Liquid foraging behaviour in leafcutting ants: The lunchbox hypothesis

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Efficient food acquisition is a central challenge faced by organisms, and optimal foraging theory (OFT) explores the ways natural selection has fine-tuned foraging strategies to meet this challenge, maximising food harvest while minimising foraging time and exposure to predators (Stephens, Brown & Ydenberg, 2007; Stephens & Krebs, 1986). OFT models typically weigh energetic dietary gains relative to energetic foraging costs and predict reduced fitness with excessively costly or unsuccessful foraging trips (Pyke, Pulliam & Charnov, 1977). However, it is often not feasible to directly observe foraging dynamics including ecological processes related to ingestion (e.g. food capture and transport) and physiological processes related to digestion (e.g. food assimilation). This means that measuring foraging success, and thus testing OFT models, can be difficult even with inferential techniques (e.g. stable isotopes; Feldhaar, Gebauer & Blüthgen, 2009).

Many animals are ‘central place foragers’ and a specific set of OFT models has helped understand how they overcome issues like local resource depletion when repeatedly returning harvested resources to a central nest or sleeping site (Orians & Pearson, 1979; Oster & Wilson 1978; Stephens & Krebs, 1986). Ant colonies have provided model systems for testing OFT predictions because they are central place foragers that use diverse collective foraging behaviours to locate, defend, and transport resources back to stationary nests (Lanan, 2014; Oster & Wilson, 1978; Roces & Núñez, 1993). Moreover, individual workers dynamically adjust foraging behaviours when harvesting resources with varied nutritional compositions (Dussutour & Simpson, 2008; Kay, 2002; Portha, Deneubourg & Detrain, 2002) and physical properties (Robson & Traniello, 1998). For instance, many ants carry protein-rich insect prey in their mandibles and transport carbohydrate-rich liquids (e.g. nectar from plants or insect symbionts) within three connected specialised storage organs (crop, midgut, hindgut; Fig. 1) (Engel, Fischer, Wäckers & Völkl, 2001; Josens, Farina & Roces, 1998).

In colonies where solid food is most commonly harvested, foragers returning full of liquids can appear ‘unladen’, or lacking harvested resources, and thus be erroneously considered energetic drains on their colonies. However, these liquid resources support an ant colony’s ‘social stomach’, with a digestive adaptation called the proventriculus (Fig. 1c), enabling workers to regurgitate liquids and share them with nestmates (Cook & Davidson, 2006; Eisner, 1957; Eisner & Brown, 1958). Leafcutter ants (Attini; genera *Atta* and *Acromyrmex*) are the most evolutionarily derived fungus-farming attine ants, cutting and transporting mostly fresh vegetation they prepare as compost to cultivate fungus crops for food in subterranean nests (Hölldobler & Wilson, 2010). Leafcutter foragers often transport loads optimised in size and shape to their individual foraging abilities (Lewis, Pollard & Dibley, 1974; Wetterer, 1994, 1995). However, foraging trips also often appear unsuccessful as foragers return without carrying any vegetation (Araújo, Lucia, Lima, Souza & Petternelli, 2002; Lewis, et al., 1974, but see Kooij, et al., 2014). While these unladen foragers appear to present efficiency problems relative to OFT predictions, they have alternatively been hypothesised to lead nestmates to high-quality resources (Bollazzi & Roces, 2011; Jaffe & Howse, 1979; Roces & Núñez, 1993), or maintain foraging trails (Lewis, et al., 1974).

Unladen leafcutter foragers have also frequently been assumed to transport, consume, and assimilate liquid resources in the form of carbohydrate-rich plant nectar (Bass & Cherrett, 1995; Littledyke & Cherrett, 1976; Mueller, Schultz, Currie, Adams & Malloch, 2001; Wirth, Herz, Ryel, Beyschlag & Hölldobler, 2003), but no direct evidence has been provided that this happens routinely in the field. While many ant lineages are known to consume nectar or similar plant secretions (Hölldobler & Wilson, 1990), it has remained underappreciated that specialised fungivory may constrain opportunities to maintain a complementary generalist feeding strategy. Indeed, since the digestive system of *Acromyrmex* leafcutter ants appears to be specialised for vectoring fungal enzymes to new

garden growth via fecal droplets (De Fine Licht, et al., 2013; Kooij, et al., 2014; Martin, 1970; Schiøtt, Rogowska-Wrzesinka, Roepstorff & Boomsma, 2010), opportunistic foraging on other liquids would likely destabilise this fine-tuned system. A likely alternative is that while nectar foraging does not occur, the possibility has remained plausible as no study has used dissections to remove the thick, opaque abdominal exoskeleton and confirm the presence or absence of liquids in storage organs of unladen workers returning to the nest (Fig. 1a).

We first used dissections of four Panamanian rainforest leafcutter species to test the OFT prediction that unladen leafcutter ants actually represent successful foraging trips because they are more likely to harvest liquids. We initially established baseline levels of liquid storage in foragers collected as they left their nests, reasoning that if returning foragers had excess liquids above this baseline, they harvested them outside the nest. This led to the surprising observation that most foragers carried liquids in their midguts when exiting their nests, which, in turn led us to perform additional experiments testing a newly formalised ‘lunchbox hypothesis’. Below, we develop this hypothesis within an OFT framework, outlining how it integrates digestive physiology, energetic foraging costs, and symbiotic stability.

The lunchbox hypothesis provides an OFT prediction that foraging leafcutter ants leave nests with full midguts, which they deplete to fuel energetically costly foraging activities. These energetic costs include the cutting of leaves, which requires extreme mandibular forces (Roces & Lighton, 1995), foraging trips extending >200 meters from the nest (Lewis, et al., 1974), and the transport of heavy loads (Lighton, Bartholomew & Feener, 1987) weighing more than double the body mass of a forager (Wetterer, 1994). Whereas nectar foraging would appear at odds with the specialised interplay between ant farmers and fungal crops, lunchbox dynamics would provide a powerful nutritional mechanism

integrating the performance of symbiotic partners, as fungi would fuel ant foragers to perform foraging tasks needed to harvest resources that fuel fungal growth. We tested the lunchbox prediction that liquid depletion reflects task performance using a series of laboratory experiments manipulating foraging distance, load mass, and leaf-cutting activity.

METHODS

Liquid Transport in Field-Collected Foragers

We observed foraging behaviour in a rainforest within Soberania National Park, Panama (N 9.15451, W 79.73583) in May 2015, during the start of the rainy season, a period of high leafcutter activity. We observed four sympatric leafcutter species: *Acromyrmex echinator* (5 colonies), *Acromyrmex octospinosus* (3 colonies), *Atta colombica* (3 colonies), and *Atta sexdens* (3 colonies). This sampling effort is similar to other studies on leafcutter foraging dynamics (e.g. Burd & Howard, 2005; Lopes, Forti & Camargo, 2004).

We counted the number of laden and unladen foragers returning to nests in focal observations that were ≥ 30 minutes for *Acromyrmex* (*Ac. echinator*, $n = 8$; *Ac. octospinosus*, $n = 4$) and 3 minutes for *Atta* (*At. colombica*, $n = 6$; *At. sexdens*, $n = 6$). This sampling effort yielded a total of 374 observation minutes for *Ac. echinator* (365 returning ants), 151 minutes for *Ac. octospinosus* (130 returning ants), 18 minutes for *At. colombica* (2 679 returning ants), and 18 minutes for *At. sexdens* (2 078 returning ants). Colonies of *Atta* (ca. 100 workers per minute) had far more workers foraging per minute than *Acromyrmex* (ca. 1 worker per minute), which necessitated both shorter foraging observation windows and the use of video recordings to accurately capture the laden vs. unladen worker foraging dynamics (Table A1). We thus counted *Acromyrmex* workers directly in the field and video recorded foraging *Atta* trails with an iPhone 4S, slowing the recordings to 15 frames per second in VLC media player (v2.2.1). We calculated mean per cent of unladen returning foragers (not

carrying any substrate in their mandibles) per observation period, per colony, per species ($\pm$ SE).

We next tested whether unladen returning foragers were more likely to transport liquid resources, collecting foragers from three categories (laden returning, unladen returning, and exiting the nest), and returning them to the lab in separate containers for dissections to determine empty/full status of liquid storage organs (crops, midguts, hindguts; Fig. 1). Soon after being returned to the lab, foragers were secured under water in wax-filled petri dishes under a dissecting microscope (160X magnification) and their abdomens opened with #5 watchmaker forceps. Full storage organs had a characteristic distended appearance and were usually filled with yellow fluid (Fig. 1). Since repeated dissections of workers were not possible, we used the following logic to infer that liquids were harvested outside the nest. First, we established baseline liquid storage dynamics by collecting exiting workers immediately upon leaving their nests for foraging trips. Second, if returning workers had more stored liquids than exiting workers, we inferred these represented liquids harvested outside the nest.

We dissected 8.33 ± 0.88 *Acromyrmex* foragers and 18.80 ± 1.29 *Atta* foragers per foraging category, per collection event, and dissected a total of 670 foragers (*Ac. echinator*: 161, *Ac. octospinosus*: 114, *At. colombica*: 200, *At. sexdens*: 195). We selected the ‘typically’ sized forager for each species (Hughes, Summer, Van Borm & Boomsma, 2003; Moreira, et al., 2010), measuring the headwidth (HW in mm) from eye to eye for all dissected ants (*Ac. echinator*: 2.2 ± 0.2 , *Ac. octospinosus*: 2.3 ± 0.3 , *At. colombica*: 2.3 ± 0.3 , *At. sexdens*: 2.1 ± 0.3). Thus, while *Atta* species are distinct from other attines in having substantial intracolony variation in worker body size corresponding to task specialisation (Hölldobler & Wilson, 2010; Wilson, 1980), we compared similarly sized workers across all colonies in the present study.

We used the GLIMMIX procedure in SAS (V 9.3) to perform mixed model analyses with an underlying Gaussian distribution testing whether the proportion of individuals with full storage organs varied with foraging status (laden, unladen, exiting), storage organ (crop, midgut, hindgut), and species identity (*Ac. echinator*, *Ac. octospinosus*, *At. colombica*, *At. sexdens*). To account for multiple sampling events for some colonies, we included colony ID as a random factor nested within species. We evaluated significant results here and in all tests described below with post-hoc multiple comparison tests, applying Holm-Tukey adjustment of observed p-values to control for Type I error.

Liquid Transport and Foraging activity: The Lunchbox Hypothesis

To test the lunchbox hypothesis, we performed a series of laboratory experiments on three intact *Ac. echinator* colonies (Ae276, Ae342, Ae480) collected from Panama, and maintained at the University of Copenhagen in a climate-controlled room (25°C and 75% RH) housed in plastic nest boxes (38 x 28 cm) with fluon-coated walls. We first dissected 102 foragers collected immediately after they exited the nests to establish baseline liquid transport frequencies (full vs. empty) in crops (41% full), midguts (91% full), and hindguts (91% full). We then tested whether foragers depleted more liquids with increasing foraging distances, load weights, and leaf-cutting activity.

In a full-factorial design, we first compared liquid transport status in foragers varying in load mass (no load vs. 10 mg load) and foraging distance (2 m vs. 10 m). Colonies were connected via a removable bridge to a ‘middle box’ that was connected to a foraging arena via clear 1 cm inner diameter tygon tubing. We manipulated foraging distance by connecting middle boxes and foraging arenas with 2 m or 10 m lengths of tubing. Laden foragers had a 10 mg clay ball affixed to their dorsal thoracic spines (Lighton, et al., 1987; Wetterer, 1995) while ‘unladen’ control foragers lacked a clay ball. The clay ball was gently affixed

immediately after removing foragers from the empty foraging arena, and they were then placed back in the arena. We collected and dissected returning foragers as soon as they reached the middle box on their way back to the colony. We quantified the effects of experimental handling on liquid storage, comparing liquid storage in workers seconds after affixing the clay ball ($n = 30$) with control workers having just left their nest to forage ($n = 30$). Handling treatment did not significantly affect the overall presence of stored liquids ($F_{2,174} = 0.01$; $p = 0.910$), nor did handling effects vary across organs ($F_{2,174} = 1.36$; $P = 0.261$).

Prior to experiments, we placed blackberry leaves (*Rubus* sp.) in the foraging arena for ≥ 5 hours so colonies would establish foraging trails inside the tube. When collecting foragers for the experiment, we removed the leaves to avoid potential liquid harvest resulting in exiting foragers arriving to an empty foraging arena. We then collected foragers as they arrived in the arena to use for load mass manipulation. Although leafcutters are known to carry loads of >10 mg (Cherrett, 1972), the load mass we used approximated the average leaf fragment weight (9.3 ± 1.03 mg, $n = 24$) transported by laden *Ac. echinator* foragers in the lab colonies. We used the GLIMMIX procedure as described above to test for differences in the proportion of individuals with full storage organs across load mass treatments (0, 10 mg), distance treatments (2, 10 m), and storage organs (crop, midgut, hindgut), with colony ID included as a random factor. As above, we modelled residual variance with storage organ as a repeated factor, with a covariance structure based on separate residual variances for each organ and residual covariance between two adjacent organs moving from the crop to midgut to hindgut.

We tested whether foragers depleted transported liquids when cutting leaves. We allowed foragers from five colonies (Ae332, Ae376, Ae430, Ae490, Ae507) to access the middle box containing a small branch with blackberry leaves. We collected foragers for

dissection immediately after they finished cutting a leaf fragment ($n = 84$) and compared their liquid status to exiting control foragers collected just after leaving the nest ($n = 78$). We used the same statistical approach described above to test for differences in proportions of individuals with full storage organs (crop, midgut, hindgut) depending on leaf-cutting activity (cutting, exiting).

Ethical Note

Dissections were necessary to test the hypothesis of liquid foraging, since leafcutter ants have thick, opaque cuticle (Fig. 1). However, sacrificed individuals represented an exceedingly small fraction of overall colony populations (e.g. 40 workers from colonies with millions of workers). Thus, the present study did not harm leafcutter ant colonies that were observed in the field (remain intact in Panama) or lab (maintained in Copenhagen), as all colonies continued to forage normally after the experiment, and in no case was the queen harmed. Prior to dissections, ants were secured unconscious underwater in a wax filled dish, and workers were transferred to 95% EtOH as soon as observations were complete.

RESULTS

Liquid Foraging Behaviour in the Field

Unladen workers without any substrate in their mandibles were common across four leafcutter species, representing $> 41\%$ of all 5 252 returning workers observed during > 500 minutes of field observations (Fig. 2). Colonies of *Ac. echinator* had 1.17 ± 0.31 returning ants per minute, 38.1% of which were unladen, while *Ac. octospinosus* had 0.96 ± 0.22 ants per minute, 31.2% of which were unladen (Fig. 2). Colonies of *At. colombica* had 148.83 ± 30.57 returning ants per minute, 48.1% of which were unladen foragers, while *At. sexdens*

had an average foraging rate of 115.44 ± 35.19 returning ants per minute, 44.7% of which were unladen (Fig. 2).

Liquid transport behaviours in the field did not vary significantly across leafcutter species ($F_{3,10.99} = 0.42$, $P = 0.744$; Table 1), but liquid transport varied across storage organs depending on foraging status ($F_{4,50.94} = 5.10$, $P = 0.002$; Table 1; Fig. 3). Specifically, foragers tended to exit nests with full midguts and return with depleted midguts when laden with vegetation (Fig. 3, Table A2). Moreover, foragers were more likely to transport liquids in their midguts than their crops (Fig. 3; Table A2), but the likelihood of transporting liquids in their midguts and hindguts was not significantly different (Fig. 3; Table A2), indicating low potential of transport for sharing with nestmates because digestion is unidirectional after passing through the proventriculus. Laden returning foragers also tended to have fuller hindguts than midguts (Fig. 3; Table 1; Table A2), suggesting foraging work involved the assimilation of liquids from the midguts and transfer of waste to the hindgut.

Liquid Transport and Foraging Behaviour: The Lunchbox Hypothesis

Liquid transport behaviour in controlled laboratory experiments likewise varied across storage organs and foraging activities. First, as in the field, foragers more frequently carried liquids in their midguts and hindguts than in their crops ($F_{2,7.91} = 202.91$, $P = 0.0001$; Table 1), with ca. 22% foragers having crop liquids compared to ca. 83% foragers having midgut liquids (Fig. 4). Second, experimentally laden workers transported liquids in all storage organs less frequently than unladen foragers ($F_{1,10.63} = 17.46$, $P = 0.002$; Table 1), regardless of whether they foraged 2 m or 10 m ($F_{1,10.63} = 2.55$, $P = 0.139$; Table 1; Fig. 4). Third, leaf-cutting activity did not significantly reduce overall liquid storage ($F_{1,12.84} = 0.99$, $P = 0.338$; Table 1), or midgut-specific liquid storage ($F_{2,7.204} = 0.61$, $P = 0.569$; Table 1).

DISCUSSION

This study tests whether the OFT prediction that individuals forage to maximise energetic returns holds when the foragers belong to a highly derived symbiosis that would likely be destabilised by such foraging decisions. Field observations and laboratory experiments confirm that leafcutter ants do not forage for liquid nectar and thus question the decades-old assumption that they acquire nutrition from sources other than their tightly co-evolved fungal cultivars. Moreover, while over 40% of the foragers in four sympatric leafcutter species returned to their nests without leaf fragments, these unladen ants were not more likely to carry liquids. Instead, we find support for the lunchbox hypothesis because most workers left nests with midguts full of liquids that were depleted (assimilated and transferred to hindguts) if workers returned with a leaf fragment in the field or transported a load in lab experiments. Thus, in contrast to the destabilising effects of external nectar foraging, these results provide a novel mechanism promoting symbiotic stability, as fungi provide fuel for foragers to harvest more substrate for fungal crop production.

Such nutritional feedbacks likely govern the ecological success of the leafcutter symbiosis, dominant herbivores in habitats across the New World tropics and subtropics (Wirth, et al., 2003). Specifically, leafcutter ants produce a highly ‘domesticated’ fungal cultivar *Leucoagaricus gongylophorus* that no longer grows freely in the wild (Mueller, et al., 2001) and depends completely on vegetation foraged by ants for its food. Thus, if lunchbox resources fuel worker foraging, they are analogous to investment in hypha production by ancestral free-living Basidiomycete fungi (Mueller, et al., 2001) that extend hyphae to forage for decaying organic matter on the forest floor. More generally, organisms engaged in symbioses often lose critical functions (Gil, Sabater-Muñoz, Latorre, Silva & Moya, 2002), and lunchbox dynamics provide a means of eliciting the replacement cooperative behaviour from symbiotic partners.

Littledyke and Cherrett (1976) performed a ground breaking study of nutritional dynamics within leafcutter farming symbioses, using radiolabeled tracers (P^{32} , C^{14}) to indirectly track the flow of nutrients from harvested substrate to fungus cultivar to ant farmers. They found some evidence of liquid ingestion when a leaf fragment was placed directly on the fungus garden and when an exposed 'leafy shoot' was provided (Littledyke & Cherrett, 1976). However, liquid ingestion was not actually detected (or barely detected) among the leafcutter workers cutting leaves in foraging arenas (Littledyke & Cherrett, 1976). It is also unclear whether any of the P^{32} , C^{14} tracers reflected liquid assimilated by adult workers or were simply processed as fecal droplets used to manure fungus gardens. And yet, the idea that foragers consume, transport, and assimilate plant exudates while cutting and preparing leaves has been frequently used to assert that ants consume large amounts of nutrients from outside of their farming symbiosis (e.g. Bass & Cherrett, 1995; Mueller, et al., 2001; Wirth, et al., 2003).

Direct consumption by workers of nutritional subsidies from plants would imply that fungi do not provide sufficient nutrition for ant farmers (Bass & Cherrett, 1995; Mueller, 2002; Mueller, et al., 2001), and run counter to major recent advances towards understanding the specialised physiological interplay between ant farmers, their fungal cultivars, and additional microbial partners. For instance, leafcutter ants receive enzymes from ingested fungus that are then passed unharmed through the digestive system and returned to the garden as faecal droplets to manure new garden substrate (De Fine Licht, et al., 2013; Kooij, et al., 2014; Martin, 1970; Schiøtt, et al., 2010). Moreover, N-fixing rhizobiales bacteria have been discovered in the leafcutter fungus gardens (Pinto-Tomás, et al., 2009) and the guts of workers (Sapountzis, et al., 2015). Comparing these specialised digestive adaptations in leafcutter workers with their highly simplified crops and proventriculi (Caetano, 1990; Fig. 1)

strongly supports that leafcutter ants in natural populations lack plant nectar harvesting as a foraging strategy.

We next review prospects for the lunchbox hypothesis, which provides a novel strategy by which departing foragers can fuel their foraging by dynamically ingesting a short-term pulse of nutrition. First, while only load carriage was associated with liquid depletion in this study, ants may indeed use midgut liquids when the cutting more recalcitrant leaves, or foraging greater distances, especially while climbing trees. Workers may also fuel energetically costly foraging activities with alternative metabolic fuels (e.g. glycogen from fat body cells; Roma, Mathias & Bueno, 2006). Second, stored liquids were not identified, as leafcutter ants have a sieve-like organ preventing particles with diameter $> 10\ \mu\text{m}$ from entering their alimentary canals (Mueller, et al., 2001), and this prevented visual inspection of partially digested foods. Third, while we confirm a within-nest origin for lunchbox liquids, a possible retention duration of 10 days (Erthal, Silva & Samuels, 2004) makes the precise source difficult to ascertain. For instance, exiting workers may directly consume fungi or solicit regurgitated liquids from larvae. It will thus be important to both identify the chemical makeup of lunchbox liquids and the behaviours preceding their acquisition.

Moving forward, lunchbox dynamics can be tested across diverse ant species facing diverse ecological challenges. For instance lunchbox liquids may be limited to ecological specialists with ample within-nest liquid supplies, like leafcutter ants with fungi, species tending exudate-producing hemipterans inside their nests (Hölldobler & Wilson, 1990), or arid climate species with liquid storage castes (Rissing, 1984). However, such ecological specialisations may also be unnecessary, as ants generally have larvae inside their nests that could provide foragers with regurgitated liquids (Hölldobler & Wilson, 1990). More generally, the lunchbox hypothesis provides a robust theoretical framework to link foraging energetics, digestive physiology, and symbiotic performance.

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440

441 **FIGURES**

442 **FIGURE 1** Leafcutter ant digestive system, highlighting the three connected storage organs
443 (crop, midgut, hindgut) described in the text. (a) Dorsal view of storage organs in a partially
444 dissected *Ac. echinator* worker abdomen showing a full hindgut and midgut and empty crop
445 (white spheres are fat body cells). (b) Lateral view of fully dissected *At. sexdens* digestive
446 system showing a midgut full with the characteristic yellow fluid flanked by an empty crop
447 and hindgut. (c) Schematic illustration of the digestive system highlighting key digestive
448 organs: E, oesophagus, C, crop; Pr, proventriculus; M, midgut; Mt, malphigian tubules; Il,
449 Ileum; H, Hindgut; Rp, Rectal pads; A, anus. Images viewed at 250X magnification.

450 **FIGURE 2** Field observations of foraging rates across four leafcutter ant species. Data points
451 indicate percentage of workers returning to their nests unladen (without carrying vegetation
452 in their mandibles). Observations were performed over 374 min for *Ac. echinator* (365
453 returning ants), 151 min for *Ac. octospinosus* (130 returning ants), 18 min for *At. colombica*
454 (2 679 returning ants), and 18 min for *At. sexdens* (2 078 returning ants). Means provided
455 $\pm$ SD. Head size scale bars represent 0.5 mm. Ant images are from antweb.org.

456 **FIGURE 3** Similarities in liquid transport dynamics across four leafcutter ant species.
457 Columns show percentage of full crops (black), midguts (light grey), and hindguts (dark
458 grey) from dissections performed on a total of 670 field-collected foragers (*Ac. echinator*:
459 161, *Ac. octospinosus*: 114, *At. colombica*: 200, *At. sexdens*: 195). Least square means
460 provided (+SE).

461 **FIGURE 4** The effects of load carrying (0 mg vs. 10 mg) and foraging distance (2 m vs. 10
462 m) on liquid transport dynamics in (a) crops, (b) midguts, and (c) hindguts of *Ac. echinator*
463 foragers in laboratory experiments. Least square means plotted $\pm$ SE.

TABLE 1 Results of mixed model analyses testing for variation in the proportion of leafcutter ant foragers with full storage organs (crop, midgut, hindgut) in both field observations in a Panamanian rainforest and in laboratory experiments.

Effect	Num DF	Denom DF	<i>F</i>	<i>P</i>
<i>Field liquid transport</i>				
Species	3	10.99	0.42	0.744
Foraging status	2	65.34	1.32	0.274
Species x foraging status	6	65.21	0.27	0.949
Organ	2	45.45	121.17	0.0001
Species x organ	6	53.83	1.44	0.215
Foraging status x organ	4	50.94	5.10	0.002
Species x foraging status x organ	12	56.51	0.59	0.839
<i>Distance x load mass experiment</i>				
Load mass treatment	1	10.63	17.46	0.002
Foraging distance treatment	1	10.63	1.16	0.306
Load mass x distance	1	10.63	2.55	0.139
Organ	2	7.91	202.91	0.0001
Load mass x organ	2	7.91	1.25	0.337
Distance x organ	2	7.91	0.02	0.980
Load mass x distance x organ	2	7.91	1.21	0.349
<i>Leaf-cutting experiment</i>				
Cutting treatment	1	12.84	0.99	0.338
Organ	2	7.204	52.95	0.0001
Cutting treatment x organ	2	7.204	0.61	0.569

The analysis is based on field observations comparing liquid storage across four leafcutter species (*At. colombica*, *At. sexdens*, *Ac. echinator*, *Ac. octospinosus*) and foraging statuses (laden: carrying a fragment of vegetation, unladen: empty mandibles, exiting: exiting nest). In the distance and load transport experiment, we compared liquid storage across load transport treatments (control, 10 mg) and distance treatments (2 m, 10 m). In the leaf-cutting experiment analysis, we compared liquid storage across leaf-cutting treatments (cutting: having just cut a leaf fragment, control: having just exited the nest).

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