

Suction samplers for grassland invertebrates: the species diversity and composition of spider and Auchenorrhyncha assemblages collected with Vortis™ and G-vac devices

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Abstract

The species composition of samples of spiders and Auchenorrhyncha obtained using a Vortis™ and a modified garden leaf-blower / vacuum 'G-vac' was compared at three sites using standard sample areas and suction times. Both devices caught scarce 'method-unique' species not found by the other, but the G-vac caught more. The G-vac also caught a larger number of specimens in total. Rarefaction and extrapolation were therefore used to quantify three measures of species diversity (Hill numbers) with standardised sample size and sample coverage. Traditional rarefaction and extrapolation curves for the two devices, based on sample size, were not significantly different, however, estimates of species richness were higher for the G-vac than the Vortis at some levels of sample coverage implying a higher efficiency at discovering additional species using the G-vac. Some individual species were more abundant in the G-vac samples, but they were not associated with a specific microhabitat. There was, therefore, no evidence that the two devices were sampling different communities. The study reveals that Vortis and G-vac devices can provide consistent inventories of the more abundant species of spiders and Auchenorrhyncha, but that more scarce species are found with the G-vac, and fewer samples may be required with this device because of its tendency to capture more specimens per sample.

Key words: Hemiptera, Araneae, vacuum sample, rarefaction, extrapolation, sample completeness, sample coverage, iNEXT.

Introduction

Suction devices are widely used for sampling of grassland invertebrates (Dietrick, 1961; Arnold, 1994; Macleod *et al.*, 1994; Samu and Sarospataki, 1995; Dogramaci *et al.*, 2011). The most commonly used are the Vortis™ and those based on modifications to garden blo-vacs (commonly referred to as G-vacs) (Stewart and Wright, 1995; Stewart, 2002). Recently Zentane *et al.* (2016) reported the first comparison between the catches of these two types of device. The G-vac caught more individuals of Araneae, Hemiptera, Thysanoptera and Hymenoptera. It was concluded that the greater catches obtained with the G-vac arose, in part, due to the differing modes of application of the two devices which mirrored standard protocols reported in the literature (Zentane *et al.*, 2016). The G-vac was applied by moving its nozzle through the vegetation within an open-ended cylinder, while the Vortis nozzle was applied flat to the ground surface. The mode of use of the G-vac may potentially have dislodged more individuals, and particularly those lower in the vegetation profile, than the Vortis. Grassland invertebrate taxa are known to be stratified within the vegetation (Cherrill and Sanderson, 1994; Stokmane and Spungis, 2016). It is therefore hypothesised that the two devices may capture different suites of species.

The present paper extends the earlier analysis of Zentane *et al.* (2016), which focussed on abundance data without consideration of species identity or richness,

and reports the first study to compare the two devices at the species-level. Specific questions addressed are whether estimates of the species richness and composition of assemblages of spiders and Auchenorrhyncha (comprising the Hemiptera suborders Fulgoromorpha and Cicadamorpha combined) are dependent on the device used, and whether any differences reflect the microhabitat of the target species. The results will be useful to inform the design of field sampling protocols where use of a suction sampler is being considered.

Methods

Study sites

Three grassland sites with flat terrain and internally homogenous vegetation were selected. Sites 1 and 2 were un-grazed and grazed mesotrophic grasslands respectively (52°46'N 2°25'W, 60 m a.s.l.), while Site 3 was heavily grazed unimproved grass heath (52°31'N 2°53'W, 420 m a.s.l.). Vegetation heights were: Site 1, mean = 12.3 cm, SD = 2.2 cm; Site 2, mean = 15.1 cm, SD = 3.4 cm; Site 3, mean = 3.7 cm, SD = 1.0 cm (n = 15 at each site), see Zentane *et al.* (2016) for height estimation method and plant community descriptions.

Suction sampling equipment

The G-vac suction sampler was a McCulloch GBV 345 garden blower/vacuum with its pipe sawn off perpendicular to its length. This gave a flat-ended collect-

ing nozzle, 0.01 m² in cross-section, in which a nylon 1 mm mesh bag was held in place by a rubber band (Stewart and Wright, 1995; Stewart, 2002). The Vortis™ was supplied by Burkard Manufacturing Company Ltd, UK, and has a collecting tube with an area of 0.02 m². Seated within the tube, and raised 3 cm above the ground, is a narrower pipe (cross-section 0.0085 m²) fitted with vanes. The resulting vortex spins material into a vessel fitted to the side of an expansion chamber. Both devices were driven by a 25 cc two-stroke petrol motor. Estimated velocity across collecting nozzles are 20.3 ms⁻¹, and 8.75 ms⁻¹ for the G-vac and Vortis respectively, although for the latter velocity is estimated at 20.6 ms⁻¹ within the inner tube (Zentane *et al.*, 2016).

Suction sampling

Suction samples were taken at sites 1, 2 and 3, on 15th, 17th and 21st July 2014 respectively when vegetation and leaf litter was dry to the touch and air temperature was at least 24 °C. At each site a grid of fifteen 4 m by 4 m contiguous squares was marked with canes. G-vac and Vortis™ samples were taken from within each grid-square; giving 15 samples with each device at each site. Samples were matched in terms of the time and area using the procedures summarised below and elaborated by Zentane *et al.* (2016).

Operation of the Vortis™

A Vortis™ sample comprised nine sub-samples, each of which was defined by holding the collecting tube flat on the ground with the motor on full-throttle for 10 seconds. After all nine sub-samples had been taken, the collecting cup was emptied into a labelled bag giving a total time of 90 s and area of 0.18 m² for each of the fifteen grid squares at a site.

Operation of the G-vac

The area of a G-vac sample was defined by the internal diameter of a 60 cm high open-ended cylinder (0.174 m²) placed in the centre of a grid square (Cherrill, 2015). Three sub-samples, each of 30 s, were taken within the cylinder. Each sub-sample was taken by first sweeping the nozzle over the surface of the vegetation for 5 s before the nozzle was repeatedly lowered and raised from the ground surface for the remaining 25 s (whilst ensuring the nozzle was still below the rim of the cylinder). Material from the sub-samples was pooled giving a total time of 90 s and area of 0.17 m² within each of the fifteen grid squares at a site.

Treatment of samples

Adult Araneae and Auchenorrhyncha were identified to species using Roberts (1993) and Biedermann and Niedringhaus (2009) and counted. These groups were selected because they are frequently sampled using suction devices in grassland (e.g. Macleod *et al.*, 1994; Samu *et al.*, 1997; Hollier *et al.*, 2005; Maczey *et al.*, 2005) and because they are functionally and numerically important constituents of grassland invertebrate communities (Nickel, 2003; Foelix, 2011). Nomenclature follows Merrett *et al.* (2014) for spiders and Wilson *et al.* (2015) for Auchenorrhyncha.

Statistical analysis

The mean numbers of individuals captured by the Vortis™ and G-vac were compared within each site using repeated measures Generalised Linear Model with Poisson error structure within IBM SPSS ver 23. Sampling device was defined as the within-subjects factor and grid-square as the between-subjects factor. At each of Sites 1 and 2, data were available from 15 grid-squares, but at Site 3 data were available for 10 pairs of samples only because some were mislaid before all specimens were identified to species.

Species diversity was compared between devices using individual-based rarefaction and extrapolation with the iNEXT programme (Chao *et al.*, 2016). Total numbers of individuals within each species were pooled across grid-squares within sites to yield the reference samples for this analysis. The number of species in reference samples was also compiled from inventories across samples within sites. Species identified by one method only are termed as 'method-unique'.

Individual-based rarefaction and extrapolation in iNEXT yields estimates for three measures of species diversity integrated into a single equation but differing in the value of a coefficient known as q (Chao *et al.*, 2016). The three measures are known as Hill numbers and equate to species richness (when $q = 0$), the exponential of the Shannon index (when $q = 1$), and Simpson's index (when $q = 2$) (Hill, 1973). These measures are widely accepted as being the most meaningful measures of species diversity (Ellison, 2010). Rarefaction and extrapolation was performed for both sample size and sample coverage. The former is the traditional method of applying rarefaction and extrapolation, but using sample coverage has recently been shown to be more reliable (Chao and Jost, 2012). While sample size is simply the number of individuals in a sample, sample coverage is the proportion of individuals in a community that belong to the species represented in the sample. Bootstrapping, with 200 iterations, was used to estimate CL₉₅ around diversity estimates (Chao *et al.*, 2016). Estimates with non-overlapping 95% Confidence Limits can be interpreted as being significantly different (at $P < 0.05$) (Colwell *et al.*, 2012; Hsieh *et al.*, 2016). The iNEXT programme was run with sample sizes (knots) specified at intervals of ten.

Results

Sample sizes, species inventories and method-unique species

The G-vac captured more specimens in total than the Vortis (table 1) and the numbers of some of the more abundant species were also greater in samples obtained with the G-vac (table 2). Site-level species inventories based on G-vac samples were longer than those obtained with the Vortis for spiders (all three sites) and for Auchenorrhyncha (Sites 1 and 3) (table 1). Both devices caught method-unique species, however, there were more method-unique species in the G-vac samples (table 1). No method-unique species was represented by more than four specimens (table 2) suggesting that their

Table 1. Summary of the number of specimens and species identified at each site. Differences in mean numbers of specimens caught by Vortis and G-vac are shown as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns non-significant ($n = 15$ pairs for Sites 1 and 2, $n = 10$ for Site 3).

	Site 1		Site 2		Site 3	
	Vortis	G-vac	Vortis	G-vac	Vortis	G-vac
Spiders						
Immature specimens	110	298 ***	168	375 ***	9	15 ns
Adults identified to species	28	81 **	117	173 **	2	13 ***
All specimens	138	379 ***	285	548 ***	11	28 *
Total number of species	11	15	6	9	2	6
Number of method-unique species	2	6	0	3	1	5
Auchenorrhyncha						
Immature specimens	266	408 *	25	26 ns	2	3
Unidentified/damaged adults	2	2	19	23 ns	0	0
Adults identified to species	203	330 **	29	36 ns	15	23 ns
All specimens	471	740 ***	73	85 ns	17	26 ns
Total number of species	7	8	3	3	1	3
Number of method-unique species	1	2	1	1	0	2
Spiders and Auchenorrhyncha combined (in iNEXT reference samples)						
Adults identified to species	231	411 ***	146	209 **	17	36 *
Total number of species	18	23	9	12	3	9
Number of method-unique species	3	8	1	4	1	7

capture is likely to be dependent on sample size. To compare the efficiency of the two devices further, rarefaction and extrapolation techniques were applied.

Rarefaction and extrapolation

Sample size and coverage based rarefaction and extrapolation curves were produced for Site 1 (figure 1) and Site 2 (figure 2) only, because too few specimens from Site 3 were identified to species (table 2). Data for spiders and Auchenorrhyncha were combined to boost sample sizes.

The sample size based rarefaction and extrapolation curves suggest that the rates at which the two devices accumulated species with increasing number of specimens did not differ at either Sites 1 or 2. Size based rarefaction and extrapolation curves for the two devices had overlapping CL_{95} for diversity estimates when $q = 0$, $q = 1$ and $q = 2$ (figures 1 and 2). The coverage based rarefaction and extrapolation curves, however, yielded higher estimates of species richness ($q = 0$) for the G-vac than the Vortis when sample coverage was relatively high (in the range 0.60 - 0.93 at Site 1, and 0.90 - 0.98 at Site 2) but below the coverage achieved in the reference samples (figures 1 and 2).

Sample coverages for the two devices at the reference sample sizes were very similar (at Site 1 for G-vac coverage = 0.99, for Vortis coverage = 0.96; at Site 2 for G-vac coverage = 0.99, for Vortis coverage = 0.97). Extrapolation for the marginally less complete Vortis samples was therefore limited. At sample coverages for the observed reference samples, there were no differences between the two devices for any of the three diversity estimators (figures 1 and 2).

Discussion

Vortis and G-vacs are the most widely used suction samplers for grassland invertebrates, but their relative performance at the species-level has not previously been investigated (Stewart, 2002; Zentane *et al.*, 2016). Important questions are whether the two devices differ in their utility to estimate the species richness and composition of invertebrate assemblages, and whether species which are under- or over-represented by either device are associated with a particular microhabitat.

Species diversity and community composition

Method-unique species were never represented by more than four individuals (table 2). Community-level studies often omit species represented by a small number of individuals on the basis that they may be transients or because they can distort the results of multivariate analyses (Legendre and Gallagher, 2001). A threshold of five individuals has been used to exclude species (e.g. Cherrill and Rushton, 1993) and here would have resulted in the two devices yielding identical site-level species inventories (table 2). Ignoring differences in sample size between devices, and considering only the more abundant species, the G-vac and Vortis therefore appear to be equally efficient when applied with the same suction duration (90 s) and sample area (0.18 m²). More generally, however, studies of species diversity will typically need to capture the scarcer species in an assemblage and not just those which are represented by large numbers of individuals.

The simplest explanation for the greater number of method-unique species in the G-vac is that this device typically captured a greater number of individuals than

Table 2. The number of specimens of each species collected using Vortis and G-vac samplers at three sites. Differences in mean numbers between devices are shown as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns non-significant (n = 15 pairs for Sites 1 and 2, n = 10 for Site 3).

Taxa	Site 1		Site 2		Site 3	
	Vortis	G-vac	Vortis	G-vac	Vortis	G-vac
Spiders						
Theridiidae						
<i>Enoplognatha latimana</i> Hippa et Oksala 1982	0	4	0	0	0	0
Linyphiidae						
<i>Dicymbium nigrum</i> (Blackwall 1834)	0	1	0	1	1	0
<i>Entelecara flavipes</i> (Blackwall 1834)	0	0	0	0	0	1
<i>Dismodicus bifrons</i> (Blackwall 1841)	1	0	0	0	0	0
<i>Oedothorax fuscus</i> (Blackwall 1834)	1	7	1	6	0	4
<i>Oedothorax retusus</i> (Westring 1851)	12	12 ns	0	2	0	0
<i>Gongyliellum vivum</i> (O.P.-Cambridge 1875)	0	2	0	0	0	0
<i>Micrargus herbigradus</i> (Blackwall 1854)	0	0	0	0	0	1
<i>Micrargus subaequalis</i> (Westring 1851)	0	1	0	0	0	0
<i>Savignia frontata</i> Blackwall 1833	1	5	2	6	0	1
<i>Erigonella hiemalis</i> (Blackwall 1841)	1	0	0	0	0	0
<i>Milleriana inerrans</i> (O.P.-Cambridge 1885)	0	1	0	0	0	0
<i>Erigone dentipalpis</i> (Wider 1834)	3	2	20	17 ns	1	3
<i>Erigone atra</i> Blackwall 1833	1	9	56	44 ns	0	3
<i>Agyneta decora</i> (O.P.-Cambridge 1871)	1	2	0	0	0	0
<i>Meioneta rurestris</i> (C.L. Koch 1836)	0	0	0	2	0	0
<i>Bathypantes gracilis</i> (Blackwall 1841)	3	18 *	19	53 ***	0	0
<i>Tenuiphantes tenuis</i> (Blackwall 1852)	3	13 *	19	42 ***	0	0
<i>Microlinyphia pusilla</i> (Sundevall 1830)	1	3	0	0	0	0
Thomsidae						
<i>Xysticus cristatus</i> (Clerck 1757)	0	1	0	0	0	0
Auchenorrhyncha						
Aphrophoridae						
<i>Neophilaenus lineatus</i> (L. 1758)	1	0	0	0	0	0
Cicadellidae						
<i>Aphrodes bicinctus</i> (Schrank 1776)	2	3	0	0	0	0
<i>Anoscopus albifrons</i> (L. 1758)	0	2	0	0	0	0
<i>Anoscopus serratulae</i> (F. 1775)	0	3	0	0	0	0
<i>Deltocephalus pulicaris</i> (Fallen 1806)	0	0	0	0	15	19 ns
<i>Arocephalus punctum</i> (Flor 1861)	0	0	0	0	0	2
<i>Arthaldeus pascuellus</i> (Fallen 1826)	50	60 ns	0	2	0	0
<i>Psammotettix nodosus</i> (Ribaut 1925)	0	0	0	0	0	2
<i>Euscelis incisus</i> (Kirshbaum 1858)	18	37 *	0	0	0	0
<i>Cicadula persimilis</i> (Edwards 1920)	2	1	1	0	0	0
<i>Zyginidia scutellaris</i> (Herrich-Schaeffer 1838)	23	22 ns	0	0	0	0
Delphacidae						
<i>Javesella obscurella</i> (Boheman 1847)	0	0	11	4 ns	0	0
<i>Javesella pellucida</i> (Boheman 1847)	107	202 *	17	30 ns	0	0

the Vortis (table 1) (see also Zentane *et al.*, 2016). By chance these samples would be more likely to contain species represented by few individuals (Scharff *et al.*, 2003). An inference is that continued sampling with the Vortis would yield more specimens, including the “missing” method-unique species detected with the G-vac. Rarefaction and extrapolation techniques can be used to investigate this problem by estimating species diversity based on standardised samples size and coverage (Chao *et al.*, 2016).

Traditional sample based rarefaction and extrapolation suggest that the rates at which species accumulated with increasing number of specimens did not differ between

devices (figures 1 and 2). In contrast, coverage based rarefaction and extrapolation showed that for samples with incomplete coverage (approximately < 0.93), species richness ($q = 0$) was higher with the G-vac than with the Vortis (figures 1 and 2). The reference samples obtained by both devices, however, had similarly high coverage (> 0.96) providing reassurance that these observed samples included all but the scarcest species. Compared to abundant species, such scarce species contribute little to sample coverage and therefore only limited rarefaction and extrapolation was necessary to permit comparison of species diversity between devices at high coverage. At the sample coverage levels achieved

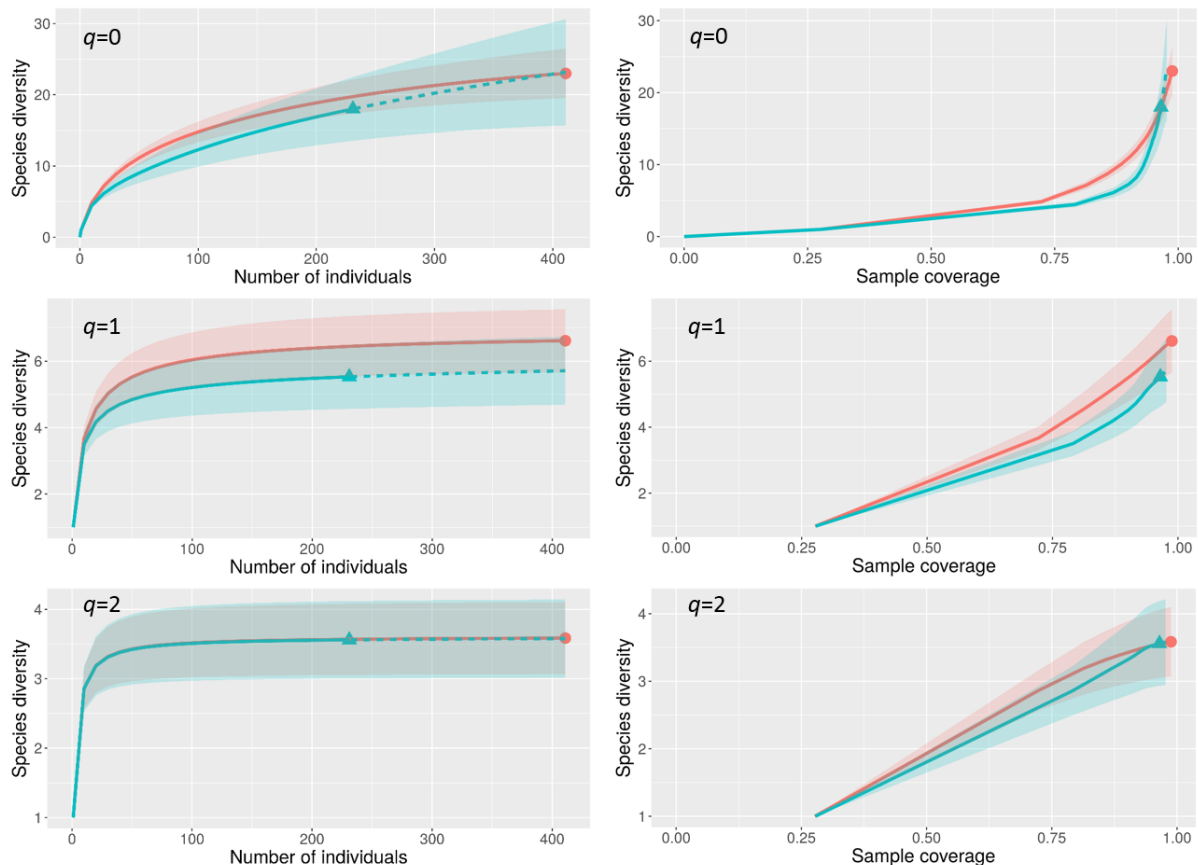


Figure 1. Rarefaction and extrapolation curves based on sample size (left) and sample coverage (right) for reference samples containing spiders and Auchenorrhyncha at Site 1 (G-vac - red line and circle; Vortis - blue line and triangle). Species diversity estimates are for species richness ($q = 0$), exponential of Shannon's Index ($q = 1$) and Simpson's Index ($q = 2$). Dashed lines represent extrapolation. Shaded areas show 95% Confidence Limits. (In colour at www.bulletinofinsectology.org)

in reference samples, species richness ($q = 0$) estimates did not differ between devices. Moreover, there were no differences between devices in species diversity estimates for $q = 1$ and $q = 2$ (figures 1 and 2) suggesting that these measures may be less sensitive to bias introduced by sampling device. This is not surprising because while an additional scarce or abundant species will contribute equally to species richness, the former will have much less influence on indices of diversity that incorporate proportional abundance (Magurran, 1998).

Species-level sampling bias and microhabitat

When using the Vortis in taller grass, leaves were sometimes trapped flat against the ground with the risk of invertebrates being shielded from the air flow. The mode of application of the G-vac may have resulted in a greater chance of invertebrates being dislodged from within tussocks and leaf litter close to the ground. Vertical stratification of spiders and Auchenorrhyncha in grassland vegetation has been demonstrated (Duffey, 1963; Andrzejewska, 1965; Richardson and Hanks, 2009; Cherrill and Sanderson, 1994; Stokmane and Spungis, 2016), hence it could be hypothesised that species occurring close to the ground may be under-represented in Vortis samples.

Relatively little information is available on the microhabitats of spiders within grasslands, although most of the species recorded in this study were Linyphiidae which exhibit ballooning behaviour (Blandenier, 2009). These species could be expected to occur high in the vegetation when dispersing, even if at other times they occur lower down. In terms of their reliance on webs for hunting and the position of their webs in the vegetation, the more abundant species of spider can be ordered as follows *Oedothorax fuscus* (Blackwall) and *Oedothorax retusus* (Westring) (active ground hunters) (Alderweireldt, 1994), *Erigone dentipalps* (Wider) and *Erigone atra* Blackwall (web-builders and active hunters, close to the ground) and, *Bathyphantes gracilis* (Blackwall) and *Tenuiphantes tenuis* (Blackwall) (web-builders, above the ground) (Harwood *et al.*, 2003; 2004). Only the latter two species were more abundant in G-vac samples (table 2), hence the hypothesis that species living lower in the vegetation are likely to be under-represented in Vortis samples is not supported.

There is also no evidence that the two devices are sampling different communities of Auchenorrhyncha. Information on the microhabitat of Auchenorrhyncha in grassland is provided by Andrzejewska (1965), Tormala (1982), Novotny (1992) and Cherrill and Sanderson (1994). At Site 1, two species of leafhopper, *Javesella*

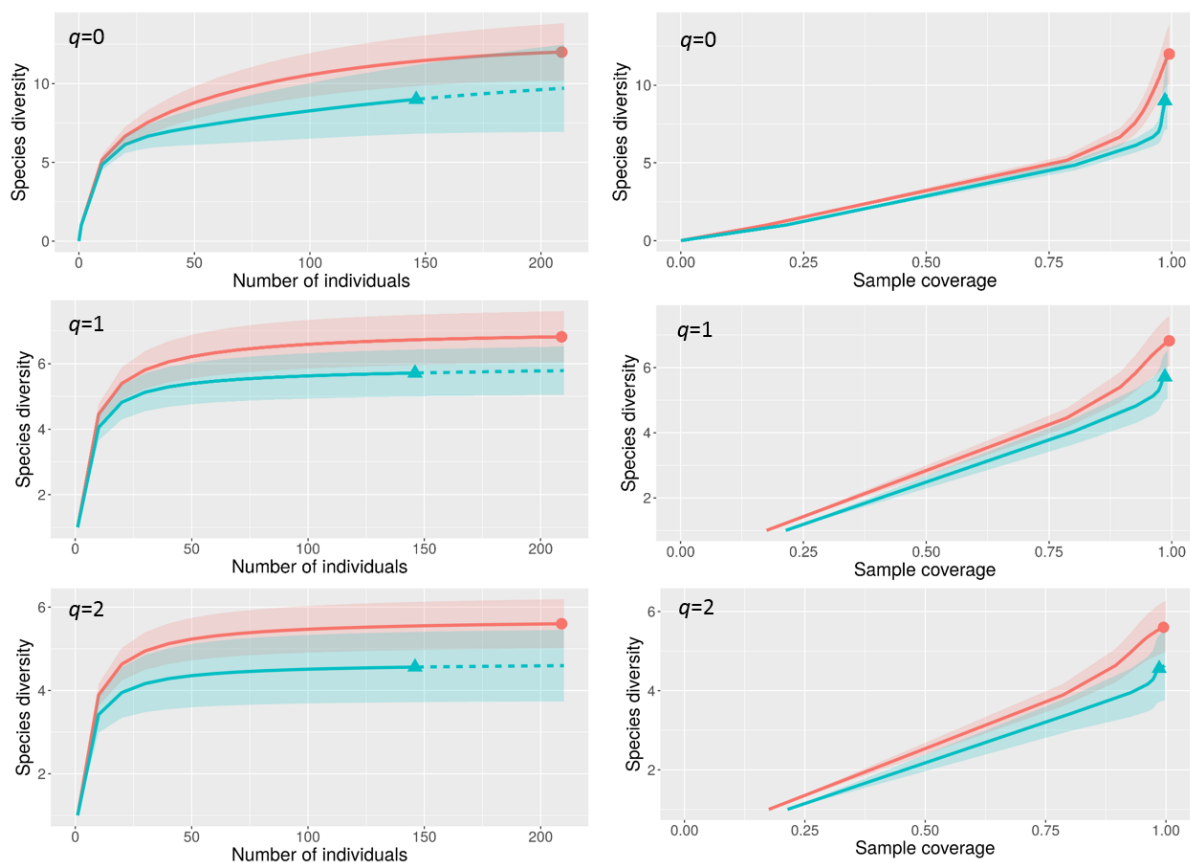


Figure 2. Rarefaction and extrapolation curves based on sample size (left) and sample coverage (right) for reference samples containing spiders and Auchenorrhyncha at Site 2 (G-vac - red line and circle; Vortis - blue line and triangle). Species diversity estimates are for species richness ($q = 0$), exponential of Shannon's Index ($q = 1$) and Simpson's Index ($q = 2$). Dashed lines represent extrapolation. Shaded areas show 95% Confidence Limits. (In colour at www.bulletinofinsectology.org)

pellucida (Boheman) and *Euscelis incisus* (Kirshbaum), were more abundant in G-vac samples than Vortis samples (table 2) but little is known about their microhabitats. Species known to occur close to the ground, *Aphrodes bicinctus* (Schrank), *Anoscopus albifrons* (L.), *Anoscopus serratulae* (F.), or high in grassland vegetation, *Neophilaenus lineatus* (L.), were not sufficiently abundant to allow a species-level analysis. Species occurring at intermediate heights in grassland vegetation, *Deltocephalus pulicaris* (Fallen), *Arthaldeus pascuellus* (Fallen), were captured equally by the two devices (table 2).

Conclusions

With sampling standardised by time and area, G-vac and Vortis samplers provided comparable species lists for the more abundant grassland Auchenorrhyncha and spiders, but the G-vac captured more scarce method-unique species. The greater number of scarce method-unique species in G-vac samples is interpreted largely as a numerical effect, because the G-vac captured more specimens in total. Rarefaction and extrapolation

showed, however, that the G-vac gave higher estimates of species richness when sample coverage was standardised, but below that achieved in the final observed reference samples, indicating a greater efficiency at finding additional species.

A factor to be included in future studies is the time required for sorting samples. In addition to capturing a greater number of specimens, the G-vac typically catches more unwanted plant debris leading to increased sorting times. There may therefore be a trade-off between sample size and sorting time which requires clarification to determine the overall cost-effectiveness of the two devices.

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