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Tool selection during foraging in two species of funnel ants

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Highlights

- Some ant species use debris as tools to transport liquid food to the nest.
- When given the choice between different kinds of potential tools ants are selective.
- They show preference for materials with optimal handling and/or soaking properties.
- They can employ artificial materials novel to them, thus tool use is flexible in ants.

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ABSTRACT

Tool use by non-human animals has received much research attention in the last couple of decades. Nonetheless, research has focused mostly on vertebrates, particularly primates and corvids, even though tool use has also been documented in insects. One of the best-documented examples involves ants using debris (e.g., sand grains, mud, leaf fragments) to collect and transport liquid food to their nest. However, little is known about the factors that determine the selection of materials to be used as tool. We investigated tool selection in two species of *Aphaenogaster* ants by giving them the choice between different kinds of potential tools (natural and artificial objects). Ant workers showed a clear preference for certain materials to be used as tool objects. Tool selection was also shaped by familiarity with the material as ants developed a preference for artificial tools with a good soaking capacity that cannot be found in their natural environment. Our results indicate that ants of this genus evolved unique foraging strategies and show plasticity in their behaviour.

Keywords: ants, *Aphaenogaster*, foraging, food transport, tool use

INTRODUCTION

Once considered unique to humans, tool use is now known to be widespread in the animal kingdom (Bentley-Condit & Smith, 2010; Shumaker, Walkup, & Beck, 2011). Moreover, in the last two decades our general understanding of the mechanisms underlying flexible tool use has significantly increased (Sanz, Call, & Boesch, 2013). Some species can make tools to meet specific task demands (e.g., Auersperg, Szabo, von Bayern, & Kacelnik, 2012; Bird & Emery, 2009; Sanz, Call, & Morgan, 2009), use multiple tools in succession to fulfil the sub-goals required to complete a task (Martin-Ordas, Schumacher, & Call, 2012; Mulcahy, Call, & Dunbar, 2005; Wimpenny, Weir, Clayton, Rutz, & Kacelnik, 2009) or select appropriate tools depending on their physical attributes or functional properties (Bird & Emery, 2009; Chappell & Kacelnik, 2002; Manrique, Gross, & Call, 2010; Visalberghi et al., 2009).

Although these findings are concerned with vertebrates, mostly primates (chimpanzees, orangutans and capuchin monkeys) and passerine birds (New Caledonian crows, rooks, woodpecker finches) (Sanz et al., 2013), tool use also occurs in invertebrates. For instance, a recently compiled catalogue reports about 50 cases of tool use in insects, involving 30 different genera (Bentley-Condit & Smith, 2010). However, little is known about the occurrence of flexible tool use in invertebrates. For instance, weaver ants use the silk produced by their larvae in nest building but this is not considered “true” tool use because ants use an animate object (Pierce, 1986). Antlions and wormlions throwing out sand to make small prey fall inside their conical pits does qualifies as tool use. Although it is a fairly stereotyped behaviour, it is not completely fixed: antlions modify the characteristics of their trap according to hunger level, prey availability, predation threat and other environmental conditions such as light and temperature; however, the influence of experience upon this flexibility is unclear (Scharf, Lubin & Ovadia, 2011). Antlions and wormlions also prefer specific substrates for pit building or ambushing prey (Devetak & Arnett, 2015). The use of pebbles to close burrows containing eggs and prey in some apoid wasps (*Ammophila*, *Sphex*) is flexible because it requires the selection of suitable pebbles (Evans & Eberhard, 1970).

One of the best documented examples of tool use by insects involves using debris to transport food by some species of ants including the harvester ant *Pogonomyrmex badius* (Morrill, 1972), *Solenopsis invicta* (Barber, Ellgaard, Thien, & Stack, 1989), and several

species of *Aphaenogaster* (Agbogba, 1985; Fellers & Fellers, 1976; McDonald, 1984; Tanaka & Ono, 1978), all belonging to the subfamily *Myrmicinae*. Many of the species from this subfamily are characterised by a very chitinous gaster (abdomen) and by the lack of a distensible crop, which prevents the transportation of large amounts of liquid food inside their bodies, a feature very common in other subfamilies, such as *Formicinae* or *Dolichoderinae* (Davidson, Cook, & Snelling, 2004; Hölldobler & Wilson, 1990). Furthermore, some myrmicine genera, namely *Aphaenogaster*, do not perform trophallaxis (Delage & Jaisson, 1969) (i.e., mouth-to-mouth exchange of liquid food, also common in other subfamilies) and as a consequence the foragers of these species cannot exchange liquids stored in their crops with in-nest workers performing other tasks, such as feeding the larvae. The characteristic anatomy of the digestive tract in myrmicine ants, in particular the absence of an expandable crop, may have favoured the evolution of tool using behaviour, which allows for an efficient gathering, transport and sharing of liquid food (Fellers & Fellers, 1976; Tanaka & Ono, 1978).

When foragers of these myrmicine species encounter liquid food sources (e.g., fruit pulp, body fluids of dead arthropods) they drop debris of various kinds (e.g., sand grains, soil particles, leaf fragments) into the food source and then transport the food-soaked debris back into the nest. Furthermore, some evidence suggests that these ants do not drop debris into non-food substances (Agbogba, 1985; Banschbach, Brunelle, Bartlett, Grivetti, & Yeaman 2006). Tool-assisted food transportation has been observed in both field and laboratory experiments with artificial baits (Agbogba, 1985; Banschbach et al., 2006; Barber et al., 1989; Fellers & Fellers, 1976; Fowler, 1982; Lőrinczi, 2014; McDonald, 1984; Morrill, 1972; Tanaka & Ono, 1978). Ants use as tools different objects found near the food source including mud clods, leaf fragments, pine needles, sand grains, or any particles of a suitable size (Banschbach et al., 2006; Fellers & Fellers, 1976; Lőrinczi, 2014; Morrill, 1972; Tanaka & Ono, 1978). So far, however, only one comprehensive study has been carried out on tool selectivity in these ants (Tanaka and Ono, 1978). Other studies have reported limited observations that might indicate selectivity in use of tools in *Aphaenogaster* species (e.g., Banschbach et al., 2006; Fellers & Fellers, 1976; Lőrinczi, 2014; Morrill, 1972).

This putative material selectivity is important because it may indicate that ants choose materials flexibly, something that has been mainly documented in vertebrates. However, little is known about the factors that determine ants' preference for various materials as suitable tools for liquid transportation. The aim of this study was to comprehensively investigate material selectivity in liquid food transportation in two *Aphaenogaster* ant species in the laboratory to assess their flexibility and establish a possible link with the literature on tool use in vertebrates. We adopted Bentley-Condit and Smith's (2010) definition of tool use, which in turn closely followed that of St Amant and Horton's (2008): "the exertion of control over a freely manipulable external object (the tool) with the goal of (1) altering the physical properties of another object, substance, surface or medium (the target, which may be the tool user or another organism) via a dynamic mechanical interaction, or (2) mediating the flow of information between the tool user and the environment or other organisms in the environment" p.1203. We chose this definition instead of Beck's (1980) or Pierce's (1986) because it provided a good balance between specificity and generality and crucially, it fully captured the behaviour that we investigated here.

We confronted ants with a liquid food source away from the nest and a set of natural or artificial (novel) objects with different weight/soaking properties. One might expect that tools with more efficient soaking properties would be preferred over alternative choices. Additionally, we expected that ants would preferentially drop debris in nutritious baits. The presentation of natural objects allowed us to link this study with previous ones while the inclusion of the artificial objects allowed us to explore the ants' flexibility in learning to use the most efficient novel materials.

METHODS

Study species and housing

We studied two monogynous Mediterranean ant species belonging to the subfamily *Myrmicinae*, *Aphaenogaster subterranea* and *Aphaenogaster senilis* (Czechowski, Radchenko, Czechowska, & Vepsäläinen, 2012; Stukalyuk & Radchenko, 2011). *A. subterranea* (Latreille, 1798) is a highly thermophilous species distributed in southern and central Europe (Czechowski et al., 2012; Seifert, 2007). It lives in moderately wet and warm

deciduous and pine forests, and builds nests under stones, in the soil, litter or occasionally in fallen branches. Colony size can vary from several hundred up to 2000 workers (Czechowski et al., 2012; Seifert, 2007; Stukalyuk & Radchenko, 2011). *A. senilis* (Mayr, 1853) inhabits open, sunny locations such as forest edges, lawns, fields and sand dunes. The nests are built into the soil, often sheltered by stones. Workers forage individually mostly at the ground level, but they can occur on shrubs and trees. Since these habitats have scarce food sources, workers can cover large areas with the help of their long legs. Colony size can vary between a few hundred to a few thousand workers (Boulay et al., 2007).

Eight medium sized colonies of *A. subterranea* (two queenright and 6 queenless, between 500-1500 workers) were collected in a black pine forest near the village of Litér (Hungary) and kept under standard conditions (temperature 24 ± 4 °C; relative humidity 42-43%; 12 L: 12 D cycle) in the laboratory. Together with some material coming from the original habitat (soil, dried pine needles and leaf fragments), the colonies were kept in plastic boxes (44 cm \times 31 cm \times 23 cm) with their cover cut in a circular shape (diameter 15 cm) and covered with a fine-wired metal mesh for ventilation and easy moistening of the nest. Every box containing a colony was connected with a 10 cm long plastic tube to an arena (60 cm \times 30 cm \times 15 cm). They were watered daily, and fed every second day with a commonly used artificial diet (Bhatkar & Whitcomb, 1970) in a distant location of the foraging arena. During the experimental period the colonies were not fed, to increase motivation for food found on the baits. Water was always provided *ad libitum*.

Three queenright colonies of *A. senilis* (colony size 500-1500 workers) were collected at Banyuls-sur-Mer (France) in a sandy area and kept in the laboratory under standard conditions (temperature 24 ± 4 °C; relative humidity 50–60%; 12 L: 12 D cycle). They were housed in artificial nests, each consisting of a cylindrical plastic box (diameter 12.5 cm) with regularly moistened plaster floor, and a hole giving access to the foraging area, which was represented by the space left in a larger plastic box (18 cm \times 25.5 cm \times 7.7 cm) containing the circular nest. The standard diet for each colony consisted of five dead crickets (*Acheta domestica*) and about 5 g of apple/honey mix two times per week. During the experimental period, to increase motivation for food, colonies were fed with only three crickets and 2 g of apple/honey mix (twice a week); water was always provided *ad libitum*.

134 *Experimental setup*

135 Ant colonies were presented with food baits and different types of potential tools in the
136 foraging arena (Fig. 1, 2). The methodology used in *A. subterranea* and *A. senilis* and
137 described below is slightly different because this study is the combined output of two initially
138 separate studies conducted independently in two different laboratories.

139 *Aphaenogaster subterranea*

140 The duration of the experiments was 3 hours, which was enough time to observe the transport
141 of tools into the nests. Each observation period lasted one minute, repeated every four minutes
142 until the end of the experiment. During this time we noted the number of tools transported to
143 the bait and from the bait into the nests. Tool preference was tested for tools found and used
144 in nature: small soil grains (diameter 1 mm), large soil grains (diameter 2-3 mm), and
145 fragments of pine needles and leaves. Additionally, we also used a fifth tool type of
146 anthropogenic origin with good soaking/weight ratio: small pieces of sponges (diameter 5
147 mm) (Fig. 1 and Fig. A1, appendix). Tools were placed at a distance of 12 cm from the baits
148 in a random order. To estimate the soaking properties, the weight of 10 tools of each type was
149 measured with a precision analytical balance (10 mg accuracy) before and after soaking them
150 in the different types of baits (Table 1). The objects were placed on the surface of the baits
151 and left there for 10 minutes. Three different baits were tested: water, honey diluted in water
152 (1:3 ratio; further honey-water) and honey (condensed with sugar powder; further honey)
153 placed in plastic plates (diameter 4 cm, Fig. 1 and A1). The baits were given to the colonies in
154 a random order.

155

156 *Aphaenogaster senilis*

157 The experiments lasted 3 hours, during which the activity of the colonies was videotaped. The
158 tapes were analysed as for *A. subterranea*. The experiments with the same setup were
159 repeated 10 times per each colony.

160 Tool use preference was tested for a total of 6 different, novel potential tools, such as pieces
161 of paper, sponges, artificial foam, twigs, string and parafilm (Fig. 2). Groups of 10 tools of

each type (average weight for each tool: 2.5 mg) were placed 12 cm from the food source (diluted honey on a piece of aluminium foil). The soaking properties were estimated by measuring the weight of 10 tools of each type with a precision analytical balance (10 mg accuracy) before and after soaking them in diluted honey (Table 2).

Ethical Note.

Ant colonies were collected with care in the field and maintained in nearly natural conditions in the laboratory. Ants were provided with suitable nesting sites, food and water thus minimizing any adverse impact on their welfare. Our experimental designs include only behavioural observations, no insect was harmed or stressed during the experimental procedure and all ant colonies were healthy at the end of the experiments.

Statistical analysis

In *A. subterranea*, we did not observe any transport of tools into the nests in the case of water baits; furthermore, the transport rate towards the water baits was much lower than towards the other baits, so we focused on the analysis of tool use in honey-water and honey baits. The effect of the bait type on the total number of tools dropped into the baits was analysed using a GLMM model (Poisson errors, maximum likelihood fit, log link). In the full model, bait type was included as a factor and colony ID as a random factor ($N = 12$) to account for within colony similarities. Differences in the number of tools dropped into baits by different colonies according to different tool types were analysed with GLMM models (Poisson errors, maximum likelihood fit, log link). In the full model, tool type was included as a fixed factor and colony ID as a random factor ($N_{A.su} = 30$, $N_{A.se} = 18$).

The transport rate of tools towards the baits for the colonies of both species was analysed with the help of Log-rank test with Montecarlo (100.000) simulations ($N_{A.su} = 60$ corpses, $N_{A.se} = 30$). The removal rate was tested until at most the 10th piece of tools was removed. The different tool types were included as dummy variables, while colony ID was included as a random factor. The transport rate of the different tools into the nests was analysed with the same model construction in both species.

In *A. subterranea*, the transport of tools to the different baits was analysed in separate models, and because 2 colonies did not transport anything to the baits, only 6 colonies were used for the analysis. In *A. senilis*, the same model constructions were used for the analysis of the 1st, the 5th and 10th experiment. The three-hour experimental period was enough in the case of both species to observe the transport of tools into the nests. In *A. subterranea*, 3 colonies in the case of honey-water, and 6 colonies in the case of honey baits were actively transporting tools into their nests, so only these colonies were used in the analysis with the same model constructions as described before ($N_{\text{honey-water}} = 15$, $N_{\text{honey}} = 30$). All statistical analyses were carried out in R (version 3.0.2, The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>). GLMMs were performed using *glmer* function in *lme4* package (Bates, Maechler, Bolker, & Walker, 2013). In the analyses, all tools used were included. Log Rank Test was carried out with the *surv_test* function in *coin* package (Hothorn, Hornik, van de Wiel, & Zeileis, 2008). Pairwise comparisons were performed with separate Log Rank Tests and the *Relevel* function was used to carry out post-hoc sequential comparisons among factor levels when performing GLMM analyses. We applied table-wide sequential Bonferroni-Holm correction to reveal the exact significance levels in these cases.

RESULTS

Aphaenogaster subterranea: transport to the baits

Workers of *A. subterranea* dropped significantly more tools into honey-water (GLMM: $z = 12.5$, $N = 24$, $P < 0.001$) and honey ($z = 11.97$, $P < 0.001$) than into water, but no difference was observed between the honey-water and honey ($z = -0.96$, NS). We therefore concentrate on honey-water and honey baits in the following analyses.

Overall, small soil grains were the most frequently transported tool to the honey-water, and their number was significantly different from the number of sponges ($z = -3.33$, $P <$

0.001) and leaf fragments (Fig. 3), with the latter being the least frequently dropped tools compared to every other tool type ($z < -3.22$, $P < 0.01$). Furthermore, leaf fragments were transported at the lowest rate (Log Rank Test: $13 < \chi^2 < 29.76$, $P < 0.001$) while sponges were transported at a slower rate than small soil grains ($\chi^2 = 29.76$, $P < 0.01$) (Fig A2).

In the case of honey baits, the preferred tools were the small soil grains and pine needles, which were transported in significantly higher numbers than leaf fragments ($z < -3.07$, $P < 0.001$) and large soil grains ($z < -2.65$, $P < 0.05$); sponges were also used more frequently than leaf fragments ($z = -2.83$, $P = 0.03$) (Fig. 3). Every tool type was transported at a faster rate than leaf fragments ($11.5 < \chi^2 < 40.29$, $P < 0.003$) (Fig. A2).

Aphaenogaster subterranea: transport into the nests

In the case of honey-water baits, small soil grains were transported into the nest in higher numbers than pine needles ($z = 3.43$, $N = 24$, $P < 0.001$), leaf fragments ($z = 3.56$, $P < 0.001$) and sponges ($z = 2.91$, $P < 0.001$) (Fig 4). Small soil grains were transported also at a faster rate than every other tool type ($11.43 < \chi^2 < 29.26$, $P < 0.003$) (Fig. A3).

In the case of honey baits, sponges were transported into the nest in highest numbers, which differed significantly from every other tool type ($z < -4.02$, $P < 0.001$). The second most preferred tools were small soil grains, which were transported significantly more often than leaf fragments ($z = -2.79$, $P = 0.03$) (Fig 4). Sponges were transported at a faster rate than every other tool type ($8.56 < \chi^2 < 37.39$, $P < 0.01$). Leaf fragments were transported at the slowest rate, which significantly differed from the small ($\chi^2 = 22.31$, $P < 0.001$) and large soil grains ($\chi^2 = 7.07$, $P < 0.05$) (Fig A3).

Aphaenogaster senilis: transport to the baits

Overall, the number of tools used by *A. senilis* workers significantly changed across trials (from the 1st, to the 5th and the 10th trials: GLMM: $z = 3.09$, $N = 36$, $P < 0.01$), and there was no significant difference between the 5th and the 10th trial ($z = 0.95$, NS), therefore the use of potential tools was analysed separately for each trail (Fig. A4).

In the 1st and 5th trials, there was no significant difference in the number of different tools transported to the bait (between every tool type 1st $z > 0.2$, $N = 18$, NS; 5th $z > -2.2$, $N = 18$, NS) (Fig. 5), although in the 1st trial paper was transported at a significantly faster rate than the artificial foam (Log Rank Test: $\chi^2 = 12.72$, $P < 0.01$; Fig. A5). In the 5th trial, the sponges (Log Rank Test: $\chi^2 = -4.33$, $P < 0.05$; Fig. A5) and the pieces of paper (Log Rank Test: $\chi^2 = -3.82$, $P < 0.05$) were transported significantly faster than every other tool types, but there was no significant difference between these two tool types (Log Rank Test: $\chi^2 = -0.28$, NS), and paper did not differ significantly from string (Log Rank Test: $\chi^2 = -2.33$, NS; Fig. S5).

In the 10th trial, the preferred tools were paper and sponges ($\chi^2 = 0.89$, NS), which were used more frequently than artificial foam ($z > 3.24$, $N = 18$, $P < 0.01$, Fig. 5) and transported at a significantly faster rate than parafilm and twigs (respectively: $\chi^2 > 8.66$, $P < 0.05$ and $\chi^2 > 8.22$, $P < 0.05$, Fig. A5). Artificial foam was transported at a significantly slower rate than every other tool type ($\chi^2 = 9.3$, $P < 0.05$, Fig. A5).

Aphaenogaster senilis: transport into the nest

Overall, the number of tools transported into the nest by *A. senilis* workers significantly changed across trials (GLMM: $z = 2.79$, $N = 36$, $P < 0.01$), and there was no significant difference between trial 5th and 10th ($z = 1.01$, NS), therefore the use of artificial tools was analysed separately for the 1st, 5th and 10th trials (Fig. A4).

In the first trial, there was no significant difference in the number (GLMM: $1.82 > z > 0.001$, NS) and transport speed (Log Rank Test: $0 > \chi^2 > 1$, NS) of different tools brought into the nest (Fig. 6 and Fig. A5). In contrast, in the 5th trial the pieces of papers and sponges were transported in a significantly higher number than the foam ($z > -3.16$, $P < 0.05$) and parafilm tools ($z > -3.38$, $P < 0.01$; Fig. 6). Moreover, paper and sponge (Log Rank Test: $\chi^2 = 0.29$, NS) were transported at a higher rate than every other tool type ($\chi^2 > 2.85$, $P < 0.05$; Fig. S5). Strings and twigs ($\chi^2 = -0.91$, NS) were transported at a higher rate than foam ($\chi^2 > 6.24$, $P < 0.01$) and parafilm ($\chi^2 > 2.48$, $P < 0.05$; Fig. A5).

In the 10th trial, paper was the most frequently transported tool to the nest, differing significantly from the number of artificial foam ($z = 2.73$, $P < 0.05$) and parafilm tools ($z = 2.73$, $P < 0.05$) (Fig. 6). The fastest transport rate was observed for paper tools, which differed significantly from every other tool type ($\chi^2 > 8.2$, $P < 0.05$, Fig. A5) except for sponges and strings ($\chi^2 > 6.78$, NS, Fig. A5). Parafilm and artificial foam ($\chi^2 = 3.35$, NS) were transported at a significantly slower rate than every other tool type ($\chi^2 > 8.66$, $P < 0.05$).

DISCUSSION

We observed the occurrence of tool use to transport liquid food in two ant species of the genus *Aphaenogaster* thus confirming previous observations (Agbogba, 1985). More importantly, ant workers were selective in both the materials they chose and the baits that they exploited. *A. subterranea* was mainly tested with natural materials. Small soil grains were the most preferred item and leaf fragments, despite having a superior soaking power than soil grains, the least preferred item. Ants showed a remarkable preference for sponges despite being novel (they were the only artificial material tested in this species). Interestingly, ant workers in more than 80% of the trials broke the sponges into smaller fragments, presumably to facilitate handling. Once tools were dropped into the baits, ants behaved differently depending on the bait's viscosity (honey-water or pure honey). Imbibed small soil grains were transported to the nest in the highest numbers and faster than other tool types from honey-water baits, while sponges were the preferred transported tools from the honey baits (small soil grains were the second preferred tool to be brought to the nest). Additionally, ants threw much fewer items into water baits compared to those baits that contained honey.

A. senilis could choose among six materials that differed in terms of soaking power (paper, sponges, artificial foam, twigs, sting and parafilm). It is likely that ants of this species, which live in sandy areas, were unfamiliar with most of these materials. Initially, *A. senilis* showed no preference for any of the tools for dropping them into the baits, although paper was transported faster to the bait compared to the artificial foam. However, along trials, ants significantly preferred dropping paper and sponges into the baits, thus choosing in accordance with the tools' soaking properties. A similar pattern was observed for tool transportation to

the nest: ants developed a preference for paper, followed by sponges, thus optimizing their foraging effort by using the tools with the best soaking properties and ease of grasping compared to other tool types.

Our results suggest that tool use to transport liquid to the nest in the two studied ant species is not behaviourally fixed. Ants incorporated novel tools, many of them made of artificial materials, into their foraging activities. Moreover, *A. senilis* learned within 10 trials to select the best tool options available based on their soaking properties and *A. subterranea* chose tools in relation to the characteristic of the food itself, e.g., food viscosity. This means that ants can select tools according to both food and tool properties. Nevertheless, other factors (besides a tool's soaking properties) may have played a role in determining ants' preferences. One possibility is that the low density of some materials in the natural habitat (e.g., leaf fragments are not abundant in the pine forest inhabited by these *A. subterranea* colonies) may have been the reason for largely ignoring this item. However, a lack of familiarity with potential tools cannot explain why *A. subterranea* used sponge pieces, which were totally novel objects. Even more compelling are the data on *A. senilis*, which developed a preference for unfamiliar materials during testing.

Handling effort is another factor that could have played an important role in tool selection, perhaps in combination with the tool's soaking properties. During our observations we noticed that *A. subterranea* workers had problems with grasping, handling and orienting the leaf fragments, while the pieces of sponges could be grasped more easily. In fact, ants may have torn apart sponges to reduce their size and facilitate handling. Although the soaking power of sponges was lower when dropped into honey than into honey-water, their relatively low weight made them more buoyant than other tool types and their irregular texture increased their potential for being grasped. We found that the preference for particular items often depended on the type of task that ants carried out. In particular, sponges may have been preferentially transported to the nest, but small soil grains were the most dropped tools into the baits. This suggests that selection among different tool types occurred both when first encountered and also at the food source after the tools had been dropped into the baits. This two-stage selection process might be especially important in the case of novel tools whose properties are still unknown. Indeed, *A. senilis* ants transported very few foam fragments to

the bait in the first trial but subsequently increased their transportation during the 5th trial. Nevertheless, foam fragments were rarely transported from the bait to the nest and by the 10th trial ant workers even reduced their transportation to the baits. This suggests that tool selection, after familiarization with novel material, may have also occurred at the baits. Other authors observed that not all the tools dropped into the baits were transported into the nest (Fowler, 1982; McDonald, 1984), corroborating our interpretation that further tool selection can occur once the tools have been dropped into the liquid food source.

Availability, weight, soaking properties, easy handling and the possibility of shaping the material to a desired form are therefore important factors in tool selection. The assessment of these characteristics appears to be the result of a familiarization and learning process, at least for the novel objects offered to the ants in our experiments. As the natural availability of certain kinds of tools varies both spatially and seasonally, plasticity in tool selection is likely to be adaptive. Tool use behaviour in ants may have evolved from the tendency shown by many species to cover with debris liquid or viscous substances, which may otherwise cause drowning or entanglement of workers near their nest (Fellers & Fellers, 1976; McDonald, 1984). Note, however, that it has been experimentally shown that *Aphaenogaster* ants typically drop debris in food substances (Agbogba 1985; Banschbach et al., 2006) and our experiments confirm this. The adaptive advantage of tool use in *Aphaenogaster* ants is that, by using tools, foragers are capable of efficiently exploiting ephemeral food sources by transporting much larger quantities of liquid nutrients than they could do by internal transport (Fellers & Fellers, 1976; Tanaka & Ono, 1978), given the non-expandable crop characteristic of myrmicine ants. Tool use might help these ants to compete with more dominant ant species which are able to monopolize food sources by numbers: if the food is quickly covered by debris, other ant species cannot exploit it and tool users can take the necessary time to bring the soaked debris back to their nest (Banschbach et al., 2006; Fellers & Fellers, 1976).

We began our paper by referring to the research on flexible tool use in vertebrates hoping to establish a link with the existing observations on invertebrate tool use. Our experiments showed some indication that ants preferred materials with good soaking power although other aspects such as ease of grasp may have also contributed to their choices. Chimpanzees create a vegetable mass with good absorbent properties by chewing leaves that

they use to extract liquid from crevices (Goodall, 1986). It is unknown, however, whether chimpanzees select certain plant species for their high soaking power. In a liquid extraction task in the laboratory, Lehner, Burkart and van Schaik (2011) reported that captive orangutans developed liquid extraction techniques based on materials with high soaking power (e.g., wood wool, paper) over those with low soaking power (e.g., leafless branch). Although this may indicate that orangutans, like ants, were sensitive to the properties of such materials, the lack of a direct and systematic comparison between different kinds of materials considerably reduces the conclusions that one may be able to draw from that study. Nevertheless, other studies with vertebrates in non-liquid extractive foraging tasks have systematically varied the materials presented and have found evidence of selectivity in terms of hardness and weight (Visalberghi et al., 2009), weight (Bird & Emery, 2009) or rigidity (Manrique et al., 2010). Our findings with ants are in some ways comparable to those studies although admittedly, our results are less clear than those reported in vertebrates. This is partly understandable because our study is only the second systematic study on material selectivity in ants (besides Tanaka and Ono, 1978) and we may have not fully considered a number of factors. For instance, dropping a substantial number of tools (e.g., pine needles) inside the liquid and abandoning them there may seem puzzling from the point of view of efficiency. Note, however, that dropping those materials on the liquid may have accomplished another function (e.g., preserving the liquid food against exploitation from other species). Also, the absorbed liquid may have altered the potential for grasping the potential tool object or increased its weight in excess. Another possibility is that ants can only identify suitable materials *after* observing its effect on the substance to be collected instead of selecting them *prior* to their use (e.g., artificial foam). Although selecting materials to collect liquid has not been described in corvids or primates, selecting and manufacturing tools prior to their use is well-documented in those taxa (e.g., Manrique et al., 2010; Sanz, Call & Morgan, 2009; Visalberghi et al., 2009; Wimpenny et al., 2009). Whether ants (or any other species) can also select new materials prior to experiencing its absorbent properties is an open question that future studies should address.

Some authors may question our use of the term tool use to describe the behaviour of the ants. Tool use is after all a contentious term in the literature typically characterized by rather long definitions, a number of clear examples, and some areas of substantial

disagreement (see Bentley-Condit & Smith, 2010; Shumaker et al., 2011). Although we would be ready to accept a less contentious terminology such as ‘material use’, it is difficult to do so when chimpanzees using leaves to extract liquid from crevices is considered a classical example of tool use (Goodall, 1986). More importantly, we think that the behaviour of the ants meets one of the most important criteria that define tool use in foraging contexts (Beck, 1980; Bentley-Condit & Smith, 2010; St Amant & Horton, 2008), i.e., the use of an external object to affect the position or location of another object or substance. Obviously one could argue that nest building materials affect the position of other nesting materials, which is why some authors object to the idea of tool use and would perhaps like to see either a more restricted use of the term, or its complete abandonment for a wider term such as construction behaviour (Hansell & Ruxton, 2008). Although we understand this position and the reasons for it, given the above considerations, we still prefer to refer to the behaviour of the ants as tool use, or the similar denomination of object use.

In conclusion, we observed *A. senilis* and *A. subterranea* using natural and artificial debris to collect and transport liquid food to their nests. We also documented the development of a preference for materials with optimal soaking properties in *A. senilis* although other factors such as familiarity and ease of grasping may have played a role in determining ants’ choices. Additional studies are needed to better characterize the extent and limits of this form of flexible tool use particularly in relation to the existing forms of flexible tool use displayed by vertebrates.

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- 505

506 **Table 1.** Soaking properties of the different tools used by *Aphaenogaster subterranea*.

Tool type	Imbibed /Initial weight (weight ratio)	
	Honey-water	Honey
Sponges	7.45	1.29
Leaf fragments	1.94	11.9
Pine needles	1.33	1.85
Small soil grains	1.1	1.48
Large soil grains	0.7	0.64

507 **Table 2.** Soaking properties of the different tools used by *Aphaenogaster senilis*.

Tool type	Imbibed /Initial weight (weight ratio)	
	Honey-water	
Paper	28.37	
Sponges	23.31	
Artificial foam	6.37	
Twigs	3.06	
String	2.00	
Parafilm	1.47	

508

509 **Figure legends**

510 **Figure 1.** Experimental setup and tool types offered to *Aphaenogaster subterranea*.

511 **Figure 2.** Experimental setup and tool types offered to *Aphaenogaster senilis*.

512 **Figure 3.** Number of different tools transported to honey-water (a) and honey (b) baits by
513 *Aphaenogaster subterranea* workers. Box plots show the median (internal line), quartiles and
514 range (whiskers). Different letters above boxes represent groups that differ significantly from
515 each other.

516 **Fig. 4.** Number of different tools transported into the nest from the honey-water (a) and honey
517 (b) baits by *Aphaenogaster subterranea* workers. Box plots show the median (internal line),
518 quartiles and range (whiskers). Different letter s above boxes represent groups that differ
519 significantly from each other.

520 **Figure 5.** Number of different tools transported to the baits in the 1st, 5th and 10th trials by
521 *Aphaenogaster senilis* workers. Box plots show the median (internal line), quartiles and range
522 (whiskers).

523 **Figure 6.** Number of different tools transported into the nest in the 1st, 5th and 10th experiment
524 by *Aphaenogaster senilis* workers. Box plots show the median (internal line), quartiles and
525 range (whiskers).

526 **Appendix - Figure legends**

527 **Figure A1.** Different tool types used for experiments with *Aphaenogaster subterranea* (a)
528 from left to right: small soil grains, sponges, pine needles, large soil grains, leaf fragments; (b)
529 the dynamics of bait covering with tools.

530 **Figure A2.** Estimated functions of tool transport time to honey-water (a) and honey (b) by
531 workers of *Aphaenogaster subterranea* (Log-rank test). Different tool types are depicted by
532 different colours.

533 **Figure A3.** Estimated functions of tool transport time into the nest from the honey-water (a)
534 and honey (b) baits by *Aphaenogaster subterranea* workers (Log-rank test). Different tool
535 types are depicted by different colours.

536 **Figure A4.** Summed number of tools transported on the baits and into the nest during the
537 three trials (box plots show: median, quartiles and range). Different letters above boxes
538 represent groups that differ significantly from each other.

539 **Figure A5.** Estimated functions of tools transport time on the baits (a; c; e) and into the nests
540 (b, d, f) in the 1st (a, b), 5th (c, d) and 10th (e, f) experiment by *Aphaenogaster senilis* workers
541 (Log-rank test). Different tool types are depicted by different colours.

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Figure

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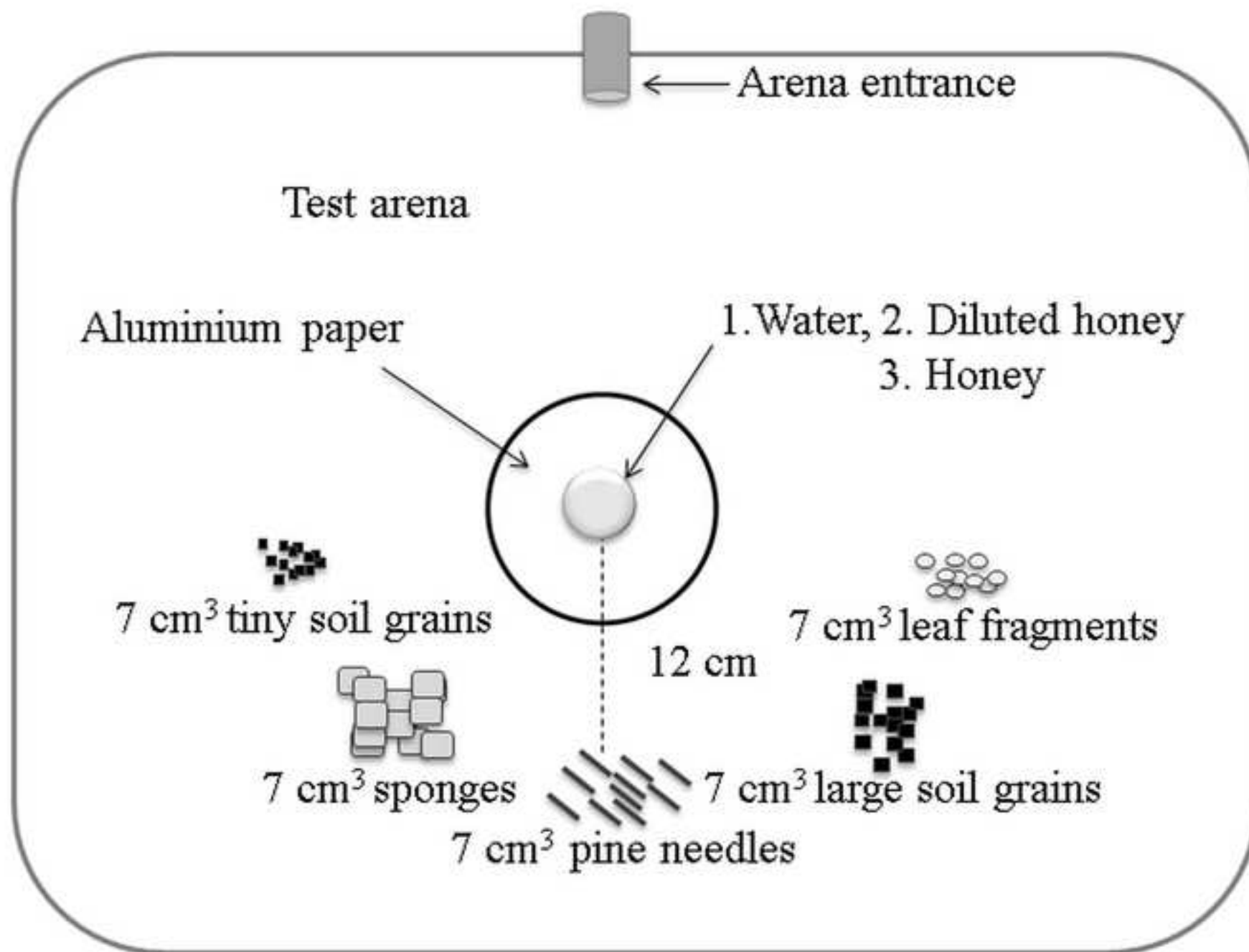


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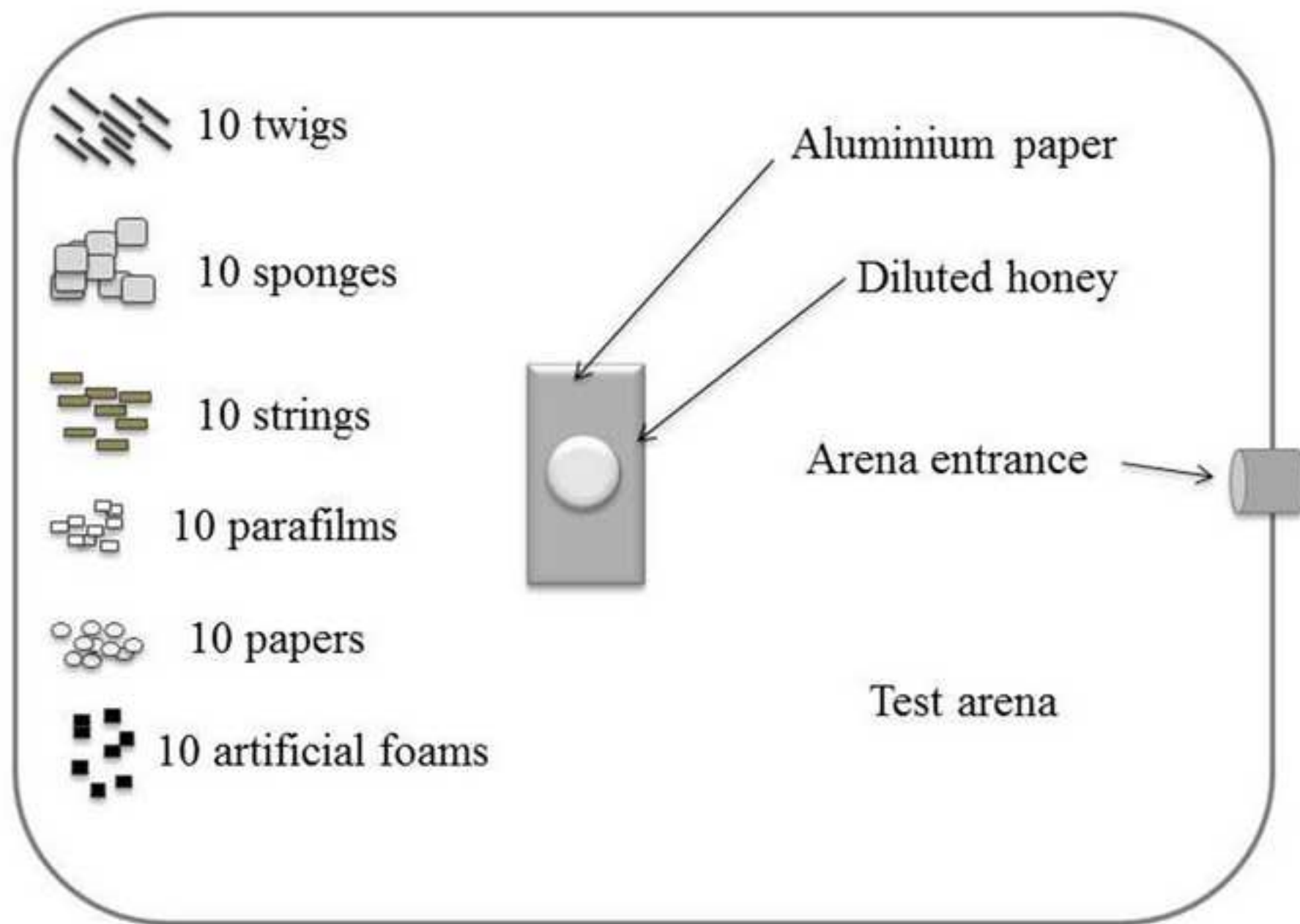


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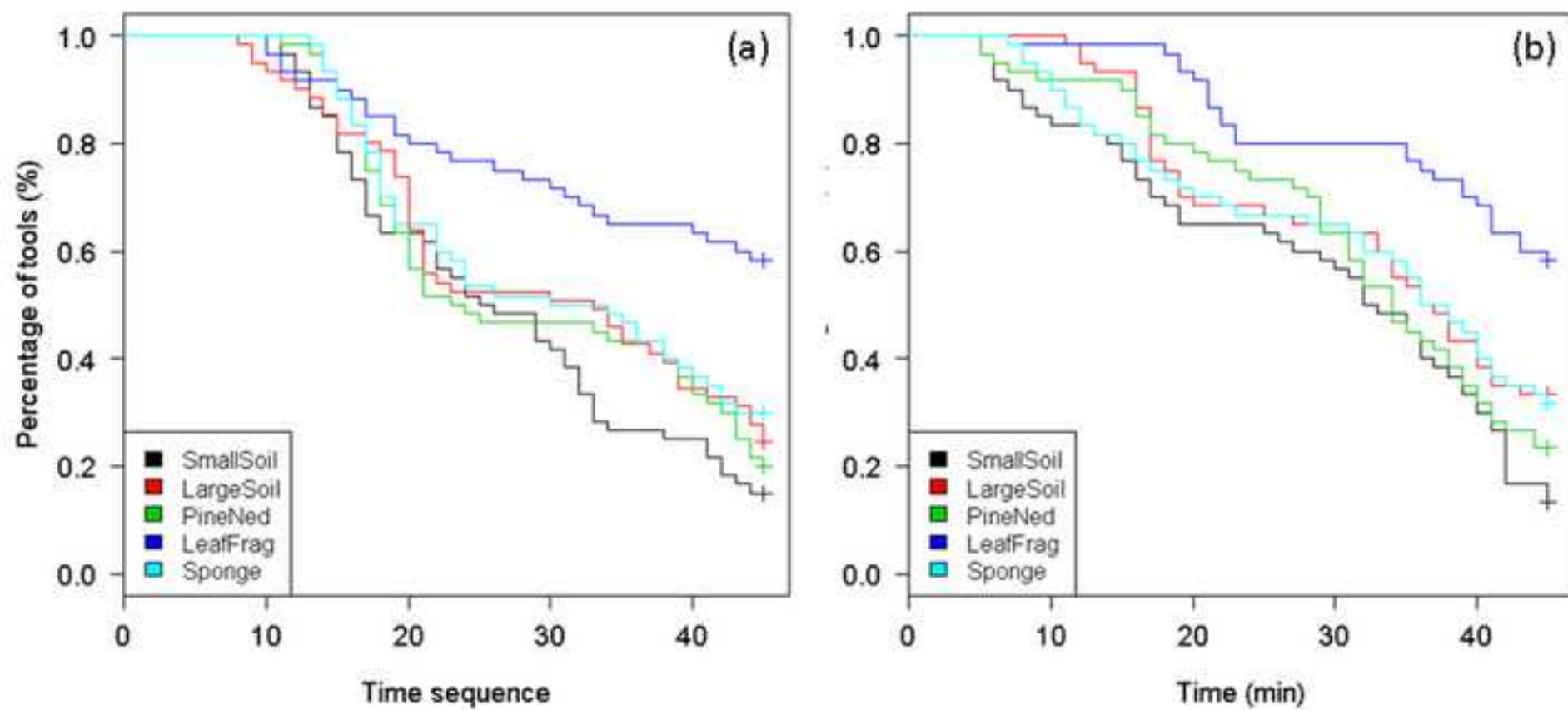


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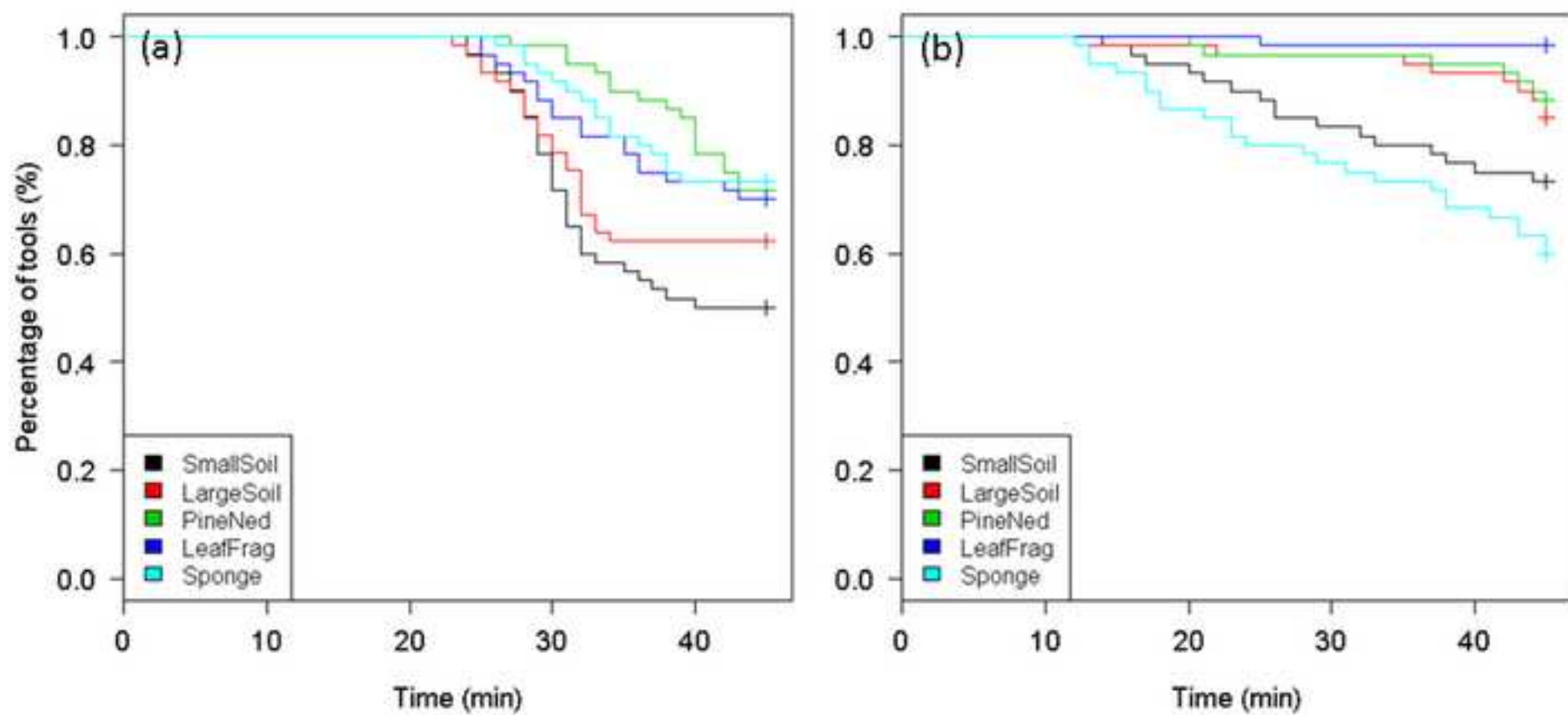


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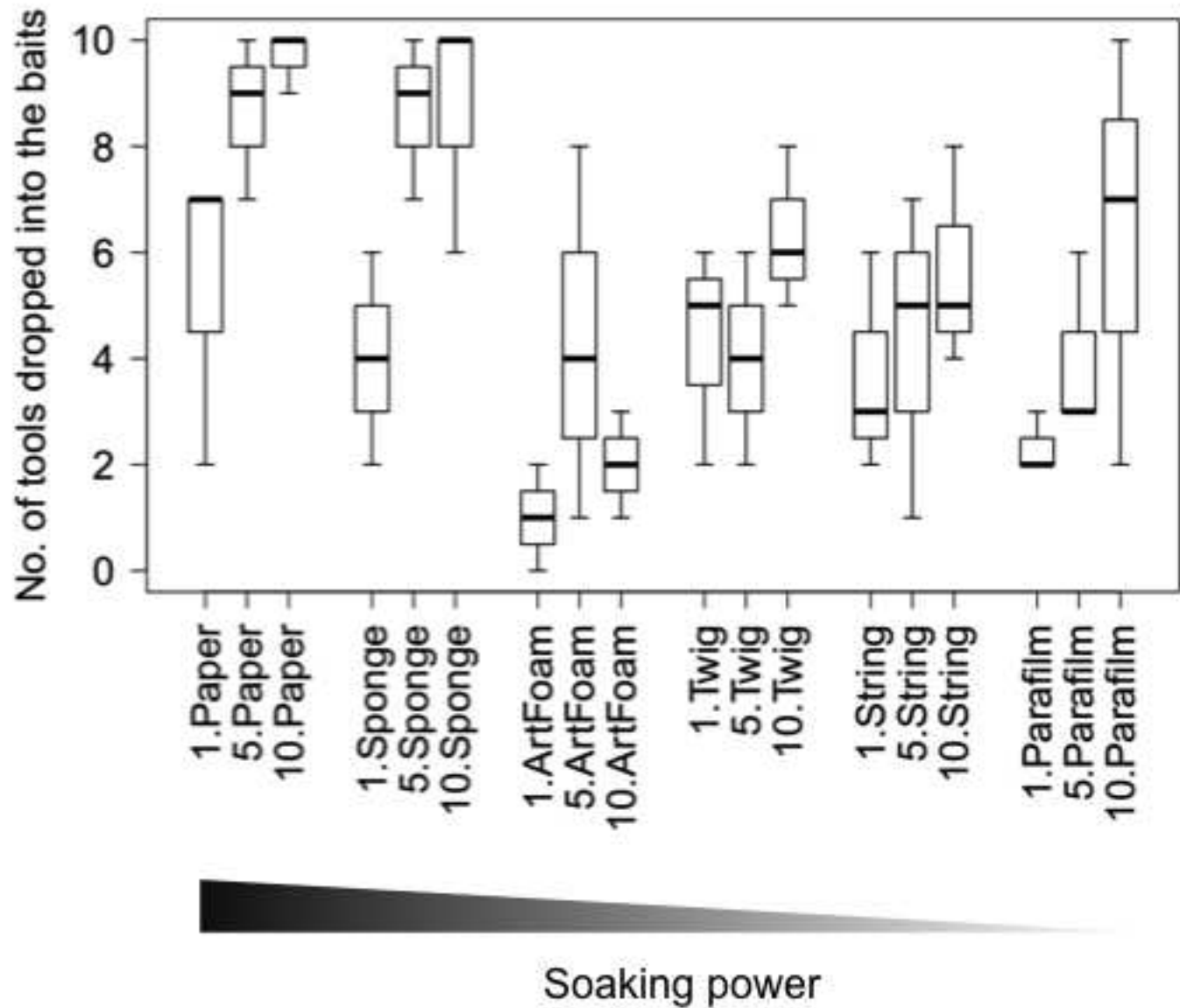


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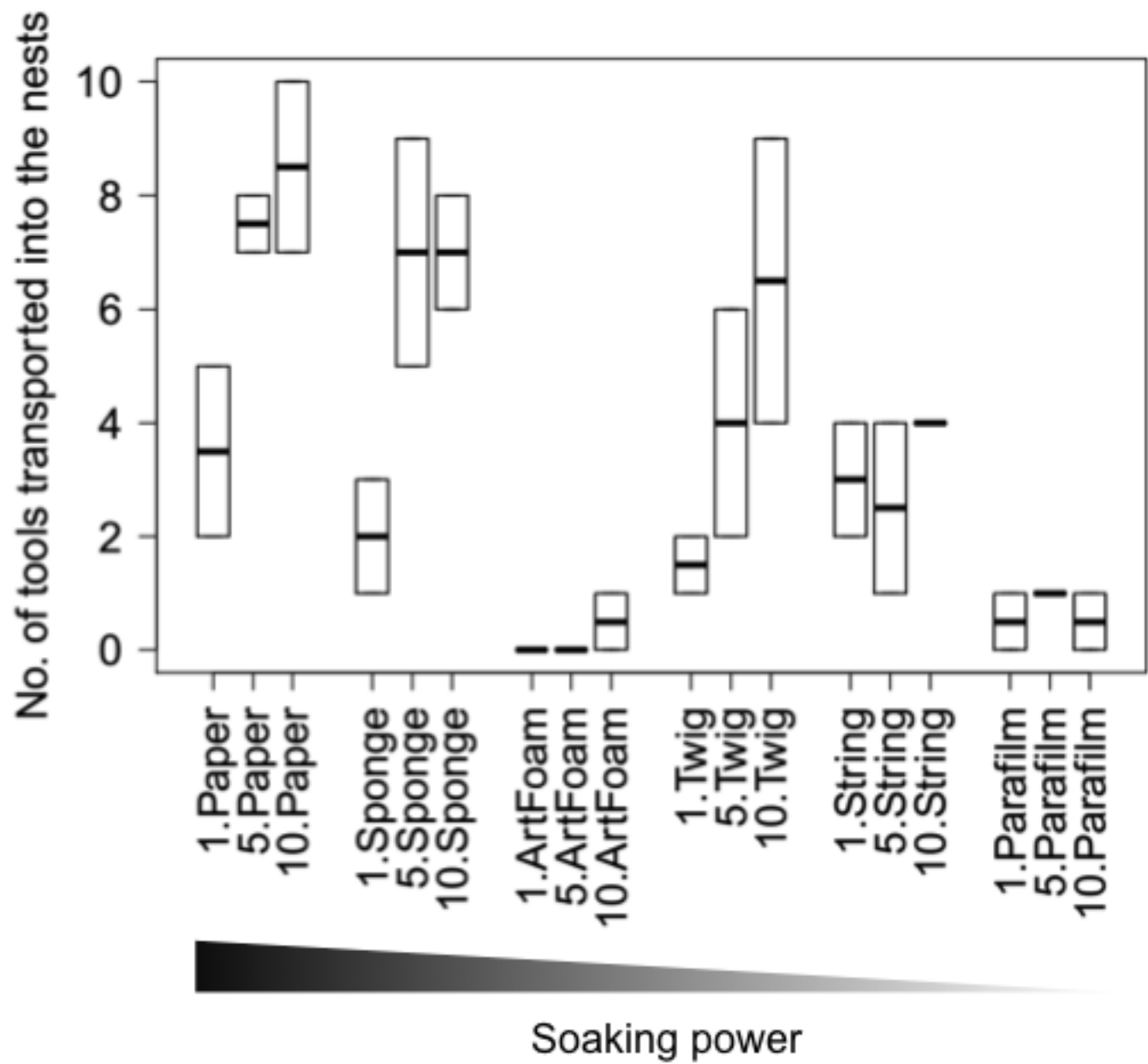


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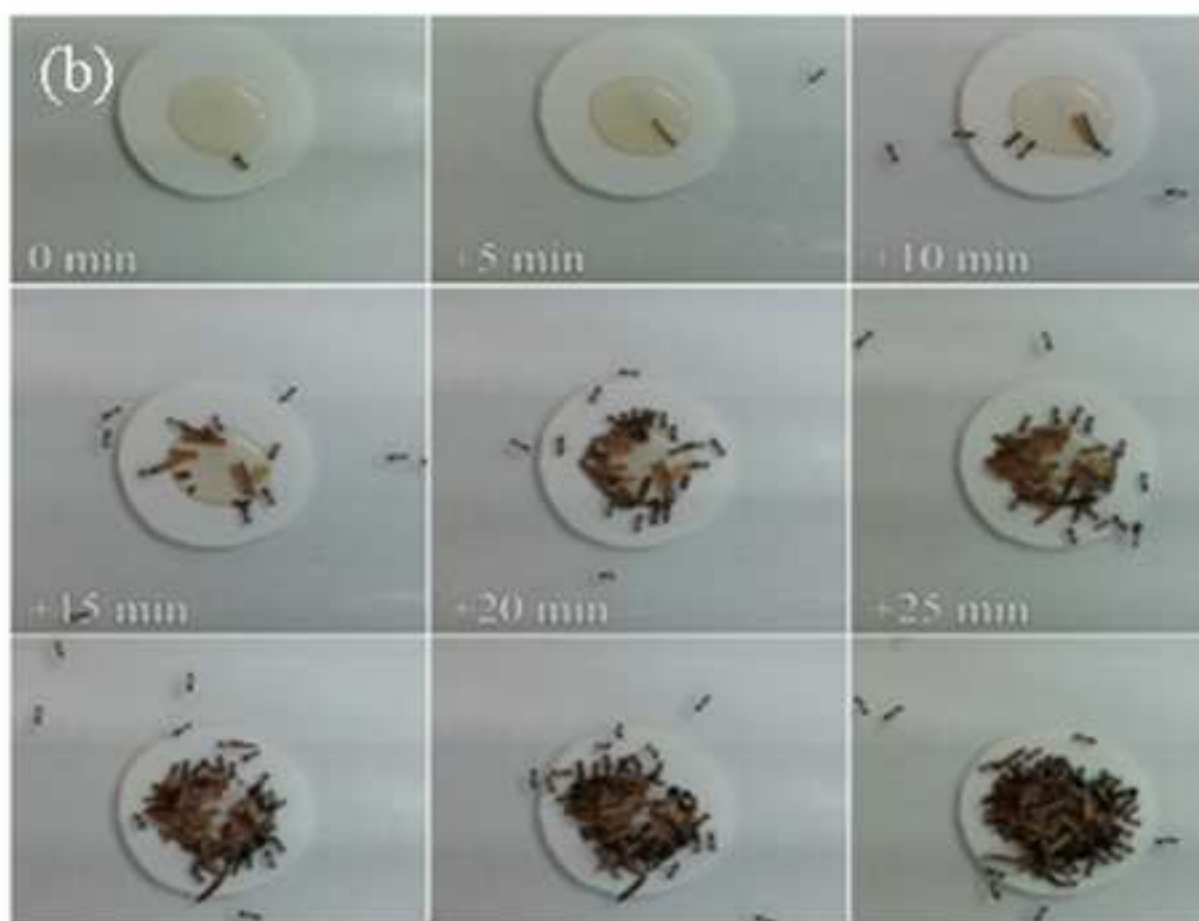


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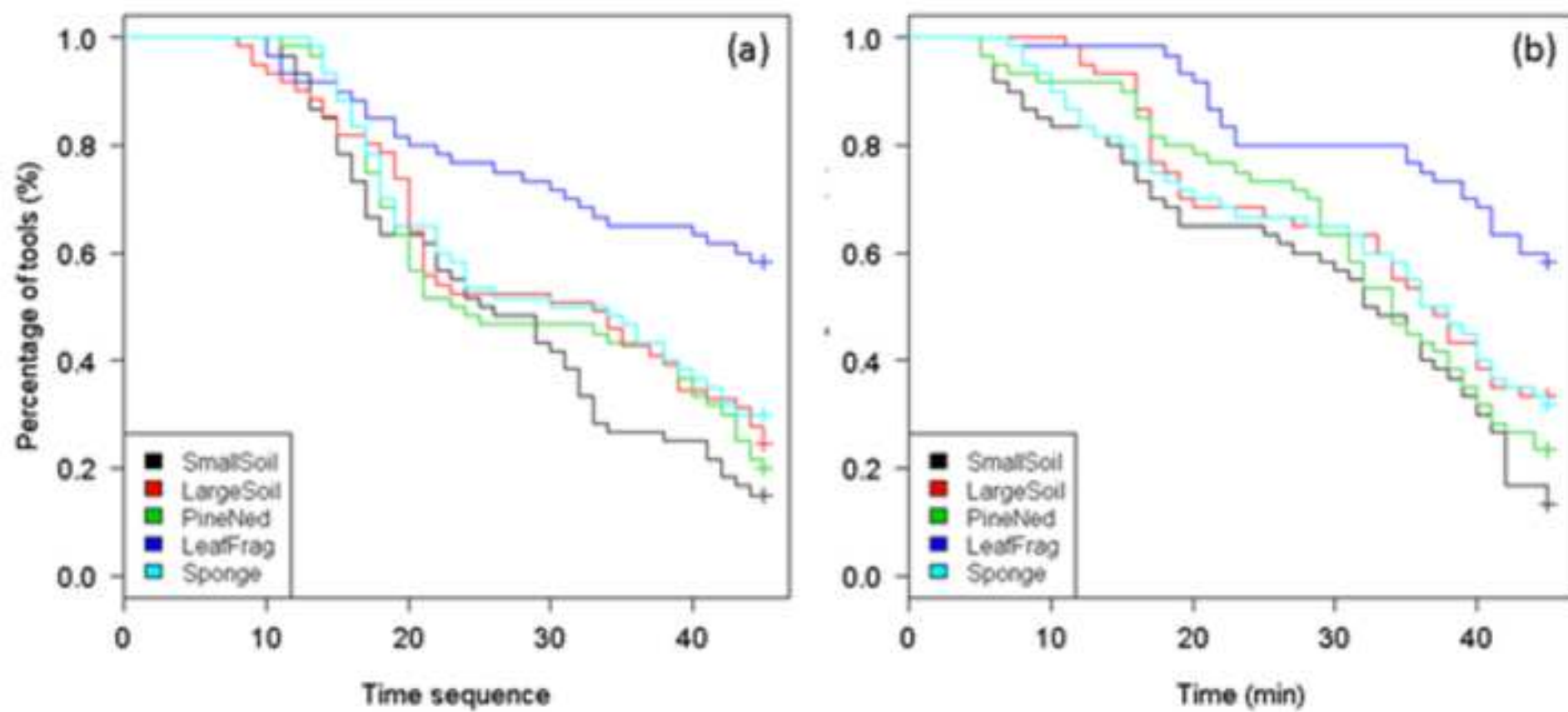


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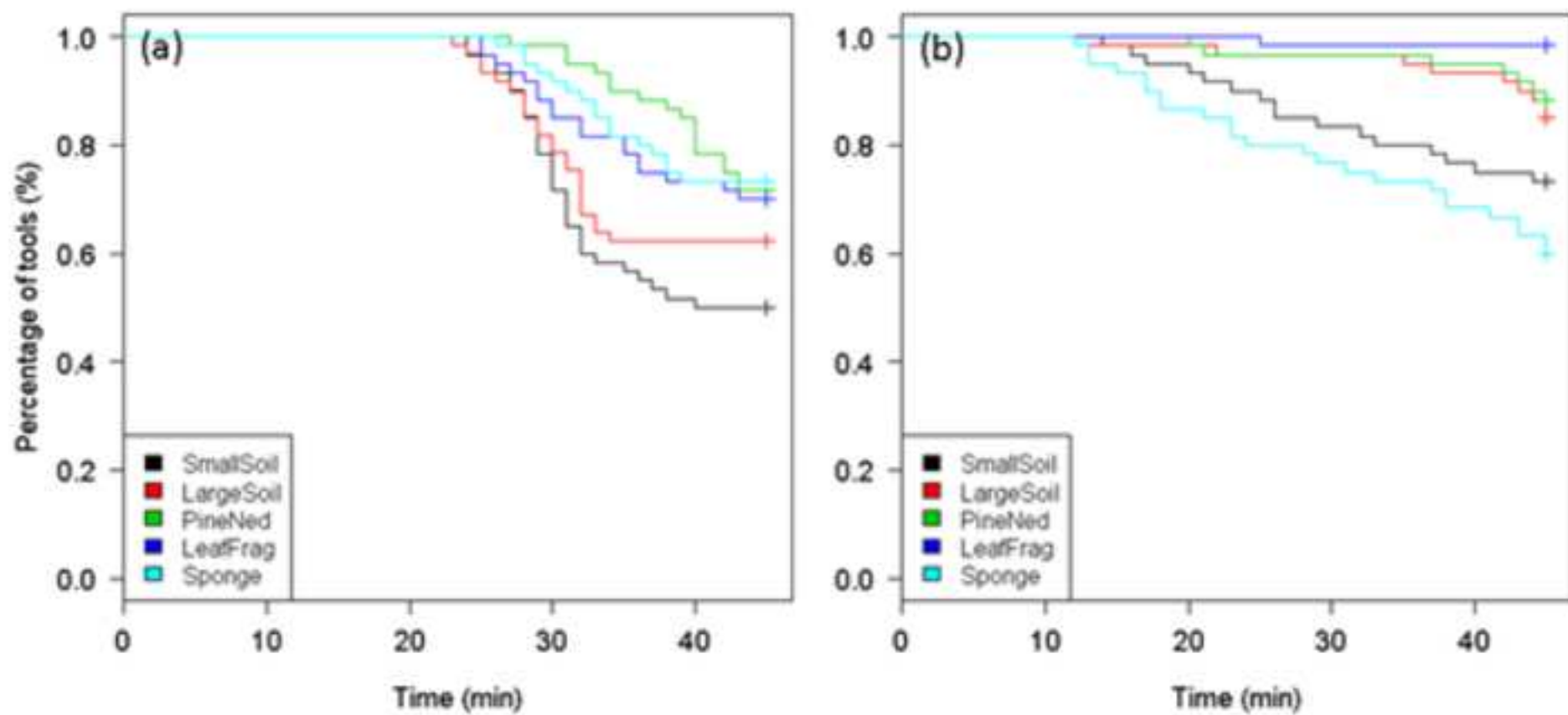


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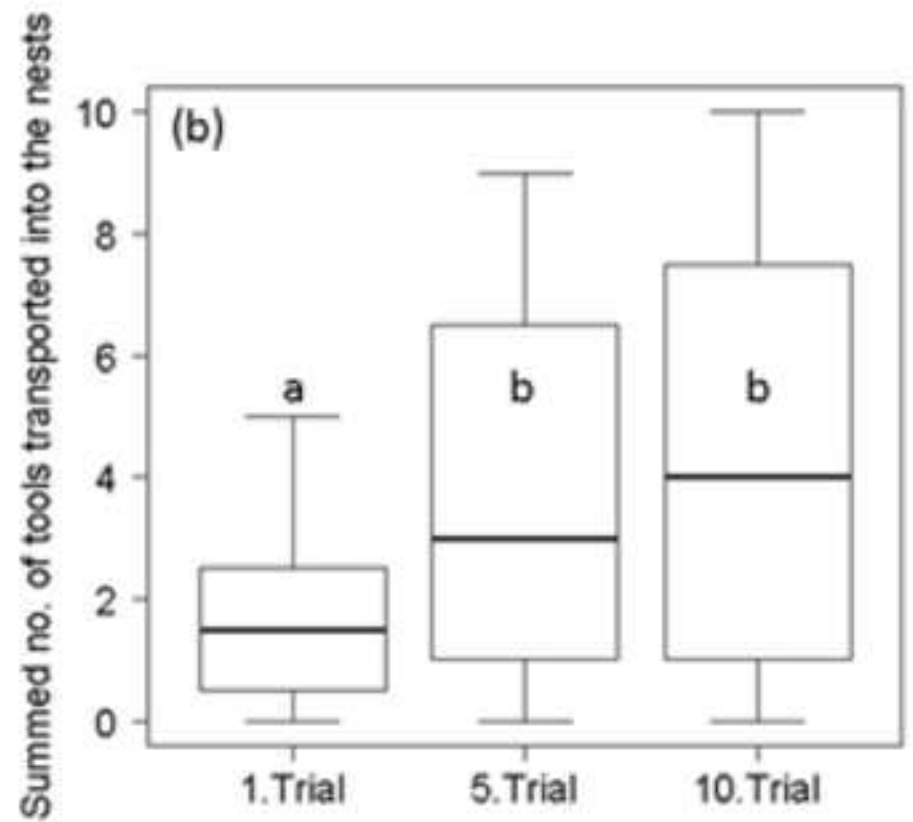
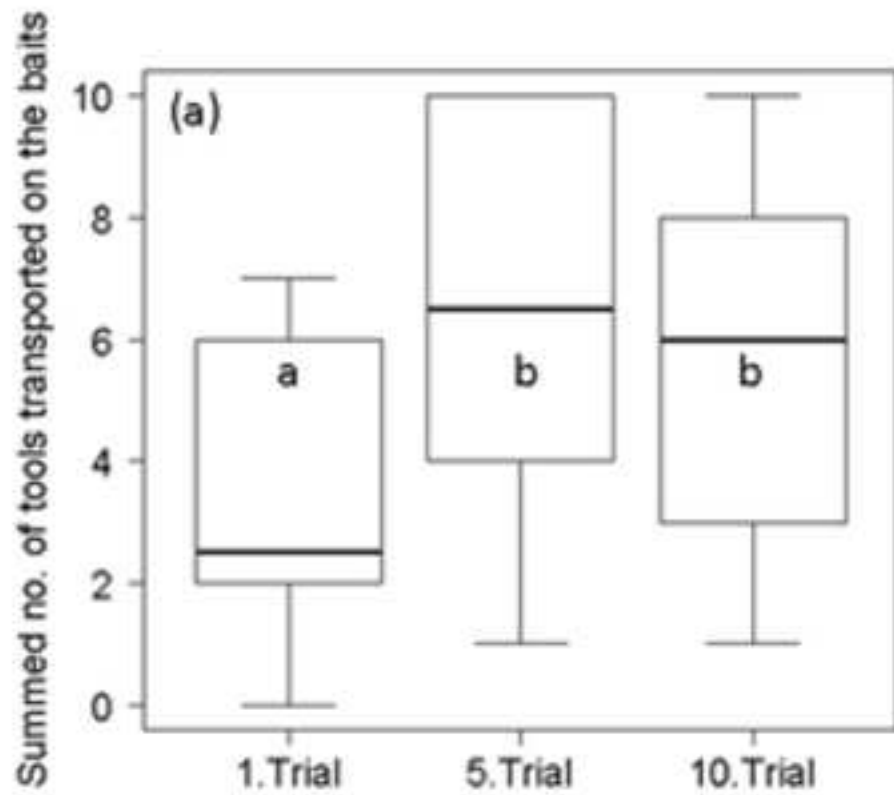
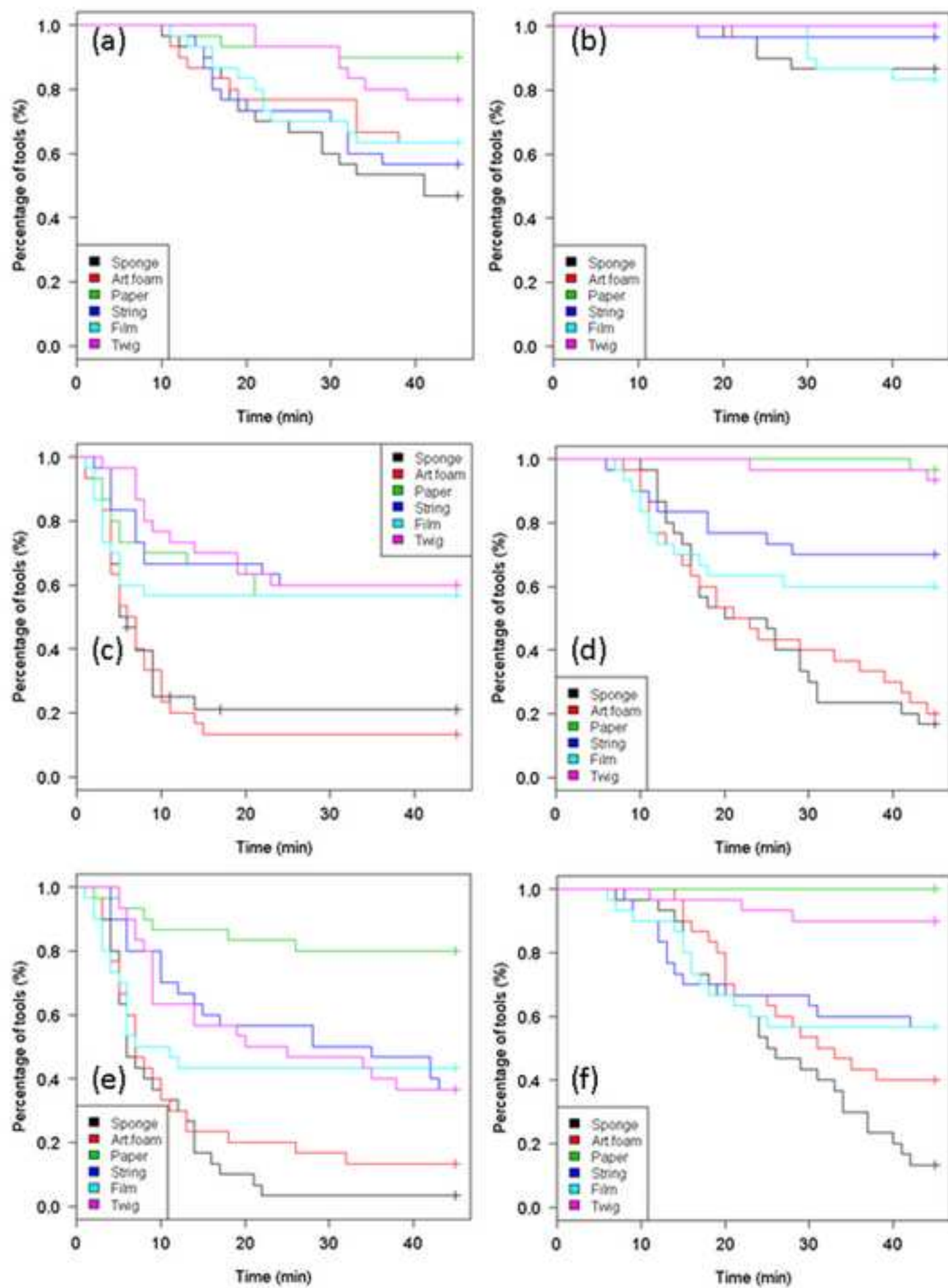


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A



B

