Nitrogen economies and trapping capacities of Australian tropical *Drosera*

Thesis submitted by

IRWAN LOVADI

In April 2020

For the degree of Doctor of Philosophy in College of Science and Engineering
James Cook University

STATEMENT OF ACCESS

I, the undersigned, author of this work, understand that James Cook University will make this thesis available for use within the University Library, via the Australian Theses Network, or by other means allow access to users in other approved libraries.

I understand that as an unpublished work, a thesis has significant protection under the Copyright Act and beyond this, I do not wish to place any restriction on access to this thesis.

Signature: Date: 29th April 2020

STATEMENT OF SOURCES

Declaration

I, the undersigned, author of this work, declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any other university or other institution of tertiary education.

Information derived from the published or unpublished work of others has been acknowledged in the text and list of references is given.

Signature: Date: 29th April 2020

STATEMENT OF CONTRIBUTION OF OTHERS

Financial support
Australian government through Australia Awards Scholarship provided financial support for my PhD study in James Cook University (JCU). I also thank Professor Holtum for providing funds to purchase equipment and to cover plant nitrogen analysis. I was also awarded a research grant from Skyrail Rainforest Foundation.

Advisors
Professor Joseph Holtum (JCU), Professor Simon Robson (University of Sydney) and Dr Charles Clarke provided advice and guidance in this project based on their scientific expertise.

Scholarship support assistance
Alex Salvador and Katherine Elliott provided support assistance during the period of my four-year scholarship.

Set up for research equipment
Wayne Morris (Laboratory and Technical Support Specialist at Division of Tropical Environments and Societies, JCU) assisted in developing equipment for adhesive force measurements.

Laboratory Facility Access
Dr. Christa Placzek granted access to the use of microbalance in the Advanced Analytical Centre (AAC), JCU.

Proofreading assistance
Dr. Leone Bielig kindly proofread the manuscript.

Insect and plant identification
Dr. Faye Christidis assisted with identification of some insects. Professor Betsy Jackes and Nanette Hooker helped identify non-carnivorous plant species.

Statistical assistance
Prof Simon Robson introduced R and R Studio to me and helped me develop skills in data analysis. Cesar Herrera Acosta assisted in multivariate data analyses with R Markdown.

Fieldwork
Brandan Espe and Bernard Bostock assisted me in getting permission to access private properties. Floris van der Leest and Andrew Crole (Seafarm Pty Ltd) kindly granted me permission to access private properties. The following people kindly helped me in the field for sample collection: Titan Ligita, Akbar Reza, Xue Kai Ngan, Abdulla Fazeel, and Firman Fachrudin Firmansyah.

ACKNOWLEDGMENTS

First of all, I thank the Government of Australia for sponsoring my PhD study in Australia through Australia Awards. I acknowledge my advisory team: Professor Joseph Holtum (JCU) who supported me from the very beginning to thesis writing, Professor Simon Robson (University of Sydney), who got me excited about R and R Studio, and Dr. Charles Clarke, also a part of the team who showed me *Drosera schizandra* in Mt. Bartle Frere, Wooroonooran National Park, QLD for the first time. I sincerely thank all three of you for assisting me to become an independent researcher.

I also want to thank the Queensland Government for granting me a permit to take, use and keep insect and plant samples for my research. I would like to acknowledge the financial support from Skyrail Rainforest Foundation so I could complete some parts of my project. Tammy Walsh, staff at the College of Science and Engineering, is thanked for helping me with paperwork for field trips. I thank Barry Williams for providing help in the use of JCU vehicles for field trips. I also thank Lexie Edwards for organising satellite phones and first aid kits for my field trips. Akbar Reza, Xue Kai Ngan, Abdulla Fazeel, and Firman Fachrudin Firmansyah are thanked for helping in data collection in the field.

Next, thank you to Titan Ligita, my wife and my special volunteer in all field trips to Mt Bartle Frere, Wooroonooran National Park, QLD. Her endless support helped me to complete this thesis. Above all, I thank God Almighty for the many blessing He bestows upon me and my family during my PhD journey.

THESIS ABSTRACT

Drosera is the most species-rich carnivorous plant genus on the planet. As with other carnivorous plants, *Drosera* can acquire nutrients via trap-based strategies. The traps often complement nutrient uptake from roots. The traps used by *Drosera* use adhesive and non-adhesive tentacles on the leaf surfaces to ensnare prey. The dynamics and extent of nutrient uptake, and the contributions of soil and prey N to a plant may vary between species and with plant habitat. As a group, *Drosera* occupy a broad range of environments, most of which have moist, low-N soils, and have reasonably open canopies. However, some *Drosera* inhabit shaded closed canopy forests. Nutrient uptake, the patterns of predation and the trapping capacity of species that grow in closed canopy forests, particularly those growing in tropical Australia, have rarely been explored.

This thesis examined predation patterns, N content and biomass allocation to plant parts and trapping capacity of *Drosera* species inhabiting different habitats. Four native Australian carnivorous *Drosera* were selected for this research: *D. adelae* F. Muell., *D. schizandra* Diels, *D. burmanni* Vahl. and *D. spatulata* Labill. *Drosera adelae* and *D. schizandra* are rainforest species endemic to north Queensland, whereas *D. burmanni* and *D. spatulata* are open-woodland/grassland species with wide distributions in Australia. The four species were chosen because they occupy different habitats in which N-use strategies might be expected to differ.

Predation patterns differed among the *Drosera* examined and across seasons. As a whole, *Drosera* principally trapped Araneae, Coleoptera, Diptera, Entomobryomorpha, Formicidae, Hemiptera, Hymenoptera and Orthoptera. Formicidae were the most common prey for *D.*

burmanni, whereas *D. adelae* frequently entrapped Diptera. *Drosera adelae* trapped a greater biomass of prey than *D. spatulata* and *D. schizandra*. The biomass of prey trapped by *D. burmanni*, an annual species of open-grasslands, was not dissimilar to that of *D. adelae*. Prey diversity was higher during the dry season in all species. Seasonal patterns in prey biomass were demonstrated in *D. adelae* and *D. spatulata* which trapped a lower biomass during the wet season than the dry season. Rainfall affected prey retention. *Drosera adelae* and *D. spatulata*, two species that inhabit reasonably open environments, lost trapped prey during rainfall, whereas *D. schizandra,* a rainforest understorey species, retained prey. Presumably, interception of rainfall by the canopy, and the channelling of water to stem-flow, prevented droplets from dissolving adhesive mucilage and from splashing prey from the leaves of *D. schizandra*. *Drosera adelae, D. schizandra* and *D. spatulata* exhibited little selectivity in prey, as the proportion and type of prey items captured matched that trapped by artificial adhesive traps placed alongside plants growing *in situ*.

Patterns in biomass allocation can differ between *Drosera* and co-existing non-carnivorous plants. *Drosera burmanni* displayed a higher biomass allocation to above-ground parts and a greater content of plant N than *Fimbristylis* sp, a co-existing non-carnivorous sedge. However, *D. adelae* and *D. spatulata* and co-existing non-carnivorous plants exhibited similar patterns in biomass allocation to roots and shoots, suggesting similar relative contributions of above- and below-ground processes to growth regardless of differences in nutrient-foraging strategies. When plant N content was compared between the *Drosera* examined, the greater content of plant N in *D. burmanni* is consistent with the argument that species growing under high-light but moist conditions have a higher N demand and the low N levels in the shaded rainforest understorey species, *D. schizandra,* supports the view of a low N requirement in species under low levels of light intensity. A relationship between prey

capture level and the contribution of prey N to total plant N was demonstrated; *D. adelae* exhibited both the greatest trapping of prey biomass and the highest contribution of prey N to total plant N.

Trapping capacity varied depending upon *Drosera* species and plant size. In this study, three components of trapping capacity were measured: tentacle density, volume of mucilage droplets and adhesive force exhibited by mucilage at the tentacular tips. A reduction of tentacle density was correlated with greater plant size among *D. adelae, D. schizandra, D. burmanni* and *D. spatulata*. However, the pattern was not demonstrated for volume of mucilage droplets and the adhesive force of mucilage at the tentacular tips. Volumes of mucilage droplets and tentacle adhesiveness for the rainforest species *D. adelae* and *D. schizandra* were not correlated with plant size. However, an increase of plant size was associated with increased volume of mucilage droplets in *D. burmanni* and *D. spatulata* and increased in tentacle stickiness in *D. burmanni*.

In a world first, nano-machined silican-tipped mico-force probes were used to quantify the adhesive force of mucilage extruded by, and attached to, individual tentacles. Mean leaf resting adhesive forces of between 654 and 3,358 $\mu N\ mm^{-2}$ were measured. The forces were compared to the maximum load lifting forces of flying insects. The *Drosera* examined here trapped prey with an upper dry mass cut-off of between 0.001 and 0.0005 g. The predicted escape capacity of the prey were between only $1/10^{th}$ and $1/25^{th}$ of the calculated potential trapping capacities of the *Drosera*. The disparity between the potential to trap prey of a certain size or mass and the smaller sizes or masses of the prey trapped indicates that not all factors that influence prey-capture were assessed. The understanding of prey trapping capacity would be improved by quantification of the adhesive capacity of mucilage under

different environmental conditions and during plant ontogeny, and by combined measurements of maximum vertical load lifting capacity and maximum horizontal load pulling capacity. The latter measurements would be of particular relevance for *D. burmanni*, which mainly trapped prey that do not fly, e.g. ants.

Table of Contents

List of Tables

List of Figures

Chapter 1. General Introduction

1.1 The diversity and characteristics of plant carnivory

Carnivory in plants has long fascinated biologists (Darwin, 1875), probably because it is an animal-associated trait and it is infrequently expressed across the plant kingdom. Recent molecular systematics revealed that carnivory has evolved in the flowering plants at least ten times at the ordinal level: once in the Nepenthales and the Oxalidales, twice in the Ericales, and three times in the Lamiales and in the Poales (Fleischmann et al., 2018a). There are *ca.* 800 carnivorous taxa (Ellison and Adamec, 2018) documented in five orders (Poales, Caryophyllales, Oxalidales, Ericales and Lamiales), 12 families and 19 genera (Givnish, 2015, Fleischmann et al., 2018a). However, carnivory has also been lost in some members of four families. The four families are Bromeliaceae, Dioncophyllaceae, Eriocaulaceae and Plantaginaceae (Fleischmann et al., 2018b). Carnivory is known in terrestrial, lithophytic, epiphytic, and aquatic lineages (e.g. *Aldrovanda*, *Utricularia* spp.).

In its broadest sense, a carnivorous plant is one that obtains nutrients and perhaps some energy from animal tissues. The organism may actively catch the animal on which it feeds, an act of predation; it may obtain nutrients from an animal that has already died, an act of scavenging; or it may digest faeces, coprophagy. In the cases of scavenging and coprophagy, nutrient uptake by a plant might be considered carnivorous rather than saprophytic if the plant exhibits behaviour that specifically seeks out dead animals or faeces.

Carnivorous plants are notable for their adaptations that trap animals, or their faeces, and digest them. Five types of trapping mechanism are recognised: pitfall traps, eel traps (also called lobster-pot traps), sticky traps (also called adhesive traps and flypaper traps), snap

traps, and suction traps (Król et al., 2012). Anatomically, all are probably modified leaves, a good example of convergent evolution.

Pitfall traps are essentially fluid-containing receptacles into which prey falls. The containers may be highly modified leaves that are shaped liked pitchers (Figure 1.1.A), as in *Cephalotus, Darlingtonia*, *Heliamphora, Nepenthes* (Figure 1.1.A) and *Sarracenia*, or tanks (phytohelmata) formed at coalescing leaf axils, as in *Brocchinia*, *Catopsis* and *Paepalanthus*. The construction of a pitcher typically involves a slick rim, a waxy region beneath the rim, and a bottom-most fluid zone containing digestive enzymes and communities of micro-organisms that assist in the breakdown of the prey. Depending upon species, the pitcher may be variously adorned with physical and chemical adaptations that attract prey or reduce their escape e.g. visual, taste and olfactory attractants, downward-pointing hairs. In a few species, pitchers may be used as repositories for faeces by vertebrates e.g. in Borneo, the treeshrew *Tupaia montana*, the woolly bat *Kerivoula hardwickii* and a nocturnal rat, *Rattus baluensis*, defecate into pitchers of various species of *Nepenthes* (Clarke et al., 2009, Grafe et al., 2011, Greenwood et al., 2011, Wells et al., 2011).

Eel-traps are subterranean or aquatic. In the genus *Genlisea* (Figure 1.1.B), the subterranean eel-traps are Y-shaped structures that support a fluid-containing digestive chamber with a long neck that may contain hairs directed towards the chamber (Taylor, 1991). The origin of the eel-traps in *Genlisia* is uncertain as, although ostensibly root-like, the evolution of carnivory in *Genlisea* is reportedly monophyletic with *Pinguicula* in which the traps are clearly derived from leaves. The aquatic eel-traps in *Sarracenia psittacina* are essentially submerged pitchers (Król et al., 2012).

Seven genera - *Byblis, Drosera* (Figure 1.1.C)*, Drosophyllum, Philcoxia, Pinguicula, Roridula* and *Triphyophyllum* (Pavlovic and Saganova, 2015) - produce sticky mucilage that entraps invertebrates. The sticky structures, usually located on photosynthetic organs, are multicellular mucilage or resin-producing glands that may be sessile, stalked or in pits (Renner and Specht, 2013, Givnish, 2015). When stalk-shaped, the glands are termed tentacles (Juniper et al., 1989, Król et al., 2012).

The closely related monotypic genera, the terrestrial *Dionaea muscipula* (Venus fly-trap) (Figure 1.1.D) and the aquatic *Aldrovanda vesiculosa*, possess snap traps which are bi-lobed traps that rapidly shut to enclose prey whenever internally-placed epidermal multicellular trigger hairs are touched.

Suction traps are physiologically complex organs exclusive to *Utricularia* (Figure 1.1.E), a rootless genus of aquatic and terrestrial taxa (Juniper et al., 1989). When triggered, the thin-walled bladder-shaped traps open and suck-in water that may contain prey, both autotrophic and heterotrophic. The bladders may contain commensal communities that exude hydrolytic enzymes. These microbes in the traps appear to be supported by organic substances excreted into the trap fluid by the host (Caravieri et al., 2014).

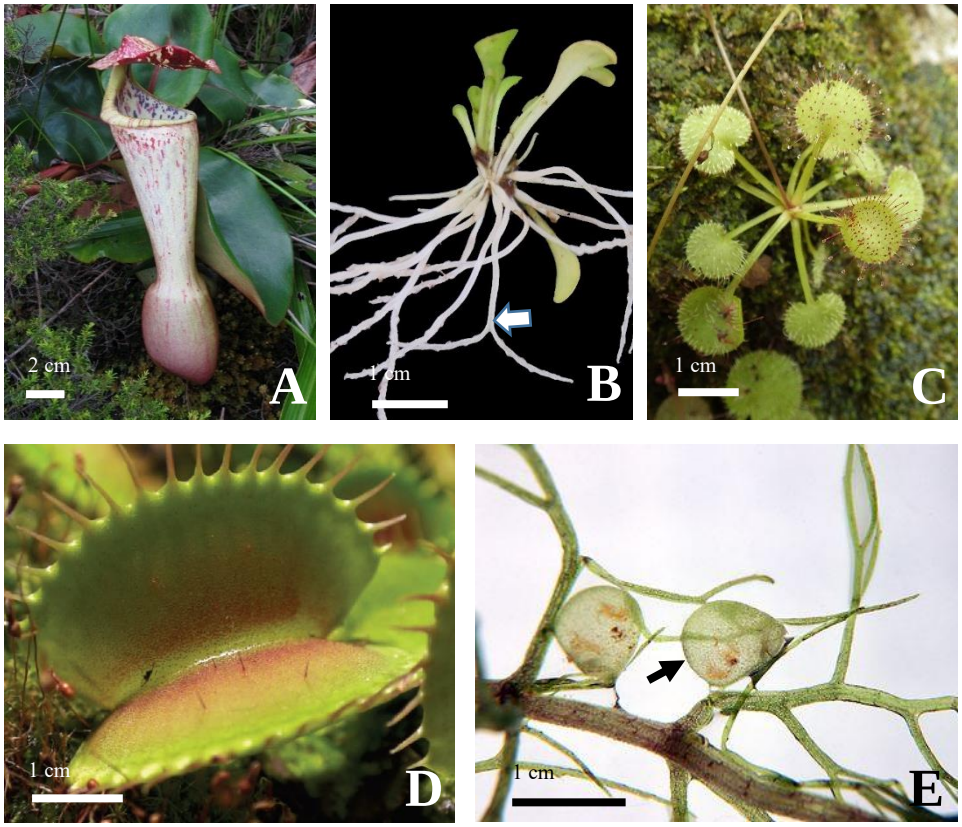

Figure 1.1 Trap diversity in carnivorous plants. A: Pitcher of *Nepenthes clipeata*, B: Eel traps of *Genlisea* – from Elhardt (2006), C: Flypaper traps of *Drosera prolifera*, D: Snap traps of *Dionaea* – from Pandolfi et al. (2014), E: Suction traps in aquatic *Utricularia* – from Rubes (2009).White and black arrows in Figure 1.1.B & E indicate traps of *Genlisea* and *Utricularia*, respectively.

The planetary centres of diversity of lineages of carnivorous plants are the Guyana Highlands of north-eastern South America, South East Asia, south-eastern United States, West Africa and Australia, particularly Western Australia (Ellison et al., 2003, Król et al., 2012). In each of these landscapes, the carnivorous species are mainly found in open and moist sites in nutrient-poor environments (Givnish et al., 1984), such as fenlands (swamps, marshes, creek and river banks), heaths, and periodically wet surfaces. Some taxa, for example several species of *Nepenthes* and *Drosera*, do however occur in low-light humid habitats of lowland and highland rainforests (Juniper et al., 1989).

The estimated 242 carnivorous angiosperm taxa in Australia constitute approximately 1 % of the flora, a level of carnivory that is roughly five times the global average (Lowrie, 2013). The high frequency of carnivory within the Australian flora may be attributed to the fact that carnivory is an adaptation that diversifies nutrient uptake and many Australian soils have low levels of macronutrients, especially nitrogen (below 0.1%) and phosphorus (below 0.01%) (Foulds, 1993, Cameron et al., 2013). Nevertheless, carnivory is relatively rare compared with other traits which enhance nutrient uptake, such as proteoid roots (*ca.* 1,600 species) and mycorrhizal associations (*ca.* 15,000 species), in Australia's nutrient-poor environments (Lamont, 2003, Lowrie, 2013, Brundrett, 2017). This is probably a result of the high metabolic cost of carnivory (e.g. production of traps, sticky substances and digestive enzymes) and the uncertainty of nutrient acquisition through prey capture (Ellison and Gotelli, 2009, Pavlovic and Saganova, 2015, Givnish et al., 2018).

1.2 Nutrient acquisition in carnivorous plants

Most vascular plants acquire mineral nutrients from their environments via their roots. The trap-based nutrient-foraging strategy of carnivorous plants enables them to complement nutrient uptake from roots with foliar uptake, facilitating competitive growth in nutrient-poor habitats (Adamec, 2013). Foliar nutrient acquisition requires attracting, trapping and digesting prey (mainly invertebrates) or nutrient-rich resources (e.g. faecal matter or leaf litter).

For many species, the capacity for prey attraction is a significant determinant for successful nutrient acquisition. Carnivorous plants employ a range of visual and olfactory cues to lure prey (Table 1.1). Visual stimuli involve trap colouration, including patterns in the ultraviolet spectrum, and trap structures that encourage visits. Olfactory and taste stimulants include the

production of fragrant organic volatiles and sugar-rich nectars (Juniper et al., 1989). For many taxa, prey attractants remain unknown, uncertain or disputed. For example, *Drosera* reportedly uses red trap-colouration to lure prey (Ichiishi et al., 1999), but Foot et al. (2014) revealed that red colouration in traps of *D. rotundifolia* has no role as a prey attractant. To date, there is a paucity of information pertaining to prey-attracting mechanisms for many *Drosera* as well as *Aldrovanda, Byblis, Paepalanthus bromelioides, Philcoxia minensis*, *Roridula* and *Utricularia*.

Table 1.1 Trap types and prey attractant mechanisms ascribed to carnivorous plant taxa.

Taxa	Trap	Prey attractants	Reference
Aldrovanda vesiculosa	Snap-trap	-	-
Brochinia	Pitfall	Fluid scent	Givnish et al. (1984)
Byblis	Flypaper	-	
Catopsis berteroniana	Pitfall	Ultraviolet marks	Frank and O'Meara (1984)
Cephalotus follicularis	Pitfall	Ultraviolet marks	Joel et al. (1985)
		Nectar	Parkes and Hallam (1984)
Darlingtonia californica	Pitfall	Nectar	Dixon et al. (2005)
Dionaea muscipula	Snap-trap	Ultraviolet marks	Joel et al. (1985)
		Blue fluorescence emissions	Kurup et al. (2013)
Drosera	Flypaper	Volatile chemicals	El-Sayed et al. (2016)
Drosophyllum lusitanicum	Flypaper	Ultraviolet marks	Joel et al. (1985)
		Scent	Bertol et al. (2015)
Genlisea	Eel-trap	Trap structure	Płachno et al. (2008)
Heliamphora	Pitfall	Ultraviolet marks	Joel et al. (1985)
		Volatiles	Jaffe et al. (1995)
Nepenthes	Pitfall	Red colouration of traps	Schaefer and Ruxton (2008)
		Blue fluorescence emissions	Kurup et al. (2013)
		Fluid scent/fragrance	Moran (1996), Di Giusto et al. (2010)
		Fragrance of the peristome	Di Giusto et al. (2008)
		Ultraviolet marks	Moran (1996)

Taxa	Trap	Prey attractants	Reference
Paepalanthus bromelioides	Pitfall	-	-
Philcoxia minensis	Flypaper	-	-
Pinguicula	Flypaper	Leaf colour	Zamora (1995)
Roridula	Flypaper	-	-
Sarracenia	Pitfall/Eel-trap	Nectar	Bennett and Ellison (2009)
		Volatiles	Jürgens et al. (2009)
		Blue fluorescence emissions	Kurup et al. (2013)
		Odour of decaying prey	Bhattarai and Horner (2009)
Triphyophyllum	Flypaper	-	-
Utricularia	Suction-trap	Remain unclear	See Albert et al. (2010)

The nutrient input from prey is also governed by patterns of prey capture and retention. Studies of prey diversity (Ellison and Gotelli, 2009), the amount of catch and catching period (Watson et al., 1982, Thum, 1989), and retention capacity (Zamora, 1995) demonstrate that predation patterns are important. In a meta-analysis of 30 studies on prey capture, Ellison and Gotelli (2009) concluded that nearly all carnivore-containing genera trap a variety of invertebrate prey (Figure 1.2). However, some groups of pitcher plants, namely *Brocchinia, Nepenthes* and *Sarracenia,* contain specialist predators.

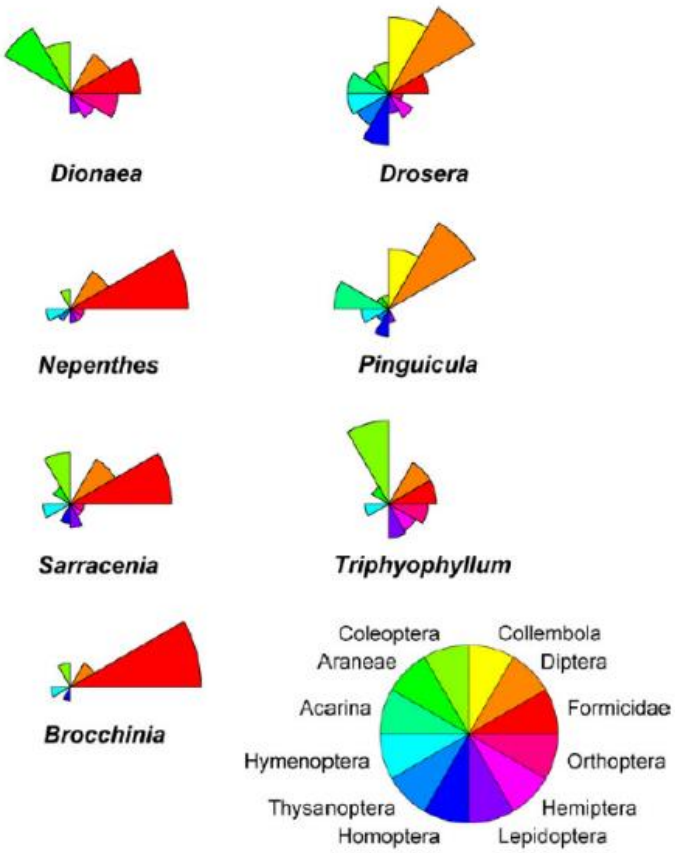

Figure 1.2 Prey diversity of terrestrial and epiphytic carnivorous plant genera. The slices of each star plot are scaled to the average proportion of each prey taxon (order except for ants - family Formicidae). The figure shows only the 11 most common prey orders and 1 family. The key to the colours is shown in the circle at lower right of the figure. The figure is from Ellison and Gotelli (2009)

Most carnivorous plants acquire nutrients, especially N, from both their prey and the soil (Christensen, 1976, Karlsson and Pate, 1992, Kruse et al., 2014). Differences in N supply from predation may be responsible for the large ranges of the relative contribution of prey N to total N within species and between relates species e.g. ranging from 20% to 60% in *Drosera rotundifolia* growing in European *Sphagnum* bogs (Millett et al., 2015), from 20 % to 54 % in *Drosera* spp from south-west Australia (Schulze et al., 1991), from 46 % to 80 % in *Dionaea* from North Carolina, USA (Schulze et al., 2001), 62 % in *Nepenthes mirabilis* from Queensland, Australia and 76 % in *Darlingtonia californica* from North Carolina, USA (Schulze et al., 1997).

Carnivorous plants have been reported to increasingly rely upon N derived from soil if the plants suffer from low prey capture (Schulze et al., 2001). Observations on photosynthetic performance on the pitcher plant *Nepenthes talangensis* supported the utilisation of N supply from soil in the absence of insect prey (Pavlovič et al., 2010). However, this concept has been challenged by studies demonstrating the lack of evidence for root nutrient uptake by *Drosera.* A study by Chandler and Anderson (1976) in *D. binata* concluded that unfed plants could not take advantage of the enriched soil environment to support optimum growth.

Despite the fact that most carnivorous plants source N from predation, the relative importance of nutrients supplied by prey may not be the same for all carnivorous plants. Insects for the pitcher plant *Sarracenia purpurea* L., for instance, are an essential source of P which is absorbed in preference to N (Wakefield et al., 2005). The similar pattern was also documented for *Drosera capensis* demonstrating effective P uptake over other nutrient elements (Pavlovič et al., 2014). As a result, N uptake from prey may vary across different taxa.

The dynamics of N acquisition is nonetheless a fundamental aspect of plant carnivory and studies of N-foraging strategies (Anderson and Midgley, 2002, Moran et al., 2003, Greenwood et al., 2011, Kruse et al., 2014), patterns of prey capture (Juniper et al., 1989, Ellison and Gotelli, 2009), mechanisms of foliar and root nutrient uptake (Adamec, 2002, Adlassnig et al., 2012, Adamec, 2013), and the contribution of prey N to total N content (Ellison and Gotelli, 2001, Gao et al., 2015, Millett et al., 2015) have revealed a variety of patterns that reflect the ways in which individual carnivorous species compete within and adjust to the complex ecological food webs they inhabit (Clarke, 1998, Sota et al., 1998). Only a small proportion of those studies have been undertaken using *Drosera* as model species.

Most studies of N uptake by *Drosera* have been on species from temperate, seasonally-cool, continually-moist swamplands typical of European and North American *Drosera* habitats. Nevertheless, studies of the forms of N acquired (Pavlovič et al., 2016), prey capture (Thum, 1986, Verbeek and Boasson, 1993), N uptake from prey (Dixon et al., 1980, Thum, 1989), contribution of prey N to total N (Schulze et al., 1991, Millett et al., 2003, Millett et al., 2012, Millett et al., 2015) and physiological responses to foliar and root uptake of N (Chandler and Anderson, 1976, Schulze and Schulze, 1990, Pavlovič et al., 2014) demonstrate plasticity in the ability of *Drosera* to draw upon both soil and prey N sources. Noticeably different N requirements and suites of prey trapped are to be expected for plants that variously may be large or small, and that live in high-energy high-light environments or in low-energy low-light environments. However, such variations are not well understood. *Drosera* growing on low-N soils occur under relatively low light-conditions in closed forests would be expected to differ in N demand (N uptake per unit time or mass) from those that inhabit more open savanna woodlands. Species under continuous low-light but moist conditions might have a low demand for N, whereas species subject to a short high-light moist growing season might grow faster and would have a higher N requirement, at least early in the growth season.

1.3 *Drosera* (the sundews)

Drosera (sundews) are herbaceous plants with diverse growth forms such as rosettes, scrambling or climbing plants and erect or self-supporting plants (Gibson and Waller, 2009). Members of the genus possess some distinct features that can be used to distinguish different functional groups. Tuberous *Drosera*, for example, produce an underground tuber (modified stolon) that allows the plant to re-grow after the dry seasons. Pygmy sundews (the smallest *Drosera*) form gemmae as a means of asexual reproduction. Another group of sundews, the *D. petiolaris* complex, has a dense cover of trichomes on their petioles that promotes

tolerance of desiccating conditions (Lowrie, 2013). Rainforest inhabiting *Drosera* tend to have broader leaves than non-rainforest species. This feature is presumably an adaptation to growth in low-light environments (Lavarack, 1979).

A distinctive characteristic of *Drosera* is the presence of adhesive-tipped stalked glands or tentacles on the upper surface of leaves. In most cases, the tentacles have some capability of movement towards prey (Juniper et al., 1989, Król et al., 2012), and may move rapidly, For example, tentacles of *D. glanduligera* can move with a maximum acceleration of 7.98 ms^{-2} (Poppinga et al., 2012).

Poppinga et al. (2013) identified four categories of tentacles: T0, T1, T2, and T3. T0 and T1-tentacles are adhesive, while the other two are non-sticky. T0-tentacles, present in all species of *Drosera*, are radially symmetric stalked glands and can bend toward prey in any direction. T1-tentacles have a similar shape to T0-tentacles, but these tentacles only bend in one plane and occur in the leaf margin of erect sundews. Some sundews also produce T2 and T3-tentacles on their leaf margin. These types of tentacles have a bisymmetric head, but they play different roles in prey capture. T2-tentacles provide the ability to retain trapped prey, while T3-tentacles are able to catapult prey to the centre of the leaf (Hartmeyer and Hartmeyer, 2010). The complement of tentacle types differs among *Drosera* species. *Drosera arcturi*, for instance, possesses T0-tentacles only, but *D. glanduligera* produces T0-, T1-, and T3-tentacles (Poppinga et al., 2013). Figure 1.3 shows the trap diversity found in four *Drosera* species: *D. arcturi, D. scorpioides, D. sessilifolia* and *D. glanduligera*.

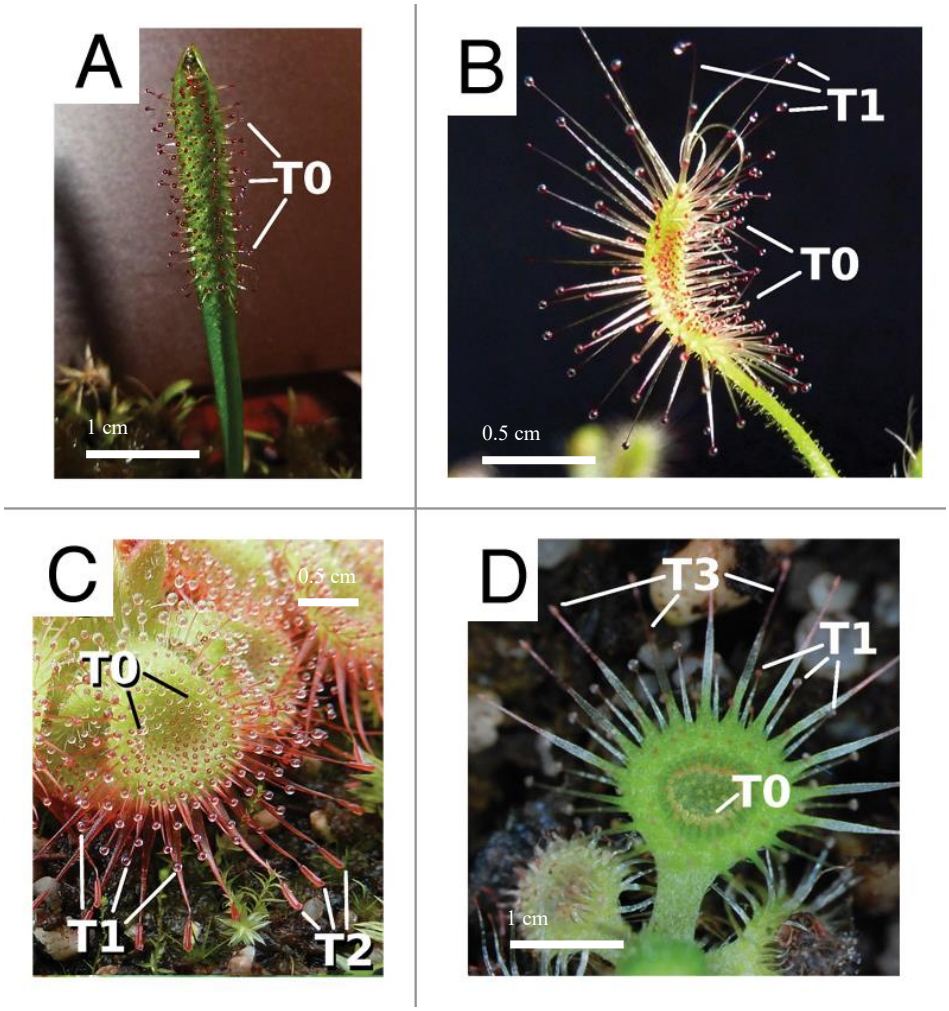

Figure 1.3 The configuration of tentacle types in four species of *Drosera*: *D. arcturi* with T0 tentacles (A), *D. scorpioides* has both T0 and T1 (B), *D. sessilifolia* feature T0, T1 and T2 tentacles (C) and *D. glanduligera* has T0, T1 and T3 (D). The image is from Poppinga et al. (2013). The arrows in each image indicate the type of tentacles as described in the labels.

Prey capture by *Drosera* is determined by the size and nature of the droplet of mucin on the head of each tentacle, by the density of tentacles (Figure 1.4) and by leaf adhesiveness. The mucin is generally an acidic polysaccharide that contains xylose, galactose, mannose, and glucuronic acid (Rost and Schauer, 1977, Gowda et al., 1982). Even though leaf adhesiveness is a determining feature of the capacity of *Drosera* to catch prey, investigations on the adhesive force of tentacles are scarce. Presumably this paucity reflects the difficulties of measuring accurately and precisely the small μNewton (μN) forces involved. The few studies on the mucin droplets and tentacle adhesiveness suggest that the adhesive force is in the order of 0.03 to 0.2 Newton cm^{-2} (Thorén et al., 2003, Cook et al., 2017). Aspects of the adhesive capacity of leaves of *Drosera* remain unclear: these include the amount of mucin produced, seasonal changes in the production of mucin, trap adhesiveness of *Drosera* species inhabiting different light regimes (sunny vs. shaded areas), and developmental and environmental factors that affect the production of the sticky mucin. Clearly, further investigations are warranted in order to increase our understanding of the adhesiveness of *Drosera* traps.

Figure 1.4 Mucin droplets on two different tentacle types (T0 and T1) on the leaf of *Drosera burmanni*.

Drosera occur in all vegetated continents. The limits of distribution are bounded by Tierra del Fuego (Argentina) and Chatham Islands (New Zealand) in the south and Alaska in the north, but the biodiversity hotspot of the genus lies in Australia (Robinson et al., 2017). Among roughly 200 known *Drosera* species worldwide, at least 163 species are found on the Australian continent and Tasmania. Although most diverse in temperate environments of Australia, particularly in south-western Western Australia, 32 species inhabit the tropics (Lowrie, 2013). Of the 22 species in Queensland, 13 have ranges that are overwhelmingly north of the Tropic of Capricorn. In addition to that, three of 13 species – *D. adelae, D. prolifera* and *D. schizandra* - are unusual as they are the only species in the world that inhabit shaded-rainforest conditions (Lavarack, 1979).

Drosera are widely distributed across north Queensland where they inhabit a range of environments from open woodlands in coastal regions to closed forests in mountainous areas (Figure 1.5). Species that occupy more open grassland encounter a wet season and followed by a relatively severe dry season, which most survive in a dormant state. By contrast, species that live under relatively low-light environments in closed woodland, such as rainforests, experience mild seasonal water-stress. One would expect *Drosera* inhabiting these differing environments might differ in N demand. Species under high light but moist conditions might grow faster and would be expected to exhibit a higher N demand, at least early in the growth season. Species grow under low-light environments in closed woodlands might be expected to exhibit lower growth rates and would thus have a low requirement for N. However, such patterns are scarcely examined in the literature. In this thesis, I will investigate N budgets and trapping capacity of *Drosera* that grow in more open and more shaded habitats.

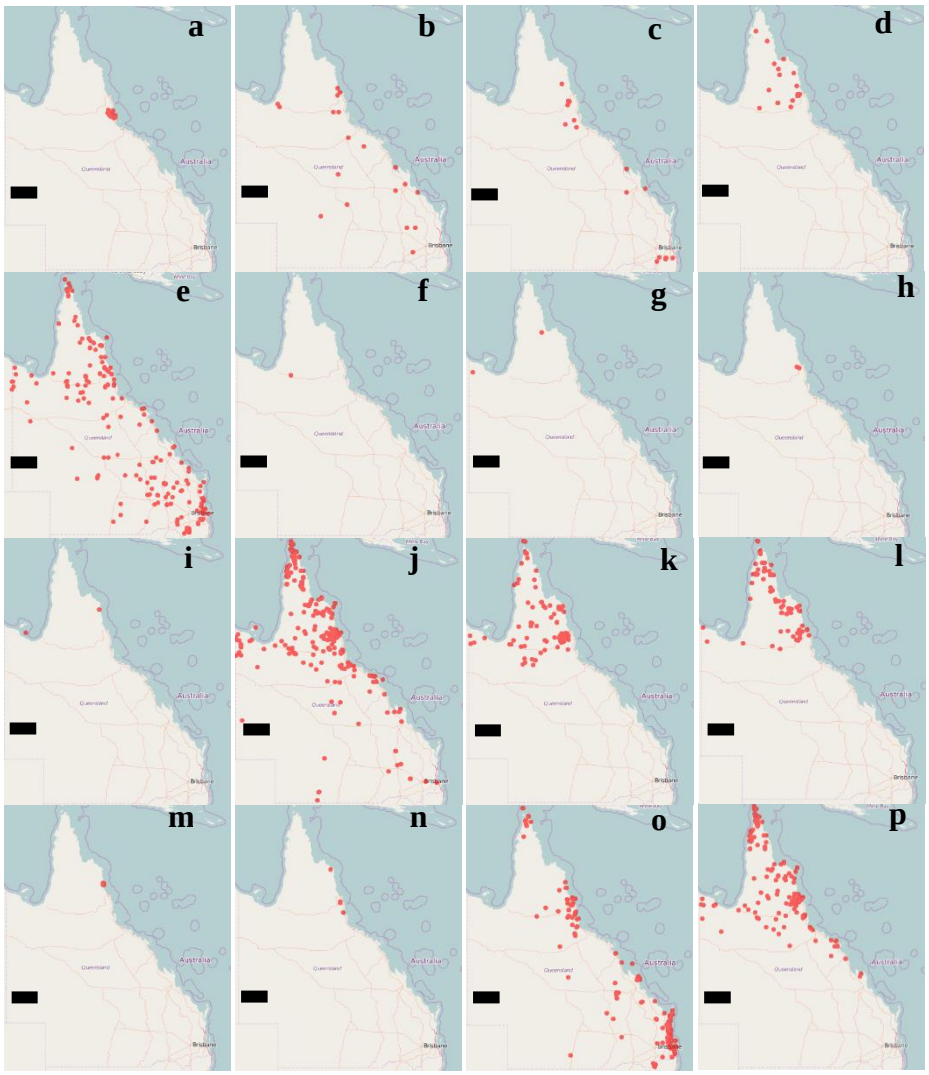

Figure 1.5 The localities of *Drosera* collected in Queensland and deposited in Australian herbaria (AVH 2016). *D. adelae* F.Muell. (a), *D. angustifolia* F.Muell. (b), *D. auriculata* Backh. ex Planch. (c), *D. banksii* R.Br. ex DC. (d), *D. burmanni* Vahl (e), *D. derbyensis* Lowrie (f), *D. dilatatorpetiolaris* Kondo (g), *D. finlaysoniana* Wall. ex Arn. (h), *D. fulva* Planch., (i), *D. indica* L. (j), *D. lanata* Kondo (k), *D. petiolaris* R.Br. ex DC. (l), *D. prolifera* C.T. White (m), *D. schizandra* Diels (n), *D. serpens* Planch. (o), and *D. spatulata* Labill. (p). Bars represent two hundred kilometres.

1.4 Research aims

In order to investigate the N economies and trapping capacities of four species of *Drosera* that inhabit open-grassland (*D. burmanni* and *D. spatulata*) or rainforests (*D. adelae* and *D. schizandra*) in north-eastern coastal Australia, I attempt to:

1) Determine insect predation patterns in four carnivorous *Drosera* of tropical north Queensland.

   This study investigated the predation patterns of two *Drosera* from open-grasslands and two from rainforests to address the following research questions: what are the relationships between *Drosera* species, seasons, and animals trapped? Do the *Drosera* studied specialise on a subset of available prey animals or does their diet simply reflect the insect community of their local habitat?

2) Document the N budgets of selected *Drosera* and co-existing non-carnivorous plants by examining N acquisition and contribution of N from prey and soil.

   I posit that the allocation of N and biomass to plant organs should differ between *Drosera* from open- and closed-woodlands, and attempt to test the hypothesis. The N contents of co-occurring non-carnivorous plants were compared and contrasted with *Drosera* from open-woodland and closed-woodland habitats to evaluate whether different N-foraging strategies are equally successful in obtaining plant N. Nitrogen stable-isotope ratios ($\delta^{15}N$) were used to quantify whether the N in the *Drosera* is prey- or soil-derived.

3) Quantify the trapping capacity of *Drosera* traps.

   Field observations and laboratory experiments were performed to measure tentacle density, volumes of mucilage droplets and adhesive force exhibited by tentacular tips. This study tested the hypotheses that trapping capacity varies with plant age and

adhesive force differs between tentacle types and light regimes. These experiments employed a nano-machined silicon-tipped micro-force sensors, a novel approach to quantify tentacle stickiness at the level of the individual tentacle.

Chapter 2. Study Species and Sites

2.1 Description of study species

This research is a study of N use in four carnivorous *Drosera*: *D. adelae* F. Muell., *D. schizandra* Diels, *D. burmanni* Vahl. and *D. spatulata* Labill. All are native to Australia. *D. adelae* and *D. schizandra* are endemic to north Queensland, whereas *D. burmanni* and *D. spatulata* have wide distributions in Australia that include north Queensland. The species were selected because they inhabit different vegetation complexes in which nitrogen-use strategies are likely to differ: *D. adelae* and *D. schizandra* are rainforest species, *D. burmanni* and *D. spatulata* are species of open grasslands and swampy areas.

2.1.1 *Drosera adelae*

Drosera adelae is one of the only three rainforest sundew species in the world (Lavarack, 1979, Lowrie, 2013). Together with *D. prolifera* and *D. schizandra, D. adelae* shares a distinct feature of rainforest understorey plants: large broad leaves (Lavarack, 1979). It is a perennial herb with an open rosette that can grow to 30 cm in diameter (Figure 2.1.A). In the juvenile stage, the leaves are obovate. As the leaves mature, they become lanceolate. Similarly, changes in leaf angle occur during development, starting from an erect orientation to a hanging position as they age. The terminal inflorescence, which is 25 - 35 cm long, bears many flowers which have green and lanceolate-sepals and red or cream petals (Lowrie, 2013).

Most sticky, mucilage-tipped tentacles on the lamina of *D. adelae* are T-0 tentacles which can bend toward trapped prey from any direction (Figure 2.1.B). A few tentacles at the leaf tips

are similar to T-1 tentacles which can move faster than other sticky tentacles on the leaf surface (Hartmeyer and Hartmeyer, 2010).

Drosera adelae is endemic to north Queensland from about Ingham north to Tully (Figure 2.2). It grows in open and shaded rainforest environments alongside creeks or on cliff faces with running water. Although mostly a species of the lowlands, some individuals have been collected from upland sites (Lavarack, 1979, Lowrie, 2013).

Figure 2.1 *Drosera adelae* growing in a shaded habitat along Arnott Creek in Bemerside, Queensland. Note the lanceolate leaves and rosette habit (A) and the sticky tentacles on the leaf surface (B).

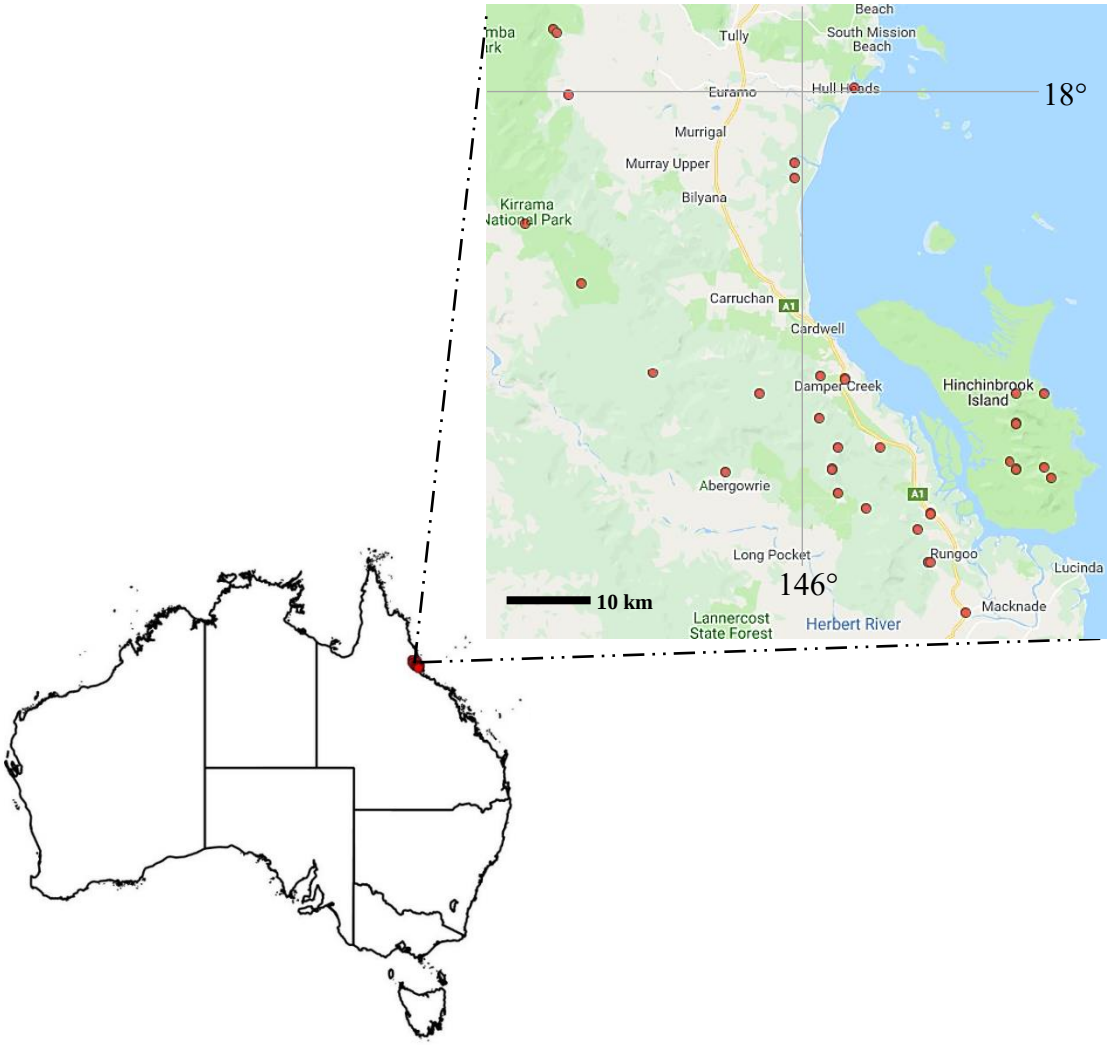

Figure 2.2 The distribution map of *Drosera adelae* (●) based upon the origins of herbarium specimens and sighting records. The map is generated from the Atlas of Living Australia website (http://www.ala.org.au)

2.1.2 *Drosera schizandra*

Drosera schizandra is a perennial herb which grows in a rosette that can reach up to 25 cm in diameter (Figure 2.3.A). The broader leaves compared to the other two rainforest sundews are consistent with deep shade-habitats in which *D. schizandra* grows (Lavarack, 1979). Leaves of mature plants are obovate and grow semi-erect. The inflorescence has a 12 – 14 cm long peduncle with several flowers which have greenish, lanceolate sepals with reddish purple petals (Lowrie, 2013).

The adhesive tentacles on the lamina of *D. schizandra* are predominately T-0 tentacles (Figure 2.3.B; Hartmeyer and Hartmeyer (2010)). The tentacles of mature plants usually remain sticky for two or three weeks and then the sundew loses its ability to catch prey. The plants regain trapping capacity when a new leaf develops (personal observation).

Drosera schizandra is confined to Mt. Bartle Frere, Wooroonoran National Park (Figure 2.4) with one outlier record specimen from nearby Bilyana (Atlas of Living Australia, 2018). Typical habitats of *D. schizandra* include creek banks and rivulets in upland rainforests (Lowrie, 2013).

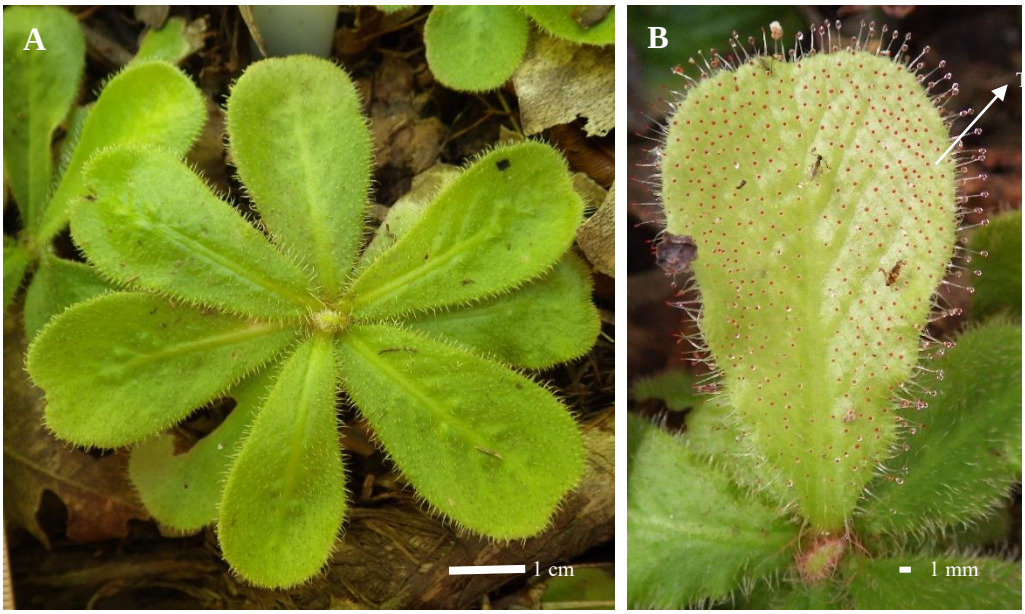

Figure 2.3 Large rosette of *Drosera schizandra* inhabiting the rainforest of Mount Bartle Frere, Wooroonooran National Park, Queensland. Note the obovate leaves and rosette habit (A), and the sticky tentacles on the lamina (B)

Figure 2.4 Distribution of *Drosera schizandra* (●) based upon the origins of herbarium specimens and sighting records. The map is generated from the Atlas of Living Australia website (http://www.ala.org.au)

2.1.3 *Drosera burmanni*

Drosera burmanni is an annual herb which produces a rosette close to the substrate surface (Figure 2.5). Leaves are obovate-spathulate, green to reddish, and have a deeply concave obovate-shaped centre (Lowrie, 2013). The length and width of leaves are about 8 - 10 mm and 5 - 6 mm, respectively. This species produces white or pale pink flowers with 5 - 20 cm long peduncle (Yanthan et al., 2017).

Drosera burmanni employs three types of tentacles to catch prey: T-0, T-1 and T-2 tentacles (Figure 2.5). The T-0 and T-1 types are sticky whereas T-2 tentacles are not (Hartmeyer and Hartmeyer, 2010, Poppinga et al., 2013). Unlike the two rainforest sundews investigated, *D. burmanni* is widely distributed in Australia, particularly in the tropics (Figure 2.6). The

sundew occupies more open habitats in wet seepage or swampy areas with various soil types: sand, peat and loam (Pandey and Saini, 2004, Lowrie, 2013).

Figure 2.5 Small rosette of *Drosera burmanni.* Note the three different tentacle types: T-0, T-1, and T-2

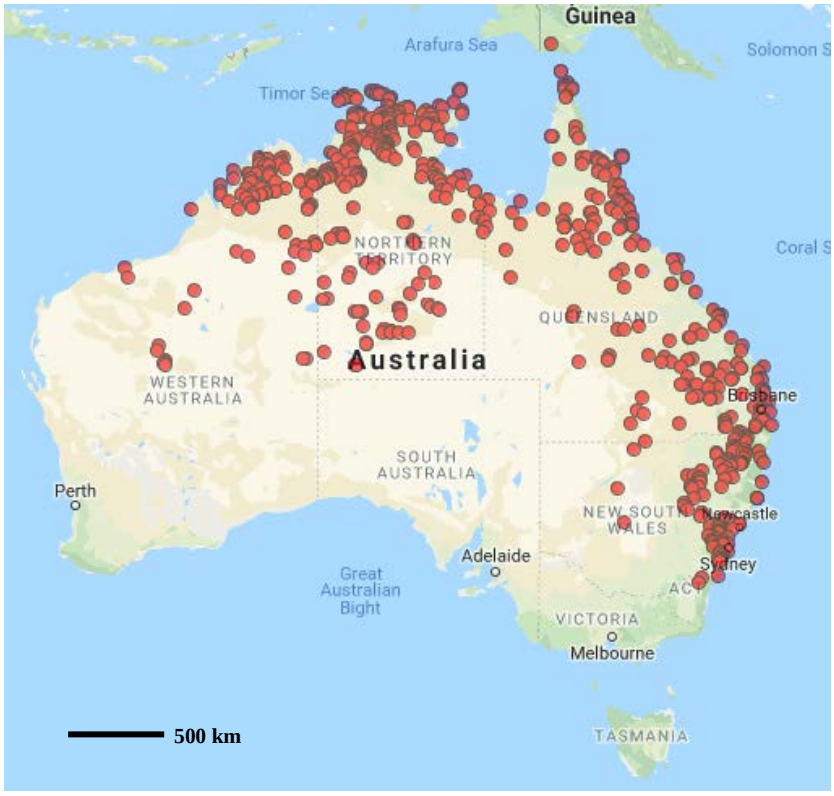

Figure 2.6 Distribution of *Drosera burmanni* (●) based upon origins of herbarium specimens and sighting records. The map is generated from the Atlas of Living Australia website (http://www.ala.org.au)

2.1.4 *Drosera spatulata*

Drosera spatulata is a perennial sundew which develops a flat rosette (Figure 2.7.A). Lowrie (2013) reported that the diameter of leafy rosettes can reach up to 7 cm, but most individuals in natural habitats are about 3 to 4 cm in diameter. Leaves are generally spathulate and either green or reddish depending upon the light intensity levels of their habitats (Lowrie, 2013). The inflorescence has one to five white or pink flowers with 6 - 20 cm long peduncle (El-Sayed et al., 2016). *Drosera spatulata* possesses two types of adhesive tentacles on the leaf surface: T-0 and T-1 (Figure 2.7.B). The T-0 tentacles are located at the centre of leaves, while T-1 tentacles are located along the leaf margin (Hartmeyer and Hartmeyer, 2010).

Drosera spatulata occurs both in temperate and tropical environments (Figure 2.8). The species inhabits open areas on swampland, heath, sandstone soils, and grasslands adjacent to ponds or marshes (Nakano et al., 2004, Lowrie, 2013).

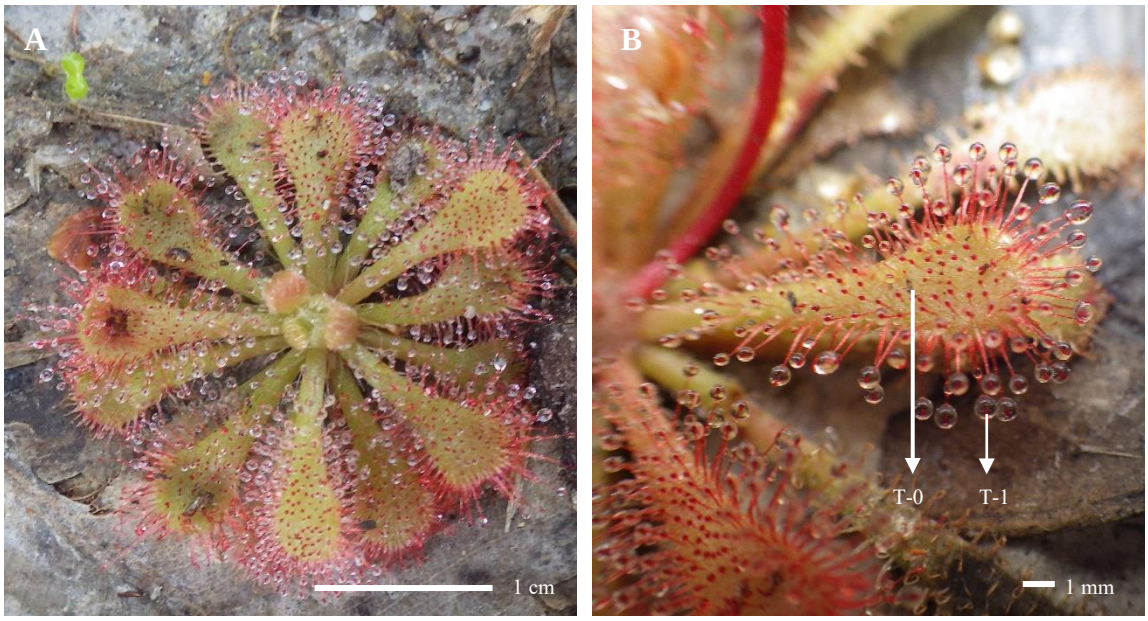

Figure 2.7 *Drosera spatulata* growing in an open coastal swampy area near Cardwell, Queensland. Note the rosette habit (A) and the two types of tentacles on the lamina (B).

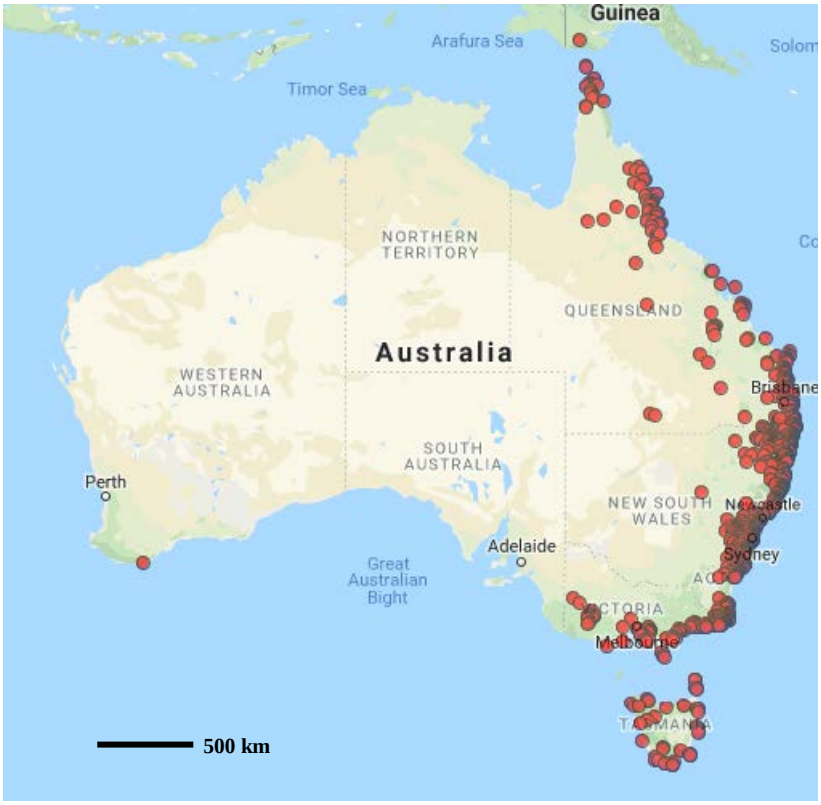

Figure 2.8 Distribution of *Drosera spatulata* (●) based upon origins of herbarium specimens and sighting records. The map is generated from the Atlas of Living Australia website (http://www.ala.org.au)

2.2 Study sites

Field work and sample collection of *Drosera* were carried out at four different locations within the Wet Tropics Management Area of north Queensland. Study of *D. schizandra* was conducted in the Mt Bartle Frere area of Wooroonooran National Park, Queensland, at approximately 17.396°S and 145.764°E, which is about 240 km north of Townsville (Figure 2.9). Tracey (1982) described the study area as complex mesophyll vine forest situated in moist uplands. Soils in the area are derived from basalts and alluvium. The annual total of rainfall for 2017 and 2018 was 3687.9 mm and 5035 mm, respectively, with 64% to 68% falling between January and April (Figure 2.10).

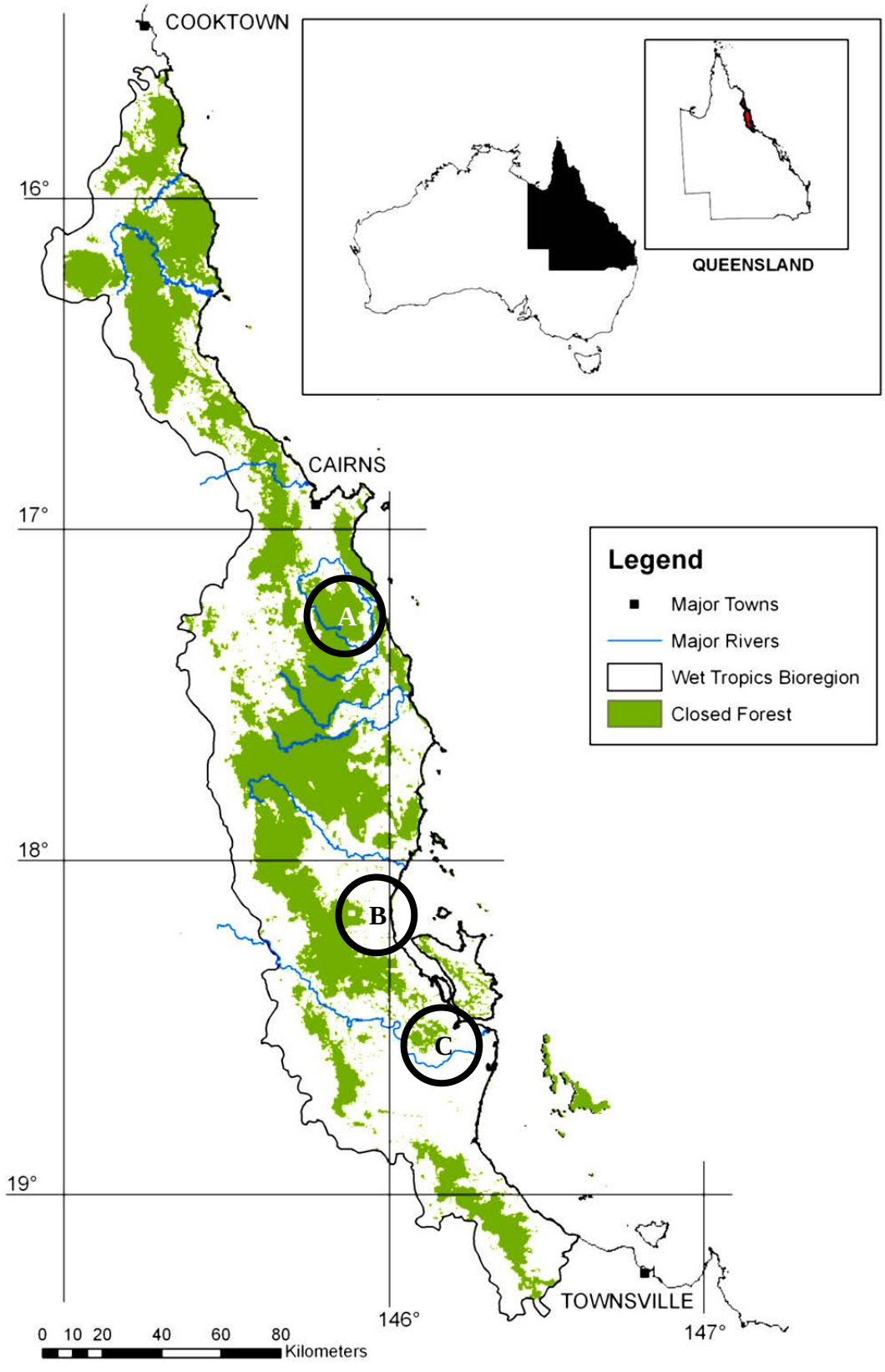

Figure 2.9 The Wet Tropics Area of north Queensland. The circles indicate the locations sites where *Drosera* were studied: *D. schizandra* (A), *D. burmanni* and *D. spatulata* (B) and *D. adelae* (C). Map modified from (Williams, 2006).

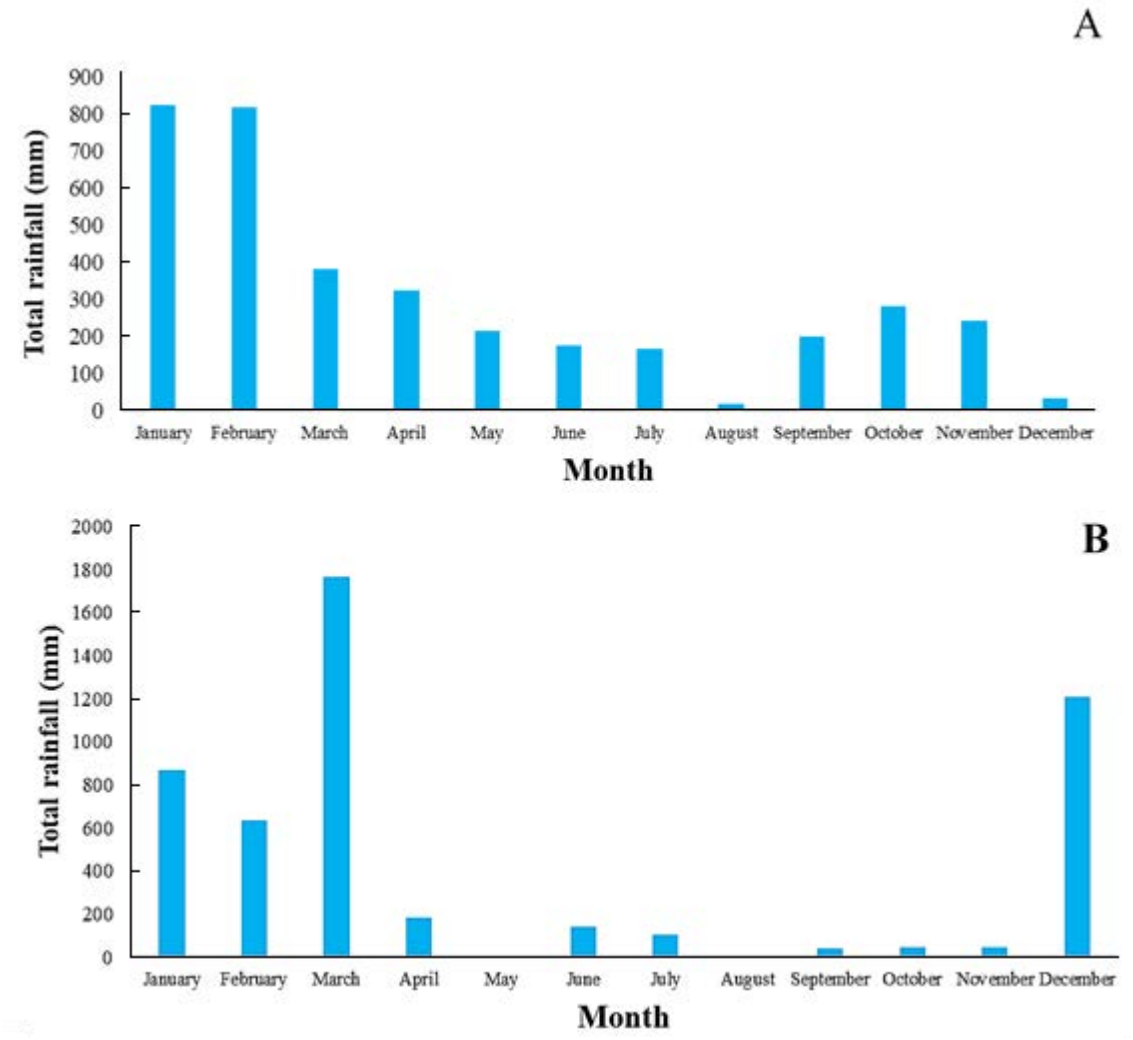

Figure 2.10 Monthly total rainfalls for the study site at Mt. Bartle Frere within the Wooroonooran National Park, Queensland for 2017 (A) and 2018 (B). Rainfall data were collected from the Russel River Station, which is about 2.5 km from Mt Bartle Frere (BOM, 2019). No published rainfall data for May 2018.

The *D. adelae* study site was at Arnott Creek, which is on private land at the foot of Mt Gardiner, Bemerside, Queensland (18.527ºS, 146.156ºE). The area is adjacent to Girringun National Park, approximately 100 km north of Townsville, Queensland (Figure 2.9). Wet Tropics Management Authority (2009) classified the area as *Syncarpia* forests and woodlands. The area is characterised by the presence of medium to tall open *Syncarpia glomulifera, Corymbia intermedia, Eucalyptus pellita* and various rainforest species in the understorey. The forests are mostly on soils derived from granites and metamorphics (Wet Tropics Management Authority, 2009). Annual total precipitation for 2017 and 2018 was

2044 mm and 3283 mm, respectively, of which 55 and 65% respectively fell between January and April (Figure 2.11).

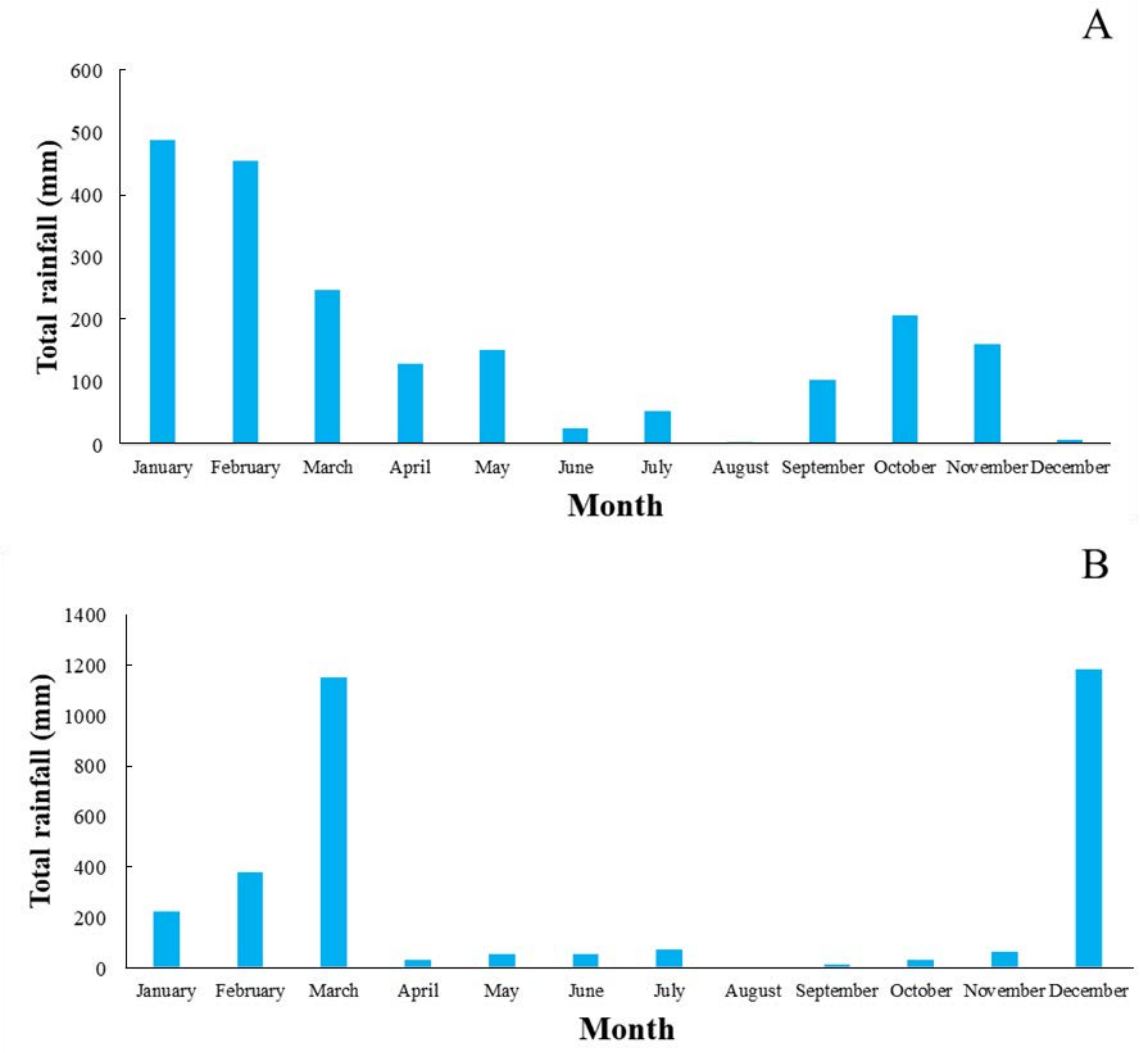

Figure 2.11 Monthly total rainfalls for the study site at Bemerside, Queensland for 2017 (A) and 2018 (B). Rainfall data were collected from the Cardwell Range Station, which is about 1.9 km from Bemerside (BOM, 2019).

The study site for *D. burmanni* was on a firebreak in Cardwell State Forest, Cardwell (18.336ºS, 146.057ºE), which is about 150 km north of Townsville, Queensland (Figure 2.9). Wet Tropics Management Authority (2009) described the area as medium *Melaleuca* forest and woodland (swamp). At this site the habitat of *D. burmanni* is a disturbed swampy

environment where seasonal flooding frequently occurs. Common plants in the firebreak where the sundews grow include sedges and *Utricularia* spp (personal observation). Annual total precipitation for 2017 and 2018 were 1910.6 mm and 2718.0 mm, respectively, of which about 65% fell between January and April (Figure 2.12).

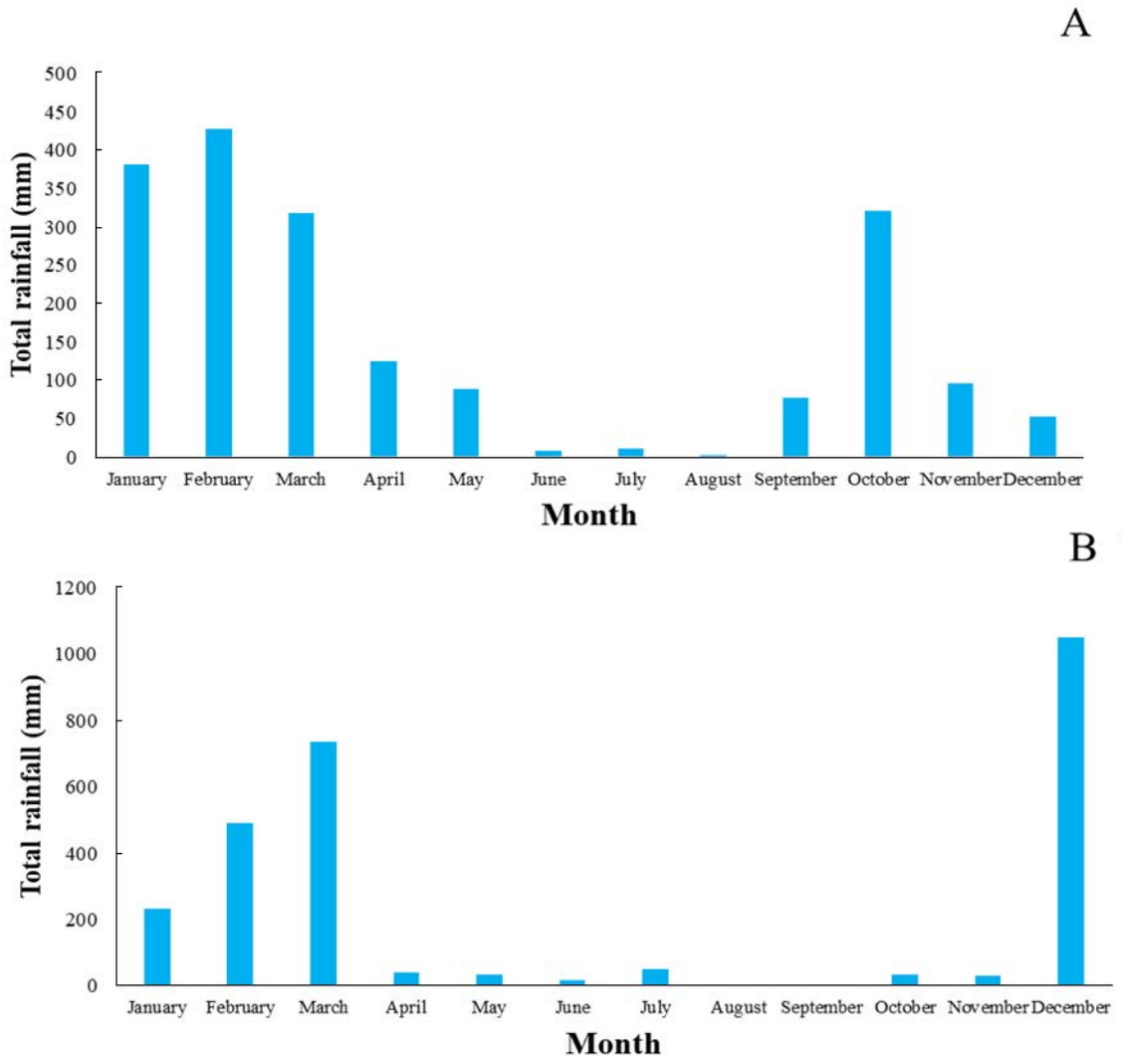

Figure 2.12 Monthly total rainfalls for the study sites at Cardwell, Queensland for 2017 (A) and 2018 (B). Rainfall data were collected from the Cardwell Marine Parade Station, which is about 1.7 km from Cardwell (BOM, 2019).

Study of *D. spatulata* was conducted on private land owned by Seafarm Pty Ltd, Cardwell, Queensland (18.330°S, 146.057°E), which is adjacent to the *D. burmanni* study site. According to Wet Tropics Management Authority (2009), the landscape is classified as low

Melaleuca woodland, which is characterised by very wet to dry lowland on alluvium. The area where the sundews grow is a former open woodland which has become grassland following the removal of the trees and which is frequently flooded during wet seasons (personal observation). Conspecifics with *D. burmanni* include sedges and salt-tolerant perennial tussock grasses. Rainfall patterns in the area are similar to those in the habitat of *D. burmanni*.

Chapter 3. Insect predation by four carnivorous sundews of tropical north Queensland

3.1 Abstract

Few studies have explored predation patterns in *Drosera* from tropical environments. Here I quantify, across seasons, the community of insects preyed upon by four Australian tropical *Drosera* species (both the relative contribution of different prey groups and the relative biomass of each prey group). As part of the study, the degree of diet specialisation was determined for two rainforest species (*D. adelae* and *D. schizandra*) and two grassland species (*D. burmanni,* and *D. spatulata*). In general, insect species consumed by the *Drosera* were Araneae, Coleoptera, Diptera, Entomobryomorpha, Formicidae, Hemiptera, Hymenoptera and Orthoptera. Diptera and Formicidae were particularly common prey. Mean dry mass of prey captured by each individual *Drosera* during a wet or dry season ranged roughly from 0.04 to 1.8 mg plant^{-1}. Prey composition and biomass differed both between the four species and seasonally. Prey diversity for example, was consistently highest in *D. adelae* and higher for all species during the dry rather than the wet season. *Drosera burmanni* consumed relatively more Formicidae than the other *Drosera* species. Seasonal patterns of prey capture were evident for *D. adelae* and *D. spatulata* when prey was assessed on a dry mass basis. In contrast, there were no differences in the insect communities consumed by *D. adelae, D. schizandra* and *D. spatulata* and the communities collected in neighbouring sticky traps, suggesting that these three species forage randomly upon the local insect community. Detailed patterns of prey consumption over time indicate that rainfall events can result in diminished foraging by *D. adelae* and *D. spatulata*, as evidenced by reduced numbers of insect prey on their leaves. However, such a pattern was not demonstrated by *D. schizandra*. Insect prey of this deep-shade rainforest species were observed to remain attached to the leaf

surface during rainfall events. The shaded, protected habitat of *D. schizandra* presumably reduces the frequency of rain droplets hitting leaves, reducing dissolution of mucilage and dislocation of prey.

3.2 Introduction

Prey capture is an essential feature of botanical carnivory. Carnivorous plants use a range of predation mechanisms (e.g. pitfall traps, sticky-traps and snap-traps) to supplement low levels of nutrients, principally N and P, in their environments (Juniper et al., 1989, Król et al., 2012, Fleischmann et al., 2018a). Differences in trapping mechanisms, plant habit and plant habitat contribute to carnivorous plants catching a variety of prey ranging in size from small aquatic invertebrates to small mammals (Harms, 1999, Harms, 2002, Wells et al., 2011, Plachno et al., 2014). However, most carnivorous plants, particularly terrestrial ones, consume insects, spiders and springtails (Ellison and Gotelli, 2009, Darnowski et al., 2018).

Drosera, the second most speciose group of carnivorous plants, employ sticky- and snap-tentacles on the leaf surface to capture their prey (Poppinga et al., 2013). The catch occurs when the prey animals land on the sticky tentacles or the snap tentacles. The latter can catapult prey animals to the more adhesive leaf centre. The *Drosera* then digest the captured prey using digestive fluids that are secreted onto the leaves (Krausko et al., 2017).

Predation patterns in *Drosera* have been investigated over the last three decades for species with different life-spans, growth forms and which inhabit a range of habitats (Watson et al., 1982, Thum, 1986, Thum, 1989, Verbeek and Boasson, 1993, Murza et al., 2006, Hagan et al., 2008, El-Sayed et al., 2016). In the main, the species investigated have been temperate species inhabiting moist swamplands in Europe and North America or dry woodlands in south-western Australia. The studies uniformly suggest that growth form, leaf shape and microhabitat all influence the composition of prey (Darnowski et al., 2018). Emergent plants (e.g. climbing and self-supporting species) predominantly capture aerial prey, while flat-rosette species catch mainly walking insects (Poppinga et al., 2013).

The goal of this chapter is to examine the variability in dietary ecology of selected Australian tropical *Drosera* by documenting the community of insects that comprise their prey, potential seasonal changes in diet, and the degree to which individual *Drosera* species may be selectively foraging on the available insect community. *Drosera*, although not common in tropical environments, occupy a range of habitats from coastal areas to mountain rainforests (Lowrie, 2013). Of the 32 tropical *Drosera* documented in Australia, 13 species are scattered in the north of Queensland.

On the basis that patterns of predation by *Drosera* might reflect not just habitat but also light regimes and the extent of seasonal water-stress, I examine here the predation patterns of two *Drosera* from rainforest habitats (*D. adelae* and *D. schizandra*) and two from open-grasslands (*D. spatulata* and *D. burmanni*). The data is used to explore 1) the influences of *Drosera* species and season on the identity of insect prey consumed, and 2) whether the *Drosera* are specialising on a subset of available insect species. The work on *D. adelae* and *D. schizandra* is the first investigation on predation patterns of rainforest sundews.

3.3 Methods

3.3.1 Patterns in prey capture

Prey capture on each *Drosera* was investigated in four locations as detailed in Chapter 2. Study of *D. adelae* was carried out at Arnott Creek, Queensland (18.527°S, 146.156°E). The study site of *D. schizandra* was in the Mt Bartle Frere area of Wooroonooran National Park, Queensland (17.396°S and 145.764°E). Study of *D. burmanni* was conducted in Cardwell State Forest, Queensland (18.336°S, 146.057°E). The study site of *D. spatulata* was on private land owned by Seafarm Pty Ltd, Queensland (18.330°S, 146.057°E). The number of observed plants and prey sampling time for each *Drosera* are presented in Table 3.1. Samples

from each *Drosera* species were collected at four-day intervals, except for *D. schizandra* which was sampled weekly. The sampling interval was chosen based on my personal observation indicated that most prey items were identifiable between four to seven days. Due to frequent National Park closures during the wet season, *D. schizandra* was sampled on only five occasions. Prey samples of the fast-growing *D. burmanni* were collected during the dry season only when the plants reached maturity. Plants died back as the dry season progressed.

Table 3.1 Timeline for fieldwork during the dry and the wet seasons

Study species	Number of observed plants	Sampling period	
		Dry season	Wet season
Drosera adelae	17	September and October 2016	February and April 2017
Drosera spatulata	30	September and October 2016	February and April 2017
Drosera schizandra	27	August and October 2017	March and April 2018
Drosera burmanni	30	September and October 2017	-

Captured prey were gently removed by forceps and transferred into 75% ethanol. Before identification, intact prey was separated from prey debris. Prey were identified to the lowest taxonomic level possible, and size was measured. Prey animals were first identified to order using a key to Australia's terrestrial invertebrates (Harvey and Yen, 1990). Further identification at superfamily and family levels were completed using a key to the insects of Australia (Naumann, 1991). Prey size is defined as the length of the head and abdomen (i.e. appendages are excluded). Prey dry mass was measured indirectly by constructing regression equations that described the correlation between prey volume and dry mass. To develop the regression equations, 50 recently trapped prey were collected from unmarked plants. The length of all samples was measured, and the volume of each prey item was calculated

following Thum (1989). After the measurements, insect samples were oven-dried for 72 hours at 60°C, and their dry mass were measured. The linear regression analysis showed that prey volumes are significantly associated with prey dry mass (R^2= 0.870, $F_{1,49}$ 321.9, p < 0.01). The regression equation for predicting prey dry mass is dry mass $_{(mg)}$ = 0.056 $_{(mg)}$ + 0.113 $_{(mg/mm3)}$ prey volume $_{(mm3)}$. Using this equation, prey dry mass per plant was also calculated.

3.3.2 Comparisons in prey capture between natural and artificial traps

To test whether *Drosera* captures prey in proportion to its local abundance, or whether they specialise in a subset of prey species, the contents of *Drosera* traps and artificial sticky traps of shape and size identical to the *Drosera* traps were compared. In this experiment, three *Drosera* species were examined: *D. adelae, D. schizandra* and *D. spatulata.* To create artificial traps, life-size images of *Drosera* species coated with waterproof glue (Tree guard, Go Natural) were mounted on flathead nails (see Appendix 3.1 for details). Each artificial trap was placed near selected plants (n = 15) and left standing for four days with the exception of *D. schizandra*. The plant models of *D. schizandra* were left standing for a week (access to the *D. schizandra* site required a multiple-hour trek). Trapped insects in natural traps were collected at the same time as those in artificial traps either at four-day or weekly intervals depending upon the species being studied. Artificial traps were replaced with new traps after prey collection. The procedure was repeated three times for both plants and traps. The trapped invertebrates were identified to the lowest taxonomic level possible, and the measurements of prey length and dry mass followed the procedures described above.

3.3.3 Statistical analyses

Prey compositional differences between *Drosera* species, seasons and trap types were examined with Non-metric Multidimensional Scaling (NMDS) and Permutational Multivariate Analysis of Variance (PERMANOVA) within the *vegan* package in R (Oksanen et al., 2019). Prior to multivariate data analyses, the number of prey groups was consolidated by grouping them into the order level except for Formicidae, with any rare orders (less than 1% of total prey items) excluded from the analysis. Dissimilarity matrices using prey species composition data used the Jaccard method, while those using prey species biomass involved the Bray-Curtis method on log-transformed data. Stress plots were used to confirm the fit of all models. Wilcoxon tests were used to further assess differences in prey dry mass as a function of plant species and trap type. Scatterplots of data visualisations were constructed using the *ggplot2* function in R (Wickham et al., 2019). Mean-standard error plots of data visualisation was carried out using the *ggpubr* function in R (Kassambara, 2019)

3.4 Results

3.4.1 The influence of species and season on prey capture in *Drosera*

3.4.1.1 The identity and species composition of insect communities consumed by Drosera

A total of 1902 prey items were collected across the four *Drosera* species, of which 63.3%, 27.7%, 0.05% were identified to family, order, and superfamily, respectively. The remaining percentage (8.9%) were digested prey items, which were classified as unidentified prey. In total, there were 74 prey groups, of which 59 groups were at the family level, 14 groups at the order level and 1 group at the superfamily level. Of the total prey items, 20.1% were Formicidae, 15.9% unknown Dipterans, 9.0% Phoridae, 8.9% unidentified prey, 8.3% Sciaridae, 4.5% Chironomidae, 3.9% Entomobryomorpha, 2.9% Hydrophilidae, 2.5%

Dolichopodidae and 2.4% Araneae. No other group exceeded 2% of the total number of prey items.

Prey composition varied between *Drosera* species and differed with season (Table 3.2). *Drosera adelae*, which trapped 55 prey groups, captured a broader spectrum of prey groups compared to the other sundews which trapped between 27 and 37 prey groups (Table 3.3). The prey of *D. adelae, D. schizandra* and *D. spatulata* were dominated by winged insects, with Diptera the most abundant. In contrast, *D. burmanni* frequently caught ants, Formicidae (Table 3.4). Prey groups that were exclusive to *D. adelae, D. burmanni* and *D. schizandra* included Culicidae, Drosophilidae, Hydrophilidae, Leptophlebiidae, Hebridae, Pseudoscorpionida and Ptiliidae.

The prey taxa captured by *Drosera* during the dry and wet seasons are shown in Table 3.2 The range of prey captured was more diverse during the dry season (Table 3.2), a phenomenon consistent with larger Shannon index values (Table 3.3).

Table 3.2 Prey composition in four *Drosera* during dry and wet seasons. The figures are the total number of prey items from all observed plants per season. n = the number of observed plants for each species.

Taxa	*D. adelae* (n = 17)		*D. schizandra* (n = 27)		*D. spatulata* (n = 30)		*D. burmanni* (n = 30)
	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	Number of individuals						
Acarina	3	0	1	2	0	0	1
Araneae	6	0	5	2	10	1	21
Coleoptera							
Cerylonidae	0	1	0	0	0	0	0
Hydraenidae	3	0	0	0	0	1	0
Hydrophilidae	0	55	0	0	0	0	0
Noteridae	0	0	0	0	1	1	0
Ptiliidae	1	0	0	0	0	0	0
Scotylidae	2	0	0	0	0	0	0
Staphylinidae	10	1	1	0	0	0	3
Unknown Coleopterans	10	1	2	0	2	0	13

Taxa	*D. adelae* (n = 17)		*D. schizandra* (n = 27)		*D. spatulata* (n = 30)		*D. burmanni* (n = 30)
	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Diptera							
Cecidomyiidae	4	6	3	3	3	1	0
Ceratopogonidae	2	2	1	0	0	1	1
Chironomidae	1	26	3	41	14	0	1
Cryptochaetidae	2	0	0	0	0	0	0
Culicidae	6	5	0	0	0	0	0
Dolichopodidae	4	34	2	0	5	1	2
Drosophilidae	21	2	0	0	0	0	0
Empididae	1	0	0	0	0	0	0
Ephydridae	1	0	0	0	0	0	0
Fanniidae	0	0	0	0	1	0	0
Lauxaniidae	1	0	0	0	0	0	0
Muscidae	0	0	0	0	1	0	0
Mycetophilidae	6	1	0	0	0	0	0
Neurochaetidae	2	0	0	0	0	0	0
Phoridae	85	25	28	22	9	0	3
Psychodidae	7	2	0	4	0	0	0
Scatopsidae	2	0	0	0	0	0	0
Sciaridae	121	4	25	5	1	0	2
Sciomyzidae	1	0	0	0	0	0	0
Tachinidae	0	0	1	0	0	0	0
Tipulidae	12	1	0	1	0	0	2
Unknown Dipterans	139	33	44	21	47	9	10
Entomobryomorpha	27	6	4	0	16	16	5
Ephemeroptera							
Leptophlebiidae	0	2	0	0	0	0	0
Hemiptera							
Aphididae	1	0	0	0	0	0	2
Cicadellidae	0	1	13	0	1	0	1
Debridae	1	0	0	0	0	0	0
Delphacidae	1	0	0	0	1	0	0
Fulgoroidea	0	0	0	0	0	0	1
Gerridae	0	0	0	0	0	0	1
Hebridae	1	0	0	0	0	0	33
Mesoveliidae	1	0	0	0	0	0	0
Mesovellidae	0	0	0	1	0	0	1
Reduviidae	0	0	2	2	0	0	0
Triozidae	1	0	0	0	0	0	0
Unknown Hemipterans	6	3	2	1	4	1	11
Hymenoptera							
Aphelinidae	0	0	0	1	0	0	0
Aulacidae	0	0	0	1	0	0	0
Bethylidae	0	0	0	1	1	0	0
Braconidae	2	0	2	1	1	0	0
Diapriidae	5	3	9	1	2	0	1
Eulophidae	0	0	1	0	0	0	0
Eupelmidae	0	0	1	1	0	0	0
Formicidae	23	7	3	6	24	1	318
Ichneumonidae	10	0	0	0	0	0	0
Megalyridae	0	0	0	1	0	0	0
Megaspilidae	0	0	3	0	0	0	0
Mymaridae	0	1	0	0	0	0	0
Platygastridae	1	0	1	0	0	0	0

Taxa	*D. adelae* (n = 17)		*D. schizandra* (n = 27)		*D. spatulata* (n = 30)		*D. burmanni* (n = 30)
	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Pteromalidae	0	0	0	1	0	0	0
Scelionidae	0	3	1	2	18	0	1
Unknown Hymenopteran	4	1	5	4	2	0	2
Lepidoptera							
Unknown Lepidopteran	2	0	1	0	0	0	0
Opilionida	1	0	1	2	0	0	1
Orthoptera							
Acrididae	2	0	0	0	2	0	3
Stenopelmatidae	0	0	0	0	3	2	2
Tetrigidae	0	1	0	0	0	0	0
Tridactylidae	0	0	0	0	5	0	3
Unknown Orthopteran	3	0	0	0	0	0	1
Pseudocorpionida	0	0	1	0	0	0	0
Psocoptera							
Unknown Psocopteran	1	0	0	0	0	0	0
Symphypleona	1	1	0	6	0	1	0
Trichoptera							
Hydroptilidae	0	1	0	0	0	0	0
Unknown Trichoptera'	1	0	0	0	0	0	0
Digested prey items							
Unidentified prey	41	23	11	17	36	5	37
Total	**589**	**252**	**177**	**150**	**210**	**41**	**483**

Table 3.3 Prey diversity of *Drosera* during observations at the dry and wet seasons. PGs: Number of Prey Groups, DS: the dry season, WS: wet season.

No	Species	Total PGs	PGs		Shannon Index	
			DS	WS	DS	WS
			Number of prey groups			
1	*D. adelae*	55	47	28	2.53	2.44
2	*D. schizandra*	37	28	25	2.49	2.37
3	*D. spatulata*	27	24	12	2.47	1.76
4	*D. burmanni*	28	28	-	1.34	-

Figure 3.1 documents seasonal changes in prey composition based on the top-five abundant prey groups captured by *D. adelae, D. schizandra* and *D. spatulata*. In *D. adelae*, captures of

unknown Dipterans, Phoridae and Sciaridae were dominant, but captures of these groups were less frequent during the wet seasons. At the same time, Hydrophilidae was the most common prey of *D. adelae* during the wet season (Figure 3.1.A). Similar patterns were also documented for *D. schizandra* and *D. spatulata* (Figures 3.1.B and 1-C). No prey were observed on the leaf surface of *D. adelae, D. burmanni* and *D. spatulata* when rain or showers occurred (Figure 3.1.A-B). By contrast, prey of *D. schizandra* remained on the traps (Figure 3.1.C).

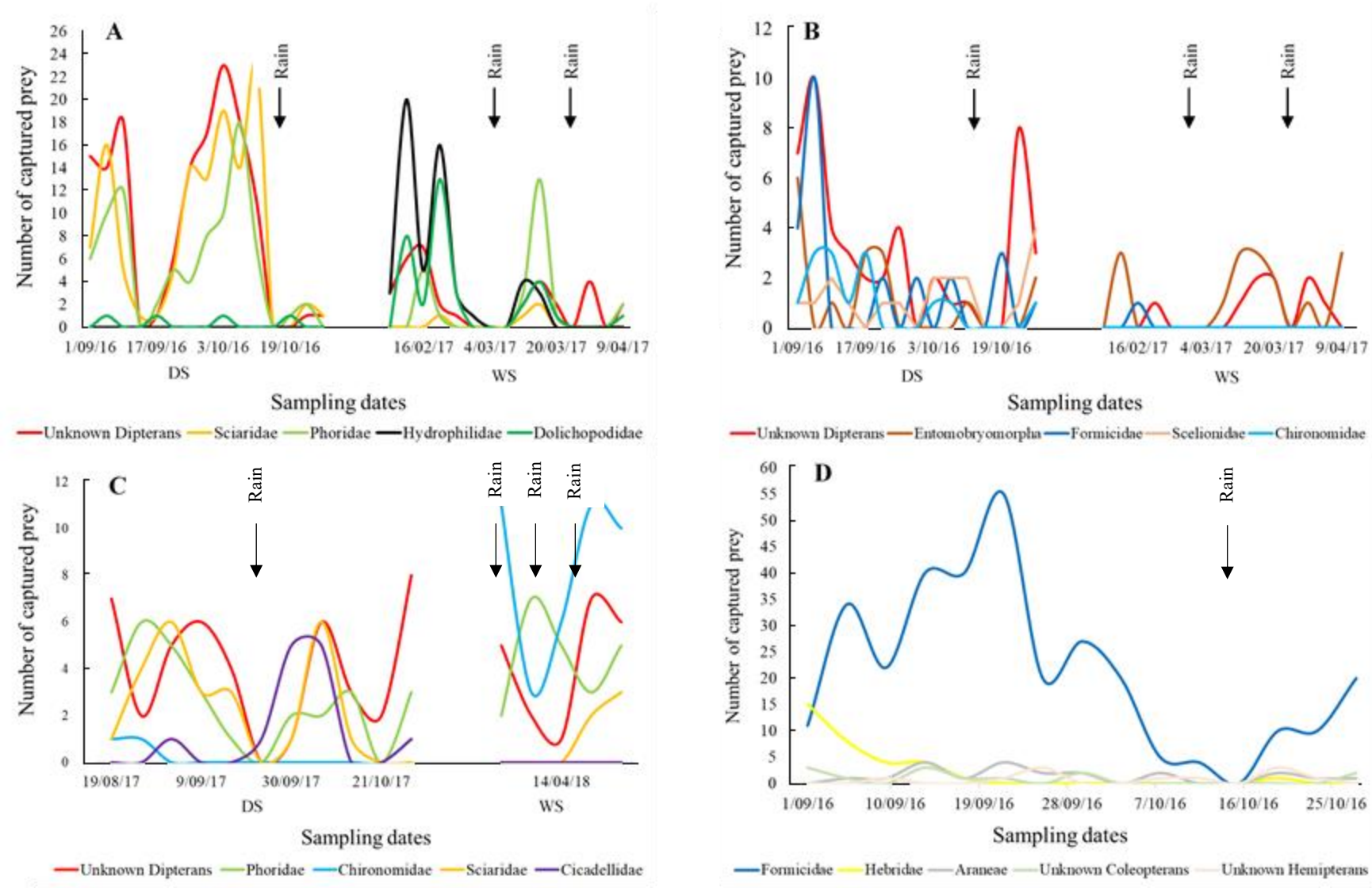

Figure 3.1 Changes in the total number of prey items of top-five abundant prey groups trapped by *D. adelae* (A), *D. spatulata* (B), *D. schizandra* (C) and *D. burmanni* (D) during dry and wet seasons. Prey collection for *D. burmanni* in the dry season only (see method section for details). DS: Dry season; WS: Wet season.

Table 3.4 A total number of captured prey items, captured by four species of *Drosera* during the dry and the wet seasons. Prey are listed at the level of order (excluding Formicidae) and rare orders are excluded. n = number of observed plants for each species.

Taxa	*D. adelae* (n = 17)		*D. schizandra* (n = 27)		*D. spatulata* (n = 30)		*D. burmanni* (n = 30)
	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	Number of prey						
Araneae	6	0	5	2	10	1	21
Coleoptera	26	58	3	0	3	2	16
Diptera	418	141	107	97	81	12	21
Entomobryomorpha	27	6	4	0	16	16	5
Formicidae	23	7	3	6	24	1	318
Hemiptera	12	4	17	4	6	1	50
Hymenoptera	22	8	23	14	24	0	4
Orthoptera	5	1	0	0	10	2	9

The NMDS ordination and PERMANOVA analysis based on data displayed in Table 3.4 confirmed differences in prey composition depending on *Drosera* species and seasons. Figure 3.2 shows separation of prey taxa assemblages caught by *Drosera* species (3.2A) and collected at different seasons (Figure 3.2.B). The vectors displayed in Figure 3.2.A indicate which prey groups are driving the underlying observed pattern in the composition of prey. These prey groups include Hemiptera, Formicidae, Hymenoptera and Entomobryomorpha. PERMANOVA analyses indicate significant differences in prey composition between *Drosera* (*Pseudo-*$F_{3,153}$= 18.020, $p < 0.01$) and between seasons (*Pseudo-*$F_{1,153}$= 4.625, $p < 0.01$). The analyses also showed a statistically significant interaction between species and seasons (*Pseudo-*$F_{2,153}$= 6.494, $p < 0.01$) indicating that season was the most important factor that affected the prey community of the *Drosera* examined (Figure 3.2.C).

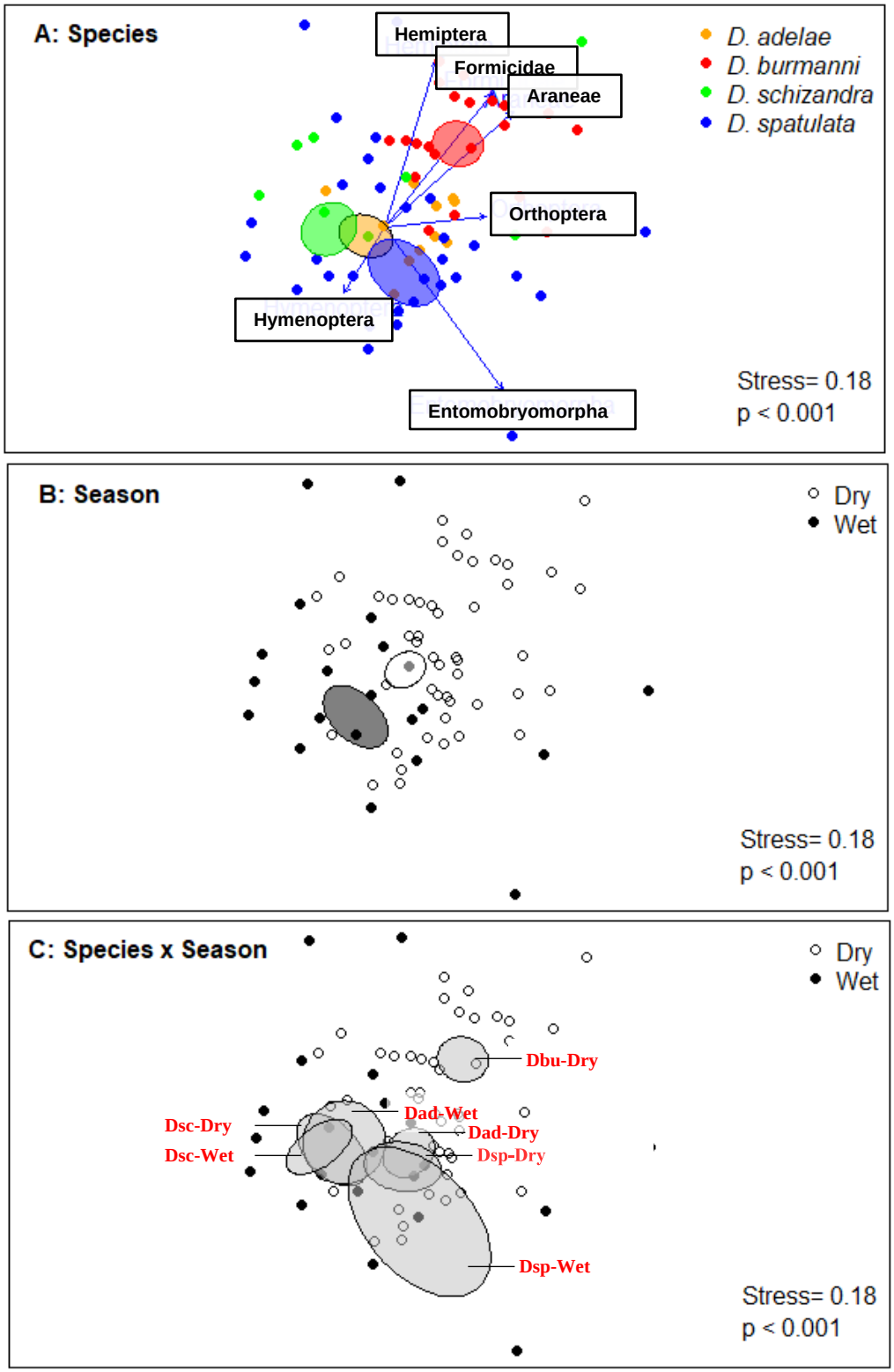

Figure 3.2 Changes in insect prey communities (based on the presence or absences of insect species consumed) between four *Drosera* species over the wet and dry season. Non-metric multidimensional scaling (NMDS) plots of distances among the centroids for two main factors: A) *Drosera* species (*D. adelae, D. burmanni, D. schizandra* and *D. spatulata*) and B) seasons (dry and wet), as well as C) interaction between factors based on Jaccard dissimilarities with presence-absence prey capture dataset. The dots with different colour represent prey communities for each *Drosera* species (Figure 3.2.A) or for each season (Figure 3.2.B-C). The coloured or transparent ellipses represent 95% intervals around the centroids and the standard errors. The *p* values indicate the level of significance based on PERMANOVA analysis. Dad-Dry: *D. adelae* - dry, Dad-Wet: *D. adelae* - wet, Dbu-Dry: *D. burmanni* -dry, Dsc-Dry: *D. schizandra* - dry, Dsc-Dry: *D. schizandra* – dry, Dsp-Dry: *D. spatulata* – Dry, Dsp-Wet: *D. spatulata* – wet.

3.4.1.2 The biomass and species composition of insect communities consumed by Drosera

Prey dry mass varied depending on *Drosera* species and according to seasons. In general, *D. adelae* and *D. burmanni* captured more prey items than *D. schizandra* and *D. spatulata* (Table 3.5). For *D. adelae,* captures of Sciaridae, Hydrophilidae, and Phoridae contributed to the majority of total prey dry mass, whereas catches of Formicidae and Hebridae contributed most of the prey dry mass for *D. burmanni*. The dominant types of captures in terms of prey dry mass across four *Drosera* species were Diptera and Formicidae (Table 3.6). This observation is consistent with the numbers of captured prey items shown in Table 3.4.

Prey dry mass changed with the season, especially for *D. adelae* and *D. spatulata* (Figure 3.3). For *D. adelae* and *D. spatulata*, mean prey dry mass per plant were significantly higher during the dry season than that during the wet season (Wilcoxon test, $p < 0.05$, Figure 3.3). However, prey dry mass per plant in *D. schizandra* do not differ between the dry and wet seasons (Wilcoxon test, $p > 0.05$, Figure 3.3). The NMDS plots and PERMANOVA results based on data presented in Table 3.6 support the observed differences. There is a clear separation in the NMDS plots between *Drosera* (Figure 3.4.A) and seasons (Figure 3.4.B). The vectors of prey animals displayed in Figure 3.4.A indicate which prey groups contribute to the observed pattern. PERMANOVA analysis shows that there are statistically significant differences in prey dry mass for two main factors: *Drosera* (*Pseudo*-$F_{3,143}$= 22.998, $p < 0.01$) and seasons (*Pseudo-*$F_{1,143}$ = 4.765, $p < 0.01$), and interaction in the effect of *Drosera* and seasons (*Pseudo-*$F_{2,153}$ = 5.169, $p < 0.01$, Figure 3.4.C).

Table 3.5 Total dry mass (mg) of prey of each taxon trapped by *Drosera* during the dry and wet seasons. n = number of observed plants for each species.

Taxa	*D. adelae* (n = 17)		*D. schizandra* (n = 27)		*D. spatulata* (n = 30)		*D. burmanni* (n = 30)
	Dry	Wet	Dry	Dry	Dry	Wet	Dry
	mg dry mass						
Acarina	0.201	0	0.074	0.132	0	0	0.099
Araneae	1.735	0	0.278	0.173	0.806	0.089	1.589
Coleoptera							
Cerylonidae	0	0.249	0	0	0	0	0
Hydraenidae	0.248	0	0	0	0	0.085	0
Hydrophilidae	0	8.855	0	0	0	0	0
Noteridae	0	0	0	0	0.101	0.101	0
Ptiliidae	0.070	0	0	0	0	0	0
Scotylidae	0.327	0	0	0	0	0	0
Staphylinidae	1.530	0.096	0.123	0	0	0	0.321
Unknown Coleopterans	0.964	0	0.171	0	0.159	0	1.356
Diptera							
Cecidomyiidae	0.390	0.300	0.074	0.195	0.207	0.068	0
Ceratopogonidae	0.214	0.163	0.077	0	0	0.080	0.064
Chironomidae	0	1.818	0.201	1.599	0.771	0	0
Cryptochaetidae	0.396	0	0	0	0	0	0
Culicidae	0.610	1.107	0	0	0	0	0
Dolichopodidae	0.418	3.169	0.322	0	0.313	0.103	0.178
Drosophilidae	2.580	0.356	0	0	0	0	0
Empididae	0.105	0	0	0	0	0	0
Ephydridae	0.113	0	0	0	0	0	0
Fanniidae	0	0	0	0	0	0	0
Lauxaniidae	0.331	0	0	0	0	0	0
Muscidae	0	0	0	0	1.114	0	0
Mycetophilidae	0.761	0.109	0	0	0	0	0
Neurochaetidae	0.203	0	0	0	0	0	0
Phoridae	7.661	2.026	1.578	1.325	0.571	0	0.179
Psychodidae	0.161	0.072	0	0.194	0	0	0
Scatopsidae	0.194	0	0	0	0	0	0
Sciaridae	13.310	0.441	1.804	0.306	0.083	0	0.496
Sciomyzidae	0.264	0	0	0	0	0	0
Tachinidae	0	0	0.340	0	0	0	0
Tipulidae	2.267	0.107	0	0.072	0	0	0.692
Unknown Dipterans	0.314	0	0	0	0.160	0.065	0
Entomobryomorpha	1.817	0.596	0.426	0	0.841	0.716	0.435
Ephemeroptera	0	0.528	0	0	0	0	0
Leptophlebiidae	0	0.528	0	0	0	0	0
Hemiptera							
Aphididae	0.066	0	0	0	0	0	0.179
Cicadellidae	0	0.439	2.024	0	0.322	0	1.184
Debridae	0.287	0	0	0	0	0	0
Delphacidae	0.243	0	0	0	0.098	0	0
Fulgoroidea	0	0	0	0	0	0	0.117
Gerridae	0	0	0	0	0	0	0.507
Hebridae	0.175	0	0	0	0	0	4.142

Taxa	*D. adelae* (n = 17)		*D. schizandra* (n = 27)		*D. spatulata* (n = 30)		*D. burmanni* (n = 30)
	Dry	Wet	Dry	Dry	Dry	Wet	Dry
Mesoveliidae	0.091	0	0	0	0	0	0
Mesovellidae	0	0	0	0.080	0	0	0.186
Reduviidae	0	0	0.061	0.183	0	0	0
Triozidae	0.067	0	0	0	0	0	0
Unknown Hemipterans	0.272	0.091	0.078	0	0	0.064	0.840
Hymenoptera							
Aphelinidae	0	0	0	0.059	0	0	0
Aulacidae	0	0	0	0.077	0	0	0
Bethylidae	0	0	0	0.058	0.062	0	0
Braconidae	0.061	0	0.183	0.091	0.120	0	0
Diapriidae	0.332	0.147	0.556	0.065	0.134	0	0.067
Eulophidae	0	0	0.060	0	0	0	0
Eupelmidae	0	0	0.065	0.094	0	0	0
Formicidae	1.813	0.574	0.238	1.184	1.764	0.152	31.613
Ichneumonidae	1.011	0	0	0	0	0	0
Megalyridae	0	0	0	0.077	0	0	0
Megaspilidae	0	0	0.197	0	0	0	0
Mymaridae	0	0.060	0	0	0	0	0
Platygastridae	0.070	0	0.066	0	0	0	0
Pteromalidae	0	0	0	0.110	0	0	0
Scelionidae	0	0.211	0.080	0.183	1.114	0	0.065
Unknown Hymenopteran	0.100	0	0	0.059	0.080	0	0.058
Lepidoptera							
Unknown Lepidopteran	0.257	0	0	0	0	0	0
Opilionida	0.074	0	0.099	0.149	0	0	0.099
Orthoptera							
Acrididae	0.350	0	0	0	0.196	0	1.388
Stenopelmatidae	0	0	0	0	0.293	0.195	0.085
Tetrigidae	0	0.164	0	0	0	0	0
Tridactylidae	0	0	0	0	0.594	0	1.038
Unknown Orthopteran	0	0	0	0	0	0	0
Pseudocorpionida	0	0	0.080	0	0	0	0
Psocoptera							
Unknown Psocopteran	0.313	0	0	0	0	0	0
Symphypleona	0.072	0.083	0	0.379	0	0.062	0
Trichoptera							
Hydroptilidae	0	0.124	0	0	0	0	0
Unknown Trichoptera'	0.171	0	0	0	0	0	0
Total	**43.009**	**21.884**	**9.254**	**6.847**	**9.902**	**1.778**	**46.976**

Table 3.6 The combined dry mass (mg) of each taxon at ordinal level (rare orders were excluded) trapped by *Drosera*. n = number of observed plants for each species.

Taxa	*D. adelae* (n = 17)		*D. schizandra* (n = 27)		*D. spatulata* (n = 30)		*D. burmanni* (n = 30)
	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	mg dry mass						
Araneae	1.735	0.000	0.278	0.173	0.806	0.089	1.589
Coleoptera	3.138	9.200	0.294	0.000	0.259	0.186	1.677
Diptera	30.292	9.667	4.397	3.692	3.220	0.315	1.608
Entomobryomorpha	1.817	0.596	0.426	0.000	0.841	0.716	0.435
Formicidae	1.813	0.574	0.238	1.184	1.764	0.152	31.613
Hemiptera	1.201	0.531	2.163	0.263	0.420	0.064	7.155
Hymenoptera	1.575	0.418	1.205	0.874	1.509	0.000	0.190
Orthoptera	0.350	0.164	0.000	0.000	1.083	0.195	2.511

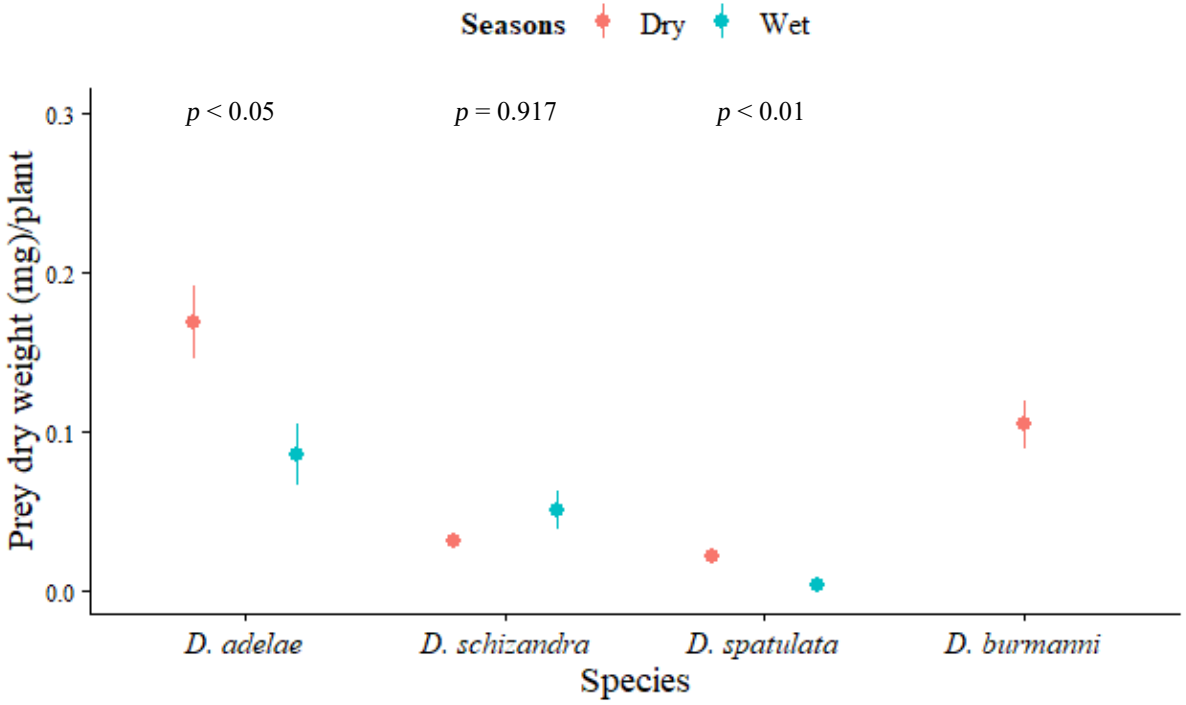

Figure 3.3 Mean (coloured dots) ± SE prey dry mass (mg)/plant in four *Drosera* during observations during the dry and wet seasons. The *p*-values lower than 0.05 or 0.01 above error mean plots for each species indicate significant differences in prey dry mass between seasons (Wilcoxon test).

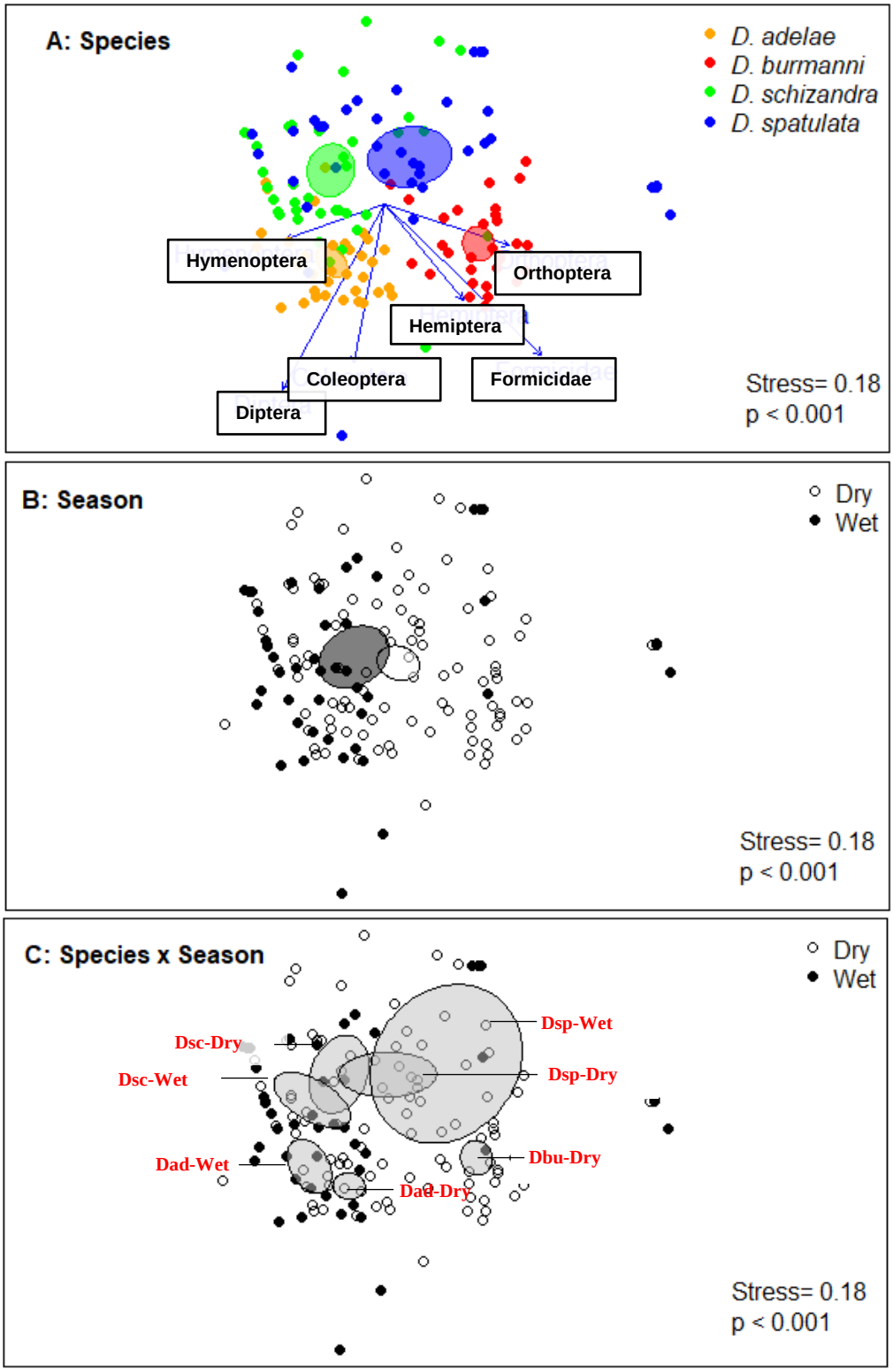

Figure 3.4 Changes in insect prey communities (based on the dry mass of insect species consumed) between four *Drosera* species over the wet and dry season. Non-metric multidimensional scaling (NMDS) plots of distances among the centroids two main factors: A) *Drosera* species (*D. adelae, D. burmanni, D. schizandra* and *D. spatulata*) and B) seasons (dry and wet), as well as C) interaction between factors based on Bray-Curtis dissimilarities of log-transformed prey-dry mass dataset displayed in Table 3.6. The dots with different colour represent prey communities for each *Drosera* species (Figure 3.4.A) or for each season (Figure 3.4.B-C). The coloured or transparent ellipses represent 95% intervals around the centroids and the standard errors. The *p* values indicate the level of significance based on PERMANOVA analysis. Dad-Dry: *D. adelae* - dry, Dad-Wet: *D. adelae* - wet, Dbu-Dry: *D. burmanni* -dry, Dsc-Dry: *D. schizandra* - dry, Dsc-Dry: *D. schizandra* – dry, Dsp-Dry: *D. spatulata* – Dry, Dsp-Wet: *D. spatulata* – wet

3.4.2 Comparisons of prey capture between natural and artificial traps

3.4.2.1 The composition of insect communities consumed by Drosera *versus artificial traps*

The prey trapped by both plants and artificial traps consisted of 19.9% Chironomidae, 17.6% Phoridae, 11.0% unknown Dipterans, 9.2% Poduromorpha, 7.5% Dolichopodidae, 6.9% Sciaridae, 6.2% Entomobryomorpha, and less than 2% for each other group. In total, there were 49 prey groups, excluding digested prey items (Table 3.7), and Diptera was the numerically dominant taxon trapped by Drosera and by the artificial traps (Table 3.8). Comparison of the number of captured prey by artificial and by natural traps, indicates variation, but no consistent pattern as shown in Table 3.7. For *D. adelae,* many more prey were caught by the plants than by the artificial traps. For *D. schizandra*, the reverse pattern was observed, the plants captured fewer prey compared to the artificial traps (Table 3.7).

A composition of prey captured by *Drosera* and the artificial traps is shown in Table 3.7. A high diversity of prey groups, the majority of which were flying insects belonging to Diptera and Hymenoptera, was captured by both the artificial and real *D. adelae* (Table 3.7, Table 3.8). The diverse range of prey groups trapped by *D. adelae* is reflected in the high values of the Shannon Index calculated for the species (Table 3.9).

The dominant prey taxa differed between the plants and their models. Common prey groups for natural traps include Phoridae, Sciaridae, and Entomobryomorpha, whereas Chironomidae and Poduromorpha were frequently collected by artificial traps, especially for *D. schizandra* (Table 3.7).

Table 3.7 Composition of prey captured by three *Drosera* species and their artificial trap analogues. The values are the total number of captured prey items from all observed traps. The number of artificial and natural traps for each species was 15.

Taxa	*D. adelae*		*D. schizandra*		*D. spatulata*	
	Artificial	Natural	Artificial	Natural	Artificial	Natural
	Number of prey captured					
Acarina	0	0	1	1	0	0
Araneae	0	3	1	0	0	1
Coleoptera						
Curculionidae	0	0	1	0	0	0
Staphilinidae	1	2	1	0	0	0
Unknown Coleopteran	1	5	4	0	1	0
Diptera						
Cecidomyiidae	1	3	0	2	0	0
Ceratopogonidae	1	0	0	0	0	1
Chironomidae	0	6	100	24	0	2
Culicidae	0	1	0	0	0	0
Dolichopodidae	3	4	0	0	36	7
Drosophilidae	0	11	0	0	0	0
Faniidae	1	0	0	0	0	0
Muscidae	1	1	0	0	1	0
Mycetophilidae	0	2	0	0	0	0
Neurochaetidae	0	1	0	0	0	0
Phoridae	29	47	38	2	0	1
Psychodidae	0	1	0	1	0	0
Sciaridae	3	24	8	9	0	2
Tachnidae	1	1	0	0	0	0
Tipulidae	2	0	0	0	0	1
Unknown Dipteran	3	29	2	11	11	17
Entomobryomorpha	5	5	14	0	5	12
Hemiptera						
Aphididae	0	1	0	0	0	0
Cicadellidae	0	0	0	0	2	1
Derbidae	1	0	0	0	0	1
Fulgoroidea	1	0	0	0	1	0
Unknown Hemipteran	1	0	0	0	1	3
Vellidae	0	1	0	0	0	0
Hymenoptera						
Braconidae	0	1	0	0	0	0
Ceraphronidae	0	0	3	0	0	1
Cynipidae	0	1	0	0	0	0
Diapriidae	2	2	0	0	0	2
Eulophidae	0	1	0	2	0	0
Formicidae	1	2	1	0	1	1
Ichneumonidae	1	0	0	0	0	0
Megalyridae	0	0	1	1	0	0
Megaspilidae	0	0	1	1	0	0
Mutillidae	0	0	0	1	0	0
Platygastridae	0	1	0	1	0	0
Scelionidae	0	1	0	0	5	3
Unknown Hymenopteran	1	0	2	1	3	1
Opilionida	0	0	2	1	0	0

Taxa	*D. adelae*		*D. schizandra*		*D. spatulata*	
	Artificial	Natural	Artificial	Natural	Artificial	Natural
Orthoptera						
Acrididae	1	0	0	0	0	0
Stenopelmatidae	1	0	0	0	1	2
Tetrigidae	1	0	0	0	0	0
Poduromorpha	0	0	58	3	0	0
Psocoptera	0	0	0	0	0	1
Symphypleona	0	1	2	0	0	0
Trichoptera						
Philopotamidae	0	1	0	0	0	0
Digested or non-intact prey items	0	3	0	5	0	4
Total	**63**	**162**	**240**	**66**	**68**	**64**

Table 3.8 The number of prey captured at the order level (rare orders are excluded) by three *Drosera* species and their artificial trap analogues. The number of artificial and natural traps for each species was 15.

Taxa	*D. adelae*		*D. schizandra*		*D. spatulata*	
	Artificial	Natural	Artificial	Natural	Artificial	Natural
	Number of prey captured					
Coleoptera	2	7	6	0	1	0
Diptera	45	131	148	49	48	31
Entomobryomorpha	5	5	14	0	5	12
Hemiptera	3	2	0	0	4	5
Hymenoptera	5	9	8	7	9	8
Opilionida	0	0	2	1	0	0
Poduromorpha	0	0	58	3	0	0

Table 3.9 Prey diversity captured by three *Drosera* species and their artificial homologues. PGs: Number of prey groups. The number of artificial and natural traps for each species was 15.

Species	Artificial traps		Natural traps	
	PGs	Shannon Index	PGs	Shannon Index
	Number of prey groups			
D. adelae	23	2.26	28	2.39
D. schizandra	18	1.72	15	1.98
D. spatulata	12	1.63	19	2.37

The NMDS ordination reveals that the taxonomic composition of captured prey differs depending on *Drosera* species (Figure 3.5.A). The vectors displayed in Figure 3.5.A indicate which prey groups are driving the underlying observed pattern in prey composition. However, there are no differences in prey composition between trap types (Figure 3.5.B). These results are supported by PERMANOVA analysis showing statistically significant differences in prey composition between *Drosera* (*Pseudo*-$F_{2,83}$= 4.001, $p < 0.01$), with no significant variation among trap types (*Pseudo*-$F_{1,83}$= 0.999, $p = 0.412$). However, the interaction between *Drosera* species and trap types was statistically significant (*Pseudo*-$F_{2,83}$= 5.714, $p < 0.01$), indicating at least one comparison between artificial and natural traps within the level of species is different (Figure 3.5.C).

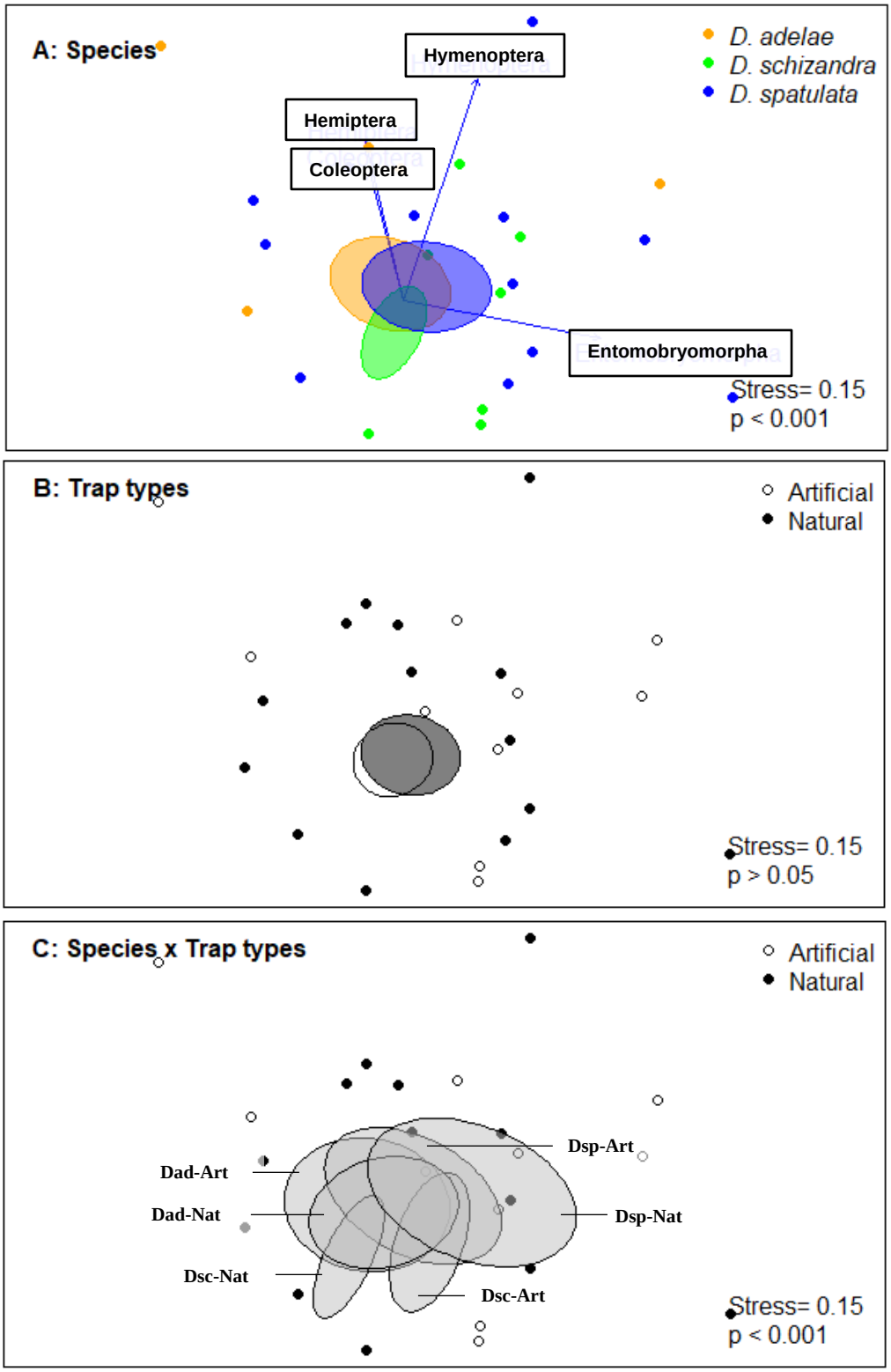

Figure 3.5 Insect prey communities (based on the presence or absences of insect species consumed) trapped by three *Drosera* species and by artificial analogue traps placed alongside them. Non-metric multidimensional scaling (NMDS) plots of distances among the centroids for two main factors: A) *Drosera* species (*D. adelae, D. burmanni, D. schizandra* and *D. spatulata*) and B) trap types (artificial and natural), as well as C) interaction between factors based on Jaccard dissimilarities with presence-absence prey capture dataset. The dots with different colour represent prey communities for each Drosera species (Figure 3.5.A) or for each trap type (Figure 3.5.B-C). The coloured or transparent ellipses represent 95% intervals around the centroids and the standard errors. The *p* values indicate the level of significance based on PERMANOVA analysis. Dad-Nat: *D. adelae* – natural traps, Dad-Art: *D. adelae* – artificial traps, Dsc-Nat: *D. schizandra* – natural traps, Dsc-Art: *D. schizandra* – artificial traps, Dsp-Nat: *D. spatulata* – natural traps, Dsp-Art: *D. spatulata* – artificial traps.

3.4.2.2 The biomass and species composition of insect communities consumed by Drosera

Both artificial and real *D. adelae* collected a greater amount of prey dry mass in comparison to the other carnivorous sundews (Table 3.10). Dolichopodidae, Drosophilidae, Phoridae, and Tipulidae were some captures that contributed to the high prey dry mass due to either frequent catch or prey sizes. Table 3.11 shows that Diptera was the most predominant capture in terms of prey dry mass across all three sundews. Even though total prey dry mass differed depending upon *Drosera* species, the Wilcoxon test revealed that only prey dry mass per trap for *D. schizandra* was significantly different between trap types (Figure 3.6). The NMDS ordinations and PERMANOVA results based on data displayed in Table 3.11 confirmed these observed differences. Figure 3.7 illustrates prey dry mass differences collected by *Drosera* (3.7A) and between trap types (Figure 3.7.B). The vectors displayed in Figure 3.7.A indicate which prey groups are driving the underlying observed pattern in prey dry mass. PERMANOVA analysis also reveals statistically significant differences in prey dry mass between *Drosera* (*Pseudo-*$F_{2,76} = 5.651$, $p < 0.01$), trap types (*Pseudo-*$F_{1,76} = 3.646$, $p < 0.01$) and interaction between *Drosera* and trap types (*Pseudo-*$F_{2,76} = 6.741$, $p < 0.01$, Figure 3.7.C).

Table 3.10 Total prey dry mass (mg) of captured prey groups by three *Drosera* species and their artificial trap analogues. Number of artificial and natural traps for each species was 15.

Taxa	*D. adelae*		*D. schizandra*		*D. spatulata*	
	Artificial	Natural	Artificial	Natural	Artificial	Natural
	mg dry mass					
Acarina	0	0	0.057	0.062	0	0
Araneae	0	0.105	0.062	0	0	0.104
Coleoptera						
Curculionidae	0	0	0.134	0	0	0
Staphylinidae	0.099	0.098	0.098	0	0	0
Unknown Coleopteran	0.242	1.232	0.301	0	0.164	0
Diptera						
Cecidomyiidae	0.089	0.182	0	0.116	0	0
Ceratopogonidae	0.096	0	0	0	0	0.060
Chironomidae	0	0.537	6.339	1.052	0	0.060

Taxa	*D. adelae*		*D. schizandra*		*D. spatulata*	
	Artificial	Natural	Artificial	Natural	Artificial	Natural
Culicidae	0	0.100	0	0	0	0
Dolichopodidae	1.583	0.626	0	0	3.504	0.586
Drosophilidae	0	1.200	0	0	0	0
Faniidae	0.421	0	0	0	0	0
Muscidae	0.349	0.891	0	0	0.765	0
Mycetophilidae	0	0.136	0	0	0	0
Neurochaetidae	0	0.228	0	0	0	0
Phoridae	2.550	4.301	3.198	0.074	0	0
Psychodidae	0	0.062	0	0.066	0	0
Sciaridae	0.526	1.721	0.693	0.418	0	0.134
Tachnidae	2.632	7.552	0	0	0	0
Tipulidae	71.402	0	0	0	0	0.147
Unknown Dipteran	2.632	0.221	0.059	0	0.587	0
Entomobryomorpha	0.741	0.250	0.905	0	0.405	0.518
Hemiptera						
Aphididae	0	0.063	0	0	0	0
Cicadellidae	0	0	0	0	0.188	0.098
Derbidae	0.322	0	0	0	0	0.525
Fulgoroidea	0.278	0	0	0	0.216	0
Unknown Hemipteran	0.130	0	0	0	0.104	0.135
Vellidae	0	0.113	0	0	0	0
Hymenoptera						
Braconidae	0	0.162	0	0	0	0
Ceraphronidae	0	0	0.179	0	0	0.058
Cynipidae	0	0.064	0	0	0	0
Diapriidae	0.171	0.127	0	0	0	0.142
Eulophidae	0	0.089	0	0.118	0	0
Formicidae	0.093	0.141	0.095	0	0	0.141
Ichneumonidae	0.141	0	0	0	0	0
Megalyridae	0	0	0.075	0.082	0	0
Megaspilidae	0	0	0.074	0.066	0	0
Mutillidae	0	0	0	0.101	0	0
Platygastridae	0	0.059	0	0.059	0	0
Scelionidae	0	0.186	0	0	0.367	0.120
Unknown Hymenopteran	0.056	0	0.113	0	0	0
Opilionida	0	0	0.116	0.121	0	0
Orthoptera						
Acrididae	0	0	0	0	0	0
Stenopelmatidae	0.131	0	0	0	0.087	0.307
Tetrigidae	0.141	0	0	0	0	0
Poduromorpha	0	0	3.590	0.133	0	0
Psocoptera	0	0	0	0	0	0.089
Unknown Psocoptera	0	0	0	0	0	0.089
Symphypleona	0	0.062	0.127	0	0	0
Trichoptera						
Philopotamidae	0	0.226	0	0	0	0
Total	**84.825**	**20.736**	**16.217**	**2.469**	**6.387**	**3.224**

Table 3.11 Total prey dry mass (mg) collected by different types of *Drosera* traps at the order level (rare orders were excluded). Number of artificial and natural traps for each species was 15.

Taxa	*D. adelae*		*D. schizandra*		*D. spatulata*	
	Artificial	Natural	Artificial	Natural	Artificial	Natural
	mg dry mass					
Coleoptera	0.342	1.330	0.533	0.000	0.164	0.000
Diptera	82.280	17.758	10.289	1.726	4.856	0.986
Entomobryomorpha	0.741	0.250	0.905	0.000	0.405	0.518
Hemiptera	0.730	0.177	0.000	0.000	0.508	0.758
Hymenoptera	0.461	0.828	0.536	0.426	0.367	0.462
Poduromorpha	0.000	0.000	3.590	0.133	0.000	0.000

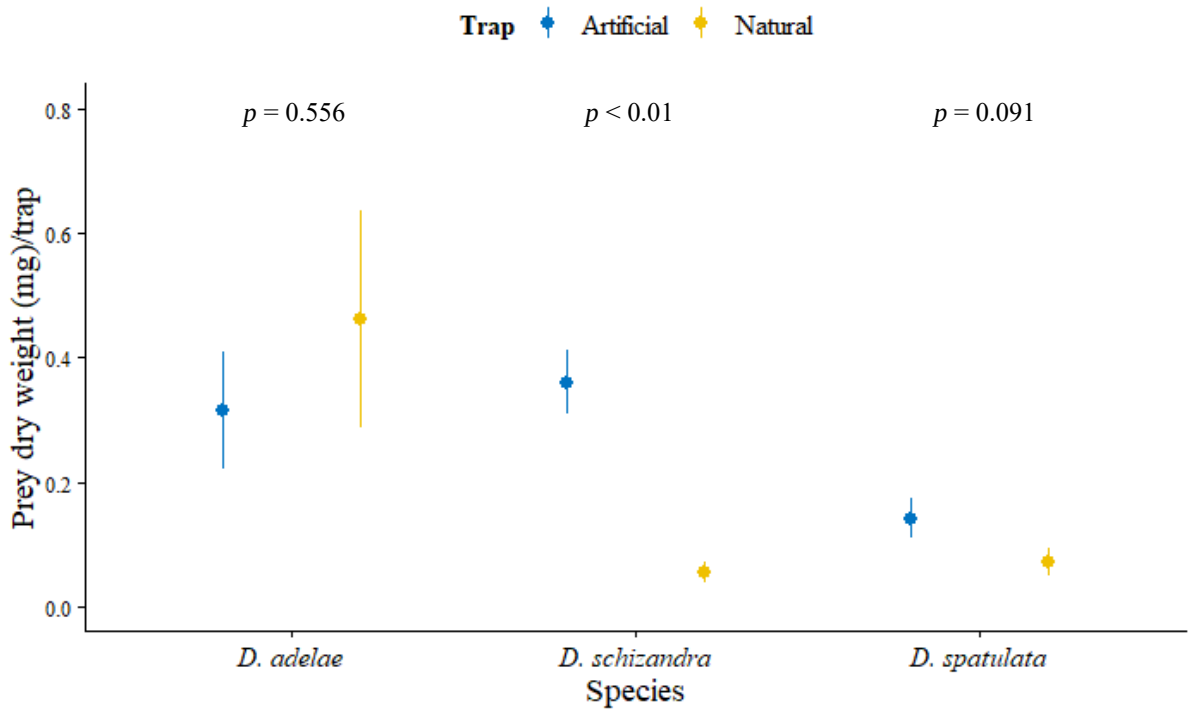

Figure 3.6 Mean (coloured dots) ± SE prey dry mass (mg)/trap collected by artificial and natural *Drosera*. The p-values lower than 0.01 above error mean plots for each species indicate significant differences in prey dry mass between seasons (Wilcoxon test).

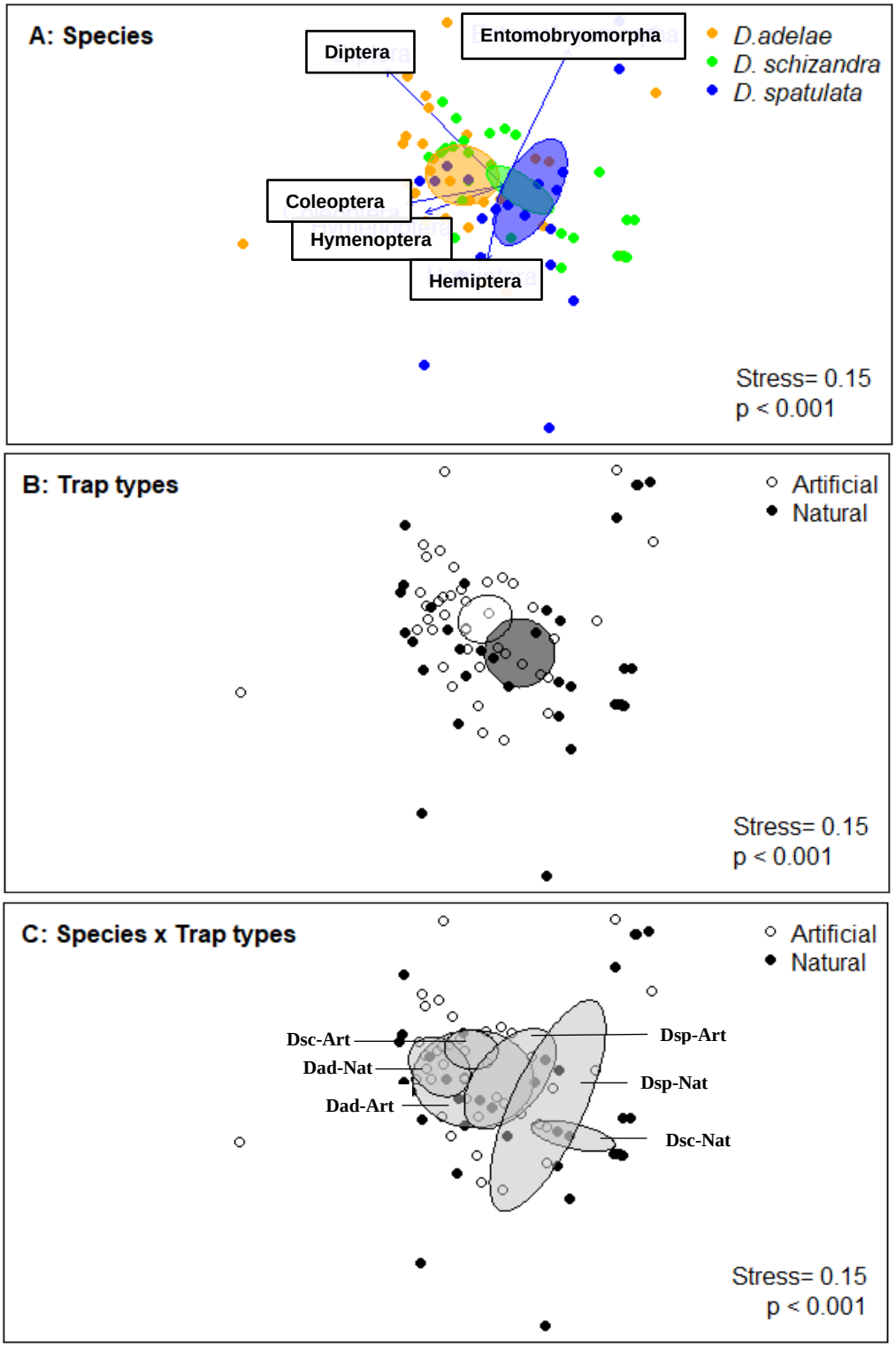

Figure 3.7 Changes in insect prey communities (based on the dry mass insect species consumed) between three *Drosera* species and neighbouring traps. Non-metric multidimensional scaling (NMDS) plots of distances among the centroids for two main factors: A) *Drosera* species (*D. adelae, D. burmanni, D. schizandra* and *D. spatulata*) and B) trap types (artificial and natural), as well as C) interaction between factors based on Bray-Curtis dissimilarities of log-transformed prey-dry mass dataset displayed in Table 3.11. The dots with different colour represent prey communities for each Drosera species (Figure 3.7.A) or for each trap type (Figure 3.7.B-C). The coloured or transparent ellipses represent 95% intervals around the centroids and the standard errors. The *p* values indicate the level of significance based on PERMANOVA analysis. Dad-Nat: *D. adelae* – natural traps, Dad-Art: *D. adelae* – artificial traps, Dsc-Nat: *D. schizandra* – natural traps, Dsc-Art: *D. schizandra* – artificial traps, Dsp-Nat: *D. spatulata* – natural traps, Dsp-Art: *D. spatulata* – artificial traps.

3.5 Discussion

3.5.1 Predation patterns

This study documents significant differences in the groups of insects consumed by the four tropical species of *Drosera* that were examined, corroborating the view that prey composition differs among *Drosera* (Verbeek and Boasson, 1993). The analysis of prey community captured by *Drosera* species revealed that different *Drosera* species captured different prey animals which are available in their habitats (Figure 3.2.A). Prey animals that contributed to differences in diet composition between *Drosera* species are Araneae, Entomobryomorpha, Formicidae, Hemiptera, Hymenoptera (excluding Formicidae), and Orthoptera (Figure 3.2.A). Compositional differences in captured prey among *Drosera* reflect their differences in habitat types (Ellison and Gotelli, 2009), growth forms (Verbeek and Boasson, 1993) and tentacle types (Poppinga et al., 2013).

The *Drosera* under examination are from rainforests under low-light but moist conditions or more open grassland habitats subjected to high-light and dry conditions. One would expect that in such different environments, the diversity and abundance of arthropods which are available for predation will differ (Cook et al. (2017). The rainforest *Drosera* tend to have more diverse prey range than *Drosera* from more open habitats, as shown by differences in Shannon Index (Table 3.3). There is also evidence that the presence of landscape features (e.g. streams, soil types, etc.) might also contribute to differences in catch composition. *Drosera adelae*, for example, occasionally trapped insects restricted to freshwater habitats (e.g. Ephemeroptera and Trichoptera) as this rainforest species occurs beside streams. By contrast, *D. schizandra,* the other rainforest sundew studied, caught no aquatic insects simply because the plants sampled did not live alongside streams.

The very high number of ants (Formicidae) in traps of *D. burmanni* and high occurrence of flying insects in *D. adelae* traps (Table 3.4) support the argument that growth forms and tentacle types influence prey composition. *Drosera burmanni* has a small flat rosette with two types of glue tentacles and snap tentacles. These features allow the plant to easily catch terrestrial prey because their leaves are close to the ground, and the presence of snap tentacles on the leaf margin facilitates trapping walking prey (Hartmeyer and Hartmeyer, 2010). On the contrary, the emergent structure of *D. adelae* with semi-erect large rosette, which contains glue tentacles only, allows the plants to access more aerial prey animals.

Differences in diet composition between seasons (Figure 3.2 and Table 3.2) might imply that prey available for predation varied with season, with peaks of prey diversity and abundance occurring in the dry season. In response, the *Drosera* studied were targeting prey that were available during each season. Differences in Shannon Indexes between seasons (Table 3.3) and high captures of particular prey groups in each season (Figure 3.1) confirmed the observed patterns. The Shannon Indexes across *D. adelae, D. schizandra,* and *D. spatulata* were consistently higher during the dry season than the wet season.

Diptera was the numerically dominant prey of *Drosera* (Table 3.4), and this observation reflects results of other studies (Murza et al., 2006, Hagan et al., 2008, Anderson, 2010). Captures of Diptera by *D. adelae*, the rainforest sundews growing alongside riverbanks, were far higher than for the other *Drosera* studied. The significant number of flying insects caught by *D. adelae* can be attributed to the fact that Diptera are the most common insects near freshwater habitats where *D. adelae* occur (Dijkstra et al., 2014).

The analyses of prey dry mass (Figure 3.4) could indicate differences in the contribution of prey capture level to N uptake by the *Drosera* studied. Such a phenomenon has been reported for *Drosera* in other environments (Pavlovič et al., 2014, Cook et al., 2017). In this research, *D. adelae* captured a greater biomass of prey than *D. spatulata* and *D. schizandra* but captures of *D. burmanni* were within the range of that of *D. adelae* (Table 3.5). Such a pattern is consistent with prey contributing more N to *D. adelae* and *D. burmanni* in comparison to *D. spatulata* and *D. schizandra*. This hypothesis will be evaluated in Chapter 4.

Differences in prey capture level among *Drosera* (Table 3.5) might be associated with trapping capacity. Several aspects of trapping capacity that could explain the variability in the prey capture level include the capability of sticky traps to cope with changes in microclimates (e.g. humidity and temperature levels in the environments) and trap stickiness, which might be related to the size of insects trapped. The research reported here indicates that *D. adelae* and *D. burmanni* capture greater numbers of prey animals than the other carnivorous sundews studied. Perhaps *D. adelae* and *D. burmanni* have greater trapping capacity than the other two species. This view will be partly tested in Chapter 5, especially regarding the effects of trap stickiness. Trapping capacity has been rarely reported in the *Drosera* literature. Further investigation of trapping capacity might provide a better understanding of the relationship between prey capture and N acquisition in carnivorous sundews.

The effect of seasons on prey capture levels (Figure 3.3 and Figure 3.4.B) is consistent with an interaction between light environment and trapping efficiency. This study found that during the wet season, *D. schizandra* could retain their prey on the leaf surface as evidenced by no statistical differences in prey dry mass between the dry and wet season (Figure 3.3).

The high retention capacity in *D. schizandra* probably occurs because this species occurs in shaded rainforests (Lavarack, 1979), protecting the plants from losing prey animals from the leaf surface. In contrast, there were no observed captures of *D. adelae* and *D. spatulata* when rain or showers occurred during the sample collection intervals (Figure 3.1). The study sites of *D. adelae* and *D. spatulata* are less-shaded in comparison with habitat of *D. schizandra.* In such environments, *D. adelae* and *D. spatulata* were highly likely to lose trapped animals during rain or showers. Other explanations for no observed captures in *D. adelae* and *D. spatulata* are that rain retarded the ability of the water-soluble mucin hydrogel to trap animals and there were no movement of potential prey during rain or showers.

3.5.2 Comparisons of captured prey and available prey

The composition of species consumed was not significantly different from the habitat (Figure 3.5.B), suggesting that *Drosera* are not selectively feeding on a subgroup of available insects. The three-study species were no better than their artificial traps at targeting and collecting prey animals from the environment as the biomass of prey captured by plants was similar or lower than that of collected by the plant models (Figure 3.6). This investigation supports previous studies (Watson et al., 1982, El-Sayed et al., 2016, Potts and Krupa, 2016) which showed the lack of prey selectivity among various carnivorous *Drosera*.

Captures of some prey groups (e.g. Chironomidae and Poduromorpha) were noticeably abundant for the models of *D. schizandra* (Table 3.7). What is more, artificial traps of *D. adelae* collected large Tipulidae, as shown in Table 3.10. Captures of these groups might indicate that artificial traps were slightly attractive for some prey taxa or retention capacity, i.e. stickiness, differed between plants and the plant models. Alternatively, large Tipulidae may have avoided the real *Drosera*.

The lack of prey selection observed during this research might also imply that the three-study species have limited attraction capacity. Studies on prey attraction in *D. adelae* and *D. spatulata* (Kurup et al., 2013, El-Sayed et al., 2016) support this argument, which found no obvious fluorescent and chemical attractants among the two species. This phenomenon suggest that some *Drosera* species probably rely on passive trapping to catch their prey, which is similar to spider webs (Potts and Krupa, 2016). This system is particularly efficient for some *Drosera* that inhabit environments with limited number of co-existing non-carnivorous plant that can act as landing perches (El-Sayed et al., 2016). In addition, passive trapping is well-known as a strategy to reduce prey-pollinator conflicts (Ellison and Gotelli, 2009) and diminishes the costs of producing attractants (Potts and Krupa, 2016).

3.6 Conclusion

In tropical environments of north Queensland, *Drosera* displays different predation patterns in terms of diet composition and biomass. Araneae, Entomobryomorpha, Formicidae, Hemiptera, Hymenoptera (excluding Formicidae), and Orthoptera are prey groups that contribute to differences in the community of insects preyed by *Drosera* species. Differences in diet composition reflect diversity and abundance of prey animals in their habitats as well as differences in growth forms and tentacle types among *Drosera* species. Prey capture levels in *D. adelae* and *D. spatulata* is strongly associated with seasons; fewer catches were evident during the wet season. However, no seasonal effects were demonstrated by *D. schizandra*. Shaded habitats permit *D. schizandra* to retain their prey when rain or showers occurred. This research also found that there is lack of evidence that *D. adelae, D. schizandra* and *D. spatulata* selectively trap or attract prey in the environments.

Chapter 4. Nitrogen content and biomass allocation in *Drosera* and co-existing non-carnivorous plants

4.1 Abstract

Nitrogen (N) uptake is expected to vary among plants that differ in nutrient-foraging strategies and habitat. Although this topic has been extensively described in the literature, comparisons of plant nutrition between root-based and trap-based N-foraging strategies are scarce. The present study compared and contrasted plant N contents and patterns of biomass allocation to above-ground and below-ground parts between four carnivorous *Drosera* species (*D. adelae, D. schizandra, D. spatulata* and *D. burmanni*) and their co-occurring non-carnivorous plants. As part of the research, here I compared plant nutrition among and the contribution of prey N to total plant N content in two rainforest *Drosera* species (*D. adelae* and *D. schizandra*) and two grassland species (*D. burmanni,* and *D. spatulata*) using N stable-isotope ($\delta^{15}N$) analysis. Patterns in biomass allocation to plant parts differed between carnivorous and conspecific non-carnivorous plants. Having a higher level of plant N content, *D. burmanni* allocated less biomass to below-ground parts than *Fimbristylis* sp, a co-existing non-carnivorous species. However, *Drosera adelae, D. spatulata* and their co-occuring non-carnivorous plants displayed similar patterns of biomass allocation and plant N content with co-existing non-carnivorous plants. The species that inhabited an extremely shaded habitat, *D. schizandra*, exhibited lower content of plant N than the species from the least shaded habitat, *D. burmanni*. The contribution of prey N to total plant N differed between the Drosera examined. The contribution of prey N to total plant N was greater in *D. adelae* than in *D. schizandra, D. spatulata* and *D. burmanni*. Prey capture levels, rather than soil N levels, may explain the variation in the level of the contribution of prey N to total N.

4.2 Introduction

Understanding the patterns in nutrient uptake, especially nitrogen (N), among roughly 350,000 species of vascular plants is a major task for plant nutritional ecology. This topic has been investigated across different nutrient-foraging strategies, i.e. specialised root structures, symbiotic structures (Aerts and Chapin III, 1999, Lambers et al., 2008) and for carnivorous plants (Ellison and Gotelli, 2009, Król et al., 2012, Ellison and Adamec, 2018). Studies of nutrient uptake suggested that the occurrence of different nutrient-foraging strategies in various landscapes exhibits patterns that, for the most part, relate to nutrient availability and soil age (Lambers et al., 2008). Specialised roots (e.g. proteoid roots), symbiotic structures (e.g. mycorrhiza and root nodules) and carnivory syndrome are more common in nutrient-poor environments (Juniper et al., 1989, Lamont, 1993, Lambers et al., 2008, Givnish, 2015, Givnish et al., 2018). When nutrients are provided to previously impoverished-soils, vascular plants tend to display traits related to efficient nutrient uptake, such as developed root systems, fast growth and rapid proliferation (Lambers et al., 2008).

The capacity for N uptake is expected to vary among plants with different nutrient-uptake strategies and habitat types because each plant will have a different N requirement for growth and other plant functions (Chapin et al., 1990). The capacity to absorb N may also be high or low depending upon factors that affect plant requirement for other nutrients. These factors include light intensity, temperature and water availability. It is expected that a higher uptake will be observed in environments where light and moisture levels are not limiting (Aerts and Chapin III, 1999). However, variations in N uptake among different nutrient-foraging strategies are not well understood.

Ellison (2006) summarised foliar nutrient content across different groups of plants, including carnivorous ones, from 17 studies. He highlighted the differences in concentrations of N and other mineral elements between carnivorous plants and other groups of plants and confirmed that N is limiting for carnivorous plants based on foliar N content. However, there are no further details described in Ellison (2006) on whether different N-foraging strategies that occupy low-N soils are equally successful in obtaining N.

To establish an understanding of patterns in N uptake between carnivorous and conspecific non-carnivorous plants, I present an analysis of N content, biomass allocation to above-ground and below-ground parts and root:shoot dry mass ratios of two *Drosera* from rainforest habitats (*D. adelae* and *D. schizandra*), two from open-grasslands (*D. spatulata* and *D. burmanni*) and their co-occurring non-carnivorous plants. The analysis is used to examine the relationship between patterns in biomass allocation to plant parts and content of plant N among plants with different foraging strategies in obtaining N. I further compare and contrast N content, and patterns in biomass allocation to plant parts among four examined *Drosera* species to investigate whether patterns in the observed variables are different or not in regard to environmental conditions where the carnivorous sundews occur. The current research also quantifies the levels of the contribution of prey-derived N among examined *Drosera* species by using N stable-isotope ($\delta^{15}N$) analysis. The quantification is used to evaluate whether the contribution of prey-derived N among studied *Drosera* species is aligned with the results of prey capture data as described in Chapter 3. This will also broaden understanding of the magnitude of the contribution of prey-derived N among carnivorous plants including *Drosera* spp (e.g. (Schulze et al., 1991, Millett et al., 2003, Millett et al., 2012).

4.3 Methods

4.3.1 Sample collection

Samples of each *Drosera* species were collected from their habitats as detailed in Table 4.1. Where it was possible, a random selection of three different non-carnivorous plants with shallow rooting system (which, depending upon site, included grasses, ferns and other herbaceous plants) were collected at each location only in the vicinity of the sundews. This sampling strategy was taken to deal with differences in the soil depth of plant roots between the sundews and non-carnivorous plants (Schulze et al., 1991). Soil samples of 250 g were collected from the root zones, between 0 - 15 cm depth, of all harvested plants. The soil samples were air-dried, sieved to 2 mm and stored in an air-tight container.

Table 4.1 Sites where *Drosera* and co-occurring non-carnivorous plants were sampled

Location	*Drosera* species	Non-carnivorous plants
Arnott Creek, Queensland (18.527°S, 146.156°E)	*D. adelae*	*Cephalomanes* sp *Doodia* sp *Carex breviscapa* C.B. Clarke*
Mt Bartle Frere area of Wooroonooran National Park, Queensland (17.396°S and 145.764°E)	*D. schizandra*	*Adiantum diaphanum* Blume *Ceratopetalum* sp
Private land owned by Seafarm Pty Ltd, Queensland (18.330°S, 146.057°E)	*D. spatulata*	*Rhynchospora* sp1 *Rhynchospora* sp2 *Xyris complanata* R. Brown
Cardwell State Forest, Queensland (18.336°S, 146.057°E)	*D. burmanni*	*Fimbristylis* sp**

* There were only two samples of each species found in the vicinity of each *Drosera* species.
** The only species with shallow rooting system found in the microhabitat

Initially, nitrogen content and $\delta^{15}N$ were assessed for potential prey and captured prey. To collect potential prey, artificial traps (10 x 10 cm) covered with transparent non-drying glue were placed in the vicinity of each *Drosera* for 24 h. Only insects with the typical length/biomass of prey of each *Drosera* were included in the analyses. However, due to the

low masses of prey captured by the artificial traps over 24 h, only captured prey on leaves of *Drosera* were used in the analyses (Table 4.2). All invertebrates captured by *Drosera* were stored in ethanol prior to the determination of $\delta^{15}N$.

Table 4.2 Insect groups captured by *Drosera* species during 24 h collecting periods

Drosera species	Insect orders
D. adelae	Diptera, Hemiptera and Hymenoptera
D. schizandra	Diptera and Hymenoptera
D. spatulata	Hymenoptera, Diptera and Araneae
D. burmanni	Hymenoptera

4.3.2 Sample analysis and determination of prey-derived N

Prior to the quantification of N, plants were separated into leaves, stem, roots and flowers. Plant and insect samples were oven-dried for 48 hours at 60°C, and their dry mass was measured. Samples were ground to a fine powder in a ball mill. Samples of *D. adelae, D. schizandra,* and *D. burmanni* were sent to the Stable Isotope Laboratory, Australian National University, Australia. Samples of *D. spatulata* were sent to the Stable Isotope Facility, University of Wyoming, USA. The former was analysed using Isoprime mass spectrometer coupled with Carlo Erba 1110 elemental analyser to determine total N concentration and $\delta^{15}N$, while the latter was analysed using a Thermo Scientific™ Delta V™ mass spectrometer coupled with a Costech elemental analyser. Natural abundance of N isotopes is expressed in parts per thousand ($^{0}/_{00}$) deviation from international standard:

$$\delta^{15}N = (Rsample / Rstandard) - 1) \times 1000.$$

All data are reported in accordance with the international standard of AIR (atmospheric N_2) for $\delta^{15}N$. The contribution of prey-derived N to the total N concentrations of *Drosera* was calculated using the model of Schulze et al. (1991):

$$\% \text{ N from prey} = (\delta^{15}N \textit{ Drosera} - \delta^{15}N \text{ Reference}) / (\delta^{15}N \text{ Insect} - \delta^{15}N \text{ Reference}) \times 100$$

where $\delta^{15}N$ *Drosera*, $\delta^{15}N$ Reference and $\delta^{15}N$ Insect are $\delta^{15}N$ values for *Drosera*, the non-carnivorous reference plant and insects, respectively.

The values of $\delta^{15}N$ *Drosera* and $\delta^{15}N$ Reference were calculated by adding isotope values of plant organs (e.g. leaves, stems and roots) based on the proportion of their dry mass.

4.3.3 Data analysis

Nitrogen contents and dry mass of *Drosera* and co-existing non-carnivorous plants were examined with factorial ANOVA followed by post-priori Tukey tests where relevant. Assumptions for factorial ANOVA were checked, and log-transformation was applied if violations of assumptions were detected. If data transformation fails to handle violation of normality, ANOVA of Aligned Rank Transformed Data within the ARTool package in R followed by pairwise comparisons with Tukey adjustment was employed (Wobbrock et al., 2011). Root:shoot dry mass ratios among three carnivorous sundews: *D. adelae, D. schizandra* and *D. spatulata* and their co-occurring non-carnivorous plants were assessed with one-way ANOVA followed by Tukey tests if assumptions of the parametric analysis were met. In the case of violations of assumptions, Kruskal-Wallis tests werr used to examine root:shoot dry mass ratios. Differences in root:shoot dry mass ratios for *D. burmanni* and the co-existing non-carnivorous plant were examined using Behrens-Fisher test (Zar, 2009). Data visualisation was carried out using the *ggpubr* function in R (Kassambara, 2019).

4.4 Results

4.4.1 Nitrogen content and biomass allocation among carnivorous sundews and non-carnivorous plants

Summaries of N content, the allocation of biomass to above-ground and below-ground parts, root:shoot dry mass ratios for *D. adelae* and three co-existing non-carnivorous plants (*Carex*

breviscapa, Cephalomanes sp and *Doodia* sp) and soil N content in their habitat are shown in Table 4.3 and Figure 4.1. Growing in soil containing 0.2% (w/w) N, N content of *D. adelae* and co-existing non-carnivorous plants ranged from 1.19% - 1.26%. Nitrogen contents of above-ground parts were greater than below-ground parts across the four species (Table 4.3).

Table 4.3 Mean nitrogen (N) content (%), dry mass (g), root:shoot dry mass ratios for *Drosera adelae* and co-occurring non carnivorous plants, and soil N content (%). Values in parentheses are number of plants sampled.

Parameters	Plant parts	*D. adelae*	*Carex breviscapa*	*Cephalomanes* sp	*Doodia* sp	Mean
N content (%)	Above-ground	1.49 (10)	1.28 (2)	1.44 (3)	1.45 (3)	1.45 (18)
	Below-ground	0.97 (10)	1.09 (2)	1.08 (3)	0.93 (3)	1.00 (18)
	Mean	1.23 (20)	1.19 (4)	1.26 (6)	1.19 (6)	1.22 (36)
Dry mass (g)	Above-ground	0.210 (10)	3.103 (2)	0.170 (3)	0.250 (3)	0.531 (18)
	Below-ground	0.073 (10)	0.640 (2)	0.077 (3)	0.137 (3)	0.147 (18)
	Mean	0.141 (20)	1.871 (4)	0.123 (6)	0.194 (6)	0.339 (36)
Root: shoot	-	0.383 (10)	0.194 (2)	0.444 (3)	0.519 (3)	0.395 (18)
Soil N (%)	Mean ± SE	0.2 ± 0.02 (n = 10)				

There were no significant differences in N content between *D. adelae* and co-existing non-carnivorous plants (*Carex breviscapa, Cephalomanes* sp and *Doodia* sp) (two-way ANOVA, $F_{3,28}$= 0.141, p = 0.934). However, above-ground N content in all observed species was significantly higher than below-ground N content (two-way ANOVA, $F_{3,28}$= 37.504, p < 0.01, Figure 4.1.A). No statistically significant interaction between species and plant parts was detected (two-way ANOVA, $F_{3,28}$= 0.753, p = 0.530).

Dry mass of *Carex breviscapa,* the non-carnivorous plant, was higher than other three examined species (Table 4.3). However, the dry mass of *D. adelae* was within the range of other two non-carnivorous plants: *Cephalomanes* sp and *Doodia* sp. The statistical analysis supports the observed patterns. Dry mass differed between *D. adelae* and co-existing non-carnivorous plants (two-way ANOVA, $F_{3,28}$= 94.575, p < 0.01). Above-ground dry mass were significantly higher than below-ground ones (two-way ANOVA, $F_{1,28}$= 43.593, p <

0.01). There was statistically significant interaction between species and plant parts (two-way ANOVA, $F_{3,28}$= 25.095, $p < 0.01$, Figure 4.1.B).

Mean root:shoot dry mass ratios of *D. adelae* and three non-carnivorous plants range from 0.194 to 0.519 (Table 4.3). Root:shoot dry mass ratios did not differ between *D. adelae* and co-occurring non-carnivorous plants (one-way ANOVA, $F_{3,14}$= 1.528, $p = 0.25$, Figure 4.1.C).

Summaries of the N content, the allocation of biomass to above-ground and below-ground parts, root:shoot dry mass ratios for *D. schizandra* and two co-existing non-carnivorous plants (*Adiantum diaphanum* and *Ceratopetalum* sp) and soil N content in their habitat are presented in Table 4.4 and Figure 4.2. Under conditions of low soil N content, N content varied depending upon species ranging from 0.58% to 0.76%. A higher N content of above-ground parts was observed across three examined species (Table 4.4).

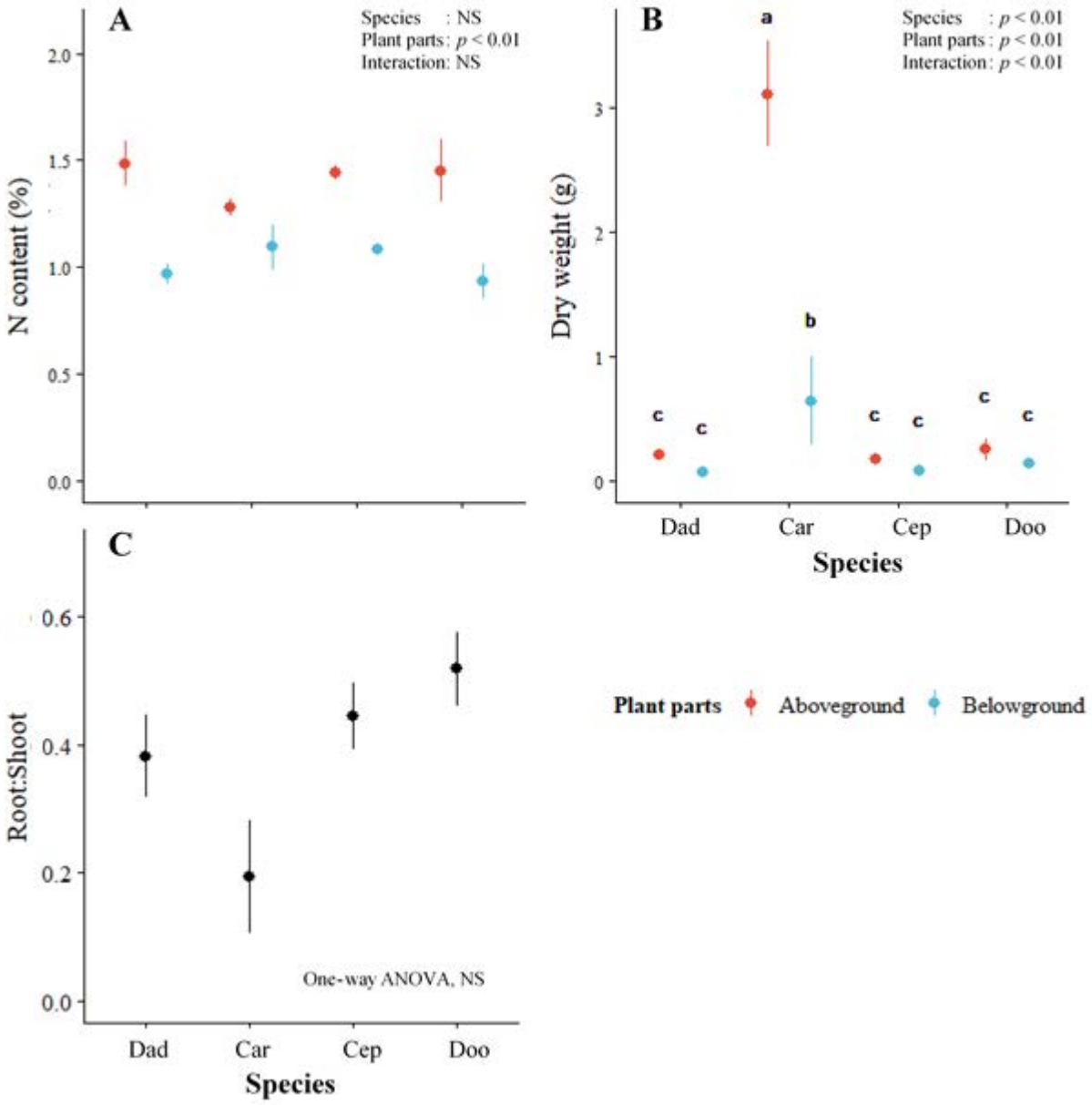

Figure 4.1 Nitrogen content (%) and biomass allocation (g) of *Drosera adelae* and non-carnivorous plants. Presented are the means (coloured and black dots) ± SE for: A) N content, B) dry mass, and C) root:shoot dry mass ratios. In Figure 4.1.A-B, all means were compared to demonstrate whether there was a significant interaction between main effects or not. The legend texts on the top right corner of Figure 4.1.A-B indicate statistical results for main effects (Species and Plant parts) and interactions between main effects. Note: S: significant, NS: non-significant. Different letters above mean plots indicate significant differences (Tukey HSD, $p < 0.05$). Dad: *Drosera adelae*, Car: *Carex breviscapa*, Cep: *Cephalomanes* sp, Doo: *Doodia* sp. Dataset for dry mass was log-transformed to meet ANOVA assumptions.

Table 4.4 Mean nitrogen (N) content (%), dry mass per plant (g), root:shoot dry mass ratios for *Drosera schizandra* and co-occurring non carnivorous plants, and soil N content. Values in parentheses are number of samples.

Parameters	Plant parts	*D. schizandra*	*Adiantum diaphanum*	*Ceratopetalum* sp	Mean
N content (%)	Above-ground	0.61 (5)	0.90 (3)	0.71 (3)	0.72 (11)
	Below-ground	0.69 (5)	0.63 (3)	0.45 (3)	0.61 (11)
	Mean	0.65 (10)	0.76 (6)	0.58 (6)	0.66 (22)
Dry mass (g)	Above-ground	0.403 (5)	0.274 (3)	0.165 (3)	0.303 (11)
	Below-ground	0.240 (5)	0.273 (3)	0.056 (3)	0.199 (11)
	Mean	0.321 (10)	0.273 (6)	0.110 (6)	0.251 (22)
Root: shoot	-	0.620 (5)	1.004 (3)	0.360 (3)	0.654 (11)
Soil N (%)	Mean ± SE	Undetectable (n = 5)			

Nitrogen content in *Drosera schizandra* significantly differed from that in co-existing non-carnivorous plants (*Adiantum diaphanum* and *Ceratopetalum* sp) (two-way ANOVA, $F_{2,16}$= 4.308, $p < 0.05$). When different plant parts were compared, there were significantly differences in N content between above-ground and below-ground (two-way ANOVA, $F_{1,16}$= 5.191, $p < 0.05$). The interaction between species (*D. schizandra* and co-existing non carnivorous plants) and plant parts (above-ground and below-ground) was statistically significant (two-way ANOVA, $F_{2,16}$= 7.138, $p < 0.01$) indicating at least one comparison of N content between above-ground and below-ground within species is different (Figure 4.2.A).

Dry mass of examined plants varied depending upon species ranging from 0.110 g to 0.321 g. Two out of three species (*D. schizandra* and *Ceratopetalum* sp) display a high dry mass of above-ground parts. In contrast, *Adiantum diaphanum* have approximately similar proportion of above-ground and below-ground parts (Table 4.4). The statistical analysis confirms the observed patterns. There were statistically significant differences in dry mass for two main factors: species ($F_{2,16}$= 19.493, $p < 0.01$) and plant parts ($F_{1,16}$= 13.550, $p < 0.01$), with no interaction between two main factors ($F_{2,16}$= 2.824, $p = 0.09$, Figure 4.3.B).

Root:shoot dry mass ratio of *D. schizandra* is within the range of the two non-carnivorous plants. However, the highest root:shoot dry mass ratio was observed in *Adiantum diaphanum*. Dry mass of *D. schizandra* differed from that of co-existing non-carnivorous plants (one-way ANOVA, $F_{2,8}$= 8.956, $p < 0.01$, Figure 4.3.C).

Summaries of the N content, the allocation of biomass to above-ground and below-ground parts, root:shoot dry mass ratios for *D. spatulata* and three co-existing non-carnivorous plants (*Rhynchospora* sp1*, Rhynchospora* sp2, *Xyris complanata*) and soil N content in their habitat are shown in Table 4.5 and Figure 4.3. Growing in very low of soil N content, N content of *D. spatulata* and co-existing non-carnivorous plants ranged from 0.42% to 0.48%. N contents of above-ground parts were greater than below-ground parts across four species (Table 4.5).

Table 4.5 Mean nitrogen (N) content (%), dry mass per plant (g), root:shoot dry mass ratios for *Drosera spatulata* and co-occurring non carnivorous plants, and soil N content (%). Values in parentheses are number of samples.

Parameters	Plant parts	*D. spatulata*	*Rhynchospora* sp1	*Rhynchospora* sp2	*Xyris complanata*	Mean
N content (%)	Above-ground	0.67 (5)	0.45 (3)	0.59 (3)	0.63 (3)	0.60 (14)
	Below-ground	0.30 (5)	0.39 (3)	0.27 (3)	0.22 (3)	0.29 (14)
	Mean	0.48 (10)	0.42 (6)	0.43 (6)	0.43 (6)	0.45 (28)
Dry mass (g)	Above-ground	0.037 (5)	0.654 (3)	1.034 (3)	1.874 (3)	0.777 (14)
	Below-ground	0.007 (5)	0.785 (3)	0.160 (3)	0.252 (3)	0.259 (14)
	Mean	0.022 (10)	0.720 (6)	0.597 (6)	1.063 (6)	0.518 (28)
Root: shoot	-	0.206 (5)	1.121 (3)	0.154 (3)	0.196 (3)	0.389 (14)
Soil N (%)	Mean ± SE	Undetectable (n = 5)				

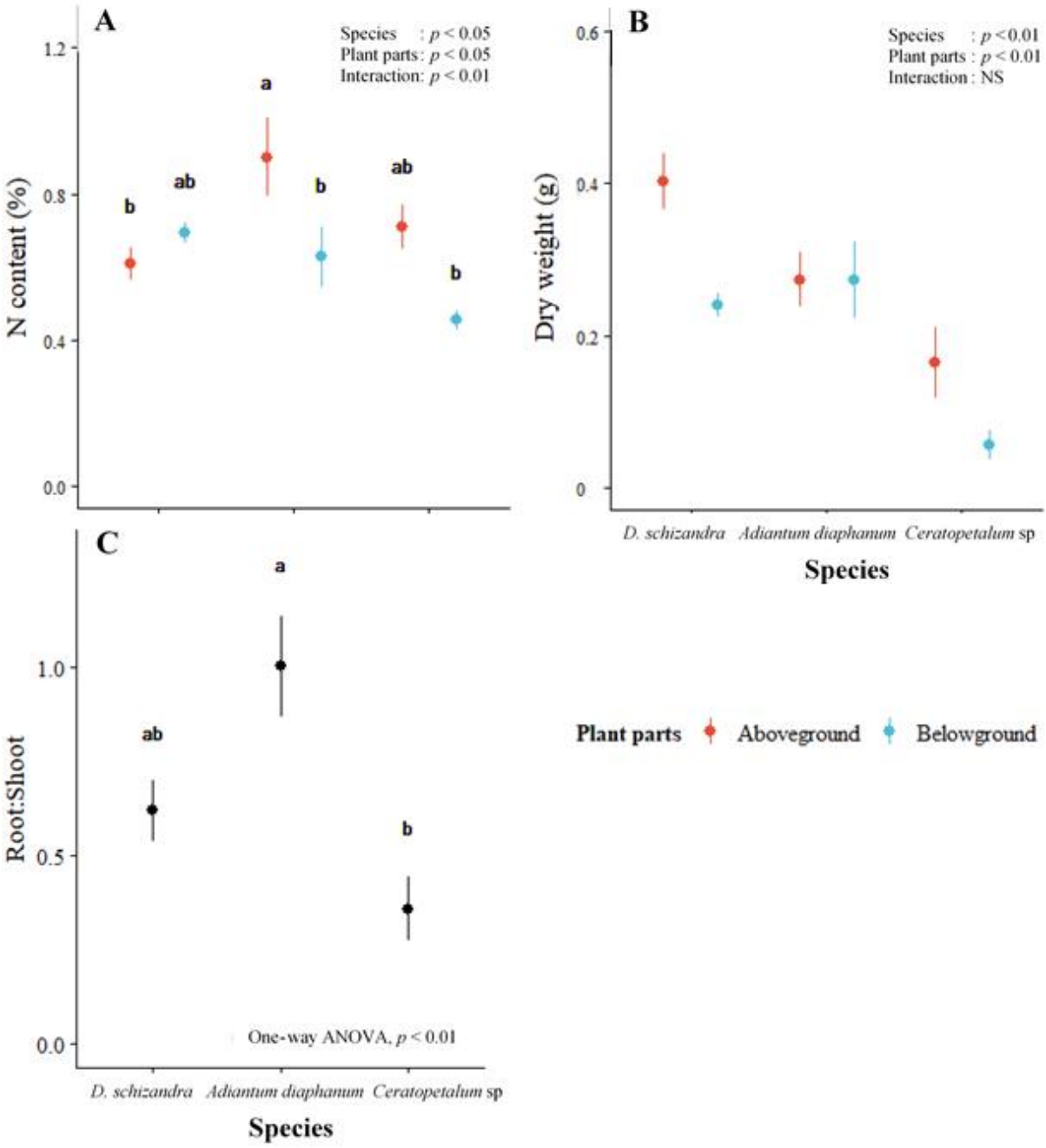

Figure 4.2 Nitrogen content (%) and biomass allocation (g) of *Drosera schizandra* and non-carnivorous plants. Presented are the means (solid circles) ± SE for: A) N content, B) dry mass, and C) root:shoot dry mass ratios. In Figure 4.2.A-B, all means were compared to demonstrate whether there was a significant interaction between main effects or not. The legend texts on the top right corner of Figure 4.2.A-B indicate statistical results for main effects (Species and Plant parts) and interactions between main effects. Note: NS: non-significant. Different letters above mean plots indicate significant differences (Tukey HSD, $p < 0.05$).

There were no statistically significant differences in the N content among *D. spatulata* and co-existing non-carnivorous plant ($F_{3,20}$= 0.969, p = 0.42). However, the amount of N content significantly differed between plant parts (two-way ANOVA, $F_{1,20}$= 81.806, $p < 0.001$)

suggesting differences in above-ground and below-ground N contents within some observed species. The analyses also showed a statistically significant interaction between species (*Drosera spatulata* and co-existing non-carnivorous plant) and plant parts (above-ground and below-ground) (two-way ANOVA, $F_{3,20}$= 5.033, $p < 0.01$) indicating at least one comparison of the amount of N content between above-ground and below-ground within species is different (Figure 4.3.A).

Dry mass of *D. spatulata* was the lowest among examined species. The majority of species in the microhabitat of *D. spatulata* have lower biomass of below-ground parts with exception for *Rhynchospora* sp1 (Table 4.5). The statistical analysis supports the observed patterns. There were statistically significant differences in dry mass among *Drosera spatulata* and co-existing non-carnivorous plant (ANOVA of Aligned Rank Transformed Data, $F_{3,20}$= 10.759, $p < 0.01$) and between plant parts (ANOVA of Aligned Rank Transformed Data, $F_{1,20}$= 9.455, $p < 0.01$), and interaction between observed species and plant parts (ANOVA of Aligned Rank Transformed Data, $F_{3,20}$= 7.265, $p < 0.01$, Figure 4.3.B).

Table 4.5 shows that root:shoot dry mass ratios are generally less than 0.3 with exception for *Rhynchospora* sp1. Root:shoot dry mass ratios did not differ between *D. spatulata* and co-existing non-carnivorous plants (Kruskal-Wallis, $\chi = 7.255$, $p = 0.06$, Figure 4.4.C).

Summaries of the nitrogen content, the allocation of biomass to above-ground and below-ground parts, root:shoot dry mass ratios for *D. burmanni* and *Fimbristylis* sp, the co-existing non-carnivorous plant, and soil N content in their habitat are presented in Table 4.6 and Figure 4.4. Under very low of soil N content, N content of *D. burmanni* and *Fimbristylis* sp

ranged from 0.98% to 1.33%. Nitrogen contents of above-ground parts were higher than that of below-ground parts across the two species (Table 4.6).

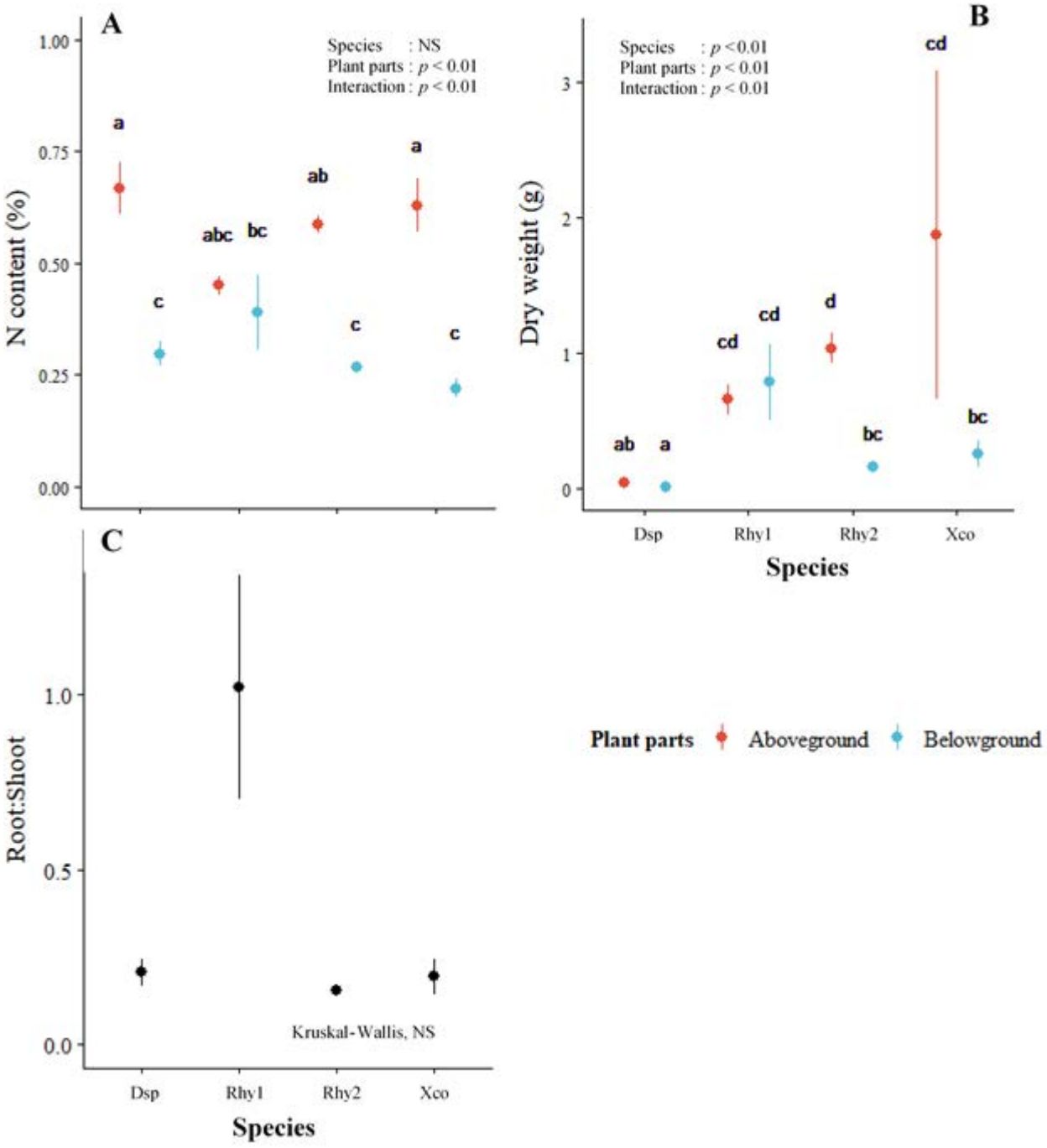

Figure 4.3 Nitrogen (N) content (%) and biomass allocation (g) of *Drosera spatulata* and non-carnivorous plants. Presented are the means (black dots) ± SE for: A) N content, B) dry mass, C) root:shoot dry mass ratios. In Figure 4.3.A-B, all means were compared to demonstrate whether there was a significant interaction between main effects or not. Texts inserted in the top-right corner of Figures 4.3.A-B indicate statistical results for main effects (species and plant parts) and interactions between main effects. Note: NS: non-significant. Different letters above mean plots indicate significant differences (Tukey HSD for N content dataset and pairwise comparisons with Tukey adjustment for dry mass dataset, $p < 0.05$). Dsp: *Drosera spatulata;* Rhy1: *Rhynhospora* sp1; Rhy2: *Rhynhospora* sp2; Xco: *Xyris complanata.*

Table 4.6 Mean nitrogen (N) content (%), dry mass per plant (g), root:shoot dry mass ratios for *Drosera burmanni* and *Fimbristylis* sp, and soil N content (%). Values in parentheses are number of plants sampled.

Parameters	Plant parts	*D. burmanni*	*Fimbristylis* sp	Mean
N content (%)	Above-ground	1.55 (5)	1.33 (5)	1.44 (10)
	Below-ground	0.97 (3)	0.62 (5)	0.75 (8)
	Mean	1.33 (8)	0.98 (10)	1.13 (18)
Dry mass (g)	Above-ground	0.039 (5)	0.192 (5)	0.116 (10)
	Below-ground	0.002 (5)	0.048 (5)	0.025 (10)
	Mean	0.025 (10)	0.120 (10)	0.070 (20)
Root: shoot	-	0.046 (5)	0.283 (5)	0.165 (10)
Soil N (%)	Mean ± SE	Undetectable (n = 5)		

There were statistically significant differences in N content between *D. burmanni* and the co-existing non-carnivorous plant, *Fimbristylis* sp (two-way ANOVA, $F_{1,14}$= 5.664, $p < 0.05$). For both *D. burmanni* and *Fimbristylis* sp, the above-ground N content was significantly higher than the below-ground N content (two-way ANOVA, $F_{1,14}$= 32.552, $p < 0.01$, Figure 4.4.A). However, there was no statistically significant interaction between species and plant parts (two-way ANOVA, $F_{1,14}$= 0.281, $p = 0.60$).

Table 4.6 indicates that *D. burmanni* is much smaller than *Fimbristylis* sp as reflected by dry mass of above-ground and below-ground parts. The statistical analysis confirms the observed pattern. Dry mass of *D. burmanni* significantly differed from that of *Fimbristylis* sp (ANOVA of Aligned Rank Transformed Data, $F_{1,16}$= 13.847, $p < 0.01$). When different plant parts were compared for both *D. burmanni* and *Fimbristylis* sp, above-ground dry mass significantly differed from below-ground dry mass (ANOVA of Aligned Rank Transformed Data, $F_{1,16}$= 7.924, $p < 0.05$, Figure 4.4.B). Significant interaction between species and plant parts (ANOVA of Aligned Rank Transformed Data, $F_{1,16}$= 4.897, $p < 0.05$).

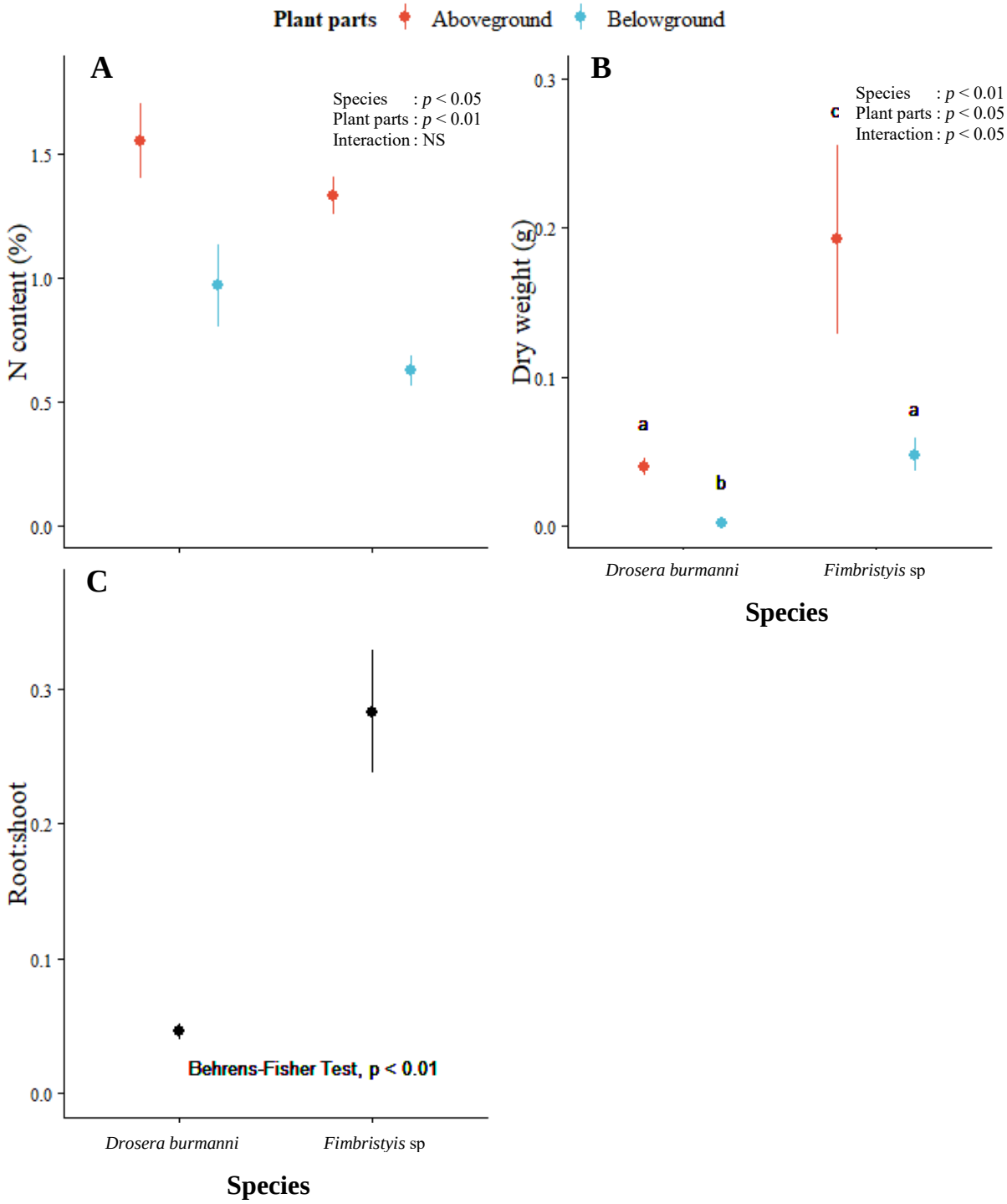

Figure 4.4 Nitrogen (N) content (%) and biomass allocation (g) of *Drosera burmanni* and the non-carnivorous plant, *Fimbristylis* sp. Presented are the means (black dots) ± SE for: A) N content (%), B) dry mass (g), and C) root:shoot dry mass ratios. In Figure 4.4.A-B, all means were compared to demonstrate whether there was a significant interaction between main effects or not. Texts inserted in the top-right corner of Figure 4.4.A-B indicate statistical results for main effects (Species and Plant parts) and interactions between main effects. Note: NS: non-significant. Different letters above mean plots indicate significant differences (Tukey HSD for N content dataset and pairwise comparisons with Tukey adjustment for dry mass dataset, $p < 0.05$).

Root:shoot dry mass ratio of *D. burmanni* were much lower than that of *Fimbristylis* sp (Table 4.6). The observed pattern was aligned with the statistical results of Behrens-Fisher test. Root:shoot dry mass ratio of *D. burmanni* were significantly lower than that of *Fimbristylis* sp ($t = 5.129$, $p < 0.01$, Figure 4.4.C).

4.4.2 Comparisons of root:shoot dry mass ratios between carnivorous sundews and non-carnivorous plants

Root:shoot dry mass ratios differ between all the *Drosera* studied and co-occurring non-carnivorous plants. In general, mean root:shoot dry mass ratios of the *Drosera* were lower than that of non-carnivorous plants (Figure 4.5).

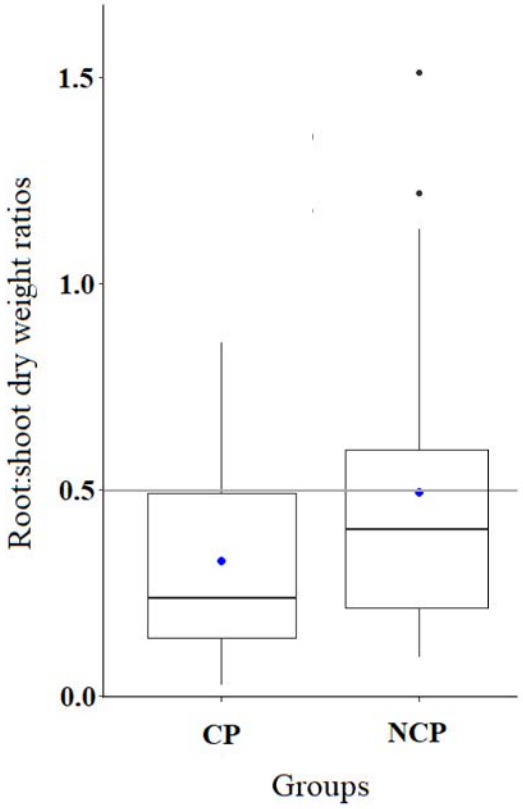

Figure 4.5 The distribution of root:shoot dry mass ratios in two different plant groups: carnivorous *Drosera* (CP) and co-existing non-carnivorous plants (NCP). The blue dots in each box are means of the root:shoot dry mass ratio for each group. The middle line in each box represents median of the observed distribution, the top and bottom parts the 25th and 75th percentiles. The horizontal line indicates equal dry masses of root and shoot.

4.4.3 Plant nutrition and contribution of prey nitrogen (N) to total plant N among examined *Drosera* species

Summaries of the N content, the allocation of biomass to above-ground and below-ground parts, root:shoot dry mass ratios across four *Drosera* species are presented in Table 4.7 and Figure 4.5. *Drosera adelae* and *D. burmanni* displayed a high content of plant N in comparison with *D. schizandra* and *D. spatulata*. N content of above-ground parts in all species were generally higher than that of below-ground parts with exception for *D. schizandra*.

Table 4.7 Mean nitrogen (N) content (%), dry mass (g), root:shoot dry mass ratios for *Drosera adelae, D. schizandra, D. spatulata* and *D. burmanni.* Values in parentheses are number of samples.

Parameters	Plant parts	*D. adelae*	*D. schizandra*	*D. spatulata*	*D. burmanni*	Mean
N content (%)	Above-ground	1.49 (10)	0.61 (5)	0.67 (5)	1.55 (5)	1.16 (25)
	Below-ground	0.97 (10)	0.69 (5)	0.30 (5)	0.97 (3)	0.76 (23)
	Mean	1.23 (20)	0.65 (10)	0.48 (10)	1.33 (8)	0.97 (48)
Dry mass (g)	Above-ground	0.210 (10)	0.403 (5)	0.037 (5)	0.039 (5)	0.180 (25)
	Below-ground	0.073 (10)	0.240 (5)	0.007 (5)	0.002 (5)	0.079 (25)
	Mean	0.141 (20)	0.321 (10)	0.022 (10)	0.025 (10)	0.129 (25)
Root: shoot	-	0.383 (10)	0.620 (5)	0.206 (5)	0.046 (5)	

The observed patterns were aligned with the statistical analysis. There were statistically significant differences in N content among *Drosera* species (two-way ANOVA, $F_{3,40}$= 54.064, $p < 0.01$), and among the two different plant parts (two-way ANOVA, $F_{1,40}$= 40.583, $p < 0.01$). The analyses also showed a statistically significant interaction between *Drosera* species and plant parts) (two-way ANOVA, $F_{3,40}$= 6.122, $p < 0.01$) indicating at least one comparison of the amount of N content between above-ground and below-ground within species is different (Figure 4.6.A).

Dry mass of *D. schizandra* and *D. adelae* were much higher than those of *D. burmanni* and *D. spatulata*. All *Drosera* species display a higher dry mass of above-ground parts in

comparison with that of below-ground parts (Table 4.7). The statistical analysis confirms the observed patterns. There were significant differences in dry mass among examined *Drosera* species (ANOVA of Aligned Rank Transformed Data, $F_{3,42}$= 76.995, $p < 0.01$). When different plant parts were compared, above-ground dry mass significantly differed from below-ground dry mass (ANOVA of Aligned Rank Transformed Data, $F_{1,42}$= 64.867, $p <$ 0.01). There was a statistically significant interaction between two factors: *Drosera* species and plant parts (ANOVA of Aligned Rank Transformed Data, $F_{3,42}$= 5.539, $p < 0.01$, Figure 4.6.B).

Root:shoot dry mass ratios varied across four *Drosera* species. Root:shoot dry mass ratios were generally less than 0.5 with exception for *D. schizandra.* The result of Welch's ANOVA supports the above pattern which showed that root:shoot dry mass ratios were significantly different among *Drosera* species ($F_{3,8.4}$= 26.308, $p < 0.01$, Figure 4.6.C).

$\delta^{15}N$ values differed between the *Drosera*, ranging from -7.33‰ to 0.15‰ (Table 4.8). $\delta^{15}N$ values of non-carnivorous plants and prey varied across different microhabitats of *Drosera*.

Table 4.8 Mean $\delta^{15}N$ values per microhabitat of *Drosera* species. The calculation of $\delta^{15}N$ values for each *Drosera* species was corrected for the masses of the plant organs (see Method section for details)

Microhabitat	$\delta^{15}N_{Drosera}$	$\delta^{15}N_{NCPs}$	$\delta 15N_{prey}$
		‰	
D. adelae	-2.56	-3.56	-2.52
D. schizandra	-7.33	-12.46	-1.98
D. spatulata	0.15	-1.32	2.02
D. burmanni	-0.87	0.38	-2.78

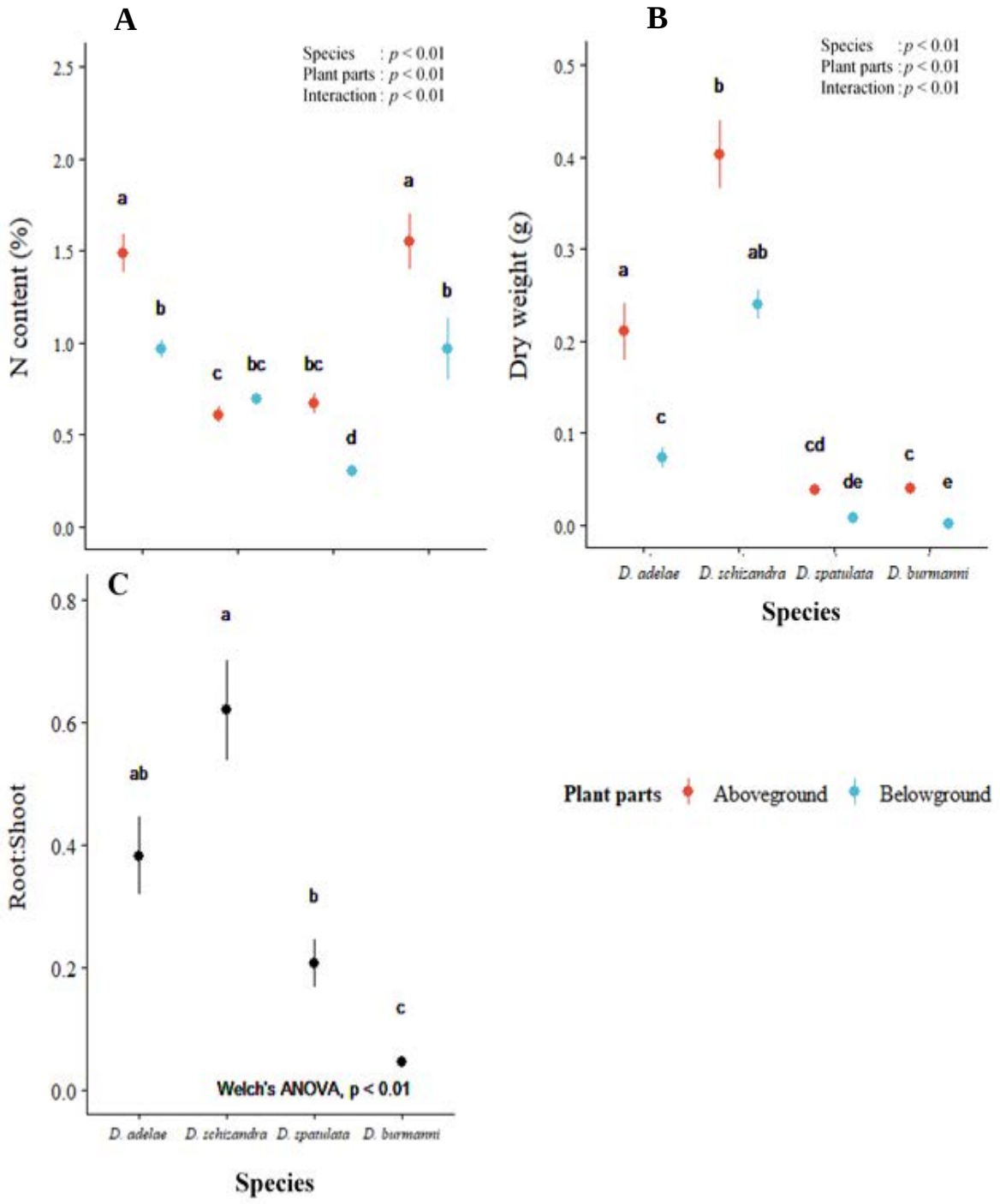

Figure 4.6 Nitrogen (N) content (%) and root:shoot biomass ratios of the *Drosera* examined. Presented are the means (black dots) ± SE for: A) N content, B) dry mass, C) root:shoot biomass ratios. In Figure 4.6.A-B, all means were compared to demonstrate whether there was a significant interaction between main effects or not. Texts inserted in the top-right corner of Figure 4.4.A-B indicate statistical results for main effects (Species and Plant parts) and interactions between main effects. Note: different letters above mean plots indicate significant differences (Tukey HSD for N content dataset and pairwise comparisons with Tukey adjustment for dry mass dataset, $p < 0.05$). Dataset for N content was log-transformed to meet ANOVA assumptions.

Contribution of prey N to the total N contained in examined *Drosera* species differed between species (Figure 4.7). In spite of high variability, contribution of prey N to total N contained in *D. adelae* was higher than that of the other three species.

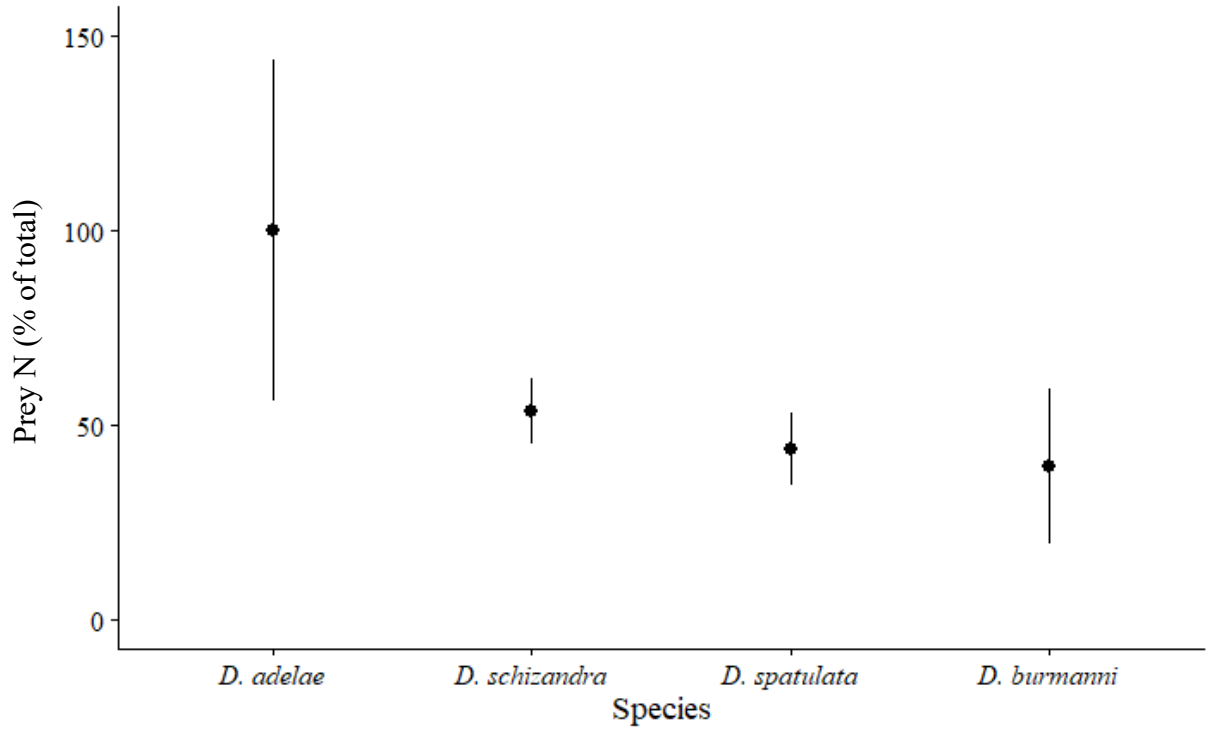

Figure 4.7 Contribution of prey N to the total N (%) of examined *Drosera* species. Presented are the means (black dots) ± SE for four *Drosera* species: *D. adelae, D. schizandra, D. spatulata* and *D. burmanni*.

4.5 Discussion

This study illustrates differences in the proportion of biomass allocated to plant parts which contribute to foraging for N among carnivorous and non-carnivorous plants. It also documents plant N contents among carnivorous *Drosera* and their co-occurring non-carnivorous plants across two vegetation complexes that vary in soil N contents. The relationship between the proportion of biomass allocated to plant parts and plant N contents reflects several responses of carnivorous and non-carnivorous plants in foraging for N. First, some carnivorous *Drosera* allocated more biomass to above-ground parts and less to below-ground parts under low soil N, whereas the contrary pattern was found in co-occurring non-

carnivorous plants. This pattern was demonstrated in *D. burmanni* which has a higher content of plant N than its non-carnivorous counterpart, *Fimbristylis* sp (Table 4.6 and Figure 4.6). However, the two species display contrasting patterns in biomass allocation to above-ground and below-ground parts. *Drosera burmanni* has a lower proportion of biomass allocation to below-ground parts than *Fimbristylis* sp as reflected by root:shoot dry mass ratios. Differences in root:shoot dry mass ratios between the two species indicate differences in the way of nutrient enter into the plant. For *D. burmanni*, the greater allocation of biomass to above-ground parts than to below-ground parts can be viewed as a strategy of foraging for N by means of traps on the above-ground part (Król et al., 2012, Ellison and Adamec, 2018). In contrast, *Fimbristylis* sp, the co-existing non-carnivorous plant, is dependent upon root uptake. This particular species has a larger proportion of below-ground parts (roots) than *D. burmanni*. This pattern supports the view that the allocation of biomass to roots and shoots is influenced by where the nutrient is usually acquired by the plant (Aerts and Chapin III (1999).

Paradoxically, of the *Drosera* studied, *D. adelae* obtained the largest proportion of plant N from prey but exhibited relatively similar plant N contents and root:shoot dry mass ratios to all of the examined non-carnivorous species in its microhabitat (Table 4.3 and Figure 4.3). This observation highlights the importance of taking into account the multifunctionality of plant organs when attempting to relate resource allocation with a particular trait, in this case N accumulation. Roots provide structural support and the habitat of *D. adelae* is often precarious. Plants, which are relatively large for *Drosera*, live alongside, or on, cliffs and near small creeks, often in places where the soil layer is thin or vagile. It is possible that *D. adelae* allocates proportionally more biomass to roots in comparison to *D. burmanni*, which

is small and which lives on more stable surfaces, because of greater demand upon its roots to act as hold-fasts.

Concentrations of N may not be equally distributed in plant tissues. The majority of plants examined store more N in above-ground parts ranging from 0.4 to 1.6% (Figure 4.1.A, Figure 4.2.A, Figure 4.3.A, and Figure 4.4.A). Such a pattern has also been reported in other plants across seven types of biome in China documenting high N contents in the above-ground parts, particularly in leaves (Tang et al., 2018). The concentrations of foliar N measured in *Drosera* and non-carnivorous conspecifics in this study lie within a range generally regarded as limiting (Ellison, 2006), but are not atypical for *Drosera* and other carnivorous plants (Figure 4.8).

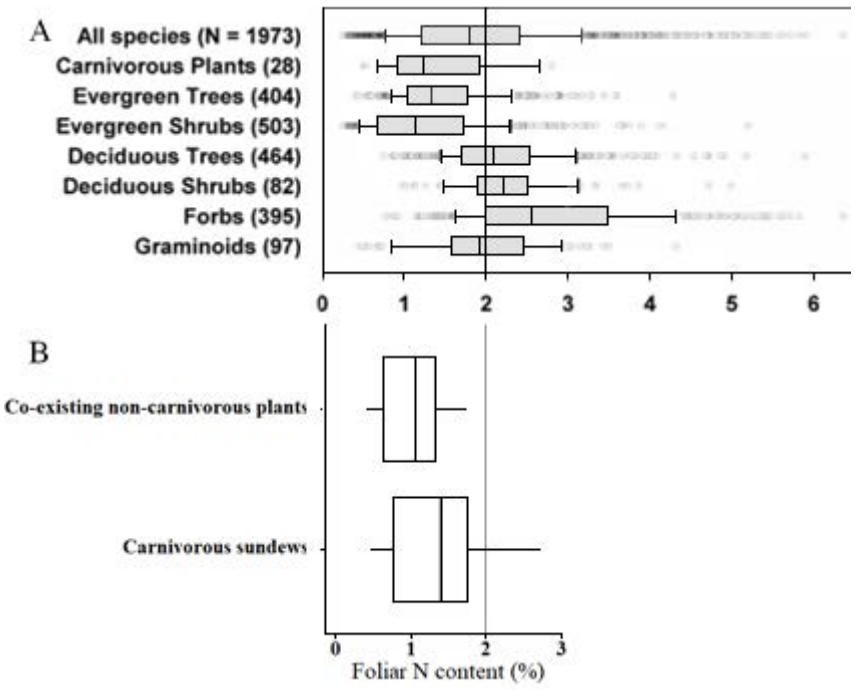

Figure 4.8 The distribution of leaf N concentrations in carnivorous and non-carnivorous plants. A. Foliar leaf N based on the GlopNet database (Wright et al., 2004) summarised in Ellison (2006). B. Foliar leaf N from the present study. The middle line in each box represents median of the observed distribution, the left and right parts the 25th and 75th percentiles. The vertical line at 2% is the concentration below which the nutrient is considered to be limiting (Aerts and Chapin III, 1999).

Nevertheless, it is not uncommon for carnivorous *Drosera* to have lower root:shoot dry mass ratios than their co-occurring non-carnivorous plants (Figure 4.5), for example, *ca.* 75% of root:shoot dry mass ratios measured in this study were lower than 0.5. This tendency to allocate resources to above-ground parts can be viewed as a strategy to invest in above-ground nutrient uptake (Ellison, 2006, Król et al., 2012). The root:shoot allocation could also respond to low light conditions (Lavarack, 1979) or, as is possible for *D. adelae*, to an increased requirement for support. The argument for nutrient foraging strategy is supported in *D. burmanni*, while the adaptation to low light situations is supported in *D. schizandra*, the rainforest sundew which has large broad leaves.

The *Drosera* studied were expected to exhibit N contents that reflected their contrasting habitats and life spans (i.e. perennial vs. annual plants). One might expect that *D. adelae* and *D. schizandra* have low demand for N as both are rainforest species occurring in shaded environments where plant growth rates and investment in Calvin-cycle proteins tend to be low. In contrast, *D. burmanni* and *D. spatulata,* which inhabit more open less-light limited environments might have higher plant N levels. The results reported here partly support the arguments. All of the species gained between *ca.* 50 % and 100 % of their N from prey. The N content in plant organs of *D. burmanni* and *D. schizandra* support the above arguments in that N levels were high in the *D. burmanni* which inhabits more open areas and is an annual and were low in *D. schizandra*, a deep rainforest species. In contrast, *D. adelae* had higher plant N levels than predicted and the open-forest *D. spatulata* had relatively low levels of plant N. The unusual pattern of plant N levels in both *D. adelae* and *D. spatulata* may simply reflect prey catching in the field. In Chapter 3 it was demonstrated that *D. adelae* trapped a greater prey biomass in comparison to its rainforest counterpart, *D. schizandra*. In

comparison, *D. spatulata*, appeared to trap fewer prey at the sites where plants were examined.

In Chapter 3, differences in biomass of captured prey were observed among *D. adelae, D. schizandra, D. spatulata* and *D. burmanni*. *Drosera adelae* captured a greater biomass of prey than *D. spatulata* and *D. schizandra* but captures by *D. burmanni* were within the range of that of *D. adelae*. The hypothesis that a high prey capture level is associated with the contribution of prey N to total plant N content is accepted by evaluating $\delta^{15}N$ values of plants and insects among the *Drosera* species examined. *Drosera adelae* exhibited a higher dependency on prey-derived N in comparison with other carnivorous sundews (Figure 4.7). This observation is supported by evidence that the N content in *D. adelae* was greater than that in *D. schizandra* and *D. spatulata* (Figure 4.6.A).

The variation of the contribution of prey N to total plant N content in the present study corroborates the view of the variability of contribution of prey N to total plant N content across different carnivorous plants (Ellison and Gotelli, 2001, Adamec and Pavlovic, 2018). Possible explanations of high variability in reliance on prey N supply include differences in the availability of soil N (Millett et al., 2015, Cook et al., 2017), prey capture level (Hanslin and Karlsson, 1996), plant size and growth form (Schulze et al., 1991), and plant size of traps (Schulze et al., 1997).

4.6 Conclusion

This research demonstrates that carnivorous and non-carnivorous plants inhabiting the same microhabitat may display different biomass allocation to plant parts depending upon their nutrient foraging strategies. Many carnivorous sundews allocate more biomass to above-

ground parts than below-ground parts, an allocation that may be associated with the above-ground biomass acting as a principal N source. On the contrary, some non-carnivorous plants allocate more biomass possibly to roots to enhance root nutrient uptake. Both carnivorous *Drosera* and co-existing non-carnivorous plants may also exhibit similar patterns in biomass allocation to above-ground and below-ground parts. The latter observation suggests that in both groups root functions such as the supply of water or other nutrients or support, are equally limiting.

Nitrogen demand differs between carnivorous sundews inhabiting different vegetation complexes is associated with light regime in environments and life spans (i.e. perennial vs. annual plants). Closed-woodland species of *Drosera* (e.g. *D. schizandra*) tend to operate with lower concentrations of plant N than open-woodland species (e.g. *D. burmanni*). However, such a pattern may not be present if abnormally high levels of prey capture occur.

The contribution of prey N to total plant N differed among carnivorous sundews. *D. adelae*, the rainforest sundews, display a greater dependency on foliar uptake than other three sundews. The variability of the contribution of prey N to total plant N was evident in each carnivorous *Drosera* species reflecting different available resources (i.e. prey and soil nutrient availability) in its environment.

Chapter 5. Trapping capacities of four carnivorous sundews of tropical Australia

5.1 Abstract

Trapping capacity, the potential of a plant to trap prey, is a key ecological parameter of carnivory among *Drosera*. Essential element uptake by D*rosera* that inhabit nutrient-poor environments depends heavily upon the ability of plants to capture prey that come into contact with them. Surprisingly, important components of trapping capacity such as tentacle density, tentacle composition, mucilage volume and mucilage adhesive force have rarely been explored. Here I report species variations in these elements that contribute to trapping capacity. Using image analysis, I compared tentacle densities and volumes of mucilage droplets within and among different plant sizes of *Drosera* species. This research used a novel approach to quantify mucilage adhesive force using nano-machined silicon-tipped micro-force sensors. The adhesive force was used to calculate leaf resting adhesive capacity (RAC), a measure of the adhesive capacity per unit leaf area. The masses and lengths of prey trapped were compared with the adhesive force capacity to trap.

Increasing plant size was associated with reduced tentacle density. However, the volume of mucilage droplets at the tip of each tentacle and the adhesive capacity of the mucilage did not show a similar pattern. Volumes of mucilage droplets and mucilage adhesiveness for the rainforest species, *D. adelae* and *D. schizandra*, were independent of plant size. In contrast, plant size, mucilage production and mucilage adhesive force were correlated positively for *D. burmanni* and *D. spatulata,* the taxa from more open habitats. Although these observations are consistent with light influencing the development of trapping capacity, size limits to prey

captured could also simply reflect the size of insects at the plant location or insect behavioural patterns.

The capacity to trap insects of a certain mass exceeded the masses of the insects trapped. It is unlikely that prey of larger mass was not available in the *Drosera* habitats. The potential for prey above certain masses to exhibit a greater capacity to escape than predicted by maximum lift load forces or for mucilage in plants under field-grown conditions to have lower adhesive capacity than measured for laboratory-grown plants is explored.

5.2 Introduction

The size of insects captured by *Drosera*, and presumably the number of insects trapped, depends upon the ability of sticky *Drosera* tentacles to adhere to prey and to restrict the prey animals from escaping until they die and can be digested. This capacity to trap will reflect features that include the density of the tentacles, the stickiness of the mucilage that coats the heads of the tentacles, the volume of mucilage on the tips of the tentacles, and the type and arrangement of tentacles. The ability of a trapped organism to escape will depend upon the number of tentacles that connect to the prey, the adhesive interaction between the plant mucilage and the exoskeleton/skin of the organism, the escape forces that legs and wings can exert, and the time over which escape forces can be generated. In the case of flying insects, escape ability is also influenced by the ability of the animals to lift body weight plus a load defined by the adhesive capacity of the mucilage (Marden, 1987). Over time, a trapped insect will tire and the escape capacity will be reduced (assuming the adhesive force of the mucilage does not change).

The role of the sticky-tentacles is to trap and retain prey. Tentacle stickiness results from a viscous mucilage secreted on the tentacle heads (Robinson et al., 2017). The interaction between the exoskeleton of the prey and mucilage in contact with it can be described by the force required to separate the two, the adhesive force.

The viscoelastic, homogenous mucilage droplets of *Drosera* exhibit characteristics typical of hydrogels (Adlassing et al., 2010, Erni et al., 2011, Huang et al., 2015), networks of hydrophilic polymers that can swell in water. While trapping a large mass of water, hydrogels can maintain their three-dimensional structure by means of chemical or physical cross-linking of individual polymer chains. The polysaccharides in *Drosera* mucilages are typically acidic

polysaccharides with molecular weights in excess of 2 x 10^6 Da (Rost and Schauer, 1977, Gowda et al., 1982, Gowda et al., 1983). The principal components of the polysaccharides include galactose, mannose, xylose and glucuronic acid. A recent NMR study also detected lipophilic methyl ester and alkyl chain-like moieties and myo-inisotol (Kokubun, 2017). These charged lipophilic moieties may provide the prey-trapping mucilage with an ability to adhere to hydrophobic insect body parts. Although reportedly non-toxic, as captured prey may escape after some time (Gaume and Forterre, 2007), and non-inhibitory to the digestive enzymes of the host, the mucilages resist microbial growth (Adlassnig et al., 2010)

Characteristics of these remarkable *Drosera* mucilages include the capacity to bind to different insect organs such as waxy exoskeletons and wings, some of which have microscopic water-repellent features (Darmanin and Guittard, 2015). Upon contact with prey, the mucilage spreads and adheres to any structures and cuticles in between. It has been suggested that adhesion of mucilage to water-repellent body parts of the prey would, by providing a hydrophilic surface, increase the area of contact for digestive enzymes (Gaume and Forterre, 2007). Being acidic hydrogels, one might expect that the adhesive properties of *Drosera* mucilages might be affected by changes in acidity, temperature (Rost and Schauer, 1977, Adlassing et al., 2010) and humidity, also by other factors that directly or indirectly affect humidity, such as exposure to sunlight.

Four types of mucilage-tipped tentacles known in *Drosera* but only two are adhesive (Poppinga et al., 2013). Tentacle types designated as T-0 and T-1 are sticky, but their movement and structure differ. T-0 tentacles can bend toward prey animals in any direction, whereas T-1 tentacles, which have longer stalk than T-0 tentacles, only bend in one plane (Hartmeyer and Hartmeyer, 2010). The complement of these mucilage-covered tentacles

differs among *Drosera*. *Drosera schizandra*, a rainforest sundew, for instance, relies only on T-0 tentacles for capturing prey. In contrast, *D. burmanni* possesses both T-0 tentacles, located in the leaf centre, and T-1 tentacles, located on the leaf margins (Figure 5.1).

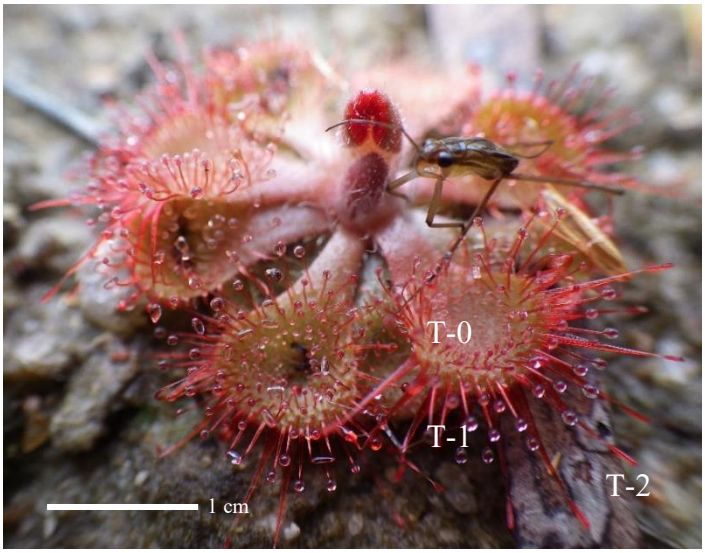

Figure 5.1 Tentacle arrangement in *Drosera burmanni*: T-0, T-1 and T-2 tentacles are indicated.

In Chapter 3, I reported differences in the number of prey captured by the *Drosera* studied. *Drosera adelae* caught relatively more prey than *D. schizandra* and *D. spatulata*. It is plausible that the differences in prey capture between the species is a function of trapping capacity. Although adhesive capacity of the mucilage is a fundamental component of the trapping capacity of *Drosera*, there are few reports of measurements of the adhesive capacity of intact tentacles *in vivo*. Mean adhesive force of *Drosera* traps reportedly ranged from 300 to 0.2 μN mm^{-2} (Thorén et al., 2003, Cook et al., 2017). It should be noted, however, these measurements were performed by attaching hydrophilic filter paper to several tentacles at once. The filter paper was attached either to a dynamometer (Thorén et al., 2003) or to handheld digital force gauge (Cook et al., 2017). The accuracy of the two methods relied upon the speed by which each instrument was pulled upwards. Not only does inconsistency in

the pulling speed lead to a high variation in measurements but no information was gained on the variation in adhesiveness between individual tentacles as the sizes of filter paper used, *ca*. 1 cm^2, were sufficiently large to stick to a number of tentacles.

In this chapter, I report the first study of adhesive forces exhibited *in vivo* by tentacle-tip mucilage for single *Drosera* tentacles. The observations are for four carnivorous sundews, two from closed Australian tropical woodlands, *D. adelae* and *D. schizandra*, and two from open Australian tropical woodlands, *D. burmanni* and *D. spatulata*. I specifically investigated 1) the relationships within and among species of plant size and tentacle region on tentacle densities and volumes of tentacle droplets, 2) the adhesive forces expressed by tentacle mucilage, 3) the influences of tentacle types on tentacle adhesive force, and 4) the influences of light regimes (low [natural] vs elevated light intensity) on adhesive force.

The technology used to measure adhesive force was novel. For the first time, state-of-the-art nano-machined silicon-tipped micro-force sensors, capable of measuring forces as low as 5 μN were used to measure the adhesive force exhibited by mucilage on the tips of individual tentacles. 5 μN is equivalent to the force exhibited by a mass of about 0.00051 g (see footnote).

Footnote

Gram-force [gf], a metric unit of force, is equal to a mass of 1 g multiplied by the standard acceleration due to gravity on earth [9.80665 ms^{-2}]. One gram-force thus
= 0.001 kg × 9.80665 ms^{-2}
= 0.00980665 kg × ms^{-2}
= 0.00980665 N
= 9,806.65 μN.
5 μN is thus equivalent to 0.0051 gf.

Development of the experimental method required the construction of a low vibration sensor delivery and monitoring system that delivered the tip of the sensor, 50 µm in width at its tip, 125 µm into the tentacle mucilage, and then withdrawing the sensor at a rate of 200 µm s^{-1}.

5.3 Methods

5.3.1 Measurements of carnivory capacity *in situ*

Tentacle density and volumes of mucilage associated with tentacles were measured *in situ* for small, medium and large plants (Tables 5.1 and 5.2) of each *Drosera* species during the dry season of 2018.

Table 5.1 Plant size definitions of *Drosera*

Plant size	Species	Plant size (rosette diameter) cm
Small	*D. adelae*	5 – 10
	D. burmanni	< 1
	D. schizandra	5 – 10
	D. spatulata	< 2
Medium	*D. adelae*	11 – 20
	D. burmanni	1 – 2
	D. schizandra	11 – 20
	D. spatulata	2 – 4
Large	*D. adelae*	21 – 30
	D. burmanni	2 – 3
	D. schizandra	20 – 30
	D. spatulata	4 – 6

High-resolution photographs (Ricoh WG-4, Japan) of each plant taken between 07:00 h and 13:00 h were used to estimate tentacle densities and volumes of mucilage droplets. For leaves with a surface area less of than 1 cm^2, all tentacles were censused. For leaves with the surface area in excess of 1 cm^2, three to six 0.25 cm^2 quadrats per leaf were assessed, depending upon the leaf shape (Figure 5.2). The estimation of mucilage volumes was performed for tentacles located close to the leaf margins. These marginal tentacles were chosen because the

resolution of the images was greatest, resulting in more accurate image analysis. Five to ten tentacle heads per plant were randomly selected and the droplet volumes were estimated using Egg Tool (Troscianko, 2014), a plug-in for ImageJ (Schneider et al., 2012).

Table 5.2 Summary of tentacular measurements undertaken upon *Drosera*

Species	Measured tentacles for density	Measured tentacles for volumes estimates
D. adelae	T-0	T-0
D. burmanni	T-0, T-1 and T-2	T-1 *
D. schizandra	T-0	T-0
D. spatulata	T-0 and T-1	T-1 *

* The estimation of mucilage volumes was performed for T-1 tentacles because the resolution of the images was greatest, resulting in more accurate image analysis.

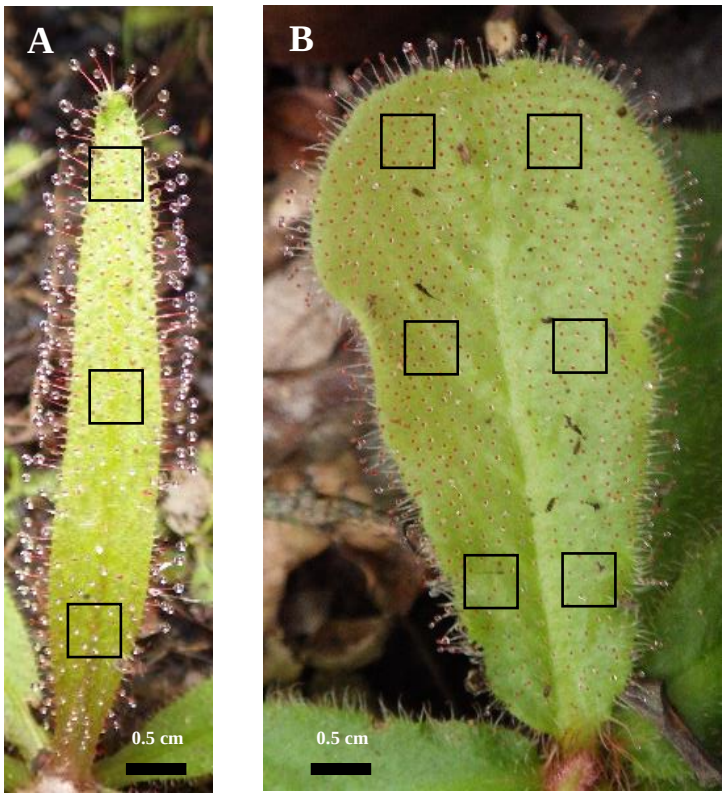

Figure 5.2 Sites of tentacle density observations on leaves of *Drosera adelae* (A) and *Drosera schizandra* (B). Basal, medial and tip sampling sites are indicated.

5.3.2 Plant material for measurements of tentacle stickiness

Five representative small and medium plants of *D. adelae* were collected from the Bemerside population (for descriptions of study species and study sites see sections 2.1 and 2.2, respectively). No large plants were collected due to their rarity, a result of the frequency of frequent flooding events in their riparian habitat. Five small, medium and large plants of *D. schizandra* were collected from Wooroonoran National Park and five small, medium and large *D. burmanni* were collected from Cardwell. Plants were individually grown in plastic pots with soil from their natural habitat.

For *D. adelae* and *D. schizandra,* pots were placed in trays filled with distilled water to a depth of 1 cm. Trays, each covered by a clear plastic dome to maintain humidity, were maintained in a controlled environment in the laboratory with temperatures between 22ºC and 24ºC and 90% relative humidity. For *D. adelae*, the trays were kept at the light intensity of ca. 8 µmol m^{-2} s^{-1}, while *D. schizandra* was grown at the light intensity of *ca.* 5 µmol m^{-2} s^{-1}. *Drosera burmanni* were grown under natural light at a maximum light intensity of 850 µmol m^{-2} s^{-1} under day/night temperatures of *ca.*30/20ºC and relative humidities of *ca.* 90/60 %.

To compare tentacle adhesive forces between plants adjusted to elevated or low (natural) light intensity, five medium plants of *D. adelae* were collected from the same habitat as those kept in the low light environment as mentioned above. The trays of these plants were kept in the same controlled chamber, but they received light intensity at 30 µmol m^{-2} s^{-1}.

5.3.3 Measurements of tentacle adhesive force

The adhesive capacity of mucilage was quantified using the system shown in Figures 5.3.A and B. The system included a 3 mm micro-force silicon sensor probe (FT-S100000,

FemtoTools, Zurich, CH), with a 125 µm 45º bevelled apex that was 50 µm in diameter. The sensor is capable of measuring forces ranging from millinewtons to nanonewtons with resolution at 1000Hz of 50 µN. The probe was attached to a motor that moved the sensor in a two-dimensional plane with a minimum thread-driven step of 3.175 µm (UMP-3 micro-pump, World Precision Instruments, FL). The motor was controlled by a micro-syringe pump controller (SYS-Micro4, World Precision Instruments, FL). The sensor was connected to a 12 V battery-powered datalogger (CR850, Campbell Scientific Australia, Qld.) by means of shielded coaxial cable. Output from the sensor was logged at 100 ms intervals. The components of the force–measuring system were each underlain by a vibration-dampening 1 cm thick playground-tile made from shredded rubber and the entire system was housed in a plastic cubicle (1400 x 700 x 2000 mm) located in a laboratory maintained at 25ºC and 50 % RH. When experiments were undertaken, the relative humidity within the cubicle was elevated to ca. 85 – 90 % by two humidifiers (Philips HU4706).

Tentacle adhesive force was measured by manoeuvring the micro-force sensor probe, with the assistance of a dissection microscope (Leica MZ6, Jena, FRG), such that the sensor tip was placed in the centre of a mucilage droplet. Optimum depth of placement of the sensor was 125 µm, the depth of the bevelled section at the tip of the probe (Figure 5.3.B). When so-placed, the contact area of the sensor tip with the tentacle droplet was 63,930 μm^2. The adhesive strength of the mucilage was determined by measuring the force (in µN) required to pull the probe tip from the droplet at a constant speed at 0.2 mm s^{-1}. After the datalogger recorded force data for each measurement, the raw data were processed to calculate the estimation of adhesive force as illustrated in Figure 5.4. The tip of the probe was cleaned between measurements by sequentially placing the tip of the probe into diluted soap liquid for

30 s, into reverse osmosis water for 30 s, and finally into 75% ethanol for 30 second prior to the next measurement.

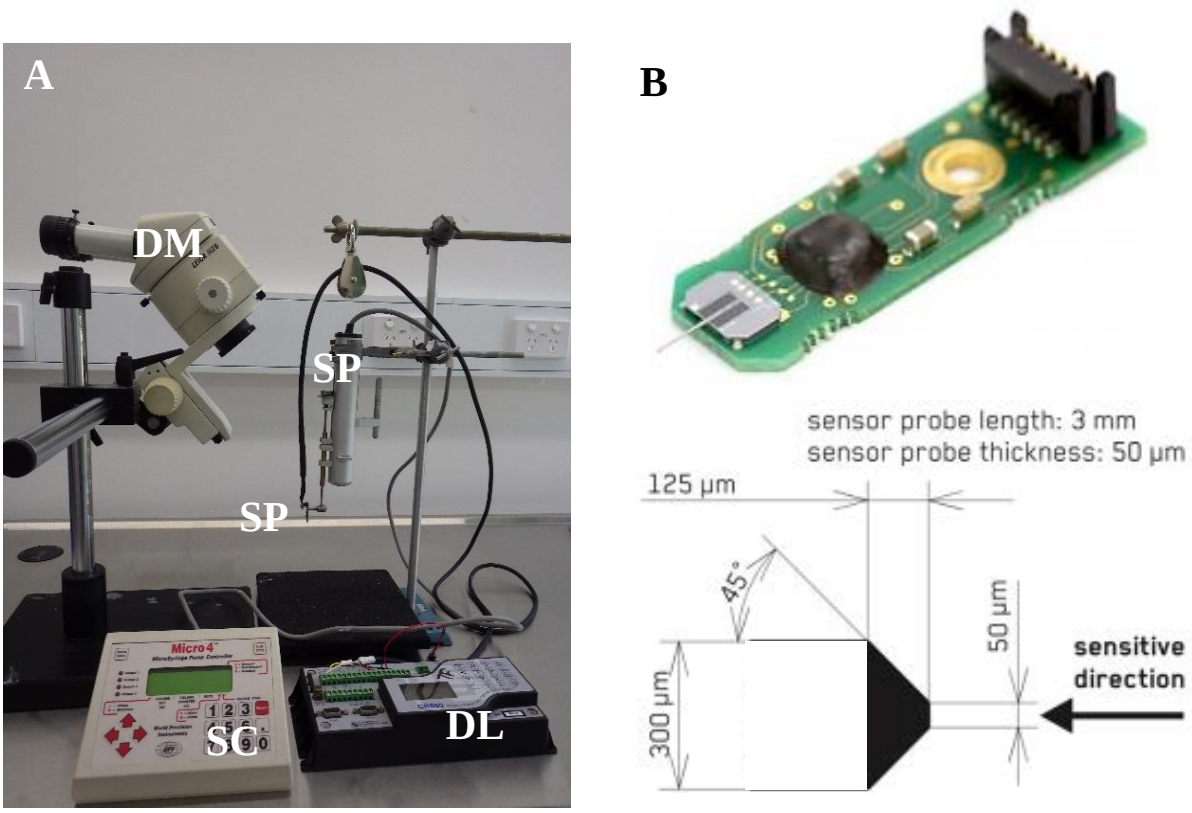

Figure 5.3 The device to measure the levels of tentacle stickiness (A) and sensor probe and contact areas between tip sensor and tentacle droplets (B). FS: Force Sensor, SP: Sensor Puller, DL: Datalogger, DM: Dissecting Microscope, SC: Sensor Controller, CA: Contact Area. Images of sensor probe and the specification of the sensor tip are from the datasheet of FT-S100000, which can be downloaded from FemtoTools website.

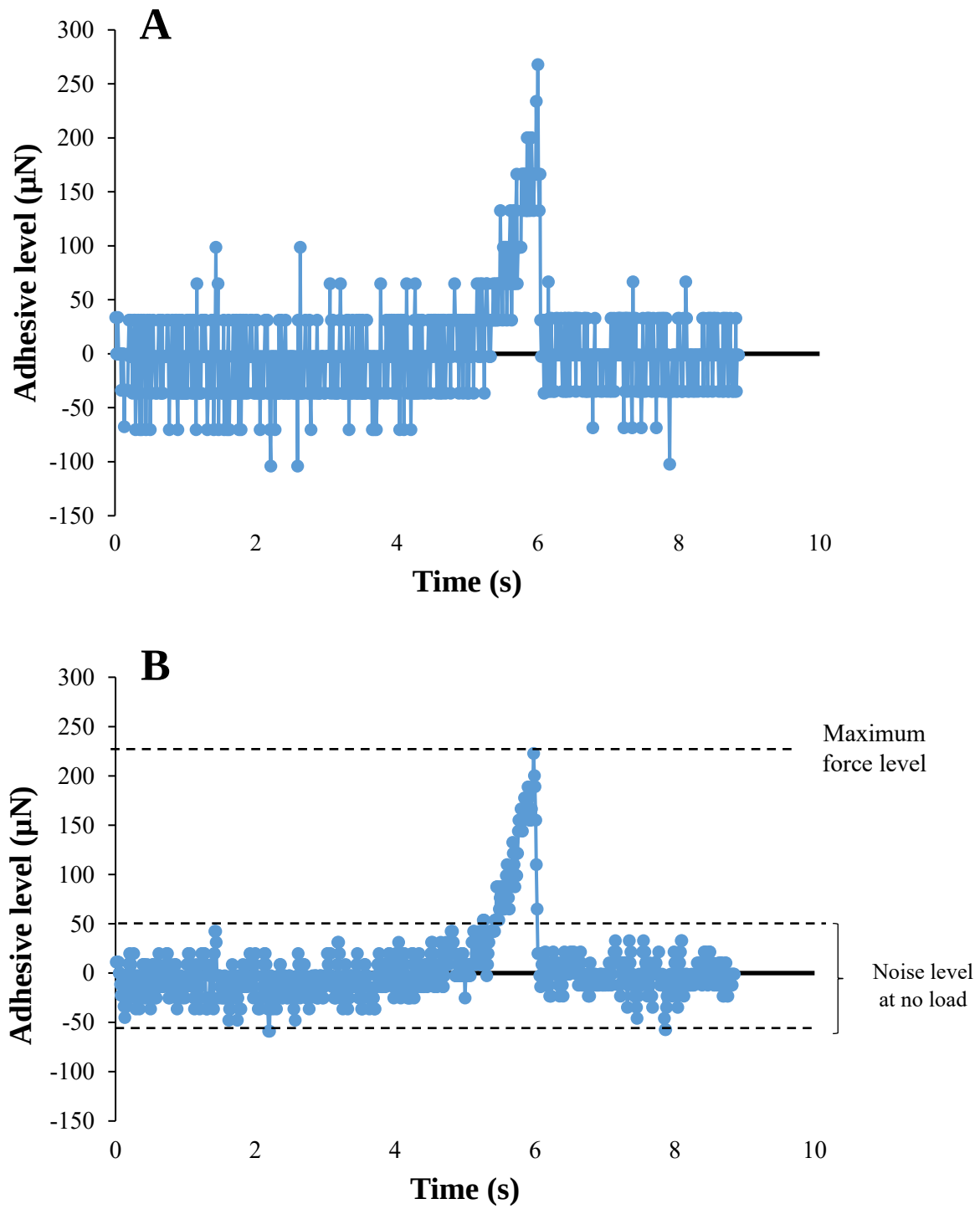

Figure 5.4 A raw data trace of the force (µN) applied to a sensor inserted into a mucilage droplet (A). At time zero, the sensor tip was extracted at 200 µm s^{-1}. The kinetics of sensor extraction reflect both adhesion of the mucilage to the sensor and the elasticity of the mucilage before the connection with the sensor breaks. Data was processed by running three moving averages before the estimation of adhesive force (B). Adhesive force was calculated by subtracting the mean of noise level at no load reading from the maximum force level.

For *D. adelae* and *D. schizandra*, measurements of the adhesive capability of the tentacle-tip mucilage were undertaken on six T-0 tentacles per leaf. Detailed positions for *D. adelae* and *D. schizandra* are displayed in Figure 5.5.A and 5.5.B, respectively. To compare adhesive forces between the two tentacle types in *D. burmanni*, three T-1 and three T-0 tentacles were selected, chosen as shown in Figure 5.5.C. Comparisons of tentacle stickiness of medium and large plants of *D. burmanni* were performed by measuring tentacle adhesive force on three T-1 tentacles per plant.

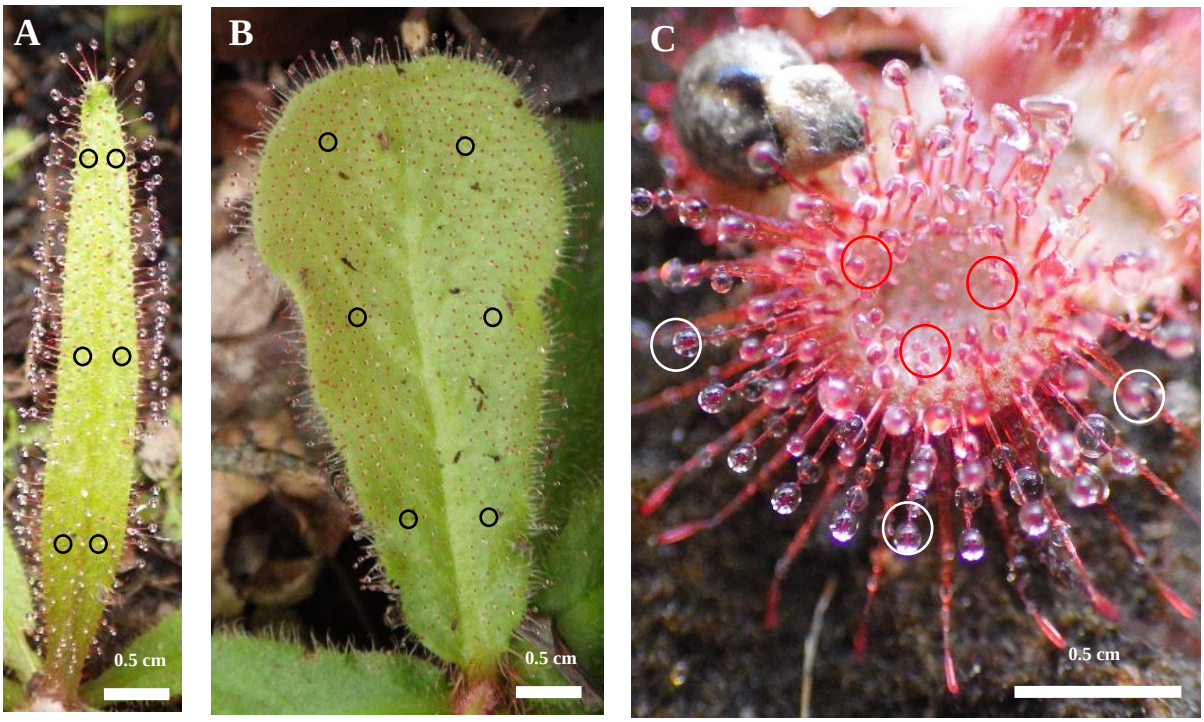

Figure 5.5 Position of measurements for the adhesive capacity of tentacular mucilage for *Drosera adelae* (A), *Drosera schizandra* (B), and *Drosera burmanni* (C). For *D. burmanni*, the white circles encircle T-1 tentacles, and the red ones encircle T-0 tentacles.

5.3.4 Conversion of sensor force data to leaf area to resting adhesive capacity (RAC)

The resting adhesive capacity (RAC) is the capacity for the surface area of mucilage on per mm^2 of leaf to stick to a sensor. The value does include a mucilage elasticity component that is observed when the mucilage stretches as the sensor is removed from a droplet. The

effective adhesive capacity may be greater than RAC as the value does not take into account increases in surface area that may come about when prey smear adhesive onto their bodies.

Calculation of leaf RAC required expressing sensor contact area, adhesive force and tentacle density data using similar units (mm^2, mm^{-2}) and calculating the surface areas of mucilage drops (mm^2) from drop volumes, assuming that the drops were spheres. Leaf RAC per unit leaf area is thus calculated as:

RAC	=	mucilage surface area per drop	x	tentacle density	x	adhesive force
$\mu N\ mm^{-2}$		mm^2		mm^{-2}		$\mu N\ mm^{-2}$

5.3.5 Estimation of potential prey size

In order to place the measurements of RAC in ecological context, it was necessary to compare RAC values of the *Drosera* with measurements of the capacity of prey to move/escape in the presence of such restrictive forces. By observing the ability of flying animals to take-off with weights attached to their legs, Marden (1987) estimated the maximum lift forces exhibited by flying animals, including bats, birds and a variety of insects from a range of orders. The lift forces were expressed on the basis of fresh mass.

In this study, due to the breakdown of prey constrained in *Drosera* traps, fresh mass data was not available as prey masses were recorded as dry mass and related to lengths by means of a regression based upon insect body volumes (Chapter 3). Fresh mass data from Marden (1987) was therefore transformed to estimate dry mass assuming a dry mass - fresh mass ratio recorded in the literature for the most common prey of the *Drosera* tested, a dipteran. The

dipteran model was *Drosophila melanogaster*, for which a dry mass – fresh mass ratio of 0.339 was assumed (Robinson et al., 2000).

5.3.6 Statistical analyses

Data analyses for *D. adelae* and *D. schizandra* were separated from *D. spatulata* and *D. burmanni* because of rosette size and tentacle differences. Tentacle density and adhesive capacity of *D. adelae* and *D. schizandra* of mucilage on tentacular tips were examined with factorial ANOVA followed by Tukey HSD. Prior to data analyses, assumptions for factorial ANOVA were checked, and log-transformation was applied if violations of assumptions were detected. Tentacle density of *D. burmanni* and *D. spatulata* and volumes of mucilage droplets of *D. adelae* and *D. schizandra* were assessed with ANOVA of Aligned Rank Transformed Data within the ARTool package in R followed by pairwise comparisons using least square means with Tukey adjustment (Wobbrock et al., 2011). This non-parametric equivalent to factorial ANOVA was used because data transformation fails to handle violations of normality. Data on tentacle density for *D. burmanni* and *D. spatulata* and on tentacle adhesiveness for *D. adelae* and *D. schizandra* were treated as the missing cell design due to the absence of T-2 tentacles for *D. spatulata* and samples of large plants for *D. adelae*, respectively (see section 5.3.1 and 5.3.2 for further details). To deal with the missing cell design in factorial ANOVA, analysing balanced subsets of data with observations in all cells was employed (Quinn and Keough, 2002). Differences in adhesive capacity of mucilage on tentacular tips between the plant size and two tentacle types in *D. burmanni* were examined using a t-test and two-tailed paired t-test, respectively. Data visualisation was carried out using the *ggpubr* function in R (Kassambara, 2019).

5.4 Results

5.4.1 Tentacle density

Tentacle density varied across the four *Drosera* species, three plant sizes, tentacular region and tentacle types (Table 5.3), with larger plants having the lowest tentacle density.

Table 5.3 Mean ± SD tentacle densities of four *Drosera* species as a function of plant size, tentacle region and tentacle type. Values in parentheses are number of samples. NA = Not Applicable

Species	Plant size	Tentacular region	Tentacle types	Density (cm^{-2})
Drosera adelae	Small (5)	Tip	T-0	73.9 ± 18.6
		Mid	T-0	73.9 ± 22.2
		Base	T-0	80.9 ± 22.4
			Mean	76.2 ± 19.9
	Medium (5)	Tip	T-0	86.0 ± 17.8
		Mid	T-0	72.0 ± 20.8
		Base	T-0	77.2 ± 20.2
			Mean	78.4 ±19.1
	Large (3)	Tip	T-0	55.6 ± 7.7
		Mid	T-0	51.1 ± 8.1
		Base	T-0	50.7 ± 6.1
			Mean	52.4 ± 6.8
			Mean at species level	**71.6 ± 20.1**
Drosera schizandra	Small (5)	Tip	T-0	109.6 ± 53.1
		Mid	T-0	86.0 ± 29.1
		Base	T-0	99.2 ± 32.9
			Mean	98.3 ± 32.9
	Medium (5)	Tip	T-0	66.0 ± 33.6
		Mid	T-0	49.6 ± 20.3
		Base	T-0	49.2 ± 18.7
			Mean	54.9 ± 24.6
	Large (2)	Tip	T-0	41.0 ± 7.1
		Mid	T-0	32.0 ± 2.8
		Base	T-0	35.0 ± 7.1
			Mean	36.0 ± 6.2
			Mean at species level	**69.8 ± 38.3**
Drosera spatulata	Small (5)	NA	T-0	444.2 ± 50.8
			T-1	401.7 ± 55.3
			Mean	422.9 ± 54.9
	Medium (5)	NA	T-0	299.5 ± 34.9
			T-1	264.2 ± 42.0
			Mean	281.9 ± 40.9
	Large (3)	NA	T-0	268.9 ± 11.1
			T-1	261.6 ± 10.5
			Mean	265.2 ± 10.5
			Mean at species level	**332.3 ± 84.2**
Drosera burmanni	Small (5)	NA	T-0	497.0 ± 69.5
			T-1	333.2 ± 51.5
			T-2	92.9 ± 13.7
			Mean	307.7 ± 178.1
	Medium (4)	NA	T-0	309.2 ± 166.8

Species	Plant size	Tentacular region	Tentacle types	Density (cm^{-2})
			T-1	162.7 ± 33.4
			T-2	38.1 ± 4.3
			Mean	170.2 ± 146.1
	Large (2)	NA	T-0	277.3 ± 24.7
			T-1	123.0 ± 4.9
			T-2	32.8 ± 1.9
			Mean	144.4 ± 111.2
			Mean at species level	**228.0 ± 169.4**

Mean tentacle density of *D. adelae* (71.6 cm^{-2}) did not differ from that of *D. schizandra* (69.8 cm^{-2}) (three-way ANOVA, $F_{1,57} = 0.405$, $p = 0.53$). When tentacle density was compared between plant size, large plants of *D. adelae* and *D. schizandra* had a lower tentacle density than small and medium plants (Table 5.3, three-way ANOVA, $F_{2,57} = 14.0$, $p < 0.01$). Mean tentacle density did not significantly differ among tentacular regions (Table 5.3, three-way ANOVA, $F_{2,57} = 1.410$, $p = 0.25$) and a significant interaction was present between species and plant size (three-way ANOVA, $F_{2,57} = 6.545$, $p < 0.01$, Figure 5.6) indicating that tentacle densities of *D. adelae* and *D. schizandra* depend upon their plant size (Table 5.3). There were no significant interactions between species and tentacle region (three-way ANOVA, $F_{2,57} = 0.382$, $p = 0.68$, Table 5.3), between plant size and tentacular regions (three-way ANOVA, $F_{4,57} = 0.159$, $p = 0.96$, Table 5.3) and among species, plant size and tentacular regions (three-way ANOVA, $F_{4,57} = 0.136$, $p = 0.97$, Table 5.3).

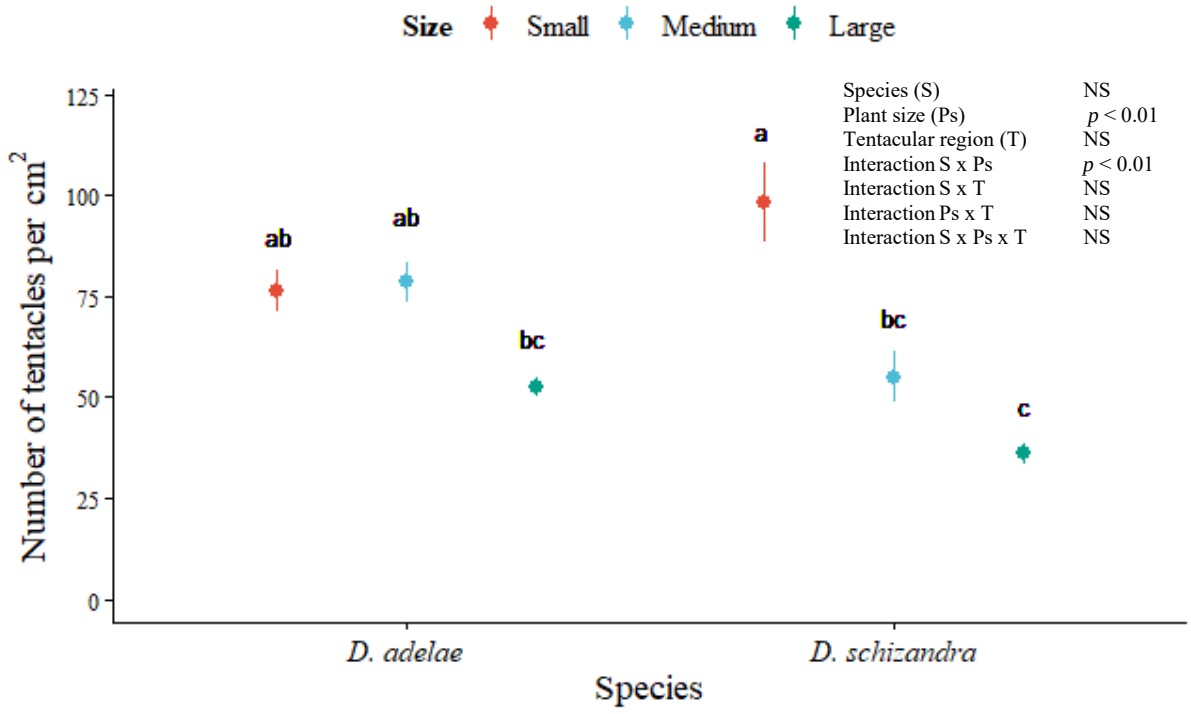

Figure 5.6 Mean (coloured dots) ± SE T-0 tentacles per cm^2 in small, medium and large *Drosera adelae* and *D. schizandra*. Text indicates statistical results for main effects (species, plant size, tentacular region) and interactions between main effects. NS: non-significant. Different letters above mean plots indicate significant differences (Tukey, $p < 0.05$). Dataset was log-transformed to meet the assumption of a factorial ANOVA.

Tentacle densities of *Drosera burmanni* and *D. spatulata* were analysed using a combination between three-way ANOVA omitting T-2 tentacles in *D. burmanni* and two-way ANOVA for *D. burmanni* only to handle missing cell design as described in the method section (see Table 5.2 for details on dataset). Mean tentacle density of *D. spatulata* (332.3 cm^{-2}) was not significantly different than that of *D. burmanni* (228.0 cm^{-2}) (three-way ANOVA of Aligned Rank Transformed Data, $F_{1,36} = 3.342$, $p = 0.08$). When tentacle density was compared with plant size, mean tentacle density of small plants (307.7 cm^{-2} for *D. burmanni* and 422.9 cm^{-2} for *D. spatulata*) was higher than medium (170.2 cm^{-2} for *D. burmanni* and 281.9 cm^{-2} for *D. spatulata*) and large plants (144.4 cm^{-2} for *D. burmanni* and 265.2 cm^{-2} for *D. spatulata*) (three-way ANOVA of Aligned Rank Transformed Data, $F_{2,36} = 35.801$, $p < 0.01$, Figure 5.7.A). Mean tentacle density of T-0 tentacles across the two species was higher than that of T-1 tentacles (three-way ANOVA of Aligned Rank Transformed Data, $F_{1,36} = 30.747$, $p <$

0.01, Table 5.3). Significant interaction was observed between species and tentacle types (three-way ANOVA of Aligned Rank Transformed Data, $F_{1,36} = 14.436$, $p < 0.01$, Table 5.3, Figure 5.7.B). There were no significant interactions between species and plant size (three-way ANOVA of Aligned Rank Transformed Data, $F_{2,36} = 1.169$, $p = 0.32$, Table 5.3), between plant size and tentacle type (three-way ANOVA of Aligned Rank Transformed Data, $F_{2,36} = 0.566$, $p = 0.57$, Table 5.3) and among species, plant size and tentacle type (three-way ANOVA of Aligned Rank Transformed Data , $F_{2,36} = 0.253$, $p = 0.78$, Table 5.3).

Another combination of factorial ANOVA for *D. burmanni* revealed that mean tentacle density of small plants (307.7 cm^{-2}) was significantly higher than that of medium (170.2 cm^{-2}) and large plants (144.4 cm^{-2}) (two-way ANOVA of Aligned Rank Transformed Data, $F_{2,24} = 22.298$, $p < 0.01$). Mean tentacle density of T-0 tentacle was consistently higher than that of T-1 and T-2 tentacles across different plant size (two-way ANOVA of Aligned Rank Transformed Data, $F_{2,24} = 66.510$, $p < 0.01$, Table 5.3). There was a significant interaction between plant size and tentacle type (two-way ANOVA of Aligned Rank Transformed Data, $F_{4,24} = 4.095$, $p < 0.05$, Table 5.3, Figure 5.8).

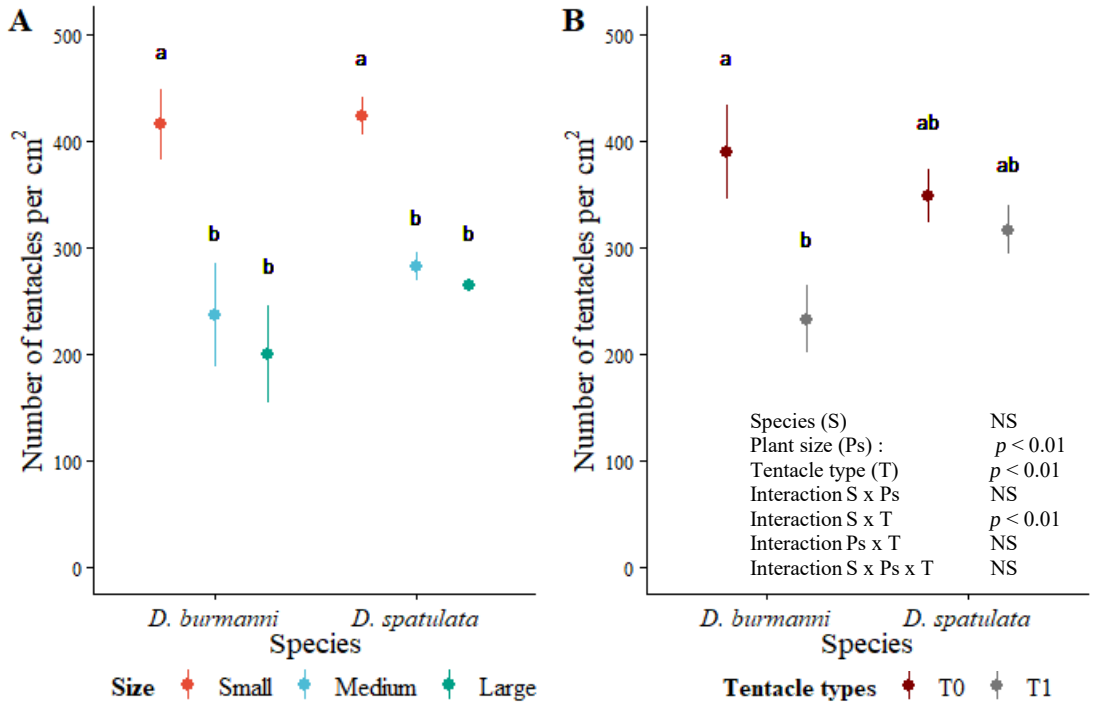

Figure 5.7 Mean (coloured dots) ± SE number of tentacles per leaf area (cm^2) in *Drosera burmanni* and *D. spatulata* with plant size (A) and tentacle type (B). The text in figure B indicates statistical results for both graphs in relation to main effects (species, plant size, tentacle type) and interactions between main effects. NS: non-significant. Different letters above mean plots indicate significant differences (least square means with Tukey adjustment, $p < 0.05$).

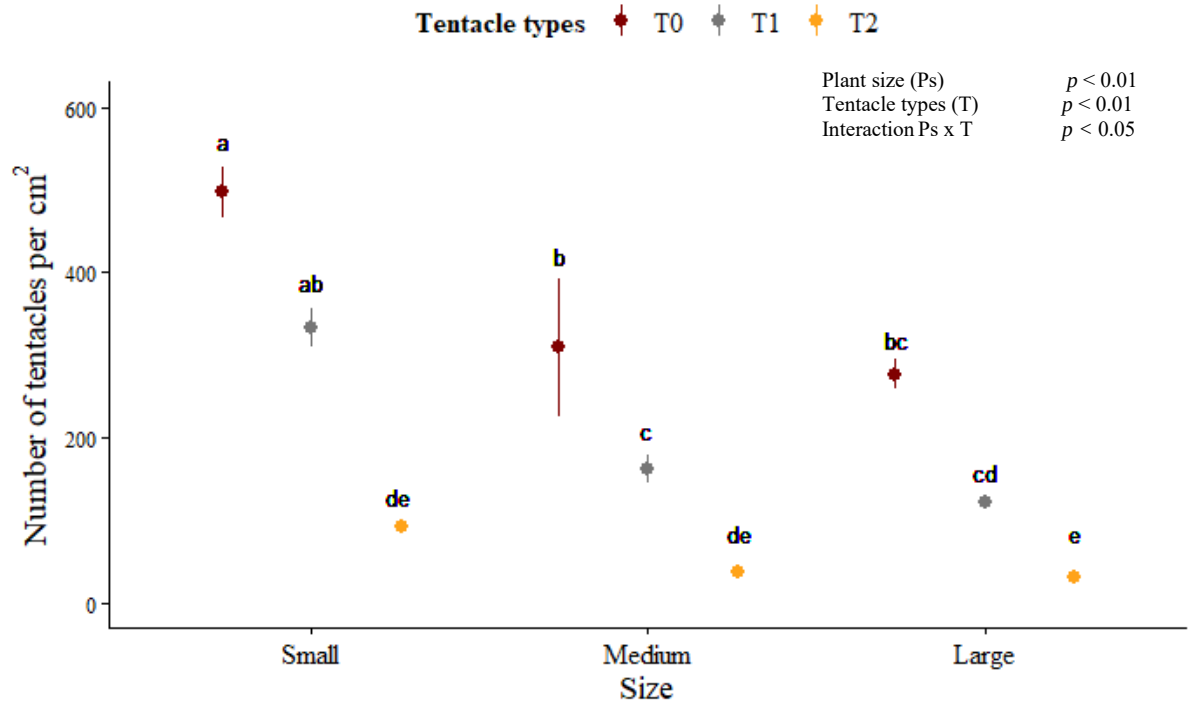

Figure 5.8 Mean (coloured dots) ± SE number of tentacles per leaf area (mm^2) in *Drosera burmanni* with different plant size and tentacle types. The text indicates statistical results for main effects (plant size and tentacle types) and interactions between main effects. NS: non-significant. Different letters above mean plots indicate significant differences (least square means with Tukey adjustment, $p < 0.05$).

5.4.2 Volumes of mucilage droplets

Summaries of volumes of mucilage droplets of four *Drosera* species are presented in Table 5.4. Volumes of mucilage droplets varied across the four carnivorous sundews. Mean volumes of mucilage droplets of *Drosera adelae* were the largest. Larger plants tended to have greater mean volumes of mucilage droplets in *D. spatulata* and *D. burmanni*. However, such a pattern was not demonstrated in *D. adelae* and *D. schizandra* (Table 5.4).

The mean volumes of mucilage droplets in *Drosera adelae* (0.1543 μL) were larger than the mean volumes of those in the other rainforest species, *D. schizandra* (0.0344 μL) (Table 5.4, two-way ANOVA of Aligned Rank Transformed Data, $F_{1,19} = 51.920$, $p < 0.01$, Figure 5.9). No effect of plant size was observed for volumes of mucilage droplets (two-way ANOVA of Aligned Rank Transformed Data, $F_{2,19} = 0.413$, $p = 0.67$, Table 5.4, Figure 5.9). There was no significant interaction between species and plant size (two-way ANOVA of Aligned Rank Transformed Data, $F_{2,19} = 1.268$, $p = 0.30$, Table 5.4, Figure 5.9).

Table 5.4 Mean ± SD volumes of mucilage droplets of four *Drosera* species of differing plant size. Values in parentheses are number of plants sampled.

Species	Plant size	Tentacle types	Volumes (μL)
Drosera adelae	Small (5)	T-0	0.1661 ± 0.0379
	Medium (5)	T-0	0.1477 ± 0.0581
	Large (3)	T-0	0.1456 ± 0.0319
		Mean at species level	**0.1543 ± 0.0432**
Drosera schizandra	Small (5)	T-0	0.0300 ± 0.0051
	Medium (5)	T-0	0.0401 ± 0.0124
	Large (2)	T-0	0.0309 ± 0.0055
		Mean at species level	**0.0344 ± 0.0097**
Drosera spatulata	Small (5)	T-1	0.0459 ± 0.0118
	Medium (5)	T-1	0.0871 ± 0.0319
	Large (3)	T-1	0.1279 ± 0.0252
		Mean at species level	**0.0807 ± 0.0396**
Drosera burmanni	Small (5)	T-1	0.0286 ± 0.0065

Species	Plant size	Tentacle types	Volumes (µL)
	Medium (4)	T-1	0.0387 ± 0.0118
	Large (2)	T-1	0.0499 ± 0.0158
		Mean at species level	**0.0361 ± 0.0124**

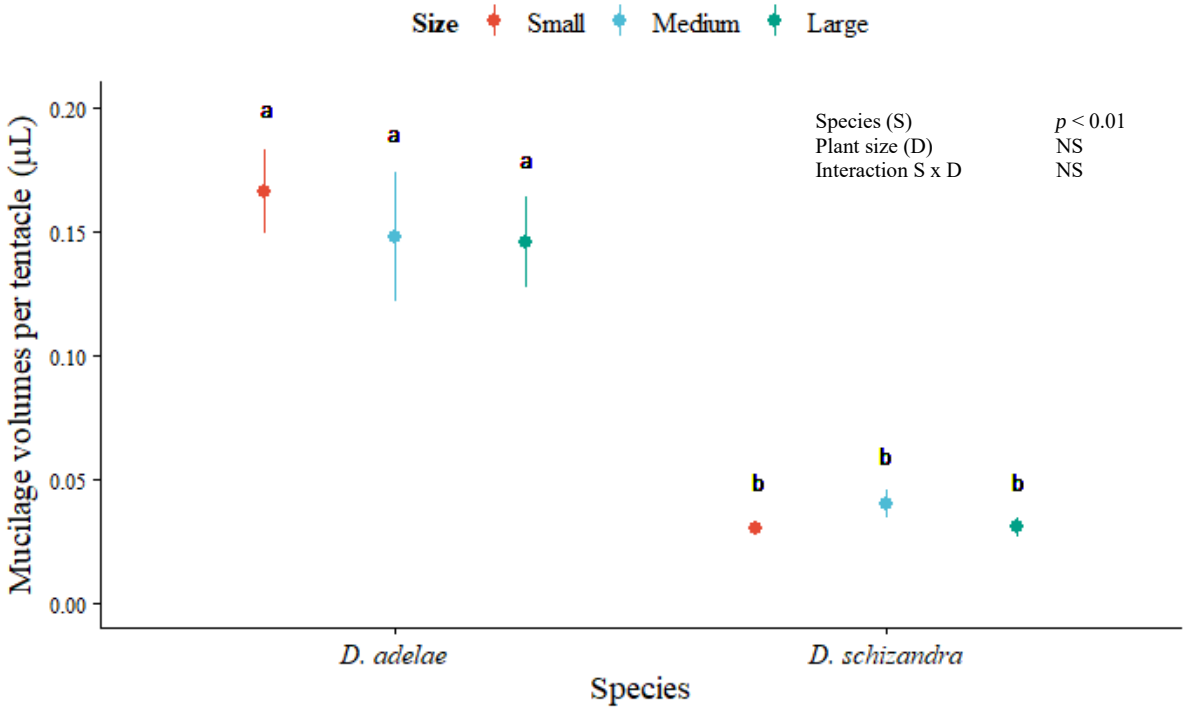

Figure 5.9 Mean (coloured dots) ± SE volumes of mucilage droplets of T-0 tentacles (µL) in small, medium and large *Drosera adelae* and *D. schizandra*. Text indicates statistical results for main effects (species and plant size) and interactions between main effects. NS: non-significant. Different letters above mean plots indicate significant differences (least square means with Tukey adjustment, $p < 0.05$).

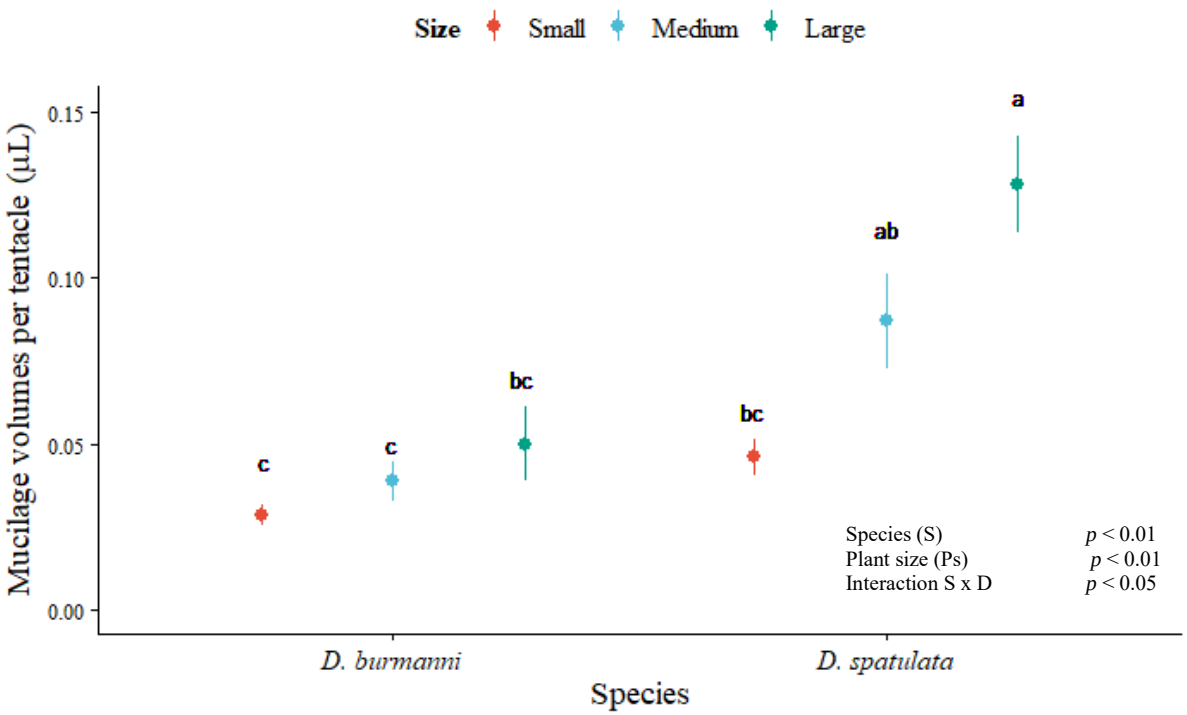

Figure 5.10 Mean (coloured dots) ± SE volumes of mucilage droplet of T-1 tentacles (µL) in small, medium and large *Drosera burmanni* and *D. spatulata*. Text indicates statistical results for main effects (species and plant size) and interactions between main effects. Different letters above mean plots indicate significant differences (Tukey, $p < 0.05$).

For the non-rainforest species, mean volumes of mucilage droplets in *Drosera burmanni* (0.0361 µL) were less than that of mucilage droplets in *D. spatulata* (0.0807 µL) (two-way ANOVA, $F_{1,18} = 26.925$, $p < 0.01$, Table 5.4). Large plants produced significantly larger mucilage droplets than small plants across the two species (Table 5.4, two-way ANOVA, $F_{2,19} = 13.946$, $p < 0.01$, Figure 5.10). A significant interaction was observed between species and plant size (two-way ANOVA, $F_{2,19} = 4.217$, $p < 0.05$, Table 5.4, Figure 5.10).

5.4.3 The adhesive capacity of tentacular mucilage

The adhesive force exhibited by mucilage at the tentacular tips varied across the three carnivorous sundews (Table 5.5). In general, *D. adelae* mucilage exhibited a larger mean adhesive force than *D. schizandra* and *D burmanni*. Differences in adhesive force between different tentacle types were observed in *D. burmanni*.

Table 5.5 Mean ± SD adhesive force (per 0.06930 mm^2 of silicon probe contact area) exhibited by mucilage at the tentacle tips of *Drosera adelae*, *D. schizandra* and *D. burmanni*. Values are calculated from data in Tables 5.3, 5.4 and 5.5. Values in parentheses are number of samples. Note: no large plants of *D. adelae* or small plants of *D. burmanni* were measured.

Species	Plant size	Tentacular region	Tentacle types	Adhesive force (μN)
Drosera adelae	Small (5)	Tip	T-0	219.9 ± 55.4
		Middle	T-0	222.4 ± 36.3
		Base	T-0	270.2 ± 36.2
		Mean	T-0	237.5 ± 46.9
	Medium (5)	Tip	T-0	226.0 ± 94.8
		Middle	T-0	163.3 ± 26.4
		Base	T-0	183.0 ± 45.0
		Mean	T-0	190.8 ± 63.9
		Mean at species level	**T-0**	**214.1 ± 60.0**
Drosera schizandra	Small (5)	Tip	T-0	119.9 ± 34.8
		Middle	T-0	122.1 ± 29.3
		Base	T-0	124.6 ± 41.3
		Mean	T-0	122.2 ± 32.9
	Medium (5)	Tip	T-0	132.4 ± 41.2
		Middle	T-0	125.3 ± 29.1
		Base	T-0	140.9 ± 16.9
		Mean	T-0	132.9 ± 29.2
	Large (5)	Tip	T-0	131.9 ± 27.7
		Middle	T-0	119.6 ± 26.1
		Base	T-0	136.3 ± 16.6
		Mean	T-0	129.2 ± 23.4
		Mean at species level		**128.1 ± 28.5**
Drosera burmanni	Medium (5)	NA	T-1	122.4 ± 36.7
	Large (5)	NA	T-0	106.2 ± 32.0
		NA	T-1	242.3 ± 49.5
		Mean at species level		**157.0 ± 73.0**

The adhesive force exhibited by mucilage on tentacular tips was analysed using a combination between three-way ANOVA omitting large plants of *D. schizandra* and two-way ANOVA for *D. schizandra* only to handle missing cell design. The mucilage on tentacles of *D. adelae* (mean 214.1 μN) was significantly more adhesive than that of *D. schizandra* (mean 128.1 μN) (three-way ANOVA, $F_{1,48} = 63.059$, $p < 0.01$, Table 5.5, Figure 5.11). However, there was no significant difference in adhesive force of mucilage from plants of differing size (Table 5.5, three-way ANOVA, $F_{1,48} = 1.268$, $p = 0.27$) or between tentacular regions (Table 5.5, three-way ANOVA, $F_{2,48} = 0.992$, $p = 0.38$). A significant interaction in adhesive force

was detected between species and plant size (three-way ANOVA, $F_{1,48} = 6.817$, $p < 0.05$, Table 5.5, Figure 5.10). No statistically significant interactions were detected between species and tentacular region (three-way ANOVA, $F_{2,48} = 0.272$, $p = 0.76$, Table 5.5), between plant size and tentacular region (three-way ANOVA, $F_{2,48} = 0.873$, $p = 0.42$, Table 5.5) and among species, plant size and tentacular region (three-way ANOVA, $F_{2,48} = 1.053$, $p = 0.36$, Table 5.5).

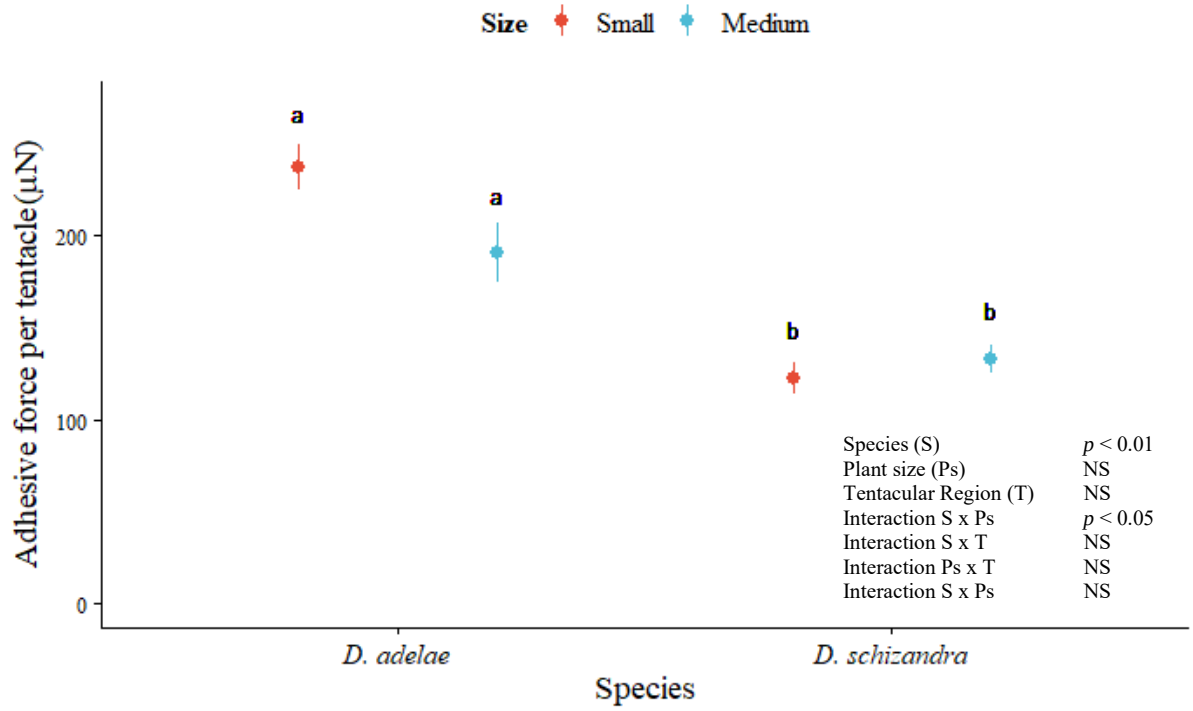

Figure 5.11 Mean (coloured dots) ± SE adhesive force (µN) of tentacular tips in small and medium-sized plants of *D. adelae* and *D. schizandra*. Text indicates statistical results for main effects (species, plant size and tentacular region) and interactions between main effects. NS: non-significant. Different letters above mean plots indicate significant differences (Tukey, $p < 0.05$).

Using two-way ANOVA for another balanced dataset which included *D. schizandra* only, mean adhesive force exhibited by mucilage on tentacles did not differ between plant size (Table 5.5, $F_{2,36} = 0.684$, $p = 0.51$, Figure 5.12) and between tentacular regions (Table 5.5, $F_{2,36} = 0.635$, $p = 0.54$, Figure 5.12). Similarly, no significant interaction was observed between plant size and tentacular regions ($F_{4,36} = 0.165$, $p = 0.95$, Table 5.5, Figure 5.12).

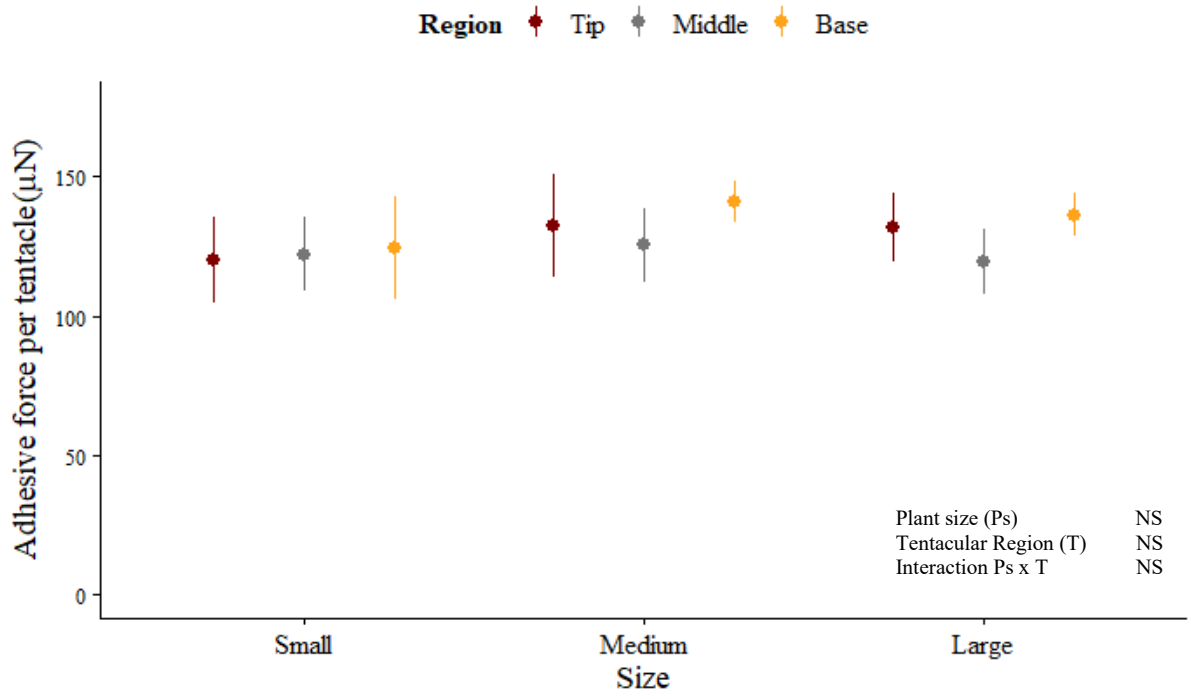

Figure 5.12 Mean (coloured dots) adhesive force (µN ± SE) of mucilage from small and large *D. schizandra* and from three tentacular regions (tip, middle, and base). Text indicates statistical results for main effects (plant size and tentacular region) and interactions between main effects. NS: non-significant.

Mean adhesive force exhibited by mucilage on T-1 tentacles in large plants of *D. burmanni* (242.3 µN) was significantly higher than that of T-1 tentacles in medium plants (122.4 µN) (Table 5.5, df = 8, t = -4.354, $p < 0.01$, t-test, Figure 5.13.A). Mucilage droplets of T-1 tentacles (mean 242.3 µN) were more adhesive than mucilage droplets of T-0 tentacles (mean 106.2 µN) (Table 5.5, two-tailed paired t-test, df = 4, t = -8.981, $p = < 0.01$, Figure 5.13.B).

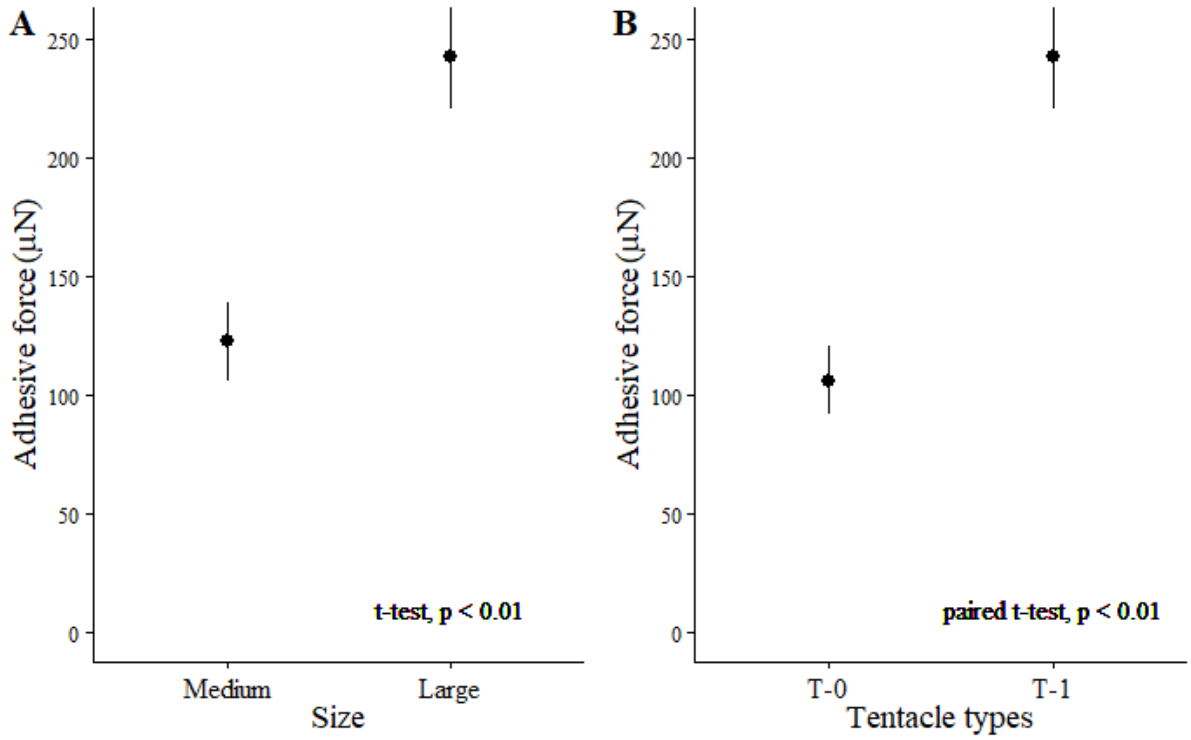

Figure 5.13 Mean adhesive force (µN ± SE) of tentacular mucilage from small and large *Drosera burmanni* (A), and from T-0 and T-1 tentacles (B).

Table 5.6 displays variations in mean adhesive force exhibited by tentacular tips of *D. adelae* under two light regimes, 8 µmol m^{-2} s^{-1} (typical light intensity in the field) and 30 µmol m^{-2} s^{-1} (an elevated light level for this species in the field). When adhesive force exhibited at the tentacular tips of *D. adelae* was compared for plants acclimated to the two light regimes, the mean adhesive force exhibited at tentacular tips of plants under the elevated light intensity (385.9 µN) was higher than that of plants at the lower intensity (190.8 µN) (Table 5.6, two-way ANOVA, $F_{1,24} = 39.146$, $p < 0.01$). However, there were no significant differences in adhesive force between tentacular regions (two-way ANOVA, $F_{2,24} = 0.286$, $p = 0.75$, Table 5.6, Figure 5.14) and no a significant interaction between light intensity and tentacular regions (two-way ANOVA, $F_{2,54} = 0.441$, $p = 0.65$, Figure 5.14).

Table 5.6 Mean ± SD adhesive force exhibited by tentacular tips of *Drosera adelae* under low (8 µmol m^{-2} s^{-1}) and elevated light levels (30 µmol m^{-2} s^{-1}). Number of plants sampled are presented in brackets.

Species	Light level	Tentacular region	Adhesive force (µN)
Drosera adelae	Low (5)	Tip	226.0 ± 94.8
		Middle	163.3 ± 26.4
		Base	183.0 ± 45.0
		Mean	**190.8 ± 63.9**
	Elevated (5)	Tip	383.7 ± 91.4
		Middle	392.5 ± 122.5
		Base	381.6 ± 93.4
		Mean	**385.9** ± 95.9

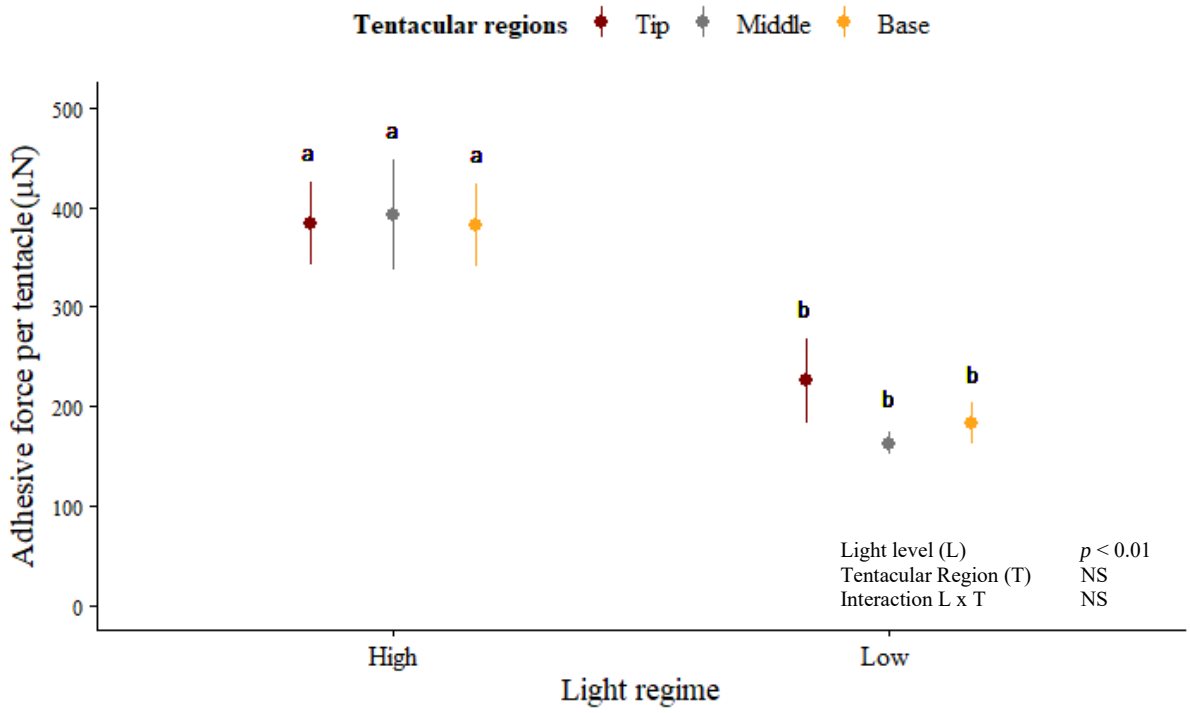

Figure 5.14 Mean (coloured dots) ± SE adhesive force (µN) of tentacular tips in *Drosera adelae* with different tentacular regions (tip, middle, and base) under low (8 µmol m^{-2} s^{-1}) and elevated (30 µmol m^{-2} s^{-1}) light intensity. Text indicates statistical results for main effects (plant size and tentacular region) and interactions between main effects. NS: non-significant. Different letters above mean plots indicate significant differences (Tukey, $p < 0.05$).

5.4.4 Leaf resting adhesive capacities (RAC) of *Drosera* leaves

The adhesive capacities of the surface areas of the mucilage drops per unit leaf area, defined as the leaf resting adhesive capacity (RAC), are shown for three species of *Drosera* in Table 5.7. Also shown are the values of the components used to calculate RAC, the density of tentacles, the size of droplets, and the adhesive force of mucilage to the silicon sensor.

The RACs of *D. adelae*, *D. schizandra* and *D. burmanni* are not the same. *Drosera adelae* and *D. burmanni* displayed the highest mean species RACs of *ca.* 3,000 and 1,914 μN mm^{-2} respectively, whereas for *D. schizandra* the mean RAC was *ca*. 650 μN mm^{-2}. *Drosera burmanni* has a smaller mean drop surface area than *D. adelae*, and a less sticky mucilage, but has more tentacles per mm^2. Of the three species, the low RAC exhibited by *D. schizandra* was a product of low tentacle density, small droplet size and mucilage that was the least adhesive.

The maximum lifting force expressed by a range of flying insect species, including species with larger body masses than those trapped by *Drosera* studied here, is linearly correlated with wet mass (Figure 5.15, data from Marden, 1987). The wet mass values can be transformed to dry mass values by assuming appropriate dry mass – wet mass ratios. Since a principal prey of *D. adelae* and *D. schizandra* were small fruit-fly-like dipterans, and the well-characterized fruit-fly, *Drosophila melanaogaste*r, has a dry mass – wet mass conversion factor of ca. 0.339 (average value for males and females from populations collected between 2.22ºS and 23.63ºS in South America; Robinson et al., 2000), Marden's data were transformed accordingly.

Table 5.7 The trapping capacity of leaves of *D. adelae, D. schizandra, D. spatulata* and *D. burmanni* of differing size. Values, which were calculated using data in Tables 5.3, 5.4 and 5.5, are mean tentacle density, mucilage surface area, adhesive force, and leaf resting adhesive capacity (RAC). Values in parentheses are number of samples. Adhesive force was not measured in *D. spatulata*, large *D. adelae* or small *D. burmanni*.

Species	Plant size	Tentacle type	Site on leaf	Tentacle density (mm^{-2})	Drop surface area (mm^2)	Adhesive force ($\mu N\ mm^{-2}$)	Leaf RAC ± S.D. ($\mu N\ mm^{-2}$)
D. adelae	Small (5)	T-0	Tip	0.739	1.4455	3440	3674
		T-0	Mid	0.739	1.4455	3479	3716
		T-0	Base	0.809	1.4455	4227	4943
			Mean	0.762	1.4455	3715	4092 ±720
	Medium (5)	T-0	Tip	0.860	1.3513	3535	4108
		T-0	Mid	0.720	1.3513	2554	2485
		T-0	Base	0.772	1.3513	2863	2986
			Mean	0.784	1.3513	2985	3162 ±831
	Large (3)	T-0	Tip	0.556	1.3384		
		T-0	Mid	0.511	1.3384		
		T-0	Base	0.507	1.3384		
			Mean	0.524	1.3384		
	Mean at species level			**0.716**	**1.4002**	**3349**	**3358 ± 858**
D. schizandra	Small (5)		Tip	1.096	0.4669	1876	960
			Mid	0.86	0.4669	1910	767
			Base	0.992	0.4669	1949	903
			Mean	0.983	0.4669	1912	877 ±99
	Medium (5)		Tip	0.66	0.5590	2071	764
			Mid	0.496	0.5590	1960	543
			Base	0.492	0.5590	2204	606
			Mean	0.549	0.5590	2079	638 ±114
	Large (3)		Tip	0.41	0.4762	2063	403
			Base	0.32	0.4762	1871	285
			Mean	0.35	0.4762	2132	355 ±59
	Mean at species level			**0.698**	**0.504**	**1988**	**654 ± 237**
D. spatulata	Small (5)	T-0		4.442	0.6100		
		T-1		4.017	0.6100		
			Mean	4.229			
	Medium (5)	T-0		2.995	0.9371		
		T-1		2.642	0.9371		
			Mean	2.819	0.9371		
	Large (3)	T-0		2.689	1.2276		
		T-1		2.616	1.2276		
		Mean		2.652	1.2276		
	Mean at species level			3.323	0.9016		
D. burmanni	Small (5)	T-0		4.97	0.4523		
		T-1		3.332	0.4523		
		T-2		0.929	0.4523		
			Mean	3.077	0.4523		
	Medium (4)	T-0		3.092	0.5533		
		T-1		1.627	0.5533	1915	1724
		T-2		0.381	0.5533		
			Mean	1.702	0.5533		
	Large (2)	T-0		2.773	0.6400	1661	2948
		T-1		1.23	0.6400	3790	2984
		T-2		0.328	0.6400		
			Mean	1.444	0.6400	2726	2966
	Mean at species level			**2.280**	**0.5273**	**1842**	**1914 ± 868**

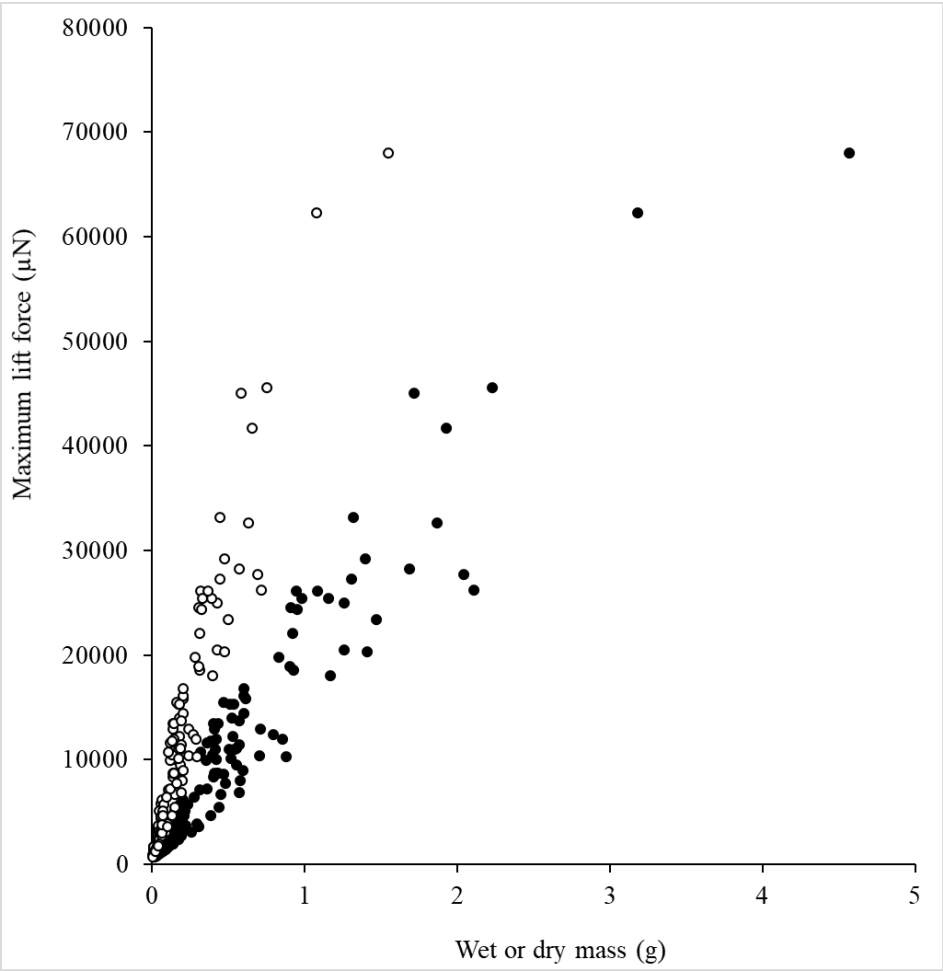

Figure 5.15 The maximum lift force expressed by a range of insect species (data from Marden, 1987). Values are for wet mass (●) and for dry mass (○). Dry mass was estimated for a *Drosophila melanaogaste*r-based dry mass – wet mass conversion factor of 0.339 (Robinson et al., 2000).

Plots of the dry mass and maximum lift force values shown in Figure 5.15 against the mean species RAC values for *D. adelae*, *D. schizandra* and *D. burmanni* (Figure 5.16) provide dry mass calibrated comparisons of insect lift force and plant adhesive force per mm^2 of leaf. For an insect of the same dry mass, *D. schizandra* requires about 5.2 times the surface area of leaf to trap the insect of the same dry mass in comparison to a *D. adelae* leaf. The values in Figure 5.16 show clearly delineated upper and lower borders for maximum lift force i.e. demonstrate upper and lower lifting limits for flying insects of defined mass. The upper and lower borders mainly reflect differences in flight muscle ratio (Marden, 1987).

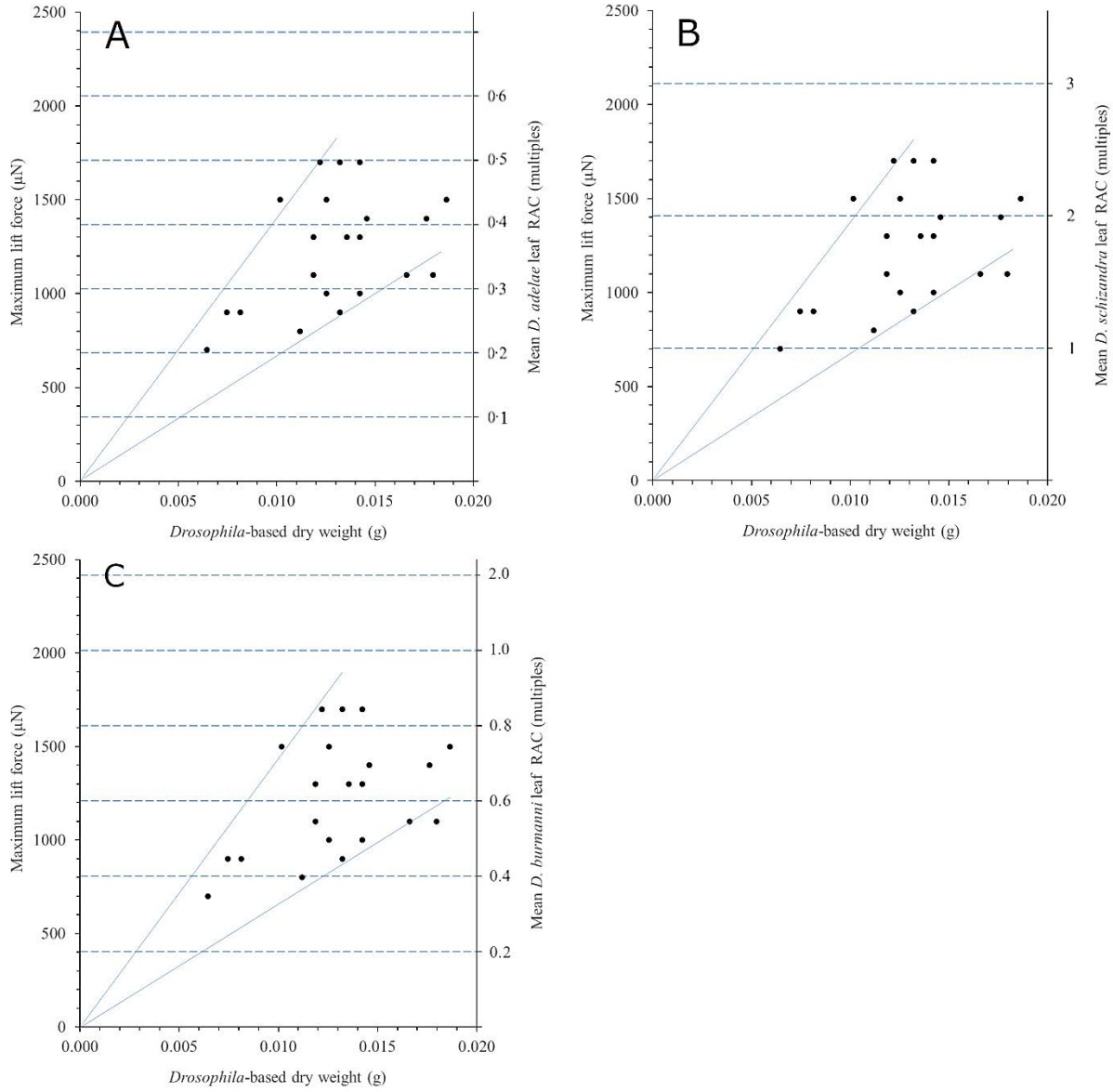

Figure 5.16 Maximum lift force as a function of dry mass and multiples of the mean species leaf RAC for *D. adelae* (A), *D. schizandra* (B), and *D. burmanni* (C). Dry mass was estimated from wet mass data assuming a dry mass – wet mass ratio of 0.339, that of the dipteran *Drosophila melanogaster* (Robinson et al., 2000). Solid lines delineate the maximum and minimum bounds of the multiple-species lift force values shown in Figure 5.15. Mass and force values for flying insects are from Marden (1987).

On the basis of differences in leaf RAC for the two closed-woodland *Drosera*, it might be expected that, on average, *D. schizandra*, which has a lower RAC, traps smaller insects than *D. adelae*, even though it has larger leaves. Comparisons of mean prey dry mass and length confirm this prediction (Figure 5.17). Mean trapped prey dry mass for *D. schizandra* was *ca.*

71% of mean trapped prey dry mass for *D. adelae*. Similarly, mean trapped prey length for *D. schizandra* was *ca.* 67.2% of mean trapped prey length for *D. adelae*.

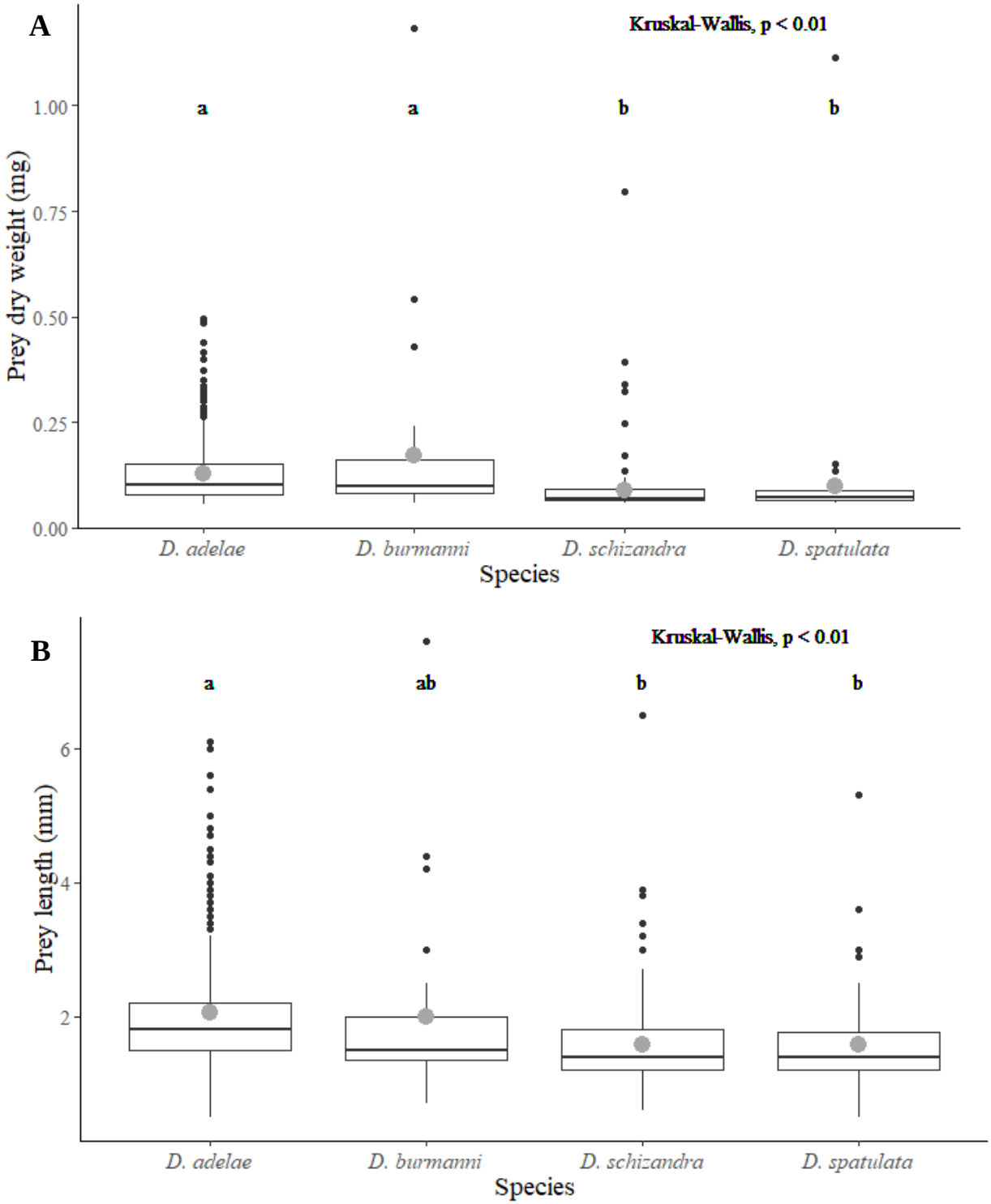

Figure 5.17 The median (-) and mean (●) dry mass (A) and length (B) of prey capable of flight trapped by four species of *Drosera*. Prey capture data were obtained during the measurements described in Chapter 3. Boxed regions are the 25th and and 75th percentile limits, vertical lines are ranges, dark closed circles are outliers. Different letters above box-plots indicate significant differences.

The leaf RAC, a measure of the leaf potential to trap flying prey of a certain size, was considerably greater than the maximum sizes of prey trapped for each of the three *Drosera* measured (Figure 5.16). The prey trapped in the field by each of examined *Drosera* species had maximum body dry masses of *ca.* 0.001g (0.0005 g for *D. adelae*) but mean dry masses of prey were roughly a factor of 10-20 less, between *ca.* 0.0001 and 0.0002 g (Figure 5.17).

The principal prey of *D. burmanni*, with its low-lying rosettes close to the soil, are ants, not flying insects. There is substantial literature on the ability of ants to carry loads (e.g (Wojtusiak et al., 1995, Nguyen et al., 2014), but only one study was uncovered that detailed the potential pushing forces of an ant (Endline and Ferderle, 2015). For the weaver ant, *Oecophylla smaragdin,* the major workers have body lengths of typically 8-10 mm and an estimated dry mass of ca. 0.0028 g, an individual leg can push maximally with a force of ca. 77 μN, consistent with a force of 462 μN if all six legs pushed at once. The value is roughly 23 % of the leaf RAC for *D. burmanni* (Table 5.7). The mean prey length for non-flying insects trapped by *D. burmanni*, 1.5 to 2 mm, is considerably smaller than a weaver ant but illustrates well the adhesive force that a leaf of *D. burmanni* can potentially exert.

It should also be noted that the surface area of the feet of many insects, such as *Drosophila melanogaster* and *Oecophylla smaragdin* illustrated in Figure 5.18, are considerable enlarged by setae and adhesive pads (pulvilli or arolium in Figure 5.18). Together, these appendages would increase appreciably the potential adhesion points for *Drosera* mucilage. Moreover, by sinking into the mucilage drops, mucilage not just at the drop surface is utilised in trapping.

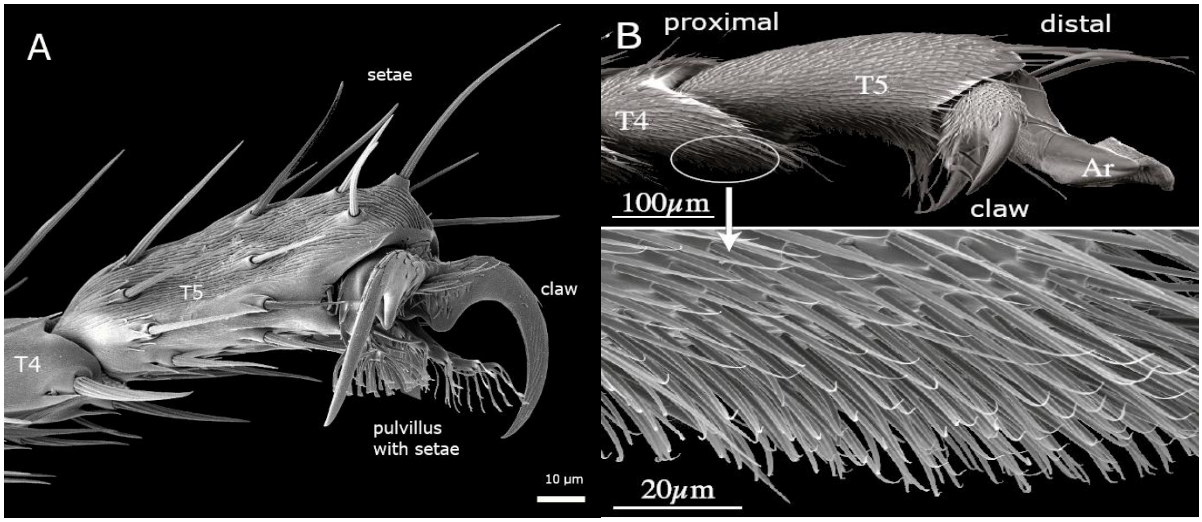

Figure 5.18 Morphology of proximal and distal tarsal appendages and hairs of the dipteran, *Drosophila melanogaster* (A), and the weaver ant, *Oecophylla smaragdina* (B). Note the large surface area that could potentially be in contact with adhesive mucilage on a *Drosera* leaf. In (B), the inset shows the morphology of setae on the underside of the tarsus. T4 and T5 are the 4th and 5th tarsomeres, respectively; Ar = arolium (foot pad equivalent of pulvillus in [A]). Images are modified from Endlein and Federle (2015).

5.5 Discussion

This study provides the first demonstration of leaf RAC values for carnivorous *Drosera*. Tentacle densities differed between *Drosera* species. T-0 tentacles, for example, were more densely spaced in small-sized sundews, such as *D. burmanni* and *D. spatulata* (Figure 5.7) in comparison with large-sized sundews, e.g. *D. adelae* and *D. schizandra* (Figure 5.6). This pattern might be associated with their differences in rosette sizes (Lowrie, 2013), tentacle function (Hartmeyer and Hartmeyer, 2010, Poppinga et al., 2013) and light environment (Zamora, 1995) which differed between species. *Drosera schizandra*, for instance, has large rosettes with leaves consisting of T-0 only and occurs in deep-shady habitats of rainforests. In contrast, *D. burmanni,* which inhabits more open sunny environments, has smaller rosettes with three different tentacle types, T-0, T-1 and T-2 tentacles.

Tentacle density was negatively correlated with plant size (Figures 5.6-8). However, such a pattern was not demonstrated in the other two components of trapping capacity: volume of

mucilage droplets and adhesive capacity of mucilage. The interaction between plant size and these carnivory traits was species-dependant. Plant size was independent of mucilage droplet volume and adhesive force of mucilage for the closed-woodland species, *D. adelae* and *D. schizandra* (Figures 5.9 and 5.11). Conversely, plant size was positively correlated the droplet volume in the open-woodland species, *D. burmanni* and *D. spatulata*, and with mucilage adhesive force in *D. burmanni* (Figures 5.10 and 5.13.A).

The micro-force sensor probes employed in this experiment, designed for micro-mechanical testing of electronic circuits, were shown to be efficient in quantifying the adhesiveness of mucilage on the tentacles of *Drosera*. The mean adhesive force to the silicon probes across three *Drosera* species ranged from *ca.* 100 μN to 400 μN (Figure 5.11, Figure 5.12, Figure 5.13.A). These values were equivalent to RACs of 654 to 3,358 μN mm^{-2} of leaf surface.

If a *Drosera* is to retain prey, the adhesive force of tentacles attached to prey should be greater than the forces exerted by the animals to escape. In the case of flying insects, escape ability is mainly dependent upon the load-lifting capacity, the amount of power required to lift body weight plus a maximum load (Dudley, 2000). Load-lifting capacity is not a constant, it will decrease with time as a trapped animal tires. The maximum load-lifting capacity among flying animals is typically linearly related to body mass as is demonstrated in Figure 5.15 (data from Marden (1987). Using Marden's data and data on prey dry mass and length from Chapter 3, I plotted the maximum lift force of flying prey in the ranges of prey dry mass trapped by *Drosera*, and compared the dry masses to the leaf RAC values measured for *D. adelae*, *D. schizandra* and *D. burmanni* (Figures 5.16 and 5.17). The calculations indicate that, for each *Drosera* species examined, the maximum lift force able to be exerted by flying prey trapped was considerably less than the adhesive force of the *Drosera* mucilage per mm^2

of leaf. For *D. adelae*, the potential lift force of a 1 mg prey was equivalent to about 1/25 of the leaf RAC. For *D. schizandra* and *D. burmanni* the values were 1/10 and 1/20 respectively.

Why are bigger insects not successfully captured if trapping capacity appears to far exceed the escape capacities of the insects trapped in the *Drosera* examined? Two, not mutually exclusive, possibilities are that larger animals escape or that the mucilage is not always highly sticky. As mucilage from different *Drosera* and the surface characteristics of prey bodies are not expected to be the same, it is to be expected that mucilage may not be equally adhesive to all prey. For example, Gibson (1991) observed that, for insects of the same body mass, the polysaccharide mucilage of *Pinguila lutea* was not as adhesive as *D. filiformis*, partially because the threads *D. filiformis* mucilage stretched over longer distances before breaking.

The data in Figure 5.16 show only the capacity for flight as a means by which prey may escape. However, prey can also walk across a leaf. The larger the prey the greater the capacity to crawl off the leaf using lateral force generated by leg muscle. Prey sizes may be evenly distributed over the flat sticky trap surface of *Drosera* when first caught, but larger insects can crawl further towards the edge of the leaf before they succumb (Gibson, 1991). One might expect that non-winged insects would have stronger leg muscles. For example, the small ant trapped in the *D. burmanni* shown in Figure 5.19 has clearly crawled from the exterior of the leaf well towards the centre of the leaf. The larger fly does not appear to have had the walking strength to crawl the same distance. The image in Figure 5.19 of a mosquito trapped on a *D. adelae* leaf illustrates well the lack of well-developed leg muscles in an insect with mainly flight locomotion.

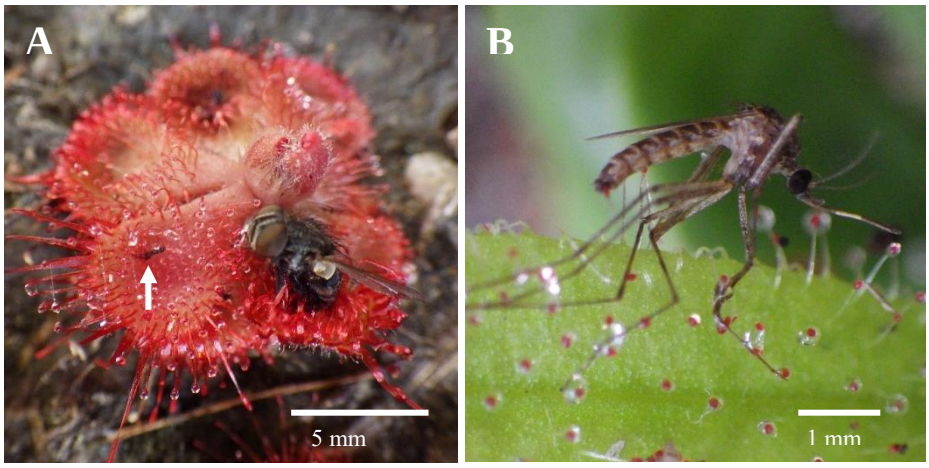

Figure 5.19 Trapping success of two *Drosera* species with different tentacle densities: a fly and an ant trapped by *D. burmanni* (A) and a mosquito trapped by *D. adelae* (B). Note the multiple points of contact between the prey and the plants. The white arrow in Figure 5.19.A indicates the ant.

For larger flying insects in particular, the lateral force of walking may be supplemented by lateral force generated by wings. Indeed, few insects take-off vertically like bees, most exhibit a take-off angle that is less than 90º which would conribute to any forward forces generated by walking. It is therefore feasible that the insect escape forces may be effectively greater than the estimates in Figure 5.16.

Although the adhesive forces measured in this study were quantified under optimal temperature and humidity, mucilage adhesive capacity is not always optimal. For example, during and after rainfall, insect trapping is reduced, and trapped prey may even be lost (Figure 3.1). Whilst the force-measuring system used here was being developed, it was noticed that adhesive force was often difficult to measure at laboratory relative humidity of 50 % or lower. This observation, although not pursued, is consistent with a reduced trapping capacity under dry conditions. One would also expect that trapping capacity might be reduced as wind increases.

At least for *D. adelae* and *D. schizandra*, there is some support for ontogenetic effects on the expression of plant adhesive force. Smaller plants exhibited greater trapping capacity per unit area than did larger plants. This may reflect the energetic costs of constructing traps or it may simply be that larger leaves are older, and on older tentacles the mucilage is less efficacious. As the construction of sticky traps requires energy, it might be expected that adhesive force might be greater for plants grown at elevated light intensities, but below light saturation. The adhesive capacity of mucilage of the rainforest *D. adelae* is greater in plants acclimated to 30 μmol m^{-2} s^{-1} in comparison to those acclimated at 10 μmol m^{-2} s^{-1}, a typical background light intensity in its habitat that is close to the light compensation point of many understorey species. Equivalent effects have been reported on the investment in carnivorous traits in other species (Zamora, 1995, Zamora et al. (1998), and perhaps are a factor in determining why carnivorous plants tend to inhabit sunny habitats (Givnish et al., 1984). *Drosera schizandra* is an exception in that it occupies shady habitats in rainforests (Lavarack, 1979).

It should be noted that the ability of the mucilage to adhere to the tentacle stalks and the mucilage-producing gland was not quantified in this study. When, during a typical measurement of adhesive force, a sensor was inserted into a mucilage droplet and then withdrawn until contact with the hydrogel was broken, it was invariably the gel strand that broke. The mucilage was never pulled off the tentacle stalk and gland, and only occasionally was the mucilage pulled off the sensor. One must therefore conclude that the adhesive force between the surface of the tentacles and the mucilage must exceed that of the force at which the mucilage connection with the sensor parted.

5.6 Conclusion

This study measured for the first time the trapping capacity of single tentacles from species of *Drosera*. Leaf RAC, the adhesiveness of a leaf per unit leaf area quantified from measurements of tentacle density, mucilage droplet size and mucilage adhesive force, differed between *Drosera* species and, in some cases, with plant size. Tentacle density was dependent upon plant size across all four *Drosera* species. All of the species displayed a reduction in tentacle density with increasing plant size, consistent with the structural cost of carnivory being a costly investment.

Each *Drosera* species exhibited a prey upper dry mass cut-off of between 0.001 and 0.0005 g. The species prey trapping upper mass limits were between $1/10^{th}$ and $1/25^{th}$ of the calculated potential trapping capacities. The disparity between the potential to trap prey of a certain size or mass and the smaller sizes or masses of the prey trapped indicates that not all factors that influence prey-capture were assessed. The understanding of prey trapping capacity would be improved by quantification of the adhesive capacity of mucilage under different environmental conditions and during plant ontogeny, and by combined measurements of maximum vertical load lifting capacity as well as the maximum horizontal load pulling capacity.

Chapter 6. General discussion

The brief of this thesis was to quantify relationships between prey capture, N economy and trapping capacity of selected north Queensland *Drosera.* Four species of *Drosera* were examined, two that inhabit open-grasslands, *D. burmanni* and *D. spatulata*, and two from rainforests, *D. adelae* and *D. schizandra*. The intention of the study was to expand the understanding of predation patterns of Australian *Drosera*, and *Drosera* in general, and to explore the interplay between plant habit, plant habitat, seasonality and N supply with aspects of the structures used to trap prey, tentacle density, volume of adhesive mucilage, and the adhesive potential of leaves. In this chapter, I discuss the relationships between the major observations reported in each chapter, attempting to place them into context, and I highlight unclear aspects of *Droserai* biology that may benefit from further investigation.

6.1 Prey nitrogen (N) and trapping capacity

Those *Drosera* that are carnivorous obtain N from prey by means of adhesive traps. Some species also obtain N from the soil. The relative uptake of soil- and prey-absorbed N varies among species (Chandler and Anderson, 1976, Schulze et al., 1991, Millett et al., 2015, Cook et al., 2017) and can be affected by soil N content and predation patterns i.e. by environment (Millett et al., 2015).

Each of the *Drosera* studied inhabited environments within which the soil N levels were extremely low, close to the limits of detection using mass spectrometry (Chapter 4). Each species was carnivorous to some extent (Chapter 3), and each species trapped prey by means of adhesive-tipped tentacles on leaf and stem surfaces (Chapter 5).

In Chapter 3, I examined predation patterns across the four *Drosera* taxa that inhabit open-grassland (*D. burmanni* and *D. spatulata*) and rainforests (*D. adelae* and *D. schizandra*) across seasons (the wet and the dry). A broad trend was that the more-erect species, *D. adelae*, *D. schizandra* and *D. spatulata* trapped mostly flying insects, in particular dipterans, whereas *D. burmanni*, a small ground-hugging species of open grasslands, trapped mostly ants. Within the broader trend, the specific composition of the prey varied between species and habitat with seasonal effects most evident in the perennials that inhabited more open areas, *D. adelae* and *D. spatulata* (*D. burmanni* is an annual).

An unpredicted observation was that, in contrast to the other *Drosera* studied, *D. schizandra,* the species that inhabited shaded rainforest understorey, retained prey during rain events. The tentacular hydrogel mucilage is water-soluble and water droplets can splash both mucilage and trapped prey from the leaves. There may have been fewer potential prey during rainfall events but the absence of already trapped prey from leaves of the other species indicates that they were washed or splashed off. Colonies of *D. schizandra* inhabited understorey locations in relatively dense forests that that were protected from direct rainfall droplets, most of the water arrived via stem-flow.

Prey capture mass might be used as an indicator of the magnitude of the contribution of prey N to total plant N. The relationship between prey capture mass as demonstrated in Chapter 3 and the contribution of prey N to total plant N as described in Chapter 4 supports this argument. *Drosera adelae* and *D. spatulata*, for instance, caught different amounts of prey biomass, and the contribution of prey N to total plant N differed between the two species. The significant biomass of prey caught by *D. adelae* was consistent with a high contribution of prey N to total plant N. Similarly, a low contribution of prey N to total plant N in *D.*

spatulata was associated with a low level of prey capture. Measurements of larger mucilage droplet size, higher tentacle density and more adhesive leaf RAC reported in Chapter 5 confirm that *D. adelae* has a greater trapping potential than *D. spatulata*. Similarly, the measurement of greater adhesive force at the tentacular tips of *Drosera adelae* than for *D. schizandra* mirrored a higher trapped prey dry mass by *D. adelae* than by its rainforest counterpart, *D. schizandra*.

The potential for trapping prey, quantified in Chapter 5 as leaf RAC from measurements of the components of the traps, *viz.* tentacle density, mucilage droplet volume and mucilage adhesive force, were generally consistent with the dry masses of prey trapped (Chapter 3), and the contribution of prey N to total plant N documented in Chapter 4. The contributions of each of the trap components to overall trapping potential varied between species but even in the species with the lowest leaf RAC quantified, together had the potential to trap insects of larger mass than typically trapped.

An aspect of trapping capacity that was not measured was the effect of leaf margin curling-in upon entrapped prey. Curling-in would increase the local tentacle density, adhesive capacity and, by increasing the number of points of contact with mucilage on two sides of the prey, would be expected to restrict further animal movement, perhaps even with mucilage blocking respiratory tracheae in the case of insects. The effect of leaf curling would be expected to be greatest in *D. burmanni*, which has a partially divide leaf that can bend as well as having moveable tentacles at the edges of leaves which can bend at speeds in excess of 1 m s^{-1}. At such speeds, prey can be catapaulted towards the leaf centre.

A stylized diagramme of the relationship between environment, prey as a source of N and the nature of the trap components is shown in figure 6.1. Prey diversity and abundance available for predation is associated with habitat (Ellison and Gotelli, 2009) and seasons as reported in Chapter 3. Trapping potential is affected by trap stickiness and tentacle density on the leaf surface. A greater trap capacity or potential would reduce prey escape (Gibson, 1991) and lead to a higher N uptake from prey that came into contact with the plant.

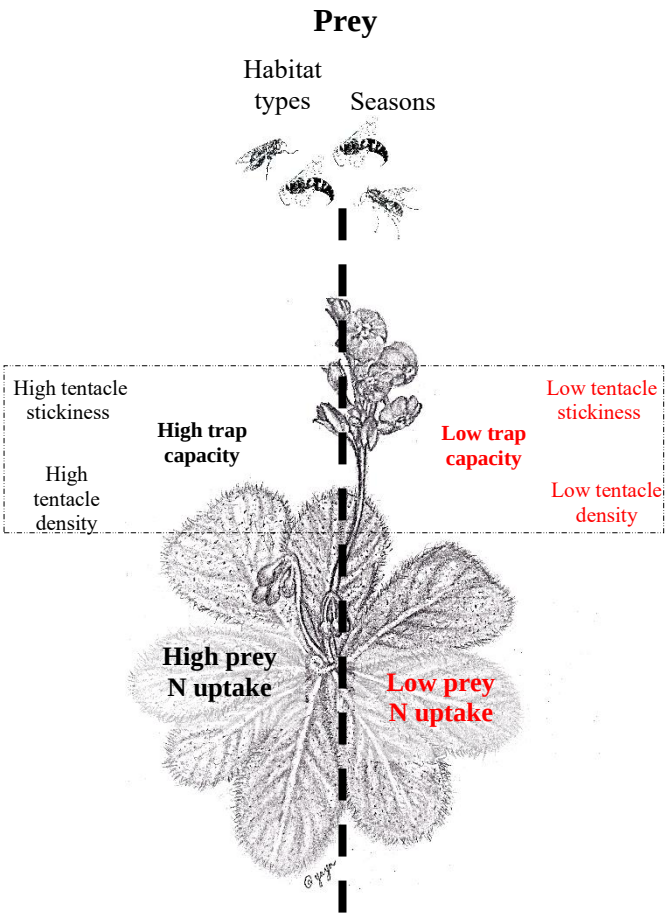

Figure 6.1 The importance of trapping capacity or potential in the nitrogen economy of a carnivorous *Drosera*. The *Drosera* illustration was prepared by Surrayal Halim. Insect images are from the Department of Entomology, University of Maryland (https://insectdrawings.umd.edu/)

6.2 Patterns in biomass allocation and nutrient uptake among different nitrogen-foraging strategies

The relative investment of resources above-ground and below-ground, particularly biomass, often reflects allocation required for adequate nutrient uptake (Aerts and Chapin III, 1999). Theories that address biomass allocation in relation to resource availability in environments include the functional equilibrium of biomass allocation (Thornley, 1972, Iwasa and Roughgarden, 1984), optimal partitioning theory (Gedroc et al., 1996) and the balanced growth hypothesis (Shipley and Meziane, 2002). In the functional equilibrium of biomass allocation model, available resources for growth determine the patterns in biomass allocation to above-ground and below-ground parts. If the limiting resources are above-ground (e.g. light), plants will allocate more biomass to above-ground parts. In the contrary, plants will allocate more biomass to below-ground parts (roots) if the limiting resources are below-ground (e.g. nutrients and water) (Iwasa and Roughgarden, 1984). The balanced growth hypothesis is derived from the same principles as the functional equilibrium, but it considers fluctuations in available resources in nature. “Plants allocate biomass to different parts to reduce any imbalance between carbon fixation by leaves and soil resource acquisition by root” (Shipley and Meziane, 2002). The optimal partitioning theory proposed that optimality in biomass allocation to above-ground and below-ground parts is present when the extent of limiting resources is equal (Gedroc et al., 1996).

The above concepts have rarely been tested in carnivorous plants. Most vascular plants acquire nutrients by root uptake whereas the majority of carnivorous plants produce traps to obtain nutrients above-ground (prey-derived nutrient), supplementing root uptake (Ellison and Adamec, 2018). Under such circumstances, patterns of biomass allocation among some carnivorous plants may differ from the above models that assume nutrients enter plants via

the roots. The results reported for *D. burmanni* provide evidence for this notion. This open-woodland species allocates more biomass to above-ground parts than below-ground parts as reported in Chapter 4 enabling plants to gain more nutrient from above-ground prey trapping (Chapter 3). In contrast, *Fimbristylis* sp, a non-carnivorous plant that grows alongside *D. burmanni*, allocates a larger proportion of biomass to roots than *D. burmanni* (Figure 4.4.C). The N-contents of the two species did not differ, an observation consistent with the two N-foraging strategies being equally successful traits in the habitat where they grow together. Comparisons of root:shoot dry mass ratios between the four species of carnivorous *Drosera* examined and co-existing non-carnivorous plants further support the argument; the *Drosera* generally have lower root:shoot dry mass ratios (Figure 4.5).

6.3 Future research

I have documented predation patterns, nutrient acquisition and trapping capacity of four carnivorous *Drosera* of tropical environments in north Queensland. These observations provide insights for future research.

For example, *D. burmanni* traps a preponderance of ants but the relative roles of its snap tentacles (T-1 and T-2) on the leaf margin and the inner T-0 tentacles to catch walking prey are unclear (Chapter 3). The present study demonstrated that the density of snap tentacles and adhesiveness of T-1 tentacles vary with plant size (Chapter 5). The contribution of the latter two trapping traits to the successful capture of walking prey, and to the mass of prey captured, is undetermined and could be measured successfully using micro-force technology.

In Chapter 4, changes in the ratios of stable N isotopes were used to calculate the dependency of *Drosera* on prey-derived N. The variations in the reliance in prey N measured may reflect

differences in the number and mass of prey captured but may also be influenced by soil N availability (Hanslin and Karlsson, 1996). As in most such studies undertaken *in vivo*, it was not feasible to sample with sufficient detail to assign the relative contributions of habitat, prey diversity and abundance, and environment to the dependency on prey-derived N. A more in-depth, single-species study that includes growing plants on media of known N content, and feeding prey of known N content, may provide the information required.

Most *Drosera* inhabit relative open habitats in which light is not limiting but soil N (and often P) is. One would therefore expect that extra nutrients gained from prey, particularly N and P, would result in an increase in photosynthesis or growth. Increases in light intensity are not expected to have a great effect on trapping potential, except perhaps if higher light is associated with greater moisture stress. For *Drosera* that live in more shaded environments, light could potentially limit grow and the allocation of resources to traps. The potential interaction between light and trap capacity in such species should be quantified by testing for correlations between light response curves, N supply and trapping capacity.

The root:shoot ratios of *Drosera* and non-carnivorous conspecifics often differ, with *Drosera* typically exhibiting higher allocation of resources to above-ground body parts as might be expected if resources typically absorbed by other plants from the soil are obtained above-ground by *Drosera*. However, at some sites investigated here, the root:shoot ratios of *Drosera* and their conspecifics are similar. Investigation of the precise reasons that enable similar root:shoot ratios would provide better understanding of the regulation of resource allocation in nutrient-limited environments.

The innovative experiments trialled and detailed in Chapter 5 demonstrate that micro-force sensors can quantify the adhesive forces of mucilage attached to plant tentacles. To my knowledge, the technique is the most accurate yet attempted for measuring adhesiveness of *Drosera* traps. Atomic force microscopy, coupled with laser nano-distancing technology, a more accurate technology, has been used to investigate hydrogel adhesiveness but only of mucilage isolated from plants and layered on glass slides (cf. Huang et al., 2015). The accuracy of the micro-force sensor technology coupled with its ability to measure directly the adhesive force of mucilage attached to plants, provides the potential to explore trapping capacity potential in *Drosera* from a much wider range of habits and environments than of the four species reported studied herein. The technique should also be relevant to measurements of adhesive force and N acquisition for other sticky-trap plant carnivores such as *Byblis* spp and *Pinguicula* spp.

The trapping potential measurements reported here, which demonstrate that the dry masses of insects trapped tend to be considerably less than what masses could be trapped, indicate that there are as yet untested effects of environment and plant ontogeny on trapping potential *in vivo*. The methodology should be used to explore the natural variation in trapping potential. Similarly, measurements have yet to be made on the escape capacity of the specific prey species trapped by the *Drosera*. Such measurements should include the determination of maximum vertical load lifting capacity as well as maximum horizontal load pulling capacity.

Bearing in mind the biomedical interest in *Drosera* mucilage that is related to its potential as a biological adhesive (Huang et al., 2015), coupled with its water solubility, the ability of the mucilage to adhere to the tentacle stalks and to the mucilage-producing gland should be quantified. As mentioned in Chapter 5, when adhesive force measurements were performed it

was invariably the gel strand that broke, not the connection between the tentacle stalk. One must therefore conclude that the adhesive force between the surface of the tentacles and the mucilage must exceed that of the force at which the mucilage connection with the sensor parted. This adhesive force should be quantified.

REFERENCES

Adamec, L. 2002. Leaf absorption of mineral nutrients in carnivorous plants stimulates root nutrient uptake. *New Phytologist,* 155**,** 89-100.

Adamec, L. 2013. Foliar mineral nutrient uptake in carnivorous plants: what do we know and what should we know? *Frontiers in Plant Science,* 4**,** 10.

Adamec, L. & Pavlovic, A. 2018. Mineral nutrition of terrestrial carnivorous plants. *In:* Ellison, A. M. & Adamec, L. (eds.) *Carnivorous plants: physiology, ecology and evolution.* Oxford: Oxford University Press.

Adlassing, W., Lendl, T., Peroutka, M. & Lang, I. 2010. Deadly glue – adhesive traps of carnivorous plants. *In:* VonByern, J. & Grunwald, I. (eds.) *Biological adhesive systems: From nature to technical and medical application.* St. Stefan im Lavanttal: Springer-Verlag/Wien.

Adlassnig, W., Koller-Peroutka, M., Bauer, S., Koshkin, E., Lendl, T. & Lichtscheidl, I. K. 2012. Endocytotic uptake of nutrients in carnivorous plants. *Plant Journal,* 71**,** 303-313.

Aerts, R. & Chapin III, F. S. 1999. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. *Advances in Ecological Research.*

Albert, V. A., Jobson, R. W., Michael, T. P. & Taylor, D. J. 2010. The carnivorous bladderwort (Utricularia, Lentibulariaceae): A system inflates. *Journal of Experimental Botany,* 61**,** 5-9.

Anderson, B. 2010. Did *Drosera* evolve long scapes to stop their pollinators from being eaten? *Annals of Botany,* 106**,** 653-657.

Anderson, B. & Midgley, J. J. 2002. It takes two to tango but three is a tangle: Mutualists and cheaters on the carnivorous plant *Roridula*. *Oecologia,* 132**,** 369-373.

Bennett, K. F. & Ellison, A. M. 2009. Nectar, not colour, may lure insects to their death. *Biology Letters,* 5**,** 469-472.

Bertol, N., Paniw, M. & Ojeda, F. 2015. Effective prey attraction in the rare *Drosophyllum lusitanicum*, a flypaper-trap carnivorous plant. *American Journal of Botany,* 102**,** 689-694.

Bhattarai, G. P. & Horner, J. D. 2009. The importance of pitcher size in prey capture in the carnivorous plant, *Sarracenia alata* Wood (Sarraceniaceae). *American Midland Naturalist,* 161**,** 264-272.

BOM. 2019. *Climate data online* [Online]. Available: http://www.bom.gov.au/climate/data/ [Accessed 13 May 2018].

Brundrett, M. C. 2017. Distribution and evolution of mycorrhizal types and other specialised roots in Australia. *In:* Tedersoo, L. (ed.) *Biogeography of Mycorrhizal Symbiosis. Ecological Studies (Analysis and Synthesis).*

Cameron, K. C., Di, H. J. & Moir, J. L. 2013. Nitrogen losses from the soil/plant system: a review. *Annals of Applied Biology,* 162**,** 145–173.

Caravieri, F. A., Ferreira, A. J., Ferreira, A., Clivati, D., de Miranda, V. F. O. & Araújo, W. L. 2014. Bacterial community associated with traps of the carnivorous plants *Utricularia hydrocarpa* and *Genlisea filiformis*. *Aquatic Botany,* 116**,** 8-12.

Chandler, G. E. & Anderson, J. W. 1976. Studies on the nutrition and growth of *Drosera* spp with reference to the carnivorous habit. *New Phytologist,* 76**,** 129-141.

Chapin, F. S., Schulze, E. D. & Mooney, H. A. 1990. The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics,* 21**,** 423-447.

Christensen, N. L. 1976. The role of carnivory in *Sarracenia flava* with regard to specific nutrient deficiencies. *Journal of the Elisha Mitchell Scientific Society,* 92**,** 144-147.

Clarke, C. 1998. A re-examination of geographical variation in *Nepenthes* food webs. *Ecography,* 21**,** 430-436.

Clarke, C. M., Bauer, U., Lee, C. C., Tuen, A. A., Rembold, K. & Moran, J. A. 2009. Tree shrew lavatories: A novel nitrogen sequestration strategy in a tropical pitcher plant. *Biology Letters,* 5**,** 632-635.

Cook, J. L., Newton, J. & Millett, J. 2017. Environmental differences between sites control the diet and nutrition of the carnivorous plant *Drosera rotundifolia*. *Plant and Soil***,** 1-18.

Darmanin, T. & Guittard, F. 2015. Superhydrophobic and superoleophobicproperties in nature. *Materials Today,* 18**,** 273–285.

Darnowski, D., Bauer, U., Mendez, M., Horner, J. & Plachno, B. J. 2018. Prey selection and specialization by carnivorous plants. *In:* Ellison, A. M. & Adamec, L. (eds.) *Carnivorous plants: Physiology, ecology, and evolution.* New York: Oxford University Press.

Darwin, C. 1875. *Insectivorous plants,* London, John Murray.

Di Giusto, B., Bessière, J., Guéroult, M., Lim, L. B. L., Marshall, D. J., Hossaert-McKey, M. & Gaume, L. 2010. Flower-scent mimicry masks a deadly trap in the carnivorous plant *Nepenthes rafflesiana*. *Journal of Ecology,* 98**,** 845-856.

Di Giusto, B., Grosbois, V., Fargeas, E., Marshall, D. J. & Gaume, L. 2008. Contribution of pitcher fragrance and fluid viscosity to high prey diversity in a Nepenthes carnivorous plant from Borneo. *Journal of Biosciences,* 33**,** 121-136.

Dijkstra, K.-D. B., Monaghan, M. T. & Pauls, S. U. 2014. Freshwater Biodiversity and Aquatic Insect Diversification. *Annual Review of Entomology,* 59**,** 143-163.

Dixon, K. W., Pate, J. S. & Bailey, W. J. 1980. Nitrogen nutrition of the tuberous sundew *Drosera erythrorhiza* with special reference to catch of arthropod fauna by its glandular leaves. *Australian Journal of Botany,* 28**,** 283-298.

Dixon, P. M., Ellison, A. M. & Gotelli, N. J. 2005. Improving the precision of estimates of the frequency of rare events. *Ecology (Washington D C),* 86**,** 1114-1123.

Dudley, R. 2000. *The biomechanics of insect flight: form, function, evolution,* Princeton, Princeton University Press.

El-Sayed, A. M., Byers, J. A. & Suckling, D. M. 2016. Pollinator-prey conflicts in carnivorous plants: When flower and trap properties mean life or death. *Scientific Reports,* 6:21065.

Elhardt, N. 2006. *Genlisea violacea giant* [Online]. Available: https://commons.wikimedia.org/wiki/File:Genlisea_violacea_giant.jpg [Accessed 28 April 2020].

Ellison, A. M. 2006. Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology (Stuttgart),* 8**,** 740-747.

Ellison, A. M. & Adamec, L. 2018. Introduction: what is a carnivorous plant? *In:* Ellison, A. M. & Adamec, L. (eds.) *Carnivorous plants: Physiology, ecology and evolution.* Oxford: Oxford University Press.

Ellison, A. M. & Gotelli, N. J. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology and Evolution,* 16**,** 623-629.

Ellison, A. M. & Gotelli, N. J. 2009. Energetics and the evolution of carnivorous plants - Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany,* 60**,** 19-42.

Ellison, A. M., Gotelli, N. J., Brewer, J. S., Cochran-Stafira, D. L., Kneitel, J. M., Miller, T. E., Worley, A. C. & Zamora, R. 2003. The evolutionary ecology of carnivorous plants. *Advances in Ecological Research.*

Endlein, T. & Federle, W. 2015. On heels and toes: How ants climb with adhesive pads and tarsal friction hair arrays. *PloS one,* 10**,** e0141269-e0141269.

Erni, P., Varagnat, M., Clasen, C., Crest, J. & McKinley, G. H. 2011. Microrheometry of sub-nanolitre biopolymer samples: non-Newtonian flow phenomena of carnivorous plant mucilage. *Soft Matter,* 7**,** 10889-10898.

Fleischmann, A., Schlauer, J., Smith, S. A. & Givnish, T. J. 2018a. Evolution of carnivory in angiosperms. *In:* Ellison, A. M. & Adamec, L. (eds.) *Carnivorous plant: Physiology, ecology, and evolution.* New York: Oxford University Press.

Fleischmann, A., Schlauer, J., Smith, S. A. & Givnish, T. J. 2018b. Evolution of carnivory in angiosperms. *In:* Ellison, A. M. & Adamec, L. (eds.) *Carnivorous plants: Physiology, ecology and evolution.* Oxford: Oxford University Press.

Foot, G., Rice, S. P. & Millett, J. 2014. Red trap colour of the carnivorous plant *Drosera rotundifolia* does not serve a prey attraction or camouflage function. *Biology Letters,* 10.

Foulds, W. 1993. Nutrient concentrations of foliage and soil in South-Western Australia. *New Phytologist,* 125**,** 529-546.

Frank, J. H. & O'Meara, G. F. 1984. The bromeliad *Catopsis berteroniana* traps terrestrial arthropods but harbors Wyeomyia larvae diptera Culicidae. *Florida Entomologist,* 67**,** 418-424.

Gao, P., Loeffler, T. S., Honsel, A., Kruse, J., Krol, E., Scherzer, S., Kreuzer, I., Bemm, F., Buegger, F., Burzlaff, T., Hedrich, R. & Rennenberg, H. 2015. Integration of trap- and root-derived nitrogen nutrition of carnivorous *Dionaea muscipula*. *New Phytologist,* 205**,** 1320-1329.

Gaume, L. & Forterre, Y. 2007. A viscoelastic deadly fluid in carnivorous pitcher plants. *Plos One,* 2**,** 7.

Gedroc, J. J., McConnaughay, K. D. M. & Coleman, J. S. 1996. Plasticity in root/shoot partitioning: Optimal, ontogenetic, or both? *Functional Ecology,* 10**,** 44-50.

Gibson, T. C. 1991. Differential escape of insects from carnivorous plant traps. *American Midland Naturalist,* 125**,** 55-62.

Gibson, T. C. & Waller, D. M. 2009. Evolving Darwin's 'most wonderful' plant: ecological steps to a snap-trap. *New Phytologist,* 183**,** 575-587.

Givnish, T. J. 2015. New evidence on the origin of carnivorous plants. *Proceedings of the National Academy of Sciences of the United States of America,* 112**,** 10-11.

Givnish, T. J., Burkhardt, E. L., Happel, R. E. & Weintraub, J. D. 1984. Carnivory in the bromeliad *Brocchinia reducta* with a cost-benefit model for the general restriction of carnivorous plants to sunny moist nutrient-poor habitats. *American Naturalist,* 124**,** 479-497.

Givnish, T. J., Sparks, K. W., Hunter, S. J. & Pavlovic, A. 2018. Why are plants carnivorous? Cost/benefit analysis, whole-plant growth, and the context-specific advantages of botanical carnivory. *In:* Ellison, A. M. & Adamec, L. (eds.) *Carnivorous plants: Physiology, ecology and evolution.* Oxford: Oxford University Press.

Gowda, D. C., Reuter, G. & Schauer, R. 1982. Structural features of an acidic polysaccharide from the mucin of *Drosera binata*. *Phytochemistry,* 21**,** 2297-2300.

Gowda, D. C., Reuter, G. & Schauer, R. 1983. Structural studies of an acidic polysaccharide from the mucin secreted by *Drosera capensis*. *Carbohydrate Research,* 113**,** 113-124.

Grafe, T. U., Schöner, C. R., Kerth, G., Junaidi, A. & Schöner, M. G. 2011. A novel resource-service mutualism between bats and pitcher plants. *Biology Letters,* 7**,** 436-439.

Greenwood, M., Clarke, C., Lee, C. i. C., Gunsalam, A. & Clarke, R. H. 2011. A unique resource mutualism between the giant Bornean pitcher plant, *Nepenthes rajah*, and members of a small mammal community. *PLoS One,* 6**,** e21114.

Hadi, A., Inubushi, K., Furukawa, Y., Purnomo, E., Rasmadi, M. & Tsuruta, H. 2005. Greenhouse gas emissions from tropical peatlands of Kalimantan, Indonesia. *Nutrient Cycling in Agroecosystems,* 71**,** 73-80.

Hagan, D. V., Grogan, W. L., Murza, G. L. & Davis, A. R. 2008. Biting midges (Diptera : Ceratopogonidae) from the English sundew, *Drosera anglica* Hudson (Droseraceae), at two fens in Saskatchewan, Canada. *Proceedings of the Entomological Society of Washington,* 110**,** 397-401.

Hanslin, H. M. & Karlsson, P. S. 1996. Nitrogen uptake from prey and substrate as affected by prey capture level and plant reproductive status in four carnivorous plant species. *Oecologia,* 106**,** 370-375.

Harms, S. 1999. Prey selection in three species of the carnivorous aquatic plant *Utricularia* (bladderwort). *Archiv fur Hydrobiologie,* 146**,** 449-470.

Harms, S. 2002. The effect of bladderwort (Utricularia) predation on microcrustacean prey. *Freshwater Biology,* 47**,** 1608-1617.

Hartmeyer, I. & Hartmeyer, S. M. H. 2010. Snap-tentacles and runway lights: Summary of comparative examination of *Drosera* tentacles. *The Carnivorous Plant Newsletter,* 39**,** 101-113.

Harvey, M. S. & Yen, A. L. 1990. *Worms to Wasps: An Illustrated Guide to Australia's Terrestrial Invertebrates,* Melbourne, Oxford University Press.

Huang, Y., Wang, Y., Sun, L., Agrawal, R. & Zhang, M. 2015. Sundew adhesive: a naturally occurring hydrogel. *Journal of the Royal Society Interface,* 12**,** 20150226.

Ichiishi, S., Nagamitsu, T., Kondo, Y., Iwashina, T., Kondo, K. & Tagashira, N. 1999. Effects of macro-components and sucrose in the medium on in vitro red-color pigmentation in *Dionaea muscipula* Ellis and *Drosera spathulata* labill. *Plant Biotechnology,* 16**,** 235-238.

Iwasa, Y. & Roughgarden, J. 1984. Shoot/root balance of plants: Optimal growth of a system with many vegetative organs. *Theoretical Population Biology,* 25**,** 78-105.

Jaffe, K., Blum, M. S., Fales, H. M., Mason, R. T. & Cabrera, A. 1995. On insect attractants from pitcher plants of the genus Heliamphora (Sarraceniaceae). *Journal of Chemical Ecology,* 21**,** 379-384.

Joel, D. M., Juniper, B. E. & Dafni, A. 1985. Ultraviolet patterns in the traps of carnivorous plants. *New Phytologist,* 101**,** 585-593.

Juniper, B. E., Ronbin, R. J. & Joel, D. M. 1989. *The carnivorous plants,* London, UK, Academic Press.

Jürgens, A., El-Sayed, A. M. & Suckling, D. M. 2009. Do carnivorous plants use volatiles for attracting prey insects? *Functional Ecology,* 23**,** 875-887.

Karlsson, P. S. & Pate, J. S. 1992. Contrasting effects of supplementary feeding of insects or mineral nutrients on the growth and nitrogen and phosphorous economy of pygmy species of *Drosera*. *Oecologia,* 92**,** 8-13.

Kassambara, A. 2019. 'ggplot2' Based Publication Ready Plots. 0.2.4 ed.

Kokubun, T. 2017. Occurrence of myo-inositol and alkyl-substituted polysaccharide in the prey-trapping mucilage of *Drosera capensis*. *The Science of Nature,* 104**,** s00114-017-1502-4.

Krausko, M., Perutka, Z., Šebela, M., Šamajová, O., Šamaj, J., Novák, O. & Pavlovič, A. 2017. The role of electrical and jasmonate signalling in the recognition of captured prey in the carnivorous sundew plant *Drosera capensis*. *New Phytologist,* 213**,** 1818-1835.

Król, E., Płachno, B. J., Adamec, L., Stolarz, M., Dziubińska, H. & Trebacz, K. 2012. Quite a few reasons for calling carnivores 'the most wonderful plants in the world'. *Annals of Botany,* 109**,** 47-64.

Kruse, J., Gao, P., Honsel, A., Kreuzwieser, J., Burzlaff, T., Alfarraj, S., Hedrich, R. & Rennenberg, H. 2014. Strategy of nitrogen acquisition and utilization by carnivorous *Dionaea muscipula*. *Oecologia,* 174**,** 839-851.

Kurup, R., Johnson, A. J., Sankar, S., Hussain, A. A., Kumar, C. S. & Sabulal, B. 2013. Fluorescent prey traps in carnivorous plants. *Plant Biology,* 15**,** 611-615.

Lambers, H., Raven, J. A., Shaver, G. R. & Smith, S. E. 2008. Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution,* 23**,** 95-103.

Lamont, B. B. 1993. Why are hairy root clusters so abundant in the most nutrient-impoverished soils of Australia? *Plant and Soil,* 155-156**,** 269-272.

Lamont, B. B. 2003. Structure, ecology and physiology of root clusters - A review. *Plant and Soil,* 248**,** 1-19.

Lavarack, P. S. 1979. Rainforest *Drosera* of north Queensland. *The Carnivorous Plant Newsletter,* 8**,** 60-64.

Lowrie, A. 2013. *Carnivorous plants of Australia, Magnum Opus,* India, Redfern Natural History Productions.

Marden, J. H. 1987. Maximum lift production during takeoff in flying animals. *Journal of Experimental Biology,* 130**,** 235 - 258.

Millett, J., Foot, G. W. & Svensson, B. M. 2015. Nitrogen deposition and prey nitrogen uptake control the nutrition of the carnivorous plant *Drosera rotundifolia*. *Science of the Total Environment,* 512**,** 631-636.

Millett, J., Jones, R. I. & Waldron, S. 2003. The contribution of insect prey to the total nitrogen content of sundews *(Drosera* spp.) determined in situ by stable isotope analysis. *New Phytologist,* 158**,** 527-534.

Millett, J., Svensson, B. M., Newton, J. & Rydin, H. 2012. Reliance on prey-derived nitrogen by the carnivorous plant *Drosera rotundifolia* decreases with increasing nitrogen deposition. *New Phytologist,* 195**,** 182-188.

Moran, J. A. 1996. Pitcher dimorphism, prey composition and the mechanisms of prey attraction in the pitcher plant Nepenthes rafflesiana in Borneo. *Journal of Ecology,* 84**,** 515-525.

Moran, J. A., Clarke, C. M. & Hawkins, B. J. 2003. From carnivore to detritivore? Isotopic evidence for leaf litter utilization by the tropical pitcher plant *Nepenthes ampullaria*. *International Journal of Plant Sciences,* 164**,** 635-639.

Murza, G. L., Heaver, J. R. & Davis, A. R. 2006. Minor pollinator-prey conflict in the carnivorous plant, *Drosera anglica*. *Plant Ecology,* 184**,** 43-52.

Nakano, M., Kinoshita, E. & Ueda, K. 2004. Life history traits and coexistence of an amphidiploid, *Drosera tokaiensis,* and its parental species, *D. rotundifolia* and *D. spatulata* (Droseraceae). *Plant Species Biology,* 19**,** 59-72.

Naumann, I. D. 1991. *The Insects of Australia : a textbook for students and research workers,* Carlton South, Victoria, Melbourne University Press.

Nguyen, V., Lilly, B. & Castro, C. 2014. The exoskeletal structure and tensile loading behavior of an ant neck joint. *Journal of Biomechanics,* 47**,** 497-504.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. & Wagner, H. 2019. Vegan: Community Ecology Package. 2.5-4 ed.

Pandey, R. K. & Saini, S. K. 2004. *Drosera burmannii*: A species under stress in natural tropical forest ecosystem in Madhya Pradesh. *Journal of Economic and Taxonomic Botany,* 28**,** 494-496.

Pandolfi, C., Masi, E., Voigt, B., Mugnai, S., Volkmann, D. & Mancuso, S. 2014. Gravity affects the closure of the traps in *Dionaea muscipula*. *BioMed Research International,* 2014.

Parkes, D. M. & Hallam, N. D. 1984. Adaptation for carnivory in the West Australian pitcher plant *Cephalotus follicularis* Labill. *Australian Journal of Botany,* 32**,** 595-604.

Pavlovič, A., Krausko, M. & Adamec, L. 2016. A carnivorous sundew plant prefers protein over chitin as a source of nitrogen from its traps. *Plant Physiology and Biochemistry,* 104**,** 11-16.

Pavlovič, A., Krausko, M., Libiaková, M. & Adamec, L. 2014. Feeding on prey increases photosynthetic efficiency in the carnivorous sundew *Drosera capensis*. *Annals of Botany,* 113**,** 69-78.

Pavlovic, A. & Saganova, M. 2015. A novel insight into the cost-benefit model for the evolution of botanical carnivory. *Annals of Botany,* 115**,** 1075-1092.

Pavlovič, A., Singerová, L., Demko, V., Šantrůček, J. & Hudák, J. 2010. Root nutrient uptake enhances photosynthetic assimilation in prey-deprived carnivorous pitcher plant *Nepenthes talangensis*. *Photosynthetica,* 48**,** 227-233.

Płachno, B. J., Kozieradzka-Kiszkurno, M., Swiątek, P. & Darnowski, D. W. 2008. Prey attraction In carnivorous Genlisea (Lentibulariaceae). *Acta Biologica Cracoviensia Series Botanica,* 50**,** 87-94.

Plachno, B. J., Wolowski, K., Fleischmann, A., Lowrie, A. & Lukaszek, M. 2014. Algae and prey associated with traps of the Australian carnivorous plant *Utricularia volubilis* (Lentibulariaceae : Utricularia subgenus Polypompholyx) in natural habitat and in cultivation. *Australian Journal of Botany,* 62**,** 528-536.

Poppinga, S., Hartmeyer, S. R. H., Masselter, T., Hartmeyer, I. & Speck, T. 2013. Trap diversity and evolution in the family Droseraceae. *Plant Signaling and Behavior,* 8**,** e24685.1-e24685.5.

Poppinga, S., Hartmeyer, S. R. H., Seidel, R., Masselter, T., Hartmeyer, I. & Speck, T. 2012. Catapulting tentacles in a sticky carnivorous Plant. *Plos One,* 7**,** 5.

Potts, L. & Krupa, J. J. 2016. Does the dwarf sundew (*Drosera brevifolia*) attract prey? *American Midland Naturalist,* 175**,** 233-241.

Quinn, G. P. & Keough, M. J. 2002. *Experimental design and data analysis for biologists,* United Kingdom, Cambridge University Press.

Robinson, A. S., Nunn, R., Bourke, G., McPherson, S. & Fleischmann, A. 2017. The Genus Drosera. *In:* Robinson, A. S. (ed.) *Drosera of the world, Volume 1: Oceania.* India: Redfern Natural History Productions.

Robinson, S. J. W., Zwaan, B. & Partridge, L. 2000. Starvation resistance and adult body composition in a latitudinal cline of *Drosophila melanogaster*. *Evolution,* 54**,** 1819-1824.

Rost, K. & Schauer, R. 1977. Physical and chemical properties of the mucin secreted by *Drosera capensis*. *Phytochemistry,* 16**,** 1365-1368.

Rubes, M. 2009. *Utricularia aurea* [Online]. Available: https://en.wikipedia.org/wiki/File:Utricularia_aurea_8_Darwiniana.jpg#file [Accessed 19 February 2020].

Schaefer, H. M. & Ruxton, G. D. 2008. Fatal attraction: Carnivorous plants roll out the red carpet to lure insects. *Biology Letters,* 4**,** 153-155.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods,* 9**,** 671-675.

Schulze, E. D., Gebauer, G., Schulze, W. & Pate, J. S. 1991. The utilization of nitrogen from insect capture by different growth forms of *Drosera* from southwest Australia. *Oecologia,* 87**,** 240-246.

Schulze, W. & Schulze, E. D. 1990. Insect capture and growth of the insectivorous *Drosera rotundifolia* L. *Oecologia (Berlin),* 82**,** 427-429.

Schulze, W., Schulze, E. D., Pate, J. S. & Gillison, A. N. 1997. The nitrogen supply from soils and insects during growth of the pitcher plants *Nepenthes mirabilis*, *Cephalotus follicularis* and *Darlingtonia californica*. *Oecologia,* 112**,** 464-471.

Schulze, W., Schulze, E. D., Schulze, I. & Oren, R. 2001. Quantification of insect nitrogen utilization by the venus fly trap *Dionaea muscipula* catching prey with highly variable isotope signatures. *Journal of Experimental Botany,* 52**,** 1041-1049.

Shipley, B. & Meziane, D. 2002. The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. *Functional Ecology,* 16**,** 326-331.

Sota, T., Mogi, M. & Kato, K. 1998. Local and regional-scale food web structure in *Nepenthes alata* pitchers. *Biotropica,* 30**,** 82-91.

Tang, Z., Xu, W., Zhou, G., Bai, Y., Li, J., Tang, X., Chen, D., Liu, Q., Ma, W., Xiong, G., He, H., He, N., Guo, Y., Guo, Q., Zhu, J., Han, W., Hu, H., Fang, J. & Xie, Z. 2018. Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to

productivity in China's terrestrial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* 115**,** 4033–4038.

Taylor, P. 1991. The Genus Genlisea. *Carniv. Pl. Newslett,* 20**,** 20-26.

Thorén, L. M., Tuomi, J., Kämäräinen, T. & Laine, K. 2003. Resource availability affects investment in carnivory in *Drosera rotundifolia*. *New Phytologist,* 159**,** 507-511.

Thornley, J. H. M. 1972. A balanced quantitative model for root: Shoot ratios in vegetative plants. *Annals of Botany,* 36**,** 431-441.

Thum, M. 1986. Segregation of habitat and prey in two sympatric carnivorous plant species *Drosera rotundifolia* and *Drosera intermedia*. *Oecologia,* 70**,** 601-605.

Thum, M. 1989. The significance of carnivory for the fitness of *Drosera* in its natural habitat. 2. The amount of captured prey and its effect on *Drosera intermedia* and *Drosera rotundifolia*. *Oecologia,* 81**,** 401-411.

Tracey, J. G. 1982. *The vegetation of the humid tropical region of North Queensland,* Melbourne, CSIRO Publication.

Troscianko, J. 2014. A simple tool for calculating egg shape, volume and surface area from digital images. *Ibis,* 156**,** 874–878.

Verbeek, N. A. M. & Boasson, R. 1993. Relationship between types of prey captured and growth form in *Drosera* in southwestern Australia. *Australian Journal of Ecology,* 18**,** 203-207.

Wakefield, A. E., Gotelli, N. J., Wittman, S. E. & Ellison, A. M. 2005. Prey addition alters nutrient stoichiometry of the carnivorous plant *Sarracenia purpurea*. *Ecology,* 86**,** 1737-1743.

Watson, A. P., Matthiessen, J. N. & Springett, B. P. 1982. Arthropod associates and macro nutrient status of the red-ink sundew *Drosera erythrorhiza*. *Australian Journal of Ecology,* 7**,** 13-22.

Wells, K., Lakim, M. B., Schulz, S. & Ayasse, M. 2011. Pitchers of *Nepenthes rajah* collect faecal droppings from both diurnal and nocturnal small mammals and emit fruity odour. *Journal of Tropical Ecology,* 27**,** 347-353.

Wet Tropics Management Authority. 2009. *Vegetation mapping of the Wet Tropics of Queensland bioregion*. Cairns: Wet Tropics Management Authority.

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C. & Woo, K. 2019. ggplot2: Create elegant data visualisations using the grammar of graphics. 3.1.1 ed.

Williams, S. E. 2006. *Vertebrates of the Wet Tropics rainforests of Australia: species distributions and biodiversity,* Cairns, Cooperative Research Centre for Tropical Rainforest Ecology and Management.

Wobbrock, J. O., Findlater, L., Gergle, D. & Higgins, J. J. 2011. The Aligned Rank Transform for nonparametric factorial analyses using only ANOVA procedures. *The ACM*

Conference on Human Factors in Computing Systems (CHI '11). Vancouver, British Columbia: ACM Press.

Wojtusiak, J., Godzińska, E. J. & Dejean, A. 1995. Capture and retrieval of very large prey by workers of the African weaver ant, *Oecophylla longinoda* (Latreille 1802). *Tropical Zoology,* 8**,** 309-318.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornellssen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., Midgley, J. J., Navas, M. L., Niinemets, Ü., Oleksyn, J., Osada, H., Poorter, H., Pool, P., Prior, L., Pyankov, V. I., Roumet, C., Thomas, S. C., Tjoelker, M. G., Veneklaas, E. J. & Villar, R. 2004. The worldwide leaf economics spectrum. *Nature,* 428**,** 821-827.

Yanthan, J. S., Kehie, M., Kumaria, S. & Tandon, P. 2017. In vitro regeneration of *Drosera burmannii* Vahl.: a carnivorous plant of north-east India. *3 Biotech,* 7: 124.

Zamora, R. 1995. The trapping success of a carnivorous plant, *Pinguicula vallisneriifolia*: The cumulative effects of availability, attraction, retention and robbery of prey. *Oikos,* 73**,** 309-322.

Zamora, R., Gomez, J. M. & Hodar, J. A. 1998. Fitness responses of a carnivorous plant in contrasting ecological scenarios. *Ecology (Washington D C),* 79**,** 1630-1644.

Zar, J. H. 2009. *Biostatistical Analysis,* New Jersey, Pearson Prentice Hall.

APPENDICES

Appendix 1.1 Permit letter to take, use, keep or interfere with cultural and natural resources (scientific purposes) issued by Department of Environment and Heritage Protection (only shown the first page of the permit letter).

This administrative form has been removed

Appendix 1.2 A screenshot of permission statement from the copyright owner of the image of *Genlisea* presented in Figure 1.1.B.

Appendix 1.3 A screenshot of permission statement from the copyright owner of the image of *Dionaea muscipula* presented in Figure 1.1.D.

Appendix 1.4 A screenshot of permission statement from the copyright owner of graphs presented in Figure 1.2.

Appendix 1.5 A screenshot of permission statement from the copyright owner of *Drosera* traps images presented in Figure 1.3.

Appendix 2.1 A screenshot of permission statement from the copyright owner of the map presented in Figure 2.9.

Appendix 3.1 Each artificial trap of *Drosera* was placed alongside a real plant of the species depicted in the image. The artificial traps were life-size coloured plastic images mounted on flathead nails (not shown in the image).

Appendix 4.1 A screenshot of permission statement from the copyright owner of Figure 4.8.

Appendix 5.1 A screenshot of permission statement from the copyright owner of FemtoTools sensor images in Figure 5.3.B.

Appendix 5.2 A screenshot of permission statement from the copyright owner of Figure 5.19.

Appendix 6.1 A screenshot of permission statement from the copyright owner of insect images in Figure 6.1.