Comparing invertebrate sampling techniques in Mill Road Cemetery

Introduction

The importance of invertebrates in ecosystem processes puts their effective surveying in the frontlines of research. Biological inventories are needed in order to focus conservation efforts adequately (Barnosky et al. 2011) and understand the effects of urbanization (Faeth et al. 2011) on invertebrate populations. The study site was Mill Road Cemetery, Cambridge (Figure 1, 2) which is a neutral-calcareous grassland area and forms a mosaic heterogeneous habitat. Cemeteries are proven to be high biodiversity (Gilbert 1991, Laske 1994) patch areas within the urban matrix. Scientists have been sampling invertebrates for a centuries and therefore, there is a great variety of techniques known. They can be grouped in two ways; (1) it can be either qualitative or quantitative or (2) passive or active. In this study, four sampling methods (pitfall, sweep netting, tree beating and suction) were used to collect invertebrates. The null hypothesises were the following: (i) there is no difference between the sampling techniques and sample sizes; and (ii) there is no difference between sampling techniques and species composition. The ecological role of each taxa, environmental factors and the vertical stratification of invertebrates is also discussed.

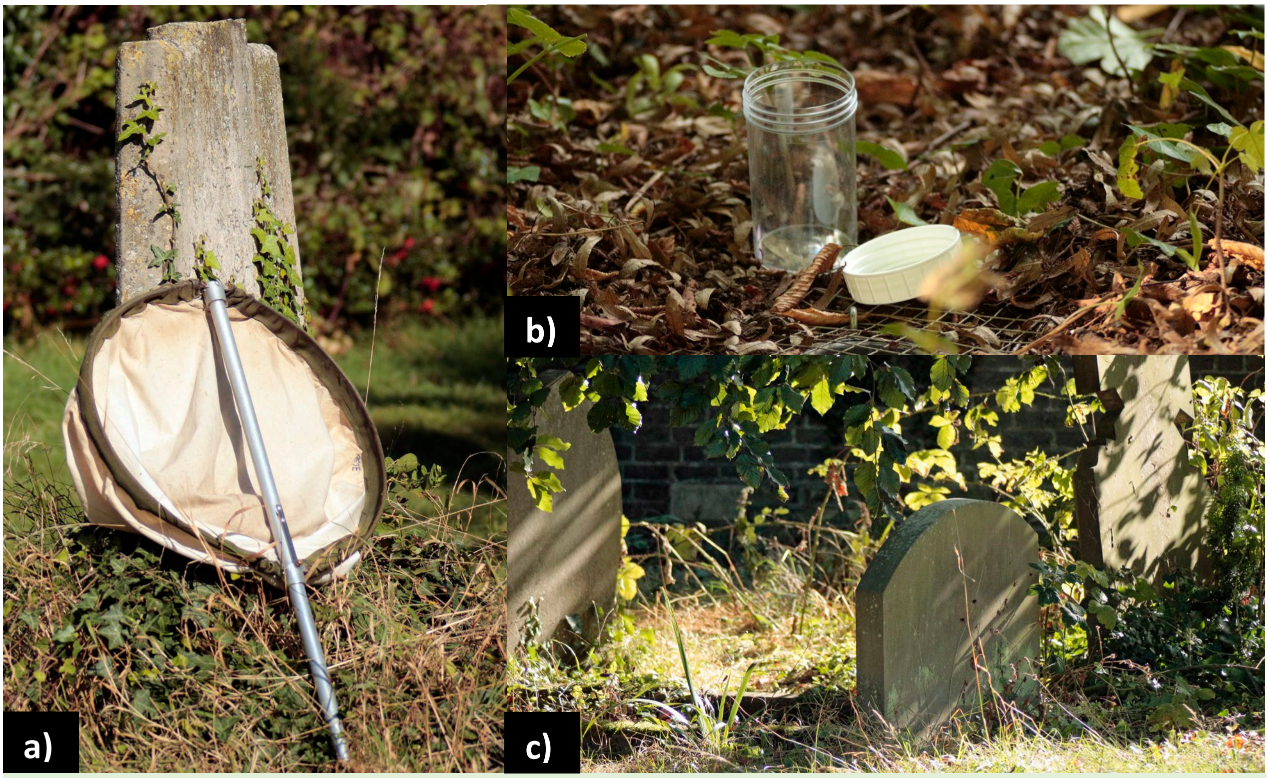


Figure 1. Pictures of the different areas of Mill Road Cemetery, Cambridge. Amenity grass is kept at short length while False-oats are not cut back surrounding the graves (a, c). Pitfall trapping (b) was set up in the northern part of the cemetery which is only cut once or twice a year (Cambridge City Wildlife Site Survey 2005). Suction sampling, tree beating and sweep netting (a) was carried out in the regularly mowed area of the cemetery.

Study Site

Mill Road Cemetery is 168 years old and is under two different management regimes. The main circular path is regularly mowed and is dominated by *Trifolium* spp., *Arrhenatherum elatius* and *Pheleum pratens*. The north end of the cemetery is only cut a few times per year. The density of trees (*Acer pseudoplatanus, Acer platanoides, Sambucus nigra*) and shrubs (*Rubus fructicosus* agg.) are higher in this area.

SITE NAME: Mill Road Cemetery
Site code: G5.1
Grid ref: TL461582
Date of survey: 5/10/2016

Habitat information

Code	Habitat type
A33	Parkland, scattered trees; mixed
B22	Grassland: neutral, semi-improved
B32	Grassland: calcareous, semi-improved
J25	Boundaries, wall

Figure 2. Phase 1 survey description of study site.

Methods

Four sampling methods were used to sample the cemetery. Pitfall trapping (Figure 2, 3) consisted of 27 plastic cups in the ground containing washing up solution. Its opening was on the ground level with a wire net on top for 3 days. Sweep netting and tree beating was carried out on the regularly managed area of the cemetery (location B). Insects were collected by pooting from the surface of the equipment. All samples were collected by two students but exact number of pairs is unknown. Samples were kept in ethanol solution. Suction sampling was carried out by an engine powered insect suction sampler (location C).

Figure 2. Map of the cemetery and its surroundings using Phase 1 habitat survey (original map by The Wildlife Trust for the Cambridge City Wildlife Site Survey 2005). The cemetery is a mosaic of different habitats (described on the right). Capital letters indicate the sampling areas.

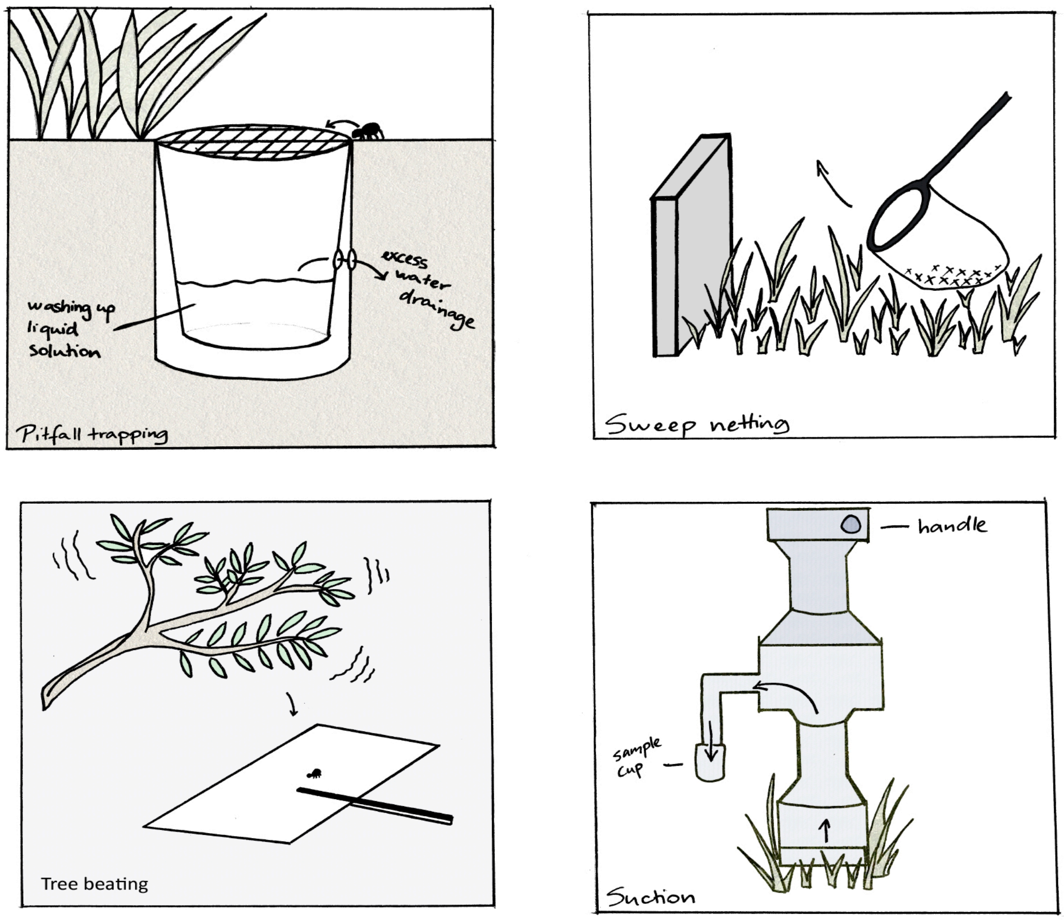


Figure 3. Four sampling methods used in this survey (illustrated by author).

Results

The total number of 860 individuals were identified to order level using compound microscopes. Suction samples were incomplete so the data was omitted (Appendix 1). Six orders contained very few individuals (Figure 4) which were also discarded. Therefore eight orders were used for the statistical analysis (Appendix 2). The three most abundant orders were the Coleoptera (n=221), Araneae (n=174) and the Diptera (n=156). For testing independence of observed frequencies, two way Chi square test was used for the nonparametric data. The test showed a highly significant difference between the sampling methods (X^2_3=156.630, N=742, $p < 0.05$) and their sample sizes (Figure 5).

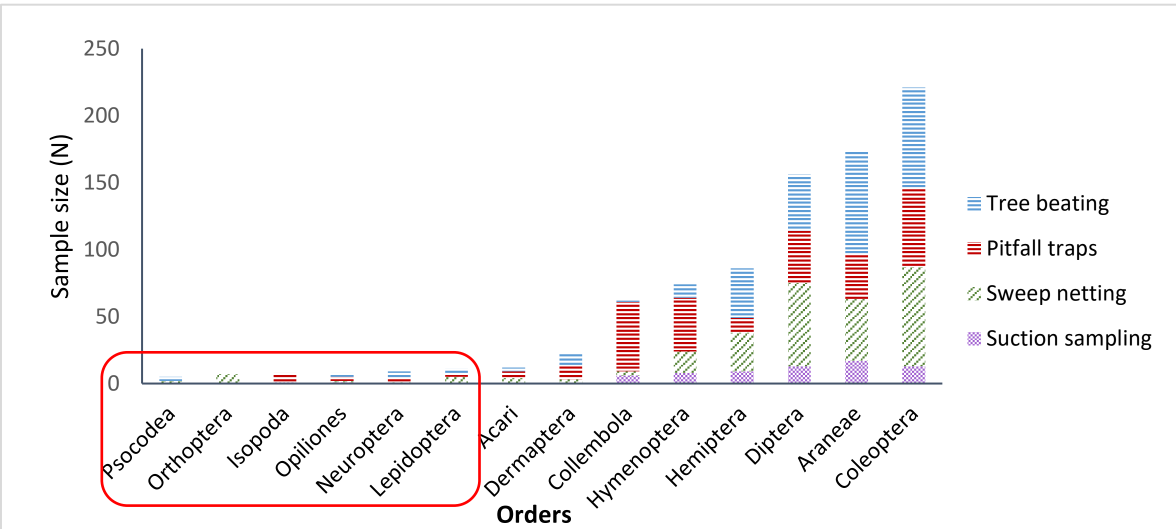


Figure 4. All samples collected in an ascending order. Marked orders had very little sample sizes and were discarded.

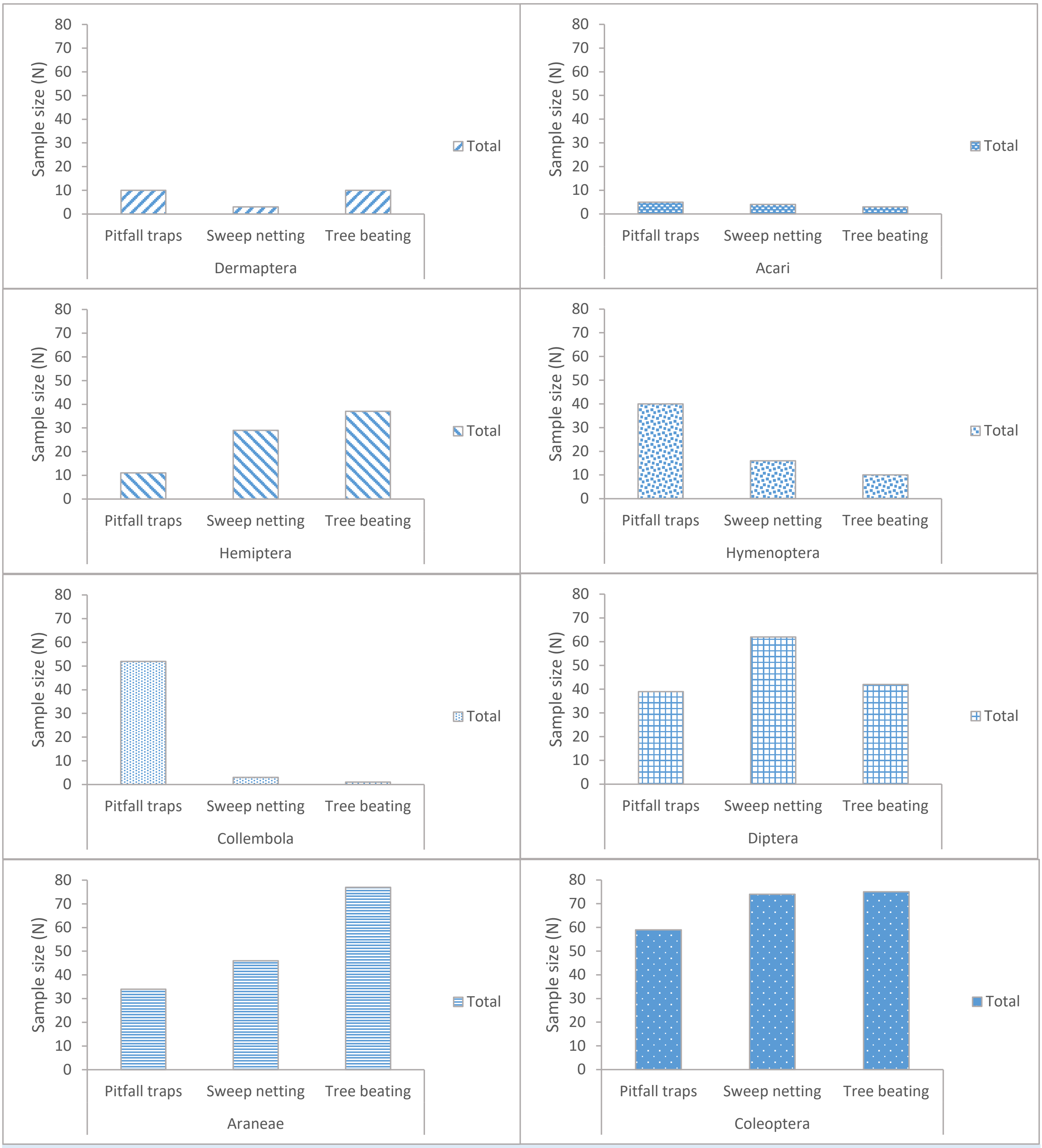
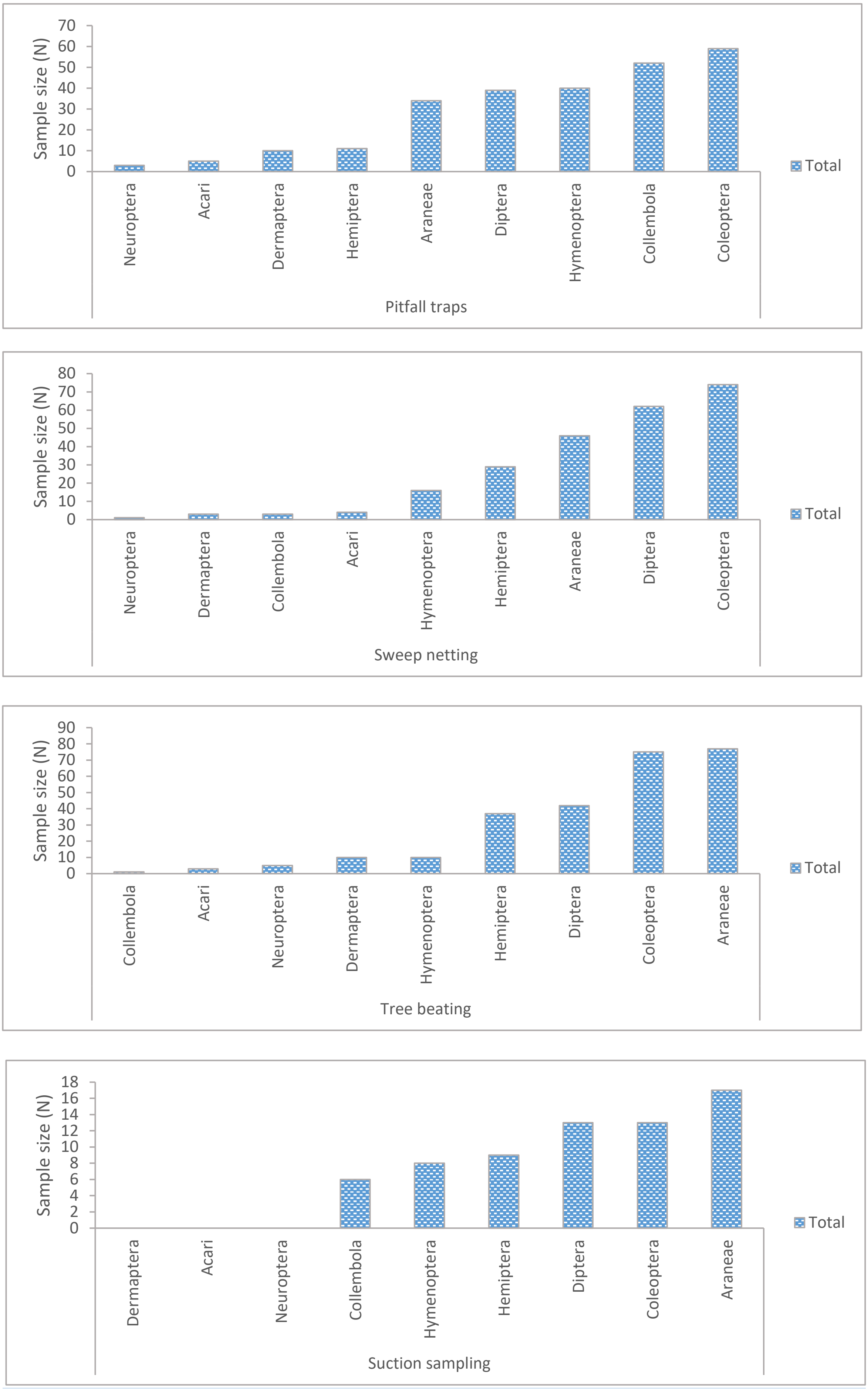


Figure 5. Sampling methods and sample sizes show significant difference within and between orders. The most significant differences are between the pitfall traps and the tree beating samples (Hemiptera, Hymenoptera, Collembola, Araneae). The difference can be explained by describing the different taxa's feeding behaviour, their habitats, dispersal strategies and development.



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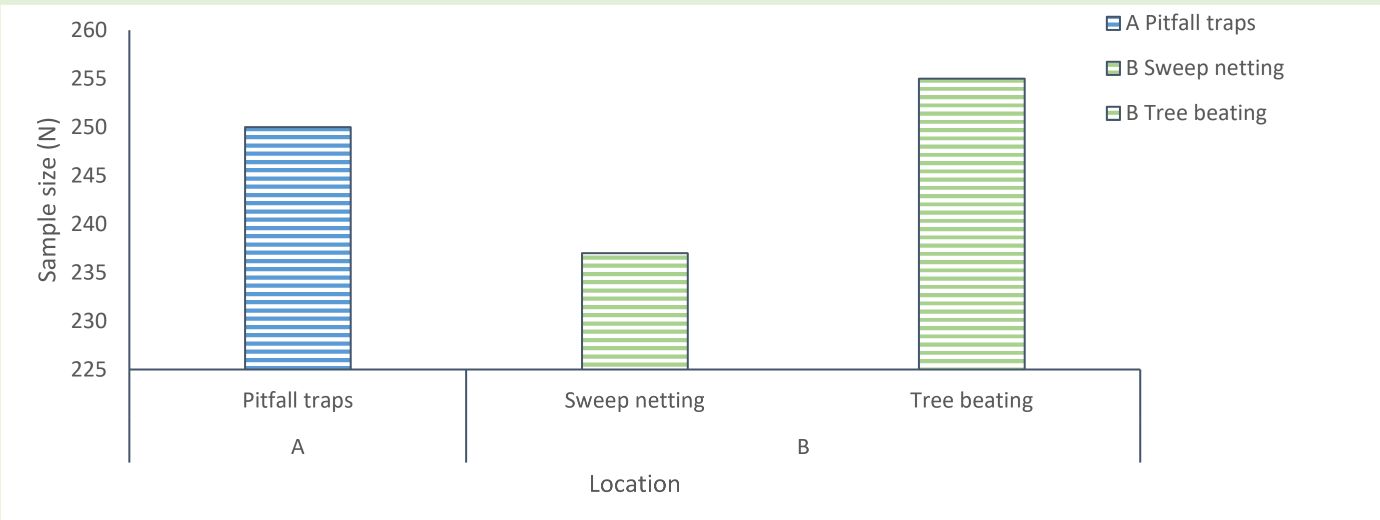
Discussion – Taxa distribution

The significant differences between sampling methods and sample composition shows that different invertebrate taxa occur in different (a) habitats and (b) on different vertical levels. **Coleoptera**, which is the largest of all insect orders, was the most abundant for pitfall trapping and sweep netting. Although beetles can fly, they mostly occupy the ground and the low vegetation which explains why their numbers greatly decreased when collected by tree beating. They have biting mouthparts and a tough elytra for physical protection which makes them a really successful species. Plant-feeders, wood-borers, scavengers, predators and parasites. Dung beetles and burying beetles are crucial links in the nitrogen cycle. Studies focusing on beetles usually use pitfall trapping for sampling (Thomas and Sleeper 1997). **Hemiptera**, compromised of Homoptera and Heteroptera suborders, is associated with roots, basal and aerial parts of vegetation which is consistent with our data, since we had more individuals from tree beating and sweep netting, than pitfall trapping. They are important agricultural pests, disease transmitters (Valente et al. 1998); mostly sap-suckers but rarely predators (Cohen 1990). 1650 species occur in the UK. **Collembola** has a world-wide distribution but they are susceptible to drying and therefore they are mostly found in soil or leaf litter. They have biting-mouthparts and their feeding varies from feeding on algae, pollen to decaying matter and nematodes (Brown 1954). Collembola the second most abundant taxa in the pitfall traps. As the traps were mostly located particularly underneath *Acer pseudoplatanus* and close to *Rubus fructicosus* the vegetation provided shade and a damp environment; ideal for springtails. Immature stages of **Diptera** can be highly abundant in the soil which explains the high number of captures in the pitfall traps. **Araneae** are mostly carnivorous (a few are phytophagous, Krantz and Lindquist, 1979), web-building species show pronounced vertical stratification with correlation to the vegetation (Duffey 1962, Enders 1974). This explains the lower numbers of pitfall trapping result compared to the tree beating (and suction) sampling method. Both Araneae and **Acari** belong to the class Arachnida. Their morphological similarities might have made it more difficult for the students to identify them correctly and that is why the Acari numbers are surprisingly low. **Hymenoptera,** containing over 100,000 known species, has a great variety of roles within ecosystems.

They carry out organic matter decomposition, soil fixation, predation, parasitism, herbivory, disease transmission and pollination.

Comparison between our sampling and the two management regimes (regularly mowed *versus* only cut once or twice per year, Figure 6) could have provided data for diversity measurement between the two areas. Because of non-uniform sampling, it cannot be tested statistically but can be hypothesized that assessing the ß-diversity between the two habitats might show difference in species richness and composition.

Our sampling methods can be compared used to compare vertical heterogeneity as opposed to horizontal one. There are numerous techniques to sample different habitats (Table 1). Many studies focus on the comparison of the effectiveness of different sampling methods used for different taxa (Corti et al. 2013, Basset et al. 1997, Hurley et al. 2015, Bouget et al. 2009, Alonso and Camargo 2010, Doxon et al. 2013). In order to model an ecosystem it is crucial to have uniform sampling effort, be qualitative and also quantitative. However, when considering the different lifestyles of animals, it is impractical to use only one method as our study has proven.



Pitfall trapping (A)		**Sweep netting and tree beating (B)**	
Habitat type		Habitat type	
A2.2	Scattered scrub	B6	Poor semi-improved grassland
A1.3.1	Mixed woodland – semi-natural	J1.2	Cultivated/ disturbed land – amenity grassland
J1.2	Cultivated/ disturbed land – amenity grassland		

Figure 6. Sampling was carried out in two different habitats (Figure 2) which will affect the invertebrate composition. Statistically cannot be compared because of non-uniform sampling effort (27 pitfall traps *versus* unknown number of individual samples of sweep netting and tree beating).

There is much debate about the significant result as only qualitative sampling methods were used for the statistical analysis. The distribution of sampling is another major issue when comparing the samples. Sweep netting and tree beating was not clearly defined, students also sampled shrubs. The lack of surveying plant coverage and composition is also decreasing the effectiveness of this survey. The mishandling the suction samples led to omitting all the data and reduced the discussion of four methods to three.

Table 1. Different habitats require different methods. Even within one habitat several complementary methods will be able to assess relatively precisely the invertebrate composition. Adapted from Samways et al. 2010.

Target habitat	Methods available
Forest canopy	Direct access via cranes, platforms, ropes Fogging/ misting using insecticide Direct foliage samples
Low canopy, shrubs	Sweep netting Beating Selective individual collection
Low vegetation	Sweep netting Suction sampling Direct examination
Ground/ surface	Pitfall Soil and litter sifting Tullgren-Berlese funnel extraction Winkler bag extraction
Flying insects	Malaise traps Window/ interception traps Suction traps Light traps Pheromone/bait traps Pan traps/water traps
Aquatic insects	Waterside vegetation Dip netting Surber sampling Benthic grabs

Discussion – Vertical distribution

The significant difference between the three sampling methods shows that for comprehensive invertebrate survey requires several sampling techniques, not just one (Table 1). Horizontal heterogeneity can be surveyed by a species-area model which requires precise distance data. The complexity of vertical heterogeneity depends on habitat structure (desert system could be less complex than a rainforest). As the vegetation strata changes, so does the arthropod assemblages (Smith 1973). Ground-to-canopy transects are crucial in order to produce eloquent data set for habitat modelling. As there is such a great variety of sampling methods, one must consider first of all the ecological question of the study and consider both (a) long-term and (b) short-term variations between the vertical stratas. A long-term example is the change of distribution throughout invertebrate development stages. Short-term example is the arthropod diel activity studied in rainforests (Basset et al. 2001, Figure 7) by using tree beating, flight-interception traps and sticky traps. Considering short-term variation, environmental variables (EV, Figure 8.) will have an effect on invertebrates which can also be (i) long-term or (ii) short-term. Short-term EV might be solar radiation, fluctuation of relative humidity, air temperature, wind speed, water condensation, canopy openness (Barker 1996, Parker 1996). Invertebrate assemblages will also differ among guilds (e.g. scavengers, deadwood eaters, herbivores, predators, pollinators, parasitoids, fungal feeders etc) (Basset 2003). Most importantly, resource quality and quantity has a strong influence on trophic structures (Price 1992) which leads to vertical strata based on different feeding guilds (such as more herbivores and their predators in the canopy and more decomposers, fungivores in the ground layer, Figure 9).

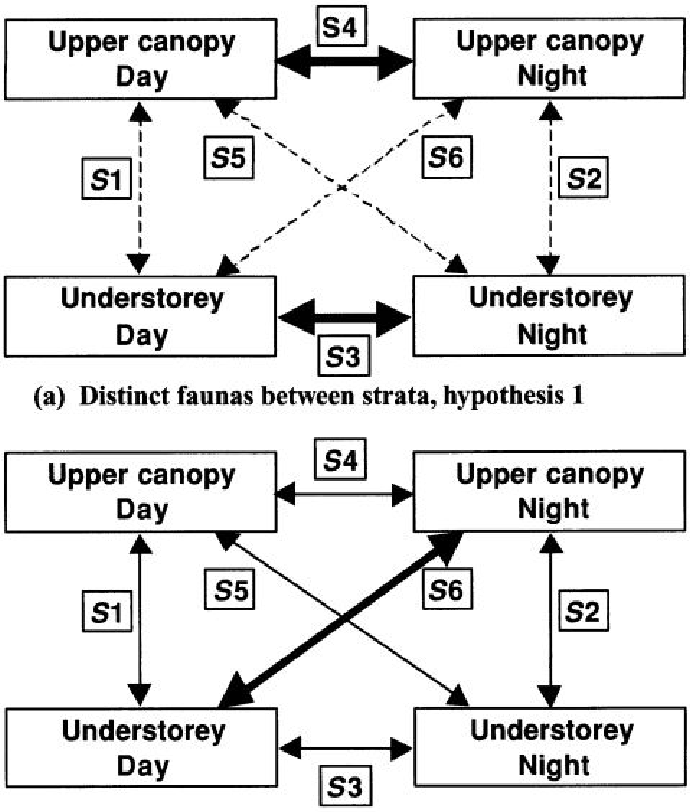


Figure 7. Hypothesis by Basset et al. (2001) of faunal correlation with invertebrate diel activity in a rainforest. S1 to S6 are coefficients of similarity between time of the day and fauna. Their data identified significant differences between day and night abundances.

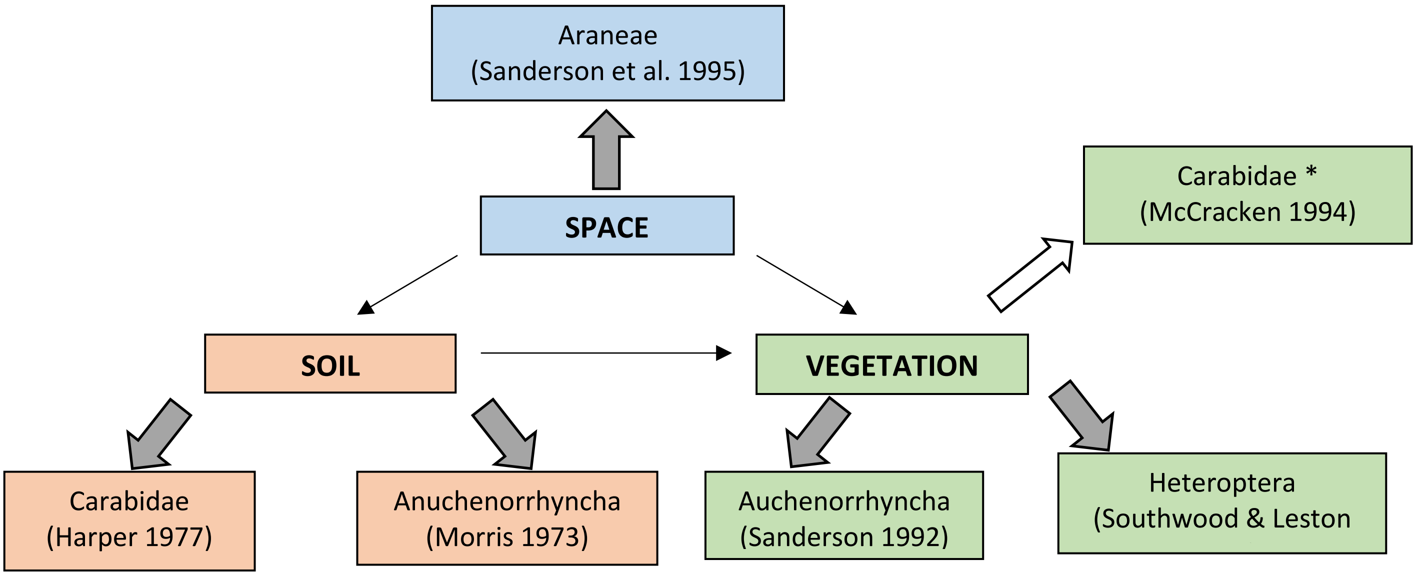


Figure 8. Environmental variables affecting different invertebrate taxa. Kowarik et al. (2016) found that plant α-diversity negatively relates to increasing tree layer and presence of *Hedera helix* in a 53 ha German cemetery. (Illustration adapted from Kowarik et al. 2016)

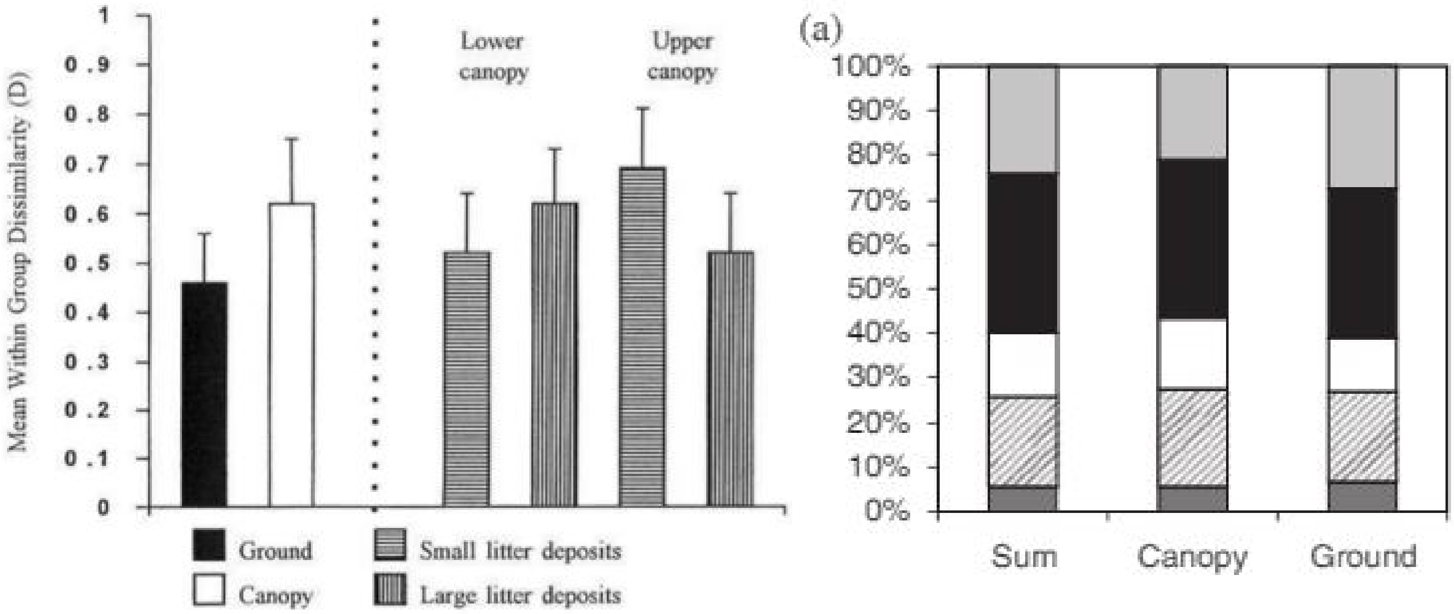


Figure 9. Collembola vertical stratification in rainforest (left) (Rodgers and Kitching 1998) showing dissimilarity between samples from the ground and the canopy and even within the lower and upper canopy in an Australian rainforest. Beetles also show segregation according to both of their feeding guilds' and body size (on the right; proportional representation by species) (Grimbacher and Stork 2007)

Conclusion

Invertebrate sampling in Mill Road Cemetery, Cambridge showed the importance of planning a survey and using multiple methods to assess the species composition of different habitats and strata of heterogeneity. The significant difference between sampling methods identified how different invertebrate taxa occupy different horizontal and vertical strata. Applying this knowledge can help healthcare professionals to track and stop the spread of diseases (e.g. dengue fever, Romero-vivas et al. 2002) or use invertebrates for forensic science (e.g. Sharanowski et al. 2008). As invertebrates are also key prey resource (Vonshak et at. 2009). IFor example, invertebrate abundance has a strong relation with chick survival (Palmer et al. 2001, Mitchell and Riegert 1994) therefore their survey is also crucial to bird conservation plans. From a global respective, reserving ecological networks require $3-11 billion per year between 2000-2030 (James et al. 2001, Pimm et al. 2001). Surveying and understanding invertebrate assemblages is in the frontlines of research and there is a great need for continuous development and testing (Meir and Possingham 2004).

Total Word Count: 1593

Appendix

Orders	**Sampling methods**			**Grand Total**
	Sweep netting	Pitfall trapping	Tree beating	
Acari	4	5	3	**12**
Araneae	46	34	77	**157**
Coleoptera	74	59	75	**208**
Collembola	3	52	1	**56**
Dermaptera	3	10	10	**23**
Diptera	62	39	42	**143**
Hemiptera	29	11	37	**77**
Hymenoptera	16	40	10	**66**
Grand Total	**237**	**250**	**255**	**742**

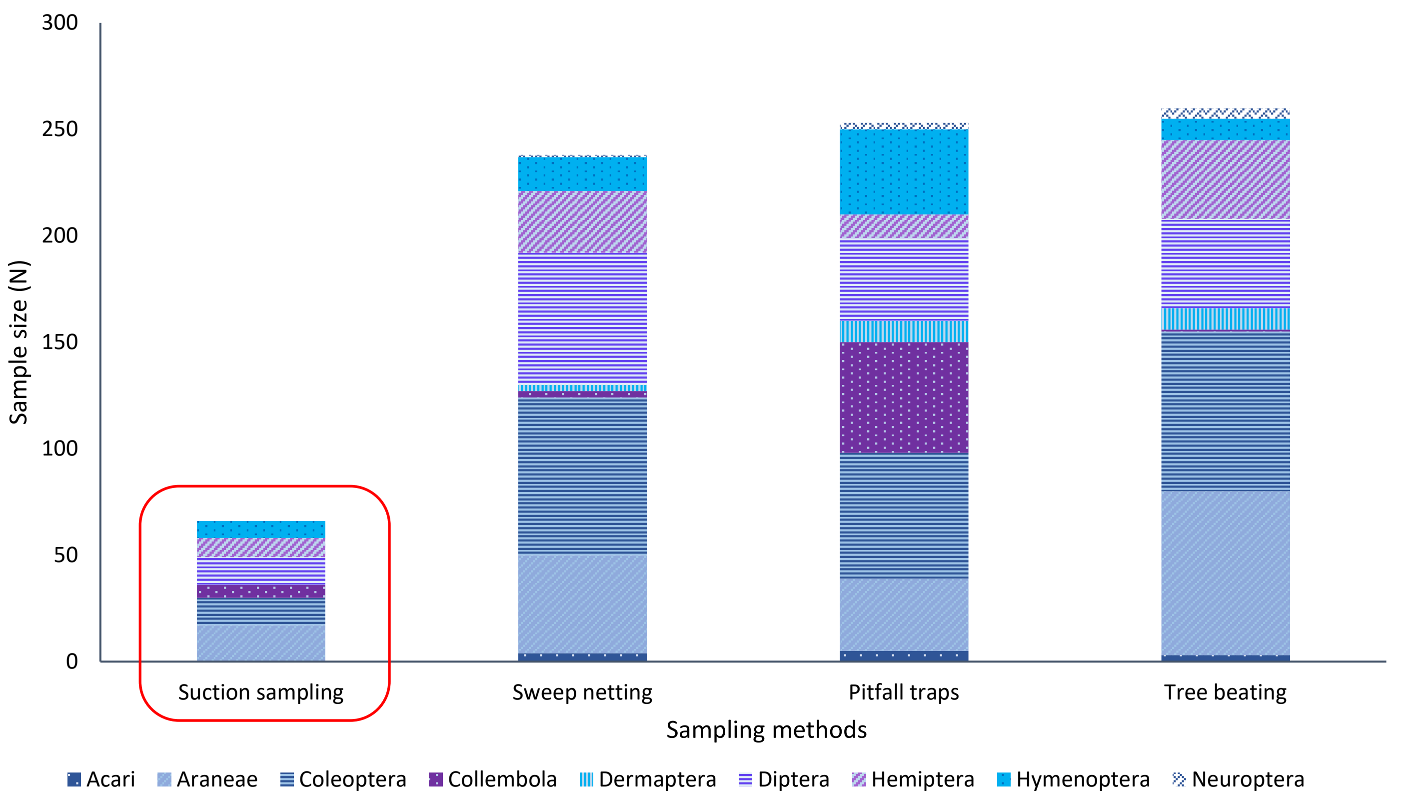


Figure 5.

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