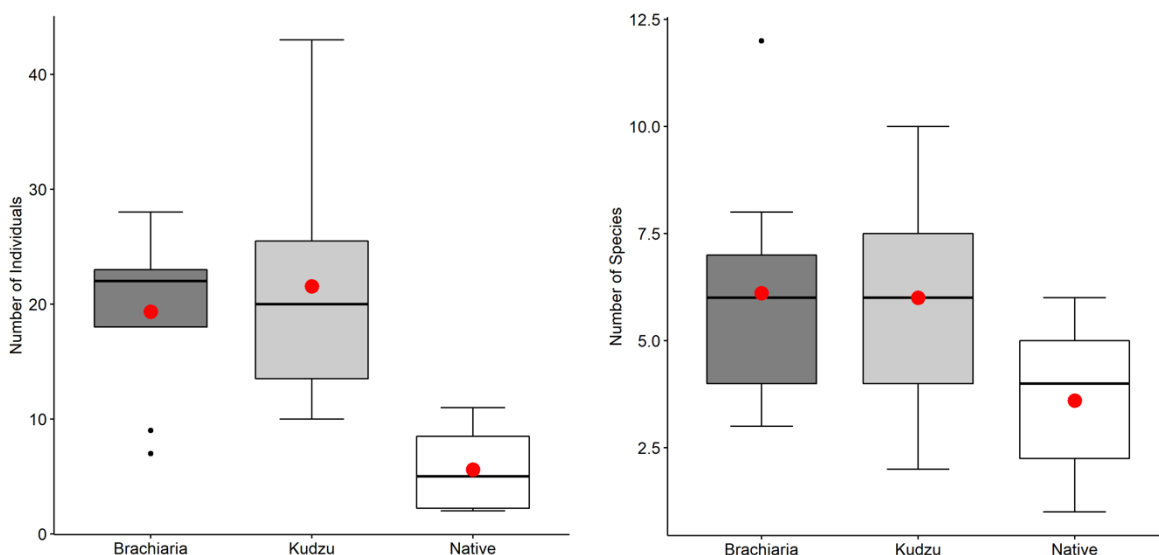


Figure 8: Boxplot of the number of species of Orthoptera present in three treatments at Finca Las Piedras.

Discussion

Through these statistical analyses, the Native treatment was seen to have a higher insect species diversity than the invasive treatments, while the invasive treatments were seen to have a higher relative abundance of insect species (Table 1 and 2). The Species-Richness Index of the Native treatment was the highest of all the treatments, which indicates that the native habitat contains the most diversity in terms of insect species. The Shannon-Wiener Index calculation reported that the Native treatment had the smallest number of individuals per species out of the three treatments. Despite the higher mean number of individual insects present in the invasive habitats, the number of insect species in such communities is lower than those in native habitats. The higher number of individual insects found in invasive habitats compared to native habitats may be due to the visibly far greater plant cover density in the invasive habitats than in the native.

The dominant and invasive nature of *Brachiaria spp.* and kudzu is characterized by monoculture vegetation communities with dense plant cover, which creates more potential viable habitat within a small geographical area for insects adapted to such habitat. Another factor potentially erroneously influencing these results is the wider diversity of plant species in the native treatment plots compared to the invasive treatment plots, which may overrepresent the native habitat's insect diversity. On the other hand, this aspect of the experimental design represents the greater diversity of plant species and unique microhabitats found in native vegetation communities, and thus may allow for a more accurate depiction of the insect community

than would an experimental design that included native vegetation communities compromised by only one native plant species.

The findings of the NMDS and Correspondence Analyses (Figure 1 and 2) illustrate that despite the lack of significant variation between the treatments in terms of the number of species and individuals (Figure 3 and 4), the insect communities present in Native, *Brachiaria spp.*, and kudzu habitats are unique from one another. These visualizations show that some insect species are strongly associated with particular habitats, and the plant habitats are partitioned among insect species and communities. These findings demonstrate that some insect species are better adapted to living among invasive plants than other insect species and may have become specialized to kudzu or *Brachiaria spp.* These results also show that the transition of a plant community from native to invasive dominant significantly changes the insect species community composition present. The invasion of non-native plants into formerly native plant communities appears likely to threaten the future survivability of strongly native-associated insect species. These findings suggest that the presence and/or expansion of invasive plant communities has a negative impact on the species diversity of local insect communities.

The results of Figures 5, 6, and 7 suggest that certain insect orders are better adapted to surviving in a particular plant habitat than other insect orders. For instance, Orthopterans appear to have adapted successfully to invasive *Brachiaria spp.* and kudzu habitats, while Hymenopterans and Blattodeans appear to have less successfully adapted to these habitats. The presence of

invasive plant habitat in Finca Las Piedras affects some insect orders more severely than others, resulting in changes to the insect community composition and the survivability of certain insect species and orders. The variation in insect order composition between habitats suggests that herbivorous insect orders, particularly Orthoptera, likely consume the invasive *Brachiaria spp.* and kudzu plants. Accordingly, it appears that the digestive systems of such herbivorous species have adapted to feeding on the invasive plants, potentially thanks to nutritional and structural similarities of the invasive plants and native plants already consumed by the insects (Harvey et al. 2010).

The findings of this study allow the expected result, that local insects would be found consuming the invasive plants, to be confirmed. The morphospecies, Red Stripe Tettigoniidae 1, was observed and filmed consuming a broken *Brachiaria spp.* stem within an invasive treatment plot. This observation confirms the expected result, and, in combination with the high abundance of Red Stripe Tettigoniidae 1 in both invasive treatments compared to its low abundance in native habitats, implicates the morphospecies' potential for use as a biological control method in invasive *Brachiaria spp.* and kudzu habitats at Finca Las Piedras. Other herbivorous insect species were also found in relatively high concentrations in the invasive habitats compared to native habitats, although they were not observed actively consuming the invasive plants. It seems appropriate to assume that such species consume the invasive plants, given that they are strongly associated with the invasive treatments and are known to be herbivorous. Given these discoveries, it seems

plausible that native herbivorous insect species could be identified and experimented with for use as a biological control method for invasive *Brachiaria spp.* and kudzu habitats in the region.

The study's other expected result, that the insect abundance and diversity in the invasive treatments would be significantly less than in the native treatment, was not confirmed by the findings. Despite this result, the study's other findings, such as those illustrated in Tables 1 and 2 and Figures 1 and 2, demonstrate that there are notable differences between the insect communities in invasive and native habitats. Native habitats generally had a smaller number of individuals present and a greater number of unique species than did the invasive plant habitats. This finding can be expanded to infer that native plant habitats in the Finca Las Piedras region generally have greater insect species diversity than the insect communities in invasive plant habitats.

Overall, the results of this study indicate the importance of native plant habitat preservation for the diversity and long-term vitality of the insect communities in the Finca Las Piedras region. Additionally, some local insect species, particularly the morphospecies "Red Striped Tettigoniidae 1," have adapted and apparently specialized to invasive plant habitats and may be suitable for use as a biological control method to assuage the growth of invasive *Brachiaria spp.* and kudzu in the region. Due to the limitations in scope, resources, and time of this study, further research on these topics is still needed. These results implicate many potential future directions for complementary research, such as identifying to species Red Stripe Tettigoniidae 1 and other observed morphospecies,

documenting the life-cycles and interspecies relationships of the selected insect species, empirically testing the dietary preferences of these insect species between kudzu, *Brachiaria spp.*, and native plants, analyzing the potential repercussions, benefits, and non-target effects of their use as a biological control method, and determining the best methods for rearing and releasing selected biological control insects. Further research that analyzes local insect species' evolutionary reactions and life-history responses to a vegetation community transitioning from native to invasive dominant is necessary to understand the mechanisms causing insect communities' compositions and dispersals. Research on the local insect community's specific roles in the region's food web might allow for insight as to how these changes in community composition due to invasive plants might affect the rest of the ecosystem. Identification of the possible long-term macro- and micro-scale effects of introducing elevated abundances of such local herbivorous insects in the ecosystem, as well as a quantified analysis of the direct effect that native insects' consumption has on the survival of these invasive plants are needed before implementing any biological control decisions based on this study (Liu et al. 2007). This study's findings demonstrate the impact of kudzu and *Brachiaria spp.* invasion on the insect community in the Finca Las Piedras region and serve as a preliminary suggestion of local herbivorous insects' potential for use as a biological control agent for the invasive plants. Further research on this topic may help to develop better land management techniques and invasive plant mitigation strategies that could contribute to the conservation of the world's most biodiverse native habitat.

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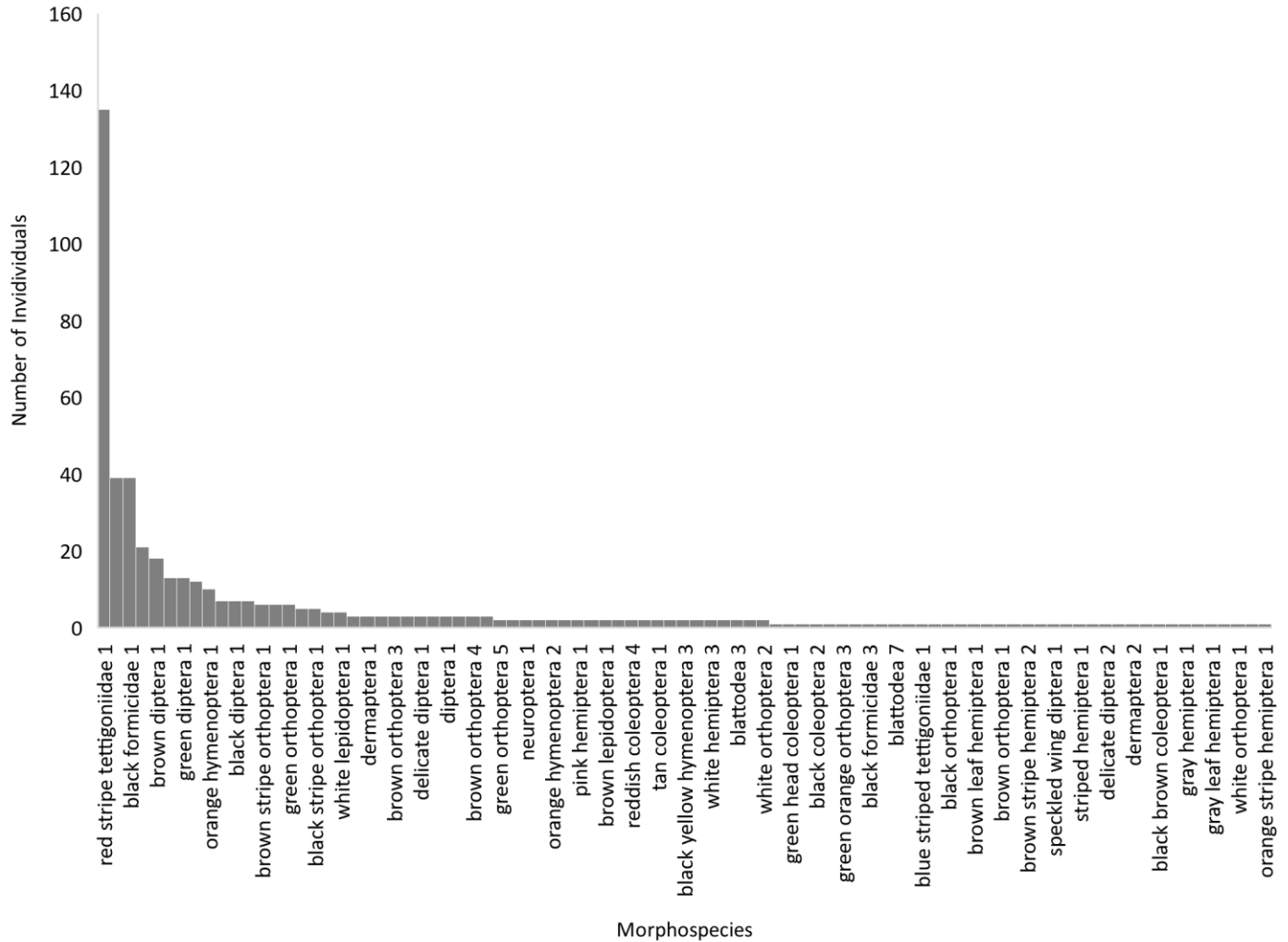
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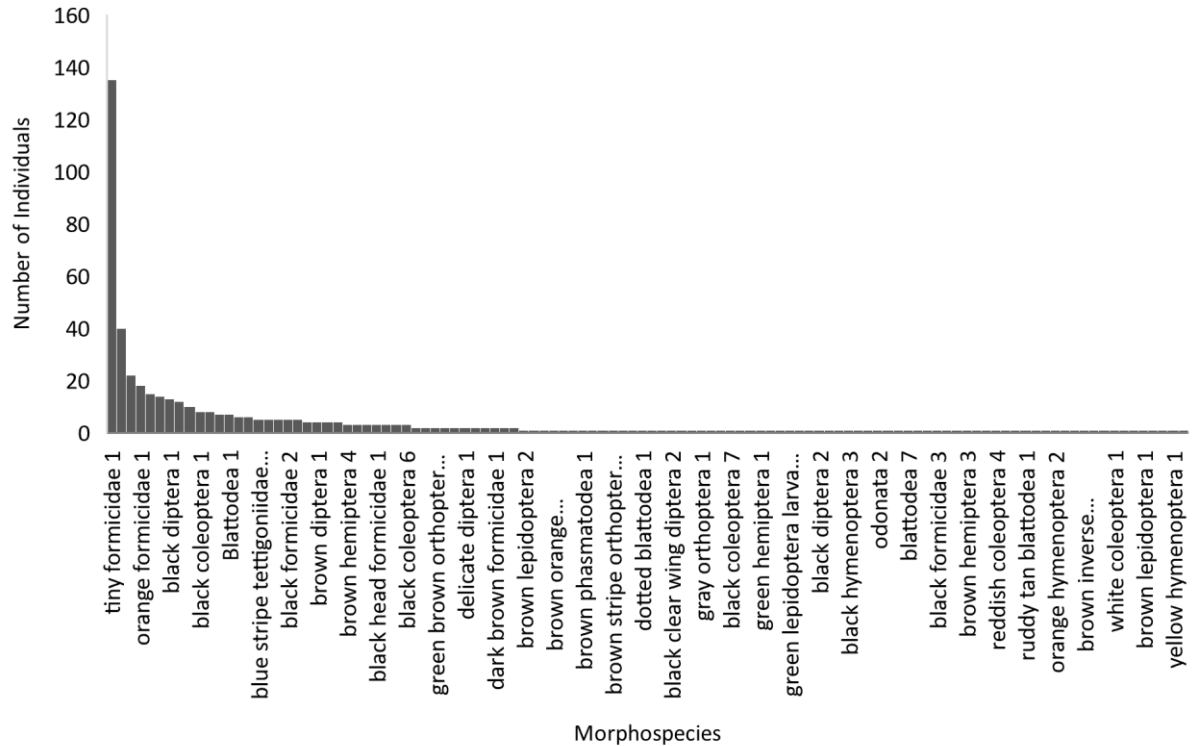
Supplementary Material

Appendix I: Number of Individuals per Morphospecies in Brachiaria spp. treatment.



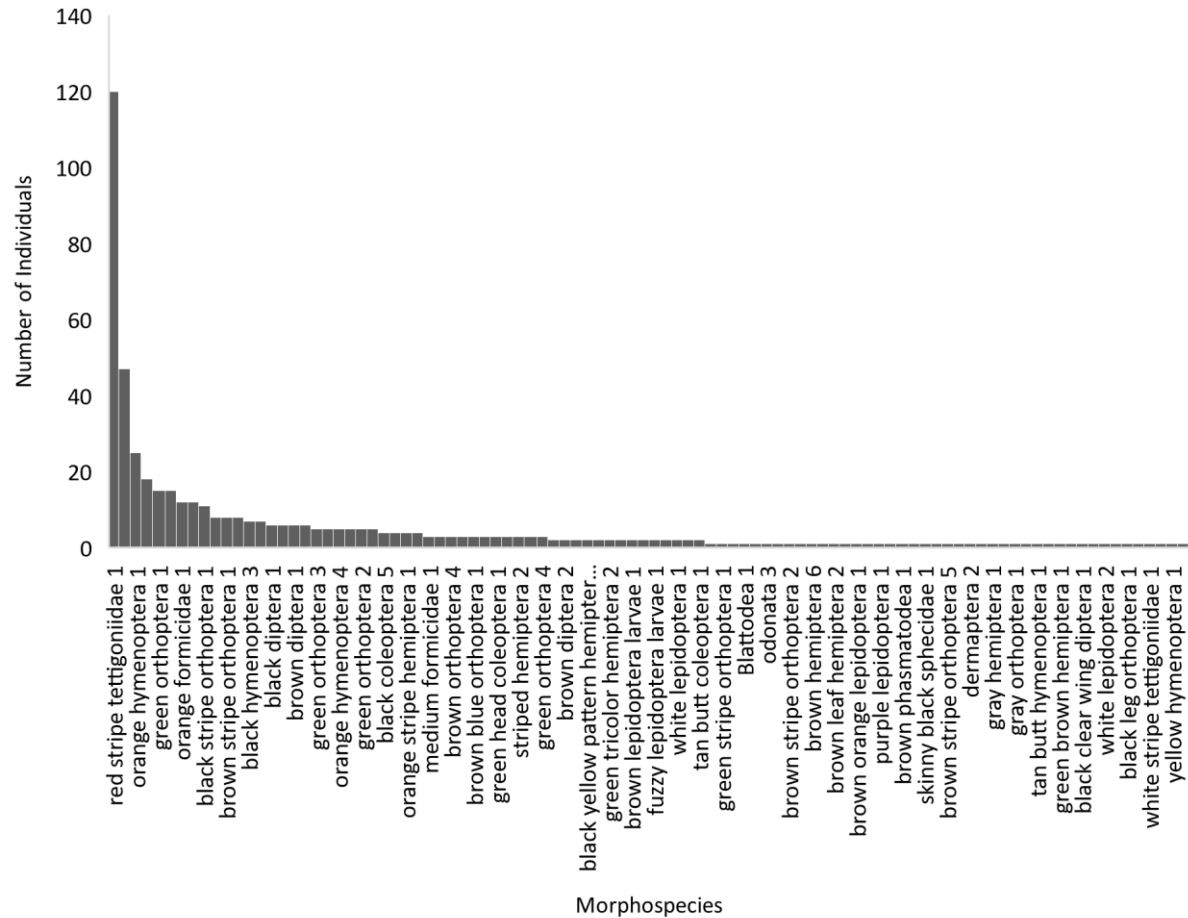
Supplementary Material

Appendix II: Number of Individuals per Morphospecies in Native Treatment.



Supplementary Material

Appendix III: Number of Individuals per Morphospecies in Kudzu Treatment.



Supplementary Material

Appendix IV: The number of different morphospecies observed across all treatments per morning and night. No significant interaction was found between time and the number of species found for any treatments. ($F_{2, 27}=0.009$, $P=0.9911$).

