

A house in the tropics: full pension for ants in *Piper* plants

Una casa en el tropico: pensión completa para hormigas en plantas de *Piper*

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Abstract: On the southern Pacific slope of Costa Rica, several species of *Piper* plants (Piperaceae) live in an obligate mutualism with *Pheidole bicornis* ants (Formicidae: Myrmicinae). These plants produce small single-celled food bodies (FBs) in the leaf domatia formed by the petiole bases and roofing leaf sheaths. FBs of four of the five *Piper* species known to live with the obligate ant mutualist *Pheidole bicornis* were analysed: *P. cenocladum*, *P. fimbriulatum*, *P. obliquum* and *P. sagittifolium*. FBs mainly consist of lipids (41% to 48% of dry mass (DM)) and proteins (17% to 24%) and are a high-energy food source (up to 23 kJ g⁻¹ DM) for the inhabiting ants. By measuring the natural abundance of the stable (i.e. non-radioactive) isotopes of carbon (¹³C) and of nitrogen (¹⁵N) in FBs of *Piper fimbriulatum* and in ants it was shown that especially the larvae of the ants mainly feed on FBs. By feeding inhabiting ants with the ¹⁵N-labelled amino acid glycine, which was supplied in sucrose solution to the ants, nutrient transfer was demonstrated not only from plants to ants via FBs but also from ants to plants via faeces. Nutrient transfer from ants to plants occurred remarkably fast. Within 6 days, up to 25% of the nitrogen ingested by the ants was incorporated by the plants. However, the provision of nitrogen by symbiotic ants to the *Piper* species accounted for a minimum daily input rate of 0.8% of the plant's above-ground nitrogen uptake, which is only of minor importance for the plant partner. On the other hand, only a minute part of the above-ground biomass is actually invested in food for ants. Hence, the energy and material investment in food bodies of *Piper* plants may be compensated by the ant-derived nitrogen.

Key words: ant-plant, mutualism, ¹⁵N and ¹³C, nutrient transfer, stable isotopes.

Resumen: En las pendientes del Pacífico meridional de Costa Rica, varias especies de plantas de *Piper* (Piperaceae) viven en un mutualismo obligado con la hormiga *Pheidole bicornis* (Formicidae-Myrmicinae). Estas plantas producen pequeños cuerpos unicelulares de comida (FBs) en el domacio de la hoja formado por la base del peciolo. Se analizaron FBs de cuatro de las cinco especies de *Piper* conocidas por vivir con la hormiga mutualista obligada *Pheidole bicornis*, ellas son *Piper cenocladum*, *P. fimbriulatum*, *P. obliquum* y *P. sagittifolium*. El FBs está formado principalmente de lípidos (41% a 48% de peso seco (PS) y proteínas (17% a 24%) y son una fuente de alimento de alta energía (hasta 23 kJ g⁻¹ PS) para las hormigas que allí habitan. Mediciones de la abundancia natural de los isótopos estables (es decir no radiactivos) de carbono ¹³C y de nitrógeno ¹⁵N in los Fbs de *Piper fimbriulatum* y en las hormigas, mostraron que especialmente la larva de las hormigas se alimenta del Fbs. Al alimentar a las hormigas con el aminoácido glicina ¹⁵N, suministrado en solución de sacarosa, se demostró que los nutrientes no sólo se transfirieron desde la planta a las hormigas vía FBs, sino también de las hormigas a las plantas a través de sus fecas. La transferencia de nutrientes desde las hormigas a la planta ocurre muy rápido. En el plazo de 6 días, hasta el 25% del nitrógeno ingerido por las hormigas fue incorporado por las plantas. Sin embargo, el suministro de nitrógeno de las hormigas simbióticas a las especies de *Piper* representa solo el 0,8% del nitrógeno de la parte aérea de la planta, que resulta de menor importancia para ella. Por otro lado, solo una mínima cantidad de la biomasa aérea es ocupada en comida para las hormigas. De esta forma, la energía y el material empleado en los cuerpos de comida por las plantas de *Piper* puede ser compensado por el nitrógeno derivado de las hormigas.

Palabras clave: hormiga-planta, mutualismo, ¹⁵N y ¹³C, transferencia de nutrientes, isótopos estables.



Fig. 1: *Piper fimbriatum*: (a) closed petiole with leaf blade, (b) entrance to the apical side of the petiole, (c) longitudinal section of the petiole with food bodies and *Pheidole bicornis* ants (minor workers and larvae).

Introduction

Interactions between ants and plants are widespread and range from very loose associations to obligate symbioses (HÖLLDOBLER & WILSON 1990). While loose associations such as myrmecochory (i.e. seed dispersal by ants) also exist in temperate habitats, symbiotic ant-plant interactions are exclusively found in the tropics, exhibiting their most impressive diversity in the neotropics (DAVIDSON & MCKEY 1993b). In this type of mutualism, plants provide special structures called “domatia”, which are used as nesting spaces, and food, either directly through food bodies or extrafloral nectar or indirectly through the excretions of trophobiotic hemipterans (BUCKLEY 1987, FIALA & MASCHWITZ 1992, GAUME et al. 1998, 2000, LEAL et al. 2006). Such plants providing nesting space for ants are called myrmecophytes. Ants protect their host plants against herbivory, and sometimes from competition with other plants (DAVIDSON & MCKEY 1993a, MOOG et al. 1998, RENNER & RICKLEFS 1998, GAUME et al. 2005b, DEJEAN et al. 2006) though it may be at the cost of decreased flower production or reduced pollination (STANTON et al. 1999, IZZO & VASCONCELOS 2002, NESS 2006). Reproduction and dispersal is independent in each of the partners, and the mutualism has to be re-established in each new generation.

Apart from protection and safe nesting sites, energy flows from plants to ants and *vice versa* are one of the main mutualistic benefits in ant-plant interactions and the species involved exhibit numerous adaptations. In particular, the food bodies (FBs) provided by myrmecophytes have been shown to play an essential role in plant-ant symbioses, e.g. in *Acacia* and *Pseudomyrmex* (JANZEN 1966), *Cecropia* and *Azteca* (JANZEN 1969) and *Macaranga* and *Crematogaster* (FIALA et al. 1989).

There have been few studies on the chemical content of FBs. RICKSON (1971, 1975, 1976) found proteins and lipids in FBs of *Acacia* and phytylglycogen in the case of *Cecropia*. In FBs of *Macaranga*, HEIL et al. (1998) found carbohydrates and proteins in similar quantities, but lipids constituted the main component. It was suggested that FBs meet the specific nutritional requirements of the ants involved, although ants are known to have different demands on their food sources during different ontogenetic stages (HOWARD & TSCHINKEL 1981).

Benefits for the plants in ant-plant symbioses are not always obvious and may vary greatly. Indirect biotic defence, i.e. ants protecting plants against herbivores, encroaching vines and fungal pathogens may constitute the major benefit for many myrmecophytes (e.g. SCHUPP 1986, VASCONCELOS 1991, GAUME et al. 1997, LETOURNEAU 1998, HEIL et al. 2001a), although plants may also benefit from provision of nutrients by ants: by storing food, discarding debris or by defecation, ants accumulate organic matter in their nesting sites and this may constitute a nutrient source for the plant host. This has been considered to be significant for myrmecophytic epiphytes (JANZEN 1974, TRESSEDER et al. 1995).

In the tropical lowland rainforests of Costa Rica, an obligate association exists between the ants *Pheidole bicornis* FOREL (Formicidae: Myrmicinae) and understory treelets of *Piper* sect. *Macrostachys* (*P. cenocladum* C. DC., *P. fimbriatum* C. DC., *P. obliquum* RUIZ & PAVON, *P. sagittifolium* C. DC, and *P. calcariformis* TEBBS; RISCH et al. 1977, LETOURNEAU 1983). In this specific interaction, *Ph. bicornis* ants defend the host plants effectively against herbivorous arthropods, fungi, stem borers and invading vines (RISCH 1982, LETOURNEAU 1983, 1998). The small *Ph. bicornis* ants, only 1-2 mm in length, are totally dependent on nesting space provided by the

Piper plants. *Ph. bicornis* is polydomous (RISCH et al. 1977), i.e. a single ant colony may inhabit several plants of the same or even different myrmecophytic *Piper* species. After mating, a *Ph. bicornis* queen colonises a “petiolar chamber” formed by the appressed margins of the petiole and tightly closed petiole sheaths with bases that clasp the stem (Fig 1.). These sheathing petioles appear to be the single most important plant character in the association between ants (TEPE et al. 2007a). As colony size increases, worker ants also excavate the stem pith to create cauline domatia and to enlarge their living space. *Piper* also provides food for the ants. The ants feed on small, single-celled FBs which are produced in the petiolar chambers (Fig. 1). The FBs have an average diameter of 300–500 µm (RISCH & RICKSON 1981) and are called ‘*Piper* bodies’ or ‘Delpinian bodies’ (JOLIVET 1996). The production of FB is exclusively stimulated by *Ph. bicornis* ants and by the beetle *Tarsobaenus le-tourneauae* BARR which acts as a parasite of this mutualism (LETOURNEAU 1990, LETOURNEAU & DYER 1998). Other ants fail to stimulate these *Piper* plants to produce FBs (RISCH & RICKSON 1981). However, the signal stimulating FB production in *Piper* is not yet known. Although FB production is thought to be costly (FOLGAR-IT & DAVIDSON 1995, HEIL et al. 1997, 2001b, HEIL 2001), the chemical composition of *Piper* bodies has not been investigated in detail and the few data that have been reported are inconsistent (RISCH et al. 1977). But in this mutualism, it is not only the plants that provide food for the inhabiting ants. RISCH et al. (1977) suggested that myrmecophytic *Piper* (Piperaceae) may gain nutrients from debris accumulated in the hollow stems by *Ph. bicornis* ants.

However, it is so far unknown if the plant can take up nutrients from the ant’s debris. Therefore, we investigated whether the *Piper* plants can take up nitrogen from the debris of the ants. To estimate the extent of nitrogen input from the ant partner to its host plant, we performed tracer studies by feeding ¹⁵N-labelled glycine to *Ph. bicornis* and by monitoring levels of ¹⁵N in the two understorey myrmecophytic species, *Piper fimbriatum* C.DC and *Piper obliquum* RUIZ & PAVON. To determine whether there is a correlation between ant distribution and intra-plant variation of incorporation of ¹⁵N (a non-radioactive isotope of nitrogen), we investigated how the food was distributed within the ant colonies and how ants were distributed within *P. fimbriatum* plants. In order to understand the whole nutrient cycle, we were interested in seeing to what extent *Ph. bicornis* ants benefit from the food offered by myrmecophytic *Piper* plants. We tested the hypothesis that FBs of myrmecophytic *Piper* are the main food source of *Ph. bicornis* ants, by using analysis of stable isotope composition to estimate the degree of the ants’ dependency on



Fig. 2: Minor workers and several major workers in a young stem of *Piper cenocladum* with excavated pith to create cauline domatia and to enlarge the living space.

Piper FBs. Moreover, we provide a thorough analysis of the chemical composition of FBs of four myrmecophytic *Piper* species, focussing on the quality of FBs as a food source for mutualistic *Ph. bicornis* ants.

Materials and methods

Study site, ant and plant material

Samples were collected in the Parque Nacional Piedras Blancas (Corcovado National Park) on the southern Pacific slope of Costa Rica at a ravine in the lowland primary rainforest (ocelot trail) near the “Estación Tropical La Gamba” in the Esquinas Forest. The vegetation has been classified as tropical wet forest (HOLDRIDGE et al. 1971). Mean annual temperature is 27.0°C near the La Gamba Research Station and 25.2°C within the forest (average of 1997-2000). Mean annual precipitation is 6.000 mm. There is no distinct dry season, although precipitation is lower from January to March (WEBER 2001).

The experimental plants, *Piper cenocladum* C. DC., *Piper fimbriatum* C. DC., *Piper obliquum* RUIZ & PAVON and *Piper sagittifolium* C. DC. (Piperaceae) are myrmecophytic understorey shrubs or treelets growing to a height of up to 5 m. All four species have long-lived leaves (at least 2 years; LETOURNEAU & DYER 1998); FBs are produced in the sheathing leaf bases (Fig. 1). These plants are inhabited by *Pheidole bicornis* Forel (Formicidae, Myrmicinae) (Fig. 2), a small (1-2 mm) ant species with dimorphic (i.e. major and minor) workers, which

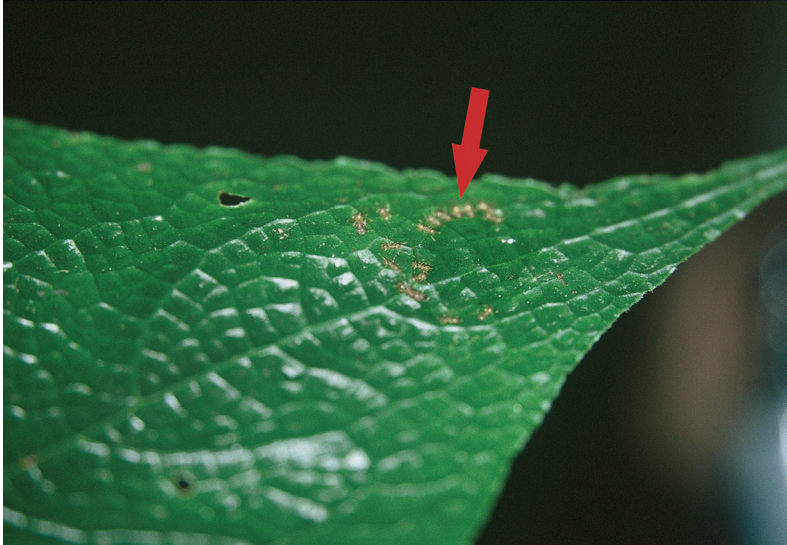


Fig. 3: *Pheidole bicornis* ants feeding on a droplet of ^{15}N -glycine in sucrose solution offered on a *Piper fimbriulatum* leaf (arrow).

feed on lipid-rich food bodies provided by the plants (RISCH et al. 1977, FISCHER et al. 2002).

Food bodies were collected from all four *Piper* species and their chemical content was analysed (see FISCHER et al. 2002).

The estimated above-ground nitrogen uptake rate (aNUR) of *P. fimbriulatum* was calculated on the basis of biometrical studies in order to determine growth rates.

Stable isotope experiments – pulse and pulse-chase experiment as well as natural abundance study

Natural abundance:

The natural abundance of the stable isotope composition of carbon (C) and nitrogen (N) was analysed in food bodies and *Ph. bicornis* ants. Additionally, non-symbiotic ants of the genus *Creumatogaster* and an undetermined *Pseudomyrmex* sp. were collected from *P. fimbriulatum* plants for comparison of stable isotope composition of carbon (C) and nitrogen (N).

Pulse and pulse-chase experiment:

Ph. bicornis ants were fed with ^{15}N -glycine (an amino acid labelled with ^{15}N , a stable isotope of nitrogen) (see Fig. 3). By this means, ^{15}N can serve as a tracer to study nutrient flows. Qualitative absorption of ^{15}N was tested in a pulse experiment with an ant colony inhabiting both a *P. fimbriulatum* and a *P. obliquum* plant. Ants were fed every second day with ^{15}N -labelled food and the plants were harvested after 15 days.

In the pulse-chase experiment, the ^{15}N -labelled food was offered only once to seven ant colonies inhab-

iting seven *P. fimbriulatum* plants and the plants were harvested after 6 days. The quantity of ^{15}N taken by the ants and the label distribution within the *Piper* plants and ant colonies was determined.

For further details concerning the extraction and chemical analysis of FBs and the experimental design of the natural abundance study as well as feeding ants with ^{15}N label to study nutrient flows from ants to plants, please refer to FISCHER et al. (2002, 2003).

Results

Chemical composition and energy content of FBs

FBs of all four myrmecophytic *Piper* species mainly contained lipids (almost 50% of the dry mass) and protein (20% of dry mass). Soluble carbohydrates constituted only about 2% of the dry mass (Fig. 4). Soluble amino acids only formed a small proportion (about 1.5%) of the dry mass (Fig. 4). Insoluble matter, such as cell wall constituents, formed about 5% of the dry mass (Fig. 4). All attempts to detect polymeric carbohydrates such as starch or phytyglycogen were unsuccessful.

FB composition was quite similar among species. Only in FBs of *P. sagittifolium* were concentrations of soluble carbohydrates and proteinaceous nitrogen significantly different from the three other species (Fig. 4). The total nitrogen content of FBs was 5.9% of dry mass for *P. obliquum* and ranged from 4.4% to 5.1% in the other species. Proteinaceous nitrogen made up the major part of total nitrogen (59% to 70%) while soluble nitrogen and cell wall nitrogen were found only in negligible quantities. The energy content of FBs was found to be high, ranging from 20.6 kJ g⁻¹ dry mass for *P. cenocladum* to 23.5 kJ g⁻¹ dry mass for *P. sagittifolium* (Table 1).

Stable isotope experiments – natural abundance study, pulse and pulse-chase experiments

To gain insight into the nutrient cycle the variation of natural occurring stable isotopes, mainly nitrogen in the form of $^{15}\text{N}/^{14}\text{N}$ and carbon in the form of $^{13}\text{C}/^{12}\text{C}$ were analysed. The heavier isotopes (^{15}N for nitrogen and ^{13}C for carbon) occur in nature in smaller quantities than the “normal” isotopes ^{14}N and ^{12}C . This relation is usually referred to as “natural abundance”. The relative abundance of the heavier isotope, e.g. ^{15}N for nitrogen and ^{13}C for carbon, is normally reported as a “delta” (δ) value in parts per thousand (denoted as ‰) enrichment or depletion relative to a standard of known composition. In our “tracer studies”, the heavier isotope is artificially introduced to the system by feeding the ants with ^{15}N -glycine. To understand the nutrient cycle

Table 1: Energy content of *Piper* food bodies. The energy content was calculated using caloric values according to Stryer (1996) and component masses of lipids, proteins, carbohydrates, amino acids and insoluble matter as shown in figure 2. Data represent means \pm SE (n = number of samples).

Energy content of FB components (kJ g ⁻¹ dry mass)	<i>Piper cenocladum</i> (n=5)	<i>Piper fimbriulatum</i> (n=3)	<i>Piper obliquum</i> (n=1)	<i>Piper sagittifolium</i> (n=3)
Lipids	15.69 \pm 0.73	18.27 \pm 0.65	16.95	18.46 \pm 0.11
Proteins	3.20 \pm 0.03	2.82 \pm 0.21	4.09	3.50 \pm 0.06
Carbohydrates	0.49 \pm 0.01	0.44 \pm 0.03	0.35	0.34 \pm 0.02
Amino acids	0.23 \pm 0.01	0.24 \pm 0.02	0.24	0.27 \pm 0.02
Insoluble matter	0.95 \pm 0.03	1.13 \pm 0.08	0.83	0.87 \pm 0.03
Total	20.56	22.90	22.46	23.44

we performed tracer studies by feeding ¹⁵N-labelled glycine (¹⁵N is a non-radioactive isotope of nitrogen) to *Ph. bicornis* ants (Fig. 3). The levels of ¹⁵N distribution from ants' faeces to the plant tissue of two understory myrmecophytic species (*Piper fimbriulatum* C.DC and *Piper obliquum* RUIZ & PAVON) was then monitored.

Natural abundance study:

Generally, the stable isotopes ¹⁵N and ¹³C show distinct patterns within food webs, higher trophic levels commonly being enriched in ¹⁵N but less so in ¹³C (PETERSON & FRY 1987). Commonly, consumers display $\delta\delta^{15}\text{N}$ values of 3.4‰ higher than their food (MINAGAWA & WADA 1984). The stable carbon isotope ¹³C is an excellent source indicator because animals that feed on the same food source display an ¹³C isotope composition similar to each other and to the food they assimilate for growth (DENIRO & EPSTEIN 1978, FRY & SHERR 1984).

FBs exhibited mean $\delta^{15}\text{N}$ values of 0.44‰ and mean $\delta^{13}\text{C}$ values of -38.84‰ (Fig. 5). The average isotope signature of *Ph. bicornis* brood was only about 1‰ more positive than that of FBs. This indicates that FBs are a major food source for ant larvae. Adult *Ph. bicornis* ants differed from FBs by approximately 2‰ in $\delta^{13}\text{C}$ and by 3‰ in $\delta^{15}\text{N}$ values. In contrast, other ant species which were not mutualists of *P. fimbriulatum* exhibited considerable differences in their $\delta^{13}\text{C}$ values: *Crematogaster* ants, living in dead trunks of the same *Piper* species were 7‰ more positive in $\delta^{13}\text{C}$ and an undetermined species of *Pseudomyrmex*, also harvested from a *P. fimbriulatum* leaf, showed a difference of 10‰ in $\delta^{13}\text{C}$ value. This indicates that these ants did not feed on FBs.

Pulse experiment:

The ¹⁵N label was fed to *Ph. bicornis* ants which inhabited a *P. fimbriulatum* and an adjacent *P. obliquum* plant. Both plants were connected by a leaf bridge (see Fig. 6) permitting one single ant colony to dwell in both plants. The ¹⁵N label was incorporated by both *Piper* species (Fig. 7) although no label was offered directly on *P. obliquum* (Fig. 7). This suggests that the ¹⁵N label was

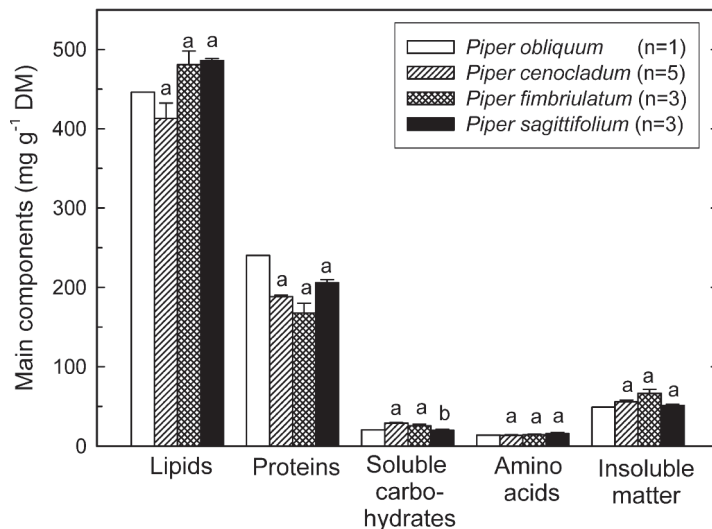


Fig. 4: Content of lipids, proteins, soluble carbohydrates (including glycerol and *myo*-inositol), amino acids and insoluble cell wall components of food bodies (FBs) of four myrmecophytic *Piper* species. Bars indicate means \pm SE. Different letters show significant differences between means of species within each component ($P < 0.01$, unifactorial ANOVA and Scheffé post hoc test). *P. cenocladum* ($n=1$) was excluded from statistical analyses.

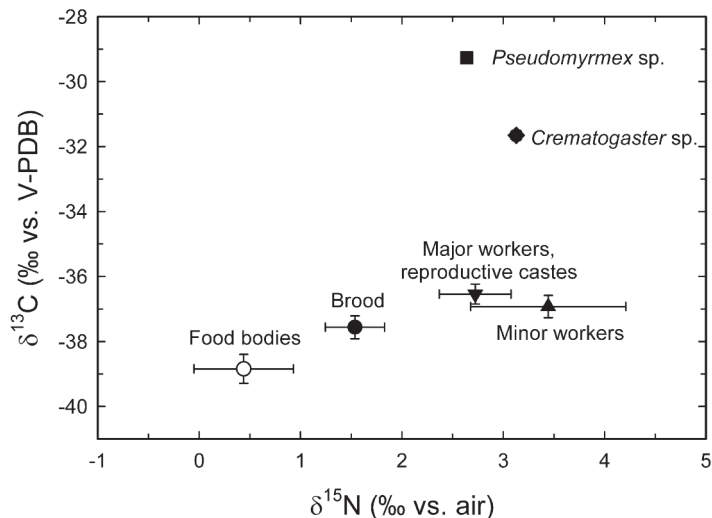


Fig. 5: Natural abundance of ¹³C and ¹⁵N in FBs of *Piper fimbriulatum* ○ ($n=9$), and different ant species and castes. ●, *Pheidole bicornis* brood ($n=14$); ▲, *Ph. bicornis* minor workers ($n=11$); ▼, *Ph. bicornis* major workers and reproductive castes (males, females, queen) ($n=10$); ■, *Pseudomyrmex* sp. ($n=1$); ◆, *Crematogaster* workers ($n=5$) living in dead trunks of *P. fimbriulatum*. Values represent means \pm SE.



Fig. 6: Leaf bridge between *Piper fimbriatum* (left) and *Piper obliquum* (right) inhabited by the same *Pheidole bicornis* ant colony. Ants are queuing up to use the leaf bridge.

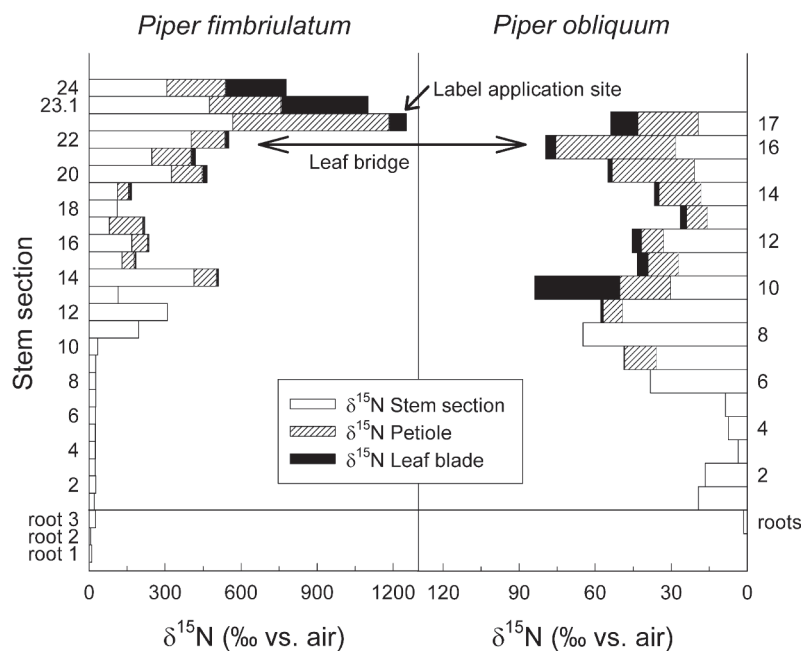


Fig. 7: Pulse experiment: $\delta^{15}\text{N}$ values of stem sections, petioles and leaf blades of two *Piper* plants inhabited by a single colony of *Pheidole bicornis* ants. Over 15 days, ants were fed 7 times with ^{15}N -glycine in sucrose solution or olive oil. The leaf where labelled solution was offered to the ants is indicated by an arrow. Between stem sections 22 (*Piper fimbriatum*) and 16 (*Piper obliquum*) a non-permanent "leaf bridge" allowed ants occasionally to pass from one plant to the other. Root 1 to root 3 indicates different root fractions (1, main roots; 2, side roots; 3, fine roots). Numbers indicate shoot sections, representing a node plus the respective internode; counting starts with the oldest, basal part. Section 23.1 represents a young side branch initiating at the 23rd storey of the main shoot. Note different $\delta^{15}\text{N}$ scales in *P. fimbriatum* and *P. obliquum*.

exclusively brought into *P. obliquum* by the ants via their faeces and debris. The ^{15}N distribution in both plant species had a peak in the youngest and in the middle-aged parts of the plants (Fig. 5). This may be due to

the fact that more nitrogen is needed where new tissue is formed.

Pulse-chase experiment:

This experiment was made to quantify the label uptake by ants and the incorporation into plant tissues in order to estimate the importance of the nitrogen supply from ants to plants.

The offered ^{15}N -glycine label was differentially allocated within the ant colony: Highest incorporation rates of the label within individual ants were found in larvae, followed by major workers, females, minor workers, queens and males (Fig. 8). Based on whole colonies, the sum of small larvae contained 44% of the total ^{15}N per colony after 6 days. Significant proportions of ^{15}N were also found in major workers (29% of total ^{15}N per colony), minor workers (15%) and large larvae (8%). Pupae of major workers and the sexual caste showed no ^{15}N incorporation, while pupae of minor workers exhibited only a slight incorporation of ^{15}N (Fig. 8).

Within four hours of giving the labelled source to the ants, *Ph. bicornis* workers acquired approximately 60% (226 $\mu\text{g } ^{15}\text{N}$) of the total ^{15}N -labelled material which was offered to them (Table 2). After 6 days, and averaging all five experimental plants of the pulse-chase experiment, 71% of total incorporated ^{15}N was retained in the ant colony, 3% remained in ant-derived detrital and faecal material which was deposited by the ant colonies within the plants and more than 25% of the ^{15}N nitrogen was found within the plants (Table 2).

This 25% of the ^{15}N label ingested by ants had been transferred into the plants within 6 days and amounted to an average of 57.5 $\mu\text{g } ^{15}\text{N}$ per plant. This approximated a daily nitrogen supply by ants of only 0.8% of the daily nitrogen demand for above-ground growth ($\text{aNUR} = 1255 \mu\text{g N plant}^{-1} \text{d}^{-1}$) of these plants.

Conclusions

We investigated a possible nutrient cycle from plants to ants and *vice versa*. The transfer from ants to plants was investigated by feeding ants ^{15}N -labelled food (Fig. 3). The food was not distributed uniformly within the colony but was allocated mainly to the larvae and to the working caste (Fig. 8). Therefore, the majority of the food flowed both into workers and into larvae to ensure the maintenance of the colony's workforce and its survival. We also found unequivocal proof for a transfer of nutrients from ants to *Piper*. We detected ^{15}N in a *P. obliquum* plant which shared an ant colony with an adjacent *P. fimbriatum*. Since labelled nitrogen was applied only on *P. fimbriatum*, all the ^{15}N found in *P. obliquum* must have been brought in by ants passing

Table 2: ^{15}N content of the *Pheidole bicornis*/*Piper fimbriatum* system 6 days after inhabiting ants were fed with ^{15}N -glycine in sucrose solution. Nitrogen incorporation is given as mean \pm SE of plant fractions, debris and ants ($n = 5$ for experimental plants, $n = 2$ for control plants).

	Nitrogen content ($\mu\text{g } ^{15}\text{N fraction}^{-1}$)	Nitrogen content (% ^{15}N of plant ^{15}N)	Nitrogen content (% ^{15}N of total ^{15}N)
Experimental plants			
Leaf blades	8.6 \pm 2.9	15.0	3.8
Petioles	10.7 \pm 2.6	18.6	4.7
Stems	34.3 \pm 4.6	59.6	15.2
Roots	3.9 \pm 2.7	6.8	1.7
Total plant (sum)	57.5 \pm 6.7	100.0	25.4
Debris	7.1 \pm 1.7		3.2
Ants	161.5 \pm 23.9		71.4
Total ant-plant system	227.1		100.0
Control plants			
Leaf blades	1.24 \pm 1.17	24.9	21.7
Petioles	2.70 \pm 2.69	54.2	47.2
Stems	0.95 \pm 0.88	19.1	16.6
Roots	0.09 \pm 0.09	1.8	1.6
Total plant (sum)	4.98 \pm 4.82	100.0	87.1
Debris	0.47 \pm 0.16		8.1
Ants	0.27		4.7
Total ant-plant system	5.72		100.0

across the leaf bridge (Fig. 6 and 7). Within six days, more than 25% of the ^{15}N collected by ants had been transferred to the host plants.

Several factors can influence the distribution of the ^{15}N label within the plants. First, plants may allocate nutrients via the phloem from the place of incorporation to other plant parts (= translocation). This is supported by significant incorporation of ^{15}N found in the youngest parts of *P. fimbriatum* plants (e.g. Fig. 7), which are not colonisable by the ant partners, and a high $\delta^{15}\text{N}$ value in very old stem sections where no ants are living.

Secondly, the place where ants predominantly stay may vary during the day and may be influenced by temperature gradients when the understorey plants are directly lit by patches of sunlight. *Ph. bicornis* may move to optimal microclimatic locations within the plants. Thirdly, the distribution of the ^{15}N -label in some plants indicated that myrmecophytic *Piper* plants may use debris as a nutrient source because we found higher label incorporation rates in stem sections where debris was deposited.

Stem sections generally showed higher ^{15}N content than did petioles and leaf blades. Anatomical analysis revealed that parenchyma cells and cell fragments lie bare on the inner surface of young hollow stems following removal of pith tissue by ants (see FISCHER et al. 2003). In newly excavated stem sections no callus or any other type of wound or boundary tissue that could hinder an uptake of nutrients was found; wound tissue is only pres-

ent in older stems (see also TEPE et al. 2007b). The wound tissue in older stem parts could hinder ^{15}N uptake and absorption could be restricted to young, recently excavated cavities and subsequently translocated within the plant. This could explain the discrepancy between ant distribution and ^{15}N incorporation pattern within the plants (data not shown, but see FISCHER et al. 2003).

In contrast, the adaxial (i.e. the inner) surface of the leaf sheaths – where ants also live and where food bodies are produced – has a cuticle regardless of its age (RISCH & RICKSON 1981). Therefore, it is most probable that nitrogen incorporation takes place primarily in the stem of *P. fimbriatum*.

However, nitrogen input from ants to plants does not necessarily represent a significant advantage for these ground-rooted plants. After six days, we found 57.5 μg of ant-derived ^{15}N in *P. fimbriatum* (Table 2). This means that plants incorporated on average 10 μg of ant-provided nitrogen per day, accounting for only 0.8% of the daily above-ground nitrogen uptake rate of the investigated plants under the experimental conditions. Under natural conditions, food bodies offered by myrmecophytic *Piper* species constitute the main food source of *Ph. bicornis* (see below and FISCHER et al. 2002). Nitrogen import through *Ph. bicornis* by food sources other than food bodies accounts for less than 5 μg N per plant per month (LETOURNEAU 1998). Therefore, the nitrogen input from ant debris and faeces may be even less important under natural conditions. Thus, nitrogen provision by *Ph. bicornis* may be interpreted as recycling of the nitrogen invested into food bodies.

Myrmecophytic *Piper* species may be more restricted by light than by nutrient availability and a nutrient supply by ants may not represent a significant evolutionary advantage for these understory myrmecophytes.

On the other hand, the nutrient supply from *Piper* plants to the mutualistic *Ph. bicornis* ants as food bodies (FB) seems to be quite important. The quite similar isotope signatures of FBs and ant brood (i.e., growing larvae and pupae) may be interpreted as the isotope difference between an animal species and its main diet. FBs seem to be the sole food source for *Ph. bicornis* brood in terms of carbon and nitrogen nutrition. The isotope signature of adult *Ph. bicornis* indicate that they feed on supplementary food sources by chance, e.g. during patrolling the host plant (Fig. 5). Non-symbiotic ants, such as *Crematogaster* sp. and *Pseudomyrmex* sp., seemed to occupy other dietary niches.

It has been proposed that the demands of ants on food sources vary among ontogenetic stages (HOWARD & TSCHINKEL 1981). Lipids are mainly used by workers and some larvae (ECHOLS 1966), carbohydrates by foragers and larvae (SORENSEN & VINSON 1981, CASSIL & TSCHINKEL 1999), proteins by growing larvae and egg-laying queens (LANGE 1967, VINSON 1968) while amino acids are shared throughout the colony (HOWARD & TSCHINKEL 1981).

It was also a matter of interest to examine whether or not FBs are able to cover the nutritional requirements of all developmental stages of *Ph. bicornis*. A detailed chemical analysis proved that FBs are an energy-rich food source (up to 23 kJ g⁻¹ DM; Table 1) which meet the requirements of all different developmental stages of an ant colony. FBs have a similar energy content to milk chocolate (22 kJ g⁻¹). However, the main components of a chocolate bar are carbohydrates while FBs of the four investigated *Piper* species consisted mainly of lipids (almost 50% of dry mass, Fig. 4). Carbohydrates were only found in minute quantities (Fig. 4) but may be metabolised by *Ph. bicornis* from lipids. FBs contained considerable amounts of soluble proteins (20% of dry mass, Fig. 4) which is needed especially by ant larvae for their growth (LANGE 1967, VINSON 1968).

Piper FBs exhibited a similar energy content to Müllerian bodies of *Cecropia*, a plant which also has inhabiting ants (FOLGARAIT & DAVIDSON 1995) (Table 1), but a higher nitrogen content, suggesting that *Piper* invests more nitrogen in FBs than does *Cecropia*. *Piper* bodies comprised twice as much lipid and four times more protein on a dry mass basis than FBs of myrmecophytic *Macaranga* species (HEIL et al. 1998), leading to higher energy content. A possible explanation for the high nutritive and energetic value of *Piper* FBs may be

that *Ph. bicornis* do not receive additional food sources from *Piper* – neither directly by extrafloral nectaries nor indirectly through sap-sucking homopterans. This is in contrast to most other ant-plant associations – *Piper* may thus have to invest more energy in FBs to meet the demands of *Ph. bicornis*. But how costly may this investment be for *Piper* plants?

As food for symbiotic ants, *Piper* FBs may be regarded as the plants' investment in indirect defence by ants. FB production has been reported to be costly for myrmecophytes (FOLGARAIT & DAVIDSON 1995, HEIL et al. 1997, 2001b, HEIL 2001). However, it might be more advantageous to invest energy in FBs to feed highly effective ant partners than to invest in perhaps more costly secondary compounds (FEDERLE et al. 1997). The *Ph. bicornis* ants are timid but effective defenders of *Piper* plants (LETOURNEAU 1998) and FBs constitute only a very small part of the plants' dry mass. Biometric studies on young *P. fimbriulatum* revealed that plants with an average height of 1.5 m had a mean above ground biomass of 32 000 mg dry mass (DM), while the total FB mass was only 3 mg DM per plant ($n = 7$, data not shown). Even taking into consideration a possible high turnover rate of FBs, only a minute part of the above ground biomass is actually invested in food for ants.

In summary, (1) the stable isotope signatures clearly reflect the dependency of *Ph. bicornis* on the FBs of its host plants, (2) FBs constitute a well balanced diet for most developmental stages of symbiotic ants, (3) ants provide nutrients to the ground-rooted *Piper* myrmecophytes although (4) the uptake of ant-derived nitrogen is only of minor importance for the plant partner. Consequently, the energy and material investment in food bodies of *Piper* myrmecophytes may be compensated by a tight cycling of nutrients.

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