STRENGTHENING AGRO-ECOSYSTEMS RESILIENCE FOR CLIMATE CHANGE ADAPTATION TO IMPROVE FOOD AND NUTRITION SECURITY (TCP/NEP/3701)

Study of the arthropods and pollinators on the mustard field in the Dang district

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Introduction

Increasing population demand for higher amount of food led to increased competition for farmland, land-use change, habitat fragmentation, changes in crop rotations and biodiversity losses (Rulli et al. 2016). The agriculture land use practices affects large number of terrestrial organisms and is the main drivers of the biodiversity loss (Habel et al 2019). The loss of the farmland biodiversity alters species interactions which lead to decline in ecosystem functions and ecosystem services (MEA 2005). The current practices of the agriculture land use change will continue to pose the threat on the biodiversity and ecosystem services (Veerkamp et al 2020). The agricultural biodiversity is critical to ensure the long term resilience which is associated with ecosystem services and functions (Tscharntke et al 2012).

Insect diversity and abundance all over the world are declining (Hallman et al 2017). For the maintenance of diverse agroecosystem, insect diversity and abundance play the important role among which arthropods are the dominant terrestrial groups (Zhang 2011). Beyond being a conservation concern, insects provide important ecosystem services. Within agriculture, biological control and pollination services provided by insect natural enemies and wild bees were estimated to be worth over $7.5 billion in the US (Losey and Vaughn 2006) and 35% of total crop production worldwide depends on pollinators (Klein et al 2007). The loss of the natural habitat and the agricultural intensification causes the decreases in the agroecosystem services such as biological control of pest and pollinators and reductions of the foraging and nesting habitat (Biesmeijer et al 2006; Potts et al 2010; Garibaldi et al 2016).

Mustard **(***Brassica campestris* Var. toria**)** is a mass flowering oilseed crop belongs to the family Brassicaceae (or Cruciferae) with the total area under cultivation in Nepal was 260,307 ha with the production of 280,530 mt with the average productivity of 1,078 kg/ha (MOAD, 2018). Various types of arthropods such as insect and spider species are found in the mustard field as they get shelter, food and oviposition site. Many of the insects and spiders have profound effect as pest, predator or pollinator and some of them just prevail in the field. Pollinators aid in pollination as well as fruit setting (Devkota et al 2021). Beneficial insects like predators, parasitoids and other arthropods maintain an ecological equilibrium. With the aims to identify the major pollinators and

arthropods in the mustard crops, the research was carried out in the three research areas of the Dang district, Nepal.

Methodology

Study location

Fieldwork was carried out in 2019 on the mass flowering crop mustard (*Brassica campestris* var. toria: Brassicaceae) in the Dang district, Nepal. We selected commercially growing areas of mustard crop in the months of December-January during the blossom period in three sites in dang: Saunepani, Bagmare and Ragaicha. Since we sampled in three localities × three repetition within mustard crops, we obtained 12 sampling units. At each sites described above, we established a quadrat of 1,250 m^2 (50 m × 25 m) for sampling flower-visiting insects following a protocol to detect and assess pollination deficits in crops as suggested by the Food and Agriculture Organization of the United Nations (Vaissière et al. 2011).

Insect sampling

Sampling began when more than 10% of the mustard crop began to bloom. In each quadrat, we sampled all insects visiting the mustard crops. The pan traps methods is widely used in the survey of the pollinators in the different plant and crops (Westphal et al 2003; Grundel et al 2010). We arranged pan traps in a sequential along the 150 m^2 transect on each oilseed and buckwheat crops in the study area. Pan traps were arranged along a transect in a linear manner, containing 3 pan traps of yellow, blue and white color being randomly placed in each transect of 25 m, spaced a minimum of 5 m apart (Droege 2010). Altogether six groups consisting 18 pan traps, of three colors yellow, blue and white were adjusted in the study area comprising six color of each in the six transect of study area as protocol suggested by the Canadian pollinator initiative. We filled each pan trap with soapy water to break the water tension placed on the ground itself as the broader scope of research to investigate the pollinator's diversity in the flooded and non-flooded areas (Abrahamczyk et al. 2010), remained exposed for 24 hrs per sampling (Westphal et al 2008). Consequently, to quantify how many insects visited the target crop, we measured by scan sampling a fixed number of open floral units in each experimental unit. The five hundred flowers or flowering units of mustard crops were assessed by scan sampling, as there was no duration attached to the observations; rather, an insect will be recorded or not depending on whether it is present at

the time a given flower is first seen, which is the most reliable way to assess the abundance of insects that reside on flowers (Levin et al. 1968; Westphal et al. 2008). The scan sampling was performed by walking slowly along each transect line and recording the numbers of flower-visiting insects seen when looking at the individual floral units one by one in sequence (Vaissière et al. 2011). Insects captured with the aerial nets were pinned, labelled and subsequently identified to the genus and species level in the entomology laboratory of the Agriculture and Forestry University in Nepal.

Predators and Parasites sampling

A total of the four quadrant of 1*1 m^2 was established within the 50m * 25m areas plot on each sites to assess the study of the predators and parasites on the mustard crops in the three research sites. The study was carried in weekly internal after the onset of the flower in the mustard. The major predators and parasites namely, lady bird beetle, ground beetle, syrphid flies, tiger beetle, tachinid flies, spider, mustard sawfly, ants, wasps, earthworm and painted bug was assessed.

Data analysis

Average number of pollinators and arthropods

The average number of pollinators and arthropods registered in each research site was plotted, here, as the mean number of pollinators and arthropods using as a measure of dispersion the confidence intervals at 95%. This approach is an accurate method to infer statistical significance once different intervals do not overlapping each other (Sim and Reid, 1999).

Biodiversity analysis

The number of unobserved species was estimated using the function (Chao *et al.* 2014) using the 'specpool' function of the *vegan* package (Oksanen *et al.* 2018). Afterward, we evaluated whether our sampling effort was enough to show the pollinator and arthropod richness. As such, we performed a rarefaction curve of the pollinator and arthropod species by calculating interpolation/extrapolation according to the number of individuals sampled.

On the other hand, we performed a diversity profile with a diversity order of Hill's series (Hill 1973). The diversity profile allows us to dynamically compare the diversity of any organism between different communities along the parameters at the scale (Rényi's diversity) (Chao *et al.* 2014). Therefore, if all the values in such scale were the greatest in a specific community, it can

be considered the most diverse among all other analysed (Tóthmérész 1995). Such an analysis was carried out by splitting the diversity matrix from each research site and posteriorly using the 'renyi' function (hill=TRUE) of the *vegan* package (Oksanen *et al.* 2018). Yet, sampling effort was estimated, and plotted, by rarefying and extrapolating samples along each research site using the *iNEXT* and *ggiNEXT* functions from homonym package (Hsieh *et al.* 2018).

To investigate the community composition of pollinators among three research sites (Bagmare, Ragaicha, Saunepani) we performed a non-metric multidimensional scaling (NMDS) analysis. As a result, our dataset was standardized by the 'rank' method. It implies in replacing the abundance values for their respective relative ranking and leaves zero values unaltered. Next, this dataset was converted into a dissimilarity matrix with Gower's method. This method divides all distances by the number of observations (rows) and scales each column to the reach unit. In this way. Therefore, the NMDS was carried out using the 'metaMDS' function of the *vegan* package, which the *stress* value was used to evaluate the ordination quality. Posteriori, the composition of the pollinator and arthropod community was tested with an ANOVA with permutations (PERMANOVA, 1,999 randomizations) controlling for sample location with the 'adonis' function of the *RVAideMemoire* package (Hervé 2020).

Finally, we performed a hierarchical clustering applying the unweighted pair group method with arithmetic mean (UPGMA), the same as average linkage, using the Euclidean method as a measure distance (rows, columns). UPGMA captures the average distance between each point in one cluster to every point in the other cluster. This data exploring was plotted as clustered heatmaps using the 'pheatmap' package (Kolde 2019). Since numbers of sampled organisms in each sampling unit drastically differ among them, such values were centered and scaled in the row direction. Posteriori, we used the 'cophenetic' and 'cor' functions from the *stats* R package to carry out the cophenetic correlation index, a way to evaluate the level of goodness-of-fit of the resulting dendrograms. All analyses were performed in R-statics (Ihaka & Gentleman 1996; R Core Team 2020).

RESULTS

Overall, we collected 427 specimens of pollinators being identified 17 pollinator taxa in our work. However, our findings suggest that the expected number of pollinator taxa should be something like 29.45 (± 28 s.d.). Three pollinator taxa showed upmost means: *Megachile* spp., *Halictus* spp.,

and *Andrena* spp. (Figure 1A). The diversity profile indicates that Ragaicha site had a highest richness (Figure 1B). Nevertheless, the pollinator community on such locality is smaller as we go to right side of scale when the abundance of organisms possesses more importance (Figure 1B). However, our rarefaction curves indicates that in Ragaicha and Saunepani sites exhibited similar behavior in diversity profile, most likely have more pollinator taxa to be observed if the number of individuals was higher than that sampled here (Figure 1C). Yet, the NMDS had a stress value of 0.06, suggesting an enough ordination fitting. However, even though three research sites seem to present different pollinator community, they somewhat overlap each other and are near to origin [0,0]. As such, our PERMANOVA test showed that, overall, they are similar in three research sites (Table 1). It can be an artifact since, at least, Ragaicha and Saunepani should have more (exclusive) pollinator taxa to be found (Figure 1C).

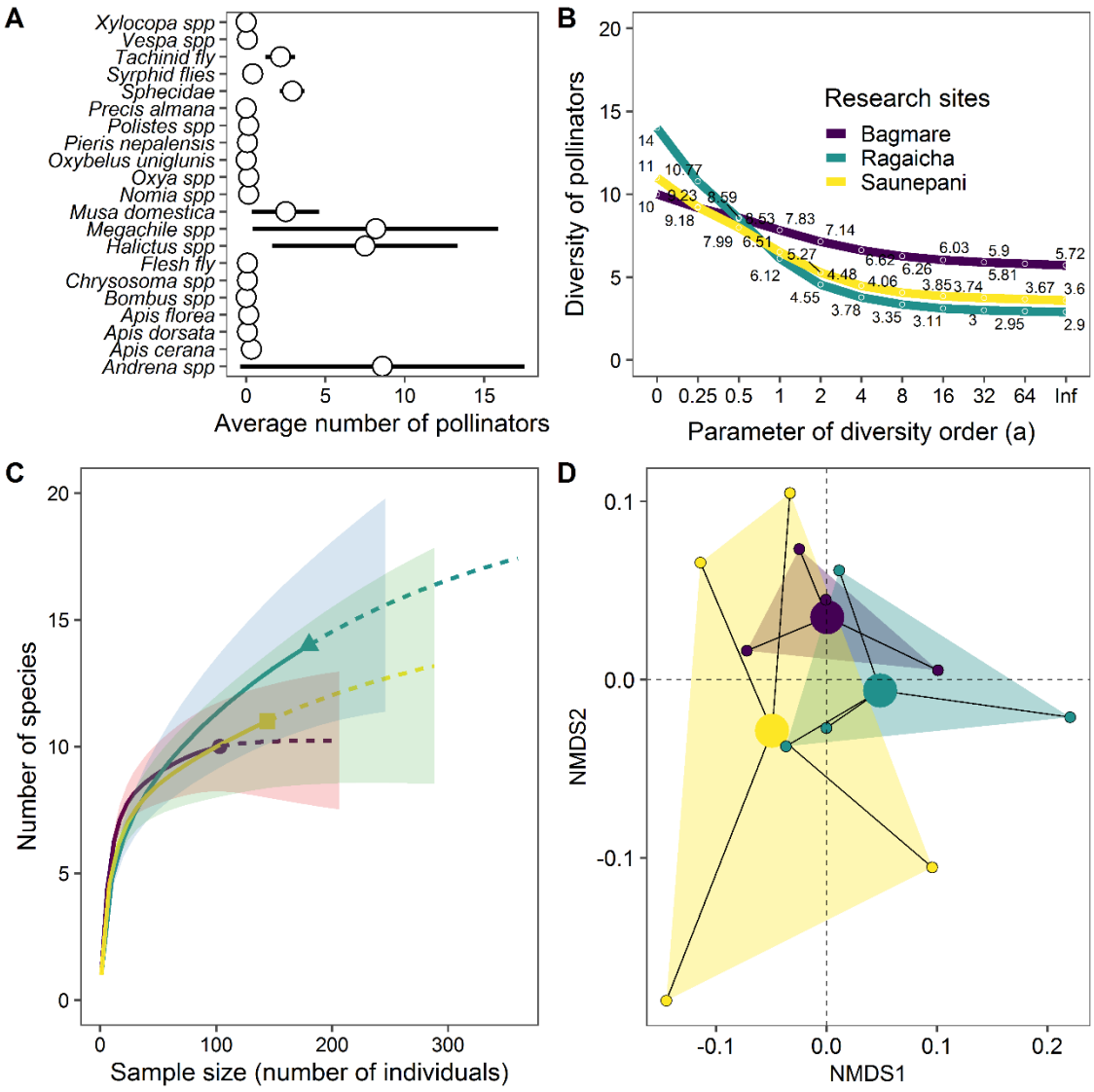

Figure 1 Pollinator diversity (A) Average number of pollinators by joying all three research sites (Bagmare, Ragaicha, Saunepani). The points indicate the mean, while horizontal black lines exhibit the confidence intervals at 95%. **(B)** Diversity profile of the Hill´s series. The *x*-axis displays the change in diversity over the parameter scale; the *y*-axis shows the level of diversity for measures on the *x*-axis. The left-hand side of the *x*-axis is more sensitive to rare species, whereas the right-hand side is more sensitive to the abundance of pollinator taxa. This continuum of values allows inferring the contributions of rare vs. abundant species in a community. Inferred diversity indices can be retrieved from such a parameter scale as: 0 = species richness; 1 = Shannon-Wiener index; 2 = Simpson index; inf = Berger-Parker index. **(C)** Rarefaction curves of pollinator taxa: *x*-axis – number of individuals per sampling unit; *y*-axis – interpolation (solid line) and extrapolation (dashed line) estimating the

expected number of putative taxa to be seen; the shadow indicates the confidence intervals (95%); **(D)** Non-metric multidimensional scaling (stress = 0.06) showing the community composition of pollinators. In D, the larger points mean the centroids, while the smaller points represent the sampling units. Note: Colors in B-D panels should be interpreted as seen in legend of the B panel.

Table 1. PERMANOVA parameters and subsequent paired comparison of the composition of the pollinator community associated with research sites in Nepal.

	Degrees of freedom	**Sum of squares**	**Mean squares**	**F**	R^2	***p*-value**
Research sites	2	0.06	0.03	0.89	0.16	0.68
Residuals	9	0.30	0.03		0.83	
Total	11	0.37			1.00	

In predators and parasites study, we collected 299 specimens of arthropods in which seven groups were identified. It appears to be an enough quantity since the expected number of was equal to seven without measure of dispersion. The two more abundant groups of arthropods were Ladybird beetles followed by Mustard saw flies (Figure 2A). The diversity profile indicates the three research sites Bagmare, Ragaicha and Saunepani showed as similar arthropod community regardless of rare (left side) or abundance (right side) of organisms receive more or less importance (Figure 2B). In the rarefaction curves, it is possible to infer that all three sites had a similar behavior displaying an enough richness when less than 50 specimens are sampled (Figure 2C). The stress value of such NMDS was of 0.15, also indicating an enough ordination fitting. Moreover, and corroborating the Figures 2B-C, the arthropod community among three research sites were sufficiently overlapped each other and are again near to origin [0,0] (Figure 2D). Consequently, the PERMANOVA test showed that there is no statistical difference among all three localities concerning to arthropod fauna (Table 2).

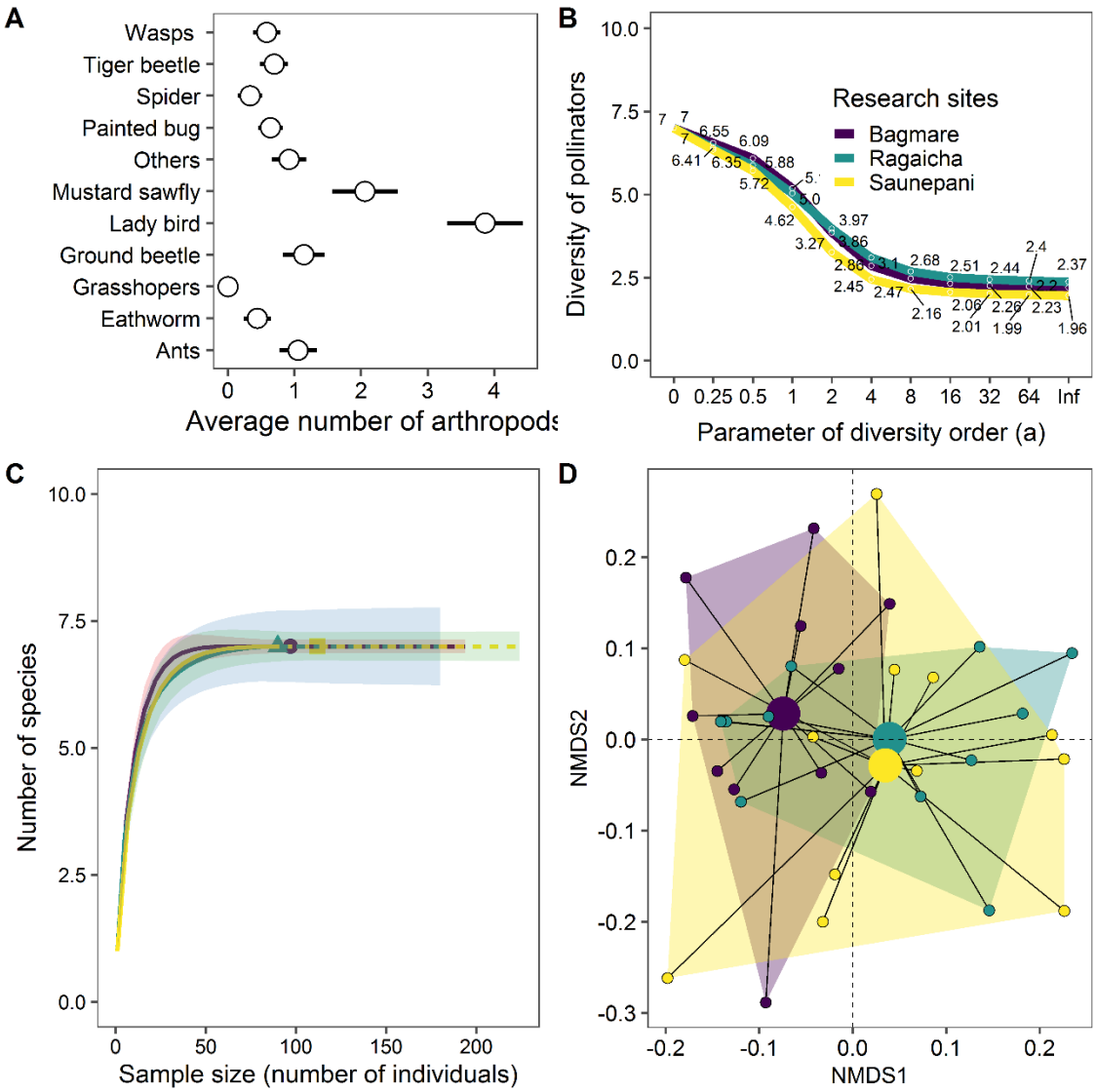

Figure 1 Comparison of trophic niche width (Shannon-Wiener index, H′) between non-eusocial and eusocial bees after phylogenetic generalized least squares (PGLS). Note: white points indicates the mean (H´ eusocial = 2.54, H´ non-eusocial = 1.37); vertical black lines show the confidence intervals (95%); horizontal red dashed line exhibits the overall mean (H´ = 2.09) combining all bee taxa. (A) Rarefaction curves of mite species: *x*-axis – number of individuals per sampling unit; *y*-axis – interpolation (before value) and extrapolation (after value) of the Hill number with order q = 0 exhibiting the species richness found and the number of expected species against the average. The shadow indicates the confidence intervals (95%); (B) Diversity profile of the Hill numbers. The *x*-axis shows the change in diversity indices over the Hill numbers; the *y*-axis displays the level of diversity for measures on the *x*-axis. The left-hand side of the *x*-axis is more sensitive to rare species, whereas

the right-hand side is more sensitive to the abundance of insect taxa. This continuum of values allows inferring the contributions of rare vs. abundant species in a community. Some diversity indices on the *x*-axis can be inferred: 0 = species richness; 1 = Shannon-Wiener index; 2 = Simpson index; inf = Berger-Parker index; (C) Number of mites sampled according to stingless bee species. Note: The total number of mites was transformed into the square root to facilitate visualization. The approximate value in the x-axis can be retrieved with the square power. (D) Non-metric multidimensional scaling (stress = 0.05): Community composition of mites sampled according to stingless bee species. Note: Larger points are the centroids; smaller points are the sampling units. Colours in all panels represent the stingless bee host (see legend in B). The full species names are shown in Table 1.

Table 2. PERMANOVA parameters and subsequent paired comparison of the composition of the arthropod community associated with research sites in Nepal.

	Degrees of freedom	**Sum of squares**	**Mean squares**	**F**	R^2	***p*-value**
Research sites	2	0.09	0.04	1.19	0.06	0.27
Residuals	33	1.36	0.04		0.93	
Total	35	1.45			1.00	

The hierarchical clusters of pollinator and arthropod communities mirror the diversity analyses described above (overlapping, no community difference) since sampling units from different research sites often grouped together or nearer each other than all sampling units belonging to same and concerning research sites (Figure 3 A-B).

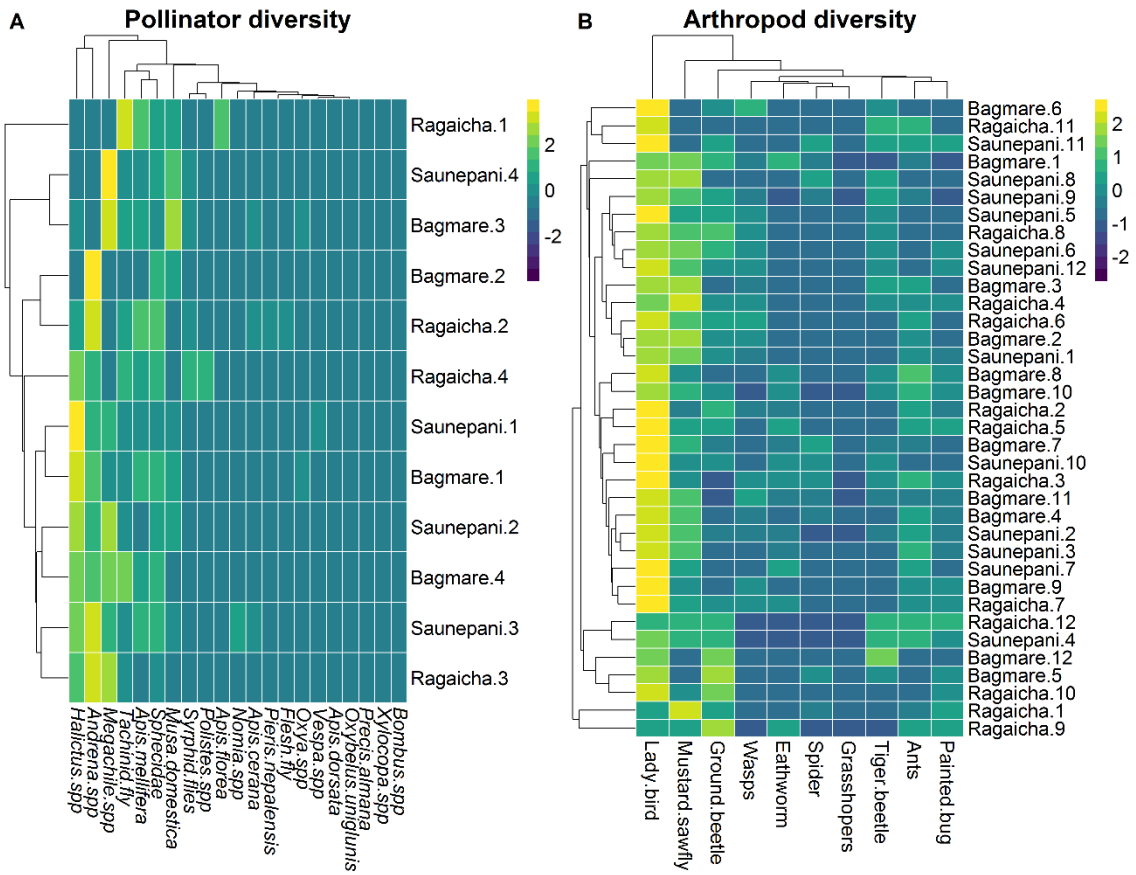

Figure 3 – Clustered heatmaps based on fauna of pollinator and arthropod communities along sampling units from three research sites (Bagmare, Ragaicha, Saunepani). Such a hierarchical cluster analysis was carried out after the UPGMA method and Euclidean as a distance measure. The coefficients of cophenetic correlation were 0.98 (pollinator) and 0.70 (arthropod), respectively. Scale color indicates the lower (purple), medium (blue) and higher (yellow) values of sampled specimens.

References

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., *et al.* (2014). Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecol. Monogr.*, 84.

Hervé, M. (2020). RVAideMemoire: Diverse basic statistical and graphical functions.

Hill, M.O. (1973). Diversity and evenness: A unifying notation and its consequences. *Ecology*, 54, 427–432.

Hsieh, T.C., Ma, K.H. & Chao, A. (2018). iNEXT: iNterpolation and EXTrapolation for species

diversity.

Ihaka, R. & Gentleman, R. (1996). R: a language for data analysis and graphics. *J. Comput. Graph. Stat.*, 5, 299–314.

Klein, A.; Vaissie, B.E.; Cane, J.H.; Steffan-dewenter, I.; Cunningham, S.A.; Kremen, C.; Tscharntke, T. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B* **2007**, *274*, 303–313.

Kolde, R. (2019). pheatmap: Pretty heatmaps.

Losey, J.E.; Vaughn, M. The economic value of ecological services provided by insects. *Bioscience* **2006**, *56*, 311–323

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., *et al.* (2018). vegan: Community Ecology Package.

R Core Team. (2020). *R: A language and environment for statistical computing*. The R Foundation for Statistical Computing, Vienna, Austria.

M.C. Rulli, D. Bellomi, A. Cazzoli, G de Carolis, P. D'Odorico, The water-land-food nexus of first-generation biofuels, Sci. Rep. 6 (2016) 22521.

Tóthmérész, B. (1995). Comparison of Different Methods for Diversity Ordering. *J. Veg. Sci.*, 6, 283–290.