Early successional colonizers both facilitate and inhibit the late successional colonizers in communities of dung-inhabiting insects

Frantisek X.J. Sladecek 1, 2, Simon T. Segar 1, 2 and Martin Konvicka 1, 2

1 University of South Bohemia in Ceske Budejovice, Faculty of Science, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic; e-mails: franzsladecek@gmail.com, konva333@gmail.com, simon.t.segar@gmail.com
2 Biology Centre, Czech Academy of Sciences, Institute of Entomology, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic

Key words. Diptera, Coleoptera, dung-inhabiting insects, competition, heterotrophic succession, successional mechanisms

Abstract. The influence of early arriving species on the establishment and activity of later ones (the priority effect) is a key issue in ecological succession. Priority effects have been extensively studied in communities subject to autotrophic succession (plants, sessile animals), but only sporadically studied in communities subject to heterotrophic succession (e.g. dung or carrion inhabiting communities). We studied the influence of early successional colonizers on late successional colonizers by manipulating the successional processes in cow dung pats via delaying, and thus lowering, colonization by early successional insects. The decreased activity of early successional insects did not affect the species richness of late successional insects, but it did lead to increased abundance of colonizers. Late successional coprophagous beetles were facilitated by early successional species while larvae of late successional coprophagous flies were inhibited, presumably, by the larvae of early successional flies. We therefore propose that both facilitation and inhibition have a role to play in the heterotrophic succession of coprophilous insects. In addition, facilitation and inhibition among taxa seems to reflect their evolutionary relationships, with facilitation being prominent between phylogenetically distant lineages (early successional Diptera and late successional Coleoptera), and inhibition being more common between closely related lineages (early vs. late successional Diptera). These patterns are strikingly reminiscent of the situation in the autotrophic succession of plants.

INTRODUCTION

Succession is one of the most studied phenomena in community ecology, yet the mechanisms behind the patterns observed are still not completely known. Successional processes either lead to the establishment of a more or less stable community (autotrophic succession, e.g., formation of forests on lava islands), or to the disintegration of the resource and loss of all species from the community (heterotrophic succession, e.g. dung decomposition) (Begon et al., 2006).

How early colonizers influence the species richness and abundance of later ones (priority effects) is a major field of study in successional theory. Priority effects typically lead to one of three outcomes and are driven by distinct processes (Connell & Slatyer, 1977). The facilitation model predicts that early successional species enable the establishment of the late successional ones; the inhibition model predicts that early successional species prevent the establishment of late successional ones; and the tolerance model predicts late successional species to establish themselves irrespective of the activity of the early successional ones. Wootton (1993) refined these ideas and added two more scenarios describing the partial effect of early successional species; i.e., that the early successional species can support or hinder the establishment of the late successional species. This stands in contrast to a strict dichotomy between enabling and disabling establishment under the facilitation and inhibition models).

Successional mechanisms have traditionally (Clements, 1916; Gleason, 1926, 1927) and primarily been studied in the context of autotrophic plant communities and sessile animals, which have been long supposed to follow the inhibition or tolerance models (Connell & Slatyer, 1977). However, in all such communities, it is more typical to see a combination of several models during the course of succession, so that facilitation and inhibition often co-occur (Callaway & Walker, 1997; van der Putten, 2009; Walker et al., 2010; Maggi et al., 2011). For example, a nurse plant only facilitates seedlings within a limited distance (Dickie et al., 2005), or facilitation applies only under high levels of environmental stress (Vázquez et al., 1998). The level of facilitation also depends on the patrastic evolutionary...
distances between plant species. Evolutionarily distant lineages tend to facilitate each other while closely related lineages tend to compete (Verdú et al., 2009; Castillo et al., 2010).

In contrast, there are still only a limited number of studies targeting the mechanisms behind heterotrophic succession, which was originally supposed to follow the facilitation model (Connell & Slatyer, 1977). The reality is, however, inevitably more complex with both facilitation and inhibition at work. Dung insect communities have shaped much of this thinking. For example, early successional dung beetles can facilitate the activity of late successional earthworms which hasten dung degradation (Holter, 1977, 1979). On the other hand, late successional dung beetles tend to be outcompeted by early successional ones to such a point that late beetles are more abundant in moths when early beetles are scarce (Davis, 1989). Moreover, early successional beetles are supposed to facilitate the invasion of late successional beetle predators and flies (Hammer, 1941; Mohr, 1943). In large carcasses, early successional larvae of blowflies (Calliphoridae) were reported to facilitate carrion degradation following the bloated stage (Pechal et al., 2014), and thus facilitate the invasion of late successional beetles (Sharanowski et al., 2008). On the other hand, elimination of some dominant early successional blowfly species increased the diversity of late successional flies from other families (Pechal et al., 2014). Furthermore, a high abundance of early successional blowfly is negatively correlated with the abundance of both early and late beetle and fly species (Kadlec et al., 2019). This cumulative evidence, however, stems from individual studies. To date, there has been no experimental focusing on interactions between early and late colonizers of ephemeral resource patches. Finally, it is not yet clear which process exactly determines the correlational trends seen between early successional and late successional species. This contrasts with studies of plant communities, where the level of facilitation depends on the patristic evolutionary distances between species (Verdú et al., 2009; Castillo et al., 2010).

Here, we present a study focusing on mechanisms that shape heterotrophic succession in communities of dung inhabiting insects. We experimentally delayed access to the dung resource for all insects for a period of five days and in doing so restricted the invasion of early successional colonizers (Sladecek et al., 2013). We then compared community development in such dung pats to those pats which were freely accessible to all insects throughout the experiment. Our approach is similar to that used to limit the dominant early successional colonizers in carrion, a comparable ephemeral resource (Kadlec et al., 2019). Any resultant increase or decrease in activity of late successional species in exclusion treatments revealed potential interactions between early and late successional species. This approach allowed an assessment of the successional mechanisms behind heterotrophic succession in dung-inhabiting communities. Communities of dung-inhabiting insects are both species-rich (Hanski & Koskela, 1977; Sladecek et al., 2013), and ecologically important for ecosystem services such as dung degradation (DeCastro-Arrazola et al., 2020). Dung insect communities are also threatened by land use changes (Buse et al., 2015) and the overuse of veterinary products with non-target effects (Tonelli et al., 2020). These threats mean that understanding dung community dynamics is of relevance to maintaining ecosystem services in conservation efforts (Cimon-Morin et al., 2013). Specifically, we address the following questions:

1. Does delaying insect access, and therefore limiting access of early successional species, enable/disable (models sensu Connell) or improve/hamper (models sensu Wootton) the activity of late successional dung-inhabiting insects?
2. Is there a preference of late successional groups of insects towards dung with previously high (controls) or low (treatment) activity of early successional species?

**MATERIAL AND METHODS**

**Study site**

The study was carried out on a 23 ha pasture, situated 10 km west of Ceske Budejovice, Czech Republic (48°59′2.4″N, 14°24′34.957″E), Central Europe. This pasture hosts a permanent herd of 30 adult cows and has been continuously grazed for decades. It is situated at 380 m a.s.l., in a region with a mean annual temperature of 8.1°C, mean annual precipitation of 620 mm, and a vegetation season spanning from March to October (Sladecek et al., 2013).

**Insect sampling**

Three sampling campaigns, covering the three main periods of coprophilous insect activity, were carried out in 2011: spring (18 April – 3 May), early summer (12–27 July) and early autumn (22 August – 6 September).

We used artificially created cow dung pats of 1.5 l volume (approx. 25 cm in diameter) to mimic naturally deposited pats. Fresh, recently defecated, un-colonized dung was obtained from a barn with permanently stalled cows. These cows were not treated with any veterinary drug that could affect insect colonization and establishment (Rodriguez-Vivas et al., 2020). No insects were found to colonize the dung in the barn, since fresh dung is cleared each day. We protected such dung from insect colonization before exposition. At the study site, we thoroughly mixed and homogenized the dung before creating the exposed pats. We artificially created dung pats (Fowler et al., 2020) because it was not possible to assure that the naturally dropped pats would not already be invaded by the eggs of earliest-occurring Diptera (Hammer, 1941). Following Barth et al. (1994), we assume that the insect communities sampled from artificially created dung pats do not substantially differ from those present in naturally dropped pats.

Insect invasion was prevented in treatment pats using simple covering constructions (Fig. 1). They consisted of a plastic basket of 30 cm diameter with 0.5 × 0.5 cm sized holes firmly covered with a 1 × 1 mm wire mesh. Similar constructions using a hard cage with a net (Pechal et al., 2014; Kadlec et al., 2019) or just a fine mesh (Lumaret & Kadiri, 1995; Lee & Wall, 2006; Tixier et al., 2015) are frequently used to exclude insects. The construction was firmly nailed to the ground to exclude crawling insects. The mesh within the basket did not touch the covered dung which eliminated potential insect (especially fly) oviposition. This covering was tested before the experiment and no insects were found to colonize covered pats before uncovering.

The sampling design consisted of treatment pats and control pats. The treatment pats were covered immediately after their
placement on the pasture and remained covered for five days. Afterwards, they were uncovered and exposed to colonizing insects for an additional one, two, three, five or seven days (exposition time). Therefore, there were five treatment pats per replication. The whole five-pat arrangement was replicated four times per sampling campaign. The four replicates were established during four successive days (one on each day) to minimize variation due to weather.

Control pats were placed on the pasture concurrently with treatment pats, but remained uncovered throughout their existence. We chose not to cover control pats with any cover device, since covering of control pats would alter the early successional species abundances. Control pats treated as such would also receive some form of insect exclusion treatment, instead of representing the natural and un-manipulated successional process. Similar uncovered controls have been used in most studies that have excluded insects in ephemeral habitats (Lumaret & Kadiri, 1995; Lee & Wall, 2006; Pechal et al., 2014; Tixier et al., 2015).

The control and treatment pats existed on the pasture for the same number of days, but differed in the lengths of exposure to early successional insects. For example, the pat covered for five days and exposed for one day (six days after creation on the pasture) was compared with the control pat also exposed for six days.

Processing the samples

We extracted insects by floating the dung pat and a small portion of underlying soil in a bucket of water. Great attention was paid to ensure capture of all quickly escaping insects, so that the percentage of potential runaways was negligible. Each dung pat was floated for at least 45 min and the floated substrate was then hand-sorted to assure that all individuals were sampled (Fowler et al., 2020). The insects were preserved in 96% ethanol and taken to the laboratory for identification.

Insect identification and classification

All adult beetles were identified to species level, or to morpho-spectra within respective genera. The larvae were identified to species whenever possible, otherwise to genus or family levels (Table S1). The genus or family morpho-species were treated as regular species in the analyses. Singleton records were excluded from the analyses. One dung pat and its inhabitants represent one sample in further analyses.

First, we placed dung-inhabiting insect species into early and late successional categories. For adult beetles, we used the temporal preferences from our previous study, in which beetles formed two distinct successional groups (Sladecek et al., 2013). Specifically, beetles occurring in dung ≤ 4 days old were classified as early successional, beetles occurring in dung ≥ 5 five or more days old as late successional. For larvae and parasitoids, we used findings reported in the literature (Hammer, 1941; Laurence, 1954; Skidmore, 1985).

Second, we established groups of dung-inhabiting insects based on a combination of their ecology and taxonomy. For this classification, we took into account their trophic strategy (coprophagous / predatory), family (coprophagous / predatory larvae of Muscidae), and in the case of Hydrophilidae beetles, if they were dung-specialists or generalist saprophages (large / small Hydrophilidae) (Hansen, 1987). Full list of our groups is provided in Table 1.

The complete list of species sampled with their successional classification and group affiliation is provided in supporting information (Table S1).

Hypotheses and analyses

(1) Does delaying insect access and therefore limiting access of early successional species enable/disable (models sensu Connell) or improve/hamper (models sensu Wootton) the activity of late successional dung-inhabiting insects?

Connell & Slatyer (1977) predicted that early successional species either directly enable or disable the late successional species establishment. It follows that a lower number of early successional species in the treatment may lead to: (1) a lower number of late successional species (facilitation), (2) a higher number of late successional species (inhibition) or to (3) no difference between treatment and control (tolerance). To compare the number of species per pat between treatment and control, we used Generalized linear models with mixed effects computed with "glmmer" function from package “lme4” (Bates et al., 2015) in R 4.0.3 (Team, 2020).

We fitted three models with one of three response variables: (a) total species richness, (b) early successional species richness and (c) late successional species richness. Each model was fitted with treatment (treatment/control) as a focal explanatory variable and with a Poisson distribution of errors. In addition, dung age (6,7,8, 10 or 12 days) and sampling campaign (spring, early summer, early autumn) were included as factors with fixed effects. Affinity to replicative line within sampling campaign (1, 2, 3, 4) was a factor with a random effect. Resulting models were validated using the package “DHARMa” (Hartig, 2019) in R 4.0.3 (Team, 2020). If overdispersion was detected, we fitted a new model with observation level as a random effect (Harrison, 2014). Statistical significance of fixed effects were retrieved using the function “Anova” in package “car” (Fox & Weisberg, 2019). Resultant figures were created using packages “effects” (Fox & Weisberg, 2019) and “ggplot2” (Wickham, 2016).

Wootton (1993) presented the idea that early successional species could not only strictly enable or prevent the activity of late successional species, but rather increase (facilitation) or decrease (inhibition) the activity of late successional species. It follows that a lower abundance of early successional species in the treatment pats should lead to: (1) lower abundance of late successional species (facilitation), (2) higher abundance of late successional species (inhibition) or to (3) no difference between treatment and control (tolerance). To compare the abundances of early and late successional species per dung between treatment and control, we used Generalized linear models with mixed effects computed
Table 1. Classification of dung-inhabiting insects used in this study. Trop. group/Order – trophic group and order; Coprophage – feeds solely on dung matter; Omnivore – switch between coprophagy (adult) and predation (larvae); Predators – feeds on living insects; Tax. group – taxonomic group (mostly families); Suc. cat. – successional category; early – mostly abundant in ≤ 4 days old dung; late – most abundant in ≥ 5 days old dung; Representative – representative genus/family; Abb. – abbreviation used in further analyses.

| Trop. group/Order | Tax. group | Suc. cat. | Representative | Abb. |
|------------------|-----------|-----------|----------------|------|
| **Coleoptera**   |           |           |                |      |
| Scarabaeidae      | early     | Onthophagus| ESca **r**     |      |
| Staphylinidae     | early     | Aphodiuss | LScara **r**   |      |
| Muscidae          | late      | Platycesthus, Mégathrus | LStaCo **r** |      |
| Anthomyiidae      | late      | Anthomyiidae larvae | LANtho **r** |      |
| **Diptera**       |           |           |                |      |
| Acalyptratae      | late      | Sepsidae, Sphaeroceridae larvae | LAcaly **r** |      |
| **Omnivores**     |           |           |                |      |
| Large Hydrophilidae | late    | Sphaeridium larvae | LSphLa **r** |      |
| Small Hydrophilidae| early    | Cercyon     | EHylSm **r**   |      |
| Muscidae          | late      | Mydaeiinae larvae | LMys **r** |      |
| **Predators**     |           |           |                |      |
| Staphylinidae     | early     | Ontholeste | ESstaPr **r** |      |
| Histeridae        | late      | Philonthus, Atheta | LStaPr **r** |      |
| **Hymenoptera**   | “Parasitica”| late     | Figitidae      | LParas |      |

Results

In total, the sampling amassed 15,814 insect individuals from 78 species and morphospecies (Table S1). Treatment pats differed visibly from control ones, yet all changes were induced by insect activity in control pats (i.e. perforated crust and galleries under the crust).

(1) Does delaying insect access and therefore limiting access of early successional species enable/disable (models sensu Connell) or improve/hamper (models sensu Wooton) the activity of late successional dung-inhabiting insects?

Both total species richness (estimate = – 0.001, standard error = 0.042, z = –0.228, p = 0.820) and species richness of late successional species (estimate = 0.044, standard error = 0.045, z = 0.976, p = 0.329) did not differ significantly between control and treatment pats (Fig. 2A, C). Species richness of early successional species was significantly lower (estimate = –0.282, standard error = 0.103, z = –2.739, p = 0.006) in treatment pats than in control pats (Fig. 2B).

Total abundance of dung-inhabiting insects did not significantly differ between control and treatment pats (estimate = 0.141, standard error = 0.099, z = 1.415, p = 0.157) (Fig. 2D). Abundance of early successional species was lower (estimate = –0.561, standard error = 0.169, z = –3.321, p < 0.001) in treatment pats than in control pats (Fig. 2E).

Abundance of late successional species was higher (estimate = 0.249, standard error = 0.103, z = 2.413, p = 0.016) in treatment pats than in control pats (Fig. 2F).

(2) Is there a preference of late successional groups of insects towards dung with previously high (controls) or low (treatment) activity of early successional species?

We tested the affinities of insect groups (sums of individuals belonging to these groups in each sample), occurring in at least ten samples, for treatment and control pats using redundancy analysis (RDA) computed in CANOCO for Windows 5 (Ter Braak & Smilauer, 2012). RDA is suitable for datasets where species display linear responses to environmental gradients (Leps & Smilauer, 2012). RDA computed in CANOCO for Windows 5 (Ter Braak & Smilauer, 2012). Data were log(x + 1) transformed prior to the analyses. Dung age, sampling campaign and af- fered crust and galleries under the crust). Were induced by insect activity in control pats (i.e. perfo-

cific line were treated as covariates (equivalent to factors with random effects). The statistical signifi-

cance of the main RDA pre-
ciput were assessed using the Monte Carlo test (999 unrestricted permutations under the reduced model).

RESULTS

In total, the sampling amassed 15,814 insect individuals from 78 species and morphospecies (Table S1). Treatment pats differed visibly from control ones, yet all changes were induced by insect activity in control pats (i.e. perforated crust and galleries under the crust).

(1) Does delaying insect access and therefore limiting access of early successional species enable/disable (models sensu Connell) or improve/hamper (models sensu Wooton) the activity of late successional dung-inhabiting insects?

Both total species richness (estimate = –0.001, standard error = 0.042, z = –0.228, p = 0.820) and species richness of late successional species (estimate = 0.044, standard error = 0.045, z = 0.976, p = 0.329) did not differ significantly between control and treatment pats (Fig. 2A, C). Species richness of early successional species was significantly lower (estimate = –0.282, standard error = 0.103, z = –2.739, p = 0.006) in treatment pats than in control pats (Fig. 2B).

Total abundance of dung-inhabiting insects did not significantly differ between control and treatment pats (estimate = 0.141, standard error = 0.099, z = 1.415, p = 0.157) (Fig. 2D). Abundance of early successional species was lower (estimate = –0.561, standard error = 0.169, z = –3.321, p < 0.001) in treatment pats than in control pats (Fig. 2E). Abundance of late successional species was higher (estimate = 0.249, standard error = 0.103, z = 2.413, p = 0.016) in treatment pats than in control pats (Fig. 2F).
DISCUSSION

Early successional species

As expected, delaying insect colonization effectively lowered the species richness and abundance of early successional species. Abundance and species richness of early successional species is generally very low under natural conditions in dung older than four days (Sladecek et al., 2013). It is therefore interesting, that treatment pats had even lower abundance and species richness of early successional species than control pats. A possible explanation could be the changes in dung-emitted volatiles (Sladecek et al., 2021a), which are the primary cue for insects inhabiting ephemeral resources when searching for their food sources (Frank et al., 2018; Weithmann et al., 2020). Dung-emitted volatiles are mostly produced by microbes (Saito et al., 2018), whose communities change with pat aging (Perez-Valera et al., 2019). Although dung beetles were always considered the prime catalyst for such changes (Lusenhop et al., 1980), it seems that any soil fauna could start this process (Perez-Valera et al., 2019). An intriguing result was the preference of early successional dung beetles and predatory Staphylinidae for treatment pats rather than controls.

For dung beetles, this could mean that they compete with two other major groups, Musca larvae and Hydrophilidae species, even though dung beetles are generally considered to be top competitors in dung, especially in comparison to fly larvae (Ridsdill-Smith et al., 1986). The earliest successional dung beetles are dung relocating beetles in our study, and therefore could avoid dung pats with higher fly larvae abundance. There is a similar relationship between relocating burying beetles and blowflies in carrion (Kadlec et al., 2019).

Predatory Staphylinidae have always been considered to be one of the primary predators of Muscidae larvae (Walsh & Posse, 2003). However, this study in combination with our recent study on food webs (Sladecek et al., 2021b)
strongly suggests that Staphylindae instead prey on other fly families (e.g., the adults and larvae of Sepsidae and Sphaeroceridae). Indeed, Staphylindae may not be the effective regulators of pestiferous (Krasfur & Moon, 1997) Calypterate flies they are assumed to be.

**Late successional species**

Connell & Slatyer (1977) emphasised the total exclusion of early successional species. This is unfortunately not completely possible in insect communities inhabiting ephemeral resource patches (Lee & Wall, 2006; Pechal et al., 2014), in contrast to sessile communities of marine organisms and plants (van der Putten, 2009; Maggi et al., 2011). Nevertheless, per pat the number of early successional species (by approximately 33%) and their abundances (by almost 50%) were significantly lower in our treatment pats. Furthermore, control pats were probably invaded by numerous early successional species after their exposition, which had already vacated the dung (Sladecek et al., 2013). We therefore consider our manipulation to have significantly reduced or even negated the activity of early successional species in treatment pats. This reduction of early successional species enabled us to make interpretations using the models of succession proposed by Connell & Slatyer (1977).

Contrary to predictions made by Connell & Slatyer (1977), and earlier studies on the dung microhabitat (e.g. Holter, 1977), we found no straightforward facilitation (exclusion of early species results in absence of late species) between early and late successional species during the heterotrophic succession of the coprophilous insect community. Lower activity of early successional insects did not translate into lower species richness of late ones. In fact, there was a trend in treatment pats to host even more late successional species than control ones (approximately two more species per pat). Based on our data, the succession of the coprophilous insect community follows the tolerance model proposed by Connell & Slatyer (1977), tending even towards the inhibition models. Our results thus contrast sharply with earlier studies from ephemeral resource patches (Lumaret & Kadiri, 1995; Lee & Wall, 2006; Pechal et al., 2014). However, the majority of such studies primarily tested the effects of early successional species on habitat degradation, rather than within-community dynamics. Previous studies tended to decrease the number of early successional colonizers, which reduced the rate of habitat degradation, which led the authors to suggest facilitation as a model for succession (Holter, 1977, 1979; Lumaret & Kadiri, 1995; Pechal et al., 2014).

Based our observations, we therefore suggest that the succession of the coprophilous insect community is better described by Wootton’s models (1993), which predicted the numerical (enhance/hinder) rather than the strict (enable/disable) effect of early successional species upon colonization of late ones. In our study, the treatment pats hosted higher abundance of late successional species (by approximately 25–30%) when compared to the control pats. This higher abundance (when taken at the community level) points toward strong inhibition of late successional species by early successional ones. The situation is, however, more complex at the level of individual groups of late successional insects.

The late successional coprophagous dung beetles (*Aphodius* spp.) and coprophagous Staphylinidae displayed a pattern suggestive of facilitation by early successional species. This may be a result of their relationship with either or both early successional larvae of Muscidae or Hydrophilidae beetles (*Sphaeridium* spp.), which are very abundant in freshly exposed dung (Sladecek et al., 2013). Despite the fact that *Sphaeridium* beetles tend to invade dung pats in high numbers during its initial exposition, their overall presence in dung is fairly limited due to their quick oviposition, as their larvae feed on the larvae of Diptera (Sowig, 1997). In contrast, larvae of *Diptera* have a more lasting presence in dung (Laurence, 1954). We therefore assume that the observed facilitative relationship between early successional species and late successional dung beetles could be attributed to high abundances of early successional larvae of Muscidae. This result is certainly controversial, since most studies to date focusing on the beetles versus fly larvae interactions have reported a negative relationship between the two (Hirschberger & Degro, 1996; Kadlec et al., 2019). Late successional dung beetles should avoid oviposition where there is a high abundance of fly larvae (Hirschberger & Degro, 1996) in sheep droppings, which are very small and provide less food and space for their inhabitants (Sowig, 1994). In contrast, in cow dung, which is large and contains high dung moisture (Gittings & Giller, 1998), adult late successional dung beetles could be facilitated by activity of fly larvae, since such beetles mostly prefer the drier parts of the dung pats (Holter, 1982). Alternatively, fly larvae might speed up the interchange between dung and soil microbiota, a process that should increase emission of beetle attractant volatile cues (Sladecek et al., 2021a). Moreover, Lee & Wall (2006) reported that numbers of *Aphodius* larvae were also highest in uncovered pats or pats covered for a very short period of time (and therefore exposed to free fly larvae activity). In combination with Lee & Wall (2006) the results of suggest that the relationships between late successional dung beetles and dung flies are probably not as straightforward as has currently thought. We encourage the collection of more manipulative data, which are needed to fully understand the relationships between *Aphodius* beetles and flies.

In common with dung beetles, coprophagous late successional Staphylinidae (Oxytelinae and Proteininae) also displayed a preference for control pats. These Staphylinidae beetles are actually saprophilous as opposed to strictly coprophilous (Koskela, 1972; Hammond, 1976). We therefore expected Staphylinidae to benefit from dung pat drying, a process hastened by the larvae of early successional *Diptera*. In addition, they may be attracted by increasing volatile emissions released by higher numbers of larvae (Sladecek et al., 2021a).

In contrast to the coprophagous beetles, the late successional small Acalypttrae *Diptera* (Sepsidae, Sphaeroceridae) larvae are most likely to be subject to competition (in-
hibitation) with the early successional larvae of Calyptratae Diptera. The large Calyptratae Diptera larvae share their main food source with small Acalyptratae Diptera (Dowding, 1967). Calyptrate Diptera, due to their size (up to 1 cm) and density in the dung (up to 400 individuals per 1.5 liters), accelerate dung desiccation, thus destroying the habitat, and also diminishing the supply of their shared food source, bacteria (Lussenhop et al., 1986). Although there is no direct evidence, competition for space is considered to be an important factor in structuring the communities of dung beetles (Hirschberger, 1998; Finn & Gittings, 2003), and it could probably also affect fly-fly relations.

Our study primarily proposed and tested successional mechanisms among species that could compete with each other, e.g., the coprophagous species. Nevertheless, ephemeral resource patches such as dung or carrion facilitate studies of the effects of early successional colonizers even on competitively unrelated species, i.e., predators and omnivores. The late successional omnivores, who were more abundant in control pats, consisted mainly of saprophilous Hydrophilidae species (Cryptopleurum spp., Cercyon spp.) (Hansen, 1987). Presumably these insects also benefited from the activity of early successional species (namely fly larvae), as did late successional dung beetles. In contrast to previous studies (Hammer, 1941; Mohr, 1943), the activity of late successional predators and parasitoids is not facilitated by the activity of early successional species, such as perforating the dung crust. The higher abundance of late successional predators and parasitoids in treatment pats could be attributable to high abundance of Acalyptratae Diptera larvae (mainly Sepsidae), which represent their main prey items (Valiela, 1974). Finally, the higher abundance of predatory Sphaeridium adults visiting the treatment pats. Another explanation could be the potential density-dependent egg laying behavior of Sphaeridium, parents typically lay eggs when the presence of their kin is low, since larvae often display harsh cannibalism (Sowig, 1997).

Based on our results, we refute facilitation as the main successional mechanism in communities of dung-inhabiting insects. Previously, facilitation has been tested for all ephemeral habitats (Connell & Slatyer, 1977) and seen as less important for dung-inhabiting communities (Holter, 1977, 1979; Slade et al., 2007). In contrast we propose that the successional processes in dung-inhabiting insects are not facilitated by the activity of early successional species (namely fly larvae), as did late successional dung beetles. —

ACKNOWLEDGEMENTS. We are very grateful to the school farm of the Faculty of Agriculture, University of South Bohemia in Ceske Budejovice, for allowing us to carry out this study on their pasture. We also thank to V. Novotny and P. Klimes for fruitful comments, P. Smilauer for advice on the statistical analyses, and M. Sweeney for language corrections. We are grateful to the host of anonymous referees for suggesting new ideas that vastly improved the manuscript. This work was supported by the Institute of Entomology, Biology Centre of the Czech Academy of Science (RVO: 60077344) and by the Grant Agency of University of South Bohemia (152/2016/P). FXJS was supported by Czech Academy of Sciences (MSM200061902) and STS acknowledges departmental support from Harper Adams University. MK acknowledges support by the Technology Agency of the Czech Republic (SS01010526).

REFERENCES

BARTH D., KARRER M., HEINZEMUTZ E. & ELSTER N. 1994: Colonization and degradation of cattle dung: aspects of sampling, fecal composition, and artificially formed pats. — Environ. Entomol. 23: 571–578.

BATES D., MAECHLER M., BOLKER B. & WALKER S. 2015: Fitting linear mixed-effects models using lme4. — J. Stat. Softw. 67: 1–49.

BEGON M., TOWNSEND C. & HARPER J. 2006: Ecology: From Individuals to Ecosystems, 4th ed. Wiley-Blackwell, Hoboken, 750 pp.

BUSE J., SLACHTA M., SLADECEK F.J., PUNG M., WAGNER T. & ENTLING M.H. 2015: Relative importance of pasture size and grazing continuity for the long-term conservation of European dung beetles. — Biol. Conserv. 187: 112–119.

CALLAWAY R. & WALKER L. 1997: Competition and facilitation: A synthetic approach to interactions in plant communities. — Ecology 78: 1958–1965.

CASTILLO J., VERDU J. & VALENTE-BANuet A. 2010: Néighbordood phylodiversity affects plant performance. — Ecology 91: 3656–3663.

CIMON-MORIN J., DARBEAU M. & POULIN M. 2013: Fostering synergies between ecosystem services and biodiversity in conservation planning: A review. — Biol. Conserv. 166: 144–154.
Sladecek et al., Eur. J. Entomol. 118: 240–249, 2021
doi: 10.14411/eje.2021.025

Clements F. 1916: Plant Succession: Analysis of the Development of Vegetation. Carnegie Institution of Washington Publication Sciences 242, Washington, 512 pp.

Connell J. & SLATER R. 1977: Mechanisms of succession in natural communities and their role in community stability and organization. — Am. Nat. 111: 119–144.

Davis A.L.V. 1989: Residence and breeding of Oniticellus (Coleoptera, Scarabaeidae) within cattle pads — inhibition by dung-burying beetles. — J. Entomol. Soc. S. Afr. 52: 229–236.

DeCastro-Arrazola I., Hortal J., Noreiga J.A. & Sanchez-Pinero F. 2020: Assessing the functional relationship between dung beetle traits and dung removal, burial, and seedling emergence. — Ecology 101: e03138, 7 pp.

Deeke I., Schindtner S., Reich P. & Hobbie S. 2005: Spatially disjoint effects of co-occurring competition and facilitation. — Ecol. Lett. 8: 1191–1200.

Dowding V. 1967: The function and ecological significance of the pharyngeal ridges occurring in the larvae of some cyclorhaphous Diptera. — Parastidology 57: 371–388.

Finn J. & Gittins T. 2003: A review of competition in northern temperate dung beetle communities. — Ecol. Entomol. 28: 1–13.

Fowler F., Wilcox T., Orr S. & Watson W. 2020: Sampling Efficiency and survival rates of Labaurus pseudolividus (Coleoptera: Scarabaeidae) and Onthophagus taurus (Coleoptera: Scarabaeidae) using flotation and sieve-separation methodology. — J. Insect Sci. 20(5): 18, 7 pp.

Fox J. & Weisberg S. 2019: An (R) Companion to Applied Regression. SAGE, Los Angeles, 572 pp.

Frank K., Bruckner A., Bluthgen N. & Schmit T. 2018: In search of cues: dung beetle attraction and the significance of volatile composition of dung. — Chemoeconomics 28: 145–152.

Gittins T. & Giller P. 1998: Resource quality and the colonization and succession of coprophagous dung beetles. — Ecography 21: 581–592.

Gleason H. 1926: The individualistic concept of the plant association. — Bull. Torrey Bot. Club 53: 7–26.

Gleason H. 1927: Further views on the succession concept. — Ecology 8: 299–326.

Hammer O. 1941: Biological and ecological investigations on flies associated with pasturing cattle and their excrement. — Vidensk. Medd. Fra Dansk. Naturh. Foren. 105: 1–257.

Hammond P.M. 1976: A review of the genus Anotylus C.G. Thomson (Coleoptera: Staphylinidae). — Bull. Br. Mus. Nat. Hist. Entomol. 33: 139–185.

Hansen M. 1987: The Hydrophiloidea (Coleoptera) of Fennoscandia and Denmark. — Fauna Entomologica Scandin. 18: 1–253.

Hanski I. & Koskela H. 1977: Niche relations among dung-inhabiting beetles. — Oecologia 28: 203–231.

Harrison X.A. 2014: Using observation-level random effects to model overlapdissipase in count data in ecology and evolution. — PeerJ 2: e416, 19 pp.

Hartig F. 2019: DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. URL: https://CRAN.R-project.org/package=DHARMa

Herschberger P. 1998: Spatial distribution, resource utilisation and intraspecific competition in the dung beetle Aphodius ater. — Oecologia 116: 136–142.

Herschberger P. & Degro H.N. 1996: Oviposition of the dung beetle Aphodius ater in relation to the abundance of yellow dungfly larvae (Scatophaga stercoraria). — Ecol. Entomol. 21: 352–357.

Holter P. 1977: An experiment on dung removal by Aphodius larvae (Scarabaeidae) and earthworms. — Oikos 28: 130–136.

Holter P. 1979: Effect of dung-beetles (Aphodius spp.) and earthworms on the disappearance of cattle dung. — Oikos 32: 393–402.

Holter P. 1982: Resource utilization and local coexistence in a guild of scarabaeid dung beetles (Aphodius spp.). — Oikos 39: 213–227.

Kadlec J., Mikatova S., Maslo P., Sipkova H., Sipek P. & Sladecek F.X.J. 2019: Delaying insect access alters community composition on small carrion: a quantitative approach. — Entomol. Exp. Appl. 167: 729–740.

Koskela H. 1972: Habitat selection of dung-inhabiting staphylinids (Coleoptera) in relation to age of the dung. — Ann. Zool. Fenn. 9: 156–171.

Krasner E.S. & Moon R.D. 1997: Bionomics of the face fly, Musca autumnalis. — Annu. Rev. Entomol. 42: 503–523.

Laurence B. 1954: The larval inhabitants of cow pats. — J. Anim. Ecol. 23: 234–260.

Lee C. & Wall R. 2006: Cow-dung colonization and decomposition following insect exclusion. — Bull. Entomol. Res. 96: 315–322.

Leps J. & Smilauer P. 2003: Multivariate Analysis of Ecological Data Using CANOCO. Cambridge University Press, Cambridge, UK, 269 pp.

Lumaret J. & Kadiri N. 1995: The influence of the first wave of colonizing insects on cattle dung dispersal. — Pedobiologia 39: 506–517.

Lussenhop J., Kumar R., Wicklow D.T. & Lloyd J.E. 1980: Insect effects on bacteria and fungi in cattle dung. — Oikos 34: 54–58.

Lussenhop J., Kumar R. & Lloyd J.E. 1986: Nutrient regeneration by fly larvae in cattle dung. — Oikos 47: 233–238.

Mang E., Bertocci I., Vaselli S. & Benedetti-Cecchi L. 2011: Connell and Slatyer’s models of succession in the biodiversity era. — Ecology 92: 1399–1406.

Mohr C.O. 1943: Cattle droppings as ecological units. — Ecol. Monogr. 13: 275–298.

Pechal J.L., Benbow M.E., Crippen T.L., Tarone A.M. & Tomberlin J.K. 2014: Delayed insect access alters carrion decomposition and necrophagous insect community assembly. — Ecology 95(4): 45, 21 pp.

Perez-Valera E., Kyselkova M., Amed E., Sladecek F.X.J., Gobena M. & Eliottova D. 2019: Native soil microorganisms hinder the soil enrichment with antibiotic resistance genes following manure applications. — Sci. Rep. 9: 6760, 10 pp.

Ridsdill-Smith T.J., Hayles L. & Palmer M.J. 1986: Competition between the bush fly and a dung beetle in dung of differing characteristics. — Entomol. Exp. Appl. 41: 83–90.

Rodriguez-Vivas R.I., Basto-Estellera G.S., Reyes-Noello E., Arco-Moran A.A., Arquila-Fuentes W.R., Ojeda-Chi M.M. & Martinez I. 2020: Evaluation of the attraction, lethal and sublethal effects of the females of ivermectin-treated cattle on the dung beetle Digitonothophagus gazella (Coleoptera: Scarabaeidae). — Aust. Entomol. 59: 368–374.

Saio Y., Sato T., Nomoto K. & Tsui H. 2018: Identification of phenol- and p-cresol-producing intestinal bacteria by using media supplemented with tyrosine and its metabolites. — FEMS Microbiol. Ecol. 94: fiy125, 11 pp.

Sharawaskis B.J., Walker E.G. & Anderson R.S. 2008: Insect succession and decomposition patterns on shaded and sunlit carrion in Saskatchewan in three different seasons. — Forensic Sci. Int. 179: 219–240.

Skidmore P. 1985: The Biology of the Muscidae of the World. Junk, Dordrecht, 550 pp.

Slade E., Mann D., Villanueva J. & Lewis O. 2007: Experimental evidence for the effects of dung beetle functional group
richness and composition on ecosystem function in a tropical forest. — J. Anim. Ecol. 76: 1094–1104.

SLADECK F.X.J., HREC J., KLIIMES P. & KONVICKA M. 2013: Interplay of succession and seasonality reflects resource utilization in an ephemeral habitat. — Acta Oecol. 46: 17–24.

SLADECK F.X.J., DOTTERL S., SCHAEFFLER I., SEGAR S.T. & KONVICKA M. 2021a: Succession of dung-inhabiting beetles and flies reflects the succession of dung-emitted volatile compounds. — J. Chem. Ecol. 47: 433–443.

SLADECK F.X.J., ZITEK T., KONVICKA M. & SEGAR T. 2021b: Evaluating the relative importance of habitat filtering and niche differentiation in shaping the food web of dung-inhabiting predators. — Acta Oecol. 112: 1–8.

SOWIG P. 1994: Resource partitioning in coprophagous beetles from sheep dung: phenology and microhabitat preferences. — Zool. Jb. Syst. 121: 171–192.

SOWIG P. 1997: Predation among Sphaeridium larvae: The role of starvation and size differences (Coleoptera: Hydrophilidae). — Ethol. Ecol. Evol. 9: 241–251.

TEAM R.C. 2020: R: A Language and Environment for Statistical Computing. Vienna, Austria. URL: http://www.r-project.org/ index.html

TER BRAAK C.J.F. & SMILAER P. 2012: Canoco Reference Manual and User’s Guide: Software for Ordination, version 5.0. Microcomputer Power, Ithaca, NY, 496 pp.

TINIER T., LUMARET J.P. & SULLIVAN G.T. 2015: Contribution of the timing of the successive waves of insect colonisation to dung removal in a grazed agro-ecosystem. — Eur. J. Soil Biol. 69: 88–93.

TONTONELLI M., VERDÚ J.R., MORELLI F. & ZUNINO M. 2020: Dung beetles: functional identity, not functional diversity, accounts for ecological process disruption caused by the use of veterinary medical products. — J. Insect Conserv. 24: 643–654.

VALIELA I. 1974: Composition, food webs and population limitation in dung arthropod communities during invasion and succession. — Am. Midl. Nat. 92: 370–385.

VAN DER PUTTE W. 2009: A multitrophic perspective on functioning and evolution of facilitation in plant communities. — J. Ecol. 97: 1131–1138.

VAZQUEZ G., MORENO-CASASOLA P. & BARRERA O. 1998: Interaction between algae and seed germination in tropical dune slack species: a facilitation process. — Aquat. Bot. 60: 409–416.

VERDU M., REY P., ALCANTARA J., SILES G. & VALIENTE-BANUET A. 2009: Phylogenetic signatures of facilitation and competition in successional communities. — J. Ecol. 97: 1117–1180.

WALKER L., LANDAU F., VELAZQUEZ E., SHELS A. & SPARROW A. 2010: Early successional woody plants facilitate andferns inhibit forest development on Puerto Rican landslides. — J. Ecol. 98: 625–635.

WALSH G. & POSE M. 2003: Abundance and seasonal distribution of predatory coprophilous Argentine rove beetles (Coleoptera: Staphylinidae), and their effects on dung breeding flies. — Coleopt. Bull. 57: 43–50.

WEITMANN S., VON HSERRMANN C., SCHMITT T., STEIGER S. & AVASSE M. 2020: The attraction of the dung beetle Anoplotrupes stercorosus (Coleoptera: Geotrupidae) to volatiles from vertebrate cadavers. — Insects 11: 476, 16 pp.

WICKHAM H. 2016: ggplot2: Elegant Graphics for Data Analysis. Springer, New York, 213 pp.

WOOTTON J. 1993: Size-dependent competition: effects on the dynamics vs. the end-point of mussel bed succession. — Ecology 74: 195–206.

Received June 15, 2021; revised and accepted August 19, 2021 Published online August 30, 2021

Table S1. List of species sampled and their respective insect group affiliations. Sum — sum of all individuals per particular group or species; Succ — successional optimum of a species or a group (early – optimum in <4 days old dung; late – optimum in >4 days old dung); Trophic — trophic strategy of a species or a group (coprophages — both adult and larva coprophagous, predators – both adult and larva predatory, omnivores – trophic shift between adults and larvae (e.g. adult coprophagous, larvae predatory)); Group – species affiliation to a particular group in the analyses, combination of successional preference, taxonomic group and trophic strategy within the taxonomic group (e.g. LStaPr – late successional Staphilinidae predators). See Table 1 for group delimitation.

| SPECIES | COLEOPTERA | SUM | SUCC | TROPIC | GROUP |
|-----------------|-------------|-----|------|--------|-------|
| Atholus duodecimstriatus | 7 | late | predators | LStaCo |
| Hister unicolor | 11 | late | predators | LStaCo |
| MARGINATOR PURPURASCENS | 9 | late | predators | LStaCo |
| MARGINATOR VENTRALIS | 3 | late | predators | LStaCo |
| Cercyon castaneipennis | 39 | early | omnivores | EHydSm |
| Cercyon haemorrhoidalis | 29 | early | omnivores | EHydSm |
| Cercyon impressus | 29 | early | omnivores | EHydSm |
| Cercyon lateralis | 1279 | late | omnivores | LHydSm |
| Cercyon melanocephalus | 20 | early | omnivores | EHydSm |
| Cercyon pygmaeus | 434 | early | omnivores | EHydSm |
| Cercyon quinquiiulus | 25 | early | omnivores | EHydSm |
| Cryptopleurum crenatum | 660 | late | omnivores | LHydSm |
| Cryptopleurum minutum | 2330 | late | omnivores | LHydSm |
| Megasternum concinum | 4 | late | omnivores | LHydSm |
| Sphaeridium bipustulatum | 44 | early | omnivores | EHydSm |
| Sphaeridium lunatum | 42 | early | omnivores | EHydSm |
| Sphaeridium scabracoeides | 4 | early | omnivores | EHydSm |
| Sphaeridium spp. larvae | 226 | late | omnivores | LStaCa |
| Scaranidae | | | | |
| Onthophagus coenobita | 3 | early | coprophages | EScara |
| Onthophagus joanne | 49 | early | coprophages | EScara |
| Onthophagus ovatus | 121 | early | coprophages | EScara |
| Aphodiinae | | | | |
| Aphodius ater | 5 | early | coprophages | EScara |
| Aphodius erraticus | 1 | early | coprophages | EScara |
| Aphodius filmetarius | 346 | late | coprophages | LScara |
| Aphodius fossor | 42 | late | coprophages | LScara |
| Aphodius granarius | 1 | late | coprophages | LScara |
| Aphodius haemorrhoidalis | 79 | late | coprophages | EStaCo |
| Aphodius pusillus | 4 | late | coprophages | EStaCo |
| Aphodius rufus | 16 | early | coprophages | EScara |
| OXYOMUS SILVESTRIS | 58 | late | coprophages | LScara |
| Staphylinidae | | | | |
| Aleocharinae | | | | |
| Allotrichia ruficollis | 312 | late | predators | LStaPr |
| Anthia longicornis | 570 | late | predators | LStaPr |
| Athela sp. 1 | 80 | late | predators | LStaPr |
| Athela sp. 2 | 691 | late | predators | LStaPr |
| Athela sp. 3 | 49 | late | predators | LStaPr |
| Athela sp. 4 | 62 | late | predators | LStaPr |
| Aleochara brevipennis | 21 | late | predators | LStaPr |
| Aleochara intricata | 188 | late | predators | LStaPr |
| Aleochara sparsa | 12 | late | predators | LStaPr |
| Oxytelinae | | | | |
| Anotylus rugosus | 25 | early | coprophages | EStaCo |
| Anotylus tetracarinatus | 1164 | late | coprophages | LStaCo |
| Platystethus arenarius | 258 | late | coprophages | LStaCo |
| Paederinae | | | | |
| Rugillis similis | 43 | late | predators | LStaPr |
| Lathrobium fulvipes | 17 | late | predators | LStaPr |
| Proetinae | | | | |
| Megarthrus denticolli | 51 | late | coprophages | EStaCo |
| Megarthrus depressus | 56 | late | coprophages | EStaCo |
| Family                  | Genus                  | Species                | Number | Type       | Subfamily | Predators  |
|------------------------|------------------------|------------------------|--------|------------|-----------|------------|
| Staphylinae: Staphyllini | Emus hirtus            | 1 early predators      | EStaPr |            |           |            |
|                        | Ontholestes murinus    | 13 early predators     | EStaPr |            |           |            |
|                        | Philonthus coprophilus | 984 late predators     | LStaPr |            |           |            |
|                        | Philonthus cruentatus  | 154 early predators    | EStaPr |            |           |            |
|                        | Philonthus marginatus  | 12 early predators     | EStaPr |            |           |            |
|                        | Philonthus politus     | 114 late predators     | LStaPr |            |           |            |
|                        | Philonthus rectangulus | 31 late predators      | LStaPr |            |           |            |
|                        | Philonthus rotundicollis | 15 late predators   | LStaPr |            |           |            |
|                        | Philonthus sanguinolentus | 156 late predators    | LStaPr |            |           |            |
|                        | Philonthus spinipes    | 1 late predators       | LStaPr |            |           |            |
|                        | Philonthus splendens   | 10 early predators     | EStaPr |            |           |            |
|                        | Philonthus varians     | 575 late predators     | LStaPr |            |           |            |
|                        | Philonthus varius      | 663 late predators     | LStaPr |            |           |            |
|                        | Quedius cinctus        | 2 late predators       | LStaPr |            |           |            |
| Staphylinae: Xantholinini | Gyrohypnus fracticornis | 75 late predators    | LStaPr |            |           |            |
|                        | Gyrohypnus angustatus  | 15 late predators      | LStaPr |            |           |            |
| Tachyporinae            | Cilea silphoides       | 6 late predators       | LStaPr |            |           |            |
|                        | Tachinus lignorum      | 1 late predators       | LStaPr |            |           |            |
|                        | Tachinus signatus      | 20 late predators      | LStaPr |            |           |            |
|                        | Tachyporus nitidulus   | 2 late predators       | LStaPr |            |           |            |
| DIPTERA                 | Anthomyiidae spp. larvae | 157 late coprophages | LAntho |            |           |            |
|                        | Muscidae               |                        |        |            |           |            |
|                        | Muscinae               |                        |        |            |           |            |
|                        | Musca autumnalis larvae | 726 early coprophages | EMusci |            |           |            |
|                        | Mesembriina meridiana larvae | 41 early omnivores | EMesem |            |           |            |
|                        | Mydeinae spp. larvae  | 38 late omnivores     | LMydae |            |           |            |
|                        | Sepsidae               |                        |        |            |           |            |
|                        | Sepsis spp. larvae     | 730 late coprophages   | LAcaly |            |           |            |
|                        | Saltella nigriceps larvae | 24 late coprophages  | LAcaly |            |           |            |
|                        | Saltella spondylii larvae | 1644 late coprophages | LAcaly |            |           |            |
|                        | Sphaeroceridae spp. larvae | 25 late coprophages | LAcaly |            |           |            |
| HYMENOPTERA             | Diapriidae spp.        | 5 late predators       | LParas |            |           |            |
|                        | Figitidae spp.         | 45 late predators      | LParas |            |           |            |
|                        | Proctotrupidae spp.    | 3 late predators       | LParas |            |           |            |
|                        | Scelionidae spp.       | 7 late predators       | LParas |            |           |            |