

# Tree diversity and nectar composition affect arthropod visitors on extrafloral nectaries in a diversity experiment

Michael Staab<sup>1,\*</sup>, Joel Methorst<sup>2</sup>, Jan Peters<sup>2</sup>, Nico Blüthgen<sup>3</sup> and Alexandra-Maria Klein<sup>1</sup>

<sup>1</sup> Faculty of Environment and Natural Resources, Chair of Nature Conservation and Landscape Ecology, University of Freiburg, Tennenbacher Straße 4, 79106 Freiburg, Germany

<sup>2</sup> Institute of Ecology, Leuphana University of Lüneburg, Scharnhorststraße 1, 21335 Lüneburg, Germany

<sup>3</sup> Ecological Network Group, Department of Biology, Technical University Darmstadt, Schnittspahnstraße 10, 64287 Darmstadt, Germany

\*Correspondence address. Chair of Nature Conservation and Landscape Ecology, Faculty of Environment and Natural Resources, University of Freiburg, Tennenbacher Straße 4, 79106 Freiburg, Germany. Tel: +49-761-203-67787; Fax: +49-761-203-3638; E-mail: michael.staab@nature.uni-freiburg.de

## Abstract

### Aims

Plants with extrafloral nectaries (EFNs) are common in tropical and subtropical habitats and, despite many other arthropods also forage for EFN, most studies solely focused on the defense mutualisms between EFN plants and ants. This study aims at a quantitative assessment of the entire arthropod community that visits EFN trees to compare visitor communities between different tree species to disentangle the mechanisms that may drive EFN visitor community composition. We also test for tree diversity effects on EFN visitors, as it is unknown if local tree species richness relates to the abundance and species richness of arthropods foraging for EFN.

### Methods

We sampled EFN-visiting arthropods in the experimental tree species richness gradient of the BEF-China Experiment, the currently largest forest diversity experiment in the world, and tested if tree species richness affects EFN visitors and if visitor community composition differs between EFN tree species. In a second step, we analyzed the EFN of *Ailanthus altissima* and *Triadica cochinchinensis*, the two EFN tree species with highest visitor abundance, for sugars and amino acids (AA) to test if tree species-specific differences in nectar chemistry translate to differing visitor communities. Lastly, we conducted a choice experiment using different artificial nectar solutions to test if nectar quality affects foraging decisions of ants, the most frequent EFN visitors in our study sites.

### Important Findings

EFN trees in young successional forests in subtropical South-East China are visited by a diverse assemblage of arthropods including ants, beetles, flies, and spiders. Albeit ants accounted for about 75% of all individuals, non-ant visitors were by far more species rich. Visitor abundance and species richness declined with increasing tree species richness, suggesting a resource dilution effect, because plots with more tree species had proportionally less EFN tree individuals and thus lower nectar availability. *Ailanthus altissima* and *T. cochinchinensis* were visited by different arthropods and their nectar had species-specific AA composition and sugar concentration, indicating that differences in visitors may, at least partly, be explained by differences in nectar chemistry. These findings are supported by the choice experiment, in which artificial nectars containing sugar solutions supplemented with essential AAs attracted more ants than pure sugar solutions or sugar solutions supplemented with non-essential AAs. Our results improve the understanding of the complex ecology of EFN trees, a plant life form that might be crucial for understanding how tree diversity influences patterns of tree growth in young successional tropical and subtropical forests.

**Keywords:** amino acids, BEF-China, choice experiment, formicidae, HPLC chemical analysis

Received: 4 September 2015, Revised: 27 January 2016, Accepted: 22 February 2016

## INTRODUCTION

Extrafloral nectaries (EFNs) are nectar-secreting glands outside reproductive plant organs and have fascinated biologists for a long time (Darwin 1876; reviewed by Heil 2015). With few exceptions such as gymnosperms and magnolids, EFNs evolved many times across the plant Tree of Life (Weber and Keeler 2013; Weber and Agrawal 2014). Being rare in temperate floras (Pemberton 1998), EFN plants are common in tropical and subtropical habitats from deserts to forests (Aranda-Rickert *et al.* 2014; Blüthgen and Reifennath 2003; Díaz-Castelazo *et al.* 2005; Fiala and Linsenmair 1995; Oliveira and Leitão-Filho 1987; So 2004).

EFN contains rich sugars and amino acids (AA) (Baker *et al.* 1978; Blüthgen *et al.* 2004a) that attracts ants, the dominant insects of forest ecosystems, (Blüthgen *et al.* 2004b), as well as other arthropods. The association between ants and EFN plants has been studied in great detail (reviewed by Bentley 1977; Heil and McKey 2003; Heil 2015): plants usually benefit from ants by reduced herbivore damage and increased reproduction (Trager *et al.* 2010); colony performances of at least some ant species benefit from EFN (Byk and Del-Claro 2011).

While ants are the most numerous EFN visitors, many other arthropod taxa such as ladybirds (Pemberton and Vandenberg 1993), flies (Heil *et al.* 2004), spiders (Taylor and Pfannenstiel 2008) or parasitic wasps (Röse *et al.* 2006), to name just a few, are known to feed on EFN (see Koptur 1992 for a detailed review). However, the large majority of studies focused on the interaction between EFN plants and ants. Quantitative assessments of entire visitor communities are rare (but see Agarwal and Rastogi 2010; Heil *et al.* 2004; Hespenheide 1985) and besides effects on dominance hierarchies of ants (Blüthgen *et al.* 2004b) and ant community composition (Camarota *et al.* 2015; Schoereder *et al.* 2010) little is known how EFN plants structure arthropod communities (Heil 2015). Surprisingly, there are no studies that compare entire EFN-visiting arthropod communities between different locally co-occurring EFN plant species, despite morphological or chemical properties of EFN plants are expected to influence their visitors (Apple and Feener 2001). For example, when an ecosystem contains several sympatric EFN-bearing plant species as in early successional tropical and subtropical forests, visitors may be able to choose between different EFN sources, with potential consequences for the organization of the associated arthropod community (Rudgers and Gardener 2004).

EFNs of different plant species vary in sugar and AA content and composition (Baker *et al.* 1978; Bixenmann *et al.* 2011; Blüthgen *et al.* 2004a; González-Teuber and Heil 2009), which influences the foraging behavior of ants (e.g. Blüthgen and Fiedler 2004b). However, it is unknown if and how EFN chemical composition affects the abundance, species richness, and composition of whole visitor communities, or if such effects are restricted to few well-studied systems (Lanza *et al.* 1993; Wilder and Eubanks 2010).

In addition to nutrients, local environmental properties may also influence EFN visitors. For example, Koptur (1985) showed that EFN plants were predominantly visited by ants at low elevations but had a changed visitor community with substantial numbers of flies and wasps at high elevations. Local plant species richness is a very important property of any given terrestrial habitat (Loreau *et al.* 2001) and biodiversity experiments demonstrated strong effects of plant diversity on arthropod abundance and species richness (Haddad *et al.* 2009, Scherber *et al.* 2010) by increasing habitat heterogeneity and food availability. To our knowledge, it has not yet been tested, if there is a relationship between EFN visitors and plant diversity.

In this paper we sampled complete EFN-visiting arthropod communities in the field sites of the Biodiversity-Ecosystem Functioning (BEF) China Experiment, which is currently the largest tree diversity experiment in the world (Bruehlheide *et al.* 2014). We first hypothesize and test if tree species richness affects the abundance and diversity of visitors, and expect a positive relationship, following general BEF principles. We also hypothesize that tree species identity has an effect on EFN visitors, resulting in differing visitor community composition between different EFN tree species. In a second step, we analyzed sugars and AAs in the EFN of the two most visited tree species, expecting tree species-specific differences which might translate to differing visitor communities. Third, we conducted a choice experiment with different artificial nectar solutions, to test the hypothesis that nectar quality affects foraging decisions of ants, the most abundant EFN visitors in our study sites. Lastly, we use our results to discuss how EFN trees can affect tree growth in young successional tropical and subtropical forests, implicating a crucial role of EFN plant species and their visitors for forest regeneration.

## MATERIAL AND METHODS

### Study site

This study was conducted in the BEF-China Experiment ([www.bef-china.de](http://www.bef-china.de)), located in South-East China near Xingangshan, Dexing, Jiangxi Province (117°54'E, 29°07'N). The climate is typically subtropical and has a pronounced seasonality with hot and humid summers contrasted by cooler and drier winters (mean annual temperature: 16.7°C/mean annual precipitation: 1821 mm; Yang *et al.* 2013). The potential natural vegetation of the area is a mixed evergreen broad-leaved forest numerically dominated by evergreen tree species (Bruehlheide *et al.* 2011). However, anthropogenic land-use is heavy and almost all suitably flat land has been converted to agricultural fields. Forests are restricted to steeper slopes and frequently consist of commercial plantations of the conifers *Pinus massoniana* Lamb. (Pinaceae) and *Cunninghamia lanceolata* (Lamb.) Hook (Cupressaceae).

The BEF-China Experiment consists of 566 plots distributed among two study sites on sloped land (Scholten *et al.* 2017; elevation: 100–300 m above sea level), respectively

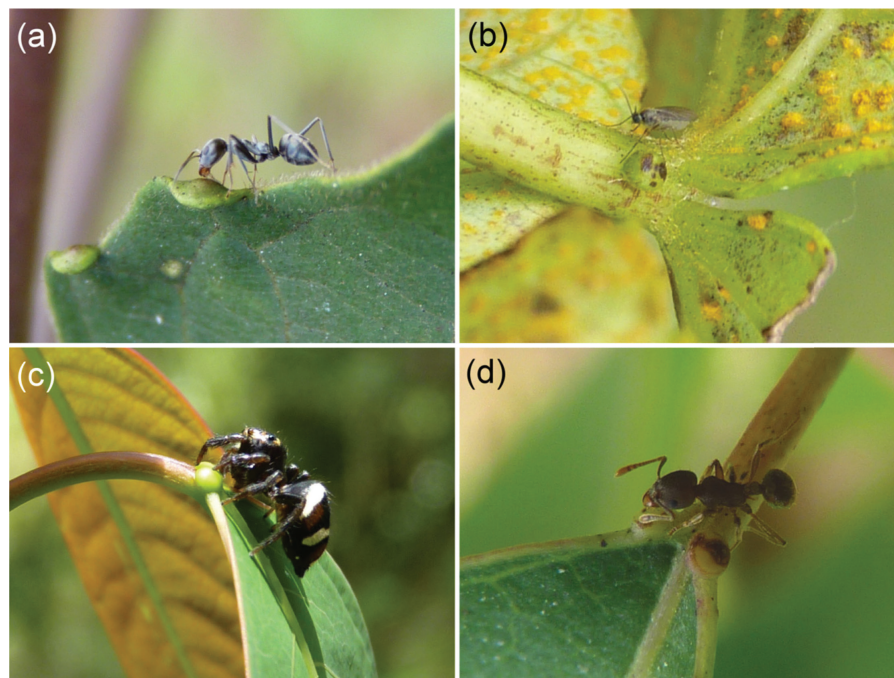
established in 2009 and 2010. Plots have a size of  $25.8 \times 25.8$  m each, which matches the traditional Chinese area unit of 1 mu. In  $20 \times 20$  regular columns and rows, 400 tree individuals were planted per plot with a distance of 1.29 m among trees. From a total pool of 42 native tree species, a tree species richness gradient from 1, 2, 4, 8, 16, to 24 species was planted. The location of trees in a plot and of plots in a site was assigned randomly. More detailed information on the BEF-China Experiment including maps and tree species lists can be found in Yang et al. (2013) and Bruelheide et al. (2014).

### EFN trees and visitor sampling

Plants with EFNs are common in South China (So 2004) and we recorded many species from several families in the secondary forests surrounding our study site, among them five tree species that were so far not known to bear EFNs (see online supplementary Table S1 for a detailed list). Six of those species are included in the species pool of the BEF-China Experiment: *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae), *Diospyros japonica* Siebold & Zucc. (Ebenaceae), *Idesia polycarpa* Maxim. (Flacourtiaceae), *Melia azedarach* L. (Meliaceae), *Triadica cochinchinensis* Lour. (Euphorbiaceae), and *Triadica sebifera* (L.) Small (Euphorbiaceae). All of those tree species are deciduous and the EFNs are not hidden in special morphological structures, generally not sheltered by ants, and easily accessible to a broad variety of arthropods (see Fig. 1).

In spring 2012 (April and May), shortly after leaf flushing, EFN-visiting arthropods were collected on all BEF-China

EFN tree species except *D. japonica* because this species has only minute EFNs that are difficult to locate. First, we selected eight individuals of each EFN tree species in monocultures only. One leaf (including the petiole) at breast height per selected tree was observed on seven different days for 20 min each, resulting in 140 min observation time per tree individual. Though limited in scale, such a detailed sampling scheme has the advantage of recording only 'true' EFN visitors, excluding species that use other resources or are 'tourists' not interacting with the EFN tree. Observations were restricted to fair weather conditions, excluding the hottest time of the day (~12:00–15:00) and rainy days. Albeit damaged leaves in the early growing season were not common, only leaves without fungal infections and herbivore damage were used because herbivory may enhance nectar production and thus attract more visitors (Heil et al. 2001). All arthropods directly interacting with the EFNs of that leaf were collected with exhausters or soft insect forceps and stored in 70% Ethanol till preparation. Due to limitations in time and workforce, it was outside the scope of this study to investigate all EFN tree species of the BEF-China Experiment along the full tree diversity gradient. Thus, after the initial survey in monocultures we selected the three tree species with the highest visitor abundance as representatives for further investigation: *A. altissima* and *I. polycarpa* were additionally surveyed in eight species mixtures. *Triadica cochinchinensis* was surveyed in 4, 8, and 16 species mixtures to conduct a case study for investigating the influence of tree species richness on visiting arthropods. At



**Figure 1** : examples of EFN visitor associations recorded in this study to illustrate the diversity of visiting arthropods: (a) *Iridomyrmex anceps* (Roger, 1863) (Formicidae: Dolichoderinae) on *A. altissima*, (b) a midge (Diptera: Nematocera) at *I. polycarpa*, (c) a jumping spider (Araneae: Salticidae) at *T. cochinchinensis* and (d) *Tetramorium wroughtonii* (Forel, 1902) (Formicidae: Myrmicinae) on *T. sebifera*. All photographs by Michael Staab.

the time of sampling, all tree individuals were 1.5–2.0 m high and had an approximately similar crown volume. Sampling intensity, the number of surveyed tree individuals per plot, and the sampling protocol were identical among tree species richness levels, resulting more than 186 h observation time on 80 tree individuals.

Arthropod species were identified to the lowest possible taxonomic level. Ants were identified to species or morphospecies within genera with primary taxonomic literature (see Guénard and Dunn 2012; Staab *et al.* 2014) and the AntWeb Database ([www.antweb.org](http://www.antweb.org)). All other arthropods were identified to morphospecies within order or family.

### Nectar collection and chemical analyses

For *A. altissima* and *T. cochinchinensis*, the two tree species with highest visitor abundance, eight samples of EFN were collected, one from each tree individual surveyed in the monocultures. To gain nectar, sticky resin (Aurum® Insektenleim, Neudorff, Emmerthal, Germany) was applied to one branch per tree and one leaf on that branch was covered with a small mesh bag (see online supplementary Fig. S1; Díaz-Castelazo *et al.* 2005) shortly before sundown (~18:00) to exclude visitors and to allow overnight accumulation of nectar. In the next morning, before sunrise (~05:00) nectar droplets were collected with graded glass capillaries. The amount of nectar (in  $\mu\text{l}$ ) was recorded and the nectar was transferred to clean filter paper, placed in envelopes, and immediately dried in tight-sealing ziplock bags filled with a plenty of silica gel (following Baker *et al.* 1978). Sealed bags were stored at  $-20^{\circ}\text{C}$  until analysis. It is unclear, if EFN chemistry might be influenced by local tree diversity. However, at the time when both, the EFN visitors and the nectar samples were collected, the trees were still rather small and not strongly interacting with each other. Thus, an influence of local tree diversity on nectar composition is highly unlikely and we are confident that our chemical data are not biased by this.

Sugars and AAs in the nectar samples were determined with high-performance liquid chromatography (HPLC). Prior to analyses, the nectar was recovered from the filter paper. First, the spot on the filter paper on which the nectar droplet had been suspended was cut out and shred with clean micro scissors. Paper pieces were transferred into standard 1.5 ml reaction tubes. Sugars and AAs were dissolved by adding 500  $\mu\text{l}$  of 100% Ethanol and shaking the sample for 4 h. Tubes were briefly centrifuged and the entire liquid phase transferred into new tubes. This procedure was repeated, both liquid phases were combined, and the Ethanol evaporated in a drying oven at  $40^{\circ}\text{C}$ . Finally, the residue was dissolved in ultrapure water and stored at  $-20^{\circ}\text{C}$  until analysis.

All analyses were done using an Agilent 1260 Infinity HPLC (Agilent Technologies, Santa Clara, CA, USA). Amino acids were separated with an Agilent Zorbax Extend-C18 column (3.0  $\times$  150 mm [inner diameter  $\times$  length], 3.5  $\mu\text{m}$  particle size) preceded by an Agilent Zorbax Extend-C18 (2.1  $\times$  12.5 mm, 5  $\mu\text{m}$ ) guard column. Before analyses, AAs were derivatized

with ortho-phthalaldehyde for non-cyclic AA and with 9-fluorenylmethyl chloroformate for cyclic AA. Quantification was done by measuring absorbance of derivatives with an Agilent 1260 Infinity diode array detector and comparison to standards of known concentrations. The following two elution buffers were used: an aqueous polar phase (1 l ultrapure water, 10 mM  $\text{Na}_2\text{HPO}_4$ , 10 mM  $\text{Na}_2\text{B}_4\text{O}_7$ , 0.5 mM  $\text{NaN}_3$ , pH 8.2) and a non-polar phase (0.45 l 99.9% acetonitrile, 0.45 l 99.9% methanol, 0.1 l ultrapure water). Flow rate and temperature were constantly kept at 1 ml/min and  $40^{\circ}\text{C}$ , respectively. The remaining protocol followed Henderson and Brooks (2010).

Sugars were separated via an Agilent Zorbax NH2 column (4.6  $\times$  250 mm, 5  $\mu\text{m}$ ) preceded by an Agilent Zorbax NH2 (4.6  $\times$  12.5 mm, 5  $\mu\text{m}$ ) guard column. Quantification was done by directly measuring refraction of the sugars with an Agilent 1260 refractive index detector and comparison to standards of known concentrations. As elution buffer we used a solution consisting of 0.78 l ultrapure water and 0.22 l 99.9% acetonitrile. Flow rate and temperature were constantly kept at 1.5 ml/min and  $30^{\circ}\text{C}$ , respectively. The analytical protocol followed the standard norm DIN 10758 (Deutsches Institut für Normung 1997).

### Choice experiment

We conducted a controlled choice experiment to test if nectar chemistry influences EFN visiting ant communities from April to June 2012. Such experiments have readily been used to investigate feeding preferences of nectar-foraging ants (Blüthgen and Fiedler 2004b; Lanza *et al.* 1993; Shenoy *et al.* 2012). For this, artificial nectaries were made from standard 1.5 ml reaction tubes with a small hole in the lid through that the content of the reaction tube was available to the outside by a wick. Twelve tubes organized in six pairs were attached to bamboo sticks (length ~50 cm) with a distance of ~5 cm between pairs as illustrated in online supplementary Fig. S2. The tubes were always filled with the following 12 artificial nectars: 0.5 mol  $\text{l}^{-1}$  solutions of the sugars sucrose, melizitose, glucose and fructose, either pure or supplemented with 0.01 mol  $\text{l}^{-1}$  of each of the ten essential or 10 non-essential canonical AAs (see Shenoy *et al.* 2012). As a broad variety of sugars are known from natural EFN (Bentley 1977; Blüthgen *et al.* 2004a), we assessed in a pilot study the preferences of the local nectar-foraging ant community for 0.5 mol  $\text{l}^{-1}$  solutions of 11 sugars. Sucrose, melizitose, glucose and fructose (in decreasing order) were most preferred, >75% of all ant individuals foraged on those four sugars that were thus used in the choice experiment. In contrast, maltose, trehalose, raffinose, xylose, mannose, melibiose and lactose (in decreasing order) were much less preferred.

Experiments were done on *Schima superba* Gardner & Champ. trees in the BEF-China Experiment. This tree species has no EFNs and we only selected tree individuals without established trophobioses to avoid potential biases from previously established ant foraging trails. We randomly selected four *S. superba* individuals in two plots each of the tree richness

levels 1, 2, 4, 8 and 16. In order to minimize multiple testing of the same ant individuals or colonies, selected trees were at least 4 m apart from each other. On days with fair weather, bamboo sticks with the 12 vials were attached at breast height to the trees between 09:00 and 10:00 in the morning. The relative position of the solutions on a bamboo stick was assigned randomly. After 2, 4 and 24 h ants feeding at the different solutions were counted and voucher specimens collected. The choice experiment was repeated four times on each tree, resulting in a total of 160 experimental trials. Ants were identified as described above.

### Statistical analyses

All statistical analyses were done with R 3.0.2 (<http://www.r-project.org>). The EFN visitor data were pooled per surveyed tree individual, resulting in eight data points per tree species  $\times$  tree species richness level combination. Sampling efficiency was assessed with first-order jackknife species richness estimators (Jack1) and sample-based species accumulation curves ( $n = 999$  permutations) in the R-package 'vegan' (Oksanen et al. 2013).

Shannon diversity of visiting arthropods was calculated as the exponential of the entropy  $H'$ , i.e.  $e^{H'}$  (following Jost 2006). The proportion of ants was calculated as the share of ant individuals on the total individual number. The relationship between the fixed effect tree species richness ( $\log_2$ -transformed) and the response variables visitor abundance (number of arthropod visitors), visitor species richness, Shannon diversity, ant species richness and proportion of ants was analyzed separately for *A. altissima* and *T. cochinchinensis* with generalized linear mixed-effect models (GLMMs) in the R-package 'lme4' (Bates et al. 2013). Poisson models were used for the count data on abundances and species richness, Gaussian models for the numeric Shannon index and binomial models for the incidence data proportion of ants. Plot identity was used as random factor to account for possible plot-specific effects on EFN visitors (see Schmid et al. 2017). All Poisson-GLMMs for visitor abundance showed signs of possible overdispersion and a single-observation random factor was added to improve the model fit (Harrison 2014). Explained variances for all significant models were calculated as marginal  $R^2$  ( $R^2_m$ ) following Nakagawa and Schielzeth (2013). The same model types were

used to test for differences between the two focal tree species *A. altissima* and *T. cochinchinensis*, to which the analyses were restricted. *Melia azedarach* and *T. sebifera* were due to low arthropod visitor frequency and limited manpower only sampled in monocultures and not further analyzed (see Table 1). Similarly, *I. polycarpa* was excluded from the statistical analyses. Despite sampling was initially done in two species richness levels, overall visitor abundance was low (Table 1) and data on nectar chemistry are not available, thus prohibiting a meaningful analysis of possible tree species richness and species identity effects for these species.

Non-metric multidimensional scaling (NMDS) was used to analyze community composition of visiting arthropods. Only trees with at least five visitors were used and the NMDS-ordination was based on the Morisita–Horn similarity index of square-root transformed, Wisconsin-double standardized abundance data. The ordination was first centered and then rotated so that the first NMDS-axis explained most of the variance. Analysis of similarity (ANOSIM,  $n = 999$  permutations) was used to test for differences between community composition on *A. altissima* and *T. cochinchinensis*. All multivariate statistics were performed in 'vegan'. Likewise, NMDS-ordination and ANOSIM were applied to AA concentration data to test for differences in AA composition between the two focal tree species. Ordinary linear models were used to test if the total concentrations of sugars and AA and the number of recorded AA differ between *A. altissima* and *T. cochinchinensis*.

For the choice experiment, all data of ants recorded at the different artificial nectar solutions and time steps were pooled per solution type (pure sugar, sugar supplemented with either non-essential or essential AA) and plot, resulting in three data points per plot. As for EFN visitors, the sampling efficiency was assessed with Jack1 estimators and sample-based species accumulation curves ( $n = 999$  permutations). For every data point, the species richness (total number of ant species) and the species occurrences (the sum of all species occurrences from the original samples taken together) were recorded. Occurrence is here used as a surrogate for abundance to prevent biases in the data that will arise if single species heavily recruit nestmates to a solution. Poisson GLMMs were used to test for the relationship between the fixed effects tree species richness ( $\log_2$ -transformed) and solution type and the

**Table 1:** summary of EFN visitor sampling on five different tree species in the BEF-China Experiment

Tree species	$n$ (trees)	Richness levels	Visitor abundance	Visitor richness (Jack1)	Ants (abundance/richness)
<i>Ailanthus altissima</i>	16	1, 8	171	23 (36 ± 5)	125/9
<i>Idesia polycarpa</i>	16	1, 8	42	23 (41 ± 6)	14/5
<i>Melia azedarach</i>	8	1	11	7 (12 ± 2)	4/2
<i>Triadica sebifera</i>	8	1	15	9 (15 ± 3)	5/2
<i>Triadica cochinchinensis</i>	32	1, 4, 8, 16	299	31 (50 ± 6)	249/11
Total	80		538	61 (96 ± 7)	397/15

Shown are the number of sampled trees, the tree species richness levels sampled, the abundance and species richness for all visiting arthropods and for ants only. Values in parentheses refer to species richness estimation  $\pm$  SE based on Jack1 estimators.

response variables ant species richness and ant occurrence. Only occurrence values without accounting for the dominant ant species *Polyrhachis dives* Smith, 1857, which commonly monopolized all artificial nectars, were used in the models. Plot identity was included as random factor and  $R^2_m$  was calculated for significant models. The community composition of ants in the choice experiment was analyzed with NMDS-ordination of occurrence data, including and excluding *P. dives*. ANOSIM was used to test for differences in ant communities among the three solution types.

## RESULTS

A diverse arthropod community visited EFNs in our study sites (Fig. 1). In total, 538 individuals from 61 species were found, of which 397 (74%) individuals belonged to 15 species of ants (Hymenoptera: Formicidae) (Table 1). Diptera was the most species-rich insect order (24 species), although individual numbers were low (44 individuals). Non-ant Hymenoptera (12 individuals/6 species), Coleoptera (78/12) and Araneae (7/4) occurred as well. A detailed list of all species and morphospecies is provided in online supplementary Table S2. Jack1 species richness estimation and species accumulation curves (online supplementary Fig. S3) showed that total sampling efficiency was intermediate, as 64% of the expected  $96 \pm 7$  (SE) species were collected. Sampling efficiency per tree species was similar and between 56% and 64%.

Neither visitor abundance and visitor species richness per tree, nor exponential Shannon diversity, ant species richness and proportion of ants were different between *A. altissima* and *T. cochinchinensis*. However, visitor species richness on both tree species was significantly lower (*A. altissima*:  $P = 0.003$ ,  $R^2_m = 0.43$ ; *T. cochinchinensis*:  $P = 0.013$ ,  $R^2_m = 0.18$ ) in plots with more planted tree species (all model parameters are given in Table 2; Fig. 2b). The same pattern was found for visitor abundance and ant species richness, albeit only significantly for *A. altissima* (abundance:  $P < 0.001$ ,  $R^2_m = 0.58$ ; ant species richness:  $P = 0.24$ ,  $R^2_m = 0.33$ ) (Fig. 2a). In both tree species, Shannon diversity and the proportion of ants were not significantly related to tree species richness; however, for *T. cochinchinensis* the relationship between proportion of ants and tree species richness was positive (estimate  $\pm$  SE:  $0.38 \pm 0.21$ ,  $Z = 1.81$ ,  $P = 0.071$ ,  $R^2_m = 0.08$ ) (online supplementary Fig. S4). Multivariate analyses revealed a large and

significant difference ( $P = 0.002$ ,  $R^2 = 0.24$ ) in visitor species composition amongst the two focal EFN tree species (Fig. 3a).

HPLC analyses found the three sugars fructose, glucose and sucrose in the nectar of *A. altissima* and *T. cochinchinensis*, albeit in varying consistency (see online supplementary Table S3 for detailed HPLC results). Total sugar concentration was significantly larger in the nectar of *T. cochinchinensis* (estimate  $\pm$  SE:  $15.22 \pm 4.06$ ,  $t = 3.75$ ,  $P = 0.002$ ,  $R^2 = 0.50$ ; Fig. 4a). All 20 proteinogenic AA were found in the EFN. There was a large difference in the concentration of single AA (online supplementary Table S3) and species-specific AA composition was very distinct ( $P = 0.001$ ,  $R^2 = 0.79$ ) as shown by the clustering in the NMDS (Fig. 3b). However, total AA concentration (Fig. 4b) and the number of AA (Fig. 4c) did not differ among tree species.

In the choice experiment, 18 ant species with a total of 2203 individuals (1136 occurrences) were recorded to forage at the artificial nectar solutions (see online supplementary Table S4 for a detailed species list). Diverse non-ant arthropods such as bees, flies and beetles also fed on the solutions but were not further studied. About two-third of all individuals and species occurrences belonged to a single species, *P. dives* (Table 3). Total sampling efficiency was 95%. For the different nectar solution types, Jack1 indicators showed that similar (71–82%) fractions of the expected species richness were found (online supplementary Fig. S5). Ant species richness increased weakly with tree species richness (estimate  $\pm$  SE:  $0.13 \pm 0.06$ ,  $Z = 2.02$ ,  $P = 0.043$ ,  $R^2_m = 0.16$ ) but was not different among nectar types (online supplementary Fig. S6). In turn, ant occurrences on solutions containing essential AA were significantly higher ( $R^2_m = 0.04$ ) than on pure sugars (estimate  $\pm$  SE:  $0.30 \pm 0.12$ ,  $Z = 2.47$ ,  $P = 0.036$ ) and sugars with-non essential AA (estimate  $\pm$  SE:  $0.38 \pm 0.13$ ,  $Z = 3.06$ ,  $P = 0.007$ , each sequential Bonferroni-corrected for multiple comparisons) while the latter two solutions did not differ from each other (Table 3, Fig. 5). The ant communities at each nectar solution type were identical, irrespective if *P. dives* was included in the dataset or not (online supplementary Fig. S7).

## DISCUSSION

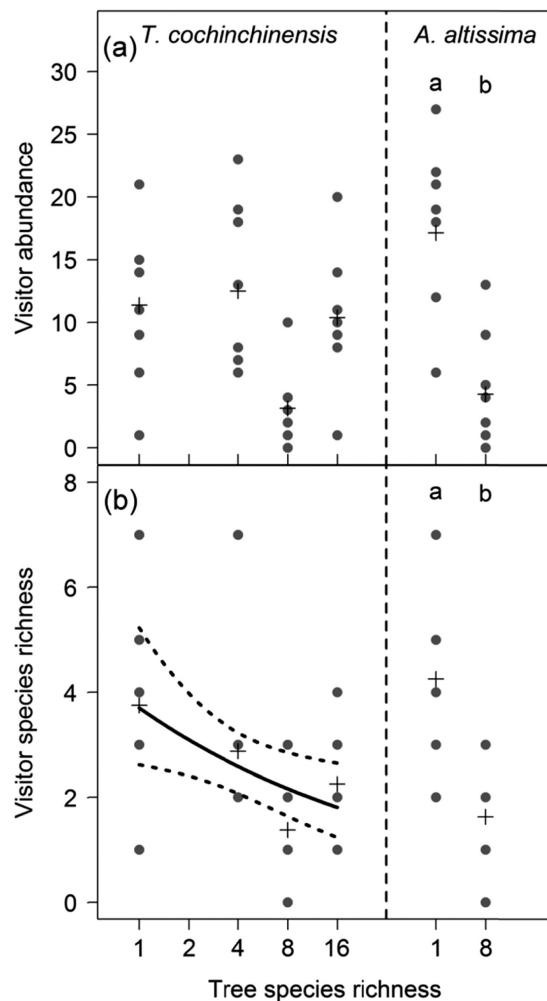
### EFN trees attract diverse non-ant visitors

To our knowledge, this is the first detailed study of EFN visitors conducted in the diverse subtropical forests of China.

**Table 2:** results of Poisson GLMMs separately testing for the effect of tree species richness on the abundance and species richness of arthropod visitors to the EFNs of *A. altissima* and *T. cochinchinensis*

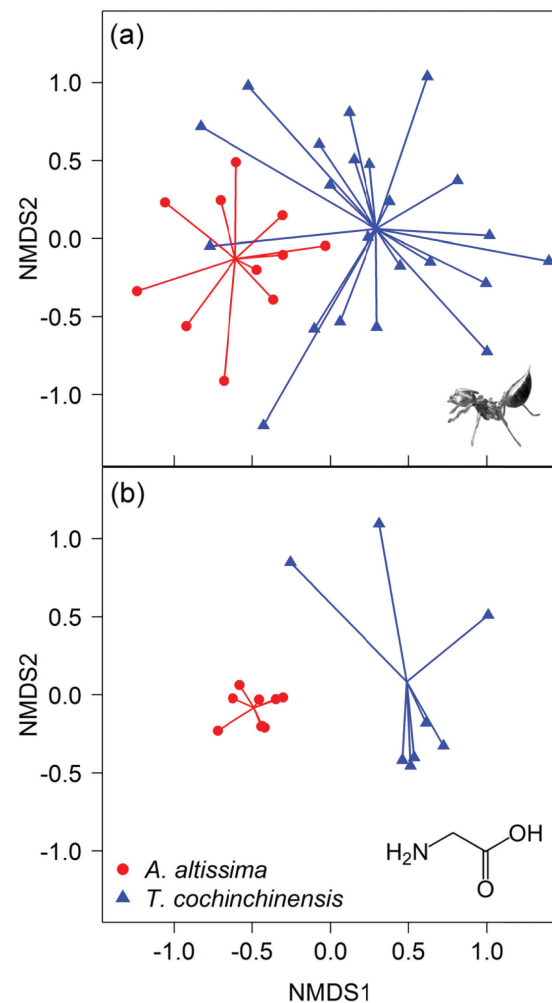
Variable	<i>Triadica cochinchinensis</i>				<i>Ailanthus altissima</i>			
	Estimate $\pm$ SE	Z	P	$R^2_m$	Estimate $\pm$ SE	Z	P	$R^2_m$
Abundance	—	—	—	—	$-1.56 \pm 0.36$	-4.32	<0.001	0.58
Total species richness	$-0.18 \pm 0.07$	-2.48	0.013	0.18	$-0.96 \pm 0.33$	-2.95	0.003	0.43
Ant species richness	—	—	—	—	$-1.00 \pm 0.44$	-2.26	0.024	0.33

Shown are model estimate  $\pm$  SE, Z-value, marginal  $R^2$  and P values of the Z-statistic.



**Figure 2:** relationship between tree species richness and (a) the abundance and (b) the species richness of arthropod visitors found on *T. cochinchinensis* (left of dashed line) and *A. altissima* (right of dashed line). Crosses indicate means. Visitor species richness on *T. cochinchinensis* decreased significantly (Poisson GLMM,  $P < 0.05$ ; shown is model prediction and CI) with increasing tree species richness; visitor abundance and species richness on *A. altissima* were significantly lower (Poisson GLMM,  $P < 0.05$ ) in eight-species mixtures compared to monocultures (as indicated by different letters). Please note that the  $x$ -axis for *T. cochinchinensis* is  $\log_2$ -scaled.

Despite the sampling was limited in scale to one leaf per tree individual, a species-rich assemblage of arthropods was found that, although numerically (almost 75%) dominated by ants, consisted taxonomically to almost 75% of non-ant species. The proportion of ant to non-ant visitors was similar among the two focal EFN tree species *A. altissima* and *T. cochinchinensis* conforming with the few other studies systematically observing entire EFN visitor communities (e.g. Agarwal and Rastogi 2010; Heil et al. 2004), indicating that commonly about a quarter of all arthropod individuals visiting EFNs are not ants. EFNs are usually open structures (Blüthgen and Reifernrath 2003; So 2004) and thus accessible to a multitude of arthropods (Koptur 1992), which will readily feed on the nectar. Some

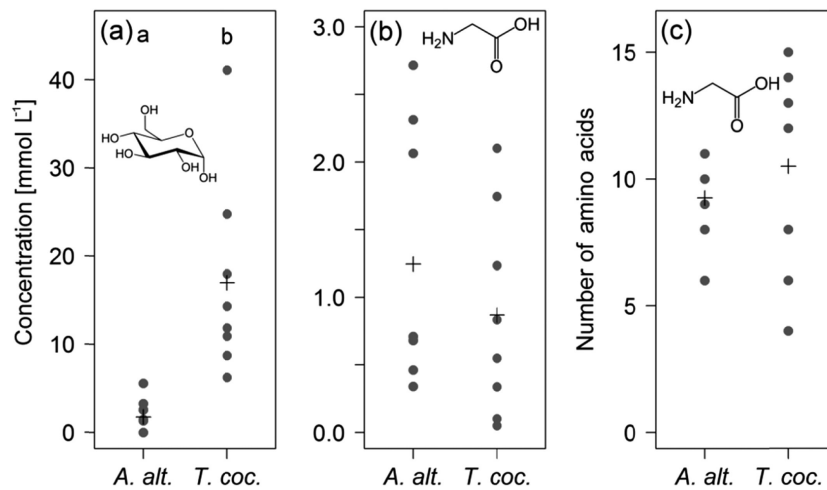


**Figure 3:** NMDS ordinations based on Morisita–Horn indices of square-root transformed, Wisconsin-double standardized data of (a) EFN visitor abundance per tree individual (stress = 0.15) and (b) AA concentrations in EFN (stress = 0.11). *Ailanthus altissima* represents circles and *T. cochinchinensis* triangles; lines mark the centroids in ordination space of each tree species. Both, visitor community and AA composition were significantly different between the two tree species (ANOSIM,  $P < 0.05$  each).

of the non-ant visitors such as flies and phytophagous beetles can be common at nectaries and may be nectar 'thieves' (Heil et al. 2004; Hespeneide 1985) without any benefit for the plant. Others, such as wasps and spiders, may be beneficial (e.g. Jamont et al. 2014; Ruhren and Handel 1999), similarly to the well-studied ant–EFN plant mutualism. Many of the non-ant visitors occurred in low individual numbers, and increased sampling effort is likely to reveal an even larger number of species. Likewise, it would be promising to repeat sampling in the night, because temporal niche differentiation is expected in ants and other EFN visitors (see e.g. Houadria et al. 2015).

#### Effect of tree species richness on EFN visitors

Plant-based resources such as EFN nourish the prevalence and ecological dominance of arboreal ants in tropical forests



**Figure 4:** total concentration of (a) sugars and (b) AA as well as (c) the number of different AA recorded in the EFN of *A. altissima* (*A. alt.*) and *T. cochinchinensis* (*T. coc.*) ( $n = 8$  samples each). The concentration of sugars was significantly (linear model,  $P < 0.05$ ) higher for *T. cochinchinensis* as indicated by different letters. Concentration and number of AA were not different. Crosses indicate means.

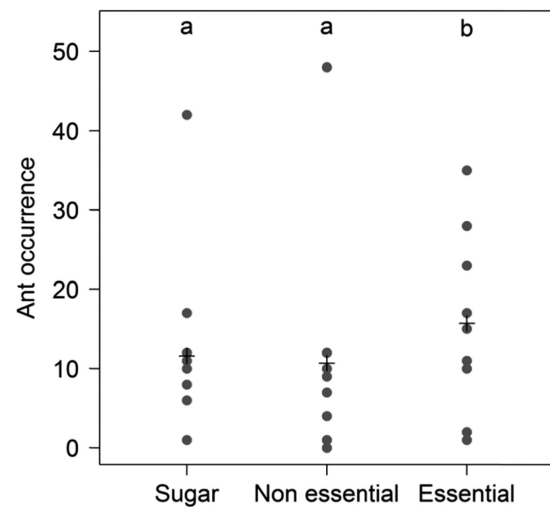
**Table 3:** summary of ant sampling with three different artificial nectar solutions (pure sugars, sugar supplemented with non-essential or essential AAs; see text for explanations)

Solution	Abundance (no <i>P. dives</i> )	Occurrence (no <i>P. dives</i> )	Species richness (Jack1)
Sugar	610 (188)	355 (126)	18 (24 ± 4)
Non-essential	685 (208)	356 (107)	15 (21 ± 4)
Essential	908 (383)	425 (157)	14 (17 ± 2)
Total	2203 (779)	1136 (386)	18 (19 ± 1)

The total abundance, occurrence and species richness of ants are shown. Values in parentheses refer to abundance and occurrences without the dominant *Polyrhachis dives* and to species richness estimation ± SE based on Jack1 estimators.

(Blüthgen *et al.* 2003; Davidson *et al.* 2003). For non-ant visitors, EFN can be an important supplemental food (Lundgren and Seagraves 2011) or even provide a substantial amount of the total nutrition (Röse *et al.* 2006).

Increasing local plant species richness increases local habitat heterogeneity and structural diversity, and positive correlations between plant species richness and arthropod abundance or richness have been shown (Basset *et al.* 2012; Haddad *et al.* 2009). However, contrary to our expectations, we found that plots with higher tree species richness had consistently lower visitor abundance and species richness. These results agree with the ‘resource concentration hypothesis’ originally postulated for herbivorous insects by Root (1973). Availability of EFN on plot level is highest in EFN tree monocultures and declines with increasing tree species richness, as the relative proportion of EFN trees gets smaller. Thus, the resource EFN visitors forage for is negatively related to tree species richness, and following a resource dilution effect (Otway *et al.* 2005; Tong *et al.* 2017), their abundance and species richness declines, supporting the predictions of



**Figure 5:** occurrences (see text for explanation) of ants per plot excluding the dominant *Polyrhachis dives* at the different artificial nectar solutions in the choice experiment. Significant differences between solution types (Poisson GLMM,  $P < 0.05$ ) are indicated by different letters. Crosses indicate means for each solution.

Rudgers and Gardener (2004). Alternatively, a higher density of EFN trees could favor few behaviorally dominant ant species, leading to lower ant and total visitor species richness due to competitive exclusion, which was not the case. In contrast, the proportion of visiting ants tended to increase with tree species richness, although not significantly. This finding supports previous evidence from the BEF-China field sites that tree species richness has a positive effect on the performance of ants, e.g. by stabilizing mutualistic Hemiptera-ant networks (Staab *et al.* 2015) or by increasing the competitive ability of ants against spiders (Schuldt and Staab 2015), notably in both cases without changing ant species richness.



## Effect of nectar chemical composition on EFN visitors

Differences in EFN visitor communities could be caused by nectar quality, nectary morphology or the presence of large-bodied and competitively superior EFN visitor species deterring other visitors (Apple and Feener 2001; Blüthgen and Fiedler 2004b; Blüthgen et al. 2004b; Heil 2015). The later possibility is unlikely in our study, because all EFN visitors were sampled directly when they appeared at the nectaries and all common ant species were of intermediate body size. Apple and Feener (2001) showed that visitation patterns of ants among different *Passiflora* species can be evoked by nectary morphology, which was also not the case in our study. All studied species had open and easily accessible nectaries. Nevertheless, as expected, visitor communities on *A. altissima* and *T. cochinchinensis* were distinct. The NMDS ordinations of visitor community composition and AA composition show a strikingly congruent pattern, suggesting an association between nectar contents and visiting arthropods. Also, the sugar concentrations in the nectar were very different between the two analyzed tree species. The results of our chemical analyses agree well with previous findings. Amino acid concentrations were about 1/10 of sugar concentrations (Heil 2015); amino acid compositions were species-specific and differed considerably among EFN tree species (Baker et al. 1978; Blüthgen et al. 2004a). EFN contains normally only the sugars fructose, glucose and sucrose with species-specific differences in quantity (Bixenmann et al. 2011; Blüthgen et al. 2004a), as it was the case in our study.

Sugar fuels nectar-foraging ants that also obtain the majority of their nitrogen from plant-based resources (Blüthgen et al. 2003; Davidson et al. 2003; Pfeiffer et al. 2014), and species-specific preferences for nectar contents seem likely. The literature on nutritional preferences of EFN visitors focuses on ants and shows that nectar content can alter foraging preferences (González-Teuber and Heil 2009; Lanza et al. 1993; Wilder and Eubanks 2010). However, results are mixed concerning the question whether sugars or AA are more important. For strict plant–ant mutualisms, such as between several *Acacia* and *Pseudomyrmex* species, a strong effect of AA composition on feeding preferences of ants has been shown. Generalist ant visitors, in contrast, responded more strongly to manipulations in sugars and the sole presence but not identity of AAs (González-Teuber and Heil 2009). This is supported by Bixenmann et al. (2011) who suggest that changes in sugar concentrations may be stronger in changing ant visitor communities than changes in AA. Nevertheless, EFN can also affect visitors by compounds other than sugars or AA such as specific proteins or vitamins (Heil 2015), on which we, unfortunately, lack data.

In our study, the differing visitor communities could either be caused by the tree species-specific AA composition or sugar content. In the choice experiment, sugar concentration was constant and the presence of essential AA promoted ant foraging, suggesting that also outside strict mutualisms the

foraging of EFN visitors can be influenced by AAs, especially by the presence of essential AAs (see Blüthgen and Fiedler 2004b; Lanza et al. 1993; Shenoy et al. 2012). In retrospective we think, however, that the choice experiment with its continuous and large provision of nectar more closely resembled trophobioses instead of EFNs. Trophobioses, the mutualistic associations between ants and honeydew-producing Hemiptera are frequently monopolized by behaviorally dominant ants, because honeydew is usually more nutritious and at the plant scale available in larger quantities (Blüthgen et al. 2000; Blüthgen and Fiedler 2004a; Blüthgen et al. 2004b). In the young successional stage of the BEF-China Experiment, this ant was *Polyrhachis dives*, which was by far the most abundant ant in the choice experiment and likewise accounted for over 50% of all ants on trophobioses (Staab et al. 2015). At EFNs, *P. dives*, a characteristic species for young habitats absent in closed-canopy forest (Staab et al. 2014) was rare. Our results support the view that at the scale of entire plant communities EFN is an opportunistic resource for all nectar-foraging ants and diverse other arthropods (see above) while honeydew is more strongly available to dominant ants (Blüthgen and Fiedler 2004a; Blüthgen et al. 2004b). Thus, the differences in nectar chemistry on our focal tree species will be more meaningful for non-dominant ants and diverse other arthropods, leading to the observed tree species-specific visitor communities, while ant communities in the choice experiment did not differ between artificial nectar types, even when for *P. dives* was accounted.

## Ecosystem consequences of EFN trees

Recently, studies conducted in the same field sites came to the result that presence and cover of EFN tree species is the best predictor of tree growth on the plot level, much better than e.g. plot-scale abiotic properties (Kröber et al. 2015; Li et al. 2017). Surprisingly, not EFN tree individuals alone but the entire tree community on a plot grew better, indicating far reaching consequences of EFN tree species on the growth and regeneration of young tropical and subtropical forests. EFN can mediate multi-species interactions across trophic levels (Rudgers and Gardener 2004) and attracts ants, which decrease herbivory and improve performance of EFN plants (meta-analysis: Trager et al. 2010). Non-ant predators and parasitoids also benefit from EFN (Lundgren and Seagraves 2011; Röse et al. 2006), which can lead to increased parasitism rates of herbivores (Jamont et al. 2014; Pemberton and Lee 1996) and ultimately to reduced herbivore abundance, lower herbivory (Rezende et al. 2014) and increased growth in co-occurring non-EFN plants. The most damaging herbivores in the BEF-China Experiment are likely generalists that can feed on several tree species and may change their host plant during their life cycle (Brezzi et al. 2017; Schuldt et al. 2015). Such herbivores might experience stronger predation and parasitism pressure on plots with EFN trees, either directly when feeding on EFN trees, or indirectly if plots with EFN trees have a priori higher populations of beneficial arthropods. Also, it is

likely that EFN trees can promote the spillover of such arthropods to non-EFN tree species. Testing this would be an interesting subject for future research.

It has not escaped our notice that the three EFN tree species *A. altissima* (tree of heaven), *M. azedarach* (chinaberry tree) and *T. sebifera* (Chinese tallow tree), which are native and locally common in South-East China, are globally invasive in warm-temperate climates (Ding *et al.* 2006). It is likely that the presence of EFNs, which are also readily visited by ants and other arthropods in non-native areas, supports the ecological success of those tree species outside their original habitat (Carrillo *et al.* 2012).

## CONCLUSION

Our study provides evidence that trees with EFNs are in South-East China and likely in other regions visited by far more arthropods than only ants. Despite ants generally account for the majority of visitor individuals, the species richness of non-ant arthropods might be much higher than those of ants. We expect that the plant species-specific association between EFN visitors and chemical composition might be widespread and found among other sympatric EFN bearing plants as well. We showed for the first time that the abundance and species number of EFN visitors is negatively related to local tree species richness, likely caused by reduced community level nectar availability and lower EFN tree density. Consequently, regenerating young successional tropical and subtropical forests and tree plantations with a high EFN tree density might grow faster due to a higher prevalence of beneficial arthropods.

## SUPPLEMENTARY MATERIAL

Supplementary material associated with this article is available at *Journal of Plant Ecology* online.

## FUNDING

German Research Foundation (DFG FOR 891/2, KL 1849/6-1); the Sino-German Center for Research Promotion (GZ 785); the German Academic Exchange Service (PROMOS scholarship to J.M.).

## ACKNOWLEDGEMENTS

We thank Keping Ma, Helge Bruelheide, Bernhard Schmid and the entire BEF-China team for setting up and maintaining this great biodiversity experiment. Fieldwork in Xingangshan would not be possible without the help of Chen Lin and numerous local helpers, to which we express our gratitude. Comments from two anonymous reviewers improved the quality of the manuscript. We especially acknowledge Fang Teng for plant identification, Angela Gronert for performing the majority of the HPLC analyses and the editors of the *Journal of Plant Ecology* for enabling this special issue.

*Conflict of interest statement.* None declared.

## REFERENCES

- Agarwal VM, Rastogi N (2010) Ants as dominant insect visitors of the extrafloral nectaries of sponge gourd plant, *Luffa cylindrica* (L.) (Cucurbitaceae). *Asian Myrmecol* **3**:45–54.
- Apple JL, Feener DH (2001) Ant visitation of extrafloral nectaries of Passiflora: the effects of nectary attributes and ant behavior on patterns in facultative ant-plant mutualisms. *Oecologia* **127**:409–16.
- Aranda-Rickert A, Diez P, Marazzi B (2014) Extrafloral nectar fuels ant life in deserts. *AoB Plants* **6**:plu068.
- Baker HG, Opler PA, Baker I (1978) A comparison of the amino acid complements of floral and extrafloral nectars. *Bot Gaz* **139**:322–32.
- Basset Y, Cizek L, Cuenoud P, *et al.* (2012) Arthropod diversity in a tropical forest. *Science* **338**:1481–4.
- Bates D, Maechler M, Bolker BM, *et al.* (2013) *Lme4: Linear Mixed-Effects Models Using Eigen and S4*. R package version 1.0–5. <http://CRAN.R-project.org/package=lme4>.
- Bentley BL (1977) Extrafloral nectaries and protection by pugnacious bodyguards. *Annu Rev Ecol Syst* **8**:407–27.
- Bixenmann RJ, Coley PD, Kursar TA (2011) Is extrafloral nectar production induced by herbivores or ants in a tropical facultative ant-plant mutualism? *Oecologia* **165**:417–25.
- Blüthgen N, Verhaagh M, Goitia W, *et al.* (2000) How plants shape the ant community in the Amazonian rainforest canopy: the key role of extrafloral nectaries and homopteran honeydew. *Oecologia* **125**:229–40.
- Blüthgen N, Reifenhath K (2003) Extrafloral nectaries in an Australian rainforest: structure and distribution. *Aust J Bot* **51**:515–27.
- 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**:426–35.
- Blüthgen N, Fiedler K (2004a) Competition for composition: lessons from nectar-feeding ant communities. *Ecology* **85**:1479–85.
- Blüthgen N, Fiedler K (2004b) Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. *J Anim Ecol* **73**:155–66.
- Blüthgen N, Gottsberger G, Fiedler K (2004a) Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. *Austral Ecol* **29**:418–29.
- Blüthgen N, Stork NE, Fiedler K (2004b) Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. *Oikos* **106**:344–58.
- Brezzi M, Schmid B, Niklaus PA, *et al.* (2017) Tree diversity increases levels of herbivore damage in a subtropical forest canopy: evidence for dietary mixing by arthropods? *J Plant Ecol* **10**:13–27.
- Bruelheide H, Böhnke M, Both S, *et al.* (2011) Community assembly during secondary forest succession in a Chinese subtropical forest. *Ecol Monogr* **81**:25–41.
- Bruelheide H, Nadrowski K, Assmann T, *et al.* (2014) Designing forest biodiversity experiments: general considerations illustrated by a new large experiment in subtropical China. *Methods Ecol Evol* **5**:74–89.
- Byk J, Del-Claro K (2011) Ant-plant interaction in the neotropical savanna: direct beneficial effects of extrafloral nectar on ant colony fitness. *Popul Ecol* **53**:327–32.

- Camarota F, Powell S, Vasconcelos HL, et al. (2015) Extrafloral nectar-ies have a limited effect on the structure of arboreal ant communities in a Neotropical savanna. *Ecology* **96**:231–40.
- Carrillo J, Wang Y, Ding J, et al. (2012) Induction of extrafloral nectar depends on herbivore type in invasive and native Chinese tallow seedlings. *Basic Appl Ecol* **13**:449–57.
- Darwin F (1876) On the glandular bodies on *Acacia sphaerocephala* and *Cecropia peltata* serving as food for ants. With an appendix on the nectar-glands of the common brake fern, *Pteris aquilina*. *Bot J Linn Soc Lond* **15**:398–409.
- Davidson DW, Cook SC, Snelling RR, et al. (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* **300**:969–72.
- Deutsches Institut für Normung (1997) *DIN Norm 10758: Untersuchung von Honig; Bestimmung des Gehaltes der Saccharide Fructose, Glucose, Saccharose, Turanose und Maltose; HPLC-Verfahren*.
- Díaz-Castelazo C, Rico-Gray V, Ortega F, et al. (2005) Morphological and secretory characterization of extrafloral nectar-ies in plants of coastal Veracruz, Mexico. *Ann Bot* **96**:1175–89.
- Ding J, Reardon R, Wu Y, et al. (2006) Biological control of invasive plants through collaboration between China and the United States of America: a perspective. *Biol Invas* **8**:1439–50.
- Fiala B, Linsenmair KE (1995) Distribution and abundance of plants with extrafloral nectar-ies in the woody flora of a lowland primary forest in Malaysia. *Biodivers Conserv* **4**:165–82.
- González-Teuber M, Heil M (2009) The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. *J Chem Ecol* **35**:459–68.
- Guénard B, Dunn RR (2012) A checklist of the ants of China. *Zootaxa* **3558**:1–77.
- Haddad NM, Crutsinger GM, Gross K, et al. (2009) Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecol Lett* **12**:1029–39.
- Harrison XA (2014) Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* **2**:e616.
- Heil M, Koch T, Hilpert A, et al. (2001) Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proc Natl Acad Sci USA* **98**:1083–8.
- Heil M, McKey D (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annu Rev Ecol Syst* **34**:425–53.
- Heil M, Hilpert A, Krüger R, et al. (2004) Competition among visitors to extrafloral nectar-ies as a source of ecological costs of an indirect defence. *J Trop Ecol* **20**:201–8.
- Heil M (2015) Extrafloral nectar at the plant-insect interface: a spotlight on chemical ecology, phenotypic plasticity, and food webs. *Annu Rev Entomol* **60**:213–32.
- Henderson JW, Brooks A (2010) *Improved Amino Acid Methods Using Agilent Zorbax Eclipse Plus C18 Columns for a Variety of Agilent LC Instrumentation and Separation Goals*. Agilent Application Note 5990-4547EN.
- Hespenheide HA (1985) Insect visitors to extrafloral nectar-ies of *Byttneria aculeata* (Sterculiaceae): relative importance and roles. *Ecol Entomol* **10**:191–204.
- Houadria M, Salas-Lopez A, Orivel J, et al. (2015) Dietary and temporal niche differentiation in tropical ants - can they explain local ant coexistence? *Biotropica* **47**:208–17.
- Jamont M, Dubois-Pot C, Jaloux B (2014) Nectar provisioning close to host patches increases parasitoid recruitment, retention and host parasitism. *Basic Appl Ecol* **15**:151–60.
- Jost L (2006) Entropy and diversity. *Oikos* **113**:363–75.
- Koptur S (1985) Alternative defenses against herbivores in *Inga* (Fabaceae, Mimosoidea) over an elevational gradient. *Ecology* **66**:1639–50.
- Koptur S (1992) Extrafloral nectar-ied mediated interactions between insects and plants. In Bernays E (ed). *Insect-Plant Interactions*, Vol. **IV**, Boca Raton, FL: CRC Press, 85–132.
- Kröber W, Li Y, Härdtle W, et al. (2015) Early subtropical forest growth is driven by community mean trait values and functional diversity rather than the abiotic environment. *Ecol Evol* **5**:3541–56.
- Lanza J, Vargo EL, Pulim S, et al. (1993) Preferences of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera, Formicidae) for amino-acid and sugar components of extrafloral nectar-ies. *Environ Entomol* **22**:411–7.
- Li Y, Kröber W, Bruelheide H, et al. (2017) Crown and leaf traits as predictors of subtropical tree sapling growth rates. *J Plant Ecol* **10**:136–45.
- Loreau M, Naeem S, Inchausti P, et al. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* **294**:804–8.
- Lundgren JG, Seagraves MP (2011) Physiological benefits of nectar feeding by a predatory beetle. *Biol J Linn Soc* **104**:661–9.
- Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining  $r^2$  from generalized linear mixed-effects models. *Methods Ecol Evol* **4**:133–42.
- Oksanen J, Blanchet GF, Kindt R, et al. (2013) *vegan: Community Ecology Package*. R package version 2.0–9. <http://cran.r-project.org/package=vegan>.
- Oliveira PS, Leitão-Filho HF (1987) Extrafloral nectar-ies - their taxonomic distribution and abundance in the woody flora of Cerrado vegetation in southeast Brazil. *Biotropica* **19**:140–8.
- Otway SJ, Hector A, Lawton JH (2005) Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. *J Anim Ecol* **74**:234–40.
- Pemberton RW, Vandenberg NJ (1993) Extrafloral nectar feeding by ladybird beetles (Coleoptera, Coccinellidae). *Proc Entomol Soc Wash* **95**:139–51.
- Pemberton RW, Lee JH (1996) The influence of extrafloral nectar-ies on parasitism of an insect herbivore. *Am J Bot* **83**:1187–94.
- Pemberton RW (1998) The occurrence and abundance of plants with extrafloral nectar-ies, the basis for antiherbivore defensive mutualisms, along a latitudinal gradient in East Asia. *J Biogeogr* **25**:661–8.
- Pfeiffer M, Mezger D, Dyckmans J (2014) Trophic ecology of tropical leaf litter ants (Hymenoptera: Formicidae) – a stable isotope study in four types of Bornean rain forest. *Myrmecol News* **19**:31–41.
- Rezende MQ, Venzon M, Perez AL, et al. (2014) Extrafloral nectar-ies of associated trees can enhance natural pest control. *Agric Ecosyst Environ* **188**:198–203.

- Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecol Monogr* **43**:95–120.
- Röse UR, Lewis J, Tumlinson JH (2006) Extrafloral nectar from cotton (*Gossypium hirsutum*) as a food source for parasitic wasps. *Funct Ecol* **20**:67–74.
- Rudgers JA, Gardener MC (2004) Extrafloral nectar as a resource mediating multispecies interactions. *Ecology* **85**:1495–502.
- Ruhren S, Handel SN (1999) Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia* **119**:227–30.
- Scherber C, Eisenhauer N, Weisser WW, et al. (2010) Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* **468**:553–6.
- Schmid B, Baruffol M, Wang ZH, et al. (2017) A guide to analyzing biodiversity experiments. *J Plant Ecol* **10**:91–110.
- Schoereder JH, Sobrinho TG, Madureira MS, et al. (2010). The arboreal ant community visiting extrafloral nectaries in the Neotropical cerrado savanna. *Terr Arthropod Rev* **3**:3–27.
- Scholten T, Goebes P, Kühn P, et al. (2017) On the combined effect of soil fertility and topography on tree growth in subtropical forest ecosystems --a study from SE China. *J Plant Ecol* **10**:111–27.
- Schuldt A, Staab M (2015) Tree species richness strengthens relationships between ants and the functional composition of spider assemblages in a highly diverse forest. *Biotropica* **47**:339–46.
- Schuldt A, Bruelheide H, Härdtle W, et al. (2015) Early positive effects of tree species richness on herbivory in a large-scale forest biodiversity experiment influence tree growth. *J Ecol* **103**:563–71.
- Shenoy M, Radhika V, Satish S, et al. (2012) Composition of extrafloral nectar influences interactions between the myrmecophyte *Humboldtia brunonis* and its ant associates. *J Chem Ecol* **38**:88–99.
- So ML (2004) The occurrence of extrafloral nectaries in Hong Kong plants. *Bot Bull Acad Sinica* **45**:237–45.
- Staab M, Schuldt A, Assmann T, et al. (2014) Ant community structure during forest succession in a subtropical forest in South-East China. *Acta Oecol* **61**:32–40.
- Staab M, Blüthgen N, Klein A-M (2015) Tree diversity alters the structure of a tri-trophic network in a biodiversity experiment. *Oikos* **124**:827–34.
- Taylor RM, Pfannenstiel RS (2008) Nectar feeding by wandering spiders on cotton plants. *Environ Entomol* **37**:996–1002.
- Tong X, Zhang Y-X, Wang R, et al. (2017) Habitat fragmentation alters predator satiation of acorns. *J Plant Ecol* **10**:67–73.
- Trager MD, Bhotika S, Hostetler JA, et al. (2010) Benefits for plants in ant-plant protective mutualisms: a meta-analysis. *PLOS ONE* **5**:e14308.
- Weber MG, Keeler KH. (2013) The phylogenetic distribution of extrafloral nectaries in plants. *Ann Bot* **111**:1251–61.
- Weber MG, Agrawal AA (2014) Defense mutualisms enhance plant diversification. *Proc Natl Acad Sci USA* **111**:16442–7.
- Wilder SM, Eubanks MD (2010) Extrafloral nectar content alters foraging preferences of a predatory ant. *Biol Lett* **6**:177–9.
- Yang XF, Bauhus J, Both S, et al. (2013) Establishment success in a forest biodiversity and ecosystem functioning experiment in subtropical China (BEF-China). *Eur J For Res* **132**:593–606.