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RESEARCH ARTICLE



Developing a future protocol for measuring spider biodiversity in pastures in New Zealand

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ABSTRACT

Arthropods are often ignored or under-sampled in biodiversity and conservation assessments because of their large diversity, small size and lack of taxonomic guides. Rapid biodiversity assessment programmes have been established to assess these groups accurately. A COBRA (Conservation Oriented Biodiversity Rapid Assessment) protocol consists of an intense sampling of a habitat using the optimal combination of sampling methods. We set a basis for future protocols of measuring spider biodiversity in exotic pastures in New Zealand. Overall, 28 spider species were collected. There was variation in species discovery for each collection method, i.e. pitfall traps (86.6% of total species found), ground hand collection (95.4%), suction sampling (85.7%), and sweeping (25%). The various collection methods were complementary in species that were found. Of the four sampling methods used pitfall traps and ground hand collection were far more efficient at collecting spider species in pastures per sample. These findings are relevant for the future development of these protocols and ultimately, these tools will be used for assessing and monitoring biodiversity on farms and the impacts of farming methods.

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Introduction

Assessing arthropod richness, abundance, composition, geographical patterns and their roles in ecosystems is logistically challenging (Ramos et al. 2001; Cardoso 2009). A lack of information about arthropod populations can hinder conservation biology, ecology, agroecology, and biogeography (Ramos et al. 2001; Gurr et al. 2004; Blanchet et al. 2015). Because arthropods are highly diverse, sampling requires an efficient and well-structured approach to maximise limited resources (New 1999). As awareness of the impact of human activities on ecosystems and organisms increases, more research and comparable data are necessary for management, and it becomes more important to have standardised protocols to collect them (Whitmore et al. 2002; Cardoso 2009; Cardoso and Leather 2019).

The most common approach for sampling arthropods is *ad hoc* sampling (non-optimised and site-specific). *Ad hoc* sampling is based on the expert judgement of the collectors, with the assumption that they will use the best combination of sampling methods to provide maximum information about the species communities in a given site in a minimum amount of time (Cardoso et al. 2009a). This approach is often used for compiling species lists (Gordon and Newton 2006; Roberts et al. 2007) for well-known taxa, such as birds (Droege et al. 1998). Because different sampling events are seldom designed in the same way, *ad hoc* sampling does not allow for reliable or repeatable comparisons, the search effort may be different between studies (Cardoso 2009). Optimised and standardised protocols, such as COBRA (Conservation Oriented Biodiversity Rapid Assessment), are not common. The COBRA protocol is a relatively new approach to sampling highly diverse arthropods, such as spiders. The COBRA protocol is designed to collect the maximum number of species, in a minimum amount of time, combining a variety of sampling methods (*optimised*) while being applicable to multiple sites (*standardised*) and currently exist only for spiders (Cardoso et al. 2008; Cardoso et al. 2008a; Cardoso 2009; Cardoso et al. 2009a; Malumbres Olarte et al. 2017; Bichuette et al. 2019).

Spiders are common in agricultural habitats and provide crucial ecosystem services (Marc et al. 1999). In agroecosystems, spider species richness is generally quite high but is dominated by only a few species (Agnew and Smith Jr 1989; Isaia et al. 2010; Michalko and Pekár 2015). Spiders are generalists and can be present even when targeted prey species are absent, capturing alternative prey as well as possessing adaptations for times of deprivation (Greenstone and Bennett 1980; Harwood et al. 2004; Michalko and Pekár 2015). Particular assemblages of spider species can reduce crop damage from pest insects in orchards and crops (Hoefer et al. 2006; Michalko and Pekár 2015).

Agriculture is a major industry in New Zealand and consists of 7–8 million hectares of pasture (Ministry of Primary Industries 2012; Pearson 2020). Most exotic pastures in New Zealand are occupied by exotic species of spiders (Vink et al. 2004). Martin (1983) sampled pastures in Nelson and identified 45 spider species. *Teniphantes tenuis* (Blackwall 1841) is one of the most common species found in New Zealand pastures (Vink et al. 2004; Malumbres-Olarte et al. 2014). This species is an exotic species and most likely originated from Europe (Millidge 1988). Linyphiidae is the dominant family found in pastures (Clark et al. 2004).

There are limited publications on spider biodiversity and abundance in agroecosystems in New Zealand (Topping and Lövei 1997; Hodge and Vink 2000; McLachlan and Wratten 2003; Clark et al. 2004; and Vink et al. 2004), and most are 10 or more years old, which is unexpected given the high spider diversity and farmland cover in New Zealand.

There are currently no efficient protocols for sampling spiders in New Zealand agricultural pastures accurately and the development of a new COBRA protocol for these pastures would be beneficial. Our aims are to develop a basis for future protocols for measuring spider diversity in exotic pastures in New Zealand and to estimate the minimum amount of time and resources to efficiently and accurately sample exotic pastures in New Zealand.

Methods

Three sites in Canterbury, New Zealand we chose for this study. These were the Lincoln University Demonstration Dairy Farm (LUDDF), the Lincoln University Research Dairy Farm (LURDF) and Lincoln University Iversen Fields. Ten sites were used and varied in size between 0.6–8.3 hectares. The average rainfall per year is 666 mm for all three sites. The LUDDF site (43°38′16.59″S 172°26′23.91″E) covers 186 ha of land with 160.1 ha of productive land. Originally a sheep farm, it was converted to dairy in 2001. The irrigation average is 37.5 ml/month to maintain an average evapo-transpiration rate of 72.5 ml/month. The pasture consists of Ronsyn/Impact ryegrass (*Lolium perenne*), Aran sustain white clover (*Trifolium repens*) and a small area of timothy (*Phleum pratense*).

The LURDF (43°38′16.29″S 172°27′38.12″E) is used for dairy research and demonstration of the best practice dairy techniques. Large areas of the pasture consist of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). There are other areas of diverse pasture that contain chicory (*Chichorium intybus*), plantain (*Plantago lanceolata*), and lucerne (*Medicago sativa*). The farm is 56 hectares and has 250 Friesian cross Jersey cows. The irrigation average is 105.1 ml/month.

Iversen fields (43°38′54.16″S 172°27′51.88″E) is an area used for multiple crops, including wheat, barley, peas, beans, oil seed rape, forage brassicas and vegetables. Fields are rotated into pasture after intensive cropping. These areas remain in pasture for up to three years depending on experiment and research requirements. The pasture is grazed by sheep and is planted with arrow ryegrass (*L. perenne*) at 20 kg/ha and white clover (*T. repens*) at 4 kg/ha. The irrigation average is 100 ml/month.

Sampling design

Common sampling methods for collecting spiders are pitfall traps, emergence traps, sweep netting, suction sampling, leaf litter extraction, ground searching, and beating (Churchill and Arthur 1999; Sutherland 2006). Not all of these sampling methods are appropriate for pastures, with leaf litter extraction and beating techniques not commonly used. Pasture grasses are short and do not have a compact structure as some native grasses do (Malumbres Olarte 2010), so foliage beating may not be suitable as a sampling method for exotic pastures. Leaf litter extraction is used mainly in forests as there is more depth in the litter (Stevenson and Dindal 1982) compared to exotic pasture (Curtis et al. 2019). Emergence traps collect insects as they emerge from a substrate, like soil, and have been known to catch spiders, but are less efficient than other traps (Malumbres Olarte 2010). Suction sampling is often used for sampling spiders in agroecosystems (McLachlan and Wratten 2003; Vink et al. 2004) but these devices are often difficult for the public to access and can be expensive (Sutherland 2006). Therefore, ground sampling, sweeping, and pitfall traps are the most appropriate sampling methods for pastures.

Pitfall trap protocol

We placed 16 pitfall traps in groups of four at each site. In the four groups, each pitfall trap was 1 m apart and 3 m from other groups. The pitfall traps were at least 100 m from the closest fence line. Plastic cups, 69 mm in diameter, were placed into the ground and

were filled with 1/2 cup of monopropylene glycol and labelled. A metal roof was placed over each pitfall trap, which follows the New Zealand Department of Conservation's guidelines for invertebrate pitfall traps (Sherley and Stringer 2016). The traps were left in the field for seven days.

Ground sampling protocol

Ground sampling consisted of collecting all spiders found below knee level (Cardoso et al. 2008). Transect lines were set using a measuring tape to create a 30-metre stripe across the pasture. The distance between transects varied due to the different sizes of the site but ranged between 10 and 20 m. Ground sampling was performed in six 10-minute periods and was five metres distant from the transect line. Each site contained five different transect lines and each line was sampled for an hour in the day and an hour in the night, for 10 hours of sampling. Therefore, each site had a total of 60 samples. Ground searching involved crawling through the pasture with an insect aspirator and searching through the grass at the root level. Four small areas (approx. 10 × 10 cm by 2 cm deep) of thick pasture were removed and searched on a beating sheet in 10-minute intervals. The labelling and placing of specimens into vials were done at 5-minute intervals, this was done at the end of the 10-minute intervals.

Sweeping protocol

Transect lines were 30 m long and followed the same design as the ground sampling protocol. Continuous sweeping was done for 12, five-minute periods and was within 10–20 m of each of the five transect lines, totalling five hours of collecting and 60 samples per site. Collecting took place once at night and once during the day, totalling 10 h of sampling. There was also a two-minute maximum collecting period to remove spiders found in the net, excluding labelling. The two-minute collecting period was not included in the five-hour collection period. The sweeping net was emptied onto a beating tray or sheet from which we collected the spiders using an aspirator and/or hand collected in vials. Specimens were placed in labelled vials with 70% ethanol.

Suction sampling protocol

The suction sampler with a sampling pipe diameter of 16.4 cm was built from a modified leaf blower. To avoid bias, the sample area was chosen by throwing a tennis ball and sampling the area where it stopped. Suction sampling was carried out within an area of 1 m² per transect. In the transect square, the sampling pipe was placed firmly on the ground and held for 30 s. A pitfall trap cup was placed onto the other end of the pipe to collect the spiders. The suction sampler was lifted and placed onto another patch of ground in the transect square and held for another 30 s. This was repeated for three minutes of sampling time. Each site had three samples totalling nine minutes of sampling. The samples were labelled and placed in 70% ethanol.

The collected spiders were sorted and identified to species level using the taxonomic literature (Dondale 1966; Forster 1967; Forster and Wilton 1968; Forster 1970; Forster

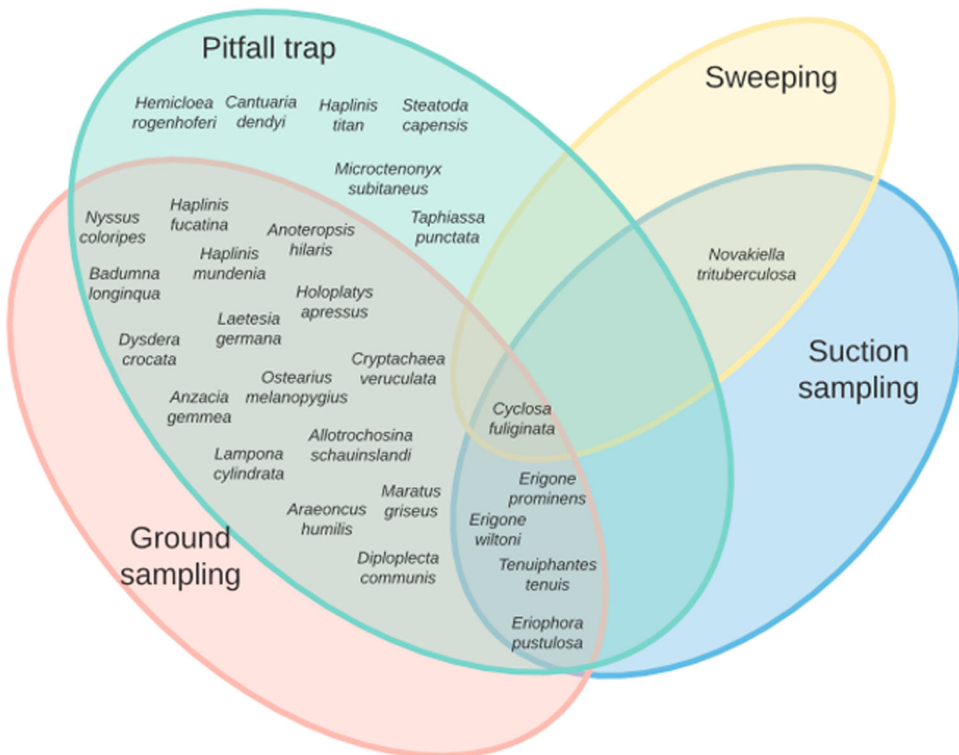
Table 1. Quantitative results and species estimates from the methods used in the sampling protocol in total.

	Pitfalls	Ground Sampling	Suction Sampling	Sweeping
Individuals	1500	1093	85	89
Species	26	21	6	2
Singletons	3	1	1	2
Doubletons	2	2	1	0
Jackknife 1	29	22	7	6
Jackknife 2	30	21	7	8
Chao 1	27	21	6	5
Chao 2	26	21.5	6	4.5
Slope S	26.9	21.7	6.3	5.01
Sampling completeness	92%	80.7%	95.2%	17.8%

and Wilton 1973; Forster and Blest 1979; Forster et al. 1988; Millidge 1988; Vink 2002; Žabka and Pollard 2002; Paquin et al. 2010; Rix and Harvey 2010).

Statistical analyses

The software package Genstat 19th Edition (VSN International 2017) was used to calculate randomised species accumulation curves for observed species richness to statistically determine if the randomised curves reached an asymptote. Four species richness estimators (Jackknife 1, Jackknife 2, Chao 1, and Chao 2) were calculated per site and per sampling method. Singletons and doubletons were calculated for each site and sampling

**Figure 1.** Unique and shared species by collecting method.

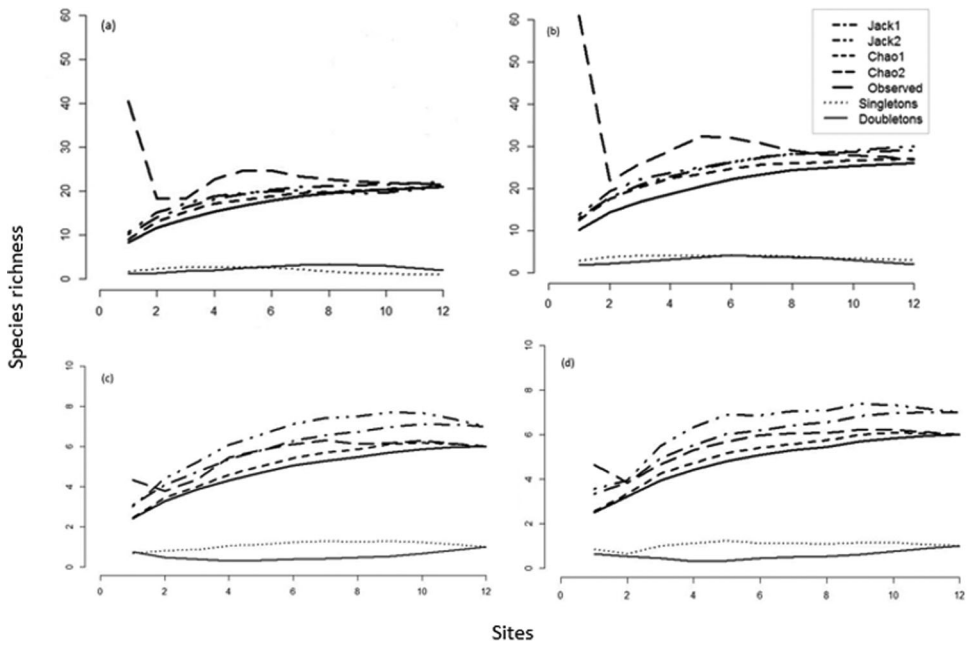


Figure 2. Randomised accumulation curves of observed species richness over increasing sampling sites for singletons, doubletons, and other estimators: **A**, ground sampling, **B**, pitfall traps, **C**, sweeping, **D**, suction sampling.

method, with 100 sample order randomisations in R package BAT (Cardoso et al. 2015). Sampling completeness was estimated using Jackknife 2 and is the number of observed species divided by the estimator (Jackknife 2) number of species.

Results

A total of 2767 spiders were caught, which included 1384 adults (50%) representing 12 families and 28 species (Table 2, Appendix). Pitfall traps collected the most with 1500 individuals, ground sampling collected 1093 individuals, suction sampling collected 89 individuals, and sweeping collected 85 individuals (Table 1). Sweeping and suction sampling only added one new species *Novakiella trituberculosa* to the total (Figure 1).

The non-parametric estimators for pitfall traps, the accumulation curves of Chao 1 and Chao 2, reached asymptote (Figure 2). Chao 1 (26 species) and Chao 2 (27 species) produced the estimation of the number of species found in pitfall traps. Jackknife

Table 2. Number of individuals, per sample, per method for each three sites. (LUDDF) Lincoln University Demonstration Dairy Farm, (LURDF) Lincoln University Research Dairy Farm, (LUIF) Lincoln University Iversen Fields, (PT) pitfall trap, (GS) ground sampling, (SW) sweeping and (SU) suction sampling.

	LUDDF				LURDF				LUIF				Totals
	PT	GS	SW	SU	PT	GS	SW	SU	PT	GS	SW	SU	
Samples	16	11	44	6	16	11	44	6	16	11	44	6	77
Individuals	376	248	2	28	211	367	1	24	913	478	86	33	2767
Species	7	9	1	3	6	6	1	2	18	12	1	5	28

2 was used to estimate sample completeness of 86.6%. Jackknife 1 (29 species) and Jackknife 2 (30 species) accumulation curves did not reach asymptote. Three species were singletons (11.1% of the total species collected using this sampling method), and two species were doubletons (7.6%) (Figure 2).

For ground sampling, the accumulation curves using Chao 1, Chao 2, and Jackknife 2 reached asymptote (Figure 2). Chao 1 and Jackknife 2 (21 species) were estimated. The accumulation curve of Jackknife 1 (22 species) did not reach asymptote. Sample completeness was 95.4% and there was a singleton (4.7%) and two doubletons (9.5%) (Figure 2).

For sweeping, the accumulation curves of Chao 1 (2 species) and Chao 2 (4.5 species) reached asymptote (Figure 2). The estimates from Jackknife 1 (6 species) and Jackknife 2 (8 species) did not reach asymptote. Sample completeness was 25% and there were two singletons (40%) and no doubletons (Figure 2).

For suction sampling, the accumulation curves of Chao 1 and Chao 2 (6 species) reached asymptote (Figure 2). Chao 2 uses data from multiple samples in total to estimate the species diversity and Figure 2 suggests it may take three sites to get an estimate of species diversity. Jackknife 1 and Jackknife 2 (7 species) were estimated, but the accumulation curves did not reach asymptote. Sample completeness was 85.7% and there was a singleton and a doubleton (15.8%) (Figure 2).

Discussion

This study is the first to estimate spider diversity in exotic pastures in New Zealand. Pitfall traps were found to be the best method for catching spider species with 26 out of the total 28 caught. Pitfall traps are commonly used to monitor ground-dwelling arthropods (Moeed and Meads 1985; Prasifka et al. 2007) and suggests most spider species found in pasture are ground-dwelling spiders. Topping and Lövei (1997), pitfall traps collected a total of nine species. Six out of the nine species collected belonged to the family Linyphiidae, including *Tenuiphantes tenuis*, which builds webs just above the ground surface. This species is unlikely to be caught in pitfall traps and often escapes (Topping 1993), e.g. three individuals were caught in pitfall traps compared to 340 individuals found with ground sampling. Only 1.5% of the individuals caught in pitfalls were *Tenuiphantes tenuis* compared to 56% when ground sampling was used. This suggests that ground sampling supplements pitfall traps. In our study, Linyphiidae was the most abundant family with a total of 11 collected species. More effort was needed for pitfall traps as three of the non-parametric estimators did not reach asymptote.

Ground sampling was the second-best method for collecting a large number of species. This method collected 21 of the 28 species found in this study. There have been no studies in New Zealand that have collected spiders by ground sampling in pastures. This method was the closest to reaching asymptote and three of the non-parametric estimators suggested that ground sampling had collected the maximum number of species. This method collected no unique species and shared all the same species found by pitfall traps. Ground sampling did however collect more individuals in the Linyphiidae family, which may be because the species of this family build webs above the ground surface (Topping 1993).

Sweeping had the lowest sampling completeness and less than one-quarter of the total number of species detected at the sites were collected by sweeping. This pattern may be

due to environmental factors, including pasture dampness and short grasses in the cropping pastures. Sweeping was designed to be used in long grasses and occasionally used in short vegetation (Sutherland 2006). The issues that arise from sweeping in pastures include the fact that sheep pasture is normally quite short, whereas dairy pasture normally has longer grass, which is more suitable for sweeping. Dairy also uses irrigation, which makes it difficult to sweep the moist grass. In six sites on dairy pastures, there was a total of three adult spider individuals from two species: *Cyclosa fuliginata* and *Novakiella trituberculosa*. In the cropping pasture a total of 86 spider individuals were caught from one species: *Cyclosa fuliginata*. The remaining individuals were all juveniles from the family Araneidae.

All five studies that have sampled spiders in agroecosystems in New Zealand used suction sampling as the only method to capture spiders, apart from Topping and Lövei (1997) who used pitfall traps and collected a total of six species. Five of the six species were captured in cropping pasture while one species was caught in dairy pasture. It is difficult to compare the five studies, as they were spread across different types of New Zealand pastures. However, Vink et al. (2004) sampled sites around Lincoln that did vary between crops of ryegrass, fescue (*Festuca arundinacea*), cocksfoot (*Dactylis glomerata* L.), prairie grass (*Bromus willdenowii* Kunth), wheat and barley, as well as samples in ungrazed pastures. Across all sites the spider abundance and richness were predominately higher in the pasture grasses compared to the cereals (Vink et al. 2004). Our study showed cropping pasture had the highest species richness compared to dairy, which is different from Vink et al. (2004) findings. Vink et al. (2004) study is nearly two decades old, and irrigation is now more common and effective in New Zealand (Ministry for the Environment and StatsNZ 2021; Whitehead et al. 2021), which may have had an impact. The species collected from suction sampling were also collected using pitfall traps or using sweeping. Although suction sampling is faster than sweeping and may collect more species, it is not cost-effective as it is not accessible for most people, and therefore, future COBRA protocols for pasture may not include it.

The common and most important factor that influenced all previous COBRA protocol studies is the methods used in the design (Cardoso 2009). This reduces the influence that the collector has on the data, although it has been recommended that at least one of the collectors has experience in quantitative sampling. It is important to recognise that although certain methods may not yield high numbers of spiders, this does not mean that a particular sampling method is inefficient, as it is the number of species found that is important (Cardoso 2009). Previous research has shown that balanced designs are misrepresentative as they do not accurately represent the population in proportion to their abundance (Cardoso 2009). Conversely, unbalanced designs may provide data that may represent populations better, as different methods overlap, as shown in Figure 1. Therefore, it is more productive to design an unbalanced design, which will often result in better sample accuracy of the focused population or communities (Cardoso 2009).

A protocol for pastures

Based on our results and those of other protocols worldwide, we recommend the following steps to design a COBRA protocol for collecting spiders in exotic pastures in New Zealand.

- (1) To be comparable with other COBRA protocols, such as COBRA-TF (Malumbres Olarte et al. 2017) and COBRA Mediterranean cork forests (Cardoso et al. 2008), there should be 12 samples with 4 pitfall traps for each sample, totalling 48 pitfall traps. Pitfall traps are to be placed 100 m away from fences or shelterbelts. This is to stop collecting spiders that are not common in pasture, as shelterbelts have a higher species richness (Bowie et al. 2014; Curtis et al. 2019). The pitfall traps may be placed in a square plot or transect line (depending on the shape of the property) that is divided into 12 groups (samples). In each of the groups, each pitfall trap should be at least one metre apart and each group should be a minimum of three metres apart. These 48 traps should be active for two weeks. It is also recommended that stock should not be present in the pasture while the pitfall traps are out, as the traps contain antifreeze, which can poison stock.
- (2) Ground sampling is to be carried out only at night between 11pm and 1am. Most spiders are nocturnal, and we found these times were the best in collecting a larger number of species. Samples should be taken at least 100 m from any fences or shelterbelts. Samples are to be taken from the base of the soil to pasture level only and from randomised sites around the pasture. Sampling should be carried out for six hours with each individual sampling event consisting of searching for spiders at pasture level for 60 min. If there is thick pasture, soil squares of 2 cm deep section should be removed and placed on a white sheet to facilitate collecting of spiders. Time taken for labelling, moving between sites, and sorting specimens into vials is not included in the hour of sampling. Pre-made labels will make this process more efficient. Specimens should be stored in 70% ethanol. Stock should not be in the field while ground sampling is taking place for health and safety reasons.
- (3) Sweeping is not recommended.
- (4) Suction sampling should only be carried out if a suction device is easily accessible. This method is an alternative to sweeping. Suction sampling is faster than sweeping for collecting samples, but it is not cost-effective if this item is not available. If the pasture is wet, do not use this method. Suction sampling should occur during the early morning and again at night. Use the suction sampler in the middle of the pasture and at least 100 m away from fences or shelterbelts. To avoid bias when selecting a site, use a method to randomise the collection point. Suction sampling should be increased to 3 h per 1 ha plot. The sampling pipe should be placed firmly on the ground and held for 60 s. A pitfall trap cup is placed onto the other end of the pipe to collect the spiders.

One of the main purposes of this research was to set the basis for the design of protocols for pastures in New Zealand. We provide fundamental information for the future design of a simplified protocol for pastures, one that is based on the COBRA for open habitats. This future protocol will allow the collecting of the highest diversity with the least amount of effort. Using a standardised protocol in pasture will allow biodiversity to be monitored over time to accurately assess whether it has increased or decreased as farming strategies or climatic conditions change. Different pasture compositions, different applications of fertiliser or insecticides, and different stocking levels are some of the farming strategies that can affect biodiversity and their impact could be measured using appropriate, effective, comparable, and efficient sampling approaches.

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Appendix: List of captured species and the collection method (PT = pitfall traps, GS = ground sampling, SW = sweeping, SU = suction sampling, * = native species).

Family	Species	Collection method PT, GS, SW, SU	
Araneidae	<i>Eriophora pustulosa</i> (Walckenaer, 1841)	GS SU	
	<i>Cyclosa fuliginata</i> (L. Koch, 1872)	PT GS SW SU	
	<i>Novakiella trituberculosa</i> (Roewer, 1942)	SW SU	
Corinnidae	<i>Nyssus coloripes</i> (Walckenaer, 1805)	PT GS	
Desidae	<i>Badumna longinqua</i> (L. Koch, 1867)	PT GS	
Dysderidae	<i>Dysdera crocata</i> (C. L. Koch, 1838)	PT GS	
Gnaphosidae	<i>Anzacia gemmea</i> (Dalmás, 1917)	PT GS	
	<i>Hemicloea rogenhoferi</i> (L. Koch, 1875)	PT	
Idiopidae	* <i>Cantuarina dendyi</i> (Hogg, 1901)	PT	
Lamponidae	<i>Lampona cylindrata</i> (L. Koch, 1866)	PT GS	
Linyphiidae	<i>Araeoncus humilis</i> (Blackwall, 1841)	PT GS	
	* <i>Diploplecta communis</i> (Millidge, 1988)	PT GS	
	<i>Erigone prominens</i> (Bosenberg and Strand, 1906)	PT GS SU	
	<i>Erigone wiltoni</i> (Locket, 1973)	PT GS SU	
	* <i>Haplinis fucatina</i> (Urquhart, 1894)	PT GS	
	* <i>Haplinis mundenia</i> (Urquhart, 1894)	PT GS	
	* <i>Haplinis titan</i> (Blest, 1979)	PT	
	* <i>Laetesia germana</i> (Millidge, 1988)	PT GS	
	<i>Microctenonyx subitaneus</i> (O. Pickard-Cambridge, 1875)	PT	
	<i>Ostearius melanopygius</i> (O. Pickard-Cambridge, 1880)	PT GS	
	<i>Tenuiphantes tenuis</i> (Blackwall, 1852)	PT GS SU	
	Lycosidae	* <i>Allotrochosina schauinslandi</i> (Simon, 1899)	PT GS
		* <i>Anoteropsis hilaris</i> (L. Koch, 1877)	PT GS
Salticidae	* <i>Holoplatys apressus</i> (Powell, 1873)	PT GS	
	<i>Maratus griseus</i> (Keyserling, 1882)	PT GS	
Micropholcommatidae	* <i>Taphiassa punctata</i> (Forster, 1959)	PT	
Theridiidae	<i>Cryptachaea veruculata</i> (Urquhart, 1886)	PT GS	
	<i>Steatoda capensis</i> (Hann, 1990)	PT	