LAÍS FERREIRA MAIA

FOUR YEAR MULTITROPHIC FOOD WEB:

SOURCE FOOD WEB DESCRIPTION,

METHODOLOGY ACCURACY

AND SPECIES DIVERSITY

LAVRAS – MG

2016

LAIS FERREIRA MAIA

FOUR YEAR MULTITROPHIC FOOD WEB: SOURCE FOOD WEB DESCRIPTION, METHODOLOGY ACCURACY AND SPECIES DIVERSITY

Dissertação apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Conservação de Paisagens Fragmentadas e Agrossistemas, para obtenção do título de Mestre.

Orientador

Dr. Lucas Del Bianco Faria

LAVRAS – MG

2016

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Maia, Lais Ferreira.
Four Year Multitrophic Food Web : Source Food Web Description, Methodology Accuracy and Species Diversity / Lais Ferreira Maia. – Lavras : UFLA, 2016.
91 p. : il.

Dissertação (mestrado acadêmico)–Universidade Federal de Lavras, 2016.
Orientador(a): Lucas Del Bianco Faria.
Bibliografia.

1. Senegalia tenuifolia. 2. rede trófica. 3. metodologia. 4. variação temporal. 5. diversidade. I. Universidade Federal de Lavras. II. Título.

LAIS FERREIRA MAIA

FOUR YEAR MULTITROPHIC FOOD WEB: SOURCE FOOD WEB DESCRIPTION, METHODOLOGY ACCURACY AND SPECIES DIVERSITY

Dissertação apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Conservação de Paisagens Fragmentadas e Agrossistemas, para obtenção do título de Mestre.

.

APROVADA em 02 de maio de 2016

Dr. Frederico de Siqueira Neves	UFMG
Dr. Julio Neil Cassa Louzada	UFLA

Dr. Lucas Del Bianco Faria

Orientador

LAVRAS – MG

2016

Aos meus pais, André e Cristina

Minha amada irmã, Clara

E ao meu noivo, Filipe

DEDICO

AGRADECIMENTOS

A Deus por me conceder a oportunidade de melhorar e servir.

Aos meus pais, pelo exemplo de vida, amor, dedicação, incentivo, formação, e por estarem sempre ao meu lado permitindo que eu chegasse até aqui, à minha amada irmã, pelo amor, companheirismo, amizade, as muitas alegrias, companhia e ajuda nos momentos difíceis. Aos meus avôs Geraldo e Athanoel e as avós Emerenciana e Alda e Cidinha, exemplo de força e sabedoria, obrigada pelo carinho, pela orações e ensinamentos sobre a vida.

Àos meus Tios e Tias, todos vocês, sem exceção, me ajudaram de alguma forma para que eu pudesse conseguir esta vitória, lembro-me de cada um de vocês com muito carinho e admiração. Obrigada pelo apoio, compreensão, momentos de alegrias e amizade. Aos meus primos, mesmo daqueles que estão distantes fisicamente, muito obrigada pela união e amizade, que fizeram de mim uma pessoa melhor e mais feliz! Ás minhas irmãs Lídia e Lívia que estão todos os dias ao meu lado, fazendo meus dias mais alegres e divertidos.

Ao meu noivo Filipe obrigada pelo carinho, compreensão e por me ajudar na vida acadêmica e pessoal, obrigada por me incentivar a alçar voos mais altos. Esteja você longe ou perto de mim eu agradeço por todo amor e felicidades que você traz à minha vida!! Obrigada por cuidar de mim e estar sempre ao meu lado, eu te amo muito.

Aos meus colegas da turma de 2009/2 os quais estarão sempre em minhas lembranças, obrigada pelas conversas e momento alegres e de tensão divididos. Aos meus amigos Mateus, Fernanda, Danielle, Ludson e Laise por todos os bons momentos vividos, pelos estudos até tarde, pela compreensão, por toda a ajuda, e pela amizade que dura até os dias de hoje. Obrigada por todo carinho!! Aos bons, “velhos” e novos amigos, Débora, Fernanda, as meninas da U.A.I, Leydiane, Nathália, Tamara, Lívia, Stefanie, Pulguinha e aqueles que

sempre estiveram presentes, mesmo através de telefonemas e mensagens no facebook. Aos amigos da University of the Fraser Valley: Pedro, Nathália, Guguinha, Caio, Jessica, Karla e Victoria pela amizade e companheirismo até os dias de hoje. Em especial a minha basement-mate Bárbara, pelo carinho e ensinamentos.

Ao professor Lucas Del Bianco Faria, pela oportunidade, paciência e aprendizado ao longo destes seis anos que me permitiram alcançar muitos dos meus objetivos e sonhos na vida. Aos colegas e amigos do Laboratório de Ecologia e Complexidade: pelas ajudas, convivência e pelos ótimos momentos de alegrias. Sou muito grata por ter encontrado pessoas tão especiais.

Agradeço imensamente aos professores do Programa de Ecologia Aplicada da UFLA por todo ensinamento e pela profissional que me tornei até agora. Minha eterna gratidão! Agradeço de coração aos amigos que conheci durante mestrado, amigos da Ecologia! Cada um de vocês fizeram meu aprendizado muito mais divertido, meu dias mais alegres e minha vida mais doce! Vocês fizeram a diferença na minha vida e os levarei para sempre dentro do meu coração!!

À UFLA pela minha formação acadêmica. Aos funcionários da Biologia e Ecologia pela atenção e dedicação aos alunos. Ao CNPq pela concessão de bolsa de estudos no exterior, à FAPEMIG pela bolsa de iniciação científica, e à CAPES pela bolsa de mestrado que contribuíram para meu desenvolvimento e avanço na ciência. E a todos professores que fizeram parte da minha formação, minha gratidão! A Juliana Tuller, Filipe França, Ricardo Solar e Luiz Magnago obrigada por todos os ensinamentos em estatística e cursos do R que me permitiram realizar as análises deste trabalho. Muito obrigada a todos!

A todos que de alguma forma me ajudaram, com um sorriso em cada manhã, com um carinho, amizade, compreensão, apoio, disponibilidade em estender a mão e em orações, a minha eterna gratidão!

“Primeiro foi necessário civilizar o homem em relação ao próprio homem. Agora é necessário civilizar o homem em relação à natureza e aos animais”

Victor Hugo

RESUMO

O uso de redes tróficas vem sendo aplicado para o entendimento das relações entre organismos de um ecossistema, no entanto a maneira como o esforço amostral poderia influenciar os padrões das redes tróficas permanece pouco compreendida. Até o momento, existe uma falta de dados amostrais de longa duração para muitos grupos de insetos, principalmente relacionado às interações entre os herbívoros e suas plantas hospedeiras. No primeiro capítulo, eu descrevo uma rede trófica do tipo *source web* baseada na planta *Senegalia tenuifolia* através da identificação dos insetos associados e das interações entre eles e com essa planta hospedeira. Além disso, eu proponho uma metodologia para checar a robustez dos dados de cada nível trófico. Os resultados desse capítulo demonstram que o conjunto de dados coletados e a metodologia de coleta utilizada são suficientes para amostrar maior parte da riqueza de uma rede trófica tipo *source web*. No total foram amostradas 27 species pertencentes a quatro níveis tróficos. No segundo capítulo, eu apresento a variação temporal na riqueza e abundancia de cada nível trófico, bem como na relação entre os diferentes níveis tróficos. Também investiguei os padrões de diversidade do segundo e terceiro níveis tróficos através da avaliação da contribuição dos componentes alpha e beta da diversidade ao longo dos anos. Esse capítulo mostra que, em nosso sistema, a abundância de parasitóides varia conforme a abundância de herbívoros, além disso, a riqueza e abundância dos quatro níveis tróficos variam ao longo do tempo. Os resultados também demonstram que a diversidade alpha contribuiu mais para a diversidade de herbívoros (2º nível trófico), enquanto que a contribuição dos componentes alpha e beta para a diversidade de parasitoides (3º nível) variou ao longo dos anos. De maneira geral, essa dissertação descreve uma rede trófica do tipo *source* e traz informações sobre os desafios relacionados ao esforço amostral suficiente para amostrar espécies de todos os níveis tróficos de uma rede trófica. Também discute-se a relação entre as comunidades associadas à diferentes níveis tróficos e a sua variação temporal e padrões de diversidade. No geral, esta dissertação contribui com o banco de dados de pesquisas em redes tróficas, na compreensão de interações entre os níveis tróficos e também dos padrões que cada nivel trofico apresenta em uma escala temporal e espacial.

Palavras chave: *Senegalia tenuifolia*, rede trófica, metodologia, variação temporal, diversidade.

ABSTRACT

Food webs have been used in order to understand the trophic relationship among organisms within an ecosystem, however the extension by which sampling efficiency could affect food web responses remain poorly understood. Still, there is a lack of long-term sampling data for many insect groups, mainly related to the interactions between herbivores and their host plants. In the first chapter, I describe a source food web based on the *Senegalia tenuifolia* plant by identifying the associated insect species and the interactions among them and with this host plant. Furthermore, I check for the data robustness from each trophic level and propose a cost-efficiently methodology. The results from this chapter show that the collected dataset and the methodology presented are a good tool for sample most insect richness of a source food web. In total the food web comprises 27 species belonging to four trophic levels. In the second chapter, I demonstrate the temporal variation in the species richness and abundance from each trophic level, as well as the relationship among distinct trophic levels. Moreover, I investigate the diversity patterns of the second and third trophic level by assessing the contribution of alfa and beta-diversity components along the years. This chapter shows that in our system the parasitoid abundance is regulated by the herbivore abundances. Besides, the species richness and abundances of the trophic levels vary temporally. It also shows that alfa-diversity was the diversity component that most contribute to the herbivore species diversity (2nd trophic level), while the contribution of alfa- and beta-diversity changed along the years for parasitoid diversity (3rd level). Overall, this dissertation describes a source food web and bring insights into some food web challenges related to the sampling effort to gather enough species from all trophic levels. It also discuss the relation among communities associated with distinct trophic levels and their temporal variation and diversity patterns. Finally, this dissertation contributes for the world food web database and in understanding the interactions among its trophic levels and each trophic level pattern along time and space

Key-words: *Senegalia tenuifolia*, food web, methodology, temporal variation, diversity.

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

Chapter 1:

1 GENERAL INTRODUCTION

1.1 Food webs

Knowing how the species interact and how those interactions influence the community structure and functioning are relevant when building and applying food web theories. Food webs are a dynamic multitrophic system in which resource, prey and predators/parasitoids interact leading to temporal and spatial changes on their abundances and species richness (MAAR et al., 2002; MOORE & RUITER, 1991). When studying food web aspects, it is very important to consider the type of food web being examined. According to Cohen (1978) there are three kinds of food webs: (i) sink, (ii) community and (iii) source food webs. **(i) Sink food webs** are those which one or more predators are selected as model organism and, from them, preys and other interacting organisms in below trophic levels are observed. Contrastingly, **(ii) community food webs** include populations of many plants, herbivores or predators; and from them all links (predator and prey links) are studied. Lastly, the **(iii) source food webs** are considered as the ones which we select one or more plant or herbivore species and from them we observe its predators and organisms in the trophic levels above them.

1.2 Temporal and spatial variation in food webs

Despite advances made by previous research, a better understanding of the relationships among different trophic levels is still necessary, mainly with focus on the influence of space and time variation on the interaction dynamics and structure of food webs. Previous research has shown that food webs' structure can vary temporally and spatially, while there are also evidence that

insect-plant associations are rarely static within a given region (COHEN et al., 1993, PILOSOF et al., 2003). As result, plant-herbivore studies at local scale could be considered as a good opportunity to evaluate whether local-scale food webs are good to predict different patters at broader scales (PRADO & LEWINSOHN 2000). Thus, a better understanding of multitrophic temporal and spatial approaches are well considered.

1.3 Studied system

In this study, I use a source food web comprising four trophic levels: the resource and its primary, secondary and tertiary consumer levels.

1.4.1 Resource level

The resource level (plant or primary level) was always represented by *Senegalia tenuifolia* plant (L.) Britton & Rose, also known as *Acacia tenuifolia* (L.) Willd, (Fabaceae: Mimosoideae), popularly called as catclaw. This plant is capable of fixing large amount of nitrogen since their roots are associated with nitrogen fixing organisms (MATTSON 1980). The species is also considered as economically important, being used for charcoal production due to its high growth rates (CARVALHO et al. 2010).

This plant is worldwide distributed but, mainly occurring in all tropical regions (ATCHISON 1948). *Senegalia tenuifolia* is frequently found at South America and its individuals vary in plant height, behavior (liana, shrub or tree), and hooklets density (QUEIROZ 2009). Its fruits are considered dehiscence dry pods (or legume), presenting a brownish color during the ripening period, when the fruits open. As other Leguminous plants, *S. tenuifolia* fruit ripe occur during some months of the year, generally from March to August. By the end of this

period, fruits have already ripen and just some of them can still be found attached to the mother plant (KINGSOLVER 2004).

1.4.2 Herbivores: primary consumers and second trophic level

Regarding the associated consumer-levels of the studied plant, the herbivore level (primary consumers and secondary trophic level) accounted for eleven insect species. Most species are considered granivorous or seed-consumers, as the beetles from the subfamily Bruchinae (Coleoptera: Chrysomelidae) and an herbivore hymenoptera belonging to the Braconidae family (Hymenoptera: Ichneumonoidea) (HULME & BENKMAN 2002, TULLER et al. 2015).

Bruchine beetles are likely found in tropical zones, usually feeding on Fabaceae plants from the *Acacia* genus (JOHNSON & ROMERO 2004; JOHNSON & SIEMENS 1997). The most abundant herbivore found in all fours years of data sample was *Merobruchus terani*, which has been reported as consumer of at least five Acacia species, such as *Acacia angustissima*, *A. berlandieri*, *A. gaumeri*, *A. picachensis* e *A. tenuifolia* (JOHNSON & SIEMENS 1997). Most of Bruchinae beetles support large parasitoid populations belonging to the Eulophidae, Eupelmidae and Pteromalidae families in the Hymenoptera order (SOUTHGATE 1979, TULLER et al. 2015).

1.4.3 Parasitoid and hyperparasitoid levels

The parasitoid level (secondary consumers and third trophic level) was represented by thirteen species associated with the sampled herbivores, most of them from the families Braconidae, Eulophidae, Eupelmidae, Pteromalidae e Bethydae (see also TULLER et al., 2015). The hyperparasitoid level (tertiary consumers and fourth trophic level) presented two species that were associated

with the parasitoid species. Due to the difficulty in known parasitoid and hyperparasitoid at species level we can seldom infer about their origin and feeding preferences. However, we could set the herbivore-parasites interactions by data on literature and observation at laboratory (as showed in Chapter 1).

1.5 Dissertation structure and research objectives

This dissertation focuses on applying concepts of ecological communities in the food web context. In particular, by describing the insect communities from different trophic levels associated with the *S. tenuifolia* plant species occurring in the south of the state of Minas Gerais, Brazil.

The structure of this dissertation is made up of two stand-alone chapters. In Chapter 1, I used a methodological approach to verify the robustness of the studied food web. In doing that, I propose a cost-efficiently sampling protocol to sample insects and to make good inferences related to source food webs at local and reginal scales. Furthermore, in Chapter 2, I investigated the existence of temporal (along years) and spatial (across sampling sites and areas) variation in community diversity patterns (abundance and richness) of the insect communities associated with distinct trophic levels.

REFERENCES

ATCHISON E. 1948. Studies in the Leguminosae. II. Cytogeography of Acacia (Tourn.) L. **American Journal of Botany** v. 35, n.10, pp. 651-655.

CARVALHO F. A., FAGG C. W. E., FELFILI M.J. 2010. Dinâmica populacional de Acacia tenuifolia (L.) Willd. Em uma floresta decidual sobre afloramentos calcários no Brasil Central. **Scientia Florestalis** v.38, n.86, pp. 297-306.

COHEN, J. E. et al. Improving Food Webs. **Ecology**, v. 74, n. 1, p. 252–258, 1993.

COHEN, J.E. 1978. **Food Webs and Niche Space**. Princenton Univeristy Press.

HULME P.E. ; BENKMAN C. W. 2002. Granivory. In: Herrera C.M. & Pellmyr O. (eds.), **Plant-animal interactions: an evolutionary approach.** Blackwell, Oxford pp .132-154.

JOHNSON C. D. ; SIEMENS H. D 1997. Oviposition Behaviour, Guilds, Host Relationships and New Host and Distribution Records for the Genus Merobruchus Bridwell (Coleoptera: Bruchidae). **The Coleopterists Bulletin** v.51, n.1, pp. 13-12

JOHNSON, C. D; ROMERO J. 2004. A review of evolution of oviposition guilds in the Bruchidae (Coleoptera). **Revista Brasileira de Entomologia** v.48, n.3, pp. 401-408.

KINGSOLVER J. G., John M. 2004. Handbook of the Bruchidae of the United States and Canada (Insecta, Coleoptera). **U.S. Department of Agriculture technical Bulletin** 1912 v.2, p.636.

MAAR, M. et al. Spatial and temporal variability of food web structure during the spring bloom in the Skagerrak. **Marine ecology progress series**, v. 239, p. 11–29, 2002.

MATTSON J. ; WILLIAM JR., 1980 Herbivory in relation to plant nitrogen content. **Annual Review of Ecology and Systematics** v.11, pp. 119-161.

MOORE, J. C.; RUITER, P. C. DE. The identification and evaluation of food webs in soil Temporal and spatial heterogeneity of trophic interactions within below-ground food webs. **Agriculture, Ecosystems & Environment**, v. 34, n. 23, p. 371–397, 1991.

PILOSOF, S., FORTUNA, M.A., VINARSKI, M.V., VINARSKAYA-KORALLO N. & KRASNOV, B.R. (2013) Temporal dynamics of direct reciprocal and indirect effects in a host-parasite network. **Journal of Animal Ecology**, 82, 987-996

PRADO PIKL, LEWINSOHN TM (2000) Associações inseto-planta no nível local e regional: Tephritidae e Vernonieae na Serra do Espinhaço. pp. 405-422. In Martins RP, Lewinsohn TM, Barbeitos MS (eds). **Ecologia e Comportamento de Insetos**. Série Oecologia Brasiliensis, vol. VIII. PPGE-UFRJ. Rio de Janeiro, Brasil.

QUEIROZ, L.P. (2009) **Leguminosas da Caatinga**. Universidade Estadual de Feira de Santana: Feira de Santana. 914 p.

SOUTHGATE, B. J. Biology of the Bruchidae. **Annual Review of Entomology**, v. 24, n. 1, p. 449–473, 1979.

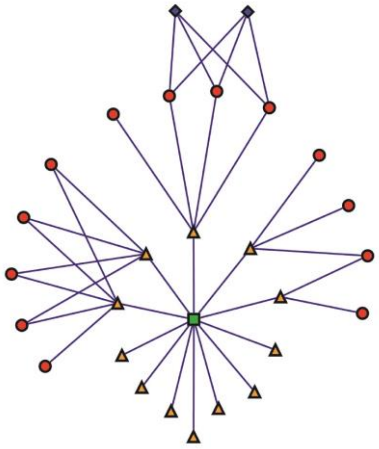
TULLER, J. et al. Seed predation food web, nutrient availability, and impact on the seed germination of Senegalia tenuifolia (Fabaceae). **Revista de biología tropical**, v. 63, n. December, p. 1149–1159, 2015.

SECOND PART: Manuscripts

Chapter 2: FIRST MANUSCRIPT

A HIGHLY RESOLVED SOURCE FOOD WEB AND ITS CHALLENGES

Publication status: *In prep.* for submission to *Ecological Entomology*



ABSTRACT

Poor sampling effort can lead to a methodological bias towards a misunderstood food web patterns. The sampling incompleteness has been shown as a major problem affecting data quality in terrestrial environments. We aim to describe a food web based in a four years interaction database and check for the dataset robustness then proposing an accurate methodology for food web samplings. We sampled *Senegalia tenuifolia* fruits in eight sampling sites distributed in three areas during four years in the Brazilian savanna. We observed 26 insect species distributed in three trophic levels. Based on the species accumulation curves we observed that all insect trophic levels, areas and years reached the asymptote, except by one area. Thus, the sampling effort used was efficient to access most of the insect species richness and provided a well-represented food web. We also used the accumulated insect species richness and abundances along years to proposed an accurately methodology and found that, in overall, 1,053 fruits are enough to access the species richness from all insect trophic levels. Although studies based on just one trophic level can use less fruits that suggested in overall. In conclusion, food web studies should be viewed with caution in respect to sampling effort in order to achieve the most complete food web. Also, studies based on multiple areas and years is enough to represent most of the food web species richness.

Key-words: sampling-effort, source food web, methodology, *Senegalia tenuifolia*, four trophic levels.

2 First manuscript: Highly Resolved Source Food Web and Its Challenges

2.1 Introduction

The use of food webs to understand the trophic relationships between organisms within ecosystems have increased significantly during the past decades (Shurin *et al.*, 2006; Ings *et al.*, 2009). There is much hope that by knowing the complexity of natural ecosystem it will be possible to predict its response to anthropogenic activities (de Sassi *et al.*, 2012; Burkle *et al.*, 2013). While food web responses are inevitably accompanied by changes within the components from each trophic level (e.g. species richness and resource availability), there is a long-standing debate about the extension that sampling efficiency could affect the accuracy in assessing such responses (Guegan & Kennedy, 1996; Novotny, 2009; Tylianakis *et al.*, 2010; Rivera-Hutinel *et al.*, 2012).

Although previous studies have controlled for uneven sampling effort (Walther *et al.*, 1995; Walther & Martin, 2001; Cao *et al.*, 2002; Clauset *et al.*, 2008) only limited information is available about how much sampling effort should be done to fully describe ecological communities (Memmott, 1999; Willott, 2001; Gibson *et al.*, 2011; Stürmer *et al.*, 2014). Poor sampling effort can demonstrate a methodological bias towards a misunderstood food web patterns (Goldwasser & Roughgarden, 1997; Vizentin-Bugoni *et al.*, 2015). The sampling incompleteness has been shown as a major problem affecting data quality in terrestrial (Chacoff *et al.*, 2012; Parmain *et al.*, 2013; Vizentin-Bugoni *et al.*, 2015) and aquatic food web metrics (Winemiller, 1990; Wood *et al.*, 2015). To date, results about the magnitude of sampling incompleteness as a major problem to understand food web properties have being investigated (Goldwasser & Roughgarden, 1997; Martinez *et al.*, 1999; Vizentin-Bugoni *et al.*, 2015).

Other important methodological issue in the food web research is the sampling scale. Different sampling scales have been shown to affect the observed food web metrics (Levin, 1992; Hewitt *et al.*, 1998; Hill & Hamer, 2004; Chave, 2013). Such scale-effects are suggested to result from the organism population dynamics changing both temporally and spatially (Teder *et al.*, 2000; Liebhold *et al.*, 2004), highlighting therefore the importance of considering multi-scale sampling designs to accurately assess the food web patterns on natural ecosystems. Indeed, long-term studies have been fundamentally important to demonstrate the accurateness of sampling methods in assessing biodiversity patterns (Morris, 1960; Brown *et al.*, 2001; Strayer *et al.*, 2006)

The examination of insect host-parasitoid source webs has been very often applied to represent food web interactions and to test theory (e.g. Memmott *et al.*, 2000; Eveleigh *et al.*, 2007). Source webs are defined as those which host-parasitoid interactions are dependent on only one food resource (Cohen 1978). Despite progress made in our understanding of source food webs (e.g**.** Hawkins *et al.*, 1997; Memmott *et al.*, 2000; Dunne *et al.*, 2002) no previous research, to our knowledge, has explored how sampling effort and scale effects could influence different insect trophic levels within source food webs. Also, there is still a problem concerning insufficient-sampling for some insect groups and interactions (Olesen *et al.*, 2010; Falcão *et al.*, 2016) and long-term datasets of herbivores and its host plant are still scarce (Lewinsohn *et al.* 2005).

The overarching aim of this study was to address this knowledge gap by examining a host-parasitoid source web dependent on the fruits of the plant species *Senegalia tenuifolia* (L.) Britton & Rose (Fabaceae). To achieve this, we took three main steps and its hypothesis when applicable: (i) we described the

insect identities and their observed and potential interactions among the four trophic levels; (ii) we examined the robustness of this food web data by analyzing accumulation curves, thus we hypothesized that studies involving multiple areas and long-term sampling are a good tool to estimate insect richness. Lastly (iii) we suggest a cost-efficiently sampling effort to sample the insects of a food web at local (each area) and reginal scale (all areas combined).

2.2 Methods

2.2.1 Study site

The study was conducted near to the Lavras and Luminárias municipalities in Minas Gerais state, Brazil. Surveys were done at eight sampling sites distant at least 400 m from each one. Sampling sites were distributed within three main areas (fragments) of Brazilian savanna (Ae, La and Lu), which were at least 6 km distant. Tuller *et al.*, (2015) provide more details of the study site.

2.2.2 Model plant

The *Senegalia tenuifolia* (L.) Britton & Rose (Fabaceae) is a widely distributed plant across tropical regions (Atchison 1948). In South America, *S. tenuifolia* individuals vary in structure (being shrub or liana) and in thorns density (Queiroz 2009), and it is present in several phytogeographic domains in almost all Brazilian territory (Barros & Morim, 2014). Their fruits are pod-shaped, generally occurring between June and August, when they are mature and start to fall on the ground, but few fruits remain attached to mother-plants (*LFM, personal observation*).

2.2.3 Data set

Insects data presented here were gathered from fruits (i.e. pods) of *S. tenuifolia* plants surveyed during four years (2011, 2012, 2013 and 2014). Fruits were collected during the plant ripening period in three monthly sampling events (June, July and August) for each year at each sampling site (Ae-1: 21°14'4.57" S - 44°57'6.38" W, Ae-2: 21°14'5.71" S - 44°57'8.66" W, Ae-3: 21°14'7.87" S - 44°58'0.06" W, La-1: 21°18'3.46" S - 44°58'0.53" W, Lu-1: 21°31'1.36" S - 44°53'1.78" W, Lu-2: 21°31'5.13" S - 44°52'6.32" W, Lu-3: 21°31'5.31" S - 44°52'3.84" W and Lu -4: 21°41'9.88" S - 44°96'7.18").

In this study we considered just the data from the second and third sampling events (July and August), since the insects from fruits collected in June were underdeveloped and not possible to identify. In each sampling event, we collect 25 fruits from all the sampling sites, thus for each year we collected 350 fruits, except for 2013 (349 fruits sampled) totalizing 1399 fruits. Moreover, there were different numbers of sampling sites for each area (please see Table 2.1 for more details). At each survey, fruits were taken to the laboratory and individually stored into PVC tubes covered by voile on both sides to enable the air circulation. Three months after the fruit collections, we made the insect sorting and identification at the lowest possible taxonomic level. The voucher specimens were deposited in the Entomological collection of the Laboratory of Ecology and Complexity at Federal University of Lavras, Minas Gerais, Brazil.

Table 2. 1 Areas and sampling sites along four years and the number of fruits gathered per area in each year.

Area	Sampling sites and *number of fruits per area* in each year			
	2011	2012	2013	2014
Ae	Ae1, Ae2, Ae3	Ae2, Ae3	Ae2, Ae3	Ae2, Ae3
	150 fruits	*100 fruits*	*99 fruits*	*100 fruits*
La	La1	La1	La1	La1
	50 fruits	*50 fruits*	*50 fruits*	*50 fruits*
Lu	Lu1, Lu2, Lu3	Lu1, Lu2, Lu3, Lu4	Lu1, Lu2, Lu3, Lu4	Lu1, Lu2, Lu3, Lu4
	150 fruits	*200 fruits*	*200 fruits*	*200 fruits*

2.2.4 Statistical Analyses

All analyses were made using R program (R Core Team). We described the examined food web and its interactions (Figure 2.1) by using the *cheddar* package (Hudson *et al.* 2013). Food web interactions included here were based on laboratory observations and literature review (Table ST1). In the laboratory, species-specific host-parasitoid relationships were set when a parasitoid was found at the place where mouthparts of the larval herbivores were (please see Tuller *et al*., 2015 for more details). Moreover, to complement our food web picture we built the Interaction Table ST2 (Supplementary Methodology SM1).

We built species-accumulation curves to assess the sampling efficiency for each year, trophic level and area. These curves are frequently used to evaluate sampling effectiveness by relating the sampling effort (here number of fruits) to the accumulated species richness. Accumulation curves were generated using the *vegan* package (Oksanen *et al.*, 2007) with the rarefaction method and non-parametric bootstrapping based on 1000 randomizations to display the confidence intervals (±95% CI). Then, to check the sampling efficiency we use the function s*pecpool* to extrapolate the species richness and then to compare with the observed species richness. We did not use the La area in the proposed methodology analysis at local scale, as this area did not reach the asymptote in the accumulation curves.

Finally, we used the *lme4* package to perform linear mixed models (GLMM) using the accumulated mean insect abundance, accumulated mean total seeds and accumulated mean insect richness for both regional and local scales followed by a contrast test. For this, years were treated as fixed explanatory variables into different GLMMs, where sampled areas were always treated as random effects. In the first GLMM, the accumulated mean of total

seeds was considered as response variable, here used to assess the responses of the first trophic level. Secondly, independent GLMMs were run with the accumulated mean insect abundance and richness as response variables to assess the patterns in the second, third and fourth trophic levels.

When dealing with accumulated data, results were interpreted as follow: (i) if the analysis previously proposed (GLMM) was significant (based on Chi-square test), we assume that more than one year should be sampled and the number of years would be indicated by the contrast test. On the contrary, (ii) if the analysis was non-significant, we could assume that only one sampling-year is enough to access all trophic levels for species richness and abundance.

2.3 Results

2.3.1 Food web description

We found 27 species distributed within four trophic levels of our source food web (Figure 2.1, Table ST1). Please, see Supplementary Methods SM2 for details about the used criteria to maintain/exclude species and interaction in this source food web (Figure 2.1). Consumer-levels and their quantitative and qualitative interactions are displayed in Table ST2 (Supplementary Material).

The first trophic level of this source food web was represented by the *S. tenuifolia* individuals, by which 12,651 seeds were found from 1,399 sampled fruits (9.04 ±2.6 seeds per fruit). The second trophic level was represented by 1,776 herbivore insects from 11 species distributed in six families and four orders. The herbivore abundance temporally varied (495, 187, 394 and 700 insects from 2011 to 2014, sequentially). From the herbivores, the third trophic level comprised 595 parasitoid individuals belonging to 13 species and distributed in six families, which also temporally varied from 2011 to 2014

(between 93 to 320 individuals, respectively). The fourth, and last trophic level, (hyperparasitoids) was represented by five individuals from the species *Brasema* sp. (Hymenoptera: Eupelmidae) and *Aprostocetus* sp. (Hymenoptera: Eulophidae). Please, see Table ST1 in Supplementary Material for all consumer-level insect species (from second and third trophic levels).

2.3.2 Data robustness and food web sampling accuracy

Overall, species-based accumulation curves demonstrated an adequate sampling effort for each year, trophic level and sampled areas in the studied source food web (Figure 2.2). The sampling efficiency was around 75% and 97.0 % for trophic levels (Figure 2.2A), between 86.36% and 94.73% for years (Figure 2.2B) and between 84% and 95.60% for sampled areas (Figure 2.2C). Except for the La area, all curves reached the asymptote.

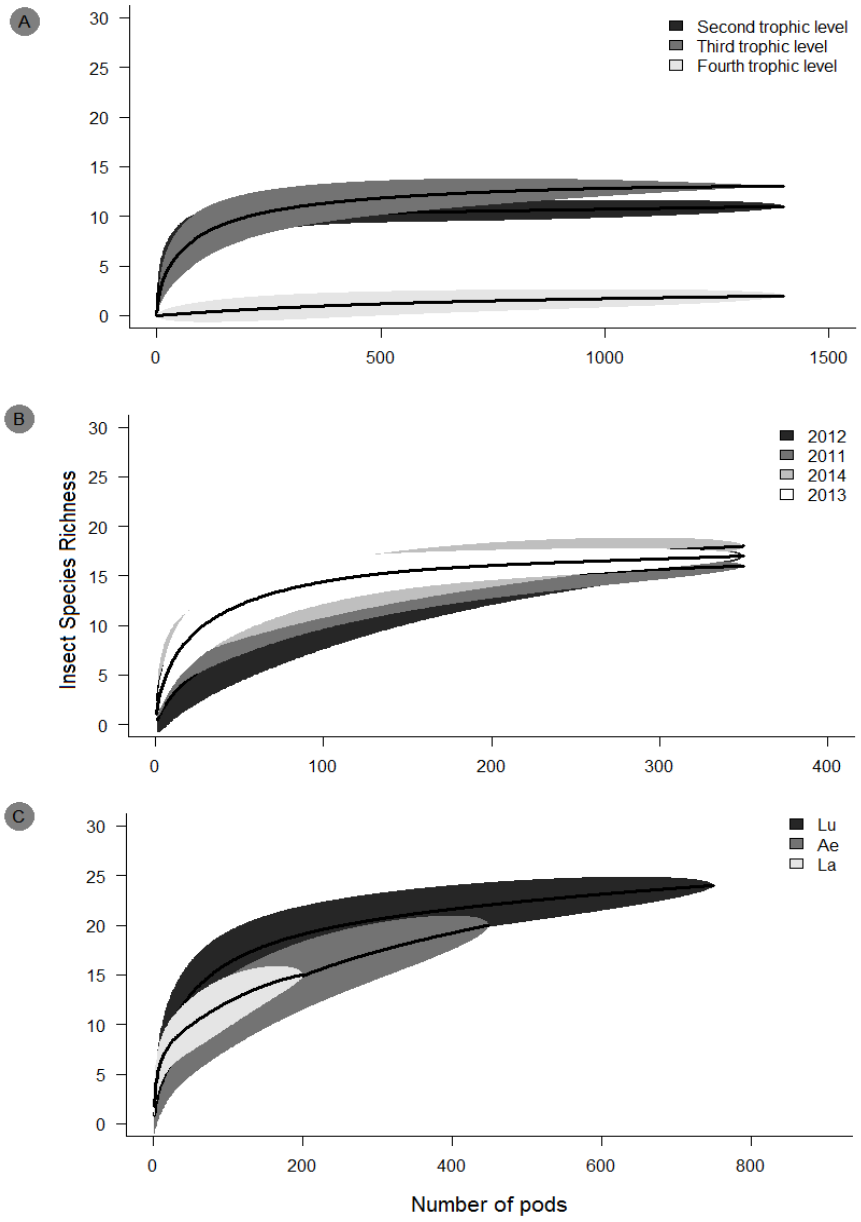


Figure 2.1 Sample-based species accumulation curves. Rarefaction curve comparing the pods between the (A) three trophic levels (TL) being second TL: herbivores, third TL: parasitoids and fourth TL: hyperparasitoids, (B) years and (C) areas sampled. The 95% CIs are shown in grey scale according to trophic levels, years and areas respectively.

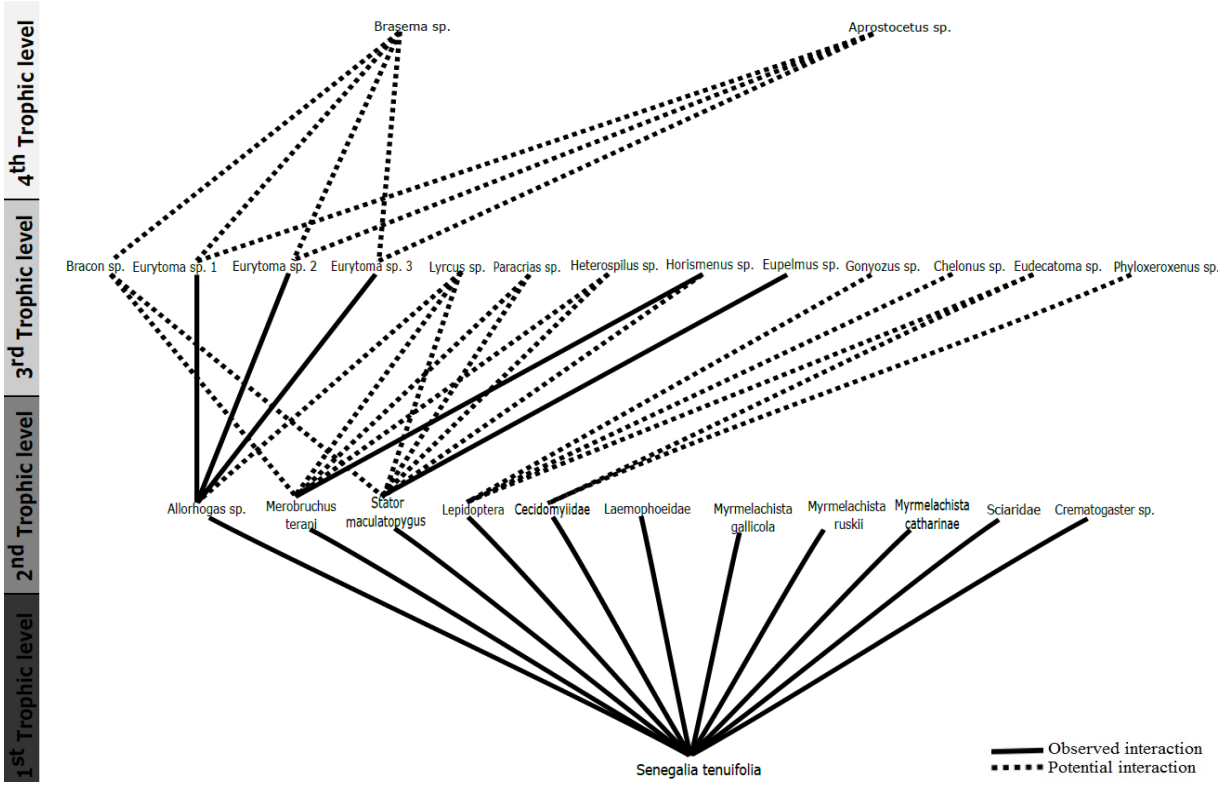


Figure 2.2 The *S. tenuifolia* food web and related trophic levels represented in grey scales. The first trophic level is represented by the resource plant, the second trophic level comprises the herbivores species, the third trophic level comprises the parasitoids species and the last trophic level are the hyperparasitoids species. The potential interactions based on the literature review is shown by the dashed lines while the observed interactions at laboratory conditions is shown by the solid lines.

2.3.3 Scales comparisons

Looking at insect and seed abundances (based on accumulated mean insect abundance and total seeds), we found that the more fruits we sample the more insect individuals or seeds we had for the resource ($p<0.001$; Figure 2.3A), herbivores ($p<0.001$; Figure 2.3B) and parasitoids ($p<0.001$; Figure 2.3C) at regional scale. Nevertheless, this did not happened for the hyperparasitoid level, which the number of individuals were independent of the number of collected fruits ($p = 0.98$; Figure 2.3D).

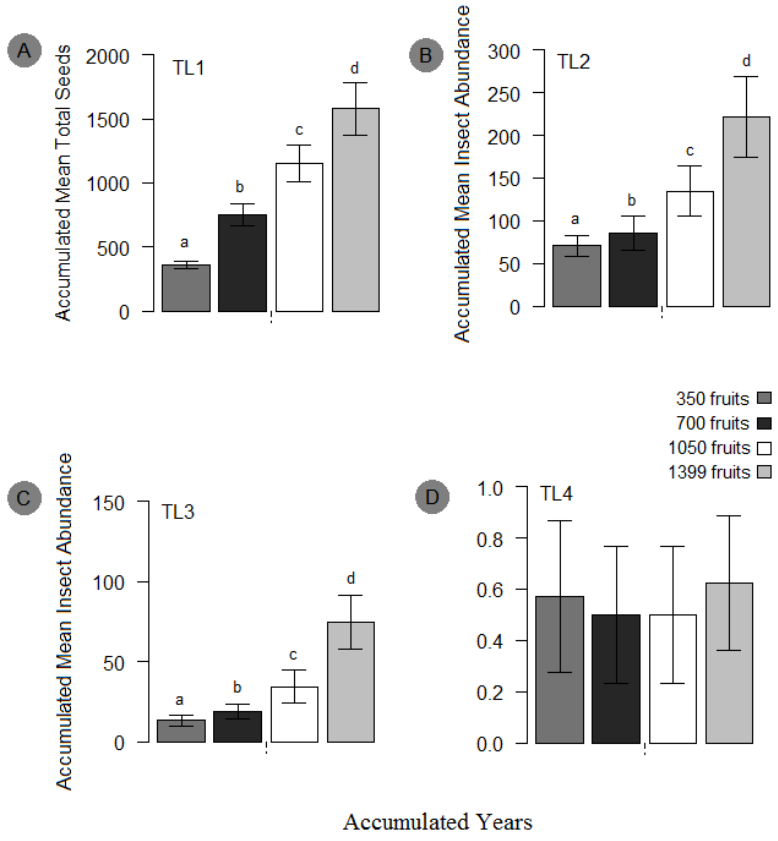


Figure 2.3 Accumulated number of insects or seeds at regional scale for each trophic level (TL) along a gradient of accumulated years (fruits summed). (A) TL1: the first

trophic level the abundance is represented by the total accumulated number of seeds along the years; (B) TL2: the second trophic level the abundance is represented by the accumulated number of total herbivore individuals along years; (C) TL3: the third trophic level the abundance is represented by the accumulated number of parasitoids individuals along years and (D) TL4: the fourth trophic level the abundance is represented by the accumulated number of hyperparasitoid individuals along years. Error bars represent the standard error.

When considering the sampling of a significant number of species to represent the studied food web (based on the accumulated mean insect richness), we should sample 1050 fruits (collected over three years) to gather all the parasitoid species ($p<0.05$; Figure 2.4B) and only 350 fruits for herbivores ($p = 0.26$; Figure 2.4A) and hyperparasitoids ($p = 0.66$; Figure 2.4C).

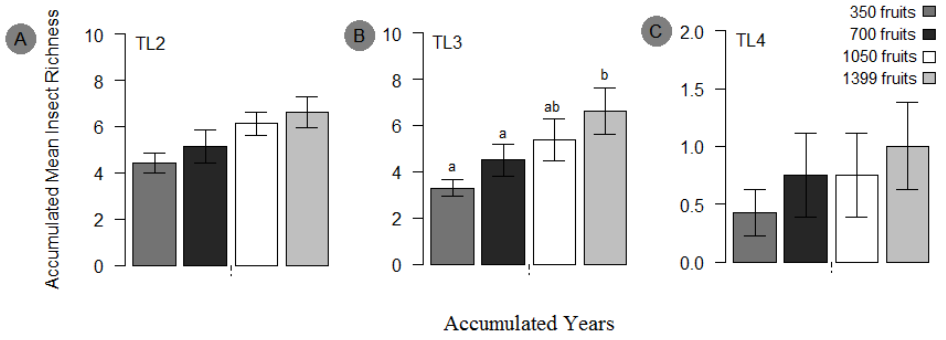


Figure 2.4 Accumulated number of insect species richness at regional scale along a gradient of accumulated years (fruits summed). (A) TL2: is the second trophic level represented by the accumulated herbivore species richness along years; (B) TL3: is the third trophic level represented by the accumulated parasitoid species richness along years (C) TL4: is the fourth trophic level represented by the accumulated hyperparasitoid species richness along years. Error bars represent the standard error.

At local scale, we found that all 1399 collected fruits would be need to accurately sample the abundance seeds, and herbivore and parasitoid insects. Thus, all the analyses for the two examined areas (Ae and Lu) were significant (all p-values <0.001) for the abundance of seeds (Figure 2.5A), herbivores (Figure 2.5B) and parasitoids (Figure 2.5C).

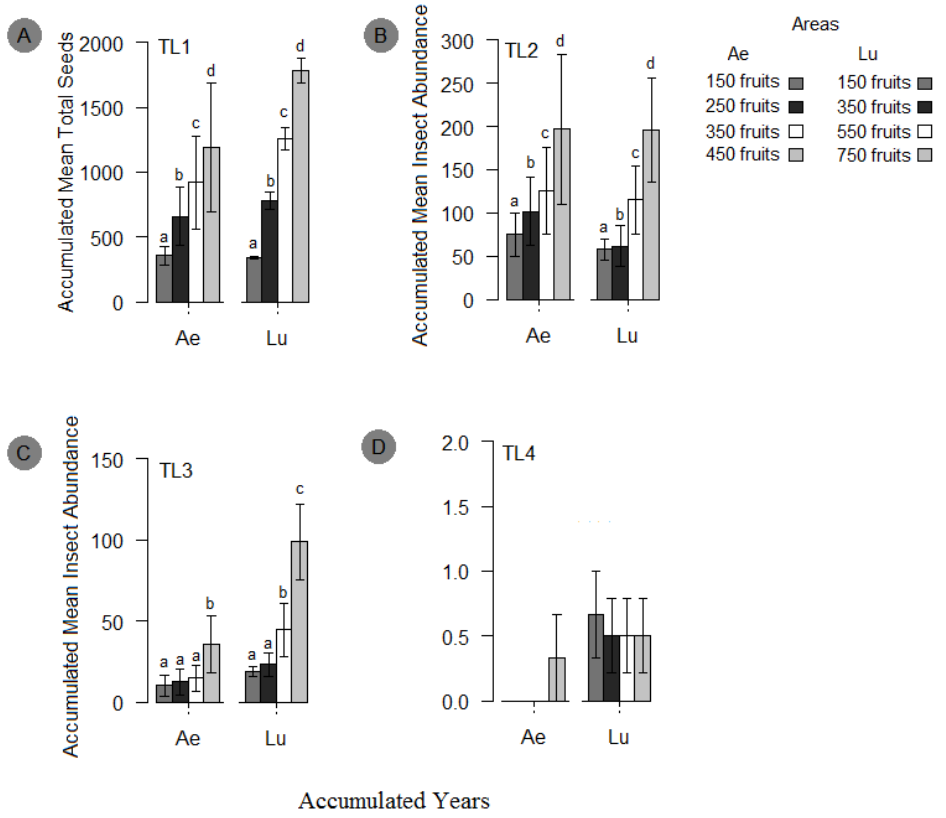


Figure 2.5 Accumulated number of insects or seeds at local scale for each trophic level (TL) in each sampling area (Ae and Lu) along a gradient of accumulated years (fruits summed). (A) TL1: the first trophic level the abundance is represented by the total accumulated number of seeds along the years; (B) TL2: the second trophic level the abundance is represented by the accumulated number of total herbivore individuals along years; (C) TL3: the third trophic level the abundance is represented by the accumulated number of parasitoids individuals along years and (D) TL4: the fourth trophic level the abundance is represented by the accumulated number of hyperparasitoids individuals along years. Error bars represent the standard error.

Table 2.2 Sampling effort needed to achieve the true trophic levels species richness and abundance in both regional and local scale

Studies at	Insect/Seed abundance				Insect Richness		
	1st TL	2nd TL	3rd TL	4th TL	2nd TL	3rd TL	4th TL
Regional Scale	as many years and fruits sample as possible				350 fruits in one year	350 fruits per year along 3 years	350 fruits in one year
Local Scale	as many years and fruits sample as possible				150 fruits in one year	150 fruits in one year	150 fruits in one year

Surprisingly, just 150 fruits would be enough when considering the species richness for each trophic levels in the two examined areas (herbivores Ae: p = 0.71, Lu: p = 0.56; parasitoids Ae: p = 0.59, Lu: p = 0.10; and hyperparasitoids Ae: p = 0.14; Lu: p = 0.84; Figure 2.6). Table 2.2 summarizes the accurate sampling effort at regional and local scale for all trophic levels.

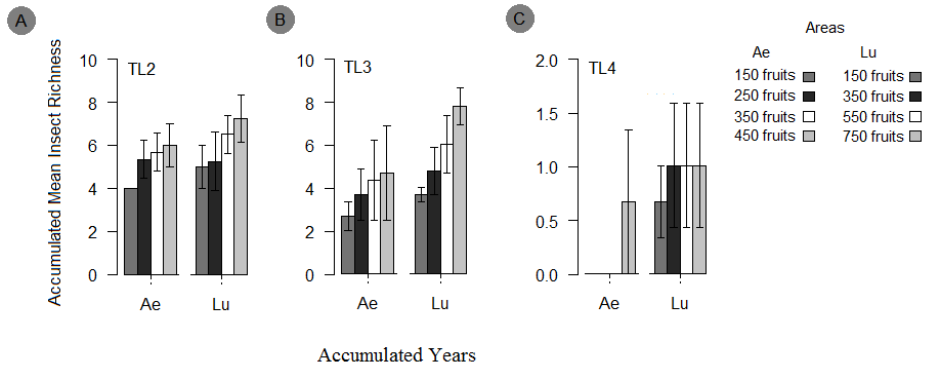


Figure 2.6 The accumulated number of insect species richness at local scale in each area (Ae and Lu) along a gradient of accumulated years (fruits summed). (A) TL2: is the second trophic level represented by the accumulated herbivore species richness along years; (B) TL3: is the third trophic level represented by the accumulated parasitoid species richness along years (C) TL4: is the fourth trophic level represented by the accumulated hyperparasitoid species richness along years. Error bars represent the standard error.

2.4 Discussion

We described a multitrophic source food web based on an extensive 4-year dataset from eight sampling sites. The source food web presented 26 species distributed in four trophic levels. The outcome of our research clearly demonstrates the robustness of our datasets, as well as brings insights about the minimal number of fruits needed to accurately sample insect communities associated with each trophic level of multitrophic source food webs.

From our knowledge, only one study (Tuller et al. 2015) have demonstrate the insects associated to *Senegalia tenuifolia* plant. However, some of the insect interactions observed in this source food web have also been reported associated with other leguminous plant in Costa Rica, Uruguay and Chile (Whitehead, 1975; Rojas-Rousse, 2006). Also, the *M. terani*, *S. maculatopygus* and *Allorhogas sp*. species feeding behaviors were similar to the ones previously reported by Southgate (1979). Although source food webs are considered as having low quality data due to the lack of links with other resources (Hawkins *et al.*, 1997), they are very applicable to many ecological fields, such as biological control (Nofemela, 2013; Gómez-Marco *et al.*, 2015), community ecology (Sigsgaard, 2002) and even to assess the stability of ecosystems (Rooney *et al.*, 2006). Because our work examined a source food web based on insects associated with just one plant species, we strongly encourage that further studies should address others links that are likely to be missing, such as fruit-pathogens (De Castro & Bolker, 2005; Lafferty *et al.*, 2006) and the presence of leaf-miners and cannibalistic interactions (e.g. Johnson, 1977; Wang & Kok, 1986). Additionally, laboratory studies should be conducted to confirm cannibalism interactions, which may result from the combination of the insects' genetic characteristics, population density-dependent factors or even due to environmental aspects (Richardson *et al.*, 2010).

We assume our sampling effort was accurate to collect all the species associated with *S. tenuifolia* fruits and seeds in each area, year and trophic level. This was reinforced by previous research, which has shown that long-term studies conducted at multiple sampling sites are good tools to estimate insect species richness (Fukami & Wardle, 2005). Therefore, we believe that species-based accumulation curves should be used by further research on multitrophic source food webs to check for the accuracy of their sampling effort. The proposed sampling effort to gather most of the source food web species and the interactions within its trophic levels is effective because it provides a cost-effective and standardized sampling effort. Besides, we believe the methodology and sampling effort (according to the summary outcome in Table 2.2) can be replicated as many times as sufficient for the potential use as a guideline to community food webs sampling which is a set of many resource plants.

Our research also reinforce the importance of long-term studies to accurately assess the diversity within each trophic level of multitrophic food webs. For example, here we show that the number of parasitoids and, consequently, the number of interactions within the examined food web increased with number of sampling years. Therefore, by sampling many years it may also be possible to assess changes in food web patterns resulting from the arisen of new species (e.g. Brown *et al.*, 2001; Alarcón *et al.*, 2008; Behl & Stibor, 2015; Gsell *et al.*, 2015). Although most of the species from the web were collected, other species would continue to be detected with additional sampling since opportunistic and rare species could appear in the subsequent years (Schoenly & Cohen, 1991; Alarcón *et al.*, 2008). Thus, a minimal sample effort to achieve the closest true food web diversity, minimizes the effects of rare and opportunistic species. For example, the species *Bracon* sp. and *Eudecatoma* sp. just appeared in the last year. Moreover, the study highlight the

importance of long-term food web researches because the web presented here were previously studied during one year sample (Tuller *et al.*, 2015), thus less abundant species links that were proposed based on sampling observations could not be identified properly as we reveled in this long term study which allowed us to achieve the food web completeness.

Another important point is sampling effort (number of fruits) and temporal gradients (number of years) varying depending on studies that were done at regional or local scales. Consequently, distinct outcomes could arise from different sampling scales (e.g. local and regional scale) as shown in this and other studies (Hill & Hamer, 2004; Chave, 2013; Wood *et al.*, 2015). For example, the pattern of increasing accumulated insect abundance along years within a trophic level is different from local to regional scales (for details compare accumulated insect abundance in Figure 2.4B and 2.6B). It demonstrates that if two studies are conducted using different scales, it can bring different interpretation for the analyzed trophic level over the years. However, by applying fast, reliable, simple and cheap methods, such studies could provide important and cost-effective insights into the food web theory in natural ecosystems.

2.5 Conclusion

Sampling effort issues pose as one of the main problems in defining biological patterns and understanding ecological interactions (Goldwasser & Roughgarden, 1997; Falcão *et al.*, 2016). Our research address this problem by using a source food web to provide insights into the minimal sampling effort, in terms of number of fruits, that further studies may need to accurately assess spatial and temporal changes in food webs. Therefore, by having robust food web datasets, researchers may be able to better investigate changes in

quantitative and qualitative food web metrics, with further better understanding of how such changes may influence the complexity and stability of ecosystems.

2.6 Acknowledgments

We are grateful to Drª C.S. Ribeiro-Costa, A.R. Nascimento, A.C.M. de Queiroz, and Drª M.S.C. Morini for insect identification support. We also thank França F. for the comments on the manuscript. We thank Universidade Federal de Lavras and PPG in Applied Ecology for logistical support, and FAPEMIG and CAPES who provide grants and funding for the project.

2.7 References

Alarcón, R., Waser, N.M. & Ollerton, J. (2008) Year-to-year variation in the topology of a plant pollinator interaction network. *Oikos*, **117**, 1796–1807.

Barros, M.J.F. & Morim, M.P. (2014) Senegalia (Leguminosae, Mimosoideae) from the Atlantic Domain, Brazil. *Systematic Botany*, **39**, 452–477.

Behl, S. & Stibor, H. (2015) Prey diversity and prey identity affect herbivore performance on different time scales in a long term aquatic food-web experiment. *Oikos*, **124**, 1192–1202.

Brown, J.H., T., W., Ernest, S. & Gehring, C. (2001) Complex species interactions and the dynamics of ecological systems: long-term experiments. *Science*, **293**, 643–649.

Burkle, L.A., Marlin, J.C. & Knight, T.M. (2013) Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurence, and Function. *Science*, **339**, 1611–1615.

Cao, Y., Williams, D.D. & Larsen, D.P. (2002) Comparison of ecological communities: the problem of sampling representativeness. *Ecological Monographs*, **72**, 41–63.

Castro, F. De & Bolker, B.M. (2005) Parasite establishment and host extinction in model communities. *Oikos*, **111**, 501–513.

Chacoff, N.P., Vázquez, D.P., Lomáscolo, S.B., Stevani, E.L., Dorado, J. & Padrón, B. (2012) Evaluating sampling completeness in a desert plant-pollinator network. *Journal of Animal Ecology*, **81**, 190–200.

Chave, J. (2013) The problem of pattern and scale in ecology: What have we learned in 20 years? *Ecology Letters*, **16**, 4–16.

Clauset, a, Moore, C. & Newman, M.E. (2008) Hierarchical structure and the prediction of missing links in networks. *Nature*, **453**, 98–101.

Cohen, J.E. (1978) *Food Webs and Niche Space*. Princenton Univeristy Press.

Dunne, J.A., Williams, R.J. & Martinez, N.D. (2002) Network structure and biodiversity loss in food webs: Robustness increases with connectance. *Ecology Letters*, **5**, 558–567.

Eveleigh, E.S., McCann, K.S., McCarthy, P.C., Pollock, S.J., Lucarotti, C.J., Morin, B., *et al.* (2007) Fluctuations in density of an outbreak species drive diversity cascades in food webs. *Proceedings of the National Academy of Sciences USA*, **104**, 16976–81.

Falcão, J.C.F., Dáttilo, W. & Rico-Gray, V. (2016) Sampling effort differences can lead to biased conclusions on the architecture of ant-plant interaction networks. *Ecological Complexity*, **25**, 44–52.

Fukami, T. & Wardle, D. a. (2005) Long-term ecological dynamics: reciprocal insights from natural and anthropogenic gradients. *Proceedings. Biological sciences / The Royal Society*, **272**, 2105–2115.

Gibson, R.H., Knott, B., Eberlein, T. & Memmott, J. (2011) Sampling method influences the structure of plant-pollinator networks. *Oikos*, **120**, 822–831.

Goldwasser, L. & Roughgarden, J. (1997) Sampling effects and the estimation of food-web properties. *Ecology*, **78**, 41–54.

Gómez-Marco, F., Urbaneja, A., Jaques, J.A., Rugman-Jones, P.F., Stouthamer, R. & Tena, A. (2015) Untangling the aphid-parasitoid food web in citrus: Can hyperparasitoids disrupt biological control? *Biological Control*, **81**, 111–121.

Gsell, A.S., Özkundakci, D., Hébert, M.-P. & Adrian, R. (2015) Quantifying

change in pelagic plankton network stability and topology based on empirical long-term data. *Ecological Indicators*, 1–13.

Guegan, J.F. & Kennedy, C.R. (1996) Parasite richness sampling effort host range: The fancy three-piece jigsaw puzzle. *Parasitology Today*, **12**, 367–369.

Hawkins, B. a, Martinez, N.D. & Gilbert, F. (1997) Source food webs as estimators of community web structure. *Acta Oecologica-International Journal of Ecology*, **18**, 575–586.

Hewitt, J.E., Thrush, S.F., Cummings, V.J. & Turner, S.J. (1998) The effect of changing sampling scales on our ability to detect effects of large-scale processes on communities. *Journal of Experimental Marine Biology and Ecology*, **227**, 251–264.

Hill, J.K. & Hamer, K.C. (2004) Determining impacts of habitat modification on diversity of tropical forest fauna the importance of spatial scale, Journal of Applied Ecology Volume 41, Issue 4. *Journal of Applied Ecology*, **41**, 744–754.

Hudson, L.N., Emerson, R., Jenkins, G.B., Layer, K., Ledger, M.E., Pichler, D.E., *et al.* (2013) Cheddar: Analysis and visualisation of ecological communities in R. *Methods in Ecology and Evolution*, **4**, 99–104.

Ings, T.C., Montoya, J.M., Bascompte, J., Bl??thgen, N., Brown, L., Dormann, C.F., *et al.* (2009) Ecological networks - Beyond food webs. *Journal of Animal Ecology*, **78**, 253–269.

Johnson, C.D. (1977) Life History of Ctenocolum Janzeni (Coleoptera: Bruchidae) in Seeds of Piscidia Mollis (Leguminosae). *the Coleopterists Bulletin*, **31**, 313–318.

Lafferty, K.D., Dobson, A.P. & Kuris, A.M. (2006) Parasites dominate food web links. *Proceedings of the National Academy of Sciences*, **103**, 11211–6.

Levin, S.A. (1992) The Problem of Pattern and Scale in Ecology. *Ecology*, **73**, 1943–1967.

Lewinsohn, T.M., Novotny, V. & Basset, Y. (2005) Insects on plants: Diversity of herbivore assemblages revisited. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 597–620.

Liebhold, A., Koenig, W.D. & Bjornstad, O.N. (2004) Spatial Synchrony in

Population Dynamics. *Annual Review of Ecology, Evolution and Systematics*, **35**, 467–490.

Martinez, N.D., Hawkins, B.A., Dawah, H.A. & Feifarek, B.P. (1999) Effects of sampling effort on characterization of food-web structure. *Ecology*, **80**, 1044–1055.

Memmott, J. (1999) The structure of a plant-pollinator food web. *Ecology Letters*, **2**, 276–280.

Memmott, J., Martinez, N.D. & Cohen, J.E. (2000) Predators, parasites and pathogens: species richness, trophic generality, and body sizes in a natural food web. *Animal Ecology*, **69**, 1–15.

Morris, R.F. (1960) Sampling Insect Populations. *Annual Review of Entomology*, **5**, 243–264.

Nofemela, R.S. (2013) The effect of obligate hyperparasitoids on biological control: Differential vulnerability of primary parasitoids to hyperparasitism can mitigate trophic cascades. *Biological Control*, **65**, 218–224.

Novotny, V. (2009) Beta diversity of plant-insect food webs in tropical forests: A conceptual framework. *Insect Conservation and Diversity*, **2**, 5–9.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Henry, M., Stevens, H., *et al.* (2007) The vegan package. *Community ecology package*, **10**, 631–637.

Olesen, J.M., Bascompte, J., Dupont, Y.L., Elberling, H., Rasmussen, C. & Jordano, P. (2010) Missing and forbidden links in mutualistic networks. *Proceedings. Biological sciences / The Royal Society*, **278**, 725–732.

Parmain, G., Dufrêne, M., Brin, A. & Bouget, C. (2013) Influence of sampling effort on saproxylic beetle diversity assessment: Implications for insect monitoring studies in European temperate forests. *Agricultural and Forest Entomology*, **15**, 135–145.

Queiroz, L.P. (2009) Leguminosas da Caatinga. Universidade Estadual de Feira de Santana: Feira de Santana. 914 p.

Richardson, M.L., Mitchell, R.F., Reagel, P.F. & Hanks, L.M. (2010) Causes and consequences of cannibalism in noncarnivorous insects. *Annual Review of Entomology*, **55**, 39–53.

Rivera-Hutinel, A., Bustamante, R.O., Marin, V.H. & Medel, R. (2012) Effects of sampling completeness on the structure of plant – pollinator networks. *Ecology*, **93**, 1593–1603.

Rojas-Rousse, D. (2006) Persistent pods of the tree Acacia caven: a natural refuge for diverse insects including Bruchid beetles and the parasitoids Trichogrammatidae, Pteromalidae and Eulophidae. *Journal of insect science (Online)*, **6**, 1–9.

Rooney, N., McCann, K., Gellner, G. & Moore, J.C. (2006) Structural asymmetry and the stability of diverse food webs. *Nature*, **442**, 265–269.

Sassi, C. de, Staniczenko, P.P. a & Tylianakis, J.M. (2012) Warming and nitrogen affect size structuring and density dependence in a host-parasitoid food web. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **367**, 3033–41.

Schoenly, K. & Cohen, J.E. (1991) Temporal Variation in Food Web Structure: 16 Empirical Cases. *Ecological Monographs*, **61**, 267–298.

Shurin, J., Gruner, D.S. & Hillebrand, H. (2006) All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proceedings of the Royal Society B*, **273**, 1–9.

Sigsgaard, L. (2002) A survey of aphids and aphid parasitoids in cereal fields in Denmark, and the parasitoids' role in biological control. *Journal of Applied Entomology*, **126**, 101–107.

Southgate, B.J. (1979) Biology of the Bruchidae. *Annual Review of Entomology*, **24**, 449–473.

Strayer, D.L., Eviner, V.T., Jeschke, J.M. & Pace, M.L. (2006) Understanding the long-term effects of species invasions. *Trends in Ecology and Evolution*, **21**, 645–651.

Stürmer, G.R., Filho, A.C., Guedes, J.V.C. & Stacke, R.F. (2014) Sample size for estimating the population of stink bugs in soybean crops. *Revista Ciencia Agronomica*, **45**, 155–167.

Teder, T., Tanhuanpää, M., Ruohomäki, K., Kaitaniemi, P. & Henriksson, J. (2000) Temporal and spatial variation of larval parasitism in non-outbreaking populations of a folivorous moth. *Oecologia*, **123**, 516–524.

Tuller, J., Paula, E.L. De, Maia, L.F., Moraes, R.A. & Faria, L.D.B. (2015) Seed predation food web, nutrient availability, and impact on the seed germination of Senegalia tenuifolia (Fabaceae). *Revista de biología tropical*, **63**, 1149–1159.

Tylianakis, J.M., Laliberté, E., Nielsen, A. & Bascompte, J. (2010) Conservation of species interaction networks. *Biological Conservation*, **143**, 2270–2279.

Vizentin-Bugoni, J., Maruyama, P.K., Debastiani, V.J., Duarte, L.D.S., Dalsgaard, B. & Sazima, M. (2015) Influences of sampling effort on detected patterns and structuring processes of a Neotropical plant-hummingbird network. *Journal of Animal Ecology*, **85**, 262-272.

Walther, B. A & Martin, J. (2001) How To Control for Sampling Effort ? *Ecology*, 413–419.

Walther, B. A., Cotgreave, P., Price, R.D., Gregory, R.D. & Clayton, D.H. (1995) Sampling Effort and Parasite Species Richness. *Parasitology Today*, **11**, 306–310.

Wang, R. & Kok, L.T. (1986) Life-history of Megacerus discoidus (Coleoptera, Bruchidae), a seedfeeder of hedge bindweed, in southwestern Virginia. *Annals Of The Entomological Society Of America*, **79**, 359–363.

Whitehead, D.R. (1975) Parasitic Hymenoptera Associated with Brichid-Infested Fruits in Costa Rica. *Journal Washington Academy of Sciences*, **65**, 108–116.

Willott, S.J. (2001) Species accumulation curves and the measure of sampling effort. *Journal of Applied Ecology*, **38**, 484–486.

Winemiller, K.O. (1990) Spatial and Temporal Variation in Tropical Fish Trophic Networks ’. *Ecological Monographs*, **60**, 331–367.

Wood, S. a., Russell, R., Hanson, D., Williams, R.J. & Dunne, J. a. (2015) Effects of spatial scale of sampling on food web structure. *Ecology and Evolution*, **5**, 3769–3782.

2.8 Supplementary Material

Table ST1 Observed and potential interactions of the *S. tenuifolia* source food web. Abbreviations: * The observed interaction at laboratory conditions is not due directly by behavior experimentation but only by isolating each fruit and observing its individuals knowing their exactly interaction; ** This interaction were based on parasitoid found within the seed with its host mouth parts, then having its interaction confirmed; † Please see Figure SF1 in Supplementary Material showing the evidence of this interaction.

Resource	Consumer	Trophic Interaction	Interaction Evidence
Senegalia tenuifolia	*Allorhogas* sp. (Hymenoptera: Braconidae)	observed	***** † Observed at laboratory conditions. This genus is found consuming seeds, or as galls (Marsh *et al.* 2000, Penteado-Dias e Carvalho 2008)
Senegalia tenuifolia	*M. terani* (Coleoptera: Bruchidae)	observed	***** Observed at laboratory conditions. These herbivores are frequently observed feeding inside seeds from the Fabaceae family (Johnson and Siemens 1997, Janzen 1969, Tuda *et al.* 2009 and Rossi *et al.* 2011)
Senegalia tenuifolia	*S. maculatopygus* (Coleoptera: Bruchidae)	observed	***** Observed at laboratory conditions. This herbivores is frequently observed feeding inside the seeds from the Fabaceae family (Johnson and Siemens 1997, Janzen 1969, Tuda *et al.* 2009 and Rossi *et al*. 2011)
Senegalia tenuifolia	Laemophloeidae (Coleoptera)	observed	***** Observed at laboratory conditions. Also called flat bark beetle, the Laemophloeidae feed on fungi and are found under bark of many trees (Andrew and Hughes 2005).
Senegalia tenuifolia	Lepidoptera	observed	***** Observed at laboratory conditions. Also found associated with *Senegalia* genus (Gupta et al. 2014, Agassiz and Harper 2009)
Senegalia tenuifolia	Cecidomyiidae (Diptera)	observed	***** Observed at laboratory conditions. Cecidomyiidae are known to induce galls forming in may Fabaceae plants (Fernandes et al. 2010, Maia 2006), including leaves of *Senegalia* species (Costa *et al.* 2014).
Senegalia tenuifolia	*Solenopsis; Myrmelachista gallicola; M. ruskii; M. catharinae; Crematogaster* sp. (Hymenoptera: Formicidae)	observed	***** Observed at laboratory conditions. Some ant species are exclusively arboreal, present in plants where resources (e.g. extrafloral nectaries) and protection can be found (Lanan et al. 2011, Inouye and Taylor Jr 1979, Perry et al. 2004, Richard et al. 2001, Longino 2003).

Resource	Consumer	Trophic Interaction	Interaction Evidence
Senegalia tenuifolia	Sciaridae (Diptera)	observed	***** Observed at laboratory conditions. Knowing as fungus gnat, the Sciaridae family is associated with many plants including the genus *Acacia*. (Gagné 1979)
Allorhogas sp. *M. terani* *S. maculatopygus*	*Lyrcus* sp. (Hymenoptera: Pteromalidae)	potential	The genus *Lyrcus* is observed as a possible parasitoid of *Allorhogas* (Marsh et al. 2000). Also, this genus can also parasitize Bruchidae (Gibson et al. 1997). Since we observe all the fruits in which *Lyrcus* sp emerged or not, we opt to place the *Lyrcus* sp. in the web as *Allorhogas* sp. and Bruchidae (*M. terani* and *S. maculatopygus*) parasitoid.
Allorhogas sp.	*Eurytoma* sp1, sp2 and sp3 (Hymenoptera: Eurytomidae)	observed	***†** Observed at laboratory conditions, and *Eurytoma* is observed as *Allorhogas* larvae ectoparasitoids (Macêdo and Monteiro 1989, Marsh et al 2000, Penteado-Dias e Carvalho 2008).
M. terani	*Paracrias* sp. (Hymenoptera: Eulophidae)	potential	Knowing to parasitize Bruchinae species (Pikart et al. 2011)
M. terani	*Horismenus* sp. (Hymenoptera: Eulophidae)	observed	****** Observed at laboratory conditions, and the *M.terani* subfamily (Bruchinae) is observed as hosts for this parasitoid genus (Bonet 2008), and found as attacking various genera of Bruchidae (Whitehead 1975).
M. terani	*Heterospilus* sp. (Hymenoptera: Braconidae)	potential	Parasitic hymenoptera associated with Bruchid-infested fruit (Whitehead 1975)
S. maculatopygus	*Paracrias* sp. (Hymenoptera: Eulophidae)	potential	Knowing to parasitize Bruchinae species (Pikart et al. 2011)
S. maculatopygus	*Horismenus* sp. (Hymenoptera: Eulophidae)	potential	The *S. maculatopygus* subfamily (Bruchinae) is observed as hosts for this parasitoid genus (Bonet 2008), and found as attacking various genera of Bruchidae (Whitehead 1975).
S. maculatopygus	*Heterospilus* sp. (Hymenoptera: Braconidae)	potential	Parasitic hymenoptera associated with Bruchid-infested fruit (Whitehead 1975)
S. maculatopygus	*Eupelmus* sp. (Hymenoptera: Eupelmidae)	observed	****** Observed at laboratory conditions, and *Eupelmus* is found in literature as Coleoptera larvae and pupae ectoparaisotids (Askew and Nieves-Aldrey 2000 and Fusu 2009). Also are observed as Bruchinae parasites (Sari and Ribeiro-Costa 2005).
Lepidoptera	*Goniozus* sp. (Hymenoptera: Bethydae)	potential	*Goniozus* is a primary parasitoid of Lepidoptera larvae (Gordh and Medved 1986. Gordh 1976)

Resource	Consumer	Trophic Interaction	Interaction Evidence
Lepidoptera	*Chelonus* sp. (Hymenoptera: Braconidae)	potential	Generally found as a Lepidoptera parasite (Wharton et al. 1997).
Lepidoptera	*Eudecatoma* sp. (Hymenoptera: Eurytomidae)	potential	*Eudecatoma* genus is commonly found as Cynipidae parasitoid. However, as in this study, we did not found any of this individuals over four years. We place *Eudecatoma* as Lepidoptera parasitoid, as shown in the literature (Force and Thompson 1984, Claridge 1959, Powell 1989). Little is known about its biology and laboratory experiments are needed.
Cecidomyiidae	*Eudecatoma* sp. (Hymenoptera: Eurytomidae)	potential	Eudecatoma were found parasitizing Cecidomyiidae galls (Claridge 1959)
Cecidomyiidae	*Phylloxeroxenus* sp. (Hymenoptera: Eurytomidae)	potential	Burks 1971
Eurytoma sp1, sp2 and sp3	*Brasema* sp. (Hymenoptera: Eupelmidae)	potential	The *Brasema* genus can parasite *Eurytoma* species (Askew and Nieves-Aldrey 2004 and 2006)
Bracon sp.	*Brasema* sp. (Hymenoptera: Eupelmidae)	potential	*Brasema* can behave as secondary parasitoid on *Bracon* sp. (Askew 1998)
Eurytoma sp1, sp2 and sp3	*Aprostocetus* sp. (Hymenoptera: Eulophidae)	potential	*Aprostocetus* is a genus that can be found behaving as egg parasitoid or hyperparasitoid of many insects, including *Eurytoma* hymenoptera (Graham 1987). Although a non-identified species from the *Aprostocetus* subfamily, Tetrastichinae were observed as *Allorhogas* parasites (Badenes-Perez and Johnson 2007). This interaction was not included as it was not confirmed and could be spurious in the food web.
S. maculatopygus	*Bracon* sp. (Hymenoptera: Braconidae)	potential	Parasites various genera of Bruchinae (Whitehead 1975, Gagnepain et al. 1989)
M. terani	*Bracon* sp. (Hymenoptera: Braconidae)	potential	Parasites various genera of Bruchinae (Whitehead 1975, Gagnepain et al. 1989)

Table ST2 Source web and its insect interactions based in the abundance and parasitism data of 2011, 2012, 2013 and 2014 years for the four trophic levels.

	Merobruchus terani	*Stator maculatopygus*	*Allorhogas* sp.	*Crematogaster* sp.	*Myrmelachista_ruszkii*	*Myrmelachista gallicola*	*Myrmelachista catharinae*	Laemophloeidae	Lepidoptera	Cecidomyiidae	Sciaridae	*Bracon* sp.	*Eurytoma* sp3	*Eudecatoma* sp.	*Horismenus* sp.	*Eurytoma* sp1	*Eurytoma* sp2	*Chelonus* sp.	*Lyrcus* sp.	*Eupelmus* sp.	*Phylloxeroxenus* sp.	*Paracrias* sp.	*Goniozus* sp.	*Heterospillus* sp.	*Aprostocetus* sp.	*Brasema* sp.
Senegalia tenuifolia	874	162	574	12	52	15	34	10	28	1	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Merobruchus terani	0	0	0	0	0	0	0	0	0	0	0	24	0	0	215	0	0	0	4	1	0	70	0	1	0	0
Stator maculatopygus	0	0	0	0	0	0	0	0	0	0	0	6	0	0	45	0	0	0	3	8	0	21	0	1	0	0
Allorhogas sp.	0	0	0	0	0	0	0	0	0	0	0	0	18	0	0	121	15	0	3	0	0	0	0	0	0	0
Crematogaster sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrmelachista_ruszkii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrmelachista gallicola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrmelachista catharinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laemophloeidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lepidoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	2	0	0	0	0	3	0	0	0
Cecidomyiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	7	0	0	0	0	0
Sciaridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bracon sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Eurytoma sp3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eudecatoma sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Horismenus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eurytoma sp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
Eurytoma sp2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Chelonus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lyrcus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eupelmus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phylloxeroxenus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paracrias sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Goniozus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heterospillus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aprostocetus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brasema sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

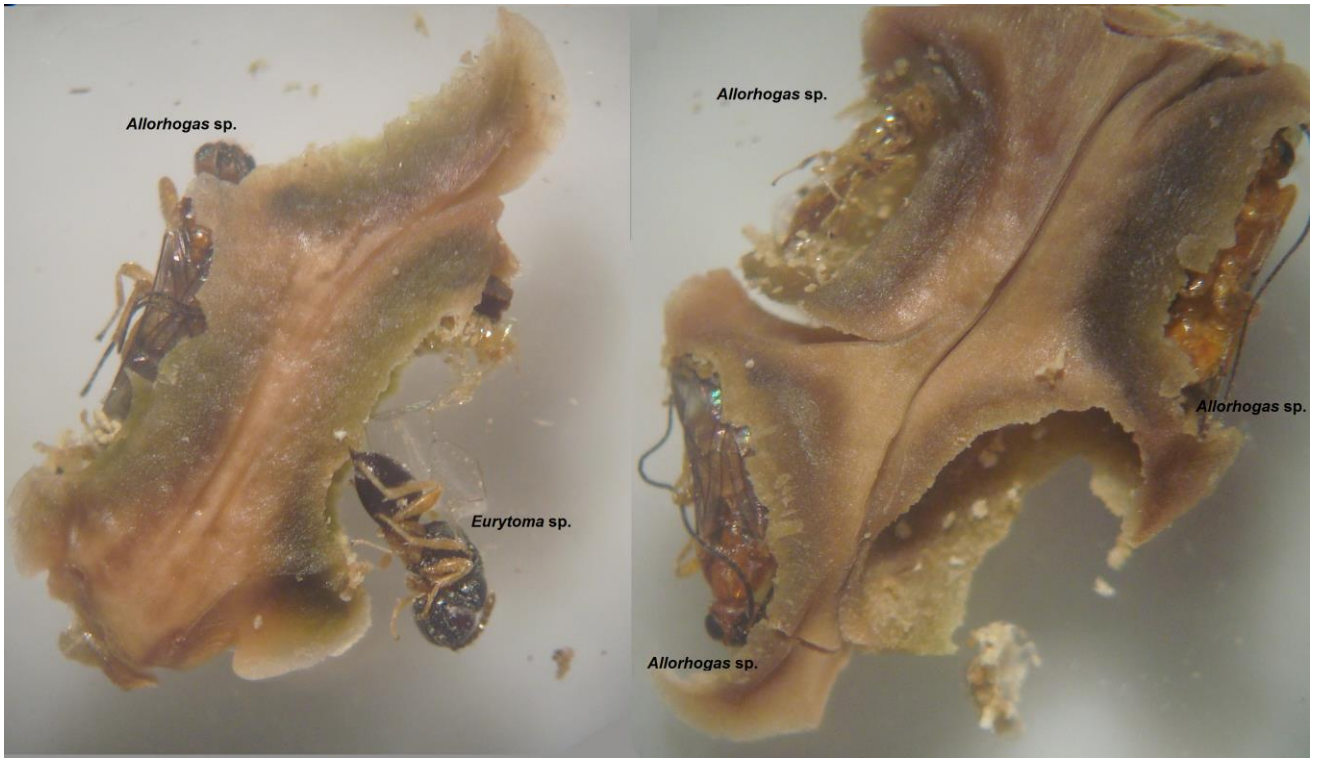


Figure SF1 Pictures taken from the fruits stored at laboratory. *S. tenuifolia* seeds and its herbivores (*Allorhogas* sp.) and parasitoid (*Eurytoma* sp.) insects. The insects in this figure did not emerged from the fruits, thus we could infer about their relation with *S. tenuifolia*. The *Allorhogas* sp. were frequently found consuming the seed edges like in this photo and its parasitoid *Eurytoma* sp were found in the same place as *Allorhogas* sp.

2.8.1 Supplementary Methods SM1

To build Table ST2 we calculate at what proportion parasitoid (P) were parasitizing the herbivores (H_1 and H_2), using the parasitoid abundance as reference. Thus, the proportion at which one parasitoid species parasites one herbivore species is the parasitoid`s absolute abundance. However, when a parasitoid species parasitized two different herbivore species we calculate the proportion of herbivores parasitism rate (H_{Pr}) based on each herbivore species total abundance. Thus,

$$H_1 * 100\% / H_1 + H_2 = Z \text{ then,}$$

$$P_{abund} - Z = H_{Pr1}$$

where, H_1 is the first herbivore abundance, H_2 is the second herbivore abundance, Z is the percentage found for the first herbivore abundance, P_{abund} is the parasitoid abundance and H_{Pr1} is the parasitism rate for the first herbivore.

2.8.2 Supplementary Methods SM2

Parasitoids from the subfamily Pteromalinae (5 individuals) and Eulophinae (2 individuals), could not be identified at genus level. We therefore excluded them from the food web because the literature describes these subfamilies as generalist (Gibson et al. 1997, Hanson and Gauld, 2006), and we could not precisely place them within specific trophic levels. We also excluded the insects with less than five individuals in the four years (assuming that they could be opportunistic species). Except by the insects that were parasitoids and hyperparasitoids, or herbivores with strict interactions with parasitoid previously placed in the web. Excluded species were *Solenopsis* sp. (Hymenoptera: Formicidae), *Prodecatoma* (Hymenoptera: Eurytomidae: Prodecatominae), six

Diptera species (one individual from each species), Psocoptera, Hemiptera, Eiphosoma sp. (Hymenoptera: Ichneumonidae: Cremastinae).

2.8.3 Supplementary References

Agassiz, D.J.L. & Harper, D.M. (2009) The Macrolepidoptera fauna of Acacia in the Kenyan Rift Valley (Part 1). *Tropical Lepidoptera Resources*, **19**, 4–8.

Andrew, N.R. & Hughes, L. (2005) Arthropod community structure along a latitudinal gradient: Implications for future impacts of climate change. *Austral Ecology*, **30**, 281–297.

Askew R.R. and Blasco-Zumeta J. (1998) Insects associated with galls of a new species of Eurytomidae (Hymenoptera: Chalcidoidea) on Ephedra nebrodensis in Spain. *Journal of Natural History*, **32**, 805-821

Askew R.R., Nieves-Aldrey J.L. (2000) The genus *Eupelmus* Dalman, 1820 (Hymenoptera, Chalcidoidea, Eupelmidae) in Peninsular Spain and the Canary Islands, with taxonomic notes and descriptions of new species. *Graellsia,* **56**, 49-61.

_____ (2004) Further observations on Eupelminae (Hymenoptera, Chalcidoidea, Eupelmidae) in the Iberian Peninsula and Canary Islands, including descriptions of new species. *Graellsia* **60**(1), 27-39.

_____ (2006) Calosotinae and Neanastatinae in the Iberian Pensinsula and Canary Islands, with descriptions of new species and a supplementary note on *Brasema* Cameron, 1884 (Hymenoptera, Chalcidoidea, Eupelmidae). *Graellsia* **62**(1), 87-100.

Badenes-Perez, F.R. & Tracy Johnson, M. (2007) Ecology and impact of Allorhogas sp. (Hymenoptera: Braconidae) and Apion sp. (Coleoptera: Curculionoidea) on fruits of Miconia calvescens DC (Melastomataceae) in Brazil. *Biological Control*, **43**, 317–322.

Bonet A. (2008) New hosts, host plants, and distribution records for *Horismenus* (Hymenoptera: Eulophidae) species in a bruchid beetle parasitoid guild attacking wild type *Phaseolus coccineus* and *P. vulgaris* in Central Mexico. *Florida Entomologist*, **91**(4), 698-701.

Burks, B. D. (1971) A synopsis of the genera of the family Eurytomidae (Hymenoptera: Chalcidoidea). *Transactions of the American Entomological Society*, **97**, 1–89.

Claridge, M. F. (1959). A contribution to the biology and taxonomy of the British species of the genus Eudecatoma Ashmead (=Decatoma auctt. Nec Spinola) (Hym., Eurytomidae) *Transactions of the Society for British Entomology*, **13** (9), 149- 168.

Costa, E.C., Fernandes, S.P.C. & Silva, J.S. (2014) Galhas entomógenas associadas à Leguminosae do entorno do riacho Jatobá, Caetité, Bahia, Brasil. *Revista Brasileira de Biociências*, **12**, 115.

Fernades, S.P.C., Maia, V.C. & Rafael, J.A. (2010) Gall midges (Diptera , Cecidomyiidae) associated with Aldina heterophylla Spr . ex Benth. (Fabaceae) from Brazil. *Biota Neotropica*, **10**, 161–166.

Force, D.C. & Thompson, M.L. (1984) Parasitoids of the immature stages of several southwestern yucca moths. *Southwestern Naturalist*, **29**, 45–56.

Fusu L. (2009) Romain Eupelmidae (Hymenoptera, Chalcidoidea): new cytogenetic, faunistic and host records. *North-West Journal of Zoology*, **5**(2), 307-320.

Gagné, W. (1979) Canopy-associated arthropods in Acacia koa and Metrosideros tree communities along an altitudinal transect on Hawaii Island. *Pacific insects*, **21**, 56–82.

Gagnepain, C. & Rasplus, J.Y. (1989) Caryedon serratus and its parasitoids in the savanna around Lamto, Ivory Coast. *Entomophaga*, **34**, 559–567.

Gibson Gary A.P., John T. Huber, and James Braden Woolley (1997) eds. *Annotated keys to the genera of Nearctic Chalcidoidea (Hymenoptera)*. Vol. 40392. Ottawa: NRC Research Press.

Gordh, G. (1976) Goniozus Gallicola Fouts, a Parasite of Moth Larvae, with Notes on Other Bethylids: (Hymenoptera: Bethylidae, Lepidoptera: Gelechiidae).

Gordh, G. & Medved, R.E. (1986) Biological notes on Goniozus pakmanus Gordh (Hymenoptera: Bethylidae), a parasite of pink bollworm, Pectinophora gossypiella (Saunders)(Lepidoptera: Gelechiidae). *Journal of the Kansas*

Entomological Society, **59**, 723–734.

Graham M.W.R.V. (1987) A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae), with a revision of certains genera. *Bulletin of British Museum* (Natural History Entomology Series), **55**(1), 1-392.

Gupta, A., Khot, R. & Chorge, S. (2014) A new species of Parapanteles Ashmead, 1900 (Hymenoptera: Braconidae: Microgastrinae) parasitic on Charaxes athamas (Drury) (Lepidoptera: Nymphalidae) in India. *Systematic Parasitology*, **88**, 273–279.

Inouye D.W., Taylor-Jr O.R. (1979) A temperate region plant-ant-seed predator system: Consequences of extra floral nectar secretion by *Helianthella quinquenervis*. *Ecology* **60**(1):1-7.

Janzen, D.H. (1969) Seed-Eaters Versus Seed Size, Number, Toxicity and Dispersal. *Evolution*, **23**, 1.

Johnson C.D., Siemens D.H. (1997) Oviposition behavior, guilds, host relationships and new host and distribution records for the genus *Merobruchus* Bridwell (Coleoptera: Bruchidae). *Coleopterists Bulletin*, **51**(1), 13-21.

Lanan M.C., Dornhaus A., Bronstein J.L. (2011) The function of polydomy: The *Crematogaster torosa* preferentially forms new nests near food sources and fortifies outstations. *Behavioral Ecology and Sociobiology*, **65**, 959-968.

Longino J.T. (2003) The *Crematogaster* (Hymenoptera, Formicidae, Myrmicinae) of Costa Rica. *Zootaxa*, **151,** 1-150.

Macêdo M.V., Monteiro R.F. (1989) Seed predation by a braconid wasp, *Allorhogas* sp. (Hymenoptera). *Journal of New York Entomological Society*, **97**(3), 358-362.

Maia, V.C. & Fernandes, G.W. (2006) A new genus and species of gall midge (Diptera, Cecidomyiidae) associated with Parkia pendula (Fabaceae, Mimosoideae). *Revista Brasileira de Entomologia*, **50**, 1–5.

Marsh P.M., Macêdo M.V., Pimental M.C.P. (2000) Descriptions and biological notes on two new phytophagous species of the genus *Allorhogas* from Brasil (Hymenoptera: Braconidae: Doryctinae). *Journal of Hymenoptera Research*, **9**(20), 292-297.

Penteado-Dias A.M., Carvalho F.M. (2008) New species of Hymenoptera associated with galls on *Calliandra brevipes* Benth. (Fabaceae: Mimosoidea) in Brazil. *Revista Brasileira de Entomologia*, **52**(2), 305-310.

Perry J.C., Mondor E.B., Addicott J.F. (2004) An indirect mutualism: ants deter seed predators from oviposition in yucca fruit. *Canadian Journal of Zoology*, **82**, 823-827.

Pikart, T.G., Souza, G.K., Costa, V. a., Hansson, C. & Zanuncio, J.C. (2011) Paracrias pluteus (hymenoptera, eulophidae) in Brazil: New distribution and host records, and with a new host group for paracrias. *ZooKeys*, **102**, 77–82.

Powell, J. a. (1989) Synchronized, mass-emergences of a yucca moth, Prodoxus Y-inversus (Lepidoptera: Prodoxidae), after 16 and 17 years in diapause. *Oecologia*, **81**, 490–493.

Richard F.J., Fabre A., Dejean A. (2001) Predatory behavior in dominant arboreal ant species: The case of *Crematogaster* sp. (Hymenoptera: Formicidae). *Journal of Insect Behavior*, **14**(2), 271-282.

Rossi M.N., Rodrigues L.M.S., Ishino M.N., Kestring D. (2011) Oviposition pattern and within-season spatial and temporal variation of pre-dispersal seed predation in a population of *Mimosa bimucronata* trees. *Arthropod-Plant Interactions*, **5**, 1-9.

Sari L.T., Ribeiro-Costa C.S. (2005) Predação de sementes de *Senna multijuga* (Rich.) H.S. Irwin & Barneby (Caesalpinaceae) por bruquíneos (Coleoptera: Chrysomelidae). *Neotropical Entomology*, **34**(3), 521-525.

Tuda M., Wu L.H., Tateishi Y., Niyomdham C., Buranapanichpan S., Morimoto K., Wu W.J., Wang C.P., Chen Z.Q., Zhu H.Y., Zhang Y.C., Murugan K., Chou L.Y., Johnson C.D. (2009) A novel host shift and invaded range of a seed predator, *Acanthocelides macrophthalamus* (Coleoptera: Chrysomelidae: Bruchinae), of an invasive weed, *Leucaena leucocephala*. *Entomological Science*, **12**, 1-8.

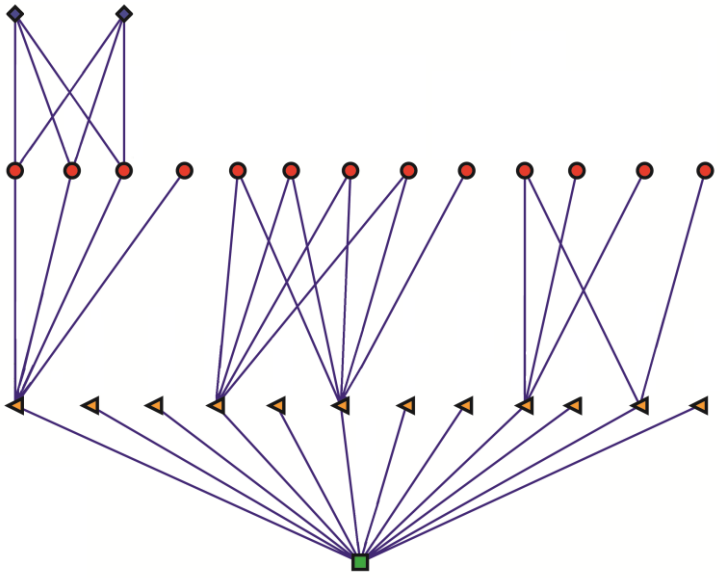
Wharton R.A., Marsh P.M., Sharkey M.J. (1997) Manual of the New World Genera of the family Braconidae (Hymenoptera). Special Publication of the International Society of Hymenopterists, no. 1.

Whitehead, D.R. (1975) Parasitic Hymenoptera Associated with Brichid-Infested Fruits in Costa Rica. *J. Washington Academy of Sciences*, **65**, 108–116.

Chapter 3: SECOND MANUSCRIPT

ECOLOGICAL COMMUNITIES REPORT USING THE FOOD WEB AND SPECIES DIVERSITY APPROACHES

Publication status: *In prep.* for submission to *Ecological Entomology*



ABSTRACT

The ecological communities can be represented through food webs and their community species diversity may change temporally and spatially. The studies on herbivore communities and their hosts are still scarce, and it is less known in the tropics where we find the highest biodiversity. We aimed explore the temporal variation of tropical insect communities of different trophic levels associated with the resource plant species *Senegalia tenuifolia*. We sampled 1,399 Senegalia tenuifolia fruits across eight sampling sites distributed in three areas collected along four years. The species richness, abundance and composition for all trophic levels escribed a temporal variation. Besides, herbivore abundance showed to influence the increases in parasitoid abundance. Based on the diversity patterns for the herbivore and parasitoid communities, the local species richness (alfa) most contribute to the total diversity for herbivores while the contribution to the total diversity vary between local species richness (alfa) and among areas species richness (beta) for parasitoids. Also, the Shannon index was most explained by alfa diversity for herbivores and parasitoids demonstrating that the most abundant species were widespread across sampling sites. The difference in species richness along the years for both herbivore and parasitoid communities are mostly due to the replacement of some species by others. In conclusion, trophic levels present different species distribution patterns, abundances and species richness in space and time, reinforcing the idea of use this dimensions when using food web approaches.

Key-words: diversity, trophic levels, temporal variation, resource, parasitoids.

3 Second manuscript: Ecological communities report using the food web and species diversity approaches

3.1 Introduction

The arrangement of ecological communities is not random (Elton, 1933) and can be represented through food webs, which consist of a number of feeding relationships within a community (Paine 1980). For example, the distribution of predator, parasites and herbivorous insects can be determined by the occurrence of the host plant (Araújo *et al.,* 2013; Heub *et al.,* 2013; Grandez-Rios *et al.,* 2015). Similarly, the resource availability can influence the above-levels diversity (Reich *et al.,* 2012). The use of food webs and species diversity metrics (i.e. species richness and composition) have been extensively used to demonstrate the structure of ecological communities in a more comprehensive way and finer detail (Allouche *et al.,* 2012; Hansson *et al.,* 2012; Mougi & Kondoh, 2012). Furthermore, the species abundance, richness and composition have been also extensively applied to understand patterns of ecological communities (McNaughton, 1977; Boulton *et al.,* 2005; MacIvor & Lundholm, 2011; Bang *et al.,* 2012; Peralta *et al.,* 2014) and are suggested to improve the traditional food web approach (Cohen *et al.,* 2003).

Despite progress made by applying the food web approach to demonstrate temporal changes in ecological communities (e.g. Gergs *et al.,* 2011; Kaartinen & Roslin, 2012; Burkle *et al.,* 2013; Lu *et al.,* 2014), two key

areas remain underexplored in the literature. First, there is a clear bias towards examining trophic relationships of aquatic systems (e.g Peacor & Werner, 1997; Marcarelli *et al.*, 2011; Shurin *et al.*, 2012). Nevertheless, terrestrial environments are extremely diverse (May, 1994; Jenkins *et al.*, 2013) and present complex species networks to be explored (Price, 2002). Second, most of the research to date have been carried out within temperate systems (e.g. Paine, 1966; Dunne *et al.*, 2002; Winemiller *et al.*, 2007; but see Layman *et al.*, 2005 for tropical). Yet tropical environments are among the most diverse in the world (Basset *et al.*, 2012; Brown, 2014) and globally, are experiencing species extinction rates caused by human activities (Cardinale *et al.*, 2012). Given that species diversity may temporally change (Tylianakis *et al.*, 2005) and there are few studies exploring such variation in tropical host-enemy relationships (but see: Pilosof *et al*., 2013), the comprehension of how tropical ecological communities are constituted may not be accurately inferred from studies conducted in temperate regions.

The Legume family (Fabaceae) has a widespread global distribution (Raub *et al.*, 2015; Schrire *et al*., 2005). Among the most diverse tropical botanic families (Judd *et al.* 2002), leguminous plants are easily recognized by their fruit shape (pod) (de Faria *et al.*, 1989). Those plants are commonly encountered in early successional stages (10 years after the disturbance has occurred), thus disappearing in very old successional stages (Heinrich *et al.*, 2004). Leguminous plants also have an ecological importance due to the role they have in fixing nitrogen (de Faria *et al.*, 1989). Furthermore, those plants also maintain high-diverse ecological communities, which include many phytophagous beetle species and associated natural enemies (Ødegaard, 2000; Baldock *et al.*, 2011; Tuller *et al.*, 2015). Among the leguminous groups with their center of diversity in the Neotropics, the *Senegalia* (Leguminosae:

Mimosoidea) is a perennial climbing shrub genus (Seigler et al. 2006), mainly distributed within the Brazilian Atlantic Domain (Barros & Morim, 2014). Although much previous research has explored the distribution of plant species from this genus (e.g. Terra *et al.*, 2014) or the associated ecological communities (e.g. (Baldock *et al.*, 2011) few studies have explored the ecological communities of different trophic levels associated with *Senegalia tenuifolia* plants in Neotropical regions (but see Tuller *et al.*, 2015).

The overarching aim of this study was to fill the above knowledge gaps by exploring the temporal variation of tropical insect communities of different trophic levels associated with the resource plant species *Senegalia tenuifolia* (L.) Britton & Rose (Fabaceae: Mimosoidea). Insect communities have been extensively considered in food web examinations due the host-specificity they present (Ødegaard, 2000; Novotny & Basset, 2005), the ease of sampling methods and quick alteration responses, such as habitat modification and temporal seasonality (e.g. Tylianakis *et al.*, 2005, 2007). We collected fruits of *S. tenuifolia* during four years, and from their seeds we gathered the associated insects to calculate the community parameters: species richness, abundance and species composition of each trophic level for each year.

Here we tested two specific hypotheses. First, in accordance with the positive relationships reported between resource availability and species diversity of upper trophic levels (Kruess & Tscharntke, 2002), we predicted that years in which more seeds were found would have higher species richness and abundance of insect species within each above-plant trophic level. Secondly, as we want to explore food web diversity patterns across a spatial (sampling sites and areas) and temporal (years) scale, we predicted that the contribution of the diversity components α- diversity (i.e. the local species richness or Shannon entropy, Chao *et al.*, 2014) and β-diversity (i.e. differences in species

assemblage composition, Whittaker, 1972; Karp *et al.*, 2012) to the total diversity would be different and temporally change for the ecological communities associated with the second (herbivores) and third (parasitoids) trophic levels. Although previous studies have investigated temporal changes in species composition of ecological communities (Le Corff *et al.*, 2000; Pearce *et al.*, 2006; Martínez-Falcón *et al.*, 2011), to our knowledge this is the first research that assesses temporal changes in species composition at different trophic levels by using a diversity partition approach (but see Cronin & Reeve, 2005 for changes in host-parasitoid using a spatial approach).

3.2 Methods

3.2.1 Field location

The study was carried out across three areas (Ae, La and Lu) subdivided into eight sampling sites (see Table ST3.1 – Supplementary Material), located in fragments of Southern Brazilian savannah (known as '*cerrado'*) around the municipalities of Lavras and Luminárias, state of Minas Gerais (Figure 1). The three areas were at least 6 km distant from each other and all sampling sites were at least 400 m apart (Figure 3.1 and Table ST3.2). Further descriptions of the sampling sites are described in Tuller et al. (2015).

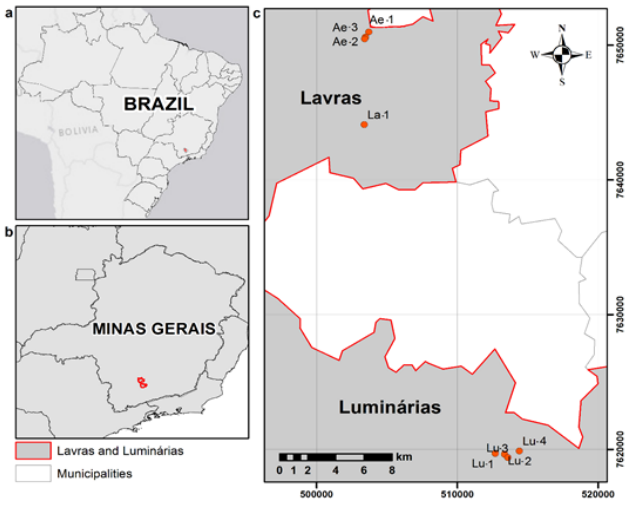


Figure 3.1 (a) The Brazilian map showing the sampled areas in red; (b) Minas Gerais state showing the sampled areas in red; (c) Both cities Lavras and Luminárias where the fruits we gathered and its eight sampling sites distant at least 400m each.

3.2.2 Fruit collection

The *S. tenuifolia* species is widely spread in South America (Queiroz, 2009), behaving as a liana or shrub species (Silva *et al*., 2007), with a reproductive period occurring from January to August and ripening period between June to August when the seeds start to be dispersed (*LFM, personal observation*). To collect *S. tenuifolia* fruits we conducted three sampling events per year, one per month, between June and August in 2011, 2012, 2013 and 2014. We collected 25 fruits per sampling site in each sampling event during the four years. All sampled fruits were previously attached to the plant at the time and then gathered into paper bags for transporting to the laboratory where each fruit was stored separately within PVC tubes (19 cm diameter, 10 cm depth) properly labeled and covered by voile to enable air circulation. In each year, we left these fruits stored for three months after each sampling event to allow the complete development and emergence of the insects associated with the seeds. All insects

(emerged from seeds or not) were identified to the lowest possible taxonomic level. We deposited the voucher specimens in the Entomological collection of the Laboratory of Ecology and Complexity at Federal University of Lavras, Minas Gerais, Brazil.

3.2.3 Statistical Analyses

In this work, we considered just the data from the second and third sampling events (July and August), since in the first sampling event (June) the *S. tenuifolia* plants were still beginning the ripening period, therefore presenting underdeveloped seeds and beetles species at the larval phase (that hindered species identification). Additionally, one sampling site (Ae-1) was burned in 2011, that impeded the data sampling for the subsequent years (please, see Table ST3.1 for details of areas sampled in each year).

We checked for temporal data independency by performing correlation analysis (*dplyr* package) between the insect abundance and species richness along the years and between each trophic level (see Figures SF3.1, SF3.2 and SF3.3 – Supplementary Material). As we observed a temporal independency along years and among trophic levels, we tested our first prediction by using generalized linear models (GLMs) (*stats* package). First, the GLMs were used to test the differences in the total seed number (first trophic level) and insects' abundance and species richness (above-plant trophic levels) along years (explanatory variable). Contrast tests among each year were conducted when significant differences were found. Furthermore, the GLMs were conducted to check for the relationship between number of seeds and insects' abundance and species richness. For this, the explanatory variables were total number of seeds, total number of herbivore insects and total number of parasitoid insects, and the

response variables were total number of herbivore insects, total number of parasitoids and total number of hyperparasitoids.

We tested the predictions that species composition and the contribution of the α- and β-diversity of the ecological communities associated with second and third trophic levels would temporally change using diversity partitioning and randomizations. The total diversity (γ) can be partitioned into the α and β components using either the additive or multiplicative approach (Veech *et al.*, 2002; Jost, 2007; Larsen *et al.*, 2015). The additive approach (γ = mean α + β) has the advantage of expressing each component in the same unit (number of taxa), but also has stronger dependence of β on both α and γ (Baselga, 2010). In particular, for each above-plant trophic level, diversity was partitioned separately for each year as follows (see also Zajac *et al.*, 2013):

$\gamma = \alpha$ *(within sampling site)* $+ \beta_1$ *(among sampling sites)* $+ \beta_2$ *(among areas)*

Diversity values were calculated by using the species richness (Hill numbers of order = 0) and the Shannon entropy (Hill numbers of order = 1) (as done by Solar *et al.*, 2015). The species richness is already a "true diversity" value, whereas the Shannon entropy weights the partitioning by the combined effect of richness and relative abundances, which reduces the influence of rare species (Jost, 2007; Chao *et al.*, 2014). We used 1,000 randomisations as implemented in the *adipart* function in the vegan R-package (Oksanen *et al.*, 2012). Randomisations allow the calculation of the null distribution of scale-specific α and β components, and to test levels of significance of the actual values against those simulated and expected by chance (Crist *et al.*, 2003; Matsuda *et al.*, 2015). Although our analyses and interpretation were based on the additive partitioning approach, in order to facilitate comparison with other studies, we also present α and β components based on the multiplicative approach, which allows an indirect comparison of α and β values. For this approach we used the *multipart* function (vegan R-package Oksanen *et al.*,

2012) with 1,000 randomizations (Figure SF3.4 and SF3.5, see Supplementary material). In general, the hierarchical diversity partition provides insight of scale effect on diversity patterns. Furthermore, to understand the differences of β diversity along years we use the R package *betapart* to perform the beta partition (β_{SOR}) into β_{SIM} (turnover) plus β_{SNE} (species gain or loss).

3.3 Results

3.3.1 Community description

Across the four years we collected 1,399 fruits of *S. tenuifolia*, from which we counted 12,651 seeds. From those, 2,766 seeds were considered consumed by 2,376 emerged and non-emerged insects. Each fruit had on average 9.04±2.67 (mean ± SD) seeds. Overall, we found three trophic levels above the *Senegalia tenuifolia* leguminous species. Table 3.1 and Figure SF3.6 summarises the number of seeds and the insect abundance and richness for each trophic level (TL) per year and Table 3.2 shows the species that were found, followed by their trophic level and respective abundance for each year. We also found a strong positive correlation between species richness and abundance within each trophic level (see Figure SF3.7 for R^2). Furthermore, there was no relationship between herbivores and seed abundances ($F_{1,26} = 0.21$, $p>0.05$) nor did hyperparasitoids increase their abundance with increasing parasitoid abundance ($p>0.05$). In contrast, there were an increase in parasitoid abundance with increasing herbivore abundance ($F_{1,26} = 11.20$, $p<0.01$).

The number of seeds (first trophic level) found in 2011 was lower than in the subsequent years ($F_{3,24}= 5.99$, $p< 0.005$ - Figure 3.2A). The herbivore level had abundance peaks in 2011 and 2014 ($F_{3,24}= 5.38$, $p< 0.005$ – Figure 3.2B), while the parasitoid abundance in 2014 was higher than in the other years

($F_{3,24}$= 7.87, p< 0.005 – Figure 3.2C). There were significant differences for hyperparasitoid abundance across the years ($F_{3,24}$= 5.67, p< 0.005 – Figure 3.2D). The herbivore and parasitoid species richness were higher in 2013 and 2014 (herbivore: $F_{3,24}$= 5.94, p< 0.005; parasitoid: $F_{3,24}$= 3.32, p< 0.05 – Figure 3.3A-B). The hyperparasitoid species richness varied across the years ($F_{3,24}$= 5.67, p< 0.005 – Figure 3.3C).

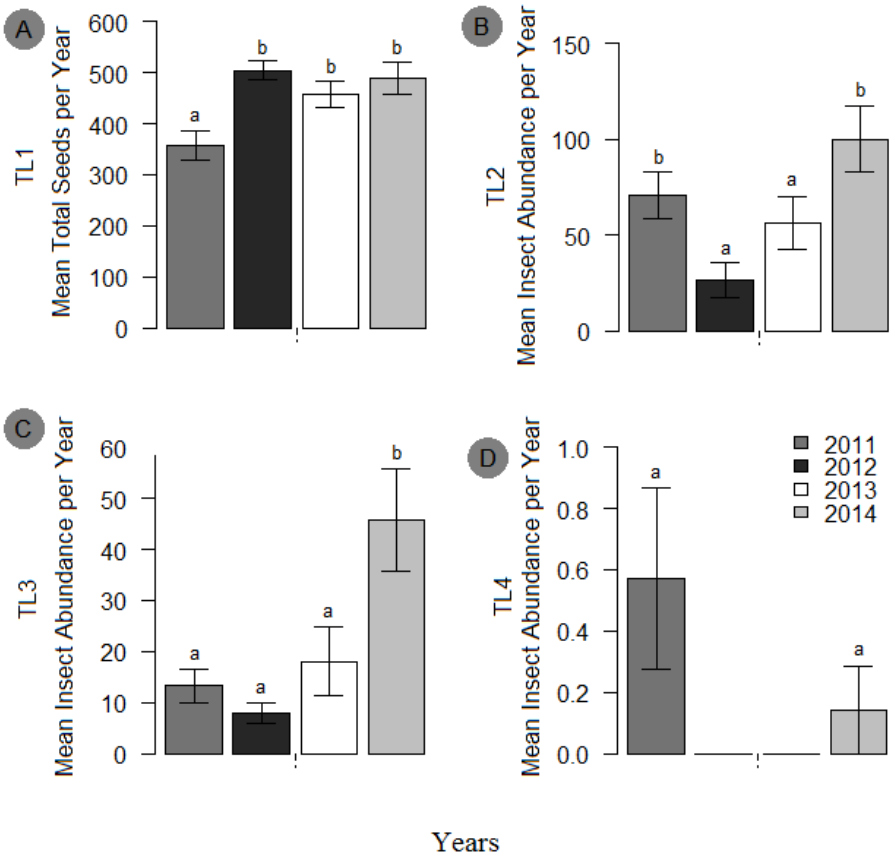


Figure 3.2 Abundance for each trophic level (TL) in each year. (A) TL1: In the first trophic level the abundance is represented by the total number of seeds (response variable) in each year (explanatory variable) ; (B) TL2: In the second trophic level the abundance is represented by the total number of herbivore individuals (response variable) in each year (explanatory variable); (C) TL3: In the third trophic level the abundance is represented by the total number of parasitoids individuals (response

variable) in each year (explanatory variable) and (D) TL4: In the fourth trophic level the abundance is represented by the total number of hyperparasitoids individuals (response variable) in each year (explanatory variable). Error bars represent the standard errors.

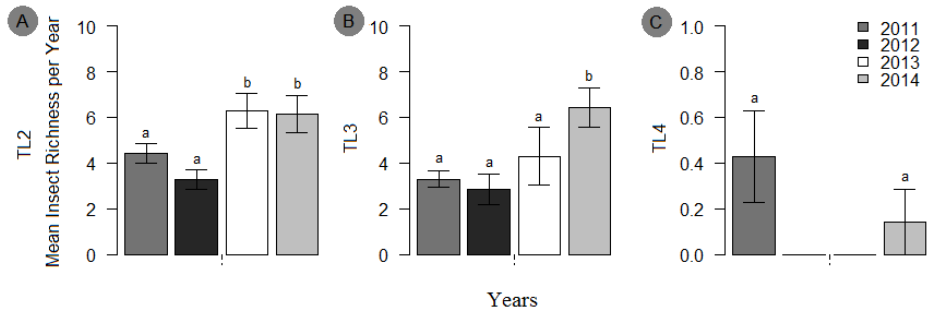


Figure 3.3 Species richness for each trophic level in each year. The response variables are (A) TL2: Second trophic level indicates the herbivore species richness; (B) TL3 Third trophic level indicates the parasitoid species richness and (C) TL4: Fourth trophic level indicates the hyperparasitoids species richness. For all the trophic levels the explanatory variable is the years sampled. Error bars represent the standard errors.

3.3.2 Diversity Partitioning

The insect diversity components based on the additive partitioning approach across different temporal scales varied for herbivore (2nd TL) and parasitoid (3rd TL) communities (Figure 3.4). The second trophic level α diversity was consistent, accounting for 41-63 % of total species richness along the years. The contributions of $\beta_{1\ (sites)}$ and $\beta_{2\ (areas)}$ components to γ diversity varied along the years. β_1 contribution ranged between 20 and 29% and it were higher than the expected from the random values in 2011. Contrastingly, the observed β_1 contribution to γ diversity in 2012 and 2013 was higher than expected from the random, while no differences were found between observed and expected values in 2014. Changes in the observed β_2 contribution were not significantly different from the random in 2011 and 2014, but were greater than expected from the random in 2012 and 2013, accounting for 29 and 33 % of total species richness, respectively.

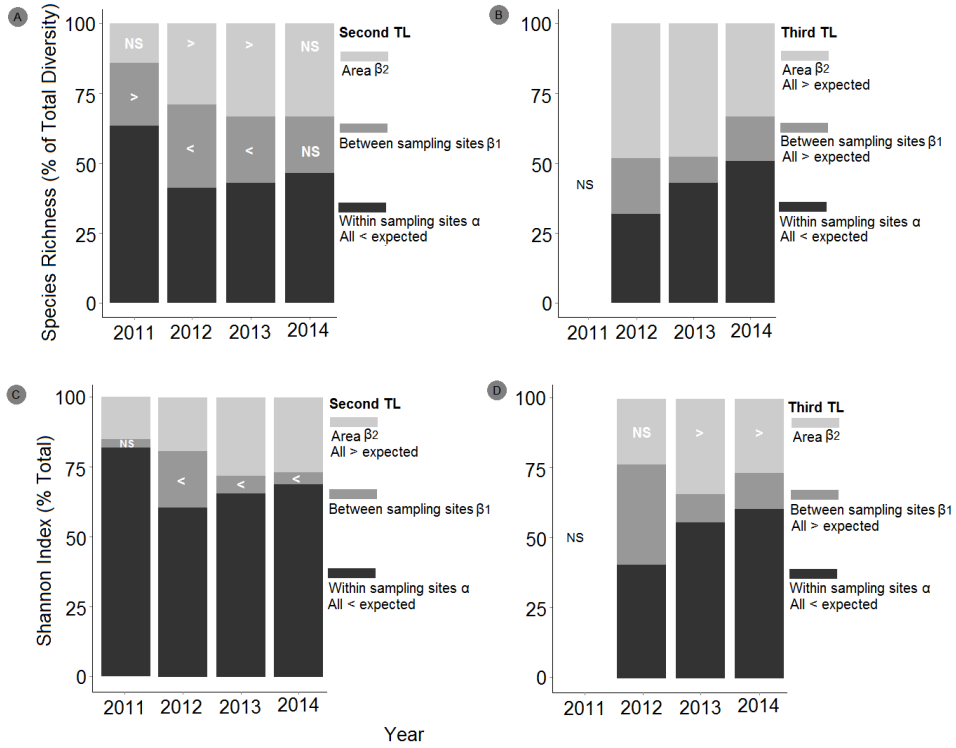


Figure 3.4 Additively portioned diversity of two insect communities (A and C) herbivores and (B and D) parasitoids along a temporal scale. Species richness estimates for (A) herbivores and (B) parasitoids, and Shannon Index for (C) herbivores and (D) parasitoids. Hierarchical components: α= diversity within sampling sites; β_1= diversity between sampling sites and β_2= diversity between areas. Randomization test results are given to the right of the figure and inside the figure when unequal results are given for each year. ">" indicates observed values presenting significant larger contributions than simulated from random, on the contrary, "<" indicates smaller contributions and "NS" indicate not significantly different from random. Statistical differences are based on the 0.05 level between observed and expected values obtained by 10,000 individual based randomizations.

The diversity partitioning of the third trophic level resulted in highly temporal variation in diversity components, with different diversity component relative contributions to the total species richness (Figure 3.4B). The α-diversity component accounted for 31 to 50% of total species richness along the years. Excepted by 2011, all observed α-diversity values were significantly lower than predicted (at $p<0.05$). Contributions of β_1 diversity ranged from 9 to 20%, and

were all greater than expected, excepted for 2011 as well. Diversity components among areas (β_2) showed the highest contribution to the total diversity in the third trophic level, accounting for 47-48% of total diversity, excepted in 2014, when its contribution was around 33%.

When considering the Shannon diversity index, the α-diversity had the highest contribution to the total diversity (γ) along the years for both second and third trophic levels, ranging around 60-81% and 40-60% for the second and third trophic levels, respectively. All α-diversity components were greater than expected from the random. In the multiplicative partitioning the α and β_1 diversity components exhibited similar degrees of variation on comparing the herbivore and parasitoid communities, considering species richness and Shannon diversity index (for more details see Supplementary Material SF3.4 and SF3.5). The beta diversity partition along the years accounted for 71% explained by β_{SIM} and 29% explained by β_{SNE} for the herbivore level and 84% explained by β_{SIM} and 16% explained by β_{SNE} for the parasitoid level.

3.4 Discussion

The species richness, abundance and composition (trough β –diversity estimations) for the resource, herbivore, parasitoid and hyperparasitoid communities presented a temporal variation. The fruits/seeds are not a limiting resource for the herbivores while the parasitoids depend on the herbivore abundance to increase their population. On the other hand, hyperparasitoids, which are less abundant in nature, revealed to be independent of their host (parasitoid) abundances. Basing on the major contributions to the herbivore richness, the local species richness (α) most contribute to the total diversity and herbivores present an intraspecific aggregate distribution (also found by Wertheim *et al.*, 2000; Veech, 2005). The α diversity values was lower than

expected, also found in 28 data from different arthropod communities (Veech, 2005). The major contribution to gamma diversity for parasitoid richness was due to the differences in species identity among areas (β_2 diversity) as a consequence of the difficult displacement of species (low turnover). However, in the last year the parasitoids changed their species richness contribution to an α diversity with species presenting an aggregate pattern. As the Shannon index was explained mostly by α diversity within sampling sites, the most abundant species were widespread, thus the same common (more abundant) species comprise most of the α diversity within sampling sites. As we can observe, the most abundant herbivore species (*Merobruchus terani*) and its parasitoid (*Horismenus* sp.) appear in all sites and years at a high abundance. Moreover, the difference in species richness along the years for the herbivores and parasitoids are mostly due to the replacement of some species by others (β_{SIM}).

The prediction that the trophic level are directly influenced by the level above was confirmed just for herbivore-parasitoid interactions. The herbivore abundance does not increase with the resource quantity available. Plant resources are often abundant and underexploited by herbivores and also the herbivores are frequently regulated by their natural enemies contributing to the host density control which contributes to plants remaining abundant (Hairston, 1960). Thus, the seeds unconsumed by the primary consumer herbivore and secondary disperser herbivore will be transformed into organic matter to be part of the detritus communities (Bardgett & Wardle, 2003) or germinate when in the soil. Even though host plant population size influences herbivore distribution (von Zeipel *et al.*, 2006), many explanations arise from the premise that herbivores are not food limited and here we propose those possible explanations. The first possible explanations for the high resource availability and low number of herbivore individuals in some years is that its populations may be reduced by

the parasitoid populations which controls it (Fretwell, 1987), the second is that herbivores can exhibit intraspecific competition at high densities keeping its population dynamic regulated. The third prediction and that more likely to happen is that herbivores are not limited by food supply but by other resources such as ovipositional sites which lead to a larval cannibalism (Murdoch, 1966; Rosenzweig, 1973; Wang & Kok, 1986) since in this system the most abundant herbivore is a Bruchinae species spending most of its life cycle inside the seed (Southgate, 1979).

Instead, herbivores and parasitoids show similar abundance patterns presenting direct relationship on each other's population dynamics with a coincident peak of abundance in the last year. The host-parasitoid interaction has many aspects to be explored, such as through host-parasitoid population dynamics, shifting sex rations in the parasitoid population and other components of this interaction. Here, as we cannot distinguish the species interactions and sex ratios, we explored only the parasitoid and host density dependence. As reported by many studies, natural enemies can regulate their host dynamic populations, which is often applied to the biological control (Hassell, 2000; Van Driesche *et al.*, 2010). As the parasitoids and herbivores are closely related because parasitoid development depends on its host, the decrease in the herbivore richness may lead to a reduction in the parasitoid species richness (Albrecht *et al.*, 2007; Osorio *et al.*, 2015), as supported by our results when the herbivore richness increased in 2013 and remained in 2014, the parasitoid richness started to increase in 2013 and reached its peak richness in 2014 (Figure 3A and 3B).

On the contrary, hyperparasitoids are known to be limited by the availability of their host population (parasitoids) leading to the extinction of small parasitoid populations sometimes disrupting biological control (Brodeur

2000; Schooler *et al.,* 2011 – cage experiment, Gómez-Marco *et al.*, 2015). However, this study does not report parasitoid-hyperparasitoid abundance dependence since here the hyperparasitoids level is composed of very few individuals and often they do not increase their abundance in synchrony with the parasitoid level not presenting control over its population (Nofemela, 2013, Schooler et al. 2011 – field experiment). Also, the hyperparasitoid species richness does not increase with higher parasitoid richness.

Although little is known about hyperparasitoids and their real impact on the four-trophic interaction (Rosenheim, 1998), it may happen because the hyperparasitoids can be reported as obligated or facultative (Rosenheim *et al.*, 1995). The obligate hyperparasitoids only reproduce in primary parasitoids, thus directly affecting their populations (Sullivan & Völkl, 1999), the facultative hyperparasitoid is not considered detrimental for the parasitoid population control (Pérez-Lachaud *et al.*, 2004) being, in general, omnivorous and part of the parasitoid or hyperparasitoid levels depending on the level of competition for hosts (Brodeur, 2000; Godfray, 1994). Here we address the importance of laboratorial experiments for a better understand of hyperparasitoid biology and behavior under different circumstances since they can behave as obligate or facultative hyperparasitoids in different systems (Nwanze *et al.*, 1998; Rosenheim, 1998; Morse, 2009; Hoddle *et al.*, 2013). Moreover, all the insect trophic levels presented variation in the abundance and richness along the years, being richer, more abundant and even presenting more trophic levels in some years than others, reinforcing the importance of a temporal approach when evaluating ecological communities.

The sampling incompleteness of plant-herbivore food webs in tropical rainforests tends to overestimate beta diversity and affect the importance of beta diversity components (Novotny, 2009). Thus, to overcome any possible bias, we

have checked the robustness of this studied food web, finding that we have sampled all insect richness for all three trophic levels (herbivore –parasitoid-hyperparasitoid) along four years sample (see Maia et al. 2016 – First Chapter). As the understanding of beta diversity dynamics (turnover and nestdness) is important for conservation in many fields, such as protected area selection (Socolar *et al.*, 2016), here we add a starting point to better understand the species diversity dynamics across scales through two different levels of one food web since here the levels display different diversity component (α and β_2) contributions changing along years and even within a year.

The need for conservation of insect diversity is important because insects provide ecological services such as biological control and pollination (Kim, 1993). This study showed herbivores displaying an intraspecific aggregation pattern within sampling sites what could be explained by the resource distribution which is also uneven (Price, 2002; Babah & Sword, 2004) and also it supports that herbivores are aggregate because female adults seek fruits (as ovipositional sites) in adjacent plants considering positive visual cues such as total number and mature fruits (Cuautle & Parra-Tabla, 2014). Moreover, the spatial context in which host-parasitoid interactions happen determine the parasitoid behaviors (such as foraging efficiency) (Bukovinszky *et al.*, 2012, Waage & Hassell, 1982). The parasitoid species richness is mostly due to species differences among areas since the parasitoids seem not to disperse from one area to another while just in 2014 they presented an intraspecific aggregation pattern within sampling sites (based on the major diversity components contributions). Conspecifics can be aggregate for many reasons such as similar habitat requirements, a shared resource base and predator avoidance. This may be explained by the high number of hosts (herbivore) displaying an aggregate pattern, decreasing the parasitoid dispersion (French &

Travis, 2001). However, when host abundance and richness increased even more in the last year, it seems that the parasitoids not only increased their abundance and richness, but their richness pattern also changed, thus the difference in parasitoid richness among areas does not exist anymore, presenting an aggregate pattern of parasitoid richness. This aggregate pattern might also be a strategy demonstrated by the predators and hyperparasitoids when host densities are high because it enables females to reduce the offspring mortality risk by spreading it over several hosts (Mackauer & Volkl, 1993). It also confirms that the third trophic level respond more sensitively to other scales than the second trophic level, as also found by other studies (Thies et al., 2003, but see Heisswolf *et al.*, 2006; Cuautle & Parra-Tabla, 2014). Moreover, in this present study, parasitoid and herbivore turnover was not observed and the decrease in abundance does not increase the turnover at any spatial scale as also found in a landscape context (Kaartinen & Roslin, 2012). However, more studies should be done in order to test the different explanations about scale patterns influencing different communities and their strict relation.

The differences in the species richness component contributions demonstrate the importance of studying not only the food web changes in a landscape context (Kaartinen & Roslin, 2011), but also how each trophic level behaves (Legendre *et al.*, 2005; Gagic *et al.*, 2012). The changes observed in the community parameters over time reinforce the need for studying a food web using temporal and spatial scales approaches, since we know that networks are not static in time (Eveleigh *et al.*, 2007; Gagic *et al.*, 2012). Although we minimize the seasonal changes by collecting the data in the same months in each year, we also address the importance of collecting environmental variables (e.g. temperature, altitudinal gradients, and resource quantity and quality) because species distributions, abundance and turnover in communities can be related to

environmental conditions (Shreeve, 1986; Legendre *et al.*, 2005; Novotny *et al.*, 2007). Finally, we have demonstrated that trophic levels in a food web present different species distribution patterns in space and time, reinforcing the necessity to consider a food web approach in future community studies to better comprehend natural communities and their distribution.

3.5 Conclusion

The herbivore and parasitoid ecological communities present different shifts in abundance and richness over time and high herbivorous abundance and richness mediate the changing in diversity distribution pattern for third trophic level. Furthermore, each trophic level differs in species diversity patterns across scales highlighting the importance of local to global landscape context in biological control planning (Tamburini *et al.*, 2015). In this study we reinforce what some authors have found important, such as ecological communities and food web studies at multiple scales and the use of temporal approaches to analyze distinct trophic levels such as hosts and their natural enemy levels (see Maia et al. 2016 - 1st Chapter, (Cronin & Reeve, 2005; Cuautle & Parra-Tabla, 2014; McMeans *et al.*, 2015). Finally, as the nature of interactions is known to shape community components, further work will investigate how this community is structured using a food web perspective (interaction strength, population dynamics, secondary extinction rates) and how spatial scales and time affect community patterns (composition, richness and abundance) through quantitative and qualitative food web analysis as was found in Kaartinen & Roslin (2011) study.

3.6 Ackowledgments

We are grateful to Drª C.S. Ribeiro-Costa, A.R. Nascimento, A.C.M. de Queiroz, and Drª M.S.C. Morini for insect identification support. We also thank França F. and Zanella L. for the suggestions and maps on the manuscript. We

thank Universidade Federal de Lavras and PPG in Applied Ecology for logistical support and the FAPEMIG and CAPES for providing grants.

3.7 References

Albrecht, M., Duelli, P., Schmid, B. & Müller, C.B. (2007) Interaction diversity within quantified insect food webs in restored and adjacent intensively managed meadows. *Journal of Animal Ecology*, **76**, 1015–1025.

Allouche, O., Kalyuzhny, M., Moreno-Rueda, G., Pizarro, M. & Kadmon, R. (2012) Area-heterogeneity tradeoff and the diversity of ecological communities. *Proceedings of the National Academy of Sciences*, **109**, 17495–17500.

Araújo, W.S., Scareli-Santos, C., Guilherme, F.A.G. & Cuevas-Reyes, P. (2013) Comparing galling insect richness among Neotropical savannas: Effects of plant richness, vegetation structure and super-host presence. *Biodiversity and Conservation*, **22**, 1083–1094.

Azeria, E.T., Ibarzabal, J., Boucher, J. & Hebert, C. (2010) *Towards a better understand of beta diversity: Decontructing composition patterns of saproxylic beetles breeding in recently burnt boreal forests*. In Pavlinov, I. (ed.), Research in Biodiversity: Models and Applications, 75-95. InTech, Rijeka

Babah, M. a O. & Sword, G. a. (2004) Linking locust gregarization to local resource distribution patterns across a large spatial scale. *Environmental Entomology*, **33**, 1577–1583.

Baldock, K.C.R., Memmott, J., Carlos Ruiz-Guajardo, J., Roze, D. & Stone, G.N. (2011) Daily temporal structure in African savanna flower visitation networks and consequences for network sampling. *Ecology*, **92**, 687–698.

Bang, C., Faeth, S.H. & Sabo, J.L. (2012) Control of arthropod abundance, richness, and composition in a heterogeneous desert city. *Ecological Monographs*, **82**, 85–100.

Bardgett, R.D. & Wardle, D. a. (2003) Herbivore mediated linkages between aboveground and belowground communities. *Ecology*, **84**, 2258–2268.

Barros, M.J.F. & Morim, M.P. (2014) Senegalia (Leguminosae, Mimosoideae) from the Atlantic Domain, Brazil. *Systematic Botany*, **39**, 452–477.

Baselga, A. (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, **19**, 134–143.

Basset, Y., Cizek, L., Cuenoud, P., Didham, R.K., Guilhaumon, F., Missa, O., *et al.* (2012) Arthropod Diversity in a Tropical Forest. *Science*, **338**, 1481–1484.

Boulton, A.M., Davies, K.F. & Ward, P.S. (2005) Species Richness, Abundance, and Composition of Ground-Dwelling Ants in Northern California Grasslands: Role of Plants, Soil, and Grazing. *Environmental Entomology*, **34**, 96–104.

Brodeur, J. (2000) Host specificity and trophic relationships of hyperparasitoids. *Parasitoid Population Biology* (eds M. E. Hochberg & A. R. Ives), pp 139-183. Princenton University Press, Princenton, New Jersey.

Brown, J.H. (2014) Why are there so many species in the tropics? *Journal of Biogeography*, **41**, 8–22.

Bukovinszky, T., Poelman, E.H., Kamp, A., Hemerik, L., Prekatsakis, G. & Dicke, M. (2012) Plants under multiple herbivory: Consequences for parasitoid search behaviour and foraging efficiency. *Animal Behaviour*, **83**, 501–509.

Burkle, L.A., Marlin, J.C. & Knight, T.M. (2013) Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurence, and Function. *Science*, **339**, 1611–1615.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., *et al.* (2012) Corrigendum: Biodiversity loss and its impact on humanity. *Nature*, **489**, 326–326.

Chao, A., Chiu, C.-H. & Jost, L. (2014) Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill N¡numbers. *Annual Review of Ecology, Evolution, and Systematics*, **45**, 297–324.

Cohen, J.E. (1978) *Food Webs and Niche Space*. Princenton Univeristy Press.

Cohen, J.E., Jonsson, T. & Carpenter, S.R. (2003) Ecological community description using the food web, species abundance, and body size. *Most*, **100**, 1781–1786.

Corff, J. Le, Marquis, R.J., Whitfield, J.B. & Corff, J.L.E. (2000) Temporal and spatial variation in a parasitoid community associated with the herbivores that feed on Missouri Quercus. *Environmental Entomology*, **29**, 181–194.

Crist, T.O., Veech, J.A., Gering, J.C. & Summerville, K.S. (2003) Partitioning Species Diversity across Landscapes and Regions: A hierarchical analysis of alfa, beta and gamma diversity. *The American Naturalist*, **162**, 734–743.

Cronin, J.T. & Reeve, J.D. (2005) Host-parasitoid spatial ecology: a plea for a landscape-level synthesis. *Proceedings of the Royal Society B: Biological Sciences*, **272**,

2225–2235.

Cuautle, M. & Parra-Tabla, V. (2014) Describing a multitrophic plant-herbivore-parasitoid system at four spatial scales. *Acta Oecologica*, **55**, 8–14.

Driesche, R.G. Van, Carruthers, R.I., Center, T., Hoddle, M.S., Hough-Goldstein, J., Morin, L., *et al.* (2010) Classical biological control for the protection of natural ecosystems. *Biological Control*, **54**, S2–S33.

Dunne, J. a., Williams, R.J. & Martinez, N.D. (2002) Food-web structure and network theory: The role of connectance and size. *Proceedings of the National Academy of Sciences*, **99**, 12917–12922.

Elton, C. (1933) *Ecology of Animals*; reprinted (1966) Methuen, London.

Eveleigh, E.S., McCann, K.S., McCarthy, P.C., Pollock, S.J., Lucarotti, C.J., Morin, B., *et al.* (2007) Fluctuations in density of an outbreak species drive diversity cascades in food webs. *Proceedings of the National Academy of Sciences USA*, **104**, 16976–81.

Faria, S.M. de, Lewis, G.P., Sprent, J.I. & Sutherland, J.M. (1989) Occurrence of nodulation in the Leguminosae. *New Phytologist*, **111**, 607–619.

French, D.R. & Travis, J.M.J. (2001) Density-dependent dispersal in host-parasitoid assemblages. *Oikos*, **95**, 125–135.

Fretwell, S.D. (1987) Food chain dynamics: the central theory of ecology? *Oikos*, **50**, 291–301.

Gagic, V., Hänke, S., Thies, C., Scherber, C., Tomanovic, Z. & Tscharntke, T. (2012) Agricultural intensification and cereal aphid-parasitoid-hyperparasitoid food webs: Network complexity, temporal variability and parasitism rates. *Oecologia*, **170**, 1099–1109.

Gergs, R., Grey, J. & Rothhaupt, K.O. (2011) Temporal variation in zebra mussel (Dreissena polymorpha) density structure the benthic food web and community composition on hard substrates in Lake Constance, Germany. *Biological Invasions*, **13**, 2727–2738.

Godfray, H. C. J. (1994) *Parasitoids: behavioral and evolutionary ecology*. Princenton University Press, Princenton, New Jersey.

Gómez-Marco, F., Urbaneja, A., Jaques, J.A., Rugman-Jones, P.F., Stouthamer, R. & Tena, A. (2015) Untangling the aphid-parasitoid food web in citrus: Can hyperparasitoids disrupt biological control? *Biological Control*, **81**, 111–121.

Grandez-Rios, J.M., Bergamini, L.L., Araujo, W.S. De, Villalobos, F. & Almeida-Neto, M. (2015) The effect of host-plant Phylogenetic isolation on species richness, composition and specialization of insect herbivores: A comparison between native and exotic hosts. *Plos One*, **10**, 1–14.

Hairston, N.G., Smith, F.E. & Slobodkin L.B. (1960) Community structure, population control and competition. *The American Naturalist*, **94**, 421-425.

Hansson, L.-A., Nicolle, A., Granéli, W., Hallgren, P., Kritzberg, E., Persson, A., *et al.* (2012) Food-chain length alters community responses to global change in aquatic systems. *Nature Climate Change*, **2**, 1–6.

Hassell, M.P. (2000) Host-Parasitoid population dynamics. *Journal of Animal Ecology*, **69**, 543–566.

Heinrich, A., Hurka, H., Biology, F.B. & Osnabrueck, S.B. (2004) Species richness and composition during sylvigenesis in a tropical dry forest in northwestern Costa Rica. *Tropical Ecology*, **45**, 43–57.

Heisswolf, A., Poethke, H.J. & Obermaier, E. (2006) Multitrophic influences on egg distribution in a specialized leaf beetle at multiple spatial scales. *Basic and Applied Ecology*, **7**, 565–576.

Heub, S.J., Seog, K.C., Taek, Y.Y., Yeong, H.S. & Chan, L.S. (2013) Annual Occurrent Pattern of Scirtothrips dorsalis (Thysanoptera : Thripidae) on Citrus Trees and Surrounding Host Plants. *The Korean Society of Applied Entomology*, **52**, 185–191.

Hoddle, C.D., Hoddle, M.S. & Triapitsyn, S. V. (2013) Marietta leopardina (Hymenoptera: Aphelinidae) and Aprostocetus (Aprostocetus) sp. (Hymenoptera: Eulophidae) are obligate hyperparasitoids of Tamarixia radiata (Eulophidae) and Diaphorencyrtus aligarhensis (Hymenoptera: Encyrtidae). *Florida Entomologist*, **96**, 643–646.

Jenkins, C.N., Pimm, S.L. & Joppa, L.N. (2013) Global patterns of terrestrial vertebrate diversity and conservation. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, E2602–10.

Jost, L. (2007) Partitioning diversity into independent alpha and beta components. *Ecology*, **88**, 2427–2439.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F., Donoghue, M.J. (2002) *Plant systematics: a phylogenetic approach*. 2nd edn.

Kaartinen, R. & Roslin, T. (2011) Shrinking by numbers: landscape context affects the species composition but not the quantitative structure of local food webs. *Journal of*

Animal Ecology, **80**, 622–631.

Kaartinen, R. & Roslin, T. (2012) High temporal consistency in quantitative food web structure in the face of extreme species turnover. *Oikos*, **121**, 1771–1782.

Karp, D.S., Rominger, A.J., Zook, J., Ranganathan, J., Ehrlich, P.R. & Daily, G.C. (2012) Intensive agriculture erodes beta-diversity at large scales. *Ecology Letters*, **15**, 963–970.

Kim, K.C. (1993) Biodiversity, conservation and inventory: why insects matter. *Biodiversity and Conservation*, **2**, 191–214.

Kruess, A. & Tscharntke, T. (2002) Grazing intensity and the diversity of grasshoppers, butterflies and trap-nest bees and wasps. *Conservation Biology*, **16**, 1570–1580.

Larsen, S., Scalici, M. & Tancioni, L. (2015) Scale dependent biodiversity patterns in Mediterranean river catchments: a multi taxa approach. *Aquatic Sciences*, **77**, 455–463.

Layman, C.A., Winemiller, K.O., Arrington, D.A. & Jepsen, D.B. (2005) Body Size and Trophic Position in a Diverse Tropical Food Web. *Ecology*, **86**, 2530–2535.

Legendre, P., Borcard, D. & Perez-Neto, P.R. (2005) Analyzing beta diversity: partitioning the spatial variation of community composition data. *Ecological Monographs*, **75**, 435–450.

Lu, Z., Fisk, A.T., Kovacs, K.M., Lydersen, C., McKinney, M. a., Tomy, G.T., *et al.* (2014) Temporal and spatial variation in polychlorinated biphenyl chiral signatures of the Greenland shark (Somniosus microcephalus) and its arctic marine food web. *Environmental Pollution*, **186**, 216–225.

MacIvor, J.S. & Lundholm, J. (2011) Insect species composition and diversity on intensive green roofs and adjacent level-ground habitats. *Urban Ecosystems*, **14**, 225–241.

Mackauer, M. & Volkl, W. (1993) Regulation of aphid populations by aphidiid wasps: does parasitoid foraging behaviour or hyperparasitism limit impact? *Oecologia*, **94**, 339–350.

Marcarelli, A.M., Baxter, C. V., Mineau, M.M. & Hall, R.O.J. (2011) Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology*, **92**, 1215–1225.

Martínez-Falcón, A.P., Marcos-García, M.Á. & Moreno, C.E. (2011) Temporal shifts and niche overlapping in Copestylum (Diptera, Syrphidae) communities reared in cactus species in a central Mexican scrubland. *Ecological Research*, **26**, 341–350.

Matsuda, J.T., Martens, K. & Higuti, J. (2015) Diversity of ostracod communities (Crustacea, Ostracoda) across hierarchical spatial levels in a tropical floodplain. *Hydrobiologia*, **762**, 113–126.

May, R.M. (1994) Biological diversity: differences between land and sea. *Philosophical transactions of the Royal Society of London. Series B*, **343**, 105–111.

McMeans, B.C., McCann, K.S., Humphries, M., Rooney, N. & Fisk, A.T. (2015) Food Web Structure in Temporally-Forced Ecosystems. *Trends in Ecology and Evolution*, **30**, 662–672.

McNaughton, S.J. (1977) Diversity and Stability of Ecological Communities: A Comment on the Role of Empiricism in Ecology. *The American Naturalist*, **111**, 515–525.

Morse, D.H. (2009) Four-level interactions: Herbivore use of ferns and subsequent parasitoid-hyperparasitoid performance. *Ecological Entomology*, **34**, 246–253.

Mougi, A. & Kondoh, M. (2012) Diversity of Interaction Types and Ecological Community Stability. *Science*, **337**, 349–351.

Murdoch, W.W. (1966) Community Structure, Population Control, and Competition - A Critique. *The American Naturalist*, **100**, 219–226.

Nofemela, R.S. (2013) The effect of obligate hyperparasitoids on biological control: Differential vulnerability of primary parasitoids to hyperparasitism can mitigate trophic cascades. *Biological Control*, **65**, 218–224.

Novotny, V. (2009) Beta diversity of plant-insect food webs in tropical forests: A conceptual framework. *Insect Conservation and Diversity*, **2**, 5–9.

Novotny, V. & Basset, Y. (2005) Host specificity of insect herbivores in tropical forests. *Proceedings of The Royal Society B*, **272**, 1083–90.

Novotny, V., Miller, S.E., Hulcr, J., Drew, R. a I., Basset, Y., Janda, M., *et al.* (2007) Low beta diversity of herbivorous insects in tropical forests. *Nature*, **448**, 692–5.

Nwanze, K.F., Reddy, Y.V.R., Nwilene, F.E., Kausalya, K.G. & Reddy, D.D.R. (1998) Tritrophic interactions in sorghum, midge (Stenodiplosis sorghicola) and its parasitoid (Aprostocetus spp.). *Crop Protection*, **17**, 165–169.

Ødegaard, F. (2000) The relative importance of trees versus lianas as hosts for phytophagous beetles (Coleoptera) in tropical forests. *Journal of Biogeography*, **27**, 283–296.

Oksanen, A.J., Blanchet, F.G., Kindt, R., Legendre, P., O`Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H. (2012) Vegan: community ecology package. Version 20-2.

Osorio, S., Arnan, X., Bassols, E., Vicens, N. & Bosch, J. (2015) Local and landscape effects in a host-parasitoid interaction network along a forest-cropland gradient. *Ecological Applications*, **25**, 1869–1879.

Paine, R.T. (1966) Food web complexity and species diversity. *The American Naturalist*, **100**, 65–75.

Paine, R.. (1980) Food Web: Linkage, Interaction Strength and Community Infrastructure. *Journal of Animal Ecology*, **49**, 666–685.

Peacor, S.D. & Werner, E.E. (1997) Trait-Mediated Indirect Interactions in a Simple Aquatic Food Web. *Ecology*, **78**, 1146–1156.

Pearce, I.G., Chaplain, M. a J., Schofield, P.G., Anderson, A.R. a & Hubbard, S.F. (2006) Modelling the spatio-temporal dynamics of multi-species host-parasitoid interactions: Heterogeneous patterns and ecological implications. *Journal of Theoretical Biology*, **241**, 876–886.

Peralta, G., Frost, C.M., Rand, T. a., Didham, R.K. & Tylianakis, J.M. (2014) Complementarity and redundancy of interactions enhance attack rates and spatial stability in host-parasitoid food webs. *Ecology*, **95**, 1888–1896.

Pérez-Lachaud, G., Batchelor, T.P. & Hardy, I.C.W. (2004) Wasp eat wasp: Facultative hyperparasitism and intra-guild predation by bethylid wasps. *Biological Control*, **30**, 149–155.

Pilosof, S., Fortuna, M.A., Vinarski, M.V., Vinarskaya-Korallo N. & Krasnov, B.R. (2013) Temporal dynamics of direct reciprocal and indirect effects in a host-parasite network. *Journal of Animal Ecology*, **82**, 987-996

Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.L., & Kaplan, I. (2011) Insect Ecology: Behavior, Populations and Communities. *Behavior, mating systems and sexual selection*. Cambridge University Press, New York.

Price, P.W. (2002) Resource-driven terrestrial interaction webs. *Ecological Research*, **17**, 241–247.

Queiroz, L.P. (2009) Leguminosas da Caatinga. Universidade Estadual de Feira de Santana: Feira de Santana. 914 p.

Raub, F., Höfer, H., Scheuermann, L., Britez, R.M. de & Brandl, R. (2015) Conserving

landscape structure – conclusions from partitioning of spider diversity in southern Atlantic forests of Brazil. *Studies on Neotropical Fauna and Environment*, **0521**, 1–17.

Reich, P.B., Frelich, L.E., Voldseth, R. a., Bakken, P. & Adair, E.C. (2012) Understorey diversity in southern boreal forests is regulated by productivity and its indirect impacts on resource availability and heterogeneity. *Journal of Ecology*, **100**, 539–545.

Rosenheim, J. a. (1998) Higher-Order Predators and the Regulation of Insect Herbivore Populations. *Annual Review of Entomology*, **43**, 421–447.

Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J. & Jaffee, B.A. (1995) Intraguild predation among Biological-Control Agents: Theory and Evidence. *Biological Control*, **5**, 303–335.

Rosenzweig, M.L. (1973) Exploitation in Three Trophic Levels. *The American Naturalist*, **107**, 275–294.

Seigler, D.S., John, E.E. & Joseph, T.M. (2006) The genus Senegalia (Fabaceae: Mimosoideae) from the new world. *Phytologia* **88**, 38-93.

Schooler, S.S., De Barro, P., Ives, A.R. (2011) The potential for hyperparasitism to compromise biological control: Why don`t hyperparasitoids drive their primary parasitoid hosts extinct?. *Biological Control*, **58**, 167-173

Schrire, B.D., Lewis, G.P., Lavin, M. (2005). "Biogeography of the Leguminosae". In Lewis, G; Schrire, G.; Mackinder, B.; Lock, M. *Legumes of the world*. Kew, England: Royal Botanic Gardens. pp. 21–54.

Shreeve, T.G. (1986) Egg-laying by the speckled wood butterfly (*Pararge aegeria*): the role of female behavior, host-plant abundance and temperature. *Ecological Entomology* **11,** 229-236.

Shurin, J.B., Clasen, J.L., Greig, H.S., Kratina, P. & Thompson, P.L. (2012) Warming shifts top-down and bottom-up control of pond food web structure and function. *Philosophical transactions of the Royal Society of London. Series B*, **367**, 3008–17.

Silva, E.F., Santos, T.R.R., Fernandes-Bulhão, C. (2007) Levantamento florístico das lianas lenhosas, arbustos e subarbustos do Cerrado do Parque do Cababa, Nova Xavantina-MT. *Revista Brasileira de Biociências* **5**, 948-950.

Socolar, J.B., Gilroy, J.J., Kunin, W.E. & Edwards, D.P. (2016) How should beta-diversity inform biodiversity conservation? *Trends in Ecology & Evolution*, **31**, 67–80.

Solar, R.R.C., Barlow, J., Ferreira, J., Berenguer, E., Lees, A.C., Thomson, J.R., *et al.* (2015) How pervasive is biotic homogenization in human-modified tropical forest

landscapes? *Ecology Letters*, **18**, 1108–1118.

Southgate, B.J. (1979) Biology of the Bruchidae. *Annual Review of Entomology*, **24**, 449–473.

Sullivan, D.J. & Völkl, W. (1999) Hyperparasitism: multitrophic ecology and behavior. *Annual review of entomology*, **44**, 291–315.

Tamburini, G., Simone, S. De, Sigura, M., Boscutti, F. & Marini, L. (2015) Conservation tillage mitigates the negative effect of landscape simplification on biological control. *Journal of Applied Ecology*, **53**, 233–241.

Terra, V., Neri, A.V. & Garcia, F.C.P. (2014) Patterns of geographic distribution and conservation of Acacieae Benth. (Leguminosae-Mimosoideae), in Minas Gerais, Brazil. *Revista Brasileira de Botanica*, **37**, 151–158.

Thies, C., Dewenter-Steffan, I., Tscharntke, T. (2003) Effects of landscape context on herbivory and parasitism at different spatial scales. *Oikos*, **101**, 18-25

Tuller, J., Paula, E.L. De, Maia, L.F., Moraes, R.A. & Faria, L.D.B. (2015) Seed predation food web, nutrient availability, and impact on the seed germination of Senegalia tenuifolia (Fabaceae). *Revista de biología tropical*, **63**, 1149–1159.

Tylianakis, J.M., Klein, A.M. & Tscharntke, T. (2005) Spatiotemporal variation in the diversity of hymenoptera across a tropical habitat gradient. *Ecology*, **86**, 3296–3302.

Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007) Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature*, **445**, 202–205.

Veech, J. A, Summerville, K.S., Crist, T.O. & Gering, J.C. (2002) The additive partitioning of species diversity: recent revival of an old idea. *Oikos*, **99**, 3–9.

Veech, J. A. (2005) Analyzing patterns of species diversity as departures from random expectations. *Oikos*, **108**, 149–155.

Waage, J. K. & Hassell, M. P. (1982). Parasitoids as biological control agents – a fundamental approach. *Parasitology*, **84**, 241-268.

Wang, R. & Kok, L.T. (1986) Life-history of Megacerus discoidus (Coleoptera, Bruchidae), a seedfeeder of hedge bindweed, in southwestern Virginia. *Annals Of The Entomological Society Of America*, **79**, 359–363.

Wertheim, B., Sevenster, J.G., Eijs, I.E.M. & JJM, A. Van. (2000) Species diversity in a mycophagous insect community: the case of spatial segregation vs. resource partitioning. *Journal of Animal Ecology*, **69**, 335–351.

Whittaker, R.H. (1972) Evolution and Measurement of Species Diversity. *Taxon*, **21**, 213–251.

Winemiller, K.O., Akin, S. & Zeug, S.C. (2007) Production sources and food web structure of a temperate tidal estuary: Integration of dietary and stable isotope data. *Marine Ecology Progress Series*, **343**, 63–76.

Zajac, R.N., Vozarik, J.M. & Gibbons, B.R. (2013) Spatial and temporal patterns in macrofaunal diversity components relative to sea floor landscape structure. *PloS one*, **8**, e65823.

Zeipel, H. von, Eriksson, O. & Ehrlén, J. (2006) Host plant population size determines cascading effects in a plant-herbivore-parasitoid system. *Basic and Applied Ecology*, **7**, 191–200.

Table 3.1 Number of collected seeds of *Senegalia tenuifolia* (1st TL) and insects abundance and richness (2nd-4th TL) in each year sampled (abundance | richness) or within each sampling site**.**

Year/Within sampling site	1st TL	2nd TL	3rd TL	4th TL
2011	2,500	495 | 7	93 | 7	4 | 2
2012	3,528	187 | 8	56 | 9	0
2013	3,200	394 | 10	126 | 7	0
2014	3,423	700 | 8	320 | 9	1 | 1

Table 3.2 Insects associated with Senegalia tenuifolia seeds sampled from 2011 to 2014. TL = Trophic level; Abund-11 = abundance in the 2011 sample; Abund-12 = abundance in the 2012 sample; Abund-13 = abundance in the 2013 sample; Abund-14 = abundance in the 2014 sample.

Species	TL	Abund-11	Abund-12	Abund-13	Abund-14	Total abundance
Merobruchus terani	TL2	315	96	157	306	874
Stator maculatopygus	TL2	67	3	24	68	162
Allorhogas sp.	TL2	62	55	145	312	574
*Crematogaste*r sp.	TL2	7	0	1	4	12
Myrmelachista ruskii	TL2	34	2	16	0	52
Myrmelachista gallicola	TL2	0	6	4	5	15
Myrmelachista catharinae	TL2	0	13	21	0	34

Species	TL	Abund-11	Abund-12	Abund-13	Abund-14	Total abundance
Laemophoeidae	TL2	4	0	4	2	10
Lepidoptera	TL2	6	5	15	2	28
Cecidomyiidae	TL2	0	0	0	1	1
Sciaridae	TL2	0	7	7	0	14
Bracon sp.	TL3	0	0	0	30	30
Eurytoma sp. 1	TL3	13	8	44	56	121
Eurytoma sp. 2	TL3	4	3	7	1	15
Eurytoma sp. 3	TL3	2	3	6	7	18
*Eudecato*ma sp.	TL3	0	0	5	22	27
Horismenus sp.	TL3	65	29	57	109	260
Chelonus sp.	TL3	0	1	1	0	2
Lyrcus sp.	TL3	0	0	6	4	10
Eupelmus sp.	TL3	1	6	0	2	9
Phylloxeroxenus sp.	TL3	6	1	0	0	7
Paracrias sp.	TL3	0	2	0	89	91
Goniozus sp.	TL3	0	3	0	0	3
Heterospilus sp.	TL3	2	0	0	0	2
Aprostocetus sp.	TL4	1	0	0	0	1
Brasema sp.	TL4	3	0	0	1	4

3.8 Supplementary Material

Table ST3.1 Areas and sampling sites sampled along four years

Area*	Sampling sites sampled in each year			
	2011	**2012**	**2013**	**2014**
Ae	Ae-1, Ae-2, Ae-3	Ae-1, Ae-2	Ae-1, Ae-2	Ae-1, Ae-2
La	La-1	La-1	La-1	La-1
Lu	Lu-1, Lu-2,Lu-3	Lu-1, Lu-2,Lu-3, Lu -4	Lu-1, Lu-2,Lu-3, Lu -4	Lu-1, Lu-2,Lu-3, Lu -4

***** More precisely Ae area was 6.350 km distant from La, which was 24.000 km distant from Lu which was 32.475 km distant from Ae area

Table ST3.2 Sampling sites GPS (Global Position System) coordinates

Sampling sites	GPS point
Ae-1	21°14'4.57" S - 44°57'6.38" W
Ae-2	21°14'5.71" S - 44°57'8.66" W
Ae-3	21°14'7.87" S - 44°58'0.06" W
La-1	21°18'3.46" S - 44°58'0.53" W
Lu-1	21°31'1.36" S - 44°53'1.78" W
Lu-2	21°31'5.13" S - 44°52'6.32" W
Lu-3	21°31'5.31" S - 44°52'3.84" W
Lu-4	21°41'9.88" S - 44°96'7.18" W

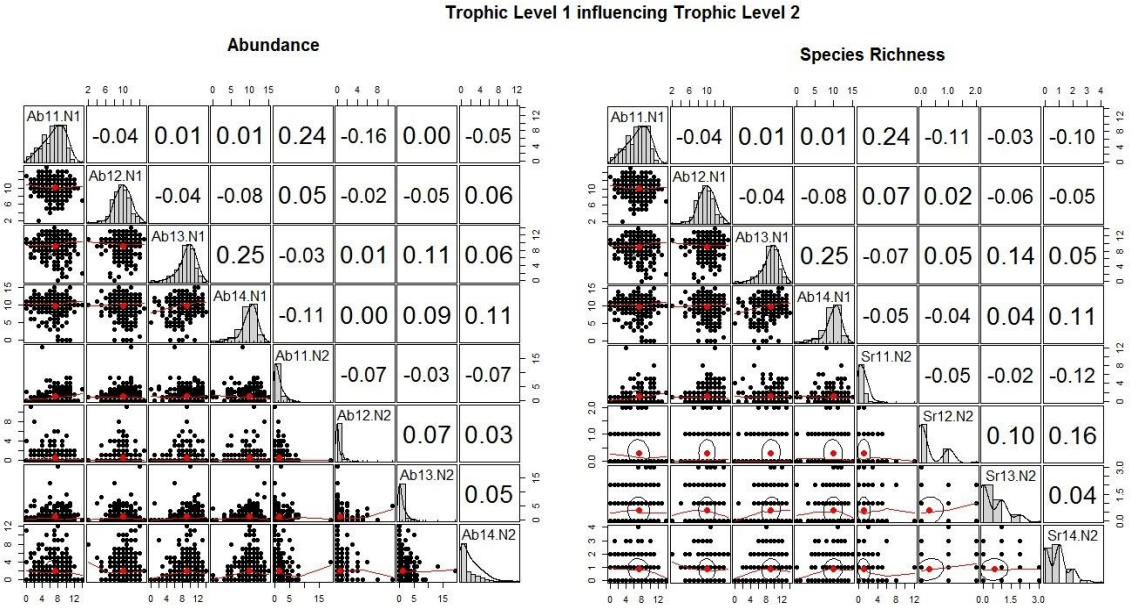


Figure SF3.1: Correlation analyses among and within each trophic level. Abundance and Richness from trophic level one influencing trophic level two. Ab x N y: Abundance year x trophic level y; Sr x N y: Species richness in year x on trophic level y. As an example, Ab11N1: Abundance in year 2011 on trophic level one (resource quantity measure as the number of total seeds). There is no correlation between the abundance and richness from trophic levels in one year and the year behind.

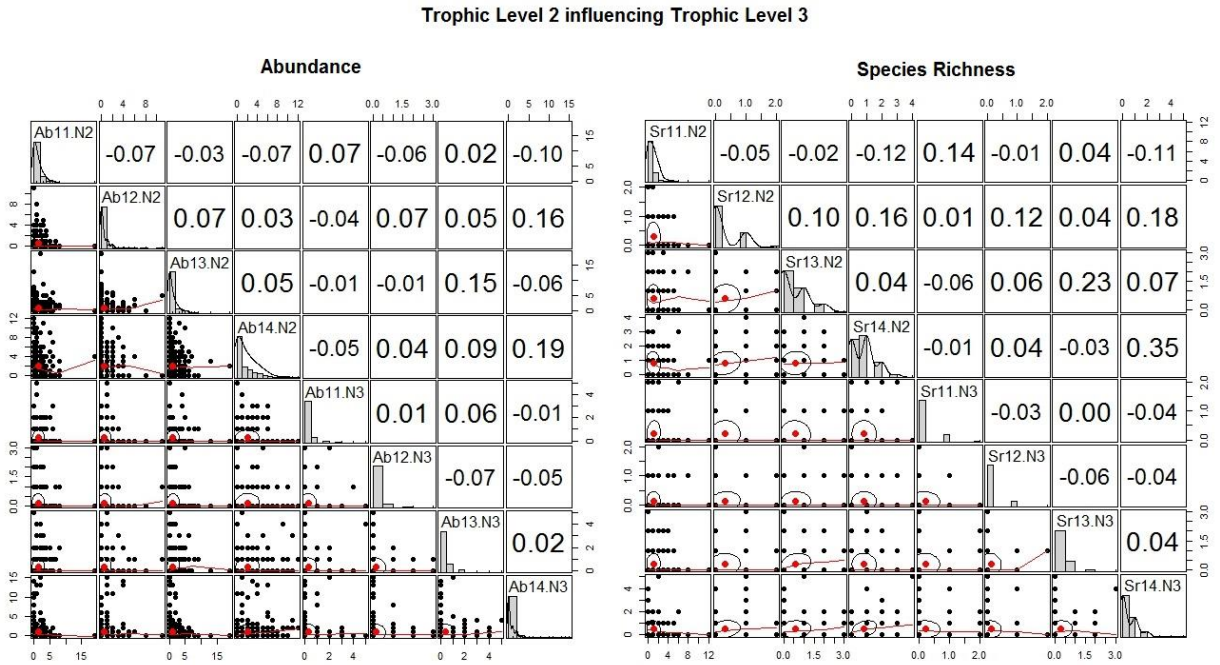


Figure SF3.2: Correlation analyses among and within each trophic level. Abundance and Richness from trophic level two influencing trophic level three. Ab x N y: Abundance year x trophic level y; Sr x N y: Species richness in year x on trophic level y. As an example, Ab11N2: Abundance in year 2011 on trophic level two (herbivores abundance). There is no correlation between the abundance and richness from trophic levels in one year and the year behind.

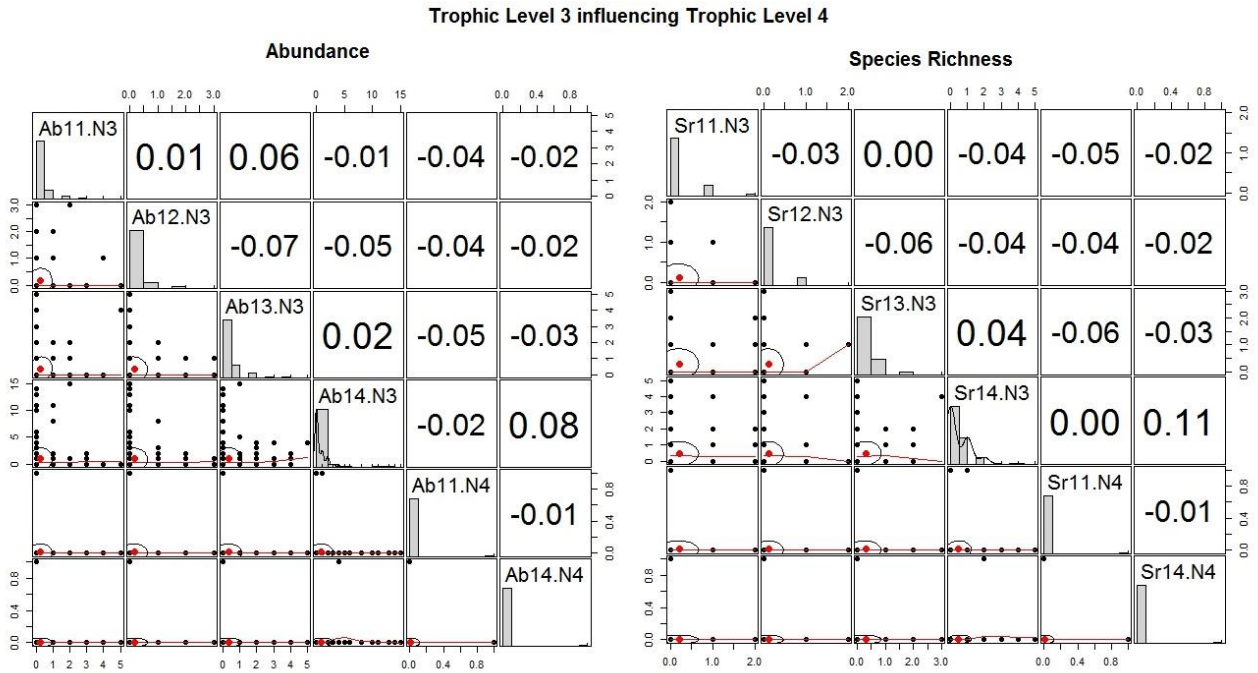


Figure SF3.3: Correlation analyses among and within each trophic level. Abundance and Richness from trophic level three influencing trophic level four. Ab x N y: Abundance year x trophic level y; Sr x N y: Species richness in year x on trophic level y. As an example, Ab11N3: Abundance in year 2011 on trophic level three (parasitoid abundance). There is no correlation between the abundance and richness from trophic levels in one year and the year behind.

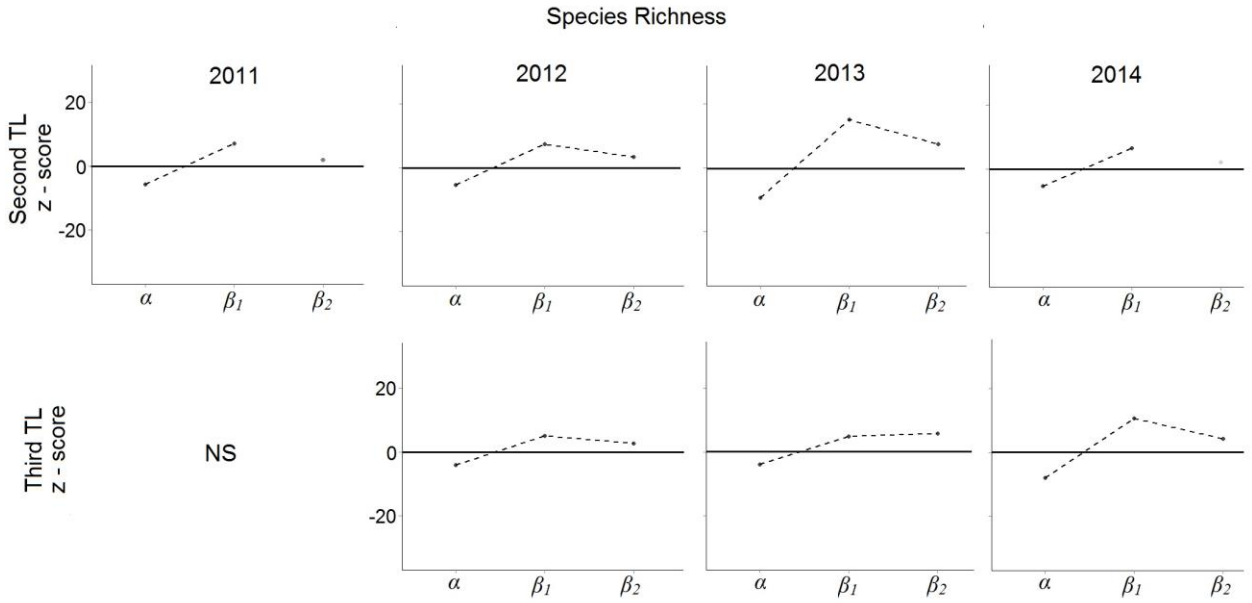


Figure SF3.4: Results from null model analyses of multiplicative partitioning diversity across scales in the second and third trophic levels associated with *S. tenuifolia* seeds according to Species Richness. Hierarchical components: α= diversity within sampling sites (n = 8); β_1= diversity between sampling sites; and β_2= diversity between areas (n = 3). The magnitude of deviation of each diversity component from the null expectation is illustrated by the z-score (=observed value – mean simulated values /SD simulated values). Black dots above and below the horizontal straight line represent larger and smaller contributions than expected by chance, respectively. "NS" indicate non-significant values. Statistical significance is based on the difference at 0.05 level between observed and simulated values obtained by 10,000 randomizations.

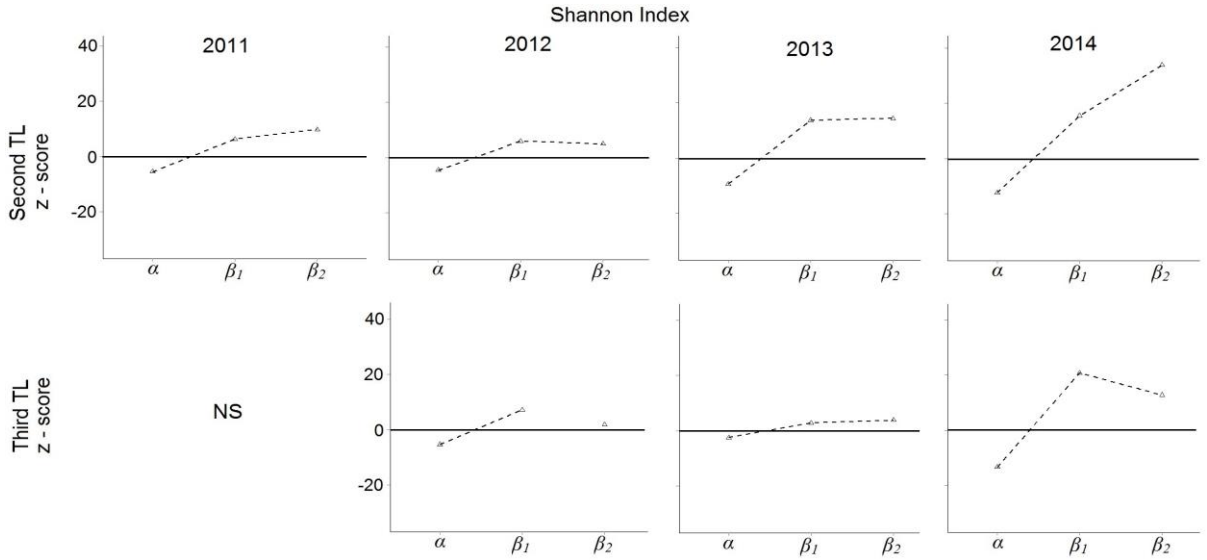


Figure SF3.5: Results from null model analyses of multiplicative partitioning diversity across scales in the second and third trophic levels associated with *S. tenuifolia* seeds according to Shannon Index. Hierarchical components: α= diversity within sampling sites (n = 8); β_1= diversity between sampling sites; and β_2= diversity between areas (n = 3). The magnitude of deviation of each diversity component from the null expectation is illustrated by the z-score (=observed value – mean simulated values/SD simulated values). Black dots above and below the horizontal straight line represent larger and smaller contributions than expected by chance, respectively. "NS" indicate non-significant values. Statistical significance is based on the difference at 0.05 level between observed and simulated values obtained by 10,000 randomizations.

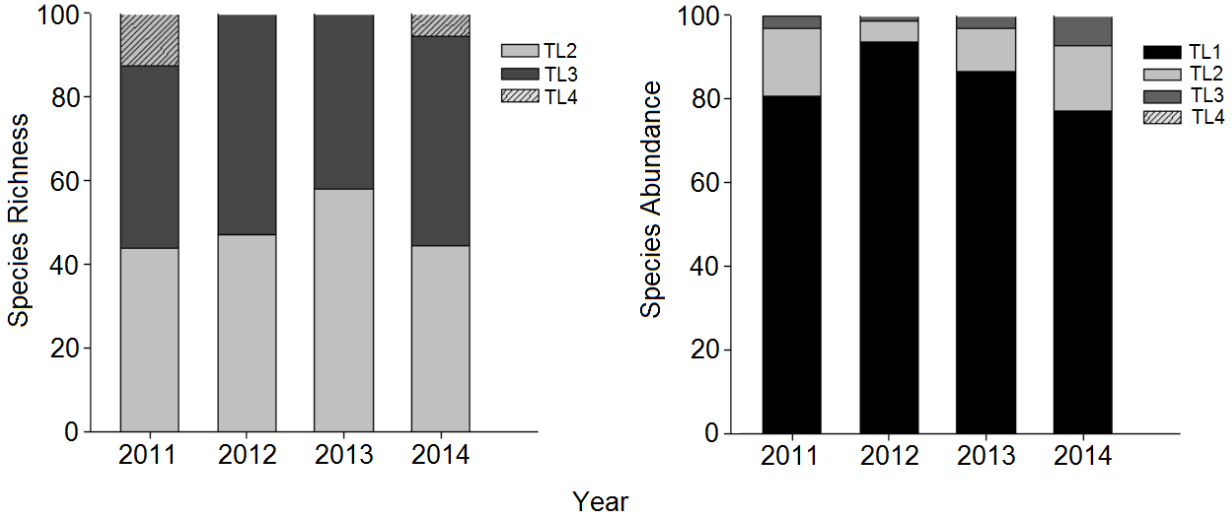


Figure SF3.6: The abundance and species richness of each trophic level in each year. TL1: First trophic level; TL2: Second trophic level; TL3: Third trophic level and TL4: Fourth trophic level. TL4 did not appear in the figure B because it accounts for less than 2% of the total abundance in the web.

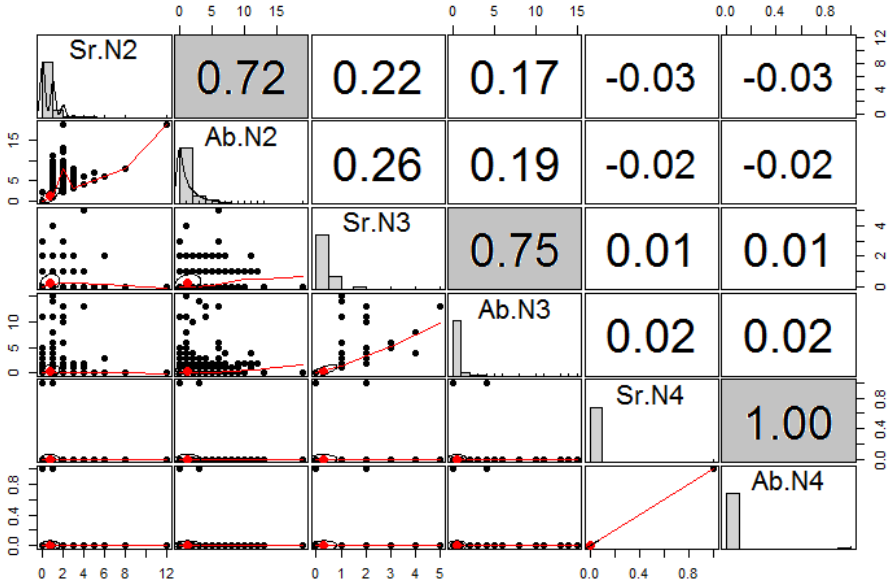


Figure SF3.7: Correlation analyses demonstrating a strong positive relationship (light grey squares) between species richness and abundance for each trophic level. Sr.Ny: Species richness on trophic level y and Ab.Ny: Abundance on trophic level y. N2: second trophic level, N3: third trophic level and N4: fourth trophic level.

4. GENERAL CONCLUSION

In Chapter 1 we identified the species associated with *Senegalia tenuifolia* plant and the interactions among them. After describe the source food web we check for the data robustness, and we found that the sampled species richness was sufficient to represent the source food web. Besides, we accurately proposed a cost-efficiently methodology to sample each trophic level of a source food web, and we believe this methodology can also be extended to community food web sampling. Then, in Chapter 2 we demonstrated based on this source food web that only the herbivore trophic level influence its trophic level above (parasitoids) while herbivores abundances are not influenced by resource quantity and hyperparasitoids seemed not to control the parasitoid community. After, we found that herbivore and parasitoid communities presented different shifts in abundance and richness over the time. Moreover, the diversity distribution patterns vary along years for parasitoid trophic levels what suggested that high herbivore abundance and richness mediate the changing in diversity distribution pattern for parasitoids.

In general, the parasitoid level seemed to present different outcomes compared to the other trophic levels. For example, the parasitoid level needed a higher sample effort to gather most of its species richness and also it presented a different diversity pattern along years. It is interesting to note that this difference could be due to differences in the feeding specialization. Although we cannot infer about feeding specialization and insect origin in parasitoids and herbivores because the parasitoid species are identified only at genus level and the herbivore biology is not well-known, herbivores are more specialized in a determined plant family or species while parasitoids tend to be more generalist. Thus, herbivores tend to be frequently linked to the resource being easier to sample most of its species richness and to determine its diversity pattern.

Finally, the dataset provided here can also be incorporated to the food web database to help to improve food web research.