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"Trophobiotic associations between ants and honeydew producers in Eastern Austria"

Verfasser

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Abstract

Field data of 239 associations between 13 ant and 15 honeydew producer species, forming 37 different trophobiotic association pairs, was collated in two regions in Eastern Austria. Data were assembled in four types of habitats which spanned the gradient from near-natural forest edges to grassland under land use. Species of *Formica* and *Lasius* were by far the most important attendants, while *Aphis fabae* and *Chaitophorus capreae* were the commonest trophobionts. The regions Thayatal and Donauauen showed a significant difference in their assembly of trophobiotic interactions. ANOVA calculations showed a strong habitat and seasonal effect with regard to the abundance of associations in hedgerows and dry semi-natural grassland. By means of a recently developed Interaction Diversity Index this difference could be allotted to the trophobionts rather than ants. Further this index well depicted a land use gradient from near-natural to more strongly impacted habitats. Measured with the classical Shannon Wiener Diversity Index as well as the Interaction Diversity Index, trophobiotic interactions tended to be more specialized and less diverse in more strongly impacted habitats, whereas these associations were less predictable and more diverse in natural habitats. The Interaction Diversity Index as quantitative tool may turn out helpful for assessing effects of land use on biotic networks, such as trophobiotic interactions.

Zusammenfassung

Im Rahmen meiner Diplomarbeit habe ich Daten zu 239 Assoziationen zwischen 13 Ameisen- und 15 Pflanzensaugerspezies, die zusammen 37 verschieden trophobiotische Assoziationspaare bildeten, gesammelt. Die Freilanderhebungen wurden in Ostösterreich (Nationalparks Donauauen und Thayatal) durchgeführt. Die untersuchten Habitate wurden in 4 Typen eingeteilt (von naturnahen Waldrändern bis zu Ackerrandstreifen), welche unterschiedliche Intensitäten der Landnutzung widerspiegeln.

Die bei weitem am häufigsten angetroffenen involvierten Ameisenspezies waren den Gattungen *Formica* und *Lasius* zuzuordnen, während auf der Seite der Trophobionten *Aphis fabae* und *Chaitophorus capreae* dominierten. Die zwei untersuchten Gebiete zeigten einen signifikanten Unterschied in der Anordnung ihrer trophobiotischen Interaktionen. ANOVA-Berechnungen zeigten sowohl einen starken Habitat- als auch Saisoneffekt bezüglich der Häufigkeit trophobiotischer Interaktionen, und zwar in Waldrandlebensräumen, Hecken und

in Halbtrockenrasen. Mit Hilfe eines neu entwickelten Interaktions-Diversitäts-Indexes (IDI) konnten diese Effekte eher den Trophobionten als den Ameisen zugerechnet werden. Weiters konnte durch diesen Index der Landnutzungsgradient von naturnahen zu stärker beeinflussten Habitaten abgebildet werden. Berechungen mit dem klassischen Shannon-Wiener-Index als auch mit dem neuen IDI zeigten einen Trend zu höherer Spezialisierung und geringerer Diversität von trophobiotischen Interaktionen in stark beeinflussten Habitaten, während diese Interaktionen in naturnahen Habitaten eine geringere Vorhersagbarbarkeit und eine höhere Diversität zu erkennen gaben.

Der IDI stellte sich als Werkzeug zur quantitativen Analyse von Landnutzungsgradienten in biotischen Netzwerken, wie trophobiotische Interaktionen, als sehr hilfreich heraus und wird höchstwahrscheinlich in Zukunft an Bedeutung gewinnen.

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1. Introduction

Many ecological processes depend on interactions between organisms as upholders of ecosystem functions [1, 4, 16, 35, 37]. Intra- and interspecific competition for the same resources, or antagonistic interactions between trophic levels, are well known processes that profoundly shape structure and function of ecosystems whereas the potential of mutualistic interactions has traditionally been underestimated. Therefore our understanding of the precise functional and ecological consequences, when disturbing mutualistic interactions, remains scarce, but has recently gained strong interest (e.g. ALARM-project of the EU [3]). Practically all species of organisms on Earth are involved in interactions that can at least potentially be mutualistic [16, 18, 19]. Mutualistic associations require reciprocal contributions from both partners, and therefore they have frequently been analysed in the frameworks of either game theory or market models. In biological markets two species reciprocally trade commodities for which they have a need, but which they cannot obtain by themselves [30]. A special form of mutualism is the trophobiosis between ants and other insects that deliver nutritious nectar-like fluids. In this type of mutualistic interaction one insect species (the trophobiont) offers to its attendant ants some modified excrements (or more rarely glandular secretions) in exchange for protection against parasitoids and predators [34, 37].

In central Europe the most common form of trophobiosis is between ants and plant sucking herbivores that produce honeydew as faeces. Among the Homoptera, trophobiotic associations with ants are known from the Aphidina [34, 40], Coccina [34, 40], Psyllina [34, 40] and Cicadina [34, 40]. Trophobiosis with phytophagous Heteroptera is far less common [16, 34, 40]. In central Europe the quantitatively most important group of trophobionts are representatives of the Aphidina. However, from the 800 different aphid species that occur in central Europe only a fraction is known to engage in trophobiotic interactions, and among these the intensity of associations with ants varies from highly obligatory associations to very loose and ephemeral interactions [34].

Trophobiotic Homoptera posses special characters both in their physical appearance and behaviour which are related to their interactions with ants [4, 5, 6, 13, 16, 17, 37, 38]. At least four different behavioural adaptations are known:

1) Honeydew producers attended by ants tend to form a cluster which makes it more efficient for the ants to collect the honeydew. 2) The honeydew is only offered upon tactile

stimulation when an ant is attending the trophobiont. 3) Several subsequent generations of aphids usually stay on the same plant (monoecic) so that more generations are available to the ants. 4) Upon disturbance the honeydew producers emit alarm or defense pheromones which cause either a reaction of the plant suckers (dropping off the plant and escape from predators), or a defensive response of the attendant ants (they attack every enemy nearby). Despite these manifold adaptations of Homopterans to trophobiosis, it must be emphasized that these interactions are rarely host specific with regard to the ants involved. Moreover, many aphid species are but facultatively trophobiotic, i.e. they do not depend on ant attendance for survival and reproduction [34, 36].

Trophobiosis pays off for the honeydew producers as well as for the ants. Ants protect their trophobionts against natural enemies (e.g. Coccinellidae, Syrphidae [4, 34, 37]) and provide shelter during bad weather conditions by building pavilions [17, 34]. They also play an important role as “waste removers”. If honeydew producers do not get rid of their sticky excretions they run in danger of lethal contamination [17, 34]. Accumulation of honeydew increases the vulnerability against fungi [34]. Whole aphid aggregations may quickly become exterminated if they are not attended by ants [17, 34]. The ants, in turn, harvest honeydew which is a rich mixture of water soluble carbohydrates (mainly glucose, fructose, and sucrose), amino acids, proteins and mineral nutrients [7, 8, 9, 14]. By using honeydew as a resource ants acquire access to plant-derived nutrients at a low trophic level [6, 7, 8, 9, 15]. Honeydew is primarily an important source of energy, but can also contribute significantly to the nitrogen budget of ants [6, 7, 8, 9, 15]. A large colony of *Formica rufa* may gather as much as 50kg per year [34]. The quality of the honeydew largely depends on the host plant and on the plant organs the trophobionts are living on [4, 6, 12].

Even though trophobiotic associations between ants and aphids are long known from Europe [2], studies that report quantitative details as to the relative intimacy and diversity of these interactions on the community level are surprisingly scarce. Most studies focused on the costs and benefits of the association for selected pairs of partners [5], or on the contribution of trophobiosis to the nutrient budget of individual ant colonies [6, 7, 8, 9, 12]. For most European ant species [33, 34] as well as homopterans [40] it is at best known that they do, or do not, engage in trophobiosis. However, information as to the relative preference or importance of different attendant ants for certain trophobionts, or vice versa, is almost non-existent.

The principal aim of this study, therefore, was to establish quantitative base-line data about trophobiotic associations in eastern Austria. This data allows first inferences on the frequency, intensity and diversity of associations between individual ant and honeydew producer species, respectively. In addition, I set out to assess whether habitat structure and human land-use affect the diversity and intensity of trophobiotic associations. To achieve this goal, I performed field surveys along gradients of land-use in two different regions. Specifically, I address the following questions:

- Which ant and homopteran species engage in trophobiotic interactions in the two selected regions, and how frequently do the various association pairs occur?
- Are there differences in the specificity and intimacy of interactions between the various ant and trophobiont species?
- Are there more trophobiotic associations per unit area in the near-natural habitats than in habitats with stronger human impact (due to higher richness of host plants and more diverse structural conditions of the vegetation)?
- Do networks comprised of ant-homopteran associations change over the course of the season? Is there a seasonal pattern in resource use by ants (e.g. higher demand at the peak of the brood cycle)?
- Do these networks vary in diversity between regions or across habitat gradients?

2. Material and Methods

(a) Study areas

Sampling was conducted in two regions of eastern Austria. The first study area is situated in and nearby the national park Thayatal (north of Lower Austria, at the border to the Czech Republic). The region is situated in the boundary zone between the atlantic and pannonic climate type. Mean annual temperatures are about 10°C (in the year 2006 10°C, between May and August 18.4°C), and average rainfall amounts to just 328 mm (336.2 mm in the year 2006, 175.3 mm between May and August) [http:\\www.wetteronline.de]. The second study area is located east of Vienna, in the national park Donauauen near the Slovakian boarder. Here the climate is subcontinental: mean annual temperatures are 11°C (12.3°C in the year 2006, 18.4 °C between May and August) with an average rainfall of 610 mm (594.4 mm in the year 2006, 319.4 mm between May and August) [http:\\www.wetteronline.de]. In each of these regions I carried out sampling in four different habitat types: dry semi-natural grassland, edges of forests and hedges, open grassland, and edges of arable farmland (intensive land-use).

(b) Data collection

Samples were taken in the habitats by intensive visual examination of the above-ground vegetation at eight transects per habitat type. Each transect had an area of 5 m^2 (5m × 1m) and was randomly chosen within the respective habitat. Examination of an individual transect site took 25-40 min. During each sampling unit I carefully inspected the entire vegetation for the presence of trophobiotic associations. Voucher specimens of ants and honeydew producers were taken with exhaustors (Ø 3 cm and 5 cm) and immediately placed in 70% ethanol. Depending on the total number of individuals involved, several worker ants as well as honeydew producers were removed. Insects were later identified to species level using the keys of Thieme & Müller [40] for aphids, and Schlick-Steiner et al. [33] and Seifert [34] for ants.

In addition to the species combination of the trophobiotic partners, I recorded the number of individuals of both species involved, the frequency of the association in the examined transect, the intensity of the interaction, the host plant species, and the position on the host plant (stem, inflorescence, leaf). Each transect site was visited five times between May and August in the year 2006.

(c) Data processing and statistical analysis

I considered every encountered homogeneous trophobiotic association per transect site as one data point. Hence, two associations between the same pair of ant × aphid were not considered independent, if they were encountered on the same host plant individual, or on plants immediately adjacent to another. Field data were collated into frequency tables in a spreadsheet program where the rows contained the ant × aphid combination and the columns the sites.

Based on these data I conducted two main types of analysis. First I compared the frequency of trophobiotic associations across regions, habitat types, and temporal replicates of surveys. These analyses were performed using standard ANOVA approaches in the software package STATISTICA 7.0 (Statsoft Inc.). Second I analysed the diversity of trophobiotic associations with standard diversity indexes (Shannon-Wiener diversity) as well as with a newly developed model to quantify interactive diversities [10, 11]. In this latter model two indexes are of special importance. *d'* describes the degree of interactive specialization on the species level which can be used to analyze variation between networks, from the perspective of the ants as well as the aphids. The second measure H_2' describes the overall degree of specialization of associations in the entire network. These indexes can be used to characterize and compare different interaction webs. A unique feature of this novel statistical model is that it is far less dependent on sampling intensity and network size than earlier methods that have traditionally been used to quantify the strength of connections in multi-species interaction webs [10, 11].

3. Results

(a) Frequency distribution and identity of trophobiotic associations

Across all eastern Austrian study sites I recorded a total number of 239 associations between 13 ant species and 15 species of honeydew producers, comprising 37 pairs of interacting species [Table 1.1].

Table 1.1: Total numbers of encountered trophobiotic associations between ants and honeydew producer in eastern Austria in 2006.

	Anuraphis farfarae	*Aphis fabae*	*Aulacorthum solani*	*Brachycaudus cardui*	*Ceruraphis eriophori*	*Chaitophorus capreae*	*Dysaphis plantaginea*	*Hormaphis betulae*	*Idiocerus populi*	*Myzus ascalonicus*	*Myzus ornatus*	*Neophilaenus albipennis*	*Phyllaphis fagi*	*Pterocoma rufipes*	*Trioza* sp.		Total
Formica fuscocinerea										12							12
Formica cunicularia		9	1		1								2				13
Formica rufa						53								15			68
Formica sanguinea						7								3	3		13
Fromica transkaukasica						1											1
Lasius emarginatus	2	12		9	1	3			1		1	2				2	31
Lasius fuliginosus				14		17		1						9			41
Lasius neglectus						9			2	3							14
Lasius niger		7								8							15
Lasius platythorax		4			3												7
Lasius psammophilus		4							5								9
Tapinoma ambiguum		4	1		5		1										11
Tetramorium caespitum							4										4
										12							12
Total	2	40	2	23	10	90	5	1	8	23	1	2	2	27	3	2	

From these 239 interacting pairs 181 where encountered in the Thayatal region and just 58 in the Donauauen.

Table 1.2: Total numbers of encountered trophobiotic associations between ants and honeydew producers in the national park Thayatal.

	Myzus ascalonicus	*Trioza* sp.	*Aphis fabae*	*Brachycaudus cardui*	*Chaitophorus capreae*	*Dysaphis plantaginea*	*Hormaphis betulae*	*Idiocerus populi*	*Pterocoma rufipes*		Total
Formica fuscocinerea	12										12
Formica rufa					53				15		68
Formica sanguinea		3			7				3		13
Fromica transkaukasica					1						1
Lasius emarginatus			12	2	3			1			18
Lasius fuliginosus				14	17		1		9		41
Lasius neglectus	3				4			2			9
Lasius niger	3										3
Lasius platythorax			3								3
Lasius psammophilus			4					5			9
Tetramorium caespitum						4					4
	12										12
Total	18	3	19	16	85	4	1	8	27		

Table 1.3: Total numbers of encountered trophobiotic associations between ants and honeydew producers in the national park Donauauen.

	Anuraphis farfarae	*Aphis fabae*	*Aulacorthum solani*	*Brachycaudus cardui*	*Ceruraphis eriophori*	*Chaitophorus capreae*	*Dysaphis plantaginea*	*Myzus ornatus*	*Neophilaenus albipennis*	*Phyllaphis fagi*		Total
Formica cunicularia		9	1		1					2		13
Lasius emarginatus	2			7	1			1	2			13
Lasius neglectus						5						5
Lasius niger		7				5						12
Lasius platythorax		1			3							4
Tapinoma ambiguum		4	1		5		1					11
Total	2	21	2	7	10	10	1	1	2	2		

The trophobiotic ant species with the largest diversity of honeydew sources was *Lasius emarginatus* which I found in association with nine different partners. The honeydew producers *Chaitophorus capreae* and *Aphis fabae* formed trophobiotic associations with six

partners each. With 22 % of all observed trophobiotic interactions, *Formica rufa* attending *Chaitophorus capreae* was the dominant combination. Theses two species formed, with various partners, also about 66 % of all encountered trophobiotic associations. [Fig. 1.1] Overall, the diversity of the interaction pairs was quite low compared to similar studies (mainly due to just one recording period) [2].

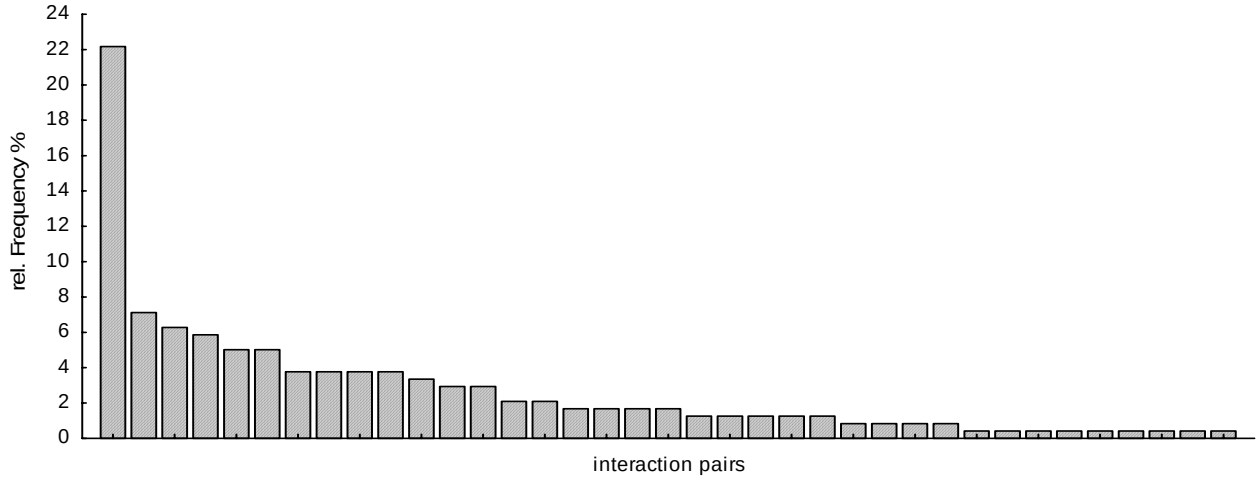

Fig. 1.1: Relative frequency distribution of eastern Austrian trophobiotic association pairs (total n=239; n interaction pairs = 37)

If viewed separately for the two study regions, interactions in the Thayatal area are again dominated by *F. rufa* attending *Ch. capreae* [29 % of the total number of recorded associations]. *Ch. capreae* alone accounted for 46 % of the honeydew producers here. The frequency distribution was particularly uneven: the four commonest trophobiotic association pairs represented over 50 % of the encountered interactions [Fig. 1.2].

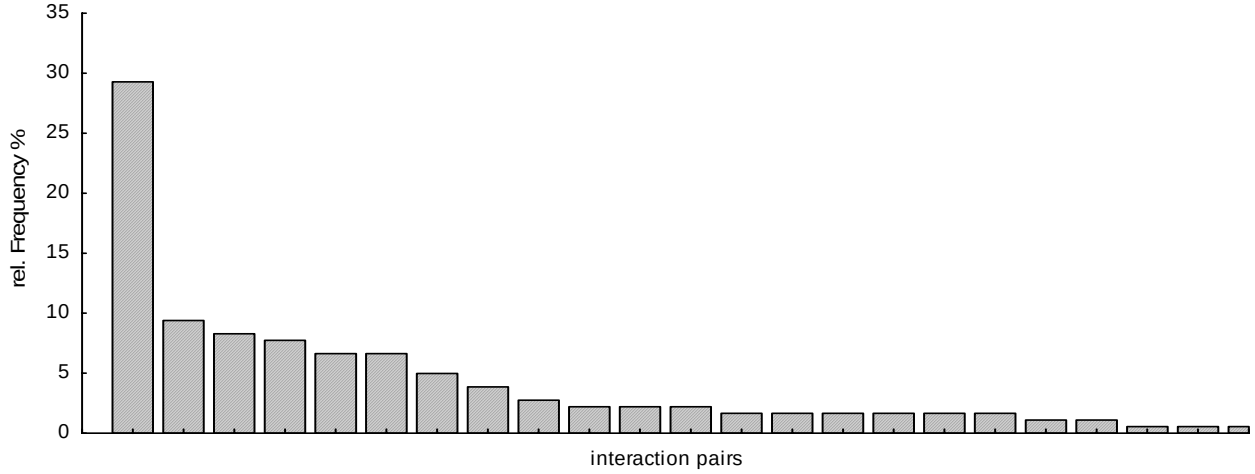

Fig. 1.2: Relative frequency distribution of national park Thayatal trophobiotic association pairs (total n=181; n interaction pairs = 23).

Here the diversity was high in respect of all encountered interaction pairs and especially in ants. In the Donauauen region the picture was quite different. The most prevalent trophobiotic ant species here were *Formica cunicularia* and *Lasius emarginatus* accounting for 44 % of all interaction pairs found. In 36 % of all associations the aphid partner was *Aphis fabae*. Nevertheless the distribution was more even with no outstandingly dominant interaction pair as in the Thayatal region [Fig. 1.3]. Diversity was lower in ant species but higher in honeydew producers. ´

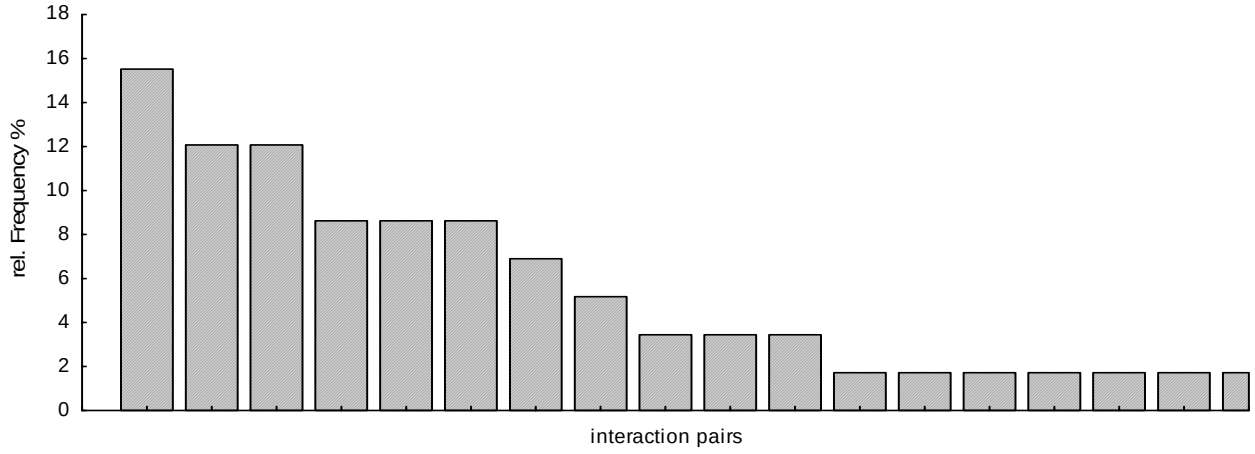

Fig. 1.3: Relative frequency distribution of national park Donauauen trophobiotic association pairs (total n = 58; n interaction pairs = 18).

I calculated a correlation to assess to what extent the trophobiotic associations in the two regions Thayatal and Donauauen consist of similar association pairs. This analysis revealed that the two regions are very complementary in their composition of trophobiotic ant-homopteran associations [Fig. 1.4].

Just four interacting pairs, all with *Lasius* as trophobiont, were in common between both regions. This strong complementarity underscores the faunistic dissimilarity of the examined regions with regard to both the ants and homopterans. As a rule, trophobiotic interaction pairs that were encountered frequently in one region were rare in the other.

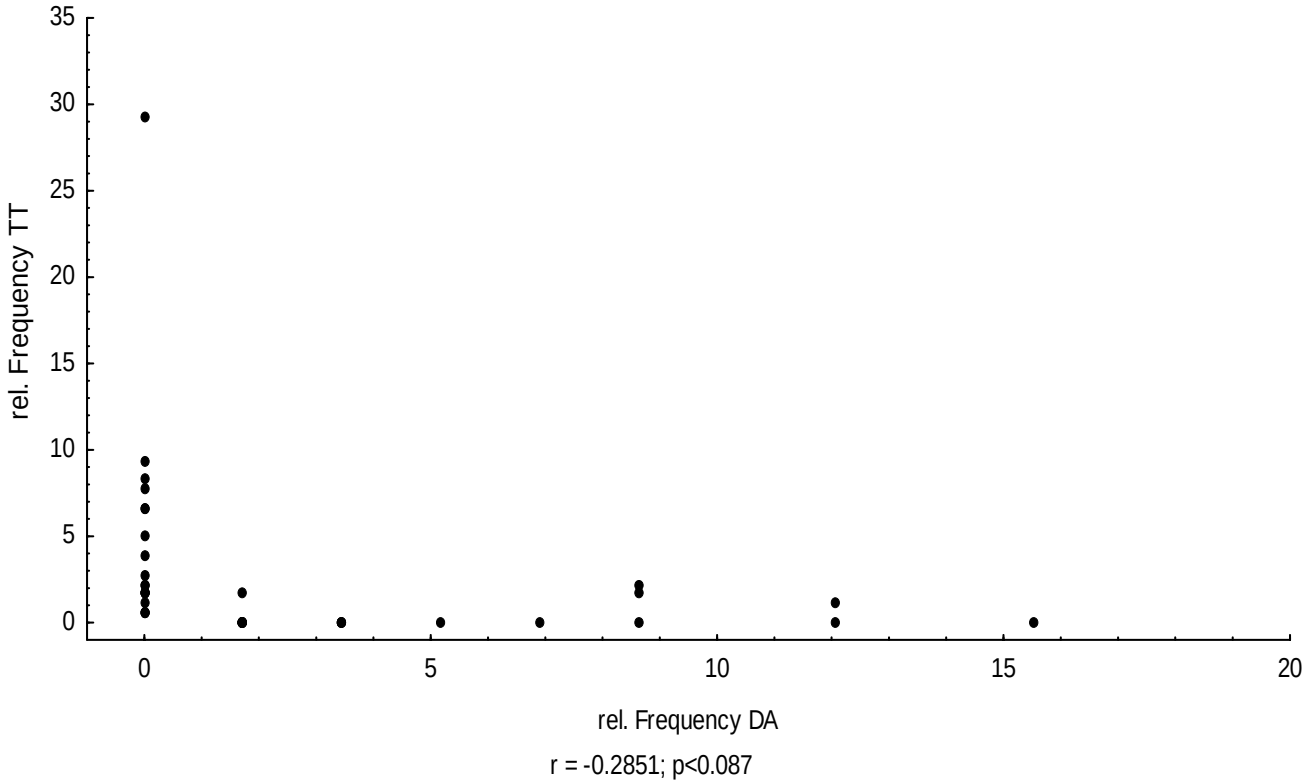

Fig. 1.4: Relation of frequencies of individual trophobiotic pairs in the national parks Thayatal (TT) and Donauauen (DA). Most interaction pairs were exclusive to either of the regions, and thus score as 0 on the first or second axis. *r* and *p* relate to Spearman rank correlation coefficient.

(b) Effects of habitat type and season on the frequency of trophobiotic associations

After this general view on the frequency and diversity of trophobiotic pairs, the next step was to examine the available data for potential habitat or seasonal effects. Accordingly, I partitioned my data according to time and habitat type, respectively. A strong habitat effect on the frequency of trophobiotic association was noted: these were more abundant along forests edges and hedgerows than in all other habitat types [Fig. 2.1].

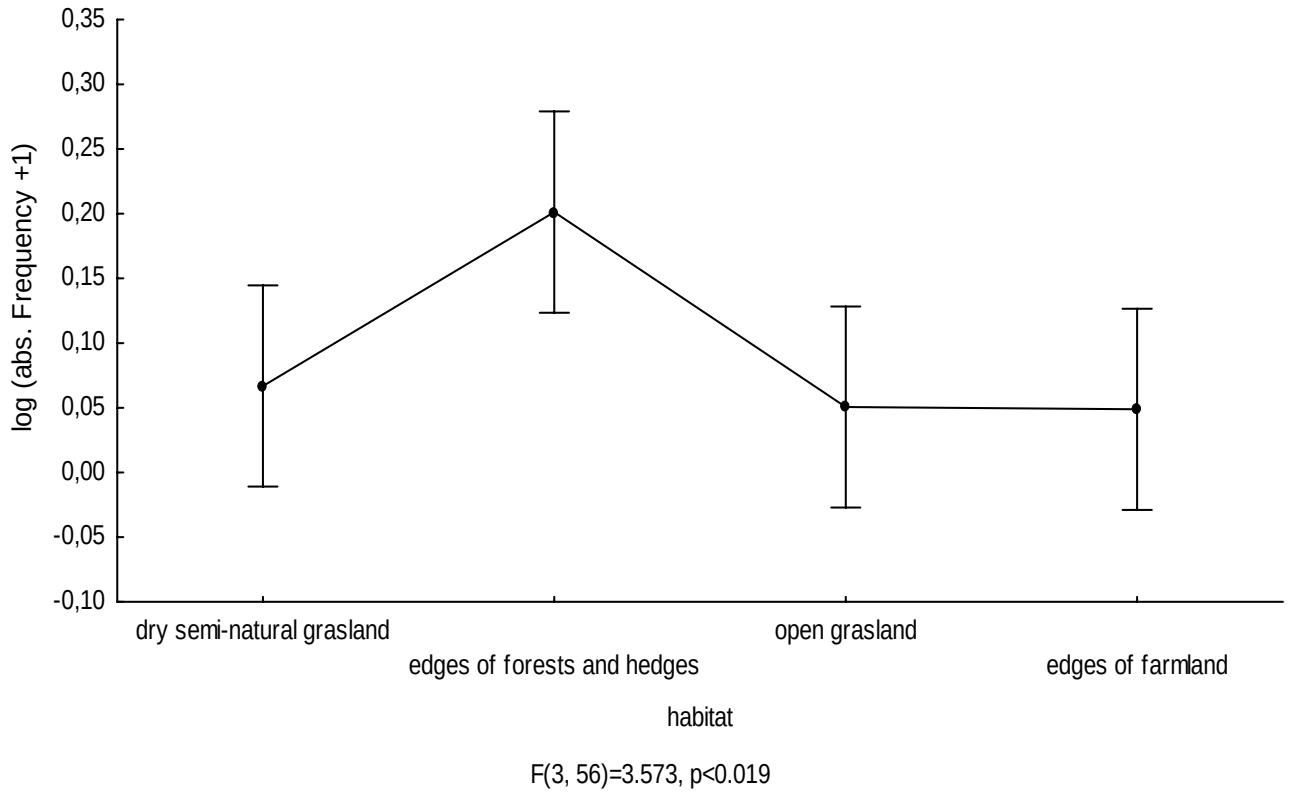

Fig. 2.1: Abundance of trophobiotic associations across four habitat types (data combined from all sites and regions). Given are means (dots) and 95% confidence intervals (whiskers). Included in the figure are the results of one-way ANOVA (*F*-statistics and associated *p*-value).

There was also a strong seasonal decline from April to August, followed by a but marginal increase towards the end of the recording period in August [Fig. 2.2]

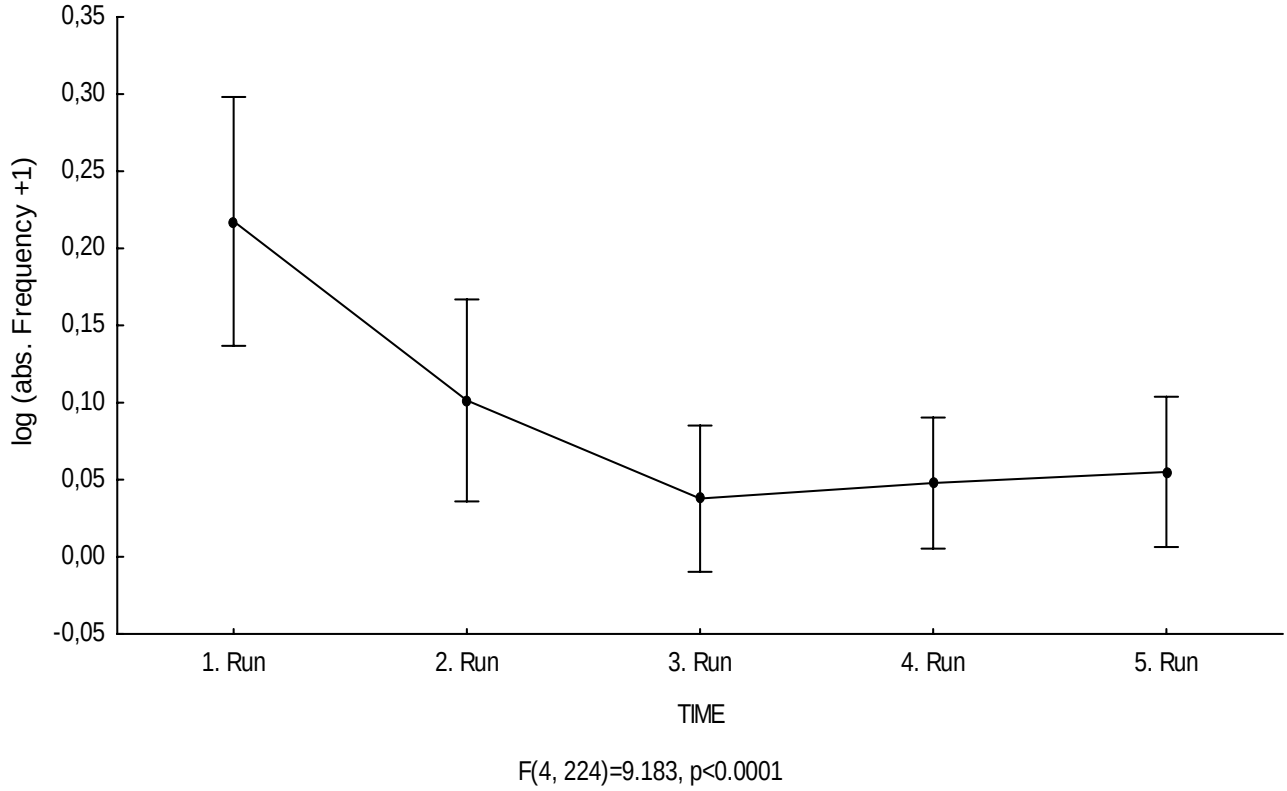

Fig. 2.2: Abundance of trophobiotic associations across five periods of data collection (data combined from all sites and regions). Given are means (dots) and 95% confidence intervals (whiskers). Included in the figure are the results of one-way ANOVA (*F*-statistics and associated *p*-value)

In the next step of analysis I combined habitat and time in a two-factorial design. A seasonal decline of trophobiosis frequency was distinct only in dry semi-natural grasslands and in forest edge/hedgerow habitats [Fig. 2.3]

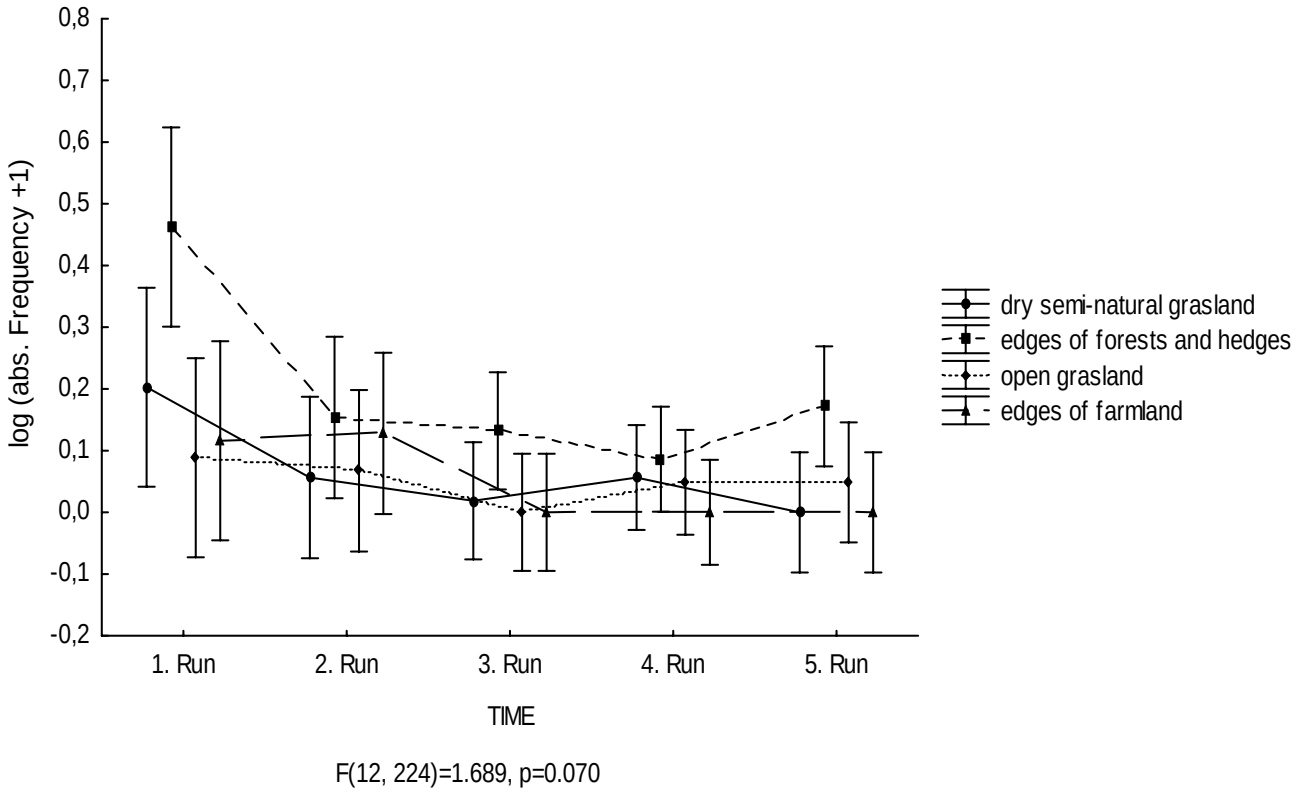

Fig. 2.3: Abundance of trophobiotic associations across four habitat types and five periods of data collection (data combined from all sites and regions). Given are means (dots) and 95% confidence intervals (whiskers). Included in the figure is the significance of the interaction term habitat × time in a two-way ANOVA (*F*-statistics and associated *p*-value)

Moreover the habitat differences noted above were pronounced only at the beginning of my recording period [April, May] and to some extent at the end [late July, August]. In a similar manner I also compared regions and habitat types in a two-factorial design. This analysis revealed that at forest edges and in hedgerows trophobiotic associations tended to be more common in the Thayatal region than in the Donauauen, whereas the opposite came true for dry semi-natural grasslands [Fig. 2.4]

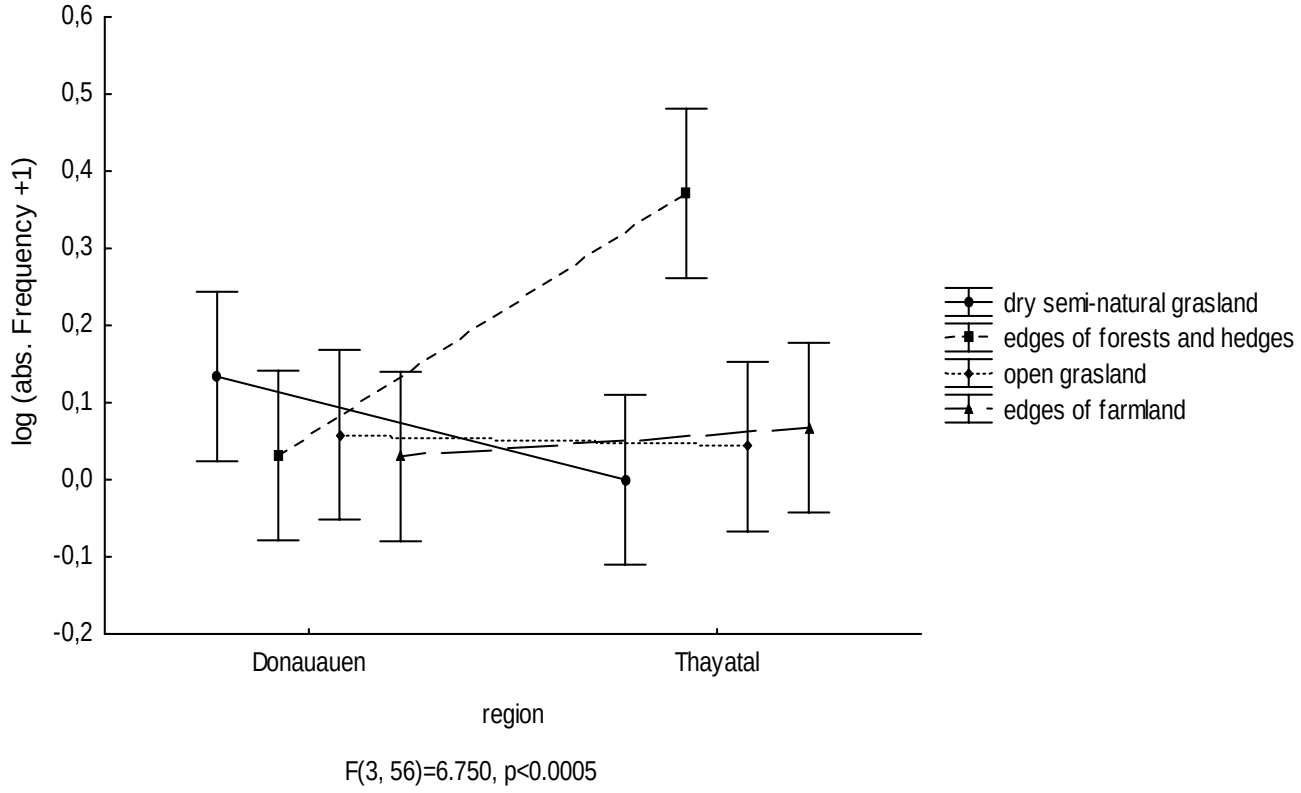

Fig. 2.4: Abundance of trophobiotic associations across four habitat types and two regions. Given are means (dots) and 95% confidence intervals (whiskers). Included in the figure is the significance of the interaction term habitat × region in a two-way ANOVA (*F*-statistics and associated *p*-value

When combining all these aspects in a three-factorial analysis, it became apparent that trophobiotic associations were equally rare in most habitats most of the time. Exceptions to that rule applied to the forest edges and hedges, which had more trophobiotic associations in the Thayatal region throughout the season, and to dry semi-natural grasslands that harboured more trophobiotic associations in the Donauauen, but only at the onset of my recording period [Fig. 2.5]

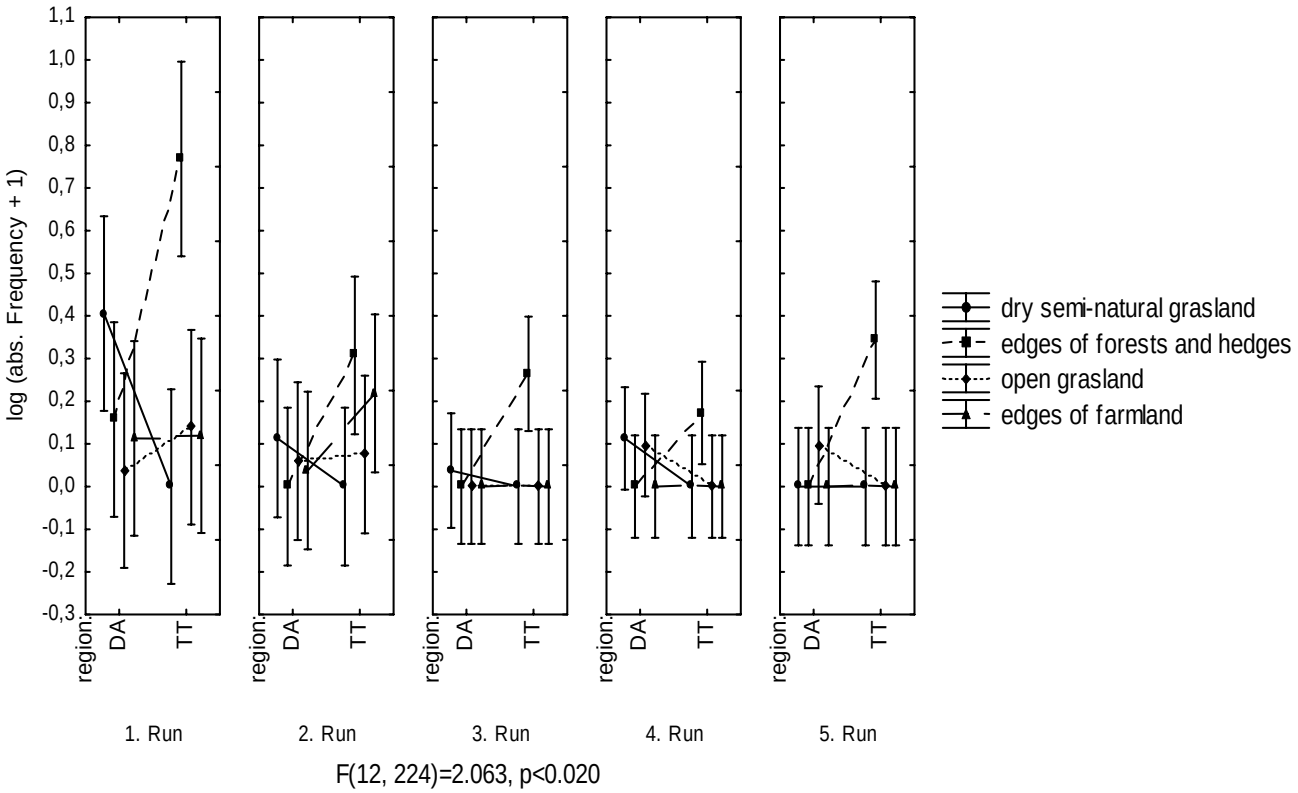

Fig. 2.5: Abundance of trophobiotic associations across five sampling rounds, four habitat types, and two regions. Given are means (dots) and 95% confidence intervals (whiskers). Included in the figure are the results of three-way ANOVA (*F*-statistics and associated *p*-value

Tab. 2: *F*-statistics and associated *p*-values of all conducted ANOVAs

	Factor	F	df	p
Fig. 2.1	Habitat type	3.573	3, 56	<0.019
Fig. 2.2	Time	9.183	4; 224	<0.0001
Fig. 2.3	Time	9.183	4; 224	<0.0001
	Habitat type	3.573	3, 56	<0.019
	Time × Habitat	1.689	12; 224	0.070
Fig. 2.4	Region	2.156	1; 56	0.148
	Habitat type	3.573	3, 56	<0.019
	Region × Habitat	6.750	3; 56	<0.0005
Fig. 2.5	Region	2.156	1; 56	0.148
	Habitat type	3.573	3, 56	<0.019
	Time	9.183	4; 224	<0.0001
	Region × Habitat	6.750	3, 56	<0.0005
	Region ×Time	0.681	4; 224	0.606
	Time × Habitat	1.689	12; 224	0.070
	Region × Habitat ×Time	2.063	12; 224	<0.020

(c) Diversity and specialization in ant-homopteran associations

One major aim of my survey was to assess possible differences in the diversity of ant-homopteran interactions across habitat types and regions in eastern Austria. Therefore I first compared the four habitat types, collating data from both study regions, using the classical Shannon-Wiener diversity index. Trophobiotic associations were most diverse in the edges of forests and hedges [Table 3].

Table 3: Shannon diversity of trophobiotic associations between ants and honeydew producers grouped according to habitat types. Habitat types, Shannon diversity index (H), theoretical maximum Shannon diversity index (H_{max}) and Evenness (E_H) are given.

	H	H_{max}	E_H
dry semi-natural grassland	**2.19**	**3.40**	**0.64**
edges of forests and hedges	**2.38**	**5.02**	**0.47**
open grassland	**1.19**	**3.33**	**0.36**
edges of farmland (intensive land use)	**1.45**	**3.37**	**0.43**

Dry semi-natural grassland showed slightly lower diversity compared to forest edges and hedgerows, whereas in more strongly impacted open habitats the diversity of ant-homopteran associations was lowest. The evenness values revealed another facet of the situation. Apparently the most dominant trophobiotic association system occurred in open grassland (E=0.36). Forest edges and hedgerows as well as edges of farmland had intermediate evenness values of about 0.45. The highest evenness was encountered in dry semi-natural grassland (E=0.64) indicating a lesser occurrence of dominant trophobiotic interaction pairs.

Since the traditional Shannon diversity index is very sensitive to sampling effects and differences in overall network size, I also applied the newly developed generalizations of this measure for biotic networks to my data on trophobiotic associations. The results are summarized in Tables 4.1-4.3.

Table 4.1: Results of the interaction diversity network analysis. Given are total numbers of encountered species (ants, honeydew producers), total interactions (*m*), weighted mean specialization of ants and honeydew producers (*d*' ants, *d*' honeydew producers) and overall specialization (H_2').

	Ants	Honeydew Producers	*m*	*d*' Ants	*d*' Honeydew Producers	H_2'
Region						
Total Eastern Austria	13	15	239	0.48	0.53	0.58
NP Thayatal	11	9	181	0.47	0.47	0.62
NP Donauauen	6	10	58	0.50	0.47	0.61
Habitat						
dry semi-natural grassland	4	6	30	0.30	0.29	0.37
edges of forests and hedges	10	10	152	0.42	0.38	0.56
open grassland	3	3	28	0.31	0.32	0.79
edges of farmland (intensive land use)	4	3	29	0.67	0.61	0.91
Time						
1. Run	10	11	117	0.55	0.64	0.72
2. Run	8	8	48	0.71	0.76	0.74
3. Run	3	4	30	0.40	0.13	0.78
4. Run	4	3	17	0.66	0.89	1.00
5. Run	3	3	27	0.59	0.56	0.81
Other Networks						
Pollination Network Azoren Flores [31]	12	10	1139	0.46	0.50	0.53
Pollination Network Mauritius [31]	13	14	1512	0.25	0.19	0.38
Pollination Network Greenland Plants [28]	26	17	149	0.44	0.48	0.48
Trophobiotic Associations Vogelsberg, Germany[2]	18	56	247	0.26	0.15	0.18
Seed dispersal Network, West Malaysia [25]	51	33	448	0.28	0.27	0.24

Table 4.2: Interaction diversity of individual honeydew producers *d*' (weighted mean specialization)

Honeydew producers	*A. farfarae*	*A. fabae*	*A. solani*	*B. cardui*	*C. eriophori*	*C. capreae*	*D. plantaginea*	*H. betulae*	*I. populi*	*M. ascalonicus*	*M. ornatus*	*N. albipennis*	*P. fagi*	*P. rufipes*	*Trioza* sp.
Region															
Eastern Austria	0.32	0.59	0.37	0.51	0.58	0.50	0.86	0.12	0.58	0.82	0.19	0.19	0.53	0.30	0.61
NP Thayatal		0.81		0.49		0.35	1.00	0.12	0.69	0.88				0.21	0.69
NP Donauauen	0.32	0.35	0.27	0.74	0.45	0.75	0.00				0.10	0.10	0.21		
Habitat															
dry semi-natural grassland	0.61	0.33	0.00		0.20		0.00					0.61			
edges of forests and hedges		0.87		0.63		0.27	1.00	0.17	0.73	0.56			1.00	0.15	0.58
open grassland		0.44								0.24	1.00				
edges of farmland (intensive land use)		0.43		0.49		1.00									

Table 4.3: Interaction diversity *d*' of individual ant species in trophobiotic associations (weighted mean specialization)

Ants	*F. cunicularia*	*F. fuscocinerea*	*F. rufa*	*F. sanguinea*	*F. transkaukasica*	*L emarginatus*	*L. fuliginosus*	*L. neglectus*	*L. niger*	*L. platythorax*	*L. psammophilus*	*T. ambiguum*	*T. caespitum*
Regions													
Eastern Austria	0.55	0.77	0.56	0.33	0.00	0.38	0.36	0.22	0.48	0.45	0.59	0.54	0.94
NP Thayatal		0.85	0.50	0.18	0.00	0.48	0.37	0.46	0.50	0.49	0.73		1.00
NP Donauauen	0.45					0.87		0.69	0.37	0.31		0.30	
Habitats													
dry semi-natural grassland	0.34					0.70				0.11		0.15	
edges of forests and hedges	1.00		0.41	0.22	0.00	0.40	0.40	0.29		0.73	0.85		1.00
open grassland		0.38				1.00			0.20				
edges of farmland (intensive land use)	0.00					0.60	1.00				0.44		

A comparison between the two study regions revealed no differences in the overall specialization of trophobiotic association networks between the Thayatal and the Donauauen. In both regions there was a tendency towards overall specialization of the networks, with H_2' values around 0.60. At the level of interacting partner guilds, median values of *d*' were also quite similar between regions, as well as for both sides of the interaction. Ants and honeydew producers both had *d*' values close to 0.50, indicating but moderate specialization. In contrast to this homogeneity across regions, I observed a strong habitat effect on interactive diversity and specialization. The H_2' measure depicted the gradient of human intervention in the habitats. There was a clear trend towards unpredictability and high diversity in natural habitats (dry semi-natural grassland H_2'=0.37), and to predictability and low interaction diversity in impacted areas (edges of farmland H_2'=0.91). Interaction diversity also changed over the season. H_2' tended to increase over the summer, i.e. networks became more selective, and only at the end of the season a decline were again observed. In a complementary approach I also compared the degree of partner specialization between regions and habitats for individual ant and honeydew producer species. The overall low sample sizes could be responsible for the results. Some species showed different specialization degrees in different habitats. *Aphis fabae* e.g. seems to be a generalistic species in dry semi-natural grasslands (*d*'=0.33) whereas in edges of forests and hedges it has a narrow range of ant attendants (*d*'=0.87). Also some ant species show this phenomenon. *Lasius emarginatus* was highly opportunistic in edges of forests and hedges (*d*'=0.4), but had just one trophobiont (the aphid *Myzus ornatus*) in open grassland (*d*'=1).

4. Discussion

(a) General features of ants and their trophobionts in Eastern Austria

My results confirm that the ecologically most dominant ant genera in Central Europe, *Lasius* and *Formica* [33, 34], are also by far the most dominant in forming trophobiotic associations in Eastern Austria. Interestingly, however, the frequency distribution of trophobiotic association pairs differed strongly between the two study regions Thayatal and Donauauen. These differences, firstly, reflect the variation in ant communities, with mound-building *Formica* species being highly prevalent in forested habitats at higher altitudes and with cooler climate (Thayatal), as opposed to lowland sites dominated by *Lasius* spp. (as in the Donauauen). Secondly, also the floristic differences may play a role in shaping different trophobiont communities, since the host plants determine which trophobiotic honeydew producers may occur at any given site. For example, trophobiotic interactions involving conifer-feeding aphids are naturally absent from lowland sites, whereas aphids with herbaceous host plants are less likely to be found in forested habitats with cooler climate.

In general the identity of the recorded trophobiotic associations confirms the current literature [34]. Ant species in the genera *Formica* and *Lasius* are known to cover much of their energy demand from honeydew through trophobiotic interactions and are by far the most important honeydew consumers in middle Europe. Also the trophobiont communities consisted, as was expected, of facultatively myremcophilous species like *Aphis fabae* (obligate trophobiotic aphid species were not likely to occur in my surveys because they e.g. feed at the roots of host plants and therefore were not covered by my recording methods). Therefore, despite the relatively small total number of recorded trophobiotic species, I assume that the major fraction of trophobiotic interaction pairs that may possibly occur in the two study regions (excluding trophobiotic interactions between ants and subterranean aphids) is represented in the data [33, 34].

Two interaction pairs deserve to be examined more closely for their relevance under a conservation perspective. The first case comprises the interaction pair of *Formica transkaukasica* with *Chaitophorus capreae*. *F. transkaukasica* is a very rare ant in Central Europe [34] and is scored as category 1 (in danger of extinction) in the Red Data Book for Lower Austria [33]. The preferred low-land habitats of this ant species are swamps and peat bogs [33, 34]; thus it was quite surprising to find this ant species participating in a

trophobiotic association in the conifer forest region of the national park Thayatal. Quite as surprising is also the trophobiont because *F. transkaukasica* is usually known to maintain trophobiotic relationships to root aphids, while zoophagy has been scored as being negligible [34]. The second interesting case is *L. neglectus* which formed trophobiotic associations with three different honeydew producers. *L. neglectus* is known to maintain intense trophobiotic associations with a wide variety of honeydew producers [34] and may establish big colonies which both can lead to damages in greenhouses and nature [34]. Through their intranidal reproduction and high degree of polygyny *L. neglectus* can spread explosively over newly conquered habitats replacing native ant species [34]. I found *L. neglectus* on several occasions in the national parks Donauauen and Thayatal which confirms their steady advance from eastern to middle Europe, and from urban into more rural habitats. Future investigations should monitor the resulting impact of *L. neglectus* on the ant diversity in the national parks and their vicinity.

(b) Frequency of trophobiotic interactions across habitat types and seasons

The frequency of trophobiotic associations between ants and honeydew producers varied across habitats and seasons (Figs. 2.1-2.5). More trophobiotic associations were found in the edge/hedgerow and dry semi-natural grassland habitats, especially at the onset of my recording period. Except at the beginning, and to a lesser extent at the end, of my recording period these effects were small because trophobiotic associations were generally rare. In the two-factorial analysis it became apparent that the seasonal effect was largely restricted to two habitat types: hedgerows in the national park Thayatal, and dry semi-natural grasslands in the national park Donauauen. In all other examined habitats there was little seasonal variation with regard to the occurrence of trophobiotic associations. Thus it is interesting to examine where these effects come from and why are they concentrated in just these two habitat types. Firstly, the overall seasonal decline in trophobiotic associations may be connected with the brood cycle of the ants. In spring all Central European ants need to gather as much energy as possible for raising their brood, thus using every food source without strong specialization. This process is expected to culminate with the production of alate sexuals, and energy demand should then decrease quickly after the swarming flight [4, 39]. Hence, it is not surprising to see that the abundance of trophobiotic association mirrors the variation in energy demand throughout the brood cycle.

Alternatively, the observed seasonal pattern may merely result from trophobiont abundance and availability. In April and May when plants start their growing period and are therefore

rich in nutrients, the abundance of honeydew producers is high [4]. In summer, especially during spells of dry weather, aphid abundance usually decreases [4]. At the season's end there is again a smaller peak in aphid density thus providing more possibilities for trophobiotic associations to be established. However, the way of data collection and analysis that I had chosen did not allow to conclusively deciding which of these two competing hypotheses (i.e. regulation by demand, or by availability) would better explain the observed temporal variation in the frequency of trophobiotic associations. If demand is the determining variable, strong opportunism in partner selection should be seen at times of high demand, whereas reciprocally at times of low demand increasing selectivity would be expected. On the other hand, if availability is crucial then only the most profitable sources should be used if there is sufficient scope for being selective, whereas opportunism should increase when resources become overall scarce. This problem I try to discuss below with the concept of offer and demand.

Trophobiotic communities in the two examined regions differed greatly with regard to their species and partner compositions [Figs. 1.4 and 2.4]. In the Thayatal, forest edges and hedgerows were the regionally optimal habitats with regard to ant and honeydew producer diversity, and thus also for the abundance and diversity of trophobiotic associations. Here the most important tropobiotic ants were *Formica rufa* and *Lasius fuliginosus* both of which are woodland ants; also the major trophobiotic aphid in the Thayatal, i.e. *Chaitophorus capreae,* feeds mostly on trees. Flood plain forests in the Donauauen, in contrast, would not offer as many favourable microhabitats because of the closed forest canopy, dense leaf litter and frequent flooding. In the Danube flood plains dry semi-natural grasslands were the more favourable habitat with regard to trophobiotic associations, with grassland ants such as *Lasius niger* and *Tapinoma ambiguum* being prevalent amongst ants, and *Aphis fabae* on bushes and herbs as trophobiont. Though the dry grasslands in the Thayatal are generally rich in diversity of different ant species (through method and selection of my data collection not assessed but observed), they are qualitatively suboptimal for trophobiotic ant-honeydew producer interactions as opposed to the ones in Donauauen.

I analysed the diversity of ant-trophobiont interactions with two different quantitative tools: the 'traditional' Shannon-Wiener diversity index and the recently developed methods to quantify interactive diversity (which is a scaled two-dimensional improvement of the Shannon measure: [10, 11]). I used both these indexes in order to compare their versatility. With the

help of these two analytical approaches I specifically intend to discuss two ideas: the concept of offer and demand throughout the season, and the effect of land-use and anthropogenic interference in habitats on trophobiotic associations.

(c) Regulation by offer or demand – insight from network diversities

To understand the seasonal effects observed it is necessary to realize the influence of ants and honeydew producers on the accumulation of trophobiotic associations separately. The question was whether the seasonal pattern is more strongly governed by the ants (through variation in their energy demand over the season), or by the honeydew producers (changes in the abundance or host change of the trophobionts). Therefore I calculated the specialization of individual ants and honeydew producers, respectively, using the interaction diversity *d'* value. As can be seen in Fig. 4.1 the honeydew producers had a much higher variation (min. 0.13, max. 0.89) over time than the ants (min. 0.40, max. 0.71). This observation suggests that the seasonal variation in the incidence and specialization of ant-honeydew producer-relationships is more strongly controlled by the availability of trophobionts rather than by the demand of ants as honeydew consumers. Obviously, the latter show rather similar and moderate levels of specialization throughout the season, whereas in the aphids the high level of opportunism as seen in spring and early summer is followed by more selective, and predictable, interactions during the driest time of the summer.

(d) Land-use gradient

Land use gradients are frequently analysed to draw conclusions about management decisions or concerning classification of habitats [27, 42]. Traditionally such analyses have been focused on individual species or species assemblages as "indicators", such as avian, mesopredator, and plant communities [29], arthropod communities [26] or termites [23]. It has commonly be observed that either species richness, or species composition, or the presence and/or abundance of selected target species respond in a predictable manner with increasing anthropogenic interference in habitats [23, 26, 27, 29]. However, for the functionality of ecosystems the integrity of interspecific interactions may be more relevant than the mere persistence of some (or all) component species. For example, mutualistic interactions are extremely widespread in all types of ecosystems and are often essential for their functioning, but the persistence of both partners in a system does not necessarily mean that their interaction also remains functional (e.g. mutualists might evolve into parasites which will then abandon their partners to live autonomously [32]).

Hence, measures for the integrity or diversity of mutualistic relationships may provide a more realistic figure by connecting two (or more) affected community modules together, thus providing deeper insight in effects of habitat disturbance. Therefore I applied the Interactive Diversity approach to express the degree of specialization for whole trophobiotic assemblages in a habitat type. Through the index of specialization degree it was possible to figure out the level of disturbance in habitats or regions. A direct comparison between the Shannon diversity *H*' and the novel H_2' measure revealed that the gradient in specialization emerged in a much more pronounced way using the network-wide index than with the traditional Shannon diversity *H*' and evenness *E* [Tab. 3, Tab. 4.1]. This can be explained by the lesser dependency of the new network measure from sample and network sizes. Overall, the more natural habitats showed a higher diversity as well as a more even distribution of trophobiotic associations than the more impacted habitats. Concomitantly, with increasing anthropogenic interference in the habitats the trophobiotic associations became more depauperate and predictable.

(e) Diversity of biotic networks – some conclusions and perspectives

The novel measures for interactive diversity proved to produce more accurate results than traditional diversity figures to analyze the effects of habitat disturbance on biotic networks. These results, that were obtained despite rather small sample sizes, suggest that the use of these newly developed tools should be recommended for assessing and monitoring purposes, especially when dealing with small (or highly variable) network sizes or with sparse data. It may also be valuable for the long-term management of conservation areas such as the national parks Thayatal and Donauauen to assess the diversity and integrity of interaction networks, rather than species communities, over longer periods of time.

Finally the two scale-independent network metrics *d*' and H_2' have the peculiar feature of allowing for quantitative comparisons between very different types of interactive networks (pollination networks, seed dispersal networks, trophobiotic networks). Even though the number of networks analyzed in that manner is still low, some first trends can be illustrated [Tab. 4.1]. Overall, biotic networks vary profoundly in their degree of species interactions and accordingly also in their overall specialization. Pollination Networks seem to have a generally higher specialization rate. This can be explained through the benefit for the plant to have specific pollinators and therefore a greater likelihood of reproductive success (generalization vs. specialization in plant pollinator systems, see [43])? On the other hand, seed dispersal

networks tend to a higher degree of generalisation, corresponding to a broader spectrum of seed dispersers (aggregated seed dispersal, see [22]).

A very interesting conclusion can be drawn from a comparison of the two outlined trophobiotic networks, the trophobiotic network from Vogelsberg and the trophobiotic network from Eastern Austria. The two networks vary considerably in their degree of specialization within the network and in their overall specialization. Whereas the Vogelsberg network shows asymmetrical network architecture (honeydew producers were far more numerous then attending ants), the Eastern Austrian on the contrary is more symmetric (small differences between honeydew producer richness and ant richness). This may suggest that network architecture (here shown as differences between d' of ants versus d' of honeydew producers) constrains the overall degree of specialization in a trophobiotic network.

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5. References

1. Bascompte J., Jordano P., Olesen J.M. (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance, Science 312: 431-433

2. Bauschmann G., Friedberg, Wenzel S. (1987) Erste Ergebnisse über trophobiotische Beziehungen zwischen Ameisen und Blattläusen (Hymenoptera:Formicidae; Homoptera: Aphidoidea) in Vogelsberg/Hessen, Hessische Faunistische Briefe 3: 41-47

3. Biesmeijer J.C., Roberts S.P.M., Reemer M., Ohlemuller R., Edwards M., Peeters T., Schaffers A.P., Potts S.G., Kleukers R., Thomas C.D., Settele J., and Kunin W.E. (2006): Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313: 351-354

4. Buckley R. (1987) Ant-plant-homopteran interactions, Advances in Ecological Research 16: 53-85

5. Blüthgen N. & Fiedler K. (2002) Interactions between weaver ants *Oecophylla smaragdina*, homopterans, trees and lianas in an Australian rain forest canopy, Journal of Animal Ecology 71: 793-801

6. Blüthgen N. & Fiedler K. (2004) Competition for composition: Lessons from nectar-feeding ant communities, Ecology 85: 1479-1485

7. Blüthgen N. & Fiedler K. (2004) Preferences for sugar and amino acids and their conditionality in a diverse nectar-feeding ant community, Journal of Animal Ecology 73: 155-166

8. Blüthgen N., Gebauer G. & Fiedler K. (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. Oecologia 137: 426-435

9. Blüthgen N., Gottsberger G. & Fiedler K. (2004) Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. Austral Ecology 29: 418-429

10. Blüthgen N., Menzel F., Blüthgen N. (2006) Measuring specialization in species interaction networks, BMC Ecology 6:9 doi: 10.1186/1472-6785-6-9

11. Blüthgen N., Menzel F., Hovestadt T., Fiala B., Blüthgen N. (2007) Specialization, Constraints and conflicting interests in mutualistic networks, Current Biology 17: 1-6

12. Blüthgen N., Stork N.E. & Fiedler K. (2004) Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. Oikos 106: 344-358

13. Buschinger A. (1999) Soziale Insekten. in Dettner K. & Peters W. (eds.) Lehrbuch der Entomologie, Gustav Fischer Verlag, Stuttgart

14. Cushman J.H. & Addicott J.F. (1989) Intra- and interspecific competition for mutualists: ants as a limited and limiting resource for aphids, Oecologia 79: 315-321

15. Daniels H., Gottsberger G. & Fiedler K. (2005) Nutrient composition of larval nectar secretions from three species of myrmecophilous butterflies, Journal of Chemical Ecology 31: 2805-2821

16. Delabie J.H.C. (2001) Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an overview, Neotropical Entomology 30: 501-516

17. Dumpert K. (1994), Das Sozialleben der Ameisen. 2. neubearb. Auflage, Verlag Paul Parey, Berlin & Hamburg

18. Eckloff W. (1978) Wechselbeziehung zwischen Pflanzenläusen und Ameisen, Biologie in unserer Zeit 2: 48-53

19. Fiedler K. (2001) Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography, Diversity and Distributions 7: 45-60

20. Fiedler K. (2006) Ant-associates of palaearctic lycaenid butterfly larvae (Hymenoptera: Formicidae; Lepidoptera: Lycaenidae) – a review, Myrmecologische Nachrichten 9: 77-87

21. Gamerith W. (1999) Donau-Auen: Naturreichtum im Nationalpark. Tyrolia Verlag, Innsbruck & Wien

22. Johnson S.D. & Steiner K.E. (2000) Generalization versus specialization in plant pollination systems, Trends in Ecology and Evolution 15: 140–143

23. Jones D.T., Susilo F.X., Bignell D.E., Hardiwinoto S., Gillison A.N., Eggleton P. (2003) Termite assemblage collapse along a land-use intensification gradient in lowland central Sumatra, Indonesia, Journal of Applied Ecology 40: 380-391

24. Kaspari M., Ward P.S. & Yuan M. (2004) Energy gradients and the geographic distribution of local ant diversity, Oecologia 140: 407-413

25. Kaufmann, E. (2002). Southeast Asian ant-gardens. PhD thesis, University of Frankfurt (Main), Frankfurt (Main), Germany

26. Klein A.M., Steffan-Dewenter I., Tscharntke T. (2002) Predator-prey ratios on cocoa along a land-use gradient in Indonesia, Biodiversity and Conservation 11: 683-693

27. Koleff P. & Gaston K.S. (2002) The relationship between local and regional species richness and spatial turnover; Global Ecology & Biogeography 11: 363-375

28. Lundgren R. & Olesen J.M (2005) The dense and highly connected world of Greenland's plants and their pollinators, Arctic, Antarctic and Alpine Research 37: 514-520

29. Maestas J. D., R. L. Knight R. L., Gilgert W.C. (2003) Biodiversity across a rural land-use gradient, Conservation Biology 17: 1425-1434

30. Noë, R. & Hammerstein P. (1995) Biological markets. Trends in Ecology & Evolution 10: 336-339

31. Olesen J.M., Eskildsen L.I., and Venkatasamy S. (2002). Invasion of pollination networks on oceanic islands: Importance of invader complexes and endemic super generalists, Diversity and Distributions 8: 181–192

32. Sachs J.L., Simms E.L. (2006) Pathways to mutualism breakdown, Trends in Ecology & Evolution 21: 585-592

33. Schlick-Steiner B.C., Steiner F.M., Schödl S. (2003) Rote Liste ausgewählter Tiergruppen Niederösterreichs – Ameisen (Hymenoptera: Formicidae). 1. Fassung 2003, Amt der NÖ Landesregierung, Abteilung Naturschutz, St. Pölten

34. Seifert B. (2007) Die Ameisen Mittel- und Nordeuropas, Lutra Verlags- und Vertriebsgesellschaft, Görlitz/Tauer

35. Stachowicz J.J. (2001) Mutualism, facilitation, and the structure of ecological communities, BioScience 51: 235-246

36. Stadler B., Dixon A.F.G. (1998) Why are obligate mutualistic interactions between aphids and ants so rare? Pp 271-278 in Nieto Nafria J.M. & Dixon A.F.G.(eds) Aphids in natural and managed ecosystems, Universidad de Leon, Spain

37. Stadler B., Dixon A.F.G. (2005) Ecology and evolution of aphid-ant-interactions, Annual Review of Ecology, Evolution and Systematics 36: 345-372

38. Stadler B., Fiedler K., Kawecki T.J. & Weisser W.W. (2001) Costs and benefits for phytophagous myrmecophiles: when ants are not always available, Oikos 92: 467-478

39. Stadler B., Kindlmann P., Šmilauer P. & Fiedler K. (2003) A comparative analysis of morphological and ecological characters of European aphids and lycaenids in relation to ant attendance, Oecologia 135: 422-430

40. Thieme T. & Müller F.P. (2005) Aphidina. Pp. 169-237 in Hannemann H.-J., Klausnitzer B. & Senglaub K. (eds.) Exkursionsfauna von Deutschland, vol. 2: Wirbellose – Insekten. 10. Auflage, Spektrum Akademischer Verlag, Heidelberg, Berlin

41. Tillberg C.V., McCarthy D.P., Dolezal A.G. & Suarez A.V. (2006) Measuring the trophic ecology of ants using stable isotopes, Insectes Sociaux 53: 65-69

42. Tylianakis J.M., Tscharntke T., Lewis O.T. (2007) Habitat modification alters the structure of tropical host-parasitoid food webs, N

43. Wheelwright N.T. & Orians G.H. (1982) Seed dispersal by animals: Contrasts with pollen dispersal, problems of terminology,and constraints on coevolution, The American Naturalist 119: 402–413